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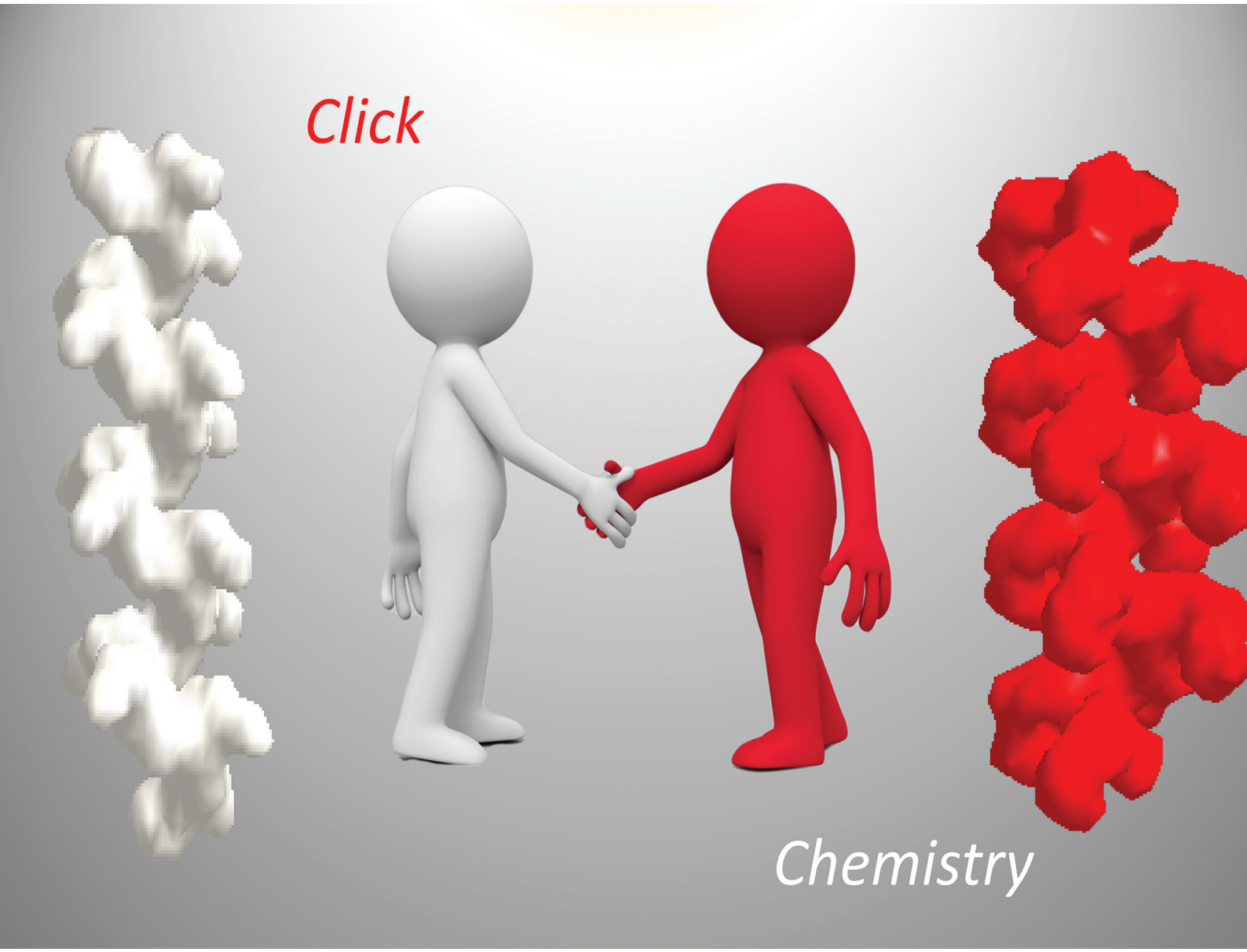

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"Clickable" bacterial poly(γ-glutamic acid)†

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Poly(γ-glutamic acid) (γ-PGA) is one of the few bacterial polymers in nature; as such, it possesses the added value of a "green birth" (fermentation) and "green death" (biodegradability), which is ideal for sustainable macromolecular platforms. Efficient functionalization strategies are required in order to transform the native bacterial biopolymer into a material with tailored properties for bulk scale or biomedical applications. We report on a novel approach to the facile functionalization of bacterial γ-PGA, realized with several levels of control: (a) the modulation of the molecular weight through sonication, and the solubilization in organic solvents through counterion exchange with suitable quaternary ammonium salts, (b) the introduction of reactive functionalities through reactions in homogeneous conditions in organic solvents, to afford homo- or copolymers; (c) a second tier functionalization using copper(I)-catalyzed alkyne–azide cycloaddition (CuAAC) click chemistry.

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Introduction

Poly(γ-glutamic acid) (γ-PGA) is a homopolymer of the naturally-occurring α-amino acid glutamic acid, and one of the only three polyaminoacids present in nature.[1] Since the amide linkages in the repeating unit are formed between the α-amino and γ-carboxylate groups, it is formally a nylon 4 derivative. Although it is still more expensive to produce than other common biobased polymers (polylactic acid, polyhydroxyalkanoates), it is used on a large scale, mainly in the cosmetic and food industries. Some of its features are indeed outstanding. In fact, γ-PGA is highly biodegradable and even edible: the popular food known as natto in Japan contains about 1% of γ-PGA. Its chemical synthesis has been approached by polycondensation with limited success, but it is especially problematic using controlled ring opening polymerizations of the corresponding *N*-carboxy anhydrides.[2] It is therefore industrially obtained by fermentation from a series of bacteria strains.[3,4]

Its "green birth" (fermentation) and "green death" (biodegradability) characteristics make it an ideal macromolecular backbone for the development of innovative biomaterials. γ-PGA has therefore been at the center of scientific attention in the last 20 years.[5–9] Its biodegradation has been studied by other authors. It has been reported that γ-PGA is resistant to normal proteases but it is biodegraded by many bacterial species.[10] On the other hand, the commercially available and isomeric counterpart α-PGA, thoroughly studied as a biomaterial and polymeric drug carrier because it can be obtained synthetically by the ring-opening polymerization of the corresponding *N*-carboxy anhydrides, is mentioned to be degraded by proteolitic enzymes.[11,12]

