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Green Chain–Shattering Polymers Based on a Self-Immolative Azobenzene Motif

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A chain-shattering polymer system consisting of nontoxic, renewable resource-based monomers via acyclic diene metathesis (ADMET) chemistry is introduced. Amphiphilic triblock copolymers with apparent molecular weights in the range from 10 to 23 kDa are synthesized using a monofunctional polyethylene glycole monoacrylate, which acts as a selective chain-transfer agent during the polymerization process. Most importantly, the functional polymers possess repetitive midchain azobenzene moieties imparting them with self-immolative properties. By virtue of the enzyme degradable azobenzene chain elements, the amphiphilic macromolecules can be efficiently degraded via a self-immolative reaction into small fragments. The construction of the macromolecules along with their degradation is evidenced by nuclear magnetic resonance spectroscopy, electrospray ionization mass spectrometry and size exclusion chromatography. In addition, the triggered degradation leads to a strong reduction in the UV absorptivity of the polymeric material.

Introduction

The current drive to sustainable processes and environmental responsibility in macromolecular science requires alternative polymers with improved degradability based on renewable source-derived materials.¹ The use of biomass, for instance castor oil or many other plant oils, has been recognized as one of the most promising routes, especially as a substitute source for chemicals which prospectively may replace the known descending petrochemicals.² Importantly, degradation is not dependent on the origin of the building blocks but only on the chemical linkages present in the polymer.³ Nevertheless, one approach to obtain a biodegradable material in a sustainable fashion is to synthesize polymers constituted of a polymer backbone based on renewable source-derived components in which one or more degradable units (hydro degradable, photodegradable or biodegradable) are embedded.⁴ However, the variety of degradation units in such materials remains limited. Thus, increasing the diversity of degradable polymers – including those derived from renewable resources – might allow for better matching soft matter materials to specific applications. The azobenzene functionality is unique as it is capable of responding to changes in environmental conditions⁵ as well as cleavable under enzymatic impact (*in vivo*) via a two-

electron reduction process.^{6,7} Despite the fact that the azo containing compounds display high structural diversity, the degradation is induced by specific enzymes, among which are azoreductases and glycosidases.⁶ While the azo bond reduction has been described in both anaerobic and aerobic bacteria, anaerobic conditions are prevalent in the human body.^{8,9} The operation of these enzymes have common mechanistic features as they all catalyse redox reactions and moreover exhibit relatively wide substrate specificities.⁶ More specifically, azoreductases – present and active both in the gut microflora and mammalian tissues (such as the skin⁹ and the liver)¹⁰ – are redox cofactor¹¹ (*i.e.* flavin mononucleotide (FMN) or flavin adenine dinucleotide (FAD)) dependent enzymes that require the addition of a cofactor such as nicotinamide adenine dinucleotide phosphate (NAD(P)H) as electron donors for the reduction of the azo linkage. Thus, the reductive cleavage of the azobenzene bond in the presence of the azoreductase results in a self-immolative aniline derivative, which degrades entirely by intramolecular 1,6-elimination reactions via iminoquinone methide intermediates to 4-aminobenzyl alcohol, a by-product with low toxicity.^{12,13} The driving force is – in addition to the increase in entropy – the irreversible formation of CO₂. Substantial efforts have been devoted to designing polymers incorporating azo moieties into the main chain including poly(ether ester),¹⁴ polyurethanes,¹⁵ and polyamides,¹⁶ however these are rarely discussed with regard to the structural features of the azo compound. The azo unit can accelerate the rate of reduction and in turn the degradation of the polymer in a self-immolative manner. Besides the typical self-immolative polymers end-capped with variable trigger responsive functional groups,¹⁷ there are rare examples of self-immolative polymeric systems which possess

