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Design, synthesis and biological evaluation of peptidyl-vinylaminophosphonates as novel cysteine protease inhibitors

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ABSTRACT

We report herein, design and synthesis of vinylaminophosphonates, a novel class of compounds as possible cysteine protease inhibitors. The synthesis of vinylaminophosphonates has been accomplished employing Tsuji–Trost reaction as a key step. The synthesized compounds were assayed against papain, a model cysteine protease and some of our synthesized compounds showed IC_{50} values in the range of 30–54 μM thereby suggesting that these chemical entities thus could constitute an interesting template for the design of potential novel protease inhibitors.

1. Introduction

Cysteine proteases are sulfhydryl protease which catalyses the hydrolysis of peptide, amide, ester, and thiol ester bonds.[1] Many normal physiological functions necessitate cysteine protease and disharmony in the activity of cysteine protease may lead to various pathologies such as rheumatoid arthritis, multiple sclerosis, neurological disorders, tumours, and osteoporosis.[1] Therefore, study directed towards design and synthesis of cysteine protease inhibitors has gained considerable importance in the field of medicinal chemistry for the development of new candidate drugs.[2]

The general mechanism of cysteine protease action has been very well studied[3] with papain as the model enzyme. Several mechanism based inhibitors have been designed, synthesized, and evaluated for the inhibition of cysteine proteases[1–6] which includes peptidyl aldehyde,[7a] semicarbazone,[7b] α-keto acids/esters,[7c] nitriles,[7d,e] halides,[7f] and epoxides[7g] (Fig. 1).

In continuation of our interest[8] in design and synthesis of aminophosphonates as novel protease inhibitors, we considered designing a novel class of cysteine protease inhibitors. Keeping in mind mechanism of proteolysis by cysteine proteases, we envisaged that peptidyl-vinylphosphonate **1** (Fig. 2) could ideally fulfil the requirement as it contains vinylic phosphonate group as electrophilic centre required for the nucleophilic attack of the cysteine thiol. The peptidyl character of the designed molecule is required for the hydrophobic interaction at the active site of the enzyme and this necessitates that R_1 and R_2 should be hydrophobic in nature. The literature search on previously reported[1a,b] cysteine protease inhibitors revealed that phenylalanine and leucine were found to be the best among all the amino acid residues at R_2 and hence, we chose these amino acid residues for the peptidyl character of the designed molecules in order to have an effective binding in the P_2 pocket.

2. Results and discussion

We opined that synthesis of peptidyl-vinylphosphonates could be realised by transforming hydroxyphosphonate to γ-amino-vinylphosphonate (Scheme 1). The conversion of hydroxyphosphonate to γ-amino-vinylphosphonate in turn could be achieved by employing either Ritter reaction[9] or Tsuji–Trost reaction.[10]

In our attempt to synthesise γ-amino-vinylphosphonate via Ritter reaction, cinnamaldehyde **2** was subjected to the hydrophosphynylation reaction in presence of triethyl amine (TEA), to form hydroxyphosphonate **3** (Scheme 2). When hydoxyphophonate **3** was subjected to the Ritter reaction in presence of Lewis acid ($BF_3{\cdot}Et_2O$) and acetonitrile as solvent at room temperature for overnight, γ-acetamino-vinylphosphonates **4** was obtained exclusively. However, the deprotection of the acetate group of the acetamide **4** was found to be very difficult. In the literature,[11] it has been reported that deprotection of the acetate group of acetamide could be achieved by following a three steps protocol, that is, Boc protection followed by acetate deprotection and Boc deprotection.

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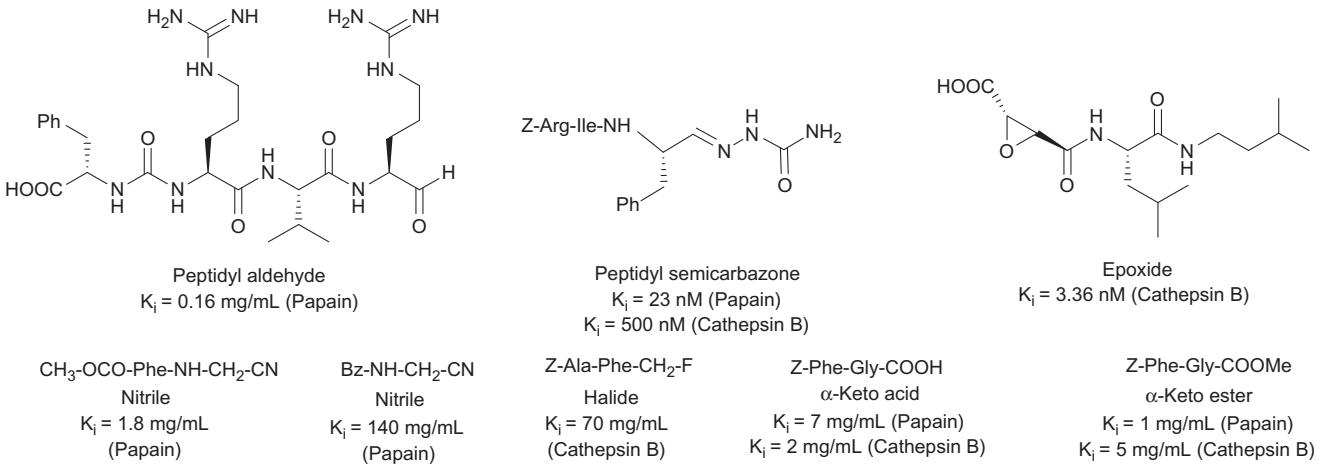


