Synthesis of Phosphonamide and Thiophosphonamide Dipeptides

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Phosphonopeptides are peptide mimics[1] that emulate high-energy tetrahedral transition states of enzyme-catalyzed peptide hydrolysis reactions. Thus, they are useful as mechanism-based inhibitors of aspartyl and metalloproteases.[2] Several phosphonopeptides have also shown powerful antibacterial activity.[3] More recently, these types of compounds have been applied to hapten design for the generation of catalytic antibodies that possess peptide ligase activity.[4] Herein we report the synthesis of phosphonamide dipeptides **1a**−**d** (R = Cbz) (Scheme 1) in isolated yields up to 30% using our P(III) one-pot activation−coupling−oxidation procedure.[5] Previous attempts to prepare the hapten precursor **1a** from P(V) phosphonochloridate **2** and D-tryptophanamide under a variety of conditions were unsuccessful, due in part to the steric bulk of the amino acid side chains.[6] The difficulty in preparing the phosphonamide **1a** exemplifies the experiences that many laboratories have encountered in preparing phosphonamidate peptides via P(V) coupling protocols. The successful preparation of **1** detailed herein using phosphonochloridite **3** [P(III)] as the key intermediate and exploration of parameters for the reaction should provide a road map to others who wish to prepare phosphonamides not accessible by P(V) protocols.

The air-stable precursor used for generation of phosphonochloridite **3** is racemic, carbamate-protected H-phosphinate amino acid **4** as a diastereomeric mixture of *p*-nitrobenzyl (*p*Nb) esters. The H-phosphinate **4**, under certain conditions, was converted to **3** with the commercially available dichlorotriphenylphosphorane (Ph_3PCl_2). Because the solvent pyridine and the solvent system CH_2Cl_2/Et_3N worked well in our model systems,[5] they were initially employed during activation of **4** with Ph_3PCl_2. However, only a small amount of **3** (^{31}P = 200 ppm; Table 1) formed (admixed with several other as yet unidentified products). This may be due to the amide functionality in **4** possibly being sensitive to the combination of Ph_3PCl_2 with Et_3N or pyridine (when used as solvent). Ph_3PCl_2 in the presence of Et_3N is known to convert secondary amides to imidoyl chlorides.[7] Mechanistically, the formation of an isocyanate from the carbamate functionality with Ph_3PCl_2/Et_3N is also possible. We therefore searched for alternative solvent conditions for the activation step. In standard P(V) chemistry, successful conversion of a phosphonate monoester to the corresponding phosphonochloridate with thionyl or oxalyl chloride is often done in the absence of base.[6,8] Therefore, in an analogous attempt to improve the activation step of our P (III) protocol,[5] treatment of **4** with Ph_3PCl_2 was performed in CH_2Cl_2 without any base present, resulting in the complete conversion of **4** to an activated P(III) species (^{31}P = 193 ppm). This P(III) species was later determined, via a study conducted on the activation step using **4** and GC-MS analysis of the products, to be lacking the *p*Nb group.[9] This study on the activation step was conducted because of problems with isolation of final products **1** by normal-phase chromatography and because of FAB-MS spectra of **1** revealing the major products to be ones in which the *p*Nb group had been apparently cleaved off. This same type of ester cleavage was also observed in other systems in our laboratory.[10] We concluded that in order to prevent *p*Nb cleavage, the presence of base to scavenge generated HCl during the activation reaction was essential.

The best solvent/base combination for activation of **4** with Ph_3PCl_2 was finally determined to be 2 equiv of pyridine (~3%) in CH_2Cl_2. With 1 equiv of pyridine nearly equal amounts of **3** (^{31}P = 200 ppm) and the P(III) species (^{31}P = 193 ppm)[9] that resulted from *p*Nb cleavage formed. Furthermore, as the number

