Polyphosphazene Based Star-Branched and Dendritic Molecular Brushes

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Abstract

A new synthetic procedure is described for the preparation of poly(organo)phosphazenes with star-branched and star dendritic molecular brush type structures, thus describing the first time it has been possible to prepare controlled, highly branched architectures for this type of polymer. Furthermore, as a result of the extremely high-arm density generated by the phosphazene repeat unit, the second-generation structures represent quite unique architectures for any type of polymer. Using two relatively straightforward iterative syntheses it is possible to prepare globular highly branched polymers with up to 30 000 functional end groups, while keeping relatively narrow polydispersities (1.2–1.6). Phosphine mediated polymerization of chlorophosphoranimine is first used to prepare three-arm star polymers. Subsequent substitution with diphenylphosphine moieties gives poly(organo)phosphazenes to function as multifunctional macroinitiators for the growth of a second generation of polyphosphazene arms. Macrosubstitution with Jeffamine oligomers gives a series of large, water soluble branched macromolecules with high-arm density and hydrodynamic diameters between 10 and 70 nm.

Keywords
biodegradable polymers; dendritic molecular brushes; macromolecular architectures; polyphosphazenes; star polymers

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1 Introduction

Polymers with a phosphorus containing backbone, such as polyphosphazenes[1] and polyphosphoesters,[2,3] have recently merited much attention, especially in the biomedical field,[4,5] due to the tunable hydrolytic stability of the phosphorus based backbone, and the adjustable rates of (bio)degradation this infers.[6,7] Polyphosphazenes are of particular interest due to their unique properties compared to many conventional carbon based polymers,[8–13] and are currently being investigated for many advanced applications including polymer therapeutics,[5,14,15] vaccine delivery,[16,17] thermoresponsive hydrogels for drug delivery,[18,19] and tissue engineering.[20–22]

The progress in living polymerization and controlled radical polymerization over recent years has resulted in higher control and a rapid expansion in the molecular architectures available, including molecular brush,[23] star branched,[24,25] dendritic,[26] and dendronized molecular brushes,[27] just to name a few. Globular highly branched polymers have gained interest in particular for their unique characteristics, which cannot be obtained by linear polymers, such as compact structure and high density of end groups.[28] Of the advanced architectures available however, most of them consist of organic polymers with aliphatic carbon backbones, although the use of phosphorus based building blocks containing organic units has yielded dendrimers with high functionality and promising properties.[29,30]

Despite much research effort, a controlled synthesis of poly(dichlorophosphazene) ([PNCl₂]ₙ), the most used precursor, remains a limiting factor for the preparation of polyphosphazenes.[31–35] The development of a living cationic polymerization of chlorophosphoranimine (Cl₃PNSi(CH₃)₃) has propelled the research on polyphosphazenes with controlled structures forward.[36–38] Indeed, using (RO)₃P = NSi(CH₃)₃ type phosphoranimines as a precursor, three-arm star polymers have been reported.[39] Successive studies by the same authors revealed this innovative grafting-from route not to be as promising as first appeared,[40] with only a more extensive route capping branched organic species with phosphoranimines being viable. Although polyphosphazenes can be successfully grafted onto branched organic polymers and dendrimers using this method,[41] it remains a challenge to prepare polyphosphazene branches via this route. Hybrid branched polyphosphazenes,[38] where organic polymers are grafted onto the phosphazene backbone, for example via atom transfer radical polymerization (ATRP),[42] have also been frequently reported. To the best of our knowledge, however, polyphosphazene branches grafted from or onto a polyphosphazene backbone have hitherto not been reported.

