Poly(organophosphazenes) with Oligopeptides as Side Groups: Prospective Biomaterials

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ABSTRACT: Poly(organophosphazenes) with pendent primary amino groups have been synthesized. The sodium salt of 2-(2-aminoethoxy)ethanol, in which the amino group was selectively protected, was allowed to react with poly(dichlorophosphazene). The resulting polymer was amino-deprotected with 80% trifluoroacetic acid to yield the [2-(2-aminoethoxy)ethoxy]phosphazene polymer. The cosubstituent polymer $[NP(OCH_2CF_3)(OCH_2CH_2OCH_2CH_2NH_2)]_n$ was also synthesized in a similar manner via the sequential treatment of poly(dichlorophosphazene) with sodium trifluoroethoxide and sodium 2-[2-((*tert*-butoxycarbonyl)amino)-ethoxy]ethoxide. The tripeptides Gly-Pro-Gly and Gly-Val-Ala were assembled at the polymer amino side group termini by step-by-step coupling reactions. The physical and thermal properties of the resultant polymers were examined. Model reactions were also carried out with the use of cyclotriphosphazene small-molecule systems.

Poly(organophosphazenes)[1-6] have a wide potential as biomaterials for the replacement of living tissues and as carriers for biologically active agents. The advantages in the use of these polymers come mainly from the wide range of physical properties than can be generated by variations in side groups. Many of the polymers prepared in our program are biostable, flexible, and durable, while a few are hydrolytically unstable and biodegradable.[7] In addition, polymers with substituents that have latent functionality may be employed in further reactions with biologically interesting compounds to provide biological activity or biocompatibility.[3]

Biocompatibility of materials requires the absence of thrombogenic, toxic, allergic, inflammatory, or carcinogenic reactions.[8] Few of the existing synthetic polymers meet all these requirements. Biopolymers such as collagen and heparin are often used along with synthetic polymers to improve biocompatibility.[9] Recently, synthetic polypeptides have attracted attention as biomaterials in spite of their high cost and synthetic difficulties when prepared on a large scale. Polypeptides with widely varying properties can be obtained synthetically from the 20 common α-amino acids. Some of these may mimic natural tissues.[10,11]

In the present work, we have explored a novel way to obtain stable functional poly(organophosphazenes) that bear primary amino groups. These functional sites formed the starting point for the assembly of Gly-Pro-Gly and Gly-Val-Ala side group sequences. These sequences are found as major components of elastin, a protein that is present in arteries, lungs, and skin.[12] The primary structure of elastin contains repeats of a tetrapeptide, Gly-Gly-Val-Pro, a pentapeptide, Pro-Gly-Val-Gly-Val, and a hexapeptide, Pro-Gly-Val-Gly-Val-Ala.[13] Recently, the polypentapeptide $(Val\text{-}Pro\text{-}Gly\text{-}Val\text{-}Gly)_n$ was reported as a model.[10] We were particularly interested in the step-by-step coupling reactions of amino acids with polyphosphazenes for comparison with liquid-phase peptide syntheses[14] that allow facile control over the amino acid sequence.

Results and Discussion

Synthesis of Spacer Unit. The *tert*-butoxycarbonyl (Boc) group is widely used for protecting amines since it decomposes readily by treatment with acid and remains intact under basic conditions.[15] The protected amino alcohol **1** was prepared as a substituent by the reaction of 2-(aminoethoxy)ethanol with di-*tert*-butyl dicarbonate (Scheme I). Species **1** was isolated as an oil by vacuum distillation and was characterized by ^1H NMR and elemental analysis. No reaction of the hydroxyl group was detected when equimolar amounts of amino alcohol and di-*tert*-butyl dicarbonate were used.

Model Compound Studies. Compound **1** was allowed to react with either sodium metal or sodium hydride in tetrahydrofuran to give sodium 2-[2-((*tert*-butoxycarbonyl)amino)ethoxy]ethoxide. This sodium salt was then allowed to react with monochloropentaphenoxycyclotriphosphazene under homogeneous conditions at room temperature. After 4 h of reaction, the ^{31}P NMR spectrum contained a new AB_2 spin system centered at 9.0 and 12.6 ppm (J = 87.8 Hz)(Scheme I). The remaining sodium salts were removed by passing the reaction mixture through silica gel. Compound **3** was isolated as an oil by column chromatography. Species **3** was then treated with 80% trifluoroacetic acid to remove the Boc group. After adjustment of the reaction mixture to pH 9 with a 5 N NaOH solution, the product was extracted with chloroform. The coupling reaction of compound **4** with *N*-Boc-glycine was carried out by use of an active ester method. The *N*-hydroxysuccinimide ester of *N*-Boc-glycine was prepared according to the literature.[16] A 50% excess of the active ester was used to complete the reaction. The remaining active esters were hydrolyzed by refluxing the reaction mixture in 50% ethanol, and *N*-hydroxysuccinimide was removed by washing with water. Product **5** was isolated as an oil by column chromatography. Characterization data for the products are summarized in Table I.

