Journal of Peptide Science
J. Peptide Sci. 2006; **12**: 303–309
Published online 24 October 2005 in Wiley InterScience (www.interscience.wiley.com). DOI: 10.1002/psc.727

JOURNAL OF PEPTIDE SCIENCE

Facile synthesis of phosphonamidate- and phosphonate-linked phosphonopeptides

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Received 7 July 2005; Accepted 2 August 2005

Abstract: A direct method for the preparation of phosphonamidate- and phosphonate-linked phosphonopeptides has been developed. Using this method, both phosphonopeptides were prepared in acceptable yields directly from simple and commercially available chemicals in one-pot reactions of benzyl carbamate, aldehydes, and methyl dichlorophosphite, followed by aminolysis with amino acid esters or alcoholysis with hydroxy esters. Copyright © 2005 European Peptide Society and John Wiley & Sons, Ltd.

Keywords: peptide; phosphonamidate; phosphonate; phosphonopeptide; synthesis

INTRODUCTION

Phosphonamidate- and phosphonate-linked peptides ('phosphonamidate peptides' and 'phosphonate peptides') are important phosphorus analogues ('phosphopeptides') of naturally occurring peptides. They have been used often as stable mimetics of tetrahedral transition states in recent years [1–14]. Transition state analogues incorporating these functionalities have been exploited as enzyme inhibitors [1–8] and as haptens for catalytic antibody research [9–14]. From both steric and electronic perspectives, they have been found to show close resemblance to the transition states of ester and amide hydrolysis and formation. Several phosphonopeptides have also been shown to possess potent antibacterial activity [15].

Phosphonamidate and phosphonate peptides **1** are generally prepared by the reaction of phosphonochloridates **3** with amino acid esters, peptide esters, or hydroxy esters. The phosphonochloridates **3** are readily prepared by the reaction of phosphonate diesters with one equivalent of phosphorus pentachloride [2,3,16] by the treatment of phosphonate monoesters **2** with thionyl chloride [1,16–18] or oxalyl chloride [1,7,16,19], or by the oxidative chlorination of phosphinate esters with carbon tetrachloride [20,21] or dichlorotriphenylphosphorane under base conditions [22]. Direct condensation of phosphonate monoesters **2** with hydroxy esters, amino acid esters, or peptide esters is an alternative method for the synthesis of phosphonopeptides, in which effective coupling reagents include DCC [23,24], BOP ((1*H*-benzotriazol-1-yloxy)tris(dimethylamino)phosphonium hexafluorophosphate) [23,25], BOP-Cl (*N*,*N*-bis(2-oxo-3-oxazolidinyl)phosphinic chloride) [26], PyBOP ((1*H*-benzotriazol-1-yloxy)tripyrrolidinophosphonium hexafluorophosphate) [23,25], HBTU (the so-called *O*-(1*H*-benzotriazol-1-yl)-*N*,*N*,*N*',*N*'-tetramethyluronium hexafluorophosphate reagent) [23], BroP (bromotris(dimethylamino)-phosphonium hexafluorophosphate) [23,27], TPyCIU (the so-called *N*,*N*,*N*',*N*'-bis(tetramethylene)-chlorouronium tetrafluoroborate reagent) [27], and Mitsunobu reagents [28–30]. Another method for the synthesis of phosphonate peptides involves DCC-DMAP-mediated condensation of phosphonous acids **6** and hydroxy esters [31], or coupling of phosphonochloridites **5** (a highly reactive trivalent species) and hydroxy esters [22], and followed by the oxidation of the resulting phosphonous monoesters **4** by sodium periodate [31], or anhydrous *tert*-butyl peroxide [22] (Scheme 1). Thiophosphonopeptides could also be prepared following sulfurization [22].

Although several methods are available for the preparation of phosphonamidate peptides and phosphonate peptides as mentioned above, all of them use phosphonic acid or phosphonous acid derivatives as starting materials [1–14]. In the most commonly employed method, phosphonochloridates are prepared from phosphonate diesters, phosphonate monoesters, or phosphonite esters. In the direct condensation method, phosphonate monoesters are generally prepared from phosphonate diesters or phosphonic acids. The overall yields from the synthesis of phosphonic acid or phosphonous acid derivatives to the desired peptide products, phosphonate and phosphonamidate peptides, are seldom satisfactory. It is therefore necessary to develop a novel synthetic method for the preparation of these two types of important phosphonopeptides. Recently, we reported novel methods for the preparation of phosphonate mixed diesters and phosphonamidates using simple and commercially available starting materials

