

# Synthesis of Water-Soluble Methoxyethoxy-Aminoarloyoxy Cosubstituted Polyphosphazenes as Carrier Molecules for Bioactive Agents

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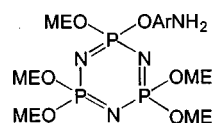
The water-soluble poly(methoxyethoxy-aminoarloyoxy phosphazene) has been synthesized and investigated as a polymeric carrier molecule for the covalent attachment of bioactive agents. The synthetic procedures were developed first through the use of cyclic trimeric model systems. These model systems were utilized for the synthesis of polymeric analogues containing bioactive side groups. The sodium salts of 2-methoxyethanol and 4-acetamidophenol were allowed to react with  $(\text{NPCl}_2)_3$  or  $(\text{NPCl}_2)_n$  or to yield derivatives of type  $[\text{NP}(\text{OCl}_2\text{CH}_2\text{CH}_2\text{OCH}_3)_x(\text{OArNHCOCH}_3)_y]_3$  or  $n$ . The 4-acetamido groups were then hydrolyzed to 4-amino-phenoxy units with potassium tert-butoxide. Coupling reactions between amino group and *N*-acetylglycine was accomplished with the use of dicyclohexylcarbodiimide. Their properties and structural characterization are discussed.

## Introduction

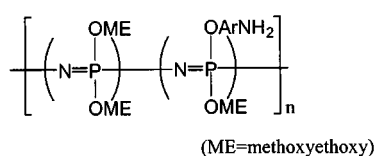
Considerable interest exists in the synthesis of biocompatible polymers as carrier molecules for the bioactive agent.<sup>1-4</sup> However, relatively few conventional polymers are suitable for this purpose because of the need for the hydrophilicity, biocompatibility, biostability, and facile side-group release mechanism in aqueous media.<sup>5-7</sup> As discussed in the previous papers,<sup>8-10</sup> poly(organophosphazenes) possess a lot of advantages as potential carriers for the controlled release of bioactive agents. Synthetic routes to polyphosphazenes are known for the introductions of a wide range of different organic side groups.<sup>11,12</sup> These features include the wide choice of side-group structures that can impart water solubility, hydrophilic or hydrophobic insolubility, or biodegradability to nontoxic molecules.<sup>13</sup> Moreover, biologically active agents can be easily linked covalently or coordinatively to the polyphosphazene systems.<sup>14</sup>

In the past we have developed several synthetic techniques to obtain water-soluble polyphosphazenes, by the reactions of poly(dichlorophosphazenes) with hydrophilic organic side groups.<sup>15-17</sup> In the present work we have described synthetic routes to water-soluble polyphosphazenes that contain both methoxyethoxy and aminoarloyoxy side groups. As discussed earlier, our preferred route to the synthesis of new phosphazene polymers involves a prior exploration of new reactions using small-molecule cyclic trimeric phosphazene models.<sup>18-20</sup>

In this study cyclic trimeric and polyphosphazene model systems were synthesized. Hence, a cyclic trimeric phosphazene was used as a preliminary model to produce high molecular weight polyphosphazene II.



I



II

## Experimental Section

**Reagents.** All experimental manipulations were performed under an atmosphere of dry nitrogen. Tetrahydrofuran (THF), and dioxane were freshly distilled under nitrogen from sodium benzophenone ketyl. Hexachlorocyclotriphosphazene (mp 110-120 °C) was obtained from a trimer-tetramer mixture (Ethyl Corp.) after two fractional vacuum sublimations at 60 °C/0.5 torr, two recrystallization from heptane and two further vacuum sublimations. Poly(dichlorophosphazene)  $[\text{NPCl}_2]_n$  was prepared by the melt polymerization of  $(\text{NPCl}_2)_3$  at 250 °C for an 24 h period in a sealed Pyrex tube. 2-Methoxyethanol (Aldrich) was dried over molecular sieves before use. Sodium hydride, as an 50% dispersion in oil (Aldrich), was washed with dry heptane before use. Acetamidophenol (Aldrich), potassium tert-butoxide (Aldrich), dicyclohexylcarbodiimide (DCC) (Aldrich), and *N*-acetylglycine (Sigma) were used without further purification.