Substitution Reactions of the High Polymers. The reaction of poly(dichlorophosphazene) with the sodium salt of **1** was carried out in tetrahydrofuran at room temperature. ^{31}P NMR spectroscopy showed a singlet at −7.9 ppm after the mixture had been stirred for 24 h. Boc groups were removed by treatment with 80% trifluoroacetic acid. No significant decomposition was detected by ^{31}P NMR, which still showed a sharp singlet at −7.9 ppm. The cosubstituent polymer **11** was prepared by deprotection of amino groups in a similar manner after the sequential treatment of poly(dichlorophosphazene) with

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Scheme I

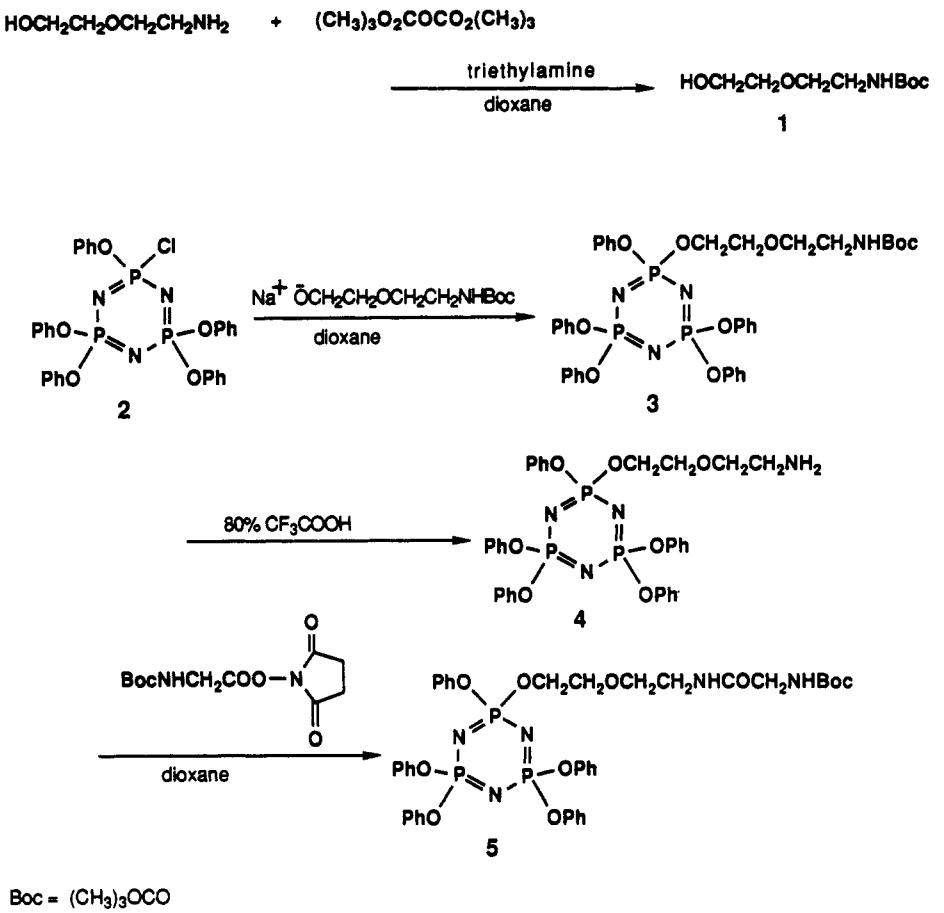


Table I
Cyclotriphosphazene Characterization Data

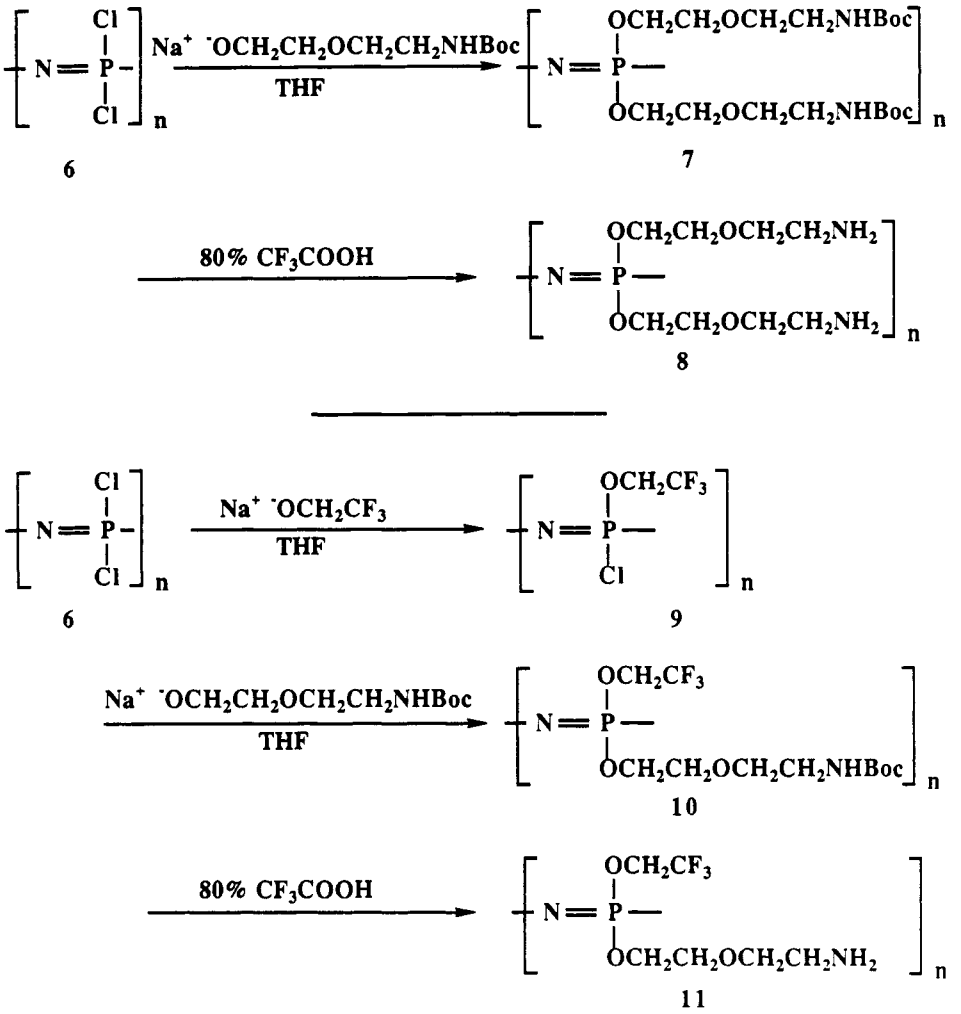
compd	^{1}H NMR,[a] ppm	^{31}P NMR,[a] ppm	elem anal., % found	elem anal., % calcd
$N_3P_3(OC_6H_5)_5(OCH_2CH_2OCH_2CH_2NHBoc)$ (3)	6.82–7.27 (m, 25 H, C_6H_5) 4.82 (s, 1 H, N*H*) 3.48 (m, 2 H, POCH_2) 3.31 (t, 2 H, CH_2OCH_2) 3.27 (t, 2 H, CH$_2$OCH_2) 3.18 (q, 2 H, CH_2N) 1.35 [s, 9 H, C(CH_3)$_3$]	ν_A = 12.6 ν_B = 9.0 J_{PNP} = 87.8 Hz	C, 58.10 H, 5.51 N, 6.90	C, 58.21 H, 5.39 N, 6.96
$N_3P_3(OC_6H_5)_5(OCH_2CH_2OCH_2CH_2NH_2)$ (4)	6.82–7.27 (m, 25 H, C_6H_5) 3.53 (m, 2 H, POCH_2) 3.30 (m, 4 H, CH_2OCH_2) 2.69 (t, 2 H, CH_2N)	ν_A = 12.5 ν_B = 9.0 J_{PNP} = 88.0 Hz	C, 57.33 H, 5.29 N, 7.68	C, 57.96 H, 5.01 N, 7.95
$N_3P_3(OC_6H_5)_5(OCH_2CH_2OCH_2CH_2NHCOCH_2NHBoc)$ (5)	6.82–7.27 (m, 25 H, C_6H_5) 6.29 (s, 1 H, N*H*COC) 5.17 (s, 1 H, N*H*COO) 3.62 (d, 2 H, COCH_2N) 3.49 (m, 2 H, POCH_2) (m, 4 H, CH_2OCH_2) 1.32 [s, 9 H, C(CH_3)$_3$]	ν_A = 12.8 ν_B = 8.9 J_{PNP} = 87.9 Hz	C, 56.95 H, 5.51 N, 8.02	C, 57.14 H, 5.38 N, 8.13

[a] The ^{1}H and ^{31}P NMR spectra were obtained in $CDCl_3$.

sodium trifluoroethoxide and the sodium salt of 1 (Scheme II).

