Straightforward Synthesis of Depsiphosphonopeptides via Mannich-Type Multicomponent Condensation

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Abstract: A straightforward method for the synthesis of depsiphosphonopeptides via a Mannich-type multicomponent condensation of simple starting materials, such as benzyl carbamate, aldehydes, and 1-carbethoxyalkyl phosphorodichloridites, was developed. Compared to previous methods, our strategy provides a more efficient, convenient, and practical route for the preparation of depsiphosphonopeptides under mild reaction conditions with good yields. Such a strategy avoids the initial synthesis of 1-aminoalkylphosphonic acid or 1-aminoalkylphosphonous acid derivatives as starting materials.

Key words: depsiphosphonopeptides, Mannich reactions, multicomponent condensations, peptides, phosphonates, phosphonopeptides

Phosphonopeptides (phosphonamidate-linked phosphonopeptides) and depsiphosphonopeptides (phosphonate-linked phosphonopeptides) are very important tetrahedral analogues of naturally occurring peptides. They are recognized as an important class of enzyme inhibitors either as transition-state analogues, or as nonhydrolyzable phosphonate surrogates,[1] and are of great interest in the development of haptens for the production of catalytic antibodies.[2]

Phosphonopeptides and depsiphosphonopeptides are generally prepared by the reaction of *N*-protected aminoalkylphosphonochloridates with amino esters or hydroxy esters (or peptide esters or hydroxy acyl esters), respectively;[3] use of the monochloridates is more common. The phosphonochloridates in turn are prepared by the reaction of N-protected aminoalkylphosphonate diesters with one equivalent of phosphorus pentachloride,[1b,1c,3f,3g] by treatment of N-protected aminoalkylphosphonic acid monoesters with thionyl chloride[1a,1c,1d,3e,4] or oxalyl chloride,[3b,3d,3h,4] or by oxidative chloridation of N-protected aminoalkylphosphinate esters with carbon tetrachloride.[5] In addition, the syntheses of phosphonopeptides from N-protected aminoalkylphosphonic acid hydroxybenzotriazole esters and phosphonochloridates under silver ion catalysis have also been reported.[6] Depsiphosphonopeptides have also been prepared from N-protected aminoalkylphosphonic acids or their monoesters using coupling reagents,[7] such as DPPA, BOP, PyBOP, BOP-Cl, HBTU, BroP, TPyCIU, etc. and Mitsunobu[8] strategies have also been developed. An alternative method for synthesizing depsiphosphonopeptides involves the DCC–DMAP-mediated condensation of N-protected aminoalkylphosphonous acids and hydroxy esters, followed by oxidation of the resulting aminoalkylphosphonous monoester by sodium periodate.[9]

Depsiphosphonopeptides are generally more stable than phosphonopeptides and have been widely used as structural analogues of phosphonopeptides. Although several methods have been developed for the synthesis of depsiphosphonopeptides, to date, all of the methods require aminoalkylphosphonic acid or aminoalkylphosphonous acid derivatives as starting materials and the overall yields from the synthesis of aminoalkylphosphonic acid or phosphonous acid derivatives to the desired depsiphosphonopeptides are not satisfactory in most cases.[1–3] Thus, it is necessary to seek a new synthetic route to prepare depsiphosphonopeptides efficiently. Herein, we present our strategy for the direct preparation of depsiphosphonopeptides from simple starting materials.

Recently, multicomponent condensations have been widely used in synthetic organic chemistry, especially in combinatorial synthesis.[10] They show high efficiency in syntheses with structurally diverse products. The three-component Mannich-type reaction of amines (or carbamates), aldehydes, and phosphites has been widely utilized in the synthesis of aminoalkylphosphonic acids[11a] and their derivatives, including monoesters[11] and diesters.[12] The Mannich-type reaction followed by alcoholysis and aminolysis has also been used in the preparation of aminoalkylphosphonic mixed diesters,[13] depsiphosphonopeptides,[14] phosphonamidates,[15] and phosphonopeptides.[14] However, the yields of the desired products are generally low because aminoalkylphosphonic acid monoester byproducts always form.[13–15] Very recently, we found that side-chain hydroxymethyl functionalized depsiphosphonopeptides can be prepared from the Mannich-type reaction of benzyl carbamate, aldehydes, and a cyclic monochlorophosphite, diethyl (*R*,*R*)-2-chloro-1,3,2-dioxaphospholane-4,5-dicarboxylate, and subsequent water-mediated ring-opening reaction of 2-oxo-1,3,2-dioxaphospholane intermediates generated in the Mannich-type reaction.

