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Oxidation Responsive Polymers with a Triggered Degradation via Arylboronate Self-Immolative Motifs on a Polyphosphazene Backbone

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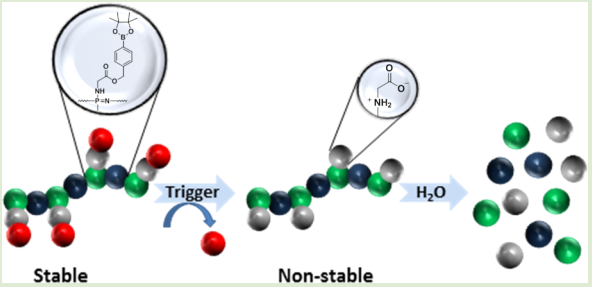
Supporting Information

ABSTRACT: Oxidation responsive polymers with triggered degradation pathways have been prepared via attachment of self-immolative moieties onto a hydrolytically unstable polyphosphazene backbone. After controlled main-chain growth, postpolymerization functionalization allows the preparation of hydrolytically stable poly(organo)phosphazenes decorated with a phenylboronic ester caging group. In oxidative environments, triggered cleavage of the caging group is followed by self-immolation, exposing the unstable glycine-substituted polyphosphazene which subsequently undergoes to backbone degradation to low-molecular weight molecules. As well as giving mechanistic insights, detailed GPC and ^{1}H and ^{31}P NMR analysis reveal the polymers to be stable in aqueous solutions, but show a selective, fast degradation upon exposure to hydrogen peroxide containing solutions. Since the post-polymerization functionalization route allows simple access to polymer backbones with a broad range of molecular weights, the approach of using the inorganic backbone as a platform significantly expands the toolbox of polymers capable of stimuli-responsive degradation.



Degradable polymers have ever-growing importance for environmental reasons, as well as for use in biomedical applications.[1] In recent years there has been significant progress toward smart responsive polymers that can undergo stimuli-controlled degradation, that is, remain stable, but then undergo spontaneous complete disintegration of the backbone only after activation by a specific stimulus. Such polymers have significant potential in a wide variety of applications, for example, in sensoring technologies,[2] on-demand drug release,[3] and nano-patterning.[4] Spontaneous main-chain disintegration can be achieved by end-to-end backbone depolymerization, so-called "self-immolative polymers".[5] Such smart polymers are designed to sequentially disassemble into their respective building blocks in response to a specific triggering event. This property is commonly achieved via incorporation of *ortho*/*para*-benzylic amines or alcohols capable of undergoing 1,4-/1,6-eliminations upon deprotection of the amine or alcohol or, alternatively, by the design of polymer main chains with urea or carbamate linkages that can undergo intramolecular cyclization.[6] Typically self-immolative polymers are prepared via step-growth mechanisms in order to incorporate the aforementioned self-immolating moieties, thus, putting constraints in terms of the molecular weight control and architectures available.[6] This field has thus also been expanded to chain shattering polymers,[3b,7] in which cleavage of pendant groups along the main chain leads to chain scission into small components. Such a design strategy is potentially open to a wider variety of chemistries and indeed recently ring-opening polymerization[8] and olefin metathesis chemistry[7b,9] have been used to prepare poly(caprolactone) and poly(carbonate)s (PCs),[10] which undergo a chain-shattering process in response to a variety of stimuli including enzymatic,[7b] photochemical,[8] and oxidative[3b] environments.

