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Degradable, Dendritic Polyols on a Branched Polyphosphazene Backbone

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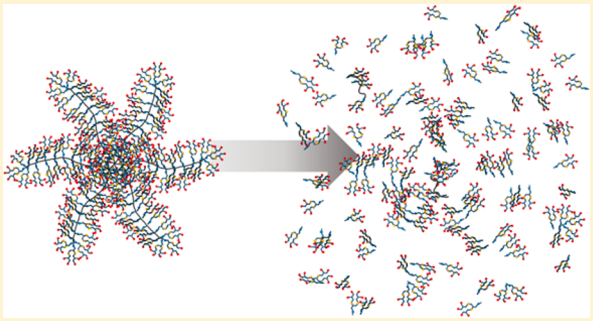
Supporting Information

ABSTRACT: Herein, we present the design, synthesis, and characterization of fully degradable, hybrid, star-branched dendritic polyols. First multiarmed polyphosphazenes were prepared as a star-branched scaffold which upon functionalization produced globular branched hydroxyl-functionalized polymers with over 1700 peripheral functional end groups. These polyols with unique branched architectures could be prepared with controlled molecular weights and relatively narrow dispersities. Furthermore, the polymers are shown to undergo hydrolytic degradation to low molecular weight degradation products, the rate of which could be controlled through postpolymerization functionalization of the phosphazene backbone.



1. INTRODUCTION

Multifunctional polyols are of academic and commercial importance for a wide range of applications ranging from adhesives and coatings[1] and as polyurethane cross-linkers[2] to biosensors and drug delivery systems.[3] Polyols are available in numerous advanced architectures, including dendritic and hyperbranched polymers[1b] and dendronized polymers,[4] and can be used to prepare supramolecular structures via self-assembly[1b] and as nanogels.[5] The branched architecture, globular structure, and peripheral functional groups of such architectures can be exploited to tailor the properties of these polyols, enhancing their versatility.[6] Among these highly branched polymers, dendritic polyesters are especially well researched, for example, bis-MPA (2,2-bismethylolpropionic acid)-based dendritic polymers[1b] and polyether-based systems.[7] Another widely investigated family are polyglycidol-based[8] polymers, including hyperbranched (hPG)[6b] or dendritic polyglycerol (dPG).[6a,9] Further interesting, but less widely investigated, branched functional polymers include hybrid inorganic phosphorus-based dendrimers[10] and hyperbranched polyphosphates.[11]

Due to their biocompatibility and multifunctionality, water-soluble polyols are of significant interest for biomedical applications, for example, drug delivery[8] where the high multivalency allows the covalent attachment of a variety of active molecules such as imaging agents,[12] drugs,[9] and/or targeting moieties in a controlled ratio.[13] In this field, as in many others,[14] it remains an important challenge to develop polymers that are fully (bio)degradable (that is undergoing chain cleavage to small molecules) while combining the multifunctionality, water solubility, and controlled structures which are much sought after in such applications.[15] Hence, there is considerable interest in biodegradable polyols, and although polyether based systems are nondegradable,[16] degradable groups like ester bonds,[6c] acetals,[17] or ketals[18] have been incorporated into the polyglycerol structure leading to (semi)degradable systems. Aliphatic polyester-based systems are meanwhile susceptible to both acid/base and enzymatic degradation at given rates.[1a] The next generation of dendritic polyols should have controllable and predictable degradation pathways which can be tuned to the required application.

It has been well reported that poly(organo)phosphazenes can be prepared with various degradation rates[19] due to the hydrolytic instability of the phosphorus-based backbone[20] and that degradation rates can be easily tuned by choice of organic substituents.[21] Furthermore, triggered and controlled degradation pathways are available.[22] Since recent advances in polyphosphazene synthesis[23] also allow highly branched architectures,[19,24] including star-shaped structures with branched polyphosphazene-based arms,[25] we proposed to prepare globular, highly branched, hybrid polyols with the known backbone degradability of the polyphosphazene scaffold. The synthesis and characterization of which are presented herein.

2. EXPERIMENTAL SECTION

Materials and Methods. Polymer synthesis was carried out under argon using a glovebox (MBRAUN). The synthesis of the N-(trimethylsilyl)-trichlorophosphoranimine was carried

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out according to literature procedures.[26] Triethylamine was distilled and dried over molecular sieves prior to use. 3-(Diphenylphosphino)-1-propylamine was purchased from abcr, and 1,1,1-tris(diphenylphosphino)methane, hexachloroethane, and D_2O are purchased from Sigma-Aldrich, CD_2Cl_2 from Fluorochem, solvents and DMSO-d_6 from VWR-Chemicals and used as received. Photochemical reactions were carried out in glass vials with septum caps in a Rayonet chamber reactor with a UV lamp from Camag at 254 nm.

