Solid-Phase Synthesis of Peptidylphosphonates

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We are currently engaged in the construction of synthetic combinatorial libraries on solid supports with a focus on pharmacophore units that have proven therapeutic value. The development of combinatorial libraries has provided researchers with a valuable source of new compounds to be screened against receptors and enzymes in the search for drug leads.[1] Combinatorial libraries augment natural product collections and chemical databases typically used in the pharmaceutical industry. Initially chemical libraries were limited to peptides,[2] but have recently been expanded to include natural and unnatural polymers[3] and nonpolymeric organic compounds.[4] Peptidylphosphonates are recognized as effective transition state analogue inhibitors of peptidases and esterases, and their use as inhibitors of metalloproteases has been well documented.[5] The insertion of a phosphonate unit in place of the scissile amide bond of a substrate provides access to the additional binding interactions available within the enzyme substrate complex as it approaches its transition-state conformation. These additional binding interactions, which are unavailable to the ground-state complex, can then be used to design potent inhibitors of the enzyme.

In this communication we report a method for the solid-phase synthesis of peptidylphosphonates (SPPPS) that is compatible with peptide synthesis using Fmoc chemistry. Although a number of useful synthetic routes are available for the solution-phase synthesis of peptidylphosphonates,[6] the advantages of a solid-phase synthesis, including high yields and ease of workup, would facilitate the synthesis of this important class of compounds. In addition, this methodology will enable the construction of combinatorial peptidylphosphonate libraries and represents an important advance in the ongoing evolution of synthetic combinatorial libraries, the incorporation of pharmacophores within a biopolymer.

(1) (a) Jung, G.; Beck-Singer, A. G. *Angew. Chem.. Int. Ed. Engl.* **1992**, *31*, 367. (b) Pavia, M. R.; Sawyer, T. K.; Moos, W. H. *Bioorg. Med. Chem. Lett.* **1993**, *3*, 387.

(2) For synthetic libraries, see: (a) Fodor, S. P.; Read, J. L.; Pirrung, M. C.; Stryer, L.; Lu, A. T.; Solas, D. *Science* **1991**, *251*, 767. (b) Lam, K. S.; Salmon, S. E.; Hersh, E. M.; Hruby, V. J.; Kazmierski, W. M.; Knapp, R. J. *Nature* **1991**, *354*, 82. (c) Houghten, R. A.; Pinilla, C.; Blondelles, S. E.; Appel, J. R.; Dooley, C. T.; Cuervo, J. H. *Nature* **1991**, *354*, 84. For recombinant peptide libraries, see: (d) Cwirla, S. E.; Peters, E. A.; Barrett, R. W.; Dower, W. J. *Proc. Natl. Acad. Sci. U.S.A.* **1990**, *87*, 6378. (e) Scott, J. K.; Smith, G. P. *Science* **1990**, *249*, 386. (f) Devlin, J. J.; Panganiban, L. C.; Devlin, P. E. *Science* **1990**, *249*, 404. (g) Cull, M. G.; Miller, J. F.; Schatz, P. J. *Proc. Natl. Acad. Sci. U.S.A.* **1992**, *89*, 1865.

(3) (a) Cho, C. Y.; Moran, E. J.; Cherry, S. R.; Stephans, J. C.; Fodor, S. P.; Adams, C. L.; Sundaram, A.; Jacobs, J. W.; Schultz, P. G. *Science*, **1993**, *261*, 1303. (b) Simon, R. J.; Kania, R. S.; Zuckermann, R. N.; Huebner, V. D.; Jewell, D. A.; Banville, S.; Ng, S.; Wang, L.; Rosenberg, S.; Marlowe, C. K.; Spellmeyer, D. C.; Tan, R.; Frankel, A. D.; Santi, D. V.; Cohen, F. E.; Bartlett, P. A. *Proc. Natl. Acad. Sci. U.S.A.* **1992**, *89*, 9367.

(4) (a) Bunin, B. A.; Ellman, J. A. *J. Am. Chem. Soc.* **1992**, *114*, 10997. (b) DeWitt, S. H.; Kiely, J. S.; Stankovic, C. J.; Schroeder, M. C.; Cody, D. M.; Pavia, M. R. *Proc. Natl. Acad. Sci. U.S.A.* **1993**, *90*, 6909.

