Article

Straightforward Enzymatic Methacrylation of Poly(Glycerol Adipate) for Potential Applications as UV Curing Systems

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Abstract: Enzymatic one-pot synthesis procedures in a one-step and two-step monomers addition were developed to obtain poly(glycerol adipate) macromers with methacrylate end-functional groups under the presence of 1 and 3 wt% of *Candida antarctica* lipase B (CALB). Glycerol, divinyl adipate, and vinyl methacrylate were enzymatically reacted (vinyl methacrylate was either present from the beginning in the monomers solution or slowly dropped after 6 h of reaction) in tetrahydrofuran (THF) at 40 °C over 48 h. Macromers with a methacrylate end groups fraction of ≈52% in a simple one-pot one-step procedure were obtained with molecular weights (M_n) of ≈7500–7900 g/mol. The obtained products under the one-pot one-step and two steps synthesis procedures carried out using 1 and 3 wt% of a CALB enzymatic catalyst were profusely characterized by NMR (^{1}H and ^{13}C), MALDI-TOF MS, and SEC. The methacrylate functional macromers obtained with the different procedures and 1 wt% of CALB were combined with an Irgacure® 369 initiator to undergo homopolymerization under UV irradiation for 10 and 30 min, in order to test their potential to obtain amorphous networks within minutes with similar properties to those typically obtained by complex acrylation/methacrylation procedures, which need multiple purification steps and harsh reagents such as acyl chlorides. To the best of our knowledge, this is the first time that it has been demonstrated that the obtention of methacrylate-functional predominantly linear macromers based on poly(glycerol adipate) is able to be UV crosslinked in a simple one-step procedure.

Keywords: poly(glycerol adipate); enzymatic synthesis; functionalization; steps; UV-curing

1. Introduction

Nowadays, there is an increasing interest in the synthesis of polyester macromers based on a variety of monomers with different functional groups. More advantageous is to control the polymer end groups' nature, since terminal functionalized polymers/macromers are very useful; for instance, they are used as blocks for new copolymers, comb polymers, and polymer networks for surface modification applications [1–6]. Conventional strategies to afford polymers with abundant functionalities are frequently based on complex processes that often involve multi-step synthesis procedures with intermediate purification steps that are tedious and time-consuming. Thus, more environmentally friendly procedures and a faster and higher implementation simplicity are desired. This demands efficient one-pot synthesis approaches to obtain the desired product in a single reaction, which, at the same time, will permit the control of the polymer structure [7–12]. To this end, the excellent chemo, regio-, and stereoselectivity of enzymes are of interest in endowing polyesters with specific end-functionalities in one-pot processes [13]. However, mostly, the aim of polyester synthesis using enzymes as catalysts has been strongly focused on obtaining high-molecular-weight polymers and its success in scaling them up, with the benefit

of mild reaction conditions, more than obtaining specific functionalities in a single-step procedure [14–17].

Polyols such as glycerol are interesting due to their high hydroxyl functionality that provides an enhanced hydrophilicity, reduced toxicity, and high susceptibility to biodegradation [18,19]. Additionally, pendant hydroxyl groups can permit further functionalization, drug encapsulation, and the conjugation of active molecules [20–22]. Typically, to achieve structural homogeneity in glycerol-based polymers, a high linearity must be ensured. Towards this aim, a common practice is to selectively protect/block secondary hydroxyl groups [23,24], although the use of enzymes as selective catalysts with a preference for a primary hydroxyl reaction is a promising but less exploited alternative. In previous works, we have demonstrated the possibility of achieving linear poly(glycerol adipate) (PGA)-based macromers enzymatically synthesized from divinyl adipate and glycerol with controlled end group functionality by the means of reagents ratios, synthesis conditions, and amounts of catalyst [25].

Nowadays, 3D printing, such as stereolithography, has aroused great interest due to the rapid and easy preparation of polymer systems based on biodegradable polymers, which mitigate the concern about the use of non-renewable resources in this field [26]. These biobased polymeric systems have potential and versatility in biomedical applications [27,28], mainly because they present mechanical properties similar to soft tissues, and biocompatibility [29–33]. Therefore, it would be of greater interest to obtain thermoset polyesters from these macromers. For polyester macromers to be rendered photocurable within minutes under the presence of a photoinitiator and the appropriate wavelength light, their functionalization with acrylate/methacrylate moieties, typically incorporated from the reaction of acyl chlorides in the presence of triethylamine, is one of the preferred procedures [34,35]. These methods imply harsh reagents that need to be neutralized during the process, resulting in a large number of undesirable side products that are difficult to remove; thus, multiple purification/washing steps are needed. Moreover, due to the high reactivity of the acyl chlorides, the structural homogeneity of the macromers is compromised unless the protection of secondary hydroxyl groups is performed. Our group also performed, for the first time, enzymatic functionalization with methacrylate moieties in the independent synthesis step of a previously enzymatically synthesized and purified PGA macromer bearing a majority of hydroxyl end groups [36].

Importantly, the amount of substitution degree of the hydroxyl end groups with methacrylate moieties allowed for film formation during the photocrosslinking of this linear macromer. However, this synthesis procedure was carried out in two well-differentiated syntheses—firstly, a PGA macromer was enzymatically synthesized from divinyl adipate and glycerol monomers into THF for 24 h. The product was then subsequently isolated from the reaction media and purified to obtain the desired macromer; secondly, the obtained PGA was subsequently redissolved in THF for its enzymatic functionalization, in an independent synthesis, under the presence of vinyl methacrylate for 48 h. Although the obtained macromers presented the desired characteristics, the synthesis protocols were time and resource demanding. These synthesis protocols could be improved if these macromers could be obtained in a one-step reaction. This improvement would mean the use of less catalyst, solvent, and time.

Therefore, our aim in this work was to study and methacrylate functionalized linear poly(glycerol adipate) macromers using one-pot step lipase catalyzed polycondensation/transesterification and functionalization. This synthetic protocol is not only a more ecological and simpler alternative to the one used to obtain the previous crosslinkable polymer, but also produced a greater modification of the macromers. Additionally, the ability of the obtained macromers under ultraviolet light radiation to undergo further crosslinking and film formation via initiated free-radical polymerization was demonstrated.

2. Experimental

2.1. Materials

Glycerol (GLY 99%, Sigma-Aldrich, St. Quentin Fallavier, France), divinyl adipate (DVA, 96.0%, TCI Europe, Paris, France), vinyl methacrylate (VMA, TCI Chemicals, Paris, France, 98.0%), acetone (99.5%, Scharlau, Sentmenat, Barcelona, Spain), diethyl ether (99.8%, Honeywell, Charlotte, NC, USA), 2-benzyl-2-dimethylamino-1-(4-morpholinophenyl)-1-butanone (Irgacure® 369, Ciba Specialty Chemicals, Basel, Switzerland), deuterated acetone (99.8%, Eurisotop, Andover, MA, USA), N,N-dimethyl formamide (DMF, Scharlau), and lithium bromide (LiBr, Sigma Aldrich, >99.9%) were used as received. Anhydrous tetrahydrofuran (THF) was obtained by passing analytical grade (Scharlab) over a solvent purification system (Innovative Technology, Inc., Newburyport, MA, USA). Lipase B from *Candida antarctica*, immobilized on microporous (CALB (Novozyme 435) 5000 U/g, Sigma-Aldrich), was dried for 24 h before its use.

2.2. Synthesis of Methacrylated Poly(glycerol Adipate) Macromers

2.2.1. One-Pot Two-Step Enzymatic Synthesis of Methacrylated Poly(glycerol Adipate)

For the two-step one-pot synthesis, DVA (0.760 g, 3.9 mmol) and GLY (0.389 g, 4.22 mmol) were dissolved in anhydrous THF (730 mg/mL relative to the total monomers weight) in a round-bottom flask. CALB was added in different amounts (1–3 wt% of the total monomers weight) and the mixture was allowed to react for 6 h under argon and magnetic stirring at 40 °C. After that time, VMA (0.258 g, 2.30 mmol) was slowly dropped over 30 min into the reaction media and allowed to react (Scheme 1). After 48 h of total reaction time, THF was added to dilute the products and the reaction medium was poured into a glass funnel with a filter plate to facilitate the separation of enzymes. After filtering, the solutions were concentrated and dried into a rotary evaporator, subsequently dissolved in acetone, and precipitated into cold diethyl ether. Precipitates were recovered using decantation and dried under vacuum at room temperature until a constant weight was obtained, so no residual solvents were detected during characterization. NMR and MALDI-TOF analyses were carried out over the obtained products.
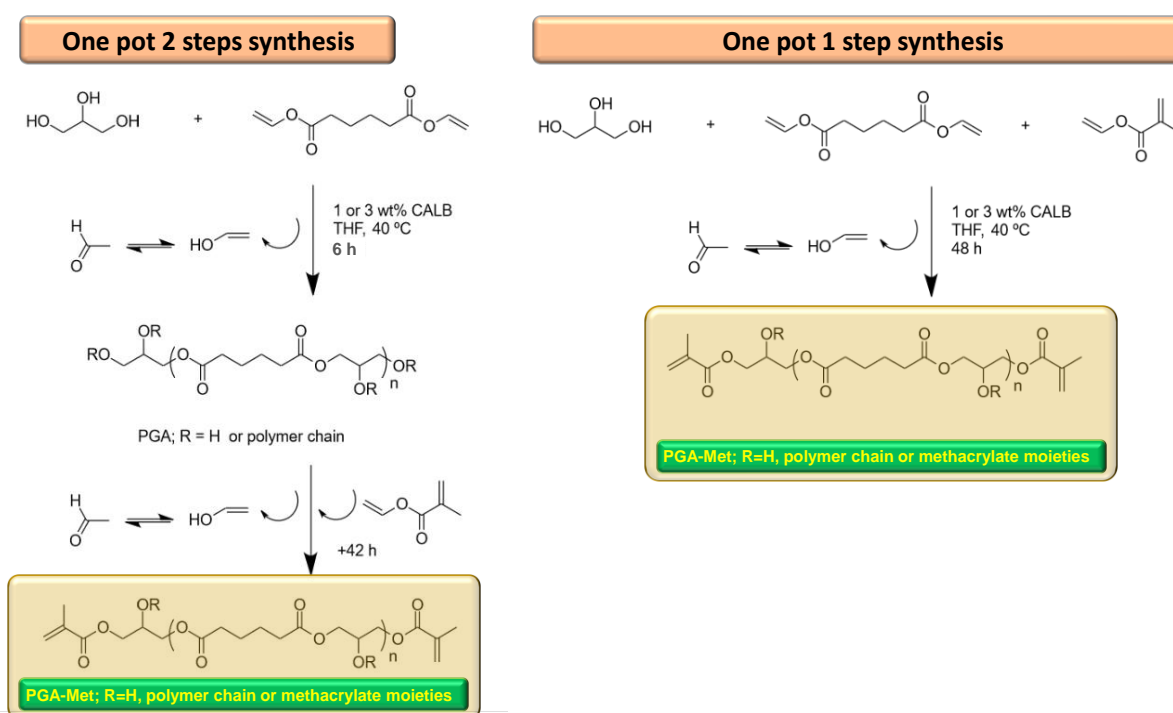

Scheme 1. Synthesis of PGA-Met macromers using one-pot two-step (**left**) and one-pot one step (**right**) synthesis procedures.

