Synthesis of Purine- and Pyrimidine-Containing Polyphosphazenes: Physical Properties and Hydrolytic Behavior

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ABSTRACT: We report here the first examples of poly(organophosphazenes) with side groups derived from the purines guanine and adenine and the pyrimidine cytosine. Polymers with these purines or pyrimidines as the only side groups proved difficult to synthesize by macromolecular substitution techniques because of the insolubility of the intermediate products. Therefore, cosubstitution reactions of the poly(dichlorophosphazene) with glycine ethyl ester, alanine ethyl ester, or diethylene glycol methyl ether, followed by the respective purine or pyrimidine, were utilized. Each pair of side groups was incorporated into the polyphosphazene in a 1:1 ratio. ^{31}P NMR spectroscopy verified the replacement of all the chlorine atoms, while ^{1}H and ^{13}C NMR techniques confirmed the presence and ratio of the different side group. DRIFT spectroscopy indicated that the attachment of the purines or pyrimidines was via the primary amino functionality. Glass transition temperatures ranged from −28 to −15 °C for the mixed-substituent polyphosphazenes with both purine or pyrimidine side groups together with glycine ethyl ester or alanine ethyl ester units. Other mixed-substituent polymers with purine- or pyrimidine-substituted polyphosphazenes with the cosubstituent diethylene glycol methyl ether yielded block type side group distributions, which were revealed by ^{31}P NMR and DSC methods. The polymers appeared to be thermally stable to at least 200 °C, with 10% mass loss at temperatures as high as 263 °C. The hydrolysis of the amino acid ester/purine or pyrimidine cosubstituted polyphosphazenes in deionized water at 37 °C followed a bulk hydrolysis profile with the initial reaction being a cleavage of the amino acid ester units from the skeleton, followed by subsequent detachment of the purine or pyrimidine. Significant hydrolytic weight loss and molecular weight declines were detected after 14 days for the amino acid/purine or pyrimidine cosubstituted polyphosphazenes.

Introduction

Polyphosphazenes are hybrid inorganic−organic macromolecular systems with a wide range of potential uses. Most polyphosphazenes are synthesized via a macromolecular substitution approach in which the reactive chlorine atoms in poly(dichlorophosphazene) (**1**) are replaced by reactions with organic nucleophiles. This method has led to the synthesis of many different molecular structures and architectures.[1] The properties of the final polymeric materials depend on the nature of the side groups, which are typically alkoxy, aryloxy, or amino units. The macromolecular substitution approach allows one, two, or more different side groups to be linked to the same polymer chain to generate different solid state or solution properties. Thus, numerous polyphosphazenes have been studied as materials for membranes,[2−4] optical materials,[5,6] and various biomedical applications.[7,8] Within the field of biomedical materials, hydrolytically sensitive polyphosphazenes and their hydrolysis mechanisms have been studied in detail.[9−12]

The hydrolytic sensitivity of a polyphosphazene matrix is achieved through the use of selected side groups that include imidizolyl,[13,14] glucosyl,[15] lactide or glycolide esters,[16] amino acid esters,[9,10] or peptides.[12] The incorporation of different side groups affects biomedically important properties, such as hydrolysis rate, hydrophobicity, molecular interaction capabilities, etc. In addition, a number of new biologically compatible side groups that possess the ability to interact through hydrogen bonding and impart hydrolytic instability to the polymers are under investigation. This combination of properties provides attractive opportunities for uses in different biomedical applications, including drug delivery[17,18] and tissue engineering.[19,20]

In principle, purine and pyrimidine side groups have the additional ability to generate interesting and potentially useful polymer−polymer interactions in polyphosphazenes, perhaps in the manner found in DNA. For example, it is possible to visualize systems in which the viscosity or gelation behavior at different pH values can be controlled through the hydrogen-bonding characteristics of the different side groups. Alternatively, the conformations of specific polymers in solution or the solid state might be controllable through the substitution pattern of the side groups. Hydrolysis of these polymers in composites with poly(glycolic−lactic acid) (PLGA) could serve to neutralize the low pH generated during the hydrolysis of the polyester.

This is the first reported synthesis of purine- and pyrimidine-substituted polyphosphazenes. Guanine, adenine, and cytosine all bear primary amino units that can participate in nucleophilic replacement of the chlorine atoms in poly(dichlorophosphazene). Initial small molecule model studies with the model cyclic trimer, $(NPCl_2)_3$, confirmed that the purine and pyrimidine substituents can indeed replace some of the chlorine atoms of hexachlorocyclotriphosphazene. However, the products become insoluble before all the chlorine atoms were replaced. Nevertheless, the feasibility of the substitution was developed through these model substitution studies. Similar difficulties were encountered with the synthesis of high polymers that bear only purine or pyrimidine side groups. Therefore, to avoid these problems, mixed-substituent polymers were synthesized with

