ChemSusChem

Research Article
doi.org/10.1002/cssc.202301841

Chemistry Europe
European Chemical Societies Publishing

www.chemsuschem.org

Controlled Enzymatic Synthesis of Polyesters Based on a Cellulose–Derived Triol Monomer: A Design of Experiment Approach

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Regioselective enzymatic polycondensation of the bio–based cellulose derived polyol, Triol–citro, and dimethyl adipate using *Candida antarctica* Lipase B (CaLB) was investigated. A Design of Experiment approach with MODDE® Pro 13 was used to determine important factors in the branching behavior of this polymer, and reactant ratio, temperature, reaction time and enzyme wt% were the studied factors. Multifunctional polyesters with pendant hydroxy groups were synthesized and fully characterized using 2D NMR techniques to determine degree of branching. Branching was minimal, with a maximum of 16% observed, and monomer ratio, temperature and reaction time were all determined to be significant factors. In this work, M_n of up to 13 kDa were achieved, while maintaining degree of branching below 15%, resulting in a linear polyester with the potential to be further functionalized.

Introduction

Since the concept first emerged in the 1990s, the drive to incorporate Green Chemistry into all aspects of the chemical industry has steadily increased. The need for sustainability requires researchers to look away from fossil resources and their growing problematic utilization, and instead turn to biomass feedstock. One platform molecule that has risen to prominence in recent years is levoglucosenone (LGO). It can be synthesized from waste cellulose, and there are many publications focusing on more sustainable production of this chemical.[1–4] This chiral molecule containing both a cyclic acetal and an α,β-unsaturated ketone has increasingly been used as a starting feedstock for solvents,[5,6] specialty drugs,[7,8] and polymers.[9–12] A recent example involves the development of a green one–pot pathway to synthesise a multifunctional triol monomer (Triol–citro) from LGO.[13] This monomer features a photocrosslinkable citronellol side group and has been employed in polymer synthesis (Scheme 1).[13–15]

Triol compounds are particularly interesting as there is the potential to synthesise polymers with pendant hydroxy groups. Such polymers often have increased hydrophilicity,[16] which can be useful in drug delivery[17] or membrane applications.[18] The presence of reactive hydroxy groups also opens the polymer to functionalisation for further tailoring of their properties. Quaternary ammonium compounds,[19] proteins[20] and antibodies,[21] have all been grafted to a polymer backbone through reaction with an available functional group. Linear polyesters can be converted to crosslinked structures to improve mechanical properties,[22,23] and these crosslinks can even be reversible to maintain processability.[24] While functional groups can be introduced at the post–polymerisation stage through surface modification techniques such as enzymatic[25] or chemical[26] hydrolysis, as well as physical methods such as plasma treatment,[27] it is important to note that these methods may lead to a reduced molecular weight polymer. Directly polymerizing a trifunctional monomer eliminates this drawback, but to avoid branching resulting from the reaction of all three hydroxy groups, protecting groups or a selective mode of catalysis must be employed.

Lipases are an example of such catalysts, as they catalyze (trans) esterification reactions and often display regioselectivity in their choice of substrate.[28] Biocatalysts are considered more sustainable compared to more traditional organic catalysts; they are safe, energy efficient and minimize the use of toxic reagents. The enzyme *Candida antarctica* Lipase B (CaLB), due to its *sn*-1,3 regioselectivity, has been used to synthesise polymers based on glycerol,[29–43] sorbitol[36,43–49] and other bio–based triols.[45,48] By changing different factors and controlling the degree of branching; crosslinked, linear and dendritic polymers can be produced enzymatically.

Overall, the controlled synthesis of linear polyesters using triol monomers, with a focus on directing the reaction towards two of the three hydroxy groups to prevent or minimize

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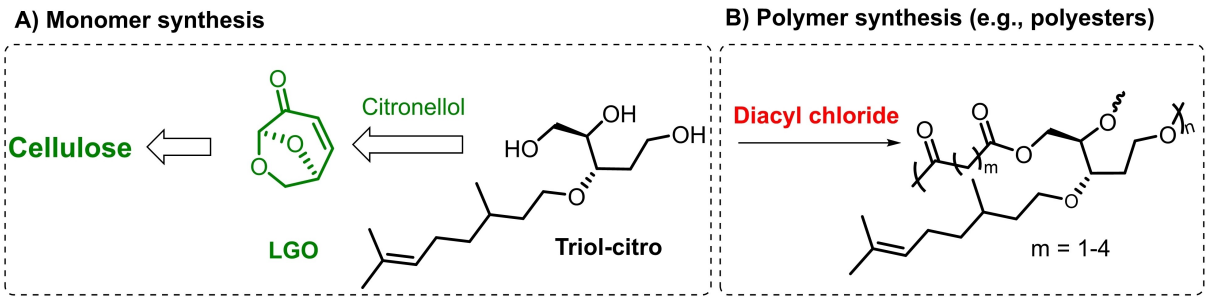
Supporting information for this article is available on the WWW under https://doi.org/10.1002/cssc.202301841



Scheme 1. A. Synthesis of Triol–citro from cellulose and B. polymerisation of Triol–citro with diacyl chlorides to form crosslinked polyesters.[13–15]

branching, is of great interest, but most research focuses on glycerol and sugar–derived compounds. The advantage of a monomer such as Triol–citro is twofold. Firstly, the presence of a second (alkene) functional group allows for more than one functionalisation of the polymer. Alternatively, this citronellol side chain can also be photocrosslinked, as shown in Triol–citro based polycarbonates.[14] Secondly, the synthesis of this molecule is theoretically extremely versatile. The oxa–Michael addition could proceed with a wide range of alkene alcohols, not only citronellol. There are therefore many potential triol based compounds that can be synthesized from LGO using this method.

Triol–citro based polyesters have been synthesized,[13] but use of diacyl chloride reagents in previous approaches resulted in insoluble polymers, due to the formation of branched/crosslinked structures. In addition, from a green chemistry perspective, diacyl chloride poses notable hazards due to its highly reactive and corrosive nature. In contrast, exploring enzymatic approaches, such as utilizing lipase CaLB with diesters, provides a greener and safer alternative for polymer synthesis. Here, the aim is to use CaLB to synthesise linear polyesters from the monomers Triol–citro and dimethyl adipate (DMA) (Scheme 2). A Design of Experiment (DoE) approach was used to investigate the influence of several factors on polymer molecular weight, conversion, and the degree of branching, with the intent of producing high molecular weight and preferentially linear polyesters.