2 Results and Discussion

2.1 Star Branched Polyphosphazenes

Herein the synthesis of a series of star dendritic molecular brush type polyphosphazenes is reported. Applying a recently established one-pot phosphine-mediated living polymerization route,[43] a polyphosphazene tri-arm star polymer (Figure 1a) was first prepared and subsequently second-generation polyphosphazene side chains added, yielding star dendritic molecular brush structures (Figure 1b). The three-arm star polymer was prepared using
1,1,1-tris(diphenylphosphino)methane as a multifunctional core for the parallel growth of three polymer chains (Figure 1a). The phosphine moieties of the core were first chlorinated with C\textsubscript{2}Cl\textsubscript{6} functioning as a mild chlorinating agent. The choice of solvent here is critical, with CH\textsubscript{2}Cl\textsubscript{2} yielding three fully ionized [RPh\textsubscript{2}PCl]\textsuperscript{+} species with Cl\textsuperscript{-} counter ions.\[44\] The living cationic polymerization was then initiated from these cationic phosphorus atoms immediately upon addition of \(n\) equivalents of the Cl\textsubscript{3}PNSi(CH\textsubscript{3})\textsubscript{3} monomer\[45\] to give three [PNCl\textsubscript{2}]\textsubscript{p} chains, linked together by the tris(diphenylphosphino)methane core. The chlorine atoms in the [PNCl\textsubscript{2}]\textsubscript{p} chains were then macrosubstituted\[1\] (the post-polymerization of the macromolecular [PNCl\textsubscript{2}]\textsubscript{p}) by Jeffamine, an amino end-capped polyalkyl oxide, yielding the desired star-brush poly(organo)phosphazenes (polymers 1 and 2), with each repeat unit of the [PNCl\textsubscript{2}]\textsubscript{p} arms having two Jeffamine oligomers. Since all of these steps are a simple addition of reactants in solution to the existing reaction mixture, this can be regarded as a one-pot synthesis.

2.2 Star Dendritic Molecular Brushes

A second series of three-arm star polymers were then prepared, this time with 3-(diphenylphosphino)-1-propanamine as the substituent on the [PNCl\textsubscript{2}]\textsubscript{p} chains (3, 4, Figure 1b). Each diphenylphosphine moiety was then chlorinated yielding the ionic [RPh\textsubscript{2}PCl]\textsuperscript{+} species (3a, 4a) acting as macroinitiators. The addition of Cl\textsubscript{3}PNSi(CH\textsubscript{3})\textsubscript{3} results in the growth of two [PNCl\textsubscript{2}]\textsubscript{p} chains per repeat unit (3b, 4b), followed by the macrosubstitution with Jeffamine, yielding second-generation poly(organo)phosphazene star dendritic molecular brushes (5, 6) (Figure 1b). The completion of each step can be followed by \({\textsuperscript{1}H}\textsuperscript{31}P\) NMR spectroscopy (Figure 2a), including the complete substitution of the P-N backbone, due to the absence of P-Cl groups from the precursor (Figure 2a (IV)).

The growth of [PNCl\textsubscript{2}]\textsubscript{p} chains from the macroinitiators (4a to 4b) was confirmed by \({\textsuperscript{1}H}\textsuperscript{31}P\) NMR spectroscopy measurements in CD\textsubscript{2}Cl\textsubscript{2} in which monomer consumption over time showed living chain growth kinetics (Figure 2c,d). The NMR measurements show the complete consumption of the monomer into the growing chains of the [PNCl\textsubscript{2}]\textsubscript{p} (Figure 2b). The kinetics derived from the measurements are slower than expected from previous studies.\[43\] Although this could be partly due to the use of macroinitiators, it is more likely due to the alkyl moiety of the 3-(diphenylphosphino)-1-propanamine. Replacement of the alkyl moiety with a substituted phenyl group would be expected to enhance reaction rates, since previous studies with fully aromatic phosphines showed shorter polymerization times.\[43\]