γ-PGA has a functional group pendant from the polymer backbone, the α-carboxylic group; from an organic chemistry point of view, this functional group is amenable to a variety of transformation, which, carried out on every other repeating unit, can be very effective into transforming and modulating the physical and chemical properties of the native biopolymer. Only a handful of synthetic methodologies have been disclosed for the synthesis of γ-PGA derivatives with reliable control of functionalization degree and substitution patterns.[13] The difficulties associated to the derivatization of γ-PGA are related to the poor solubility of the starting material in organic solvents and the reactivity of the carboxylate functionalities, hampered by the proximity of the amide group. Whereas early work focused on transesterification protocols on preformed, fully functionalized γ-PGA methyl or ethyl ester derivatives,[14] alkylation protocols on the biopolymer carboxylate groups have also been addressed.[15] A recent approach made use of low molecular weight γ-PGA (*ca.* 2×10^4 Da), and of activation of the carboxylate in organic solvents using oxalyl chloride.[16] Our group has reported a "two tier approach" to the functionalization of γ-PGA: in a first step, clickable (allyl or propargyl) functional groups are introduced by alkylation

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† Electronic supplementary information (ESI) available: Additional experimental procedures, additional GPC graphs, copies of NMR spectra of new polymers. See DOI: 10.1039/d0py00843e

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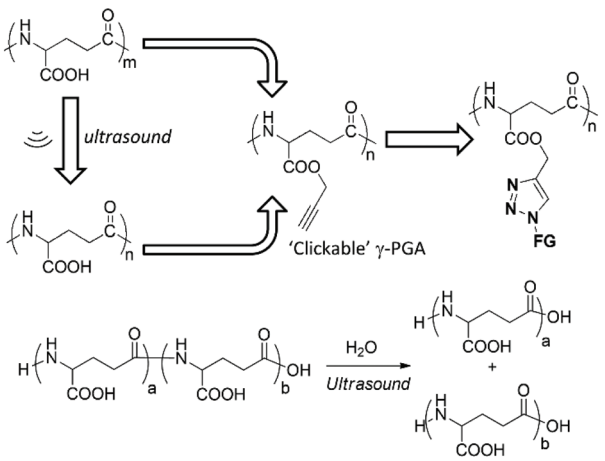
under heterogeneous conditions (DMSO as a solvent, and potassium carbonate as the base).[17] Although promising for the broad scope potential, since clicking of different functionalities through CuAAC or thiol–ene reactions was demonstrated, the approach suffered from several drawbacks and demonstrated to be rather "uncontrolled". It afforded a good degree of functionalization on a low molecular weight native γ-PGA (*ca.* 2×10^4 Da) only when an iterative procedure was used, but it consistently failed to give high coverage of substitution using higher molecular weight native γ-PGA. Furthermore, the use of bases in the presence of high polarity solvents such as DMSO generated unpleasant sulfide byproducts, and the presence of colored impurities were difficult to remove from the final polymeric products.

In this paper, we investigate the controlled reduction of the molecular weight of the native polymer by inputs of mechanical force; we investigate and report a remarkably more efficient functionalization methodology by alkylation, carried out in homogeneous conditions through a counterion exchange approach, allowing the synthesis and characterization of a series of γ-PGA ester derivatives, either homo or copolymers, containing allyl or propargyl groups. We also demonstrate that, in the case of propargyl esters, CuAAC click chemistry can be applied with high fidelity to generate functionality-rich γ-PGA backbones. The overall strategy is illustrated in Scheme 1.

Results and discussion

Controlled reduction of native γ-PGA molecular weight

One of the potential advantages of γ-PGA is that the relatively high molecular weight of the native, bioproduced polymer can be modulated by a mechanochemical procedure.[18] In order to address and potentially control the physico-chemical and mechanical properties of the derivatives obtained after functionalization, we report further about the reduction of the molecular weight characteristics and dispersity of γ-PGA aqueous solutions through sonication.



Scheme 1 Controlled molecular weight reduction and a representative 'click' chemistry based modification of γ-PGA biopolymer.

The gradual reduction of the molecular weight of the polymer with the sonication procedure could be achieved successfully and with full reproducibility of the results. The samples were characterized by GPC in buffered H_2O, and molecular weight parameters were determined against a pullulan-based calibration curve. In fact, two different sonication procedures have been adopted and compared, either with interrupted sonication using 5 minutes pulses, or uninterrupted sonication for an equivalent amount of time. The results are essentially superimposable (Fig. 1, black and grey dots for the two procedures, respectively). The reduction of the polymer chains molecular weight reaches a plateau around average number molecular weight M_n = 100 kDa, independently from the concentration of the polymer solution. In fact, sonication is a mechanical input of force, and it is efficient in polymer chains to activate scission of covalent bonds when the chains are long enough to absorb the force at the molecular level.[19,20] The $Đ$ index does not seem to be affected by the reduction of the molecular weight.

Further confirmation of the controlled reduction and of the nature of the chain scission activity came from ^{1}H NMR spectroscopy (Fig. 2). The ^{1}H NMR spectra is coherent with the proposed structure with broad signals and no evident fine structure, typical of polymeric systems.

Upon sonication, the reduction of molecular weight, detected and quantified by the GPC experiments, is confirmed by the attenuation of the broadening of all the peaks. Furthermore, NMR spectroscopy proves that chain scission occurs within middle of chains (where the applied mechanical force is effective), rather than unzipping monomers or short oligomers from chain ends, since monomeric or oligomeric species are not present in the NMR spectra. $[\alpha]_D^{rt}$ and circular dichroism spectra of γ-PGA aqueous solutions before and after sonication (20 min) showed to be essentially superimposable, excluding any possibility of racemization of the polymer backbone induced by the mechanical force (see Experimental section and Fig. S2†). Other methods can be used to further reduce the molar masses of PGGA below 100 kDa; enzymatic degradation is reported to cleave high molecular weight γ-PGA

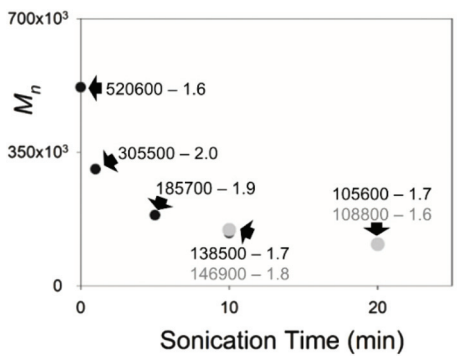


Fig. 1 Molecular weight reduction as a function of sonication time for a 0.033 M γ-PGA solution in H_2O. Numbers indicate M_n and $Đ$, for each respective dot. Grey dots: sonication time of 5 + 5 min and 10 + 10 min.