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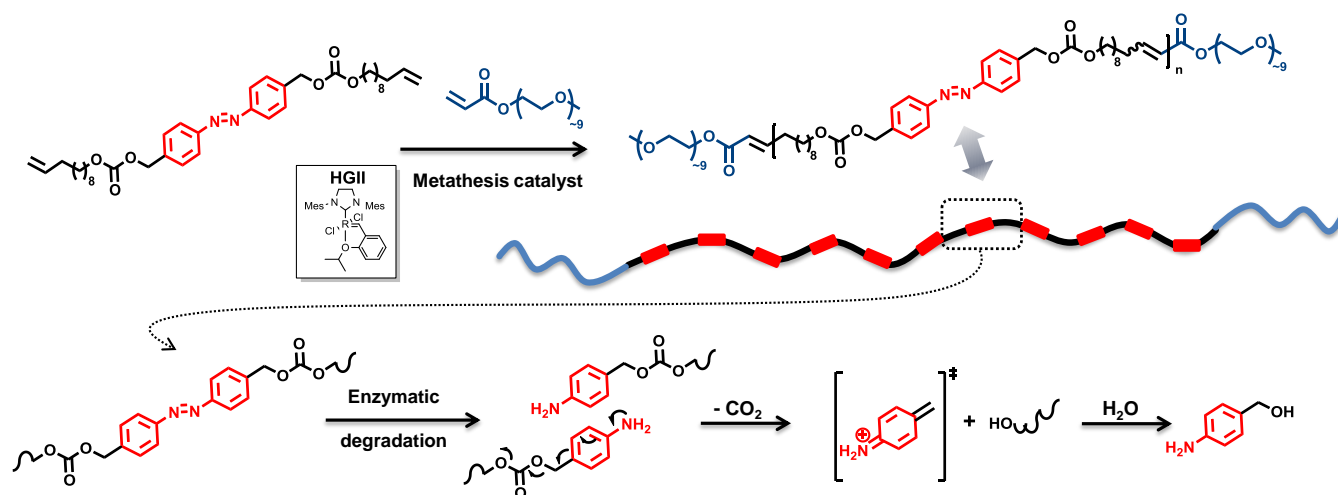


Fig. 1. Enzyme-responsive chain-shattering amphiphilic ADMET triblock copolymer containing multiple self-immolative degradable azobenzene moieties as a repetitive constraint.

multiple triggers linked either as side chain motifs or directly within the backbone, termed chain-shattering polymers.

Chain-shattering polymers spontaneously disintegrate along the main chain into small components only after activation by a specific stimulus. The systems whose multiple triggers are connected as pendant groups are generally synthesized using classical step-growth copolymerization. Typically, activated bifunctional self-immolative linkers capped by a trigger are reacted in step-growth manner with bifunctional comonomers to yield polymers which degrade either through a consecutive quinone methide rearrangement (*via* 1,4-/1,6-elimination) and/or intramolecular cyclization upon a specific stimulus.¹⁸ Alternatively, the toolbox for the synthesis of chain-shattering polymer was expanded by employing chain-growth ring-opening polymerization (ROP)¹⁹ and olefin metathesis chemistry²⁰ (specifically acyclic diene metathesis). However, none of the systems described above were targeted to develop a chain-shattering polymer whose enzyme-labile self-immolative triggers are directly incorporated as repetitive chain motifs within the polymer backbone. Thus, with the aim to critically diversify the available chain-shattering polymer toolbox and enlarge the variety of self-immolative monomers, we describe the development of the strategy represented in Fig. 1.

Results and Discussion

The construction of polymers conjugated with triggers in the backbone from a building block with two terminal alkenes and an azo moiety serving as an enzyme-responsive group, will result in elimination reactions at each repeating unit after the application of a trigger – here an enzyme. Thus, a depolymerisation reaction will occur by means of a chain-shattering mechanism, thereby accelerating the depolymerisation. The challenge and innovation inherent to the current approach is the design of the enzyme-responsive polymer segment. Inspired by the synthetic possibilities to

introduce repetitive constraining elements *via* acyclic diene metathesis (ADMET) polymerization,²¹ a precision polyolefin containing a degradable azobenzene moiety as a repetitive constraint (Fig. 1) is introduced. Indeed, Wagener has explored the use of this polymerization technique to elegantly synthesize precision polyolefins containing diverse functional groups.²² Moreover, Meier and colleagues used the ADMET polymerization of renewable bio-based α,ω -dienes incorporating hydrodegradable linkages (such as anhydride, esters)²³ to yield (bio)degradable polymers. Undoubtedly, fatty acids and their derivatives are suitable components for the preparation of (bio)degradable polymers since they are hydrophobic entities that naturally occur in the body, and their application as building blocks for such polymers introduces additional pliability.²⁴ Thus, 10-undecene-1-ol, a subsidiary product of castor oil,² was used as renewable-based alcohol derivative for the synthesis of the self-immolative α,ω -diene monomer **M1** (Fig. 2). The synthesis of a suitable monomer for ADMET polymerization was achieved by placing a carbonate moiety as a leaving group at the benzylic position of the azobenzene functionality in the hydroxyl alkene-based **M1** (for preparative details refer to the Supporting Information section). Our efforts for ensuring an environmentally benign nature of **M1** were not limited to biomass as a source. We employed glucose as a 'green' reducing agent for the synthesis of the azo derivative, 4,4'-bis(hydroxymethyl)-azobenzene (Fig. 2) starting from 4-nitrobenzyl alcohol. Moreover, the aforementioned nitro benzyl derivative was obtained *via* the oxone mediated oxidation of 4-aminobenzyl alcohol.²⁵ In addition, 1,1-carbonyl diimidazole (CDI)²⁶ was used as a safe and non-toxic reagent to couple the azo diol moiety with 10-undecen-1-ol yielding the self-immolative α,ω -diene monomer **M1** (Fig. 2).

