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Chemo-Enzymatic Derivatization of Glycerol-Based Oligomers: Structural Elucidation and Potential Applications

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Switching from oil-based to bio-based feedstocks to ensure the green transition to a sustainable and circular future is one of the most pressing challenges faced by many industries worldwide. For the cosmetics and personal and house care industries there is a strong drive to accelerate this transition from the customers that starts favoring the purchase of naturally derived and bio-degradable products over the traditionally available products. In this work we developed a series of fully biobased macromolecules constituted of a glycerol-based oligoester backbone. Based on the subsequent derivatization with fatty acids or peptides, the resulting products may find application as emulsifiers, wetting agents, and potential vectors for the delivery of bioactive peptides. All steps of the resulting macromolecules were conducted following the green chemistry principles with no toxic or environmentally damaging compounds that were used in the overall production process.

1. Introduction

The life expectancy of the World's population increased dramatically from the 1950s (47 years) to the 2020s (73.2 years)[1] leading, among other things, to a great development of the cosmetics industry with a special focus in developing novel anti-aging solutions and treatments. For this kind of applications, protein and peptide-based formulations have a prominent role. In fact, ageing is characterized by the accumulation of molecular damage and progressive failure of the maintenance and repair systems. Skin is one of the tissues where these effects are tangible and measurable, showing loss of elasticity due to collagen crosslinking, flattening of the dermo-epidermal junction, decreasing barrier function and change in colouring.[2] The human skin is however an excellent barrier versus molecules that are hydrophilic, charged and have a certain molecular weight. For this reason, an optimal diffusion of the cosmetic peptides to the dermis, the layer of the skin that has a critical importance for wound healing and the maintenance of a healthy skin must be insured to maximize their effect.[3] To ensure the appropriate delivery, three main systems were developed and are the use of 1) chemical and physical penetration enhancers; 2) chemical modification of the peptide/protein; and 3) modification of the used formulation.[2]

For reaching the dermis, one of the most studied approaches is the use of lipopeptides (LP), molecules where a hydrophilic peptide is covalently linked with a fatty acid that acts as a carrier. When released in the extracellular environment, these amphiphilic molecules increase the permeability of the stratum corneum, therefore enhancing the bioavailability of the peptides which can then reach their biological target more easily.[4]

LP are commonly produced by various microorganisms, have a cyclic structure, and can possess different surface activities. One of the most found LP is surfactin that greatly reduces the surface tension of water when used at very low concentrations.[5] These natural cyclic LP can also have biological activities such as antibacterial and antiviral functionality and can be used by scientists as templates for the design of synthetic amphiphilic peptides.[6] The commonly used techniques to join a lipid to a peptide involve the reaction of an amine with an N-hydroxy succinimide ester to form an amide bond, the reaction of an amine with an aldehyde to produce a Schiff base that can be reduced by borohydrides to produce a secondary amine linkage, and the reaction of a thiol with a maleimide derivative that produces a thioether bond. These methods, among many others that were summarized by Ian Hamley in 2014, are the classical PEGylation strategies used to derivatize peptides for obtaining an improved water solubility, stability, and bioavailability.[7] Poly(ethylene glycol) (PEG) is in fact a non-ionic, nontoxic, biocompatible, and highly hydrophilic polymer which, coupling to various peptides, is now a standard procedure to produce peptide-based drug candidates.[7] The drawback of utilizing PEG as the hydrophilic part of the molecule is that this polymer (widely used in sunscreens, hair products, shower gels, etc.) is derived from petrol-based sources and its backbone, formed by C–C bonds, is not easily degraded in Nature making it one of the so called oxo-degradable plastics.[8] Poly(glycerol adipate) (PGA) was coupled with a small number of aromatic N-acetyl amino acids, developing a novel class of biodegradable grafted polyesters with tunable physical properties. These structures widen the

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range of possible interactions with drugs and biological macromolecules.[9] Utilizing a similar polymer modification approach, Suksiriworapong et al. reported a polymer-anticancer drug conjugate based on PGA through the successful conjugation of methotrexate, avoiding the use of intermediate linkers.[10] Other glycerol-based polyesters such as poly(glycerol sebacate) have applications as scaffolds for hard tissue engineering, control drug delivery devices and tissue adhesives.[11]

Getting inspired by the existing literature on LPs and PEGylated peptides, and looking for alternative ways to green the cosmetics industry, an environmentally friendly method based on enzymatic catalysis and green organic media for the synthesis of derivatized polyesters was developed. Fatty acid methyl esters were used as model molecules and fatty acid-polyester-peptides conjugates, as macromolecular structures of potential interest for skin-delivery applications, were the final target molecules of this work. Building up from previous literature on the enzymatic synthesis of glycerol-based polyesters having a highly controlled degree of branching,[12,13] a bio-based and biodegradable linear linker based on a glycerol-based polyester (substituting the non-biodegradable and non-biobased PEG) was used to link a dipeptide (glycyl-glycine) with the hydrophobic tail constituted by a fatty acid molecule. The resulting system, being amphiphilic, constitutes a potential model vector molecule for the delivery of bioactive peptide sequences to the cell's cytoplasm or a potential additive for home and personal care products.

2. Results and discussion

The overall goal of this work was to synthesize, using only environmentally friendly procedures, a three-component macromolecule (Figure 1) comprising a short peptide chain linked *via* a biobased and biodegradable spacer to a fatty acid molecule.

2.1. Biocatalyzed synthesis of glycerol-based oligoesters

In the first instance the enzymatic synthesis of the hydrophilic spacer was investigated and optimized. Glycerol (GLY) was selected as the functional biobased monomer on which the spacer should be based as its secondary polar hydroxy groups could give the desired hydrophilicity properties to the resulting molecule. In fact, the aim of this spacer is to have characteristics similar to the petrol-based and non-degradable poly(ethylene glycol) currently used in the cosmetics industry.[14,15] Starting from previous reports, a detailed study investigating various reaction temperatures (40 °C < T < 60 °C), diester:glycerol molar ratios (1 : 1 < dd:GLY < 1 : 2) and monomer's carbon chain length (4, 6 or 10 carbon atoms) was carried out, as reported in Table 1.

Due to the presence of both primary and secondary hydroxy groups, a 2D-NMR investigation of the reaction products has been necessary to determine the degree with which the secondary hydroxy group of GLY was reacting, as the final goal was to obtain mostly linear oligomers. As we can see from Figure 2, after assigning all signals in the HSQC spectra (Figure 2a), we were able to assign the corresponding signals in the ^{1}H-NMR spectra (Figure 2b). When comparing our results with the ones previously reported by other authors using different monomer combinations,[16,17] this led to significant discrepancies in the spectra interpretation. Only the recent paper from Todea et al.,[18] that uses azelaic acid as the comonomer for the GLY polycondensation, reports a spectra interpretation (including COSY and TOCSY analysis) that is