Results and Discussion

Polymer Synthesis and Characterization

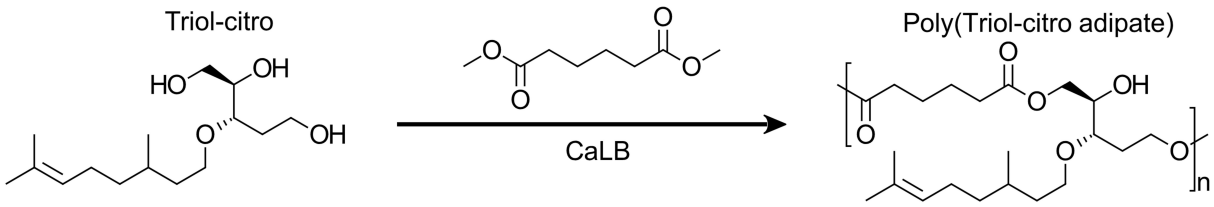
In order to identify the factors that affect branching in the enzymatic polycondensation of polyols, a literature search was conducted. Numerous publications that synthesized polyesters from polyol monomers were found.[29,31–44,46–49] Subsequently, temperature, enzyme wt%, reaction time and monomer ratio were identified as pertinent factors for investigation in this work (Table 1). To streamline and optimize the experimental process, a DoE approach was used.

A central composite design model generated 27 experiments (N1–N27), seen in Table S1. The polymers produced pre–washing were liquids of varying viscosity and degrees of precipitation (see Table S2). Conversions and molecular weights were obtained directly from unwashed sample and were determined using ^{1}H-NMR and GPC analysis, respectively, with Equation (1) used to calculate conversion.

$$Conv.\ (\%) = 100 - \left(\left(\frac{(I_a/N_a)}{(I_a/N_a) + (I_b/N_b)} \right) \times 100 \right) \quad (1)$$

Determining the degree of branching proved to be challenging. ^{1}H-NMR analysis clearly showed the presence of unreacted monomer in the obtained products, as along with other impurities (Figures S1–S27). Attempts were made to wash the crude polymer with MeOH, however a significant portion of the polymer mass consisted of short chain oligomers that were also soluble in MeOH at this concentration. For example, N6 was washed with 2×5 mL MeOH and the mass of the polymer obtained was insufficient for ^{13}C-NMR and any 2D techniques.

Subsequently, three experiments (N28–N30) were performed in a longer timeframe in an attempt to increase



Scheme 2. CaLB–catalyzed polycondensation reaction of Triol–citro and dimethyl adipate to produce linear polyesters.

Table 1. Factors that have been shown to affect branching and the observed effect in the enzymatic polycondensation of polyols in previous literature.

Factor	Effect on branching
Temperature	Increased or decreased branching depending on solvent.[29] Increased branching with increasing temperature.[32]
Enzyme wt%	Increased enzyme wt% results in increased branching.[29,31]
Reaction time	Increased reaction time results in increased branching.[31,39] Increased reaction time increases molecular weight and has no effect on branching.[32]
Monomer ratio	Increasing the amount of polyol monomer results in both increased branching[31], and decreased branching.[29] Excess (other) monomer results in increased branching.[29,35,40]

molecular weight and induce some branching of the polymer, as it has done for other polyol based polyesters in the literature.[31,39] The conditions of experiment N15 (1:1 monomer ratio, 20 wt% enzyme, 90 °C, 24 h) were selected as this was the highest molecular weight polymer produced, with the reaction time extended from 24 h to 96 h. Immediately after the workup, it was evident that the polymer had branched/crosslinked structures to some extent as experiment N28 was mostly insoluble in tetrahydrofuran (THF). Chloroform ($CHCl_3$) dissolved a larger proportion, leaving a small amount of insoluble residue in the glassware. N29 and N30 were soluble in THF and dimethyl sulfoxide (DMSO) which is attributed to the fact that these polymers were synthesized using a different batch of Triol–citro monomer (Figure 1 which was flash purified using a slightly different method (see ESI). NMR analysis for unwashed N28 was performed in $CDCl_3$, which produced spectra of sufficient resolution so as to assign peaks. The remaining amount of N28 was washed (2×1 mL), and an attempt was made to dissolve it in DMSO-d_6 and $CDCl_3$ for analysis, which was unsuccessful. This is likely due to the washing step having removed a lot of the short linear chains and increasing the proportion of branched/crosslinked structures present.

Observing the ^{1}H-NMR spectrum for unwashed N28 (Figure 2), there is a considerable decrease in signal intensity from 3.2–3.6 ppm and the formation of new signals from 3.7–5.0 ppm are observed when compared to that of the Triol–citro monomer. These new signals relate to the formation of ester groups, and the corresponding shift of the signals. To definitively assign signals in the ^{1}H-NMR spectrum, ^{13}C-NMR analysis was also performed, as well as HSQC, HMBC and COSY (Figure 2 and Figures S28–29). From the HSQC, HMBC and COSY, the majority of peaks could be definitively assigned to protons in the polymer structure. It was determined that Triol–citro protons 2, 4 and 7 (Figure 2A), if reacted, will show a shift towards a higher ppm on the ^{1}H-NMR spectra, but not necessarily in the ^{13}C-NMR one. Reacted forms of 2, 4 and 7 are noted as 2′, 4′ and 7′. When reacted, proton 2 shows a shift from 4.07 ppm to 4.22 ppm on ^{1}H, while the ^{13}C peak stays at 66 ppm. Proton 7 shows a shift from 3.76 ppm to 4.22 ppm on ^{1}H, and the ^{13}C stays at 61 ppm. However, proton 4 shows a shift from 3.76 ppm to 4.94 ppm on ^{1}H spectra, and also shows a shift in the ^{13}C spectra, from 71 ppm to around 77 ppm. It is difficult to determine exactly where the ^{13}C peak falls for 4′, as peaks in this region overlap with the $CDCl_3$ signals; both signals for carbons 4′ and 9 are expected to fall in this area. DMSO-d_6 was therefore used as an NMR solvent for samples N29 and N30.