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Table 1. Structural and Physical Characterization Data for Polymers 6−14

polymer	^{1}H NMR (ppm)	^{31}P NMR (ppm)	^{13}C NMR (ppm)	$M_w \times 10^{-3}$ (g/mol)	T_g (°C)	T_d (°C)
6	1.1 (3H), 3.8 (2H), 4.0 (2H), 8.1 (1H)	−0.76	14.1 (CH_3), 39.4 (CH_2), 61.2 (CH_2), 134−161 (4 Ar), 171.3 (2 C═O)	103	−24.8	192,[a] 554[b]
7	1.3 (3H), 3.8 (2H), 4.1 (2H), 8.3 (1H), 8.6 (1H)	−0.22	14.0 (2 CH_3), 40.1 (CH_2), 60.5 (CH_2), 130−155 (5 Ar), 172.4 (C═O)	110	−24.1	207[a]
8	1.3 (3H), 3.8 (2H), 4.1 (2H), 4.7 (1H) 7.4 (1H)	−0.89	15.2 (CH_3), 41.5 (CH_2), 62.6 (CH_2), 103−168 (4 Ar), 174.2 (C═O)	109	−27.7	213,[a] 606[b]
9	1.1 (6H), 3.7 (1H), 4.4 (2H), 8.5 (1H)	−0.18	13.2 (CH_3), 19.5 (CH_3), 43.6 (CH), 138−165 (4 Ar), 171.2 (2 C═O)	118	−16.8	207,[a] 593[b]
10	1.4 (6H), 3.9 (1H), 4.1 (2H), 8.0 (1H), 8.6 (1H)	−1.1	13.5 (2 CH_3), 41.8 (CH_2), 62.2 (CH_2), 128−159 (5 Ar), 171.0 (C═O)	111	−20.2	213[a]
11	1.2 (6H), 3.7 (1H), 4.3 (2H),4.7 (1H), 8.2 (1H)	−1.3	15.1 (2 CH_3), 46.3 (CH), 61.4 (CH_2), 101−165 (4 Ar), 173 (C═O)	120	−15.5	263,[a] 458[b]
12	3.1 (3H), 3.6 (6H), 3.9 (2H), 8.1 (1H)	−7.8,−12.3	54.0 (CH_3, CH_2), 72.4 (3 CH_2), 139−164 (4 Ar), 170.6 (C═O)	263	−70.6, 25.7	229,[a] 408[b]
13	3.2 (3H), 3.6 (6H), 3.8 (2H), 8.3 (1H), 8.8 (1H)	−7.5,−13.6	55.0 (CH_3, CH_2), 72.1 (3 CH_2), 131−157 (5 Ar)	302	−69.0, 23.4	176,[a] 396[b]
14	3.2 (3H), 3.5 (6H), 3.7 (2H), 4.6 (1H), 7.5 (1H)	−8.5,−14.1	55.3 (CH_3, CH_2), 72.5 (3 CH_2), 100−162 (4 Ar)	288	−72.6, 30.2	190,[a] 500[b]

[a] Temperature at which 10% mass loss was detected. [b] Temperature at which 50% mass loss occurred.

purine or pyrimidine side groups together with glycine ethyl ester, alanine ethyl ester, or ethylene glycol methyl ether units to maintain the solubility of the final products. The molecular structure of each polymer was studied with the use of multinuclear NMR and DRIFT techniques. Molecular weights and thermal properties were examined by the use of GPC, DSC, and TGA methods. Hydrolysis studies confirmed the hydrolytic sensitivity of the polyphosphazenes through the detection of molecular weight decline and weight loss analysis. The presence of phosphates from the polymer backbone was evident from ^{31}P NMR analyses.

