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Analogs of poly(L-phosphoserine) via living polymerization of phosphonate containing NCA monomers

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ABSTRACT: We have synthesized new phosphonate containing α-amino acid N-carboxyanhydride (NCA) monomers, and used these to prepare well-defined phosphonate containing polypeptides and block copolypeptides. These NCAs were found to undergo living polymerization to high molecular weights with narrow chain length distributions. The methods described here demonstrate that phosphono polypeptides are also readily incorporated into block copolymers with controlled architecture and composition. One polymer, poly(L-phosphonohomoalanine), poly(Pha), is an isosteric analog of poly(L-phosphoserine) and was found to display a pH responsive conformational change. The pH responsive α-helical conformation of poly(Pha) is unprecedented, and may prove to be useful in preparation of stimuli responsive block copolypeptides containing poly(Pha) segments.

Phosphate containing polymers have attracted recent attention for their utility in a wide range of applications, including as dental adhesives, adhesion promoters, ion-exchange resins, and flame retardants.[1] For medical uses, such as in bone regeneration, it would be advantageous for these polymers to mimic the features of natural phosphoproteins including their bioactivity and biodegradability. Phosphoserine (Scheme 1) is a key component of phosphoproteins that mediates many diverse processes such as protein-protein interactions,[2] protein activation and inhibition,[3] and biomineralization.[4] For example, serine-rich osteopontin has been shown to nucleate hydroxyapatite deposition, but only when highly phosphorylated.[5] Synthetic polypeptide mimics of phosphoproteins have been studied for many years, yet have been limited by inefficient synthesis and inability to be incorporated into well-defined block copolymers. In response to these issues, we synthesized new phosphonate containing α-amino acid N-carboxyanhydride (NCA) monomers, and used these to prepare well-defined phosphonate containing polypeptides and block copolypeptides. These NCAs were found to undergo living polymerization to high molecular weights with narrow chain length distributions. One polymer, poly(L-phosphonohomoalanine), poly(Pha), is an isosteric analog of poly(L-phosphoserine) and was found to display a pH responsive conformational change.

Homopolymers of L-phosphoserine have been prepared via polymerization of diphenylphosphoserine NCA.[6] While this method was able to give poly(L-phosphoserine) in good yield, there was no demonstration of chain length control or incorporation into copolymers. Removal of protecting groups also required an expensive catalyst, lessening the practical utility and applicability of this approach. The poly(L-phosphoserine) obtained was found to be conformationally disordered over a pH range of 1.3 to 12. Recently, new potential mimics of poly(L-phosphoserine), based on phosphate and phosphonate containing S-alkyl-L-cysteine derivatives, were prepared from the corresponding functionalized NCA monomers.[7] In this system, phosphorous containing homopolypeptides could be prepared with controlled chain lengths up to degrees of polymerization of 40, and with defined C-terminal end-groups. However, the phosphorylated polypeptide could not be deprotected without dephosphorylation, and no block copolypeptides were prepared.[7] The polyphosphonate derivative could be deprotected, and gave a water soluble homopolypeptide with a disordered conformation at pH 7.2. One key feature of this system is the length of the side chains in these polypeptides, which are significantly longer than in phosphoserine (5 versus 2 atoms between backbone and phosphorous, respectively), which may limit their ability to mimic natural phosphorylated proteins.

Scheme 1: Structures of Phosphoserine and Phosphonate Analogs

Our lab became interested in preparation of phosphoserine analogs, especially for incorporation into self-assembling block copolypeptides for biomaterials uses.[8] Due to difficulties in preparing phosphoserine based NCAs with suitable protecting groups, we began to explore phosphonate derivatives of amino acids as viable mimics of phosphoserine. It is known that alkyl phosphonates typically have similar pK_as to their alkyl phosphate counterparts. Phosphonates thus have similar properties to phosphates, yet are more easily protected and deprotected, as shown by previous work. Protection of these functional groups is essential for their use in living NCA polymerizations. In order to obtain phosphono amino acids that would be structurally similar to phosphoserine, we synthesized

NCA monomers based upon the isosteric phosphonohomoalanine (Pha) and the smaller phosphonoalanine (Pal) (Scheme 1).