Figure 1. Some of the reported cysteine protease inhibitors.

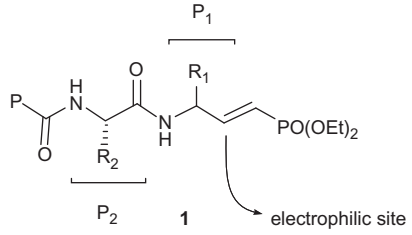
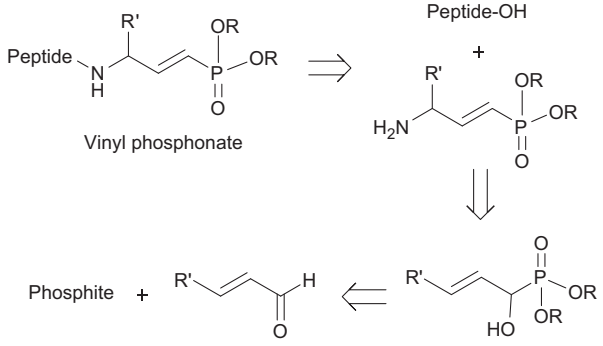
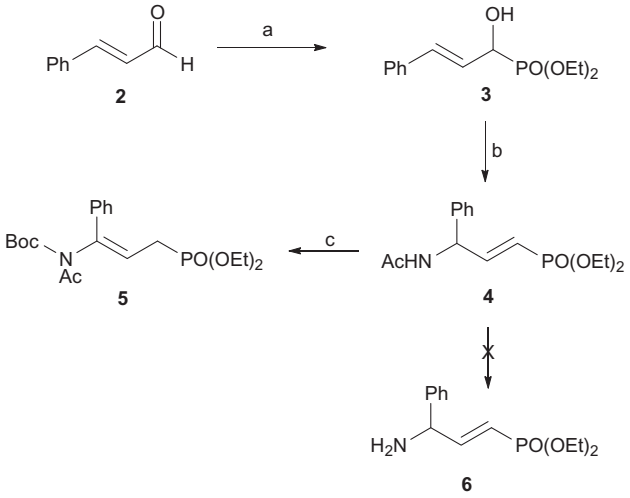


Figure 2. Designing of the cysteine protease inhibitors.



Scheme 1. Retrosynthesis of peptidyl-vinylphosphonates.



Scheme 2. Reagents and conditions: (a) DEP, TEA, 0 °C to rt, 3 h, 77%; (b) $BF_3{\cdot}Et_2O$, MeCN, 0 °C to rt, 24 h, 70%; (c) Boc_2O, DMP, MeCN, rt, 12 h, 83%.

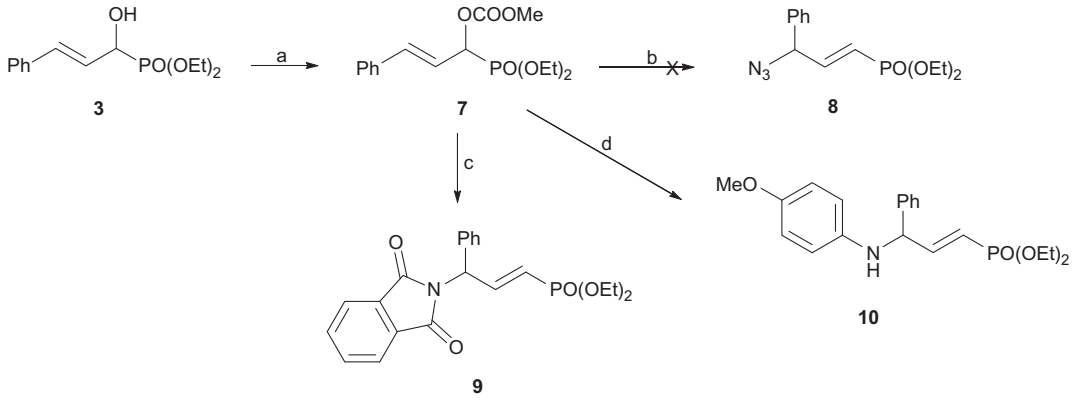
However, in our case the very first step, that is, Boc protection resulted in the double bond migration to furnish product **5**. Since other reported[12] methods of deprotection of the acetate group required harsh reaction conditions which may result in the hydrolysis of the phosphonate ester, therefore we looked at Tsuji–Trost reaction as an alternative method to achieve synthesis of the target compound.