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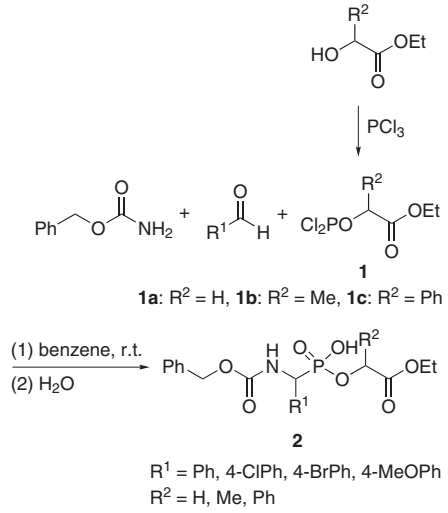
In our current strategy, *N*-Cbz-protected depsiphosphonopeptides were prepared via the Mannich-type multiple component condensation of benzyl carbamate, aldehydes, and 1-carbethoxyalkyl phosphorodichloridites, followed by hydrolysis. Such a strategy for the preparation of depsiphosphonopeptides avoids the initial synthesis of phosphonic acid or phosphonous acid derivatives found in conventional depsiphosphonopeptide synthesis.[3]

1-Carbethoxyalkyl phosphorodichloridites **1** were readily prepared from the corresponding hydroxy esters and phosphorous trichloride according to a known procedure.[16] They were mixed and stirred with benzyl carbamate and an aldehyde in anhydrous benzene. After stirring for six hours, hydrolysis furnished the desired depsiphosphonopeptides in good yields following crystallization or chromatographic separation (Scheme 1 and Table 1). Thus, depsiphosphonopeptides were synthesized in a straight forward manner from simple starting materials with the Mannich-type multicomponent condensation as a key step. Unlike previous synthetic methods where they were obtained by carefully selective hydrolysis of phosphonate diester linkage,[3b] by condensation of phosphonic acid with hydroxy esters,[7] or by oxidation of phosphonous monoester linkage depsiphosphonopeptides;[9] the current strategy provides a fast and practical way to prepare depsiphosphonopeptides in one pot under mild reaction conditions with good yields, and could be considered as a pseudo-four-component condensation as hydrolysis occurs in situ after the Mannich reaction. To the best of our knowledge, this is the shortest route to depsiphosphonopeptides to date. The reaction works very well for aromatic aldehydes, however, for aliphatic aldehydes, only low yields were obtained.



Scheme 1 Straightforward synthesis of depsiphosphonopeptides **2** via Mannich-type multicomponent condensation

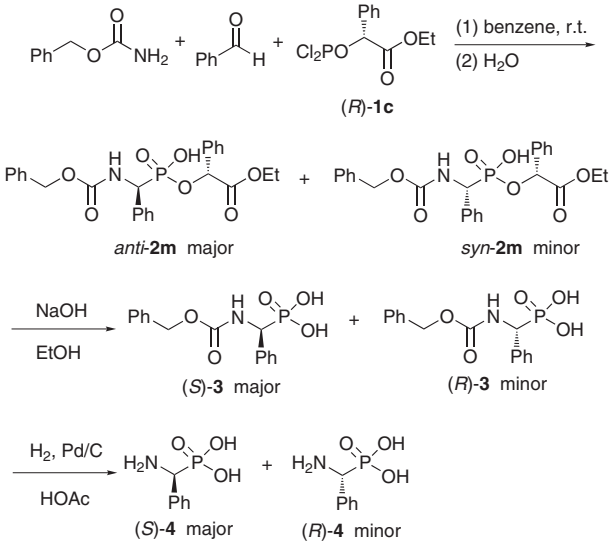
In order to identify the relative configuration of the two chiral carbon atoms in depsiphosphonopeptides **2e–l**, an optically pure hydroxyl ester, ethyl (*R*)-mandelate was employed in the three-component reaction. The results indicated that depsiphosphonopeptide **2m** was obtained as two diastereomers in a 85:15 ratio on the basis of ^{31}P NMR analysis. After saponification, 1-Cbz-amino-1-phenylmethylphosphonic acid **3** was obtained with a negative optical rotation. From a previous publication,[17] we concluded that the major isomer of *N*-Cbz-amino phosphonic acid **3** should have an *S*-configuration. To further confirm this, *N*-Cbz-amino phosphonic acid **3** was deprotected under hydrogenolysis in the presence of Pd/C to yield free 1-amino-1-phenylmethylphosphonic acid **4** with a positive optical rotation, which also indicates that (*S*)-1-amino-1-phenylmethylphosphonic acid **4** is the major component on the basis of the reported results.[18] Thus, the (*S*,*R*)-diastereomer is the major depsiphosphonopeptide **2m** isomer formed, with the (*R*,*R*)-isomer as the minor component (namely 1,4-*anti*/1,4-*syn*, 85:15) (Scheme 2). For peptides **2e–l**, 1,4-*anti* isomers predominate with the *anti*/*syn* ratio ranging from 84:16 to 88:12 on the basis of ^{31}P NMR analyses.