^1H NMR and ^{13}C NMR spectroscopy measurements were recorded on a Bruker Ultra Shield 300 device at 300 and 75 MHz, respectively. $^{31}P\{^1H\}$ NMR spectra were recorded in a decoupled mode on the same spectrometer at 121 MHz, using 85% phosphoric acid as an external standard. An Agilent Technologies mass spectrometer LC/MSD TrapSL (Agilent Technologies, Vienna, Austria) equipped with an electrospray ionization (ESI) interface operated in positive mode, and the samples injected from 1% formic acid were used for mass spectrometry. Size exclusion chromatography (SEC) was measured with a Viscothek GPCmax instrument (Malvern Instruments, Malvern, UK) equipped with a D1000 (300 mm × 8 mm, 6 μm particle size) and D4000 (300 mm × 8 mm, 7 μm particle size) column from Malvern (Malvern Istruments, Malvern, UK). The samples were eluted with DMF containing 10 mM LiBr at a flow rate of 0.75 mL/min at 60 °C. The molecular weights were estimated with a calibration method against linear polystyrene standards. ATR-FTIR spectra were measured on a PerkinElmer Spectrum 100 FTIR spectrometer (Waltham, Massachusetts, USA). A Zetasizer Nano ZSP (Malvern Instruments, Malvern, UK) was used for dynamic light scattering (DLS) measurements. The measurements were carried out in buffer solutions (0.7 mg/mL), and all samples were filtered through a 0.2 μm PTFE filter and measured in a disposable polystyrene microcuvette at 25 °C and a backscatter angle of 173°. UV−vis spectra were recorded on a PerkinElmer Lambda 25 UV/vis spectrophotometer (Waltham, Massachusetts, USA).

Syntheses. *Three-Arm Poly(propargylamino)-phosphazene (Polymer **1**).* 1,1,1-Tris(diphenylphosphino)-methane (100 mg, 0.16 mmol) and the chlorinating agent C_2Cl_6 (3.3 eq, 125 mg) were dissolved in anhydrous CH_2Cl_2 (0.5 mL) and stirred overnight. To this solution, the monomer N-(trimethylsilyl)-trichlorophosphoranimine (50 eq, 1.8 g, 8 mmol) was added and stirred overnight. Propargylamine (1.1 g, 20 mmol) in excess was dissolved in anhydrous THF containing Et_3N (2.8 mL, 2g, 20 mmol) as a base and added to the three-arm poly(dichloro)phosphazene precursor solution. The reaction solution was stirred at room temperature overnight. After filtration and removal of the solvent under vacuum, the polymer was purified by precipitation in diethyl ether and cyclohexane from THF, resulting in a yellow solid.

Yield: 601 mg (44%). ^1H NMR (300 MHz, CD_2Cl_2, δ[ppm]): 2.34 (br, 1H, −NH), 3.81 (br, 3H, CH_2−C≡CH), 7.47−7.97 (br, 0.7H, −PPh_2) ppm; $^{31}P\{^1H\}$ −NMR (121.4 MHz, CD_2Cl2, δ[ppm]): 0.76 ((−P=N−)$_n$), 10.03−14.76 (−PPh2−). ^{13}C NMR (75.432 MHz, CD_2Cl_2, δ[ppm]): 31.33 (CH_2−C≡CH), 71.42 (CH_2−C≡CH), 84.20 (CH_2−C≡CH). FTIR (solid): ν_{max} = 3170 (C≡C−H), 3080 (C−H), 2850 (C−H), 2121 (C≡C), 1184 (P = N).

Hexa-initiator. 3-(Diphenylphosphino)-1-propylamine (554.2 mg, 2.278 mmol) was dissolved in 3 mL anhydrous THF containing Et_3N (0.3 mL, 2.278 mmol), added to hexachlorocyclotriphosphazene (120 mg, 0.345 mmol), and left overnight at room temperature. After filtration, the solvent was concentrated and the polymer precipitated three times in heptane followed by washing the precipitate three times with 2-propanol. The precipitate was dried under vacuum to yield the product as a colorless solid.

Yield: 356 mg (65%). ^1H NMR (300 MHz, CD_2Cl_2, δ[ppm]): 1.43 (br, 2H), 2.00 (br, 2H), 2.78 (br, 2H), 7.29 (br, 10H) ppm. $^{31}P\{^1H\}$ −NMR (121.4 MHz, CD_2Cl_2, δ[ppm]): −17.44 (−PPh_2), 13.23 (−P = N−).

2-Amino-N-(prop-2-yn-1-yl)acetamide. Propargylamine (1 eq, 1 g, 18.16 mmol) was added to a suspension of BOC-Gly-OH (1.1 eq, 3.5 g, 19.97 mmol) in dry CH_2Cl_2 containing N-methylmorpholine (1.1 eq, 2.0 g, 19.97 mmol), HOBt (1.1 eq, 2.7 g, 19.97 mmol), and EDC·HCl (1.1 eq, 3.8 g, 19.97 mol). The resulting solution was stirred at room temperature overnight. Ethyl acetate (40 mL) was added to the reaction mixture, and the resulting suspension was washed twice with 5% HCl (30 mL), H_2O (30 mL), 5% Na_2CO_3 (30 mL), and brine (30 mL). The organic layer was separated and dried with $MgSO_4$, and the solvent removed under vacuum.

Yield: 2.224 g (58%). ^1H NMR (300 MHz, $CDCl_3$, δ[ppm]): 1.47 (s, 9H, CH_3), 2.25 (t, 1H,−C≡CH), 3.82 (d, 2H, −CH_2−NH_2), 4.08 (dd, 2H, −CH_2−C≡CH), 5.14 (s, 1H, −NH), 6.44 (s, 1H, −NH).

For Boc deprotection, the product was dissolved in a 2:1 mixture of CH_2Cl_2:CF_3COOH and stirred for 3 h. The solvent was removed under vacuum, and the product precipitated from CH_2Cl_2 with diethyl ether to yield a white solid.

Yield: 1.024 g (87%). ^1H NMR (300 MHz, D_2O, δ[ppm]): 2.61 (t, 1H, −C≡CH), 3.79 (s, 2H, −CH_2−NH_2), 4.0 (d, 2H, −CH_2−C≡CH).