2.2.2. One-Pot One-Step Enzymatic Synthesis of Methacrylated Poly(glycerol Adipate)

For the one-step one-pot synthesis, DVA (0.760 g, 3.83 mmol), GLY (0.389 g, 4.22 mmol), and VMA (0.258 g, 2.30 mmol), (1.0:1.1:0.6) were dissolved in anhydrous THF (730 mg/mL relative to the total monomers weight) in a round-bottom flask. CALB was added in different amounts (1–3 wt% of the total monomers weight) and the reaction was left under argon and magnetic stirring for 48 h at 40 °C (Scheme 1). After the reaction time, the products were recovered as those in the previously described one-pot two-step enzymatic synthesis description were. NMR and MALDI-TOF analyses were carried out over the obtained products.

2.3. UV Curing and Film

To prepare photo-crosslinkable systems, PGA-Met obtained macromers were dissolved in acetone (263 mg/mL), and 3 wt% of a photoinitiator (Irgacure® 369), concerning the total mass of the PGA-Met, was added to the solutions (Scheme 2). The obtained mixtures were protected from light and stirred for 5 min at room temperature. The Crosslinked samples were prepared on glass plates (ø = 12 mm), adding 10 μL of mixture and drying them for 10 min in a fume hood protected from light at room temperature, allowing the solvent to evaporate. Subsequently, they were exposed to a UV lamp using a UVPTM CL–1000 short-wave photo-crosslinker (λ = 313 nm) for 10 and 30 min. The photo-crosslinked samples were named as n-mPGA-Metx, n being the amount of CALB, m the number of steps, and x the time of UV irradiation during crosslinking. Thus, 1-2PGA-Met10 indicates the crosslinked sample from a PGA-Met macromer obtained with 1 wt% of CALB in a one-pot two-step synthesis procedure and crosslinked by exposure to UV radiation for 10 min.
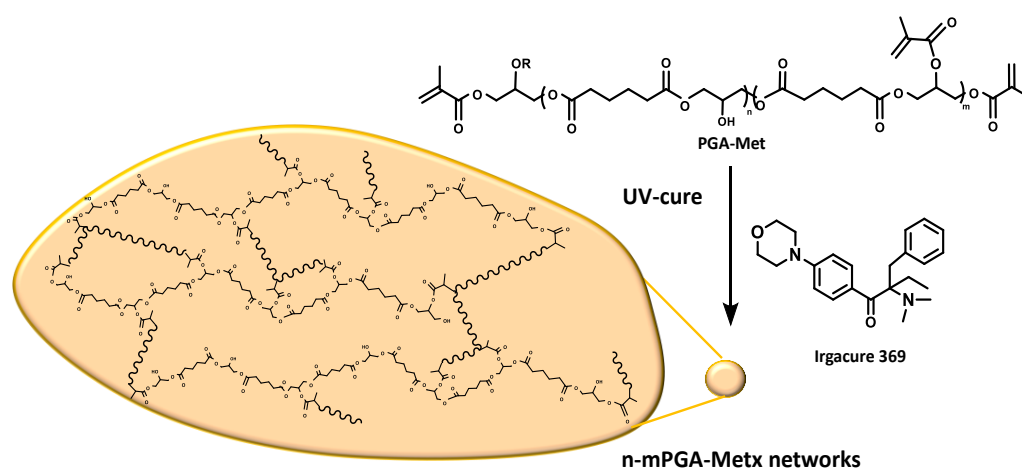
Scheme 2. Film synthesis through UV irradiation crosslinking in the presence of initiation of obtained PGA-Met macromers.

2.4. Characterization

2.4.1. Nuclear Magnetic Resonance, ^{1}H and ^{13}C NMR analyses

[1]H (128 scans, 1 s relaxation delay) and ^{13}C NMR (5120 scans, 1 s relaxation delay) spectra were recorded on a TM Bruker DPX 400 (400 MHz) spectrometer in deuterated acetone (≃40 mg/mL for ^{1}H NMR and ≃80 mg/mL for ^{13}C NMR), containing tetramethylsilane (TMS) as an internal standard. The amount of 1,2,3-substituted (branching degree) and 1,2-substituted units were calculated from the ^{1}H NMR, following Equations (1) and (2):

$$1,2,3 - \text{substituted units} = \frac{\frac{Ie''}{ne''}}{\frac{Ia}{na}} \cdot 100 \tag{1}$$

$$1, 2 - \text{substituted units} = \frac{\frac{Ie'}{ne'}}{\frac{Ia}{na}} \cdot 100 \qquad (2)$$

where I is the intensity and n is the number of protons.

The degree of branching was calculated using the following Equation (3):

$$\text{Degree of Branching (DB)} = 2D/(2D + L) \cdot 100 \qquad (3)$$

2.4.2. Matrix-Assisted Laser Desorption/Ionization-Time-of-Flight Mass Spectrometry (MALDI-TOF-MS)

A matrix-assisted laser desorption/ionization-time-of-flight mass spectrometry (MALDI-TOF-MS) analysis of the synthesized macromers was performed using a Voyager-DE PRO time-of-flight mass spectrometer (Applied Biosystems, Foster City, CA, USA) equipped with a nitrogen laser emitting at $\lambda = 337$ nm. All the spectra were acquired in positive ion mode and the delayed extraction and instrumental settings were tuned to the parameters that optimized the signal intensity and resolution. The samples were dissolved in acetone and 1 µL of matrix (2,5-dihydroxybenzoic acid (DHB)) and 1 µL of cesium chloride were mixed with 1 µL of each dissolved sample (1 mg/mL).

2.4.3. Size Exclusion Chromatography (SEC)

The obtained macromers were analyzed using Size Exclusion Chromatography (SEC) to estimate the number-average molecular weight (M_n), weight-average molecular weight (M_w), and polydispersity index (Đ). DMF stabilized with 0.1 M LiBr was used as an eluent at a temperature of 50 °C and 1 mL min^{-1} flow rate. A Waters Division Millipore system equipped with a Waters 2414 refractive index detector and Styragel packed columns (HR2, HR3, and HR4, Waters Division Millipore, Billerica, MA, USA) was used. The calibration curve was obtained using poly(methyl methacrylate) standards (Polymer Laboratories LTD) ranging from 1.4×10^6 to 5.5×10^2 Da. The samples were dissolved in DMF and filtered through a 0.45 µm Teflon filter before the analysis.

2.4.4. Differential Scanning Calorimetry (DSC)

The thermal behavior of the photocrosslinked films was studied with a TA Instruments (New Castle, DE, USA) DSC Q2000 differential scanning calorimeter, equipped with a refrigerated cooling system (RCS) under an N_2 atmosphere. The measurements were carried out in a three-step procedure of heating–cooling–heating over the temperature range from −70 to 180 °C. After being equilibrated at −70 °C, the samples were heated to 150 °C at 10 °C/min, then rapidly cooled to −70 °C, and heated again to 180 °C at the same rate. The glass transition temperatures (T_g) were estimated from the first and second heating runs and taken as the temperature at the midpoint of the calorific capacity change.

2.4.5. Fourier-Transform Infrared Spectroscopy with Attenuated Total Reflectance (FTIR-ATR)

The crosslinking process was confirmed by analyzing the samples in a Perkin Elmer Spectrum Two instrument equipped with an attenuated total reflection module (ATR). A background spectrum was acquired before every sample and all the spectra were recorded between the 400 and 4000 cm^{-1} spectral range with a 4 cm^{-1} resolution.

3. Results and Discussion

3.1. Molecular Weight and Structural Characterization

3.1.1. ^{1}H NMR, ^{13}C NMR and SEC

The one-pot procedure was carried out by reacting GLY, DVA, and VMA dissolved in anhydrous THF with CALB at 40 °C under N_2 and continuous stirring. For the two-step one-pot procedure, the VMA monomer was added to the mixture after 6 h of the DVA and GLY reaction (see Scheme 1).

Figure 1A,B show the ^{1}H NMR assignments and spectra of the products, respectively. The assignments and calculations were made based on our previous studies of enzymatically synthesized PGA with different reagent ratios, catalyst amounts, and others [25]. CH_2 adipic protons (a, b) appeared in the spectral range between 1.6 and 2.5 ppm, while all the protons related to glyceride repeating units were found between 3.5 and 5.3 ppm; (c, d) and (e) protons corresponded to a 1,3-substituted linear glycerol unit; (h, i), (f, g), and (e′) protons correspond to a 1,2-substituted linear glycerol unit; and (h′, i′), and (e″) corresponded to a 1,2,3-substituted dendritic glycerol unit. The last peak (dendritic, e″) at 5.3 ppm due to the 1,2,3-substituted glycerides was produced due to the lack of regioselectivity of the enzyme during the reaction. Glycerol terminal units gave multiplets in the region of 3.4-3.7 ppm, (m, n), (j, k), and (l) protons corresponded to terminal glycerol that was 1 or 3 substituted, while (m′, n′) and (l′) protons corresponded to terminal glycerol that was 2 substituted. Resonances at 1.8, 5.5, and 6 ppm were attributed to the (p), (o′), and (o) protons of the vinyl component from the methacrylate group, respectively.

Figure 2A,B show the ^{13}C NMR assignments and a representative spectrum of the product obtained in the one-pot one-step synthesis with 3 wt% of CALB. The ^{13}C NMR of the macromers obtained in the one-pot one-step synthesis with 1 wt% of CALB and two-step synthesis with 1 and 3 wt% of CALB are shown in Appendix A in Figures A1 and A2, respectively.

^{1}H NMR and ^{13}C NMR confirmed that the methacrylate end groups were bounded to the poly(glycerol adipate) polymer when 3 wt% of CALB was present, either for the one-step or two-steps one-pot syntheses. For smaller amounts of CALB (1 wt%), methacrylate end groups were only found in the one-pot synthesis strategy. When VMA was added after a 6 h reaction of DVA and GLY, resonances from the methacrylate group protons/carbons were absent, while vinyl protons/carbons from the DVA appeared. This fact can be indicative of the VMA reacting at the latest stages of the synthesis, when most of the DVA was incorporated into the polymer chains. This could be due to the slower reaction rate of VMA, which acts as an end-capper terminating the polymeric chains' growth, as well as to the presence of the higher amount of vinyl groups suffering from potential hydrolysis, which could slow down the kinetics hydrolysis of the DVA, making it able to participate for longer in the chain growth and not lose the vinyl end groups.

