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# Lipase-catalysed polycondensation of levulinic acid derived diol-diamide monomers: access to new poly(ester-*co*-amide)s†

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Research toward bio-based polymers is an expanding field due to environmental concerns. A library of new aliphatic diol-diamide monomers with different chain lengths between the two amido groups was synthesized from sustainable levulinic acid and various linear aliphatic diamines ($C_2$–$C_{10}$). The monomers were prepared by diacylation of the diamines followed by reduction of the ketones to alcohols. These secondary diols were successfully recognized by an enzyme and polymerised in solution through a lipase-catalysed polycondensation. Poly(ester-*co*-amide)s with number-average molecular weights ($M_n$) in the range of 1300–7200 g mol$^{-1}$ were obtained, with dispersities between 1.5 and 1.8. An improvement of the $M_n$ value was observed upon increasing the monomer chain length. The variation of the aliphatic diol allows modulating the thermal properties of the final polymers. The glass transition temperatures were found to be between –23 °C and 0 °C. The polymers containing a long aliphatic segment ($C_8$–$C_{10}$) were able to crystallize (melting temperature of 90–97 °C). TGA analyses showed that the ester linkages degrade at lower temperatures than the amide bonds. The stability of the latter was found to be higher when the number of methylene units increased from 2 (355 °C) to 10 (378 °C). This kind of biopolymer could be used as a drug delivery system or for tissue engineering applications.

## Introduction

Research toward bio-based polymers is a growing and important field due to environmental concerns and fossil resource depletion. This encourages the transformation of renewable biomass, *i.e.* starch, oil and lignocelluloses, into valuable chemical molecules, especially monomers.[1,2] Alternatively, the polysaccharide feedstock gives access to different green chemical building blocks such as 5-hydroxymethylfurfural (HMF), and lactic, succinic, glutaric and levulinic acids.[3,4] As being recognized as one of the top 12 building blocks, levulinic acid is a promising bio-based platform due to its competitive cost and large scale production (around 2600 tons per year) from renewable feedstocks. Generally, mineral acid (sulfonic, hydrochloric and methanesulfonic acids) catalysed treatment of sugars (glucose, fructose, galactose, xylose), obtained from the hydrolysis of polysaccharides such as cellulose, lignocellulose, hemicellulose or starch, gives access to HMF or furfural (transforms in furfuryl alcohol by reduction), which upon rehydration leads to levulinic acid (Scheme 1).[5–7] This process yields 34 to 80% levulinic acid depending on precursors.[8] Formic acid is formed as a by-product which can be used as a commodity chemical in the production of formaldehyde, rubber and plasticizers.[9] The use of heterogeneous catalysts, such as Amberlite IR 120, clay minerals, and zeolites, was reported as an alternative to mineral acids, notably due to the difficulty in recovering the former, with yields up to 70%.[8]

Levulinic acid can be converted into value-added products, finding application in several fields such as fuels, additives, pharmaceutical intermediates, solvents, plasticizers and polymers.[8,10–12] For example, a library of biobased ketal–diester derivatives of levulinic acid plasticizers was synthesized and improved the properties of the poly(vinyl chloride)

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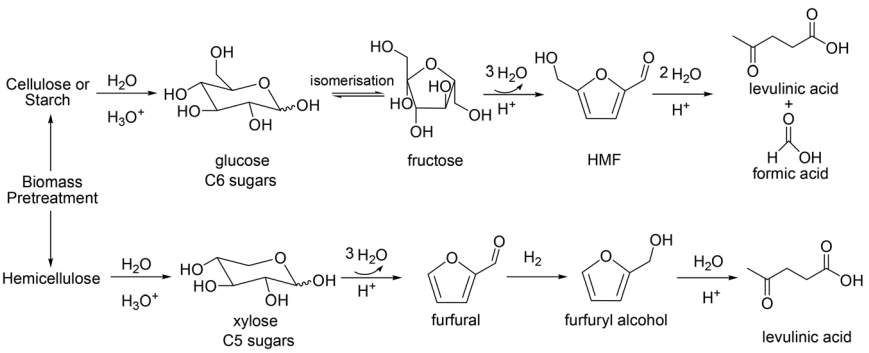



**Scheme 1** Different pathways to access levulinic acid from biomass.[5,7,8]

material.[13] The conversion of levulinic acid into valuable monomers is also an interesting way to obtain renewable specialty polymers. For instance, poly(amine-*co*-ester)s,[14] and polyesters bearing a pendant lactam group,[15,16] were recently prepared from levulinic acid *via* reductive amination or *via* a multicomponent reaction. The condensation of levulinic acid with glycerol and erythritol to form ketals has also been exploited to access polyesters and polyurethanes.[17–19] Levulinic acid can also be directly converted into γ-valerolactone, which can be directly polymerised or further transformed into diol-diamide valuable precursors for polyurethane[20–22] and poly(ester-*co*-amide)[23] syntheses.

Bio-based poly(ester-*co*-amide)s have been less investigated than bio-based polyesters or polyamides.[24–27] Poly(ester-*co*-amide)s are biodegradable polymers with great potential in the biomedical field notably. They have been incorporated in controlled drug delivery systems,[28] hydrogels,[29] tissue engineering[30] and adhesives.[31] The presence of ester and amide moieties in their structure confers them the degradable character of the ester linkage and good thermal and mechanical properties due to the hydrogen-bonding abilities of the amide groups. The incorporation of the amide bond into the backbone of polyesters (polyethylene succinate, polypropylene succinate, polybutylene succinate...) was reported *via* copolymerisation of crystallizable symmetrical diols containing two internal amide bonds (from for example the reaction of ε-caprolactone/γ-butyrolactone and different diamines), diols (*e.g.* ethylene glycol, 1,3-propanediol, 1,4-butanediol) and diacids/diesters. The purpose was to enhance the thermal properties of polyesters *via* the introduction of amide groups.[32–34] The mechanical properties were also improved, with a higher elastic modulus and stress at break.[35] The properties can be tuned by changing the ratio of the comonomers.

