Versatile Building Block for the Synthesis of Phosphine-Containing Peptides: The Sulfide of Diphenylphosphinoserine

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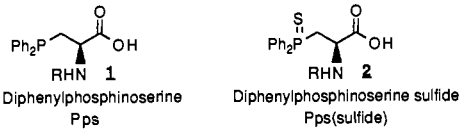
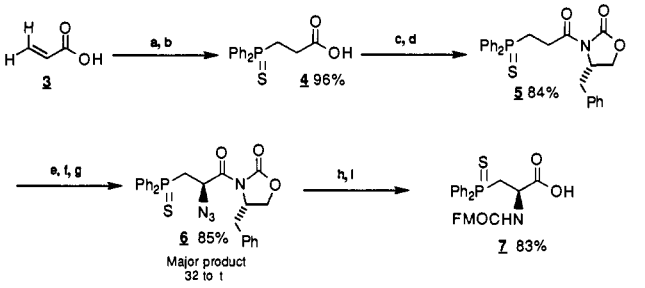
Peptide chemistry has seen the de novo synthesis of a number of protein structural motifs,[1,2] artificial β-sheets, and β-turns;[3] three-, four-, and six-helix bundles[4] have all recently been reported. Metal-binding peptides using both natural and unnatural amino acids have been built to purify enzymes, influence protein structure, or control enzyme activity.[5] Work has been reported using proteins to deliver radioactive or paramagnetic metals for medical imaging.[6] Peptide-based structures will eventually be used to build man-made enzymes for both biological and non-biological reactions. To take full advantage of the structural aspects of peptides, methods must be developed that allow for the incorporation of unique functionality into these biological systems. We are interested in developing methodology to facilitate the synthesis of peptides that contain phosphine ligands. The chemistry of transition metal phosphine complexes is diverse, and consequently the successful development of a general route to such compounds will yield interesting protein–metal conjugates.[7] Phosphine-containing peptides are potentially useful in a number of areas. One might imagine using the secondary and tertiary structure of peptides to control reactivity of transition metals bound to the phosphines. Phosphine-containing peptides could be used to position and immobilize transition metals in membranes. Commercial technetium medical imaging agents often contain phosphine ligands.[8] By using phosphines to bind technetium to a protein one should be able to target specific organs or disease states for imaging. This paper reports the first system that allows for the incorporation of phosphine-containing amino acids into any peptide sequence attainable by solid-phase peptide synthesis. The synthesis and incorporation of this amino acid into a 12-residue peptide and the binding of rhodium to this fundamentally new phosphine ligand are illustrated.

Chart 1



Scheme 1[a]



[a] (a) Ph_2PH, $Me_4N^+ {}^-OH$, CH_3CN, room temperature, 12 h; (b) $Na_2S_2O_3$, CH_3OH, 46 °C, 12 h; (c) ClC(O)OtBu, THF, −78 to 0 °C, 1.5 h; (d) lithium, (*S*)-(−)-4-benzyl-2-oxazolidinone, THF, −78 to 0 °C, 2 h; (e) KHMDS, THF, −78 °C, 30 min; (f) Tris-N_3, THF, −78 °C, 2 min; (g) HOAc, −78 to 0 °C, 8 h; (h) LiOH, THF/H_2O, 0 °C; (i) $SnCl_2$, CH_3OH, 0 °C, 1.5 h; (j) FMOC-Cl, $NaHCO_3$, $dioxane/H_2O$, 0 °C to room temperature, 12 h.

The first type of amino acid we chose as our target was a phosphine derivative of serine (**1**, diphenylphosphinoserine) (Chart 1). To be useful any amino acid of this type must be amenable to solid-phase peptide synthesis. We found that, as expected, alkyldiarylphosphines readily oxidize under the conditions used in solid-phase peptide synthesis. To prevent formation of the unwanted phosphine oxide, it was necessary to find a method to protect the phosphine group in a manner that allowed it to be deprotected once the entire peptide was assembled. The conversion of the phosphine to the phosphine sulfide (**2**)[9] gave rise to an amino acid that could be used in standard peptide coupling procedures. Once the desired peptide was assembled, the phosphine could be regenerated by desulfurization with Raney nickel.[10]

The best route to the required amino acid involved the use of Evans' chiral oxazolidinone chemistry (Scheme 1).[11] Addition of diphenylphosphine to acrylic acid proceeds smoothly, using tetramethylammonium hydroxide as base.[12] Treatment of the crude reaction mixture with sodium thiosulfate converts the

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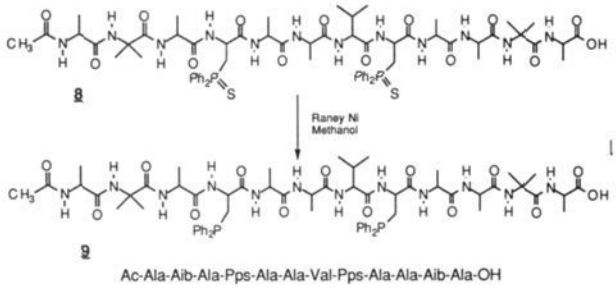
Scheme 2