Initially, the hydroxyl group of compound **3** was converted to carbonate derivative **7** (Scheme 3). The carbonate **7** was then subjected to Tsuji–Trost reaction conditions with various nucleophiles, for example, sodium azide, potassium pthalimide and *p*-anisidine. In case when carbonate **7** was reacted with sodium azide, no corresponding product formation was observed. The Tsuji–Trost reaction with potassium pthalimide resulted in the formation of corresponding product **9**, albeit in low yield. However, the Tsuji–Trost reaction of **7** with *p*-anisidine was successful furnishing the corresponding product **10** in excellent yield.

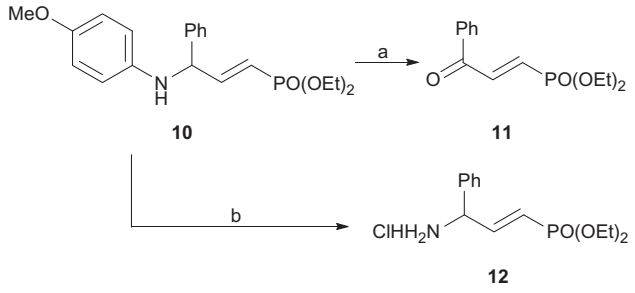
The deprotection of the PMP group was attempted using CAN[13] however, by-product **11** was obtained as the sole product in low yield. The deprotection of PMP group in compound **10** was finally achieved by treating it with trichloroisocyanuric acid (TCCA)[14] in acidic medium to furnish free γ-amino-vinylphosphonate **12** which was obtained as its hydrochloride salt due to acidic work-up (HCl/ethyl acetate) (Scheme 4).

The salt **12** when subjected to peptide coupling reaction with Cbz protected phenylalanine **13** using DCC/HOBt as coupling reagent[15] resulted in the formation of product **14** which was found to be allylic phosphonate by its spectral data instead of the expected vinylic phosphonate (Scheme 5). The formation of allylic phosphonate **14** could be due to the use of triethylamine as base which resulted in the isomerisation of the double bond since triethylamine was used to form free amine in situ from the hydrochloride salt **12**.

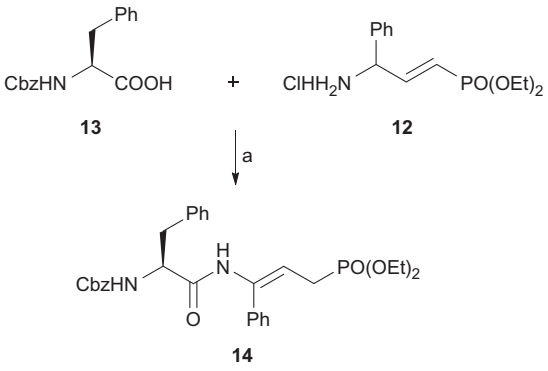
Since coupling reaction of hydrochloride salt **12** resulted in isomerisation of the double bond therefore, we opined that deprotection of PMP group can be carried out using the earlier used deprotection condition, that is, with TCCA however, no acidic work



Scheme 3. Reagents and conditions: (a) MeOCOCl, Py, MeCN, 0 °C to rt, 12 h, 70%; (b) NaN_3, THF/H_2O, $Pd(OAc)_2$, PPh_3, rt/reflux; (c) *K*-phthalimide, DMF, $Pd(OAc)_2$, PPh_3, 80 °C, 3 h, 33%; (d) *p*-anisidine, THF, $Pd(OAc)_2$, PPh_3, rt, 1 h, 95%.



Scheme 4. Reagents and conditions: (a) CAN, MeCN/H_2O, rt, 10%; (b) TCCA, MeCN/H_2O, 1 M H_2SO_4, 12 h then HCl, 57%.