Six-Arm Poly(dichloro)phosphazene. The chlorination agent C_2Cl_6 (23.6 mg, 0.1 mmol) was dissolved in anhydrous CH_2Cl_2 (3 mL) and added to a solution of the hexa-initiator (24 mg, 0.05 mmol) and stirred for 1 h. To this solution, the monomer N-(trimethylsilyl)-trichlorophosphoranimine (1 g, 4.5 mmol) was added and stirred overnight to obtain the poly(dichloro)phosphazene six-arm star.

$^{31}P\{^1H\}$ NMR (121 MHz, CD_2Cl_2, δ[ppm]): −18.03 (−P=N−), 13.08 ((−P=N−)$_3$), 20.14 (−PPh_2).

*Six-Arm Polymers **3** and **5**.* An excess of propargylamine (613.6 mg, 11.14 mmol) was dissolved in 5 mL anhydrous THF containing Et_3N (1.13 g, 11.14 mmol) and dropped slowly into a cold poly(dichloro)phosphazene solution and stirred overnight at room temperature. After filtration and concentration of the solution under vacuum, the polymer was precipitated two times in cyclohexane and dried under vacuum to yield a yellow sticky solid. For the preparation of polymer **5**, 2-amino-N-(prop-2-yn-1-yl)acetamide (1.25 g, 11.14 mmol) was used instead of propargylamine as substituent.

*Polymer **3**.* Yield: 464 mg (57%). ^1H NMR (300 MHz, CD_2Cl_2, δ[ppm]): 2.36 (br, 1H, −CH), 3.83 (br, 3H, −CH_2-C≡CH, −NH), 7.2−8.2 (br, 0.11H, −PPh_2) ppm. $^{31}P\{^1H\}$ NMR (121.4 MHz, CD_2Cl_2, δ[ppm]): 0.6 (−P=N−), 10−14 ((−P=N−)$_3$), 17.7 (−PPh2−). ^{13}C NMR (75.432 MHz, CD_2Cl_2, δ[ppm]): 31.36 (CH_2−C≡CH), 71.57 (CH_2−C≡CH), 83.95 (CH_2−C≡CH). FTIR (solid): ν_{max} = 3286 (C≡C−H), 2918 (C−H), 2854 (C−H), 2115 (C≡C), 1182 (P=N).

*Polymer **5**.* Yield: 898 mg (69%). ^1H NMR (300 MHz, DMSO-d_6, δ[ppm]): 3.04 (br, 1H, CH_2-C≡CH), 3.35 3.55 (br, 2H, CH_2), 3.89 (br, 2H, CH_2) 7.4 (br, −PPh_2), 8.3 (br, −PPh_2) ppm. $^{31}P\{^1H\}$ NMR (121.4 MHz, CD_2Cl2, δ[ppm]):

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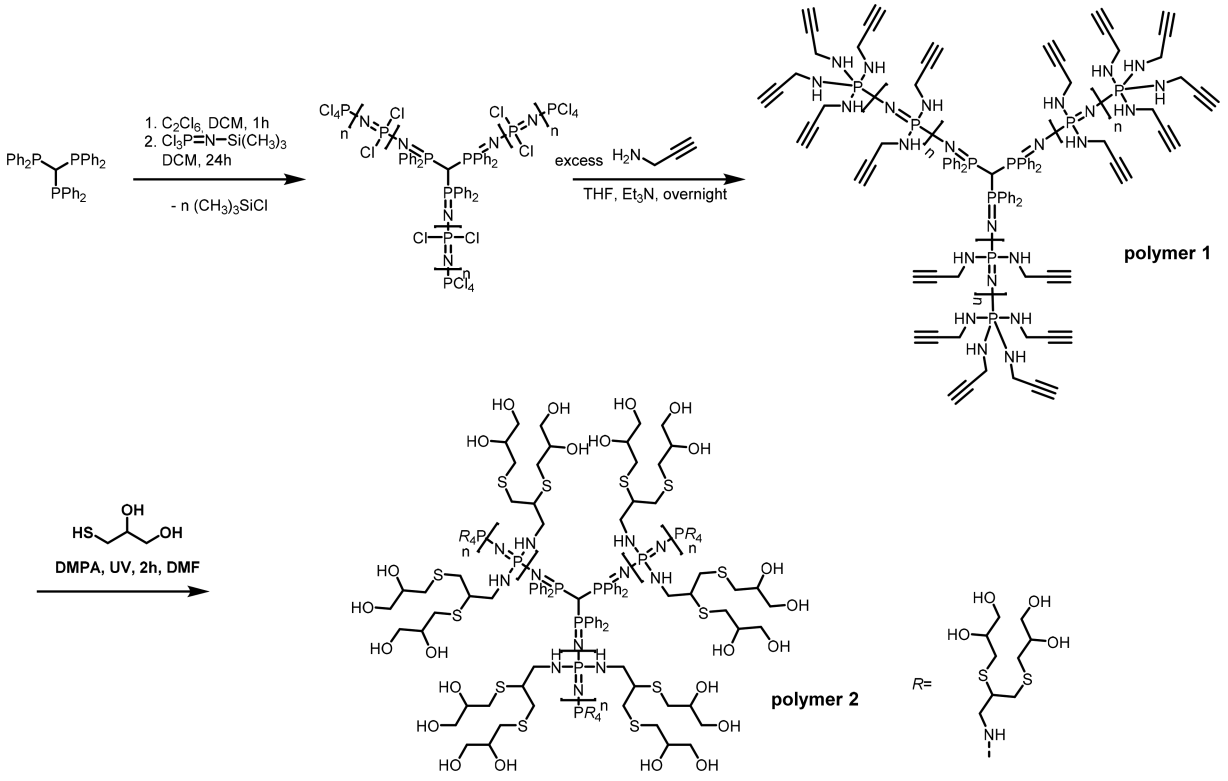
Scheme 1. Synthesis of Poly[di(propargylamino)phosphazene] Tri-Arm Stars (Polymer 1) followed by Postpolymerization Functionalization with 1-Thioglycerol To Give Dendritic Polyols



2.7 (−P=N−) 11.00 ((−P=N−)$_3$), 15.68 (−PPh2−). FTIR (solid): ν_{max} = 3232 (C≡), 2979 (C−H), 2947 (C−H), 2120 (C≡C), 1652 (−CO−NH−), 1131 (P=N).