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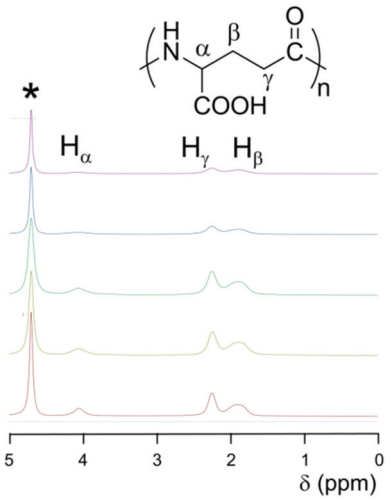

Fig. 2 ^{1}H NMR (200 MHz, D_2O, 25 °C) of γ-PGA solutions (0.3–0.5 M concentration of repeating units) related to the sonication experiments reported above. From top to bottom (sonication time): native polymer, 1 min, 5 min, 5 + 5 min, 10 min. The asterisk denotes the solvent residual in the deuterated D_2O.

into fragments between 1000 Da to 20 kDa,[21] as well as hydrolytic degradation at different temperatures and pH's.[22,23]

γ-PGA solubilization in organic solvents and derivatization

γ-PGA, either as the carboxylic acid or the carboxylate salt, is soluble in water, and essentially insoluble in organic solvents. We have verified that by exchanging the sodium cationic of our anionic high molecular weight bacterial γ-PGA with quaternary ammonium cations with very long side chains such as cetyltrimethylammonium, it is possible to solubilize the polymer in common organic solvents.[24] The exchange reaction takes place in the aqueous phase: two separate solutions in H_2O of the organic salt, cetyltrimethylammonium bromide (CTAB), and of the polymer **1** as the sodium salt are mixed and the precipitation of the exchanged polymer **2** is observed. Washings of the precipitate with MeCN were crucial in order to remove residual coprecipitated CTAB, and the full counterion exchange and the 1 : 1 stoichiometry (the organic cation associated to each carboxylate functionality) could be verified by integration of the respective peaks in the NMR spectra in $CDCl_3$. The exchanged polymer **2** was soluble in chlorinated organic solvents such as CH_2Cl_2 or $CHCl_3$. We also found that, using shorter alkyl chain quaternary ammonium salts, such as tetrabutylammonium bromide, effective counterion exchange and precipitation from H_2O did not occur with the γ-PGA sample used in our study.

Interestingly, solutions of polymer **2** in $CHCl_3$, when exposed to the same sonication protocol described above, did not show any variation of the molecular weight. We attribute this phenomenon to the absence of H_2O as the solvent for the sonication; H_2O is indeed a reagent in the depolymerization event associated to the molecular weight reduction, and its absence hampers the depolymerization process.

The conjugation of γ-PGA with suitable molecular fragments or other biopolymers is necessary for its transformation into a tailored material. Suitable reactive functionalities need therefore to be placed on the γ-PGA backbone, by means of reliable synthetic and purification protocols.

The functionalization of polymer **2** in organic solvents proved to be controllable, reliable and reproducible. We focused on the controlled introduction of propargyl esters, and we performed several experiments in order to verify the possibility to address the substitution pattern with high fidelity on the γ-PGA backbone. Indeed, using less than stoichiometric amounts of propargyl bromide, we achieved partial esterification and we observed good agreements between the feed ratio of propargyl bromide in the reaction mixture and the observed ratio of ester coverage on the γ-PGA backbone, as determined by ^{1}H NMR spectroscopy (Scheme 2, polymers **3a–c**). Using an excess (5 equiv.) of benzyl or allyl bromides in homogeneous conditions afforded complete functionalization giving polymers **4** and **5** in essentially quantitative yields (Scheme 2). ^{1}H NMR spectroscopy confirmed the full degree of the substitution pattern achieved.

The crude products **3–5** were purified by precipitation from DMF to aqueous HCl, and eventually reprecipitated from DMF/ether. All samples appeared as white powders. In the case of the propargyl esters, full coverage could not be achieved even in the case of **3d**, obtained by using the same excess used for allyl and benzyl bromide. In any case, the synthetic methodology proposed here for the propargyl derivative **3** is vastly outperforming the results previously available in the literature.[15] We believe that the lack of complete coverage in the case of γ-PGA is a consequence of steric factors, and of the higher steric hindrance of the propargyl groups, rather than the lower reactivity of propargyl bromide *vs.* allyl or benzyl groups in nucleophilic substitutions. In fact, in the propargyl substituent, the locked linear sequence of three carbon atoms and the terminal hydrogen, freely rotating around the sp^3 carbon atom, may define a larger cone than the cone defined by the rotation of the allyl or the benzyl substituents.

This observation finds confirmation from a far from random sequence distribution of the propargyl substituents in polymers sample **3a** (*vide infra* NMR spectroscopy). Indeed, when a partial functionalization of γ-PGA with propargyl esters was conducted, a second round of functionalization with an excess of allyl bromide did not achieve full coverage (polymer **6** in Scheme 2), since presumably propargyl groups already placed on the γ-PGA backbone prevent achievement of full coverage by the allyl substituents.

The click chemistry philosophy develops the much-needed concepts of modularity and orthogonality in macromolecular functionalization.[25,26] The click approach is synthetically efficient, and it has already been shown useful and able to provide alternatives for the postmodification of γ-PGA. The prototypical and perhaps still most popular click reaction is the CuAAC reaction, and it was our selected choice to explore, making the propargyl derivatives **3** the benchmark γ-PGA "first tier" derivative.

