Notes

Synthesis of *O*-Phosphorylserine-Terminated Poly(*tert*-butyl acrylate) and Poly(acrylic acid)

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Introduction

We are interested in ways to control the solubility of peptides and amino acids. The use of telechelic polymers and oligomers for the modification of the solubility of peptides has been previously described by Mutter using poly(ethylene glycol) moieties.[1] In a first paper, we described a synthetic method to link peptides to a polymer chain which can be either hydrophilic or hydrophobic.[2] The polymer used for coupling with the peptide was poly(*tert*-butyl acrylate), which renders the peptide oil-soluble. Hydrolysis of the *tert*-butyl ester functions yields a peptide linked to poly(acrylic acid) and results in a peptide soluble in dilute alkali. Thus the polymer chain determines the solubility of the attached peptide.

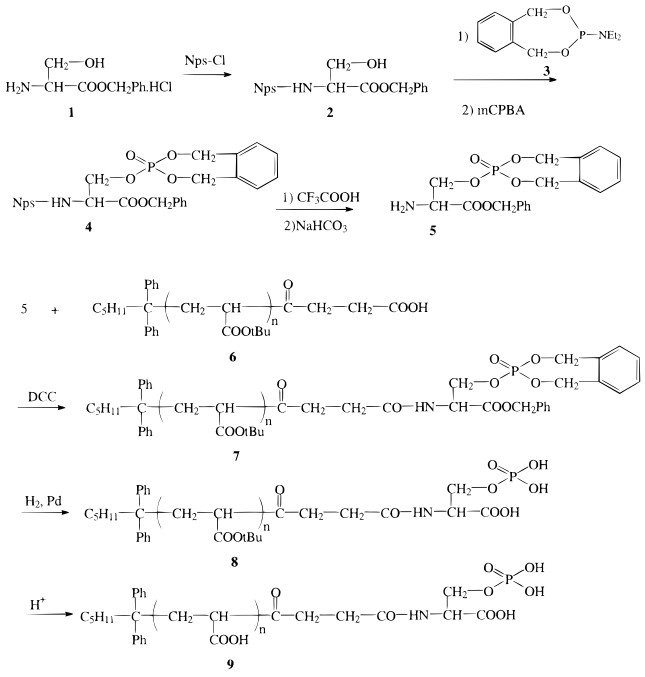
In this paper, we describe the synthesis of *O*-phosphorylserine ester linked to either poly(*tert*-butyl acrylate) or poly(acrylic acid) to demonstrate further the generality of our concept to control the solubility of a more functionalized amino acid.

Results and Discussion

The controlled synthesis of poly(*tert*-butyl acrylate) terminated with a carboxylic acid functionality was described in our previous paper.[2] Anionic polymerization of *tert*-butyl acrylate is carried out in LiCl−tetrahydrofuran mixture. *n*-Butyllithium and 1,1-diphenylethylene are used to initiate the polymerization. Termination by succinic anhydride followed by acidification results in a carboxylic acid-terminated polymer chain of controlled molecular weight. The molecular weight used in this study was 1300.

The reaction sequence for the synthesis of the title compounds is shown in Scheme 1. Serine was obtained as the benzyl ester hydrochloride **1**, and the amine functionality was protected as the (2-nitrophenyl)-sulfenyl derivative **2**. The *O*-phosphoryl functionality was introduced by reaction of **2** with *N,N*-diethyl-1,5-dihydro-2,4,3-benzodioxaphosphepin-3-amine (**3**),[3] followed immediately by oxidation with *m*-chloroperbenzoic acid, to yield the serine derivative **4** with a protected phosphonic acid function on O.[4] The Nps protecting

Scheme 1



group on N was easily removed with trifluoroacetic acid to give the trifluoroacetate salt of **5**. The free base **5**, *O*-(*o*-xylene-α,α′-diyl)phosphorylserine benzyl ester, was generated immediately before the coupling reaction by treatment with sodium bicarbonate.