Experimental Section

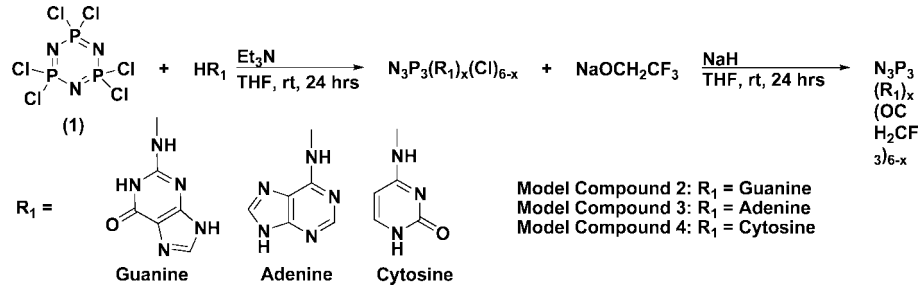
Reagents and Equipment. All reactions were carried out under a dry argon atmosphere using standard Schlenk line techniques. Tetrahydrofuran (EMD) and triethylamine (EMD) were dried using solvent purification columns.[21] Adenine, cytosine, guanine, glycine ethyl ester hydrochloride (Aldrich), and alanine ethyl ester hydrochloride (ChemImpex) were used as received. Ethylene glycol methyl ether and 2,2,2-trifluoroethanol (Aldrich) were distilled from CaH_2 and were stored under argon. Poly(dichlorophosphazene) was prepared by the thermal ring-opening polymerization of recrystallized and sublimed hexachlorocyclotriphosphazene (Fushimi Chemical Co., Japan) in evacuated Pyrex tubes at 250 °C. ^{31}P, ^{13}C, and ^{1}H NMR spectra were obtained with use of a Bruker 360 WM instrument operated at 145, 90, and 360 MHz, respectively. Glass transition temperatures were measured with a TA Instruments Q10 differential scanning calorimetry apparatus with a heating rate of 10 °C/min and a sample size of ca. 10 mg. Thermogravimetric analyses were performed using a Perkin-Elmer TGA 7 thermogravimetric analyzer. Samples of ca. 10 mg were heated at 10 °C/min in a nitrogen atmosphere. Gel permeation chromatograms were obtained using a Hewlett-Packard HP 1100 gel permeation chromatograph equipped with two Phenomenex Phenogel linear 10 columns and a Hewlett-Packard 1047A refractive index detector. The samples were eluted at 1.0 mL/min with a 10 mM solution of tetra-*n*-butylammonium nitrate in THF. The elution times were calibrated with polystyrene standards. DRIFT samples were analyzed using a Digilab FTS 7000 spectrometer, with 32 scans per sample. pH measurements were obtained using a Beckman Φ 31 pH meter.

Synthesis of Model Compounds 2−4. Model compounds **2−4** were synthesized in a similar fashion. The preparation of compound **2** is described as an example. Hexachlorocyclotriphosphazene (2.00 g, 5.75 mmol) was dissolved in THF (50 mL). Guanine (2.61 g, 17.3 mmol) and triethylamine (1.81 g, 17.8 mmol) were added simultaneously to the solution. This mixture was stirred at room temperature for 24 h. In a separate reaction vessel, sodium hydride (60%) (1.38 g, 34.5 mmol) was suspended in THF (100 mL) and 2,2,2-trifluoroethanol (3.51 g, 35.1 mmol) was added to the suspension. After complete addition of the alcohol, the resultant alkoxide salt solution was added to the hexachlorocyclotriphosphazene/guanine solution. The mixture was then stirred at room temperature for 24 h. Filtration and solvent removal under vacuum yielded a white solid. The product was then recrystallized from acetone, and its molecular structure studied by ^{31}P and ^{1}H NMR techniques as well as by mass spectrometry. The product yields were in the range of 64−68%.

Synthesis of Polymers 6−11. Polymers **6−11** were synthesized in a similar manner, with the synthesis of polymer **7** described as an example. Poly(dichlorophosphazene) (2.00 g, 0.0345 mol) was dissolved in 200 mL of dry THF. Glycine ethyl ester hydrochloride (2.65 g, 0.0190 mol) was suspended in 100 mL of dry THF, and triethylamine (4.19 g, 0.0414 mol) was added. This suspension was refluxed for 24 h, then filtered, and added dropwise to the polymer solution. The mixture was stirred at room temperature for 24 h, and the progress of the reaction was monitored by ^{31}P NMR spectroscopy. Triethylamine (4.19 g, 0.0414 mol) and adenine (4.66 g, 0.0345 mol) were then added simultaneously to the polymer solution. The mixture was stirred at room temperature for 24 h, and the reaction was monitored by ^{31}P NMR spectroscopy. After complete replacement of the chlorine atoms was confirmed, the mixture was centrifuged (10K rpm) for 30 min, and the solution was decanted, concentrated in vacuo, and precipitated into hexanes. Polymer **7** was purified by dialysis against methanol (3 days) and, after solvent removal, was isolated as a white, adhesive polymer (yield of 76%). Characterization data for polymers **6−11** are shown in Table 1.

Synthesis of Polymers 12−14. Polymers **12−14** were synthesized in a similar manner, and the synthesis of polymer **12** is described. Poly(dichlorophosphazene) (2.00 g, 0.0345 mol) was dissolved in 200 mL of dry THF. Sodium hydride (60% in mineral oil) (0.690 g, 0.0173 mol) was suspended in 100 mL of dry THF, and the mixture was cooled to 0 °C before diethylene glycol methyl ether (2.16 g, 0.0180 mol) was added slowly. After complete addition of the diethylene glycol methyl ether, the alkoxide salt solution was slowly warmed to room temperature and was stirred for 24 h. The solution of the side group reagent was then added dropwise to the polymer solution, and the resulting mixture was stirred at room temperature for 24 h as the reaction was monitored by ^{31}P NMR spectroscopy. Triethylamine (7.68 g, 0.0759 mol) and solid adenine (4.66 g, 0.0345 mol) were then added simultaneously to the polymer solution. The final polymer mixture was stirred at room temperature for 24 h and then refluxed for 24 h while monitored by ^{31}P NMR spectroscopy. After complete replacement of the chlorine atoms was confirmed, the mixture was centrifuged (10K rpm) for 30 min, and the solution was decanted, concentrated under vacuum, and precipitated into hexanes. Polymer **12** was purified by dialysis against methanol (3 times) and was isolated as

