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Water-Soluble Poly(phosphonate)s via Living Ring-Opening Polymerization

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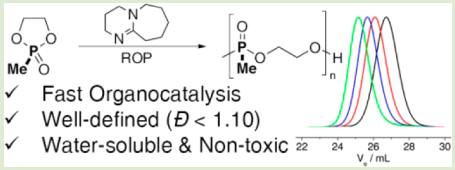
Supporting Information

ABSTRACT: A small difference brings high control: In poly(phosphonate)s a stable carbon−phosphorus linkage attaches a side chain to a degradable poly(phosphoester)-backbone. A novel cyclic phosphonate monomer was developed to generate water-soluble aliphatic poly(ethylene methylphosphonate)s. The monomer is accessible via a robust three-step protocol that can be easily scaled-up. Polymerization was initiated by a primary alcohol, mediated by 1,8-diazabicyclo[5.4.0]undec-7-ene (DBU) in less than 2 h at 0 °C. The molecular weight distributions were monomodal and very narrow (below 1.1) in all cases and molecular weights up to about 20000 g/mol have been prepared, proving the living nature of this polymerization. The resulting polymers were characterized in detail via NMR spectroscopy, size exclusion chromatography, and differential scanning calorimetry. Also, the reaction kinetics have been evaluated for several monomer/initiator ratios and found to guarantee a living behavior in all cases superior to other poly(phosphate)s reported earlier. The polymers are all highly water-soluble without a lower critical solution temperature and are nontoxic against HeLa cells.



Poly(phosphoester)s (PPEs) are one of the most versatile class of materials due to their modular synthesis and broad range of possible applications.[1] The most prominent PPE is deoxyribose nucleic acid (DNA), the storage of biological information, and the basis for life. In the lab solid phase oligonucleotide synthesis or polymerase chain reaction are normally used for the synthesis of DNA segments.[2] Other synthetic PPEs are biodegradable polymers with the phosphoesters forming the backbone and are prepared via various polymerization techniques. PPEs have gained increasing interest in polymer science since the pioneering works of Penczek,[3−6] Wang,[1,7,8] Leong,[9] Iwasaki,[10,11] and others. Several features render these phosphorus-based polyesters to highly promising materials for future applications: one first major difference to carboxylic acid polyesters is the versatility that the pentavalent phosphorus offers in designing a wide range of polymers altering the main and the side chains. This allows attaching labels, functional or solubilizing groups and further is another handle on degradability, a very important feature of polyesters. During the pioneering works initiated by Penczek and co-workers in the 1970s, various synthetic routes have been investigated to synthesize polyphosphates and polyphosphites by polycondensation, polyaddition, and ring-opening polymerization (ROP).

A very interesting subclass are poly(phosphonate)s, which are more or less forgotten in the academic world in spite of some highly promising flame-retarding properties of ill-defined oligomeric poly(phosphonate)s, that are used commercially. In poly(phosphonate)s, two phosphoesters build up the main-chain polyester, while the side chain is based on directly linked alkyl or aryl groups (polyesters of alkyl- or aryl-phosphonic acid, Figure 1); this makes them usually more stable than the corresponding polyphosphates with three ester groups.[12]

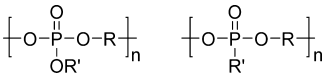


Figure 1. Structural representation of poly(phosphate)s (left) and poly(phosphonate)s (right).

As poly(phosphonate)s can be synthesized only by classical polycondensation routes to date,[13−21] research interest has faded because of the relatively expensive synthesis and the low molecular weights that were accessible via the traditional synthetic pathways. Especially aliphatic poly(phosphonate)s may find application besides flame-retardant materials in the biomedical field, since they are potentially biodegradable and biocompatible. Further, the lack of the hydrolyzable side chain could be beneficial, as no polyanion can be generated during the degradation process (note: the side chains of poly(phosphate)s are usually hydrolyzed first).

Typical poly(phosphonate)s are hydrophobic, however, increasing the hydrophilicity by short alkyl spacers in the main chain or the introduction of oligoethylene glycol units in the backbone was only possible by polycondensation

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techniques to date with broad molecular weight distributions ($Đ \geq 2.0$), which is unfavorable for biomedical applications. Administrative regulations require well-defined structures to be used in patients; therefore, classical polycondensation cannot be employed to synthesize hydrophilic poly(phosphonate)s.