Different routes have been explored for the synthesis of poly(ester-*co*-amide)s.[36,37] The ring-opening polymerisation (ROP) of cyclic depsipeptides, mediated *via* either stannous octoate[38] or an enzyme,[39] has been described. The ring-opening polymerisation of *N*-acylated-1,4-oxazepan-7-one monomers catalysed by the 1,5,7-triazabicyclo[4.4.0]dec-5-ene (TBD)/thiourea combination was also described to access poly(ester-*co*-amide)s *via* controlled/living polymerisation.[40] Base-catalysed step-growth reactions involving activated acyl chloride/dicarboxylic acid derivatives by incorporation of a leaving group agent (*e.g.*, acyl chloride or *p*-nitrophenol, *p*-chlorophenol-*N*-hydroxysuccinimide esters)[41,42] or interfacial polycondensation[43] were reported as well as metal-catalysed melt polycondensation.[43]

Enzyme-catalysed polycondensation has also been reported as an efficient and eco-friendly strategy to prepare poly(ester-*co*-amide)s.[44] For example, an amide-amino-alcohol monomer bearing a primary alcohol was polymerised with diethyl sebacate in diphenyl ether under vacuum.[45] Furan-aliphatic poly(ester-*co*-amide)s were also synthesized from primary diols, diamines (or amino-alcohols) and dimethyl 2,5-furandicarboxylate by enzymatic polycondensation in toluene or in an ionic liquid solvent.[46] The synthesis of poly(ester-*co*-amide)s with polydimethylsiloxane blocks was also successfully performed in bulk *via* lipase catalysis under mild conditions using the association of three monomers: diethyl adipate, 1,8-octanediol, and α,ω-(diaminopropyl)polydimethylsiloxane.[47] As a matter of fact, enzymatic polycondensation was significantly less reported for the synthesis of poly(ester-*co*-amide)s[44] compared to the impressive amount of research devoted to polyesters[48–52] and polyamides.[45,53–55] Among the lipases, Novozyme 435 (immobilized *Candida antarctica* lipase B) is probably the most widely applied. However, due to the low reactivity of lipases towards secondary alcohols, most of the polycondensations were conducted from primary alcohols. It was shown for instance that the enzymatic polyesterification of sorbitol or glycerol with adipic acid leads to polyesters with unreacted pendant secondary hydroxyl groups (respectively 95% and 66%).[56] The enzymatic polycondensation of sebacic acid with aliphatic diols containing both primary and secondary alcohol groups was also reported with the number average molecular weight ($M_n$) up to 5700 g mol$^{-1}$.[57] In addition to steric hindrance, the presence of an asymmetric center has proven to have an impact on the reactivity of lipase toward secondary diols as well. As an illustration of this, it has been highlighted that the enantioselectivity of the lipase towards the *R*-configured centers of racemic α,α′-dimethyl-1,4-benzenedimethanol leads to reduction in the total conversion (reactivity ratio $R/S = 10^6$).[58] It is finally worth mentioning that the syn-

thesis of polyesters from a bicyclic secondary diol, isosorbide (1,4 : 3,6-dianhydro-D-sorbitol), with molecular weights up to $M_n$ = 20 000 g mol$^{-1}$ has been reported in the literature.[59]

Due to the poor recognition of secondary alcohols by lipases, it is challenging to obtain a polymer from secondary diols by enzymatic polycondensation. As discussed previously, only a few studies presented the synthesis of poly(ester-*co*-amide)s *via* enzymatic catalysis,[45–47] and to our knowledge, we report herein the first example of a step-growth synthesis of poly(ester-*co*-amide) polymers from a secondary diol *via* biocatalysis. The synthesis of new secondary diol-diamide monomers from the renewable levulinic acid and diamines was reported. These diols were successfully polymerised with diethyl adipate *via* enzymatic polycondensation, giving access to a library of new bio-based biodegradable poly(ester-*co*-amide)s which could be used in the medical field.

# Experimental

## Materials

Levulinic acid (98%), 1,4-diaminobutane (98%), 1,5-diaminopentane (98%), and 1,10-diaminodecane (98%) were supplied by Alfa Aesar. Triethylamine (99%), ethyl chloroformate (99%), 1,2-diaminoethane (99%), 1,3-diaminopropane (99%), and 1,8-diaminooctane (98%) were provided by Acros. Sodium borohydride (99%), 1,6-diaminohexane (98%), adipic acid (99%), sebacic acid (99%), and diethyl adipate (99%) were supplied by Aldrich. Diethyl sebacate (98%) was supplied by TCI Europe. Novozyme 435 was kindly provided by Novozymes A/S. All the reactants and solvents (from VWR) were used as received. The synthesized monomers were obtained with high purity after purification (silica gel column chromatography and then recrystallization) as assessed by NMR analysis (ESI section†). Analytical thin layer chromatography (TLC) was performed on commercial silica gel 60 Å with a fluorescent indicator with UV absorbance at 254 nm (Merck). Detection was accomplished by treatment of the plate with dying reagents (potassium permanganate, vanillin or anisaldehyde). Chromatographic purifications of compounds **2a–g** were performed on silica gel columns (silica 60 Å, 40–63 μm).

## General procedure for diketone-diamide (1a–g) syntheses (Scheme 2)

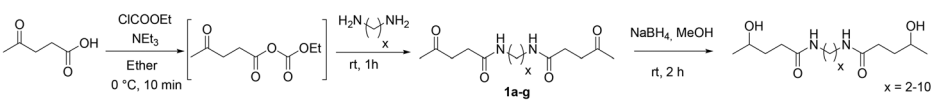
In a 1 L round bottom flask, levulinic acid (10 g, 86.1 mmol) was dissolved in diethyl ether (300 mL). The resulting mixture was ice cooled and triethylamine (12 mL, 86.1 mmol) was added. After stirring for 5 min at room temperature, ethyl chloroformate (8.2 mL, 86.1 mmol) was quickly added, resulting in the formation of a white solid. After 15 min of additional stirring, the precipitate was filtered off and washed once with diethyl ether (200 mL). The filtrate was transferred into a 1 L round bottom flask and vigorously stirred in an ice bath. The chosen diamine (43.1 mmol, 0.5 eq.) was added at once and the heterogeneous mixture was stirred for 1–2 h at room temperature. The operating procedure slightly differed for ethylene diamine (product **1a**) and propane diamine (product **1b**). In those cases, the diamine was dissolved in 50 mL of dichloromethane (DCM) and was added dropwise to the reaction mixture *via* a dropping funnel over 30 min. In all cases, the white precipitate was filtered off and washed twice with diethyl ether (2 × 100 mL), and then dried under vacuum for several hours, yielding the pure desired products **1a–g**.

## General procedure for diol-diamide (2a–g) syntheses (Scheme 2)

Compounds **1a–g** (32 mmol) were suspended in methanol (200 mL), and the mixture was ice cooled. Then sodium borohydride (3.63 g, 96 mmol) was added by portion to the mixture over a period of 10 min. After complete addition, the reaction mixture was stirred at room temperature for 2–5 h and monitored by TLC ($SiO_2$ plates, DCM/MeOH 95/5, $KMnO_4$ stain). As soon as the starting material was totally consumed, the reaction mixture was concentrated under vacuum. The resulting residue was purified by silica gel column chromatography (DCM/MeOH, from 95/5 to 85/15), yielding the product as a white solid. A further recrystallization can be performed from tetrahydrofuran to ensure the optimal purity.