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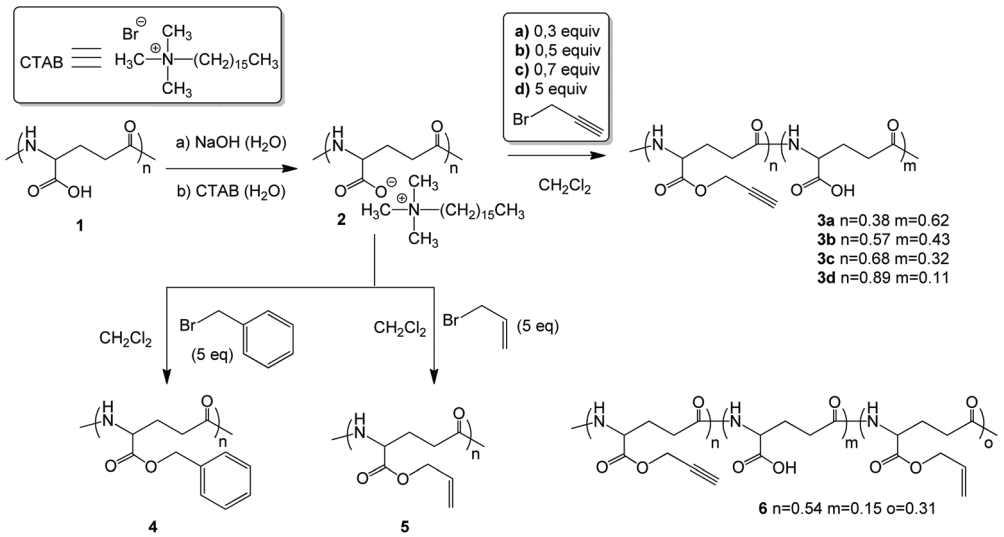
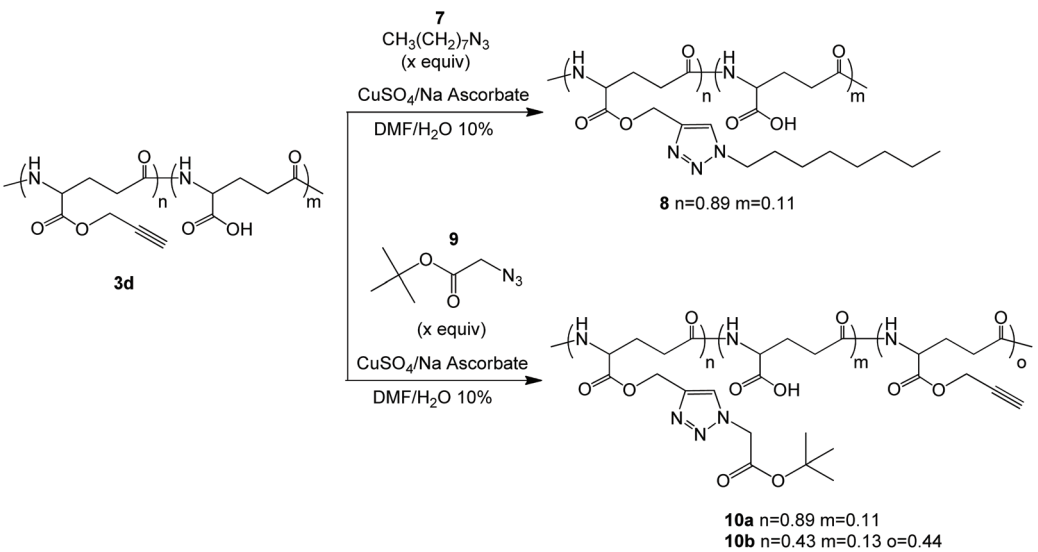


Scheme 2 Synthesis by first tier postfunctionalization of γ-PGA derivatives. Estimated copolymer composition determined by NMR spectroscopy of the purified products.

They have been subjected to a "second tier" postfunctionalization with two different azides (Scheme 3): a linear alkyl chain (compound **7**), and a more sterically hindered azide with a protected carboxylate unit. In order to find suitable conditions for the clicking reactions, some optimization experiments were carried out with **7** on the model substrate propargyl acetate (see ESI†). It was found that DMF alone was not a good solvent for the click reaction, neither at room temperatures or elevated temperature, and that the reaction worked more efficiently by generating the catalytically-active Cu(I) species *in situ* rather than introducing it directly. Azide **9** was chosen since it is a key spacer to give, when clicked, hydrophilic functionalities, in their deprotected form, which are indeed very suitable for conjugating in a controlled manner short peptides. In order to verify the control of the functionalization, two experiments were carried out with **9**, working either in excess or in less than a stoichiometric ratio with respect to the triple bond coverage present in polymer **3d**. In both case fidelity in the substitution pattern has been achieved.

NMR and GPC characterization of derivatives

NMR spectroscopy was effective in order to confirm the structure of the homopolymer and to determine the composition of the co-and ter-polymers. For achieving better resolution and reduce the broadening of the signals, spectra acquisition was



Scheme 3 Second tier postfunctionalization of γ-PGA derivatives. Estimated copolymer composition determined by NMR spectroscopy of the purified products.

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performed at 80 °C in deuterated DMSO. As an example, the 4–9 ppm region of partially functionalized polymers **3a**, **3d**, fully functionalized polymer **5** and terpolymer **6** are shown in Fig. 3. For example, NH proton signals of functionalized (at *ca.* 8.0 ppm) and unfunctionalized (at *ca.* 7.8 ppm) units of the copolymers were clearly distinguishable. Efficient clicking could be verified from the partial or complete disappearance of the ^{1}H NMR spectra of the unique signals of the propargyl ester groups (for example, the proton resonances of the $CH_2C{\equiv}C$ groups at *ca.* 4.7 ppm), and from the appearance of the newly formed aromatic triazole proton resonances at *ca.* 8.0 ppm (Fig. 4).

Important information was also gathered from the ^{13}C NMR spectroscopy. In the case of the partially esterified derivative **3a**, the signal due to α-methylene main chain carbon is sensitive to sequence distribution at the level of dyads. Four signals were observed and assigned to the four possible dyads. The Lorentzian deconvolution of these peaks afforded the quantification and using simple expressions[27] the degree of randomness could be estimated to be 0.5 (Fig. 5) essentially indicating a blocky microstructure for these series.

GPC chromatography was carried out on selected derivatives in dimethylacetamide (DMAc) as the solvent, which solubilized well all the samples analyzed. The results are reported in Table 1.

We performed postfunctionalization experiments using both the high molecular weight native polymer (entry 1), and the reduced molecular weight sample (entry 2) obtained at the end of the sonication sequence shown in Fig. 1. The two samples were separately exchanged of the counterions, and then subjected to the same functionalization protocol. In the former case, the reaction conditions used for the introduction brought a reduction in the molecular weight of the resulting polymer **3d**, which fell from an initial average degree of polymerization of *ca.* 3000 to around 600 (entry 3).

After clicking compound **7**, the degree of polymerization remained high (entry 4). In the latter case, after the click reaction with compound **7** or **9**, a lower degree of polymerization was observed (entries 5 and 6), although the reduction is rather marginal. The $Đ$ values remained remarkably similar during the whole synthetic process.