Reaction of Poly[bis(propargylamino)phosphazene] with 1-Thioglycerol. In the following, the procedure used for the synthesis of polymer **4** is described. Polymers **2** and **6** were synthesized from the precursor polymers **1** and **5**, respectively.

In a glass vial, the six-arm poly[bis(propargylamino)-phosphazene] (60 mg, 2.1 μmol), 1-thioglycerol (8 eq per alkyne group, 640 mg, 512 μL, 5.9 mmol), and 15.2 mg 2,2-dimethoxy-2-phenylacetophenone (DMPA, 0.06 mmol) were dissolved in 2 mL of DMF and degassed with argon for 20 min to remove oxygen. Afterward, the mixture was exposed to UV light for 2 h. The resulting polymer was purified by dialysis against water (3.5 kDa cutoff) for 2 days and lyophilized to yield a slightly yellow solid.

Polymer 2. Yield: 184 mg (approximately 90%). ^{1}H NMR (300 MHz, D_2O, δ[ppm]): 2.66 and 2.74 (br, 4H, −S−C$\underline{H}_2$−), 2.93−3.30 (br, 2H, −NH−C$\underline{H}_2$−), 3.54 and 3.58 (br, 4H, OH−C$\underline{H}_2$−), 3.81 (br, 2H, OH−C$\underline{H}$−). ^{31}P{^{1}H} NMR (121.4 MHz, D_2O, δ[ppm]): 3.59 (br, −P=N−), 12.2 (br, (−P=N−)$_3$). ^{13}C NMR (75.432 MHz, D_2O, δ[ppm]): 34.2 (−S−$\underline{C}H_2$−), 36.1 (−S−$\underline{C}H_2$−), 48.2 (−$\underline{C}H_2$−NH−), 65.2 (OH−$\underline{C}H_2$), 72.0 (, OH−$\underline{C}$H−). FTIR (solid): ν_{max} = 3295 (O−H), 2915 (C−H), 2868 (C−H), 1637 (O−H), 1179 (P=N).

Polymer 4. Yield: 70 mg (approximately 30%). ^{1}H NMR (300 MHz, D_2O, δ[ppm]): 2.69 and 2.75 (br, 4H, −S−C$\underline{H}_2$−), 2.93 and 3.07 (br, 2H, −NH−C$\underline{H}_2$−), 3.55 and 3.63 (br, 4H, OH−C$\underline{H}_2$−), 3.83 (br, 2H, OH−C$\underline{H}$−). ^{31}P{^{1}H} NMR (121.4 MHz, D_2O, δ[ppm]): 2.9 (br, −P=N−), 12.1 (br, (−P=N−)$_3$). ^{13}C NMR (75.432 MHz, D_2O, δ[ppm]): 34.1 (−S−$\underline{C}H_2$−), 36.2 (−S−$\underline{C}H_2$−), 44.4 (−$\underline{C}H_2$−NH−), 47.8 (−S−$\underline{C}H_2$−), 65.2 (OH−$\underline{C}H_2$), 71.9 (OH−$\underline{C}$H−). FTIR (solid): ν_{max} = 3276 (O−H), 2918 (C−H), 2872 (C−H), 1666 (O−H), 1165 (P=N).

Polymer 6. Yield: 152 mg (approximately 50%). ^{1}H NMR (300 MHz, D_2O, δ[ppm]): 2.5 and 3.27 (br, 6H, −S−C$\underline{H}_2$−, −NH−C$\underline{H}_2$−), 3.58 and 3.64 (br, 4H, OH−C$\underline{H}_2$−), 3.85 (br, 2H, OH−C$\underline{H}$−). ^{31}P{^{1}H} NMR (121.4 MHz, D_2O, δ[ppm]): 3.3 (br, −P=N−), 12.2 (br, (−P=N−)$_3$). ^{13}C NMR (75.432 MHz, D_2O, δ[ppm]): 34.1 (−S−$\underline{C}H_2$−), 36.2 (−S−$\underline{C}H_2$−), 44.3 (−$\underline{C}H_2$−NH−), 48.2 (−S−$\underline{C}H_2$−), 65.2 (OH−$\underline{C}H_2$), 71.9 (OH−$\underline{C}$H−). FTIR (solid): ν_{max} = 3280 (O−H), 2924 (C−H), 2827 (C−H), 1690 (HN−CO−), 1652 (O−H), 1121 (P=N).

Degradation Studies. For ^{1}H NMR and ^{31}P{^{1}H} NMR degradation studies, 10 mg of each polymer was incubated in acidified D_2O using HCl (pH 2, enhanced degradation conditions) and incubated at 37 °C. The changes of the proton and phosphorus signals were monitored in regular time intervals. Between each measurement, the samples were stored at 37 °C.

