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Efficient synthesis of phosphonodepsipeptides derived from norleucine

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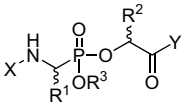
ABSTRACT

In the present work, we describe in detail an efficient solution synthesis of norleucine-derived phosphonopeptides mimicking the peptide sequences Nle-Gly(Ala) and Nle-Gly(Ala)-Val. The most efficient strategy involved use of the benzyl group. The synthesis was achieved through BOP-catalysed coupling of the monobenzyl ester of the *N*-Cbz-protected phosphonate derivative of norleucine with the hydroxyl moieties of derivatised L-lactic or glycolic acid. Subsequently, complete deprotection of the products was achieved in good yields by one-step Pd-catalysed hydrogenolysis. We also prepared the Fmoc-Nle-Ψ[PO(OH)O]-CH_2-COOH synthon and demonstrated that this precursor is a suitable building block for the solid-phase synthesis of cysteine-containing phosphonopeptides.

1. Introduction

Phosphinopeptides[1,2] and phosphonamidopeptides[3] belong to the family of pseudopeptidic compounds, in which one peptide bond is substituted by a non-hydrolysable phosphinate[4] –PO(OH)–CH_2– or phosphonamidate[3] –PO(OH)–NH– group. Phosphonopeptides,[3,5] also called depsiphosphonopeptides,[6] bearing the motif –PO(OH)–O–, are another type of mimic in which a phosphonate ester replaces the peptide bond. The resemblance of these phosphorus-containing moieties to the structure of the peptide bond during enzyme-catalysed hydrolysis enables effective mimicry of the structure of the peptidic substrate in the transition state[7–9] (TS). Thus, these compounds may be potent inhibitors of peptidases.[4] Phosphonopeptides were found to be efficacious inhibitors of leucine aminopeptidase,[10] pepsin,[11,12] carboxypeptidase A[9,13–15] and B,[16] chymotrypsin,[17] serine proteases,[18] thermolysin[19] and penicillopepsin.[20–22] Recently, it was shown that vancomycin-resistant bacteria are able to produce D-Ala-D-lactate dipeptide instead of D-Ala-D-Ala dipeptide. D-Ala-D-lactate dipeptide binds much more weakly to vancomycin, and can be incorporated into the peptidoglycan layer. This resulted in the synthesis of new phosphonate dipeptides for use as inhibitors of VanX D,D-dipeptidase.[6,23–26] The preparations of some phosphonate compounds designed as potential antihypertensive agents are protected by patents.[27–31]

The most common strategy to prepare depsiphosphonopeptides of Structure I (see below) consists of two crucial steps: (i) elongation of the C- and/or N-terminus of mixed esters, followed by (ii) selective cleavage of the protecting group R^3.[11,12]



R^1, R^2 = alkyl, aryl
R^3 = methyl, benzyl
X, Y = amino acids

The synthesis of the above-mentioned mixed diesters can be accomplished by the condensation of an *N*-protected phosphonate monoester with the hydroxyl moiety of a derivative of a glycolic, lactic or mandelic acid using coupling agents such as DIAD/PPh_3,[31–34] BOP or PyBOP.[35,36] The same products have also been prepared from phosphonochloridates by treatment of monoesters with thionyl chloride[10–12,14,23,24] or by oxidative chlorination with carbon tetrachloride.[11,18] Next, the demethylation of unsymmetrical diesters using TMSBr,[12,20,22,31,32,34,35] LiSPr,[10,23,24] LiOH[14,19] or by hydrogenolysis of the protecting benzyl group[6,29] led to the desired phosphonic acid monoesters. Alternatively, direct monoesterification starting from phosphonic acid using thionyl chloride,[16,25,37] BroP, TPyClU[38] or DCC[27,30] has also been reported. Similar results were obtained by the esterification of the corresponding phosphorous acid with DCC followed by oxidation with sodium periodate.[28,39,40] A new approach to the one-pot preparation of depsiphosphonopeptides involves a multicomponent condensation of an aldehyde, benzylcarbamate and carboethoxyalkyl phosphorodichloridate.[41] However, this method

Abbreviations: BOP, (benzotriazol-1-yloxy)tris(dimethylamino)phosphonium hexafluorophosphate; BroP, bromotris(dimethylamino)phosphonium hexafluorophosphate; Cbz, benzyloxycarbonyl; DABCO, 1,4-diazabicyclo[2.2.0]octane; DBU, 1,8-diazabicyclo[5.4.0]undec-7-ene; DCC, *N,Ñ*-dicyclohexylcarbodiimide; DIAD, diisopropyl azodicarboxylate; DIC, *N,N′*-diisopropylcarbodiimide; DIPEA, *N,N*-diisopropylethylamine; Fmoc, 9-fluorenylmethyloxycarbonyl; HBTA, 1-hydroxybenzotriazole; PyBOP, (benzotriazol-1-yloxy)tripyrrolidinophosphonium hexafluorophosphate; TEA, triethylamine; TFA, trifluoroacetic acid; TMSBr, trimethylsilyl bromide; TPyClU, 1,1,3,3-bis(tetramethylene)chlorouronium tetrafluoroborate; TrisCl, 2,4,6-triisopropylbenzenesulfonyl chloride.

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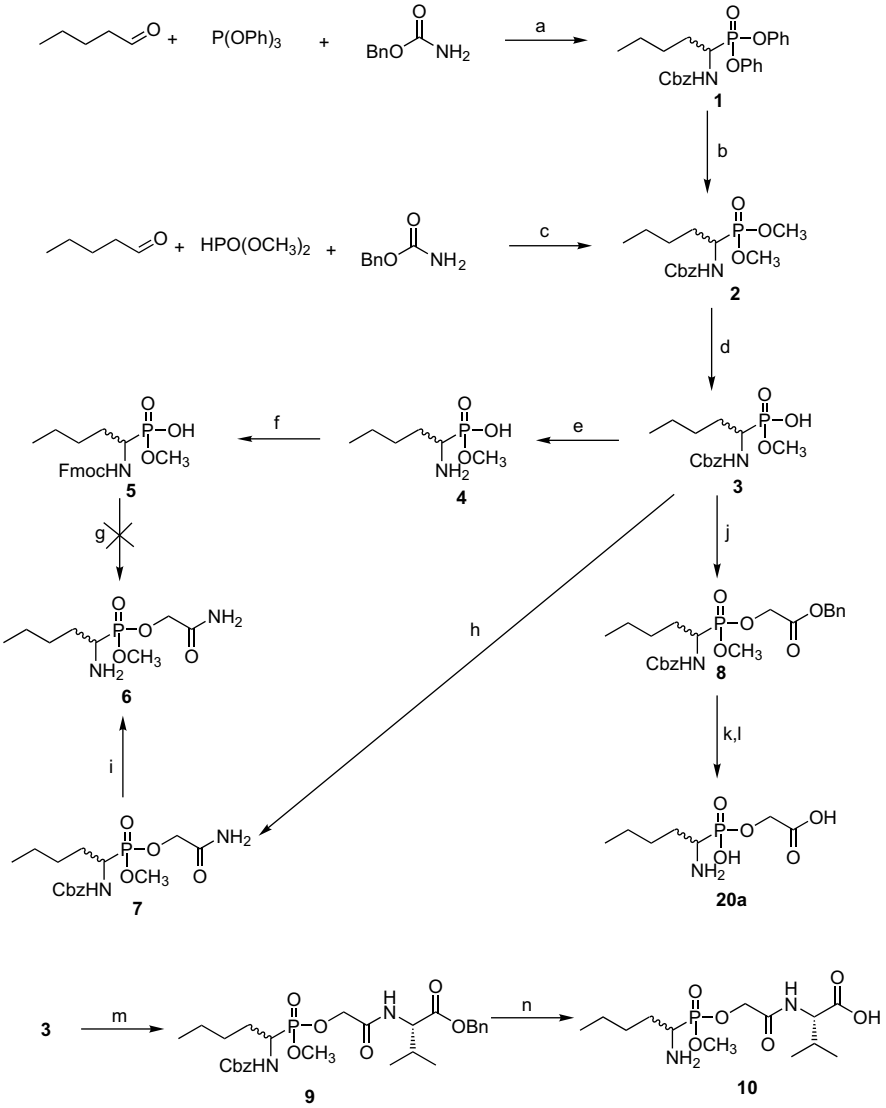
is suitable only for aromatic aldehydes. Campagne et al.[42] described a methodology for the preparation of phosphonopeptides from *N*-Fmoc-protected monoesters of phosphonic acids by solid-phase synthesis using BOP as a condensation agent.

Herein, we present a detailed description of the synthesis of new types of phosphonodepsipeptide, NleΨ[PO(OH)O]-Gly(Ala) and NleΨ[PO(OH)O]-Gly(Ala)-Val, mimicking the peptide sequences Nle-Gly(Ala) and Nle-Gly(Ala)-Val and, in all cases, having a phosphonate analogue of norleucine at the N-terminus. Recently, we published[43] a preliminary report of this synthesis. The presented compounds may represent potential inhibitors of methionine or leucine aminopeptidases. The proposed pseudopeptide sequences should fulfil the structural requirements of methionine aminopeptidases; Nle or Met at P_1 and a small aliphatic residue, such as Ala or Gly, in the P_1' position. The C-terminal Val at the P_2' position was chosen based on our recent results with statine pseudopeptides as inhibitors of methionine aminopeptidases.[44] We also prepared an Fmoc-Nle-Ψ[PO(OH)O]-CH_2-COOH synthon. We used this building block for the efficient solid-phase synthesis of Tyr-Nle-Ψ[PO(OH)O]-Gly-Cys-NH_2 pseudopeptide, and demonstrated that this precursor is a suitable building block for the solid-phase synthesis of cysteine-containing phosphonopeptides.

2. Results and discussion

First, we decided to synthesise target compounds **6**, **10** and **20a** using the methodology outlined in Scheme 1. The first step of our effort consisted of the preparation of phosphonate monomethyl ester **3**, which was further transformed in three synthetic steps: (i) condensation with benzyl glycolate followed by (ii) selective demethylation or (iii) use in solid-phase synthesis.

Thus, diphenyl ester **1** was prepared through the one-pot condensation of valeraldehyde, triphenyl phosphite and benzyl carbamate according to the procedure described by Oleksyszyn et al.,[45] followed by a slightly modified procedure of transesterification[46] with sodium methanolate to yield dimethyl ester **2**. Alternatively, we also prepared compound **2** using a one-pot reaction of valeraldehyde, dimethyl phosphite and benzyl carbamate in acetyl chloride,[47] which gave a moderate yield of 36%. The procedure of Oleksyszyn et al.[45] is somewhat longer, but more convenient with



Scheme 1. Reagents, conditions, yields: (a) AcOH, 80 °C, 2 h (75%); (b) $NaOCH_3$, methanol and dioxane, rt, overnight (77%); (c) AcCl, −5 to 0 °C, 1 h then rt overnight (36%); (d) NaOH, water and methanol, 80 °C, 4 h (84%); (e) 10% Pd–C, H_2, methanol, rt, 15 psi, overnight (87%); (f) Fmoc-OSu, $NaHCO_3$, water and dioxane, rt, overnight; (g) TrisCl, 1-methylimidazole, glycolyl-resin (Sieber Amide); (h) glycolamide, BOP, TEA, DMF, rt, overnight (81%); (i) 10% Pd–C, H_2, methanol, rt, 15 psi, overnight (59%); (j) benzyl glycolate, BOP, TEA, DMF, rt, overnight (89%); (k) TMSBr, dichloromethane, rt, 3 h; (l) 10% Pd–C, H_2, methanol, rt, overnight (3% over two steps); (m) $HOCH_2CONHCH(S\text{-}i\text{Pr})COOBn$ **17a**, BOP, TEA, DMF, rt, overnight (67%); (n) 10% Pd–C, H_2, methanol, rt, 15 psi, overnight (50%).

regard to the yield (58% of **2** over two steps). The hydrolysis of **2** with NaOH afforded the important monomethyl ester intermediate **3**.

Coupling of **3** with benzyl glycolate afforded mixed diester **8**, which was treated with TMSBr. Subsequent hydrogenolysis gave target compound **20a**, but in an unsatisfactory yield (3% after RP-HPLC purification). The main problem with this method was the low selectivity of the demethylation agent (TMSBr) and difficulty in purifying crude compound **20a** by flash chromatography (due to the presence of the polar P–OH group).

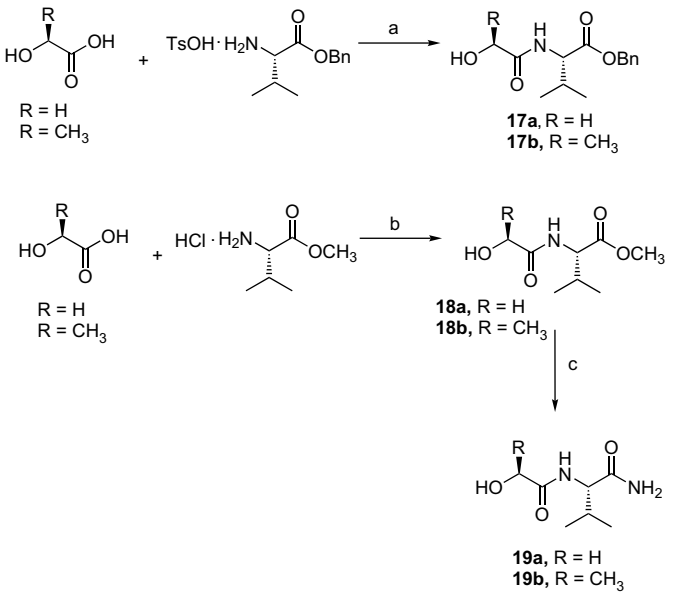
Concerning the solid-phase synthesis (SPS) pathway, removal of the Cbz group through catalytic hydrogenation[48] afforded zwitterion **4**, which was converted into protected phosphonate **5** by treatment with Fmoc-OSu.[49] We tried to use phosphonate synthon **5** in a condensation reaction with the hydroxyl group of glycolic acid attached via its carboxylic group to Sieber Amide resin using a mixture of TrisCl and 1-methylimidazole,[50] but all attempts failed. Later, we found that compound **6** was easily available by coupling **3** with glycolamide followed by removal of the Cbz group. Phosphonate **10**, having the Gly-Val dipeptide sequence at the C-terminus, was obtained in a similar manner.

Taking into account the synthetic problems we encountered with methyl ester protection of the phosphoryl moiety, we decided to focus our next effort on the development of a new method, one that would overcome the disadvantages of the synthetic procedures described above.

In this work, we modified previously published methodologies for the preparation of phosphonodepsipetides reported by Campagne et al.[42] and Isomura et al.[6] Instead of a phosphonic methyl ester moiety, which we used for compound **3**, we introduced benzyl ester protection, which is easily removable through one-step catalytic hydrogenolysis together with the Cbz protecting group. Scheme 2 describes the synthesis of key intermediate **16**, which we proposed as a precursor for the synthesis of the target compounds.

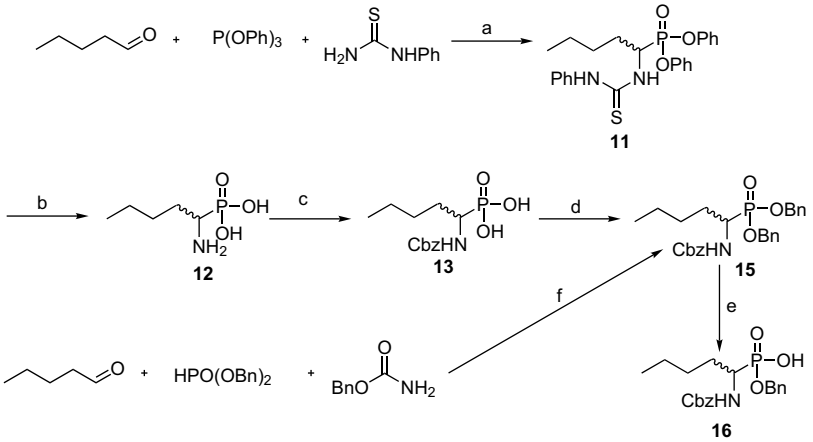
Diphenylester **11** was prepared as described by Kudzin and Stec[51] using a three-component condensation of valeraldehyde, triphenyl phosphite and *N*-phenyl thiourea, which after heating in concentrated hydrochloric acid afforded free 1-aminophosphonic acid **12**. Reaction of **12** with benzyloxycarbonyl chloride furnished Cbz-protected acid **13**, and after esterification[52,53] proceeding under mild conditions with 2 equiv of *N*,*N*-diisopropyl-*O*-benzylisourea[54] **14**, we isolated the corresponding dibenzyl ester **15**. Key intermediate **16** was obtained through the base-catalysed hydrolysis of **15** using DABCO.[55] Surprisingly, the condensation of valeraldehyde, dibenzyl phosphite and benzyl carbamate performed analogously to the standard method[47] (Scheme 2, reaction f) furnished dibenzyl ester **15** in only 5%.

Glycolic and L-lactic acid-derived dipeptides, necessary for coupling with phosphonic acid **16**, were synthesised as published earlier[56] (Scheme 3). Due to great differences in the reactivity of amino and alcohol groups, the hydroxyl functions of glycolic and L-lactic acids did not need to be protected. Glycolic or L-lactic acid reacted with L-valine benzyl ester *p*-toluenesulfonate using DIC as a coupling reagent to afford **17a** or **17b**, respectively. Compounds **18a** and **18b** were prepared in the same manner starting from L-valine methyl ester hydrochloride, but DBU was used instead of TEA for liberating the product from the salt. Aminolysis of **18a** and **18b** with ammonia in methanol afforded amides **19a** and **19b**, respectively.