Scheme 1. Synthesis of Small Molecule Model Compounds 2−4



a light brown, adhesive polymer (yield 82%). Characterization data for polymers **12**−**14** are shown in Table 1.

Hydrolysis Studies of Polymers 6−14. Polymers **6**−**14** (200 mg) were dissolved in 2 mL of chloroform (10% solution g/mL). Films were solution-cast and air-dried for 24 h and then dried under reduced pressure (30 mTorr) for 7 days. Each film had a thickness of ∼500 μm. Samples were distributed into 15 different test tubes containing 10 mL of deionized water (pH = 6.0). The tubes were contained in a constant shaker bath at 37 °C. Three samples were removed at the time points 7, 14, 21, 28, and 35 days. The weight loss and molecular weight declines were monitored for each solid sample. In addition, the pH of each hydrolysis medium was analyzed. ^{31}P and ^{1}H NMR techniques were utilized to follow the formation of any small molecule hydrolysis products dissolved in the aqueous medium. Silver nitrate and ninhydrin tests were employed to qualitatively determine the presence of phosphates and ammonia and/or amino acids, respectively.

Results and Discussion

Synthesis of Model Compounds 2−4. Hexachlorocyclotriphosphazene (**1**) was utilized as a small molecule model for the introduction of the appropriate purine or pyrimidine. Initially, attempts were made to produce a cyclic phosphazene that contained six covalently linked purine or pyrimidine substituents. Thus, the reaction of hexachlorocyclotriphosphazene with an excess of guanine, adenine, or cytosine in the presence of triethylamine was monitored with ^{31}P NMR techniques. Analysis of the reaction mixtures after 24 h at ambient temperature showed peaks indicative of several different degrees of substitution, but no fully substituted compound. The solution was then refluxed in THF for 24 h, during which time the products precipitated from solution, and no phosphorus nuclei were detected by ^{31}P NMR analysis due to the insolubility of the product in the solvent. Triethylamine hydrochloride salts were extract with boiling methanol, and the residue was analyzed by ^{1}H and ^{31}P NMR techniques. However, the cyclic phosphazene species remained insoluble in organic solvents.

To monitor the reaction pattern before the product became insoluble, a cosubstituent synthesis reaction was utilized, with an initial addition of a limited amount of the appropriate purine or pyrimidine, followed by use of a second nucleophile to replace the remaining chlorine atoms. Thus, after the initial reaction had proceeded for 24 h under ambient conditions, an alkoxide salt solution of 2,2,2-trifluoroethanol was added, as outlined in Scheme 1. This completed the replacement of all the chlorine atoms. These trial reactions were carried out to ensure that only the primary amino function of the purine or pyrimidine was covalently linked to the phosphorus atoms in the cyclic model species. Analysis of the products showed that di- to tetrasubstitutions by the purine or pyrimidine had occurred. This was confirmed by mass spectral analysis that showed the presence of various substituted phosphazene rings. Infrared spectral analysis presented additional evidence that the primary amino function had reacted with the phosphazene core through the loss of the primary amino vibrational shift.