## General procedure for polymer syntheses

The experimental procedure is adapted from a study on the enzyme-catalysed polycondensation of a secondary diol.[59] Diols **2a–g** (2 mmol), a diacid or a diester (2 mmol) and Novozyme 435 (10 wt%) were charged in a 50 or 100 mL round bottom flask containing 20–60 mL of cyclohexane. A Dean Stark apparatus was connected to the round bottom flask. In the case of reactions with diester, the Dean Stark apparatus was filled with molecular sieves (4 Å) to ensure trapping of ethanol (cyclohexane: $d$ = 0.779, ethanol: $d$ = 0.789). The molecular sieves were replaced every 48 hours. It is worth noting that it was not necessary to use molecular sieves in the case of diacids (cyclohexane: $d$ = 0.779, water: $d$ = 0.999). The reaction was carried out under reflux for 7 days. At the end of the reaction, the final mixture containing the insoluble product was dissolved into an excess of chloroform and then filtered off to remove the supported enzyme and eventual residues of diol or diacid. The solvent was evaporated, and the product was dried under vacuum for 24 hours.




Scheme 2 Synthesis of diol-diamides (**2a–g**) from levulinic acid and diamines.

### Analytical methods

$^1$H NMR and $^{13}$C NMR spectra were recorded on a Bruker AC 300 spectrometer at room temperature in DMSO-$d_6$. If necessary, to improve the solubility of the polymer, 0.1 mL of $CDCl_3$ was added into the NMR tube. Chemical shifts were reported in ppm relative to the residual solvent peak. Multiplicities were given as s (singlet), d (doublet), t (triplet) and m (multiplet). Coupling constants *J* were reported in Hz. If required, chemical shifts were fully attributed using a COSY experiment. High resolution mass spectra (HRMS) were recorded on a Waters Synapt G2–Si (mode ESI(+) with Leucine Enkephalin ions – *m*/*z* 556.2771 – as the lock mass). The samples were first dissolved in acetonitrile/methanol 1/1 (HPLC grade) at a concentration of 0.1 mg ml$^{-1}$ and the solutions were directly infused (5 μl min$^{-1}$) in the ESI source (capillary voltage: 3.1 kV). Positive-ion Matrix assisted LASER Desorption/Ionization-Mass Spectrometry (MALDI-MS) experiments were performed using a Waters QToF Premier mass spectrometer equipped with a Nd:YAG laser operating at 355 nm (third harmonic) with a maximum output of 65 μJ delivered to the sample in 2.2 ns pulses at 50 Hz repeating rate. Time-of-flight mass analysis was performed in the reflectron mode at a resolution of about 10k (*m*/*z* 569). All samples were analysed using *trans*-2-[3-(4-*tert*-butylphenyl)-2-methylprop-2-enylidene]malononitrile (DCTB) as a matrix. Polymer samples were dissolved in a mixture of chloroform/THF (50/50) to obtain a 1 mg mL$^{-1}$ solution. Additionally, 40 μL of 2 mg mL$^{-1}$ NaI solution in acetonitrile was added to the polymer solution. The number-average molecular weight ($M_n$) and the molar mass dispersity ($Đ_M$) were determined by Size Exclusion Chromatography (SEC). The apparatus (Agilent Technologies) was equipped with a refractive index detector calibrated with polystyrene standards in chloroform as the eluent (1 mL min$^{-1}$, 25 °C). Differential Scanning Calorimetry (DSC) analyses were carried out on a DSC Q20 instrument calibrated according to standard procedures using a high purity indium sample. For the analyses, samples (5 mg) were placed onto aluminium hermetic pans and heated from −70 °C to 160 °C, cooled to 160 °C from −70 °C, and then heated from −70 °C to 160 °C at a rate of 10 °C min$^{-1}$ under a nitrogen atmosphere. Thermogravimetry (TGA) analyses were performed on a Discovery TA Q 5500 instrument. Samples (10 mg) were heated from 30 °C to 800 °C with a heating rate of 10 °C min$^{-1}$ under nitrogen gas (balance: 10 mL min$^{-1}$, sample: 25 mL min$^{-1}$).

## Results and discussion

### Monomer syntheses

The diol-diamides **2a–g** were synthesized by a two-step procedure starting from levulinic acid and linear aliphatic diamines of different chain lengths (Scheme 2). In the first step, levulinic acid was converted into a mixed anhydride using ethyl chloroformate and was then allowed to react with the diamines to yield the diketones **1a–g**. In the second step, the two keto groups were reduced with sodium borohydride, affording the targeted monomers **2a–g**. The best overall yield of 73% was obtained from butane-1,4-diamine (compound **2c**, Table 1). It is noteworthy that the preparation of **2c** and **2e** was scaled up to 20 g. The structure of compounds **2a–g** was confirmed by NMR spectroscopy ($^1$H and $^{13}$C) and by high resolution mass spectrometry (ESI section†). The thermal properties, *i.e.* the melting, crystallization and degradation temperatures, were measured by DSC and TGA, respectively (Table 1).

An even–odd effect can be noticed with lower melting temperatures and the absence of crystallization for the odd number of carbons in the central segment. Such even–odd behaviours are well known for amides containing monomers and polymers.[23] The degradation temperatures define the polymerisation temperature window, which should not exceed 180 to 200 °C depending on the size of the monomer. It can be seen that the longer the monomer, the higher the thermal stability.

### Poly(ester-*co*-amide) syntheses

A first set of experiments was performed to assess the feasibility of the lipase-catalysed polymerisation and to identify the best reactions conditions. For this purpose the $C_6$ diol **2e** and the $C_6$ and $C_{10}$ diacids and diesters were selected as monomers (Scheme 3). The different results are summarized in Table 2.