Coupling Reactions of the Polymers with Amino Acids. A liquid-phase peptide synthesis method[14] was employed for the grafting of amino acids onto the side chains of polymers 8 and 11 (Scheme III). Since polymers 8 and 11 were not soluble in aprotic solvents, they were first dissolved in methanol, and the solutions were diluted with dimethylformamide. N-Protected amino acids were activated by using *N*-hydroxysuccinimide and dicyclohexylcarbodiimide.[16] A onefold excess of the active esters was used to complete the reactions. All coupling reactions proceeded under homogeneous conditions. The Boc groups of the polymers were removed by treatment with 80% trifluoroacetic acid, and the resultant polymers were isolated by dialysis against water and methanol. An important advantage of the liquid-phase peptide synthesis method over a solid-phase method is that each step of the coupling reaction can be monitored, and products of high purity are obtained because the reaction is carried out under homogeneous conditions. Figure 1 shows ^{1}H NMR spectra of polymers 8, 13, 15, and 17 in D_2O or methanol-d_4. The spectra of polymers 8 and 13 show relatively sharp peaks, and, as a side chain is lengthened via amide bonds,

Scheme II[a]



[a]Note that the mixed-substituent structures shown in 9–11 are schematic only. Geminally substituted units are probably also present.

the peaks become broad (Figures 1–3). These results can be attributed to a decrease of polymer chain flexibility by hydrogen bonding between the side chains, together with the high torsional barrier within the amide groups. The coupling reaction, as determined from the peak area ratios, was almost quantitative for the reaction of the glycine active ester. It was 70–80% efficient for the reaction of the proline active ester. ^{31}P NMR spectra obtained during the deprotection and coupling reactions showed singlets around −7.9 ppm. No signal that might be attributed to decomposition was observed.