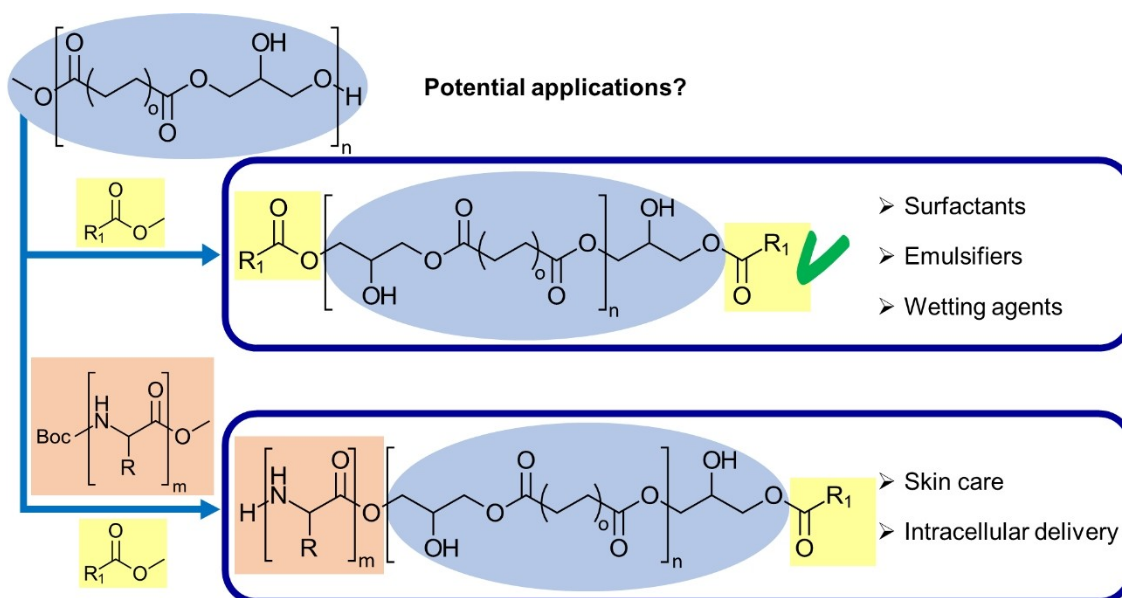

Figure 1. Structures of the glycerol-based macromolecules (blue circles) targeted in the present work and their potential applications based on the derivatizations using fatty acids (yellow squares) or peptides (red squares).

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Table 1. Biocatalyzed derivatization of GLY-based oligoesters using fatty acids methyl esters (FA) having various chain length.

Entry	Diester[c]	FA[d]	GLY:FA ratio	M_n[a]	M_w[a]	Đ[a]	HLB[b]	Appearance
1	C_4	–	–	700	900	1.2	9.5	Colorless oil
2	C_6	–	–	800	1000	1.4	8.3	White oil
3	C_{10}	-	–	1100	3000	2.5	6.6	White wax
4	C_6	C_6	1:2	1000	1400	1.8	7.3	Colorless oil
5	C_6	C_6	1:6	1500	4200	1.7	6.3	Colorless oil
6	C_6	C_{16}	1:2	1400	2500	1.1	5.8	White wax
7	C_6	C_{16}	1:6	1700	2900	2.8	5.1	White wax

[a] Calculated via GPC
[b] Calculated using the formula: $20\frac{\text{Mass of hydrophilic groups}}{\text{Mass of molecule}}$
[c] C_4: dimethyl succinate, C_6: dimethyl adipate, C_{10}: dimethyl sebacate.
[d] C_6: hexanoic acid, C_{16}: palmitic acid.
Reactions carried out using 10% w w^{-1} CaLB calculated on the total amount of monomers in a solventless system stirring the reaction mixture magnetically at 60°C at 400 rpm.

consistent with the assignments performed in the current work. This discrepancy is most likely due to the deeper investigation performed by Todea et al. by using COSY and TOCSY and in this work by using 2D and quantitative NMR techniques that allowed a better understanding of the enzymatic selectivity of primary vs secondary -OH groups of GLY. The operational temperature for the enzymatic polymerization of GLY was selected based on various reports that indicated that an optimal temperature to obtain almost fully linear oligomers was between 30 and 60°C.[19–21]

From this interpretation we can observe how, using an immobilized preparation of *Candida antarctica* lipase B (CaLB) as the catalyst at very mild temperatures (60°C ≤ T), it is possible to obtain GLY-based (end capped) oligomers using various dimethyl esters, having a degree of branching (DB) between 7% < DB < 33% and number average molecular weights (M_n) between 600 g mol^{-1} < M_n < 1100 g mol^{-1} (see ESI, Table S1).

2.2. Derivatization of the GLY-based oligomers with fatty acids

After the successful synthesis of the short GLY-based oligomers, a first derivatization using fatty acids methyl esters (FA), having a different carbon chain length, was carried out using enzymatic catalysis. By varying the FA:GLY-based oligoesters molar ratio (considering in the calculation the unreacted hydroxy groups of GLY still available) it was possible to synthesize a series of derivatives where the FA was selectively coupled only to the primary (1:2 ratio) or on both primary and secondary (1:6 ratio) GLY hydroxy groups. Working with oligomers having various chain lengths the reaction was monitored by ^{1}H-NMR following the disappearance of the methoxy group protons of the FA at 3.58 ppm upon reaction workup. Is it interesting to notice that, after the coupling of the FA hydrophobic structure, the obtained macromolecules are not anymore soluble in DMSO-d_6 (like the parental ones) but must be solubilized in $CDCl_3$ for NMR analysis. Moreover, looking at the M_ns values of the oligomers before and after reaction (Table 1), it is possible to observe how, reacting the DMA-GLY oligomer having a M_n of 800 g·mol^{-1} with 2 equivalents of the C_6 FA (methyl hexanoate), a resulting macromolecule having a M_n of 1000 g mol^{-1} was obtained. Considering that from the ^{1}H-NMR spectra we observed a complete disappearance of the methoxy signal from the C_6 FA and that the M_n of the hexanoyl radical is 99 g mol^{-1}, we can conclude that the coupling was successful. The same calculations can be done for all other macromolecules reported in Table 1 and always result in a quantitative conversion of the GLY oligomer substrate.

For all the obtained macromolecules, derivatized and non-, the hydrophilic-lipophilic balance (HLB) value was calculated. The HLB is a measure of the degree of hydrophilicity or lipophilicity of a molecule that is determined by calculating the percentages of molecular weights for the hydrophilic and lipophilic portions of the structure as first described by Griffin in 1949.[22] As we can observe from Figure 3, already for the oligomers synthesized only from GLY and a diester having a C_4, C_6 or C_{10} carbon chain length the HLB values significantly differ, leading to macromolecules having HLB values between 8.3 and 9.5 that are water soluble and have oil/H_2O emulsifying properties (for the C_4 and C_6 GLY-based oligomers) to an HLB value of 6.6 for the C_{10} GLY-based oligomers that, being more hydrophobic, act more like a H_2O/oil emulsifier. Adding the FA having various chain lengths it was possible to obtain a library of molecules spacing from HLB values from 4.9 to 9.5, therefore covering a broad spectrum of potential applications in the cosmetics and personal care industries.