The synthesized azo derivative α,ω -diene monomer **M1** enabled the introduction of a degradable constraint every 20 carbons in the polymer obtained by ADMET chemistry. Polymer **P1**, with apparent molar mass of 25 kDa and with a dispersity of 2.0 (as determined by size exclusion

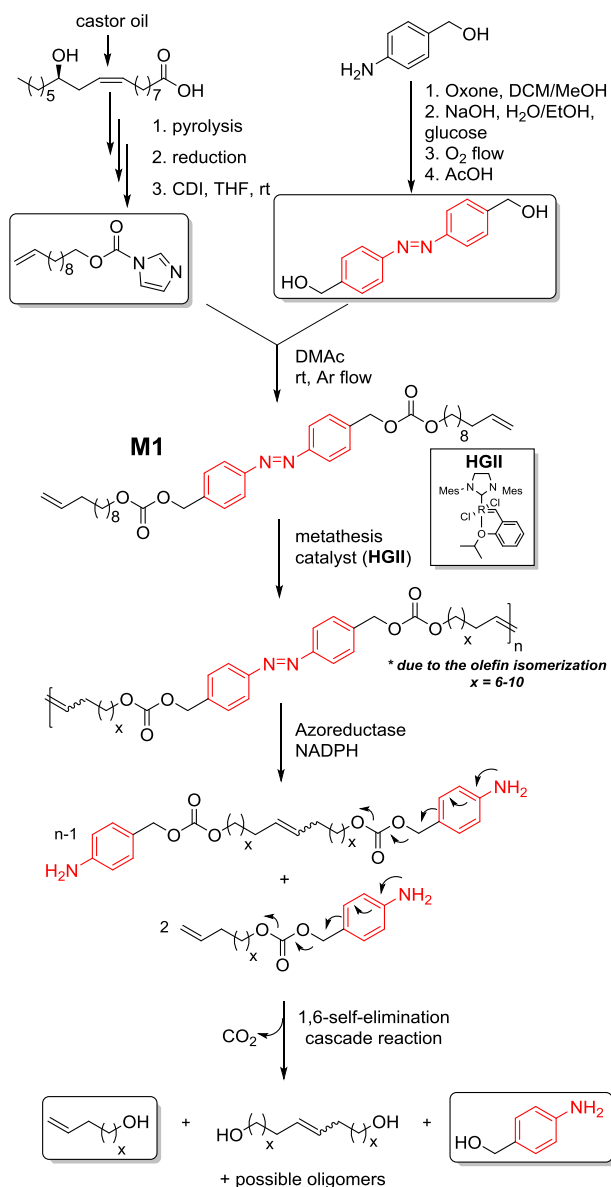


Fig. 2. Synthesis of the monomer, **M1**, possessing self-immolative character and the respective ADMET polymer **P1**. The plausible cleavage of azobenzene linkages in **P1** upon enzymatic action and subsequent 1,6-self elimination to smaller molecular weight species.

chromatography (SEC) relative to polystyrene standards), was generated in the presence of 1 mol% second-generation Hoveyda-Grubbs (HGII) catalyst per double bond (*i.e.* 2 mol% HGII catalyst/molecule) under reduced pressure (compare Fig. 2 and Table S1 in the Supporting Information section). HGII was the choice of catalyst due to its outstanding tolerance and activity toward coordinative and non-coordinative heteroatom containing dienes.²⁷

To remove the quenched metathesis catalyst from the crude polymer reaction mixture, the mixture was eluted with chloroform and passed through a short plug of silica/basic alumina. In comparison with the ¹H NMR of the corresponding monomer **M1** (Fig. 3a), a clean and complete transformation of the α,ω -diene to unsaturated polymer **P1** was observed (Fig. 3b). ¹H NMR spectroscopic analysis of the polymer revealed resonances characteristic of the newly formed internal double

bond at δ H 5.35 ppm. Furthermore, it is noteworthy to highlight that along ¹H NMR, ¹³C NMR analysis also demonstrated that the azo functionality remained intact, indicating the compatibility of ADMET chemistry towards the azobenzene unit (compare Fig. S2 in the Supporting Information section).