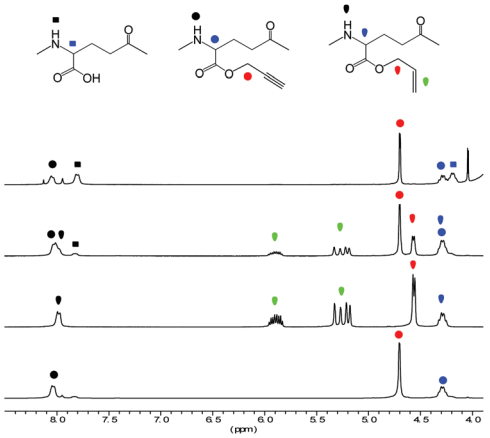

Fig. 3 Partial ^{1}H NMR spectra (from 4 to 9 ppm, DMSO-d_6, 300 MHz, 80 °C) of representative polymer samples. From top to bottom: polymer **3a**, **5**, **6** and **3d**.

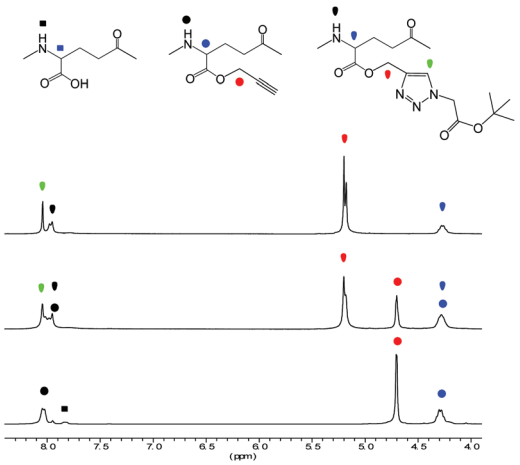

Fig. 4 Partial ^{1}H NMR spectra (from 4 to 9 ppm, DMSO-d_6, 400 MHz, 80 °C) of representative polymer samples. From top to bottom: polymers **10a**, **10b** and **3d**.

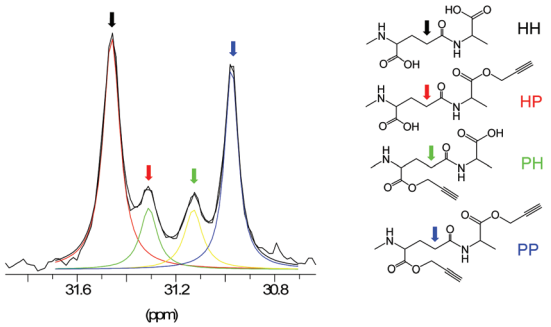

Fig. 5 ^{13}C NMR spectra in the region of the α-methylene carbon (*ca.* 31 ppm) (75.5 MHz, DMSO-d_6) of copolymer **3a** with dyads assignments (right).

Table 1 Molecular weight characteristics of selected synthesized copolymers[a]

Entry	Polymer	M_n	$Đ$	DP[b]
1[c]	**1** (Na^+ salt)	520 600	1.6	2900
2[c]	**1** (Na^+ salt)	106 600	1.7	590
3[d]	**3d**	121 000	1.8	630
4[d]	**8**	138 000	1.8	450
5[d]	**8**[e]	113 000	1.7	370
6[d]	**10a**[e]	134 000	1.5	440

[a] For analysis method, see experimental part. $Đ$ = dispersity index. [b] Degree of polymerization; calculated on the basis of M_n values, using the weighted (according to composition determined by ^{1}H NMR spectroscopy) average repeating unit molecular weight. [c] GPC in H_2O. [d] GPC in DMAc. [e] Obtained from starting polymer **1** in entry 2.

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Conclusions

We have presented a thorough study addressing the functionalization of bacterial γ-PGA in a controlled manner. The biopolymer has several extremely interesting characteristics, which are unheard of in state of the art biobased polymeric platforms. We have demonstrated that it is possible to reduce, in a controlled manner, the molecular weight of the native biopolymer using a mechanochemical approach, and we have demonstrated that the chain-breaking mechanism occurring within the macromolecular chains maintains the polymer integrity and the original molecular weight dispersities. The synthetic modification, involving reactions in the homogeneous phase in organic solvents, allow for an unprecedented control of the degree of substitution of the reactive functional groups installed within a first tier approach. Clicking with high fidelity allowed the installment through the second tier approach of lipophilic or hydrophilic functionalities. One can anticipate that in future studies clicking of fluorescent probes or drugs will advance the evaluation of the biopolymer for therapeutic applications. In the case of the clicked hydrophilic protected carboxylate functionalities, it is foreseeable a straightforward grafting of biobased oligomeric products (*i.e.* peptides), which is otherwise not possible to achieve in a controlled manner on the carboxylate groups of the native γ-PGA. The degrees of polymerization of the elaborated macromolecular derivatives presented in this study are unprecedented, and they are certainly promising for applications in the biomaterials and polymers for healthcare arena. We will report studies towards this end in the near future.

Experimental section

General experimental

γ-PGA sample **1** was purchased from Biological Technology (declared molecular weight 700 000 Da, free acid form, D : L enantiomeric ratio 77 : 23) and used as received. Compounds **7**[28] and **9**[29] were prepared as previously described. All other commercially available compounds were purchased from Sigma-Aldrich, Alfa Aesar and FluoroChem and used as received. Analytical thin layer chromatography was performed on silica gel, chromophore loaded, commercially available plates. Flash chromatography was carried out using silica gel (pore size 60 Å, 40–63 μm). ^{1}H and ^{13}C NMR spectra were recorded on Bruker 300 and 400 MHz instruments and calibrated with the solvent residual proton signal or tetramethylsilane.

Reduction of molecular weight of γ-PGA solutions by ultrasonication

Ultrasonic treatment was carried out on aqueous γ-PGA solutions by an ultrasonic probe with diameter of 12 mm and a 500 W high intensity ultrasonic processor (Sonics Vibracell, Sonic & Materials Inc., USA). The ultrasonication protocol was as follows: 25 mL of γ-PGA aqueous solutions (5.25 mg mL^{-1}) were cooled down to 15 °C in an ice bath and then sonicated at 80% power. Ultrasonication was performed either continuously for increasing time spans of 1, 5, 10, 20 minutes respectively, or discontinuously, with 2 min intervals between 5 minutes pulses. All samples were kept in ice bath continuously during treatment.

Evaluation of γ-PGA molecular weight: gel permeation chromatography (GPC)

γ-PGA molecular weight and dispersity was evaluated by GPC analysis using a system equipped with a refractive index detector, with two universal columns (Ultrahydrogel 1000 and 2000) in series, thermostated at 40 °C, and using a 50 mM phosphate buffer at pH = 7 (0.8 mL min^{-1}) as the mobile phase. The samples were dissolved in the mobile phase, filtered (nylon filters with a 0.20 μm cutoff) and injected.[30] The relevant data (M_n, M_w and $Đ$) were obtained after elaboration with software Breeze, using a calibration curve built with narrow polydispersity pullulane standards (9 standards at M_n = 670, 5500, 10 500, 20 000, 45 500, 100 000, 189 000, 356 000 and 636 000 Da). GPC in DMAc was performed using two universal columns, thermostated at 30 °C, and calibrated against PMMA standards.