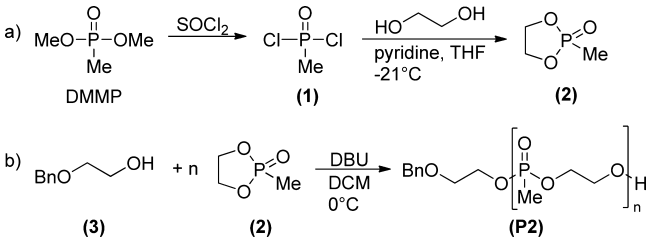
Novel strategies to synthesize PPEs in a controlled manner have been investigated in recent years. Polyphosphates were synthesized by olefin metathesis via acyclic diene metathesis (ADMET) polymerization and ring-opening metathesis polymerization (ROMP), respectively.[22−24] However, the anionic ring opening of cyclic phosphates, so-called phospholanes, has found the most attention so far since ultrafast organic catalytic systems have been developed by Iwasaki and were explored in some recent elegant works by Wooley and co-workers.[11,25−27] In spite of the high control at the beginning of the polymerization, conversion is limited to about 50% as transesterification reactions become dominant at higher conversions and broaden the molecular weight distribution distinctively. This results in unreacted and generally non-recoverable monomer loss. Only for sterically demanding[11] or functional alkoxy residues,[25] conversions as high as 80−90% are reported, yielding polymers with low molecular dispersities. Similarly, very narrow molecular weight distributions for polyphosphoramidates are only obtained for conversions less than 68%.[27]

Herein we report the first living ROP of a cyclic phosphonate, that is, 2-methyl-1,3,2-dioxaphospholane 2-oxide (MeEP, **2**) as a robust protocol for the synthesis of highly water-soluble PPEs. For the first time, high molecular weight poly(phosphonate)s are accessible with narrow molecular weight distributions via a chain growth mechanism. Up to very high conversions (above 90%), no pronounced transesterification was observed, yielding hydrolytically degradable polymers with promising applications in the biomedical field and materials science.

ROP of five-membered dioxaphospholane oxides (i.e., cylic phosphates) was established by Penczek and co-workers in the 1970s and can be conducted via several catalytic systems to produce rather well-defined polyphosphates.[28−31] A great benefit of this route is the fast access to water-soluble polyphosphates.[32,33] However, this protocol was not used for the synthesis of well-defined polyphosphonates to date. As early as 1957, Korshak et al. reported the preparation of low molecular weight oligomers from alkyl-dioxaphospholane oxides at high temperature.[34] Different catalysts (water, hydrochloric, and acetic acid) were used to facilitate the polymerization, but the degree of polymerization was always very low (typically between 3 and 4).

In contrast to most reported five-membered cyclic phosphate monomers, which are synthesized in one simple step from the commercially available 2-chloro-2-oxo-1,3,2-dioxaphospholane by condensation with an alcohol of choice, the corresponding phosphonate monomers need to be synthesized from scratch. As a first proof of principle, we have chosen to synthesize 2-methyl-1,3,2-dioxaphospholane 2-oxide (MeEP, **2**). Methyl dichloro phosphonate (**1**) is used as precursor which is converted into the 5-membered cyclic phosphonate via esterification with ethylene glycol. The monomer (**2**) can be purified by distillation and was stable at −28 °C for several months (Scheme 1a). Many phosphonates are known to inhibit acetylcholinesterase irreversibly, arising toxicity issues during the synthesis of **2**. Following the guidelines published by Schrader,[35] **2** is not expected to be a biologically active phosphonate because of the lack of a substituent like fluoride, cyanide, rhodanide, or enolate. Furthermore, the vapor pressure of **2** and its polymers is negligible, so that intoxication by inhalation is unlikely. Nevertheless, care must be taken when working with compounds **1** and **2**.

Scheme 1. (a) Synthesis of Monomer 2 by Condensation of Ethylene Glycol with 1; (b) Polymerization of 2, Initiated by 2-(Benzyloxy)ethanol (3), and Catalyzed by DBU at 0 °C in Dichloromethane



Purity of the monomer was verified by ^{1}H and ^{13}C NMR spectroscopy (Figures 2a and S1). ^{1}H NMR gives two groups of signals corresponding to the diastereotopic protons of the ethylene bridge and a doublet at 1.66 ppm corresponding to the methyl group connected to the phosphorus with a reasonable ^{2}J coupling of 17.5 Hz. A single resonance in ^{31}P NMR at 48.8