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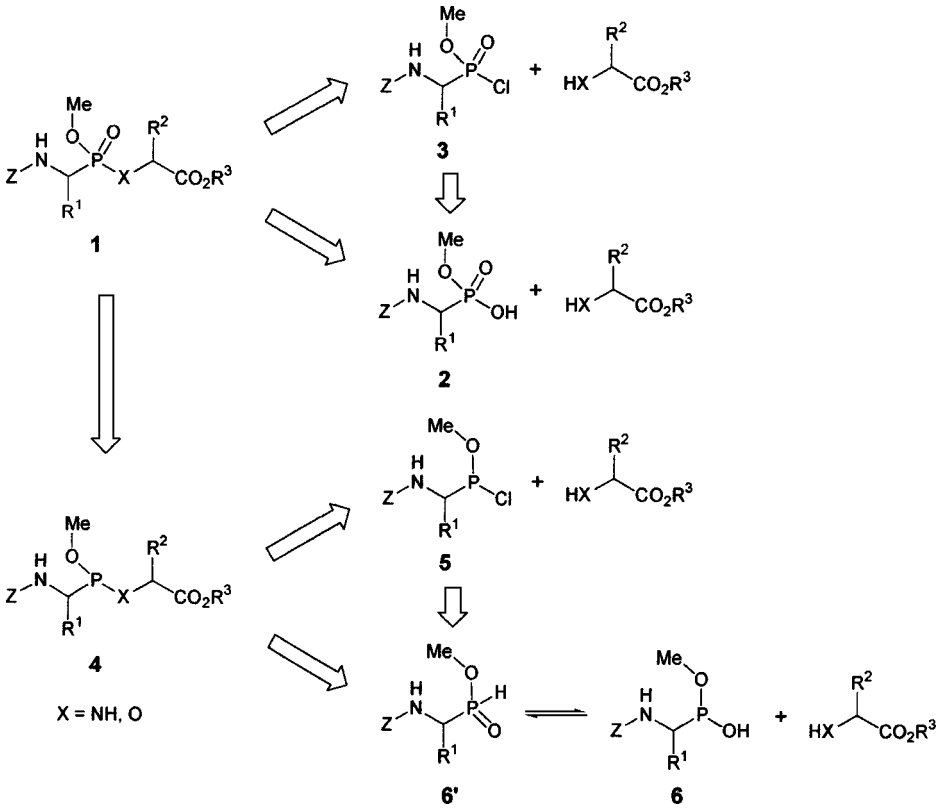
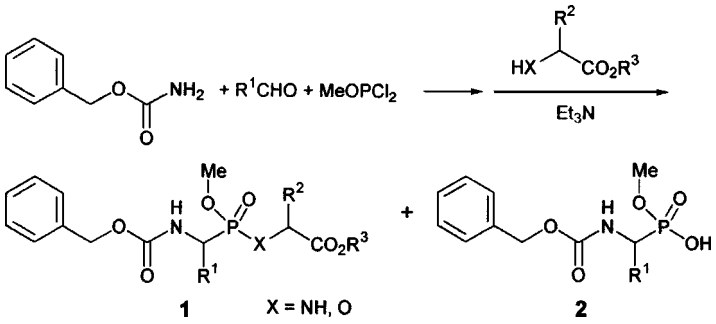


Scheme 1 General methods for the synthesis of phosphonamidate peptides and phosphonate peptides.



Scheme 2 Direct synthesis of phosphonate peptides and phosphonamidate peptides.

[32–34]. We envisaged that this method could offer a new and direct synthetic route to phosphonate- and phosphonamidate-linked phosphonopeptides. We therefore decided to investigate this reaction and to undertake a survey to ascertain the generality of the reaction. We herein present our results.

RESULTS AND DISCUSSION

On the basis of our previous success with the synthesis of phosphonate mixed diesters and phosphonamidates by a one-pot reaction protocol [[32–34], we postulated that this method could be extended to the synthesis of phosphonamidate and phosphonate peptides directly if amino acid esters and hydroxy esters are used instead of amines and alcohols in the aminolysis or alcoholysis step, respectively. The desired phosphonamidate peptides were obtained by reaction of benzyl carbamate, benzaldehyde, and methyl dichlorophosphite, followed by aminolysis with methyl or ethyl glycinate hydrochloride salt in the presence of triethylamine, in yields of 40 and 35%, respectively (Scheme 2). Although the yields are not high, they are comparable with those obtained by both the phosphonochloridate and direct condensation methods. The former method requires the preparation of aminoalkylphosphonic acid derivatives followed by chlorination and then aminolysis [1–8,16–22]. Similarly, the latter method also needs the preparation of aminoalkylphosphonic acid monoesters and then condensation with amino acid esters [23–30]. In contrast, our present method is highly convenient and

Table 1 Synthesis of phosphonamidate peptides **1a–k** and phosphonate peptides **1l–r**

Entry	X	R^1	R^2	R^3	Phosphopeptide **1** Yield (%)		Phosphonate monoester **2** Yield (%)	
1	NH	Ph	H	Me	**1a**	40	**2a**	31
2	NH	4-ClPh	H	Me	**1b**	38	**2b**	32
3	NH	4-MeOPh	H	Me	**1c**	36	**2c**	27
4	NH	Ph	H	Et	**1d**	35	**2a**	44
5	NH	4-ClPh	H	Et	**1e**	42	**2b**	33
6	NH	4-MeOPh	H	Et	**1f**	35	**2c**	25
7	NH	4-MePh	H	Et	**1g**	37	**2d**	23
8	NH	Ph	$CHMe_2$	Et	**1h**	43	**2a**	38
9	NH	Ph	CH_2CHMe_2	Et	**1i**	39	**2a**	28
10	NH	Ph	CH_2Ph	Et	**1j**	40	**2a**	37
11	NH	Ph	CH_2CH_2SMe	Et	**1k**	43	**2a**	28
12	O	Ph	Me	Et	**1l**	58	**2a**	23
13	O	4-ClPh	Me	Et	**1l**	64	**2b**	26
14	O	4-MeOPh	Me	Et	**1n**	51	**2c**	25
15	O	Ph	CH_2CO_2Et	Et	**1o**	69	**2a**	27
16	O	4-ClPh	CH_2CO_2Et	Et	**1p**	63	**2b**	15
17	O	4-MeOPh	CH_2CO_2Et	Et	**1q**	68	**2c**	21
18	O	4-MePh	CH_2CO_2Et	Et	**1r**	47	**2d**	19

practical and no significant difference was observed during aminolysis with either methyl or ethyl glycinate hydrochloride salts. Dichloromethane is a better solvent to dissolve amino acid hydrochloride salt than benzene which was previously used as a solvent for this one-pot reaction [32]. A series of phosphonamidate peptides were synthesized by the use of different aldehydes and naturally occurring L-amino acid esters other than glycine esters. The results are summarized in Table 1 (entries 1–11). An attempt to synthesize phosphonate peptides was also successful when hydroxy esters were used in alcoholysis. Two hydroxy esters, lactate and malate, which could be considered as oxygen analogues of alanine and aspartic acid, were selected and used in alcoholysis. The results are tabulated in Table 1 (entries 12–18).

