Amino Acids (2010) 39:1265–1280
DOI 10.1007/s00726-010-0561-z

ORIGINAL ARTICLE

Synthesis of α-carboxyphosphinopeptides derived from norleucine

Jan Pícha · Miloš Buděšínský · Pavel Fiedler · Miloslav Šanda · Jiří Jiráček

Received: 6 January 2010 / Accepted: 9 March 2010 / Published online: 28 March 2010

Abstract In the present study, we describe in detail the synthesis of a relatively rare class of phosphorus compounds, α-carboxyphosphinopeptides. We prepared several norleucine-derived α-carboxyphosphinic pseudopeptides of the general formula Nle-Ψ[PO(OH)]-Gly. These compounds could have important applications as transition state-mimicking inhibitors for methionine or leucine aminopeptidases or other enzymes. For the preparation of the key α-carboxyphosphinate protected precursors, we investigated, compared and improved two different synthetic methods described in literature: the Arbuzov reaction of a silylated *N*-protected phosphinic acid with a bromoacetate ester and the nucleophilic addition of a mixed *O*-methyl *S*-phenyl *N*-protected phosphonic acid or a methyl *N*-protected phosphonochloridate with *tert*-butyl lithioacetate. We also prepared two *N*-Fmoc protected synthons, Fmoc-Nle-Ψ[PO(OH)]-Gly-COOH and Fmoc-Nle-Ψ[PO(OAd)]-Gly-COOH, and demonstrated that these precursors are suitable building blocks for the solid-phase synthesis of α-carboxyphosphinopeptides.

Keywords Phosphinopeptides · Pseudopetides · Phosphinate · Phosphinic pseudopeptides · Solid-phase synthesis · Norleucine

Abbreviations

AdBr	1-Bromoadamantane
Ad	1-Adamantyl
BOP	(Benzotriazol-1-yloxy)tris(dimethylamino) phosphonium hexafluorophosphate
BSA	*N*,*O*-bis(trimethylsilyl)acetamide
Cbz	Benzyloxycarbonyl
DIC	*N*,*N*′-Diisopropylcarbodiimide
DIPEA	*N*,*N*-Diisopropylethylamine
Fmoc	9-Fluorenylmethyloxycarbonyl
Fmoc-Cl	9-Flurenylmethoxycarbonyl chloride
Fmoc-OSu	*N*-(9-fluorenylmethoxycarbonyloxy) succinimide
LiSPr	Lithium *n*-propyl mercaptide
LDA	Lithium diisopropylamide
TEA	Triethylamine
TFA	Trifluoroacetic acid
TMSCl	Trimethylsilyl chloride

J. Pícha · M. Buděšínský · P. Fiedler · M. Šanda · J. Jiráček (✉)
Institute of Organic Chemistry and Biochemistry, v. v. i., Academy of Sciences of the Czech Republic, Flemingovo náměstí 2, 166 10 Prague 6, Czech Republic
e-mail: jiracek@uochb.cas.cz

Introduction

Phosphinopeptides (Dive et al. 2004; Kafarski and Lejczak 2000a, b; Yiotakis et al. 2004), also called phosphinic pseudopeptides, fall into a class of peptide isosteres in which a scissile peptide bond is replaced by a chemically stable, non-hydrolysable moiety. This structural change, considered to fulfil the requirements of a transition state mimetic (Phillips et al. 1992; Schramm et al. 1994; Wolfenden and Radzicka 1991), enables the rational design of phosphinate inhibitors for enzymes, mainly peptidases and transferases (Collinsova and Jiracek 2000).

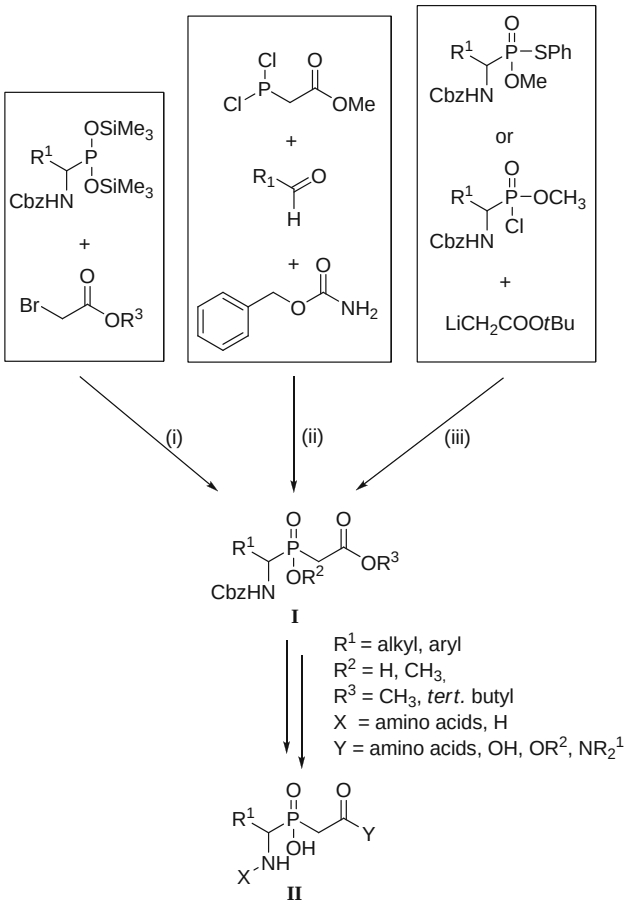
The formation of the P–C bond in *β*-carboxyphosphinopeptides, which bear the motif –PO(OH)CH_2CH(R)CO–, is easily achieved by the Michael addition of silyl phosphonites to acrylates (Georgiadis et al. 2001b; Yiotakis

et al. 1996). The β-carboxyphosphinodipeptides can be incorporated into peptide chains by standard methods of peptide synthesis. The application of *N*-Fmoc-protected derivatives of β-carboxyphosphinodipeptides, having the hydroxyphosphinyl function protected as a convenient ester (e.g. adamantyl) (Yiotakis et al. 1996), enabled the use of respective β-carboxyphosphinodipeptide synthons in the solid-phase synthesis of combinatorial libraries of metalloenzyme inhibitors (Collinsova et al. 2003; Dive et al. 1999; Jiracek et al. 1995, 1996).

In contrast to β-carboxyphosphinopeptides (Collinsova and Jiracek 2000), the number of scientific reports concerning α-carboxyphosphinopeptides, which bear the motif –PO(OH)CH(R)CO–, is much smaller, and many of them are patents. Nevertheless, different α-carboxyphosphinopeptides have been studied as inhibitors of aspartic acid peptidases, including pepsin (Bartlett and Kezer 1984) and penicillopepsin (Bartlett et al. 1990; Fraser et al. 1992), HIV protease (Dreyer et al. 1989; Jensen et al. 2005; Vidossich and Carloni 2006), endothiapepsin (Bailey and Cooper 1994; Beveridge et al. 1995; Coates et al. 2002; Lunney et al. 1993) and renin (Allen et al. 1989). Some compounds that are active as inhibitors of metallopeptidases (such as angiotensin-converting enzyme or endothelin-converting enzyme) or viral enzymes appear as potential therapeutic agents and were patented for the treatment of hypertension (Boger et al. 1986; Boger and Bock 1985; Matsueda et al. 1984; Patchett et al. 1985; Petrillo 1982; Shiosaki et al. 1991) or HIV (Jensen et al. 2005) and HCV (Casarez et al. 2008) infections.

In general, there are three possible pathways for the preparation of an α-carboxyphosphinate intermediate of the general formula **I** (Scheme 1), which represents the key compound for the synthesis of α-carboxyphosphinopeptides of the general structure **II**: (1) an Arbuzov reaction of a silylated *N*-protected phosphinic acid with a bromoacetate ester (Allen et al. 1989; Jensen et al. 2005; Patchett et al. 1985); (2) a three-component condensation of methyl dichlorophosphinoacetate, an aldehyde and benzyl carbamate (Matsueda et al. 1984; Morita et al. 1987); or (3) nucleophilic addition of a mixed *O*-methyl *S*-phenyl *N*-protected phosphonic acid (Bartlett et al. 1990; Bartlett and Kezer 1984; Boger et al. 1986; Boger and Bock 1985; Shiosaki et al. 1991) or a methyl *N*-protected phosphonochloridate (Georgiadis et al. 2001a; Patchett et al. 1985) with *tert*-butyl lithioacetate.

Concerning the synthesis of **II** from **I**, Bartlett's approach (Bartlett and Kezer 1984) represents a typical synthetic strategy for the rather complicated case where X and Y in **II** are amino acids. Briefly, the treatment of **I** (where R^2 is CH_3 and R^3 is *tert*-butyl) with TFA affords a free carboxylic group that enables elongation of the C-terminus of **I** by an amino acid sequence. Then the



Scheme 1 Summary of some possible pathways, (*i*)–(*iii*), for the preparation of an α-carboxyphosphinate intermediate of the general formula **I**, which represents the key compound for the synthesis of α-carboxyphosphinopeptides of the general structure **II**

benzyloxycarbonyl group is removed from the *N*-terminus by catalytic hydrogenolysis, thereby allowing subsequent attachment of an *N*-terminal peptide chain. Finally, the selective demethylation of the phosphinic ester by LiSPr leads to the desired products.

Herein, we present the synthesis of several α-carboxyphosphinic pseudopeptides of the general formula Nle-Ψ[PO(OH)]-Gly derived from a phosphinic analogue of norleucine. Recently, we published (Liboska et al. 2008) a preliminary report of this study. The target compounds were designed as potential inhibitors of methionine or leucine aminopeptidases. We built up the basic phosphinic scaffold using the above-described methods (1) or (3); the latter method we improved with the use of benzyl protection. We also prepared and successfully used two protected precursors for solid-phase synthesis of pseudopeptides bearing the α-carboxyphosphinic synthon Nle-Ψ[PO(OH)]-Gly: phosphinyl unprotected Fmoc-Nle-Ψ[PO(OH)]-CH_2COOH and adamantyl (Ad) protected Fmoc-Nle-Ψ[PO(OAd)]-CH_2COOH. We successfully used both building

blocks for the solid-phase synthesis of a model α-carboxyphosphinopeptide Tyr-Nle-Ψ[PO(OH)]-Gly-Cys-NH_2.

Materials and methods

General

The chemicals, reagents and solvents (Sigma-Aldrich, Fluka) were of analytical grade and were used without further purification. TLC on silica gel-coated aluminium plates (Fluka) was performed in the following systems (v/v): isopropyl alcohol—concentrated aqueous ammonia 10/1 (S1), chloroform–methanol–acetic acid 6/2/0.5 (S2), chloroform–methanol 95/5 (S3), chloroform–methanol 98/2 (S4) and chloroform–methanol 9/1 (S5). The compounds were visualized by exposure to UV light at 254 nm by ninhydrin spraying (dark blue colour of amines) and by spraying with a 1% ethanolic solution of 4-(4-nitrobenzyl)pyridine followed by heating and treatment with gaseous ammonia (blue colour of esters of phosphinic acid or esters of carboxylic acid). Flash chromatography purifications were carried out on silica gel (40–63 μm, Fluka). Preparative reverse-phase chromatography was carried out on a C18 Luna column (Phenomenex, 250 × 21.2 mm, 10 μm) at a flow rate of 9 mL/min with the following solvents: solvent A = 0.1% (v/v) TFA, solvent B = 80% (v/v) CH_3CN and 0.1% (v/v) TFA. The following elution conditions and gradients were used: G1: 55% B, isocratic elution; G2: $t = 0$ min (0% B), $t = 35$ min (50% B), $t = 40$ min (100% B), $t = 41$ min (0% B); G3: $t = 0$–15 min (0% B), $t = 30$ min (20% B), $t = 40$ min (40% B), $t = 45$ min (100% B), $t = 46$ min (0% B). Eluted peaks were detected at 218 nm and also at 276 nm for **24**, **25a**, and **25b**. Melting points were determined on a Boetius block and are uncorrected. ^{1}H and ^{13}C NMR spectra were measured on a Bruker AVANCE-600 spectrometer (^{1}H at 600.13 MHz, ^{13}C at 150.9 MHz) in $CDCl_3$, DMSO-d_6, CD_3OD or D_2O solution at 300 K. The 2D-H,H-COSY, 2D-H,C-HSQC and 2D-H,C-HMBC spectra were recorded and used for the structural assignment of proton and carbon signals. IR spectra were recorded on a Bruker IFS 55 Equinox apparatus. HRMS mass spectra were obtained on a FTMS mass spectrometer LTQ-orbitrap XL (Thermo Fisher, Bremen, Germany) in electrospray ionization mode.

Diphenylmethylamine hypophosphite (**1**)

Benzhydrylamine (26.7 g, 0.146 mol) in 100 mL of ethanol was slowly added dropwise to an ice-cooled 50% solution of hypophosphorous acid (9.64 g, 0.146 mol). The mixture was allowed to stand overnight at room temperature, and then the solvent was evaporated under reduced pressure and co-evaporated with toluene (2× 100 mL). Crystallization from ethanol afforded the pure product. Yield was 31.2 g (86%). White solid, mp 178–180°C (lit. 179–180°C) (Baylis et al. 1984). $R_f = 0.57$ (S2). ^{1}H NMR (600 MHz; DMSO): 5.53 (1H, s, CH), 7.32 (2H, m, Ar–H), 7.39 (4H, m, Ar–H), 7.51 (4H, m, Ar–H), 9.30 (3H, vb, NH_3^+). ^{13}C NMR (150.9 MHz; DMSO): 57.26 (N–CH<), 127.49 (4× Ar–CH), 128.34 (2× Ar–CH), 128.96 (4× Ar–CH), 139.23 (2× Ar–C). IR (KBr, ν_{max} cm^{-1}) 3,200–2,400 br, 2,340, 2,309, 1,571, 1,175, 1,158, 1,151, 1,040, 700. HRMS (ESI) calculated for $C_{13}H_{14}N$ $[M + H]^+$ 184.1121; found: 184.1113.