Table 1 Straightforward Synthesis of Depsiphosphonopeptides **2**

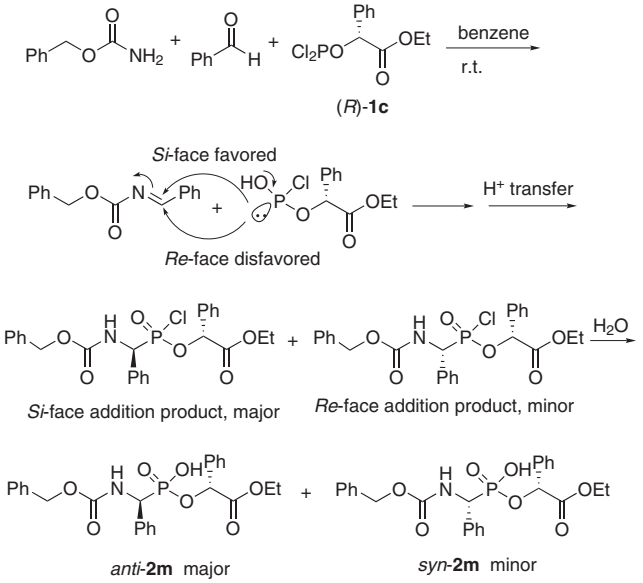
Entry	Peptide	R^1	R^2	Yield[a] (%)	*anti*/*syn*
1	**2a**	Ph	H	86	
2	**2b**	4-ClPh	H	87	
3	**2c**	4-BrPh	H	87	
4	**2d**	4-MeOPh	H	78	
5	**2e**	Ph	Me	72	88:12
6	**2f**	4-ClPh	Me	81	88:12
7	**2g**	4-BrPh	Me	86	87:13
8	**2h**	4-MeOPh	Me	66	87:13
9	**2i**	Ph	Ph	89	86:14
10	**2j**	4-ClPh	Ph	86	84:16
11	**2k**	4-BrPh	Ph	82	84:16
12	**2l**	4-MeOPh	Ph	87	NA
13	**2m**	Ph	Ph	86	85:15

[a] Isolated yield after crystallization or column chromatography.

Next we looked at the mechanism, 1-carbethoxyalkyl phosphorodichloridites **1** are dehydrating agents and could promote the formation of imines from benzyl carbamate and aldehydes. They are converted into hydroxyl phosphorochloridite intermediates, which then undergo addition to the imines formed, proton transfer, and hydrolysis yield depsiphosphonopeptides **2**. For 1-carbethoxyalkyl phosphorodichloridites **1b** and **1c**, for example, (*R*)-**1c**, *Si* face addition is more favorable than *Re* face addition due to the steric hindrance imposed by the methyl or phenyl group. Thus, the 1,4-*anti*-depsiphosphonopeptides *anti*-**2e–l** are the major products along with **2m** possessing a newly formed *S* chiral center (Scheme 3).



Scheme 2 Determination of the relative configuration of depsiphosphonopeptides **2e–l** via chemical correlation



Scheme 3 Proposed formation mechanism of depsiphosphonopeptide **2m**

In summary, a straightforward method for the synthesis of depsiphosphonopeptides using Mannich-type multiple component condensation of simple starting materials, benzyl carbamate, aldehydes, and 1-carbethoxyalkyl phosphorodichloridites was developed. Compared to previous methods, our strategy provides an efficient, convenient, and practical route for the preparation of depsiphosphonopeptides in one-pot under mild reaction conditions with good yields. It can also be applied to prepare optically active depsiphosphonopeptides with optically active hydroxyl esters. Such a strategy avoids the need for the synthesis of aminoalkylphosphonic acid or phosphonous acid derivatives first as starting materials. The desired depsiphosphonopeptides could be used directly as anionic type tetrahedral analogues of naturally occurring peptides, esters, and amides because they are easily converted into their anionic forms when they are dissolved in a weak basic buffer. This method could find applicability in the fields of chemistry, biochemistry, and enzymology.

Mps were obtained on a Yanaco melting point apparatus and are uncorrected. ^{1}H NMR and ^{31}P NMR spectra were recorded on a Varian Mercury 300 Plus spectrometer with TMS as an internal standard or 85% H_3PO_4 as an external standard in $CDCl_3$. The IR spectra were taken on a Bruker Vector 22 FT-IR spectrophotometer. Mass spectral data were recorded on a VG-ZAB spectrometer or a Bruker ESQUIRE-LC^{TM} ESI ion trap spectrometer. CHN analyses were recorded on an Elementar Vario EL analyzer. Optical rotations were measured on a Perkin-Elmer Model 341LC polarimeter with a thermally jacketed 10 cm cell (concentration *c* given as g/100 mL). TLC were performed on silica gel GF_{254} plates and were visualized with UV light. Benzene was refluxed with sodium and freshly distilled prior to use. All reactions were performed under a nitrogen atmosphere. Petroleum ether with a bp range 30–60 °C was used.

1-Carbethoxyalkyl Phosphorodichloridites (1); General Procedure

Hydroxy ester (0.15 mol) was added dropwise to PCl_3 (42 g, 0.30 mol) in an ice–water bath over 2 h. After addition, the resulting mixture was stirred for 6 h, and then allowed to warm to r.t. After removal of excess PCl_3, the residue was distilled to afford 1-carbethoxyalkyl phosphorodichloridite as a colorless liquid.