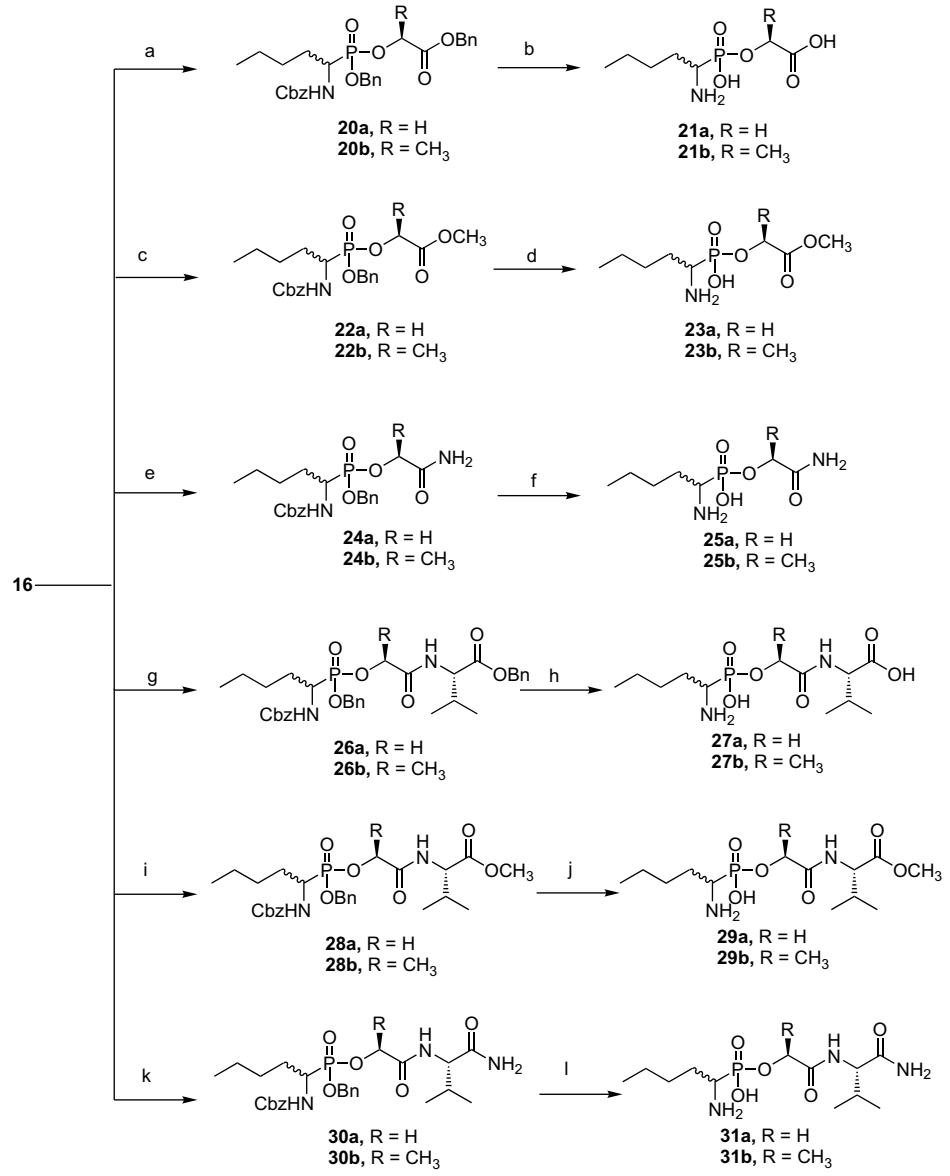


Scheme 3. Reagents, conditions, yields: (a) TEA, HBTA, DIC, dichloromethane, 0 °C, 1 h then rt overnight R=H (80%), R=CH_3 (88%); (b) DBU, HBTA, DIC, dichloromethane, 0 °C, 1 h then rt overnight R=H (61%), R=CH_3 (69%); (c) NH_3, CH_3OH, rt, 6 days, R=H (60%), R=CH_3 (79%).

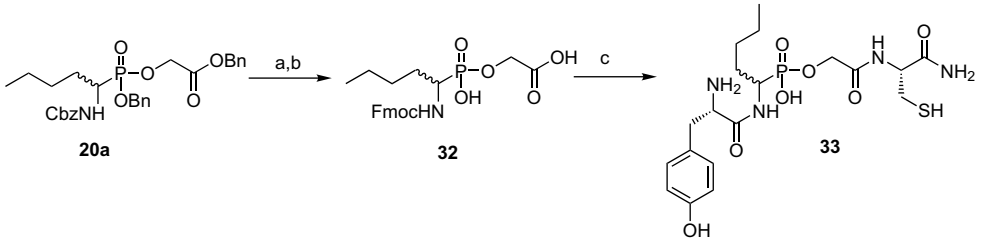
The key intermediate **16** and commercially available benzyl glycolate, benzyl (*S*)-lactate, methyl glycolate, methyl (*S*)-lactate, glycolamide or (*S*)-lactamide were coupled using BOP[35] to afford the corresponding mixed phosphonic acid diesters **20a**, **20b**, **22a**, **22b**, **24a** and **24b**, respectively (Scheme 4). Phosphonic acid diesters **26a**, **26b**, **28a**, **28b**, **30a** and **30b** were obtained by reaction of



Scheme 2. Reagents, conditions, yields: (a) AcOH, 80 °C, 3 h (81%); (b) 35% HCl and AcOH, reflux for 20 h, then 1,2-epoxypropane, ethanol (58%); (c) benzyl chloroformate, Na_2CO_3, water and dioxane, 0 °C, 2 h then at rt overnight (88%); (d) *N*,*N*′-diisopropyl-*O*-benzylisourea **14**, benzene and DMF, 80 °C, 8 h (75%); (e) DABCO, toluene, 80 °C, 8 h (92%); (f) AcCl, −5 to 0 °C, 1 h then rt overnight (5%).



Scheme 4. Reagents, conditions, yields: (a) benzyl glycolate or benzyl (*S*)-lactate, BOP, TEA, DMF, rt, overnight, R=H (88%), R=CH_3 (86%); (b) 10% Pd–C, H_2, methanol, rt, overnight, R=H (70%), R=CH_3 (69%); (c) methyl glycolate or methyl (*S*)-lactate, BOP, TEA, DMF, rt, overnight, R=H (85%), R=CH_3 (82%); (d) 10% Pd–C, H_2, methanol, rt, overnight, R=H (78%), R=CH_3 (73%); (e) glycolamide or (*S*)-lactamide, BOP, TEA, DMF, rt, overnight, R=H (74%), R=CH_3 (70%); (f) 10% Pd–C, H_2, methanol, rt, overnight, R=H (77%), R=CH_3 (64%); (g) $HOCH_2CONHCH(S\text{-}i\text{-}Pr)COOBn$ **17a** or $HOCH(S\text{-}CH_3)CONHCH(S\text{-}i\text{-}Pr)COOBn$ **17b**, BOP, TEA, DMF, rt, overnight, R=H (70%), R=CH_3 (72%); (h) 10% Pd–C, H_2, methanol, rt, overnight, R=H (65%), R=CH_3 (56%); (i) $HOCH_2CONHCH(S\text{-}i\text{-}Pr)COOCH_3$ **18a** or $HOCH(S\text{-}CH_3)CONHCH(S\text{-}i\text{-}Pr)COOCH_3$ **18b**, BOP, TEA, DMF, rt, overnight, R=H (83%), R=CH_3 (78%); (j) 10% Pd–C, H_2, methanol, rt, overnight, R=H (66%), R=CH_3 (77%); (k) $HOCH_2CONHCH(S\text{-}i\text{-}Pr)CONH_2$ **19a** or $HOCH(S\text{-}CH_3)CONHCH(S\text{-}i\text{-}Pr)CONH_2$ **19b**, BOP, TEA, DMF, rt, overnight, R=H (76%), R=CH_3 (82%); (l) 10% Pd–C, H_2, methanol, rt, overnight, R=H (62%), R=CH_3 (71%).



Scheme 5. Reagents, conditions, yields: (a) 10% Pd–C, H_2, methanol, rt, overnight; (b) Fmoc-OSu, $NaHCO_3$, water–dioxane, rt, overnight (22% through two steps); (c) (i) Cys(Trt)-resin Sieber Amide (0.9 equiv), BOP (2 equiv)–DIPEA (4 equiv) in DMF, rt overnight, 8 h; (ii) piperidine–DMF; (iii) Fmoc-Tyr(O*t*-Bu) (3 equiv), BOP (3 equiv)–DIPEA (6 equiv), rt, 2 and 1 h; (iv) piperidine–DMF; (v) TFA (2%), ethanedithiol (2%), water (1%), triisopropylsilane (2%) in dichloromethane, rt for 20 min, evaporation of the filtrate to dryness, (vi) TFA (95%), ethanedithiol (2%), water (1%) and triisopropylsilane (2%), evaporation of the filtrate to dryness (45%, yield is given for compound **33** purified by RP-HPLC as a mixture of diastereoisomers).

16 under the same conditions with dipeptides **17a**, **17b**, **18a**, **18b**, **19a** and **19b**, respectively. In all cases, the reactions proceeded smoothly and in high preparative yields (72–86%). The final step of the synthesis involved the removal of the benzyl and Cbz groups by classical catalytic hydrogenation. The final target compounds **21a**, **21b**, **23a**, **23b**, **25a**, **25b**, **27a**, **27b**, **29a**, **29b**, **31a** and **31b** were purified using RP-HPLC in good yields.

To extend the versatility of our approach, we prepared *N*-protected phosphonate synthon **32** by hydrogenolysis of **20a** followed by reaction with Fmoc-OSu, as illustrated in Scheme 5. The presence of the lypophilic Fmoc group and free phosphonic acid in the molecule resulted in a difficult purification and rather low preparative yield. On the other hand, synthon **32** appeared to be a very useful building block for the solid-phase synthesis of pseudopeptidic phosphonate inhibitors of various metallopeptidases. As an example, using a protocol described by Raguin et al.,[57] we successfully prepared phosphonodepsipeptide **33**, which mimics the peptide sequence of Tyr-Nle-Gly-Cys-amide. Synthon **32** may be useful in the synthesis of cysteine-containing pseudopeptides, which are hardly accessible (due to the poisoning effect of sulfur) by solution-synthesis approaches requiring a final catalytic hydrogenolysis.

3. Conclusion

In conclusion, we present herein an efficient method for the solution synthesis of phosphonodepsipeptides. In comparison to previously published methods, our approach offers several improvements: (i) masking of the amino and hydroxyl moieties by non-polar protecting groups permits convenient and simple work-up of intermediates, (ii) the condensation of protected phosphonic acid **16** with the hydroxyl moiety of glycolic or L-lactic acid derivatives proceeds easily and without sensitivity to steric hindrance and (iii) the final step of the synthesis, catalytic hydrogenation, enables the complete removal of the protecting groups under mild conditions. In addition, *N*-Fmoc-protected precursor **32** provides a convenient building block for the solid-phase synthesis of pseudopeptide phosphonates without any restriction of sulfur-containing amino acids.

4. Experimental

4.1. General

Reagents and solvents (Sigma–Aldrich–Fluka) used in this study were of analytical grade. TLC on silica gel coated aluminium plates (Fluka) was performed in the following systems (v/v): chloroform–ethanol 98:2 (S1), chloroform–ethanol 95:5 (S2), isopropyl alcohol–acetic acid–water 12:3:5 (S3), chloroform–ethanol 90:10 (S4), isopropyl alcohol–concentrated aqueous ammonia–water 7:1:2 (S5). The compounds were visualised by exposure to UV light at 254 nm, by ninhydrin spraying (dark blue colour of amines) and by spraying with a 1% (v/v) ethanolic solution of 4-(4-nitrobenzyl)pyridine followed by heating and treating with gaseous ammonia (blue colour of diesters of phosphonic acids or esters of carboxylic acids). Dipeptides **19a** and **19b** were visualised by spraying with a solution of 10% $CuSO_4$ $5H_2O$ in 10% phosphoric acid followed by heating at 200 °C. Flash chromatography purifications were carried out on silica gel (40–63 μm, Fluka). Preparative RP-HPLC chromatography was carried out on a C18 Luna column (Phenomenex, 250×21.2 mm, 10 μm) at a flow rate 9 mL/min. Solvent A: 0.1% TFA. Solvent B: 80% CH_3CN, 0.1% TFA. The following gradients were used; G1: *t*=0–15 min (0% B), *t*=30 min (20% B), *t*=40 min (40% B), *t*=45 min (100% B); G2: *t*=0–15 min (0% B), *t*=30 min (20% B), *t*=40 min (80% B), *t*=45 min (100% B). Analytical RP-HPLC chromatography was carried out at a flow rate of 1 mL/min on a C18 Nucleosil column (250×4 mm, 5 μm) from Watrex (Praha) using the same gradients and solvents. Eluted compounds were detected at 218 nm. Melting points were determined on a Boetius block and are uncorrected. ^1H and ^{13} NMR spectra were measured on a Bruker AVANCE-600 spectrometer (^1H 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 Bruker IFS 55 Equinox apparatus. HRMS spectra were obtained on an FTMS mass spectrometer LTQ-orbitrap XL (Thermo Fisher, Bremen, Germany) in electrospray ionisation mode. Optical rotation values were measured on polarimeter AUTOPOL IV (Rudolph Research Analytical, USA) for the sodium D line at 20 °C.

4.2. Synthesis of compounds

*4.2.1. Diphenyl [(R,S)-1-(benzyloxycarbonylamino)-pentyl]phosphonate (**1**)*

Benzyl carbamate (6.04 g, 0.04 mol), triphenyl phosphite (12.41 g, 0.04 mol) and valeraldehyde (5.17 g, 0.06 mol) were stirred for 2 h at 80 °C in glacial acetic acid (20 mL). The acetic acid was evaporated in vacuo and the residue was dissolved in 15 mL of methanol. The solution was left to stand overnight at 5 °C to afford colourless crystals, which were filtered off and washed with petroleum ether. The pure product was obtained by recrystallisation from a mixture chloroform–methanol. Yield 13.5 g (75%). Colourless solid, mp 97–98 °C (lit.[58] 95–97 °C). R_f=0.62 (S1). ^1H NMR (600 MHz, DMSO): 0.86 (3H, t, *J*=7.3, CH_3), 1.26 and 1.34 (2H, m, CH_2), 1.34 and 1.45 (2H, m, CH_2), 1.80 and 1.90 (2H, m, CH_2), 4.30 (1H, m, N–CH–P), 5.09 and 5.13 (2H, d, J_{gem}=12.6, –CH_2–O), 7.15–7.39 (15H, m, 3×C_6H_5), 8.08 (1H, d, *J*=9.5, NH). ^{13}C NMR (150.9 MHz, DMSO): 13.99 (CH_3), 21.67 (CH_2), 27.74 (d, *J*(C,P)=14.4, CH_2), 28.23 (d, *J*(C,P)=3.3, CH_2), 48.48 (d, *J*(C,P)=158.3, N–CH–P), 66.05 (O–CH_2), 120.62 (d, *J*(C,P)=4.0, 2×Ar–CH), 120.83 (d, *J*(C,P)=3.8, 2×Ar–CH), 125.38 (Ar–CH), 125.53 (Ar–CH), 127.93 (2×Ar–CH), 128.10 (Ar–CH), 128.57 (2×Ar–CH), 130.09 (2×Ar–CH), 130.13 (2×Ar–CH), 137.17 (Ar–C), 150.12 (d, *J*(C,P)=9.6, Ar–C), 150.40 (d, *J*(C,P)=9.7, Ar–C), 156.54 (d, *J*(C,P)=5.0, O–CO–N). IR (KBr, ν_{max} cm^{-1}) 3270, 1711, 1591, 1544, 1491, 1455, 1253, 1218, 1201, 1163, 1071, 1028, 950, 772, 758, 701, 692, 688. HRMS (ESI) calcd for $C_{25}H_{28}NNa\ O_5P$ $[M+Na]^+$ 476.1603; found: 476.1597.

*4.2.2. Dimethyl [(R,S)-1-(benzyloxycarbonylamino)-pentyl]phosphonate (**2**)*

Sodium methoxide (5.6 g, 0.011 mol) was added in one portion to a stirred solution of compound **1** (9.5 g, 0.021 mol) dissolved in 100 mL of anhydrous methanol and 100 mL of dioxane. The flask was equipped with a calcium dichloride tube and the reaction was allowed to proceed at rt overnight. The solvents were evaporated in vacuo, the residue was taken up in 100 mL of ethyl acetate and washed with 100 mL of water and twice with 100 mL of a 1% solution of NaOH. The organic layer was separated, dried over Na_2SO_4, filtered and concentrated in vacuo. The crude product was purified by flash chromatography on silica gel using elution with a linear gradient of ethyl acetate in toluene. Yield 5.3 g (77%).

Alternatively, we also prepared compound **2** as follows. A mixture of benzyl carbamate (6.05 g; 0.04 mol) and dimethyl phosphite (4.4 g; 0.04 mol) in 50 mL of acetyl chloride was cooled to −5 °C, and valeraldehyde (4.31 g, 0.05 mol) was added dropwise over 10 min. The reaction mixture was stirred for 1 h for at 0 °C and then left to react at rt overnight. The acetyl chloride 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. Yield 4.8 g (36%). Colourless oil. R_f=0.67 (S2). ^1H NMR

(600 MHz, $CDCl_3$): 0.89 (3H, t, J=7.2, CH_3), 1.32 (2H, m, CH_2), 1.34 and 1.45 (2H, m, CH_2), 1.57 and 1.84 (2H, m, CH_2), 3.71 (3H, d, J(H,P)=10.6, OCH_3), 3.75 (3H, d, J(H,P)=10.6, OCH_3), 4.10 (1H, m, N–CH–P), 5.11 and 5.15 (2H, d, J_{gem}=12.3, –CH_2–O), 5.15 (1H, d, J=9.5, NH), 7.31–7.37 (5H, m, C_6H_5). ^{13}C NMR (150.9 MHz, $CDCl_3$): 13.79 (CH_3), 22.12 (CH_2), 27.78 (d, J(C,P)=12.5, CH_2), 29.34 (d, J(C,P)=2.7, CH_2), 47.08 (d, J(C,P)=156.0, N–CH–P), 52.93 (d, J(C,P)=6.6, OCH_3), 53.10 (d, J(C,P)=7.2, OCH_3), 67.07 (O–CH_2), 127.94 (2×Ar–CH), 128.12 (Ar–CH), 128.44 (2×Ar–CH), 136.20 (Ar–C), 156.03 (d, J(C,P)=5.6, O–CO–N). IR ($CHCl_3$, ν_{max} cm^{-1}): 3433, 3020, 2958, 1721, 1511, 1246, 1059, 1042, 836, 698. HRMS (ESI) calcd for $C_{15}H_{25}NO_5P$ $[M+H]^+$ 330.1470; found: 330.1467.