**Equipment.** <sup>31</sup>P NMR and <sup>1</sup>H NMR spectra were obtained in the Fourier transform mode with a Varian Gemini-2000 FT spectrometer. Infrared spectra were obtained with a Bio-Rad FTS-165 spectrometer. Polymer molecular weight approximations were determined by Spectra-Physics PI000 instrument. Mass spectra were obtained with an AEI MS 902 mass spectrometer operated at an ionization potential of 20 eV. Elemental analyses were obtained with Carlo-Erba EA1108 elemental analyzer.

**Synthesis of Trimer 2.** Sodium hydride (28 g, 0.7 mol) was suspended in dry THF 50 mL. To this solution was added 2-methoxyethanol (22 g, 0.288 mol) dissolved in THF at 0 °C. This solution was stirred for 3 h. The sodium methoxyethoxide solution was added to the solution of  $(\text{NPCl}_2)_3$  (20 g, 0.056 mol) at 0 °C. The reaction mixture was stirred for 48 h. The <sup>31</sup>P NMR spectrum of the mixture consisted of a singlet (hexasubstituted) and AB<sub>2</sub> pattern (pentasubstituted). Solvent was removed under reduced pressure to yield oily products. Two trimers were separated by column chro-

matography which used methylene chloride and THF (90 : 10) as eluting solvents. Trimer 2 was confirmed by TLC,  $^{31}\text{P}$  NMR,  $^1\text{H}$  NMR, GC/MS, and elemental analysis.

**Synthesis of Trimer 3.** 4-Acetamidophenol (2.5 g, 0.0165 mol) was dissolved in THF (250 mL). This solution was added slowly to a suspension of sodium hydride (0.8 g, 0.0167 mol) in THF (100 mL). When the reaction was progressed completely, the mixture was heated to 50 °C. The hot solution was filtered and the filtrate was transferred to a 1000-mL capacity vessel. To this solution was added rapidly a sample of trimer 2 (4.5 g, 0.008 mol) dissolved in THF (100 mL). The reaction mixture was refluxed for 72 h. The solvent was then removed using a rotary evaporator, and methylene chloride (100 mL) was added to the residue. The solution was extracted twice with water (250 mL). The organic layer was dried with magnesium sulfate and the solution was concentrated by means of a rotary evaporator. The final product was filtered through silica gel to remove sodium salts. The yellowish oily product was obtained and characterized by instrumental analysis.

**Synthesis of Trimer 4.** Potassium tert-butoxide (4.43 g, 0.043 mol) was suspended in 100 mL of dry ether. This mixture was cooled to 0 °C, and 0.2 mL (0.11 mol) of water was added *via* syringe. After 5 min of stirring at 0 °C, trimer 3 (0.5 g, 0.76 mmol) was added. The ice bath was removed, and the mixture was allowed to react at room temperature. Thin-layer chromatography tests showed that the starting trimer disappeared completely after 20 h. A large excess of ice water was then added, and the isolated aqueous solution was neutralized with hydrochloric acid. Water was removed by evaporator and the final product was purified by column chromatography. Trimer 4 was characterized by instrumental analysis.

**Synthesis of Trimer 5.** Trimer 4 (1 g, 0.0016 mol) was dissolved in dry methylene chloride (25 mL). The solution was cooled with an ice bath, and *N*-acetyl glycine (0.37 g, 0.0032 mol) was added. DCC (0.49 g, 0.0024 mol) was dissolved in dry methylene chloride (10 mL) and was added in one portion. A copious white precipitate formed within 1 hr. The solution was allowed to warm down to room temperature and was stirred for 24 h. The mixture was reduced in volume to 10 mL using rotary evaporator and was filtered through a coarse fritted funnel to remove DCC-urea. The solution was extracted with water, dried with magnesium sulfate, and evaporated to dryness to yield a yellowish oil. This was purified by column chromatography on silica gel. The  $^{31}\text{P}$  NMR spectra,  $^1\text{H}$  NMR spectra, and microanalytical data for this compound were consistent with the structure postulated.