{(*R,S*)-1-[(Diphenylmethyl)amino]pentyl}phosphinic acid (**2**)

Benzhydrylamine (11.8 g; 0.178 mol) in 250 mL of dried dioxane was slowly added to anhydrous phosphonous acid (21.8 g; 0.12 mol). The suspension of salt was vigorously stirred and heated under reflux, and valeraldehyde (12.3 g; 0.14 mol) in 50 mL of dried dioxane was added dropwise within 15 min. The solution became homogenous after adding about a third of the aldehyde, almost immediately followed by the formation of dense crystalline slurry. Approximately half of the solvent was evaporated and the reaction mixture was allowed to cool to room temperature. Then, 150 mL of ethanol was added and the reaction mixture was allowed to stand at 5°C overnight. The crystals were filtered off through sintered glass, washed successively with 200 mL of ethanol and with 200 mL of diethyl ether and dried in vacuo. Yield 25 g (58%). White powder, mp 212–214°C (lit. 209–210°C) (Dingwall et al. 1977). $R_f = 0.52$ (S1). ^{1}H NMR (600 MHz; D_2O + NaOD): 0.84 (3H, t, $J = 7.4$, CH_3), 1.20 (2H, m, CH_2), 1.35 (2H, m, CH_2), 1.57 and 1.70 (2H, two m, CH_2), 2.50 (1H, m, N–CH–P), 5.19 (1H, s, CH–N), 6.95 (1H, dd, $J = 563.5$ and 1.5, P–H), 7.24, 7.33 and 7.43 (2H, 4H and 4H, m, 2× C_6H_5). ^{13}C NMR (150.9 MHz; D_2O + NaOD): 13.93 (CH_3), 22.76 (CH_2), 28.10 (CH_2), 28.59 (d, J(C,P) = 7.7, CH_2), 55.41 (d, J(C,P) = 101.6, N–CH–P), 64.85 (d, J(C,P) = 8.6, N–CH<), 127.96 (2× Ar–CH), 128.03 (4× Ar–CH), 129.43 (2× Ar–CH), 129.48 (2× Ar–CH), 143.22 (Ar–C), 143.53 (Ar–C). IR (KBr, ν_{max} cm^{-1}) 2,800–2,400 br, 2,337, 1,597, 1,497, 1,455, 1,178, 1,056, 1,044, 744, 702. HRMS (ESI) calculated for $C_{18}H_{23}NO_2P$ $[M - H]^+$ 316.1461; found: 316.1472.

Alternatively, compound **2** was also prepared as follows. Valeraldehyde (10.68 g; 0.124 mol) in 100 mL of dioxane was added dropwise to a refluxing slurry of diphenylmethylamine hypophosphite (**1**) (30.8 g; 0.124 mol) in 300 mL of dried dioxane. The rest of the procedure was the same as described above. Yield 20 g (51%).

((*R,S*)-1-Aminopentyl)phosphinic acid (**3**)

Phosphinic acid **2** (22 g; 0.069 mol) was refluxed for a period of 6 h with 50 mL of 48% hydrobromic acid. The reaction mixture was allowed to cool to room temperature and the upper layer of diphenylmethyl bromide was extracted with diethyl ether (3× 100 mL). The aqueous layer was separated, and the solvent was evaporated under reduced pressure. The residual brown oil was dissolved in 40 mL of ethanol, and then methyloxirane was added until a pH of about 6 was reached. The precipitate was filtered off and washed with ethanol and diethyl ether. Yield 7.2 g (69%). $R_f = 0.10$ (S1). White powder, mp 235°C (lit. 230–232°C) (Dingwall et al. 1977); ^{1}H NMR (D_2O + NaOD) δ 0.90 (t, $J = 7.3$ Hz, 3H, CH_3), 1.38 (m, 2H, CH_2), 1.33 and 1.47 (two m, 2H, CH_2), 1.56 and 1.80 (two m, 2H, CH_2), 2.90 (m, 1H, N–CH–P), 6.87 (dd, $J = 519.0$ and 1.4 Hz, P–H). ^{13}C NMR (D_2O + NaOD) δ 13.68 (CH_3), 22.37 (CH_2), 27.64 (CH_2), 28.00 (d, J(C,P) = 9.8 Hz, CH_2), 51.09 (d, J(C,P) = 95.5 Hz, N–CH–P); IR (KBr, ν_{max} cm^{-1}): 3,200–2,500 br, 1,546, 1,192, 1,057, 971; HRMS (ESI) calculated for $C_5H_{13}NO_2P$ $[M - H]^+$ 150.0689; found: 150.0690.

((*R,S*)-1-{[(Benzyloxy)carbonyl]amino}pentyl) phospinic acid (**4**)

Phosphinic acid **3** (7.2 g; 0.048 mol) was dissolved in 100 mL of 10% (v/v) aqueous sodium carbonate, and a solution of benzyloxycarbonyl chloride (9.7 g; 0.057 mol) in 50 mL of dioxane was added dropwise at 0°C within 1 h. After stirring for an additional 2 h at 0°C, the mixture was allowed to stand overnight at room temperature. The dioxane was evaporated under reduced pressure, and the acidity of the residual aqueous solution was adjusted with diluted hydrochloric acid to a pH of about 1, whilst efficiently cooling with ice. The precipitate was filtered off, washed with water until the pH of the filtrate was neutral and dried in vacuo. Yield 11.1 g (81%). White powder, mp 132–134°C. $R_f = 0.48$ (S1). ^{1}H NMR (600 MHz; DMSO): 0.84 (3H, t, $J = 7.1$, CH_3), 1.26 (2H, m, CH_2), 1.25 and 1.36 (2H, two m, CH_2), 1.49 and 1.64 (2H, two m, CH_2), 3.56 (1H, m, N–CH–P), 5.03 and 5.06 (2H, two d, $J = 12.5$, CH_2–O), 6.77 (1H, dd, $J = 531$ and 1.0, P–H), 7.31–7.37 (5H, m, C_6H_5), 7.58 (1H, d, $J = 9.0$, NH). ^{13}C NMR (150.9 MHz; DMSO): 14.05 (CH_3), 21.90 (CH_2), 26.23 (d, J(C,P) = 4.1, CH_2), 27.87 (d, J(C,P) = 11.9, CH_2), 50.61 (d, J(C,P) = 105.3, N–CH–P), 65.82 (–CH_2–O), 127.89 (2× Ar–CH), 128.08 (Ar–CH), 128.61 (2× Ar–CH), 137.26 (Ar–C), 156.56 (d, J(C,P) = 3.9, O–CO–N). IR (KBr, ν_{max} cm^{-1}) 3,293, 2,425, 1,721, 1,546, 1,457, 1,248, 1,158, 1,027, 1,013, 751, 699. HRMS (ESI) calculated for $C_{13}H_{19}NO_4P$ $[M - H]^+$ 284.1046; found: 284.1056.

Methyl [((*R,S*)-1-{[(benzyloxy)carbonyl]amino}pentyl) (hydroxy)phosphoryl]acetate (**5**)

TEA (0.87 g; 8.6 mmol) and TMSCl (0.93 g; 8.6 mmol) were added successively to an ice-cooled solution of phosphinic acid **4** (1.3 g; 4.3 mmol) in 25 mL of dried THF under nitrogen atmosphere. After 1 h, methyl bromoacetate (0.65 g; 4.3 mmol) was added dropwise and the reaction mixture was allowed to stand at room temperature overnight. The solvent was removed under reduced pressure and the residue was partitioned between 1 M HCl (30 mL) and ethyl acetate (2× 30 mL). The combined organic layers were dried with Na_2SO_4 and concentrated in vacuo. The pure product was obtained by trituration of the resulting oil in diethyl ether and petroleum ether at −20°C. Yield 1.3 g (79%). White powder, mp 75–77°C. $R_f = 0.58$ (S1). For ^{1}H, ^{13}C NMR see Ref. (Liboska et al. 2008). IR (KBr, ν_{max} cm^{-1}) 3,294, 1,736, 1,720, 1,541, 1,245, 1,003, 985, 743, 698. HRMS (ESI) calculated for $C_{16}H_{23}NO_6P$ $[M - H]^+$ 356.1258; found: 356.1264.

Alternatively, compound **5** was also prepared as follows. BSA (4.27 g; 21 mmol) was added dropwise to an ice-cooled slurry of phosphinic acid **4** (1 g; 3.5 mmol) in 20 mL of dried dichloromethane. As soon as the reaction mixture clarified (10 min), methyl bromoacetate (0.73 g; 4.7 mmol) was added in one portion. After 1 h, the cooling bath was removed, and the reaction was allowed to proceed at room temperature overnight. The reaction was quenched by carefully pouring the mixture into 50 mL of chilled 1 M HCl. Then the reaction mixture was extracted with chloroform (2× 30 mL). The combined organic layers were dried with Na_2SO_4 and evaporated in vacuo. Trituration of the residual oil in a mixture of diethyl ether and petroleum ether afforded the pure product. Yield 0.95 g (76%).

[((*R,S*)-1-{[(Benzyloxy)carbonyl]amino}pentyl) (hydroxy)phosphoryl]acetic acid (**6**)

Ester **5** (0.8 g; 2.1 mmol) was dissolved in a mixture of 20 mL of methanol and 10 mL of water with 0.2 g of NaOH (5 mmol) and stirred at room temperature overnight. The methanol was evaporated in vacuo, and the aqueous layer was acidified (pH 1) using 1 M HCl. The separated oil was extracted three times with 50 mL of ethyl acetate, and the organic phase was dried with Na_2SO_4 prior to filtration and evaporation under reduced pressure. The pure product was obtained by trituration of the oily residue at −20°C in a mixture of ethyl acetate and petroleum ether. Yield 0.63 g (85%). White powder, mp 109–112°C. $R_f = 0.44$ (S1). ^{1}H NMR (600 MHz; DMSO): (mixture of two isomers ~7:3; only signals of the major isomer are given): 0.84 (3H, t, $J = 7.1$, CH_3), 1.21 and 1.29 (2H, two m, CH_2), 1.21 and 1.34 (2H, two m, CH_2), 1.47 and 1.72

(2H, two m, CH_2), 2.74 and 2.77 (2H, two dd, $J = 15.4$ and 14.0, P–CH_2), 3.75 (1H, m, N–CH–P), 5.02 and 5.07 (2H, two d, $J = 11.7$, CH_2–O), 7.30–7.36 (5H, m, C_6H_5), 7.39 (1H, d, $J = 9.6$, NH). ^{13}C NMR (150.9 MHz; DMSO): 14.10 (CH_3), 21.84 (CH_2), 27.24 (d, J(C,P) = 3.2, CH_2), 28.08 (d, J(C,P) = 12.2, CH_2), 35.81 (d, J(C,P) = 76.1, P–CH_2), 50.85 (d, J(C,P) = 110.5, N–CH–P), 65.75 (–CH_2–O), 127.77 (2× Ar–CH), 128.02 (Ar–CH), 128.59 (2× Ar–CH), 137.42 (Ar–C), 156.62 (d, J(C,P) = 5.1,O–CO–N), 168.22 (d, J(C,P) = 6.0, COOH). IR (KBr, ν_{max} cm^{-1}) 3,304, 1,717, 1,536, 1,301, 1,248, 994, 975, 696. HRMS (ESI) calculated for $C_{15}H_{21}NO_6P$ $[M - H]^+$ 342.1101; found: 342.1111.

Alternatively, compound **6** was also prepared by the treatment of **14** (1.6 g; 4.3 mmol) with NaOH (0.4 g; 10 mmol) as described above. Yield 1.3 g (88%).

[((*R,S*)-1-{[(Benzyloxy)carbonyl]amino}pentyl)(hydroxy)phosphoryl]acetamide (**7**)

Compound **5** (2.1 g; 5.6 mmol) was stirred for 2 days in 50 mL of a saturated solution of ammonia in methanol. The solvent was evaporated in vacuo, and the aqueous layer was acidified (pH about 1) with 1 M HCl. The separated oil was extracted three times with 50 mL ethyl acetate, and the organic phase was dried with Na_2SO_4 prior to filtration and evaporation under reduced pressure. The pure product was obtained by trituration of the residue at −20°C in a mixture of ethyl acetate and petroleum ether. Yield 1.3 g (66%). White powder, mp 136–139°C. $R_f = 0.16$ (S1). ^{1}H NMR (600 MHz; DMSO): 0.84 (3H, t, $J = 7.1$, CH_3), 1.22 and 1.30 (2H, two m, CH_2), 1.22 and 1.34 (2H, two m, CH_2), 1.48 and 1.73 (2H, two m, CH_2), 2.61 (1H, dd, J(P,H) = 15.2 and J(H,H) = 14.2, P–C$\underline{H_a}H_b$), 2.68 (1H, dd, J(P,H) = 16.3 and J(H,H) = 14.2, P–C$H_a\underline{H_b}$), 3.73 (1H, m, N–CH–P), 5.02 and 5.08 (2H, two d, $J = 12.6$, CH_2–O), 7.10 and 7.43 (2H, two bd, $J = 2.2$, CO–NH_2), 7.36 (1H, d, $J = 10.0$, NH), 7.30–7.37 (5H, m, C_6H_5). ^{13}C NMR (150.9 MHz; DMSO): 14.11 (CH_3), 21.87 (CH_2), 27.84 (d, J(C,P) = 3.8, CH_2), 28.17 (d, J(C,P) = 12.1, CH_2), 36.24 (d, J(C,P) = 78.6, P–CH_2), 50.97 (d, J(C,P) = 108.1, N–CH–P), 65.80 (−CH_2−O), 127.80 (2× Ar–CH), 128.04 (Ar–CH), 128.61 (2× Ar–CH), 137.37 (Ar–C), 158.65 (d, J(C,P) = 5.0, O–CO–N), 167.78 (d, J(C,P) = 4.7, −CO−NH_2). IR (KBr, ν_{max} cm^{-1}) 3,390, 3,309, 2,937, 1,697, 1,659, 1,532, 1,455, 1,427, 1,245, 961, 696. HRMS (ESI) calculated for $C_{15}H_{22}N_2O_5P$ $[M - H]^+$ 341.1272; found: 341.1275.