4.2.3. Methyl hydrogen [(R,S)-1-(benzyloxycarbonylamino)-pentyl]phosphonate (**3**)

Phosphonate **2** (3.9 g; 0.012 mol) was dissolved in 25 mL of methanol and 25 mL of 1 M NaOH. The reaction mixture was heated for 4 h at 80 °C. The methanol was evaporated in vacuo and the aqueous layer was extracted with 25 mL of ethyl acetate. The separated aqueous layer was acidified (pH=1) using 1 M HCl. The resulting clear oil was extracted twice with 25 mL of ethyl acetate and the organic phase was dried over Na_2SO_4 prior to filtering and evaporating under reduced pressure. The pure product was obtained by trituration of the residue at −20 °C from a mixture of ethyl acetate and petroleum ether. Yield 3.14 g (84%). Colourless solid, mp 131–133 °C. R_f=0.71 (S5). ^{1}H NMR (600 MHz, $CDCl_3$): 0.87 (3H, t, J=7.2, CH_3), 1.27 and 1.34 (2H, m, CH_2), 1.32 and 1.42 (2H, m, CH_2), 1.52 and 1.87 (2H, m, CH_2), 3.70 (3H, d, J(H,P)=10.8, OCH_3), 4.09 (1H, m, N–CH–P), 5.11 and 5.13 (2H, d, J_{gem}=12.3, –CH_2–O), 5.22 (1H, d, J=10.2, NH), 7.29–7.35 (5H, m, C_6H_5). ^{13}C NMR (150.9 MHz, $CDCl_3$): 13.84 (CH_3), 22.17 (CH_2), 27.87 (d, J(C,P)=12.8, CH_2), 29.23 (d, J(C,P)=2.7, CH_2), 47.32 (d, J(C,P)=158.0, N–CH–P), 52.59 (d, J(C,P)=6.9, OCH_3), 67.14 (O–CH_2), 127.95 (2×Ar–CH), 128.13 (Ar–CH), 128.48 (2×Ar–CH), 136.18 (Ar–C), 156.19 (d, J(C,P)=5.7, O–CO–N). IR (KBr, ν_{max} cm^{-1}): 3280, 2952, 1692, 1547, 1286, 1257, 1221, 1047, 1034, 978, 835, 754, 695. HRMS (ESI) calcd for $C_{14}H_{23}NO_5P$ $[M+H]^+$ 316.1314; found: 316.1323.

4.2.4. Methyl hydrogen [(R,S)-1-aminopentyl] phosphonate (**4**)

Monoester **3** (2 g, 0.006 mol) was dissolved in 80 mL of methanol, and then 10% Pd–C (0.15 g) was added. The reaction mixture was vigorously stirred and left to react under an atmosphere of hydrogen (15 psi) at rt overnight. The catalyst was filtered off through Celite and the filter was washed with 100 mL of methanol. The solvent was evaporated in vacuo, and pure product was obtained by crystallisation from a mixture of methanol and petroleum ether. Yield 1 g (87%). Colourless solid, mp 240–242 °C. R_f=0.75 (S3). ^{1}H NMR (600 MHz, CD_3OD): 0.96 (3H, t, J=7.3, CH_3), 1.40 (2H, m, CH_2), 1.47 and 1.52 (2H, m, CH_2), 1.71 and 1.94 (2H, m, CH_2), 3.09 (1H, m, N–CH–P), 3.64 (3H, d, J(H,P)=10.3, OCH_3). ^{13}C NMR (150.9 MHz, CD_3OD): 14.15 (CH_3), 23.55 (CH_2), 29.50 (d, J(C,P)=8.5, CH_2), 29.91 (d, J(C,P)=2.0, CH_2), 49.41 (d, J(C,P)=145.6, N–CH–P), 52.08 (d, J(C,P)=6.2, OCH_3). IR (KBr, ν_{max} cm^{-1}): 3008 br, 2959, 1645, 1539, 1469, 1205, 1087, 1039, 782. HRMS (ESI) calcd for $C_6H_{17}NO_3P$ $[M+H]^+$ 182.0946; found: 182.0941.

4.2.5. Methyl hydrogen [(R,S)-1-(9H-fluoren-9-ylmethoxycarbonylamino)-pentyl] phosphonate (**5**)

Fmoc-OSu (1.63 g, 0.0048 mol) in 10 mL of dioxane was added dropwise to a solution of monoester **4** (0.8 g, 4.4 mmol) in 15 mL of saturated $NaHCO_3$. The reaction mixture was left to react at rt overnight, and then the dioxane was evaporated in vacuo and the solution was acidified (pH=1) using 1 M HCl. The resulting precipitate was decanted and stirred in 20 mL of ethyl acetate. The crystals were filtered off and the pure product was recrystallised from a mixture of methanol and ethyl acetate. Yield 0.95 g (51%). Colourless solid, mp 176–178 °C. R_f=0.59 (S5). ^{1}H NMR (600 MHz, DMSO): 0.84 (3H, t, J=7.0, CH_3), 1.22 and 1.29 (2H, m, CH_2), 1.22 and 1.34 (2H, m, CH_2), 1.56 and 1.68 (2H, m, CH_2), 3.54 (3H, d, J(H,P)=10.5, OCH_3), 3.73 (1H, m, N–CH–P), 4.26 (1H, dd, J=10.4 and 6.8, –C*H*aHb-O), 4.28 (1H, dd, J=10.4 and 7.8, –CHa*H*b–O), 4.31 (1H, m, CH (Fmoc)), 7.31 (2H, m, Ar–H), 7.41 (2H, m, Ar–H), 7.56 (1H, d, J=9.6, NH), 7.74 (2H, m, Ar–H), 7.88 (2H, m, Ar–H). ^{13}C NMR (150.9 MHz, DMSO): 14.09 (CH_3), 21.84 (CH_2), 27.99 (d, J(C,P)=13.1, CH_2), 28.57 (d, J(C,P)=3.0, CH_2), 46.91 (>CH– (Fmoc)), 47.64 (d, J(C,P)=154.7, N–CH–P), 52.20 (d, J(C,P)=6.2, OCH_3), 65.88 (O–CH_2), 120.34 (2×Ar–CH), 125.61 (Ar–CH), 125.63 (Ar–CH), 127.25 (Ar–CH), 127.28 (Ar–CH), 127.88 (2×Ar–CH), 140.94 (2×Ar–C), 143.95 (Ar–C), 144.13 (Ar–C), 156.37 (d, J(C,P)=5.1, O–CO–N). IR (KBr, ν_{max} cm^{-1}): 3285, 2957, 1688, 1543, 1451, 1294, 1258, 1216, 1044, 756, 739. HRMS (ESI) calcd for $C_{21}H_{27}NO_5P$ $[M+H]^+$ 404.1627; found: 404.1612.

4.2.6. General procedure for the preparation of compounds **7**, **8** and **9**

Monoester **3** (0.5 g, 1.6 mmol), BOP (1.04 g, 2.4 mmol) and the hydroxy component (2.4 mmol) were dissolved in 10 mL of dried DMF and TEA (0.64 g, 6.3 mmol) was added in one portion. The reaction mixture was allowed to react at rt overnight, and the solvent was evaporated to provide a black oil that was purified by column chromatography.

*4.2.6.1. {[(R,S)-1-(Benzyloxycarbonylamino)-pentyl(methoxy)phosphoryl]-oxy}acetamide (**7**).* Compound **7** was prepared by the reaction of **3** with glycolamide. Flash chromatography: linear gradient of ethanol in chloroform. Yield 0.49 g (81%). Colourless oil, R_f=0.47 (S4). ^{1}H NMR (600 MHz, $CDCl_3$): mixture of diastereoisomers (~3:2), only signals of the major isomer are given: 0.90 (3H, t, J=7.2, CH_3), 1.30 and 1.36 (2H, m, CH_2), 1.34 and 1.47 (2H, m, CH_2), 1.58 and 1.92 (2H, m, CH_2), 3.75 (3H, d, J(H,P)=10.6, OCH_3), 4.11 (1H, m, N–CH–P), 4.36 (1H, dd, J=15.3 and 3.8, O–C*H*aHb–CO), 4.65 (1H, dd, J=15.3 and 8.0, O–CHa*H*b–CO), 5.10 and 5.13 (2H, d, J_{gem}=12.2, –CH_2–O), 5.94 (1H, d, J=9.8, NH), 6.07 and 6.98 (2H, br, $CONH_2$), 7.31–7.37 (5H, m, C_6H_5). ^{13}C NMR (150.9 MHz, $CDCl_3$): 13.76 (CH_3), 22.02 (CH_2), 27.86 (d, J(C,P)=13.3, CH_2), 28.70 (d, J(C,P)=2.9, CH_2), 48.22 (d, J(C,P)=156.6, N–CH–P), 53.33 (d, J(C,P)=7.4, OCH_3), 63.78 (d, J(C,P)=7.4, O–CH_2–CO), 67.37 (O–CH_2–C), 128.02 (2×Ar–CH), 128.33 (Ar–CH), 128.53 (2×Ar–CH), 136.09 (Ar–C), 156.56 (d, J(C,P)=4.1, O–CO–N), 170.14 (d, J(C,P)=7.5, N–*C*O–C). IR (KBr, ν_{max} cm^{-1}) 3470, 3410, 3279, 3220, 1716, 1696, 1685, 1662, 1545, 1498, 1456, 1377, 1246, 1071, 1033, 982, 753, 701. HRMS (ESI) calcd for $C_{16}H_{25}N_2NaO_6P$ $[M+Na]^+$ 395.1348; found: 395.1343.

*4.2.6.2. Benzyl {[(R,S)-1-(benzyloxycarbonylamino)-pentyl(methoxy)-phosphoryl]oxy}acetate (**8**).* Compound **8** was prepared by the reaction of **3** with benzyl glycolate. Flash chromatography: linear gradient of ethyl acetate in toluene. Yield 0.64 g (89%). Colourless oil, R_f=0.67 (S2). ^{1}H NMR (600 MHz, $CDCl_3$): mixture of diastereoisomers (59:41), only signals of the major isomer are given: 0.88 (3H, t, J=7.2, CH_3), 1.28 and 1.34 (2H, m, CH_2), 1.34 and 1.43 (2H, m, CH_2), 1.57 and 1.87 (2H, m, CH_2), 3.76 (3H, d, J(H,P)=10.9, OCH_3), 4.18 (1H, m, N–CH–P), 4.63 (1H, dd, J=16.2 and 11.0, O–C*H*aHb–CO), 4.67 (1H, dd, J=16.2 and 11.5, O–CHa*H*b–CO), 5.09 and 5.13 (2H, d, J_{gem}=12.2, –CH_2–O), 5.19 and 5.21 (2H, d, *Jgem*=12.0, –CH_2–O), 5.25 (1H, d, J=9.5, NH), 7.17–7.37 (10H, m, 2×C_6H_5). ^{13}C NMR (150.9 MHz, $CDCl_3$): 13.83 (CH_3), 22.16 (CH_2), 27.89 (d, J(C,P)=12.5, CH_2), 29.53 (d, J(C,P)=2.3, CH_2), 47.90 (d, J(C,P)=156.8, N–CH–P), 52.78 (d, J(C,P)=7.5, OCH_3), 62.37 (d, J(C,P)=6.0, O–CH_2–CO), 67.09 (O–CH_2–C), 67.36 (O–CH_2–C), 127.97 (Ar–CH), 128.10 (Ar–CH), 128.45 (2×Ar–CH), 128.46 (2×Ar–CH), 128.51 (2×Ar–CH), 128.64 (2×Ar–CH), 134.75 (Ar–C), 136.27 (Ar–C), 156.14 (d, J(C,P)=5.7, O–CO–N), 168.55 (d, J(C,P)=4.2, O–*C*O–C). IR ($CHCl_3$, ν_{max} cm^{-1}) 3431, 3068, 3031, 1758, 1721, 1511, 1456, 1240, 1099, 1046, 698. HRMS (ESI) calcd for $C_{23}H_{30}NNaO_7P$ $[M+Na]^+$ 486.1658; found: 486.1652.

*4.2.6.3. Benzyl (2S)-2-[({[((R,S)-1-benzyloxycarbonylamino)-pentyl-(methoxy)phosphoryl]oxy}acetyl)amino]-3-methylbutanoate (**9**).* Compound **9** was prepared by the reaction of **3** with dipeptide **16a**. Flash chromatography: linear gradient of ethyl acetate in toluene. Yield 0.70 g (79%). Pale yellow oil, R_f=0.33 (S2). Mixture of four diastereomers; assignment of individual isomers was not possible; only groups and/or regions of signals are given: ^{1}H NMR (600 MHz, $CDCl_3$): 0.88–0.90 (3H, t, J=7.3, CH_3), 0.91–0.94 (6H, d, J=7.0, 2×CH_3 (Val)), ~1.29 and ~1.36 (2H, m, CH_2), ~1.36 and ~1.46 (2H, m, CH_2), ~1.60 and ~1.87 (2H, m, CH_2), 2.20–2.25 (1H, m, CH (Val)), 3.75, 3.755, 3.79 and 3.80 (3H, d, J(H,P)=10.7, OCH_3), 4.14–4.19 (1H, m, N–CH–P), 4.47–4.65 (2H, m, O–CH_2–CO), 4.59–4.61 (1H, dd, N–CH–CO), 5.09–5.19 (4H, m, 2×O–CH_2), 5.19, 5.23, 5.34 and 5.38 (1H, d, J=10.0, O–CO–NH), 7.11, 7.13 and 7.22 (1H, d, J=8.5, CO–NH), 7.15–7.36 (10H, m, 2×C_6H_5). ^{13}C NMR (150.9 MHz, $CDCl_3$): 13.76 (CH_3), 17.52–17.88 and 18.83–18.94 (2×CH_3 (Val)), 22.03–22.06 (CH_2), 27.33–27.85 (CH_2), 28.72–29.13 (CH_2), 30.96–31.22 (CH (Val)), 47.02–48.46 (N–CH–P), 53.34–53.66 (OCH_3), 56.78–56.98 (N–***C***H–CO), 63.90–64.19 (O–***C***H_2–CO), 66.95–67.36 (2×O–***C***H_2–C_6H_5), 127.86–137.79 (2×C_6H_5), 156.02–156.19 (O–CO–NH), 167.10–167.25 (CO–NH), 171.22–171.34 (CO–O). IR ($CHCl_3$, ν_{max} cm^{-1}) 3431, 1722, 1685, 1511, 1456, 1393, 1382, 1235, 1069, 1043, 978, 698. HRMS (ESI) calcd for $C_{28}H_{39}N_2NaO_8P$ $[M+Na]^+$ 585.2342; found: 585.2338.

*4.2.7. Diphenyl [(R,S)-1-(3-phenylthioureido)-pentyl]-phosphonate (**11**)*

The compound was prepared according to Kudzin and Stec.[51] A solution of triphenyl phosphite (37.2 g, 0.12 mol), valeraldehyde (12.9 g, 0.15 mol) and *N*-phenyl thiourea (18.2 g, 0.12 mol) was heated for 4 h at 80 °C in 100 mL of glacial acetic acid. After cooling to rt, water was added (20 mL) and the solution was allowed to stand overnight at rt. The precipitate was filtered off, washed with a mixture of acetic acid–water (40 mL, 1:1) and dried over P_2O_5. Recrystallisation from a mixture of chloroform and methanol yielded pure product. Yield 44 g (81%). Colourless solid, mp 174–175 °C (lit.[51] 161–162 °C). Calcd for $C_{24}H_{27}N_2O_3PS$ (454.52): C 63.24%, H 5.99%, N 6.16%. Found: 63.37%, 6.03%, 6.08%. R_f=0.48 (S1). ^{1}H NMR (600 MHz, DMSO): 0.87 (3H, t, J=7.2, CH_3), 1.31 and 1.47 (2H, m, CH_2), 1.40 (2H, m, CH_2), 1.84 and 1.98 (2H, m, CH_2), 5.63 (1H, m, N–CH–P), 7.13, 7.33 and 7.49 (1H, 2H and 2H, m, N–C_6H_5), 7.19, 7.23 and 7.39 (2H, 1H and 2H, m, O–C_6H_5), 7.24 and 7.41 (3H and 2H, m, O–C_6H_5), 8.25 (1H, d, J=9.8, NH), 9.72 (1H, s, NH). ^{13}C NMR (150.9 MHz, DMSO): 14.02 (CH_3), 22.08 (CH_2), 27.48 (d, J(C,P)=12.8, CH_2), 29.31 (d, J(C,P)=3.2, CH_2), 50.58 (d, J(C,P)=155.1, N–CH–P), 120.70 (d, J(C,P)=3.8, 2×Ar–CH), 120.87 (d, J(C,P)=3.9, 2×Ar–CH), 123.20 (2×Ar–CH), 124.65 (Ar–CH), 125.58 (Ar–CH), 125.68 (Ar–CH), 128.79 (2×Ar–CH), 130.11 (2×Ar–CH), 130.21 (2×Ar–CH), 139.48 (Ar–C), 150.00 (d, J(C,P)=9.6, Ar–C), 150.26 (d, J(C,P)=9.7, Ar–C), 181.79 (d, J(C,P)=8.1, N–CS–N). IR (KBr, ν_{max} cm^{-1}) 3291, 3070, 3057, 3041, 1597, 1589, 1488, 1337, 1235, 1209, 1159, 955, 945, 767, 709, 691, 487. HRMS (ESI) calcd for $C_{24}H_{27}N_2NaO_3PS$ $[M+Na]^+$ 477.1378; found: 477.1364.