**Synthesis of Polymer 8.** Polydichlorophosphazene (5 g, 0.04 mol) was dissolved in dry THF (100 mL), and to the polymer solution was added sodium hydride (2.79 g, 0.068 mol) and 2-methoxyethanol (5.2 g, 0.068 mol). The reaction mixture was stirred for 48 h. The  $^{31}\text{P}$  NMR spectrum of this solution possessed two singlets at 7.8 and 11.9 ppm respectively. This milky solution was added to the solution of sodium 4-acetamidophenoxy prepared from sodium hydride

(6.4 g, 0.16 mol) and 4-acetamidophenol (24.2 g, 0.16 mol). This mixture was consequently stirred at reflux temperature for 72 h. The  $^{31}\text{P}$  NMR spectrum of final product possessed two singlets at 7.9 and 14.29 ppm. The solution was concentrated under reduced pressure and dissolved in deionized water. The polymers were purified by dialysis. The polymer was then dried *in vacuo*. Characterization data of the polymers are listed in Table 2.

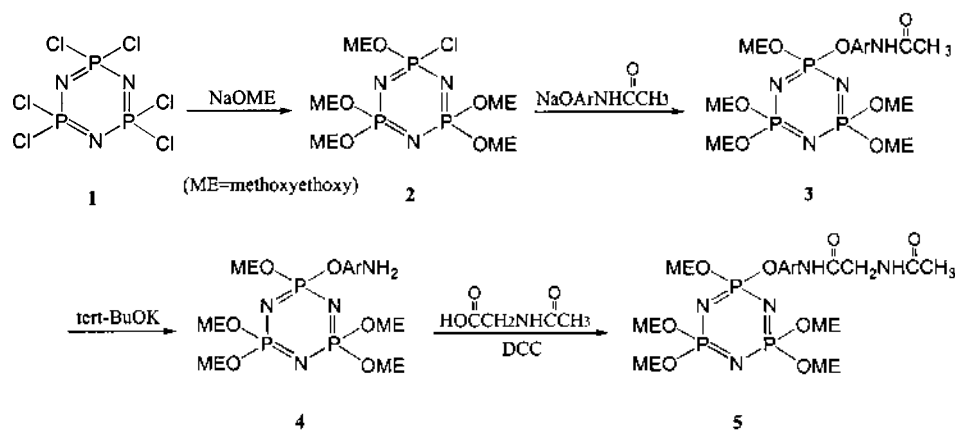
**Synthesis of Polymer 9.** Polymer 8 (1 g, 0.002 mol) was dissolved in dry THF (50 mL). The solution was added slowly to a mixture of potassium tert-butoxide (4 g, 0.04 mol) and 0.2 mL (0.011 mol) of water in dry THF (100 mL). For the first 5 min the mixture was cooled to 0 °C and then stirred at room temperature for 40 h. A large excess of ice water (300 mL) was added, and the solution was concentrated by evaporation. The solution was dialyzed through a cellulose tube against deionized water. After dialysis for 72 h, the polymer was dried *in vacuo* and characterized by instrumental analysis.

**Synthesis of Polymer 10.** Polymer 9 (1 g, 0.0025 mol) was dissolved in dry THF (50 mL). The solution was cooled to 0 °C and *N*-acetyl glycine (0.491 g, 0.0042 mol) was added quickly. DCC (0.6 g, 0.0029 mol) dissolved in THF (20 mL) was added to the polymer 9/*N*-acetyl glycine solution. This solution was kept at room temperature. This was accompanied by the formation of a white precipitate of DCC-urea. The solution was stirred at room temperature for 24 h. The polymer solution was concentrated by a rotary evaporator and then dissolved in deionized water. The aqueous polymer solution was dialyzed for 72 h. The final polymer was dried *in vacuo*. Characterization data are listed in Table 2.

## Results and Discussion

**Synthesis of Cyclic Trimeric Model Systems.** The specific reaction sequences used for phosphazene cyclic trimers are described in Scheme 1. Hexachlorocyclotriphosphazene reacted with sodium methoxyethoxide to yield trimer 2. Treatment of trimer 2 with excess sodium 4-acetamidophenoxy yielded trimer 3.<sup>21</sup> The 4-acetamidophenoxy unit of trimer 3 was hydrolyzed to a 4-aminophenoxy group with potassium tert-butoxide<sup>10</sup> or other strong bases such as potassium hydroxide. The latter reagent was preferred because it never generates any back-bone breakdown, and it is considered that the use of stronger bases might decrease the molecular weight of polymeric analogues for a successful application of this approach to the high-polymeric analogues.<sup>10</sup> Trimer 4 coupled with *N*-acetyl glycine to yield trimer 5, under the influence of DCC in organic media.<sup>22</sup> Trimer 5 was yellowish-colored oil which was very stable in air or water.