As we previously reported [25], the ^{13}C NMR spectra of PGAs facilitate the verification of the presence of terminal glycerol units, as well as the hydrolysis of double bonds from terminal divinyl adipate, which are not clearly revealed in ^{1}H NMR spectra. Figure 2A,B display the carbon peak assignments, based on previous studies, as well as expanded regions of 61–77 ppm, 125–138 ppm, and 173–174 ppm [37–40]. Importantly, the spectrum for the one-pot two-step protocol with the highest amount of CALB (3 wt%) shows a clear, intense signal centered at 174.6 ppm, which corresponds to an acid carbonyl carbon due to the DVA end-group hydrolysis. Therefore, the low M_n and M_w achieved in this polymer could be strongly related to the hydrolysis of the vinyl groups at the early synthesis stages, which was somehow prevented when VMA was present as a starting monomer (one-pot synthesis procedure). In such a situation, an increase in the availability of vinyl groups from different species in the reaction mixture would permit DVA to contribute to the polymer chain growth to a higher extent. For a lower amount of CALB (1 wt%) in the two-step protocol, the ^{13}C NMR spectrum confirms the slower reaction rate of the VMA. In agreement with the ^{1}H NMR results, the peaks at 142 ppm and 97 ppm were due to the

reacted DVA vinyl end groups being preserved, while the peaks centered at 18.4 ppm (Q), 125.8 ppm (O), and 137.3 ppm (P) were due to methacrylate end groups being absent.
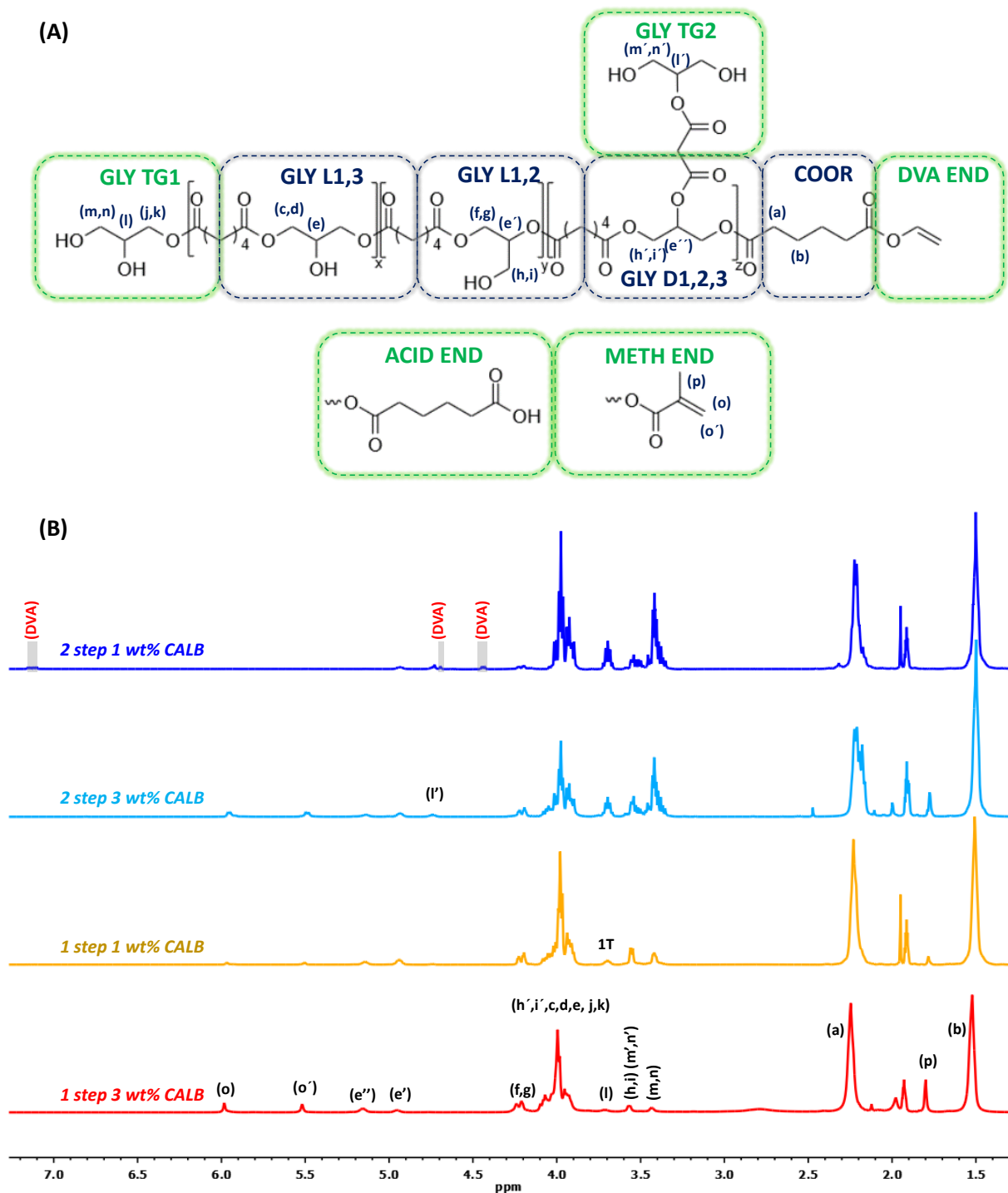

Figure 1. (**A**) ¹H NMR proton assignments for linear, terminal, and dendritic glycerol units contained in the polymers, as well as for DVA, acid, and methacrylate possible terminations. (**B**) ¹H NMR spectra of one-pot synthesis carried out. From up to down; in two-step with 1 and 3 wt% of CALB and one-step with 1 and 3 wt% of CALB.
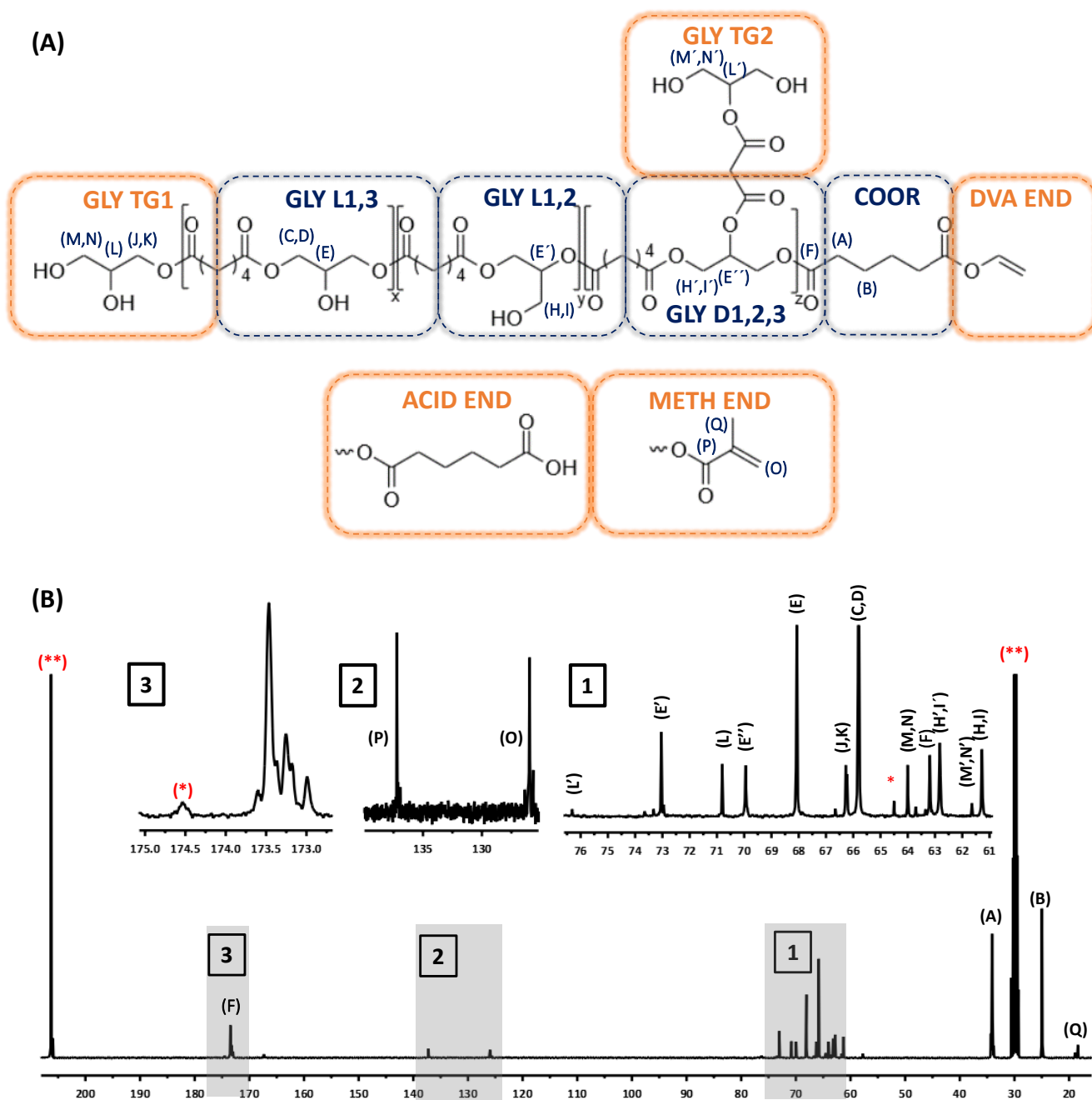
(A)

(B)

Figure 2. (**A**) ^{13}C NMR carbon assignments for linear, terminal, and dendritic glycerol units contained in the polymers, as well as for DVA, acid, and methacrylate possible terminations. (**B**) ^{13}C NMR spectra of one-pot synthesis one-step macromer obtained with 3 wt% CALB. (*) Adipic Acid Termination, (**) Deuterated Solvent, * Glycerol. Boxes 1, 2, and 3, show the expansion areas of selected peaks.

In line with this, the data analysis of Table 1 shows that there is a significant difference among the M_n and M_w achieved in the one-pot one-step and two-step synthesis procedures, resulting in the highest values when 3 wt% of CALB was added to the reaction mixture. When VMA was present as a starting monomer, a higher M_n and M_w were reached (M_n; 7450–7900 g/mol and M_w; 12,100–13,600 g/mol for 1 and 3 wt%, respectively), while adding this monomer when the DVA and GLY were already reacted for 6 h considerably diminished the M_n and M_w values, being more noticeable for the lowest amount of CALB (M_n; 1150 g/mol and M_w; 1220 g/mol). This confirms that a higher presence of potential

hydrolyzable groups from the beginning of the synthesis helped to preserve the reactive ends of the DVA, and therefore to achieve higher molecular weights. In addition to this, the $Đ_{SEC}$ values indicate the formation of branched polymers by the incorporation of DVA at the beginning of the reaction.

Table 1. SEC molecular weight values of polymers synthesized at 40 °C in THF solution.

Synthesis Procedure (Steps)	CALB (wt%)	$M_{w, SEC}$ (g/mol)	$M_{n, SEC}$ (g/mol)	$Đ_{SEC}$ M_w/M_n	Yield (%)
2 steps	1	1220	1150	1.1	61.9
2 steps	3	2090	1890	1.1	66.8
1 step	1	12,100	7450	1.6	58.7
1 step	3	13,600	7900	1.7	46.4

The relative percentages of TG (1-substituted), TG2 (2-substituted), L1-3 (1,3-substituted), L1-2 (1,2-substituted), and D (1,2,3-substituted), and the end group relative abundance in terms of the % of glycerol, divinyl, and methacrylate end groups, as well as the regioselectivity, degree of branching, and degree of methacrylation, are given in Table 2. The results compiled there show that all the synthesized polymers were mainly linear, as DB was retained at really low values, which is consistent with our previous report on enzymatically synthesized PGA polyesters with a low excess of vinyl groups [25]. Thus, the regioselectivity of the enzyme was retained and methacrylate moieties were preferentially located at the end positions of the polymeric chains. Interestingly, the higher the amount of VMA end groups, the lower the 2-substituted glycerol end groups; thus, it seems that most of the VMA groups were incorporated by reacting with primary the hydroxyl groups and that the regioselectivity lack was mainly due to the late incorporation into the secondary hydroxyl groups from glycerol in the polymer chains.