The free base **5** was coupled with carboxylic acid-terminated poly(*tert*-butyl acrylate) using dicyclohexylcarbodiimide (DCC) and 1-hydroxybenzotriazole (HOBt) as described in the previous paper.[2] The NMR spectrum of the polymer **7** after purification indicates that about 70% of the polymer chain has the serine end group. Both the benzyl ester group and the cyclic phosphonic ester group can be removed simultaneously by catalytic hydrogenation under very mild conditions to yield polymer **8**. The presence of the *O*-phosphonic acid functionality in this polymer was verified by ^{31}P NMR. Polymer **8** was hydrolyzed in formic acid to yield polymer **9**, the poly(acrylic acid) with *O*-phosphorylserine as the end group. Both of these last two reactions proceed in quantitative yield based on the NMR spectra.

Other reaction pathways were examined. Protecting the amine functionality with a carbobenzoxy (Cbz) group did lead to unsatisfactory results, because the amine could not be regenerated without partial hydrolysis at the phosphonic ester functionality. As far as the phosphate ester function is concerned, the cyclic *o*-xylene-α,α′-diester is much less bulky than the corresponding dibenzyl phosphonic ester equivalent, which greatly simplifies the removal of this protecting group by hydrogenation after the coupling with the polymer.

There is a clear difference in the solubilities of polymers **8** and **9**. The poly(*tert*-butyl acrylate)-linked *O*-phosphorylserine is soluble in common organic sol-

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vents such as hexane, dichloromethane, and chloroform, while the hydrolyzed poly(acrylic acid)-linked *O*-phosphorylserine is soluble in dilute alkaline water.

Experimental Section

Information about general methods and instrumentation can be found in the first paper of this series.[2] Serine benzyl ester hydrochloride (**1**) was obtained from Sigma and used as received.

***N*-((2-Nitrophenyl)sulfenyl)serine Benzyl Ester (2).** Serine benzyl ester hydrochloride (**1**) (2.32 g, 10 mmol) was mixed with triethylamine (2.02 g, 20 mmol) in 18 mL of methanol. (2-Nitrophenyl)sulfenyl chloride (Nps-Cl) (1.90 g, 10 mmol) was added dropwise over 10 min at 5−10 °C under a nitrogen atmosphere. After 24 h, water was added to the reaction mixture and the mixture was extracted with ethyl acetate. The extract was dried over anhydrous sodium sulfate and placed under reduced pressure to remove the solvent. The residue was charged on a silica gel column using ethyl acetate as eluent. After the first band was collected, the eluent was changed to 1/1 v/v CH_2Cl_2/EtOAc and the second yellow band was collected to give 2.68 g (77%) of **2** as a viscous yellow oil. ^{1}H NMR ($CDCl_3$) δ 8.27 (d, 1H), 7.98 (d, 1H), 7.60 (t, 1H), 7.41 (s, 5H), 7.25 (t, 1H), 5.28 (s, 2H), 4.7 (br s, 1H), 4.0 (m, 2H), 3.77 (d, 1H), 3.7 (m, 1H) ppm.

***N*-((2-Nitrophenyl)sulfenyl)-*O*-((*o*-xylene-α,α′-diyl)phosphoryl)serine Benzyl Ester (4).** Serine derivative **2** (1.34 g, 3.8 mmol) and 1*H*-tetrazole were dissolved in 12 mL of CH_2Cl_2 and *N*,*N*-diethyl-1,5-dihydro-2,4,3-benzodioxaphosphepin-3-amine (**3**) (1.45 g, 6.1 mmol), synthesized according to Arbuzov,[3] was added under nitrogen. The reaction mixture was stirred for 1 h at room temperature. Water (0.35 mL) was added, the mixture was cooled to −40 °C, and 1.75 g of *m*-chloroperbenzoic acid was added. The reaction mixture was allowed to warm to room temperature, diluted with EtOAc, and washed with 10% Na_2SO_3 solution, saturated $NaHCO_3$, and water. The organic layer was dried over anhydrous sodium sulfate and concentrated. The residue was charged on a silica gel column using 2/1 v/v CH_2Cl_2/EtOAc, and the first yellow band was collected to give 1.69 g (83%) of **4** as a yellow waxy material. ^{1}H NMR ($CDCl_3$) δ 8.25 (d, 2H), 7.60 (t, 1H), 7.4−7.2 (m, 10H), 5.3−5.0 (m, 6H), 4.5 (m, 2H), 3.8 (m, 1H), 3.78 (d, 1H) ppm.