Scheme 2: Synthesis of Phosphono Polypeptides

(a) PPh_3, I_2, imidazole, CH_2Cl_2 (*ca.* 90% yield); (b) $(iPrO)_3P$, 100 °C (**2a**: 85% yield; **2b**: 65% yield); (c) H_2, Pd/C, MeOH (**3a**: 77% yield; **3b**: 88% yield); (d) $COCl_2$, THF, 40 ºC (**4a**: 80% yield; **4b**: 68% yield); (e) $Co(PMe_3)_4$, THF, See **Table 1** for yields; (f) TMSBr, $CHCl_3$, 60 °C. **6a**: n=1, poly(Pal); **6b**: n=2, poly(Pha).

NCA monomer synthesis began with the commercially available serine derivative **1a** or the previously described **1b** (Scheme 2).[12] The alcohols were converted to the iodides **2a** and **2b** followed by their conversion to phosphonates via the Arbuzov reaction with triisopropylphosphite. It is noteworthy that the reaction was successful at 100 ºC, which is lower than expected for an unactivated iodide. The carboxylate and amine groups were then deprotected via hydrogenolysis. NCA synthesis was then accomplished using phosgene in dry THF at 40 °C. The monomers were purified using flash chromatography[13] and then crystallized from THF/Hexanes to afford NCAs of suitable purity for polymerization. While diisopropylphosphonohomoalanine NCA, **4b**, was optically pure, diisopropylphosphonoalanine NCA, **4a**, was found to be partially racemized (see Supporting Information (SI)). Optical rotations of the precursors to **4a** showed that the racemization occurs during the phosgenation step. This result is unprecedented for NCA synthesis via phosgenation, which typically results in no loss of optical purity.[14] A possible explanation is that at elevated temperatures during **4a** synthesis, fast, reversible elimination of diisopropyl phosphite occurs creating a dehydroalanine intermediate that racemizes the stereocenter (See SI Figure S1).[15]

Polymerizations of NCAs **4a** and **4b** using $Co(PMe_3)_4$ in THF proceeded readily at ambient temperature to give the corresponding homopolypeptides **5a** and **5b** with complete monomer conversions and no reactions at the side-chain phosphonate groups.[16] Residual Co salts were readily removed by washing polypeptides with water. To see if chain length could be controlled, both **4a** and **4b** were separately polymerized to complete monomer conversions at different monomer to initiator ratios (M:I), and the active chains were then end-capped with isocyanate terminated PEG (M_n = 2000 Da).[17] Compositional analysis of purified, end-capped polypeptides by ^1H NMR gave number average chain lengths that increased linearly with M:I stoichiometry (Table 1, Figure 1a, see SI Figure S2). Chain length distributions of the poly(**5b**) samples were obtained by GPC/LS analysis and polydispersity indices (M_w/M_n) were found to be between 1.03 and 1.28, indicating well-defined polypeptides were formed (Figure 1b). Due to lack of a refractive index (RI) difference from solvent, poly(**5a**) gave no RI signal in GPC analysis, and polydispersity indices could not be determined for these samples. Both poly(**5a**) and poly(**5b**) were obtained in high yields with precisely controlled chain lengths to over 150 residues long. These high molecular weight phosphono polypeptides were obtained in reaction times of less than 12 hours, as compared to previous studies where much longer reaction times (36 hours) were necessary to obtain shorter chains (40 residues).[7] These chain lengths, e.g. 25 to 150 residues, also cover a desirable range for many polypeptide materials applications.[8] Overall, these data show that NCAs **4a** and **4b**, similar to other NCAs,[16] are able to undergo living polymerization when initiated with $Co(PMe_3)_4$.