To confirm the trends observed with the HLB calculations, water contact angle (WCA) analysis of the obtained materials was carried out. From Figure 4 it is possible to observe how increasing the % w·w^{-1} of the polymer in the water solution the contact angle of the drop tends to steadily decrease from 85° to 68° for the 2.5 mg·mL^{-1} solution. After this concentration a

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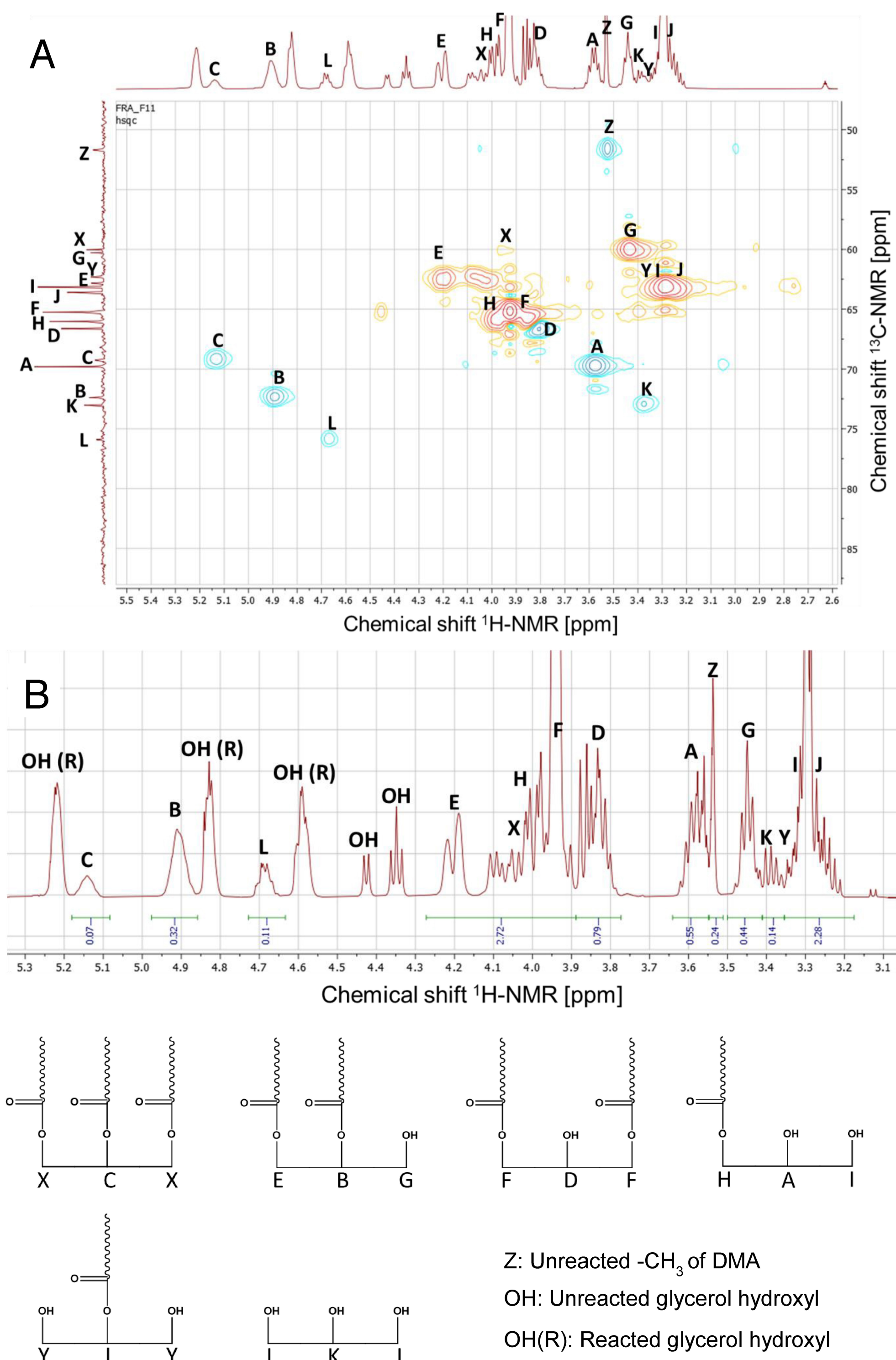

Figure 2. NMR characterization of the obtained poly(glycerol adipate) oligomers. A) 2D HSQC assignment; B) ^{1}H-NMR and structures of the observed reaction products.

flattening of the trend is observed with the WCA stabilizing around 65–66°. This trend shows additional proof of the potential of these oligomers as water dispersible wetting and emulsifying agents. When changing the used diester from the C6-adipate to the C4-succinate the WCA of a solution having the same concentration increases from 75° to 81° due to the shorter carbon chain length of the polymer backbone.

The esterification reaction can be easily followed by comparing the FT-IR-ATR spectra of the various samples before and after the functionalization reaction. From Figure 5 we can observe how the broad -OH stretching signal at 3400 cm^{-1} gets

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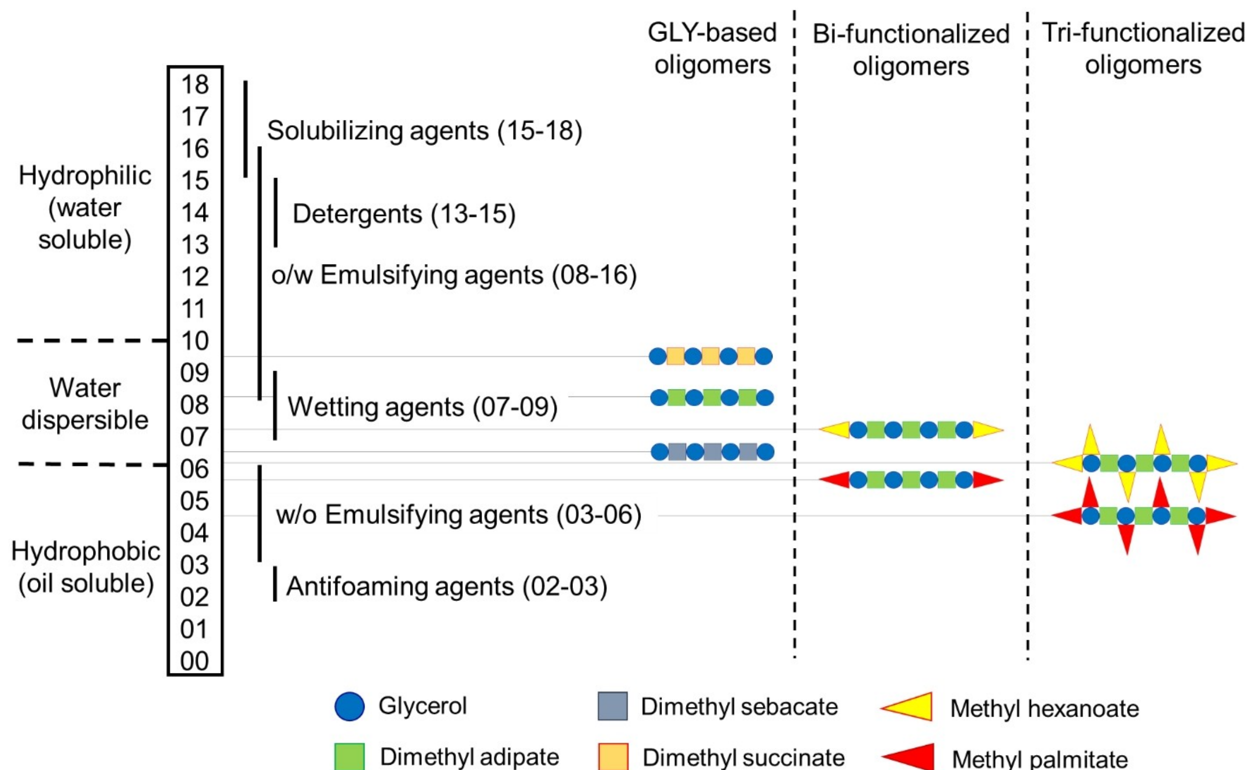

Figure 3. HLB values for the most common hydrophilic and hydrophobic molecules of industrial interest and relative values for the enzymatically synthesized GLY-based oligomers before and after FA derivatization.

