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# Synthetic Communications: An International Journal for Rapid Communication of Synthetic Organic Chemistry

Publication details, including instructions for authors and subscription information: <u>http://www.tandfonline.com/loi/lsyc20</u>

# SYNTHESIS OF N-PHOSPHOPEPTIDES COUPLED BY DICHLOROTRIPHENYLPHOSPHORANE

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Published online: 09 Nov 2006.

To cite this article: Shu-Zhi Dong , Hua Fu & Yu-Fen Zhao (2001) SYNTHESIS OF N-PHOSPHOPEPTIDES COUPLED BY DICHLOROTRIPHENYLPHOSPHORANE, Synthetic Communications: An International Journal for Rapid Communication of Synthetic Organic Chemistry, 31:13, 2067-2075, DOI: <u>10.1081/SCC-100104428</u>

To link to this article: <u>http://dx.doi.org/10.1081/SCC-100104428</u>

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#### SYNTHETIC COMMUNICATIONS, 31(13), 2067–2075 (2001)

# SYNTHESIS OF N-PHOSPHOPEPTIDES COUPLED BY DICHLOROTRIPHENYL-PHOSPHORANE

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## ABSTRACT

Coupling of N-phosphoamino acids 2 and amino acid methyl esters by dichlorotriphenylphosphorane 3 produced N-phosphopeptide methyl esters 5, which were sequentially hydrolyzed in triethylamine or dilute NaOH aqueous solution. The target products N-phosphodipeptides 6 were obtained in reasonable yields and high purity after isolation using extraction method. The convenient and efficient approach could be generally used for synthesis of polypeptides.

Derivatives of N-phosphopeptides are of pharmaceutical and biologically active interest.<sup>1–4</sup> They are continuing to be increasingly important as mechanistic probes for proteases,<sup>5</sup> potent inhibitors of peptidases as tetrahedral transition-state or high-energy intermediate analogs,<sup>6</sup> useful tools for

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the investigation of phosphorylation, or as important posttranslational modification of peptides and proteins.<sup>7</sup>

In order to decipher the role of many phosphopeptides and explore their biological activities, a facile method to acqurie multi-gram quantities of phosphopeptides is in high demand. In general, N-(dialkoxyphosphoryl)peptides were prepared by phosphorylating the peptides obtained by the deprotection of N-protected peptides such as N-Boc-peptides.<sup>4</sup> However, N-(dialkoxyphosphoryl)amino acids have hardly been used for the synthesis of N-(dialkoxyphosphoryl)peptides, probably because it is difficult to synthesize and purify them. Fortunately, an efficient method to prepare highly pure and large quantities of N-phosphoamino acids has been developed in our lab.<sup>8</sup>

Furthermore, it has been proved that the phosphinic carboxylic mixed anhydride method had the superiority in peptide synthesis<sup>9</sup> than the mixed carboxylic anhydride method for the faster coupling rate, more regiospecificity in nucleophilic attack and more thermal stability to disproportionation. Hence, the methodology of N-phosphopeptide synthesis starting from N-phosphoamino acid and using dichlorotriphenylphosphorane **3** as the coupling reagent was investigated.

#### **RESULTS AND DISCUSSION**

## Synthesis of N-(Diisopropyloxyphosphoryl)dipeptide Methyl Esters 5 (N-DIPP-dipeptide Methyl Esters)

Dichlorotriphenylphosphorane 3, which was assigned to the ionised form according to <sup>31</sup>P NMR spectrum ( $\delta_P = 61.1 \text{ ppm}$ ), is either commercially available or can readily be prepared by the reaction of triphenylphosphine with hexachloroethane without any undesirable phosphorus by-product (Scheme 1).<sup>10</sup>

$$Ph = P \begin{pmatrix} Ph \\ Ph \end{pmatrix} + C_2 Cl_6 \longrightarrow \left[ Ph_3 \stackrel{+}{P} - Cl \right] Cl + C_2 Cl_4$$

Scheme 1. Synthetic Pathway of Dichlorotriphenylphosphorane 3.