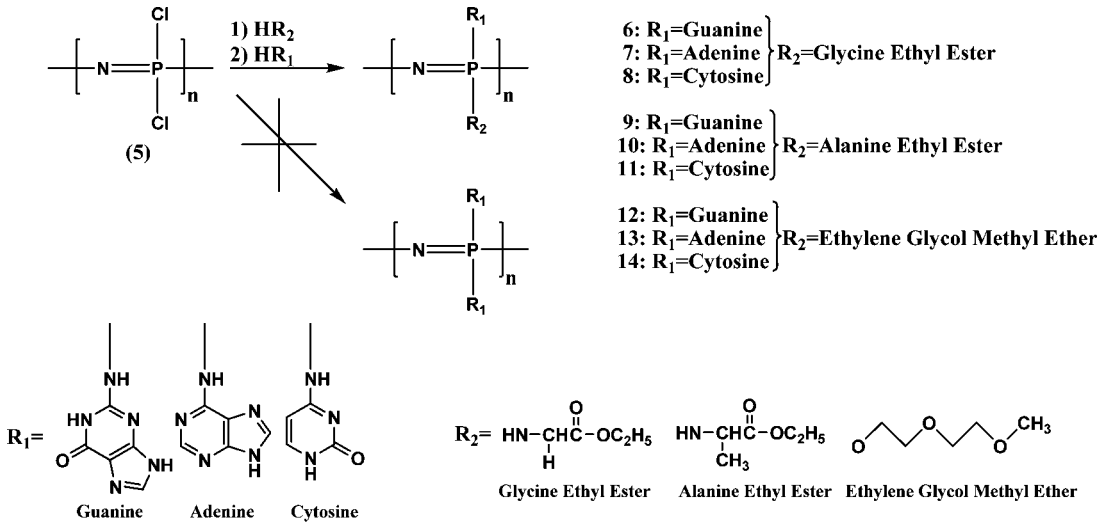
Synthesis and Structural Characterization of Polymers 6−14. All the polymers were synthesized via macromolecular substitution (i.e., chlorine replacement) of poly(dichlorophosphazene). The initial attempts focused on the synthesis of polyphosphazenes that bear only purine or pyrimidine side groups. However, after 24 h of reaction at room temperature, ^{31}P NMR analysis showed that only ∼70% of the chlorine atoms in the polymer had been replaced. Moreover, after reflux of the solution in THF for 24 h, no phosphorus nuclei could be detected in solution due to precipitation of the product. Changes in the solvent system (to dioxane or benzene) did not improve the solubility of either the intermediate or the final products. The precipitated solids were insoluble in water, DMSO, ethanol, methanol, chloroform, dichloromethane, DMF, methyl ethyl ketone, acetone, acetontrile, glyme, and diglyme. Thus, it was concluded that the insoluble species formed during the final 30% of chlorine replacement were unsuitable for further investigation.

The problems of product insolubility with single-substituent polyphosphazenes led to a focus on mixed-substituent polyphosphazenes that contained both a purine or pyrimidine side group and glycine ethyl ester, alanine ethyl ester, or ethylene glycol methyl ether side groups. During the synthesis of these polymers, a stoichiometric amount of the amino acid ester or ethylene glycol methyl ether was added first to polymer **5** to maintain the solubility of the intermediate, and then an excess of purine or pyrimidine and triethylamine was added to complete the substitution, as described in Scheme 2.

To ensure that the side groups were randomly distributed along the polymer backbone, the amino acid ester or alkoxy ether, R_2, was added dropwise to the poly(dichlorophosphazene) solution. The random substitution of R_2 was monitored by ^{31}P NMR spectroscopy. Polymers **6**−**11** contained 50% glycine ethyl ester or 50% alanine ethyl ester and showed a single chemical shift at approximately −8 ppm after the addition of the amino acid ester side group. This corresponds to phosphorus atoms with one chlorine atom and one organic side group. Subsequent addition of the purine or pyrimidine yielded a single peak in the ^{31}P NMR spectrum at ∼0 ppm for polymers **6**−**11**. Thus, the NMR evidence indicated that all the chlorine atoms had been replaced. A single glass transition temperature (T_g) was detected for polymers **6**−**11**, which supports the existence of a randomly substituted polymer. ^{1}H and ^{13}C NMR techniques were used to confirm the presence and the composition of the side groups on the polyphosphazene backbone (Table 1). Analysis of 2D NMR experiments comparing ^{1}H and ^{31}P support the conclusion that attachment of the purine or pyrimidine substituent occurred only through the primary amino site.

By contrast, polymers **12**−**14** had a different substitution pattern. After the addition of the methoxyethoxyethoxide sodium salt to poly(dichlorophosphazene), the polymers showed two ^{31}P NMR shifts at −9 and −18 ppm. This suggests the presence of some phosphorus atoms with two organic side groups and others with two remaining chlorine atoms in an approximately 1:1 ratio. Subsequent addition of the appropriate purine or

Scheme 2. Structures of Polymers 5−14[a]



[a] The ratio of purine or pyrimidine to glycine ethyl ester, alanine ethyl ester, and ethylene glycol methyl ether was maintained at a 1:1 ratio.

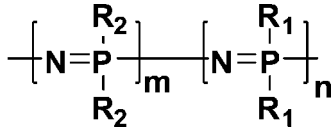


Figure 1. Proposed block structure of polymers **12−14**.

pyrimidine, together with triethylamine, completed the substitution. The final ^{31}P NMR spectra contained two peaks (Table 1), with chemical shifts that would be expected from a polyphosphazene with a block substitution pattern, as shown in Figure 1. This phenomenon has been seen before in our laboratory when methoxyethoxyethoxy side groups are linked to the skeleton first and are then followed by a second organic substituent. It may be connected with the tendency for the initial alkyl ether substitution reaction to undergo gelation, followed by degelation as more nucleophile is added. The gelation process could reflect an initial labile ionic cross-linking between sodium ions from the sodium alkoxide and the oxygen atoms in the side groups. Subsequent precipitation of sodium chloride as the reaction proceeds would then remove the cross-link sites and allow the polymer to redissolve. The ionic cross-linking of MEEP though lithium ions is well established. However, the details of how this process might lead to a block substitution pattern has yet to be established, and the average length of each block segment (*n* and *m*) is not yet known.