All ^{1}H spectra have the peak characteristic of the methyl groups of DMA (3.56 ppm in DMSO-d_6), meaning unreacted Triol–citro will also likely be present. Furthermore, many experiments were run with an excess of Triol–citro (Table S1), and this unreacted monomer is expected to contribute significantly to the signals for protons 2 and 7. While substituted terminal units may also be responsible for 2′, 4′ and 7′ proton signals, the presence of the 4 substituted terminal unit (as shown in Figure 2A) was deemed improbable due to enzyme regioselectivity. In previous works investigating CaLB synthesized glycerol–based polyesters, glycerol units that had reacted only on the secondary hydroxy were a very small proportion of the polymer,[29,31] and in some cases not observed at all.[31]

Unwashed N29 and N30 were not characterized, instead they were washed with MeOH, which produced sufficient polymer for comprehensive characterization using NMR. DMSO-d_6 was used, resulting in spectra with noticeably fewer impurity peaks and the absence of a sharp peak at 3.56 ppm attributed to the methyl ester group of DMA. Although terminal group analysis of this polymer would be of interest due to its post–polymerisation functionalisation potential, this is not possible due to overlapping peaks in this region. Similar to the ^{1}H spectra of N28, a peak was observed at 4.93 ppm, attributed to 4′ (Figure S32). The ^{13}C-NMR spectra no longer had obscured peaks in the range of 75–80 ppm (Figure S31). The positioning of peaks in DMSO-d_6 spectra differed from $CDCl_3$ spectra slightly, which can be seen when comparing Figure 2B and Figure S32. DMSO-d_6 was therefore used as a solvent for ^{1}H-

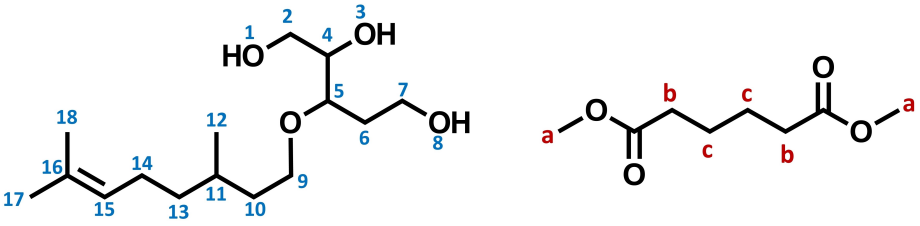


Figure 1. Monomers with labels pertaining to equations (1) and (2).

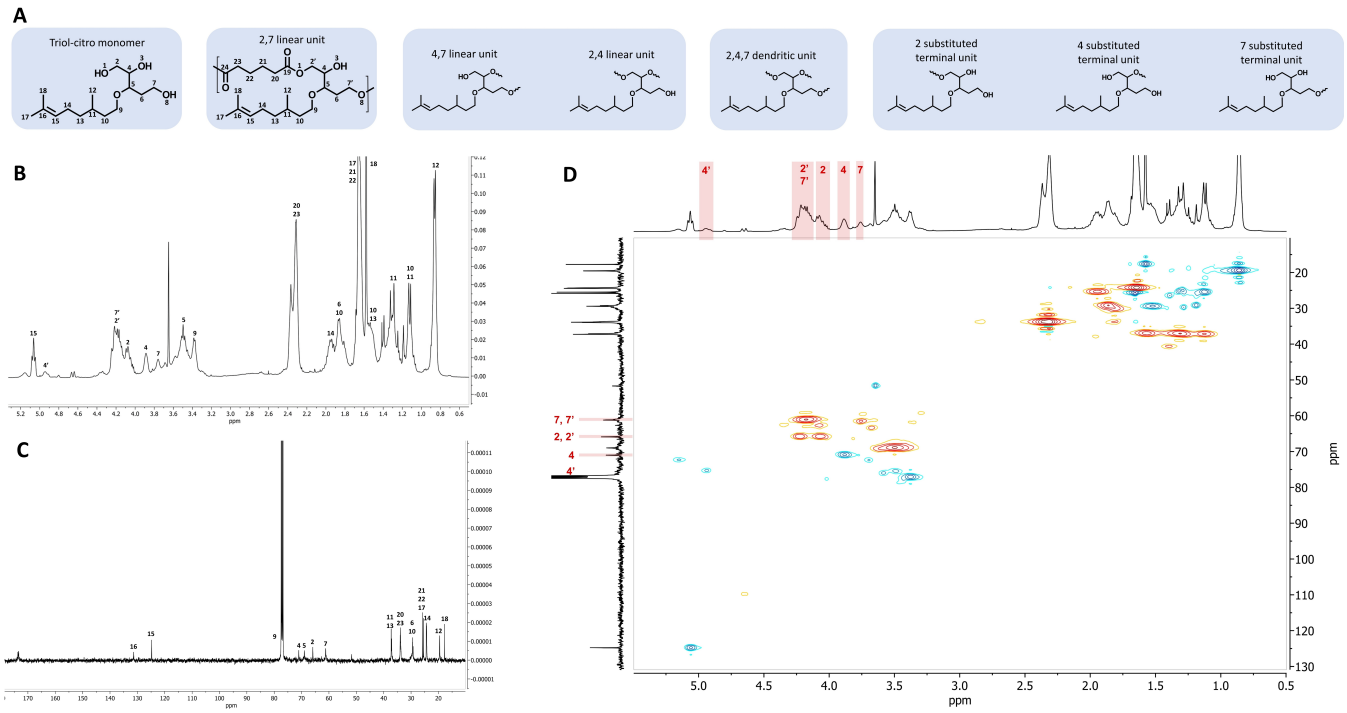


Figure 2. A. Labelled Triol–citro and possible linear, dendritic and terminal structures of the Triol–citro unit within the polyester, with atom labelling of the 2,7 linear unit, B. ^{1}H-NMR spectra of N28 in $CDCl_3$ with peaks fully assigned, C. ^{13}C-NMR spectra of N28 in $CDCl_3$ with peaks fully assigned, and D. HSQC spectra of N28 in $CDCl_3$ with peaks for 2, 2′, 4, 4′, 7 and 7′ highlighted and labelled in red. Peaks labelled with′ are from the reacted form of that atom.

NMR analysis to determine branching of N1–N27. All 27 spectra can be found in the ESI.