(5) (a) Morgan, B. P.; Scholtz, J. M.; Ballinger, I. D.; Zipkin, I. D.; Bartlett, P. A. *J. Am. Chem. Soc.* **1991**, *113*, 297. (b) Kaplan, A. P.; Bartlett, P. A. *Biochemistry* **1991**, *30*, 8165. (c) Bartlett, P. A.; Marlowe, C. K. *Science* **1987**, *235*, 569. (d) Bartlett, P. A.; Marlowe, C. K. *Biochemistry* **1987**, *26*, 8553. (e) Thorsett, E. D.; Harris E. E.; Peterson, E. R.; Greenlee, W. J.; Patchett, A. A.; Ulm, E. H.; Vassil, T. C. *Proc. Natl. Acad. Sci. U.S.A* **1982**, *79*, 2176.

(6) (a) Karanewsky, D. S.; Badia, M. C. *Tetrahedron Lett.* **1986**, *27*, 1751. (b) Campbell, D. A. *J. Org. Chem.* **1992**, *57*, 6331. (c) Campbell, D. A.; Bermak, J. C. *J. Org. Chem.*, in press. (d) Campagne, J. M.; Coste, J.; Jouin, P. *Tetrahedron Lett.* **1993**, *34*, 6743.

Table 1. Time (Hours) Required for >90% Coupling Yields of the Phosphonic Acid and Resin-Bound Alcohol.[a] Scheme 1, Intermediate 5, without Any Intervening Peptide Sequence between the Support and the α-Hydroxy Acid

	alcohol; R_1			
phosphonate; R_2	H	(R)-CH_3	(R)-$CH_2(C_6H_5)$	(R)-$CH_2CH(CH_3)_2$
H	<0.5	<1.5	12	6.4
(R)-CH_3	<0.5	<1.5	12	8.4
(R,S)-$CH_2(C_6H_5)$	<0.5	<1.5	13	10
(R,S)-$CH_2CH(CH_3)_2$	<0.5	<1.5	14	10
(R,S)-$CH(CH_3)_2$	<0.5	<1.5	10	20

[a] Determined by spectrophotometric assay of released 4-nitrostyrene upon deprotection of the tethered phosphonate.

We have recently described the synthesis of phosphonic acid esters using a modified Mitsunobu condensation under conditions in which the alcohol is the limiting reagent.[6c] These modifications include addition of an exogenous base as a general base catalyst and the use of tris(4-chlorophenyl)phosphine instead of triphenylphosphine. Successful application of this methodology to the solid-phase synthesis of peptidylphosphonates has been achieved and is described below.

SPPPS begins with the construction of the carboxy terminus peptide sequence **1** (Scheme 1) using Fmoc chemistry and HOBT/HBTU coupling. After removal of the N-terminal Fmoc protecting group from **1** with piperidine in NMP, it is coupled with an α-*O*-Fmoc-hydroxy acid **2** using HOBT/HBTU. Treatment of **3** with piperidine in NMP removes the Fmoc group, yielding a free α-hydroxyl group. The alcohol is condensed with a methyl α-[*N*-(4-nitrophenethyloxycarbonyl)amino] alkylphosphonic acid **4** using the modified Mitsunobu coupling procedure,[6c] providing the peptidylphosphonate **5** with inversion of configuration at the carbinol carbon. The betaine formed between the phosphine and DIAD was basic enough to cause β-elimination of an Fmoc group, necessitating the use of the more stable 4-nitrophenethyloxycarbonyl (NPEOC) group.[7] Removal of the NPEOC group in **3** was accomplished with 5% DBU in NMP.

In order to determine the coupling yield of the α-hydroxy acid and phosphonic acid segments, the deprotection products were assayed spectrophotometrically. Upon treatment of **3** with piperidine, a dibenzofulvene–piperidine adduct (301 nm, $\epsilon = 7800$ $M^{-1}{\cdot}cm^{-1}$)[8] is released, while treatment of **5** with DBU releases 4-nitrostyrene (308 nm, $\epsilon = 13\,200$ $M^{-1}{\cdot}cm^{-1}$). UV analysis allowed the extent of coupling to be calculated for both steps. As expected, the coupling rate was pseudo first order with respect to the resin-bound alcohol and exhibited more sensitivity to the steric bulk of the hydroxy component's side chain **2** than the phosphoryl component's side chain **4** (Table 1). Coupling yields of greater than 90% were obtained for the examples shown in Table 1.

Following removal of the NPEOC group, extension of the peptidylphosphonate **5** can be continued using standard peptide coupling chemistry or the peptidylphosphonate can be deprotected and cleaved from the support. A two-step deprotection and cleavage sequence is used. Selective demethylation of the phosphonic acid diester with triethylammonium phenoxide in dioxane[9] is followed by simultaneous side chain deprotection and cleavage from the support in TFA containing the appropriate scavengers. Purification of the peptidylphosphonate is accomplished by aqueous workup and/or reverse phase chromatography.