Table 2. Relative abundance of different glycerol structural units, end group functionality of PGA-Met polymers obtained at 40 °C in THF solution, and regioselectivity of the enzyme as calculated by ^{1}H NMR.

Synthesis Procedure	CALB (wt%)	Glycerol Structural Unit Relative Abundance (%)					End Group Relative Abundance (%)			Regioselectivity (%)	DB (%)
		TG (1-sub.)	TG$_2$ (2-sub.)	L$_{1,3}$ (1,3-sub.)	L$_{1,2}$ (1,2-disub.)	D (1,2,3-sub.)	GLY	VMA	DVA		
2 steps	1	45.5	7.4	42.4	4.7	--	95.1	--	4.9	91.8	--
2 steps	3	38.8	8.5	34.6	6.3	11.7	80.8	19.2	--	83.8	0.4
1 step	1	11.8	1.1	63.1	15.2	8.8	81.1	18.9	--	87.2	0.2
1 step	3	10.1	--	65.2	8.8	16.0	38.4	61.6	--	88.0	0.3

3.1.2. MALDI-TOF-MS

A representative MALDI-TOF spectrum from a PGA-Met obtained in the one-pot synthesis with 3 wt% of CALB is shown in Figure 3. The analysis showed several distributions corresponding to different degrees of polymerization of the PGA terminated with various groups (see Figures 3 and A3–A6 and Table A2): n_{gv} stands for HO-(PGA)-Vinyl ($202n + 44$), n_{vv} stands for Vinyl-(PGA)-Vinyl ($202n + 198$), n_{gg} stands for HO-(PGA)-OH ($202n + 92$), n_{gm} stands for HO-(PGA)-Met ($202n + 86$), n_{mm} stands for Met-(PGA)-Met ($202n + 170$), and n_{mv} stands for Met-(PGA)-Vinyl ($202n + 156$) structures. Due to the hydrolysis reactions of the vinyl groups during the growth of the polymers, free acid ends were also present, where: n_{ga} stands for HO-(PGA)-Acid ($202n + 18$), n_{aa} stands for

Acid-(PGA)-Acid (202n + 146), n_{va} stands for Vinyl-(PGA)-Acid structures (202n + 172), and n_{ma} stands for Met-(PGA)-Acid (202n + 158) structures.
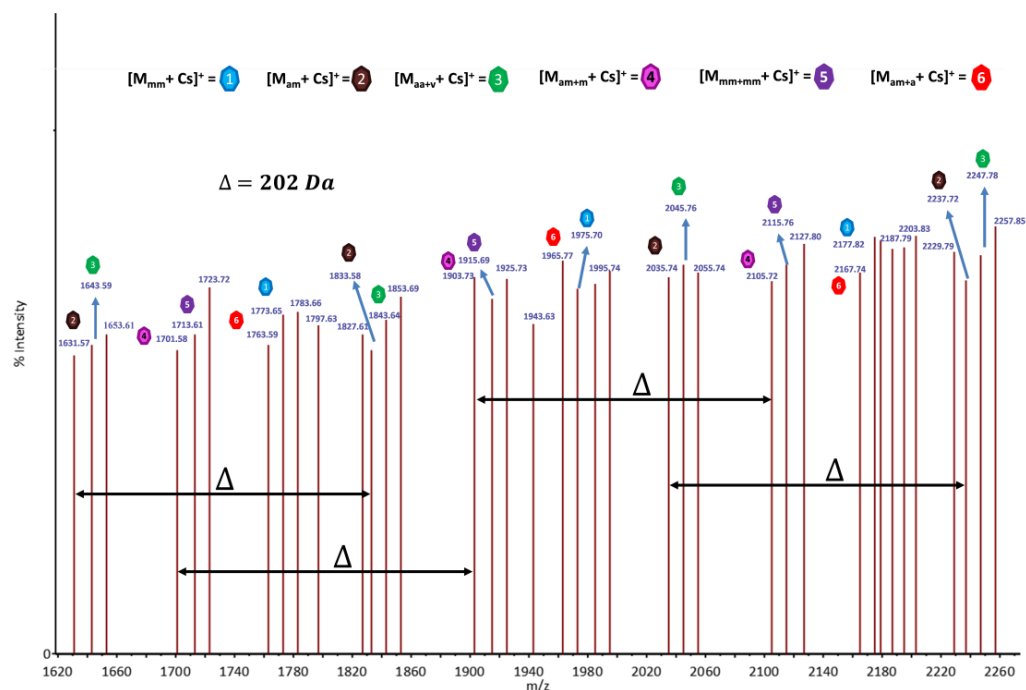

Figure 3. Detailed region of MALDI-TOF spectrum from 1600 to 2300 m/z for one-pot synthesis one-step macromer obtained with 3 wt% CALB.

Wherein, n is the number of peaks per polymer chain. The repeating mass difference between any given series of peaks is 202 Da, which is the molecular weight for a linear hydroxyl-substituted polyester from GLY and DVA.

Figure 4 and Table 3 show the end group composition of the polyesters obtained in the one-pot one-step and two-step synthesis procedures under the presence of 1 and 3 wt% of an enzymatic catalyst. Interestingly, the one-step synthesis procedure allowed for the highest proportion of Met-(PGA)-Met (% n_{mm}) end-capped-type chains (~52–55%), without a marked influence on the enzyme concentration. When polymers were obtained in the one-pot two-step synthesis procedure, the number of Met-(PGA)-Met species significantly decreased, being higher when CALB was present in a higher amount (3 wt%), in accordance with the ^{1}H NMR results.

Table 3. Effect of enzyme concentration and one-pot synthesis (one step and two steps) in end group composition relative abundance as calculated from MALDI-TOF-MS and its comparison with ^{1}H NMR results.

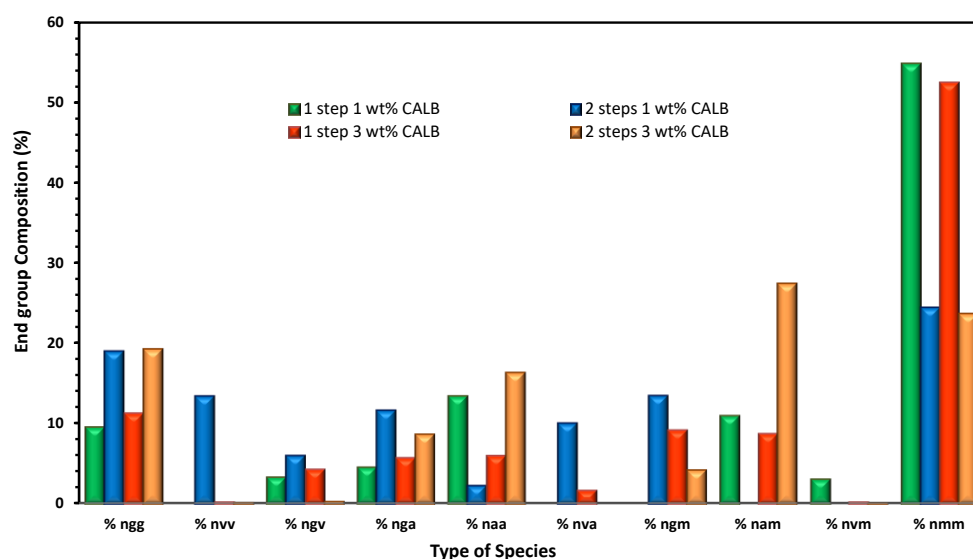| Synthesis Procedure	CALB (wt%)	End Group Relative Abundance (%) MALDI-TOF-MS				End Group Relative Abundance (%) ^{1}H NMR		
		GLY	VMA	DVA	AC	GLY	VMA	DVA
2 steps	1	57.0	23.8	11.8	7.4	95.1	--	4.9
2 steps	3	29.8	30.6	1.4	38.2	80.8	19.2	--
1 step	1	15.2	53.1	9.5	22.2	81.1	18.9	--
1 step	3	21.4	52.0	8.8	17.8	38.4	61.6	--

Figure 4. Effect of one-pot synthesis steps and enzymatic catalyst amount on the composition of synthesized polyesters, as calculated from MALDI-TOF-MS.

As shown in Table 3, importantly, the terminal carboxylic groups that were not evidenced in the ^{1}H NMR were evidenced by MALDI-TOF-MS, confirming the ^{13}C NMR results. The quantitative determination of the acid end groups using MALDI-TOF shows that the maximum acid chain ends were obtained for the highest amount of catalyst in the one-pot two-step synthesis procedure. For 3 wt% of CALB and the one-pot two-step synthesis procedure, it seems that the highest amount of catalyst hydrolysed the DVA end groups, increasing the amount of acid terminal chains.

3.2. Thermal Properties of UV Crosslinked Films

Having in mind that the polymers synthesized with 1 wt% of CALB presented a lower hydrolysis and had a similar percentage to those obtained with 3 wt% of CALB, the evaluation of their applicability as photo-crosslinking systems was performed with polymers synthesized with a lower amount of lipase and considered as less costly. The thermal properties of the PGA-Met macromers obtained in the one-pot one-step and two-step synthesis procedures after UV irradiation for 10 and 30 min (see Scheme 2), 1-1PGA-Met10, 1-1PGA-Met30, 1-2PGA-Met10, and 1-2PGA-Met30, respectively, were analyzed using DSC and the resulting T_gs are collected in Table 4.

Table 4. Thermal properties of the PGA-Met macromers obtained networks as determined by DSC first and second heating runs. The errors of the temperature are equal to ± 1 °C.

Network	UV Light Exposure Time (min)	1st Heating T_g (°C)	2nd Heating T_g (°C)
1-2PGA-Met10	10	-50	-41
1-2PGA-Met30	30	-52	-44
1-1PGA-Met10	10	-28	-20
1-1PGA-Met30	30	-29	-20

The crosslinking of selected macromers resulted in amorphous networks, as only glass transitions were visible in DSC, with no evident melting or crystallization. Importantly, the T_g in the second heating run increased at around 10 °C for all the samples, which was indicative of the presence of some uncured fraction that was suppressed after the first heating run. With increasing the amount of VMA end groups (see Table 3), as well as the molecular weight of the macromers (see Table 1), the T_g value also increased, which was

indicative of the higher crosslinking degree obtained for the macromer synthesized in the one-pot one-step procedure with 1 wt% of CALB. Additionally, no noticeable changes in the transitions were observed after 30 min of UV irradiation in comparison to the values obtained at 10 min. This indicates that curing longer times were not able to crosslink the remaining fractions; therefore, the shorter time was enough to obtain the UV curing of the samples. Moreover, the samples synthesized in two steps did not produce consistent films, since their molecular weights were small and percentage of VMA end groups was lower.