1.5 equivalents of in-situ generated dichlorotriphenylphosphorane **3** was added to 1 equivalent of N-(diisopropyloxyphosphoryl)amino acid **2** (N-DIPP-amino acid **2**), amino acid methyl ester and triethylamine (Et<sub>3</sub>N) in CH<sub>2</sub>Cl<sub>2</sub> at room temperature. The mixture was stirred for 1 h,

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and compound 2 and amino acid ester almost transferred into N-DIPPdipeptide methyl ester 5 in quantitative amount (indicated by the single signal at about <sup>31</sup>P NMR 6.8 ppm of compound 5) (Scheme 2). The activation mechanism was proposed that dichlorotriphenylphosphorane 3 phosphorylated N-DIPP-amino acid 2 to give the phosphinic carboxylic mixed anhydride 4, followed by the nucleophilic attack of amino acid methyl ester resulting into the formation of peptide bond.<sup>11</sup> Excessive dichlorotriphenylphosphorane 3 would consume the remnant water to guarantee the anhydrous environment and make the coupling reaction complete. Moreover, it would not bring in further by-products because 3 can react with H<sub>2</sub>O to form triphenylphosphine oxide, which is the same as the product yielded in coupling reaction.



Scheme 2. Synthetic Pathway of N-DIPP-dipeptide Methyl Esters 5.

### Hydrolysis of N-DIPP-dipeptide Methyl Esters 5

In order to avoid the side reactions on N-protecting group DIPP (diisopropyloxyphosphoryl), triethylamine, as a weak base, was the first choice to catalyze the hydrolysis of compound **5** (Scheme 3).<sup>12</sup> Yet it was only efficient for the hydrolysis of compound **5a**, **5b** and **5c**. Hydrolysis of compound **5d**, **5e**, **5f**, however, was completed in a stronger base, dilute NaOH aqueous solution, with a longer time as shown in **Table 1**. **Table 1** shows that the only difference between **5a**, **5b**, and **5d**, **5e**, **5f** lies in the side chain of the amino acid residue on C-terminal. And the nuance between compound **5c** and **5e** is the addition of phenyl on the side-chain of the first amino acid residue. These show that the hydrolysis reaction is strongly dependent upon the dual side-chains.

The hydrolysis reaction was carried out in aqueous-organic mixed solvents to facilitate the tracing of reaction process by <sup>31</sup>P NMR techniques. N-DIPP-dipeptide methyl esters **5** are organic soluble species, while the



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Final Poduct <b>6</b>	$R^1$	$R^2$	Base	Time (h)	Overall Yield (%)
DIPP-Phe-Gly a	-CH <sub>2</sub> -Ph	-H	Et <sub>3</sub> N	3.5	85.2
DIPP-Phe-Ala b	-CH <sub>2</sub> -Ph	-CH <sub>3</sub>	Et <sub>3</sub> N	3.5	60.7
DIPP-Ala-Phe c	-CH <sub>3</sub>	-CH <sub>2</sub> -Ph	Et <sub>3</sub> N	3.5	60.0
DIPP-Phe-Leu d	-CH <sub>2</sub> -Ph	$-CH_2-Pr^1$	NaOH (0.17 N)	7.0	80.3
DIPP-Phe-Phe e	-CH <sub>2</sub> -Ph	-CH <sub>2</sub> -Ph	NaOH (0.17 N)	7.0	75.8
DIPP-Phe-Val f	-CH <sub>2</sub> -Ph	$-Pr^1$	NaOH (0.65 N)	9.0	88.2

Table 1. Hydrolysis of N-DIPP-dipeptide Methyl Esters 5

basic hydrolyzed products **6** are the corresponding salts which are aqueous soluble. As the hydrolysis proceeding the signal at about <sup>31</sup>P NMR 6.8 ppm of compound **5** in organic phase decreased. At the end point there was no compound **5** left, therefore no <sup>31</sup>P NMR signal of compound **5** could be detected in the organic phase. Whilst triphenylphosphine oxide was imprisoned in organic phase (the signal at about <sup>31</sup>P NMR 28.0 ppm of Ph<sub>3</sub>P = O only existed in the organic phase). So the major impurity was removed completely. Dichlorotriphenylphosphorane **3** gives cleaner reactions due to the high sensitivity towards the atmosphere and the exclusive resultant triphenylphosphine oxide. Hence after eliminating the main impurity Ph<sub>3</sub>P = O, we got highly pure N-phosphodipeptides **6a–f** using simple extraction workup.