Carbethoxymethyl Phosphorodichloridite (1a)

Colorless liquid; yield: 66%; bp 69–70 °C/10 mmHg (Lit.[16] 91–92 °C/13 mmHg).

^{1}H NMR (300 MHz, $CDCl_3$): δ = 4.72 (d, J_{PH} = 9.3 Hz, 1 H, $POCH_2$), 4.29 (q, *J* = 6.9 Hz, 2 H, OCH_2), 1.32 (t, *J* = 6.9 Hz, 3 H, CH_3).

^{31}P NMR (121.5 MHz, $CDCl_3$): δ = 180.1.

EI-MS: *m*/*z* = 204 (M^+).

HRMS: *m*/*z* calcd for $C_4H_7Cl_2O_3P$: 203.9510; found: 203.9512.

1-Carbethoxyethyl Phosphorodichloridite (1b)

Colorless liquid; yield: 54%; bp 113–115 °C/10 mmHg.

IR (KBr): 1718 (C=O), 1097, 1025 (POC) cm^{-1}.

^{1}H NMR (300 MHz, $CDCl_3$): δ = 5.16 (sext, *J* = 6.9 Hz, 1 H, POCH), 4.25 (q, *J* = 7.0 Hz, 2 H, OCH_2), 1.60 (d, *J* = 6.9 Hz, 3 H, CH_3), 1.31 (t, *J* = 7.0 Hz, 3 H, CH_3).

^{31}P NMR (121.5 MHz, $CDCl_3$): δ = 178.7.

EI-MS: *m*/*z* = 218 (M^+).

Anal. Calcd for $C_5H_9Cl_2O_3P$ (219.00): C, 27.42; H, 4.14. Found: C, 27.13; H, 4.32.

1-Carbethoxy-1-phenylmethyl Phosphorodichloridite (1c)

Colorless liquid; yield: 48%; bp 133–135 °C/10 mmHg.

IR (KBr): 1720 (C=O), 1098, 1024 (POC) cm^{-1}.

^{1}H NMR (300 MHz, $CDCl_3$): δ = 7.53–7.37 (m, 5 H, Ph), 6.01 (d, J_{PH} = 14.4 Hz, 1 H, POCH), 4.25 (d, *J* = 7.0 Hz, 2 H, OCH_2), 1.25 (t, *J* = 7.0 Hz, 3 H, CH_3).

^{31}P NMR (121.5 MHz, $CDCl_3$): δ = 168.1.

EI-MS: *m*/*z* = 280 (M^+).

Anal. Calcd for $C_{10}H_{11}Cl_2O_3P$ (281.07): C, 42.73; H, 3.94. Found: C, 42.67; H, 4.08.

Depsiphosphonopeptides (2); General Procedure

2-Carbethoxyalkyl phosphorodichloridite **1** (2 mmol) was slowly added dropwise to a stirred mixture of benzyl carbamate (0.30 g, 2 mmol) and aldehyde (2 mmol) in anhyd benzene (10 mL) in an ice–water bath and then allowed to warm to r.t. under stirring. After stirring the reaction mixture for 6 h at r.t., H_2O (0.1 mL) was added dropwise and the resulting reaction mixture was allowed to stir for 2 h. After removal of the solvent, the residue was crystallized from petroleum ether–EtOAc to afford depsiphosphonopeptides **2** except for **2i** and **2l**, which were separated by column chromatography (petroleum ether–acetone, 5:1).

Ethyl 2-[({1-[(Benzyloxycarbonyl)amino]phenylmethyl}hydroxyphosphoryl)oxy]acetate (2a)

Colorless crystals; yield: 86%; mp 113–115 °C; R_f 0.25 (EtOAc–MeOH, 2:1).

IR (KBr): 3313 (NH, POH), 1719 (C=O), 1236 (P=O), 1099, 1025 (POC) cm^{-1}.

^1H NMR (300 MHz, $CDCl_3$): δ = 10.27 (br s, 1 H, POH), 7.37–7.17 (m, 10 H, Ph), 6.55 (br s, 1 H, NH), 5.29 (d, J_{PH} = 21.0 Hz, 1 H, PCH), 5.08 (s, 2 H, OCH_2), 4.42–4.24 (m, 2 H, OCH_2), 4.19 (q, J = 7.0 Hz, 2 H, CH_2), 1.24 (t, J = 7.0 Hz, 3 H, CH_3).

^{31}P NMR (121.5 MHz, $CDCl_3$): δ = 21.61.

ESI-MS: m/z = 408 (MH^+).

Anal. Calcd for $C_{19}H_{22}NO_7P$ (407.35): C, 56.02; H, 5.44; N, 3.44. Found: C, 55.78; H, 5.56; N, 3.62.

Ethyl 2-[({1-[(Benzyloxycarbonyl)amino]-(4-chlorophenyl)methyl}hydroxyphosphoryl)oxy]acetate (2b)

Colorless crystals; yield: 87%; mp 137–139 °C; R_f 0.16 (EtOAc–MeOH, 2:1).