*4.2.8. [(R,S)-1-Aminopentyl]phosphonic acid (**12**)*

The compound was prepared according to Kudzin and Stec.[51] Ester **11** (44 g, 0.10 mol) was heated for 20 h at reflux with 200 mL of 35% HCl and 30 mL of glacial acetic acid. The solvents were evaporated under reduced pressure and the oily residue was taken up in 200 mL of ethanol. The ethanolic solution of the crude product was treated with methyloxirane until pH≈6 was reached. The precipitate of the product was filtered off, and then washed with ethanol and diethyl ether. Yield 9.4 g (58%). Colourless solid, mp 260–264 °C (lit.[51] 262–264 °C). R_f=0.68 (S3). ^{1}H NMR (600 MHz, D_2O+NaOD): 0.89 (3H, t, J=7.0, CH_3), 1.28–1.36 (2H, m, CH_2), 1.30 and 1.49 (2H, m, CH_2), 1.32 and 1.74 (2H, m, CH_2), 2.49 (1H, td, J=10.4, 10.4 and 3.3, N–CH–P). ^{13}C NMR (150.9 MHz, D_2O+NaOD): 14.06 (CH_3), 22.74 (CH_2), 29.52 (d, J(C,P)=12.5, CH_2), 31.89 (CH_2), 50.61 (d, J(C,P)=138.7, N–CH–P). IR (KBr, ν_{max} cm^{-1})≈3000 br, 2295 br, 1650, 1601, 1530, 1469, 1178, 1032, 924, 564, 511, 493. HRMS (ESI) calcd for $C_5H_{15}NO_3P$ $[M+H]^+$ 168.0790; found: 168.0782.

*4.2.9. [(R,S)-1-(Benzyloxycarbonylamino)-pentyl]phosphonic acid (**13**)*

Phosphonic acid **12** was dissolved in a 20% aqueous solution of sodium carbonate (150 mL) and a solution of benzyloxycarbonyl chloride (11.46 g, 0.067 mol) in 50 mL of dioxane was added dropwise at 0 °C over 1 h. After stirring for 2 h at 0 °C, the mixture was allowed to stand overnight at rt. The dioxane was evaporated under reduced pressure and the acidity of the aqueous solution was adjusted to pH=1 while efficiently ice-cooling. The solution was extracted three times with 200 mL of ethyl acetate, and the combined organic layers were dried over Na_2SO_4 before filtering and removing the solvent in vacuo. Trituration of the residue from a mixture of ethyl acetate and petroleum ether at −20 °C afforded the pure product. Yield 14.9 g (88%). Colourless solid, mp 100–102 °C. R_f=0.45 (S5). ^{1}H NMR (600 MHz, D_2O+NaOD): 0.85 (3H, t, J=7.0, CH_3), 1.21–1.37 (4H, m, 2×CH_2), 1.52 and 1.82 (2H, m, CH_2), 3.69 (1H, ddd, J=15.8, 11.5 and 3.0, N–CH–P), 5.04 and 5.19 (2H, d, J_{gem}=12.6, –CH_2–O), 7.37–7.43 (5H, m, C_6H_5). ^{13}C NMR (150.9 MHz, D_2O+NaOD): 14.03 (CH_3), 22.20 (CH_2), 28.55 (d, J(C,P)=12.8, CH_2), 29.90 (d, J(C,P)=2.1, CH_2), 50.29 (d, J(C,P)=149.9, N–CH–P), 67.51 (O–CH_2), 128.16 (2×Ar–CH), 128.90 (Ar–CH), 129.38 (2×Ar–CH), 137.25 (Ar–C), 158.79 (d, J(C,P)=6.0, O–CO–N). IR (KBr, ν_{max} cm^{-1}) 3343, 3282, 2900 br, 1694, 1531, 1456, 1378, 1277, 1040, 736, 699. HRMS (ESI) calcd for $C_{13}H_{19}NO_5P$ $[M-H]^+$ 300.1001; found: 300.1007.

*4.2.10. N,N′-Diisopropyl-O-benzylisourea (**14**)*

The compound was prepared according to Mathias et al.[54] Benzyl alcohol (17.95 g, 0.17 mol) was added dropwise to a stirred mixture of *N*,*N*′-diisopropylcarboimide (20.9 g, 0.17 mol) and CuCl (0.5 g) at 0 °C over a period of 30 min. The cooling was removed and then stirring was continued overnight at rt. Thereafter, the reaction mixture was taken up in 100 mL of hexane and the cuprous salts were filtered off over a pad of neutral alumina. The solvent was evaporated under reduced pressure and the rest of the hexane was removed under high vacuum. Yield 37 g (95%). Colourless oil, R_f=0.30 (S4). ^{1}H NMR (600 MHz, $CDCl_3$): 1.12 (12H, d, J=6.5, 4×CH_3), 3.20 and 3.47 (2H, m, 2×>CH–N), 5.10 (2H, s, –CH_2–O), 7.28 (1H, m, Ar–H), 7.34 (2H, m, Ar–H), 7.38 (2H, m, Ar–H). ^{13}C NMR (150.9 MHz, $CDCl_3$): 24.00 (2×CH_3), 24.25 (2×CH_3), 43.42 (>CH–N), 46.24 (>CH–N), 66.66 (O–CH_2), 127.32 (Ar–CH), 127.56 (2×Ar–CH), 128.19 (2×Ar–CH), 137.89 (Ar–C), 151.50 (N–C(O)=N). IR (CCl_4, ν_{max} cm^{-1}) 3446, 3091, 3067, 3034, 2967, 1669, 1467, 1388, 1366, 1320, 1306, 1124, 1029, 732, 696. HRMS (ESI) calcd for $C_{14}H_{23}N_2$ $[M+H]^+$ 235.1810; found: 235.1805.

*4.2.11. Dibenzyl [(R,S)-1-(benzyloxycarbonylamino)-pentyl]phosphonate (**15**)*

Phosphonic acid **13** (14.9 g, 0.049 mol) and isourea **14** (23.19 g, 0.099 mol) were heated at 80 °C in a mixture of benzene (200 mL) and DMF (60 mL) over a period of 12 h. The solvents were evaporated in vacuo to afford the crude product, which was purified by flash chromatography on silica gel using elution with a linear gradient of ethyl acetate in toluene. Trituration at −20 °C from a mixture of ethyl acetate and petroleum ether furnished pure product. Yield 17.9 g (75%). Colourless solid, mp 65–67 °C. R_f=0.64 (S2). ^{1}H NMR (600 MHz, $CDCl_3$): 0.85 (3H, t, J=7.2, CH_3), 1.24 and 1.30 (2H, m, CH_2), 1.32 and 1.42 (2H, m, CH_2), 1.53 and 1.84 (2H, m, CH_2), 5.63 (1H, dtd, J=16.4, 10.6, 10.6 and 3.8, N–CH–P), 4.95 (1H, d, J=10.6, NH), 4.97 (2H, m, O–CH_2), 4.98 (1H, dd, J=11.7 and 7.4, O–C*H*a*H*b), 5.03 (1H, dd, J=11.7 and 8.5, O–CHa*H*b), 5.06 (2H, s, O–CH_2), 7.29–

7.33 (15H, m, 3×C_6H_5). ^{13}C NMR (150.9 MHz, $CDCl_3$): 13.80 (CH_3), 22.12 (CH_2), 27.80 (d, J(C,P)=12.5, CH_2), 29.45 (d, J(C,P)=2.9, CH_2), 47.78 (d, J(C,P)=155.4, N–CH–P), 67.05 (O–CH_2), 67.75 (d, J(C,P)=6.5, O–CH_2), 67.86 (d, J(C,P)=7.1, O–CH_2), 127.96 (6×Ar–CH), 128.13 (2×Ar–CH), 128.40 (Ar–CH), 128.42 (Ar–CH), 128.46 (2×Ar–CH), 128.52 (Ar–CH), 128.54 (2×Ar–CH), 135.98 (d, J(C,P)=6.0, Ar–C), 136.04 (d, J(C,P)=5.7, Ar–C), 136.18 (Ar–C), 155.97 (O–CO–N). IR (KBr, ν_{max} cm^{-1}): 3208, 3063, 3048, 1722, 1550, 1497, 1455, 1290, 1260, 1222, 1215, 1046, 1018, 748, 732, 701, 696, 548, 536, 461, 456. HRMS (ESI) calcd for $C_{27}H_{32}NNaO_5P$ $[M+Na]^+$ 504.1916; found: 504.1908.

Alternatively, compound **15** was prepared as follows. A mixture of benzyl carbamate (3.02 g, 0.02 mol) and dibenzyl phosphite (5.25 g, 0.02 mol) in 50 mL of acetyl chloride was cooled to −5 °C and valeraldehyde (2.15 g, 0.025 mol) was added dropwise over 10 min. The reaction mixture was stirred for 1 h at 0 °C and left to react at rt overnight. The acetyl chloride 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. Yield 0.45 g (5%).

4.2.12. Benzyl hydrogen [(R,S)-1-(benzyloxycarbonylamino)-pentyl]phosphonate (**16**)

Dibenzyl ester **15** (17.9 g, 0.037 mol) and DABCO (6.23 g, 0.055 mol) in toluene (150 mL) were heated at 80 °C over a period of 12 h. The solvent was removed in vacuo, and the residue was taken up in 100 mL of ethyl acetate and washed with 30 mL of 1 M HCl. The organic phase was dried over Na_2SO_4 prior to filtration and removal of the solvent in vacuo. Trituration of the crude compound at −20 °C from a mixture of ethyl acetate and petroleum ether afforded pure product. Yield 13.4 g (92%). Colourless solid, mp 98–100 °C. (lit.[39] 154–156 °C). Calcd for $C_{20}H_{26}NO_5P$ (391.39): C 61.37%, H 6.70%, N 3.58%. Found: 61.48%, 6.80%, 3.53%. R_f=0.86 (S5). ^{1}H NMR (600 MHz, DMSO): 0.83 (3H, t, J=7.1, CH_3), 1.22 and 1.29 (2H, m, CH_2), 1.22 and 1.35 (2H, m, CH_2), 1.55 and 1.70 (2H, m, CH_2), 3.80 (1H, dddd, J=15.3, 11.6, 9.7 and 3.4, N–CH–P), 4.91 (1H, dd, J=12.1 and 7.1, O–C*Ha*Hb), 4.95 (1H, dd, J=12.1 and 6.9, O–CHa*Hb*), 5.04 (2H, s, O–CH_2), 7.30–7.35 (10H, m, 2×C_6H_5), 7.49 (1H, d, J=9.7, NH). ^{13}C NMR (150.9 MHz, DMSO): 14.08 (CH_3), 21.82 (CH_2), 28.03 (d, J(C,P)=13.3, CH_2), 28.55 (d, J(C,P)=3.0, CH_2), 48.09 (d, J(C,P)=155.3, N–CH–P), 65.63 (O–CH_2), 66.35 (d, J(C,P)=5.7, O–CH_2), 127.57 (2×Ar–CH), 127.77 (2×Ar–CH), 127.89 (Ar–CH), 128.04 (Ar–CH), 128.54 (2×Ar–CH), 128.56 (2×Ar–CH), 137.44 (Ar–C), 137.60 (d, J(C,P)=6.5, Ar–C), 156.49 (d, J(C,P)=5.0, O–CO–N). IR (KBr, ν_{max} cm^{-1}) 3291, 2800–2500 br, 1724, 1711, 1685, 1541, 1497, 1456, 1244, 1218, 1052, 1035, 1025, 737, 697. HRMS (ESI) calcd for $C_{20}H_{25}NO_5P$ $[M-H]^+$ 390.1470; found: 390.1478.

4.2.13. Benzyl (2S)-2-[(hydroxyacetyl)amino]-3-methylbutanoate (**17a**)

A mixture of glycolic acid (0.52 g, 6.8 mmol), L-valine benzyl ester *p*-toluensulfonate (2.58 g, 6.8 mmol), 1-hydroxybenzotriazole (0.91 g, 6.8 mmol) and triethylamine (0.69 g, 6.8 mmol) in 50 mL of dichloromethane was cooled to 0 °C and DIC (1.72 g, 13.6 mmol) was added dropwise. The reaction mixture was stirred at 0 °C for 2 h and then at rt overnight. The solvent was removed in vacuo and the residue was dissolved in 50 mL of cold ethyl acetate. Crystals of diisopropylurea were filtered off and the filtrate was washed successively with a saturated solution of citric acid and then with a saturated solution of sodium hydrogencarbonate. The separated organic layer was dried over Na_2SO_4 and concentrated to dryness. The crude product was purified by flash chromatography on silica gel using elution with a linear gradient of ethyl acetate in toluene. Yield 1.45 g (80%). Semisolid, R_f=0.31 (S2). ^{1}H NMR (600 MHz, $CDCl_3$): 0.88 (3H, d, J=6.8, CH_3), 0.93 (3H, d, J=6.8, CH_3), 2.22 (1H, m, J=6.8(6×) and 4.9, >CH–), 4.13 (2H, s, O–CH_2–CO), 4.62 (1H, dd, J=9.2 and 4.9, N–CH–CO), 5.16 and 5.20 (2H, d, J_{gem}=12.2, O–CH_2), 7.13 (1H, br d, J=9.2, NH), 7.32–7.38 (5H, m, C_6H_5). ^{13}C NMR (150.9 MHz, $CDCl_3$): 17.53 (CH_3), 18.93 (CH_3), 31.18 (>CH–), 56.60 (N–CH<), 62.06 (CH_2OH), 67.11 (O–CH_2), 128.32 (2×Ar–CH), 128.46 (Ar–CH), 128.57 (2×Ar–CH), 135.13 (Ar–C), 171.73 (CO–O), 172.08 (CO–N). IR ($CHCl_3$, ν_{max} cm^{-1}) 3410, 2970, 1736, 1674, 1526, 1500, 1456, 1393, 1384, 1196, 1071, 1003, 698. HRMS (ESI) calcd for $C_{14}H_{19}NNaO_4$ $[M+Na]^+$ 288.1212; found: 288.1207. $[\alpha]_D^{20}$ −3.0 (*c* 0.710, $CHCl_3$).

4.2.14. Benzyl (2S)-2-{[(2S)-2-hydroxypropanoyl]amino}-3-methylbutanoate (**17b**)

Dipeptide **17b** was prepared in the same manner as **17a** by the reaction of L-lactic acid (0.28 g, 3.1 mmol), L-valine benzyl ester *p*-toluensulfonate (1.17 g, 3.1 mmol), 1-hydroxybenzotriazole (0.42 g, 3.1 mmol), triethylamine (0.31 g, 3.1 mmol) and DIC (0.78 g, 6.2 mmol). Yield 0.76 g (88%). Semisolid, R_f=0.40 (S2). ^{1}H NMR (600 MHz, $CDCl_3$): 0.87 (3H, d, J=6.9, CH_3), 0.92 (3H, d, J=6.9, CH_3), 1.43 (3H, d, J=6.8, CH_3), 2.22 (1H, m, J=6.9(6×) and 4.9, >CH–), 3.37 (1H, d, J=4.8, OH), 4.27 (1H, qd, J=6.8(3×) and 4.8, >CH–), 4.59 (1H, dd, J=9.1 and 4.9, N–CH–CO), 5.14 and 5.20 (2H, d, J_{gem}=12.3, O–CH_2), 7.09 (1H, br d, J=9.1, NH), 7.32–7.38 (5H, m, C_6H_5). ^{13}C NMR (150.9 MHz, $CDCl_3$): 17.48 (CH_3), 18.95 (CH_3), 21.27 (CH_3), 31.22 (>CH–), 56.54 (N–CH<), 67.08 (O–CH_2), 68.48 (>CH–O), 128.32 (2×Ar–CH), 128.45 (Ar–CH), 128.58 (2×Ar–CH), 135.18 (Ar–C), 171.87 (CO–O), 174.70 (CO–N). IR ($CHCl_3$, ν_{max} cm^{-1}) 3413, 2970, 1736, 1672, 1522, 1456, 1392, 1382, 1374, 1265, 1195, 1082, 1030, 1002, 698. HRMS (ESI) calcd for $C_{15}H_{21}NNaO_4$ $[M+Na]^+$ 302.1368; found: 302.1362. $[\alpha]_D^{20}$ −12.5 (*c* 0.584, $CHCl_3$).

4.2.15. Methyl (2S)-2-[(hydroxyacetyl)amino]-3-methylbutanoate (**18a**)

Dipeptide **18a** was prepared in the same manner as dipeptide **17a** by the reaction of glycolic acid (0.52 g, 6.8 mmol), L-valine methyl ester hydrochloride (1.14 g, 6.8 mmol), 1-hydroxybenzotriazole (0.92 g, 6.8 mmol), DBU (1.03 g, 6.8 mmol) and DIC (1.03 g, 8.2 mmol). Yield 0.79 g (61%). Semisolid, R_f=0.40 (S4). ^{1}H NMR (600 MHz, $CDCl_3$): 0.94 (3H, d, J=6.8, CH_3), 0.96 (3H, d, J=6.8, CH_3), 2.20 (1H, m, J=6.8(6×) and 5.1, >CH–), 3.75 (3H, s, OCH_3), 4.12 (1H, dd, J=15.0 and 5.5, O–C*Ha*Hb), 4.15 (1H, dd, J=15.0 and 5.0, O–CHa*Hb*), 4.22 (1H, br t, J=5.5 and 5.0, OH), 4.56 (1H, dd, J=9.1 and 5.1, N–CH–CO), 7.21 (1H, br d, J=9.1, NH). ^{13}C NMR (150.9 MHz, $CDCl_3$): 17.69 (CH_3), 18.90 (CH_3), 31.12 (>CH–), 52.19 (OCH_3), 56.63 (N–CH<), 62.02 (O–CH_2), 172.26 and 172.27 (CO–O and CO–N). IR ($CHCl_3$, ν_{max} cm^{-1}) 3409, 2970, 1739, 1673, 1527, 1439, 1393, 1374, 1272, 1153, 1074. HRMS (ESI) calcd for $C_8H_{15}NNaO_4$ $[M+Na]^+$ 212.0899; found: 212.0893. $[\alpha]_D^{20}$ +13.0 (*c* 0.432, $CHCl_3$).