Scheme 1

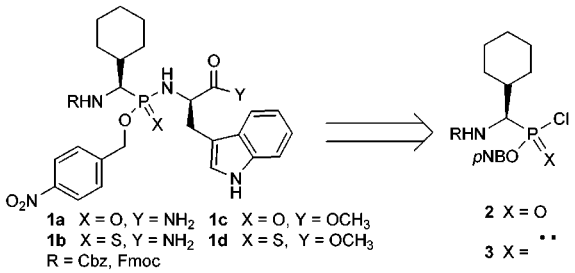
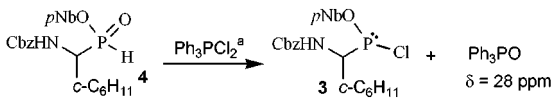


Table 1. Phosphonochloridite Generation under Various Solvent and Base Conditions



entry	solvent	Ph_3PCl_2 equiv	base (equiv)	^{31}P δ ppm (ratio)[c]
1	pyridine	2.5[b]	solvent	200 (0.25), 180 (0.05), 165 (0.04), 158 (0.15), 120 (0.18), 115 (0.05), 39−41 (0.08), 28 (1))
2	CH_2Cl_2	2.5[b]	Et_3N (2)	same as above
3	CH_2Cl_2	2.0[b]	none	193 (0.4), 40[d] (1)
4	CH_2Cl_2	1.5[b]	pyridine (1)	200 (0.3), 193 (0.4), 28 (1)
5	CH_2Cl_2	1.2[b]	pyridine (2)	200 (0.85), 28 (1)

[a] Contains 10−20% Ph_3PO. [b] Number of equivalents needed to consume **4**. [c] Value in parentheses represents intensity of each peak relative to Ph_3PO. [d] $Ph_3PO^+HCl^-$.

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(9) We propose that activation in the absence of base forms **3** but quickly converts, after *p*Nb cleavage, to the respective phosphonodichloridite, with concomitant *p*-nitrobenzyl chloride formation.

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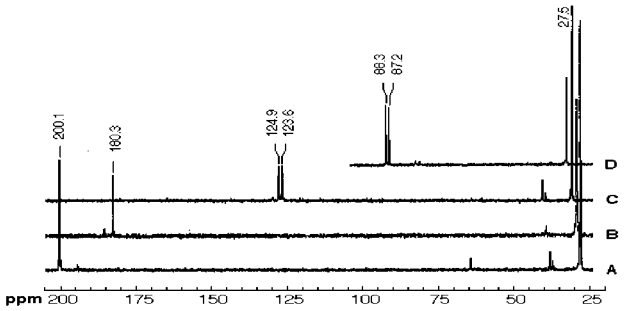
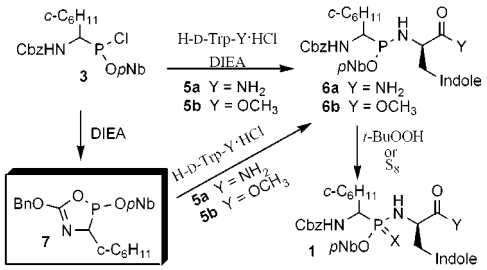


Figure 1. ^{31}P spectra: (A) species **3** (200 ppm), Ph_3PO (27.5 ppm), and unreacted **4** (36, 37 ppm) and Ph_3PCl_2 (64 ppm); (B) proposed oxazaphospholine **7** (180.3 ppm); (C) species **6a** (123.6, 124.9 ppm); and (D) target **1b** (87.2 and 88.3 ppm).

Scheme 2



of equivalents of pyridine increased from two to large excess (e.g., pyridine as solvent), the number and amount of side-products increased (Table 1, entry 1 vs entries 4 and 5). With the optimized solvent/base conditions, very few side products were evident from ^{31}P NMR (Table 1, entry 5; Figure 1A).

The next step of the one-pot procedure was the coupling of **3** to D-tryptophanamide **5a** or D-tryptophan methyl ester **5b** to form the phosphonamidite **6** (Scheme 2). The major problem initially encountered with the amine coupling of **5** to **3** was the formation of an unknown substance (^{31}P, ≈180 ppm) that competed with the formation of **6**. If **5** was added in one portion to the phosphonochloridite **3**, only the peak ≈180 ppm would be observed. However, if **5** was added over 15−20 min, the intensity of the peak ≈180 ppm and the expected four diastereomeric peaks (^{31}P, 123, 124, 126, 128 ppm) would be nearly equal. We therefore directed our investigation toward elucidating the identity or properties of that unknown species (^{31}P, ≈180 ppm.).