Trimer 2-5 were characterized by a combination of  $^{31}\text{P}$  NMR,  $^1\text{H}$  NMR, and infrared spectroscopy, mass spectrometry, and elemental microanalysis. The characterization data were listed in Table 1. For example, the  $^{31}\text{P}$  NMR spectra were  $\text{AB}_2$  patterns for trimers 2 to 5 which indicated pentasubstitution.<sup>23</sup> Trimer 3 to 5 showed the almost same chemical



Scheme 1

shifts because phosphorous environments were not seriously changed. The  $^1\text{H}$  NMR spectra were a little bit complicated, but the specified chemical shifts for different type of protons corresponded to the expected shift positions. Especially trimer 3 showed the chemical shift for acetyl protons at 2.1 ppm, but it disappeared after the hydrolysis. Trimer 5 possessed again the chemical shift for acetyl proton at 1.9 ppm.

Infrared spectra showed medium peaks at in the 1200–1250  $\text{cm}^{-1}$  range suggesting the presence of the cyclophosphazene ring. Carbonyl stretching peak of trimer 3 as observed at 1665  $\text{cm}^{-1}$ , but it disappeared after the hydrolysis.<sup>25</sup> After the reaction of trimer 4 and *N*-acetylglycine, a new peak appeared at around 1675  $\text{cm}^{-1}$  showed the successful coupling reaction. The mass spectra of trimers 2 to 5 show parent peaks that correspond to the expected molecular weights (see Table 1).

**Synthesis and Structural Characterization of the High Polymers.** Poly(dichlorophosphazene) (6) was prepared by the well-established thermal polymerization of the cyclic trimer 1.<sup>26,27</sup> Polymer 6 was then allowed to react with the sodium salts of methoxyethanol to form polymer 7 similar to that described for the cyclic trimers. Polymer 7 was then treated with the sodium salt of *p*-acetamidophenol to pro-

duce polymer 8. Polymer 8 was hydrolyzed with potassium tert-butoxide to form polymer 9. The coupling reaction of polymer 9 with *N*-acetylglycine was carried out in THF solution with the aid of DCC.<sup>22</sup> The coupled polymer 10 were readily soluble in THF or dioxane. These reactions are summarized in Scheme 2.

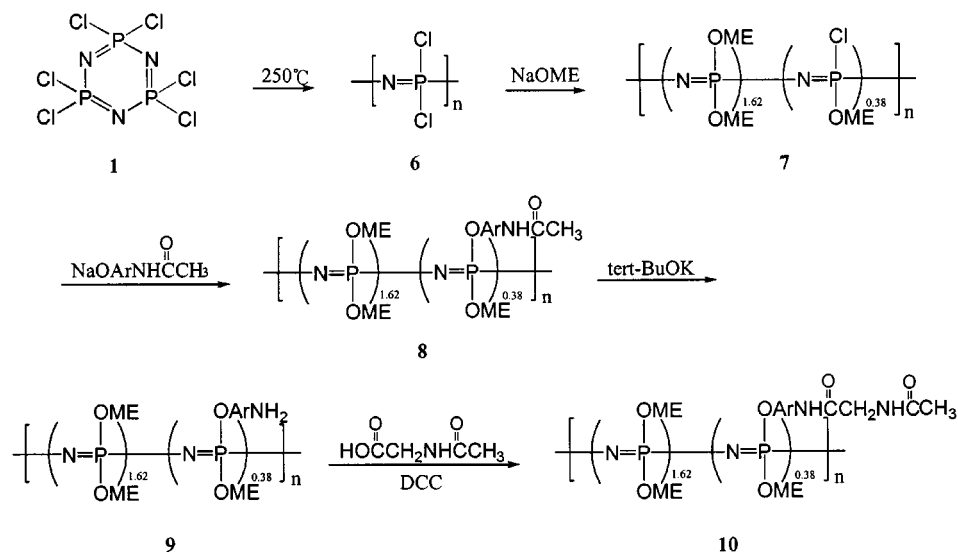
Substitution percents of the side groups in polymer 7 to 10 were deduced from the  $^{31}\text{P}$  NMR spectra of each polymers. These were also confirmed by the microanalytical data (Table 2). All the polymers possessed two singlets. Only polymer 7 showed a slightly different shift position at 7.9 and 11.9 ppm, because polymer 7 possessed unreacted P-Cl bonds. Polymer 8 to 10 showed two singlet at 7.8 to 8.1 ppm and 13.7 to 14.3 ppm. The  $^1\text{H}$  NMR spectra from the polymers were identical to those from the cyclic trimers. The infrared spectra of the high polymers were consistent with the chemical of polymer 8 to 10.