4.2.16. Methyl (2S)-2-{[(2S)-2-hydroxypropanoyl]amino}-3-methylbutanoate (**18b**)

Dipeptide **18b** was prepared in the same manner as dipeptide **18a** by the reaction of L-lactic acid (0.61 g, 6.8 mmol), L-valine methyl ester hydrochloride (1.14 g, 6.8 mmol), 1-hydroxybenzotriazole (0.92 g, 6.8 mmol), DBU (1.03 g, 6.8 mmol) and DIC (1.03 g, 8.2 mmol). Yield 0.95 g (69%). Semisolid, R_f=0.47 (S4). ^{1}H NMR (600 MHz, $CDCl_3$): 0.92 (3H, d, J=6.9, CH_3), 0.95 (3H, d, J=6.9, CH_3), 1.45 (3H, d, J=6.9, CH_3), 2.20 (1H, m, J=6.9(6×) and 5.0, >CH–), 3.75 (3H, s, OCH_3), 4.28 (1H, q, J=6.9(3×), >CH–O), 4.54 (1H, dd, J=9.1 and 5.0, N–CH–CO), 7.10 (1H, br d, J=9.1, NH). ^{13}C NMR (150.9 MHz, $CDCl_3$): 17.64 (CH_3), 18.92 (CH_3), 21.25 (CH_3), 31.17 (>CH–), 52.21 (OCH_3), 56.57 (N–CH<), 68.48 (O–CH<), 172.52 (CO–O), 174.74 (CO–N). IR ($CHCl_3$, ν_{max} cm^{-1}) 3413, 2970, 1738, 1672, 1523, 1439, 1373, 1270, 1154, 1119. HRMS (ESI) calcd for $C_9H_{17}NNaO_4$ $[M+Na]^+$ 226.1055; found: 226.1050. $[\alpha]_D^{20}$ +3.2 (*c* 0.437, $CHCl_3$).

*4.2.17. (2S)-2-[(Hydroxyacetyl)amino]-3-methylbutanamide (**19a**)*

Compound **18a** (1.5 g, 7.9 mmol) was stirred over one week 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. Yield 0.83 g (60%). Colourless solid, mp 148–151 °C. R_f=0.22 (S4). ^{1}H NMR (600 MHz, DMSO): 0.80 (3H, d, J=6.7, CH_3), 0.85 (3H, d, J=6.7, CH_3), 1.96 (1H, m, J=6.7(6×) and 6.1, >CH–), 3.81 (1H, dd, J=16.0 and 5.8, O–C*H*aHb), 3.86 (1H, dd, J=16.0 and 5.8, O–CHa*H*b), 4.17 (1H, dd, J=9.2 and 6.1, N–CH–CO), 5.66 (1H, t, J=5.8, OH), 7.42 (1H, d, J=9.2, NH), 7.14 and 7.56 (2H, d, J_{gem}=1.8, $CONH_2$). ^{13}C NMR (150.9 MHz, DMSO): 18.01 (CH_3), 19.56 (CH_3), 31.32 (>CH–), 56.73 (N–CH<), 61.55 (CH_2–OH), 171.75 (CO–NH), 173.02 (CO–NH_2). IR (KBr, ν_{max} cm^{-1}) 3370, 3206, 2973, 2965, 1680, 1617, 1550, 1426, 1390, 1372, 1252, 1230, 1082, 666. HRMS (ESI) calcd for $C_7H_{14}N_2NaO_3$ $[M+Na]^+$ 197.0902; found: 197.0897. $[\alpha]_D^{20}$ +3.2 (*c* 0.495, CH_3OH).

*4.2.18. (2S)-2-{[(2S)-2-Hydroxypropanoyl]amino}-3-methylbutanamide (**19b**)*

Dipeptide **19b** was prepared from compound **18b** (1.5 g, 7.3 mmol) in the same manner as compound **19a**. Yield 1.1 g (79%). Colourless solid, mp 140–143 °C. R_f=0.25 (S4). ^{1}H NMR (600 MHz, DMSO): 0.79 (3H, d, J=6.7, CH_3), 0.84 (3H, d, J=6.7, CH_3), 1.22 (3H, d, J=6.8, CH_3), 1.94 (1H, m, J=6.7(6×) and 6.2, >CH–), 3.97 (1H, qd, J=6.8(3×) and 5.2, O–CH–CO), 4.16 (1H, dd, J=9.4 and 6.2, N–CH–CO), 5.72 (1H, d, J=5.2, OH), 7.40 (1H, d, J=9.4, NH), 7.14 and 7.55 (2H, d, J_{gem}=1.8, $CONH_2$). ^{13}C NMR (150.9 MHz, DMSO): 17.89 (CH_3), 19.53 (CH_3), 21.56 (CH_3), 31.48 (>CH–), 56.54 (N–CH<), 67.67 (O–CH<), 173.03 (CO–NH_2), 174.41 (CO–NH). IR (KBr, ν_{max} cm^{-1}) 3389, 3361, 3300, 3201, 2976, 2961, 1667, 1648, 1625, 1550, 1432, 1236, 1142, 1125, 661. HRMS (ESI) calcd for $C_8H_{16}N_2NaO_3$ $[M+Na]^+$ 211.1059; found: 211.1053. $[\alpha]_D^{20}$ −13.2 (*c* 0.608, CH_3OH).

*4.2.19. General procedure for the preparation of compounds **20a**, **20b**, **22a**, **22b**, **24a**, **24b**, **26a**, **26b**, **28a**, **28b**, **30a** and **30b***

Monoester **16** (0.5 g, 1.3 mmol), the hydroxy component (1.95 mmol) and BOP (0.86 g, 1.95 mmol) were dissolved in 10 mL of dry DMF, and TEA (0.53 g, 5.2 mmol) was added in one portion. The reaction mixture was stirred at rt overnight, the solvent was evaporated and the resulting black oil was purified by flash chromatography on silica gel.

*4.2.19.1. Benzyl {[(R,S)-1-(benzyloxycarbonylamino)-pentyl(benzyloxy)-phosphoryl]oxy}acetate (**20a**).* Compound **20a** was prepared by the reaction of **16** and benzyl glycolate (0.32 g, 1.95 mmol). Flash chromatography: linear gradient of ethyl acetate in toluene. Yield 0.60 g (88%). Colourless oil, R_f=0.76 (S2). ^{1}H NMR (600 MHz, $CDCl_3$): mixture of diastereoisomers (~2:1), only signals of the major isomer are given: 0.86 (3H, t, J=7.1, CH_3), 1.25 and 1.32 (2H, m, CH_2), 1.32 and 1.42 (2H, m, CH_2), 1.56 and 1.87 (2H, m, CH_2), 4.18 (1H, m, N–CH–P), 4.50 (1H, dd, J=16.3 and 10.8, O–C*H*aHb–CO), 4.62 (1H, dd, J=16.3 and 11.5, O–CHa*H*b–CO), 5.06–5.19 (6H, m, 3×–CH_2–O), 5.18 (1H, d, J=9.5, NH), 7.29–7.36 (15H, m, 3×C_6H_5). ^{13}C NMR (150.9 MHz, $CDCl_3$): 13.84 (CH_3), 22.15 (CH_2), 27.85 (d, J(C,P)=12.8, CH_2), 29.56 (d, J(C,P)=2.1, CH_2), 48.21 (d, J(C,P)=156.3, N–CH–P), 62.21 (O–CH_2–CO), 67.08 (O–CH_2), 67.36 (O–CH_2), 67.80 (d, J(C,P)=7.4, O–CH_2), 127.99–128.67 (15×ArCH), 125.85 (d, J(C,P)=5.9, Ar–C), 136.30 (2×Ar–C), 156.14 (d, J(C,P)=5.7, O–CO–N), 168.57 (–CO–O). IR ($CHCl_3$, ν_{max} cm^{-1}) 3431, 3034, 1759, 1721, 1510, 1456, 1381, 1252, 1098, 1039, 1010, 1002, 698. HRMS (ESI) calcd for $C_{29}H_{34}NNaO_7P$ $[M+Na]^+$ 562.1971; found: 562.1964.

*4.2.19.2. Benzyl (2S)-2-{[(R,S)-1-(benzyloxycarbonylamino)-pentyl-(benzyloxy)phosphoryl]oxy} propanoate (**20b**).* Compound **20b** was prepared by the reaction of **16** and benzyl (*S*)-lactate (0.39 g, 1.95 mmol). Flash chromatography: linear gradient of ethyl acetate in toluene. Yield 0.64 g (86%). Colourless oil, R_f=0.84 (S2). ^{1}H NMR (600 MHz, $CDCl_3$): mixture of four diastereomers, assignment of individual isomers was not possible, only regions of signals are given: ~0.85 (3H, t, J=7.3, CH_3), 1.42–1.44 (3H, d, J=7.0, CH_3), ~1.25 (2H, m, CH_2), 1.30–1.40 (2H, m, CH_2), ~1.55 and ~1.85 (2H, m, CH_2), 4.10–4.25 (1H, m, N-CH-P), ~4.32 (1H, br q, J=7.0, O–CH–CO), 4.10–4.25 and 5.05–5.45 (4H, m, 2×O–CH_2–CO), 4.92–5.00 (2H, m, P–O–CH_2), 4.90, 5.21, 5.28 and 5.45 (1H, br dd, NH–CO), 7.29–7.39 (15H, m, 3×C_6H_5). ^{13}C NMR (150.9 MHz, $CDCl_3$): ~13.8 (CH_3), 18.9–20.3 (CH_3), ~22.1 (CH_2), 27.7–28.0 (CH_2), 29.3–29.8 (CH_2), 47.6–48.9 (N–CH–P), 66.77 (O–*C*H–CO), 66.9–68.0 (2×O–CH_2), 70.54, 70.71, 70.88 and 71.06 (P–O–CH_2), 127.79–128.62 (15×Ar–CH), 134.9–136.4 (3×Ar–C), 155.9–156.25 (O–CO–NH), 175.5 and 170.75–171.1 (CO–O). IR ($CHCl_3$, ν_{max} cm^{-1}) 3430, 3033, 1757, 1729, 1510, 1456, 1380, 1250, 1098, 1041, 999, 698. HRMS (ESI) calcd for $C_{30}H_{36}NNaO_7P$ $[M+Na]^+$ 576.2127; found: 576.2121.

*4.2.19.3. Methyl {[(R,S)-1-(benzyloxycarbonylamino)-pentyl(benzyloxy)-phosphoryl]oxy}acetate (**22a**).* Compound **22a** was prepared by the reaction of **16** and methyl glycolate (0.18 g, 1.95 mmol). Flash chromatography: linear gradient of ethyl acetate in toluene. Yield 0.51 g (85%). Colourless oil, R_f=0.54 (S2). ^{1}H NMR (600 MHz, $CDCl_3$): mixture of diastereoisomers ~2:1, only signals of the major isomer are given: 0.87 (3H, t, J=7.1, CH_3), 1.27 and 1.34 (2H, m, CH_2), 1.34 and 1.44 (2H, m, CH_2), 1.57 and 1.88 (2H, m, CH_2), 3.75 (3H, s, OCH_3), 4.20 (1H, m, N–CH–P), 4.47 (1H, dd, J=16.2 and 11.0, O–C*H*aHb–CO), 4.60 (1H, dd, J=16.2 and 11.7, O–CHa*H*b–CO), 5.08 and 5.12 (2H, d, J_{gem}=12.2, OCH_2), 5.13 (2H, m, OCH_2), 5.24 (1H, br dd, J=10.2 and 1.5, NH), 7.30–7.38 (10H, m, 2×C_6H_5). ^{13}C NMR (150.9 MHz, $CDCl_3$): 13.81 (CH_3), 22.14 (CH_2), 27.84 (d, J(C,P)=12.8, CH_2), 29.54 (CH_2), 48.17 (d, J(C,P)=156.2, N-CH-P), 52.39 (OCH_3), 62.07 (d, J(C,P)=6.3, O–CH_2), 67.04 (O–CH_2), 67.79 (d, J(C,P)=7.4, O–CH_2), 127.92–128.59 (10×Ar–CH), 135.85 (d, J(C,P)=5.9, Ar–C), 136.28 (Ar–C), 156.14 (d, J(C,P)=5.9, O–CO–N), 169.10 (d, J(C,P)=4.4, O–CO). IR ($CHCl_3$, ν_{max} cm^{-1}) 3431, 3031, 1762, 1721, 1510, 1456, 1442, 1384, 1234, 1101, 1039, 1010, 697. HRMS (ESI) calcd for $C_{23}H_{29}NO_7P$ $[M-H]^+$ 462.1682; found: 462.1674.

*4.2.19.4. Methyl (2S)-2-{[(R,S)-1-(benzyloxycarbonylamino)-pentyl-(benzyloxy)phosphoryl]oxy} propanoate (**22b**).* Compound **22b** was prepared by the reaction of **16** and methyl (*S*)-lactate (0.20 g, 1.95 mmol). Flash chromatography: linear gradient of ethyl acetate in toluene. Yield 0.50 g (82%). Colourless oil, R_f=0.61 (S2). ^{1}H NMR (600 MHz, $CDCl_3$): mixture of four diastereomers, assignment of individual isomers was not possible, only regions of signals are given: 0.86–0.87 (3H, t, J=7.0, CH_3), 1.41–1.49 (3H, d, J=7.0, CH_3), 1.25–1.33 (2H, m, CH_2), 1.33–1.43 (2H, m, CH_2), ~1.55 and ~1.85 (2H, m, CH_2), 3.67–3.74 (3H, s, OCH_3), 4.12–4.25 (1H, m, N–CH–P), 4.88–4.98 (1H, m, O–CH–CO), 5.05–5.21 (4H, m, 2×O–CH_2), 4.88, 5.16, 5.29 and 5.44 (1H, dd, NH–CO), 7.30–7.37 (10H, m, 2×C_6H_5). ^{13}C NMR (150.9 MHz, $CDCl_3$): 13.81–13.84 (CH_3), 18.96, 19.02, 19.08 and 19.37 (CH_3), 22.0–22.2 (CH_2), 27.7–28.0 (CH_2), 29.3–29.9 (CH_2), 47.6–48.8 (N-CH-P), 52.6–52.4 (OCH_3), 66.9–68.1 (2×O–CH_2), 70.49, 70.67, 70.81 and 70.93 (d, J~6.5, O–*C*H–CO), 127.8–128.6 (10×Ar–CH), 135.8–136.4 (2×Ar–C), 155.9–156.2 (O–CO–NH), 171.4–171.7 (CO–O). IR ($CHCl_3$, ν_{max} cm^{-1}) 3430, 3031, 1751, 1721, 1511, 1456, 1439, 1380, 1247, 1055, 1041, 997, 697. HRMS (ESI) calcd for $C_{24}H_{32}NNaO_7P$ $[M+Na]^+$ 500.1814; found: 500.1807.

*4.2.19.5. {[((R,S)-1-Benzyloxycarbonylamino)-pentyl(benzyloxy)-phosphoryl]oxy}acetamide (**24a**).* Compound **24a** was prepared by the reaction of **16** and glycolamide (0.15 g, 1.95 mmol). Flash chromatography: linear gradient of ethanol in chloroform. Yield 0.42 g (74%). Pale yellow oil, R_f=0.23 (S2). ^{1}H NMR (600 MHz, $CDCl_3$): mixture of diastereoisomers ~2:1, only signals of the major isomer are given: 0.87 (3H, t, J=7.3, CH_3), 1.24–1.32 (2H, m, CH_2), 1.31–1.44 (2H, m, CH_2), 1.49 and 1.89 (2H, m, CH_2), 4.11 (1H, m, N–

CH–P), 4.14 (1H, dd, *J*=15.2 and 5.6, O–C*Ha*Hb–CO), 4.53 (1H, dd, *J*=15.2 and 7.8, O–CHa*Hb*–CO), 5.08 (4H, m, 2×O–CH_2), 5.93 (1H, d, *J*=9.8, NH), 5.96 and 6.88 (2H, br, $CONH_2$), 7.30–7.36 (10H, m, 2×C_6H_5). ^{13}C NMR (150.9 MHz, $CDCl_3$): 13.74 (CH_3), 21.99 (CH_2), 27.82 (d, *J*(C,P)=13.4, CH_2), 28.55 (d, *J*(C,P)=3.0, CH_2), 48.41 (d, *J*(C,P)=156.4, N–CH–P), 63.42 (d, *J*(C,P)=7.6, O–CH_2), 67.33 (O–CH_2), 68.51 (d, *J*(C,P)=7.2, O–CH_2), 127.93–128.92 (10×Ar–CH), 135.57 (d, *J*(C,P)=4.6, Ar–C), 136.08 (Ar–C), 156.37 (d, *J*(C,P)=3.5, O–CO–N), 169.86 (d, *J*(C,P)=8.8, CO–NH_2). IR ($CHCl_3$, ν_{max} cm^{-1}) 3484, 3431, 1718, 1694, 1579, 1511, 1456, 1403, 1380, 1241, 1066, 1037, 970, 698. HRMS (ESI) calcd for $C_{22}H_{29}N_2NaO_6P$ $[M+Na]^+$ 471.1661; found: 471.1651.

*4.2.19.6. (2S)-2-{[((R,S)-1-Benzyloxycarbonylamino)-pentyl(benzyloxy)-phosphoryl]oxy} propionamide (**24b**).* Compound **24b** was prepared by the reaction of **16** and (*S*)-lactamide (0.17 g, 1.95 mmol). Flash chromatography: linear gradient of ethanol in chloroform. Yield 0.45 g (70%). Pale yellow oil, R_f=0.29 (S2). ^{1}H NMR (600 MHz, DMSO): mixture of four diastereomers, assignment of individual isomers was not possible, only regions of signals are given: 0.82–0.83 (3H, t, *J*=7.3, CH_3), ~1.22 (2H, m, CH_2), 1.28–1.35 (3H, d, *J*=6.8, CH_3), ~1.32 (2H, m, CH_2), 1.55 and 1.69 (2H, m, CH_2), 3.90–4.02 (1H, m, N–CH–P), 4.57–4.66 (1H, m, O–CH–CO), 5.01–5.09 (4H, m, O–CH_2), 7.30–7.38 (10H, m, 2×C_6H_5), 7.40–7.46 (2H, CO–NH_2), 7.72–7.78 (1H, d, *J*=9.5, NH). ^{13}C NMR (150.9 MHz, DMSO): 14.01–14.03 (CH_3), 19.98–20.32 (CH_3), ~21.70 (CH_2), 27.76–28.22 (2×CH_2), 47.11–48.39 (N–CH–P), 65.84–66.02 (O–CH_2), 67.16–67.36 (O–CH_2), 71.70–72.16 (O–*C*H–CO), 127.77–128.68 (10×Ar–CH), 136.66–137.23 (2×Ar–C), 156.46–156.67 (O–CO–NH), 172.43–172.66 ($CONH_2$). IR ($CHCl_3$, ν_{max} cm^{-1}) 3430, 1712, 1697, 1587, 1511, 1456, 1378, 1088, 1038, 1007, 999, 980, 698. HRMS (ESI) calcd for $C_{23}H_{31}N_2NaO_6P$ $[M+Na]^+$ 485.1817; found: 485.1810.