In the presence of tertiary amines urethane-protected amino acids and peptides are known to cyclize to form oxazolones,[11] which are themselves reactive acylating agents. We reasoned that similar cyclization could be occurring with our urethane-protected phosphonochloridite **3** to form an oxazaphospholine[12] (^{31}P, ≈180 ppm). We generated the proposed oxazaphospholine **7** by adding 1.5 equiv of the tertiary base, diisopropylethylamine (DIEA), to **3**. Next amine nucleophile **5a** or **5b** and DIEA (2 equiv each) were added in one portion. After about 15 min, two phosphonamidite diastereomers preferentially formed, which, after sulfurization or oxidation, resulted in compound **1** (Table 2). Leucine methyl ester and glycine ethyl ester were also coupled to **7** followed by sulfurization, resulting in isolated yields of 28% and 26%, respectively (see Supporting Information). As NMR spectra indicate almost complete conversion of starting H-phosphinates to final phosphonamides, the moderate yields obtained here are likely indicative of losses during purification due to the hydrolytic instability of phosphonamides.[13]

Table 2. Synthesis of Phosphonamide and Thiophosphonamide Dipeptides[a]

entry	Y	X	product[b]	% yield[c]	^{31}P
1	NH_2	O	**1a**	18	28.8, 29.4
2	NH_2	S	**1b**	14	85.3, 86.6
3	OCH_3	O	**1c**	15	30.5, 30.8
4	OCH_3	S	**1d**	30	87.3, 88.4

[a] **1a**−**d** were prepared by first generating the proposed phosphitylating agent **7** from **3** and 2 equiv of DIEA. Then **5** was coupled to **7** to form the phosphonamidite **6a** or **6b** followed by sulfurization or oxidation with elemental S_8 or *t*-BuOOH, respectively. [b] **1a**−**d** were isolated using HPLC and characterized by ^{31}P NMR, ^{1}H NMR, and FAB-MS. [c] Isolated yield.

We also explored an alternative structure for the phosphitylating agent. We speculated that the observed reactive species could be analogous to the phosphonyltrialkylammonium salts observed by Hirschmann and co-workers in phosphonate activation.[6b,c] To test that hypothesis, we generated the phosphitylating agent (^{31}P, ≈180 ppm) by adding the phosphonochloridite **3** to PS-DIEA resin (DIEA attached to a polystyrene resin). We reasoned that if a reactive phosphinyltrialkylammonium salt forms, then it would remain attached to the resin. However, after the reaction mixture was filtered from the resin, the newly formed phosphitylating agent was found only in solution, and no ^{31}P signal was detected on the solid support by gel ^{31}P NMR. Therefore, in our case, we concluded that the species that resonated around 180 ppm was not a phosphinyltrialkylammonium salt but the oxazaphospholine **7**.

In conclusion, the P(III) approach to phosphonopeptides described here provides a route to phosphonamide **1a** (and **1c**) that was previously unattainable by P(V) methods. Additionally the use of reduced phosphorus intermediates allowed preparation of thiophosphonamide peptides **1b** and **1d.** Further improvements in this method using nonparticipating amino protection, milder activation reagents, and solid-supported approaches are currently under investigation.

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Supporting Information Available: Experimental details for the synthesis and characterization of phosphonopeptides (PDF). This material is available free of charge via the Internet at http://pubs.acs.org.

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(12) For an example of a P(III) oxazaphospholine formed from Cl_2POEt and $RCONHCH_2CO_2Et$ with Et_3N see: Malenko, D.; Nesterova, L.; Lukyanenko, S.; Sinitsa, A. *Zh. Obshch. Khim.* **1989**, *59*, 2626. Oxazaphospholine formation has also been proposed as an intermediate in phosphonate [P(V)] activation and coupling, but was discounted in favor of phosphonyltrialkylammonium salts (see refs 6b,c).

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