Herein we present an alternative approach toward polymers with stimuli-controlled degradation by utilizing the hydrolytically instable inorganic phosphorus nitrogen backbone of polyphosphazenes as a platform.[11] Polyphosphazenes are commonly prepared via the highly reactive precursor $[NPCl_2]_n$, the facile postpolymerization functionalization of which allows the insertion of a wide range of pendant groups along the polymer backbone.[12] The polymeric precursor $[NPCl_2]_n$ can be prepared by ring-opening or living polymerization methods,[13] thus, allowing high molecular weights and controlled M_n with narrow dispersities and potentially a variety of architectures, including highly branched structures[14] and block copolymers.[15] The precursor $[NPCl_2]_n$ can be readily substituted with amino acid esters,[11b,16] giving rise to poly(amino acid ester)phosphazenes, a family of materials that are of great promise for biomedical applications due to their easily tunable degradation rates.[11b,16a,17] The backbone degradation mechanisms are well-studied[16c,d,17b,18] and known to involve hydrolysis of the backbone phosphorus, resulting in cleavage of the amino acid ester[16c,d] with the main chain

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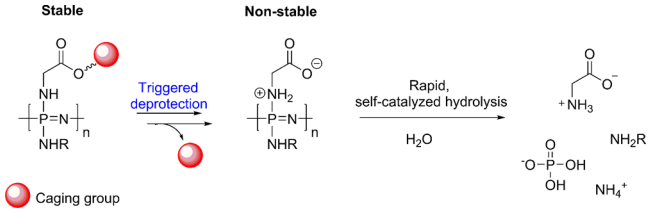
degradation products shown to be a benign buffered mixture of amino acid (ester), phosphates, and ammonium salts.[16d] It is also well-established that the degradation is acid-catalyzed[17c,d] and indeed that the presence of acidic groups in proximity to the backbone phosphorus accelerate hydrolysis rates.[17b] Indeed, in early studies into poly(amino acid ester)phosphazenes, Allcock and co-workers described the inability to isolate the glycine-substituted polyphosphazene $[NP(NHCH_2COOH)_2]_3$ due to its extremely rapid hydrolysis.[17b] Thus, we proposed that through essentially caging poly(glycine)phosphazene via the addition of stimuli-responsive protection groups, it should be possible to prepare a stable polymer that, upon removal of the caging moiety, produces this hydrolytically unstable glycine-substituted polyphosphazene, which will spontaneously and rapidly disintegrate into small molecules (Scheme 1), an effect similar to that of a chain-shattering polymer.

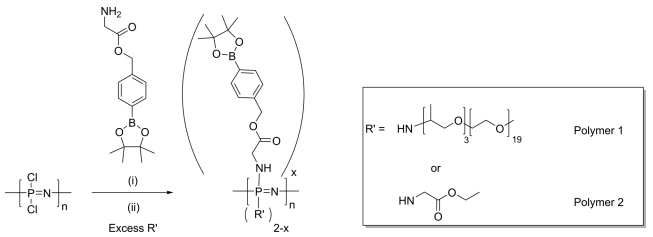
Scheme 1. Proposed Concept for Caged Polyphosphazenes with Triggered Degradation[a]



[a]Upon triggered decaging, the hydrolytically sensitive poly(glycine)-phosphazene is produced, which undergoes a rapid self-catalyzed degradation to phosphates and ammonia.

Herein the hydrolytically sensitive $[NP(NHCH_2COOH)(R')]_n$ polymer was prepared with arylboronate pincacol ester as a self-immolative caging group (Scheme 2). The phenyl-

Scheme 2. Synthesis of Polymer 1 and Polymer 2 with Arylboronic Acid Pinacol Ester as a Caging Group[a]



[a]Reagents and conditions: (i) glycinate arylboronic acid pinacol ester, THF, rt, 16 h; (ii) excess of R′ (Jeffamine M1000 for polymer **1** and glycine ethyl ester for polymer **2**), THF, rt, 16 h.