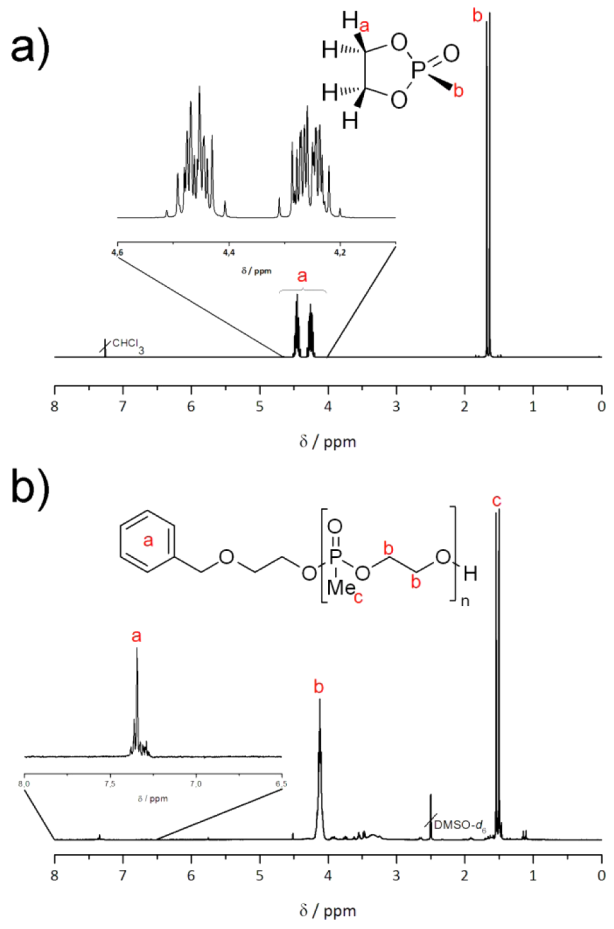


Figure 2. (a) ^{1}H NMR spectrum of **2** in $CDCl_3$ at 400 MHz. Region of diastereotopic protons of the ethylene bridge is magnified for clarity. (b) ^{1}H NMR spectrum of poly(**2**) in DMSO-d_6 at 400 MHz. Region of aromatic protons for end group analysis is magnified for clarity. All spectra were measured at 298 K.

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ppm (Figure S2) further suggests the successful formation of a strained phosphonate ring.

2-Methyl-1,3,2-dioxaphospholane-2-oxide (**2**) was investigated with respect to its polymerization behavior in an organo-catalyzed anionic ROP initiated by 2-(benzyloxy)-ethanol and catalyzed by DBU at 0 °C (Scheme 1b). DBU has been employed in the synthesis of polyesters and polyamides due to its capability of activating a suitable initiator and the propagating species leading to a controlled polymerization.[36] However, for polyphosphates with small side chains, for example, methoxy or ethoxy residues, this system suffers from relatively broad molecular weight distributions for conversions above 50% due to pronounced transesterifications with the pendant and the main-chain esters at longer reaction times and decreasing monomer supply. The reaction time (and therefore the probability for transesterification reactions) can be reduced by the addition of an organic Lewis acid thiourea.[37] This additive was found to be able to activate the phosphate monomer by increasing the electrophilicity of the phosphorus center and facilitating the attack of the nucleophile. With this approach well-defined polyphosphates ($Đ$ < 1.15) were synthesized and reported by different groups and studied intensively by Lecomte and co-workers recently.[38] The major disadvantages of this method are the use of high quantities of *co*-catalyst (usually 5 mol %) and the difficulties arising during the removal of both catalysts from the polymer.

Monomer **2**, however, is effectively polymerized with DBU as single catalyst up to very high conversions (above 90%) without pronounced transesterification. In contrast to polyphosphates with four possible pathways for transesterification,[38] poly(phosphonate)s can undergo only two transesterification reactions (Scheme S1). Two major side reactions leading to broad molecular weight distributions can be excluded because the side chain in linear poly(phosphonate)s cannot be exchanged by transesterification reactions (as it is observed for polyphosphates). From the ^{1}H NMR spectra of the polymers, fast determination of the number average of the molecular weight is possible by end-group analysis. It was found that the calculated molecular weight is very close to the theoretical molecular weight, underlining the high control of the polymerization (Table S1). Polymers between 1500 and 17200 g·mol^{-1} were obtained. All polymers exhibited a very narrow monomodal molecular weight distribution which remained narrow even at high conversion (Figure 3). After polymerization of **2**, a clear shift in the ^{31}P NMR spectra