As noted in Table 1, we found that the yields of phosphonate peptides were higher than those of phosphonamidate peptides. Although the amino group generally shows stronger nucleophilicity than the hydroxy group during acylation with a carboxylic chloride or carboxylic anhydride, the situation is different in acylation with phosphonic chloridate and phosphonic anhydride. The results are in agreement with those in the literature that the formation of phosphonates is more predominant than that of phosphonamidates as observed in the direct condensation method [23] and the phosphonochloridate method [35,36]. According to our previous mechanistic investigation [32–34], both phosphonochloridate and phosphonic anhydride are formed as intermediates in this reaction. The aminoalkylphosphonic acid monoesters **2** always form as by-products and cannot be avoided. It is for this reason that the yields are not high when using this method. However, the low yields are offset by the highly practical and convenient method in which all starting materials are commercially available and the protocol is both very simple and practical.

The structures of phosphonopeptides **1** were characterized by ^{1}H NMR, ^{31}P NMR, mass spectrometry (MS), and elemental analysis. In most cases, the methoxy and other groups in the ^{1}H NMR spectra and phosphorus in the ^{31}P NMR spectra appeared at different chemical shifts, which correspond to different diastereomers generated by one or two chiral carbon atom(s) and a chiral phosphorus atom. Even though the current method produces racemic products, the ratios of each pair of diastereomers could be roughly determined by ^{31}P NMR integrals in some cases. The method is thus a direct synthetic route to these valuable peptide mimetics and will be very useful for the preparation of a phosphonopeptide library containing structurally diverse phosphonamidate and phosphonate peptide members for some enzyme inhibitor screening.

CONCLUSION

In conclusion, a direct method for the one-pot preparation of phosphonate- and phosphonamidate-linked phosphonopeptides has been developed. Using this approach, phosphonate and phosphonamidate peptides can be conveniently prepared in acceptable yields directly from simple and commercially available

chemicals. The method should prove valuable for organic and biological chemists interested in and/or requiring the preparation of these two types of phosphorus containing peptides.

EXPERIMENTAL

General

Melting points were obtained on a Yanaco melting point apparatus and were uncorrected. Elemental analyses were carried out on an Elementar Vario EL elemental analyzer. The ^{1}H NMR and ^{31}P NMR spectra were recorded in $CDCl_3$ solution on a Varian Mercury Plus 300 (300 MHz) spectrometer with TMS as an internal standard. ^{31}P NMR spectra were obtained with use of broadband ^{1}H decoupling; chemical shifts are reported as ppm referenced to 85% phosphoric acid with positive downfield shift. The IR spectra were recorded on a Brucker Vector 22 FT-IR spectrophotometer in KBr or film for oil samples. Mass spectra were obtained on a VG ZAB-HS mass spectrometer or a Brucker ESQUIRE–LC ESI ion-trap spectrometer. TLC separations were performed on silica gel G plates with petroleum ether (60–90 °C)/ethyl acetate (1 : 1, v/v), and the plates were viewed with UV light.

General Procedure for the Synthesis of Phosphonamidate Peptides and Phosphonate Peptides

Methyl dichlorophosphite (3 mmol) was slowly added dropwise to a stirred mixture of benzyl carbamate (0.45 g, 3 mmol) and aldehyde (3 mmol) in 15 ml of anhydrous dichloromethane in an icewater bath and allowed to warm to room temperature under stirring. After stirring the reaction mixture for 6 h at room temperature, amino acid ester hydrochloride was added in portions (or hydroxy ester (3 mmol) was added dropwise). After stirring for 15 min, triethylamine (0.92 ml, 0.67 g, 6.6 mmol, 3.3 mmol for hydroxy ester) was added dropwise and the resulting reaction mixture was stirred continuously overnight. After addition of ethyl acetate (20 ml), the resulting reaction mixture was washed with saturated aqueous sodium bicarbonate twice and dried over anhydrous sodium sulfate. After removal of solvent, the residue was separated on a silica gel column with a mixture of petroleum ether (30–60 °C) and ethyl acetate (1 : 1) as an eluent to give desired products **1**. (Caution: The desired peptides show very weak fluorescent intensity under UV light. It is better to monitor collective fractions in the column separation after concentration). The aqueous phase was acidified with 10% HCl to give precipitates that were recrystallized from ethanol to give phosphonic monoesters **2**. Their analytical data are in accordance with literature values [32–34].

***N*-(((*R,S*)-1-Benzyloxycarbonylamino-1-phenylmethyl)-methoxyphosphinyl)glycine methyl ester (1a).** Colorless crystals; m.p. 172–176 °C; yield 40%; $R_f = 0.14$ (hexane: AcOEt 1 : 1, v/v); ^{1}H NMR (300 MHz, $CDCl_3$) δ 2.93 (s, br, 1H, PNH), 3.46–3.59 (m, 2H, CH_2CO_2), 3.70 (d, $J_{PH} = 10.8$ Hz, 3H, $POCH_3$), 3.70 (s, 3H, CO_2Me), 5.04–5.22 (m, 3H, $PhCH_2O$ and CHP), 6.23 (br, 1H, NHCO), 7.27–7.41 (m, 10H, ArH); ^{31}P NMR (121.5 MHz, $CDCl_3$) δ 27.62; IR (KBr) ν_{max}: 3314 (NH), 1718 (C=O), 1245 (P=O), 1030 (P–O–C) cm^{-1}; ESI-MS (*m/z*): 407 (MH^+, 23). Anal. calcd. for $C_{19}H_{23}N_2O_6P$ (406.37): C, 56.16; H, 5.70; N, 6.89. Found: C, 56.35; H, 5.81; N, 6.69.