The purity and structure of compounds **6a–f** were determined by <sup>1</sup>H NMR, <sup>13</sup>C NMR, <sup>31</sup>P NMR and ESI-MS methods. Each target product gave only one <sup>31</sup>P NMR signal implying that no racemization occurred during this peptide bond formation.<sup>13</sup> These highly optical pure N-DIPP-dipeptides **6a–f** could be used for elongation of N-phosphopeptides or/and test for biological activities.

In conclusion, dichlorotriphenylphosphorane **3** is an effective coupling reagent for synthesis of N-phosphopeptides. The present method enables us to obtain N-phosphopeptides on a large-scale and highly pure production because of its availability, convenience and low racemization. Further, the N-phosphopeptides could be deprotected under acid conditions<sup>14</sup> to yield the corresponding peptides. Thus the convenient and efficient approach could be also applied for the synthesis of polypeptides.



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#### EXPERIMENTAL

### **General Procedure**

All glassware was dried in an oven for at least 3 h at 120°C prior to use. Air sensitive materials were transferred under a nitrogen atmosphere. Dichloromethane and triethylamine were dried over  $P_2O_5$  and  $CaH_2$  respectively. <sup>1</sup>H MNR and <sup>13</sup>C NMR spectra were recorded on Bruker AM 500 spectrometer. TMS ( $\delta$ =0.0) and CDCl<sub>3</sub> ( $\delta$ =77.0 ppm) were references for <sup>1</sup>H and <sup>13</sup>C NMR spectra respectively. <sup>13</sup>C NMR spectra were all taken under <sup>1</sup>H decoupled and <sup>31</sup>P coupled conditions. <sup>31</sup>P NMR spectra were taken on Bruker AC 200 spectrometer at 81 MHz under <sup>1</sup>H decoupled conditions. <sup>31</sup>P NMR chemical shifts are reported in ppm downfield (+) or upfield (-) from external 85% H<sub>3</sub>PO<sub>4</sub> as reference. Mass spectra were conducted on Bruker Esquire-LC mass spectrometer in positive and negative ion mode.

#### Synthesis of Diisopropyl Phosphite (DIPPH) 1

The starting material DIPPH **1** was prepared according to the published procedure.<sup>15</sup> Yield: 75%. <sup>31</sup>P NMR: 4.4 ppm.

#### Synthesis of N-(Diisopropyloxyphosphoryl)amino Acids 2

N-DIPP-amino acid 2 was synthesized by adding diisopropyl phosphite 1 to an aqueous-organic mixture containing the amino acid, water, carbon tetrachloride, triethylamine and ethanol, well stirring the mixture at  $0-20^{\circ}$ C for a few hours, and quenching the reaction by acidification.<sup>8</sup> Yields: DIPP-Ala, 89%; DIPP-Phe, 93%. <sup>31</sup>P NMR: 7.1, 6.9 ppm respectively.

#### Synthesis of Dichlorotriphenylphosphorane 3

According to literature,<sup>10</sup> under a nitrogen atmosphere, 2 mmol triphenylphosphine in 3 mL dry  $CH_2Cl_2$  was added dropwise to a stirred 2 mmol hexachloroethane in 3 mL dry  $CH_2Cl_2$  in an ice-water bath. After five minutes, the bath was removed. The reaction was stirred continuously for 1 h at room temperature, then dichlorotriphenylphosphorane **3** was produced. Yield: 90% (by <sup>31</sup>P NMR spectral integral). <sup>31</sup>P NMR: 61.1 ppm.