***O*-((*o*-Xylene-α,α′-diyl)phosphoryl)serine Benzyl Ester (5).** This compound was made by modification of a literature procedure.[4] A mixture of compound **4** and 2 mL of trifluoroacetic acid was stirred under nitrogen for 30 min, and the excess CF_3COOH was evaporated under reduced pressure. The residue was charged on a silica gel column using EtOAc as eluent. After the first yellow band was collected, the eluent was changed to methanol. The second band was collected to give the trifluoroacetate salt of **5** as a colorless oil. The oil was dissolved in CH_2Cl_2 and washed with $NaHCO_3$ solution. The organic layer was dried over sodium sulfate and placed under reduced pressure to give free **5**, which was immediately used for the coupling reaction.

Protected *O*-((*o*-Xylene-α,α′-diyl)phosphoryl)serine-Coupled Poly(*tert*-butyl acrylate) (7). A mixture of freshly prepared **5**, DCC (0.10 g, 0.5 mmol), 1-hydroxybenzotriazole (HOBt) (0.077 g, 0.5 mmol), carboxyl-terminated poly(*tert*-butyl acrylate) (**6**) (M_n = 1300, 0.65 g, 0.5 mmol), and 2 mL of CH_2Cl_2 was stirred at room temperature for 48 h and diluted with CCl_4, and the precipitate was filtered off. The filtrate was washed successively with 1 N H_2SO_4, water, 5% $NaHCO_3$, and water and dried over anhydrous sodium sulfate. Placing this mixture under reduced pressure yielded 0.8 g of **7** as a sticky material: ^{1}H NMR ($CDCl_3$) δ 7.5−7.2 (m, phenyls of end terminal), 7.2−7.0 (m, phenyl of head group), 6.6 (m, NH), 5.3−5.0 (m, 4.8−4.4 (m, OCH and OCH_2), 2.5 (m, CH_2), 2.2 (br, CH), 2.0−1.5 (m, CH_2), 1.4 (s, *t*-Bu) ppm. From the NMR spectrum, the coupling yield was calculated to be 69%.

Deprotected *O*-Phosphorylserine-Coupled Poly(*tert*-butyl acrylate) (8). 10% Pd/C (0.2 g) was added to a solution of 0.66 g of polymer **7** in 50 mL of methanol. Hydrogen gas was bubbled through the solution for 8 h at room temperature. The reaction mixture was filtered, and the filtrate was placed under reduced pressure to remove the solvent. The residue was dissolved in CH_2Cl_2 and dried over sodium sulfate. After evaporation of the solvent under reduced pressure, 0.50 g of **8** was obtained as a sticky material. Conversion: ∼100%. ^{1}H NMR ($CDCl_3$) δ 7.2−7.0 (m, phenyl of head group), 2.3 (br, CH), 2.0−1.5 (m, CH_2), 1.4 (s, *tert*-Bu) ppm. ^{31}P NMR ($CDCl_3$) −1.64 ppm (HPO_3H in D_2O, δ 0.0 ppm).

***O*-Phosphorylserine-Coupled Poly(acrylic acid) (9).** Polymer **8** (0.31 g) was placed in 10 mL of formic acid and heated to 60 °C for 20 h. Excess formic acid was removed under reduced pressure, and the residue was washed with hexane. Polymer **9** (0.17 g) was obtained as a sticky material. Conversion: ∼100%. ^{1}H NMR (acetone-d_6) δ 8.0 (very broad, COOH), 7.2−7.0 (m, phenyl of head group), 2.3 (br, CH), 2.0−1.5 (m, CH_2) ppm.

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