***N*-{((*R,S*)-1-Benzyloxycarbonylamino-1-(4-chlorophenyl)-methyl)methoxyphosphinyl}glycine methyl ester (1b).** Colorless crystals; m.p. 151–156 °C; yield 38%; $R_f = 0.13$ (hexane: AcOEt 1 : 1, v/v); ^{1}H NMR (300 MHz, $CDCl_3$) δ 2.94 (dt, $J_{PH} = 13.2$ Hz, $J = 6.6$ Hz, 1H, PNH), 3.60 (dd, $J_{PH} = 9.3$ Hz, $J = 6.6$ Hz, 2H, CH_2CO), 3.70 (d, $J_{PH} = 11.1$ Hz, 3H, $POCH_3$), 3.72 (s, 3H, CO_2Me), 5.09 (d, $J_{PH} = 6.6$ Hz, 2H, CH_2O), 5.04–5.19 (m, 1H, CHP), 6.26 (s, br, 1H, NHCO), 7.27–7.34 (m, 9H, ArH); ^{31}P NMR (121.5 MHz, $CDCl_3$) δ 26.54; IR (KBr) ν_{max}: 3247 (NH), 1708, 1719 (C=O), 1245 (P=O), 1038 (P–O–C) cm^{-1}; FAB-MS (*m/z*): 441 (MH^+, 23). Anal. calcd for $C_{19}H_{22}ClN_2O_6P$ (440.81): C, 51.77; H, 5.03; N, 6.35. Found: C, 51.73; H, 4.83; N, 6.17.

***N*-{((*R,S*)-1-Benzyloxycarbonylamino-1-(4-methoxyphenyl)methyl)methoxyphosphinyl}glycinemethyl ester (1c).** Colorless crystals; m.p. 142–146 °C; yield 36%; $R_f = 0.12$ (hexane: AcOEt 1 : 1, v/v); ^{1}H NMR (300 MHz, $CDCl_3$) δ 2.94 (dt, $J_{PH} = 13.2$ Hz, $J = 6.6$ Hz, 1H, PNH), 3.57 (dd, $J_{PH} = 9.6$ Hz, $J = 6.6$ Hz, 2H, CH_2CO), 3.70 (d, $J_{PH} = 11.2$ Hz, 3H, $POCH_3$), 3.71 (s, 3H, CO_2Me), 3.79 (s, 3H, CH_3O), 5.03–5.15 (m, 1H, CHP), 5.09 (d, $J = 2.5$ Hz, 2H, CH_2O), 6.17 (s, br, 1H, NHCO), 6.87 (d, $J = 9.0$, 2H, ArH), 7.27–7.34 (m, 7H, ArH); ^{31}P NMR (121.5 MHz, $CDCl_3$) δ 27.44; IR (KBr) ν_{max}: 3314 (NH), 1718 (C=O), 1245 (P=O), 1030 (P–O–C) cm^{-1}; FAB-MS (*m/z*): 437 (MH^+, 13), 459 (MNa^+, 2.6). Anal. calcd for $C_{20}H_{25}N_2O_7P$ (436.40): C, 55.05; H, 5.77; N, 6.42. Found: C, 54.80; H, 5.65; N, 6.20.

***N*-(((*R,S*)-1-Benzyloxycarbonylamino-1-phenylmethyl)-methoxyphosphinyl)glycine ethyl ester (1d).** Colorless crystals; m.p. 108–103 °C; yield 35%; $R_f = 0.14$ (hexane: AcOEt 1 : 1, v/v); ^{1}H NMR (300 MHz, $CDCl_3$) δ 1.23 (t, $J = 7.1$ Hz, 3H, CH_3), 3.01 (s, br, 1H, PNH), 3.40–3.76 (m, 2H, CH_2CO), 3.55 and 3.66 (d, $J_{PH} = 11.1$ Hz, 3H, $POCH_3$), 4.15 (q, $J = 7.2$ Hz, 2H, OCH_2), 5.04–5.29 (m, 1H, CHP), 5.06 (d, $J_{PH} = 2.8$ Hz, 2H, CH_2O), 6.36 (s, br, 1H, NH), 7.24–7.40 (m, 10H, ArH); ^{31}P NMR (121.5 MHz, $CDCl_3$) δ 27.44; IR (KBr) ν_{max}: 3252 and 3223 (NH), 1745 and 1715 (C=O), 1242 (P=O), 1030 (P–O–C) cm^{-1}; ESI-MS (*m/z*): 421 (MH^+, 11), 443 (MNa^+, 100). Anal. calcd for $C_{20}H_{25}N_2O_6P$ (420.40): C, 57.14; H, 5.99; N, 6.66. Found: C, 57.33; H, 6.03; N, 6.67.