Polymer 2. NaOH (48 mL, 48 mmol) was added to a solution of γ-PGA **1** (5.2 g, 40 mmol) in H_2O (1 L). The mixture was stirred for 20 min and an aqueous solution of cetyltrimethylammonium bromide (CTAB) (14.6 g, 40 mmol) was added. The suspension was filtered on Buchner funnel and washed with CH_3CN (several portions). The polymer was dissolved in $CHCl_3$ (700 mL) and dried (Na_2SO_4). The solvent was removed *in vacuo* to give purified **2** (14.0 g, 85%). ^{1}H NMR ($CDCl_3$, 200 MHz) δ = 7.88 (bs, 1H; functionalized NH, 100%), 4.16 (bs, 1H; –C<u>H</u>CH$_2$CH$_2$– polymeric chain), 3.28 (bs, 11 H; (C<u>H</u>$_3$)$_3$N$^+$C<u>H</u>$_2$), 2.5–1.8 (bm, 4H; –C<u>H</u>$_2$– polymeric chain). 1.72 (bs, 2H; –NCH$_2$C<u>H</u>$_2$– CTAB), 1.26 (bs, 26H; –C<u>H</u>$_2$– CTAB chain), 0.89 (bt, 3H; C<u>H</u>$_3$– terminal of CTAB).

Optical activity and circular dichroism measurements

Optical rotations were measured on a polarimeter with a sodium lamp (λ = 589 nm) and are reported as follows: $[\alpha]_D^{rt}$ (c = g per 100 mL solvent). The values obtained for a γ-PGA solution before and after the sonication protocol reported above (20 min sonication) were as follows: $[\alpha]_D^{rt}$ = −12.5 (c = g per 100 mL H_2O) and −11.9 (c = 1.05 g per 100 mL H_2O), respectively. The value can be compared with $[\alpha]_D^{rt}$ = −30.3 (c = 2 g per 100 mL H_2O) reported for D-glutamic acid.[31] CD spectroscopy was performed using a spectropolarimeter; spectra were recorded at 25 °C at a scanning speed of 50 nm min^{-1} and were background corrected.

Polymers 3. Representative procedure for the synthesis of polymers 3. Polymer 3a. Propargyl bromide (71 μL, 0.72 mmol) was added to polymer **2** (1.0 g, 2.4 mmol) dissolved in CH_2Cl_2 (40 mL). The solution was stirred for 46 h at 40 °C. The solvent was removed *in vacuo* and the solid crude product was washed with CH_3CN, filtered and dried under vacuum for several hours. The polymer was dissolved in *N*,*N*-dimethylformamide

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(DMF) (10 mL) and precipitated into an aqueous HCl solution (1 N, 100 mL). The product was filtrated and dried to give purified **3a** (650 mg, 73%). ^{1}H NMR (DMSO-d_6, 400 MHz) δ = 8.06 (bs, 1H; functionalized NH, 20%), 7.80 (bs, 1H; unfunctionalized NH), 4.70 (bs, 2H; –C$\underline{H}_2$ propargylic), 4.15–4.09 (bs, 1H; –C$\underline{H}$– polymer chain), 3.02 (bs, 1H; –C$\underline{H}$ triple bond), 2.19–1.68 (bm, 4H; –C$\underline{H}_2$– polymer chain). ^{13}C NMR (DMSO-d_6, 100 MHz, 80 °C) δ = 171.2, 171.0, 170.6, 77.8, 77.0, 73.9, 51.7, 51.5, 51.3, 48.6, 34.1, 31.4, 31.3, 31.1, 31.0, 28.5, 26.9, 26.4. **Polymer 3d**. Propargyl bromide (24.6 mL, 220.6 mmol) was added to polymer **2** (18.2 g, 44.1 mmol) dissolved in CH_2Cl_2 (300 mL). The solution was stirred for 3 days at 40 °C. The solvent was removed *in vacuo* and the crude product was dried under vacuum. The polymer was dissolved in DMF (300 mL) and precipitated into an aqueous HCl solution (0.1 N, 1.6 L). The product was filtered and dried to give purified **3d** (7.1 g, 96%). ^{1}H NMR (DMSO-d_6, 400 MHz, 80 °C) δ = 8.06 (bs, 1H; functionalized NH), 7.80 (bs, 1H; unfunctionalized NH), 4.70 (bs, 2H; –C$\underline{H}_2$ propargyl), 4.15–4.09 (bs, 1H; –C$\underline{H}$– polymer chain), 3.02 (bs, 1H; –C$\underline{H}$ triple bond), 2.19–1.68 (bm, 4H; –C$\underline{H}_2$– polymer chain). ^{13}C NMR (DMSO-d_6, 100 MHz, 80 °C) δ = 172.6, 171.4, 171.2, 171.0, 170.6, 77.0, 76.0, 51.6, 51.4, 31.2, 30.9, 28.5, 26.8, 26.4.

Polymer 4. Benzyl bromide (19.9 g, 110.5 mmol) was added to polymer **2** (9.1 g, 22.1 mmol) dissolved in CH_2Cl_2 (300 mL). The solution was stirred for 3 days at 40 °C. The solvent was removed *in vacuo* and the crude product was dried under *vacuum*. The polymer was dissolved in DMF (100 mL) and precipitated into an aqueous HCl solution (0.1 N, 1 L). The product was filtered and dried to give purified **4** (5.3 g, 96%). ^{1}H NMR (DMSO-d_6, 300 MHz) δ = 8.03 (bs, 1H; functionalized NH), 7.30 (bs, 5H; aromatic CH), 5.08 (s, 2H; benzyl CH_2), 4.27–4.24 (bs, 1H; –C$\underline{H}$– polymer chain), 2.07–1.77 (m, 4H; bm, 4H; –C$\underline{H}_2$– polymer chain). The spectra were consistent with those already published.[17]