Figure 5 shows the FTIR spectra of the systems before and after exposure to UV light at 10 and 30 min. It is easily appreciable that the band at 1660 cm^{-1}, corresponding to the stretching vibration of the methacrylate double bond, disappeared, while a new band at 1595 cm^{-1}, corresponding to -CH$_2$-CH$_2$-, appeared. It is also observed that still there were double bonds available due to the elevated percentage of functionalization, which was not able to fully cure itself. However, it could be a promising candidate material for stereolithography.
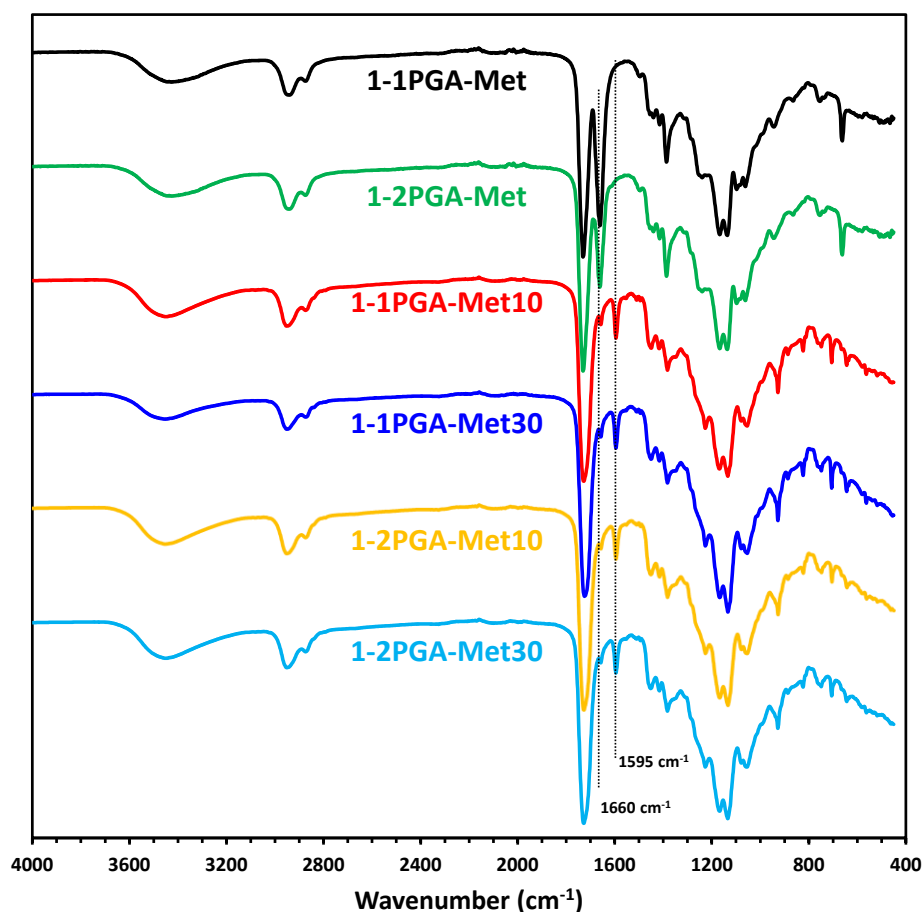

Figure 5. FTIR spectra of 1-1PGA-Met and 1-2PGA-Met before and after photocuring for 10 and 30 min.

4. Conclusions

Our main goal was to create enzymatically synthesized methacrylated end functional poly(glycerol adipate) linear macromers in a one-pot synthesis procedure, which were able to undergo film formation under UV light irradiation when a photoinitiator was added. Polyester macromers with a high methacrylate content were obtained in a simple one-pot one-step enzymatic synthesis ($\approx$52% of methacrylate end groups moieties in comparison to $\approx$25% for two-step enzymatic synthesis, as calculated using MALDI-TOF-MS). The ^{13}C NMR analysis confirmed the presence of acid end groups due to the DVA hydrolysis, which was quantified using MALDI-TOF-MS to be in the range of $\approx$20% for the one-pot

one-step-obtained macromers. The highest amount of methacrylate functionality of one-pot one-step synthesized macromers with 1 wt% of CALB allowed for the formation of higher crosslinking networks. Thus, the synthesized macromers included polyesters with different combinations of methacrylate, vinyl, hydroxyl, and carboxyl acid end groups, which could be shifted towards obtaining preferentially methacrylate end-capped species in a higher concentration by using a one-pot one-step synthesis method. This easy and clean synthesis approach towards methacrylate functional polyesters that is useful for photo-crosslinking processes has not been previously reported and constitutes, to date, the most efficient procedure, opening up a promising strategy for industrially applicable new biobased materials for 3D printing.

Appendix A

Table A1. ^{1}H NMR Signal integrals for different CALB amounts and one-pot synthesis steps to obtain PGA-Met (40 °C in THF).

Proton	Explanation	Number of H [n]	Integral [I] 1 Step 3wt%	Integral [I] 1 Step 1wt%	Integral [I] 2 Steps 3wt%	Integral [I] 2 Steps 1wt%
b	Aliphatic RO-CH=CH–CH$_2$–CH$_3$ (m)	4	40.0_0	40.0_0	40.0_0	40.0_0
p	Terminal ROC=CH$_2$CH$_3$ (m)	3	4.8_1	0.9_0	3.2_7	-
a	Aliphatic ROCH$_2$–CH$_2$–CH$_2$–CH$_2$OR′ (m)	4	40.4_4	39.7_8	39.8_8	38.4_7
m, n	Terminal 1,3 ROCH$_2$–CHOH–CH$_2$OH (ddd)	2	2.0_0	2.1_7	6.3_6	10.6_0
h, i m′, n′	Lineal 1,2 ROCH$_2$–CH(OR′)–CH$_2$OH (m) Terminal 1,2 HOCH$_2$–CH(OR)CH$_2$OH (m)	2 2	2.0_0	3.0_7	4.9_4	4.3_1
l	Terminal 1,2 ROCH$_2$–CHO–CH$_2$OH (m)	1	1.0_0	1.0_9	3.1_6	5.2_8
j, k c, d, e h′, i′	Lineal 1,3 ROCH$_2$–CHOH–CH$_2$OR′ (m) Terminal 1,3 ROCH$_2$–CHOH–CH$_2$OH′ (ddd) Dendritic ROCH$_2$–CH(OR′)–CH$_2$OR″ (dd–dd)	2 5 2	40.5_9	34.3_6	24.3_6	35.2_9
DVA	Terminal ROCH$_2$–CH$_2$–CH$_2$–CH$_2$OCHCH$_2$ (dd)	1	-	-	-	0.3_6
DVA	Terminal ROCH$_2$–CH$_2$–CH$_2$–CH$_2$OCHCH$_2$ (dd)	1	-	-	-	0.3_6
f, g	Lineal 1,2 ROCH$_2$–CH(OR′)–CH$_2$OH (ddd)	2	4.6_8	2.6_7	2.1_5	0.8_8
l′	Terminal 1,2 ROCH–CH$_2$OH (m)	1	-	-	-	0.4_1

Table A1. *Cont.*

Proton	Explanation	Number of H [n]	Integral [I] 1 Step 3wt%	Integral [I] 1 Step 1wt%	Integral [I] 2 Steps 3wt%	Integral [I] 2 Steps 1wt%
e′	Linear 1,2; $ROCH_2$–CH(OR′)–CH_2OH (m)	1	0.8_7	1.3_9	0.5_2	0.5_5
e″, e	Dendritic; $ROCH_2$–CH(OR′)–CH_2OR''(m)	1	1.6_0	0.3_1	1.0_9	-
o′	Terminal ROC=CH_2CH_3 (s)	1	1.6_0	0.3_1	1.0_9	-
o	Terminal ROC=CH_2CH_3 (s)	1	1.6_0	0.3_1	1.0_9	-
DVA	Terminal $ROCH_2$–CH_2–CH_2–CH_2OCHCH_2 (dd)	1	-	-	-	0.3_7

Integrals of protons (b), (p), (a), (m,n), (l), (f,g), (e′), (e″), (o′), and (o) were directly obtained from the ^{1}H NMR spectra. Integral values for (h,i), (m′,n′), (h′,i′), (j,k), (c,d), and (c,d,e) protons where obtained indirectly based on the equalities previously reported [25].

The total glycerol integral was calculated as follows:

$$I_{GLY} = \frac{8 \cdot (I_{e''} + I_{e'} + I_e) + 4 \cdot \left(I_{(m,n)}\right) + 2 \cdot \left(I_{(m',n')}\right)}{8} \tag{A1}$$

Therefore, % of terminal glycerol, linear 1,3- and 1,2-substituted, as well as dendritic % of glycerol in the polymers can be calculated using the following equations:

$$\%TG = \frac{\frac{I_{m,n}}{2}}{I_{GLY}} \cdot 100 \tag{A2}$$

$$\%TG2 = \frac{\frac{I_{m',n'}}{2}}{I_{GLY}} \cdot 100 \tag{A3}$$

$$\%L\,1,3 = \frac{I_e}{I_{GLY}} \cdot 100 \tag{A4}$$

$$\%L\,1,2 = \frac{I_{e'}}{I_{GLY}} \cdot 100 \tag{A5}$$

$$\%D = \frac{I_{e''}}{I_{GLY}} \cdot 100 \tag{A6}$$

where, %TG refers to terminal glycerol 1- or 3-substituted units; %TG2 refers to terminal glycerol 2-substituted units; %L 1,3 refers to 1,3-substituted linear glycerol units; %L 1,2 refers to 1,2 linear glycerol units; and %D refers to dendritic or 1,2,3-substituted glycerol units.

Total amount of GLY, DVA, and MET end groups were calculated as follows:

$$I_{END\,GROUPS} = \frac{I_{m,n} + I_{m',n'} + 3I_p}{2} \tag{A7}$$

*when DVA signals were present and MA signals were absent, the term $3I_p$ was substituted by $2I_p$ being "p" a signal due to terminal vinyl protons of DVA ($ROCH_2$-CH_2-CH_2-CH_2OCHCH_2).

$$\%GLY\,END\,GROUPS = \frac{\frac{I_{m,n} + I_{m',n'}}{2}}{I_{END\,GROUPS}} \cdot 100 \tag{A8}$$

$$\%MA\,END\,GROUPS = \frac{I_p}{I_{END\,GROUPS}} \cdot 100 \tag{A9}$$

*when DVA signals were present and MA signals were absent, the term I_p was related to a signal due to terminal vinyl protons of DVA (ROCH$_2$-CH$_2$-CH$_2$-CH$_2$OC*H*CH$_2$).

