

Synthesis of Water-Soluble Aminoaryloxy-Methylamino Cosubstituted Polyphosphazenes as Carrier Species for Biologically Active Agents

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The water-soluble poly(aminoaryloxy-methylamino phosphazene) has been synthesized and investigated as a polymeric carrier species for the covalent attachment of biologically active agents. The cyclic trimeric model systems were utilized for the synthesis of polymeric analogues containing bioactive side groups. The sodium salt of 4-acetamidophenol was first allowed to react with $(\text{NPCl}_2)_3$ or $(\text{NPCl}_2)_n$ and was then treated with excess methylamine to yield derivatives of type $[\text{NP}(\text{NHCH}_3)_x(\text{OArNHCOCH}_3)_y]_3$ or $[\text{NP}(\text{NHCH}_3)_x(\text{OArNHCOCH}_3)_y]_n$. The 4-acetamido groups were then hydrolyzed to 4-aminophenoxy 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.

Keywords : Aminoaryloxy, Methylamino, Polyphosphazenes.

Introduction

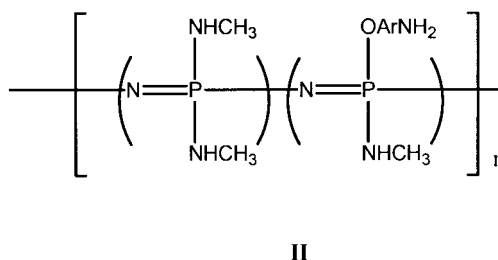
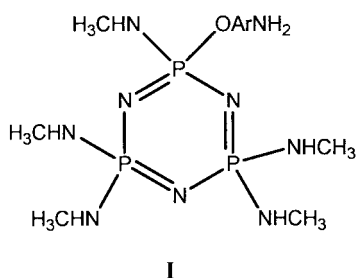
The use of polymers as carrier molecules for bioactive agents is a subject of growing interest.¹⁻⁶ One aspect of the field involves the attachment of bioactive small species to polymers in order to confer, for example, chemotherapeutic activity on the polymer themselves⁷⁻⁹ or to generate a controlled-release system as the bioactive small species is released from the polymer.¹⁰⁻¹² However, few conventional polymers are appropriate for this purpose, primarily because of their bioincompatibility or resistance to hydrolytic breakdown.^{13,14}

Poly(organophosphazenes) are of particular interest as carrier molecules for bioactive agents.¹⁵ Poly(organophosphazenes) possess a number of almost unique characteristics that may favor their use as carrier molecules.^{16,17} These features include the ease with which biologically active agents can be linked covalently or coordinatively to the polymeric system and the wide choice of side-group structures that can impart water solubility, hydrophilic or hydrophobic insolubility, or, in special cases, biodegradability to nontoxic molecules.¹⁸⁻²¹ In the past, several synthetic techniques to obtain water-soluble polyphosphazenes, by the reactions of poly(dichlorophosphazenes) with hydrophilic organic side groups have been developed.²²⁻²⁴ In this paper we have described synthetic routes to water-soluble polyphosphazenes that contain both aminoaryloxy and methylamino side groups.

As discussed in the previous papers, our preferred route to the synthesis of new phosphazene polymers involves a prior exploration of new reactions using small-molecule cyclic trimeric phosphazene models.²⁵⁻²⁷ In this study cyclic trimeric and polyphosphazene model systems were synthesized. Hence, a cyclic trimeric phosphazene **I** was used as a preliminary model to produce high molecular weight polyphosphazene **II**.

Experimental Section

Reagents. All experimental manipulations were performed under an atmosphere of dry nitrogen. Tetrahydrofuran (THF) 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. Methylamine (Matheson) was dried and distilled from metallic sodium before use. Sodium hydride, as a 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. ^{31}P NMR and ^1H 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 P1000 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 3. A solution of 4-acetamidophenol (3 g, 0.02 mol) in THF (50 mL) was added dropwise under nitrogen to a stirred suspension of excess sodium hydride (1.2 g, 0.025 mol) in THF (100 mL). After 2 h at 25 °C the mixture was filtered, and the filtrate was added dropwise to a stirred solution of hexachlorocyclotriphosphazene (1) (10 g, 0.028 mol) in THF (100 mL). The mixture was stirred for 2 h and then cooled with an ice bath. Methylamine (150 mL) was condensed into the reaction vessel by using dry ice. After 2 h, the reaction mixture was allowed to return to 25 °C under nitrogen, while unreacted methylamine gas was permitted to escape through a silicone oil bubbler. Stirring was maintained for 60 h. Triethylamine (150 mL) was then added as a hydrochloride acceptor and the solvent was removed with a rotary evaporator. The ^{31}P NMR spectrum of the mixture consisted of a singlet (hexamethylamino substitution) and AB_2 pattern (pentamethylamino-monoacetaminophenoxy substitution). Two trimers were separated by column chromatography which used methylene chloride and hexane (80:20) as eluting solvents. Trimer 3 was the yellowish oily products and confirmed by TLC, ^{31}P NMR, ^1H NMR, GC/MS, and elemental analysis.