***N*-{((*R,S*)-1-Benzyloxycarbonylamino-1-(4-chlorophenyl)-methyl)methoxyphosphinyl}glycine ethyl ester (1e).** Colorless crystals; m.p. 81–85 °C; yield 42%; $R_f = 0.13$ (hexane: AcOEt 1 : 1, v/v); ^{1}H NMR (300 MHz, $CDCl_3$) δ 1.24 (t, $J = 6.8$ Hz, 3H, CH_3), 3.44–3.76 (m, 6H, $POCH_3$ and $NHCH_2CO$), 4.14 (q, $J = 6.8$ Hz, 2H, OCH_2), 4.96–5.22 (m, 1H, CHP), 5.06 (d, $J_{PH} = 2.8$ Hz, 2H, CH_2O), 6.42 (s, br, 1H, NH), 7.10–7.40 (m, 9H, ArH); ^{31}P NMR (121.5 MHz, $CDCl_3$) δ 27.56; IR (KBr) ν_{max}: 3267 (NH), 1745 and 1715 (C=O), 1245 (P=O), 1041 (P–O–C) cm^{-1}; ESI-MS (*m/z*): 455 (MH^+, 15), 477 (MNa^+, 100). Anal. calcd for $C_{20}H_{24}ClN_2O_6P$ (454.84): C, 52.81; H, 5.32; N, 7.79. Found: C, 52.93; H, 5.43; N, 7.67.

***N*-{((*R,S*)-1-Benzyloxycarbonylamino-1-(4-methoxyphenyl)methyl)methoxyphosphinyl}glycine ethyl ester (1f).** Colorless crystals; m.p. 106–110 °C; yield 35%; $R_f = 0.12$ (hexane: AcOEt 1 : 1, v/v); ^{1}H NMR (300 MHz, $CDCl_3$) δ 1.26

(t, $J = 6.0$ Hz, 3H, CH_3), 3.49–3.75 (m, 6H, $POCH_3$ and $NHCH_2CO$), 3.78 (s, 3H, CH_3O), 4.15 (q, $J = 6.0$ Hz, 2H, OCH_2), 5.02–5.22 (m, 1H, CHP), 5.08 (d, $J_{PH} = 2.8$ Hz, 2H, CH_2O), 6.44 (s, br, 1H, NH), 6.85 (d, $J = 8.1$ Hz, 2H, ArH), 7.20–7.31 (m, 7H, ArH); ^{31}P NMR (121.5 MHz, $CDCl_3$) δ 28.35; IR (KBr) ν_{max}: 3285 (NH), 1719 (C=O), 1248 (P=O), 1034 (P–O–C) cm^{-1}; ESI-MS (m/z): 451 (MH^+, 73), 473 (MNa^+, 53). Anal. calcd for $C_{21}H_{27}N_2O_7P$ (450.42): C, 56.00; H, 6.04; N, 6.22. Found: C, 55.89; H, 5.95; N, 6.23.

***N*-{*(R,S)*-1-Benzyloxycarbonylamino-1-(4-methylphenyl)-methyl)methoxyphosphinyl}glycine ethyl ester (1g).** Colorless crystals; m.p. 104–107 °C; yield 37%; $R_f = 0.15$ (hexane: AcOEt 1 : 1, v/v); ^{1}H NMR (300 MHz, $CDCl_3$) δ 1.22 (t, $J = 6.5$ Hz, 3H, CH_3), 2.30 (s, 3H, CH_3), 3.07 (s, br, 1H, NH), 3.40–3.84 (m, 5H, CH_2CO and $POCH_3$), 4.13 (q, $J = 6.5$ Hz, 2H, OCH_2), 4.91–5.22 (m, 1H, CHP), 5.06 (d, $J_{PH} = 2.8$ Hz, 2H, CH_2O), 6.40 (s, br, 1H, NH), 7.13–7.30 (m, 9H, ArH); ^{31}P NMR (121.5 MHz, $CDCl_3$) δ 27.87; IR (KBr) ν_{max}: 3278 (NH), 1720 (C=O), 1244 (P=O), 1040 (P–O–C) cm^{-1}; ESI-MS (m/z): 435 (MH^+, 7.3), 457 (MNa^+, 100). Anal. calcd for $C_{21}H_{27}N_2O_6P$ (434.42): C, 58.06; H, 6.26; N, 6.45. Found: C, 58.13; H, 6.23; N, 6.61.

***(S)*-*N*-(((*R,S*)-1-Benzyloxycarbonylamino-1-phenylmethyl)-methoxyphosphinyl)valine ethyl ester (1h).** Colorless crystals; mp 56–61 °C; yield 43%; $R_f = 0.21$ (hexane: AcOEt 1 : 1, v/v); ^{1}H NMR (300 MHz, $CDCl_3$) δ 0.89 (m, 6H, $2CH_3$), 1.21 (t, $J = 7.6$ Hz, 3H, CH_3), 1.82 (m, 1H, CH), 3.44 and 3.69 (d, $J_{PH} = 11.0$ Hz, 3H, $POCH_3$), 3.65 (s, br, 1H, NH), 3.94–4.26 (m, 1H, CHCO), 4.12 (q, $J = 7.6$ Hz, 2H, OCH_2), 4.90–5.28 (m, 1H, CHP), 5.06 (d, $J_{PH} = 2.9$ Hz, 2H, CH_2O), 6.05 and 6.32 (s, br, 1H, PNH), 7.21–7.60 (m, 10H, ArH); ^{31}P NMR (121.5 MHz, $CDCl_3$) δ 26.72, 27.63 (1.0: 2.1); IR (KBr) ν_{max}: 3258 (NH), 1725 (C=O), 1251 (P=O), 1043 (P–O–C) cm^{-1}; ESI-MS (m/z): 463 (MH^+, 26), 485 (MNa^+, 31). Anal. calcd for $C_{23}H_{31}N_2O_6P$ (462.48): C, 59.73; H, 6.76; N, 6.06. Found: C, 59.62; H, 6.68; N, 6.27.