To determine the distribution and the degree of polymerization (DP_n) of the polymer, **P1** was subjected to matrix assisted laser desorption ionization time-of-flight mass spectrometry. However, due to the broad dispersity of **P1**, initial attempts to obtain mass spectra representative of the complete distribution of the polymer failed. On the other hand, the undesired isomerization side reaction²⁸ which usually occurs during ADMET polymerization facilitated to define the DP_n by ¹H NMR *via* end-group resonance integration, yielding a DP_n of 42 for **P1** (M_n of 25.5 kDa, refer to the NMR discussion part in the Supporting Information). The end groups associated with the olefin isomerization are indicated as **E1**, **E2**, and **E3**, respectively (refer to the ¹H NMR assignments in Fig. 3). Commonly, additives such as benzoquinone²⁹ are used to suppress the isomerization, however any attempts to employ benzoquinone during the ADMET polymerization reactions failed. It has been reported that the azobenzene group does not undergo any 1,4-cycloadditions with benzoquinone,³⁰ yet the polymers obtained from the polymerization of **M1** in the presence of 1,4-benzoquinone were not completely soluble in common organic solvents, suggesting that some limited crosslinking reactions might have taken place.

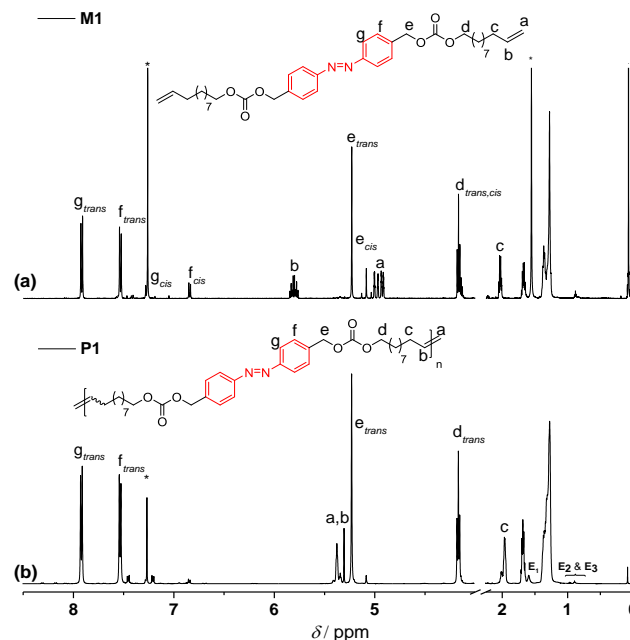


Fig. 3. ¹H NMR (500 MHz, CDCl₃) spectra of (a) the azo derivative α,ω -diene monomer **M1** and (b) of a representative homopolymer **P1** (shown in Table S1 in the Supporting Information section). The end groups associated with the olefin isomerization in the homopolymer **P1** are indicated as **E1**, **E2**, and **E3**, respectively.

In order to evaluate the efficiency of the anticipated elimination reactions in the new polymer system, the reductive cleavage of the azo functionality within **M1** itself was

assessed by NMR spectroscopic methods. $\text{Na}_2\text{S}_2\text{O}_4$, a substitute that mimics the azoreductase enzyme, was employed as the reducing agent.³¹ Upon treatment with 1 equivalent of the reducing agent in phosphate-buffered saline (PBS, pH 7.4),³² the reaction was incubated at 37 °C for several hours. ^1H NMR analysis of the final reaction mixture showed far different results than expected, *i.e.* no degradation. The limited solubility of the hydrophobic **M1** in PBS is identified as the main reason for the incomplete reduction.⁸ However, this attempt also clearly evidenced the hydrolytic stability of **M1**. To facilitate a complete reduction, a mixture of dichloromethane:methanol:water (3:20:20), which has proven to be a useful solvent for the conversion of azobenzenes into *N,N'*-diarylhydrazines,³³ was employed to drive the reduction to the corresponding aniline derivative. By using 5.5 equivalents of $\text{Na}_2\text{S}_2\text{O}_4$, a loss of the characteristic strong yellow colour of **M1** is detected within 40 min, and the reduction was confirmed by UV-Vis and ^1H NMR (Fig. 4 and S3 in the Main Text and Supporting Information section, respectively). The UV-visible absorption spectra of **M1** was characterized by a band at 330 nm, which intensity decreased gradually with time during the degradation process (Fig. 4). For the complete reduction, the necessary reaction time was 5 h in total.