Upon observing N1–N27, a peak at 4.93–4.94 ppm is noticeable in the majority of ^{1}H spectra. In many spectra, this peak clearly overlaps with an impurity peak (see Figures S1–27), consequently, selected samples underwent washing in an attempt to eliminate this issue. Polymers with several different molecular weights were selected (N13, N9 and N27) and approx. 100 mg were washed with 2×1 mL MeOH. The solubility of the short chain oligomers in MeOH is evident, as these 2 washes resulted in up to 96% mass loss (Table S3). After washing, a white powder was obtained; a contrast to the samples before washing, which were viscous liquids. These samples proved insoluble in DMSO-d_6, suggesting that this solid fraction is the part of the polymer that has crosslinked or branched. This is supported by the fact that N2, a polymer lacking a peak at 4.93 ppm, was fully soluble in 1 mL of MeOH. Degree of branching was therefore calculated with the spectra of the unwashed polymer using equation (2); with′ denoting the reacted form of the atom (Figure 1), and integrals normalized at the CH_3 peak (0.82 ppm).

$$Branching\ (\%) = \left(\frac{(I_{4'}/N_{4'})}{(I_{4'}/N_{4'}) + (I_{2'+7'}/N_{2'+7'})} \right) \times 100 \qquad (2)$$

The results for those polymers discussed in the text are summarized in Table 2, and a more complete table can be found in the ESI (Table S1). Overall, conversion was consistently high (always >68%), and degree of branching never exceeded 16%, but there was significant variability in molecular weight. Higher molecular weight polymers also exhibited considerably greater dispersity (*Đ*), with a highest observed *Đ* of 37.3 (polymer N30). Such high dispersities are unusual for polycondensations conducted in bulk,[50] especially for reactions N29 and N30, which were run for 96 h. This can likely be attributed to a combination of molecular weight and branching behavior of these polymers, as branched structures exhibit a much larger diversity in shape compared to linear structures. Once a structure has branched, it has more sites where polycondensation can occur, further increasing molecular weight in the branched chains. There are many examples in the literature of highly disperse branched polymers to the extent that many researchers focus on limiting *Đ* in these structures.[51,52]

To gain a better understanding of factors that significantly affected these parameters, as well as any interaction effects, the results were analyzed using DoE software.

DoE Analysis

The raw data was analyzed using MODDE®. Data for N28–N30 were not included as N28 had a considerable amount of insoluble residue which could not be tested and N29 and N30 were washed with methanol, removing a significant amount of short chain oligomers. This would affect the degree of branching and molecular weight respectively. Data for molecular weight and conversion required a transformation to obtain a normal data distribution; a logarithm (Log (10 Log (Y)) and negative logarithm (−10 Log (1–Y)) transformation respectively.

Table 2. Effect of several reaction conditions (highlighted in grey) on the conversion, molecular weight and degree of branching (DB) observed in the synthesis of poly (Triol–citro adipate).

Reaction	Triol/DMA [moles]	Enzyme [wt%]	Temp. [°C]	Reaction time [h]	Conv. [%][a]	Mn [Da][b]	Mw [Da][b]	Đ[b]	DP[b]	DB [%][a]
N2	2	5	40	6	68	2,058	4,792	2.33	5.8	0
N9	1	5	40	24	88	735	869	1.18	2.1	13
N13	1	5	90	24	92	960	1,335	1.39	2.7	12
N15	1	20	90	24	92	2,789	8,044	2.88	7.8	16
N27	1.5	12.5	65	18	93	1,161	2,130	1.84	3.3	9
N28	1	20	90	96	92	–	–	–	–	13
N29	1	20	90	96	100[c]	13,300	157,400	11.82	37.4	13
N30	1	20	90	96	100[c]	13,000	484,500	37.3	36.4	12

[a] Determined through ^{1}H NMR. [b] Determined through GPC analysis (All chromatograms available in ESI, Figures S35–63). N28 was not analyzed due to lack of sample so is marked with [c] Calculated after washing with MeOH to remove DMA.

Selection of significant terms for the coefficients plot was performed using the auto tune function.

The summary of fits for each factor tested can be seen in Figure 3A, where a value of 1 is optimal. The model for the branching factor is statistically significant, with a $R^2 > 0.5$, a $Q^2 > 0.1$ and a difference of < 0.3 between the two parameters. The conversion and molecular weight factors, however, have a low validity and a large difference between the R^2 and Q^2 parameters. R^2 is a measure of how well the model fits to the original data, while Q^2 shows how well this model can be used to predict future datasets. Although Q^2 for molecular weight and conversion is above 0.1, the rather low model validity indicates statistically significant issues for both conversion and molecular weight. Nevertheless, the main objective of the DoE was to maximize the limitation of the polymer branching, and the model for this response was both valid and reproducible.

From the graph (Figure 3C), although there are many factors that look to have an effect on the molecular weight, the p values (Table S4) show that only temperature is significant (at a confidence value of 0.95). Temperature is well known to affect molecular weight in enzymatic polycondensations; by increasing reaction temperature higher molecular weights are achieved. This has been observed for many different polymer systems,[53,54] although temperature is limited by the thermo-