As an example of the usefulness of this methodology a number of thermolysin inhibitors described by Bartlett[5] have been

(7) Pfleiderer, W.; Schwarz, M.; Schirmeister, H. *Chem. Scr.* **1986**, *26*, 147.

(8) Bernatowicz, M. S.; Daniels, S. B.; Koster, H. *Tetrahedron Lett.* **1989**, *30*, 4645.

(9) Norbeck, D. W.; Kramer, J. B.; Lartey, P. A. *J. Org. Chem.* **1987**, *52*, 2174.

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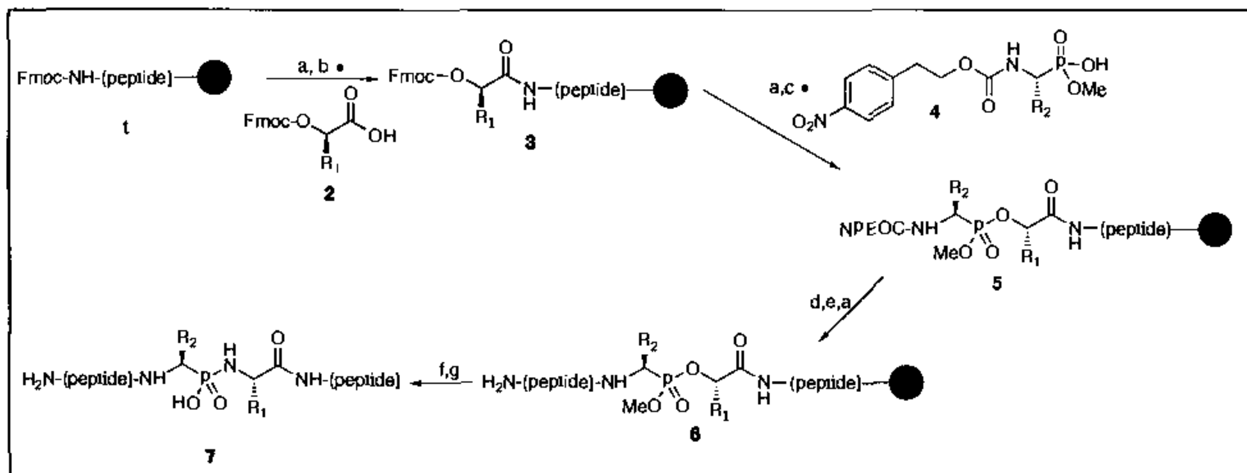
Scheme 1. Solid-Phase Peptidylphosphonate Synthesis Cycle[a]



[a] (a) 30% piperidine/NMP; (b) HBTU, HOBT, DIEA, NMP; (c) tris(4-chlorophenyl)phosphine, DIAD, DIEA, THF; (d) 5% DBU/NMP; (e) *N*-Fmoc-amino acid, HOBT, HBTU, DIEA, NMP; (f) 1:2:2 thiophenol–triethylamine–dioxane; (g) scavengers, TFA.

synthesized. After cleavage from the resin and aqueous workup, yields of >95% were obtained for the following peptidylphosphonates: Cbz-G^P(O)-L-A(OH); Cbz-(R)-A^P(O)-L-A(OH); Cbz-(R,S)-F^P(O)-L-A(OH); Cbz-(R,S)-L^P(O)-L-A(OH); Cbz-(R,S)-V^P(O)-L-A(OH).

In conclusion, the solid-phase synthesis of peptidylphosphonates using a modified Mitsunobu condensation has been described. The condensation is compatible with peptide synthesis using Fmoc chemistry and routinely provides coupling yields >90%. This represents the first successful solid-phase synthesis of this important class of enzyme inhibitors. Characterization of the scope and limitations of this solid phase synthesis methodology, as well as expansion of the monomer basis set to include functionalized side chains, is currently in progress. In addition, the construction and screening of peptidylphosphonate libraries for the discovery of inhibitors of various metalloproteases is in progress and will be reported in due course.

Supplementary Material Available: Listings of experimental procedures for the synthesis of the monomer basis set and for the solid phase synthesis of the peptidylphosphonates, including analytical data for a representative sample of the peptidylphosphonates and intermediates (8 pages). This material is contained in many libraries on microfiche, immediately follows this article in the microfilm version of the journal, and can be ordered from the ACS; see any current masthead page for ordering information.