Synthesis of Trimer 4. Potassium *tert*-butoxide (5.15 g, 0.05 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, 1.1 mmol) was added. The ice bath was removed, and the mixture was allowed to react at room temperature. Thin-layer chromatography 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 ^1H NMR, GC/MS, and elemental analysis.

Synthesis of Trimer 5. Trimer 4 (1 g, 0.0025 mol) was dissolved in dry methylene chloride (25 mL). The solution was cooled with an ice bath, and N-acetylglycine (0.58 g, 0.005 mol) was added. DCC (0.78 g, 0.0038 mol) was dissolved in dry methylene chloride (12 mL) and was added in one portion. A copious white precipitate formed within 1 h. The solution was allowed to warm up to room temperature and was stirred for 24 h. The mixture was reduced 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}P NMR spectra, ^1H NMR spectra, and microanalytical data for this compound were consistent with the structure postulated.

Synthesis of Polymer 8. 4-Acetamidophenol (1.51 g, 0.01 mol) was dissolved in THF (50 mL), and excess sodium hydride was added. After 5 h at 25 °C, the suspension was filtered under nitrogen, and the filtrate was added to poly (dichlorophosphazene) (5 g, 0.04 mol) dissolved in THF (100 mL). Constant stirring was maintained for the duration of the reaction for 24 h. Methylamine gas (300 mL) was condensed into the reaction vessel and maintained there for 2 h with a dry ice condenser. The milky polymer solution which was previously cooled with an ice bath was added to liquid methylamine. After 5 h, the reaction mixture was allowed to return to 25 °C under nitrogen while unreacted methylamine gas was permitted to escape through a silicone oil bubbler. Stirring was maintained for 72 h. The solvent was removed with a rotary evaporator, and liquid methylamine (200 mL) was added to the residue. Triethylamine (200 mL) was then added as a hydrochloride acceptor and the methylamine was removed with a rotary evaporator. The polymeric residue was collected and was dissolved in THF. After filtration and reprecipitation from THF into hexane, a white polymeric product was obtained. The polymer was dissolved in deionized water and then dried in vacuo. Characterization data of the polymers are listed in Table 2.

Synthesis of Polymer 9. Polymer 8 (1 g, 0.004 mol) was dissolved in dry THF (50 mL). The solution was added slowly

Table 1. Characterization data for cyclic trimers

Trimer		Microanalysis			^{31}P NMR ^a ppm	^1H NMR ppm	IR cm ⁻¹	M.W (mass spec) m/e
		%C	%H	%N				
3	calcd	35.86	6.44	28.97	AB_2 at +18.6, +9.4	2.0 (s), 2.8 (s) 6.8-7.8 (m)	P=N at 1250 C-N at 1180 C=O at 1670	435
	found	35.24	6.52	29.14				
4	calcd	33.59	6.62	32.06	AB_2 at +18.8, +9.3	2.7 (s), 4.4 (s) 6.5-7.6 (m)	P=N at 1250 C-N at 1180 N-H at 3300-3500	393
	found	33.75	6.48	32.45				
5	calcd	36.59	6.30	28.46	AB_2 at +18.5, +9.5	1.8 (s), 2.8 (s) 3.9 (s), 6.7-7.2 (m)	P=N at 1250 C-N at 1180 C=O at 1675	492
	found	36.34	6.25	28.72				

^aChemical shift positions were relative to aqueous 85% H_3PO_4 . A D_2O capillary lock was used.

Table 2. Characterization data for polymers

Polymer		Microanalysis			³¹ P NMR ^a ppm	¹ H NMR ppm	IR cm ⁻¹	M.W (GPC) 10 ⁶
		%C	%H	%N				
8	calcd	28.54	7.10	35.18	+1.76 (s)	2.0 (s), 2.7 (s)	P=N at 1250	1.1
	found	28.47	7.21	34.87	-1.13 (s)	6.7-7.6 (m)	C-N at 1180 C=O at 1670	
9	calcd	27.28	7.21	36.73	+7.8 (s)	3.4 (s), 4.3 (s)	P=N at 1250	0.9
	found	27.06	7.27	37.12	-1.09 (s)	6.6-7.4 (m)	C-N at 1180 N-H at 3300-3500	
10	calcd	29.28	7.00	34.60	+7.3 (s)	1.9 (s), 2.8 (s)	P=N at 1250	1.0
	found	29.41	6.92	34.17	-1.25 (s)	3.8 (s), 6.8-7.2 (m)	C-N at 1180 C=O at 1675	

^aChemical shift positions were relative to aqueous 85% H₃PO₄. A D₂O capillary lock was used.