The initial trials of enzymatic polycondensation were performed in bulk (solvent-free) under reduced pressure to remove ethanol and at a temperature over 100 °C to melt the diol monomer ($T_{melting}$ > 95 °C except for **2b**, Table 1). A representative example is presented in entry 1. Such conditions led to a low $M_n$ oligomer (800 g mol$^{-1}$) which may be ascribed to the high viscosity of the mixture hampering the removal of the

**Table 1** Yields and thermal properties of the monomers

| Products | Number of carbons *x* | Isolated yields (%) of **1a–g** | Overall yields (%) of **2a–g** | $T_{melting}$ [a] (°C) | $T_{crystallization}$ [b] (°C) | $T_{degradation}$ [c] (°C) |
|---|---|---|---|---|---|---|
| **2a** | 2 | 75 | 51 | 116 | 64 | 199 |
| **2b** | 3 | 57 | 41 | 79 | — | 183 |
| **2c** | 4 | 98 | 73 | 98 | 77 | 212 |
| **2d** | 5 | 82 | 57 | 95 | — | 223 |
| **2e** | 6 | 93 | 71 | 110 | 78 | 211 |
| **2f** | 8 | 87 | 62 | 108 | 95 | 239 |
| **2g** | 10 | 78 | 33 | 116 | 99 | 225 |

[a] Determined by DSC in the heating step. [b] Determined by DSC in the cooling step. [c] TGA temperature at which 3% of the weight was lost.


**Scheme 3** Synthesis of poly(ester-*co*-amide)s by enzymatic polycondensation.

**Table 2** Screening of reaction conditions with the $C_6$ diol-diamide **2e** to access poly(ester-*co*-amide)s[a]

| Entry | Comonomer | Experimental conditions | Concentration (mol $L^{-1}$) *vs.* diol | Yield (%) | $M_{n\ SEC}$[b] (g $mol^{-1}$) | $Đ_M$[b] | Main peak[b,c] wt (%) | Corrected yield[d] (%) |
|---|---|---|---|---|---|---|---|---|
| 1 | Diethyl adipate | 105 °C, 6 h ($P_{atm}$) 105 °C, 60 h (2 mbar) | – | 82 | 500 | 1.7 | — | 82 |
| 2 | Diethyl adipate | 81 °C, 3 days ($P_{atm}$) | 0.033 | 85 | 800 | 2.0 | 90 | 77 |
| 3 | Diethyl adipate | 81 °C, 7 days ($P_{atm}$) | 0.033 | 86 | 1800 | 1.5 | 91 | 78 |
| 4 | Diethyl adipate | 81 °C, 10 days ($P_{atm}$) | 0.033 | 86 | 2200 | 1.6 | 92 | 80 |
| 5 | Diethyl sebacate | 81 °C, 7 days ($P_{atm}$) | 0.033 | 50 | 5600 | 1.7 | 92 | 46 |
| 6 | Adipic acid | 81 °C, 7 days ($P_{atm}$) | 0.033 | 24 | 800 | 1.5 | 88 | 21 |
| 7 | Sebacid acid | 81 °C, 7 days ($P_{atm}$) | 0.033 | 26 | 2600 | 1.8 | 93 | 24 |
| 8 | Diethyl adipate | 81 °C, 7 days ($P_{atm}$) | 0.025 | 80 | 1900 | 1.6 | 77 | 62 |
| 9 | Diethyl adipate | 81 °C, 7 days ($P_{atm}$) | 0.050 | 72 | 1700 | 1.5 | 92 | 67 |
| 10 | Diethyl adipate | 81 °C, 7 days ($P_{atm}$) | 0.10 | 90 | 3300 | 1.5 | 92 | 83 |

[a] Diol-diamide/diester or diacid (2 mmol/2 mmol), enzyme N435 (10 wt% weight), reaction in solution in Dean Stark apparatus (with molecular sieves 4 Å replaced every 48 h for diester), solvent: cyclohexane [from 20 mL (0.1 mol $L^{-1}$ *vs.* diol) to 80 mL (0.025 mol $L^{-1}$ *vs.* diol)]. [b] SEC in chloroform at 25 °C (polystyrene standards), and $M_n$ and $Đ_M$ for the main peak. [c] Main peak and secondary peak distributions represent high $M_n$ oligomers and small oligomers ($M_n$ = 400–600 g $mol^{-1}$, $Đ_M$ = 1–1.2), respectively. % by weight main peak = [area of main peak/(area of main peak + area of secondary peaks)] × 100. [d] Corrected yield = yield × main peak wt(%)/100.

ethanol by-product and reducing the stirring efficiency. It was not possible to decrease the viscosity at a higher temperature without denaturing the enzyme.

The enzyme-catalysed polycondensation was then conducted in solution (entries 2–10). The choice of solvent is crucial for these reactions, as it can influence the activity, stability (denaturation) and selectivity of the lipase. As already described in the literature to allow the polycondensation of a secondary diol with diacids or diesters in cyclohexane,[59] we decided to set the polymerisation at reflux with a Dean Stark trap (the scheme of the apparatus in ESI S17†). Furthermore, it was shown previously that hydrophobic solvents, like cyclohexane, can promote enzymatic activity.[60]

The reaction was conducted at different times (3, 7, 10 days) in boiling cyclohexane (81 °C) using Novozyme 435 as the catalyst and diethyl adipate as the comonomer (entries 2–4). Seven days were required to improve the $M_n$ value with a yield of 78%. The molecular weight distribution (entry 3, 7 days – Fig. 1) was composed of two populations. The main was related to a high $M_n$ oligomer fraction (1800 g $mol^{-1}$). It was composed of small peaks below the main signal due to the presence of intermediate growing chains as the reaction was not complete after 7 days. The secondary population was related to a very small oligomer, probably a dimer ($M_n$ = 400 g $mol^{-1}$, 9% by weight). The weight ratio of each population was determined from the SEC area of the two zones. Unfortunately, attempts to remove the low $M_n$ oligomeric fraction from the product by precipitation in various solvents were unsuccessful. Decreasing the reaction time to 3 days reduced the number

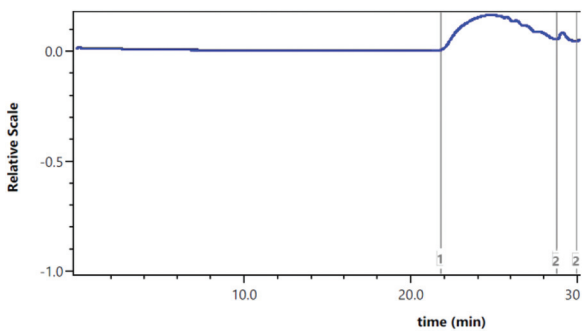



**Fig. 1** SEC chromatogram of the polycondensation product (entry 3) obtained from diol-diamide **2e** ($x$ = 6) and diethyl adipate ($CHCl_3$, 25 °C, standard PS).

average molecular weight by a factor 2. When the polymerisation was conducted for 10 days (entry 4) the $M_n$ value was not substantially improved compared to the 7 days reaction. The dispersities around 1.5–1.6 and the yield extent may be ascribed to a slow and uncompleted conversion kinetics of secondary diols *via* the enzymatic catalyst in 7–10 days.