The ^{1}H NMR spectral patterns of the cosubstituent polymers **11**, **19**, **21**, and **23** are similar to those of the corresponding monosubstituent polymers except for the peaks for trifluoroalkoxy groups at 4.4 ppm (Figure 2). In the ^{31}P NMR spectra of the polymers, the peaks appeared as singlets around 7.4 ppm. Here, too, no signal that might indicate decomposition was detected.

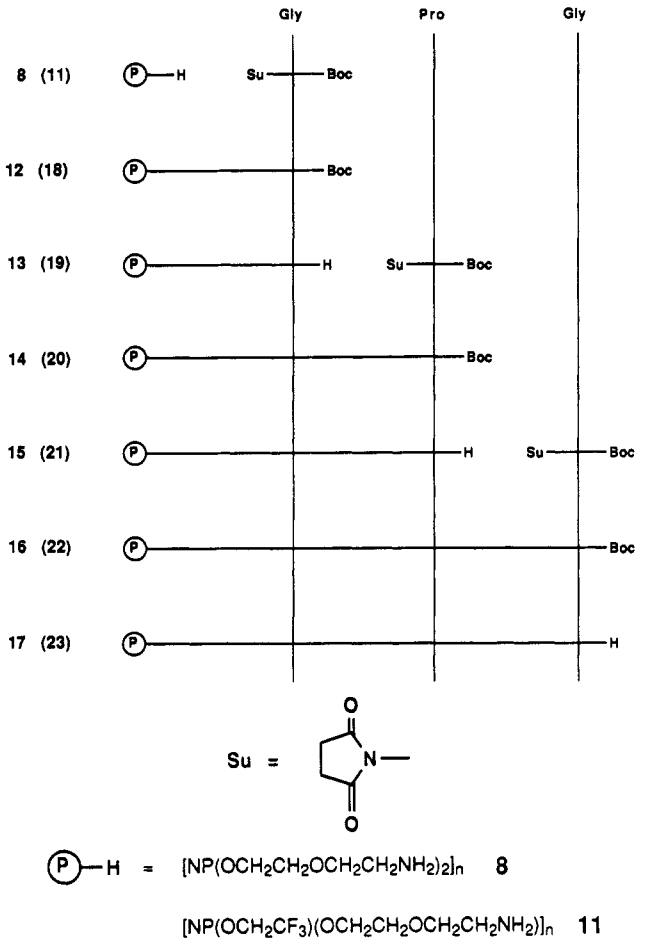
The coupling reactions of polymer **13** with the more sterically hindered amino acids valine and alanine were carried out in a similar manner (Scheme IV). The percent coupling efficiency, determined from the peak ratios, was 60–70% in the reaction of the valine active ester and almost quantitative for the reaction of the alanine active ester (Figure 3).

Properties of the Polymers. The protected polymers were used to determine the molecular weights. The results are summarized in Table II. Since the deprotection reaction was carried out under acidic conditions, chain cleavage was considered to be a possible side reaction. However, no decrease of molecular weight was detected after deprotection. These results are consistent with the ^{31}P NMR spectra of the polymers, in which the peak patterns remained unchanged during the coupling and deprotection reactions.

All the deprotected polymers were hygroscopic and soluble in methanol but were insoluble in tetrahydrofuran and dioxane. They were leathery at room temperature. They could be fabricated into films in the presence of traces of water or methanol. However, following complete drying by heating in vacuo, the polymers lost elasticity and became insoluble in methanol, probably due to hydrogen bonding. Polymers **8**, **11**, **13**, **15**, **17**, and **19** were fairly soluble in water. The other polymers dissolved slightly in water.

The glass transition temperatures (T_g) of polymers **8** and **11** were found to be much higher than that of $[NP(OCH_2CH_2OCH_2CH_2OCH_3)_2]_n$ (T_g − 75 °C),[6] which indicates an influence by hydrogen bonding between side groups or between a side group and nitrogen atoms on the polymer backbone (Table III). The T_g's of the deprotected polymers varied significantly according to sample history. The T_g of polymer **8** was −18 °C when the polymer was dried in vacuo at 35 °C for 4 days after dialysis against methanol. It increased to 8 °C after storage for 3 months under air at room temperature. The measured glass transition temperatures under the same conditions increased as the side chains lengthened. The deprotected

Scheme III



polymers could be kept as solutions in methanol for several months without decomposition.