The degree of branching can be calculated using the following equation:

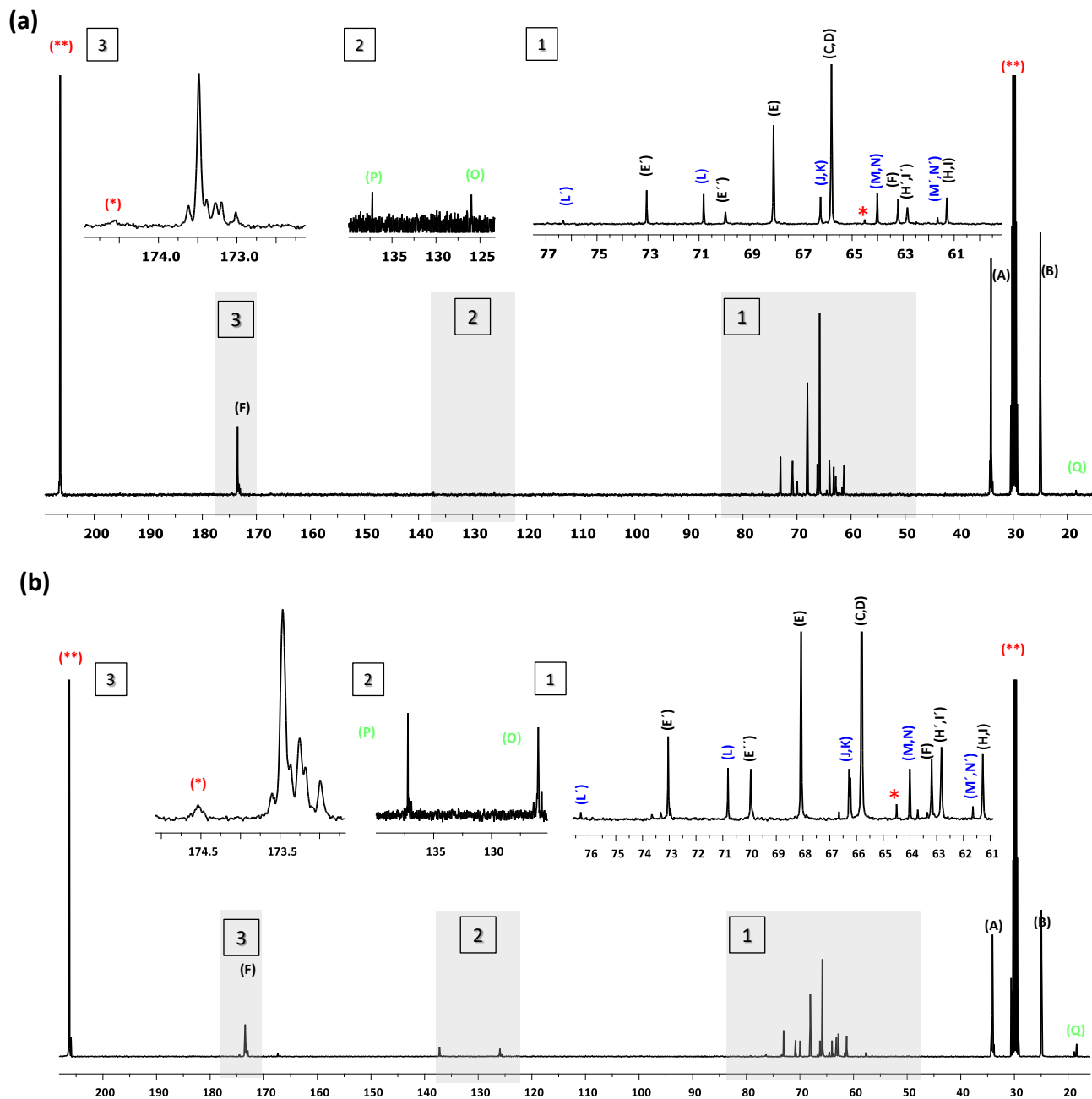$$\text{Degree of Branching (DB)} = \frac{2D}{2D + L} \cdot 100 \qquad (A10)$$

Figure A1. ^{13}C NMR spectra of one-step one-pot synthesis of PGA-Met at 40 °C in THF with (**a**) 1 wt% CALB and (**b**) 3 wt% CALB. (*) Adipic Acid Termination, (**) Deuterated Solvent, * Glycerol.
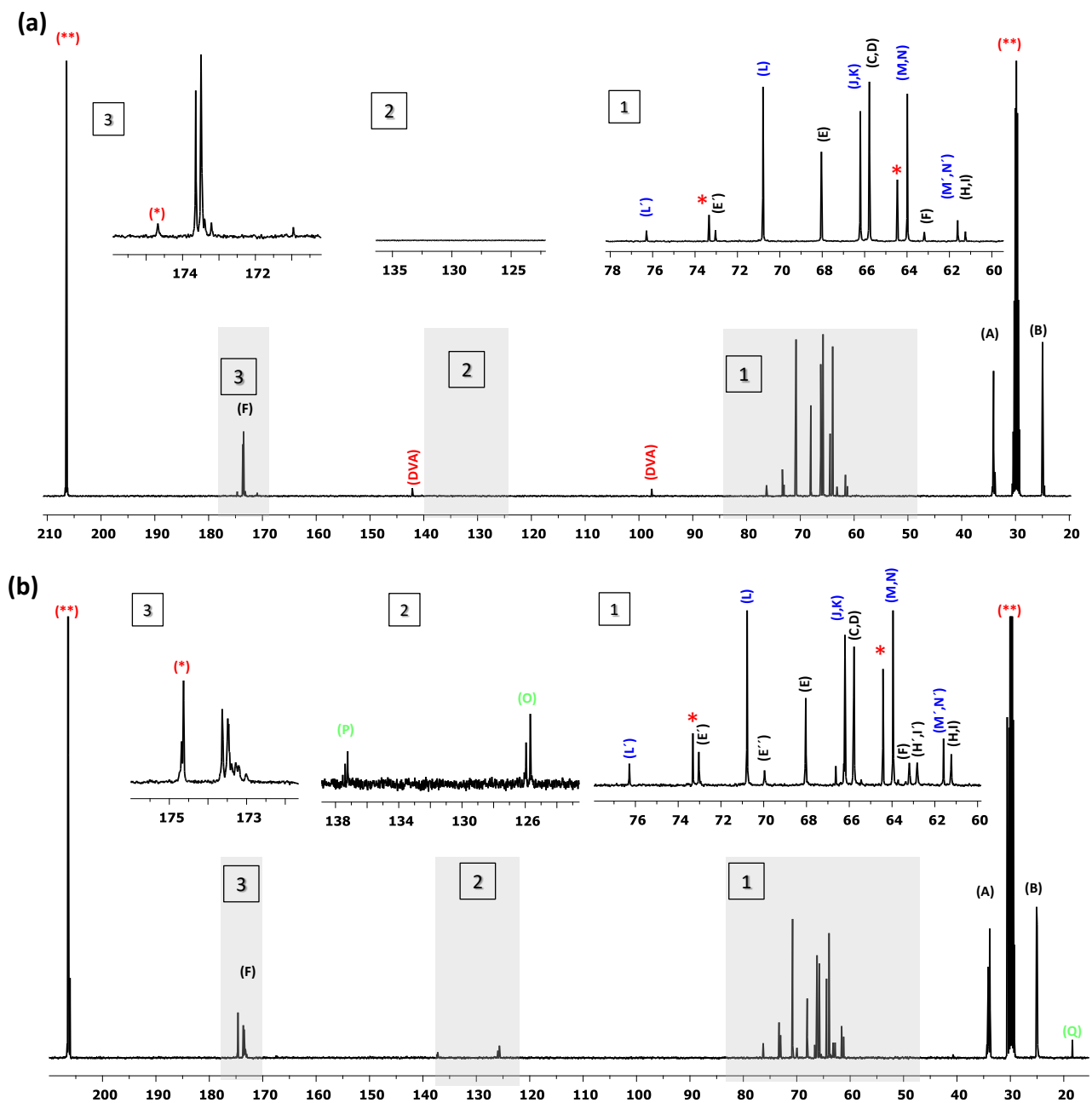
Figure A2. ^{13}C NMR spectra of two steps one-pot synthesis of PGA-Met at 40 °C in THF with (**a**) 1 wt% CALB and (**b**) 3 wt% CALB. (*) Adipic Acid Termination, (**) Deuterated Solvent, * Glycerol.
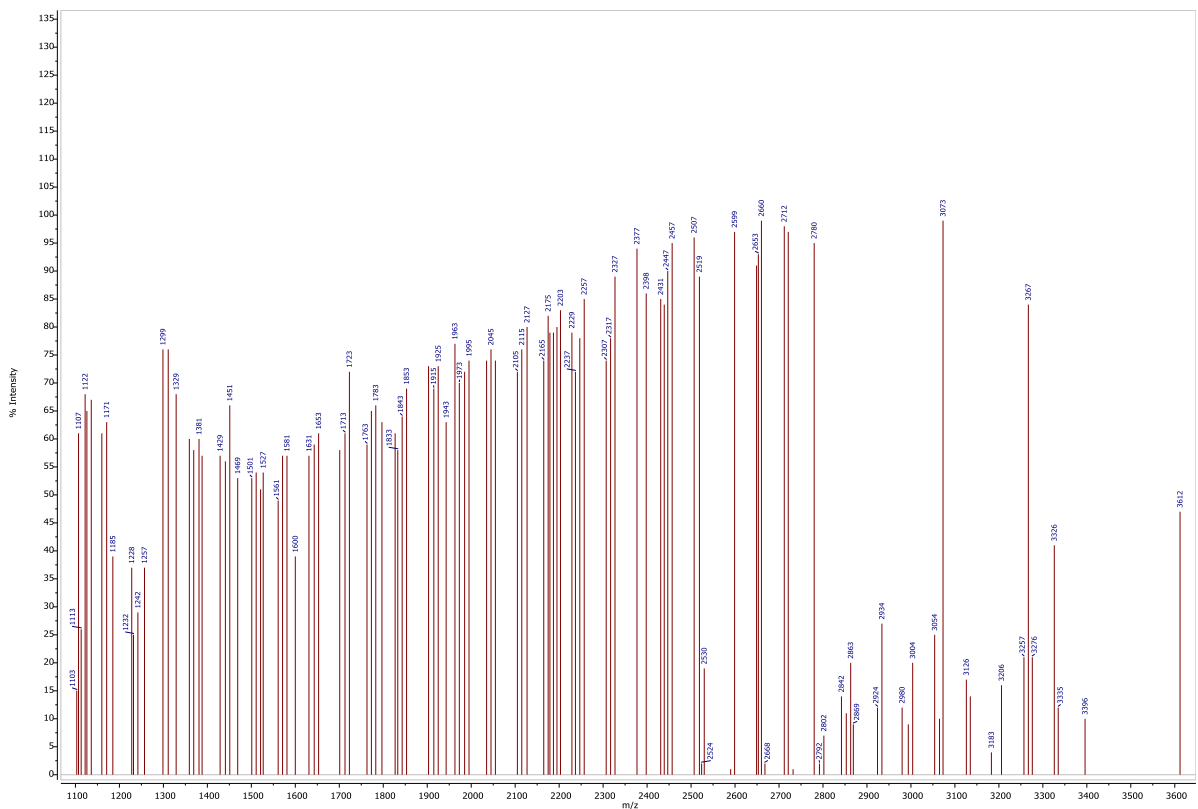
Figure A3. Full MALDI-TOF spectrum for PGA-Met synthesized from one-pot one-step procedure in the presence of 3 wt% CALB.
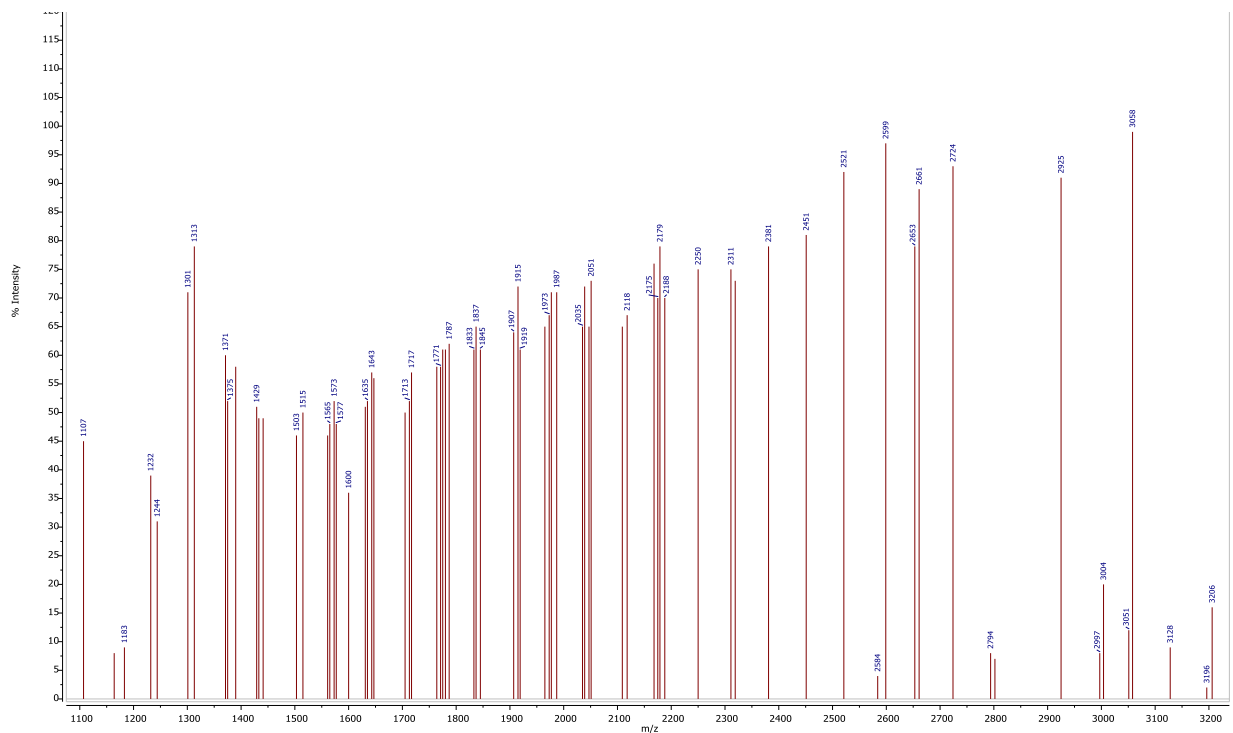