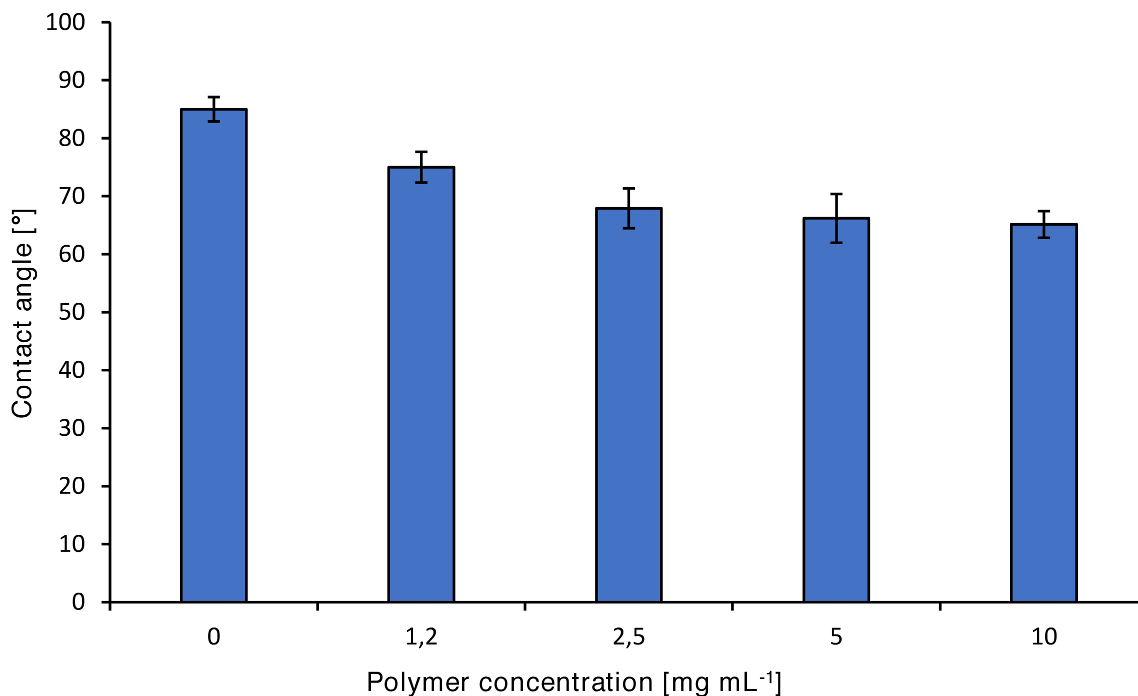

Figure 4. Water contact angle analysis of poly(glycerol adipate) solutions having polymer concentrations varying from 0 mg mL^{-1} (distilled water) to 10 mg·mL^{-1}.

significantly reduced after the coupling to produce the bifunctional molecule (2:1 FA:free OH ratio) and disappears completely after the coupling performed to prepare the trifunctional molecule (6:1 FA:free OH ratio). Moreover, the esterification of the free OH groups of the oligomers is further confirmed by the progressive disappearance of the shoulder under the signal

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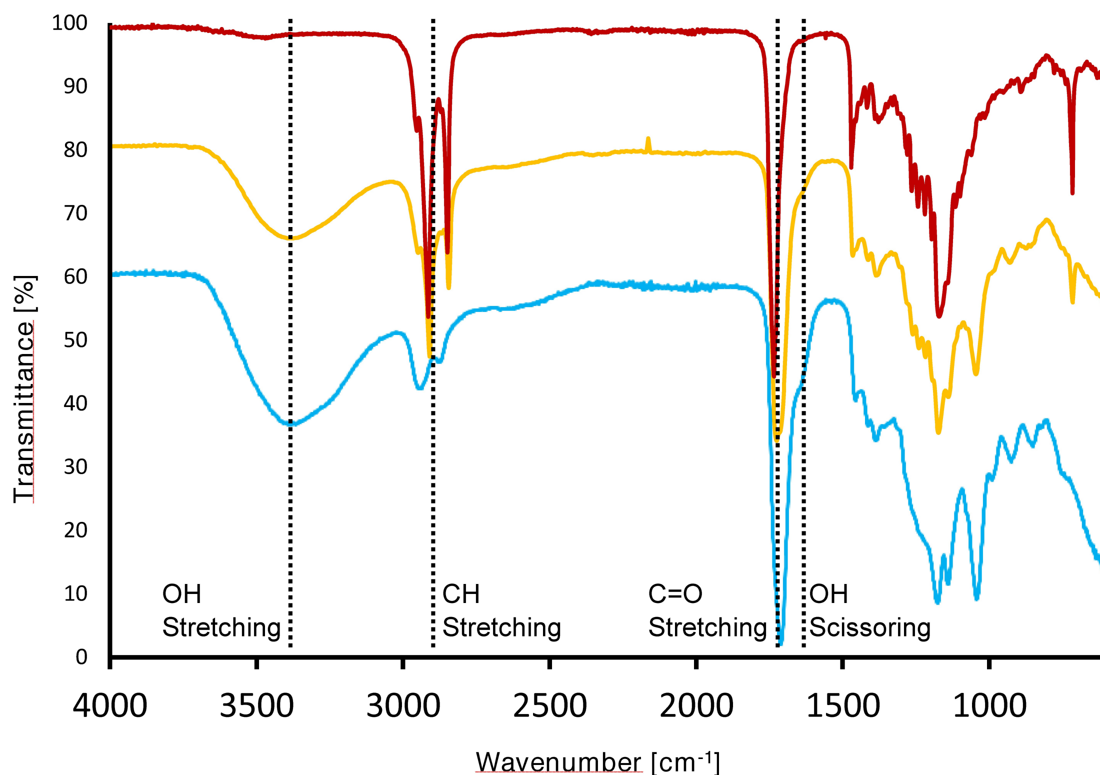

Figure 5. IR spectra of DMA-GLY polymers: unfunctionalized (cyan), functionalized with 2 equivalents of palmitic acid (yellow), functionalized with 6 equivalents of palmitic acid (red). Equivalents of the added fatty acids methyl esters were calculated on the total amount of free OH groups quantified *via* NMR after the enzymatic oligomers' synthesis.

observable at 1700 cm^{-1} that is due to the OH bond scissoring. It is also evident how at 2900 and 2850 cm^{-1} there is an increase of the observable signals relative to the CH_2 and CH_3 groups stretching that are forming the long carbon chains of the fatty acid methyl esters used for the functionalization reaction.

2.3. Derivatization of the GLY-based oligomers with activated peptides

After the successful derivatization of the GLY-based oligomers with the FA, a second derivatization using short peptides was carried out to prepare an amphiphilic molecule of potential interest for the cosmetic industry. Glycine (G) and its di- (GG) and tripeptides (GGG) were selected as simple model molecules to carry out the work as it consists of 5–12% of the amino acid composition of commercial saltwater or freshwater fish waste, tilapia filleting residue and fish silages,[23] and is therefore an excellent substrate from a circular economy perspective. Unfortunately, the use of unprotected aminoacid methyl esters was not suitable, because of concurrent amidation reactions. Thus, the aminoacids or oligopeptides were protected as the tert-butyl urethanes (Boc).

Following the do-not-significant-harm dogma we chose to employ route B of Figure 6, that is introducing first the Boc protection, followed by esterification. However, due to solubility issues, the Boc-protection of aminoacids or oligopeptides is usually carried out in the presence of an organic solvent, such as DMF[24,25] or 1,4-dioxane.[26] Moreover, the esterification reaction usually employs toxic reagents such as MeI[27] or $SOCl_2$.[28] Therefore, we tried to optimize this process from the point of view of green chemistry.