Phosphinic acid **7** was also prepared by the treatment of **15** (1.27 g; 3.6 mmol) with NaOH (0.14 g; 3.6 mmol) as described for **6**. Yield 0.93 g (76%).

General procedure for the hydrogenolysis of compounds **8–10**

The protected precursor was dissolved in 80 mL of methanol, and then 10% Pd/C (0.15 g) was added. The mixture was vigorously stirred and allowed to react under the atmosphere of hydrogen (15 psi) at room temperature overnight. The catalyst was filtered off through Celite, and the pad was washed with 100 mL of methanol. The filtrate was evaporated in vacuo and the crude product was purified using preparative RP-HPLC. Finally, the target compounds were lyophilized from water.

Methyl [((*R,S*)-1-aminopentyl)(hydroxy)phosphoryl]acetate (**8**)

Phosphinate **8** was prepared by hydrogenolysis of **5** (0.2 g; 0.56 mmol). Gradient G3 was used for RP-HPLC purification. Yield 55 mg (44%). White powder. ^{1}H NMR (600 MHz; DMSO): 0.87 (3H, t, $J = 7.2$, CH_3), 1.29 (2H, m, CH_2), 1.34 and 1.45 (2H, two m, CH_2), 1.59 and 1.80 (2H, two m, CH_2), 2.98 and 3.04 (2H, two dd, $J = 16.5$ and 14.1, P–CH_2), 3.24 (1H, m, N–CH–P), 3.61 (3H, s, OCH_3), 8.10 (2H, b, NH_2). ^{13}C NMR (150.9 MHz; DMSO): 13.94 (CH_3), 22.09 (CH_2), 27.40 (CH_2), 27.87 (d (C,P) = 8.5, CH_2), 36.95 (d, J(C,P) = 80.8, P–CH_2), 49.03 (d, J(C,P) = 100.4, N–CH–P), 52.18 (OCH_3), 168.06 (d, J(C,P) = 5.2, –CO–O). IR (KBr, ν_{max} cm^{-1}) 2,960 vbr, 2,875, 1,631, 1,541, 1,439, 1,239, 1,169, 1,052, 1,030. HRMS (ESI) calculated for $C_8H_{19}NO_4P$ $[M + H]^+$ 224.1046; found: 224.1047.

Phosphinate **8** was also prepared by hydrogenolysis of **19** (0.4 g; 0.89 mmol). Yield 119 mg (60%).

[((*R,S*)-1-Aminopentyl)(hydroxy)phosphoryl]acetic acid (**9**)

Phosphinate **9** was prepared by hydrogenolysis of **6** (0.15 g; 0.44 mmol). Gradient G3 was used for RP-HPLC purification. Yield 44.2 mg (48%). White powder. ^{1}H NMR (600 MHz; DMSO): 0.87 (3H, t, $J = 7.3$, CH_3), 1.27 (2H, m, CH_2), 1.33 and 1.45 (2H, two m, CH_2), 1.56 and 1.78 (2H, two m, CH_2), 2.77 and 2.70 (2H, two dd, $J = 16.4$ and 14.2, P–CH_2), 3.08 (1H, m, N–CH–P), 8.03 (2H, b, NH_2). ^{13}C NMR (150.9 MHz; DMSO): 14.00 (CH_3), 22.18 (CH_2), 27.75 (CH_2), 28.11 (d, J(C,P) = 8.3, CH_2), 37.86 (d, J(C,P) = 77.7, P–CH_2), 49.60 (d, J(C,P) = 97.9, N–CH–P), 170.19 (d, J(C,P) = 3.6, COOH). IR (KBr, ν_{max} cm^{-1}) 2,900vbr, 1,710, 1,624, 1,517, 1,208, 1,184, 1,050. HRMS (ESI) calculated for $C_7H_{15}NO_4P$ $[M - H]^+$ 208.0744; found: 208.0746.

Phosphinate **9** was also prepared by hydrogenolysis of **18** (0.5 g; 0.95 mmol). Yield 140 mg (70%).

Alternatively, compound **9** was also prepared as follows. Phosphinic acid **6** (1.1 g; 3.2 mmol) was stirred for 2 h in 33% HBr/AcOH (3 mL). The solvent was evaporated under reduced pressure, and the oily residue was taken up in 1.5 mL of ethanol. The ethanolic solution was treated with methyloxirane until a pH of about six was reached. The precipitated product was filtered off and washed with diethyl ether. Yield 0.55 g (82%). White powder, mp 195–198°C.

[((*R,S*)-1-Aminopentyl)(hydroxy)phosphoryl] acetamide (**10**)

Phosphinate **10** was prepared by hydrogenolysis of **7** (0.3 g; 0.87 mmol). Gradient G3 was used for RP-HPLC purification. Yield 122 mg (66%). White powder. ^{1}H NMR (600 MHz; DMSO): 0.87 (3H, t, $J = 7.2$, CH_3), 1.26 and 1.31 (2H, two m, CH_2), 1.34 and 1.46 (2H, two m, CH_2), 1.62 and 1.84 (2H, two m, CH_2), 2.97 (1H, dd, J(P,H) = 18.0 and J(H,H) = 15.1, P–C$\underline{H_a}$H$_b$), 3.00 (1H, dd, J(P,H) = 18.8 and J(H,H) = 15.1, P–CH$_a$$\underline{H_b}$), 3.38 (1H, m, N–CH-P), 7.46 and 7.92 (2H, b, CO–NH_2), 8.33 (3H, b, NH_3). ^{13}C NMR (150.9 MHz; DMSO): 13.93 (CH_3), 22.05 (CH_2), 26.73 (d, J(C,P) = 1.3, CH_2), 27.92 (d, J(C,P) = 9.0, CH_2), 38.26 (d, J(C,P) = 85.9, P–CH_2), 49.21 (d, J(C,P) = 100.9, N–CH–P), 169.86 (d, J(C,P) = 3.3, –CO–NH_2). IR (KBr, ν_{max} cm^{-1}) 3,415, 3,205, 3,059 br, 2,968, 2,938, 1,676, 1,622, 1,429, 1,386, 1,227, 1,193, 1,044, 819. HRMS (ESI) calculated for $C_7H_{17}N_2NaO_3P$ $[M + Na]^+$ 231.0869; found: 231.0866.

Methyl ((*R,S*)-1-{[(benzyloxy)carbonyl]amino}pentyl) phosphinate (**11**)

Phosphinic acid **4** (10.7 g; 37.5 mmol) in a mixture of anhydrous methanol (20 mL) and dried THF (100 mL) was cooled to 0°C, and DIC (5.3 g; 41 mmol) was added dropwise. The reaction was allowed to proceed at 0°C for 2 h and then at room temperature overnight. The solvents were evaporated in vacuo and the residue was dissolved in 50 mL of cold ethyl acetate. The precipitate of diisopropyl urea was removed by filtration, and the filtrate was concentrated under reduced pressure. After evaporation, the crude product was purified by flash chromatography on silica gel using elution with a linear gradient of ethanol in chloroform. Yield 7.1 g (63%). Colourless oil. $R_f = 0.61$ (S3). ^{1}H NMR (600 MHz; $CDCl_3$): 0.90 (3H, t, $J = 7.2$, CH_3), 1.31 and 1.37 (2H, two m, CH_2), 1.36 and 1.47 (2H, two m, CH_2), 1.62 and 1.83 (2H, two m, CH_2), 3.80 (3H, d, J(H,P) = 11.4, OCH_3), 3.97 (1H, m, N–CH–P), 5.12 (2H, s, CH_2–O), 5.31 (1H, d, $J = 9.7$, NH), 6.97 (J(H,P) = 550.6, OCH_3), 7.32–7.38 (5H, m, C_6H_5). ^{13}C NMR (150.9 MHz; $CDCl_3$): 13.76 (CH_3), 22.16 (CH_2), 26.65 (d, J(C,P) = 1.3, CH_2), 27.62 (d, J(C,P) = 10.9, CH_2), 49.59 (d, J(C,P) = 107.8, N–CH–P), 53.16 (d, J(C,P) = 7.5, OCH_3), 67.30 (–CH_2–O), 128.12 (2× Ar–CH), 128.24 (Ar–CH), 128.51 (2× Ar–CH), 135.96 (Ar–C), 156.20 (d, J(C,P) = 4.3, O–CO–N). IR ($CHCl_3$, ν_{max} cm^{-1}) 3,431, 3,028, 2,361, 1,718, 1,508, 1,456, 1,240, 1,047, 698. HRMS (ESI) calculated for $C_{14}H_{21}NO_4P$ $[M - H]^+$ 298.1214; found: 298.1213.

Methyl hydrogen [(*R,S*)-1-(benzyloxycarbonylamino)-pentyl]phosphonate (**12**)

$NaIO_4$ (2.04 g; 9.6 mmol) in water (20 mL) was added to phosphinic ester **11** (2.6 g; 8.7 mmol) dissolved in 250 mL of dioxane. After stirring for 12 h at room temperature, the solvents were evaporated in vacuo, the residue was taken up into 200 mL of ethyl acetate and the solid particles were filtered off. The filtrate was washed successively with 50 mL of 2% aqueous $KHSO_4$ and with 100 mL of freshly prepared aqueous solution of sulphur dioxide and brine. The separated organic layer was dried with Na_2SO_4 prior to filtration and evaporation under reduced pressure. The pure product was obtained by trituration of the residue at −20°C in a mixture of ethyl acetate and petroleum ether. Yield 2.3 g (84%). For spectra, R_f and melting point see Ref. (Picha et al. 2009).

O-Methyl *S*-phenyl ((*R,S*)-1-{[(benzyloxy)carbonyl] amino}pentyl)phosphonothiate (**13**)

Monomethyl phosphonic ester **12** (8.3 g; 26 mmol) suspended in 150 mL of dried dichloromethane was treated with thionyl chloride (9.1 g; 76 mmol). After 2 h at room temperature, the solvent was evaporated under reduced pressure, and the residual oil was taken up with 100 mL of dichloromethane, which was re-evaporated to remove the residual sulphur dioxide and hydrochloric acid. The resulting oil was again re-suspended in 100 mL dichloromethane, ice cooled, and then triethylamine (7.9 g; 78 mmol) and thiophenol (8.59 g; 78 mmol) were added. The reaction mixture was kept overnight at room temperature. The solution was washed with 100 mL of water and brine, then dried with Na_2SO_4 and evaporated under reduced pressure. The crude product was purified by flash chromatography on silica gel using elution with a linear gradient of ethanol in chloroform followed by trituration in a mixture of diethyl ether and petroleum ether at −20°C. Yield 5.7 g (53%). White powder, mp 97–99°C. $R_f = 0.55$ (S3). ^{1}H NMR (600 MHz; $CDCl_3$): mixture of two diastereoisomers ~2:1, only signals of the major isomer are given: 0.84 (3H, t, $J = 7.2$, CH_3), 1.23 and 1.30 (2H, two m, CH_2), 1.28 and 1.40 (2H, two m,

CH_2), 1.55 and 1.77 (2H, two m, CH_2), 3.85 (3H, d, J(H,P) = 11.8, OCH_3), 4.22 (1H, m, N–CH–P), 5.07 (1H, dd, J = 10.6 and 1.5, NH), 5.14 and 5.18 (2H, two d, J = 12.2, CH_2–O), 7.30–7.38 (5H, m, C_6H_5). ^{13}C NMR (150.9 MHz; $CDCl_3$): 13.78 (CH_3), 22.17 (CH_2), 27.75 (d, J(C,P) = 12.2, CH_2), 29.24 (d, J(C,P) = 2.7, CH_2), 52.26 (d, J(C,P) = 118.4, N–CH–P), 52.44 (d, J(C,P) = 8.3, OCH_3), 67.17 (–CH_2–O), 125.98 (d, J(C,P) = 5.6, Ar–C), 127.96 (2× Ar–CH), 128.17 (2× Ar–CH), 128.50 (Ar–C), 129.23 (d, J(C,P) = 2.4, Ar–CH), 129.39 (d, J(C,P) = 1.8, 2× Ar–CH), 135.30 (d, J(C,P) = 4.1, 2× Ar–CH), 136.21 (Ar–C), 156.03 (d, J(C,P) = 6.7, O–CO–N). IR (KBr, ν_{max} cm^{-1}) 3,280, 3,233, 1,716, 1,685, 1,544, 1,456, 1,255, 1,246, 1,226, 1,214, 1,029, 1,023, 749, 699, 689. HRMS (ESI) calculated for $C_{20}H_{26}NNaO_4PS$ $[M + Na]^+$ 430.1212; found: 430.1210.

Alternatively, compound **13** was also prepared as follows. Thiophenol (0.55 g; 4.95 mmol) and triethylamine (0.83 g; 8.25 mmol) were added to a solution of phosphinic ester **11** (1 g; 3.3 mmol) in 25 mL of dried tetrachloromethane. The reaction mixture was allowed to react overnight at room temperature. The solvent was evaporated in vacuo, and the crude product was purified by flash chromatography on silica gel using elution with a linear gradient of ethyl acetate in toluene followed by trituration in a mixture of diethyl ether and petroleum ether at −20°C. Yield 0.95 g (80%).