***(S)*-*N*-(((*R,S*)-1-Benzyloxycarbonylamino-1-phenylmethyl)-methoxyphosphinyl)leucine ethyl ester (1i).** Colorless viscous oil; yield 39%; $R_f = 0.21$ (hexane: AcOEt 1 : 1, v/v); ^{1}H NMR (300 MHz, $CDCl_3$) δ 0.76–0.79 and 0.86–0.94 (m, 6H, $2CH_3$), 1.23 (dt, $J_{PH} = 3.9$ Hz, $J = 7.0$ Hz, 3H, CH_3), 1.32–1.43 (m, 1H, CH), 1.44–1.59 (m, 1H, H in CH_2), 1.60–1.80 (m, 1H, H in CH_2), 2.80, 2.92 and 3.12 (s, br, 1H, NH), 3.52, 3.65 and 3.69 (d, $J_{PH} = 10.8$ Hz, 3H, $POCH_3$), 3.78–3.92 (m, 1H, CHCO), 4.04–4.19 (m, 2H, OCH_2), 5.02–5.84 (m, 3H, CHP and CH_2O), 5.84, 6.01 and 6.25 (s, br, 1H, NH), 7.24–7.42 (m, 10H, ArH); ^{31}P NMR (121.5 MHz, $CDCl_3$) δ 27.26, 26.60 (1.8 : 1.0); IR (neat) ν_{max}: 3287 (NH), 1719 (C=O), 1242 (P=O), 1046 (P–O–C) cm^{-1}; ESI-MS (m/z): 477 (MH^+, 1.2), 499 (MNa^+, 100). Anal. calcd for $C_{24}H_{33}N_2O_6P$ (476.50): C, 60.49; H, 6.98; N, 5.88. Found: C, 60.33; H, 7.07; N, 5.67.

***(S)*-*N*-(((*R,S*)-1-Benzyloxycarbonylamino-1-phenylmethyl)-methoxyphosphinyl)phenylalanine ethyl ester (1j).** Colorless crystals; m.p. 136–140 °C; yield 40%; $R_f = 0.18$ (hexane: AcOEt 1 : 1, v/v); ^{1}H NMR (300 MHz, $CDCl_3$) δ 1.18 (t, $J = 7.2$ Hz, 3H, CH_3), 2.61 (dd, $J = 5.7$, 13.2 Hz, 1H, CH), 2.76 (dd, $J = 6.6$, 13.2 Hz, 1H, H in CH_2), 2.86–3.04 (m, 2H, NHCO and H in CH_2), 3.38 and 3.56 (d, $J_{PH} = 11.0$ Hz, 3H, $POCH_3$), 4.04–4.13 (m, 2H, CO_2CH_2), 4.96–5.12 (m, 3H, CHP and CH_2O), 5.66, 6.01 and 6.27 (s, br, 1H, PNH), 7.00–7.33 (m, 15H, ArH); ^{31}P NMR (121.5 MHz, $CDCl_3$) δ 26.54, 26.05 (5.2 : 1.0; IR (KBr): ν_{max}: 3229 (NH), 1734 (C=O), 1248 (P=O), 1030 (P–O–C) cm^{-1}; ESI-MS (m/z): 511 (MH^+, 3.23). Anal. calcd for $C_{27}H_{31}N_2O_6P$ (510.52): C, 63.52; H, 6.12; N, 5.49. Found: C, 63.33; H, 6.03; N, 5.67.

***(S)*-*N*-(((*R,S*)-1-Benzyloxycarbonylamino-1-phenylmethyl)-methoxyphosphinyl)methionine ethylester (1k).** Colorless crystals; m.p. 78–82 °C; yield 43%; $R_f = 0.18$ (hexane: AcOEt 1 : 1, v/v); ^{1}H NMR (300 MHz, $CDCl_3$) δ 1.24 (t, $J = 7.1$ Hz, 3H, CH_3), 1.89–2.04 (m, 2H, CH_2), 2.03 (s, 3H, SCH_3), 2.46 (t, $J = 7.2$ Hz, CH_2S), 3.16 (s, br, 1H, NH), 3.54 and 3.69 (d, $J_{PH} = 11.1$ Hz, 3H, $POCH_3$), 3.99–4.28 (m, 1H, CHCO), 4.12 (q, $J = 7.1$ Hz, 2H, OCH_2), 4.99–5.26 (m, 1H, CHP), 5.07 (d, $J_{PH} = 2.8$ Hz, 2H, CH_2O), 6.02 and 6.12 (s, br, 1H, NH), 7.27–7.41 (m, 10H, ArH); ^{31}P NMR (121.5 MHz, $CDCl_3$) δ 27.02, 26.56 (1.9 : 1.0); IR (KBr) ν_{max}: 3229 (NH), 1721 (P=O), 1250 (P=O), 1041 (P–O–C) cm^{-1}; ESI-MS (m/z): 495 (MH^+, 2), 517 (MNa^+, 100). Anal. calcd for $C_{23}H_{21}N_2O_6$ PS (494.54): C, 55.86; H, 6.32; N, 5.66. Found: C, 55.59; H, 6.06; N, 5.57.