Experimental Section

Materials and Instrumentation. Hexachlorocyclotriphosphazene (Ethyl Corp.) was purified by fractional vacuum sublimation at 60 °C (0.5 Torr). 2-(2-Aminoethoxy)ethanol (Aldrich) was vacuum distilled. Triethylamine was distilled over BaO. All the other reagents were purchased from Aldrich or Sigma and were used without further purification. Poly(dichlorophosphazene) was prepared by the thermal polymerization of $(NPCl_2)_3$ at 250 °C. An average of 40–50% conversion to the linear polymer was obtained. Proton-decoupled ^{31}P NMR spectra were obtained with the use of a JEOL FX-90Q or a Bruker WM-360 spectrometer. Chemical shifts were reported in ppm relative to 85% H_3PO_4 at 0 ppm. ^1H NMR spectra were recorded on a Bruker WM-360 spectrometer. Gel permeation chromatography was carried out with a Hewlett-Packard HP-1090 liquid chromatograph fitted with an HP-1037A refractive index detector and a Polymer Laboratories gel 10-μm column. Tetrahydrofuran with 0.1% tetra-*n*-butylammonium bromide was used as the eluent. Approximate calibration of the columns was accomplished by means of narrow molecular weight polystyrene standard obtained from Waters Associates. Glass transition temperatures were recorded with the use of Perkin-Elmer 7 thermal analysis equipment, with a heating rate of 10 °C/min under a nitrogen atmosphere. Elemental analyses (Tables I and III) were obtained by Galbraith Laboratories.

Preparation of 2-[2-((*tert*-Butoxycarbonyl)amino)ethoxy]ethanol (1). To a solution of 2-(2-aminoethoxy)ethanol (5.26 g, 0.05 mol) and triethylamine (7.00 mL, 0.05 mol) in dioxane (100 mL) was added dropwise a solution of di-*tert*-butyl dicarbonate (11.49 mL, 0.05 mol) in dioxane (50 mL) over 2 h at room temperature, and the reaction mixture was stirred for 15 h at the same temperature. The solvent was evaporated under reduced pressure, and the residue was dissolved in chloroform (60 mL).

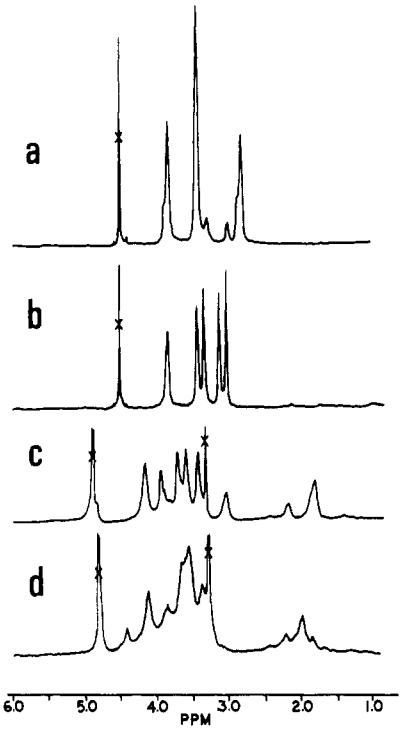


Figure 1. ^1H NMR spectra of polymers 8 in D_2O (a), 13 in D_2O (b), 15 in methanol-d_4 (c), and 17 in methanol-d_4 (d).

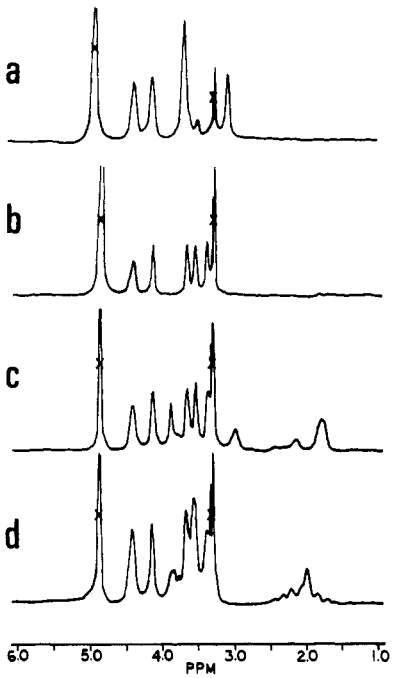


Figure 2. ^1H NMR spectra of polymers 11 (a), 19 (b), 21 (c), and 23 (d), all in methanol-d_4.

The solution was washed with water (2 × 30 mL) and dried over anhydrous magnesium sulfate. After filtration and evaporation, the desired product was isolated by column chromatography on silica gel, R_f 0.47 (17% ethanol in ethyl acetate), and was further purified by vacuum distilation, bp 128–132 °C at 0.2 mmHg (yield 55%).

^1H NMR ($CDCl_3$): δ 5.06 (s, 1 H, N*H*), 3.71 (t, 2 H, HOCH_2), 3.57 (m, 4 H, CH_2OCH_2), 3.31 (q, 2 H, CH_2N), 2.49 (s, 1 H, O*H*), 1.40 (s, 9 H, CCH_3).