After some initial attempts using these classical methods (see ESI, Table S2), that unsurprisingly led to excellent results in terms of conversion (99% yields for the Boc-protection and of 75% for the esterification) a 2-step procedure for the green derivatization of aminoacids was developed (Figure 6). We could not avoid the use of an organic cosolvent for the Boc protection, but we substituted DMF or dioxane with the non-toxic DMSO, whereas the esterification was carried out using dimethyl carbonate (DMC) as reagent. The use of DMC has a great advantage as the reaction can be carried out in the liquid phase, at temperatures of 120–220°C) and, unlike chloroformates, DMC is only mildly irritating to the eyes, skin, and mucous membranes.[29] As a future perspective would be interesting to develop alternative greener solvents that could substitute DMF and dioxane while allowing the solubility of unprotected amino acids, one of the main bottlenecks that many scientists have encountered when trying to couple these amphoteric molecules.

As shown in Table 2, this methodology leads to the isolation of Boc-glycine methyl ester in a 78% yield. Good results were obtained also for the GG dipeptide that was isolated with a 61% overall yield. On the other hand, the GGG derivatization allowed to isolate the expected product with a poor 2–12%

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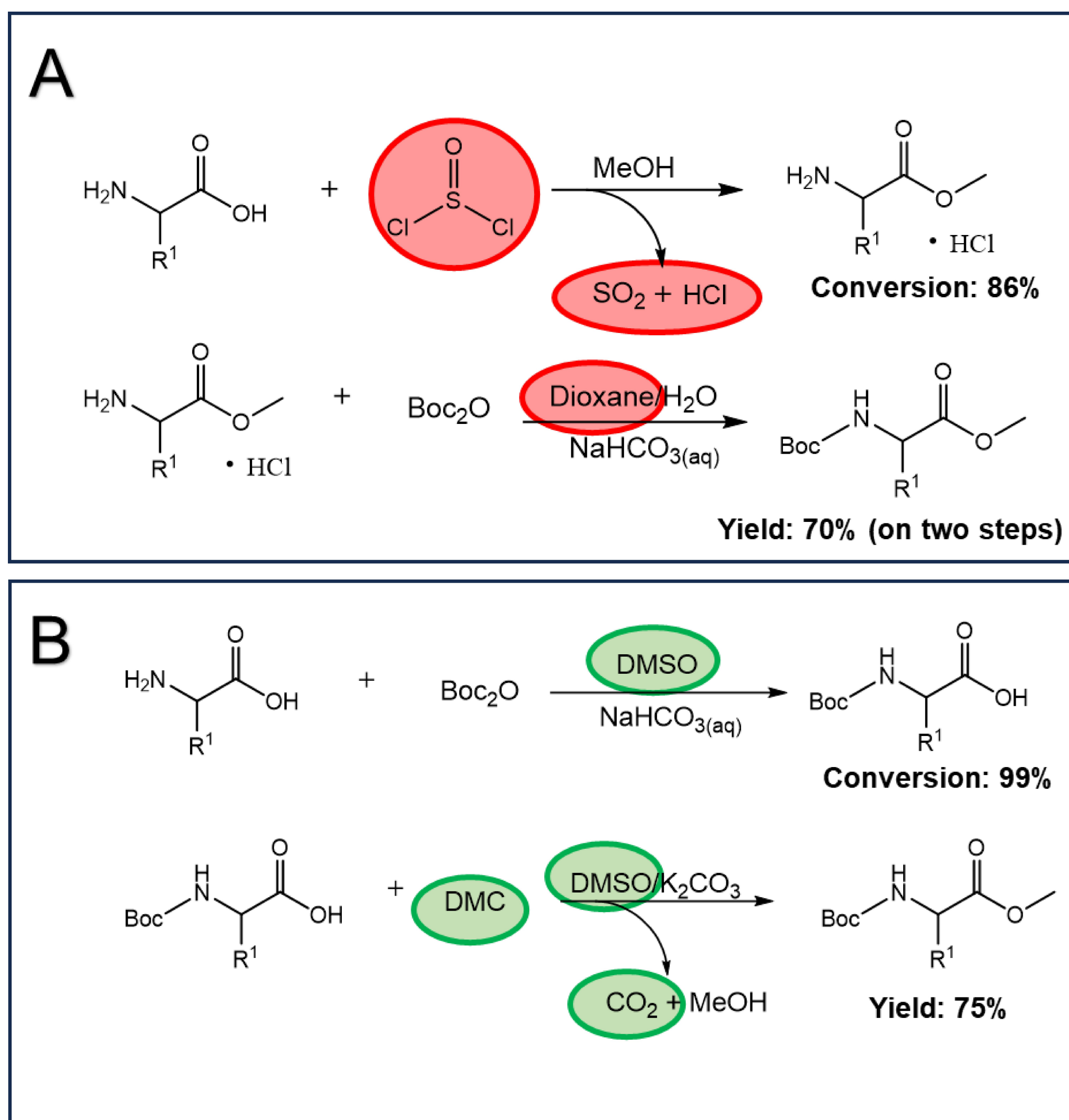

Figure 6. Synthetic pathway for Boc-protected amino acids. R[1] = H. A) Classic methodology (esterification with $SOCl_2$ and protection) and B) The newly developed green methodology (protection and esterification with dimethyl carbonate (DMC)).

overall yield. Indeed, the comparison between the esterification of tripeptides using DMC or MeI,[27] clearly demonstrates that also the latter is not very effective as the yield is only around 10%.

Therefore, we decided to functionalize the GLY oligomers with the Boc-GG-OMe activated dipeptide.

Before working on the GLY oligomers, some model esterification and transesterifications reactions using 1-butanol and Boc-amino acids or Boc-amino acids methyl esters were carried out using CaLB as the catalyst to test its suitability for this kind of reactions (see ESI, Table S3). These model couplings helped to understand the faster kinetic of the enzymatic reaction when starting from the methyl esters of the amino acids. This ensured a quantitative conversion of the substrate to the desired esterification product. In fact, the best reaction starting from the the non-esterified amino acid led to a maximum conversion of 44% while starting from the methyl ester a conversion of 99% was obtained using the same biocatalyst amount (10% $w \cdot w^{-1}$) and reaction time (24 h).

The last step consisted in the coupling of the three segments of the macromolecule (activated peptide, GLY-spacer, and FA). A first coupling was carried out using the esterified peptide that was mixed with the GLY oligomer to conduct the transesterification reaction. The monitoring of the peptide reactivity was not straightforward as the signals of the GLY-oligomer and the peptide have similar chemical shifts therefore overlapping in the ^{1}H-NMR spectra. For this reason, 2D NMR HSQC analysis was carried out to monitor (once again) the disappearance of the –COOMe protons of the Boc-GG-OMe

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Table 2. Protection and esterification of glycine and small glycine peptides using the newly developed method B (Boc protection in DMSO and esterification using DMC) depicted in Figure 6.

Entry	Substrate	Reagent	Solvent	Catalyst	T [°C]	Time [h]	Yield %
1	GG	$(Boc)_2O$	DMF	$NaHCO_{3\ sat.}$	r.t.	30	99
2	GG	$(Boc)_2O$	DMSO	$NaHCO_{3\ sat.}$	r.t.	30	[a]
3	GGG	$(Boc)_2O$	DMF	$NaHCO_{3\ sat.}$	r.t.	30	99
4	GGG	$(Boc)_2O$	DMSO	$NaHCO_{3\ sat.}$	r.t.	30	[b]
5	Boc-G	DMC	DMSO	K_2CO_3	120	2	78
6	Boc-GG	DMC	DMSO	K_2CO_3	120	2	46
7	Boc-GG	DMC	DMSO	K_2CO_3	120	2	52[c]
8	Boc-GG	DMC	DMSO	K_2CO_3	120	2	61[d]
9	Boc-GGG	DMC	DMSO	K_2CO_3	120	2	0
10	Boc-GGG	DMC	DMSO	K_2CO_3	90	48	12[e]

[a] The unpurified raw product (99% conversion) was used to carry out the reaction 8; [b] The unpurified raw product (99% conversion) was used to carry out the reaction 10; [c] Reaction 7 was carried out adding 1 gram of NaCl in addition to the K_2CO_3 catalyst; [d] Yield calculated on the overall two step reaction of protection (entry 2) and esterification (entry 8); [e] Yield calculated on the overall two step reaction of protection (entry 4) and esterification (entry 10).

peptide by comparing the spectra at time 0 h and after 24 h of reaction (Figure 7).