Once the reduction of **M1** had been established, the subsequent step was to perform reduction studies on the polymer itself. While $\text{Na}_2\text{S}_2\text{O}_4$ is a specific, bio-compatible³⁴ and effective chemical stimulus that may be useful in some applications, it was also of interest to demonstrate that the reduction of the specified system is possible under biologically

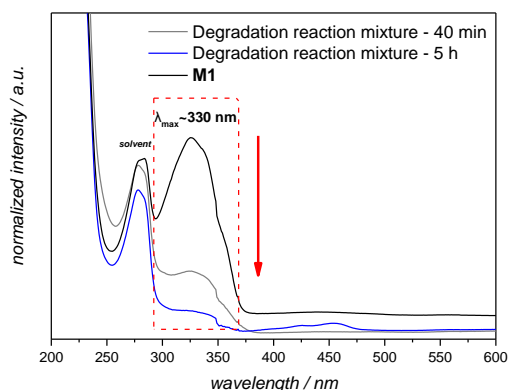


Fig. 4. UV-visible spectra illustrating the degradation of **M1** in the presence of 5.5 equivalents of $\text{Na}_2\text{S}_2\text{O}_4$ in a mixture of dichloromethane:methanol:water at reflux temperature (for details see Section A.8 in the Supporting Information). Spectra recorded in THF at 25 °C.

relevant conditions, as this would significantly expand the utility of the polymer. Thus, the following experiments were conducted with the enzyme azoreductase (commercially known as DT-diaphorase) in the presence of the coenzyme NADPH.

The initial degradation studies were performed in the same manner as those previously reported by Khan *et al.*, by suspending the ADMET polymer **P1** in PBS, and subsequently

exposing it to a mixture of the enzyme azoreductase and the coenzyme NADPH, followed with incubation at 37 °C for a period of 12 h. As noted, a crucial point in the reduction reactions is to avoid oxygen due to the fact that dioxygen can serve as an alternative electron acceptor, thus inhibiting the azoreductase activity.³⁵ Accordingly, the reduction was performed under anaerobic conditions. To remove the cofactor and its by-products, the solution was dialyzed against water. Unexpectedly, ^1H NMR and SEC analysis of **P1'** (Fig. S4 and S5 in the Supporting Information section, respectively) did not indicate any degradation, moreover no specific colour change was observed during the reaction. On the basis of the ^1H NMR analysis, the *trans-cis* isomerisation of the azo bond was detected as the only significant change (compare Fig. S4 in the Supporting Information section). Indeed, this transformation can be of particular interest for future studies in the context of the reduction-sensitive moiety due to the fact that *cis* azobenzene has an increased rate of reduction relative to the *trans* isomer.³⁶ Nevertheless, having in mind that the specified enzyme can show 'degressive' prolonged stability at the aforementioned conditions, the influence of the reaction time was subsequently investigated. However, even after monitoring the degradation reaction over a period of 11 days (**P1''**), there was a minimal decrease in the molecular weight of the polymer (due to a possibly competing hydrolytic mechanism) as observed in the SEC chromatogram of the isolated product (Fig. S6 in the Supporting Information section).

The ineffective degradation of **P1** clearly indicates that to achieve the desired degradability, the polymer structure needs to be adjusted. Indeed, an advantage of the ADMET polymerization approach is the possibility to use olefins other than terminal ones not only to introduce defined end groups but also as a way to obtain ABA triblock copolymers with finely selected solubility properties. Thus, a poly(ethylene glycol) (PEG) moiety possessing a methyl ether acrylate functionality, considered to be inert and possessing a low level of toxicity,³⁷ can provide the desired water solubility. By incorporation of water soluble blocks in the polymer system, one can avoid the potential steric inhibition of the azoreductase. Thus, an amphiphilic copolymer was prepared using similar conditions as for the ADMET homopolymer, yet with the addition of a selective and irreversible chain-stopper (**CS**), a monofunctional PEG methyl ether acrylate ($M_n = 480$ Da) (compare Fig. 1, 2 and S7, respectively in the Main Text and the Supporting Information section).³⁸ The addition of variable amounts of **CS** (2, 5, 10 and 20 mol%, respectively) to the polymerization mixture led to an efficient control of the molecular weight of the resulting polymers **P2-P5** (see Fig. 5 as well as Table S1, entries 2-5 in the Supporting Information section), thus resulting in defined amphiphilic ABA triblock copolymer architectures. The M_n values determined for the copolymers by SEC are slightly different compared with the theoretical ones (Table S1 in the Supporting Information section), since SEC molecular weights were determined relative to polystyrene (PS) standards. The ABA triblock copolymers were tested for their degradation behaviour. The triblock **P4**,