Verification of the level of purine and pyrimidine incorporation into the polyphosphazene structure via the primary amino function was obtained from diffuse reflectance infrared Fourier transform (DRIFT) techniques, with verification of the loss of the primary amine absorption by the side group at 3500 cm^{-1}. This confirmed that the purine and pyrimidine substituents had been linked to polymer **1** through the primary amino, rather than through a secondary amino unit.

Thermal Characterization of Polymers 6−14. The glass transition temperatures (T_g) of polymers **6−14** are shown in Table 1. The relatively low glass transition temperatures of polymers **6−8** are probably caused by the presence of glycine ethyl ester side groups. Poly[bis(ethyl glycinato)phosphazene] has a glass transition temperature of −20 °C.[10] Polymers **9−11** show a similar trend because poly[bis(ethyl alanato)phosphazene] (PNEA) has a glass transition temperature at −15 °C, and polymers **9−11** have similar glass transition temperatures to PNEA.[10]

Polymers **12−14** had two distinct thermal transitions, which supports the ^{31}P NMR data, and strongly suggests the presence of a block substitution pattern. The lowest glass transition temperatures were measured from the set of polymers, **12−14**, that contain diethylene glycol methyl ether units. Poly[bis(methoxyethoxyethoxy)phosphazene] has a T_g of −80 °C.[22] The higher glass transition temperatures, at 25, 23, and 30 °C for polymers **12−14**, respectively, are associated with the purine- or pyrimidine-substituted segment of the macromolecules. Polymers **12−14** also showed evidence of melting temperatures at −37 and −27 °C. The melting temperatures are possibly indicative of interactions of the purine or pyrimidine side groups, since poly[bis(methoxyethoxyethoxy)phosphazene] is completely amorphous. The block nature of polymers **12−14** is almost certainly responsible for the melting transition. The higher degree of molecular order within the purine or pyrimidine blocks of polymers **12−14**, coupled with their low solubility, could explain the difficulties encountered with the synthesis of single-substituent purine or pyrimidine polyphosphazenes. Thermal decomposition temperatures (T_d) for polymers **6−14** are shown in Table 1. Polymers **11** and **12** proved to be the most thermally stable species, with a 10% mass loss detected at 263 and 258 °C, respectively. However, it is recognized that T_{10} measurements are inaccurate measures of thermal stability. Polymer **7** had a 50% mass loss above 700 °C, which is higher than the detection limit of the instrument. These data provide some support for the idea that polymers **6−14** are stable at moderate temperatures and are more thermally stable than some classical organic polymers.

Hydrolytic Degradation of Polymers 6−14. Polymers **12−14** were soluble in water. The media that contained the dissolved polymers remained at a pH of 5.5 throughout each experiment. The ^{1}H and ^{31}P NMR analyses showed no evidence of hydrolysis over the 35 day period at 37 °C. Thus, polymers **12−14** appear to be essentially hydrolytically stable in solution, at least during this period of time.

Solution-cast films of water-insoluble polymers **6−11** were analyzed for hydrolysis over a 35 day period. The initial weight of each film was recorded prior to submersion in water. The samples were removed at specific time points, and the films were dried in vacuo for a minimum of 1 week. Figure 2 shows that polymers **6−8** all underwent a significant weight loss during 7 days. Polymer **8** is the only polymer that retained more than 50% of its original weight after 7 days. After 35 days, films of

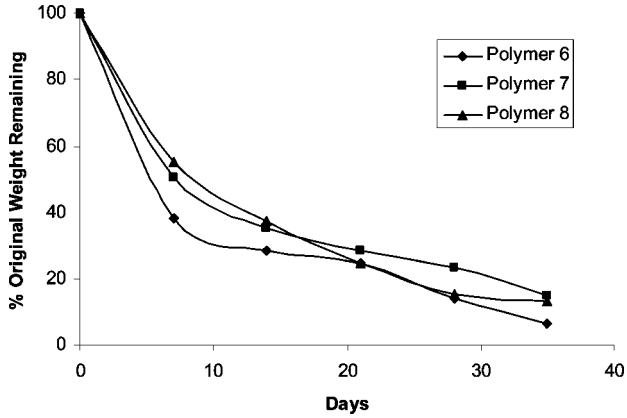


Figure 2. Weight loss profile during hydrolysis of polymers **6**−**8** over a 35 day period.