The degradation rate of the presented polymers was studied by inorganic phosphate determination monitored by UV−vis spectroscopy. The polymers were incubated in TRIS buffer (pH 7.4), sodium acetate buffer (pH 5), or acidified H_2O (pH 2, enhanced degradation conditions) in a concentration of 0.7 mg mL^{-1} at 37 °C during the time of analysis. Aliquots of the degradation medium (0.2 mL) were taken in regular time intervals and mixed with a reagent solution (0.5 mL) consisting of ammonium molybdate, ascorbic acid, sulfuric acid, and potassium antimonyl tartrate.[27] UV−vis analyses at 885 nm of the mixtures were performed after 15 min of incubation time. The concentration of phosphate was calculated from a calibration curve using potassium dihydrogen phosphate.

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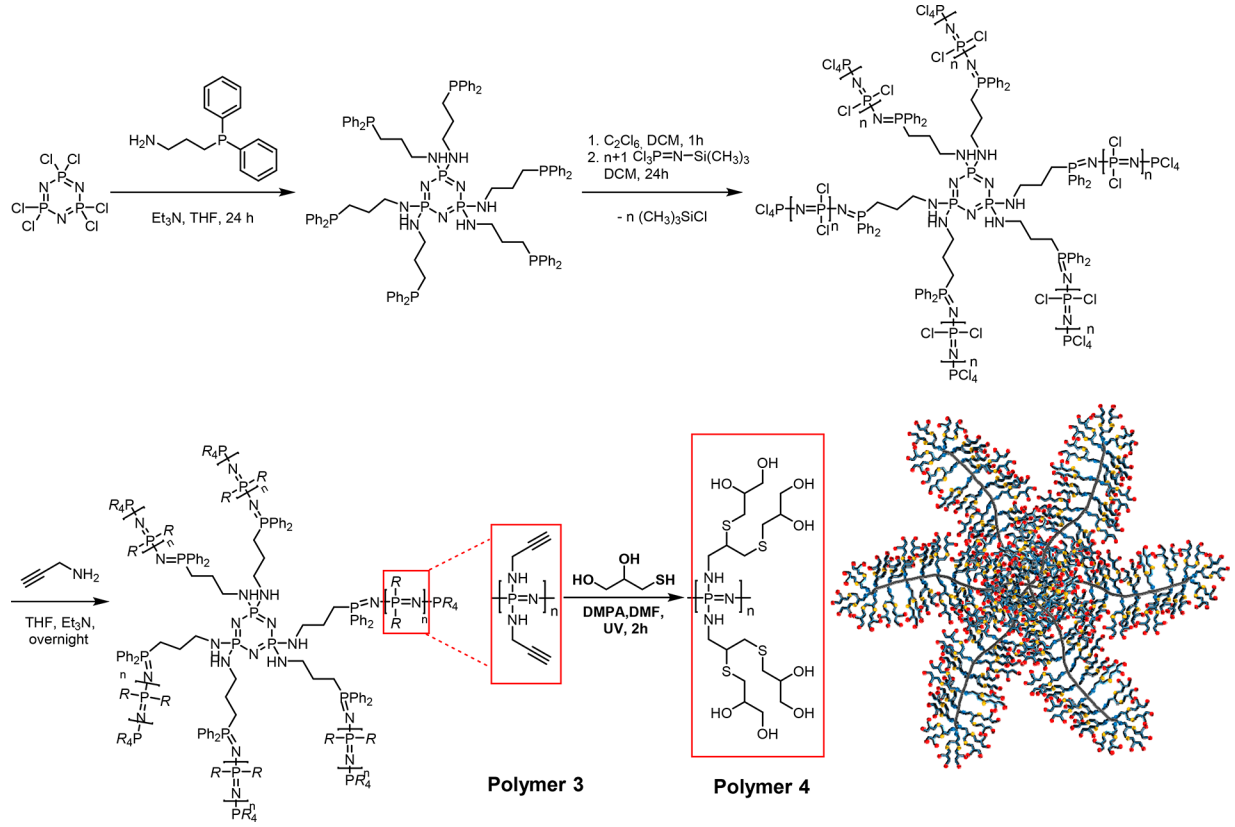
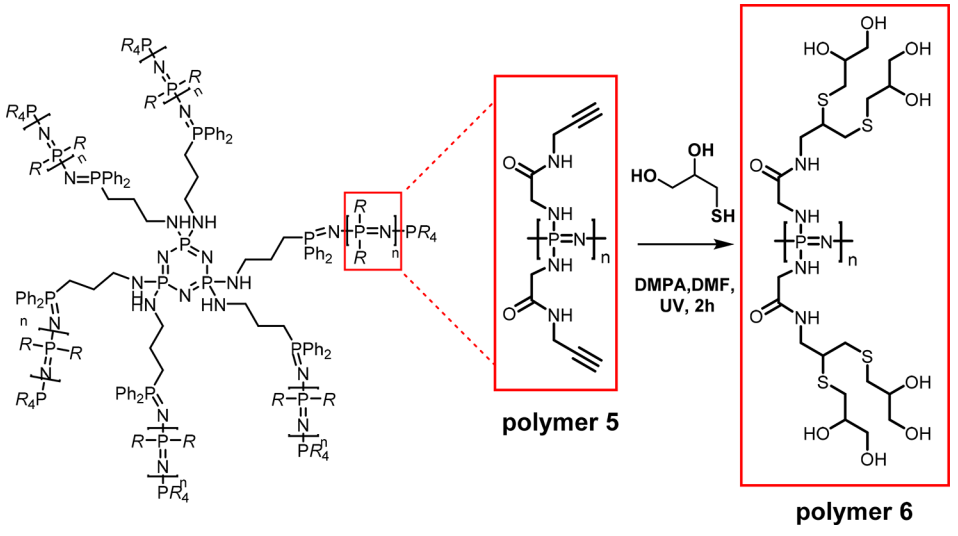
Scheme 2. Synthesis of Six-Arm Polymer via a Hexachlorocyclotriphosphazene Core Substituted with 3-(Diphenylphosphino)-1-propylamine as Starting Material (hexa-initiator)[a]



[a]Chlorination and polymerization produces a six-arm $[NPCl_2]_n$. Postpolymerization substitution with propargylamine (polymer **3**) is followed by addition of 1-thioglycerol via thiol–yne addition chemistry (polymer **4**).