The $^1$H NMR spectra resulting from the experiment depicted in entry 4 and from the monomers are presented in Fig. 1. The assignments were confirmed with a COSY experiment (ESI S15†). The formation of the polycondensation product was evidenced by the appearance of new signals in the spectrum, *i.e.* the b signal at 4.8 ppm and the c signal at 1.7 ppm attributed respectively to the protons at the α and β positions of the ester

oxygen of the polymer backbone [C***H***$_2$C***H***(CH$_3$)OCO]. The integrations were in accordance with the structure of the polymer presented in Fig. 2C. It should be noted that the signals characteristic of the terminal hydroxyl group (denoted as m at 4.4 ppm), the protons at the α and β positions of this latter group (denoted as *b′* and *a′* at 3.6 and 1.0 ppm, respectively) and the ethyl adipate moiety (CH$_3$C***H***$_2$OCO denoted as *k* at 4.1 ppm) were observed in the polymer spectrum (Fig. 2C). They could be assigned to the chain-ends in view of the integral values ($I_{a+a'}$ = 5.90 for 6H, $I_{b+b'}$ = 2.00 for 2H, $I_m = I_{b'}$).

The structure was confirmed by MALDI-ToF analyses (ESI S16†). Different combinations of chain ends were observed: alcohol–alcohol, ester–ester and alcohol–ester associations and in a negligible way alcohol–acid (non-observable by NMR). The formation of a cyclic structure was also observed in negligible quantity.

Lipase N435 has generally better affinity with long chain monomers for polyesterification reactions. Indeed, it has been shown that increasing the diol and/or the diacid/diester length leads to a higher molecular weight.[59,61,62] Thus, the polycondensation reaction was conducted with diethyl sebacate (C$_{10}$, entry 5) instead of diethyl adipate (C$_6$, entry 3). The longer diester chain led to a yield of 46% (*vs.* 78% for entry 3), but an improvement of $M_n$ was observed, 5600 *vs.* 1800 g mol$^{-1}$ for entries 5 and 3, respectively. The reactions from diacids as monomers instead of diesters were also explored (entries 6 and 7) but led to lower yields (21–24%). As observed for diesters, increasing the length of the diacid from C$_6$ (adipic) to C$_{10}$ (sebacic) results in a higher $M_n$ (2600 g mol$^{-1}$ for C$_{10}$ *vs.* 800 g mol$^{-1}$ for C$_6$). Additionally, the isolated product contains in this case a substantial amount of γ-valerolactone (26–28% by weight, $^1$H NMR, ESI S18†). It was reported in previous studies that immobilized *Candida antarctica* lipase B can catalyse lactonization from hydroxy-ester toward γ-valerolactone *via* intramolecular cyclisation. A similar mechanism can be proposed in the case of diol-diamides (Scheme 4).[63,64] This side-product was not detected when the reaction was conducted with diesters. It may be explained by an enhanced electrophili-

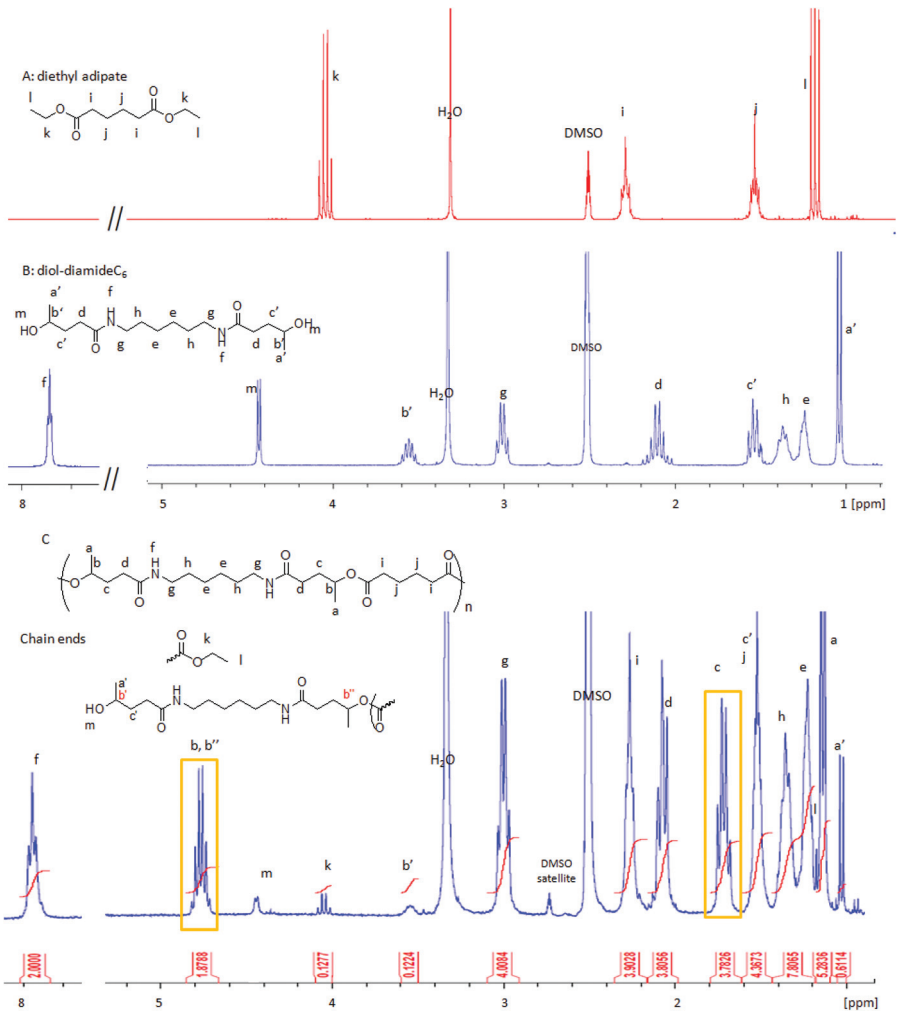
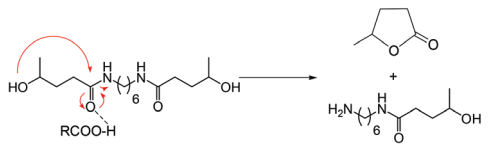



**Fig. 2** $^1$H NMR spectra of diethyl adipate (A), the diol diamide monomer (*x* = 6, B) and the polymer obtained after polycondensation (C, entry 4) (DMSO-d$_6$, 300 MHz).