In a second reaction step the coupling with 1 equivalent of FA (calculated on the primary unreacted OH groups of glycerol) was performed as, based on the previous results on FA coupling, the esterification of the two species via enzymatic catalysis was not considered to be a challenge. This reaction lead in fact to a quantitative conversion of the FA that was monitored via ^{1}H-NMR observing the disappearance on the -COOMe of the FA that is released as MeOH upon trans-esterification.

To confirm what was evident in the HSQC spectra and validate the method used for the determination of the conversion, quantitative ^{13}C-NMR analyses were also carried out on selected samples and were compared to the HSQC calculations (Table 3).

As it is possible to observe from Table 3, the two used methods lead to very similar results, therefore validating the HSQC calculations that can be made running simpler and

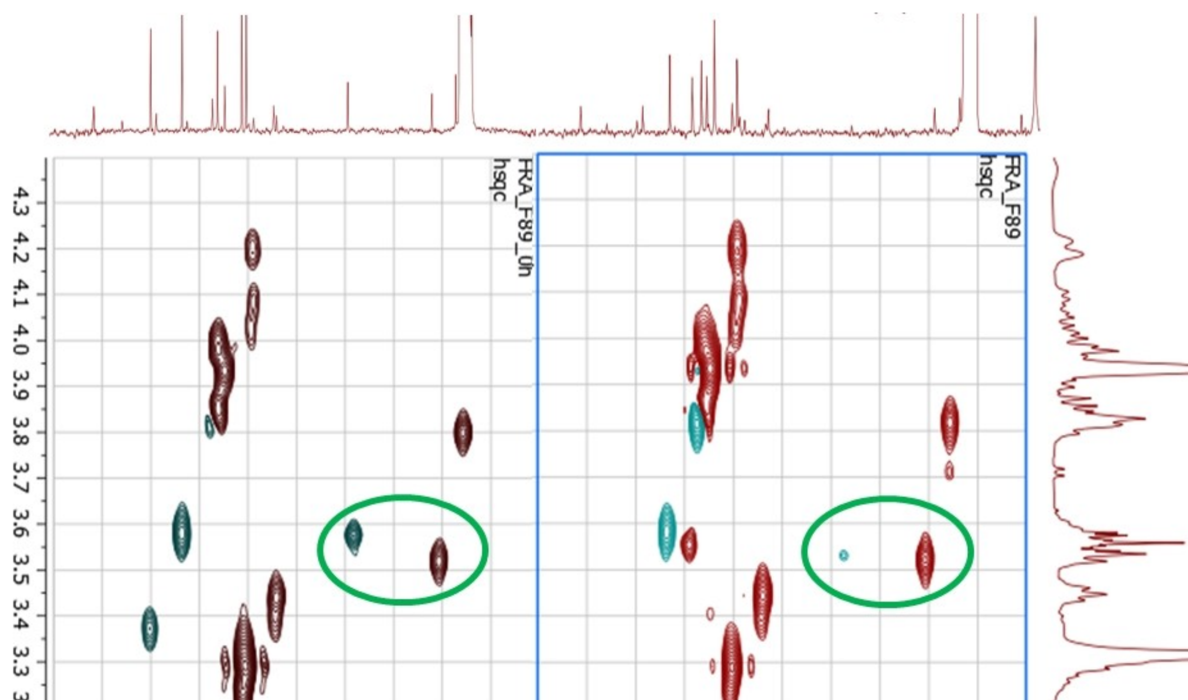

Figure 7. Evolution of 2D HSQC spectra of polymer-peptide coupling, at the beginning (left) and after 24 h of enzymatic reaction (right). The disappearance of the -OCH_3 group of DMA is highlighted with a green circle.

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Table 3. Comparison of conversions regarding the coupling between Boc-GG-OMe and poly(glycerol adipate) using HSQC and quantitative ^{13}C-NMR.

Entry	HSQC	^{13}C-NMR	Time [h]
1	72 %	66 %	12
2	80 %	74 %	18
3	92 %	91 %	24

Three reactions were performed to test the accuracy of the methodology.

shorter (1.5 h for the 2D HSQC versus the 22 h analysis needed for the ^{13}C quantitative NMR).

The esterification reaction can again be followed comparing the FT-IR-ATR spectra before and after the functionalization reaction. In Figure 8 is possible to observe the simultaneous reduction of the free –OH stretching signal at 3400 cm^{-1} and the concomitant increase in intensity of the shoulder at 1700 cm^{-1} caused by the -NH bending. Furthermore, the presence of the C–N stretching above 1500 cm^{-1} fully confirms the reaction between polymer and amino ester has occurred.

3. Conclusions

In this work we presented an environmentally friendly method for the synthesis and the post-polymerization functionalization of bio-based oligoesters. The aliphatic, -OH rich polymers were synthesized via enzymatic catalysis at mild temperatures (40–60 °C) in the absence of solvent. The obtained molecular weights ranged from 660 to 1150 g mol^{-1} and had DPs ranging from 3 to 5 units. These short oligomers were then reacted with fatty acids methyl esters with conversions up to 95 % and showing very promising properties as wetting and emulsifying agents as highlighted by the HLB calculations. Finally, the Boc-protection and green esterification of amino acids and short peptides was also carried out using the dimethyl carbonate chemistry. This step was carried out as an activation procedure to allow a semi-quantitative coupling of these aminoacidic derivatives with the previously synthesized oligoesters and the fatty acid methyl esters to prepare amphiphilic molecules of interest for the cosmetics industry.

4. Materials and methods

4.1. Materials

4.1.1. Reagents and solvents

Commercial products were used directly without further purifications. Reactions under inert atmosphere were carried out using anhydrous solvents and *luer-lock* syringes.

Dimethyl adipate (Alfa Aesar 99 %), dimethyl sebacate (Merck 99 %), dimethyl succinate (Merck 99 %), glycerol (Carl Roth 99 %.), DL-phenylalanine (Acros Organics 99 %), Boc-