Polymer 5. Allyl bromide (2.1 mL, 24.2 mmol) was added to polymer **2** (2.0 g, 4.85 mmol) dissolved in CH_2Cl_2 (150 mL). The solution was stirred for 3 days at 40 °C. The solvent was removed *in vacuo* and the crude product was dried under vacuum. The polymer was dissolved in DMF (140 mL) and precipitated into an aqueous HCl solution (0.1 N, 0.75 L). The product was filtered and dried to give purified **5** (0.8 g, 97%). ^{1}H NMR (DMSO-d_6, 400 MHz, 80 °C) δ = 8.03 (bs, 1H; NH), 5.95–5.79 (bm, 1H; –C$\underline{H}$=C$\underline{H}_2$), 5.33–5.17 (bm, 2H; –CH=C$\underline{H}_2$), 4.56–4.54 (bd, 2H; –C$\underline{H}_2$CH=CH$_2$), 4.24–4.21 (bs, 1H; –C$\underline{H}$– polymer chain), 2.21–1.78 (m, 4H; bm, 4H; –C$\underline{H}_2$– polymer chain). ^{13}C NMR (DMSO-d_6, 100 MHz, 80 °C) δ = 171.1, 170.9, 131.0, 117.0, 64.8, 51.5, 51.4, 31.0, 26.5. The spectra were consistent with those already published.[14]

Polymer 6. Propargyl bromide (1.4 g, 12 mmol) was added to polymer **2** (1.0 g, 2.4 mmol) dissolved in CH_2Cl_2 (150 mL). The solution was stirred for 6 hours at 40 °C. Allyl bromide (1.4 g, 12 mmol) was then added and the homogeneous reaction mixture was then stirred for further 72 hours at 40 °C. The solvent was removed *in vacuo* and the crude product was dried under vacuum. The polymer was dissolved in DMF (70 mL) and precipitated into an aqueous HCl solution (0.1 N, 0.55 L). The product was filtered and dried to give purified **6** (450 mg, 97%). ^{1}H NMR (DMSO-d_6, 400 MHz, 80 °C) δ = 8.03 (bs, 1H; NH), 7.80 (bs, 1H; unfunctionalized NH), 5.95–5.79 (bm, 1H; –C$\underline{H}$=CH$_2$), 5.33–5.17 (bm, 2H; –CH=C$\underline{H}_2$), 4.70 (bs, 2H; –C$\underline{H}_2$ propargyl), 4.56–4.54 (bd, 2H; –C$\underline{H}_2$CH=CH$_2$), 4.24–4.21 (bs, 1H; –C$\underline{H}$– polymer chain), 3.02 (bs, 1H; –C$\underline{H}$ triple bond), 2.21–1.78 (m, 4H; bm, 4H; –C$\underline{H}_2$– polymer chain). ^{13}C NMR (DMSO-d_6, 100 MHz, 80 °C) δ = 171.1, 170.9, 170.6, 132.0, 117.3, 77.7, 77.0, 64.3, 51.7, 51.4, 51.3, 31.0, 28.5, 26.8, 26.5, 26.4.

Polymer 8. Compound **7** (249 mg, 1.6 mmol) was added to polymer **3d** (185 mg, 1.1 mmol) dissolved in DMF (10 mL). $CuSO_4$ (35 mg, 0.2 mmol), sodium ascorbate (88 mg, 0.4 mmol), and, slowly, H_2O (1 mL, 10% relative to DMF), were added to the solution. The solution was stirred for 65 h at room temperature. The crude product was precipitated into an aqueous HCl solution (0.1 N, 300 mL). The product was filtered and dried to give purified **8** (280 mg, 79%). ^{1}H NMR (DMSO-d_6, 400 MHz, 80 °C) δ = 8.09 (bs, 1H; –C$\underline{H}$– triazole), 8.05 (s, 1H; functionalized NH), 7.90 (bs, 1H; unfunctionalized NH), 5.12 (bs, 2H; –COOC$\underline{H}_2$–), 4.34 (bm, 2H; –NC$\underline{H}_2$–), 4.20 (bs, 1H; –C$\underline{H}$– polymer chain), 2.18–1.76 (bm, 4H; –C$\underline{H}_2$– polymer chain), 1.21 (bs, 10H; –C$\underline{H}_2$– chain), 0.82 (bs, 3H; –CH$_2$C$\underline{H}_3$). ^{13}C NMR (DMSO-d_6, 100 MHz, 80 °C) δ = 171.4, 141.7, 124.1, 57.9, 51.9, 51.8, 49.5, 31.4, 31.0, 29.5, 28.3, 28.2, 26.9, 25.8, 21.9, 13.6.

Polymer 10a. Compound **9** (2.8 g, 18 mmol) was added to polymer **3d** (2.5 g, 15 mmol) dissolved in DMF (75 mL). $CuSO_4$ (481 mg, 3 mmol), sodium ascorbate (1.2 g, 6 mmol), and, slowly, H_2O (7.5 mL, 10% relative to DMF), were added to the solution. The solution was stirred for 18 h at room temperature. The polymer was precipitated into an aqueous HCl solution (0.01 N, 1.1 L). The product was filtered and then it was dissolved in DMF (95 mL) and reprecipitated into an aqueous HCl solution (0.01 N, 1.6 L). The product was filtered and dried to give purified **10a** (4.3 g, 88%). ^{1}H NMR (DMSO-d_6, 400 MHz, 80 °C) δ = 8.26 (bs, 1H; functionalized NH), 8.10 (bs, 1H; –C$\underline{H}$– triazole), 5.25 (bs, 2H; –NC$\underline{H}_2$OOC–), 5.15 (bs, 2H; –OC$\underline{H}_2$–), 4.21 (bs, 1H; –C$\underline{H}$– polymer chain), 2.18–1.76 (bm, 4H; –C$\underline{H}_2$– polymer chain), 1.40 (bs, 9H; C$\underline{H}_3$ ^{13}C NMR (DMSO-d_6, 100 MHz, 80 °C) δ = 171.5, 171.4, 165.8, 141.8, 125.7, 82.5, 57.7, 51.8, 51.2, 31.4, 27.6, 26.9.