These star branched and star dendritic molecular brushes have high effective degrees of polymerization (DP) for polyphosphazenes prepared from Cl\textsubscript{3}PNSi(CH\textsubscript{3})\textsubscript{3} and an unprecedented large number of end groups due to the multiplying dendritic effect of two arms emanating from each repeat unit. Since \({\textsuperscript{1}H}\textsuperscript{31}P\) NMR studies show near complete consumption of the monomer into the growing molecules (as previously shown for this polymerization type\[43\]), the DP for the star polymer 2 is calculated as 150, i.e., three times \(n = 50\). The second generation consists of two [PNCl\textsubscript{2}]\textsubscript{p} arms per repeat unit, each with \(p = 50\), thus giving an expected overall DP of \(\approx 15\,000\) for polymer 6. Furthermore, with two organic substituents per repeat unit, polymer 6 gives an estimated 30 000 end groups (Table 1). The molecular weights obtained from size
exclusion chromatography measurements confirmed an increase in molecular weight upon increasing generation and chain lengths (Table 1). The values received are, as expected, much lower than the theoretical values, due to the reliance on linear polystyrene standards. The obtained polydispersities range from 1.2 to 1.6, suggesting relatively good control of the polymerization. The hydrodynamic volumes of the polymers were also measured by dynamic light scattering (DLS) (Figure 3), with a similar increase in hydrodynamic diameter upon increasing chain lengths and number of generations being observed. Jeffamine oligomer side chains render the poly(organo) phosphazenes not only water soluble but also augment the hydrodynamic diameter of the resulting polymers. DLS measurements in H$_2$O showed only a slight increase when increasing the number of repeat units of the star from 10 to 50 per arm (polymer 1 vs 2). However, on going from the first generation to the second generation, with short ($n = 10$) polyphosphazene side-arms (5), the hydrodynamic volume doubles. When extending each side-arm to 50 repeat units, the hydrodynamic volume is quadrupled (6) and reaches a value of 70 nm (Figure 3), thus, large, unimolecular, water soluble nanostructures are obtained, as also confirmed by atomic force microscopy (AFM) (Figure SI-1, Supporting Information).

3 Conclusions

Phosphine-mediated living polymerization was used to prepare star-branched polyphosphazenes emanating from a central core containing three phosphine moieties. These three-armed star polymers could be substituted with two phosphine moieties per repeat unit. Upon chlorination, the phosphine moieties subsequently act as macroinitiators for the preparation of second-generation polyphosphazene side chains, yielding star dendritic molecular brush structures. Monomer consumption (and chain growth) was tracked by $^1$H$^{31}$P NMR spectroscopy and showed linear kinetics and after macromutation with Jeffamine, DLS and AFM measurements confirmed water soluble globular unimolecular structures in the region of 70 nm. The term star dendritic molecular brushes is used to describe these unique macromolecules due to their high degree of branching (two branches per repeat unit) emanating from a central core. Since these structures do not fall under the definition of classical hyperbranched polymers, nor dendritic polymers, the description dendritic molecular brushes was chosen, due to the similarity to such recently reported structures.[46] With two simple one-pot syntheses, it was possible to reach a degree of polymerization of up to 15 000 and thus ≈30 000 end groups in a relatively simple synthesis. Moreover, the limit of further generations that could be synthesized remains to be explored. Furthermore, due to the high number of functional groups which can be easily introduced by mixed substitution of the poly(dichlorophosphazene) backbone, it should be a simple task to introduce a variety of functional groups, for example for catalyst or drug loading. The hydrodynamic volumes in the 10–70 nm range, in combination with the proven biocompatibility and degradability of similar poly(organo)phosphazenes, as well as their good aqueous solubility,[7] render these materials particularly interesting candidates as polymer therapeutics,[4,5,15] where highly branched, controlled structures could be highly valuable.[47,48]
Supporting Information

Refer to Web version on PubMed Central for supplementary material.