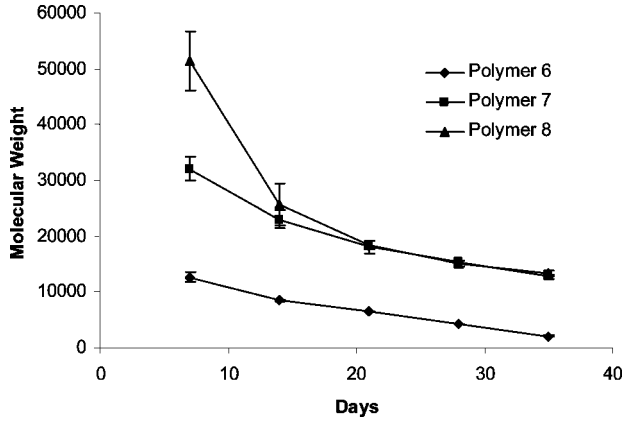


Figure 3. Molecular weight (M_w) decline of polymers **6**−**8** over 35 days. The polymers had the initial molecular weights at day zero, as shown in Table 1.

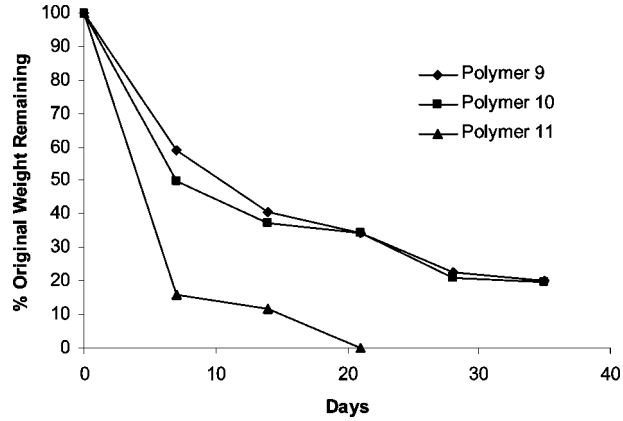


Figure 4. Weight loss profile during hydrolysis of polymers **9**−**11** over a 35 day period.

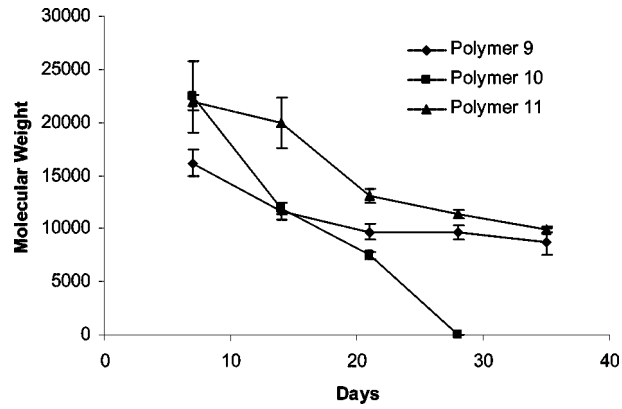


Figure 5. Molecular weight (M_w) decline of polymers **9**−**11** over 35 days. All the polymers had the initial molecular weight at day zero, as represented in Table 1.

polymers **7** and **8** had lost 80% of their initial weight, while polymer **6** had lost more than 90% of its weight. The molecular weight of each polymer declined in similar way (Figure 3). Polymer **6** lost over 90% of its initial molecular weight within 7 days, with a decline in M_w from 103 000 to 10 300 g/mol after 7 days. Polymers **7** and **8** had lost 70% and 50%, respectively, of their initial molecular weights during the same time.

A comparison between the hydrolysis of polymers **9**−**11** and polymers **6**−**8** revealed some interesting conclusions. The best example is the comparison between polymers **8** and **11**. Polymer **11** hydrolyzed completely during 21 days, and no solid material was recovered from the medium after this time. This polymer had lost almost 85% of its original weight after 7 days (Figure 4) in deionized water at 37 °C, whereas polymer **8** still retained 55% of its original mass (Figure 3). Polymer **8** underwent a molecular weight decline to 52 kg/mol, which was about 50% of the original molecular weight, as shown in Figure 4, whereas polymer **11** had lost over 80% of its initial molecular weight, down to 22 kg/mol (Figure 5). This is an unexpected result because previous work had shown that polyphosphazenes that contain alanine residues degrade at a slower rate than glycine-containing polyphosphazenes.[10,11] The films of polymers **9**−**11** swelled considerably when immersed in deionized water. Thus, a higher water uptake occurred for polymer **11** than with polymer **8**, and this increased water uptake might explain the increased hydrolysis rate.

The hydrolysis media were analyzed in an attempted to assess the degradation mechanism for polymers **6**−**11**. ^{31}P NMR spectroscopy was used to identify the presence of phosphates dissolved in the aqueous medium. After 7 days of hydrolysis, phosphates were detected from a sharp peak at 0 ppm from the aqueous media that contained polymers **6**−**11**. These conclusions were reinforced by the addition of silver nitrate, which resulted in a yellow precipitate of silver phosphate. ^{1}H NMR analysis of the hydrolysis medium for polymers **6**−**11** showed evidence for the free amino acid and ethanol after 7 days, followed by detection of the respective purine or pyrimidine base after 14 days. A positive ninhydrin test was positive for the presence of ammonia and/or the amine terminus of the amino acid.