Figure A4. Full MALDI-TOF spectrum for PGA-Met synthesized from one-pot one-step procedure in the presence of 1 wt% CALB.
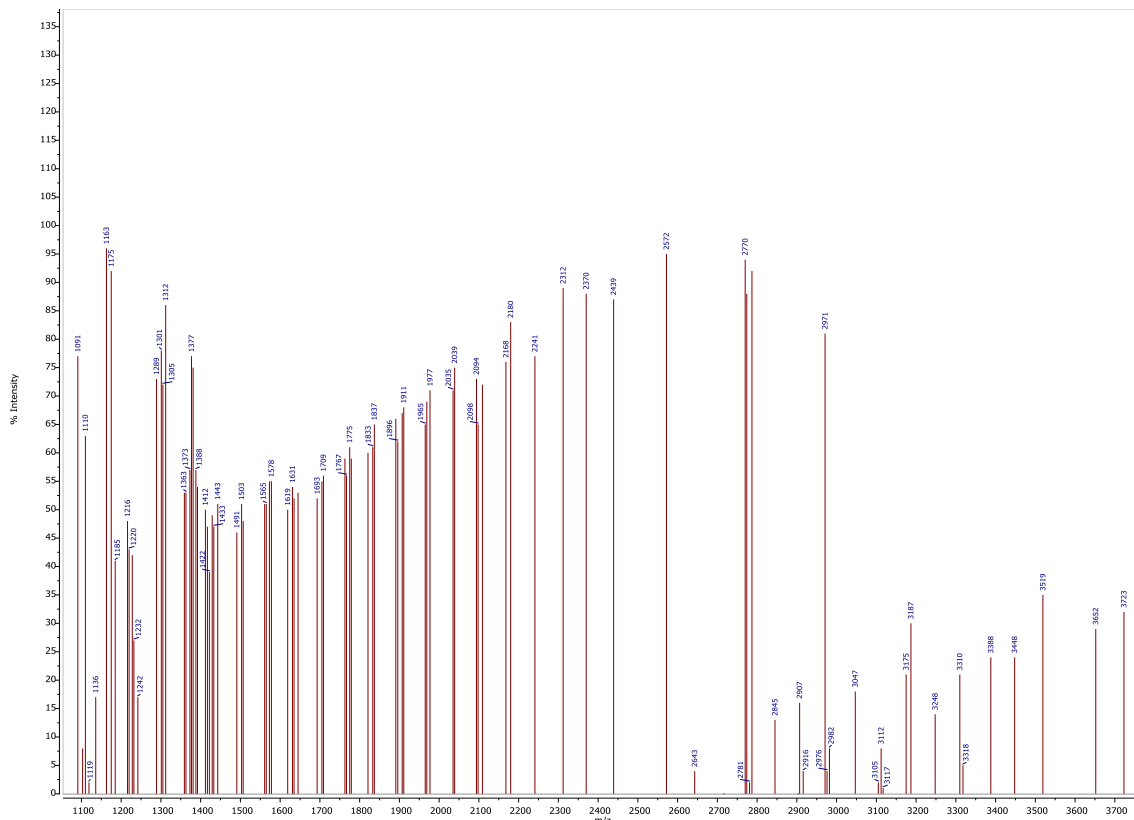
Figure A5. Full MALDI-TOF spectrum for PGA-Met synthesized from one-pot two-step procedure in the presence of 3 wt% CALB.
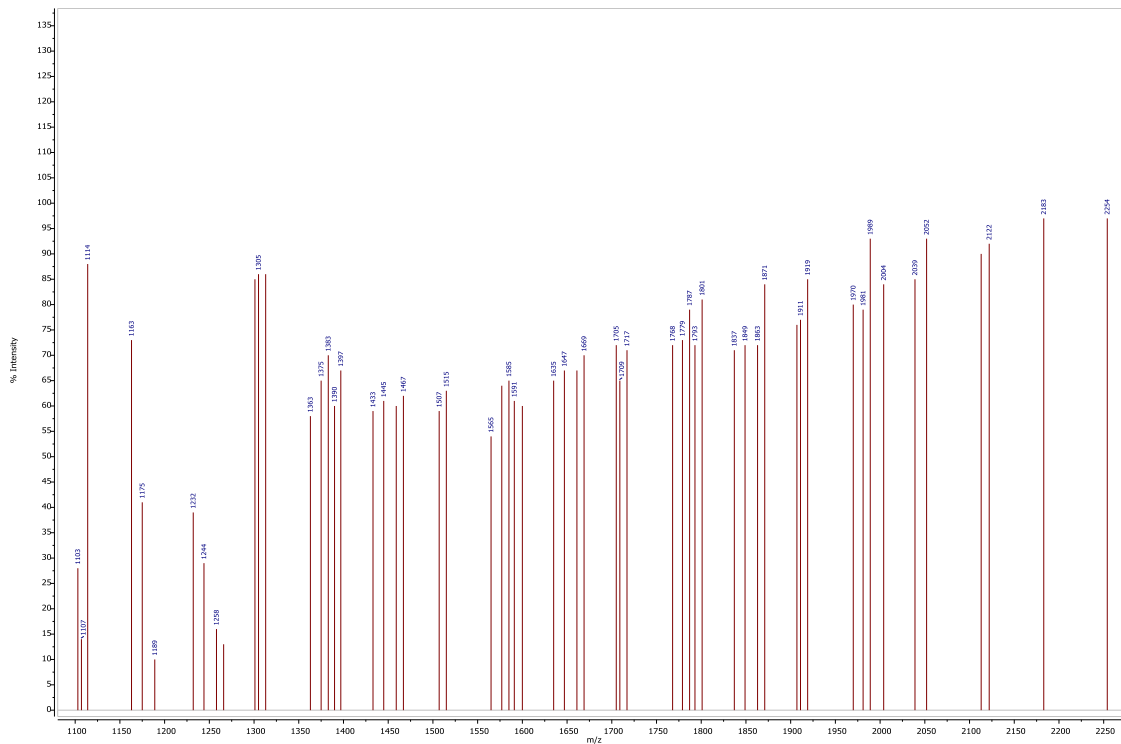

Figure A6. Full MALDI-TOF spectrum for PGA-Met synthesized from one-pot two-step procedure in the presence of 1 wt% CALB.

Table A2. Effect of synthesis procedure (one-pot number of steps) on product end group composition.

CALB (wt%)		End Group	Composition (%)	
			One-Pot Synthesis Steps	
			1 Step	2 Steps
1	n_{gg}	HO-(PGA)-OH	11.3	19.3
	n_{vv}	Vinyl-(PGA)-Vinyl	0.2	0.1
	n_{gv}	HO-(PGA)-Vinyl	4.3	0.3
	n_{ga}	HO-(PGA)-Acid	5.8	8.7
	n_{aa}	Acid-(PGA)-Acid	6.0	16.3
	n_{va}	Vinyl-(PGA)-Acid	1.7	0
	n_{gg}	HO-(PGA)-Met	9.2	4.2
	n_{vv}	Acid-(PGA)-Met	8.8	27.4
	n_{ga}	Vinil-(PGA)-Met	0.2	0.1
	n_{aa}	Met-(PGA)-Met	52.5	23.6
3	n_{gg}	HO-(PGA)-OH	9.6	19.0
	n_{vv}	Vinyl-(PGA)-Vinyl	-	13.4
	n_{gv}	HO-(PGA)-Vinyl	3.3	6.0
	n_{ga}	HO-(PGA)-Acid	4.6	11.6
	n_{aa}	Acid-(PGA)-Acid	13.5	2.3
	n_{va}	Vinyl-(PGA)-Acid	-	10.0
	n_{gg}	HO-(PGA)-Met	-	13.4
	n_{vv}	Acid-(PGA)-Met	11.0	0
	n_{ga}	Vinil-(PGA)-Met	3.1	0
	n_{va}	Met-(PGA)-Met	54.9	24.3