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Synthesis of N-(Diisopropyloxyphosphoryl)dipeptide Methyl Esters 5

Under a nitrogen atmosphere, 1.2 mmol dichlorotriphenylphosphorane **3** in CH<sub>2</sub>Cl<sub>2</sub> was added dropwise to a stirred dry CH<sub>2</sub>Cl<sub>2</sub> (5 mL) solution of 0.8 mmol N-(diisopropyloxyphosphoryl)amino acid **2**, 0.8 mmol amino acid methyl ester and  $350 \,\mu$ L dry Et<sub>3</sub>N at room temperature. Extra addition of adequate Et<sub>3</sub>N was followed after half an hour. The stirring was continued for 1 h. After the removal of solvent the resultant residue was dissolved in 15 mL ethyl acetate, then followed by the filtration of residue triethylamine hydrochloride. The filtrate was washed with 4 mL 1 mol/L citric acid, 4 mL saturated NaCl solution, 4 mL 5% NaHCO<sub>3</sub> solution, and 4 mL H<sub>2</sub>O.

Synthesis of N-(Diisopropyloxyphosphoryl)dipeptides 6

**Method A**: The above filtrate by ethyl acetate was evaporated to dryness. A mixture of  $3.5 \text{ mL Et}_3\text{N}$  and  $3.5 \text{ mL H}_2\text{O}$  was added to the resulting residue containing N-(diisopropyloxyphosphoryl)dipeptide methyl ester **5** and stirred at 40°C for 3.5 h. The water layer was washed with Et<sub>3</sub>N ( $3 \times 4 \text{ mL}$ ) followed by addition of 1.5 equivalents NaOH. The mixture was stirred for ten minutes, then washed with CH<sub>2</sub>Cl<sub>2</sub> ( $3 \times 5 \text{ mL}$ ) and acidified to pH 3 with 1 mol/L HCl in an ice-water bath. The nepheloid solution was extracted with ethyl acetate ( $3 \times 15 \text{ mL}$ ). The organic layer was washed with water, brine and dried over anhydrous MgSO<sub>4</sub>. Removal of the solvent afforded the pure products **6a–c** as white solid.

Spectral data for compounds 6a-c

**N-DIPP-Phe-Gly 6a**, yield: 85.2%. <sup>31</sup>P NMR (81.0 MHz, AcOEt), δ (ppm): 6.95. <sup>1</sup>H NMR (500 MHz, CDCl<sub>3</sub>), δ (ppm): 1.09–1.25 (m, 12H, (P(OCH(C<u>H</u><sub>3</sub>)<sub>2</sub>)<sub>2</sub>), 2.97–3.11 (m, 2H, C<u>H</u><sub>2</sub>Ph), 3.78–3.83 (m, 2H, C<u>H</u><sub>2</sub>COOH), 4.04–4.16 (br, 1H, CO-NH), 4.19 (q, J=5.7 Hz, <sup>3</sup>J<sub>P-H</sub>=18.6 Hz, 1H, C<u>H</u>CH<sub>2</sub>Ph), 4.27–4.38 (m, 2H, P(OC<u>H</u>(CH<sub>3</sub>)<sub>2</sub>)<sub>2</sub>), 7.18–7.29 (m, 6H, P-NH, Ph). <sup>13</sup>C NMR (125 MHz, CDCl<sub>3</sub>), δ (ppm): 23.52 (br, OCH(C<u>H</u><sub>3</sub>)<sub>2</sub>), 40.30 (d, <sup>3</sup>J<sub>P-C</sub>=5.0 Hz, CH<sub>2</sub>Ph), 41.49 (C<u>H</u><sub>2</sub>COOH), 56.78 (CHCH<sub>2</sub>Ph), 71.07 (q, <sup>2</sup>J<sub>P-C</sub>=6.1 Hz, P(OC<u>H</u>(CH<sub>3</sub>)<sub>2</sub>)<sub>2</sub>), 126.80–136.53 (Ph), 172.23 (COOH), 172.40 (d, <sup>3</sup>J<sub>P-C</sub>=4.4 Hz, CO-NH). Positive-ion ESI-MS (m/z): 387.1 (M + H)<sup>+</sup>. Negative-ion ESI-MS (m/z): 385.1 (M-H)<sup>-</sup>.