IR (KBr): 3320 (NH, POH), 1708 (C=O), 1239 (P=O), 1093, 1015 (POC) cm^{-1}.

^1H NMR (300 MHz, $CDCl_3$): δ = 11.13 (br s, 1 H, POH), 7.34–7.24 (m, 9 H, Ph), 6.75 (br s, 1 H, NH), 5.28 (d, J_{PH} = 22.5 Hz, 1 H, PCH), 5.07 (s, 2 H, OCH_2), 4.44–4.28 (m, 2 H, OCH_2), 4.19 (q, J = 7.0 Hz, 2 H, CH_2), 1.24 (t, J = 7.0 Hz, 3 H, CH_3).

^{31}P NMR (121.5 MHz, $CDCl_3$): δ = 21.00.

ESI-MS: m/z = 442 (MH^+).

Anal. Calcd for $C_{19}H_{21}ClNO_7P$ (441.80): C, 51.65; H, 4.79; N, 3.17. Found: C, 51.89; H, 5.00; N, 2.96.

Ethyl 2-[({1-[(Benzyloxycarbonyl)amino]-(4-bromophenyl)methyl}hydroxyphosphoryl)oxy]acetate (2c)

Colorless crystals; yield: 87%; mp 139–40 °C; R_f 0.28 (EtOAc–MeOH, 2:1).

IR (KBr): 3344 (NH, POH), 1722 (C=O), 1240 (P=O), 1098, 1021 (POC) cm^{-1}.

^1H NMR (300 MHz, $CDCl_3$): δ = 9.95 (br s, 1 H, POH), 7.45–7.28 (m, 9 H, Ph), 6.65 (br s, 1 H, NH), 5.27 (d, J_{PH} = 22.2 Hz, 1 H, PCH), 5.08 (s, 2 H, OCH_2), 4.49–4.24 (m, 2 H, OCH_2), 4.21 (q, J = 7.2 Hz, 2 H, CH_2), 1.25 (t, J = 7.2 Hz, 3 H, CH_3).

^{31}P NMR (121.5 MHz, $CDCl_3$): δ = 20.94.

ESI-MS: m/z = 486 (MH^+).

Anal. Calcd for $C_{19}H_{21}BrNO_7P$ (486.25): C, 46.93; H, 4.35; N, 2.88. Found: C, 47.09; H, 4.03; N, 3.12.

Ethyl 2-[({1-[(Benzyloxycarbonyl)amino]-(4-methoxyphenyl)methyl}hydroxyphosphoryl)oxy]acetate (2d)

Colorless crystals; yield: 78%; mp 115–117 °C; R_f 0.14 (EtOAc–MeOH, 2:1).

IR (KBr): 3333 (NH, POH), 1720 (C=O), 1246 (P=O), 1098, 1028 (POC) cm^{-1}.

^1H NMR (300 MHz, $CDCl_3$): δ = 10.26 (br s, 1 H, POH), 7.30–6.82 (m, 9 H, Ph), 6.48 (br s, 1 H, NH), 5.24 (d, J_{PH} = 20.1 Hz, 1 H, PCH), 5.08 (s, 2 H, OCH_2), 4.45–4.27 (m, 2 H, OCH_2), 4.19 (q, J = 7.2 Hz, 2 H, CH_2), 3.76 (s, 3 H, CH_3O), 1.24 (t, J = 7.2 Hz, 3 H, CH_3).

^{31}P NMR (121.5 MHz, $CDCl_3$): δ = 20.88.

ESI-MS: m/z = 438 (MH^+).

Anal. Calcd for $C_{20}H_{24}NO_8P$ (437.38): C, 54.92; H, 5.53; N, 3.20. Found: C, 55.14; H, 5.30; N, 3.01.

Ethyl 2-[({1-[(Benzyloxycarbonyl)amino]phenylmethyl}hydroxyphosphoryl)oxy]propionate (2e)

Colorless crystals; yield: 72%; mp 122–125 °C; *anti*/*syn*, 88:12; R_f 0.18 (EtOAc–MeOH, 2:1).

IR (KBr): 3319 (NH, POH), 1726 (C=O), 1239 (P=O), 1053, 1009 (POC) cm^{-1}.

^1H NMR (300 MHz, $CDCl_3$): δ = 8.22 (br s, 1 H, POH), 7.41–7.27 (m, 10 H, Ph), 6.41, 6.25 (br s, 1 H, NH), 5.30–5.10 (m, 1 H, CHP), 5.10 (d, J_{PH} = 2.9 Hz, 2 H, CH_2O), 4.66, 4.53 (dq, J_{PH} = 7.0 Hz, J = 7.2 Hz, 1 H, POCH), 4.18, 4.15 (q, J = 7.2 Hz, 2 H, OCH_2), 1.38, 1.31 (d, J = 7.2 Hz, 3 H, CH_3), 1.24, 1.22 (t, J = 7.0 Hz, 3 H, CH_3).