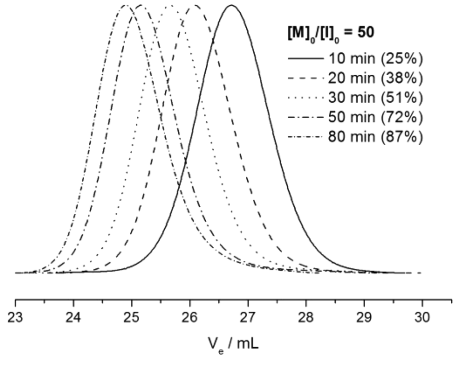

Figure 3. SEC elugrams of linear poly(**2**)s prepared by anionic ring-opening polymerization in dichloromethane using DBU as catalyst for $[M]_0/[I]_0$ = 50 (for 100 and 200, see Supporting Information).

resonances to about 32 ppm was observed indicating complete ring-opening of the monomer (Figure S4). No complex microstructure of the final polymer was expected as no chiral groups are incorporated. Still, high resolution ^{31}P NMR spectroscopy revealed a second signal at 31.5 ppm besides the broad backbone signal which was assigned by ^{1}H-^{31}P HMBC NMR spectra (Figure S5). A cross relaxation between the signal at 31.5 ppm in ^{31}P NMR and a multiplet at 3.97–3.88 ppm in ^{1}H NMR indicates that this signal corresponds to the terminal unit of the polymer chain, as this phosphonate group is expected to be in a different chemical environment than the phosphonate groups in the backbone. In the same manner, the terminal methylene group is shifted to higher field and can be detected as a triplet at 3.62 ppm. Similar observations were already been reported by our group recently concerning the microstructure of poly(phosphate)s synthesized from a racemic monomer.[33] Moreover, it can be excluded that this cross relaxation originates from the first monomer unit ring-opened by the initiator, since 2-(benzyloxy) ethanol was chosen to initiate the polymerization. This initiator has the advantage that the initiating species is chemically equivalent to the propagating species and also carries multiple protons for end group analysis. This ensures that the initiator has a similar reactivity toward the nucleophilic phosphorus in **2** compared to the resulting growing chain end. A falsification of the polymerization kinetics by a more (or less) reactive initiator can therefore be excluded.

To prove the high control over the molecular weight distribution, the polymerization kinetics was studied. Figure 4 shows the plot of $\ln([M]_0/[M])$ versus time and the number

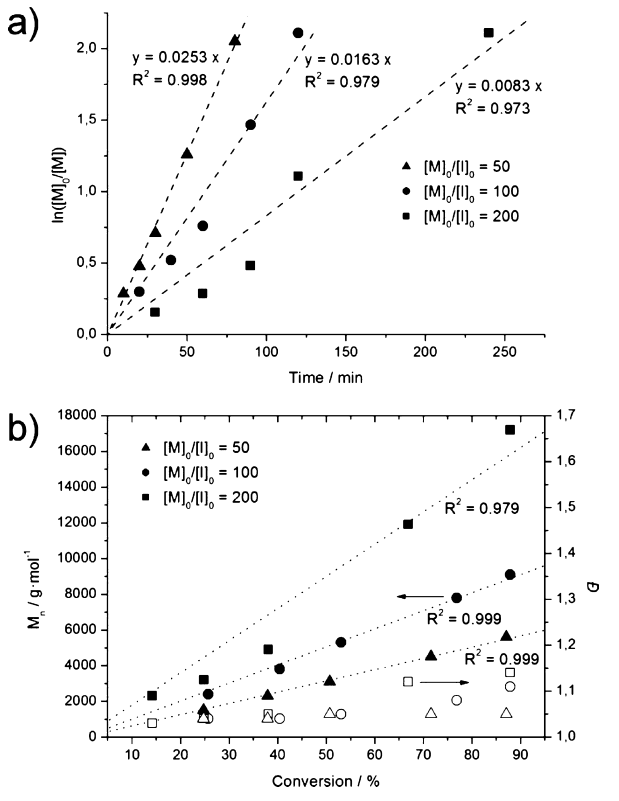

Figure 4. Kinetic studies: Plots of (a) $\ln([M]_0/[M])$ vs time and (b) M_n and $Đ$ vs conversion for the ring-opening polymerization of **2** in DCM at 0 °C using DBU as catalyst and 2-(benzyloxy)ethanol as initiator.