Methyl [((*R,S*)-1-{[(benzyloxy)carbonyl]amino}pentyl) (methoxy)phosphoryl]acetate (**14**)

Methyl acetate (3.1 g; 42 mmol) in 50 mL of dried THF was added dropwise under nitrogen atmosphere to 23.3 mL of LDA (1.8 M) cooled to −78°C (bath with a mixture of solid CO_2 in acetone). After 15 min, the solution of **13** (5.6 g; 14 mmol) in 50 mL of THF was added dropwise. The reaction mixture was stirred at −78°C for 1 h and then allowed to warm to room temperature. The reaction was quenched by the addition of 20 mL of 0.1 M HCl. The mixture was extracted with chloroform (5× 30 mL), and the combined organic layers were washed with brine, dried with Na_2SO_4 and evaporated in vacuo. The product was isolated by flash chromatography on silica gel using elution with a linear gradient of ethanol in chloroform. Yield 2.1 g (41%). Colourless oil. R_f = 0.36 (S3). ^{1}H NMR (600 MHz; $CDCl_3$): mixture of two diastereoisomers ~77:23, only signals of the major isomer are given : 0.89 (3H, t, J = 7.2, CH_3), 1.31 and 1.37 (2H, two m, CH_2), 1.36 and 1.46 (2H, two m, CH_2), 1.60 and 1.88 (2H, two m, CH_2), 3.00 (2H, m, P–CH_2), 3.71 (3H, s, CO–OCH_3), 3.80 (3H, d, J(H,P) = 10.8, P–OCH_3), 4.16 (1H, m, N–CH–P), 5.14 (2H, s, CH_2–O), 5.32 (1H, d, J = 10.3, NH), 7.32–7.37 (5H, m, C_6H_5). ^{13}C NMR (150.9 MHz; $CDCl_3$): 13.82 (CH_3), 22.16 (CH_2), 27.90 (d, J(C,P) = 11.7, CH_2), 28.15 (d, J(C,P) = 2.9, CH_2), 34.03 (d, J(C,P) = 77.3, P–CH_2), 49.94 (d, J(C,P) = 111.2, N–CH–P), 52.43 (d, J(C,P) = 7.0, P–OCH_3), 52.76 (OCH_3), 67.18 (–CH_2–O), 127.90 (2× Ar–CH), 128.18 (Ar–CH), 128.50 (2× Ar–CH), 136.19 (Ar–C), 156.19 (d, J(C,P) = 5.9, O–CO–N), 166.76 (d, J(C,P) = 4.5, –CO–O). IR ($CHCl_3$, ν_{max} cm^{-1}) 3,424, 3,029, 1,723, 1,509, 1,438, 1,243, 1,180, 1,044, 698. HRMS (ESI) calculated for $C_{17}H_{26}NNaO_6P$ $[M + Na]^+$ 394.1390; found: 394.1388.

Alternatively, compound **14** was also prepared as follows. Thionyl chloride (1.73 g; 14.6 mmol) was added to a solution of compound **12** (2.3 g; 7.3 mmol) in 25 mL of dichloromethane. The mixture was stirred for 2 h and then evaporated in vacuo. The residual oil was dissolved twice in dichloromethane (25 mL) and re-evaporated. After 15 min, the resulting phosphochloridate of **12** (in 50 mL of THF) was added dropwise to a mixture (prepared as described above) of LDA (12.2 mL, 1.8 M) and methyl acetate (1.6 g; 21.9 mmol) in 50 mL of dried THF. The following procedures were the same as described above. Yield 1.65 g (60%).

[((*R,S*)-1-{[(Benzyloxy)carbonyl]amino}pentyl) (methoxy)phosphoryl]acetamide (**15**)

Compound **14** (2.1 g; 5.6 mmol) was stirred over 2 days in 50 mL of a saturated solution of ammonia in methanol. The solvent was evaporated in vacuo and the crude product was purified by flash chromatography on silica gel using elution with a linear gradient of ethanol in chloroform followed by trituration in a mixture of ethyl acetate and petroleum ether at −20°C. Yield 1.5 g (73%). White powder, mp 120–122°C. R_f = 0.55 (S5). ^{1}H NMR (600 MHz; $CDCl_3$): mixture of two diastereoisomers ~73:27, only signals of the major isomer are given: 0.89 (3H, t, J = 7.2, CH_3), 1.29 and 1.34 (2H, two m, CH_2), 1.32 and 1.47 (2H, two m, CH_2), 1.63 and 1.85 (2H, two m, CH_2), 2.86 (2H, m, P–CH_2), 3.80 (3H, d, J(H,P) = 10.8, P–OCH_3), 4.10 (1H, m, N–CH–P), 5.13 (2H, s, CH_2–O), 5.80 and 7.33 (2H, two b, CO–NH_2), 6.16 (1H, d, J = 9.8, NH), 7.32–7.37 (5H, m, C_6H_5). ^{13}C NMR (150.9 MHz; $CDCl_3$): 13.80 (CH_3), 22.05 (CH_2), 27.12 (d, J(C,P) = 1.2, CH_2), 27.95 (d, J(C,P) = 11.5, CH_2), 34.10 (d, J(C,P) = 73.5, P–CH_2), 49.26 (d, J(C,P) = 112.0, N–CH–P), 52.59 (d, J(C,P) = 7.3, P–OCH_3), 67.44 (–CH_2–O), 127.96 (2× Ar–CH), 128.33 (Ar–CH), 128.55 (2× Ar–CH), 135.97 (Ar–C), 157.06 (d, J(C,P) = 4.0, O–CO–N), 166.92 (d, J(C,P) = 3.5, –CO–O).IR (KBr, ν_{max} cm^{-1}) 3,373, 3,336, 3,208, 2,957, 2,932, 1,704, 1,658, 1,533, 1,419, 1,373, 1,290, 1,247, 1,214, 1,046, 743, 697. HRMS (ESI) calculated for $C_{16}H_{25}N_2NaO_5P$ $[M + Na]^+$ 379.1393; found: 379.1392.

Benzyl ((*R,S*)-1-{[(benzyloxy)carbonyl]amino}pentyl) phosphinate (**16**)

Benzyl ester **16** was prepared in a similar way to **11** by the reaction of **4** (1 g; 3.5 mmol) with benzyl alcohol (0.37 g; 3.5 mmol) and DIC (0.48 g; 3.8 mmol). Trituration in a mixture of ethyl acetate and petroleum ether at −20°C afforded the pure product. Yield 1.1 g (84%). White powder, mp 62–65°C. $R_f = 0.34$ (S4). IR (KBr, ν_{max} cm^{-1}) 3,193, 2,933, 1,711, 1,542, 1,455, 1,273, 1,244, 1,206, 1,027, 738. 697. HRMS (ESI) calculated for $C_{20}H_{27}NO_4P$ [M + H] 376.1672; found: 376.1674. ^{1}H NMR (600 MHz; $CDCl_3$): mixture of two diastereoisomers, only signals of the major isomer are given: 0.88 (3H, t, $J = 7.2$, CH_3), 1.25–1.37 (2H, m, CH_2), 1.35 and 1.43 (2H, two m, CH_2), 1.53 and 1.87 (2H, two m, CH_2), 4.03 (1H, dddd, $J = 10.5$, 9.8, 8.0 and 4.2, N–CH–P), 5.02–5.16 (4H, m, 2× CH_2–O), 5.02 (1H, d, $J = 9.8$, NH), 7.07 (1H, d, J(H,P) = 553.1, P–H), 7.28–7.38 (10H, m, 2× C_6H_5). ^{13}C NMR (150.9 MHz; $CDCl_3$): 13.78 (CH_3), 22.17 (CH_2), 27.11 (d, J(C,P) = 3.2, CH_2), 27.80 (d, J(C,P) = 1.5, CH_2), 49.65 (d, J(C,P) = 103.7, N–CH–P), 67.36 (–CH_2–O), 68.20 (d, J(C,P) = 6.0, P–OCH_2), 126.95–128.86 (10x Ar–CH), 135.22 (d, J(C,P) = 5.7, Ar–C), 135.93 (Ar–C), 156.07 (d, J(C,P) = 4.8, O–CO–N).

Benzyl hydrogen [(*R,S*)-1-(benzyloxycarbonylamino)-pentyl]phosphonate (**17**)

Phosphonic acid **17** was prepared in a similar way to **12** by the reaction of **16** (3.27 g; 8.7 mmol) with $NaIO_4$ (2.04 g; 9.6 mmol). Yield 3.05 g (90%). For spectra, R_f and melting point see Ref. (Picha et al. 2009).

Benzyl [((*R,S*)-1-{[(benzyloxy)carbonyl]amino}pentyl) (benzyloxy)phosphoryl]acetate (**18**)

Dibenzyl ester **18** was prepared in a similar way to compound **14** (Scheme 3, f) by the reaction of **17** (2 g; 5.1 mmol) with thionyl chloride (1.2 g; 10.2 mmol), 8.5 mL LDA (1.8 M) and benzylacetate (2.3 g; 15.3 mmol). The product was isolated by flash chromatography on silica gel using elution with a linear gradient of ethyl acetate in toluene. Yield 1.5 g (56%). Colourless oil. $R_f = 0.30$ (S4). ^{1}H NMR (600 MHz; $CDCl_3$): mixture of two diastereoisomers ~ 1:1, many signals are doubled and those clearly identified are given connected with symbol + in data below: 0.89 (3H, t, $J = 7.1$, CH_3), 1.23 and 1.29 (2H, two m, CH_2), 1.29 and 1.40 (2H, two m, CH_2), 1.41 + 1.56 and 1.81 + 1.90 (2H, two m, CH_2), 3.06 (2H, m, P–CH_2), 4.21 (1H, m, N–CH–P), 4.99–5.13 (6H, m, 3× CH_2–O), 4.91 + 5.25 (1H, d, $J = 10.3$, NH), 7.28–7.34 (15H, m, 3× C_6H_5). ^{13}C NMR (150.9 MHz; $CDCl_3$): 13.78 + 13.81 (CH_3), 22.11 (CH_2), 27.75 + 27.86 (d, J(C,P) = 12.1 + 11.7, CH_2), 28.10 + 28.19 (d, J(C,P) = 3.0 + 2.9, CH_2), 34.91 + 35.22 (d, J(C,P) = 76.6 + 75.9, P–CH_2), 50.29 + 50.48 (d, J(C,P) = 110.2 + 108.4, N–CH–P), 67.12 + 67.20 (d, J(C,P) = 6.9 + 6.4, CH_2–O), 67.18 + 67.30 (CH_2–O), 67.54 (CH_2–O), 127.93–128.61 (15× Ar–CH), 134.98 + 135.04 (Ar–C), 135.82 + 135.88 (d, J(C,P) = 6.0 + 5.5, Ar–C), 136.01 + 136.12 (Ar–C), 155.95 + 156.17 (d, J(C,P) = 6.0 + 5.8, N–CO–O), 165.72 + 166.14 (d, J(C,P) = 4.7 + 4.6, –CO–O). IR ($CHCl_3$, ν_{max} cm^{-1}) 3,425, 2,961, 2,934, 1,724, 1,508, 1,456, 1,243, 1,000, 697. HRMS (ESI) calculated for $C_{29}H_{34}NNaO_6P$ $[M + Na]^+$ 546.2016; found: 546.2014.

Methyl [((*R,S*)-1-{[(benzyloxy)carbonyl]amino}pentyl) (benzyloxy)phosphoryl]acetate (**19**)

Diester **19** was prepared in a similar way to **14** by the reaction of compound **17** (2 g; 5.1 mmol) with thionyl chloride (1.2 g; 10.2 mmol), 8.5 mL LDA (1.8 M) and methyl acetate (1.1 g; 15.3 mmol). The product was isolated by flash chromatography on silica gel using elution with a linear gradient of ethanol in chloroform. Yield 1.5 g (65%). Colourless oil. $R_f = 0.32$ (S4). ^{1}H NMR (600 MHz; $CDCl_3$): 0.89 (3H, t, $J = 7.2$, CH_3), 1.26 and 1.33 (2H, two m, CH_2), 1.33 and 1.44 (2H, two m, CH_2), 1.59 and 1.84 (2H, two m, CH_2), 3.03 (2H, m, P–CH_2), 3.64 (3H, s, OCH_3), 4.21 (1H, m, N–CH–P), 5.11 (4H, m, 2× CH_2–O), 5.32 (1H, d, $J = 10.4$, NH), 7.31–7.37 (10H, m, 2× C_6H_5). ^{13}C NMR (150.9 MHz; $CDCl_3$): 13.78 (CH_3), 22.13 (CH_2), 27.85 (d, J(C,P) = 11.6, CH_2), 28.08 (d, J(C,P) = 2.7, CH_2), 34.71 (d, J(C,P) = 76.3, P–CH_2), 50.27 (d, J(C,P) = 110.7, N–CH–P), 52.66 (OCH_3), 67.18 (CH_2–O), 67.27 (d, J(C,P) = 13.0, CH_2–O), 127.92–128.64 (10x Ar–CH), 135.85 (d, J(C,P) = 5.6, Ar–C), 136.15 (Ar–C), 156.19 (d, J(C,P) = 6.0, N–CO–O), 166.69 (d, J(C,P) = 4.7, –CO–O). IR ($CHCl_3$, ν_{max} cm^{-1}). 3,426, 2,958, 2,934, 2,875, 1,723, 1,508, 1,438, 1,289, 1,243, 1,039, 1,009, 697. HRMS (ESI) calculated for $C_{23}H_{30}NNaO_6P$ $[M + Na]^+$ 470.1703; found: 470.1700.

tert-Butyl [((*R,S*)-1-{[(benzyloxy)carbonyl]amino} pentyl)(benzyloxy)phosphoryl]acetate (**20**)

Diester **20** was prepared in a similar way to **14** by the reaction of compound **17** (5 g; 13 mmol) with thionyl chloride (3.1 g; 26 mmol), 21.6 mL LDA (1.8 M) and *tert*-butylacetate (4.5 g; 39 mmol) except that the reaction mixture was quenched with citric acid instead of HCl. The product was isolated by flash chromatography on silica gel using elution with a linear gradient of ethanol in chloroform followed by trituration in a mixture of ethyl acetate

and petroleum ether at −20°C. Yield 3.3 g (52%). White powder, mp 74–77°C. $R_f = 0.29$ (S4). ^{1}H NMR (600 MHz; $CDCl_3$): mixture of two diastereoisomers ~ 76:24, only signals of the major isomer are given: 0.87 (3H, t, $J = 7.2$, CH_3), 1.28 and 1.35 (2H, two m, CH_2), 1.38 (9H, s, *t*-Bu), 1.36 and 1.47 (2H, two m, CH_2), 1.62 and 1.90 (2H, two m, CH_2), 2.90–3.00 (2H, m, P–CH_2), 4.26 (1H, m, N–CH–P), 5.10 (4H, m, 2× CH_2–O), 5.39 (1H, d, $J = 10.0$, NH), 7.30–7.35 (10H, m, 2× C_6H_5). ^{13}C NMR (150.9 MHz; $CDCl_3$): 13.80 (CH_3), 22.16 (CH_2), 27.75 (3× CH_3 (t-Bu)), 28.04 (d, J(C,P) = 11.8, CH_2), 28.57 (d, J(C,P) = 4.1, CH_2), 36.13 (d, J(C,P) = 76.5, P–CH_2), 50.28 (d, J(C,P) = 107.4, N–CH–P), 66.99 (d, J(C,P) = 6.6, CH_2–O), 67.06 (CH_2–O), 82.82 (>C <(t-Bu)), 127.88 (2× Ar–CH), 127.93 (2× Ar–CH), 128.10 (Ar–CH), 128.37 (Ar–CH), 128.44 (2× Ar–CH), 128.48 (2× Ar–CH), 135.99 (d, J(C,P) = 7.0, Ar–C), 136.20 (Ar–C), 156.17 (d, J(C,P) = 5.8, N–CO–O), 165.82 (d, J(C,P) = 4.4, –CO–O). IR (KBr, ν_{max} cm^{-1}) 3,202, 3,089, 3,036, 2,975, 1,719, 1,550, 1,394, 1,365, 1,369, 1,294, 1,259, 1,211, 1,165, 1,045, 1,007,742, 733, 698. HRMS (ESI) calculated for $C_{26}H_{36}NNaO_6P$ $[M + Na]^+$ 512.2172; found: 512.2170.