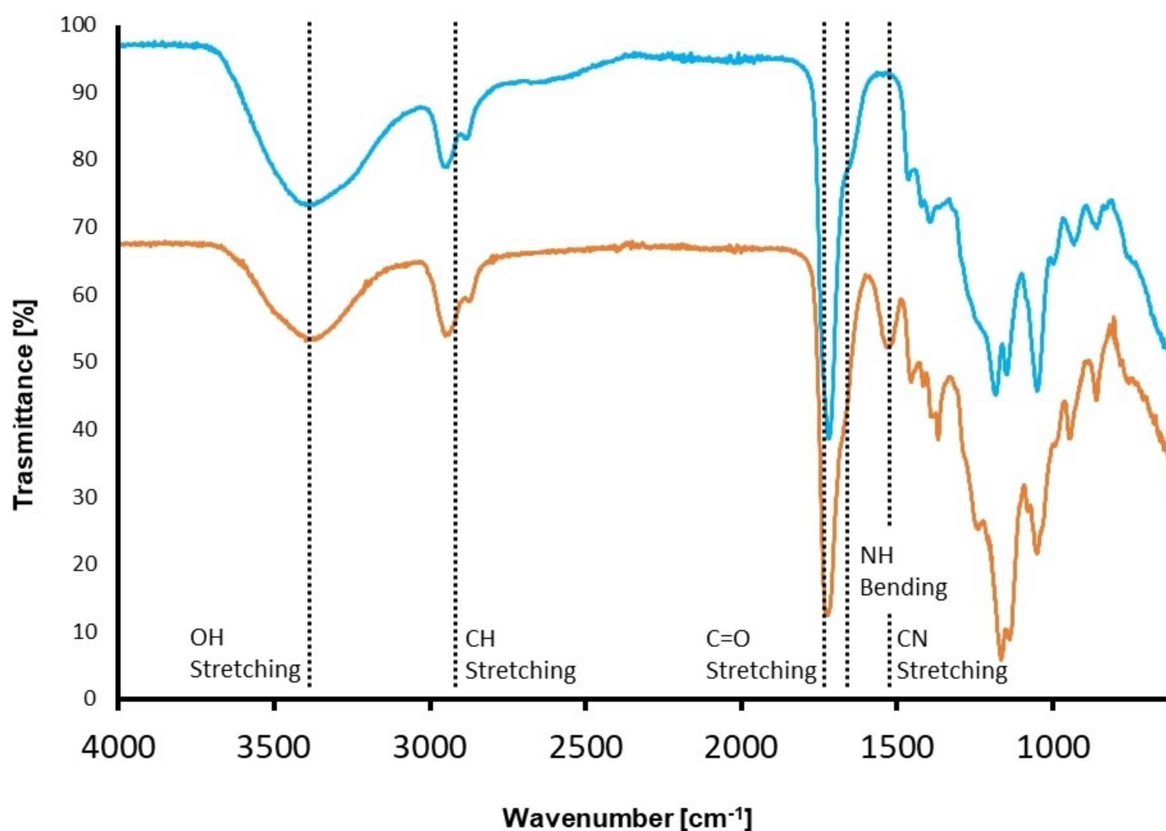

Figure 8. IR spectra of DMA-GLY polymer: unfunctionalized (cyan), functionalized with Boc-Gly-Gly (orange).

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phenylalanine (Merck 99%), thionyl chloride (Fluka 99%), dimethylcarbonate (Fluka 99%), di-*tert*-butyl dicarbonate ($(Boc)_2O$, Merck 99%), methyl hexanoate (Merck 99%), methyl palmitate (Merck 99%), glycine (Merck 99%), Boc-glycine (Merck 99%), glycilglycine (Merck 98%), glycilglycilglicine (Merck 99%).

THF (Riedel-de Haen 99%, stabilized with 250 ppm of BHT), toluene (Merck 99%), Et_2O (Honeywell 99%), petroleum ether (Carlo Erba 99%), DCM (Merck 99%), EtOAc (Merck 99%), DMSO (Merck 99%), MeOH (VWR Chemicals 99%), EtOH (Carlo Erba 99%), 1,4-dioxane (VWR 99%), 2-Me-THF (Merck 99%), DMF (Riedel de Haen 99%), $CDCl_3$ (Euristop 99.8% D), DMSO-d_6 (Euristop 99.8% D). Methyl esters were preferred instead of acids and diacids as the stripping of the condensation by-product (MeOH vs H_2O) was more efficient at the used operational temperatures (85 °C and 10 mbar).

4.1.2. Enzymes

Candida antarctica lipase B (CaLB) on acrylic support (Novozym 435) was purchased from Merck (product code: L4777). Measured synthetic activity: 5280 U/g (propyl laurate assay).

4.1.3. Routine analysis

NMR spectra were recorded on a JEOL JNM-ECZ400R (400 MHz for ^1H e 100 MHz for ^{13}C) with a Royal HFX probe. $CDCl_3$, DMSO-d_6 or D_2O were used as solvents, employing tetramethylesilane (TMS) as internal standard (0.00 ppm) and the solvent peak for carbon spectra. Chemical shifts (δ) were reported in parts per million (ppm) and coupling constants (J) in Hertz. Peak assignments were also made with the aid of 2D HSQC experiments comparing the signal of the methoxy end group of the starting oligomer ($-OC\underline{H_3}$) deriving from the used dimethyl ester with the same signal after the enzymatic coupling reaction. Quantitative carbon (Q-POMMIE) was recorded using 1536 acquisitions with a delay of 45 seconds.

IR spectra were recorded with a Perkin Elmer Spectrum 65 FT-IR (Fourier Transform Infrared) in ATR (Attenuated Total Reflectance) mode, range 4000–600 cm^{-1}, 16 scans for spectra, resolution: 1 cm^{-1}.

Gel permeation chromatography (GPC) was performed at 30 °C on an Agilent Technologies HPLC System (Agilent Technologies 1260 Infinity) connected to a column 17369 6.0 mm ID ×40 mm L HHR–H, 5 μm Guard column and a liquid chromatography column 18055 7.8 mm ID ×300 mm L GMHHR–N, 5 μm TSKgel (Tosoh Bioscience, Tessenderlo, Belgium), and dissolving samples in THF (BHT 250 ppm), filtering the solution on Whatman 595 $\frac{1}{2}$ GmbH and using THF (BHT 250 ppm) to elute. The detector was reflex index Agilent Technologies G1362 A, and calibration was performed with polystyrene (400–2 000 000 Da).

GC-MS analyses were performed on a Shimadzu GC-MS QP2010 SE using a HI-5 ms column (0.25 mm, 0.25 mm i.d., and 30 m). Analysis conditions are as follows: solvent delay 2.5 min, mass range 35–600, injector temperature 250 °C, detector temperature 250 °C, MS temperature around 250 °C, starting temperature 150 °C, starting time 3 min, temperature gradient 25 °C min^{-1}, final temperature 300 °C, flux through column 1.0 mL min^{-1}, split ratio 1:10, sample concentration 0.1 mg mL^{-1}, and amount injected 1 mL.

TLC analyses were carried out on glass supported silica gel plates (Merck 60 F254 0.25 mm) and viewed at UV (λ = 254 nm), developed with basic potassium permanganate (solution prepared dissolving 1.5 g $KMnO_4$, 10 g K_2CO_3, 1 mL NaOH 10% in 200 mL water).

Column chromatography was done using the "flash" methodology using Ge Duran SI 60 Å (230–400 mesh).

All reactions using dry solvents were carried out under a nitrogen atmosphere.

Contact angles were measured at r.t. using Attension Theta Tensiometer, plastic covered microscope slides as surface and water as reference. For each sample at least three drops were analyzed.

4.2. Materials & Methods

4.2.1. Biocatalyzed synthesis of glycerol-based oligoesters

A mixture of glycerol (0.6631 g, 7.200 mmol) and a dimethyl ester (1.0452 g adipate (DMA), 0.8768 g succinate (DMS), 1.3818 g sebacate (DMSe), 6.000 mmol) was heated to 60 °C under magnetic stirring (400 rpm) using a cross shaped stirrer. After complete homogenization of the monomers, CaLB Novozym 435 (10% w·w^{-1} calculated on the total amount of monomers) was added to the flask. After 6 hours the reaction was put under vacuum (11 mbar) and left stirring for 18 more hours at 200 rpm. The workup was carried out solubilizing the product in 2-Me-THF and filtering out the enzyme beads using a WHATMAN 595.5 cellulose filter. Solvent was removed under reduced pressure and residue further dried under reduced pressure to give the desired polyester (1.402 g, 97% yield DMA) (1.202 g, 95% yield DMS) (1.665 g, 98% yield DMSe), as a colorless oil (adipic and succinic) or white wax (sebacic).