*4.2.19.7. Benzyl (2S)-2-[({[((R,S)-1-benzyloxycarbonylamino)-pentyl-(benzyloxy)phosphoryl]oxy} acetyl)amino]-3-methylbutanoate (**26a**).* Compound **26a** was prepared by the reaction of **16** and dipeptide **17a** (0.51 g, 1.95 mmol). Flash chromatography: linear gradient of ethyl acetate in toluene. Yield 0.58 g (70%). Colourless oil, R_f=0.43 (S2). ^{1}H NMR (600 MHz, $CDCl_3$): mixture of four diastereomers, assignment of individual isomers was not possible; only regions of signals are given: ~0.87 (3H, t, *J*=7.0, CH_3), 0.84–0.93 (6H, d, *J*=7.0, CH_3), ~1.26 and ~1.32 (2H, m, CH_2), ~1.33 and ~1.44 (2H, m, CH_2), ~1.82 and ~1.89 (2H, m, CH_2), 2.17–2.24 (1H, m, –$CH<$), 4.15–4.20 (1H, m, N–CH–P), 4.25–4.43 (2H, O–CH_2–CO), ~4.57 (1H, m, N–CH–CO), 4.93–5.12 (1H, br d, CO–NH), 5.06–5.18 (6H, m, O–CH_2–CO), 7.07–7.23 (1H, br d, CO–NH), 7.30–7.37 (15H, m, 3×C_6H_5). ^{13}C NMR (150.9 MHz, $CDCl_3$): ~13.8 (CH_3), 17.6–19.0 (2×CH_3), 22.0–22.1 (CH_2), 27.7–27.9 (CH_2), 28.7–29.2 (CH_2), 30.9–31.2 (–$CH<$), 47.4–48.0 (N–CH–P), 56.9–57.1 (N–*C*H–CO), 63.6–64.0 (O–CH_2–CO), 66.9–68.9 (3×O–CH_2), 127.9–129.0 (15×Ar–CH), 135.2–136.1 (3×Ar–C), 156.0–156.2 (O–CO–NH), 167.1–167.2 (CO–NH), 171.2–171.4 (CO–O). IR ($CHCl_3$, ν_{max} cm^{-1}) 3431, 1735, 1723, 1685, 1510, 1456, 1393, 1376, 1247, 1198, 1148, 1067, 1040, 1009, 1000, 970, 698. HRMS (ESI) calcd for $C_{34}H_{43}N_2NaO_8P$ $[M+Na]^+$ 661.2655; found: 661.2650.

*4.2.19.8. Benzyl (2S)-2-{[(2S)-2-{[((R,S)-1-benzyloxycarbonylamino)-pentyl(benzyloxy) phosphoryl]oxy}propanoyl]amino}-3-methylbutanoate (**26b**).* Compound **26b** was prepared by the reaction of **16** and dipeptide **17b** (0.55 g, 1.95 mmol). Flash chromatography: linear gradient of ethyl acetate in toluene. Yield 0.60 g (72%). Colourless oil, R_f=0.33 (S2). ^{1}H NMR (600 MHz, $CDCl_3$): mixture of four diastereomers, assignment of individual isomers was not possible; only regions of signals are given: 0.80–0.83 (3H, t, *J*=7.0, CH_3), 0.83–0.85 (6H, d, *J*=7.0, CH_3), 1.27–1.31 (3H, d, *J*=6.8, CH_3), ~1.22 (2H, m, CH_2), ~1.19 and ~1.33 (2H, m, CH_2), ~1.57 and ~1.68 (2H, m, CH_2), 2.05–2.12 (1H, m, –$CH<$), 3.88–3.99 (1H, m, N–CH–P), 4.20–4.26 (1H, m, N–CH–CO), 4.86–4.90 (1H, m, O–CH–CO), 5.00–5.17 (6H, m, 3×O–CH_2), 7.30–7.39 (15H, m, 3×C_6H_5), 7.60, 7.70, 7.72 and 7.73 (1H, d, *J*~9.5, CO–NH), 8.16, 8.21, 8.23 and 8.28 (1H, d, *J*~8.2, CO–NH). ^{13}C NMR (150.9 MHz, $CDCl_3$): 14.0–14.3 (CH_3), 18.1–19.1 (2×CH_3), 19.8–20.2 (CH_3), ~21.7 (CH_2), ~27.9 (CH_2), ~28.2 (CH_2), 30.0–30.4 (–$CH<$), 47.2–48.7 (N–CH–P), 56.87, 57.52, 57.60 and 57.68 (N–*C*H–CO), 65.8–67.2 (3×O–CH_2), 71.3–71.7 (O–*C*H–CO), 127.7–128.7 (15×Ar–CH), 136.0–137.2 (3×Ar–C), 156.4–156.6 (O–CO–NH), 170.7–171.5 (CO–NH and CO–O). IR ($CHCl_3$, ν_{max} cm^{-1}) 3429, 3033, 1736, 1724, 1682, 1514, 1456, 1392, 1376, 1247, 999, 698. HRMS (ESI) calcd for $C_{35}H_{45}N_2NaO_8P$ $[M+Na]^+$ 675.2811; found: 675.2808.

*4.2.19.9. Methyl (2S)-2-[({[((R,S)-1-benzyloxycarbonylamino)-pentyl-(benzyloxy)phosphoryl]oxy} acetyl)amino]-3-methylbutanoate (**28a**).* Compound **28a** was prepared by the reaction of **16** and dipeptide **18a** (0.37 g, 1.95 mmol). Flash chromatography: linear gradient of ethyl acetate in toluene. Yield 0.60 g (83%). Colourless oil, R_f=0.36 (S2). ^{1}H NMR (600 MHz, $CDCl_3$): mixture of four diastereomers, assignment of individual isomers was not possible, only regions of signals are given: 0.86–0.89 (3H, t, *J*=7.3, CH_3), 0.89–0.96 (6H, d, *J*=7.0, CH_3), ~1.26 and ~1.33 (2H, m, CH_2), ~1.33 and ~1.45 (2H, m, CH_2), 1.56–1.60 and 1.82–1.90 (2H, m, CH_2), 2.15–2.23 (1H, m, –$CH<$), 3.70–3.72 (3H, s, OCH_3), 4.15–4.20 (1H, m, N–CH–P), 4.25–4.57 (2H, dd, *J* ~ 15.0 and 8.0, O–CH_2–CO), 4.47–4.52 (1H, N–CH–CO), 5.06–5.17 (4H, m, 2×O–CH_2), 4.96, 5.01 and 5.13 (1H, br d, *J*~10.0, O–CO–NH), 7.06, 7.07, 7.17 and 7.21 (1H, br d, *J*~9.0, CO–NH), 7.31–7.39 (10H, m, 2×C_6H_5). ^{13}C NMR (150.9 MHz, $CDCl_3$): 13.75–13.83 (CH_3), 17.79–18.07 and 18.84–19.01 (2×CH_3), 22.06–22.13 (CH_2), 27.75–27.87 (CH_2), 28.74–29.15 (CH_2), 30.87–31.11 (–$CH<$), 47.44–48.75 (N–CH–P), 52.08–52.18 (OCH_3), 56.89–57.14 (N–*C*H–CO), 63.57–64.00 (O–CH_2–CO), 67.25–67.45 and 68.61–68.87 (2×O–CH_2), 127.94–128.92 (10×Ar–CH), 135.46–136.05 (2×Ar–C), 156.00–156.20 (O–CO–NH), 167.03–167.20 (CO–NH), 171.80–171.99 (CO–O). IR ($CHCl_3$, ν_{max} cm^{-1}) 3431, 1740, 1722, 1684, 1510, 1456, 1439, 1375, 1067, 1039, 1009, 1000, 698. HRMS (ESI) calcd for $C_{28}H_{38}N_2O_8P$ $[M-H]^+$ 561.2366; found: 561.2355.

*4.2.19.10. Methyl (2S)-2-{[(2S)-2-{[((R,S)-1-benzyloxycarbonylamino)-pentyl(benzyloxy) phosphoryl]oxy}propanoyl]amino}-3-methylbutanoate (**28b**).* Compound **28b** was prepared by the reaction of **16** and dipeptide **18b** (0.39 g, 1.95 mmol). Flash chromatography: linear gradient of ethyl acetate in toluene. Yield 0.58 g (78%). Colourless oil, R_f=0.44 (S2). ^{1}H NMR (600 MHz, $CDCl_3$): mixture of four diastereomers, only regions of signals are given: ~0.86 (3H, t, *J*=7.3, CH_3), 0.88–0.94 (6H, d, *J*=7.0, CH_3), 1.54, 1.48, 1.45 and 1.40 (3H, d, *J*=7.0, CH_3), ~1.25 and ~1.33 (2H, m, CH_2), ~1.32 and ~1.46 (2H, m, CH_2), ~1.50 and ~1.83 (2H, m, CH_2), 2.15–2.22 (1H, m, –$CH<$), 3.72, 3.70, 3.68 and 3.66 (3H, s, OCH_3), 4.15–4.22 (1H, m, N–CH–P), 4.46–4.50 (1H, m, N–CH–CO), 4.81–4.90 (1H, br d, O–CH–CO), 5.05–5.15 (4H, m, O–CH_2), 5.05, 5.10, 5.25 and 5.45 (1H, br d, *J* ~ 10.0, O–CO–NH), 6.99, 7.06, 7.09 and 7.39 (1H, br d, *J*~9.0, CO–NH), 7.30–7.38 (10H, m, 2×C_6H_5). ^{13}C NMR (150.9 MHz, $CDCl_3$): 13.74–13.78 (CH_3), 17.67–17.89 and 18.86–18.95 (2×CH_3), 19.86–20.17 (CH_3), 22.02–22.08 (CH_2), 27.73–27.93 (CH_2), 28.84–29.44 (CH_2), 30.90–31.19 (–$CH<$), 47.61–49.14 (N–CH–P), 52.09–52.25 (OCH_3), 56.74–57.03 (N–*C*H–CO), 67.12–67.25 and 68.25–68.64 (2×O–CH_2), 72.95–73.35 (O–CH–CO), 127.91–128.72 (10×Ar–CH), 135.61–136.22 (2×Ar–C), 155.94–156.22 (O–CO–NH), 170.20–170.47 (CO–NH), 171.93–172.39 (CO–O). IR ($CHCl_3$, ν_{max} cm^{-1}) 3430, 1741, 1722, 1684, 1512, 1456, 1439, 1375, 1088, 1026, 998, 697. HRMS (ESI) calcd for $C_{29}H_{41}N_2NaO_8P$ $[M+Na]^+$ 599.2498; found: 599.2492.

*4.2.19.11. (2S)-2-[({[((R,S)-1-Benzyloxycarbonylamino)-pentyl(benzyloxy)phosphoryl]oxy}acetyl) amino]-3-methylbutanamide (**30a**).* Compound **30a** was prepared by the reaction of **16** and dipeptide

19a (0.34 g, 1.95 mmol). Flash chromatography: linear gradient of ethanol in chloroform. Yield 0.53 g (76%). Semisolid, R_f=0.40 (S4). ^{1}H NMR (600 MHz, DMSO): mixture of four diastereomers, assignment of individual isomers was not possible, only regions of signals are given: 0.80–0.86 (9H, m, 3×CH_3), ~1.22 (2H, m, CH_2), ~1.22 and ~1.34 (2H, m, CH_2), ~1.58 and ~1.71 (2H, m, CH_2), 1.97–2.02 (1H, m, –CH<), 3.95–4.02 (1H, N–CH–CO), 4.16–4.20 (1H, m, N–CH–P), 4.40–4.50 (2H, dd, O–CH_2–CO), 5.03–5.11 (4H, m, 2×O–CH_2), ~7.14 and ~7.52 (2H, d, J=1.8, $CONH_2$), 7.30–7.38 (10H, m, 2×C_6H_5), 7.73, 7.74, 7.75, 7.76, 7.81, 7.83, 7.84 and 7.88 (2H, d, 2×CO–NH). ^{13}C NMR (150.9 MHz, DMSO): 14.01–14.02 (CH_3), 17.95–18.03 and 19.49 (2×CH_3), 21.74 (CH_2), 27.89–28.39 (2×CH_2), 30.62–30.68 (–CH<), 47.84, 47.88, 47.90 and 47.96 (d, J(C,P)~156, N–CH–P), 57.36 and 57.39 (N–*C*H–CO), 63.80–63.97 (O–CH_2–CO), 65.89–65.96 and 67.11–67.28 (2×O–CH_2), 127.83–128.67 (10×Ar–CH), 136.68–137.23 (2×Ar–C), 156.44–156.50 (O–CO–NH), 167.25–167.43 (CO–NH), 172.74 and 172.76 (CO–NH_2). IR ($CHCl_3$, ν_{max} cm^{-1}) 3428, 3410, 3323, 1717, 1695, 1676, 1593, 1512, 1456, 1391, 1375, 1066, 1041, 1009, 1000, 698. HRMS (ESI) calcd for $C_{27}H_{37}N_3O_7P$ [M-H]$^+$ 546.2369; found: 546.2375.

*4.2.19.12. (2S)-2-{[(2S)-2-{[((R,S)-1-Benzyloxycarbonylamino)-pentyl-(benzyloxy)phosphoryl]oxy} propanoyl]amino}-3-methylbutanamide (**30b**).* Compound **30b** was prepared by the reaction of **16** and dipeptide **19b** (0.37 g, 1.95 mmol). Flash chromatography: linear gradient of ethanol in chloroform. Yield 0.59 g (82%). Semisolid, R_f=0.21 (S2). ^{1}H NMR (600 MHz, DMSO): mixture of four diastereomers; assignment of individual isomers was not possible, only regions of signals are given: ~0.82 (3H, t, J=7.3, CH_3), 0.81–0.86 (6H, d, J=7.0, CH_3), ~1.22 (2H, m, CH_2), ~1.21 and ~1.33 (2H, m, CH_2), 1.30–1.35 (3H, d, J=6.8, CH_3), 1.50–1.69 (2H, m, CH_2), 1.95–2.01 (1H, m, –CH<), 3.90–4.01 (1H, m, N–CH–P), 4.15 (1H, dd, J=9.0 and 6.4, N–CH–CO), 4.82 (1H, m, O–CH–CO), 5.01–5.09 (4H, m, O–CH_2), 7.11–7.15 and 7.47–7.51 (2H, d, J~2.0, $CONH_2$), 7.30–7.38 (10H, m, 2×C_6H_5), 7.64, 7.68, 7.70, 7.72, 7.73, 7.74, 7.75 and 7.79 (2H, d, J~9–10, O–CO–NH+CO–NH). ^{13}C NMR (150.9 MHz, DMSO): 13.98–14.05 (CH_3), 17.99–18.15 and 19.46–19.51 (2×CH_3), 19.92–20.29 (CH_3), 21.69–21.74 (CH_2), 27.72–28.40 (2×CH_2), 30.72–30.97 (–CH<), 47.28–48.57 (N–CH–P), 57.24–57.45 (N–*C*H–CO), 65.86–65.98 and 67.02–67.34 (2×O–CH_2), 71.95–72.43 (O–*C*H–CO), 127.72–129.16 (10×Ar–CH), 136.75–137.61 (2×Ar–C), 156.43–156.51 (O–CO–NH), 170.10–170.26 (CO–NH), 172.69–172.75 (CO–NH_2). IR ($CHCl_3$, ν_{max} cm^{-1}) 3430, 3410, 3329, 1717, 1691, 1680, 1512, 1456, 1392, 1375, 1242, 1086, 1038, 1025, 1007, 999, 697. HRMS (ESI) calcd for $C_{28}H_{40}N_3NaO_7P$ [M+Na]$^+$ 584.2502; found: 584.2502.

*4.2.20. General procedure for the preparation of compounds **6**, **10**, **21a**, **21b**, **23a**, **23b**, **25a**, **25b**, **27a**, **27b**, **29a**, **29b**, **31a** and **31b***

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 an atmosphere of hydrogen (15 psi) at rt overnight. The catalyst was filtered off through Celite and the filter was washed with 100 mL of methanol. The filtrate was concentrated in vacuo and the crude product was purified using preparative RP-HPLC. Finally, the target compounds were lyophilised from water.

*4.2.20.1. {[(R,S)-1-Aminopentyl(methoxy)phosphoryl]oxy}acetamide (**6**).* Phosphonate **6** was prepared by hydrogenolysis of **7** (0.3 g, 0.81 mmol). Yield 113.5 mg (59%). Semisolid, t_R=34.8 min, G1. ^{1}H NMR (600 MHz, DMSO): mixture of diastereoisomers (~2:1), only signals of the major isomer are given: 0.87 (3H, t, J=7.3, CH_3), 1.29 (2H, m, CH_2), 1.37 and 1.43 (2H, m, CH_2), 1.68 and 1.79 (2H, m, CH_2), 3.74 (1H, m, N–CH–P), 3.78 (3H, d, J(H,P)=10.9, OCH_3), 4.50 (2H, m, CO–CH_2–O), 7.58 and 7.72 (2H, br, $CONH_2$), 8.47 (2H, br, NH_2). ^{13}C NMR (150.9 MHz, DMSO): 13.83 (CH_3), 21.92 (CH_2), 27.37 (d, J(C,P)=8.6, CH_2), 27.76 (d, J(C,P)=2.2, CH_2), 46.34 (d, J(C,P)=154.0, N–CH–P), 53.50 (d, J(C,P)=7.0, OCH_3), 64.26 (d, J(C,P)=6.5, O–CH_2–CO), 169.80 (d, J(C,P)=4.4, N–*C*O–C). IR (KBr, ν_{max} cm^{-1}) 3190, 1685, 1627, 1205, 1178, 1044, 801. HRMS (ESI) calcd for $C_8H_{19}N_2NaO_4P$ [M+Na]$^+$ 261.0980; found: 261.0974.