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References

[1]. Allcock, HR. Chemistry and Applications of Polyphosphazenes. Wiley; Hoboken, USA: 2003.
[2]. Zhang S, Li A, Zou J, Lin LY, Wooley KL. ACS Macro Lett. 2012; 1:328. [PubMed: 22866244]
[3]. Steinbach T, Ritz S, Wurm FR. ACS Macro Lett. 2014; 3:244.
[4]. Teasdale, I.; Brüggemann, O. Polyphosphazenes for Medical Applications. Smithers RAPRA; Shrewsbury, UK: 2014.
[5]. Teasdale I, Brüggemann O. Polymers. 2013; 5:161. [PubMed: 24729871]
[6]. Steinbach T, Wurm FR. Angew Chem. 2015; 127:6196.
[7]. Wilpert S, Iturmendi A, Schoeberger W, Kryeziu K, Heffieter P, Berger W, Brüggemann O, Teasdale I. J Polym Sci Part A: Polym Chem. 2014; 52:287.
[8]. Soto AP, Gilroy JB, Winnik MA, Manners I. Angew Chem Int Ed. 2010; 49:8220.
[9]. Suárez-Suárez S, Carriedo GA, Tarazona MP, Presa Soto A. Chem Eur J. 2013; 19:5644. [PubMed: 23460313]
[10]. Monge S, Canniccioni B, Graillot A, Robin J-J. Biomacromolecules. 2011; 12:1973. [PubMed: 21553908]
[11]. Zhang Q, Yan Y, Li S, Feng T. Mater Sci Eng C. 2010; 30:160.
[12]. Deng M, Kumbar SG, Nair LS, Weikel AL, Allcock HR, Laurencin CT. Adv Funct Mater. 2011; 21:2641.
[13]. Nichol JL, Morozowich NL, Decker TE, Allcock HR. J Polym Sci Part A: Polym Chem. 2014; 52:2258.
[14]. Feinweber D, Verwanger T, Bruggemann O, Teasdale I, Krammer B. Photochem Photobiol Sci. 2014; 13:1607. [PubMed: 25257955]
[15]. Teasdale I, Wilpert S, Nischang I, Brüggemann O. Polym Chem. 2011; 2:828.
[16]. Palmer CD, Ninković J, Prokopowicz ZM, Mancuso CJ, Marin A, Andrianov AK, Dowling DJ, Levy O. Biomaterials. 2014; 35:8876. [PubMed: 25023392]
[17]. Andrianov AK, DeColliibus DP, Gillis HA, Kha HH, Marin A, Prausnitz MR, Babiuk LA, Townsend H, Mutwiri G. Proc Natl Acad Sci USA. 2009; 106:18936. [PubMed: 19864632]
[18]. Park M-R, Seo B-B, Song S-C. Biomaterials. 2013; 34:1327. [PubMed: 23149013]
[19]. Kim Y-M, Park M-R, Song S-C. ACS Nano. 2012; 6:5757. [PubMed: 22663194]
[20]. Morozowich NL, Nichol JL, Monedschein RJ, Allcock HR. Polym Chem. 2012; 3:778.
[21]. Deng M, Kumbar SG, Wan Y, Toci US, Allcock HR, Laurencin CT. Soft Matter. 2010; 6:3119.
[22]. Rothemund S, Aigner TB, Iturmendi A, Rigau M, Husár B, Hildner F, Oberbauer E, Prahmabauer M, Olwale G, Forstner R, Liska R, et al. Macromol Biosci. 2015; 15:351. [PubMed: 25355036]
[23]. Sheiko SS, Sumerlin BS, Matyjaszewski K. Prog Polym Sci. 2008; 33:759.
[24]. Khanna K, Varshney S, Kakkar A. Polym Chem. 2010; 1:1171.
[25]. Hirao A, Sugiyama K, Tsunoda Y, Matsuo A, Watanabe T. J Polym Sci Part A: Polym Chem. 2006; 44:6659.
[26]. Deng Y, Zhang S, Lu G, Huang X. Polym Chem. 2013; 4:1289.
[27]. Frauenrath H. Prog Polym Sci. 2005; 30:325.
[28]. Voit BI, Lederer A. Chem Rev. 2009; 109:5924. [PubMed: 19785454]
[29]. Katir N, El Brahmi N, El Kadib A, Mignani S, Caminade A-M, Bousmina M, Majoral JP. Chem Eur J. 2015; 21:6400. [PubMed: 25754619]
[30]. Maraval V, Caminade A-M, Majoral J-P, Blais J-C. Angew Chem Int Ed. 2003; 42:1822.
[31]. Wang B. Macromolecules. 2005; 38:643.
[32]. Carriedo GA, García Alonso FJ, Gómez-Elipe P, Fidalgo JJ, García Álvarez JL, Presa-Soto A. Chem Eur J. 2003; 9:3833. [PubMed: 12916107]
[33]. Andrianov AK, Chen J, LeGolvan MP. Macromolecules. 2004; 37:414.
[34]. Zhang Y, Huynh K, Manners I, Reed CA. Chem Commun. 2008; 4:494.
[35]. Blackstone V, Presa Soto A, Manners I. Dalton Trans. 2008; 33:4363. [PubMed: 18698437]
[36]. Allcock HR, Crane CA, Morrissey CT, Nelson JM, Reeves SD, Honeyman CH, Manners I. Macromolecules. 1996; 29:7740.
[37]. Honeyman CH, Manners I, Morrissey CT, Allcock HR. J Am Chem Soc. 1995; 117:7035.