**N-DIPP-Phe-Ala 6b**, yield: 60.7%. <sup>31</sup>P NMR (81.0 MHz, AcOEt),  $\delta$  (ppm): 7.21. <sup>1</sup>H NMR (500 MHz, CDCl<sub>3</sub>),  $\delta$  (ppm): 1.12 (d, J = 6.0 Hz, 3H, CHC<u>H<sub>3</sub></u>), 1.15–1.31 (m, 12H, (P(OCH(C<u>H<sub>3</sub></u>)<sub>2</sub>)<sub>2</sub>), 3.01–3.08 (m, 2H, C<u>H<sub>2</sub></u>Ph), 3.74 (t, 1H, C<u>H</u>CH<sub>3</sub>), 4.06 (br, 1H, CO-NH), 4.28–4.41 (m, 2H



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(P(OC<u>H</u>(CH<sub>3</sub>)<sub>2</sub>)<sub>2</sub>), 4.41–4.54 (m, 1H, C<u>H</u>CH<sub>2</sub>Ph), 7.19–7.48 (m, 6H, P-NH, Ph). <sup>13</sup>C NMR (125 MHz, CDCl<sub>3</sub>),  $\delta$  (ppm): 18.13 (CH<u>C</u>H<sub>3</sub>), 23.51–23.60 (m, (P(OCH(<u>C</u>H<sub>3</sub>)<sub>2</sub>)<sub>2</sub>), 39.59 (d, <sup>3</sup>J<sub>P-C</sub> = 5.5 Hz, <u>C</u>H<sub>2</sub>Ph), 48.14 (<u>C</u>HCOOH), 56.54 (<u>C</u>HCO-NH), 72.00 (q, <sup>2</sup>J<sub>P-C</sub> = 5.8 Hz, (P(O<u>C</u>H(CH<sub>3</sub>)<sub>2</sub>)<sub>2</sub>), 126.83–136.25 (Ph), 171.99 (d, <sup>3</sup>J<sub>P-C</sub> = 4.4 Hz, CO-NH), 175.16 (COOH). Positive-ion ESI-MS (m/z): 401 (M + H)<sup>+</sup>. Negative-ion ESI-MS (m/z): 399 (M-H)<sup>-</sup>.

**N-DIPP-Ala-Phe 6c**, yield: 60.0%. <sup>31</sup>P NMR (81.0 MHz, AcOEt), δ (ppm): 6.85. <sup>1</sup>H NMR (500 MHz, CDCl<sub>3</sub>), δ (ppm): 1.21 (d, J = 4.5 Hz, 3H, CHC<u>H</u><sub>3</sub>, 1.26–1.30 (m, 12H, (P(OCH(C<u>H</u><sub>3</sub>)<sub>2</sub>)<sub>2</sub>), 2.93 (d, J = 7.5 Hz, 2H, C<u>H</u><sub>2</sub>Ph), 4.54 (m, 1H, C<u>H</u>COOH), 4.54 (br, 1H, CO-NH), 4.58–4.61 (m, 2H, (P(OC<u>H</u>(CH<sub>3</sub>)<sub>2</sub>)<sub>2</sub>), 4.80 (m, 1H, C<u>H</u>CH<sub>3</sub>), 7.19–7.26 (m, 6H, P-NH, Ph). <sup>13</sup>C NMR (125 MHz, CDCl<sub>3</sub>), δ (ppm): 20.67 (CHC<u>H</u><sub>3</sub>), 23.57–23.73 ((P(OCH(<u>C</u>H<sub>3</sub>)<sub>2</sub>)<sub>2</sub>), 37.45 (<u>C</u>H<sub>2</sub>Ph), 51.42 (<u>C</u>HCOOH), 53.27 (<u>C</u>HCH<sub>3</sub>), 72.06 (q, <sup>2</sup>J<sub>P-C</sub> = 5.3 Hz, (P(O<u>C</u>H(CH<sub>3</sub>)<sub>2</sub>)<sub>2</sub>), 126.77–136.55 (Ph), 173.12 (d, <sup>3</sup>J<sub>P-C</sub> = 5.5 Hz, CO-NH), 174.20 (COOH). Positive-ion ESI-MS (m/z): 401 (M + H)<sup>+</sup>. Negative-ion ESI-MS (m/z): 399 (M-H)<sup>-</sup>.