As was expected, polymer 8 possessed carbonyl stretching peak at 1665  $\text{cm}^{-1}$  but the peak disappeared after the hydrolysis. After the coupling reactions between polymer 9 and *N*-acetylglycine, carbonyl stretching peak appeared again at 1675  $\text{cm}^{-1}$ . All the polymers 8 to 10 possessed the high molecular weight ( $M_w$ : around  $10^6$ ) and were monitored by GPC (Table 2).

Table 1. Characterization Data for Cyclic Trimers

Trimer		Microanalysis			$^{31}\text{P}$ NMR <sup>a</sup> ppm	$^1\text{H}$ NMR ppm	IR $\text{cm}^{-1}$	M.W (mass spec) m/e
		%C	%H	%N				
2	calcd	33.03	6.42	7.71	$\text{AB}_2$ at	3.3(s), 3.5(t), 3.7(t)	P=N at 1250	545
	found	32.94	6.61	7.45	+15.9, +18.6		C-O at 1050	
3	calcd	41.82	6.52	8.48	$\text{AB}_2$ at	2.1(s), 3.4(s), 3.5(t)	P=N at 1250	660
	found	40.93	6.51	8.24	+14.4, +17.5	3.6(t), 6.8-7.5(m)	C-O at 1050 C=O at 1665	
4	calcd	40.78	6.63	9.06	$\text{AB}_2$ at	3.4(s), 3.5(t), 3.6(t)	P=N at 1250	618
	found	40.35	6.53	8.92	+15.3, +18.2	4.3(s), 6.5-7.2(m)	C-O at 1050 N-H at 3300-3500	
5	calcd	41.84	6.42	9.76	$\text{AB}_2$ at	1.9(s), 3.3(s), 3.5(t)	P=N at 1250	717
	found	41.35	6.38	9.65	+14.8, +17.9	3.6(t), 3.8(s), 6.8-7.2(m)	C-O at 1050 C=O at 1667	

<sup>a</sup>Chemical shift positions were relative to aqueous 85%  $\text{H}_3\text{PO}_4$ . A  $\text{D}_2\text{O}$  capillary lock was used.

**Table 2.** Characterization Data for Polymers

Polymer	Microanalysis			<sup>31</sup> P NMR <sup>a</sup> ppm	<sup>1</sup> H NMR ppm	IR cm <sup>-1</sup>	M.W (GPC) × 10 <sup>6</sup>	
	%C	%H	%N					
8	calcd	33.03	6.42	7.71	-7.9(s)	2.1(s), 3.3(s), 3.4(t)	P=N at 1250	1.2
	found	32.94	6.61	7.45	-14.29(s)	3.6(t), 6.7-7.6(m)	C-O at 1050 C=O at 1665	
9	calcd	41.82	6.52	8.48	-8.1(s)	3.4(s), 3.5(t), 3.6(t)	P=N at 1250	0.9
	found	40.93	6.51	8.24	-13.8(s)	4.2(s), 6.6-7.3(m)	C-O at 1050 N-H at 3400	
10	calcd	40.78	6.63	9.06	-7.8(s)	1.9(s), 3.3(s), 3.4(t)	P=N at 1250	1.1
	found	40.35	6.53	8.92	-13.7(s)	3.7(t), 3.8(s), 6.7-7.3(m)	C-O at 1050 C=O at 1675	

<sup>a</sup>Chemical shift positions were relative to aqueous 85% H<sub>3</sub>PO<sub>4</sub>. A D<sub>2</sub>O capillary lock was used.

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