References

1. Kobayashi, S. Recent Developments in Lipase-Catalyzed Synthesis of Polyesters. *Macromol. Rapid Commun.* **2009**, *30*, 237–266. [CrossRef] [PubMed]
2. Peeters, J.W.; Palmans, A.R.A.; Meijer, E.W.; Koning, C.E.; Heise, A. Chemoenzymatic Synthesis of Branched Polymers. *Macromol. Rapid Commun.* **2005**, *26*, 684–689. [CrossRef]
3. Okabayashi, R.; Ohta, Y.; Yokozawa, T. Control of Molecular Weight and End-Functional Groups of Polyester from A2 + B2 Poly-condensation via Cross-Metathesis of Cyclic Unsaturated Polyester with Difunctional Olefin. *Macromolecules* **2017**, *50*, 9589–9597. [CrossRef]
4. Seo, K.S.; Castano, M.; Casiano, M.; Wesdemiotis, C.; Becker, M.L.; Puskas, J.E. Enzyme-Catalyzed Quantitative Chain-End Functionalization of Poly(Ethylene Glycol)s under Solventless Conditions. *RSC Adv.* **2014**, *4*, 1683–1688. [CrossRef]
5. Alvarez Albarran, A.; Silantyeva, E.; Seo, K.S.; Puskas, J.E. Synthesis of Functionalized Polyisobutylenes Using the Propylene Epoxide/TiCl4 Initiating System. *Polym. Chem.* **2014**, *5*, 4710–4714. [CrossRef]
6. Castano, M.; Seo, K.S.; Guo, K.; Becker, M.L.; Wesdemiotis, C.; Puskas, J.E. Green Polymer Chemistry: Synthesis of Symmetric and Asymmetric Telechelic Ethylene Glycol Oligomers. *Polym. Chem.* **2015**, *6*, 1137–1142. [CrossRef]
7. Eriksson, M.; Hult, K.; Malmström, E.; Johansson, M.; Trey, S.M.; Martinelle, M. One-Pot Enzymatic Polycondensation to Telechelic Methacrylate-Functional Oligoesters Used for Film Formation. *Polym. Chem.* **2011**, *2*, 714–719. [CrossRef]
8. Nameer, S.; Semlitsch, S.; Martinelle, M.; Johansson, M. One-Pot Enzyme-Catalyzed Synthesis of Dual-Functional Polyester Macromers towards Surface-Active Hydrophobic Films. *RSC Adv.* **2017**, *7*, 50294–50299. [CrossRef]
9. Hrsic, E.; Keul, H.; Möller, M. One-Pot Synthesis of Multifunctional Polymers by Light-Controlled Radical Polymerization and Enzymatic Catalysis with *Candida antarctica* Lipase B. *Macromol. Rapid Commun.* **2015**, *36*, 2092–2096. [CrossRef]
10. Takwa, M.; Simpson, N.; Mcilmsiträm, E.; Hult, K.; Martinelle, M. One-Pot Difunctionalization of Poly(ω-Pentadecalactone) with Thiol-Thiol or Thiol-Acrylate Groups, Catalyzed by *Candida antarctica* Lipase Ba. *Macromol. Rapid Commun.* **2006**, *27*, 1932–1936. [CrossRef]
11. Qian, X.; Wu, Q.; Xu, F.; Lin, X. Amphiphilic MPEG-Block-Poly (Profen Amide-Co-Esters) Copolymers: One Pot Biocatalytic Synthesis, Self-Assembly in Water and Drug Release. *Polymer* **2011**, *52*, 5479–5485. [CrossRef]

12. Rao, Z.K.; Ni, H.l.; Liu, Y.; Li, Y.; Zhu, H.Y.; Hao, J.Y. Dual Thermo-Responsive Amphiphilic Alternating Copolymers: One-Pot Synthesis and the Temperature-Induced Self-Assembly. *J. Mater. Sci.* **2020**, *55*, 10910–10921. [CrossRef]

13. Hevilla, V.; Sonseca, A.; Echeverría, C.; Muñoz-Bonilla, A.; Fernández-García, M. Enzymatic Synthesis of Polyesters and Their Bioapplications: Recent Advances and Perspectives. *Macromol. Biosci.* **2021**, *21*, 2100156. [CrossRef] [PubMed]

14. Sonseca, A.; El Fray, M. Enzymatic Synthesis of an Electrospinnable Poly(Butylene Succinate-Co-Dilinoleic Succinate) Thermoplastic Elastomer. *RSC Adv.* **2017**, *7*, 21258–21267. [CrossRef]

15. Korupp, C.; Weberskirch, R.; Müller, J.J.; Liese, A.; Hilterhaus, L. Scaleup of Lipase-Catalyzed Polyester Synthesis. *Org. Process Res. Dev.* **2010**, *14*, 1118–1124. [CrossRef]

16. Sonseca, A.; McClain, A.; Puskas, J.E.; El Fray, M. Kinetic Studies of Biocatalyzed Copolyesters of Poly(Butylene Succinate) (PBS) Containing Fully Bio-Based Dilinoleic Diol. *Eur. Polym. J.* **2019**, *116*, 515–525. [CrossRef]

17. Binns, F.; Harffey, P.; Roberts, S.M.; Taylor, A. Studies Leading to the Large Scale Synthesis of Polyesters Using Enzymes. *J. Chem. Soc. Perkin Trans. 1* **1999**, 2671–2676. [CrossRef]

18. Ding, X.; Chen, Y.; Chao, C.A.; Wu, Y.L.; Wang, Y. Control the Mechanical Properties and Degradation of Poly(Glycerol Sebacate) by Substitution of the Hydroxyl Groups with Palmitates. *Macromol. Biosci.* **2020**, *20*, 2000101. [CrossRef]

19. Kline, B.J.; Beckman, E.J.; Russell, A.J. One-Step Biocatalytic Synthesis of Linear Polyesters with Pendant Hydroxyl Groups. *J. Am. Chem. Soc.* **1998**, *120*, 9475–9480. [CrossRef]

20. De Espinosa, L.M.; Meier, M.A.R.; Ronda, J.C.; Galià, M.; Cádiz, V. Phosphorus-Containing Renewable Polyester-Polyols via ADMET Polymerization: Synthesis, Functionalization, and Radical Crosslinking. *J. Polym. Sci. Part A Polym. Chem.* **2010**, *48*, 1649–1660. [CrossRef]

21. Kallinteri, P.; Higgins, S.; Hutcheon, G.A.; St. Pourçain, C.B.; Garnett, M.C. Novel Functionalized Biodegradable Polymers for Nanoparticle Drug Delivery Systems. *Biomacromolecules* **2005**, *6*, 1885–1894. [CrossRef] [PubMed]

22. Parshad, B.; Kumari, M.; Khatri, V.; Rajeshwari, R.; Pan, Y.; Sharma, A.K.; Ahmed, I. Enzymatic Synthesis of Glycerol, Azido-Glycerol and Azido-Triglycerol Based Amphiphilic Copolymers and Their Relevance as Nanocarriers: A Review. *Eur. Polym. J.* **2021**, *158*, 110690. [CrossRef]

23. Wurm, F.; Dingels, C.; Frey, H.; Klok, H.A. Squaric Acid Mediated Synthesis and Biological Activity of a Library of Linear and Hyperbranched Poly(Glycerol)-Protein Conjugates. *Biomacromolecules* **2012**, *13*, 1161–1171. [CrossRef] [PubMed]

24. Thomas, A.; Müller, S.S.; Frey, H. Beyond Poly(Ethylene Glycol): Linear Polyglycerol as a Multifunctional Polyether for Biomedical and Pharmaceutical Applications. *Biomacromolecules* **2014**, *15*, 1935–1954. [CrossRef] [PubMed]

25. Sonseca, Á.; Hevilla, V.; López, D.; Gimenez, E.; Fernández-García, M. Glycerol-Based Enzymatically Synthesized Renewable Polyesters: Control of Molecular Weight, Degree of Branching and Functional Endgroups. *Eur. Polym. J.* **2022**, *170*, 111173. [CrossRef]

26. Arif, Z.U.; Khalid, M.Y.; Noroozi, R.; Hossain, M.; Shi, H.H.; Tariq, A.; Ramakrishna, S.; Umer, R. Additive manufacturing of sustainable biomaterials for biomedical applications. *Asian J. Pharm. Sci.* **2023**, *18*, 100812. [CrossRef]

27. Ding, R.; Du, Y.; Goncalves, R.B.; Francis, L.F.; Reineke, T.M. Sustainable near UV-curable acrylates based on natural phenolics for stereolithography 3D printing. *Polym. Chem.* **2019**, *10*, 1067–1077. [CrossRef]

28. Dai, L.; Song, J.; Qu, S.; Xiao, R. Triple-shape memory effect in 3D-printed polymers. *Express Polym. Lett.* **2020**, *14*, 1116–1126. [CrossRef]

29. Chen, S.; Wu, Z.; Chu, C.; Ni, Y.; Neisiany, R.E.; You, Z. Biodegradable Elastomers and Gels for Elastic Electronics. *Adv. Sci.* **2022**, *9*, 2105146. [CrossRef]

30. Pashneh-Tala, S.; Owen, R.; Bahmaee, H.; Rekštytė, S.; Malinauskas, M.; Claeyssens, F. Synthesis, Characterization and 3D Micro-Structuring via 2-Photon Polymerization of Poly(glycerol sebacate)-Methacrylate–An Elastomeric Degradable Polymer. *Front. Phys.* **2018**, *6*, 41. [CrossRef]

31. Kirillova, A.; Yeazel, T.R.; Asheghali, D.; Petersen, S.R.; Dort, S.; Gall, K.; Becker, M.L. Fabrication of Biomedical Scaffolds Using Biodegradable Polymers. *Chem. Rev.* **2021**, *121*, 11238–11304. [CrossRef]

32. Momeni, F.; Liu, X.; Ni, J. A Review of 4D Printing. *Mater. Des.* **2017**, *122*, 42–79. [CrossRef]

33. Ifkovits, J.L.; Burdick, J.A. Review: Photopolymerizable and Degradable Biomaterials for Tissue Engineering Applications. *Tissue Eng.* **2007**, *13*, 2369–2385. [CrossRef]

34. Nijst, C.L.E.; Bruggeman, J.P.; Karp, J.M.; Ferreira, L.; Zumbuehl, A.; Bettinger, C.J.; Langer, R. Synthesis and Characterization of Photocurable Elastomers from Poly(Glycerol-Co-Sebacate). *Biomacromolecules* **2007**, *8*, 3067–3073. [CrossRef] [PubMed]

35. Wu, Y.L.; D'Amato, A.R.; Yan, A.M.; Wang, R.Q.; Ding, X.; Wang, Y. Three-Dimensional Printing of Poly(Glycerol Sebacate) Acrylate Scaffolds via Digital Light Processing. *ACS Appl. Bio Mater.* **2020**, *3*, 7575–7588. [CrossRef] [PubMed]

36. Hevilla, V.; Sonseca, A.; Echeverría, C.; Muñoz-Bonilla, A.; Fernández-García, M. Photocuring of Aliphatic-Lineal Poly(Glycerol Adipate) with a Monomer Bearing Thiazolium Groups as a Promising Approach for Biomedical Applications. *Eur. Polym. J.* **2023**, *186*, 111875. [CrossRef]

37. Rabiller, C.; Maze, F. Quantitative Analysis and Determination of the Enantiomeric Purity of Glycerides by 13C NMR Spectroscopy. Application to the Lipase-catalysed Transesterification of Triacylglycerides. *Magn. Reson. Chem.* **1989**, *27*, 582–584. [CrossRef]

38. Wyatt, V.T.; Strahan, G.D. Degree of Branching in Hyperbranched Poly(Glycerol-Co-Diacid)s Synthesized in Toluene. *Polymers* **2012**, *4*, 396–407. [CrossRef]

39. Sunder, A.; Hanselmann, R.; Frey, H.; Mülhaupt, R. Controlled Synthesis of Hyperbranched Polyglycerols by Ring-Opening Multibranching Polymerization. *Macromolecules* **1999**, *32*, 4240–4246. [CrossRef]

40. Kulshrestha, A.S.; Gao, W.; Gross, R.A. Glycerol Copolyesters: Control of Branching and Molecular Weight Using a Lipase Catalyst. *Macromolecules* **2005**, *38*, 3193–3204. [CrossRef]