synthesized with 10 mol% **CS** in the presence of 1 mol% second-generation Hoveyda-Grubbs (HGII) catalyst per double bond (*i.e.* 2 mol% HGII catalyst/molecule) under reduced pressure, was subjected to the same degradation conditions as the homopolymer **P1** for reaction times of 12, 24 and 36 h, respectively. The degradation mixture of the polymer incubated for 36 h indicated a similar degree of degradation as that after 24 h (compare Fig. S8 in the Supporting Information section). We suggest that changes in the pH (from initial pH value of 7.4 to 6.5) during the degradation due to increased CO₂ levels in the sealed vials likely affected the degree of degradation. Indeed, CO₂ is acidic by nature, thus a high CO₂ saturation will lead to low pH and consequently reduce the activity of azoreductase, which is mainly active in the pH range of 5.5 to 8.3 (optimal reduction conditions being structure specific).³⁹

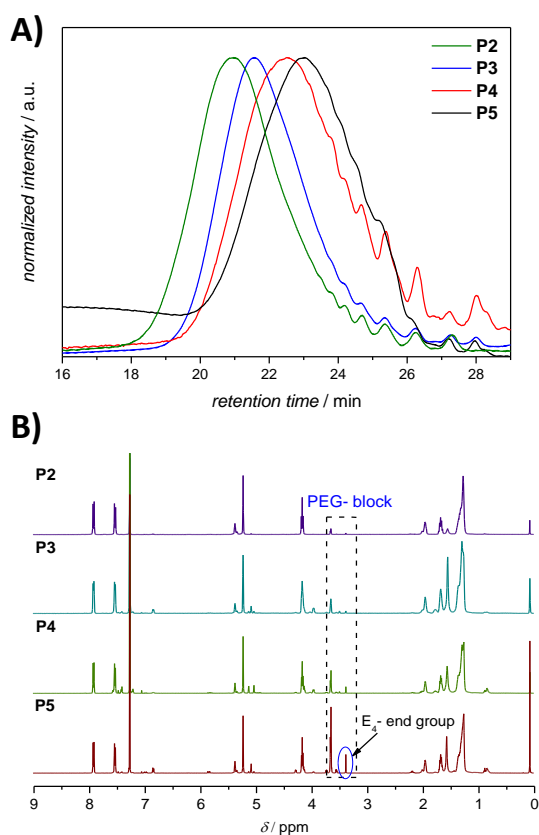


Fig. 5. A) SEC chromatograms for ADMET amphiphilic triblock copolymers **P2-P5** presented in Table S1 in the Supporting Information section. The SEC traces were measured in THF at 30 °C. B) ¹H NMR (500 MHz, CDCl₃) spectra of the amphiphilic triblock copolymers **P2-P5** presented in Table S1, respectively. Refer to Fig. 3 for the assignment of key resonances associated within the aromatic region. The major end group associated with the olefin isomerization in the copolymers **P2-P5** is indicated as **E4**.

UV-vis spectroscopy analysis established a broad adsorption band at 330 nm for the amphiphilic triblock copolymer **P4**, which is typical for the azobenzene chromophore having a dihydroxy substitution pattern in para-position.⁴⁰ Examination of the purified degradation mixture after 24 h treatment, **P4**^{''} by UV-vis spectroscopy confirmed that the azobenzene linkages were cleaved, as no absorption signal could be

observed at 330 nm after the enzymatic treatment of polymer **P4** (refer to Fig. 6B).

In addition, in the ¹H NMR spectra, resonances corresponding to the expected depolymerization products are observed to increase in intensity, while the resonances in the aromatic region associated with polymer decreased (Fig. S9 in the Supporting Information section). Although SEC, UV-Vis and ¹H NMR spectroscopy evidence the successful degradation of the amphiphilic triblock copolymer, these data alone do not provide insight into the exact composition of the small molecule degradation fragments. Therefore, additional high resolution ESI-MS allowed assigning the degradation products, evidencing all possible fragments which can arise (Fig. 7). Analysis of a solution of **P4**^{''} in CH₂Cl₂:MeOH (3:1, v/v) by a Q-Exactive Orbitrap mass spectrometer indicated that **F1-F4**