Scheme 3. Chemical Structure of Polymer 6 Incorporating a Glycine-Based Linkage between Thioglycerol Moieties and Phosphazene Backbone



After complete degradation, the sample solutions stored at pH 2 were further investigated by ESI-MS.

3. RESULTS AND DISCUSSION

Syntheses. First, a poly(dichloro)phosphazene $[NPCl_2]_n$ tri-arm star was synthesized via the recently developed core first, room temperature, living cationic polymerization of trichlorophosphoranimine ($Cl_3PNSi(CH_3)_3$ starting from 1,1,1-tris(diphenylphosphino)methane chlorinated with C_2Cl_6[24b,28] (Scheme 1). A postpolymerization substitution reaction was conducted with an excess of propargylamine to give the alkyne-substituted star polymer **1** with on average seven repeat units per arm (calculated by ^1H NMR end group analysis). The addition of 1-thioglycerol via photochemical

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Table 1. Selected Polymer Characterization

polymer	number of arms	est. number of repeat units per arm[a] (M:I ratio)	M_n, g mol^{-1} (SEC) kDa[c]	$Đ$ (SEC)[c]	D_h, nm (Z-Ave.)	D_h, nm (volume)
2	3	7 (16)	27.37	1.17	18.2 ± 0.19	6.6 ± 0.33
4	6	36 (50)	35.87	1.30	15.8 ± 0.05	9.7 ± 0.54
6	6	–[b] (50)	39.22	1.27	36.7 ± 0.67	9.9 ± 0.69

[a]Calculated from ^{1}H NMR measurements of the corresponding poly[di(propargylamino)]polyphosphazene. [b]For this polymer, end group calculation is not possible due to overlapping signals. [c]SEC analysis in DMF (+10 mM LiBr) with conventional calibration using linear polystyrene standards. $Đ$ refers to dispersity (M_w/M_n).

thiol–yne addition led to a star-shaped hydroxyl-polyphosphazene (polymer **2**). The thiol–yne addition method provides a fast and convenient alternative for the insertion of thio-glyceryl moieties onto a $[NPCl_2]_n$ backbone,[29] which otherwise would require extensive protecting group strategies.[30] The substitution of two groups per alkyne moiety further multiplies the functional groups to eight per phosphazene repeat unit.

A six-arm variant was also prepared (Scheme 2), thus further increasing the functionality and branching. First, hexachlorocyclotriphosphazene $[NPCl_2]_3$ was substituted with (diphenylphosphino)-1-propylamine to give a central core with six initiating species upon chlorination. Polymerization with $Cl_3PNSi(CH_3)_3$ gave a six-arm star $[NPCl_2]_n$ which was converted to the propargyl-functionalized polymer **3** and subsequently polymer **4** after reaction with 1-thioglycerol.

Furthermore, as is known from previous work,[31,21] the local environment of the backbone phosphorus is critical in determining the hydrolytic stability of the resulting poly(organo)phosphazenes. For this reason, 2-amino-N-(prop-2-yn-1-yl)acetamide was prepared and coupled to the $[NPCl_2]_n$ six-arm star to give polymer **5** and consequently polymer **6** after reaction with 1-thioglycerol (Scheme 3).

Characterization. ^{31}P NMR, ^{1}H NMR, ^{13}C NMR, and FTIR spectroscopy DLS and SEC measurements were used to confirm successful preparation of the presented polymers (summarized in Table 1). The synthesis route could be followed by ^{31}P NMR spectroscopy, as shown for polymer **4** (Figure 1). Starting from the hexa-initiator, a sharp signal at −17.4 corresponding to the PPh_2 group and the signal of cyclotriphosphazene at 13.2 ppm are observed. After polymerization, a signal at 18.0 ppm is observed corresponding to the backbone phosphorus in $[NPCl_2]_n$. The small signal at 13.1 ppm corresponds to the cyclotriphosphazene core, and a signal from the resulting PPh_2R groups can be found at 20.1 ppm. After substitution with propargylamine (polymer **3**), a signal at around 0 ppm appears, corresponding to the poly[di(propargylamino)phosphazene] backbone phosphorus, as well as small signals at 10–14 and 17.7 ppm corresponding to the phosphine-functionalized cyclotriphosphazene core. No peaks were resolved from P–Cl units, suggesting quantitative substitution of $[NPCl_2]_n$ to $[NP(NHR)_2]_n$.[32] ^{1}H and ^{13}C NMR spectroscopy were also used to confirm the structure of the propargylamine substituted polymers **1**, **3**, and **5**. The average number of phosphazene repeat units per arm was estimated from the ^{1}H NMR measurement integrating the phenyl protons (7.47–7.97 ppm) versus the CH-proton of the propargylamine moiety 2.34 ppm (shown for polymer **1** in Figure S1).