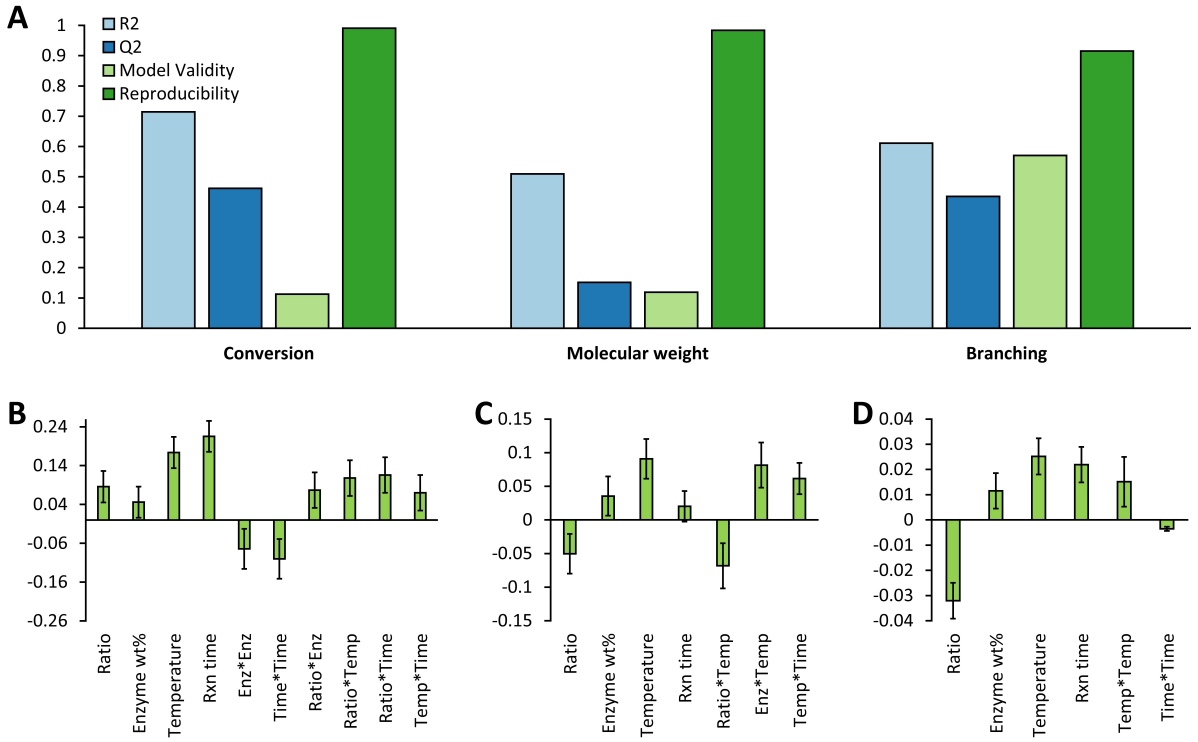


Figure 3. A. Summary of fit for all responses showing the degree of fit (R2), prediction power (Q2) model validity and reproducibility, and the coefficients plot for *B. conversion*, *C. molecular weight* and *D. branching.*

stability of the enzyme. Interaction effects for temperature are also significant; namely Enz*Temp and Temp*Time. Mahapatro *et al.* also observed some interaction between reaction time and temperature, as no significant effect of temperature on M_n was observed up to 24 h, but at 48 h there was a significant difference in the M_n between 65 and 90 °C.[53] This should be further investigated, as GPC analysis of N29 and N30, which were run for 96 h, gave much higher molecular weights.

At a 0.95 confidence, temperature and reaction time are significant factors for the conversion, which agrees with established literature. The monomer ratio is significant only when it interacts with either temperature or time (Ratio*Temp and Ratio*Time). Conversion did not differ greatly between conditions, ranging from $\geq$68% up to 99%, with the lowest conversion obtained at 40 °C run for 6 h. Enzyme wt% seems to have little effect on the conversion, indeed, with all other conditions kept the same, an increase from 1 wt% to 25 wt% of enzyme actually resulted in a decrease in conversion (from 93% to 90%) seen in Table S1.

Ratio, temperature, reaction time and Time*Time are all significant factors ($p = 0.95$) that determine the degree of branching (Figure 3D). The predictive contour plot in Figure 4. gives an overview of this, and it can clearly be seen that a higher temperature, lower ratio and a longer reaction time are associated with a higher degree of branching, while enzyme wt% is much less significant. Increasing amounts of Triol–citro, increasing temperature and increasing reaction time all result in more branched structures. The most branched structure obtained (N15) had a 16% degree of branching, was run at the highest temperature (90 °C), had the longest reaction time (24 h) and had a 1:1 ratio of triol to diester.

The fact that enzyme wt% is an insignificant parameter in predicting degree of branching supports the hypothesis that branching is mainly due to acyl migration rather than enzymatic coupling on the secondary hydroxy group. This phenomenon is well studied in carbohydrates, and is an intramolecular reaction that allows the exchange of ester groups among adjacent hydroxy groups,[55] independent of the enzyme.[56] This reaction has been shown to be affected by temperature, reaction time, reaction solvent, material used to immobilize the enzyme, pH and acyl species.[57] Temperature is particularly significant, both in literature and in this work, which is to be expected as this reaction is a thermodynamic process.[58]

Perin *et al.*, determined that in the enzymatic synthesis of esterification of poly (glycerol sebacate), the esterification of primary hydroxy groups was mainly due to acyl migration. When compared to our work, they observed much higher degrees of branching, which the authors hypothesized could be due to the use of solvent or carboxylic acids as reagents.[29] Solvent affects acyl migration in different ways. Polarity has been shown to have an effect, with low polarity solvents dispersing the charge of the transition state, lowering its energy state and decreasing the rate of the migration reaction.[57] However when compared to solventless systems, Wang *et al.* found that all solvents tested had an inhibitory effect on acyl migration (albeit to different degrees).[59] The use of solvent may also have a beneficial effect on molecular weight, as solventless polycondensations often lead to a highly viscous reaction matrix. This was most obvious in reactions N28–N30, where polymers were viscous and gel–like, and had visibly hindered stirring. Use of diesters as an alternative to dicarboxylic acids would also reduce the rate of acyl migration, due to the different leaving groups. Although both water and methanol have been found to increase the rate of acyl migration,[57,60] methanol is easier to remove, and is done so via reduced pressure in this work.

Aside from this, there are quite a number of factors affecting acyl migration that were not investigated here. The CaLB used was immobilized on an acrylic resin, which is a polar carrier, and more polar materials have been known to increase acyl migration.[57]