Ac-Ala-Aib-Ala-Pps-Ala-Ala-Val-Pps-Ala-Ala-Aib-Ala-OH

phosphine to the phosphine sulfide (**4**).[9] Acid **4** is then converted to the amino acid by formation of the oxazolidinone (**5**), reaction of its enolate with trisyl azide, and chromatographic separation of the diasteromeric azides. Cleavage of the chiral auxiliary and reduction of the azide (**6**) with tin(II) chloride gives the amino acid. Reaction of that product with 9-fluorenylmethyl chloroformate yields the FMOC-protected amino acid (**7**) ready for peptide synthesis.[13]

The peptide chosen to contain diphenylphosphinoserine was designed to possess high helix content (Scheme 2).[14] The phosphine-containing amino acids were positioned i, $i + 4$ so as to be able to bind one metal atom between them, assuming an α-helical structure for the peptide (**9**). The peptide was synthesized by standard FMOC chemistry on Wang resin.[15] Diphenylphosphinoserine was incorporated as a dimer with alanine (FMOC-Pps(sulfide)-Ala-OPfp), allowing for determination of the optical purity of the phosphine sulfide amino acid and facile purification by recrystallization[16] before incorporation into the peptide.

^{31}P NMR of the HPLC-purified peptide displayed two resonances for the inequivalent phosphine sulfides (Figure 1a).[17a] Treatment of the peptide with Raney nickel overnight converted the phosphine sulfides to the free phosphines (Figure 1b).[17b] This bis phosphine ligand could then be metalated by stirring with $RhCl(NBD)^+$ $^-ClO_4$, giving the cationic rhodium-containing peptide (Figure 1c).[17c] This reaction was conveniently monitored by ^{31}P NMR. Upon treatment with $RhCl(NBD)^+$ $^-ClO_4$ the chemical shift of the phosphorus resonances shifted downfield and coupling between spin $^1/_2$ ^{103}Rh and the phosphines was observed.[18]

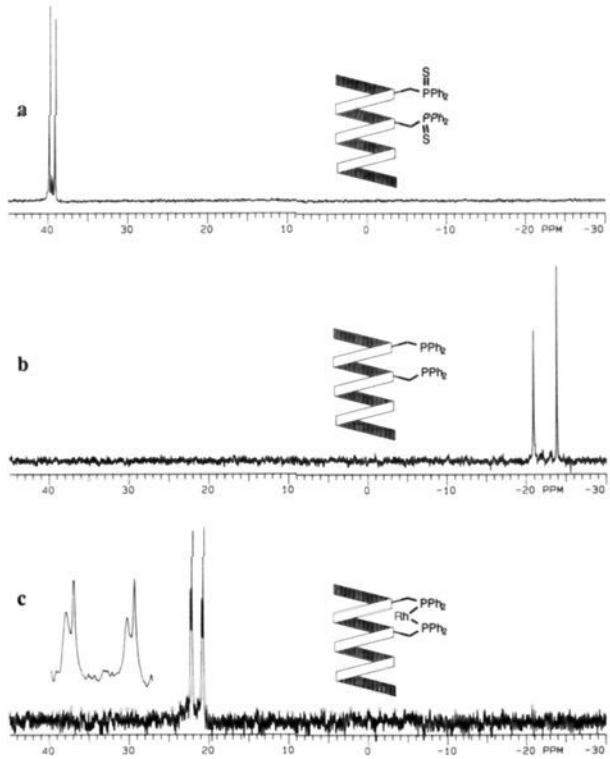


Figure 1. ^{31}P NMR of phosphorus containing peptides.

This is the first peptide–phosphine–metal complex of this type. We are presently working on the characterization of this peptide's secondary structure, the synthesis of a series of phosphine peptides, and the study of metal–peptide complexes. In conclusion, we have developed a general method, compatible with solid-phase peptide synthesis, for peptides that contain phosphine ligands, and we have shown that a metal can be coordinated to the two phosphines to form a new type of metal–peptide conjugate.

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Supplementary Material Available: Spectral data for **4–9** and a listing of NMR assignments for **8** (3 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.

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(17) Representative ^{31}P chemical shifts are as follows: (a) Ph_3PS, δ = 39.9–43.5 ppm; (b) CH_3PPh_2, δ = –28.0 ppm (Gorenstein, D. G. *Prog. NMR Spectrosc.* **1983**, *16*, 1). (c) $[Ph_2(CH_2)P]_2RhCOD$, δ = 14–18 ppm (Descotes, G.; Lafont, D.; Sinou, D.; Brown, J. M.; Chaloner, P. A.; Parker, D. *Nouv. J. Chim.* **1981**, *5*, 167).

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