[((*R,S*)-1-{(9H-Fluoren-9-ylmethoxycarbonyl]amino} pentyl)(hydroxy)phosphoryl]acetic acid (**21**)

Phosphinic acid **9** (0.25 g; 1.2 mmol) was dissolved in 3 mL of 10% aqueous Na_2CO_3 and a solution of Fmoc-Cl (0.31 g; 1.2 mmol) in 3 mL of dioxane was added dropwise at 0°C. After stirring for 2 h at 0°C, the mixture was allowed to stand overnight at room temperature. The dioxane was evaporated under reduced pressure and the acidity of the aqueous solution was adjusted to a pH of about 1 whilst efficiently cooling with ice. The solution was extracted with ethyl acetate (3× 10 mL), and the combined organic layers were dried with Na_2SO_4 before filtering and removing the solvent in vacuo. The residual oil was purified by RP-HPLC (G1, see General). Yield 185 mg (36%). White powder. $R_f = 0.32$ (S2). ^{1}H NMR (600 MHz; DMSO): 0.84 (3H, t, $J = 7.0$, CH_3), 1.22 and 1.30 (2H, two m, CH_2), 1.22 and 1.34 (2H, two m, CH_2), 1.51 and 1.73 (2H, two m, CH_2), 2.72 (2H, m, P–CH_2), 3.73 (1H, m, N–CH–P), 4.22 (1H, t, $J = 7.2$, CH (Fmoc)), 4.30 (1H, dd, $J = 10.6$ and 7.2, –CHaHb–O (Fmoc)), 4.33 (1H, dd, $J = 10.6$ and 7.2, –CHaHb–O (Fmoc)), 7.30 (1H, m, Ar–CH), 7.32 (1H, m, Ar–CH), 7.40 (1H, m, Ar–CH), 7.41 (1H, m, Ar–CH), 7.48 (1H, d, $J = 9.5$, NH), 7.72 (1H, m, Ar–CH), 7.74 (1H, m, Ar–CH), 7.88 (2H, m, 2× Ar–CH). ^{13}C NMR (150.9 MHz; DMSO): 14.09 (CH_3), 21.86 (CH_2), 27.09 (d, J(C,P) = 2.8, CH_2), 28.05 (d, J(C,P) = 12.1, CH_2), 35.81 (d, J(C,P) = 76.5, P–CH_2), 47.01 (> CH- (Fmoc)), 50.80 (d, J(C,P) = 110.1, N–CH–P), 65.80 (CH_2–O), 120.35 (Ar–CH), 120.36 (Ar–CH), 125.52 (Ar–CH), 125.64 (Ar–CH), 127.26 (Ar–CH), 127.32 (Ar–CH), 127.88 (Ar–CH), 127.89 (Ar–CH), 140.96 (Ar–C), 140.98 (Ar–C), 143.95 (Ar–C), 144.15 (Ar–C), 156.54 (d, J(C,P) = 4.7, N–CO–O), 168.24 (d, J(C,P) = 5.7, COOH). IR (KBr, ν_{max} cm^{-1}) 3,294, 1,720, 1,678, 1,534, 1,239, 987, 758, 741. HRMS (ESI) calculated for $C_{22}H_{27}NO_6P$ $[M + H]^+$ 432.1571; found: 432.1569.

Methyl[((*R,S*)-1-{[(benzyloxy)carbonyl]amino}pentyl) (adamantyloxy)phosphoryl]acetate (**22**)

Phosphinic acid **5** (0.95 g; 2.7 mmol) and 1-bromoadamantane (0.58 g; 2.7 mmol) were refluxed in 20 mL of chloroform. Silver oxide (1.25 g; 5.4 mmol) was added in five equal portions during 1 h, and the reaction mixture was refluxed for an additional 1 h. The solvent was evaporated under reduced pressure, the residue was treated with diethyl ether and filtered through a Celite pad. The filtrate was concentrated in vacuo to give a brown oil, which was purified by flash chromatography on silica gel using elution with a linear gradient of ethanol in chloroform. Yield 1 g (76%). Semisolid. $R_f = 0.39$ (S4). ^{1}H NMR (600 MHz; $CDCl_3$): mixture of two diastereoisomers ~ 1:1, many signals are doubled—those clearly identified are given connected with symbol + in data below: 0.89 (3H, t, $J = 7.2$, CH_3), 1.28 and 1.31 (2H, two m, CH_2), 1.36 and 1.46 (2H, two m, CH_2), 1.56 and 1.91 (2H, two m, CH_2), 1.61 (6H, m, 3× CH_2, (Ada)), 2.06 (6H, m, 3× CH_2 (Ada)), 2.16 (3H, m, 3× CH (Ada)), 2.90 and 3.10 (2H, dd, $J = 14.3$, 19.0 and dd, $J = 14.3$, 15.0, P–CH_2) + 3.00 and 3.03 (2H, dd, $J = 14.3$, 17.8 and dd, $J = 14.3$, 15.8, P–CH_2), 3.70 (3H, s, OCH_3), 4.12 (1H, m, N–CH–P), 5.12 and 5.15 (2H, 2× d, $J = 12.3$) + 5.13 (2H, s, CH_2–O), 4.95 + 5.30 (1H, 2× d, $J = 10.6$, NH), 7.30–7.38 (5H, m, C_6H_5). ^{13}C NMR (150.9 MHz; $CDCl_3$): 13.87 +13.90 (CH_3), 22.23 + 22.29 (CH_2), 27.86 + 27.92 (d, J(C,P) = 11.6, CH_2), 28.29 + 28.42 (2× d, J(C,P) = 2.6 + 3.6, CH_2), 31.16 + 31.17 (3× CH (Ada)), 35.57 + 36.02 (3× CH_2 (Ada)), 36.94 + 37.45 (2× d, J(C,P) = 76.0 + 75.3, P–CH_2), 44.03 + 44.15 (2× d, J(C,P) = 3.6 + 3.5, 3× CH_2 (Ada)), 50.82 + 50.89 (2× d, J(C,P) = 111.4 + 115.2, N–CH–P), 52.48 + 52.50 (OCH_3), 66.98 + 67.13 (CH_2–O), 84.36 + 84.68 (2× d, J(C,P) = 10.4 + 10.4, >C <(Ada)), 127.95–128.51 (5× Ar–CH), 136.22 + 136.39 (Ar–C), 156.08 + 156.27 (2× d, J(C,P) = 6.2 + 7.2, N–CO–O), 166.73 + 167.26 (2× d, J(C,P) = 4.2 + 4.5, –CO–O). IR ($CHCl_3$, ν_{max} cm^{-1}) 2,919, 2,857, 1,723, 1,509, 1,456, 1,277, 1,241, 1,048, 999, 984, 698. HRMS (ESI) calculated for $C_{26}H_{39}NO_6P$ $[M + H]^+$ 492.2510; found: 492.2508.

[((*R,S*)-1-{[(Benzyloxy)carbonyl]amino}pentyl) (adamatyloxy)phosphoryl]acetic acid (**23**)

Diester **22** (0.9 g; 1.8 mmol) was stirred overnight at room temperature with sodium hydroxide (0.17 g; 4.25 mmol) in a mixture of water (5 mL) and methanol (10 mL). The methanol was evaporated under reduced pressure, and then 10 mL of water was added to the residual aqueous phase. The reaction mixture was ice cooled and carefully acidified with 0.01 M HCl (pH about 2). The precipitate was quickly taken up in diethyl ether, the organic layer was washed with water and then dried with Na_2SO_4 before filtering and removing the solvent in vacuo. The product was isolated by flash chromatography on silica gel using elution with a linear gradient of ethanol in chloroform. Yield 0.6 g (88%). White foam. $R_f = 0.32$ (S5). ^{1}H NMR (600 MHz; CD_3OD): mixture of two diastereoisomers ~ 63:37, only signals of the major isomer are given: 0.91 (3H, t, $J = 7.1$, CH_3), 1.30 and 1.46 (2H, two m, CH_2), 1.31 and 1.38 (2H, two m, CH_2), 1.60 and 1.81 (2H, two m, CH_2), 1.65 (6H, m, 3× CH_2 (Ada)), 2.08 (6H, m, 3× CH_2 (Ada)), 2.15 (3H, m, 3× CH (Ada), 2.94 (2H, m, P–CH_2), 3.99 (1H, ddd, $J = 12.0$, 9.0 and 3.0, N–CH–P), 5.17 (2H, s, CH_2–O), 7.30–7.38 (5H, m, C_6H_5). ^{13}C NMR (150.9 MHz; CD_3OD): 14.34 (CH_3), 23.15 (CH_2), 29.00 (d, J(C,P) = 3.1, CH_2), 29.52 (d, J(C,P) = 13.0, CH_2), 32.75 (3× CH (Ada)), 36.68 (3× CH_2 (Ada)), 38.70 (d, J(C,P) = 79.0, P–CH_2), 45.20 (d, J(C,P) = 3.4, 3× CH_2 (Ada)), 52.63 (d, J(C,P) = 110.0, N–CH–P), 68.20 (CH_2–O), 85.52 (d, J(C,P) = 11.2, >C <(Ada)), 128.86 (2× Ar–CH), 129.19 (Ar–CH), 129.54 (2× Ar–CH), 138.01 (Ar–C), 159.00 (d, J(C,P) = 5.1, N–CO–O), 174.25 (COOH). IR (KBr, ν_{max} cm^{-1}) 3,436 br, 2,915, 2,856, 1,718, 1,596, 1,379, 1,202, 1,049, 1,004, 985, 697. HRMS (ESI) calculated for $C_{25}H_{35}NO_6P$ $[M - H]^+$ 476.2207; found: 476.2207.

[((*R,S*)-1-{(9H-Fluoren-9-ylmethoxycarbonyl]amino} pentyl)(adamantyloxy)phosphoryl] acetic acid (**24**)

N-Cbz-protected compound **23** (0.6 g; 1.3 mmol), $NaHCO_3$ (0.5 g; 6.5 mmol) and Fmoc-OSu (0.66 g; 1.95 mmol) were dissolved in 80 mL of methanol, and then 10% Pd/C (0.15 g) was added, and the mixture was allowed to react under an atmosphere of hydrogen (15 psi) with vigorous stirring at room temperature overnight. The catalyst was filtered off through Celite, and the filter pad was washed with 100 mL of methanol. The filtrate was evaporated under reduced pressure, and the residue was taken up with 20 mL of ethyl acetate. The organic layer was washed successively with citric acid and water and dried with Na_2SO_4. The pure product was obtained by flash chromatography on silica gel using elution with a linear gradient of ethanol in chloroform. Yield 0.3 g (42%). White foam. $R_f = 0.20$ (S3). ^{1}H NMR (600 MHz; DMSO): of two diastereoisomers ~ 2:1, only signals of the major isomer are given: 0.85 (3H, t, $J = 7.0$, CH_3), 1.23 and 1.30 (2H, two m, CH_2), 1.22 and 1.34 (2H, two m, CH_2), 1.52 and 1.77 (2H, two m, CH_2), 1.53 (6H, m, 3× CH_2 (Ada)), 1.98 (6H, m, 3× CH_2 (Ada)), 2.07 (3H, m, 3× CH (Ada)), 2.70 (2H, d, J(H,P) = 15.0, P–CH_2), 3.74 (1H, m, N–CH–P), 4.23 (1H, t, $J = 7.2$, CH (Fmoc)), 4.28 (2H, d, $J = 7.2$, CH_2–O (Fmoc)), 7.31 (2H, m, 2× Ar–CH), 7.40 (2H, m, 2× Ar–CH), 7.71 (2H, m, 2× Ar–CH), 7.89 (2H, m, 2× Ar–CH), 8.20 (1H, b, NH). ^{13}C NMR (150.9 MHz; DMSO): 14.25 (CH_3), 22.01 (CH_2), 28.21 (CH_2), 28.36 (d, (C,P) = 12.7, CH_2), 30.89 (3× CH (Ada)), 35.63 (3× CH_2 (Ada)), 39.05 (d, J(C,P) = 65.8, P–CH_2), 43.76 (d, J(C,P) = 3.1, 3× CH_2 (Ada)), 52.06 (d, J(C,P) = 99.1, N–CH–P), 66.13 (CH_2–O), 81.90 (d, J(C,P) = 8.6, >C <(Ada)), 120.47 (2× Ar–CH), 125.70 (2× Ar–CH), 127.38 (2× Ar–CH), 127.98 (2× Ar–CH), 141.04 (2× Ar–C), 144.01 (2× Ar–C), 156.76 (d, J(C,P) = 5.0, N–CO–O), 177.62 (COOH). IR (KBr, ν_{max} cm^{-1}) 3,433 br, 2,914, 2,855, 1,721, 1,610, 1,451, 1,356, 1,051, 1,002, 985, 758, 740. HRMS (ESI) calculated for $C_{32}H_{39}NO_6P$ $[M - H]^+$ 564.2520; found: 564.2522.