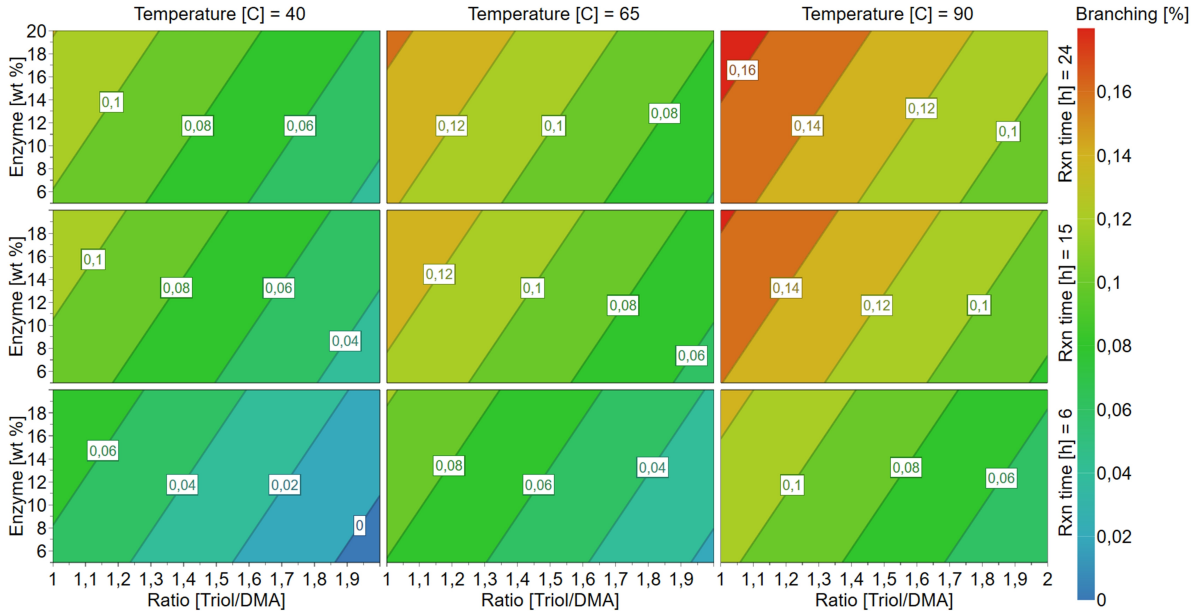


Figure 4. 4D contour response plot showing the effect of monomer ratio, enzyme wt%, temperature and reaction time on the degree of polymer branching.

Furthermore, the structure of Triol–citro may also have an influence. Unlike glycerol or other sugars such as sorbitol, only two hydroxy groups are adjacent in this monomer; instead of an α-β-γ there is an α-β-ε triol structure. A previous work attempted to induce acyl migration over the same number of C atoms in mannopyranoside structures, but were unsuccessful, suggesting that two hydroxy groups must be spatially close for this reaction to occur.[61]

Conclusions

Overall the desired outcome must be carefully considered. For example, reaction N15 gave the highest molecular weight, but also resulted in the most branched structure, at 16%. It is worth performing several more experiments to obtain a valid model for both molecular weight and conversion. In this way, all three responses can be predicted, and parameters can be carefully selected based on the desired properties of the final polymer. With respect to obtaining a high molecular weight, the design space with regard to reaction time should be extended to 96 hours, and the use of solvent should be considered.

The utility of CaLB in selectively polymerizing bulky triol monomers has been clearly shown. This work investigated the use of MODDE to determine significant factors in the branching behavior of poly (Triol–citro adipate), and determined that monomer ratio, temperature and reaction time are all significant factors. It was clearly demonstrated that branching was minimal, with a maximum of 16% observed, and this was attributed to acyl migration rather than enzyme catalyzed transesterification. Extending the reaction time to 96 h resulted in a polymer with a number average molecular weight of up to 13 kDa and only 13% of branching. CaLB has been shown to be a suitable biocatalytic alternative to traditional metal catalysts in this reaction. Polyesters produced using this method can be further functionalized through reactions with pendant hydroxy groups for more specialized purposes.

Experimental Section

Materials

Tetrahydrofuran (inhibitor–free, for HPLC, ≥99.9%), Methanol (for HPLC, gradient grade, ≥99.9%), Chloroform (HiPerSolv CHROMANORM), and *Candida antarctica* lipase B immobilized on acrylic resin (CaLB, code: L4777, ≥5,000 U/g) were purchased from Sigma Aldrich. Dimethyl adipate (99%) was purchased from Alfa Aesar. All deuterated solvents were purchased from Eurisotop.

Trio–Citro was Synthesised According to a Previously Published Procedure[13]

Design of Experiment (DoE)

The software MODDE® Pro 13 (Sartorius) was used to optimize polycondensation conditions. Reactant ratio, enzyme wt%, temperature and reaction time were selected as quantitative factors. Conversion, number average molecular weight (M_n) and degree of branching were the responses measured, with target for conversion set to 99%, M_n target set high and degree of branching set at 1%. A central composite orthogonal (CCO) design (star distance: 1.54671) was chosen with a quadratic model, to generate a set of experiments for optimisation.

Enzymatic Polycondensations

Triol–citro (233.2 mg, 8.5×10^{-4} mol) and the appropriate amount of dimethyl adipate (6.4×10^{-4} mol–1.9×10^{-3} mol) were added to a 25-mL round bottom flask with CaLB (1–25% by weight of monomers). The flask was heated (25–100 °C) and stirred at 400 rpm for 6 hours, at which point the system was placed under vacuum (20 mbar) in order to remove the methanol by-product. Experiments were run from 1–30 hrs, and experiments that were run for ≤6 hrs were not placed under vacuum.

Upon completion of the reaction, the polymers were washed with approx. 2 mL THF and filtered through cotton packed into a glass Pasteur pipette to remove the insoluble enzyme pellet. The flask was washed with 1 mL THF a further two times, and all fractions were filtered and collected. THF was removed via a rotary evaporator, and the resulting polymer was characterized by NMR and GPC analysis.

If a wash step was performed, the polymer was transferred into a 1.5 mL or 5 mL Eppendorf, precipitated through addition of ice–cold methanol (amount as specified), and centrifuged (10 min at 4 °C, 3700 rpm for 5 mL Eppendorfs and 12700 rpm for 1.5 mL Eppendorfs). The supernatant was removed and the washing step repeated as specified. The resulting polymer was characterized by NMR analysis.

Characterization

Gel permeation Chromatography (GPC)

Polymers were dissolved in $CHCl_3$ or THF to a concentration between 2.0 and 2.5 mg/mL and filtered through cotton. The analysis was performed at 30 °C on an Agilent Technologies HPLC System (Agilent Technologies 1260 Infinity) and an Agilent Technologies G1362 A refractive index detector was employed for detection. Linear polystyrene calibration standards (250–70,000 Da) purchased from Sigma–Aldrich were used to calculate the molecular weights of the polymers.

For those polymers dissolved in $CHCl_3$, a 17,369 6.0 mm ID×40 mm LHHR–H, 5 μm Guard column and a 18,055 7.8 mm ID×300 mm L GMHHR–N, 5 μm TSK gel liquid chromatography column (Tosoh Bioscience, Tessenderlo, Belgium) was used, with $CHCl_3$ as an eluent (at a flow rate of 1 mL min^{-1} for 20 min). For those polymers dissolved in THF, a guard column and chromatography column of the same specification was used with THF as the eluent (at a flow rate of 1 mL min^{-1} for 20 min).