Table 1: Synthesis of Phosphono Homopolypeptides

Monomer[a]	M_n[b]	M_w/M_n[c]	DP[d]	yield (%)[e]
20 Pal NCA	12 000	n/a	51	98
30 Pal NCA	17 600	n/a	75	99
40 Pal NCA	24 000	n/a	102	98
50 Pal NCA	30 600	n/a	130	97
60 Pal NCA	38 300	n/a	163	95
10 Pha NCA	5 480	1.07	22	94
20 Pha NCA	8 960	1.28	36	93
40 Pha NCA	17 900	1.17	72	91
60 Pha NCA	28 100	1.04	113	98
80 Pha NCA	41 800	1.03	168	93

[a] Number of equivalents of monomer per $Co(PMe_3)_4$. [b] Molecular weight determined for PEG end-capped samples using ^1H NMR. [c] Polydispersity index determined by GPC/LS analysis. [d] DP = degree of polymerization. [e] Total isolated yield of purified polypeptide. Pal NCA = **4a**; Pha NCA = **4b**. n/a = not applicable.

To further showcase the synthetic utility of these monomers, block copolypeptides were prepared using the phosphono NCAs 4a or 4b combined with N_ε-carbobenzyloxy-L-lysine NCA (Lys NCA) as a model comonomer (Figure 1c, Table 2, see SI Figures S3, S4). All of these copolymerizations gave block copolypeptides in high yields and with low polydispersity indices. The low polydispersity indices of the block copolymers of Lys NCA and 4a NCA also provide indirect evidence that polymerization of 4a NCA gives polypeptides with narrow chain length distributions. Block copolypeptides were prepared using different orders of monomer addition (Figure 1d), allowing the synthesis of copolymers

Table 2. Synthesis of Phosphonate Containing Diblock Copolypeptides

First Monomer[a]	Second Monomer[a]	First Segment[b]			Diblock Copolymer[c]			
		M_n	M_w/M_n	DP[d]	M_n	M_w/M_n	DP[d]	yield (%)[e]
20 Lys NCA	10 Pal NCA	12 600	1.12	48	19 600	1.02	78	97
20 Lys NCA	15 Pal NCA	12 600	1.12	48	22 400	1.07	90	94
20 Lys NCA	20 Pal NCA	12 600	1.12	48	24 300	1.01	98	97
20 Lys NCA	30 Pal NCA	12 600	1.12	48	27 400	1.05	111	90
20 Pal NCA	15 Lys NCA	12 000	n/a	51	25 600	1.13	100	90
20 Pal NCA	30 Lys NCA	12 000	n/a	51	33 100	1.13	151	92
20 Pal NCA	45 Lys NCA	12 000	n/a	51	55 100	1.05	209	90
20 Lys NCA	10 Pha NCA	14 900	1.12	57	21 400	1.14	83	94
20 Lys NCA	30 Pha NCA	14 900	1.12	57	36 100	1.10	142	91
20 Lys NCA	40 Pha NCA	14 900	1.12	57	43 800	1.06	173	94
40 Pha NCA	20 Lys NCA	17 900	1.15	72	24 300	1.07	96	94
40 Pha NCA	40 Lys NCA	17 900	1.15	72	32 300	1.05	127	99
40 Pha NCA	60 Lys NCA	17 900	1.15	72	39 900	1.06	156	95
40 Pha NCA	80 Lys NCA	17 900	1.15	72	42 800	1.19	167	98

[a] Number of equivalents of monomer per $Co(PMe_3)_4$. [b] Molecular weight of first segments determined for PEG end-capped samples using ^1H NMR and polydispersity index determined by GPC/LS analysis where applicable. [C] Molecular weight of diblock copolymers determined for using ^1H NMR and polydispersity index determined by GPC/LS analysis. [d] DP = degree of polymerization. [e] Total isolated yield of purified diblock copolypeptide. n/a = not applicable.