[38]. Henke H, Wilfert S, Iturmendi A, Brüggemann O, Teasdale I. J Polym Sci Part A: Polym Chem. 2013; 51:4467.
[39]. Nelson JM, Allcock HR. Macromolecules. 1997; 30:1854.
[40]. Allcock HR, Powell ES, Maher AE, Prange RL, de Denus CR. Macromolecules. 2004; 37:3635.
[41]. Cho SY, Allcock HR. Macromolecules. 2007; 40:3115.
[42]. Liu X, Tian Z, Chen C, Allcock HR. Macromolecules. 2012; 45:1417.
[43]. Wilfert S, Henke H, Schoeberl W, Brüggemann O, Teasdale I. Macromol Rapid Commun. 2014; 35:1135. [PubMed: 24700544]
[44]. Godfrey SM, McAuliffe CA, Sheffield JM. Chem Commun. 1998; 8:921.
[45]. Huynh K, Rivard E, LeBlanc W, Blackstone V, Lough AJ, Manners I. Inorg Chem. 2006; 45:7922. [PubMed: 16961385]
[46]. Li S, Gao C. Polym Chem. 2013; 4:4450.
[47]. Duro-Castano A, Movellan J, Vicent MJ. Biomater Sci. 2015; 3:1321. [PubMed: 26266272]
[48]. Fox ME, Szoka FC, Fréchet JM. Acc Chem Res. 2009; 42:1141. [PubMed: 19555070]
Figure 1.
a) Synthetic route to three-arm poly(organo)phosphazene stars via successive chlorination, polymerization, and macrosubstitution with \( n_{\text{arm}} = 10 \) (1) and \( n_{\text{arm}} = 50 \) (2). Reagents and conditions: (i) \( \text{CH}_2\text{Cl}_2 \), Ar, r.t., overnight, (ii) \( \text{CH}_2\text{Cl}_2 \), Ar, r.t., 24 h, (iii) \( \text{Et}_3\text{N} \) (1 eq.), THF, Ar, r.t., 24 h. b) The preparation of second-generation star dendritic molecular brushes (5 and 6). Diphenylphosphine substituted first-generation polyphosphazene stars (3,4) with \( n = 25 \) (3) and \( n = 50 \) (4), second-generation \([\text{PNCI}_2]_p\) (3b, 4b) and second-generation poly(organo)phosphazene star dendritic molecular brushes (5,6) with \( p = 10 \) (5) and \( p = 50 \) (6). End groups, not shown for clarity, contribute four propagating chains per arm. Reagents and conditions: (i) \( \text{C}_2\text{Cl}_6, \text{CH}_2\text{Cl}_2 \), Ar, r.t., overnight, (ii) \( n\text{Cl}_3\text{PNSi(CH}_3)_3, \text{CH}_2\text{Cl}_2 \), Ar, r.t., 24 h, (iii) \( \text{RNH}_2, \text{Et}_3\text{N} \) (1 eq.), THF, Ar, r.t., 24 h. c) A schematic representation for the preparation of second-generation star dendritic molecular brushes.
Figure 2.
a) Completion of each synthesis step followed by {\textsuperscript{1}H}{\textsuperscript{31}}P NMR spectroscopy in CDCl\textsubscript{3}. (I) RPPh\textsubscript{2} substituted three-arm star polyphosphazene with PN backbone phosphorus at 3 ppm and the RPPh\textsubscript{2} side groups at −17 ppm (3), (II) [RPPh\textsubscript{2}PCl]\textsuperscript{+}Cl\textsuperscript{−} side groups at 77 ppm (macroinitiator) (3a), (III) second-generation [PNCl\textsubscript{2}]\textsubscript{p} chains at −19 ppm (3b), (IV) star dendritic molecular brush poly(organo)phosphazenes fully substituted with Jeffamine M-1000 at 0 ppm (5). b) {\textsuperscript{1}H}{\textsuperscript{31}}P NMR measurements showing the complete consumption of the monomer. (I) Cl\textsubscript{3}PNSi(CH\textsubscript{3})\textsubscript{3} signal at −56 ppm, (II) [PNCl\textsubscript{2}]\textsubscript{p} signal at −18 ppm without the Cl\textsubscript{3}PNSi(CH\textsubscript{3})\textsubscript{3} signal. c,d) The kinetic study of the polymerization of 4a monitored by {\textsuperscript{1}H}{\textsuperscript{31}}P NMR spectroscopy. The linearity of ln (M\textsubscript{t}/M\textsubscript{0}) versus time indicates a pseudo-first-order reaction, and thus, a living polymerization.
Figure 3.
Hydrodynamic diameter of polymer stars (1 and 2) and star dendritic molecular brushes (5 and 6) measured in H$_2$O with dynamic light scattering (DLS).
Table 1