Phosphinopeptides **25a** and **25b** (diastereoisomers of **25**)

Compound **21** (47 mg; 0.11 mmol) was added to a mixture of Cys(Trt)-resin (Sieber Amide, Merck Novabiochem, 0.1 mmol) and BOP (89 mg; 0.2 mmol) in DMF (1 mL). The reaction was initiated by the addition of DIPEA (51 mg; 0.4 mmol). The reaction with **21** was allowed to proceed at room temperature overnight and for 8 h under the same conditions. The resin was filtered and washed, and the *N*-terminal Fmoc group was cleaved off with 30% (v/v) piperidine in DMF (1 mL for 5 and 20 min). Fmoc-Tyr(OtBu) (138 mg; 0.3 mmol) was added to the mixture of resin and BOP (0.133 g; 0.3 mmol) in DMF (1 mL). The reaction was initiated by the addition of DIPEA (76 mg; 0.6 mmol). The reaction was allowed to proceed at room temperature for 2 h and for 1 h under the same conditions. The resin was filtered and washed, and the *N*-terminal Fmoc group was cleaved off with 30% (v/v) piperidine in DMF (1 mL for 5 and 20 min). The resin was thoroughly washed with dichloromethane. Then a mixture (2 mL) of TFA (2%), ethanedithiol (2%), water (1%) and triisopropylsilane (2%) in dichloromethane was added for 20 min at room temperature. The resin was filtered and washed, and the filtrate was evaporated to dryness. A mixture (2 mL) of TFA (95%), ethanedithiol (2%), water (1%) and triisopropylsilane (2%) was added to the filtrate for 1 h at room temperature. The reaction mixture was evaporated to dryness. The crude compound was triturated

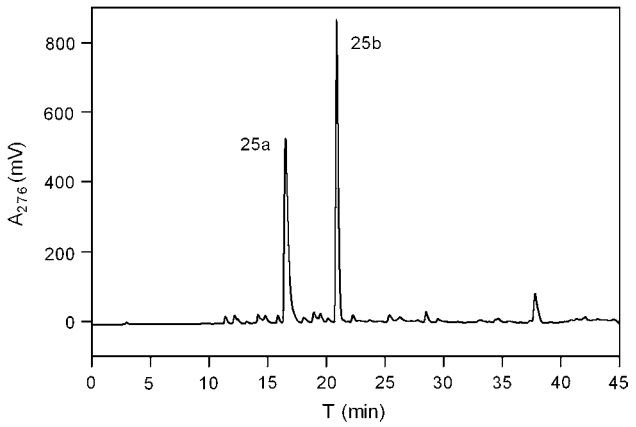


Fig. 1 The representative RP-HPLC chromatogram of the purification of crude compound **25**. Peaks **25a** and **25b** represent the two diastereoisomers of **25**

repeatedly in diethyl ether. The diethyl ether was removed and compounds **25a**, **25b** (diastereoisomers of **25**) were purified with RP-HPLC (G2, see General, and Fig. 1) and lyophilized. Yield (22 mg, 47%, for a mixture of both diastereoisomers, 11 mg for **25a** and 11 mg for **25b**). White powder.

The synthesis of phosphinopeptide **25** starting from compound **24** (62 mg; 0.11 mmol) was done similarly, except that a third additional coupling with **24** was performed overnight. Yield (16 mg, 33%, for a mixture of both diastereoisomers, 9 mg for **25a** and 7 mg for **25b**). White powder.

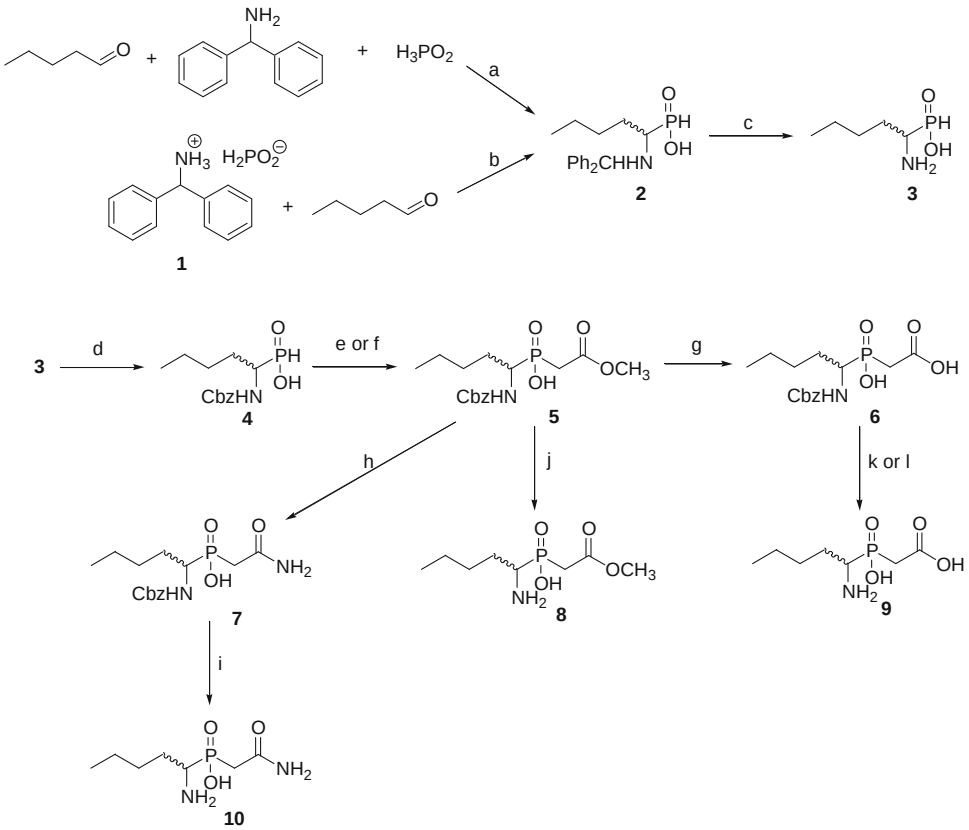
Phosphinopeptide **25a**. ^{1}H NMR (600 MHz; DMSO): 0.87 (3H, t, $J = 7.2$, CH_3), 1.24 and 1.32 (2H, two m, CH_2), 1.24 and 1.41 (2H, two m, CH_2), 1.52 and 1.82 (2H, two m, CH_2), 2.40 (1H, dd, $J = 9.1$ and 7.7, SH), 2.62 and 2.75 (2H, two dd, $J = 16.6$, 13.6 and 16.0, 13.6, P–CH_2), 2.73 (1H, dt, $J = 13.6$, 7.7 and 7.7, S–CHa,Hb–), 2.76 (1H, dd, $J = 14.4$ and 8.8, –CHaHb–), 2.85 (1H, ddd, $J = 13.6$, 9.1 and 4.3, S–CHa,Hb–), 3.06 (1H, dd, $J = 14.4$ and 4.7, –CHaHb–), 4.00 (1H, b, N–CH–CO), 4.13 (1H, m, N–CH–P), 4.31 (1H, td, $J = 8.3$, 7.7 and 4.3, N–CH–CO), 6.71 (2H, m, 2× Ar–CH), 7.11 (2H, m, 2× Ar–CH), 7.22 and 7.76 (2H, b, $CONH_2$), 8.05 (2H, b, NH_2), 8.63 (1H, d, $J = 9.3$, CONH), 9.39 (1H, b, OH). ^{13}C NMR (150.9 MHz; DMSO): 14.03 (CH_3), 22.01 (CH_2), 26.23 (S–CH_2), 27.43 (CH_2), 28.00 (d, J(C,P) = 11.7, CH_2), 36.58 (CH_2), 37.01 (d, J(C,P) = 77.0, P–CH_2), 49.04 (d, J(C,P) = 107.9, P–CH), 53.95 (CH–NH_2), 55.25 (N–CH), 115.64 (2× Ar–CH), 125.08 (Ar–C), 130.76 (2× Ar–CH), 156.82 (Ar–C), 168.69 (d, J(C,P) = 4.6, NH–CO), 171.95 ($CONH_2$). IR (KBr, ν_{max} cm^{-1}) 3,405, 3,287, 1,674, 1,518, 1,203, 1,142. HRMS (ESI) calculated for $C_{19}H_{32}N_4O_6PS$ $[M + H]^+$ 475.1775; found: 475.1773.

Phosphinopeptide **25b**. ^{1}H NMR (600 MHz; DMSO): 0.81 (3H, t, $J = 7.2$, CH_3), 0.99 and 1.07 (2H, two m, CH_2), 1.15 and 1.22 (2H, two m, CH_2), 1.40 and 1.71 (2H, two m, CH_2), 2.45 (1H, dd, $J = 9.7$ and 7.5, SH), 2.74 (1H, dt, $J = 13.5$, 7.5 and 7.5, S–CHa,Hb–), 2.82 (2H, m, P–CH_2), 2.83 (1H, dd, $J = 13.8$ and 6.5, –CHa**Hb**–), 2.84 (1H, dd, $J = 13.5$, 9.7 and 4.4, S –CHaHb–), 2.97 (1H, ddd, $J = 13.6$, 9.1 and 4.3, –CHa,Hb–), 3.99 (1H, b, N–CH–CO), 4.07 (1H, m, N–CH–P), 4.32 (1H, td, $J = 8.2$, 7.5 and 4.4, N–CH–CO), 6.70 (2H, m, 2× Ar–CH), 7.08 (2H, m, 2× Ar–CH), 7.22 and 7.62 (2H, b, $CONH_2$), 8.14 (2H, b, NH_2), 8.56 (1H, d, $J = 9.4$, CONH), 9.38 (1H, b, OH). ^{13}C NMR (150.9 MHz; DMSO): 13.90 (CH_3), 21.77 (CH_2), 26.27 (S–CH_2), 26.84 (CH_2), 27.66 (d, J(C,P) = 11.4, CH_2), 36.50 (CH_2), 37.00 (d, J(C,P) = 77.0, P–CH_2), 48.53 (d, J(C,P) = 107.0, P–CH), 54.16 (CH–NH_2), 55.30 (N–CH), 115.50 (2× Ar–CH), 124.95 (Ar–C), 130.64 (2× Ar–CH), 156.82 (Ar–C), 168.52 (d, J(C,P) = 4.6, NH–CO), 171.82 ($CONH_2$). IR (KBr, ν_{max} cm^{-1}) 3,384, 1,675, 1,518, 1,203, 1,142. HRMS (ESI) calculated for $C_{19}H_{32}N_4O_6PS$ $[M + H]^+$ 475.1775; found: 475.1771.

Results and discussion

First, we focused on the preparation of the important intermediate **4** (Scheme 2), which was used as a starting material for all syntheses. For the key synthetic step, the formation of the P–C bond, we employed the method of (Allen et al. (1989) for the introduction of the methyl carboxymethyl moiety to **4** through a trivalent phosphonous ester.

Thus, norleucine-derived phosphinic acid **2** was prepared by a method described in literature (Baylis et al. 1984; Dingwall et al. 1977) involving condensation of valeraldehyde, benzhydrylamine and anhydrous phosphonous acid (Fitch 1964) in dioxane (Scheme 2, a). In a parallel attempt to prepare **2**, we heated benzhydrylamine hypophosphite **1** with valeraldehyde under the same reaction conditions to obtain the product with a similar yield (Scheme 2, b). Cleavage of the diphenylmethyl group was achieved by refluxing **2** with concentrated aqueous hydrobromic acid to yield compound **3** (as a zwitterion), which was then converted to Cbz protected phosphinic acid **4** by a reaction with benzyl chloroformate (Scheme 2, c, d). Silylation of **4** with trimethylsilyl chloride or *N*,*O*-bis(trimethylsilyl)acetamide afforded the bis(trimethylsilyl)phosphinate of **4** that was converted to **5** by an Arbuzov reaction (Allen et al. 1989) with methyl bromoacetate and standard acidic workup (Scheme 2, e, f). α-Carboxyphosphinic acid **5** was then transformed to intermediates **6** or **7** by alkaline hydrolysis (Scheme 2, g) or by aminolysis of the carboxylic ester function (Scheme 2, h). Catalytic hydrogenolysis of compounds **5, 6** and **7** furnished desired products **8**, **9** and **10**, respectively (Scheme 2, i–l).