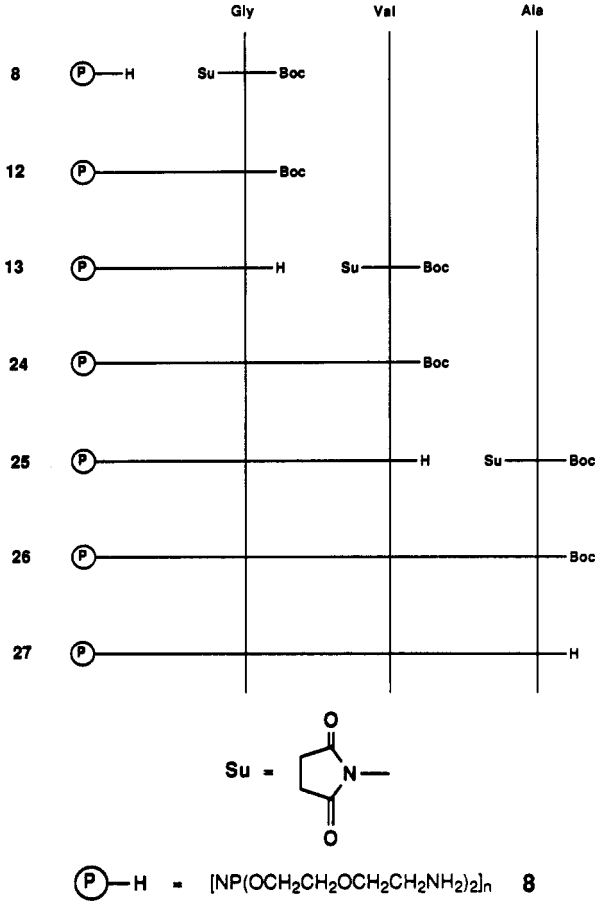
Anal. Calcd for $C_9H_{19}O_4N$: C, 52.67; H, 9.33; N, 6.82. Found: C, 52.55; H, 9.53; N, 6.60.

Preparation of $N_3P_3(OPh)_5(OCH_2CH_2OCH_2CH_2NHBoc)$ (3). $N_3P_3(OPh)_6Cl$ (2) was prepared by a published method.[17] To a solution of sodium hydride (60% dispersion, 12.5 mmol) in

Table II
Gel Permeation Chromatography Results

compd	$M_w \times 10^{-5}$	$M_n \times 10^{-4}$
$[NP(OCH_2CH_2OCH_2CH_2NHCOCH_2NHBoc)_2]_n$ (12)	7.2 7.5	4.9 5.0
$[NP(OCH_2CH_2OCH_2CH_2NHCOCH_2NHCO$-(N, $COCH_2NHBoc$)-$)_2]_n$ (16)		
$[NP(OCH_2CH_2OCH_2CH_2NHCOCH_2NHCOCHNHCOCHNHBoc)_2]_n$ (26) ($(CH_3)_2CH$, CH_3)	8.0	5.3
$[NP(OCH_2CF_3)(OCH_2CH_2OCH_2CH_2NHCOCH_2NHBoc)]_n$ (18)	15 16	24 23
$[NP(OCH_2CF_3)(OCH_2CH_2OCH_2CH_2NHCOCH_2NHCO$-(N, $COCH_2NHBoc$)-$)]_n$ (22)		

Scheme IV



tetrahydrofuran (100 mL) was added 1 (3.0 g, 14.6 mmol), and the mixture was stirred for 2 h at room temperature to form a pale yellow solution. To the solution was added dropwise a solution of **2** (3.8 g, 6.0 mmol) in tetrahydrofuran (30 mL). The reaction mixture was stirred for 30 h at 25 °C and was then filtered through a layer of silica gel (5-cm depth). Tetrahydrofuran was removed by evaporation under reduced pressure. The desired product was isolated as an oil by column chromatography on silica gel, R_f 0.23 (30% ethyl acetate in hexane) (yield 75%).

Preparation of $N_3P_3(OPh)_5(OCH_2CH_2OCH_2CH_2NH_2)$ (4). Compound **3** (2.41 g, 3 mmol) was dissolved in trifluoroacetic acid (10 mL), and the resultant solution was stirred for 30 min at room temperature. The solution was then made alkaline (pH 9) with a 5 N NaOH solution while cooled in an ice bath. The product was extracted with chloroform (2 × 60 mL). The organic layer was washed with water (2 × 50 mL) and dried over anhydrous magnesium sulfate. After filtration and evaporation, the desired product was obtained as an oil (yield 75%).

Preparation of $N_3P_3(OPh)_5(OCH_2CH_2OCH_2CH_2NHC(O)CH_2NH_2)$ (5). The *N*-hydroxysuccinimide ester of *N*-(*tert*-butoxycarbonyl)glycine was prepared by reaction of the corresponding amino acid with *N*-hydroxysuccinimide in the presence of dicyclohexylcarbodiimide.[16] To a solution of **4** (2.8 g, 4 mmol) and triethylamine (0.56 mL, 4 mmol) in dioxane (50 mL) was added the active ester (1.6 g, 6 mmol) in one portion, and the reaction mixture was stirred for 30 h at room temperature. After evaporation of the solvent, the residue was dissolved in 50% ethanol (50 mL), and the solution was refluxed for 30 min. The solvent was removed by evaporation under reduced pressure, and the resultant residue was dissolved in chloroform (100 mL). The solution was washed with water (2 × 50 mL) and dried over anydrous magnesium sulfate. After filtration and evaporation, the oily residue was purified by column chromatography on silica gel to give the desired product as an oil, R_f 0.30 (30% ethyl acetate in hexane) (yield 72%).