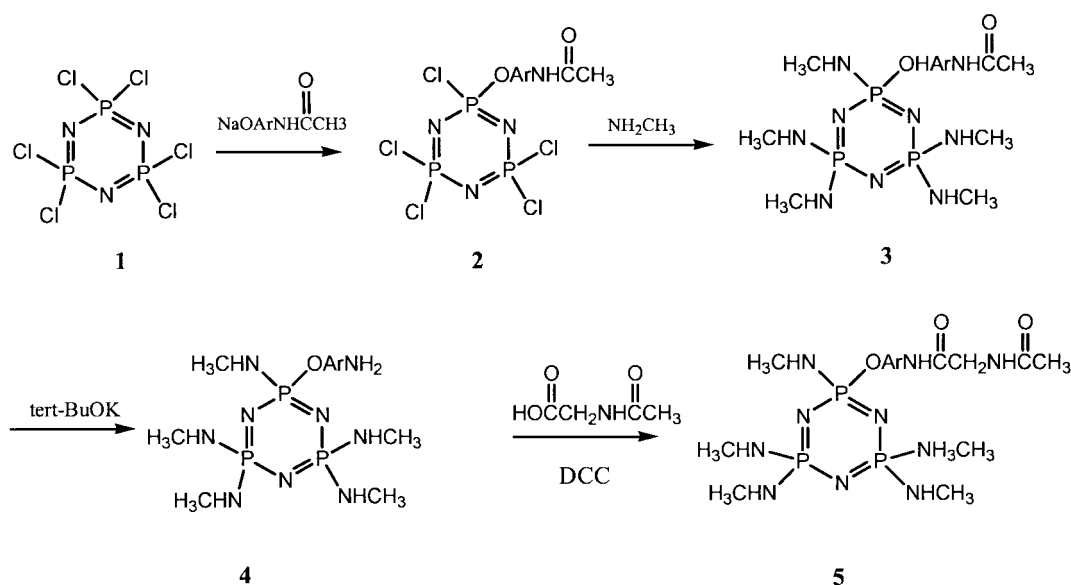
to a mixture of potassium *tert*-butoxide (8 g, 0.08 mol) and 0.4 mL (0.022 mol) of water in dry THF (150 mL). For the first 5 min the mixture was cooled to 0 °C and then stirred at room temperature for 40 h. A large excess of ice water (400 mL) was added, and the solution was concentrated by evaporation. The solution was dialyzed through a cellulose tube against deionized water. After dialysis for 72 h, the polymer was dried in vacuo. Characterization data are listed in Table 2.