boronic acid ester is known to oxidize to the corresponding phenol in biologically relevant concentrations of the reactive oxygen species (ROS) H_2O_2.[19] ROS are important in cell signaling, but the imbalance of oxidative and reducing species causes oxidative stress that contributes to several diseases, such as cancer,[20] cardiovascular disorders,[21] and Alzheimer's disease.[22] The self-immolative motif was first prepared from the reaction of 4-(hydroxymethyl)benzene boronic acid pinacol ester with Boc-gly-OH (Figure S1), followed by selective deprotection of the amine in CF_3CO_2H (Figure S2). The precursor $[NPCl_2]_n$ was prepared separately via a recently developed phosphine-mediated, living cationic polymerization[23] of $Cl_3PNSiMe_3$.[24] Glycinate arylboronic acid pinacol ester was then added to partially substitute the backbone. In a second step, an excess of a second amine substituent was added to completely substitute the phosphorus atoms in the backbone. The phosphorus main chain has a quite unique pentavalent nature, and thus, the second substituent can be used to modulate the chemical and physical properties of the resulting polymer.[12a] For this work, a Jeffamine M1000 (amino-functionalized polyalkylene oxide) was chosen as the secondary substituent, to give the water-soluble polymer **1**. Jeffamine substituents are known to augment the water solubility, biodegradability, and biocompatibility of the polymers.[17d] The resulting polymer was purified by dialysis and shown by ^1H and ^{31}P NMR experiments (Figure S3) to have a complete backbone substitution (within the NMR detection range, absence of peaks associated with non and partially substituted phosphorus atoms in the ^{31}P NMR spectrum) in a ratio of approximately 50:50, glycinate arylboronic acid pinacol ester to Jeffamine substituents (≈25 wt % boronic acid ester according to UV−vis spectroscopy, Figure S4). Thus, on average, each phosphorus atom bears one boron-containing cleavable unit. The polymer was further characterized by GPC in DMF containing 10 mM LiBr (Figure S5, $M_{n,GPC}$ = 68500 g mol^{-1}, M_w/M_n = 1.5, measured against linear polystyrene standards) and DLS (Figure S6, d = 11.23 ± 0.44 nm in H_2O).

To investigate the sensitivity of the polymer toward oxidative environments, the polymer was subjected to 10 mM aqueous solution of H_2O_2 at room temperature. Analysis by size exclusion chromatography (SEC) showed that polymer **1** degrades selectively in the oxidative environments (Figure 1a), with a decrease in the polymer peak clearly visible. This was accompanied by an increase in the low molecular weight region below approximately 1000 g mol^{-1} due to the ejection of the Jeffamine oligomers from the hybrid polymer and the formation of low molecular weight compounds. Selective backbone degradation of the polymer was further confirmed by ^{31}P NMR spectroscopy (Figure 2a) in which a reduction in the broad polymer peak is observed accompanied by the appearance of peaks associated with hydroxyphosphazene (≈−10 ppm) and a sharp peak due to phosphate formation (≈0 ppm). Both species are known degradation products for the hydrolysis of the polyphosphazene main-chain.[17b,d,18,25] Meanwhile, the polymer could be stored in aqueous solution, that is, in the absence of H_2O_2, at room temperature for the same time frame (Figure 1b and Figure 2b) and, indeed, for several weeks thereafter, before any visible signs of degradation could be detected by ^{31}P NMR spectroscopy (Figure S7), thus, confirming the selectivity of the degradation toward the oxidative environment.

After this, successful proof-of-principle, a further series of polymers were prepared with glycine ethyl ester cosubstituents. Non-water-soluble, poly(amino acid ester)phosphazenes belong to the most important polyphosphazenes for biomedical applications.[11b] Furthermore, the comparatively low molar mass of the organic substituent facilitates mechanistic studies of the backbone cleavage mechanism by ^1H NMR spectroscopy, for which many relevant peaks are obscured in polymer **1**. Polymer **2** (Figure 3a) was hence prepared with approximately 50 mol % of glycinate arylboronic acid pinacol ester substituent and 50 mol % glycine ethyl ester, as calculated by ^1H NMR spectroscopy (Figure S8) and ≈60 wt % by UV−vis (Figure S4) spectroscopy. Upon exposure to 10 mM acetone solution of H_2O_2, ^1H NMR studies of a sample of polymer **2** (23 mg mL^{-1}) revealed the oxidation of the pinacol ester to be fast,