Method B: The above filtrate by ethyl ecetate was evaporated to dryness. A mixture of 6 mL NaOH aqueous solution (0.17 mol/L for 6d and 6e, 0.65 mol/L for 6f) and 3 mL CH<sub>2</sub>Cl<sub>2</sub> was added to the resulting residue containing N-(diisopropyloxyphosphoryl)dipeptide methyl ester 5 and stirred at room temperature for 7 h. The water layer was washed with CH<sub>2</sub>Cl<sub>2</sub> ( $3 \times 4$  mL). The following isolation procedure was the same as that of method A.

Spectral data for compounds 6d-f.

**N-DIPP-Phe-Leu 6d**, yield: 80.3%. <sup>31</sup>P NMR (81.0 MHz, AcOEt), δ (ppm): 7.28. <sup>1</sup>H NMR (500 MHz, CDCl<sub>3</sub>), δ (ppm): 0.89 (q, J = 6.5, 16.5 Hz, 6H, CH<sub>2</sub>CH(C<u>H</u><sub>3</sub>)<sub>2</sub>), 1.13–1.25 (m, 12H, P(OCH(C<u>H</u><sub>3</sub>)<sub>2</sub>)<sub>2</sub>), 1.44–1.55 (m, 2H, C<u>H</u><sub>2</sub>CH(CH<sub>3</sub>)<sub>2</sub>), 1.59–1.64 (m, 1H, CH<sub>2</sub>C<u>H</u>(CH<sub>3</sub>)<sub>2</sub>), 3.00–3.15 (m, 2H, C<u>H</u><sub>2</sub>Ph), 3.58 (t, 1<u>H</u>, CHCOOH), 4.07 (br, 1H, CO-NH), 4.32–4.43 (m, 2H, P(OC<u>H</u>(CH<sub>3</sub>)<sub>2</sub>)<sub>2</sub>), 4.59 (q, J = 7.8 Hz, <sup>3</sup>J<sub>P-H</sub> = 13.8 Hz, 1H, C<u>H</u>CH<sub>2</sub>PH), 7.19–7.38 (m, 6H, P-NH, Ph). <sup>13</sup>C NMR (125 MHz, CDCl<sub>3</sub>), δ (ppm): 22.29, 22.65 (CH<sub>2</sub>CH(CH<sub>3</sub>)<sub>2</sub>), 23.50–23.65 (P(OCH(CH<sub>3</sub>)<sub>2</sub>)<sub>2</sub>), 24.52 (CH<sub>2</sub>CH(CH<sub>3</sub>)<sub>2</sub>), 39.30 (d, <sup>3</sup>J<sub>P-C</sub> = 4.5 Hz, CH<sub>2</sub>Ph), 41.51 (CH<sub>2</sub>CH(CH<sub>3</sub>)<sub>2</sub>), 50.83 (CHCOOH), 56.62 (CHCH<sub>2</sub>Ph), 71.92–72.13 (q, <sup>2</sup>J<sub>P-C</sub> = 5.9 Hz, P(OCH(CH<sub>3</sub>)<sub>2</sub>)<sub>2</sub>), 126.84–136.26 (Ph), 171.85 (d, <sup>3</sup>J<sub>P-C</sub> = 5.4 Hz, CO-NH), 175.26 (COOH). Positive-ion ESI-MS (m/z): 443.3 (M+H)<sup>+</sup>. Negative-ion ESI-MS (m/z): 441.3 (M-H)<sup>-</sup>.