Postpolymerization functionalization with 1-thioglycerol leads, as expected, to a broadening of the signal at around 0 ppm due to the increased bulkiness of the substituents (Figure 1d). Quantitative conversion of the alkyne groups of the functionalized polymers during the photoinitiated reaction with

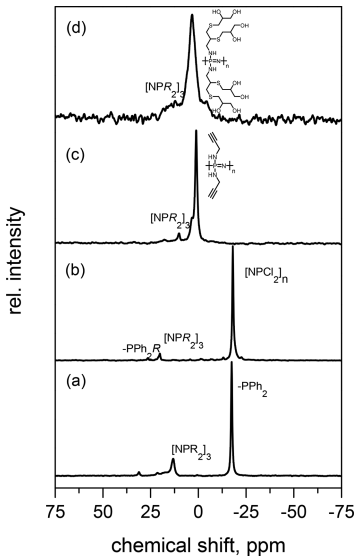

Figure 1. ^{31}P NMR study in D_2O of the synthesis of six-arm poly(dichloro)phosphazene using hexachlorocyclotriphosphazene substituted with 3-(diphenylphosphino)-1-propylamine as hexa-initiator: (a) Hexa-initiator. (b) Polymerization of trichlorophosphoranimine. (c) Propargylamine-substituted polymer **3**. (d) ^{31}P NMR spectrum after thiol–yne addition of 1-thioglycerol (polymer **4**).

1-thioglycerol was confirmed via the disappearance of the associated peaks in the ^{1}H and ^{13}C NMR spectra (shown for polymer **4** Figure S2) and FTIR spectroscopy (Figure S3). The unique combination of star-branched polymerization, quantitative phosphorus functionalization, and multiplying "dendritic effect" of the thiol–yne addition gives an extraordinary multivalency for these macromolecules. For example, polymer **2** with three arms of just n ≈ 7 repeat units has approximately 180 hydroxyl groups per macromolecule, whereas polymer **4** with six arms of 36 repeat units and eight hydroxyl groups per repeat unit is calculated as having >1700 OH groups.

The hydrodynamic volumes of the star-shaped polymer series were investigated by dynamic light scattering (DLS) measurements to determine the hydrodynamic diameter in aqueous solutions: Figure 2a shows the size distribution volume of the water-soluble polymers **2**, **4**, and **6** in acetate buffer. For the intensity distribution of the presented polymers, a bimodal or relatively broad distribution was obtained, hinting at some aggregation of the polymers (Figure S4); however, the volume and number distributions (Figure 2 and Figure S4, respectively) suggest the aggregates are minor species. Size exclusion chromatography showed M_n 25–40 kg mol^{-1} (Figure 2b) with a relatively narrow $Đ$ < 1.3 suggesting a uniform growth of the polymer arms.

Degradation Studies. The hydrolytic degradation of the presented branched polymers was also investigated. Polyphosphazenes are known to degrade to phosphates,[21] and

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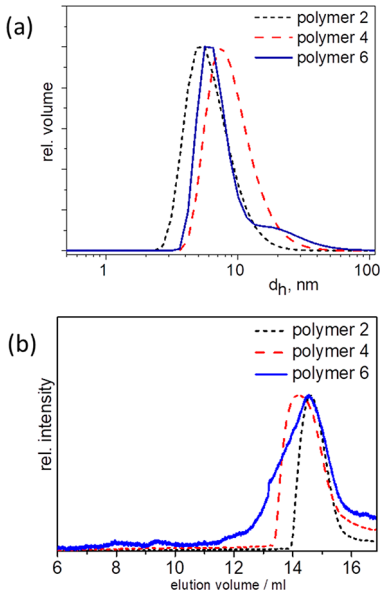

Figure 2. Molecular size distribution by volume (a) as detected by dynamic light scattering for polymers **2**, **4**, and **6** in acetate buffer at pH 5 (polymer concentration 0.7 mg/mL, d_h = hydrodynamic diameter) and (b) SEC elugram for polymers **2**, **4**, and **6** in DMF containing 10 mM LiBr.

hence, the generation of phosphate from the polymers was investigated at 37 °C over a period of 40 days via a photometric molybdate assay[33] (Figure 3). The polymers were tested under

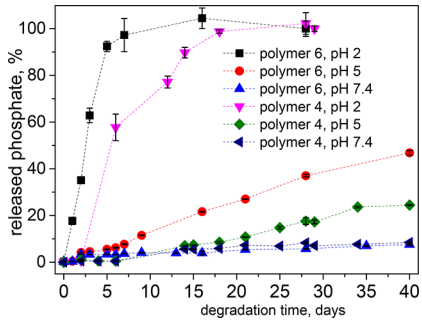

Figure 3. Phosphate determination by a molybdate assay of polymers **4** and **6** quantitatively determined by UV−vis analysis in aqueous conditions at pH 2, 5, and 7.4 at 37 °C. Similar trends are observed for polymer **2** (shown in Figure S5).

enhanced degradation conditions in water at pH 2, pseudolysosomal conditions at pH 5, and physiological conditions at pH 7.4. As expected from previous studies with polyphosphazenes, the rate of degradation was observed to be significantly faster at lower pH values.[20a,33,34] Acid degradable polymers are of particular interest for biomedical applications, for example, in endosomal−lysosomal targeting.[35,36] Indeed, all presented polymers show a full degradation of the polyphosphazene backbone to inorganic phosphate within 18 days at pH 2. Slower degradation rates were observed for all polymers at pH 5 and 7.4. Polymer **6** incorporating glycine moieties showed significantly faster phosphate generation than the other polymers (Figure 3) with full degradation within 5 days at pH 2. The ability to tune degradation rates is an important property. Different applications require different degradation rates, which is a necessity for the very definition of degradable polymers as those which degrade within the expected service life or shortly thereafter.[37] For intravenous drug delivery, for example, where retention times are in the range of several hours, polymer degradation rates should allow clearance shortly thereafter.