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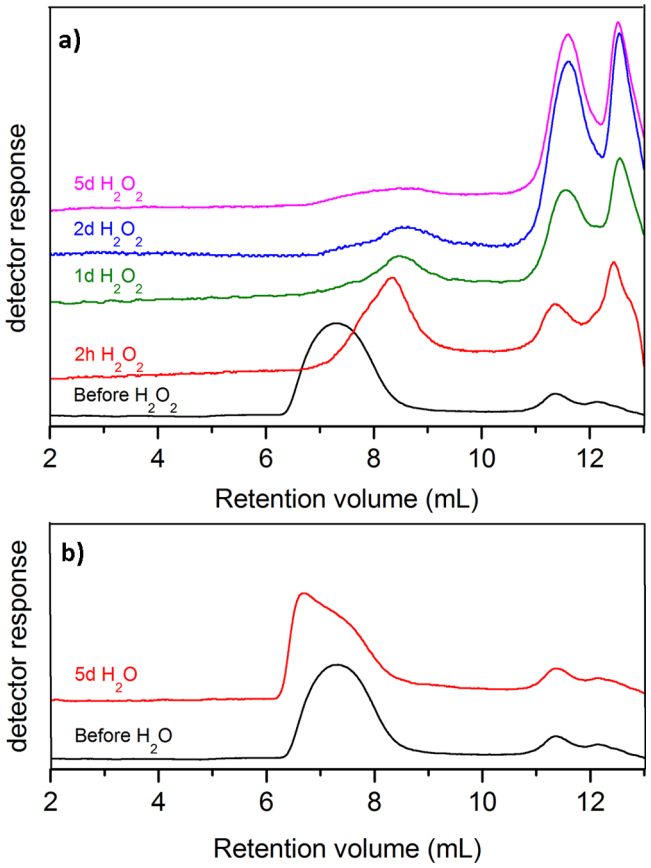

Figure 1. SEC analysis of polymer 1 stored in aqueous solution at room temperature (a) in the presence of 10 mM H_2O_2 and (b) in the absence of H_2O_2.