Polymer 10b. Compound **9** (237 mg, 1.5 mmol) was added to polymer **3d** (465 mg, 2.8 mmol) dissolved in DMF (44 mL). $CuSO_4$ (96 mg, 0.6 mmol), sodium ascorbate (238 mg, 1.1 mmol), and, slowly, H_2O (4 mL of, 10% relative to DMF), were added to the solution. The solution was stirred for 48 h at room temperature. The polymer was precipitated into an aqueous HCl solution (0.01 N, 400 mL). The product was filtered and then it was dissolved in DMF (15 mL) and reprecipitated into an aqueous HCl solution (0.01 N, 150 mL). The product was filtered and dried to give purified **10b** (403 mg, 58%). ^{1}H NMR (DMSO-d_6, 400 MHz, 80 °C) δ = 8.05 (s, 1H; –C$\underline{H}$– triazole), 8.01 (bs, 1H; functionalized NH), 7.91 (bs, 1H; unfunctionalized NH), 5.18 (bs, 2H; –COOC$\underline{H}_2$–), 4.70 (bs, 2H; –C$\underline{H}_2$– unfunctionalized propargylic group), 4.54 (bs, 2H; –NC$\underline{H}_2$OOC–), 4.25 (bs, 1H; –C$\underline{H}$– polymer chain), 3.34 (bs, 1H;

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–CH unfunctionalized triple bond), 2.18–1.76 (bm, 4H; –CH_2– polymer chain), 1.41 (bs, 9H; –$C(CH_3)_3$). ^{13}C NMR (DMSO-d_6, 100 MHz, 80 °C) δ = 171.5, 171.4, 171.0, 165.8, 141.8, 125.7, 82.5, 78.1, 77.4, 57.7, 52.1, 51.8, 51.2, 31.4, 27.6, 26.9.

Conflicts of interest

There are no conflicts to declare.

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Notes and references

1 B. Rehm, *Nat. Rev. Microbiol.*, 2010, **8**, 578–592.
2 F. Sanda, T. Fujiyama and T. Endo, *J. Polym. Sci., Part A: Polym. Chem.*, 2001, **39**, 732–741.
3 J. M. Buescher and A. Margaritis, *Crit. Rev. Biotechnol.*, 2007, **27**, 1–19.
4 V. Scoffone, D. Dondi, G. Biino, G. Borghese, D. Pasini, A. Galizzi and C. Calvio, *Biotechnol. Bioeng.*, 2013, **110**, 2006–2012.
5 I. Bajaj and R. Singhal, *Bioresour. Technol.*, 2011, **102**, 5551–5561.
6 L. Zhang, X. Geng, J. Zhou, Y. Wang, H. L. Gao, Y. Zhou and J. Huang, *J. Drug Targeting*, 2015, **23**, 453–461.
7 S. Sakuma, T. Sagawa, Y. Masaoka, M. Kataoka, S. Yamashita, Y. Shirasaka, I. Tamai, Y. Ikumi, T. Kida and M. Akashi, *J. Controlled Release*, 2009, **133**, 125–131.
8 M. Matsusaki, T. Serizawa, A. Kishida and M. Akashi, *Biomacromolecules*, 2005, **6**, 400–407.
9 D. Puppi, C. Migone, A. Morelli, C. Bartoli, M. Gazzarri, D. Pasini and F. Chiellini, *J. Bioact. Compat. Polym.*, 2016, **31**, 531–549.
10 F. B. Oppermann, S. Fickaitz and A. Steinbüchel, *Polym. Degrad. Stab.*, 1998, **59**, 337–344.
11 W. G. Miller, *J. Am. Chem. Soc.*, 1961, **83**, 259–265.
12 W. G. Miller, *J. Am. Chem. Soc.*, 1964, **86**, 3913–3918.
13 S. Muñoz-Guerra, M. García-Alvarez and J. A. Portilla-Arias, *J. Renewable Mater.*, 2013, **1**, 42–60.
14 M. Morillo, A. Martínez de Ilarduya and S. Muñoz-Guerra, *Polymer*, 2003, **44**, 7557–7564.
15 H. Kubota, Y. Nambu and T. Endo, *J. Polym. Sci., Part A: Polym. Chem.*, 1993, **31**, 2877–2878.
16 M. Biagiotti, G. Borghese, P. Francescato, C. F. Morelli, A. M. Albertini, T. Bavaro, D. Ubiali, R. Mendichi and G. Speranza, *RSC Adv.*, 2016, **6**, 43954–43958.
17 A. Pacini, M. Caricato, S. Ferrari, D. Capsoni, A. Martínez de Ilarduya, S. Muñoz-Guerra and D. Pasini, *J. Polym. Sci., Part A: Polym. Chem.*, 2012, **50**, 4790–4799.
18 G. Pérez-Camero, F. Congregado, J. J. Bou and S. Muñoz-Guerra, *Biotechnol. Bioeng.*, 1999, **63**, 110–115.
19 J. Li, C. Nagamani and J. S. Moore, *Acc. Chem. Res.*, 2015, **48**, 2181–2190.
20 C. E. Diesendruck, G. I. Peterson, H. J. Kulik, J. A. Kaitz, B. D. Mar, P. A. May, S. R. White, T. J. Martínez, A. J. Boydston and J. S. Moore, *Nat. Chem.*, 2014, **6**, 623–628.
21 Z. Luo, Y. Guo, J. Liu, H. Qiu, M. Zhao, W. Zou and S. Li, *Biotechnol. Biofuels*, 2016, **9**, 134.
22 T. Xu, S. Zhan, M. Yi, B. Chi, H. Xu and C. Mao, *Polym. Adv. Technol.*, 2018, **29**, 1966–1973.
23 A.-M. Cromwick and R. Gross, *Int. J. Biol. Macromol.*, 1995, **17**, 259–267.
24 A. Gamarra, A. Martínez de Ilarduya, M. Vives, J. Morató and S. Muñoz-Guerra, *Polymer*, 2017, **116**, 43–54.
25 C. Barner-Kowollik, F. Du Prez, P. Espeel, C. J. Hawker, T. Junkers, H. Schlaad and W. Van Camp, *Angew. Chem., Int. Ed.*, 2011, **50**, 60–62.
26 L. Beria, T. N. Gevrek, A. Erdog, R. Sanyal, D. Pasini and A. Sanyal, *Biomater. Sci.*, 2014, **2**, 67–75.
27 J. C. Randall, *Polymer Sequence Determination*, Academic Press, New York, 1977, p. 71.
28 L. Diaz, J. Bujons, J. Casas, A. Llebaria and A. Delgado, *J. Med. Chem.*, 2010, **53**, 5248–5255.
29 D. Dou, G. He, Y. Li, Z. Lai, L. Wei, K. R. Alliston, G. H. Lushington, D. M. Eichhorn and W. C. Groutas, *Bioorg. Med. Chem.*, 2010, **18**, 1093–1102.
30 D. Pasini, J. M. Klopp and J. M. J. Fréchet, *Chem. Mater.*, 2001, **13**, 4136–4146.
31 J. R. Parikh, J. P. Greenstein, M. Winitz and S. M. Birnbaum, *J. Am. Chem. Soc.*, 1958, **80**, 953–957.