^{31}P NMR (121.5 MHz, $CDCl_3$): δ = 21.49, 20.90 (88:12).

FAB-MS: m/z = 422 (MH^+).

Anal. Calcd for $C_{20}H_{24}NO_7P$ (421.38): C, 57.01; H, 5.74; N, 3.32. Found: C, 57.22; H, 5.63; N, 3.10.

Ethyl 2-[({1-[(Benzyloxycarbonyl)amino]-(4-chlorophenyl)methyl}hydroxyphosphoryl) oxy]propionate (2f)

Colorless crystals; yield: 81%; mp 127–129 °C; *anti*/*syn*, 88:12; R_f 0.14 (EtOAc–MeOH 2:1).

IR (KBr): 3319 (NH, POH), 1725 (C=O), 1239 (P=O), 1053, 1014 (POC) cm^{-1}.

^1H NMR (300 MHz, $CDCl_3$): δ = 8.59 (br s, 1 H, POH), 7.38–7.27 (m, 9 H, Ph), 6.56, 6.36 (br s, 1 H, NH), 5.32–5.07 (m, 1 H, CHP), 5.09 (d, J_{PH} = 2.7 Hz, 2 H, CH_2O), 4.72, 4.48 (dq, J_{PH} = 7.0 Hz, J = 6.9 Hz, 1 H, POCH), 4.19, 4.17 (q, J = 7.2 Hz, 2 H, OCH_2), 1.40, 1.36 (d, J = 6.9 Hz, 3 H, CH_3), 1.24, 1.23 (t, J = 7.2 Hz, 3 H, CH_3).

^{31}P NMR (121.5 MHz, $CDCl_3$): δ = 20.58, 19.60 (88:12).

FAB-MS: m/z = 456 (MH^+).

Anal. Calcd for $C_{20}H_{23}ClNO_7P$ (455.83): C, 52.70; H, 5.09; N, 3.07. Found: C, 52.53; H, 5.13; N, 3.10.

Ethyl 2-[({1-[(Benzyloxycarbonyl)amino]-(4-bromophenyl)methyl}hydroxyphosphoryl)oxy]propionate (2g)

Colorless crystals; yield: 86%; mp 124–126 °C; *anti*/*syn*, 87:13; R_f 0.21 (EtOAc–MeOH, 2:1).

IR (KBr): 3316 (NH, POH), 1719 (C=O), 1238 (P=O), 1099, 1053 (POC) cm^{-1}.

^1H NMR (300 MHz, $CDCl_3$): δ = 10.45 (br s, 1 H, POH), 7.44–7.25 (m, 9 H, Ph), 6.64, 6.44 (br s, 1 H, NH), 5.30–5.13 (m, 1 H, PCH), 5.08 (d, J_{PH} = 2.1 Hz, 2 H, OCH_2), 4.73, 4.58 (m, 1 H, OCH), 4.16 (q, J = 7.0 Hz, 2 H, CH_2), 1.40, 1.35 (d, J = 6.9 Hz, 3 H, CH_3), 1.23, 1.21 (t, J = 7.0 Hz, 3 H, CH_3).

^{31}P NMR (121.5 MHz, $CDCl_3$): δ = 20.40, 19.63 (87:13).

ESI-MS: *m/z* = 500 (MH^+).

Anal. Calcd for $C_{20}H_{23}BrNO_7P$ (500.28): C, 48.02; H, 4.63; N, 2.80. Found: C, 47.82; H, 4.89; N, 3.02.

Ethyl 2-[({1-[(Benzyloxycarbonyl)amino]-(4-methoxyphenyl)methyl}hydroxyphosphoryl)oxy]propionate (2h)
Colorless crystals; yield: 66%; mp 116–119 °C; *anti*/*syn*, 87:13; R_f 0.24 (EtOAc–MeOH, 2:1).

IR (KBr): 3319 (NH, POH), 1720 (C=O), 1245 (P=O), 1053, 1009 (POC) cm^{-1}.

^{1}H NMR (300 MHz, $CDCl_3$): δ = 7.37–7.27 (m, 7 H, Ph), 6.85 (d, *J* = 8.6 Hz, 2 H, Ph), 6.37, 6.20 (br s, 1 H, NH), 5.13–5.04 (m, 1 H, CHP), 5.09 (d, J_{PH} = 2.4 Hz, 2 H, CH_2O), 4.71, 4.56 (dq, J_{PH} = 7.0 Hz, *J* = 6.9 Hz, 1 H, POCH), 4.18, 4.16 (q, *J* = 7.0 Hz, 2 H, OCH_2), 3.78 (s, 3 H, MeO), 1.40, 1.33 (d, *J* = 6.9 Hz, 3 H, CH_3), 1.24, 1.23 (t, *J* = 7.2 Hz, 3 H, CH_3).

^{31}P NMR (121.5 MHz, $CDCl_3$): δ = 21.61, 20.70 (87:13).