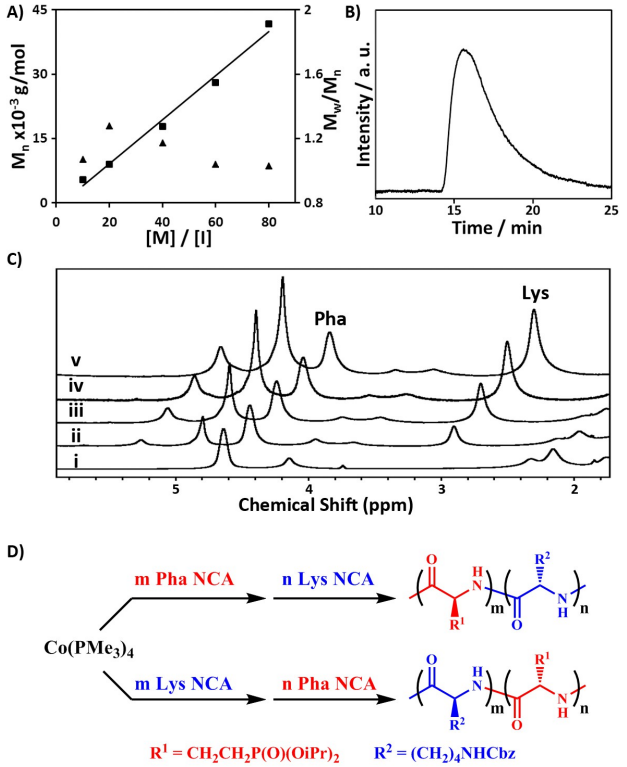

Figure 1. (A) Molecular weight (M_n, ■) and polydispersity index (M_w/M_n, ▲) of poly(**5b**) as a function of monomer to initiator ([M]/[I]) ratio using $Co(PMe_3)_4$ in THF at ambient temperature. (B) GPC chromatogram (RI intensity in arbitrary units (a.u.) versus elution time) of a poly(**5b**) sample (**Table 1**, 40 Pha NCA). (C) ^{1}H NMR data showing different Lysine to Pha compositions obtained in block copolymerizations of **4b** NCA and Lys NCA from **Table 2**. **Pha** = PC$\underline{H}_2$ resonance of **5b** segment; **Lys** = NC$\underline{H}_2$ resonance of lysine segment; i = 40 Pha NCA; ii = 40 Pha NCA:20 Lys NCA; iii = 40 Pha NCA:40 Lys NCA; iv = 40 Pha NCA:60 Lys NCA; v = 40 Pha NCA:80 Lys NCA. (D) Schematic showing different block copolymer synthesis sequences.

with a polyphosphonate segment on either the C- or N-terminal end (Table 2). Such versatility is useful for incorporation of phosphono polypeptide domains at defined locations within block copolypeptide assemblies for controlled presentation of this functionality.

Deprotection of homopolymers poly(**5a**) and poly(**5b**) was accomplished using TMSBr in chloroform at 60 °C. Following dialysis against water and lyophilization, both poly(*rac*-phosphonoalanine) (**6a**), poly(Pal), and poly(L-phosphonohomoalanine) (**6b**), poly(Pha), were obtained in >90% yields. Both poly(Pal) and poly(Pha) were found to have good water solubility over a wide pH range. The solution conformations of both polypeptides were examined by circular dichroism (CD) spectroscopy. As expected, poly(Pal) gave no CD signal since the stereocenters were racemized during monomer synthesis. Optically pure, poly(Pha) was found to give a CD spectrum consistent with a disordered chain conformation at pH 7.4 (Figure 2), similar to literature reports for poly(L-phosphoserine)[6] and the previously reported phosphono polypeptide described above.[7] This result is not surprising since all these polypeptides are highly charged polyelectrolytes at this pH. Since phosphonate groups have two acidic protons with pK_a values of *ca.* 2.5 and 8.0, we also examined CD spectra of poly(Pha) samples) at pH values above and below these transitions. Although poly(Pha) with partially or fully deprotonated phosphonate groups were found to possess disordered conformations, as described above, the fully protonated, non-ionic poly(Pha) obtained at pH 1.0 was found to be predominantly α-helical under these conditions, with characteristic minima at 208 and 222 nm (Figure 2).[18] Helical poly(Pha) was soluble in water and interconversion between α-helical and disordered conformations was readily accomplished by adjusting solution pH.

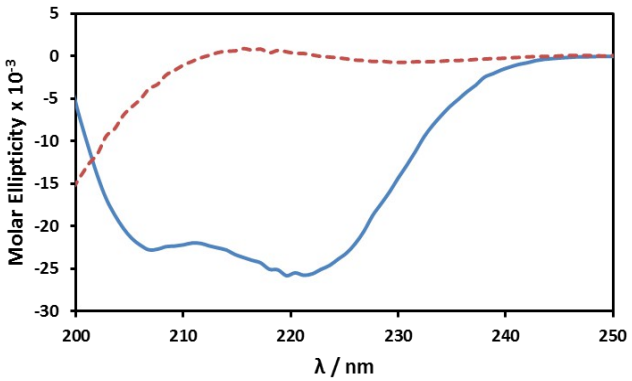

Figure 2. Circular dichroism spectra of poly(Pha)$_{36}$ (1 mg/ml) at 20 ºC in water at pH 1.0 (0.1 M HCl, solid blue line) and pH 7.4 (10 mM PBS, dotted red line).