Polymer characterization.

| Polymer | $n_{\text{arm}}$ | $p$ | Substituent | $d_{\text{h, water}}$ | $M_{n, \text{theo}}$ | $M_{n, \text{GPC}}$ | $M_w/M_n$ |
|---------|----------------|----|-------------|----------------------|-----------------|-----------------|-----------|
| 1       | 10             | -  | Jeffamine M-1000 | 8.20                 | 6.2 × 10$^4$   | 17 440          | 1.28      |
| 2       | 50             | -  | Jeffamine M-1000 | 9.55                 | 3.1 × 10$^5$   | 62 535          | 1.59      |
| 3       | 25             | -  | 3-(diphenylphosphino)-1-propanamine | -                  | 4.0 × 10$^4$ | -               | -         |
| 4       | 50             | -  | 3-(diphenylphosphino)-1-propanamine | -                  | 8.0 × 10$^4$ | -               | -         |
| 5       | 25             | 10 | Jeffamine M-1000 | 20.43                | 3.1 × 10$^6$   | 143 571         | 1.53      |
| 6       | 50             | 50 | Jeffamine M-1000 | 69.64                | 3.1 × 10$^7$   | 306 226         | 1.23      |

$a)$ $n_{\text{arm}} = \text{theoretical number of repeat units per arm of the three-arm star assuming total incorporation of the added monomer;}$

$b)$ $p = \text{theoretical number of repeat units of the second-generation polyphosphazene chain grown from the diphenylphosphine moiety assuming total incorporation of the added monomer;}$

$c)$ Hydrodynamic diameter measured by dynamic light scattering in H$_2$O;

$d)$ Theoretical molecular weights assuming total incorporation of the added monomer;

$e)$ Apparent molecular weight as measured by SEC in DMF versus linear polystyrene standards.