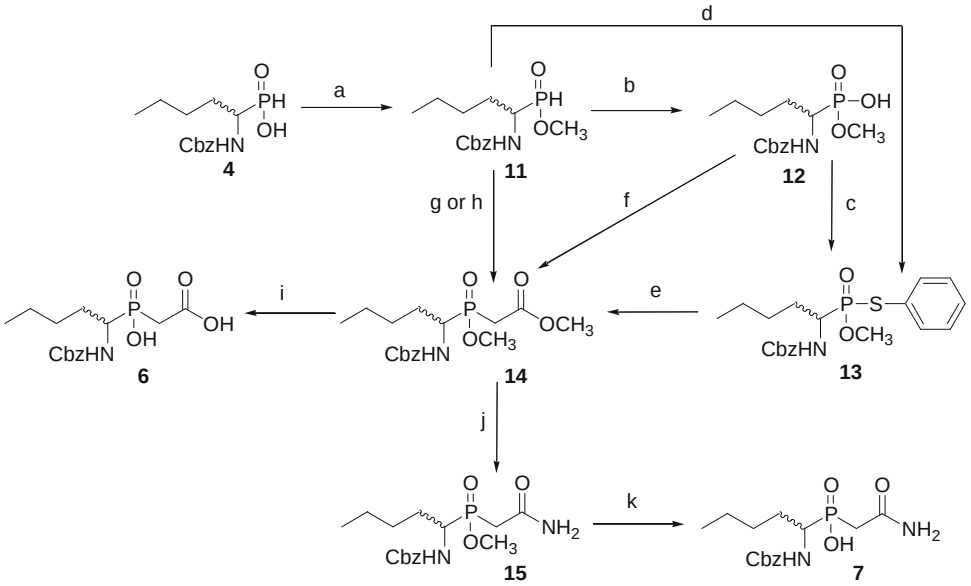


Scheme 2 Reagents, conditions, yields: (*a*), (*b*) reflux, dioxane, 1 h, 58% for (*a*), 51% for (*b*); (*c*) 48% HBr, reflux, 6 h then epoxypropane, ethanol (69%); (*d*) benzyl chloroformate, Na_2CO_3, water and dioxane, 0°C, 2 h then at room temperature overnight (81%); (*e*) TMSCl, TEA, THF, 0°C, 1 h then methyl bromoacetate at room temperature overnight (79%); (*f*) BSA, methyl bromoacetate, dichloromethane, room temperature, overnight (76%); (*g*) NaOH, methanol and water, room temperature, overnight (85%); (*h*) NH_3, methanol, room temperature, 2 days (66%); (*i*), (*j*), (*k*) 10% Pd/C, H_2 (15 psi), methanol, room temperature, overnight, for **8** (44%), for **9** (48%), for **10** (66%); (*l*) 33% HBr/AcOH, room temperature, overnight then epoxypropane, ethanol (82%)

It was found that the reaction of a methyl ester of a phophonous acid with activated bromo derivatives in the presence of TMSCl and TEA (Casarez et al. 2008; Malachowski and Coward 1994; Thottathil et al. 1984) or NaH (Orain and Mattes 2006; Patel et al. 1995) yielded the corresponding α-carboxyphosphinates. We investigated these two methods (Scheme 3, g, h) for the conversion of *N*-protected aminophosphinate **11** (prepared by esterification of **4** by DIC in methanol) to compound **14**. However, despite our best efforts, we detected only trace amounts of **14** in both reaction mixtures. Therefore, we decided to prepare this compound by a method (iii) (see "Introduction") using a mixed ester of phosphonate or phosphonochloridate. Oxidation of **11** with sodium periodate (Scheme 3, b) proceeded smoothly in a high yield in agreement with literature (Dumy et al. 1992) Treatment of monomethylester **12** with thionyl chloride yielded a phosphonochloridate, which after addition of thiophenol afforded mixed phosphonic ester **13** (Scheme 3, c). We also successfully investigated a one-step method for the preparation of **13** (Scheme 3, d) involving reaction of **11** with carbon tetrachloride, TEA and thiophenol under Atherton–Todd reaction conditions (Sampson and Bartlett 1988).

The dimethylester of α-carboxyphosphinic acid (**14**) was prepared by reaction of in situ generated lithiomethylacetate either with the phosphonochloridate (Georgiadis et al. 2001a) of **12** (Scheme 3, f) or with mixed ester **13** (Scheme 3, e) (Bartlett and Kezer 1984) The use of the phosphonochloridate appears to be the most convenient with regard to higher cumulative yield of the two steps (Scheme 3, b and f). After standard alkaline hydrolysis (Scheme 3, i) of both methyl ester groups of **14**, we obtained compound **6**, which is the precursor for preparation of **9**. For the preparation of amide **15**, as in the case of compound **10** (Scheme 2), we took advantage of the different sensitivities of the methyl esters of the phosphinic and carboxylic acids in **14** to ammonia. Amide **15** was then treated with sodium hydroxide to afford intermediate **7**. The disadvantage of dimethylester protection is that the universal precursor **5** (Scheme 2) cannot be prepared by alkaline hydrolysis of **14** due to the low chemoselectivity for phosphinic versus carboxylic methyl ester groups.

As a next step, to improve the procedures for the preparation of target compounds **8**, **9** and **10**, we decided to modify and employ our previously reported methodology of benzyl protection used for the synthesis of phosphonodepsipeptides (Picha et al. 2008, 2009) Masking of the phosphinic acid moiety by a benzyl group and the presence of an *N*-Cbz group in the molecules of precursors enable the removal of both protecting groups in one step under mild conditions, without use of any acids or bases, and also facilitate the purification due to the lipophilic character of the protected compound.



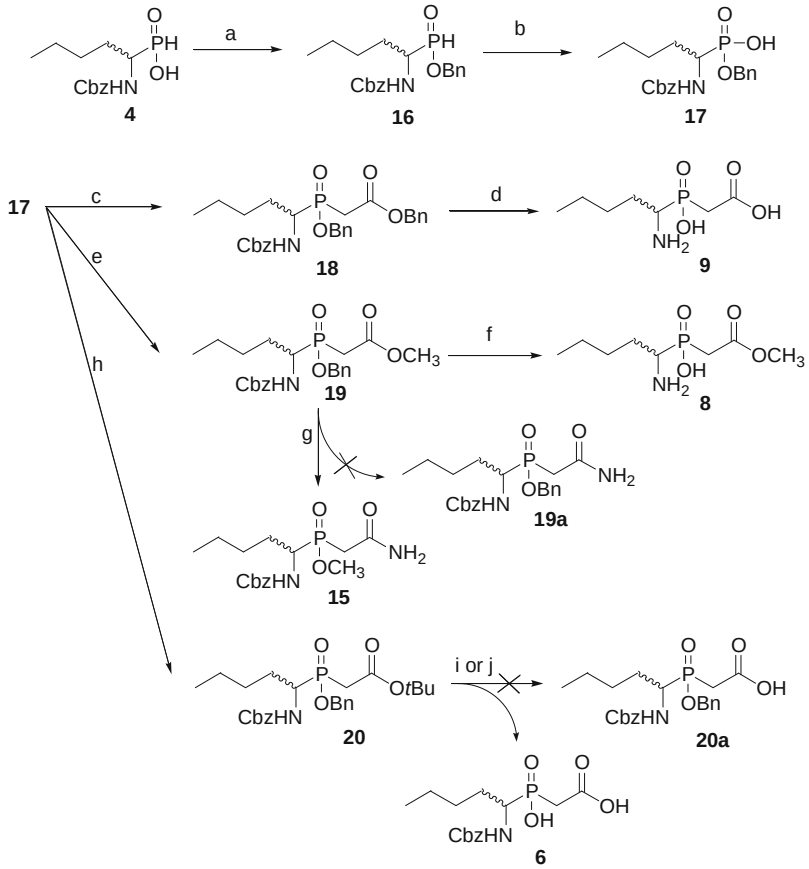
Scheme 3 (*a*) DIC, methanol, THF, 0°C, 1 h then at room temperature overnight (63%); (*b*) $NaIO_4$, dioxane and water, room temperature, overnight (84%); (*c*) $SOCl_2$, dichloromethane, room temperature, 2 h then thiophenol, TEA, 0°C, 1 h, then room temperature overnight (53%); (*d*) thiophenol, TEA, tetrachloromethane, room temperature overnight (80%); (*e*) LDA, methyl acetate, THF, −78°C, 1 h (41%); (*f*) $SOCl_2$, dichloromethane, room temperature, 2 h, then LDA, methyl acetate, THF, −78°C, 1 h (60%); (*g*) TMSCl, TEA, dichloromethane, 0°C, 1 h then methyl bromoacetate at room temperature overnight; (*h*) NaH, THF, room temperature, 2 h then methyl bromoacetate, room temperature, overnight; (*i*) NaOH, methanol and water, room temperature, overnight (88%); (*j*) NH_3, methanol, room temperature, 2 days (73%); (*k*) NaOH, methanol and water, room temperature, overnight (76%)

Esterification of **4** with benzyl alcohol afforded ester **16,** which was oxidized with $NaIO_4$ to furnish **17** (Scheme 4, a, b). Condensation of the phosphinochloridate of **17** with benzyl acetate, methyl acetate or *tert*-butyl acetate in the presence of LDA afforded the respective protected phosphinates **18**, **19** and **20** (Scheme 4, c, e, h). As expected, hydrogenolysis of **18** and **19** afforded target compounds **9** and **8**, respectively.

In the next step, we attempted to prepare carboxyamide derivative **19a** (Scheme 4). The direct reaction of acetamide with the phosphinochloridate of **17** is unfeasible; therefore, we intended to bypass this problem by aminolysis of **19**. However, the reaction of **19** with ammonia in dried methanol furnished phosphinic acid methylester **15** in a high yield of 90% (Scheme 4, g). A similar phenomenon has already been described by Li and Eakin (1955), who observed the transformation of a dibenzylphosphoryl ester under the same conditions to the corresponding dimethyl ester. Neither the use of dioxane instead of methanol nor reacting at room temperature or higher led to the desired product.

Petrillo described in a patent (Petrillo EW Jr 1982) an innovative method starting from dichloromethylphosphine for the synthesis of **20a** (Scheme 4), an excellent synthon for the preparation of some antihypertensive agents. In this study, we intended to prepare the same compound, which could be a suitable precursor for the synthesis of α-carboxyphosphinopeptides elongated at the C-terminus by different amino acids, taking advantage of the protection of the phosphoryl moiety by a benzyl ester group. Recently, we used a similar approach for the efficient synthesis of phosphonodepsipeptides (Picha et al. 2008, 2009). We chose compound **20** as a precursor for the synthesis of **20a** to avoid handling toxic organometallic reagents (Petrillo EW Jr 1982) during preparation of dichloromethylphosphine. If the phosphinic benzyl ester moiety of **20** remains stable under acidic conditions, Bartlett's methodology (Bartlett and Kezer 1984) ("Introduction") could be used for the synthesis of **20a**. However, the treatment of **20** with a mixture of TFA/dichloromethane/water or with pure TFA (Scheme 4, i or j) always resulted not only in the cleavage of the *tert*-butyl group, but also in the simultaneous cleavage of the benzyl group to form the product **6**. Taking into account the study of Abbas and Cook (1977) on the stability of benzyl diethyl phosphinates in sulphuric acid, we can assume that acid-catalysed hydrolysis of benzyl phosphinate **20** proceeds likely by a unimolecular mechanism ($A_{a1}1$) with cleavage of the C–O bond.

Finally, we prepared two synthons, **21** and **24**, tailored for the solid-phase synthesis of α-carboxyphosphinopeptides using the *N*-Fmoc strategy (Fields and Noble 1990), as outlined in Scheme 5. Reaction of **9** with Fmoc-Cl resulted in one-step in synthon **21** (Scheme 5, d). Unfortunately, the presence of the lipophilic Fmoc group and the



Scheme 4 (*a*) DIC, benzyl alcohol, 0°C, THF, 1 h then at room temperature overnight (84%); (*b*) $NaIO_4$, dioxane and water, room temperature, overnight (90%); (*c*) $SOCl_2$, dichloromethane, room temperature, 2 h, then LDA, benzyl acetate, THF, −78°C, 1 h (56%); (*d*) 10% Pd/C, H_2 (15 psi), methanol, room temperature, overnight (70%); (*e*) $SOCl_2$, dichloromethane, room temperature, 2 h, then LDA, methyl acetate, THF, −78°C, 1 h (65%); (*f*) 10% Pd/C, H_2 (15 psi), methanol, room temperature (60%); (*g*) NH_3, methanol, room temperature, 7 days (90%); (*h*) $SOCl_2$, dichloromethane, room temperature, 2 h, then LDA, *tert*-butyl acetate, THF, −78°C, 1 h (52%); (*i*) 33% (v/v) TFA, 1% (v/v) water, 66% (v/v) dichloromethane, room temperature, overnight; (*j*) TFA, room temperature, 1 h

Scheme 5 Reagents, conditions, yields: (*a*) AdBr, Ag_2O, reflux, $CHCl_3$, 2 h (76%); (*b*) NaOH, methanol/water, room temperature, overnight (88%); (*c*) 10% Pd/C, H_2 (15 psi), Fmoc-OSu, $NaHCO_3$, methanol, room temperature, overnight (42%); (*d*) Fmoc-Cl, Na_2CO_3, water/dioxane, room temperature, overnight (36%); (*e*), (i) Cys(Trt)-resin Sieber Amide resin (0.9 equiv.), BOP (1.8 equiv.)/DIPEA (3.6 equiv.) in DMF, room temperature overnight and 8 h, (ii) piperidine/DMF, (iii) Fmoc-Tyr(O*t*Bu) (2.7 equiv.), BOP (2.7 equiv.)/DIPEA (5.5 equiv.), room temperature, 2 and 1 h, (iv) piperidine/DMF, (v) TFA (2%), ethanedithiol (2%), water (1%), triisopropylsilane (2%) in dichloromethane, room temperature for 20 min, evaporation of the filtrate to dryness, (vi) TFA (95%), ethanedithiol (2%), water (1%) and triisopropylsilane (2%), room temperature for 1 h, evaporation of the filtrate to dryness and RP-HPLC, yield 47% for a mixture of of diastereoisomers **25a** and **25b**; (*f*) similar as (*e*) except that a third additional coupling with **24** was performed overnight, yield 33%

polar P(O)-OH moiety in **21** makes difficult standard purification by flash chromatography on silica. Therefore, we decided to purify compound **21** using RP-HPLC. This procedure resulted in highly pure compound **21**, but with a rather low yield of 36%.