4.2.2. Enzymatic activity assay

The enzymatic activity was determined by using the propyl-laurate assay as previously reported.[30]

4.2.3. Derivatization of the glycerol-based oligomers with fatty acids

A mixture of poly(glycerol adipate) (0.1920 g, 0.2500 mmol) and fatty acid methyl ester (palmitate or hexanoate) (0.2500, 0.5000 or 1.500 mmol depending on the desired product and calculated based on the free OH groups of the starting oligomers) was heated to 60 °C under magnetic stirring (200 rpm). After complete homogenization CaLB Novozym 435 (10% w·w^{-1} calculated on the total amount of monomers) was added to the

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flask. After 6 h the reaction was put under vacuum (11 mbar) and left stirring for 18 more hours. The workup was carried out solubilizing the product in 2-Me-THF, filtering out the enzyme beads using a WHATMAN 595.5 cellulose filter. Solvent was removed under reduced pressure and residue further dried under reduced pressure to give the desired product as a colorless-yellow oil (hexanoate derivatives) or white wax (palmitate derivatives) with a 97% conversion.

4.2.4. Esterification and protection of phenylalanine

Inside a two-necked round bottom flask phenylalanine (1.000 g, 6.05 mmol) was dissolved in anhydrous MeOH (10 mL) under inert atmosphere. The system was cooled to 0 °C and, under magnetic stirring, $SOCl_2$ (0.615 mL, 8.20 mmol) was added dropwise in about one minutes using an addition funnel. After 30 min the temperature was allowed to rise to r.t. and the reaction was left stirring for 16 hours. After removal of solvent and volatile byproducts under reduced pressure, the white phenylalanine methyl ester hydrochloride was used without further purification for the following N-protection.

A saturated solution of $NaHCO_3$ (10 mL) was added to the product. Then, under magnetic stirring, a solution of $(Boc)_2O$ (1.7550 g, 8.41 mmol) in dioxane (10 mL) was slowly added in a span of a couple minutes through an addition funnel at r.t.. After 16 hours the aqueous solution was extracted three times using Et_2O and, after further drying with $MgSO_4$, the solvent was evaporated under reduced pressure. The product was then purified using "flash" chromatography, solubilizing the sample in DCM, and eluting with PE-Et_2O 1:1. After removing solvent with vacuum, Boc-Phenylalanine-OMe was recovered as an off-white solid (70% yield).

4.2.5. Protection of peptides as carbamate

To a solution of peptide (2.61 mmol) in a mixture of DMF (10 mL) and saturated aqueous solution of $NaHCO_3$ (13 mL) was added Boc_2O (0.706 g, 3.24 mmol) under magnetic stirring, at 0 °C. After 15 min the temperature was allowed to rise to r.t. and the reaction was stirred for 30 hours. The workup was carried out lowering the pH to 5 using 6 M HCl. Solvents and volatile byproducts were then removed under reduced pressure and the solid was further dried under vacuum. Ethanol (20 mL) was then added to the residue and the inorganic salt removed by filtration. After solvent evaporation under reduced pressure the Boc-protected peptides were obtained as a white solid (99% yield).

4.2.6. Esterification of Boc-glycine-OH with dimethyl carbonate

A solution of Boc-glycine (0.3000 g, 1.712 mmol) in a mixture of DMSO (8.6 mL) and dimethyl carbonate (DMC) (3.00 mL, 34.2 mmol) was flushed with N_2 inside a pressure tube; K_2CO_3 (0.9500 g, 6.873 mol) was then added along with a magnetic stirrer. The tube was then closed and heated to 120 °C for 2 h under magnetic stirring. After that the tube was cooled to r.t. and the workup was performed. The suspension was diluted with EtOAc (30 mL), extracted two times with 20 mL of water and once with 20 mL of brine. The organic phase was then dried using Mg_2SO_4. After solvent evaporation under reduced pressure the N-Boc-glycine methyl ester was obtained as a yellow oil (78% yield).

4.2.7. Protection and esterification of glycilglycine

To a solution of glycilglycine (0.0750 g, 0.5677 mmol) in a mixture of DMSO (2.2 mL) and saturated aqueous solution of $NaHCO_3$ (2.8 mL) was added Boc_2O (0.1536 g, 0.7038 mmol) under magnetic stirring, at 0 °C. After 15 min the temperature was allowed to rise to r.t. and the reaction was stirred for 30 hours. The workup was carried out lowering the pH to 5 using 6 M HCl, water and volatile byproducts were then removed using reduced pressure. The heterogenous solution comprising of product and NaCl in DMSO was used without further purification to carry out the esterification reaction.

The suspension containing the N-Boc-peptide, DMSO, salt and magnetic stirrer was transferred inside a pressure tube, and DMSO (1 mL) was then added along with DMC (1.00 mL, 11.9 mmol). The vessel was then flushed with N_2 and, after that, K_2CO_3 (0.3150 g, 2.291 mol) was added. The tube was then closed and heated to 120 °C under magnetic stirring. After 2 h the reaction was stopped and the tube cooled to r.t, the mixture was then diluted with EtOAc (15 mL), extracted two times with water (10 mL each) and once with brine. The organic phase was then further dried using Mg_2SO_4 and, after solvent evaporation under reduced pressure, the N-Boc-peptide methyl ester was obtained as a yellow oil (61% yield).

4.2.8. Derivatization of the GLY-based oligomers with activated peptides

A mixture of poly(glycerol adipate) (0.415 g, 0.500 mmol) and Boc-protected peptide methyl ester (0.500 mmol) was heated to 85 °C under magnetic stirring (200 rpm) using a cross shaped stirrer. After complete homogenization CaLB Novozym 435 (10% w w^{-1} on the total amount of monomers) was added to the flask. After 6 hours the reaction was put under vacuum (11 mbar) and stirred for 18 more hours. The workup was carried out solubilizing the product in 2-Me-THF, filtering out the enzyme beads using a WHATMAN 595.5 cellulose filter. Solvent was removed under reduced pressure and residue further dried under reduced pressure to give the desired product as a yellow oil (92% conversion).

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4.2.9. Esterification of amino acid and derivatives with 1-butanol

A mixture of amino acid or amino ester (1.000 mmol) and 1-butanol (12.00 mmol, 0.9350 g) was heated to 85 °C under magnetic stirring (400 rpm) using a cross shaped stirrer, after complete homogenization CaLB Novozym 435 (10% w/w) was added to the flask. The reaction was left stirring for 24 hours, adding more butanol if the one in the flask evaporated. The workup was carried out solubilizing the product in THF, filtering out the enzyme beads using a WHATMAN 595.5 cellulose filter. Solvent ad residual butanol was removed under reduced pressure and residue further dried under reduced pressure to give the desired product.

Author Contributions

F.R. carried out the synthetic work and conducted the analysis of the materials. A.G. developed the quantitative ^{13}C-NMR method. A.P. and R.R. supervised the work. All authors discussed the collected data. F.R. and A.P. wrote the manuscript. R.R., L.B. and A.P. revised the manuscript. A.P. acquired the funding.

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Conflict of Interests

The authors declare no conflict of interest.

Data Availability Statement

The data that support the findings of this study are available in the supplementary material of this article.

Keywords: poly(glycerol adipate) · emulsifying agents · bioactive peptides delivery · cosmetics polymers · chemo-enzymatic synthesis · functional macromolecules

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