Nuclear Magnetic Resonance (NMR)

^{1}H-NMR, ^{13}C-NMR, COSY, HMBC and HSQC spectroscopy was performed using a JEOL ECZ400R/S3 at a frequency of 400 MHz, with some ^{1}H-NMR performed on a Bruker Avance II 400 (resonance frequencies 400.13 MHz for ^{1}H) equipped with a 5 mm N_2-cooled cryo probe (Prodigy) with z–gradients at room temperature with standard Bruker pulse programs. Samples were dissolved in either $CDCl_3$ or DMSO-d_6 as a solvent.

Acknowledgements

Dr. Louis M. M. Mouterde (URD ABI) is acknowledged for his contribution to the original synthesis of Triol–citro (reference[13]).

This work is supported by European Union's Horizon 2020 research and innovation programme under grant agreement No 953073, project UPLIFT (sUstainable PLastIcs for the Food and drink packaging indusTry). S. F. and F. A. thank Grand Reims, Département de la Marne and Grand Est Region for their financial support. Open Access publishing facilitated by Università degli Studi di Genova, as part of the Wiley - CRUI-CARE agreement.

Conflict of Interests

The authors declare no conflict of interest.

Data Availability Statement

The data that support the findings of this study are available from the corresponding author upon reasonable request.

Keywords: Bio–based · Enzyme catalysis · Green Chemistry · NMR spectroscopy · Polyesters