**Scheme 4** Proposed side-reaction leading to the formation of γ-valerolactone in the presence of a diacid comonomer.

city of the amide in the presence of the diacid monomer. The loss of a γ-valerolactone unit was confirmed by MALDI-ToF analyses (ESI S19†). In view of these results, diesters were selected for the next steps of the study. Even if diethyl sebacate allowed achieving a higher $M_n$, diethyl adipate was selected as a reference co-monomer as it gave the best yield/$M_n$ compromise.

The so formed poly(ester-*co*-amide)s were barely soluble in cyclohexane, thus affecting the polymerisation process. The influence of the concentration on the polymerisation was further studied with the aim to improve the polymer formation (entries 8–10 *vs.* 3 in Table 2). Generally, an increase of the number average molecular weight and yield could be noticed in a concentrated medium [1900 g mol$^{-1}$, yield = 62% for 0.025 mol L$^{-1}$ (entry 8) *vs.* 3300 g mol$^{-1}$, yield = 83% for 0.1 mol L$^{-1}$ (entry 10)]. This result may be ascribed to a better interaction between the reagents and enzyme (insoluble in cyclohexane) in concentrated medium. The by-product of the reaction (ethanol) may also be more easily removed in a lower volume of solvent *via* the Dean Stark process which could improve the conversion.

The enzymatic polycondensation of **2e** with diesters as comonomers and Novozyme 435 as the catalyst allowed oligomer formation (1800–5600 g mol$^{-1}$ in 7 days of reaction) with interesting yields in the case of diethyl adipate as the co-monomer (62–83%). The secondary alcohol was recognized by the lipase Novozyme 435, and the amide function did not interfere with the process.

The conditions described in entry 10, the concentration 0.1 mol L$^{-1}$, offering the best yield and $M_n$, were selected for the polymerisation of the other diol-diamides (**2a–g**, $x$ = 2–10) and are reported in Table 3 (experiments with a concentration of 0.033 mol L$^{-1}$ were also realised and are provided in ESI Table S1†). The enzyme-catalysed polycondensation led to oligo(ester-*co*-amide)s for all levulinic acid based diol-diamide monomers, with the number-average molecular weight ranging from 1500 to 7200 g mol$^{-1}$. $^1$H NMR and SEC chromatograms can be found in the ESI section (S20–26 and S27 respectively).† The polymerisation of diols with short chain lengths ($x$ = 2, 3, 4, 5) gave moderate $M_n$ (1500–2100 g mol$^{-1}$, entries 11–14). The molecular weight can be improved (3300–7200 g mol$^{-1}$) by increasing the chain length ($x$ = 6, 8, 10) probably due to the better enzyme affinity for longer monomer chains.

The oligo(ester-*co*-amide)s were characterized by differential scanning calorimetry (DSC) (see ESI S28–34† for the DSC curves). The DSC curves showed a heat capacity jump between −23 °C and 0 °C (Table 3). The range of $T_g$ obtained was in accordance with a previous study where similar poly(ester-*co*-amide)s had a $T_g$ of −11 °C.[23] It was also observed that the increase of the number of carbons in the monomer leads to a decrease of the glass transition temperature due to an increased mobility of the chains.[59,65] We observed similar trends, but the evolution was not monotonous probably due to the presence of low $M_n$ oligomers that could influence slightly the $T_g$ value. For $x$ = 8 and $x$ = 10, the oligomers recrystallized during the second heating, and the melting temperature was observed to be around 90–97 °C (ESI S31–32†). This observation showed that the high number of methylene units in the repeating units tended to improve the ability of the oligomer to crystallize.

In the TGA curves (ESI 35–41†), two main degradation stages were observed and attributed to the ester and amide bonds respectively. Amide groups are known to be more stable than ester groups due to the partial double bond character of the C–N bond.[66,67] The first degradation attributed to the ester part was found to be around 180–205 °C (3% mass loss). The degradation temperature attributed to the amide part

**Table 3** Influence of the diol-diamide chain length on polycondensation reactions and thermal analyses[a]

| Entry | Diol diamide number of carbons $x$ | Yield (%) | $M_{n\ SEC}$[b] (g mol$^{-1}$) | $Đ_M$[b] | Main peak[b,c] wt (%) | Corrected yield[d] (%) | $T_g$[e] (°C) | $T_c$[e] (°C) | $T_m$[e] (°C) | $i_{deg\ 1}$[f] (°C) | $T_{deg\ 2}$[g] (°C) |
|---|---|---|---|---|---|---|---|---|---|---|---|
| 11 | 2 | 81 | 1500 | 1.4 | 88 | 71 | −5 | — | — | 187 | 355 |
| 12 | 3 | 92 | 2300 | 1.4 | 91 | 84 | −9 | — | — | 198 | 360 |
| 13 | 4 | 65 | 2300 | 1.6 | 86 | 56 | 0 | — | — | 201 | 365 |
| 14 | 5 | 88 | 1800 | 1.5 | 91 | 80 | −10 | — | — | 202 | 373 |
| 10 | 6 | 90 | 3300 | 1.5 | 92 | 83 | −12 | — | — | 205 | 375 |
| 15 | 8 | 85 | 4200 | 1.8 | 91 | 78 | −23 | 72 | 90 | 180 | 376 |
| 16 | 10 | 71 | 7200 | 1.6 | 91 | 65 | −14 | 28 and 49 | 97 | 203 | 378 |

[a] Reaction at 81 °C for 7 days in 20 mL or 60 mL of cyclohexane (0.10 mol L$^{-1}$ *vs.* diol), diol-diamide/diester (2 mmol/2 mmol), Novozyme 435 (10% weight *vs.* monomers), Dean Stark apparatus with molecular sieves (replaced every 48 h). [b] SEC in chloroform at 25 °C (polystyrene standards), and $M_n$ and $Đ_M$ for the main peak. [c] Main peak and secondary peak distributions represent high $M_n$ oligomers and small oligomers ($M_n$ = 300–800 g mol$^{-1}$, $Đ_M$ = 1–1.3), respectively. % by weight main peak = [area of main peak/(area of main peak + area of secondary peaks)] × 100. [d] Corrected yield = yield × main peak wt(%)/100. [e] $T_g$, $T_c$ (crystallization) and $T_m$ (melting) determined by DSC in the second heating step. [f] TGA temperatures at which 3% of the mass was lost. [g] TGA temperatures for the start of the second degradation (inflection point of weight = $f(T)$).

increased when the number of methylene units increased from 2 (355 °C) to 10 (378 °C).