**N-DIPP-Phe-Phe 6e**, yield: 75.8%. <sup>31</sup>P NMR (81.0 MHz, AcOEt),  $\delta$  (ppm): 6.93. <sup>1</sup>H NMR (500 MHz, CDCl<sub>3</sub>),  $\delta$  (ppm): 1.03–1.23 (m, 12H, (P(OCH(CH<sub>3</sub>)<sub>2</sub>)<sub>2</sub>), 2.89–3.07 (m, 4H, 2(CH<sub>2</sub>Ph)), 3.83 (t, J = 10.8 Hz, 1H,

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CHCOOH), 3.99 (br, 1H, CO-NH), 4.26–4.38 (m, 2H, (P(OCH(CH<sub>3</sub>)<sub>2</sub>)<sub>2</sub>), 4.79 (q, J = 6.5 Hz,  ${}^{3}J_{P-C}$  = 12.5 Hz, 1H, CHCO-NH), 7.07–7.39 (m, 11H, P-NH, 2Ph).  ${}^{13}C$  NMR (125 MHz, CDCl<sub>3</sub>),  $\delta$  (ppm): 23.45–23.54 ((P(OCH(CH<sub>3</sub>)<sub>2</sub>)<sub>2</sub>), 37.52 (PhCH<sub>2</sub>CHCOOH), 39.51 (d, {}^{3}J\_{P-C} = 4.9 Hz, PhCH<sub>2</sub>CHCONH), 53.46 (CHCOOH), 56.83 (CHCONH), 71.00 (q, {}^{2}J\_{P-C} = 6.1 Hz, (P(OCH(CH<sub>3</sub>)<sub>2</sub>)<sub>2</sub>), 126.69–126.48 (2 Ph), 172.06 (d, {}^{3}J\_{P-C} = 5.1 Hz, CO-NH), 173.70 (COOH). Positive-ion ESI-MS (m/z): 477 (M + H)<sup>+</sup>. Negative-ion ESI-MS (m/z): 475 (M-H)<sup>-</sup>.

**N-DIPP-Phe-Valf 6f**, yield: 88.2%. <sup>31</sup> P NMR (81.0 MHz, AcOEt),  $\delta$  (ppm): 6.89. <sup>1</sup>H NMR (500 MHz, CDCl<sub>3</sub>),  $\delta$  (ppm): 0.86 (dd, J = 7.0, 14.0 Hz, 6H, CHCH(CH<sub>3</sub>)<sub>2</sub>), 1.08–1.19 (m, 12H, (P(OCH(CH<sub>3</sub>)<sub>2</sub>)<sub>2</sub>), 2.13–2.18 (m, 1H, CHCH(CH<sub>3</sub>)<sub>2</sub>), 2.96–3.04 (m, 2H, CH<sub>2</sub>Ph), 4.10 (m, 1H, CHCOOH), 4.12 (br, 1H, CO-NH), 4.18–4.37 (m, 2H, (P(OCH(CH<sub>3</sub>)<sub>2</sub>)<sub>2</sub>), 4.45 (q, J = 5.0 Hz, <sup>3</sup>J<sub>P-H</sub> = 8.5 Hz, 1H, CHCH<sub>2</sub>Ph), 7.12–7.40 (m, 6H, P-NH, Ph). <sup>13</sup>C NMR (125 MHz, CDCl<sub>3</sub>),  $\delta$  (ppm): 17.72, 18.53 (CHCH(CH<sub>3</sub>)<sub>2</sub>), 23.35–23.46 ((P(OCH(CH<sub>3</sub>)<sub>2</sub>)<sub>2</sub>), 30.92 (CHCH(CH<sub>3</sub>)<sub>2</sub>), 39.67 (d, <sup>3</sup>J<sub>P-C</sub> = 5.1 Hz, CH<sub>2</sub>Ph), 56.58 (CHCOOH), 57.34 (CHCH<sub>2</sub>Ph), 71.64 (q, <sup>2</sup>J<sub>P-C</sub> = 5.8 Hz, (P(OCH(CH<sub>3</sub>)<sub>2</sub>)<sub>2</sub>), 126.59– 136.41 (Ph), 172.85 (d, <sup>3</sup>J<sub>P-C</sub> = 4.3 Hz, CO-NH), 174.96 (COOH). Positive-ion ESI-MS (m/z): 429 (M+H)<sup>+</sup>. Negative-ion ESI-MS (m/z): 427 (M-H)<sup>-</sup>.

#### ACKNOWLEDGMENTS

The authors would like to thank the financial supports from the Chinese National Natural Science Foundation (No. 29902003), the Ministry of Science and Technology, the Chinese Education Ministry and Tsinghua University.

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Received in the Netherlands September 14, 2000



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