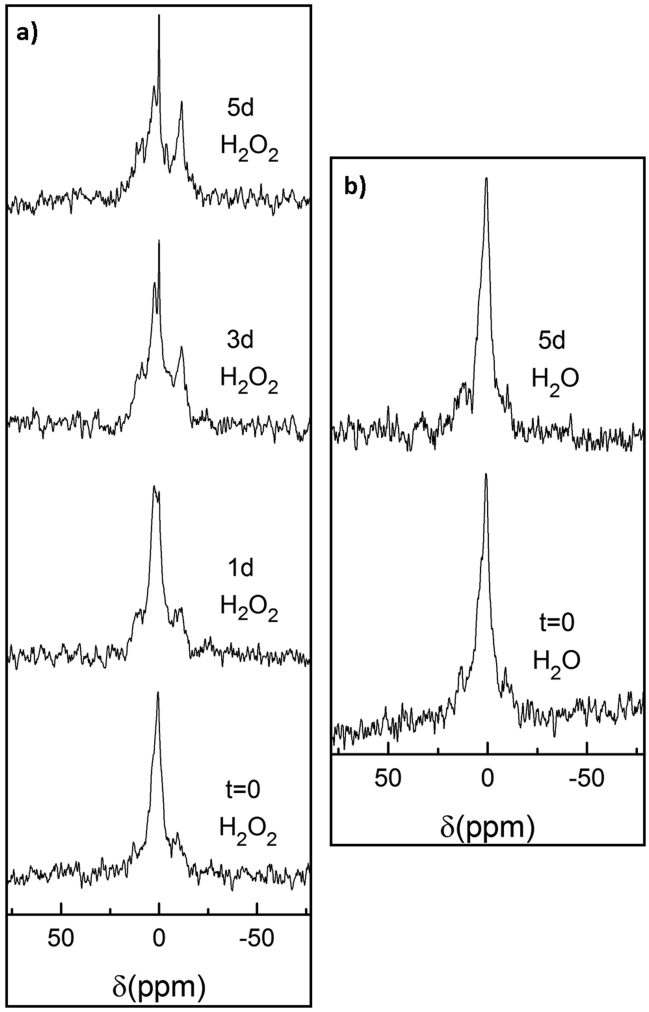

Figure 2. (a) ^{31}P NMR spectroscopy of polymer **1** in D_2O in the presence of 10 mM H_2O_2 and (b) in the absence of H_2O_2.

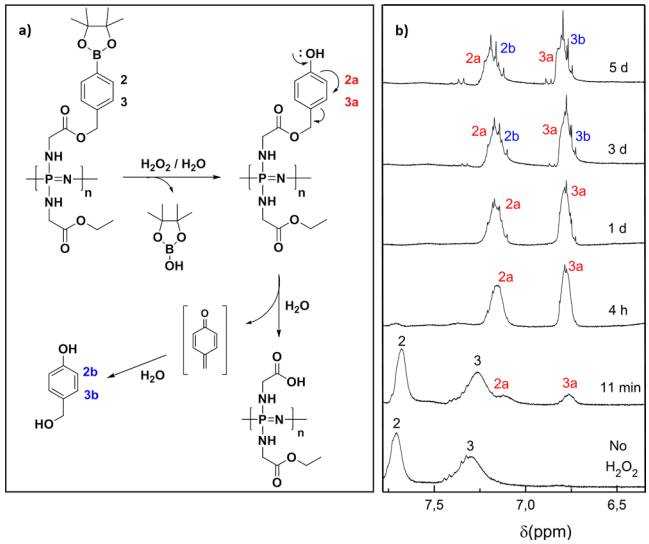

Figure 3. (a) Proposed self-immolation mechanism of polymer **2** upon H_2O_2 exposure and (b) ^1H NMR tracking of the self-immolation pathway of polymer **2** in 10 mM acetone solution of H_2O_2. Entire ^1H NMR spectra are shown in Figure S10.

with complete formation of the phenol (still bound to the polymer) in 4 h (for this particular polymer and under these conditions, see Figures 3b and S9). However, the self-immolation proved to be the rate limiting step, with the phenol intermediate remaining stable for some days. Once self-immolation has started, sharp resonance peaks begin to appear, which correspond to low molecular weight degradation products. These observations correlate with the ^{31}P NMR studies of the same sample (Figure 4b), in which no degradation is observed until a time frame in which self-immolation has occurred. Thereafter, polymer **2** showed a rapid degradation, as indicated by the presence of peaks associated with the primary chain degradation products, hydroxphosphazenes and phosphazane, as well as phosphates due to its self-catalyzed degradation (Figure 4a), in accordance with previous studies into the degradation mechanism of polyaminophosphazenes.[17b,18,25] Over a longer time period, this was seen to degrade fully to phosphates (Figure S11a). The kinetics were observed to be slower than for the water-soluble polymer **1**, which can be explained by the use of an organic solvent for this non-water-soluble polymer.[26] The nature of the degradation products could further be characterized by ESI-MS, with the detection of glycine ethyl ester and 4-hydroxybenzyl alcohol (Figure S12).