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average molecular weight (M_n) versus conversion. A linear increase of the $\ln([M]_0/[M])$ for $[M]_0/[I]_0$ feed ratios up to 200 was found. This indicates that the concentration of growing chains remains constant throughout the polymerization. M_n increases linearly up to conversions above 90%. This observation suggests that the number of polymer chains remains also constant during the reaction. The molecular weight dispersity ($Đ$) was found to be below 1.10 for conversions up to 87% ($[M]_0/[I]_0 = 50$), which is a very low $Đ$ value reported for PPEs with such high monomer conversion. This high control over the molecular weight and its distribution, even for high conversions, makes the phosphonate monomer **2** superior over all previously reported phosphate monomers,[11] as the polymerization is usually terminated after 50% conversion to achieve a narrow distribution. Monomer **2** can thus be polymerized in a highly controlled manner using a alcohol/DBU system which provides high molecular weight linear poly(ethylene methylphosphonate)s with very low molecular weight dispersities in less than two hours.

Poly(ethylene methylphosphonate)s synthesized from **2** are amorphous materials exhibiting a glass transition temperature of −35 to −40 °C independent of the molecular weight (Table S1), as determined by differential scanning calorimetry and is exemplary shown for poly(**2**)-**n** in Figure S9. While poly(phosphate)s with short side chains typically show a reduced water-solubility at elevated temperatures (LCST), all polymers prepared from **2** in this study are highly water-soluble (above 10 mg·mL^{-1} in Milli-Q water) and do not show any LCST behavior (10 mg·mL^{-1} in PBS 0.1 M pH 7.4) up to temperatures of 90 °C. The degradation of poly(**2**) was investigated employing aqueous SEC. The degradation proceeds faster for basic pH values (pH 9.0) whereas no change of the elution volume was observed for a slightly acidic (pH 5.0) aqueous environment within several days (Figures S10,11). Further degradation studies in biological media are currently under investigation. This makes this novel class of water-soluble polyesters suitable for many biomedical applications, where excellent water-solubility and very well-defined structures are necessary to meet government regulations. Due to the structural similarity to other nonionic PPEs, which have been reported to be nontoxic,[33] the herein prepared poly(**2**) were expected to be also nontoxic. Since the degradation products are relatively harmless compounds and prevent any polymer accumulation in the body this is a highly important property. The cytotoxicity of poly(phosphonate)s was investigated in vitro against a human cervical cancer cell line (HeLa) in a concentration range of 1−1000 μg·mL^{-1} by measuring the metabolic activity as ATP content of viable cells in relation to untreated cells. The results are displayed in Figure 5 and prove a very good biocompatibility for the novel polymers. No adverse effects on the viability were observed, indicating good biocompatibility comparable to other PPEs (for details please see the Experimental Section).

In summary, the first chain-growth polymerization for the synthesis of poly(phosphonate)s is presented. Fast access to water-soluble poly(ethylene methylphosphonate)s via the first metal-free organocatalytic living anionic ROP of a five-membered cyclic phosphonate was established. Due to the stable methyl phosphonate side chain, transesterifications are limited and only observed for very high conversions (>90%) and poly(phosphonate)s with high molecular weights and narrow molecular weight distributions ($Đ$ < 1.10) were obtained. The living nature of this polymerization was proven by detailed kinetic studies with different monomer to initiator ratios. The metal-free nature of this technique makes these highly water-soluble (no LCST) and potentially biodegradable poly(phosphonate)s very attractive candidates for biomedical applications. Therefore, initial studies on the biocompatibility were carried out by treating HeLa cells with various concentrations of the novel linear poly(phosphonate)s and excellent cell viability was observed.

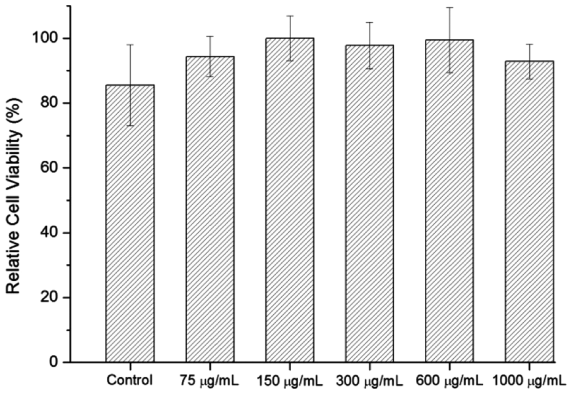

Figure 5. In vitro cell viability of HeLa cells treated with poly(**2**)-**e** after 48 h of incubation. The experiments were carried out as six independent replicates and repeated twice.

■ ASSOCIATED CONTENT

Ⓢ Supporting Information

Detailed experimental procedures as well as analytical and spectral characterization data. This material is available free of charge via the Internet at http://pubs.acs.org.

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Notes

The authors declare no competing financial interest.

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