***Ethyl(R,S)*-2-{(((*R,S*)-1-Benzyloxycarbonylamino-1-phenylmethyl)methoxyphosphinyl)oxy}propionate (1l).** Colorless crystals; m.p. 81–86 °C; yield 58%; $R_f = 0.15$ (hexane: AcOEt 1 : 1, v/v); ^{1}H NMR (300 MHz, $CDCl_3$) δ 1.03, 1.37 and 1.51 (d, $J = 6.9$ Hz, 3H, CH_3), 1.25 and 1.27 (t, $J = 7.5$ Hz, 3H, CH_3), 3.59, 3.69 and 3.84 (d, $J_{PH} = 11.1$ Hz, 3H, $POCH_3$), 4.13–4.25 (m, 2H, OCH_2), 4.50, 4.79 and 4.85 (dq, $J_{PH} = 7.0$ Hz, $J = 6.9$ Hz, 1H, POCH), 5.04–5.15 (m, 2H, CH_2O), 5.16–5.38 (m, 1H, CHP), 6.04 and 6.29 (s, br, 1H, NH), 7.27–7.46 (m, 10H, ArH); ^{31}P NMR (121.5 MHz, $CDCl_3$) δ 23.41, 22.87 (1.5 : 1.0); IR (KBr) ν_{max}: 3250 (NH), 1721 (C=O), 1256 (P=O), 1041 (P–O–C) cm^{-1}; ESI-MS (m/z): 436 (MH^+, 4.9), 458 (MNa^+, 100). Anal. calcd for $C_{21}H_{26}NO_7P$ (435.41): C, 57.93; H, 6.02; N, 3.22. Found: C, 57.80; H, 6.15; N, 3.20.

***Ethyl(R,S)*-2-{(((*R,S*)-1-Benzyloxycarbonylamino-1-(4-chlorophenyl)methyl)methoxyphosphinyl)oxy}propionate (1m).** Colorless crystals; yield 64%; m.p. 116–121 °C; $R_f = 0.14$ (hexane: AcOEt 1 : 1, v/v), ^{1}H NMR (300 MHz, $CDCl_3$) δ 1.14 (d, $J = 6.9$ Hz, 3H, CH_3), 1.26 (t, $J = 7.2$ Hz, 3H, CH_3), 3.83 (d, $J_{PH} = 11.1$ Hz, 3H, POMe), 4.19 (dq, $J_{PH} = 1.8$ Hz, $J = 6.9$ Hz, 2H, OCH_2), 4.57 (dq, $J_{PH} = 7.0$ Hz, $J = 7.2$ Hz, 1H, POCH), 5.04–5.21 (m, 3H, CH_2O and CHP), 5.90 (s, br, 1H, NH), 7.27–7.40 (m, 9H, ArH); ^{31}P NMR (121.5 MHz, $CDCl_3$) δ 22.92, 22.30 (1.5 : 1.0); IR (KBr) ν_{max}: 3250 (NH), 1953 and 1720 (C=O), 1253 (P=O), 1042 (P–O–C) cm^{-1}; FAB-MS (m/z): 470 (MH^+, 40). Anal. calcd for $C_{21}H_{25}ClNO_7P$ (469.85): C, 53.68; H, 5.36; N, 2.98. Found: C, 53.80; H, 5.15; N, 3.20.

***Ethyl(R,S)*-2-{(((*R,S*)-1-Benzyloxycarbonylamino-1-(4-methoxyphenyl)methyl)methoxyphosphinyl)oxy}propionate (1n).** Colorless crystal; m.p. 131–136 °C; yield 51%; $R_f = 0.14$ (hexane: AcOEt 1 : 1, v/v); ^{1}H NMR (200 MHz, $CDCl_3$) δ 1.07 (d, $J = 7.3$ Hz, 3H, CH_3), 1.25 (t, $J = 6.9$ Hz, 3H, CH_3), 3.80 (s, 3H, CH_3OAr), 3.84 (d, $J_{PH} = 11.4$ Hz, 3H, POMe), 4.18 (dq, $J_{PH} = 2.7$ Hz, $J = 7.2$ Hz, 2H, OCH_2), 4.52 (dq, $J_{PH} = 7.5$ Hz, $J = 7.3$ Hz, 1H, POCH), 5.04–5.18 (m, 3H, CH_2O and CHP), 5.81 (br, 1H, NH), 6.89 (d, $J = 8.7$ Hz, 2H, ArH), 7.27–7.37 (m, 7H, ArH); ^{31}P NMR (121.5 MHz, $CDCl_3$) δ 23.97, 23.31 (1.5 : 1.0); IR (KBr) ν_{max}: 3251 (NH), 1754 and 1719 (C=O), 1246 (P=O), 1037 (P–O–C) cm^{-1}; FAB-MS (m/z):

466 (MH^+, 28). Anal. calcd for $C_{22}H_{28}NO_8P$ (465.43): C, 56.77; H, 6.06; N, 3.01. Found: C, 56.90; H, 6.05; N, 3.20.

Diethyl(R,S)-2-{(((R,S)-1-Benzyloxycarbonylamino-1-phenylmethyl)methoxyphosphinyl)oxy}succinate (1o). Yellowish viscous oil; yield 69%; $R_f = 0.14$ (hexane: AcOEt 1 : 1, v/v); ^{1}H NMR (300 MHz, $CDCl_3$) δ 1.24 (t, $J = 6.8$ Hz, 3H, CH_3), 1.26 (t, $J = 6.8$ Hz, 3H, CH_3), 2.65 (ddd, 2H, CH_2CO), 3.58, 3.68 and 3.82 (d, $J_{PH} = 11.0$ Hz, 3H, $POCH_3$), 4.04–4.32 (m, 5H, POCH and $2OCH_2$), 5.08 (d, $J_{PH} = 2.7$ Hz, 2H, CH_2O), 4.93–5.48 (m, 1H, CHP), 6.12, 6.33 and 6.76 (s, br, 1H, NHCO), 7.27–7.50 (m, 10H, ArH); ^{31}P NMR (121.5 MHz, $CDCl_3$) δ 23.29, 21.73 (2.1 : 1.0); IR (neat) ν_{max} : 3332 (NH), 1736 (C=O), 1240 (P=O), 1030 (P-O-C) cm^{-1}; ESI-MS (m/z): 508 (MH^+, 13), 530 (MNa^+, 100). Anal. calcd for $C_{24}H_{30}NO_9P$ (507.47): C, 56.80; H, 5.96; N, 2.76. Found: C, 56.97; H, 6.04; N, 3.00.