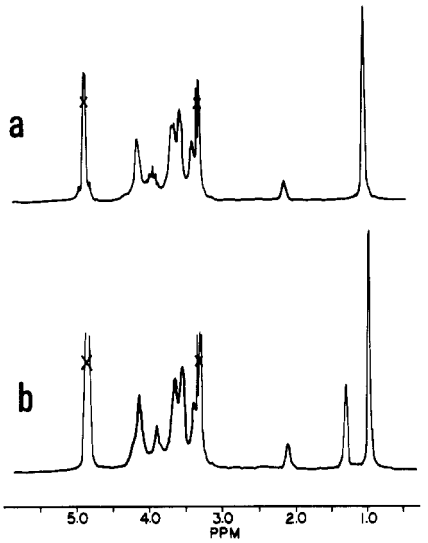
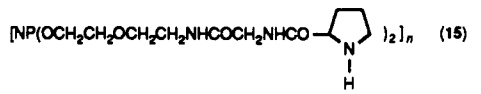
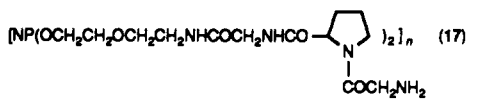
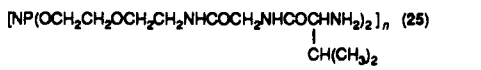
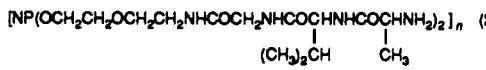
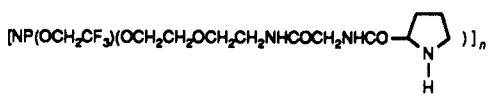
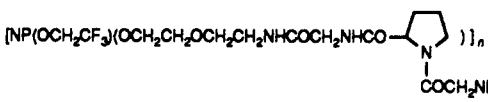


Figure 3. ^1H NMR spectra of polymers **25** (a) and **27** (b), both in methanol-d_4.

Synthesis of $[NP(OCH_2CH_2OCH_2CH_2NHBoc)_2]_n$ (7). A mixture of 1 (4.0 g, 19.5 mmol) and sodium metal (0.3 g, 13.0 mmol) in tetrahydrofuran (100 mL) was stirred for 2 days at 30 °C to form a pale yellow solution. To the solution was added dropwise a solution of poly(dichlorophosphazene)(0.3 g) in tetrahydrofuran (30 mL), and the reaction mixture was stirred for 60 h at room temperature. The polymer was precipitated in hexane and was further purified by precipitation from tetrahydrofuran into water (yield 80%).

Preparation of $[NP(OCH_2CH_2OCH_2CH_2NH_2)_2]_n$ (8). A mixture of polymer **7** (2.0 g) in 80% trifluoroacetic acid (50 mL) was stirred for 2 h at room temperature to yield a clear solution. This solution was stirred for an additional 24 h at the same temperature and was then cooled in an ice bath and adjusted to pH 9 with a 5 N NaOH solution. The aqueous solution was dialyzed against water for 3 days and against methanol for 1 day by using a cellulose membrane (MW cutoff 6000–8000) and was concentrated to dryness under reduced pressure to give the desired polymer.

Table III
Poly(organophosphazene) Characterization Data

compd	yield, %	elem anal.,[a] % found	elem anal.,[a] % calcd[b]	^{31}P NMR,[c] ppm	T_g, °C
$[NP(OCH_2CH_2OCH_2CH_2NH_2)_2]_n$ (8)	55	C, 35.92 H, 6.36 N, 14.45 Cl, 0.02	C, 37.95 H, 7.96 N, 16.60 Cl, 0.00	−7.9 (s)	−18
$[NP(OCH_2CH_2OCH_2CH_2NHCOCH_2NH_2)_2]_n$ (13)	62	C, 39.49 H, 7.27 N, 14.93	C, 39.24 H, 7.13 N, 19.06	−7.9 (s)	7
 (15)	60	C, 42.25 H, 7.02 N, 14.90	C, 47.05 H, 7.18 N, 17.46	−7.9 (s)	29
 (17)	64	C, 40.70 H, 6.67 N, 15.09	C, 46.22 H, 6.86 N, 18.66	−7.9 (s)	62
 (25)	65	C, 40.18 H, 6.62 N, 13.20	C, 46.72 H, 7.84 N, 17.33	−7.9 (s)	67
 (27)	63	C, 43.47 H, 7.41 N, 15.21	C, 47.52 H, 7.69 N, 17.81	−7.9 (s)	69
$[NP(OCH_2CF_3)(OCH_2CH_2OCH_2CH_2NH_2)]_n$ (11)	52	C, 26.89 H, 4.10 N, 7.85	C, 29.04 H, 4.87 N, 11.29	−7.4(s)[d]	−20
$[NP(OCH_2CF_3)(OCH_2CH_2OCH_2CH_2NHCOCH_2NH_2)]_n$ (19)	67	C, 31.36 H, 5.26 N, 11.72	C, 31.48 H, 4.95 N, 13.77	−7.4(s)[d]	−10
 (21)	63	C, 42.25 H, 7.02 N, 14.90	C, 47.05 H, 7.18 N, 17.46	−7.4(s)[d]	10
 (23)	64	C, 35.36 H, 5.32 N, 12.96	C, 39.22 H, 5.49 N, 15.25	−7.4(s)[d]	59