[1] F. Cao, T. J. Schwartz, D. J. McClelland, S. H. Krishna, J. A. Dumesic, G. W. Huber, *Energy Environ. Sci.* **2015**, *8*, 1808–1815.
[2] S. Saragai, S. Kudo, J. Sperry, U. P. M. Ashik, S. Asano, J. Hayashi, *Bioresour. Technol.* **2022**, *344*, 126323.
[3] A. M. Sarotti, R. A. Spanevello, A. G. Suárez, *Green Chem.* **2007**, *9*, 1137–1140.
[4] J. Mazarío, M. P. Romero, P. Concepción, M. Chávez-Sifontes, R. A. Spanevello, M. B. Comba, A. G. Suárez, M. E. Domine, *Green Chem.* **2019**, *21*, 4769–4785.
[5] J. Sherwood, M. De bruyn, A. Constantinou, L. Moity, C. R. McElroy, T. J. Farmer, T. Duncan, W. Raverty, A. J. Hunt, J. H. Clark, *Chem. Commun.* **2014**, *50*, 9650–9652.
[6] C. M. Warne, S. Fadlallah, A. C. Whitwood, J. Sherwood, L. M. M. Mouterde, F. Allais, G. M. Guebitz, C. R. McElroy, A. Pellis, *Green Chem. Lett. Rev.* **2023**, *16*, 2154573.
[7] J. E. Camp, B. W. Greatrex, *Front. Chem.* **2022**, *10*, 902239. doi: 10.3389/fchem.2022.902239.
[8] B. T. Sharipov, A. N. Davidova, A. S. Ryabova, N. F. Galimzyanova, F. A. Valeev, *Chem. Heterocycl. Compounds* **2019**, *55*, 31–37.
[9] S. Fadlallah, A. A. M. Peru, L. Longé, F. Allais, *Polym. Chem.* **2020**, *11*, 7471–7475.
[10] S. Fadlallah, L. M. M. Mouterde, G. Garnier, K. Saito, F. Allais, in *ACS Symp. Ser.* (Eds.: H. N. Cheng, R. A. Gross), American Chemical Society, Washington, DC, **2020**, pp. 77–97.
[11] P. Ray, T. Hughes, C. Smith, G. P. Simon, K. Saito, *ACS Omega* **2018**, *3*, 2040–2048.
[12] M. G. Banwell, X. Liu, L. A. Connal, M. G. Gardiner, *Macromolecules* **2020**, *53*, 5308–5314.
[13] A. Kayishaer, S. Fadlallah, L. M. M. Mouterde, A. A. M. Peru, Y. Werghi, F. Brunois, Q. Carboué, M. Lopez, F. Allais, *Molecules* **2021**, *26*, 7672.
[14] S. Fadlallah, A. Kayishaer, M. Annatelli, L. M. M. Mouterde, A. A. M. Peru, F. Aricò, F. Allais, *Green Chem.* **2022**, *24*, 2871–2881.
[15] A. Kayishaer, M. Annatelli, C. M. Hansom, L. M. M. Mouterde, A. A. M. Peru, F. Aricò, F. Allais, S. Fadlallah, *Macromol. Rapid Commun.* **2023**, *n/a*, 2300483.
[16] M. Y. Lee, W. H. Park, R. W. Lenz, *Polymer* **2000**, *41*, 1703–1709.
[17] M. Anwar, F. Pervaiz, H. Shoukat, S. Noreen, K. Shabbir, A. Majeed, S. Ijaz, *Polym. Bull.* **2021**, *78*, 59–80.
[18] X. Yang, L. Yan, J. Ma, Y. Bai, L. Shao, *J. Membr. Sci.* **2019**, *591*, 117353.
[19] R. Wang, T. Xiang, W.-F. Zhao, C.-S. Zhao, *Mater. Sci. Eng. C* **2016**, *59*, 556–564.
[20] P. Jonkheijm, D. Weinrich, M. Köhn, H. Engelkamp, P. C. M. Christianen, J. Kuhlmann, J. C. Maan, D. Nüsse, H. Schroeder, R. Wacker, R. Breinbauer, C. M. Niemeyer, H. Waldmann, *Angew. Chem. Int. Ed.* **2008**, *47*, 4421–4424.
[21] Y. Bai, C. G. Koh, M. Boreman, Y.-J. Juang, I.-C. Tang, L. J. Lee, S.-T. Yang, *Langmuir* **2006**, *22*, 9458–9467.
[22] G. X. De Hoe, M. T. Zumstein, B. J. Tiegs, J. P. Brutman, K. McNeill, M. Sander, G. W. Coates, M. A. Hillmyer, *J. Am. Chem. Soc.* **2018**, *140*, 963–973.
[23] J. Tang, Z. Zhang, Z. Song, L. Chen, X. Hou, K. Yao, *Eur. Polym. J.* **2006**, *42*, 3360–3366.
[24] A. Tanaka, M. Kohri, T. Takiguchi, M. Kato, S. Matsumura, *Polym. Degrad. Stab.* **2012**, *97*, 1415–1422.
[25] F. Quartinello, K. Kremser, S. Vecchiato, H. Schoen, R. Vielnascher, L. Ploszczanski, A. Pellis, G. M. Guebitz, *Front. Chem.* **2019**, *7*.
[26] M. Getnet, R. Chavan, *Int. J. Sci.* **2015**, *22*.
[27] D. Djidi, N. Mignard, M. Taha, *Ind. Crops Prod.* **2015**, *72*, 220–230.
[28] J. Yang, Y. Liu, X. Liang, Y. Yang, Q. Li, *Macromol. Biosci.* **2018**, *18*, 1800131.
[29] G. B. Perin, M. I. Felisberti, *Macromolecules* **2020**, *53*, 7925–7935.
[30] G. B. Perin, M. I. Felisberti, *Biomacromolecules* **2022**, *23*, 2968–2975.
[31] Á. S. Olalla, V. H. Talavera, D. L. García, E. G. Torres, M. F. García, *Eur. Polym. J.* **2022**, *170*, 111173.
[32] V. Taresco, R. G. Creasey, J. Kennon, G. Mantovani, C. Alexander, J. C. Burley, M. C. Garnett, *Polymer* **2016**, *89*, 41–49.
[33] T. Tsujimoto, H. Uyama, S. Kobayashi, *Biomacromolecules* **2001**, *2*, 29–31.
[34] H. Uyama, K. Inada, S. Kobayashi, *Macromol. Biosci.* **2001**, *1*, 40–44.
[35] B. J. Kline, E. J. Beckman, A. J. Russell, *J. Am. Chem. Soc.* **1998**, *120*, 9475–9480.
[36] A. Kumar, A. S. Kulshrestha, W. Gao, R. A. Gross, *Macromolecules* **2003**, *36*, 8219–8221.
[37] P. Kallinteri, S. Higgins, G. A. Hutcheon, C. B. St Pourçain, M. C. Garnett, *Biomacromolecules* **2005**, *6*, 1885–1894.
[38] C. Korupp, R. Weberskirch, J. J. Müller, A. Liese, L. Hilterhaus, *Org. Process Res. Dev.* **2010**, *14*, 1118–1124.
[39] Z.-K. Rao, H.-L. Ni, Y. Li, H.-Y. Zhu, Y. Liu, J.-Y. Hao, *Macromol. Chem. Phys.* **2019**, *220*, 1900048.
[40] Y. Yang, W. Lu, J. Cai, Y. Hou, S. Ouyang, W. Xie, R. A. Gross, *Macromolecules* **2011**, *44*, 1977–1985.
[41] Y.-R. Zhang, S. Spinella, W. Xie, J. Cai, Y. Yang, Y.-Z. Wang, R. A. Gross, *Eur. Polym. J.* **2013**, *49*, 793–803.
[42] A. S. Kulshrestha, W. Gao, R. A. Gross, *Macromolecules* **2005**, *38*, 3193–3204.
[43] M. Jbeily, T. Naolou, M. Bilal, E. Amado, J. Kressler, *Polym. Int.* **2014**, *63*, 894–901.
[44] H. Uyama, E. Klegraf, S. Wada, S. Kobayashi, *Chem. Lett.* **2000**, *29*, 800–801.
[45] M. H. Bilal, M. Prehm, A. E. Njau, M. H. Samiullah, A. Meister, J. Kressler, *Polymer* **2016**, *8*, 80.
[46] L. Gustini, B. A. J. Noordover, C. Gehrels, C. Dietz, C. E. Koning, *Eur. Polym. J.* **2015**, *67*, 459–475.
[47] L. Gustini, C. Lavilla, A. M. de Ilarduya, S. Muñoz-Guerra, C. E. Koning, *Biomacromolecules* **2016**, *17*, 3404–3416.
[48] D.-Y. Kim, J. S. Dordick, *Biotechnol. Bioeng.* **2001**, *76*, 200–206.
[49] Y. Mei, A. Kumar, W. Gao, R. Gross, S. B. Kennedy, N. R. Washburn, E. J. Amis, J. T. Elliott, *Biomaterials* **2004**, *25*, 4195–4201.
[50] A. Mahapatro, B. Kalra, A. Kumar, R. A. Gross, *Biomacromolecules* **2003**, *4*, 544–551.
[51] R. K. Roy, S. Ramakrishnan, *Macromolecules* **2011**, *44*, 8398–8406.
[52] Y. Lu, T. Nemoto, M. Tosaka, S. Yamago, *Nat. Commun.* **2017**, *8*, 1863.
[53] A. Mahapatro, A. Kumar, B. Kalra, R. A. Gross, *Macromolecules* **2004**, *37*, 35–40.
[54] W.-X. Wu, J. Li, X.-L. Yang, N. Wang, X.-Q. Yu, *Eur. Polym. J.* **2019**, *121*, 109315.
[55] R. Lassfolk, M. Pedrón, T. Tejero, P. Merino, J. Wärnå, R. Leino, *Chem. Eur. J.* **2022**, *28*, DOI 10.1002/chem.202200499.
[56] W. Li, W. Du, Q. Li, T. Sun, D. Liu, *J. Mol. Catal. B* **2010**, *63*, 17–22.
[57] Y. Mao, Y.-Y. Lee, X. Xie, Y. Wang, Z. Zhang, *J. Funct. Foods* **2023**, *106*, 105616.
[58] T. Yang, M.-B. Fruekilde, X. Xu, *Food Chem.* **2005**, *92*, 101–107.
[59] X. Wang, X. Zhao, Z. Yang, X. Wang, T. Wang, *J. Agric. Food Chem.* **2020**, *68*, 12358–12364.
[60] S. Hardison, S. T. Weintraub, A. Giuffrida, *Prostaglandins Other Lipid Mediat.* **2006**, *81*, 106–112.
[61] F. S. Ekholm, A. Ardá, P. Eklund, S. André, H.-J. Gabius, J. Jiménez-Barbero, R. Leino, *Chem. Eur. J.* **2012**, *18*, 14392–14405.

Manuscript received: December 7, 2023
Revised manuscript received: March 26, 2024
Accepted manuscript online: March 28, 2024

Version of record online: April 30, 2024