Diethyl(R,S)-2-{(((R,S)-1-Benzyloxycarbonylamino-1-(4-chlorophenyl)methyl)methoxyphosphinyl)oxy}-succinate (1p). Yellowish viscous oil; yield 63%; $R_f = 0.14$ (hexane: AcOEt 1 : 1, v/v); ^{1}H NMR (300 MHz, $CDCl_3$) δ 1.23 (t, $J = 6.8$ Hz, 3H, CH_3), 1.24 (t, $J = 6.8$ Hz, 3H, CH_3), 2.65 (ddd, 2H, CH_2CO), 3.61, 3.67 and 3.84 (d, $J_{PH} = 11.0$ Hz, 3H, $POCH_3$), 4.05–4.36 (m, 5H, POCH and $2OCH_2$), 5.08 (d, $J_{PH} = 2.7$ Hz, 2H, CH_2O), 5.16–5.44 (m, 1H, CHP), 6.40 (s, br, 1H, NHCO), 7.27–7.50 (m, 9H, ArH); ^{31}P NMR (121.5 MHz, $CDCl_3$) δ 22.99, 21.30 (1.6 : 1.0); IR (neat) ν_{max}: 3329 (NH), 1736 (C=O), 1240 (P=O), 1029 (P–O–C) cm^{-1}; ESI-MS (m/z): 542 (MH^+, 81), 564 (MNa^+, 100). Anal. calcd for $C_{24}H_{29}ClNO_9P$ (541.92): C, 53.19; H, 5.39; N, 2.58. Found: C, 53.30; H, 5.15; N, 2.29.

Diethyl(R,S)-2-{(((R,S)-1-Benzyloxycarbonylamino-1-(4-methoxyphenyl)methyl)methoxyphosphinyl)oxy}-succinate (1q). Yellowish viscous oil; yield 68%; $R_f = 0.13$ (hexane: AcOEt 1 : 1, v/v); ^{1}H NMR (300 MHz, $CDCl_3$) δ 1.22 (t, $J = 6.8$ Hz, 3H, CH_3), 1.24 (t, $J = 6.8$ Hz, 3H, CH_3), 2.86 (ddd, 2H, CH_2CO), 3.77 (s, 3H, CH_3O), 3.59, 3.72 and 3.84 (d, $J_{PH} = 11.0$ Hz, 3H, $POCH_3$), 4.05–4.35 (m, 5H, POCH and $2OCH_2$), 5.08 (d, $J_{PH} = 2.7$ Hz, 2H, CH_2O), 4.98–5.41 (m, 1H, CHP), 6.03 (s, br, 1H, NHCO), 6.81–7.48 (m, 9H, ArH); ^{31}P NMR (121.5 MHz, $CDCl_3$) δ 23.47, 22.03 (2.3 : 1.0); IR (neat) ν_{max} :3329 (NH), 1736 (C=O), 1248 (P=O), 1032 (P–O–C) cm^{-1}; ESI-MS (m/z): 538 (MH^+, 9.7), 560 (MNa^+, 100). Anal. calcd for $C_{25}H_{32}NO_{10}P$ (537.50): C, 55.86; H, 6.00; N, 2.61. Found: C, 55.92; H, 6.18; N, 2.80.

Diethyl(R,S)-2-{(((R,S)-1-Benzyloxycarbonylamino-1-(4-methylphenyl)methyl)methoxyphosphinyl)oxy}-succinate (1r). Yellowish viscous oil; yield 47%; $R_f = 0.14$ (hexane: AcOEt 1 : 1, v/v); ^{1}H NMR (300 MHz, $CDCl_3$) δ 1.20 (t, $J = 6.8$ Hz, 3H, CH_3), 1.24 (t, $J = 6.8$ Hz, 3H, CH_3), 2.30 (s, 3H, CH_3), 2.90 (ddd, 2H, CH_2CO), 3.59, 3.71 and 3.84 (d, $J_{PH} = 11.0$ Hz, 3H, $POCH_3$), 4.04–4.32 (m, 5H, POCH and $2OCH_2$), 5.08 (d, $J_{PH} = 2.7$ Hz, 2H, CH_2O), 5.01–5.44 (m, 1H, CHP), 6.32 and 6.72 (s, br, 1H, NHCO), 7.05–7.44 (m, 9H, ArH); ^{31}P NMR (121.5 MHz, $CDCl_3$) δ 23.35, 21.73 (2.4 : 1.0); IR (neat) ν_{max}: 3328 (NH), 1737 (C=O), 1240 (P=O), 1038 (P–O–C) cm^{-1}; ESI-MS (m/z): 522 (MH^+, 10), 544 (MNa^+, 100). Anal. calcd for $C_{25}H_{32}NO_9P$ (521.50): C, 57.58; H, 6.18; N, 2.69. Found: C, 57.76; H, 6.05; N, 2.88.

Acknowledgements

The project was supported partly by National Natural Science Foundation of China (No. 20272002 and 20472005), Ministry of Education of P. R. China (SRF for ROCS and EYTP), and Peking University.

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