The anticipated degradation mechanism of the poly(organo)phosphazenes involves statistical hydrolytic cleavage of the organic substituents, followed by backbone degradation, leading eventually to phosphates and ammonium salts, which are reported to be biologically benign.[21,22b,31,34] We next sought to investigate the nature (size and chemistry) of the degradation products, which is important information for their application as degradable polymers. To this end, the breakdown of the polymer was followed by ^{1}H NMR and ^{31}P NMR spectroscopy under accelerated degradation conditions (pH 2) to monitor the degradation products. The ^{31}P NMR series shown in Figure 4a shows a sharp signal at around 0 ppm growing within 7 days, which is associated with the formation of inorganic phosphate. Moreover, two further signals appeared associated with triphosphate (Figure 4a).[38] In addition, a clear peak sharpening in the ^{1}H NMR spectra (Figure 4b) appeared within 1 week indicating the cleavage of the organic substituents and the (3-aminopropyl)diphenylphosphine oxide released from the core. Furthermore, three signals at around 7 ppm, corresponding to ammonium chloride, were observed to increase as degradation progressed. Moreover, ESI-MS was carried out to further confirm character of the degradation products (Figure 4c). These studies are exemplarily shown for the glycine modified six-arm-star (polymer **6**) with similar results being obtained for polymers **2** and **4** (Figures S6 and S7). The masses of the expected organic substituents cleaved from the backbone and core were found in the ESI-MS spectra. In the case of polymer **6**, the organic components with and without glycine could be detected, indicating partial cleavage of the amide bond, as well as the P−N linkage.

4. CONCLUSION

A series of globular branched hydroxyl-phosphazenes are reported. The water-soluble polymers were synthesized by a phosphine-mediated polyphosphazene synthesis route, starting from multifunctional initiators with three and six initiating sites. Quantitative postpolymerization functionalization with propargyl amine was followed by photochemically initiated thiol−yne addition to give eight hydroxyl functional groups per repeat unit. Successful synthesis was confirmed by SEC, FTIR-spectroscopy, DLS, and ^{31}P NMR, ^{1}H NMR, and ^{13}C NMR spectroscopy. The presented polymers show degradation to small molecules with sizes below 350 g mol^{-1} and pH-dependent degradation rates with significantly higher degradation rates being observed at low pH. Moreover, the degradation rates could be tuned by incorporation of a glycine moiety between the polyphosphazene backbone and the organic substituent. These unique branched architectures could be prepared with controlled molecular weights and relatively narrow dispersities. They represent highly functionalized polyols, with >1700 moieties per macromolecule, which are degradable due to the hydrolytically instable inorganic main chain. Hence, these materials exhibit significant potential for the replacement of branched polyols in a wide range of applications where tunable degradation rates are desired.

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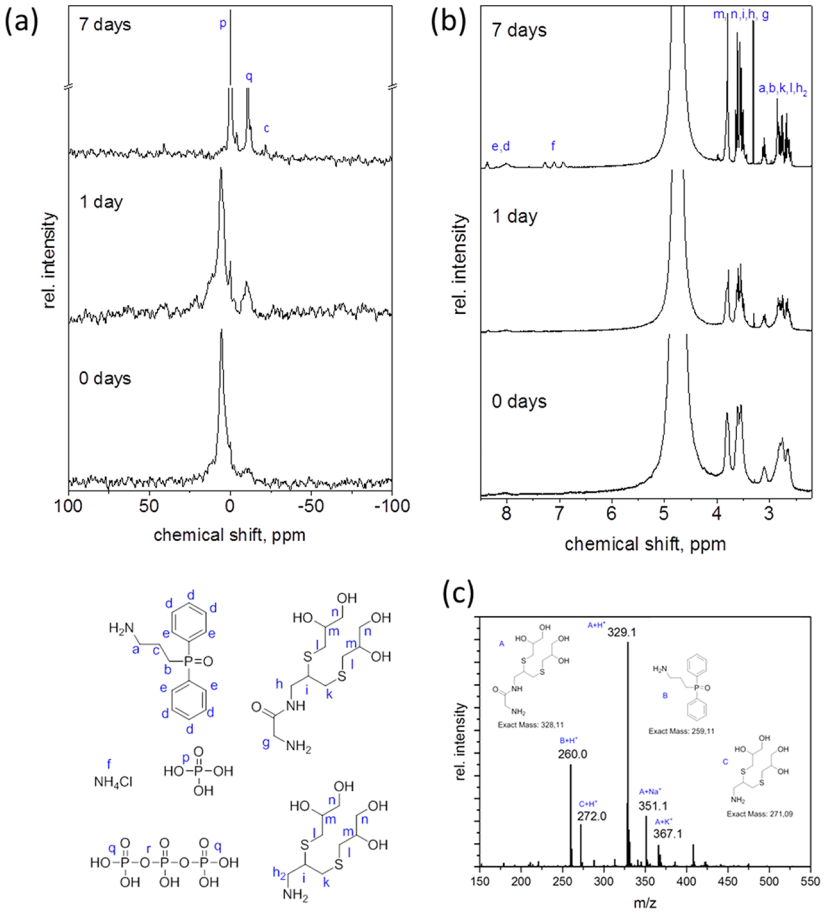

Figure 4. Degradation of polymer **6** followed with ^{31}P NMR spectra indicating the formation of phosphates (a), ^{1}H NMR spectra confirming the cleavage of the substituents and the formation of NH_4Cl (b), and ESI-MS of the degraded polymer showing the degradation products (c).

■ ASSOCIATED CONTENT

Supporting Information

The Supporting Information is available free of charge on the ACS Publications website at DOI: 10.1021/acs.iecr.7b05301..

Additional polymer characterization(PDF)

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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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