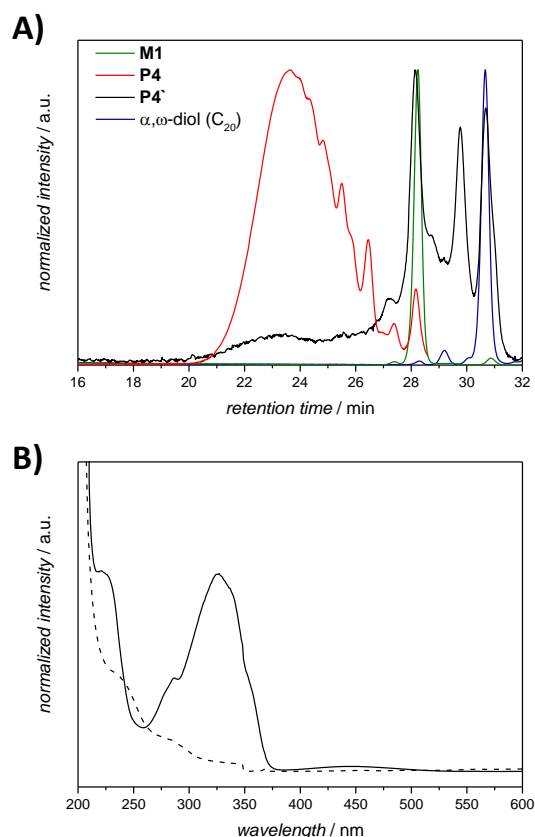


Fig. 6. A) SEC chromatograms obtained in THF for monomer **M1** (green line), polymer **P4** (red line, due to an artefact the measurement, the elugram is finished at 28.6 min), the resulting mixture after treatment with enzyme for 24 h - **P4**^{''} (black line), and model degradation product α,ω -diol (blue line), respectively. B) UV-Vis spectra of polymer **P4** (solid line) and reaction mixture **P4**^{''} after the enzymatic reaction and purified (dashed line). Sample concentration 0.4 mg/mL in CH₂Cl₂. Solvent cut-off wavelengths L₀: 245 and L₁: 230 nm, respectively (λ_{max} : 253 nm for CH₂Cl₂).

(shown in Fig. 7) were the major degradation products, suggesting that the desired degradation occurred at the majority of the self-immolative repeating units. Surprisingly, both the α,ω -diol (**F4**, $m/z_{\text{calc}} = 335.29$, [M+Na]⁺) and the unstable species **F2** ($m/z_{\text{calc}} = 633.38$, [M+Na]⁺), before its 1,6-elimination to form **F4**, were detected. Moreover, we were able to detect fragment **F1** ($m/z_{\text{calc}} = 1253.77$, [M+Na]⁺) before

its self-immolation to **F3** ($m/z_{\text{calc}} = 647.40$, $[M+Na]^+$), which is the hydrophilic block of the polymer **P4**. Therefore, the mass difference between parent ion m/z 1253.7646 and product ion m/z 647.3977 can be tentatively assigned to the repeating unit of the hydrophobic part of the triblock copolymer **P4** with a nominal mass of 606 Da. The fragments **F1** and **F3** show the typical PEG pattern with mass distributions of adjacent peaks separated by 44.08 Da, corresponding to the mass of the repeat unit, ethylene oxide.⁴¹ In addition, the expected compound with the m/z 123.07 (4-aminobenzyl alcohol) was not detected. However, as demonstrated by using a standard of 4-aminobenzyl alcohol, this compound is not stable under the experimental conditions (MS), and several coupling reactions could have taken place.

reductive/self-immolative mechanism is dominant in comparison with a possibly competing hydrolytic mechanism, which is largely inoperational unless the exposure time is very long (compare Fig. S6 in the Supporting Information section).

CONCLUSIONS

In summary, we introduce a strategy for the synthesis of renewable resource-based amphiphilic ADMET polymers which possess self-immolative degradable enzyme-triggered azobenzene units, thus establishing a new class of chain-shattering polymers. Upon treatment with the enzyme and the coenzyme, the azo units were transformed into amine groups,