Taking into account the difficulties with isolation of **21**, we decided to overcome this problem by protection of the phosphoryl moiety by an adamantyl ester. This strategy was developed for β-carboxyphosphinates by (Yiotakis et al. (1996). The introduction of the adamantyl group was easily achieved by refluxing of phosphinic acid **5** with adamantyl bromide and silver oxide in chloroform (Scheme 5, a). The methyl ester of the resulting compound **22** was saponified with sodium hydroxide followed by careful adjustment of pH by dilute hydrochloric acid (Scheme 5, b). The compound **24** was prepared by a one-pot reaction involving removal of the Cbz group from **23** by catalytic hydrogenation overnight with simultaneous in situ protection of the generated free amino group by Fmoc-*O*Su (Scheme 5, c). The advantage of the simultaneous hydrogenation/protection reaction is that it eliminates the necessity of the difficult isolation of the *N*-deprotected adamatylated synthon (Yiotakis et al. 1996).

Synthons **21** and **24** were used for the solid-phase synthesis of model pseudopeptide **25** (Scheme 5, e, f). The use of BOP as a coupling reagent enables the utilization of synthon **21** with a non-protected phosphoryl moiety (Raguin et al. 2005). Interestingly, the use of Ad-protected synthon **24** resulted in a lower yield of **25** (33%) compared to the use of non-protected synthon **21** (47%). The reason may be the steric hindrance of the bulky adamantyl group, the effect of which is more pronounced in the case of α-carboxyphosphinates (our case) than of the β-carboxyphosphinates developed previously (Yiotakis et al. 1996). However, if we compare the cumulative yields of pseudopeptide **25** starting from **5**, the results for both synthons are comparable: 12% for **21** and 9% for **24**. Using RP-HPLC we succeeded in separating both diastereoisomers of pseudopeptide **25** (Fig. 1, compounds **25a** and **25b**). Both compounds **25a** and **25b** were characterized (see Experimental), but despite some subtle differences in the NMR spectra, we were not able to assign the absolute configuration.

Conclusions

Herein, we present the synthesis of a relatively rare class of phosphorus compounds, α-carboxyphosphinopeptides. These compounds may represent an interesting alternative to more frequently used β-carboxyphosphinopeptides, which have found important applications as transition state-mimicking inhibitors for different enzymes. For the preparation of key α-carboxyphosphinate precursors, we investigated and utilized methods (i) and (iii) as mentioned in "Introduction". The latter method was modified and improved by the introduction of benzyl protection of the phosphinyl moiety. We found that intermediate **5** is a convenient precursor for the preparation of norleucine-derived target compounds **8**, **9** and **10**, which may represent potential inhibitors of methionine or leucine aminopeptidases. The synthesis of *N*-Fmoc protected synthons **21** and **24** and their successful application in the solid-phase synthesis of pseudopeptide **25** also opens the possibility of the preparation of a large series of α-carboxyphosphinopeptides in the form of combinatorial libraries.

Acknowledgments This project was supported by a grant from the Grant Agency of the Czech Republic (203/06/1405, to J.J.), by the Chemical Genetics Consortium of the Ministry of Education, Youth and Sports of the Czech Republic (LC060777, to J.J.) and by the Research Project Z4 055 0506 of the Academy of Sciences of the Czech Republic (to IOCB).

References

Abbas KA, Cook RD (1977) Acid-catalyzed hydrolysis of phosphinatesIII. The mechanism of hydrolysis of methyl and benzyl dialkylphosphinates. Can J Chem 55:3740–3750

Allen MC, Fuhrer W, Tuck B, Wade R, Wood JM (1989) Renin inhibitors—synthesis of transition-state analog inhibitors containing phosphorus-acid derivatives at the scissile bond. J Med Chem 32:1652–1661

Bailey D, Cooper JB (1994) A structural comparison of 21-inhibitor complexes of the aspartic proteinase from *Endothia parasitica*. Protein Sci 3:2129–2143

Bartlett PA, Kezer WB (1984) Phosphinic acid dipeptide analogs—potent, slow-binding inhibitors of aspartic peptidases. J Am Chem Soc 106:4282–4283

Bartlett PA, Hanson JE, Giannousis PP (1990) Potent inhibition of pepsin and penicillopepsin by phosphorus-containing peptide analogs. J Org Chem 55:6268–6274

Baylis EK, Campbell CD, Dingwall JG (1984) 1-Aminoalkylphosphonous acids. 1. Isosteres of the protein amino-acids. J Chem Soc Perkin Trans 1:2845–2853

Beveridge AJ, Dealwis C, Cooper JB (1995) A theoretical-study of the active-site complexes formed between endothiapepsin and three potent inhibitors—pepstatin-A, and peptide analogs containing difluorostatone and phosphostatine—implications for inhibitor design. Theochem J Mol Struct 333:87–97

Boger JS, Bock MG (1985) Renin inhibitors containing peptide isosteres. European patent application 85103367.0 (0156322A2)

Boger JS, Bock MG, Freidinger R, Veber DF, Patchett AA, Greenlee WJ, Parsons WH (1986) Peptide enzyme inhibitors. European patent application B6110125.1 (0 209 897 A2)

Casarez A, Chaudhary K, Cho A, Clarke M, Doerffler E, Fardis M, Kim CU, Pyun H, Sheng XC, Wang J (2008) Antiviral phosphinate compounds. International patent application PCT/US2007/015664 (WO 2008/005565 A2)

Coates L, Erskine PT, Crump MP, Wood SP, Cooper JB (2002) Five atomic resolution structures of endothiapepsin inhibitor complexes: implications for the aspartic proteinase mechanism. J Mol Biol 318:1405–1415

Collinsova M, Jiracek J (2000) Phosphinic acid compounds in biochemistry, biology and medicine. Curr Med Chem 7:629–647

Collinsova M, Castro C, Garrow TA, Yiotakis A, Dive V, Jiracek J (2003) Combining combinatorial chemistry and affinity chromatography: highly selective inhibitors of human betaine: homocysteine *S*-methyltransferase. Chem Biol 10:113–122

Dingwall JG, Baylis EK, Campbell CD (1977) Alpha aminophosphonous acid for inhibiting bacteria and yeast. United States patent 799,428 (4,147,780)

Dive V, Cotton J, Yiotakis A, Michaud A, Vassiliou S, Jiracek J, Vazeux G, Chauvet MT, Cuniasse P, Corvol P (1999) RXP 407, a phosphinic peptide, is a potent inhibitor of angiotensin I converting enzyme able to differentiate between its two active sites. Proc Natl Acad Sci USA 96:4330–4335

Dive V, Georgiadis D, Matziari M, Makaritis A, Beau F, Cuniasse P, Yiotakis A (2004) Phosphinic peptides as zinc metalloproteinase inhibitors. Cell Mol Life Sci 61:2010–2019

Dreyer GB, Metcalf BW, Tomaszek TA, Carr TJ, Chandler AC, Hyland L, Fakhoury SA, Magaard VW, Moore ML, Strickler JE, Debouck C, Meek TD (1989) Inhibition of human immunodeficiency virus-1 protease invitro—rational design of substrate-analog inhibitors. Proc Natl Acad Sci USA 86:9752–9756

Dumy P, Escale R, Girard JP, Parello J, Vidal JP (1992) A convenient synthetic approach to new alpha-(9-fluorenylmethoxycarbonylamino)alkylphosphonic acid-derivatives. Synthesis, pp 1226–1228

Fields GB, Noble RL (1990) Solid-phase peptide-synthesis utilizing 9-fluorenylmethoxycarbonyl amino-acids. Int J Pept Prot Res 35:161–214

Fitch SJ (1964) Synthesis of hypophosphite esters from orthocarbonyl compounds. J Am Chem Soc 86:61–64

Fraser ME, Strynadka NCJ, Bartlett PA, Hanson JE, James MNG (1992) Crystallographic analysis of transition-state mimics bound to penicillopepsin—phosphorus-containing peptide analogs. Biochemistry 31:5201–5214

Georgiadis D, Dive V, Yiotakis A (2001a) Synthesis and comparative study on the reactivity of peptidyl-type phosphinic esters: intramolecular effects in the alkaline and acidic cleavage of methyl beta-carboxyphosphinates. J Org Chem 66:6604–6610

Georgiadis D, Matziari M, Yiotakis A (2001b) A highly efficient method for the preparation of phosphinic pseudodipeptidic blocks suitably protected for solid-phase peptide synthesis. Tetrahedron 57:3471–3478

Jensen CE, Bondebjerg J, Naerum L (2005) HIV protease inhibitors. International patent application PCT/DK2005/000296 (WO 2005/105843 A2)

Jiracek J, Yiotakis A, Vincent B, Lecoq A, Nicolaou A, Checler F, Dive V (1995) Development of highly potent and selective phosphinic peptide inhibitors of zinc endopeptidase—24–15 using combinatorial chemistry. J Biol Chem 270:21701–21706

Jiracek J, Yiotakis A, Vincent B, Checler F, Dive V (1996) Development of the first potent and selective inhibitor of the zinc endopeptidase neurolysin using a systematic approach based on combinatorial chemistry of phosphinic peptides. J Biol Chem 271:19606–19611

Kafarski P, Lejczak B (2000a) Synthesis of phosphono- and phosphinopeptides. In: Kukhar VP, Hudson HR (eds) Aminophosphinic and aminophosphonic acids. Chemistry and biological activity. Wiley, Chichester, pp 173–203

Kafarski P, Lejczak B (2000b) The biological activity of phosphono- and phosphinopeptides. In: Kukhar VP, Hudson HR (eds) Aminophosphinic and aminophosphonic acids. Chemistry and biological activity. Wiley, Chichester, pp 407–442

Li SO, Eakin RE (1955) Synthesis of *N*-phosphorylated derivatives of amino acids. J Am Chem Soc 77:1866–1870

Liboska R, Picha J, Hanclova I, Budesinsky M, Sanda M, Jiracek J (2008) Synthesis of methionine- and norleucine-derived phosphinopeptides. Tetrahedron Lett 49:5629–5631

Lunney EA, Hamilton HW, Hodges JC, Kaltenbronn JS, Repine JT, Badasso M, Cooper JB, Dealwis C, Wallace BA, Lowther WT, Dunn BM, Humblet C (1993) Analyses of ligand-binding in five endothiapepsin crystal complexes and their use in the design and evaluation of novel renin inhibitors. J Med Chem 36:3809–3820

Malachowski WP, Coward JK (1994) The chemistry of phosphapeptides—investigations on the synthesis of phosphonamidate, phosphonate, and phosphinate analogs of glutamyl-gamma-glutamate. J Org Chem 59:7625–7634

Matsueda R, Yabe Y, Yamazaki M, Kokobu T, Hiwada T (1984) New renin-inhibitory peptides and their use. European patent application 85300738.3 (0152255 A2)

Morita Y, Hoshide Y, Taniguchi M, Ando R, Takashima J, Parsons WH (1987) 1-Aminoethylphosphinic acid derivative. Japanese patent application 62–166939 (64-013096)

Orain D, Mattes H (2006) Synthesis of imidazole phosphinic acids as I4AA analogues. Tetrahedron Lett 47:1253–1255

Patchett AA, Greenlee WJ, Parsons WH (1985) Phosphorous containing enzyme inhibitors. European patent application 86109764.0 (0210545A2)

Patel DV, Gordon EM, Schmidt RJ, Weller HN, Young MG, Zahler R, Barbacid M, Carboni JM, Gullobrown JL, Hunihan L, Ricca C, Robinson S, Seizinger BR, Tuomari AV, Manne V (1995) Phosphinyl acid-based bisubstrate analog inhibitors of Ras farnesyl-protein transferase. J Med Chem 38:435–442

Petrillo EW Jr (1982) Amino and substituted amino phosphinyl–alkanoyl compositions. European patent application 82301923.7 (0 063896 A1)

Phillips MA, Kaplan AP, Rutter WJ, Bartlett PA (1992) Transition-state characterization—a new approach combining inhibitor analogs and variation in enzyme structure. Biochemistry 31:959–963

Picha J, Budesinsky M, Sanda M, Jiracek J (2008) Synthesis of norleucine-derived phosphonopeptides. Tetrahedron Lett 49:4366–4368

Picha J, Budesinsky M, Hanclova I, Sanda M, Fiedler P, Vanek V, Jiracek J (2009) Efficient synthesis of phos phonodepsipeptides derived from norleucine. Tetrahedron 65:6090–6103

Raguin O, Fournie-Zaluski MC, Romieu A, Pelegrin A, Chatelet F, Pelaprat D, Barbet J, Roques BP, Gruaz-Guyon A (2005) A labeled neutral endopeptidase inhibitor as a potential tool for tumor diagnosis and prognosis. Angew Chem Int Ed 44:4058–4061

Sampson NS, Bartlett PA (1988) Synthesis of phosphonic acid-derivatives by oxidative activation of phosphinate esters. J Org Chem 53:4500–4503

Schramm VL, Horenstein BA, Kline PC (1994) Transition state analysis and inhibitor design for enzymatic reactions. J Biol Chem 269:18259–18262

Shiosaki K, Tasker AS, Opgenorth TJ (1991) Endothelin converting enzyme inhibitors. International patent application PCT/US91/08364 (WO92/13545)

Thottathil JK, Przybyla CA, Moniot JL (1984) Mild arbuzov reactions of phosphonous acids. Tetrahedron Lett 25:4737–4740

Vidossich P, Carloni P (2006) Binding of phosphinate and phosphonate inhibitors to aspartic proteases: a first-principles study. J Phys Chem B 110:1437–1442

Wolfenden R, Radzicka A (1991) Transition-state analogues. Curr Opin Struct Biol 1:780–787

Yiotakis A, Vassiliou S, Jiracek J, Dive V (1996) Protection of the hydroxyphosphinyl function of phosphinic dipeptides by adamantyl Application to the solid-phase synthesis of phosphinic peptides. J Org Chem 61:6601–6605

Yiotakis A, Georgiadis D, Matziari M, Makaritis A, Dive V (2004) Phosphinic peptides: synthetic approaches and biochemical evaluation as Zn-metalloprotease inhibitors. Curr Org Chem 8:1135–1158