FAB-MS: *m/z* = 452 (MH^+).

Anal. Calcd for $C_{21}H_{26}NO_8P$ (451.41): C, 55.88; H, 5.81; N, 3.10. Found: C, 55.80; H, 5.65; N, 3.22.

Ethyl 2-[({1-[(Benzyloxycarbonyl)amino]phenylmethyl}hydroxyphosphoryl)oxy]phenylacetate (2i)
Colorless crystals; yield: 89%; mp 45–48 °C; *anti*/*syn*, 86:14; R_f 0.11 (EtOAc–MeOH, 2:1).

IR (KBr): 3348 (NH, POH), 1734 (C=O), 1221 (P=O), 1058, 1026 (POC) cm^{-1}.

^{1}H NMR (300 MHz, $CDCl_3$): δ = 8.62 (br s, 1 H, POH), 7.41–6.10 (m, 15 H, Ph), 6.51, 6.33 (br s, 1 H, NH), 5.60 (d, J_{PH} = 8.4 Hz, 1 H, OCH), 5.37 (d, J_{PH} = 7.2 Hz, 1 H, OCH), 5.05 (s, 2 H, OCH_2), 5.29, 5.24 (d, *J* = 20.4 Hz, 1 H, CHP), 4.10 (q, *J* = 6.9 Hz, 2 H, OCH_2), 1.12, 1.11 (t, *J* = 6.9 Hz, 3 H, CH_3).

^{31}P NMR (121.5 MHz, $CDCl_3$): δ = 23.56, 22.40 (86:14).

ESI-MS: *m/z* = 484 (MH^+).

Anal. Calcd for $C_{25}H_{26}NO_7P$ (483.45): C, 62.11; H, 5.42; N, 2.90. Found: C, 62.00; H, 5.68; N, 3.13.

Ethyl 2-[({1-[(Benzyloxycarbonyl)amino]-(4-chlorophenyl)methyl}hydroxyphosphoryl)oxy]phenylacetate (2j)
Colorless crystals; yield: 86%; mp 147–150 °C; *anti*/*syn*, 84:16; R_f 0.18 (EtOAc–MeOH, 2:1).

IR (KBr): 3330 (NH, POH), 1725 (C=O), 1220 (P=O), 1090, 1015 (POC) cm^{-1}.

^{1}H NMR (300 MHz, $CDCl_3$): δ = 10.41 (br s, 1 H, POH), 7.28–7.20 (m, 14 H, Ph), 6.45 (br s, 1 H, NH), 5.40 (d, J_{PH} = 7.5 Hz, 1 H, OCH), 5.22 (d, J_{PH} = 23.4 Hz, 1 H, PCH), 5.05 (s, 2 H, OCH_2), 4.11 (q, *J* = 7.0 Hz, 2 H, CH_2), 1.11 (t, *J* = 7.0 Hz, 3 H, CH_3).

^{31}P NMR (121.5 MHz, $CDCl_3$): δ = 20.94, 19.80 (84:16).

ESI-MS: *m/z* = 518 (MH^+).

Anal. Calcd for $C_{25}H_{25}ClNO_7P$ (517.90): C, 57.98; H, 4.87; N, 2.70. Found: C, 58.17; H, 4.99; N, 2.61.

Ethyl 2-[({1-[(Benzyloxycarbonyl)amino]-(4-bromophenyl)methyl}hydroxyphosphoryl)oxy]phenylacetate (2k)
Colorless crystals; yield: 82%; mp 140–143 °C; *anti*/*syn*, 84:16; R_f 0.30 (EtOAc–MeOH, 2:1).

IR (KBr): 3335 (NH, POH), 1720 (C=O), 1223 (P=O), 1058, 1011 (POC) cm^{-1}.

^{1}H NMR (300 MHz, $CDCl_3$): δ = 9.50 (br s, 1 H, POH), 7.40–7.17 (m, 14 H, Ph), 6.45 (br s, 1 H, NH), 5.39 (d, J_{PH} = 7.5 Hz, 1 H, OCH), 5.20 (d, J_{PH} = 24.9 Hz, 1 H, PCH), 5.05 (s, 2 H, OCH_2), 4.10 (q, *J* = 7.5 Hz, 2 H, CH_2), 1.10 (t, *J* = 7.5 Hz, 3 H, CH_3).

^{31}P NMR (121.5 MHz, $CDCl_3$): δ = 20.88, 19.60 (84:16).

ESI-MS: *m/z* = 562 (MH^+).

Anal. Calcd for $C_{25}H_{25}BrNO_7P$ (562.35): C, 53.40; H, 4.48; N, 2.49. Found: C, 53.19; H, 4.59; N, 2.66.

Ethyl 2-[({1-[(Benzyloxycarbonyl)amino]-(4-methoxyphenyl)methyl}hydroxyphosphoryl)oxy]phenylacetate (2l)
Colorless viscous oil; yield: 87%; R_f 0.13 (EtOAc–MeOH, 2:1).