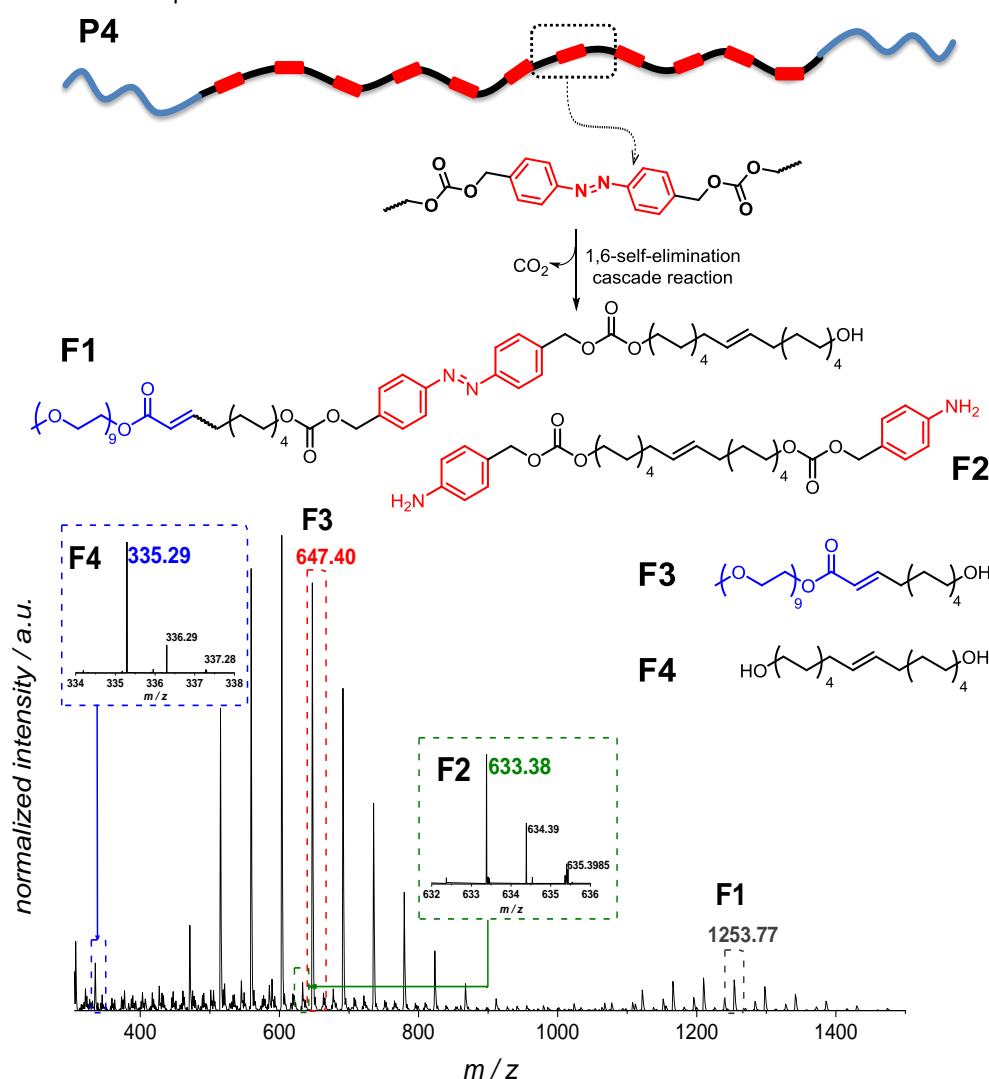


Fig. 7. High resolution ESI-MS analysis of the degradation fragments of **P4** after enzymatic treatment for 24 h (**P4**⁺).

To confirm that the observed degradation products are associated with the proposed 1,6-elimination reaction, control reactions were performed with polymer **P4** in water without any reducing agent for a period of 24 h (compare the SEC traces in Fig. S10 in the Supporting Information section). Although the control reaction indicates a slight shift compared to the initial polymer **P4**, it is clear that the proposed

which triggered the sequential self-immolative process to degrade the polymer main chain. The *in-situ* formed quinone methide intermediates were quenched by water molecules to form stable and safe hydroxymethylphenol derivatives. The degradation of the introduced system contrasts with conventional self-immolative polymers, which degrade from one chain end to the other. The chemistry of the system is

unique since the polymer degrades from the trigger cleavage side to its tail. In addition, the enzyme employed for the degradation, DT-diaphorase, does not form any free-radical or reactive oxygen intermediates during the degradation process. The developed systems thus opens the prospect to mimic a delivery vehicle in cancer cells/tissues in human body which show higher turnovers of NADPH than in normal cells/tissues.⁴² The introduced system is a prime example of a stimuli responsive biodegradable polymer and future studies will investigate the applicability of the new polymeric material for single chain nanoparticle synthesis⁴³ for applications in imaging and delivery.

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TOC Figure