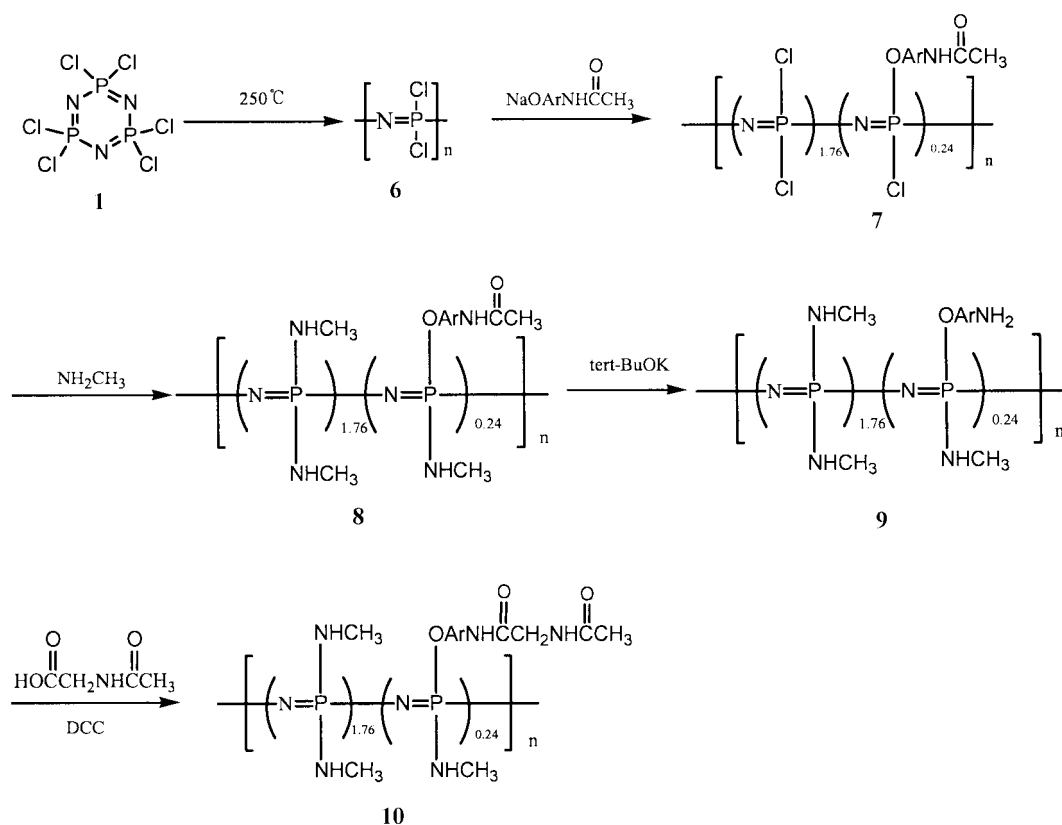
Synthesis of Polymer 10. Polymer **9** (1 g, 0.0044 mol) was dissolved in dry THF (50 mL). The solution was cooled to 0 °C and N-acetylglycine (0.94 g, 0.008 mol) was added quickly. DCC (1.24 g, 0.006 mol) dissolved in THF (30 mL) was added to the polymer **9**/N-acetylglycine solution. This solution 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 and characterized by instrumental analysis. 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 acetamidophenoxy to yield trimer **2**. Treatment of trimer **2** with excess methylamine yielded trimer **3**. The 4-acetamidophenoxy unit of trimer **3** was hydrolyzed to a 4-aminophenoxy group with potassium *tert*-butoxide 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.²⁰ Trimer **4** coupled with N-acetylglycine to yield trimer **5**, under the influence of DCC in organic media.¹³ Trimer **5** was yellowish-colored oil which was very stable in air or water.

Trimer **3-5** were characterized by a combination of ³¹P NMR, ¹H NMR, and infrared spectroscopy, mass spectrometry, and elemental microanalysis. The characterization data were

**Scheme 1**



Scheme 2

listed in Table 1. For example, the ^{31}P NMR spectra were AB_2 patterns for trimers **3** to **5** which indicated pentasubstitution.²⁸ Trimers **3** to **5** showed the almost same chemical shifts because phosphorous environments were not seriously changed. The ^1H NMR spectra were a little complicated, but the specified chemical shifts for different type of protons corresponded to the expected shift positions. Especially trimers **3** showed the chemical shift for acetyl protons at 2.0 ppm, but it disappeared after the hydrolysis. Trimer **5** possessed again the chemical shift for acetyl proton at 1.9 ppm. Trimers **3** to **5** showed almost the same chemical shifts for methylamine protons at 2.7–2.8 ppm. Infrared spectra showed medium peaks at in the $1220\text{--}1250\text{ cm}^{-1}$ range suggesting the presence of the cyclophosphazene ring. Trimers **3** to **5** also showed medium peaks at in the 1180 cm^{-1} from C–N stretching. Carbonyl stretching peak of trimer **3** was observed at 1670 cm^{-1} , but it disappeared after the hydrolysis. After the reaction of trimer **4** and N-acetylglycine, a new peak appeared at around 1675 cm^{-1} showed the successful coupling reaction. The mass spectra of trimers **3** to **5** showed parent peak 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**.²⁹ Polymer **6** was then allowed to react with the sodium salt of 4-acetamidophenol to form polymer **7** similar to that described for the cyclic trimers. Polymer **7** was then treated with excess methylamine to produce 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.¹³ The coupled polymer **10** was readily soluble in THF or dioxane. These reactions are summarized in Scheme 2.

Substitution percents of the side groups in polymers **8** to **10** were deduced from the ^{31}P NMR spectra of each polymers. These were also confirmed by the microanalytical data (Table 2). Polymers **8** to **10** showed two singlets at 7.3 to 7.8 ppm and -1.09 to -1.25 ppm. The ^1H NMR spectra of the polymers were identical to those of the cyclic trimers. The infrared spectra of the high polymers were consistent with the chemical structure of polymers **8** to **10**. As was expected, polymer **8** possessed carbonyl stretching peak at 1670 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 cm^{-1} . All the polymers **8** to **10** possessed the high molecular weight (MW: around 10^6) and were monitored by GPC (Table 2).

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