## Conclusions

We have synthesized herein a new family of poly(ester-*co*-amide) polymers. A library of bio-based aliphatic diol-diamide monomers with different chain lengths was synthesized from levulinic acid and linear aliphatic diamines through an *N*-acylation/reduction procedure. These secondary diol based monomers were polymerised under mild conditions *via* enzymatic catalysis to access oligo(ester-*co*-amide)s. The polycondensation in bulk at $T > 100$ °C led to low $M_n$ oligomers (800 g mol$^{-1}$), while the reactions conducted in refluxing cyclohexane with a Dean Stark apparatus led to a higher $M_n$ product. The nature and length of the comonomer (diacid/diester, $C_6$/$C_{10}$) were found to have a significant effect on the polymerisation. When the polymerisations were performed with diacids, γ-valerolactone was formed as a side product. The $M_n$ value increased when the reaction was conducted with both $C_{10}$ diacid and diester instead of $C_6$ analogs. But concerning the diester, the yield was divided by a factor close to 2 with $C_{10}$ compared to $C_6$. The polycondensation of the different diol-diamide monomers with diethyl adipate catalysed with Novozyme 435 allowed the preparation of oligo(ester-*co*-amide)s with number average molecular weights in the range of 1500–7200 g mol$^{-1}$, dispersities between 1.4 and 1.8 and the yield between 56–84%. The aliphatic secondary diol was recognized by the lipase Novozyme 435 whichever the number of methylene units, when in fact lipases are usually known to be poorly reactive with secondary hydroxyl groups. This is, to our knowledge, the first example of a step-growth synthesis of poly(ester-*co*-amide) polymers from a secondary diol *via* biocatalysis. This biocatalysed synthesis of levulinic acid based poly(ester-*co*-amide) is complementary to our previous work on metal catalysed polycondensations allowing the access to poly(amine-*co*-ester)s[14] and polyesters bearing a pendant lactam group[15] from levulinic acid based monomers obtained *via* reductive amination. The molecular weight could be improved (3300–7200 g mol$^{-1}$) by increasing the chain length of the diol ($x$ = 6, 8, 10). All polycondensation products exhibited a glass transition temperature between −23 °C and −0 °C. The stability of the amide part was slightly higher when the number of methylene units increased from 2 (355 °C) to 10 (378 °C). Such polymers could serve as drug delivery systems or for tissue engineering applications, as an alternative to amino acid based poly(ester-*co*-amide)s.

## Author contributions

Conception and design of the study: JM, YB, SP, TB, LP, AFH and PZ; monomer synthesis: YB and SP; monomer analysis: YB, SP and PG; polymer synthesis *via* enzymatic catalysis: JM and TC; polymer analysis: JM, JDW, VG, JFT, FC and JMR; writing: JM, YB and PZ; reading of the manuscript and critical discussion: all authors.

## Conflicts of interest

There are no conflicts to declare.

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## Notes and references

1 I. Delidovich, P. J. C. Hausoul, L. Deng, R. Pfützenreuter, M. Rose and R. Palkovits, *Chem. Rev.*, 2016, **116**, 1540.
2 L. T. Mika, E. Cséfalvay and Á. Németh, *Chem. Rev.*, 2017, **118**, 505.
3 P. F. H. Harmsen, M. M. Hackmann and H. L. Bos, *Biofuels, Bioprod. Biorefin.*, 2014, **8**, 306.
4 T. Werpy and G. Petersen, *Top Value Added Chemicals from Biomass: Volume I - Results of Screening for Potential Candidates from Sugars and Synthesis Gas*, 2004.
5 C. Antonetti, D. Licursi, S. Fulignati, G. Valentini and A. M. Raspolli Galletti, *Catalysts*, 2016, **6**, 196.
6 B. Girisuta, L. P. B. M. Janssen and H. J. Heeres, *Green Chem.*, 2006, **8**, 701.
7 M. A. Mellmer, J.-M. R. Gallo, D. M. Alonso and J. A. Dumesic, *ACS Catal.*, 2015, **5**, 3354.
8 F. D. Pileidis and M. Titirici, *ChemSusChem*, 2016, **9**, 562.
9 J.-F. L. Silva, R. Grekin, A. P. Mariano and R. M. Filho, *Energy Technol.*, 2018, **6**, 613.
10 L. Yan, Q. Yao and Y. Fu, *Green Chem.*, 2017, **19**, 5527.
11 K. Huang, S. Ma, S. Wang, Q. Li, Z. Wu, J. Liu, R. Liu and J. Zhu, *Green Chem.*, 2019, **21**, 4964.
12 W. Xuan, M. Hakkarainen and K. Odelius, *ACS Sustainable Chem. Eng.*, 2019, **7**, 12552.
13 A. Sinisi, M. D. Esposti, M. Toselli, D. Morselli and P. Fabbri, *ACS Sustainable Chem. Eng.*, 2019, **7**, 13920.
14 Y. Bernhard, L. Pagies, S. Pellegrini, T. Bousquet, A. Favrelle, L. Pelinski, P. Gerbaux and P. Zinck, *Green Chem.*, 2019, **21**, 123.

 