IR (KBr): 3333 (NH, POH), 1729 (C=O), 1248 (P=O), 1058, 1028 (POC) cm^{-1}.

^{1}H NMR (300 MHz, $CDCl_3$): δ = 8.84 (br s, 1 H, POH), 7.44–7.75 (m, 14 H, Ph), 6.50, 6.35 (br s, 1 H, NH), 5.63 (d, J_{PH} = 8.1 Hz, OCH), 5.40 (d, J_{PH} = 6.9 Hz, 1 H, OCH), 5.26, 5.19 (d, J_{PH} = 20.1 Hz, 1 H, PCH), 4.10 (q, *J* = 7.2 Hz, 2 H, OCH_2), 3.74 (s, 3 H, CH_3O), 1.12 (t, *J* = 7.2 Hz, 3 H, CH_3).

^{31}P NMR (121.5 MHz, $CDCl_3$): δ = 20.82, 20.30 (inseparable).

ESI-MS: *m/z* = 514 (MH^+).

Anal. Calcd for $C_{26}H_{28}NO_8P$ (513.48): C, 60.82; H, 5.50; N, 2.73. Found: C, 60.71; H, 5.72; N, 2.80.

Ethyl 2-[([1-[(Benzyloxycarbonyl)amino]-(*S*)-phenylmethyl]hydroxyphosphoryl)oxy]-(*R*)-phenylacetate (2m)
Colorless crystals; yield: 86%; *anti*/*syn*, 85:15; mp 47–48 °C; R_f 0.11 (EtOAc–MeOH, 2:1).

IR (KBr): 3348 (NH, POH), 1734 (C=O), 1221 (P=O), 1058, 1026 (POC) cm^{-1}.

^{1}H NMR (300 MHz, $CDCl_3$): δ = 8.62 (br s, 1 H, POH), 7.41–6.10 (m, 15 H, Ph), 6.51, 6.33 (br s, 1 H, NH), 5.60 (d, J_{PH} = 8.4 Hz, 1 H, OCH), 5.37 (d, J_{PH} = 7.2 Hz, 1 H, OCH), 5.05 (s, 2 H, OCH_2), 5.29, 5.24 (d, *J* = 20.4 Hz, 1 H, CHP), 4.10 (q, *J* = 6.9 Hz, 2 H, OCH_2), 1.12, 1.11 (t, *J* = 6.9 Hz, 3 H, CH_3).

^{31}P NMR (121.5 MHz, $CDCl_3$): δ = 23.56, 22.40 (85:15).

ESI-MS: *m/z* = 484 (MH^+).

Anal. Calcd for $C_{25}H_{26}NO_7P$ (483.45): C, 62.11; H, 5.42; N, 2.90. Found: C, 62.09; H, 5.19; N, 3.07.

1-Benzyloxycarbonylamino-1-Phenylmethylphosphonic Acid (3)
Depsiphosphonopeptide **2m** (1.69 g, 3.5 mmol) was dissolved in a solution of NaOH (1 M, EtOH; 10 mL) and stirred at r.t. overnight. The pH was adjusted to 2–3 by the addition of HCl (2 M), the resulting mixture was extracted with Et_2O (3 × 50 mL), and dried over anhyd Na_2SO_4. After removal of the solvent, the residue was crystallized from $CHCl_3$.

Colorless crystals; yield: 0.71 g (63%); mp 143–145 °C; $[\alpha]_D^{20}$ –27 (*c* 1.0, DMSO-d_6), {Lit.[17a] mp 142–143 °C; $[\alpha]_D^{20}$ –19.4 (*c* 2.0, 1 M NaOH)}.

IR (KBr): 3394 (NH, POH), 1717 (C=O), 1184 (P=O) cm^{-1}.

^{1}H NMR (300 MHz, DMSO-d_6): δ = 7.52–7.10 (m, 10 H, Ph), 5.49 (br s, 1 H, NH), 4.90 (br s, 3 H, OCH_2, CHP).

^{31}P NMR (121.5 MHz, DMSO-d_6): δ = 17.85.

ESI-MS: *m/z* = 322 (MH^+).

Anal. Calcd for $C_{15}H_{16}NO_5P$ (321.27): C, 56.08; H, 5.02; N, 4.36. Found: C, 56.09; H, 5.29; N, 4.17.

(*S*)-1-Amino-1-phenylmethylphosphonic Acid (4)

To a solution of phosphonic acid **3** (0.64 g, 2 mmol) in AcOH (2.5 mL) was added Pd/C (10%; 0.1 g), and the resulting mixture was stirred under a hydrogen atmosphere at r.t. for 14 h. After filtration and removal of the solvent, the residue was crystallized from H_2O.

Colorless crystals; yield: 0.337 g (90%); mp 282–284 °C (Lit.[18] 287–288 °C); $[\alpha]_D^{20}$ +12 (*c* 1.0, 1 M NaOH) {Lit[18] $[\alpha]_D^{20}$ +18 (*c* 2, 1 M NaOH)}.

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