*4.2.20.2. (2S)-2-[({[(R,S)-1-Aminopentyl(methoxy)phosphoryl]oxy} acetyl)amino]-3-methylbutanoic acid (**10**).* Phosphonate **10** was prepared by hydrogenolysis of **9** (0.6 g, 1.1 mmol). Yield 179.7 mg (50%). Semisolid, t_R=43.4 min, G1. ^{1}H NMR (600 MHz, $CDCl_3$): Mixture of four diastereomers, assignment of individual isomers was not possible, only regions of signals are given: 0.90–0.91 (3H, t, J=7.3, CH_3), 0.94–0.99 (6H, d, J=7.0, 2×CH_3), ~1.35 (2H, m, CH_2), 1.43–1.58 (2H, m, CH_2), 1.87–2.00 (2H, m, CH_2), 3.60–3.70 (1H, m, N–CH–P), 3.82–3.89 (3H, d, J(H,P)~11.0, OCH_3), 4.28–4.43 (1H, m, N–CH–CO), 4.63–5.03 (2H, m, O–CH_2–CO), 7.51, 7.61, 7.93 and 8.04 (1H, br d, J~7.5, CO–NH). ^{13}C NMR (150.9 MHz, $CDCl_3$): ~13.3 (CH_3), 17.5–18.8 (2×CH_3), ~21.8 (CH_2), ~27.3 (CH_2), ~27.6 (CH_2), 47.2–48.2 (N–CH–P), 53.0–54.1 (OCH_3), 57.7–58.8 (N–*C*H–CO), 64.0–66.0 (O–CH_2), 168.5–170.0 (CO–NH), ~175.0 (COOH). IR (KBr, ν_{max} cm^{-1}) 3300, 3185, 1730, 1670, 1554, 1203, 1047, 799. HRMS (ESI) calcd for $C_{13}H_{28}N_2O_6P$ [M+H]$^+$ 339.1685; found: 339.1679.

*4.2.20.3. {[(R,S)-1-Aminopentyl(hydroxy)phosphoryl]oxy}acetic acid (**21a**).* Phosphonate **21a** was prepared by hydrogenolysis of **20a** (0.35 g, 0.81 mmol). Yield 102.3 mg (70%). Colourless powder, t_R=28.4 min, G1. ^{1}H NMR (600 MHz, DMSO): 0.86 (3H, d, J=7.3, CH_3), 1.26 (2H, m, CH_2), 1.36 and 1.42 (2H, m, CH_2), 1.59 and 1.77 (2H, m, CH_2), 3.07 (1H, m, N–CH–P), 4.40 (2H, d, J=10.9, O–CH_2–CO), 8.02 (2H, br, NH_2). ^{13}C NMR (150.9 MHz, DMSO): 14.01 (CH_3), 22.22 (CH_2), 27.95 (d, J(C,P)=8.1, CH_2), 28.55 (CH_2), 47.79 (d, J(C,P)=145.2, N–CH–P), 61.91 (O–CH_2), 172.35 (COOH). IR (KBr, ν_{max} cm^{-1}) 3265, 3200–2700 br, 2658, 2568, 1720, 1620, 1535, 1204, 1180, 1058, 1012, 949, 893, 817. HRMS (ESI) calcd for $C_7H_{15}NO_5P$ [M−H]$^+$ 224.0688; found: 224.0693.

Alternatively, phosphonate **21a** was prepared from diester **8**. Compound **8** (0.5 g, 1.2 mmol) was dissolved in 5 mL of dry dichloromethane and TMSBr (0.59 g, 3.9 mmol) was added in one portion. The reaction mixture was stirred for 3 h at rt until the starting material disappeared (TLC monitoring). The reaction was quenched by adding 5 mL of 1 M HCl. The organic phase was separated, washed with brine and dried over Na_2SO_4. The solvent was removed in vacuo to give 0.5 g of an oil, which was hydrogenated according to the method described above. The crude product was purified by RP-HPLC. Yield 2.8 mg (3% over two steps).

*4.2.20.4. (2S)-2-{[(R,S)-1-Aminopentyl(hydroxy)phosphoryl]oxy}propanoic acid (**21b**).* Phosphonate **21b** was prepared by hydrogenolysis of **20b** (0.25 g, 0.45 mmol). Yield 74.5 mg (69%). Colourless powder, t_R=31.5 min, G1. ^{1}H NMR (600 MHz, D_2O): 0.90 (3H, d, J=7.3, CH_3), 1.53 (3H, d, J=7.0, CH_3), 1.36 (2H, m, CH_2), 1.38 and 1.48 (2H, m, CH_2), 1.73 and 1.92 (2H, m, CH_2), 3.33 (1H, m, N–CH–P), 4.86 (1H, dq, J=8.3 and 7.0(3×), O–CH–CO). ^{13}C NMR (150.9 MHz, D_2O): 15.67 (CH_3), 22.03 (d, J=4.4, CH_3), 24.32 (CH_2), 30.24 (d, J=9.2, CH_2), 30.28 (CH_2), 51.32 (d, J=148.7, N–CH–P), 72.11 (d, J=6.0, O–CH<), 179.21 (d, J=4.3, COOH). IR (KBr, ν_{max} cm^{-1}) 3200–2700 br, 2629, 2594, 1719, 1699, 1639, 1536, 1190, 1177, 1049, 989, 806. HRMS (ESI) calcd for $C_8H_{17}N_2O_5P$ [M−H]$^+$ 238.0844; found: 238.0849.

*4.2.20.5. Methyl {[(R,S)-1-Aminopentyl(hydroxy)phosphoryl]oxy}acetate (**23a**).* Phosphonate **23a** was prepared by hydrogenolysis of **22a** (0.45 g, 0.97 mmol). Yield 182 mg (78%). Colourless powder, t_R=33.2 min, G1. ^{1}H NMR (600 MHz, DMSO): 0.86 (3H, t, J=7.3, CH_3), 1.25 (2H, m, CH_2), 1.25 and 1.42 (2H, m, CH_2), 1.55 and 1.74 (2H, m, CH_2), 2.91 (1H, m, N–CH–P), 3.65 (3H, s, OCH_3), 4.41 (1H, dd, J=16.4 and 9.8,

O–C*Ha*Hb–CO), 4.43 (1H, dd, *J*=16.4 and 9.8, O–CHa*Hb*–CO), 7.83 (2H, br, NH). ^{13}C NMR (150.9 MHz, DMSO): 14.04 (CH_3), 22.28 (CH_2), 28.11 (d, *J*(C,P)=8.0, CH_2), 28.85 (CH_2), 48.25 (d, *J*(C,P)=141.3, N–CH–P), 51.95 (OCH_3), 61.62 (d, *J*(C,P)=5.3, O–CH_2), 171.43 (d, *J*(C,P)=4.6, O–CO). IR (KBr, ν_{max} cm^{-1}) 3200–2700 br, 2647, 1759, 1735, 1630, 1536, 1230, 1107, 1073. HRMS (ESI) calcd for $C_8H_{19}NO_5P$ $[M+H]^+$ 240.1001; found: 240.0996.

*4.2.20.6. Methyl (2S)-2-{[(R,S)-1-aminopentyl(hydroxy)phosphoryl]-oxy}propanoate (**23b**).* Phosphonate **23b** was prepared by hydrogenolysis of **22b** (0.4 g, 0.84 mmol). Yield 154.2 mg (73%). Colourless powder, t_R=36.6 min, G1. ^{1}H NMR (600 MHz, $CDCl_3$): mixture of diastereoisomers ~3:2, only signals of the major isomer are given: 0.90 (3H, t, *J*=7.3, CH_3), 1.36 (2H, m, CH_2), 1.40–1.48 (2H, m, CH_2), 1.51 (3H, d, *J*=7.0, CH_3), 1.74 and 1.91 (2H, m, CH_2), 3.29 (1H, ddd, *J*=13.2, 9.0 and 5.2, N–CH–P), 3.79 (3H, s, OCH_3), 4.88 (1H, m, O–CH–CO). ^{13}C NMR (150.9 MHz, $CDCl_3$): 15.69 (CH_3), 21.80 (d, *J*(C,P)=4.7, CH_3), 24.35 (CH_2), 30.30 (d, *J*(C,P)=8.9, CH_2), 30.44 (d, *J*(C,P)=1.9, CH_2), 51.24 (d, *J*(C,P)=146.6, N–CH–P), 55.73 (OCH_3), 72.69 (d, *J*(C,P)=6.0, O–CH<), 177.70 (d, *J*(C,P)=3.8, O–CO). IR (KBr, ν_{max} cm^{-1}) 3200–2700 br, 2608, 1755, 1739, 1638, 1532, 1439, 1380, 1212, 1162, 1072, 1057, 982. HRMS (ESI) calcd for $C_9H_{20}NNaO_5P$ $[M+Na]^+$ 276.0977; found: 276.0972.

*4.2.20.7. {[(R,S)-1-Aminopentyl(hydroxy)phosphoryl]oxy}acetamide **25a**.* Phosphonate **25a** was prepared by hydrogenolysis of **24a** (0.25 g, 0.56 mmol). Yield 95.1 mg (77%). Colourless powder, t_R=21.5 min, G1. ^{1}H NMR (600 MHz, DMSO): 0.86 (3H, t, *J*=7.4, CH_3), 1.27 (2H, m, CH_2), 1.37 and 1.43 (2H, m, CH_2), 1.60 and 1.77 (2H, m, CH_2), 3.14 (1H, m, N–CH–P), 4.29 (2H, d, *J*=9.6, O–CH_2–CO), 7.52 and 7.66 (2H, br, $CONH_2$), 8.16 (2H, br, NH_2). ^{13}C NMR (150.9 MHz, DMSO): 13.95 (CH_3), 22.13 (CH_2), 27.82 (d, *J*(C,P)=8.4, CH_2), 28.30 (CH_2), 47.52 (d, *J*(C,P)=146.4, N–CH–P), 67.20 (d, *J*(C,P)=5.9, O–CH_2), 172.11 (d, *J*(C,P)=5.3, CO–NH_2). IR (KBr, ν_{max} cm^{-1}) 3383, 3238, 3205, 3200–2700 br, 2694, 2621, 2584, 1677, 1639, 1541, 1213, 1166, 1088, 1060. HRMS (ESI) calcd for $C_7H_{16}N_2O_4P$ $[M-H]^+$ 223.0848; found: 223.0852.

*4.2.20.8. (2S)-2-{[(R,S)-1-Aminopentyl(hydroxy)phosphoryl]oxy}-propionamide (**25b**).* Phosphonate **25b** was prepared by hydrogenolysis of **24b** (0.4 g, 0.87 mmol). Yield 128.2 mg (64%). Colourless powder, t_R=29.4 min, G1. ^{1}H NMR (600 MHz, DMSO): 0.86 (3H, t, *J*=7.3, CH_3), 1.27 (2H, m, CH_2), 1.39 (2H, m, CH_2), 1.39 (3H, d, *J*=6.8, CH_3), 1.61 and 1.77 (2H, m, CH_2), 3.26 (1H, m, N–CH–P), 4.67 (1H, qd, *J*=6.8(3×) and 6.6, O–CH–CO), 7.47 and 7.58 (2H, br, $CONH_2$), 8.28 (3H, br, NH_3). ^{13}C NMR (150.9 MHz, DMSO): 13.92 (CH_3), 20.46 (d, *J*(C,P)=4.0, CH_3), 22.07 (CH_2), 27.67 (d, *J*(C,P)=9.2, CH_2), 28.00 (CH_2), 47.54 (d, *J*(C,P)=150.7, N–CH–P), 70.63 (d, *J*(C,P)=6.3, O–CH<), 174.31 (d, *J*(C,P)=4.3, CO–NH_2). IR (KBr, ν_{max} cm^{-1}) 3389, 3242, 3200–2700 br, 2696, 2615, 1679, 1655, 1607, 1530, 1205, 1072, 973. HRMS (ESI) calcd for $C_8H_{19}N_2NaO_4P$ $[M+Na]^+$ 261.0980; found: 261.0975.

*4.2.20.9. (2S)-2-[({[(R,S)-1-Aminopentyl(hydroxy)phosphoryl]oxy}acetyl)amino]-3-methylbutanoic acid (**27a**).* Phosphonate **27a** was prepared by hydrogenolysis of **26a** (0.3 g, 0.47 mmol). Yield 99 mg (65%). Colourless powder, t_R=39.7 min, G1. ^{1}H, ^{13}C NMR and HRMS see lit. 18. IR (KBr, ν_{max} cm^{-1}) 3360, 3200–2700 br, 2659, 1714, 1648, 1542, 1224, 1203, 1072.

*4.2.20.10. (2S)-2-{[(2S)-2-{[(R,S)-1-Aminopentyl(hydroxy)phosphoryl]-oxy}propanoyl]amino}-3-methylbutanoic acid (**27b**).* Phosphonate **27b** was prepared by hydrogenolysis of **26b** (0.35 g, 0.54 mmol). Yield 101.6 mg (56%). Colourless powder, t_R=42.3 min, G1. ^{1}H, ^{13}C NMR and HRMS see lit. 18. IR (KBr, ν_{max} cm^{-1}) 3419, 3200–2700 br, 2587, 1723, 1665, 1538, 1394, 1376, 1209, 1168, 1055, 1029, 989.

*4.2.20.11. Methyl (2S)-2-[({[(R,S)-1-aminopentyl(hydroxy)phosphoryl]-oxy}acetyl)amino]-3-methylbutanoate (**29a**).* Phosphonate **29a** was prepared by hydrogenolysis of **28a** (0.4 g, 0.71 mmol). Yield 158.7 mg (66%). Colourless powder, t_R=43.3 min, G1. ^{1}H NMR (600 MHz, DMSO): two diasteroisomers ca. 1:1: 0.86 (3H, t, *J*=7.3, CH_3), 0.88 and 0.89 (6H, d, *J*=7.0, CH_3), 1.27 (2H, m, CH_2), ~1.40 (2H, m, CH_2), 1.59 and 1.77 (2H, m, CH_2), 2.06 (1H, m, –CH<), 3.13 (1H, m, N–CH–P), 3.65 (3H, s, OCH_3), 4.21 and 4.22 (1H, dd, *J*~8.0 and 6.2, N–CH–P), 4.44 (2H, d, *J*=10.8, O–CH_2–CO), 8.09 (2H, br, NH_2), 8.52 and 8.53 (1H, br d, *J*~8.0, CO–NH). ^{13}C NMR (150.9 MHz, DMSO): 13.96 (CH_3), 18.32 and 19.14 (2×CH_3), 22.16 (CH_2), 27.86 (d, *J*(C,P)=8.1, CH_2), 28.36 and 28.38 (CH_2), 30.22 and 30.23 (–CH<), 47.67 (d, *J*(C,P)=145.2, N–CH–P), 52.14 (OCH_3), 57.59 and 57.61 (N–*C*H–CO), 62.95 (d, *J*(C,P)=5.6, O–CH_2–CO), ~170.48 (CO–NH), 171.85 (CO–O). IR ($CHCl_3$, ν_{max} cm^{-1}) 3420, 3200–2700 br, 2565, 1736, 1674, 1534, 1440, 1394, 1376, 1171, 1057, 985. HRMS (ESI) calcd for $C_{13}H_{27}N_2NaO_6P$ $[M+Na]^+$ 361.1504; found: 361.1498.

*4.2.20.12. Methyl (2S)-2-{[(2S)-2-{[(R,S)-1-aminopentyl(hydroxy)-phosphoryl]oxy}propanoyl] amino}-3-methylbutanoate (**29b**).* Phosphonate **29b** was prepared by hydrogenolysis of **28b** (0.4 g, 0.6 mmol). Yield 188.1 mg (77%). Colourless powder, t_R=44.7 min, G1. ^{1}H NMR (600 MHz, DMSO): two diasteroisomers ca. 1:1: 0.85 (3H, t, *J*=7.3, CH_3), 0.87–0.89 (6H, d, *J*=7.0, CH_3), 1.26 (2H, m, CH_2), 1.36 and 1.41 (2H, m, CH_2), 1.38 (3H, d, *J*=6.8), 1.61 and 1.76 (2H, m, CH_2), 2.07 (1H, m, –CH<), 3.10 and 3.21 (1H, m, N–CH–P), 3.64 (3H, s, OCH_3), 4.17 and 4.18 (1H, dd, *J*~8.0 and 6.5, N–CH–CO), 4.85 (1H, m, O–CH–CO), 8.09 and 8.16 (2H, br, NH_2), 8.35 and 8.37 (1H, br d, *J*~8.0, CO–NH). ^{13}C NMR (150.9 MHz, DMSO): 13.91 and 13.92 (CH_3), 18.45, 18.46 and 19.17 (2×CH_3), 20.35 and 20.38 (d, *J*(C,P)=2.0, CH_3), 22.08 and 22.10 (CH_2), 27.72 and 27.77 (d, *J*(C,P)~5.0, CH_2), 28.14 and 28.32 (d, *J*(C,P)=1.8, CH_2), 30.13 and 30.15 (–CH<), 47.62 (d, *J*(C,P)=148.3, N–CH–P), 52.10 (OCH_3), 57.72 and 57.73 (N–*C*H–CO), 70.34 and 70.84 (d, *J*(C,P)~6.0, O–CH–CO), 171.84 and 171.88 (CO–O), 172.33 and 172.43 (d, *J*(C,P)~3.8, CO–NH). IR ($CHCl_3$, ν_{max} cm^{-1}) 3420, 3200–2700 br, 2565, 1736, 1674, 1534, 1440, 1394, 1376, 1171, 1057, 985. HRMS (ESI) calcd for $C_{14}H_{29}N_2NaO_6P$ $[M+Na]^+$ 375.1661; found: 375.1655.