This suggests the existence of a bulk erosion mechanism in which the amino acid ester is cleaved from the polyphosphazene backbone first to give the amino acid and ethanol. It was not possible to determine if the amino group cleaved first from the backbone or if the ester group was hydrolyzed first. Cleavage of the backbone is suggested by the presence of phosphates in solution, and this could explain the significant molecular weight loss shown in Figures 3 and 5. The weight loss shown in Figures 2 and 4 also reinforces this argument because the dramatic weight loss at each time point was accompanied by a significant uptake of water into the polymer films.

The pH of each medium was analyzed (Figures 6 and 7) and showed a slight decrease in pH of the solutions in contact with polymers **7**−**11** from approximately pH 5.5 to 4.7. Phosphates and ammonia are released during the hydrolysis of these polymers, and this causes the solutions to become buffered. The pH values measured were within the range of an ammonium phosphate buffer solution. However, the hydrolysis of polymer **6** was unusual because it caused a significant decrease in pH to

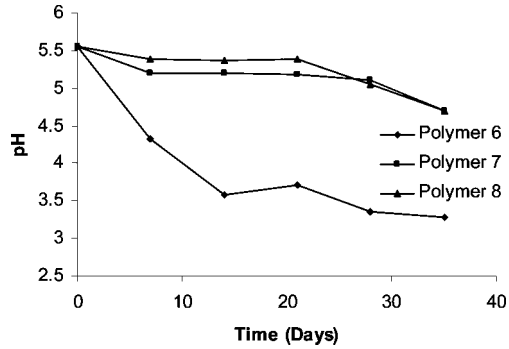


Figure 6. pH measurements of hydrolysis media of polymers **6**−**8**.

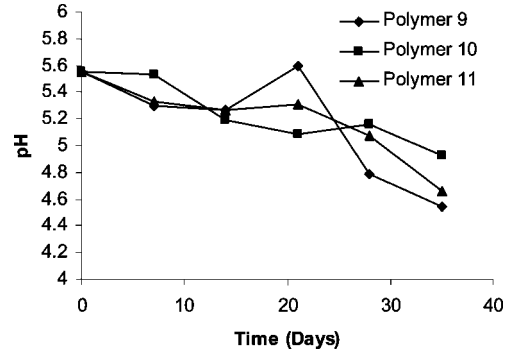


Figure 7. pH measurements of hydrolysis media of polymers **9**−**11**.

below 3.5. We attribute this anomaly to the presence of small amounts of hydrogen chloride complexed with the basic components of the polymer—either the backbone nitrogen atoms or the side groups. The removal of hydrochloride salts after aminolysis reactions with phosphazenes is difficult due to the basicity of the phosphazene which competes with triethylamine for the hydrogen chloride released during the substitution reactions. This problem is well-known from small molecules model compound studies.[1] Thus, it is conceivable that such a salt might not be removed completely during the aqueous dialysis of the final product. This would cause traces of hydrochloric acid to be liberated during aqueous hydrolysis. If this is the cause of the acidic medium, only one molecule of HCl for ∼213 polymer repeat units would be sufficient to explain the pH change, and this low concentration would not be detected by ^{1}H NMR analysis of the purified polymer.

Conclusions

To the best of our knowledge this is the first reported synthesis of poly(organophosphazenes) that contain guanine, adenine, or cytosine side groups. This study was preceded by a model compound study in which a mixture of di- to tetrasubstitution of hexachlorocyclotriphosphazene was achieved with the purine or pyrimidine bases. Polymeric products were obtained via the replacement of the chlorine atoms in poly(dichlorophosphazene) by the purine or pyrimidine bases plus cosubstituents such as as glycine ethyl ester, alanine ethyl ester, or diethylene glycol methyl ether. The mixed-substituent polymers had low glass transition temperatures and good thermal stability for amino acid-substituted polymers. The polymers with alkyl ether cosubstituents were water-soluble but showed no detectable hydrolysis after 35 days. With the exception of polymer **6**, polyphosphazenes with purine or pyrimidine side groups plus amino acid ester cosubstituent groups were hydrolytically sensitive but maintained a consistent pH in the hydrolysis medium. Polymer **6** proved difficult to purify from traces of hydrogen chloride bound as a salt complex. These polymers can be tuned for different rates of hydrolytic degradation in ways that may prove useful in drug delivery or tissue engineering. Those polyphosphazenes with ethylene glycol methyl ether side groups and the purine or pyrimidine base had a block substitution pattern that was identified by ^{31}P NMR and DSC techniques.

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