15 Y. Bernhard, S. Pellegrini, T. Bousquet, A. Favrelle, L. Pelinski, F. Cazaux, V. Gaucher, P. Gerbaux and P. Zinck, *ChemSusChem*, 2019, **12**, 3370.
16 M. Hartweg and C. R. Becer, *Green Chem.*, 2016, **18**, 3272.
17 A. S. Amarasekara, U. Ha and N. C. Okorie, *J. Polym. Sci., Part A: Polym. Chem.*, 2018, **56**, 955.
18 R. D. Ashby, D. K. Y. Solaiman, G. D. Strahan, C. Zhu, R. C. Tappel and C. T. Nomura, *Bioresour. Technol.*, 2012, **118**, 272.
19 J. A. Moore and T. Tannahill, *High Perform. Polym.*, 2001, **13**, S305.
20 M. Chalid, H. J. Heeres and A. A. Broekhuis, *Polym.-Plast. Technol. Eng.*, 2015, **54**, 234.
21 M. Chalid, H. J. Heeres and A. A. Broekhuis, *Adv. Mater. Res.*, 2013, **789**, 274.
22 M. Chalid, *Adv. Mater. Res.*, 2011, **277**, 112.
23 H. R. Stapert, P. J. Dijkstra and J. Feijen, *Macromol. Symp.*, 1998, **130**, 91.
24 K. M. Zia, A. Noreen, M. Zuber, S. Tabasum and M. Mujahid, *Int. J. Biol. Macromol.*, 2016, **82**, 1028.
25 Y. Lee, E. E. Kwon and J. Lee, *Rev. Environ. Sci. Bio/Technol.*, 2019, **18**, 317.
26 Y. Lee, K. A. Lin, E. E. Kwon and J. Lee, *J. Cleaner Prod.*, 2019, **227**, 624.
27 Y. Jiang and K. Loos, *Polymers*, 2016, **8**, 243.
28 K. Guo and C. C. Chu, *J. Biomed. Mater. Res., Part B*, 2009, **89**, 491.
29 K. Guo and C. C. Chu, *J. Biomater. Sci., Polym. Ed.*, 2007, **18**, 489.
30 P. Karimi, A. S. Rizkalla and K. Mequanint, *Materials*, 2010, **3**, 2346.
31 H. Dodiuk-Kenig, K. Lizenboim, I. Eppelbaum, B. Zalsman and S. Kenig, *J. Adhes. Sci. Technol.*, 2004, **18**, 1723.
32 S. Bera and Z. Jedliński, *Polymer*, 1992, **33**, 4331.
33 M. Kluge, D. N. Bikiaris and T. Robert, *Eur. Polym. J.*, 2019, **120**, 109195.
34 M. Kluge, H. Rennhofer, H. C. Lichtenegger, F. W. Liebner and T. Robert, *Eur. Polym. J.*, 2020, **129**, 109622.
35 P. A. M. Lips, R. Broos, M. J. M. Van Heeringen, P. J. Dijkstra and J. Feijen, *Polymer*, 2005, **46**, 7823.
36 A. Rodriguez-Galan, L. Franco and J. Puiggali, *Polymers*, 2011, **3**, 65.
37 A. C. Fonseca, A. C. Serra, J. F. J. Coelho, M. H. Gil and P. N. Simões, *Polym. Int.*, 2013, **62**, 736.
38 J. Helder, F. E. Kohn, S. Sato, J. W. Van den Berg and J. Feijen, *Makromol. Chem., Rapid Commun.*, 1985, **6**, 9.
39 Y. Feng, J. Knüfermann, D. Klee and H. Höcker, *Macromol. Rapid Commun.*, 1999, **20**, 88.
40 X. Wang and N. Hadjichristidis, *ACS Macro Lett.*, 2020, **9**, 464.
41 R. Katsarava, *Macromol. Symp.*, 2003, **199**, 419.
42 R. Katsarava, V. Beridze, N. Arabuli, D. Kharadze, C. C. Chu and C. Y. Won, *J. Polym. Sci., Part A: Polym. Chem.*, 1999, **37**, 391.
43 L. Asín, E. Armelin, J. Montané, A. Rodríguez-Galán and J. Puiggalí, *J. Polym. Sci., Part A: Polym. Chem.*, 2001, **39**, 4283.
44 A. Douka, S. Vouyiouka, L. Papaspyridi and C. D. Papaspyrides, *Prog. Polym. Sci.*, 2018, **79**, 1.
45 L. Ragupathy, U. Ziener, R. Dyllick-Brenzinger, B. Von Vacano and K. Landfester, *J. Mol. Catal. B: Enzym.*, 2012, **76**, 94.
46 D. Maniar, F. Silvianti, V. M. Ospina, A. J. J. Woortman, J. Van Dijken and K. Loos, *Polymer*, 2020, **205**, 122662.
47 B. Sharma, A. Azim, H. Azim, R. A. Gross, E. Zini, M. L. Focarete and M. Scandola, *Macromolecules*, 2007, **40**, 7919.
48 A. Mahapatro, B. Kalra, A. Kumar and R. Gross, *Biomacromolecules*, 2003, **4**, 544.
49 T. Debuissy, E. Pollet and L. Avérous, *Eur. Polym. J.*, 2017, **97**, 328.
50 A. Mahapatro, B. Kumar, B. Kalra and R. Gross, *Macromolecules*, 2004, **37**, 35.
51 D. Maniar, Y. Jiang, A. J. J. Woortman, J. Van Dijken and K. Loos, *ChemSusChem*, 2019, **12**, 990.
52 S. Baraldi, G. Fantin, G. Di Carmine, D. Ragno, A. Brandolese, A. Massi, O. Bortolini, N. Marchetti and P. P. Giovannini, *RSC Adv.*, 2019, **9**, 29044.
53 E. Stavila, R. Z. Arsyi, D. M. Petrovic and K. Loos, *Eur. Polym. J.*, 2013, **49**, 834.
54 Y. Jiang, D. Maniar, A. J. J. Woortman and K. Loos, *RSC Adv.*, 2016, **6**, 67941.
55 M. Finnveden, P. Hendil-Forssell, M. Claudino, M. Johansson and M. Martinelle, *Polymers*, 2019, **11**, 1730.
56 A. Kumar, A. S. Kulshrestha, W. Gao and R. A. Gross, *Macromolecules*, 2003, **36**, 8219.
57 K. R. Yoon, S. Hong, B. Kong and I. S. Choi, *Synth. Commun.*, 2012, **42**, 3504.
58 I. Hilker, G. Rabani, G. K. M. Verzijl, A. R. A. Palmans and A. Heise, *Angew. Chem., Int. Ed.*, 2006, **45**, 2130.
59 D. Juais, A. F. Naves, C. Li, R. A. Gross and L. H. Catalani, *Macromolecules*, 2010, **43**, 10315.
60 E. Wehtje, J. Kaur, P. Adlercreutz, S. Chand and B. Mattiasson, *Enzyme Microb. Technol.*, 1997, **21**, 502.
61 Y. Linko, Z. Wang and J. Seppälä, *Enzyme Microb. Technol.*, 1995, **17**, 506.
62 M. Hunsen, A. Azim, H. Mang, S. R. Wallner, A. Ronkvist, W. Xie and R. A. Gross, *Macromolecules*, 2007, **40**, 148.
63 K. Götz, A. Liese, M. Ansorge-Schumacher and L. Hilterhaus, *Appl. Microbiol. Biotechnol.*, 2013, **97**, 3865.
64 G. C. Clososki, C. E. Costa, L. J. Missio, Q. B. Cass and J. V. Comasseto, *Synth. Commun.*, 2004, **34**, 817.
65 C. Girard, M. Gupta, A. Lallam, D. V. Anokhin, P. V. Bovsunovskaya, A. F. Akhyamova, A. P. Melnikov, A. A. Piryazev, A. I. Rodygin, A. A. Rychkov, K. N. Grafskaya, E. D. Shabratova, X. Zhu, M. Möller and D. A. Ivanov, *Polym. Bull.*, 2019, **76**, 495.
66 Y. P. Ge, D. Yuan, Z. L. Luo and B. B. Wang, *eXPRESS Polym. Lett.*, 2014, **8**, 50.
67 Z. Qian, S. Li, Y. He, C. Li and X. Liu, *Polym. Degrad. Stab.*, 2003, **81**, 279.