*4.2.20.13. (2S)-2-[({[(R,S)-1-Aminopentyl(hydroxy)phosphoryl]oxy}-acetyl)amino]-3-methylbutanamide (**31a**).* Phosphonate **31a** was prepared by hydrogenolysis of **30a** (0.25 g, 0.46 mmol). Yield 91.5 mg (62%). Colourless powder, t_R=23.4 min, G2. ^{1}H NMR (600 MHz, DMSO): mixture of two diastereomers (1:1), some signals were doubled: 0.835 and 0.865 (6H, d, *J*=6.8, 2×CH_3), 0.86 (3H, t, *J*=7.3, CH_3), 1.27 (2H, m, CH_2), 1.38 and 1.42 (2H, m, CH_2), 1.60 and 1.77 (2H, m, CH_2), 2.01 (1H, m, –CH<), 3.16 (1H, br, N–CH–P), 4.155+4.165 (1H, dd, *J*=9.2 and 6.1, N–CH–CO), 4.41 (1H, dd, *J*=15.4 and 10.9, O–C*Ha*Hb–CO), 4.46 (1H, dd, *J*=15.4 and 10.9, O–CHa*Hb*–CO), 7.14 and 7.60 (2H, d, *J*=1.5, $CONH_2$), 8.11+8.12 (1H, d, *J*=9.0, CO–NH), 8.17 (3H, br, NH_3). ^{13}C NMR (150.9 MHz, DMSO): 13.94 (CH_3), 17.90+17.93 and 19.49+19.52 (2×CH_3), 22.14+22.15 (CH_2), 27.84 (d, *J*(C,P)=8.1, CH_2), 28.38 (CH_2), 30.56+30.63 (–CH<), 47.54+47.58 (d, *J*(C,P)=146.2+146.4, N–CH–P), 57.54+57.56 (N–*C*H–CO), 63.14 (d, *J*(C,P)=5.0, O–CH–CO), 169.89+169.91 (d, *J*(C,P)=7.4+7.2, CO–NH), 172.70 (CO–NH_2). IR (KBr, ν_{max} cm^{-1}) 3333, 3195, 3200–2700 br, 1671, 1540, 1431, 1207, 1172, 1071, 981. HRMS (ESI) calcd for $C_{12}H_{26}N_3NaO_5P$ $[M+Na]^+$ 346.1508; found: 346.1502.

*4.2.20.14. (2S)-2-{[(2S)-2-{[(R,S)-1-Aminopentyl(hydroxy)phosphoryl]-oxy}propanoyl]amino}-3-methylbutanamide (**31b**).* Phosphonate **31b** was prepared by hydrogenolysis of **30b** (0.25 g, 0.44 mmol). Yield 106.6 mg (71%). Colourless powder, t_R=25.7 min, G2. ^{1}H NMR (600 MHz, DMSO): mixture of two diastereomers (1:1), most of the signals were doubled: 0.835+0.866 (6H, br d, *J*=6.8, 2×CH_3), 0.854+0.856 (3H, t, *J*=7.3, CH_3), 1.26 (2H, m, CH_2), 1.36 (3H, d, *J*=6.8,

CH_3), 1.38 and 1.42 (2H, m, CH_2), 1.60 and 1.76 (2H, m, CH_2), 2.05 (1H, m, –CH<), 3.05+3.13 (1H, br, N–CH–P), 4.11+4.115 (1H, dd, *J*=9.2 and 5.9, N–CH–CO), 4.73+4.735 (1H, p, *J*=6.8 (O–CH–CO)), 7.12+7.135 and 7.715+7.73 (2H, d, *J*=1.7, $CONH_2$), 7.82+7.83 (1H, d, *J*=9.2, CO–NH), 8.05+8.085 (3H, br, NH_3). ^{13}C NMR (150.9 MHz, DMSO): 13.92+13.94 (CH_3), 17.82+17.83 and 19.52 (2×CH_3), 20.46+20.49 (d, *J*(C,P)=5.0, CH_3), 22.10+22.12 (CH_2), 27.82+27.85 (d, *J*(C,P)=7.5+8.4, CH_2), 28.48+28.58 (CH_2), 30.48+30.49 (–CH<), 47.71+47.74 (d, *J*(C,P)=147.3+145.8, N–CH–P), 57.47+57.48 (N–CH–CO), 70.64+71.07 (O–CH–N), 172.07+172.09 (d, *J*(C,P)=3.8, CO–NH), 172.96+173.00 (CO–NH_2). IR (KBr, ν_{max} cm^{-1}) 3406, 3194, 3200–2700 br, 1671, 1533, 1206, 1171, 1071, 982. HRMS (ESI) calcd for $C_{13}H_{28}N_3NaO_5P$ $[M+Na]^+$ 360.1664; found: 360.1660.

4.2.21. {[(R,S)-1-(9H-Fluoren-9-ylmethoxycarbonylamino)-pentyl(hydroxy)phosphoryl]oxy} acetic acid (**32**)

Compound **22a** (1 g, 1.85 mmol) was dissolved in 80 mL of methanol and then 10% Pd–C (0.15 g) was added, and the reaction mixture was vigorously stirred and allowed to react at rt overnight under an atmosphere of hydrogen (15 psi). The catalyst was filtered off through Celite and the filter was washed with 100 mL of methanol. The filtrate was evaporated in vacuo to give 400 mg of crude compound **21a**, which was directly used for the next reaction without further purification. Compound **21a** (0.4 g, 1.8 mmol) was dissolved in 5 mL of saturated $NaHCO_3$ and Fmoc-OSu (0.61 g, 1.8 mmol) in 10 mL of dioxane was added dropwise. The reaction mixture was allowed to react at rt overnight, the dioxane was evaporated in vacuo and the solution was acidified (pH=1) using 1 M HCl. The separated semisolid was extracted with 2×20 mL of ethyl acetate. The combined organic layers were dried over Na_2SO_4 before filtration and removal of the solvent in vacuo, which gave an oil that was purified by reverse-phase chromatography. Yield 183.5 mg (22%). Colourless powder, t_R=46.9 min, G2. ^1H NMR (600 MHz, DMSO): 0.85 (3H, t, *J*=7.1, CH_3), 1.23 and 1.30 (2H, m, CH_2), 1.22 and 1.35 (2H, m, CH_2), 1.55 and 1.72 (2H, m, CH_2), 3.76 (1H, dtd, *J*=16.0, 9.6(2×) and 3.2, N–CH–P), 4.21 (1H, m, CH (Fmoc)), 4.24 (1H, dd, *J*=10.2 and 5.4, –C*Ha*Hb–O), 4.27 (1H, dd, *J*=10.2 and 8.0, –CHa*Hb*–O), 4.38 (1H, dd, *J*=16.2 and 9.4, CO–C*Ha*Hb–O), 4.43 (1H, dd, *J*=16.2 and 9.4, CO–CHa*Hb*–O), 7.31 (1H, m, Ar–H), 7.33 (1H, m, Ar–H), 7.41 (2H, m, Ar–H), 7.52 (1H, d, *J*=9.6, NH), 7.72 (1H, m, Ar–H), 7.74 (1H, m, Ar–H), 7.89 (2H, m, Ar–H). ^{13}C NMR (150.9 MHz, DMSO): 14.08 (CH_3), 21.83 (CH_2), 27.98 (d, *J*(C,P)=13.4, CH_2), 28.59 (d, *J*(C,P)=2.3, CH_2), 46.89 (>CH– (FMOC)), 48.16 (d, *J*(C,P)=155.9, N–CH–P), 61.83 (d, *J*(C,P)=5.3, O–CH_2), 65.98 (O–CH_2), 120.35 (2×Ar–CH), 125.59 (Ar–CH), 125.64 (Ar–CH), 127.26 (Ar–CH), 127.31 (Ar–CH), 127.89 (2×Ar–CH), 140.93 (Ar–C), 140.94 (Ar–C), 143.92 (Ar–C), 144.19 (Ar–C), 156.39 (d, *J*(C,P)=5.3, O–CO–N), 170.28 (d, *J*(C,P)=5.4, COOH). IR (KBr, ν_{max} cm^{-1}) 3394, 3299, 3200–2700 br, 3042, 1725, 1537, 1451, 1295, 1243, 1214, 1098, 1033, 993, 759, 740. HRMS (ESI) calcd for $C_{22}H_{25}NO_7P$ $[M+Na]^+$ 446.1369; found: 446.1365.

4.2.22. Phosphonodepsipeptide **33**

Compound **32** (49 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 was allowed to proceed at rt 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 rt 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 rt. 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 rt. The reaction mixture was evaporated to dryness. The crude compound was triturated repeatedly in diethyl ether. The diethyl ether was removed and compound **33** purified with RP-HPLC at a flow rate of 3 mL/min on a C18 Nucleosil column (250×8 mm, 5 μm) from Watrex (Praha) using the following gradient (solvents are the same as described above): *t*=0 min (0% B), *t*=35 min (50% B), *t*=40 min (100% B), *t*=41 min (0% B). Yield (20 mg, 45%, for a mixture of two diastereoisomers). Colourless powder. HRMS (ESI) calcd for $C_{19}H_{32}N_4O_7PS$ $[M+H]^+$ 491.1729; found: 419.1727.

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References and notes

1. Dive, V.; Georgiadis, D.; Matziari, M.; Makaritis, A.; Beau, F.; Cuniasse, P.; Yiotakis, A. *Cell. Mol. Life Sci.* **2004**, *61*, 2010–2019.
2. Yiotakis, A.; Georgiadis, D.; Matziari, M.; Makaritis, A.; Dive, V. *Curr. Org. Chem.* **2004**, *8*, 1135–1158.
3. Kafarski, P.; Lejczak, B. Aminophosphinic and Aminophosphonic Acids. Chemistry and Biological Activity. In *Synthesis of Phosphono- and Phosphinopeptides*; Kukhar, V. P., Hudson, H. R., Eds.; John Wiley: Chichester, UK, 2000; pp 173–203.
4. Collinsova, M.; Jiracek, J. *Curr. Med. Chem.* **2000**, *7*, 629–647.
5. Kafarski, P.; Lejczak, B. Aminophosphinic and Aminophosphonic acids. Chemistry and Biological Activity. In *The Biological Activity of Phosphono- and Phosphinopeptides*; Kukhar, V. P., Hudson, H. R., Eds.; John Wiley: Chichester, UK, 2000; pp 407–442.
6. Isomura, S.; Ashley, J. A.; Wirsching, P.; Janda, K. D. *Bioorg. Med. Chem. Lett.* **2002**, *12*, 861–864.
7. Wolfenden, R.; Radzicka, A. *Curr. Opin. Struct. Biol.* **1991**, *1*, 780–787.
8. Schramm, V. L.; Horenstein, B. A.; Kline, P. C. *J. Biol. Chem.* **1994**, *269*, 18259–18262.
9. Phillips, M. A.; Kaplan, A. P.; Rutter, W. J.; Bartlett, P. A. *Biochemistry* **1992**, *31*, 959–963.
10. Giannousis, P. P.; Bartlett, P. A. *J. Med. Chem.* **1987**, *30*, 1603–1609.
11. Bartlett, P. A.; Giangiordano, M. A. *J. Org. Chem.* **1996**, *61*, 3433–3438.
12. Bartlett, P. A.; Hanson, J. E.; Giannousis, P. P. *J. Org. Chem.* **1990**, *55*, 6268–6274.
13. Hanson, J. E.; Kaplan, A. P.; Bartlett, P. A. *Biochemistry* **1989**, *28*, 6294–6305.
14. Song, J. B.; Hah, S. H.; Suh, J. *Bull. Korean Chem. Soc.* **2004**, *25*, 1703–1706.
15. Kim, H. D.; Lipscomb, W. N. *Biochemistry* **1991**, *30*, 8171–8180.
16. Buckman, B. O.; Emayan, K.; Islam, A.; May, K.; Bryant J.; Mohan, R.; West, C. L.; Yuan, S. Plasma carboxypeptidase B inhibitors. U.S. and international patent application PCT/US03/08587 (WO 03/080631 A2), 2003.
17. Bartlett, P. A.; Lamden, L. A. *Bioorg. Chem.* **1986**, *14*, 356–377.
18. Sampson, N. S.; Bartlett, P. A. *Biochemistry* **1991**, *30*, 2255–2263.
19. Morgan, B. P.; Scholtz, J. M.; Ballinger, M. D.; Zipkin, I. D.; Bartlett, P. A. *J. Am. Chem. Soc.* **1991**, *113*, 297–307.
20. Meyer, J. H.; Bartlett, P. A. *J. Am. Chem. Soc.* **1998**, *120*, 4600–4609.
21. Ding, J. H.; Fraser, M. E.; Meyer, J. H.; Bartlett, P. A.; James, M. N. G. *J. Am. Chem. Soc.* **1998**, *120*, 4610–4621.
22. Smith, W. W.; Bartlett, P. A. *J. Am. Chem. Soc.* **1998**, *120*, 4622–4628.
23. Yang, K. W.; Brandt, J. J.; Chatwood, L. L.; Crowder, M. W. *Bioorg. Med. Chem. Lett.* **2000**, *10*, 1085–1087.
24. Ellsworth, B. A.; Tom, N. J.; Bartlett, P. A. *Chem. Biol.* **1996**, *3*, 37–44.
25. Chang, Y. P.; Tseng, M. J.; Chu, Y. H. *Anal. Biochem.* **2006**, *359*, 63–71.
26. Bussiere, D. E.; Pratt, S. D.; Katz, L.; Severin, J. M.; Holzman, T.; Park, C. H. *Mol. Cells* **1998**, *2*, 75–84.
27. Karanewsky, D. S.; Petrillo, E. W., Jr. Phosphonyl hydroxyacyl amino acid derivatives as antihypersensives. European patent application 83303592.6 (0 097 534 A2), 1983.
28. Loots, M. J.; Karanewsky, D. S. Substituted aminoalkanoylaminoalkyl phosphonate angiotensin converting enzyme inhibitors. European patent application 87305099.1 (0 249 445 A2), 1987.
29. Karanewsky, D. S. and Petrillo, E. W., Jr. Orally active phosphonyl hydroxyacyl prolines and processes for preparing phosphonyl hydroxyacyl prolines. European patent application 86310043.4 (0 229 520 A1), 1986.

30. Karanewsky, D. S.; Petrillo, E. W., Jr. Phosphonyl hydroxyacyl amino acid derivatives as antihypersensitive. U.S. patent 4,703,043, 1987.
31. Campbell, D. A. Methods for the synthesis of phosphonate esters; U.S. patent 5,420,328, **2008**.
32. Campbell, D. A. *J. Org. Chem.* **1992**, *57*, 6331–6335.
33. Campbell, D. A.; Bermak, J. C. *J. Org. Chem.* **1994**, *59*, 658–660.
34. Wagner, J.; Lerner, R. A.; Barbas, C. F. *Bioorg. Med. Chem.* **1996**, *4*, 901–916.
35. Campagne, J. M.; Coste, J.; Jouin, P. *J. Org. Chem.* **1995**, *60*, 5214–5223.
36. Campagne, J. M.; Coste, J.; Jouin, P. *Tetrahedron Lett.* **1993**, *34*, 6743–6744.
37. Hoffmann, M. *Aust. J. Chem.* **1988**, *41*, 605–607.
38. Galeotti, N.; Coste, J.; Bedos, P.; Jouin, P. *Tetrahedron Lett.* **1996**, *37*, 3997–3998.
39. Karanewsky, D. S.; Badia, M. C. *Tetrahedron Lett.* **1986**, *27*, 1751–1754.
40. Sampson, N. S.; Bartlett, P. A. *J. Org. Chem.* **1988**, *53*, 4500–4503.
41. Xu, J. X.; Gao, Y. H. *Synthesis* **2006**, 783–788.
42. Campagne, J. M.; Coste, J.; Jouin, P. *Tetrahedron Lett.* **1995**, *36*, 2079–2082.
43. Picha, J.; Budesinsky, M.; Sanda, M.; Jiracek, J. *Tetrahedron Lett.* **2008**, *49*, 4366–4368.
44. Mitra, S.; Dygas-Holz, A. M.; Jiracek, J.; Zertova, M.; Zakova, L.; Holz, R. C. *Anal. Biochem.* **2006**, *357*, 43–49.
45. Oleksyszyn, J.; Subotkowska, L.; Mastalerz, P. *Synthesis-Stuttgart* **1979**, 985–986.
46. Sellergren, B.; Karmalkar, R. N.; Shea, K. J. *J. Org. Chem.* **2000**, *65*, 4009–4027.
47. Yuan, C. Y.; Wang, G. H.; Chen, S. J. *Synthesis* **1990**, 522–524.
48. King, A. O.; Shinkai, I. Handbook of Reagents for Organic Synthesis. Oxidizing and Reducing Agents. In *Palladium on carbon*; Burke, S. D., Danheiser, R. L., Eds.; John Wiley: Chicester, UK, 2005; pp 280–285.
49. Raillard, S. P. Handbook of Reagents for Organic Synthesis. Reagents for Glycoside, Nucleotide, and Peptide Synthesis In *N-(9-Fluorenylmethoxycarbonyl)oxysuccimide*; Crich, D., Ed.; John Wiley: Chichester, UK, 2005; pp 359–360.
50. Reese, C. B.; Zhang, P. Z. *J. Chem. Soc., Perkin Trans. 1* **1993**, 2291–2301.
51. Kudzin, Z. H.; Stec, W. J. *Synthesis* **1978**, 469–472.
52. Hoffmann, M. *J. Prakt. Chem.* **1988**, *330*, 820–824.
53. Mucha, A.; Kafarski, P.; Plenat, F.; Cristau, H. J. *Phosphorus Sulfur* **1995**, *105*, 187–193.
54. Mathias, L. J.; Fuller, W. D.; Nissen, D.; Goodman, M. *Macromolecules* **1978**, *11*, 534–539.
55. Hoffmann, M. *Phosphorus Sulfur* **1998**, *134*, 109–118.
56. Toth, I.; Anderson, G. J.; Hussain, R.; Wood, I. P.; Fernandez, E. D.; Ward, P.; Gibbons, W. A. *Tetrahedron* **1992**, *48*, 923–930.
57. Raguin, O.; Fournie-Zaluski, M. C.; Romieu, A.; Pelegrin, A.; Chatelet, F.; Pelaprat, D.; Barbet, J.; Roques, B. P.; Gruaz-Guyon, A. *Angew. Chem., Int. Ed.* **2005**, *44*, 4058–4061.
58. Consiglio, G. A.; Failla, S.; Finocchiaro, P.; Siracusa, V. *Phosphorus Sulfur* **1998**, *143*, 159–166.