[a] After dialysis against water and methanol, the polymer samples were dried at 0.2 mmHg at 35 °C for 3 days. ^{1}H NMR showed that the samples contained traces of water and methanol. [b] The values were obtained by calculation based on quantitative coupling in each step. [c] The ^{31}P NMR spectra were obtained in methanol-d_4. [d] Two small peaks corresponding to geminally substituted phosphorus nuclei appeared at −6.5 and −8.9 ppm.

Typical Procedure for Synthesis of Coupling Products 12, 14, 16, 24, and 26: Preparation of 12. To a solution of *N*-hydroxysuccinimide ester of *N*-(*tert*-butoxycarbonyl)glycine (5.5 g, 20 mmol) in dimethylformamide (30 mL) was added dropwise a solution of polymer 8 (1.3 g, 10 mmol/NH_2) and triethylamine (1.4 mL, 10 mmol) in methanol (30 mL), and the resultant solution was stirred for 2 days at room temperature. After concentration of the reaction mixture to 20 mL, the polymer was precipitated in hexane and was washed with water. This polymer was employed in deprotection reactions without further purification.

Typical Procedure for Synthesis of Polymers 13, 15, 17, 25, and 27 by the Deprotection Reaction: Preparation of 13. Polymer 12 (1.0 g) was dissolved in 80% trifluoroacetic acid (30 mL) at room temperature. The solution was stirred for 15 h at 25 °C and was made alkaline (pH 9) with a 5 N NaOH solution in an ice bath. The aqueous solution was dialyzed against water for 3 days and against methanol for 1 day by using a cellulose membrane (MW cutoff 6000–8000). The solution was then concentrated to dryness under reduced pressure to give the desired polymer.

Preparation of $[NP(OCH_2CF_3)(OCH_2CH_2OCH_2CH_2NH_2)]_n$ (11). Poly(dichlorophosphazene) (0.5 g) dissolved in tetrahydrofuran (30 mL) was added slowly to a stirred solution of sodium trifluoroethoxide prepared from trifluoroethanol (0.52 g, 5.2 mmol) and sodium metal (0.11 g, 5.0 mmol) in tetrahydrofuran (30 mL). The reaction mixture was stirred for 30 h at room temperature and was then treated with a solution of sodium 2-[2-((*tert*-butoxycarbonyl)amino)ethoxy]ethoxide prepared from 1 (1.03 g, 5 mmol) and sodium hydride (60% suspension, 4.8 mmol). The reaction mixture was stirred for an additional 48 h at the same temperature. After concentration of the reaction mixture to 30 mL, the polymer was precipitated in hexane and was further purified by precipitation from tetrahydrofuran into water (yield 75%). This polymer (2.0 g) was dissolved in 80% trifluoroacetic acid. The solution was stirred for 24 h at room temperature and was adjusted to pH 9 with a 5 N NaOH solution while cooled in an ice bath. It was then dialyzed against water for 3 days and against methanol for 1 day by using a cellulose membrane (MW cutoff 6000–8000). The solution was concentrated to dryness under reduced pressure to give the desired polymer.

Typical Procedure for Synthesis of Coupling Products 18, 20, and 22: Preparation of 22. Polymer 21 (1.0 g, 2.5 mmol/NH_2) was dissolved in methanol (20 mL), and the solution was added dropwise to a solution of *N*-hydroxysuccinimide ester of *N*-(*tert*-butoxycarbonyl)glycine (1.36 g, 5.0 mmol) and triethylamine (0.35 mL, 2.5 mmol) in dimethylformamide (20 mL). The resulting solution was stirred for 2 days at room temperature. After concentration of the reaction mixture to 15 mL, the polymer was precipitated in hexane and was washed with water. This polymer was employed in the deprotection reactions without further purification.

Typical Procedure for Synthesis of Polymers 19, 21, and 23 by Deprotection: Preparation of 23. Polymer 22 (1.0 g) was dissolved in 80% trifluoroacetic acid (30 mL) at room tem-

perature. The solution was stirred for 15 h at the same temperature and was made alkaline (pH 9) with a 5 N NaOH solution while cooled in an ice bath. The precipitated polymer was collected by centrifugation and was dissolved in methanol. This solution was combined with the aqueous solution before dialysis against water for 3 days and against methanol for 1 day by using a cellulose membrane (MW cutoff 6000–8000). It was concentrated to dryness under reduced pressure to give the desired polymer.

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