The pH responsive α-helical conformation of poly(Pha) is unprecedented for phosphate or phosphonate containing polypeptides, and may prove to be useful in subsequent efforts to direct self-assembly of block copolypeptides containing poly(Pha) segments. Since Pha is isosteric with L-phosphoserine, poly(Pha) and its copolymers might also prove useful in mimicking properties of phosphorylated proteins. The methods described here show that Pha and Pal based phosphono polypeptides can be prepared efficiently with controlled chain lengths, and are also readily incorporated into block copolymers with controlled architecture and composition. Future studies will explore use of these methods for development of phosphonate containing biomaterials for medical and dental applications.

ASSOCIATED CONTENT

Supporting Information Experimental procedures and spectral data for all new compounds, as well as polymerization data, ^{1}H, ^{13}C, ^{31}P NMR spectra, M_n vs. [M]/[I] data, and GPC data. This material is available free of charge via the Internet at http://pubs.acs.org.

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Author Contributions

The manuscript was written through contributions of all authors.

Notes

The authors declare no competing financial interest.

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REFERENCES

(1) Monge, S.; Canniccioni, B.; Graillot, A.; Robin, J.-J. *Biomacromolecules* **2011**, *12*, 1973-1982.

(2) Yaffe, M. B.; Elia, A. E. H. *Curr. Opin. Cell Biol.* **2001**, *13*, 131-138.

(3) Zolnierowicz, S. *Biochem. Pharmacol.* **2000**, *60*, 1225-1235.

(4) Bleek, K.; Taubert, A. *Acta Biomater.* **2013**, *9*, 6283-6321.

(5) Gericke, A.; Qin, C.; Spevak, L.; Fujimoto, Y.; Butler, W. T.; Sørensen, E. S.; Boskey, A. L. *Calcif. Tissue Int.* **2005**, *77*, 45-54.

(6) Ohkawa, K.; Saitoh, A.; Yamamoto, H. *Macromol. Rapid Commun.* **1999**, *20*, 619-621.

(7) Das, S.; Kar, M.; Gupta, S. S. *Polym. Chem.* **2013**, *4*, 4087-4091.

(8) Deming, T. J. *Prog. Polym. Sci.* **2007**, *32*, 858-875.

(9) Freedman, L. D.; Doak, G. O. *Chem. Rev.* **1957**, *57*, 479-523.

(10) Kumler, W. D.; Eiler, J. J. *J. Am. Chem. Soc.* **1943**, *65*, 2355-2361.

(11) David, G.; Negrell-Guirao, C.; Iftene, F.; Boutevin, B.; Chougrani, K. *Polym. Chem.* **2012**, *3*, 265-274.

(12) Koseki, Y.; Yamada, H.; Usuki, T. *Tetrahedron: Asymmetry* **2011**, *22*, 580-586.

(13) Kramer, J. R.; Deming, T. J. *Biomacromolecules* **2010**, *11*, 3668-3672.

(14) Cheng, J. Deming, T. J. *Top. Curr. Chem.* **2012**, *310*, 1-26.

(15) Rich, D. H.; Tam, J. P. *J. Org. Chem.* **1977**, *42*, 3815-3820.

(16) Deming, T. J. *Macromolecules* **1999**, *32*, 4500-4502.

(17) Brzezinska, K. R.; Curtin, S. A.; Deming, T. J. *Macromolecules* **2002**, *35*, 2970-2976.

(18) Morrow, J. A.; Segall, M. L.; Lund-Katz, S.; Phillips, M. C.; Knapp, M.; Rupp, B.; Weisgraber, K. H. *Biochemistry* **2000**, *39*, 11657-11666.

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