In the absence of H_2O_2, no main-chain degradation was observed in the ^{31}P NMR spectrum over same period of time (Figure 4c), with a slow hydrolytic degradation occurring thereafter (Figure S11b). ^1H NMR studies meanwhile showed a partial hydrolysis of the boronic acid ester to cleave the pinacol

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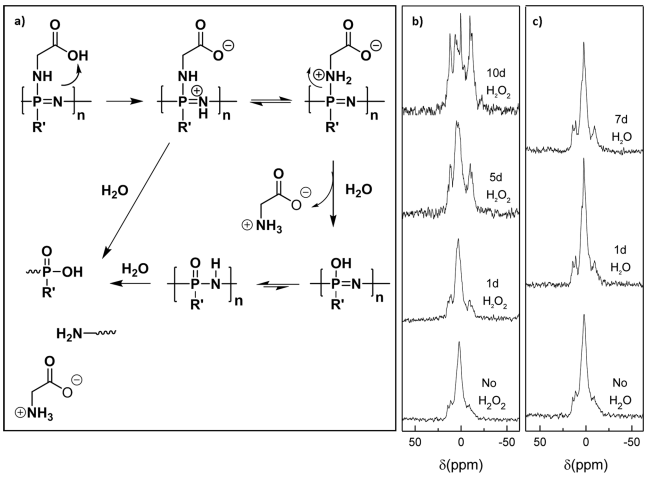

Figure 4. (a) Probable backbone chain-cleavage mechanisms of the hydrolytically sensitive glycine-substituted polyphosphazene; ^{31}P NMR spectroscopy of polymer **2** (b) in 10 mM acetone solution of H_2O_2 and (c) in acetone/water solution without H_2O_2.

(Figure S13).[27] In order to exclude the presence of an oxidative reduction pathway for poly(amino acid ester)phosphazenes, polymer **3** (poly(glycine ethyl ester)phosphazene, Figure S14), with no boronic acid ester caging groups, was also exposed to oxidative conditions. Studies of the sample by ^{31}P NMR spectroscopy (Figure S15) indicated the stability of poly(glycine ethyl ester)phosphazene even to a higher concentration of H_2O_2 (100 mM), thus, confirming that any H_2O_2 triggered degradation effect is exclusively due to the presence of the self-immolative boronate ester moiety.

In summary, a new type of polymer based on a polyphosphazene with phenylboronate moieties along the main chain has been prepared. While such unique boron-containing polymers may have many interesting properties,[28] the linkage of the boronate group via a self-immolative motif allowed the preparation of polymers stable in ambient conditions but with a stimulus-responsive degradation pathway in oxidative environments. The second substituent on the phosphorus atom was used to introduce water solubilizing groups and amino acid ester substituents to the hybrid polymers. Self-immolation of the boronate upon exposure to H_2O_2 exposed the hydrolytically sensitive glycine-substituted phosphazene main chain which subsequently underwent a rapid hydrolytic degradation to small molecules. Although the mutually exclusive responsive nature of the degradation in the different environments was clearly shown, degradation rates (hours/days) were slower than may be desired for some applications. Proton NMR studies showed that, while boron oxidation and phosphazene main chain degradation are both rapid, the rate-limiting step is the self-immolation of the phenol to present the free acid. Thus, future generations of these polymers will look to vary the type of cage and backbone linkage and further enhance the degradation kinetics in the presence of stimuli, without affecting the inherent stability of the polymer. The approach of using the hydrolytically instable inorganic backbone as a platform significantly expands the toolbox of selective degradable polymers, as the post-polymerization functionalization allows the preparation of polymer backbones with a broad range of molecular weights and advanced architectures, before insertion of the self-immolative moieties. It is thus envisaged that these novel polymers could be used as the basis of a range of new responsive materials. In particular, the unique multivalency of the phosphorus could be used for the addition of functional moieties to prepare, for example, sensory materials, while the known biocompatibility of the degradation products also makes them potentially useful polymers for the preparation of biomedical materials, for example, in diagnostics or therapeutics.

■ ASSOCIATED CONTENT

Supporting Information

The Supporting Information is available free of charge on the ACS Publications website at DOI: 10.1021/acsmacrolett.7b00015.

Experimental section, additional data for polymer characterization, and full NMR spectra (PDF).

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Author Contributions

The manuscript was written through contributions of all authors. All authors have given approval to the final version of the manuscript.

Notes

The authors declare no competing financial interest.

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