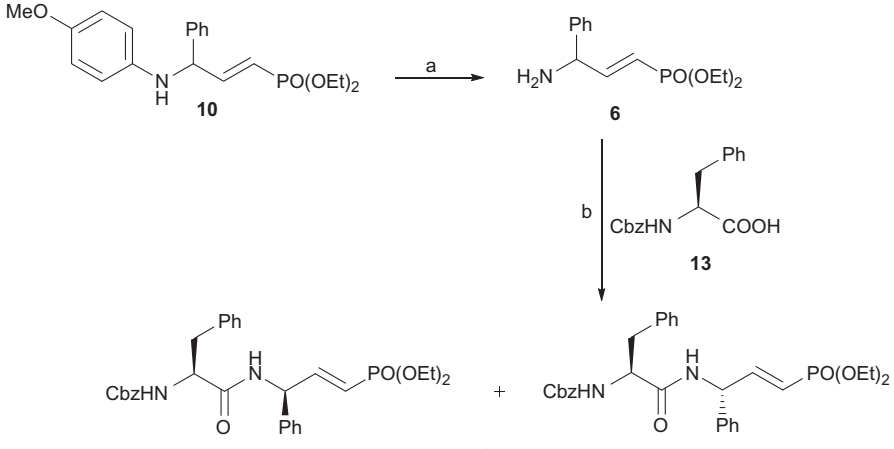


Scheme 5. Reagents and conditions: (a) Et_3N, DCC, HOBt, THF 0 °C to rt, 52%.

up will be carried out to get the free amine **6** (Scheme 6). Afterwards, compound **6** was subjected to peptide coupling reaction with benzyl carbamate protected phenylalanine to furnish peptidyl-vinylaminophosphonate **15a** as a mixture of diastereomers (1:1).

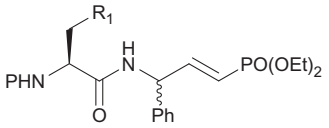
Similar peptide coupling reactions were carried out with various protected amino acids and dipeptides to furnish peptidyl-vinylaminophosphonate **15b–j** as shown in Figure 3. All these peptidyl-vinylaminophosphonate **15a–j** were isolated as a mixture of diastereomers in the ratio of 1:1 and our all attempts to separate these diastereomers by employing various chromatographic techniques were unsuccessful.

The synthesized peptidyl-vinylaminophosphonates **15a–j** were assayed for their in vitro protease inhibition activity against papain a model cysteine protease and the IC_{50} values are compiled in Table 1. The activity data revealed that peptidyl-vinylaminophosphonates having phenylalanine in P_2 pocket (compounds **15a** and **15c**) were found to be more active than corresponding peptidyl-vinylaminophosphonates with leucine and other amino acids in

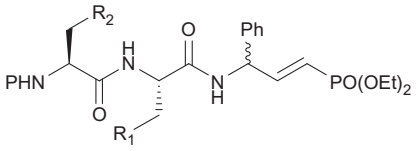


(1:1 mixture of diastereomers)

Scheme 6. Reagents and conditions: (a) TCCA, MeCN/H_2O, 1 M H_2SO_4, 12 h, 59%; (b) DCC, HOBt, THF 0 °C to rt, 53%.



	P	R_1
15a	Cbz,	Ph
15b	Cbz	^{i}Pr
15c	Boc	Ph
15d	Boc	^{i}Pr
15e	Cbz	4-OH-C_6H_4
15f	Cbz	3-indol
15g	Fmoc	^{i}Pr



	P	R_2	R_1
15h	Boc,	Ph,	^{i}Pr
15i	Boc,	Ph,	Ph
15j	Cbz,	Ph,	Ph

Figure 3. Peptidyl-vinylaminophosphonates synthesized as mixture of diastereomers (1:1).

Table 1
In vitro protease inhibition of peptidyl-vinylaminophosphonates (**15a–j**)

Entry	Compound	IC_{50} (μM)
1	**15a** (Cbz-Phe-Vp)	30
2	**15b** (Cbz-Leu-Vp)	>200
3	**15c** (Boc-Phe-Vp)	40
4	**15d** (Boc-Leu-Vp)	>200
5	**15e** (Cbz-Tyr-Vp)	54
6	**15f** (Cbz-Trp-Vp)	> 200
7	**15g** (Fmoc-Leu-Vp)	>200
8	**15h** (Boc-Phe-Leu-vp)	132
9	**15i** (Boc-Phe-Phe-Vp)	83
10	**15j** (Cbz-Phe-Phe-Vp)	125

P_2 pocket (compounds **15b**, **15d**, **15e**, and **15f**). However, it is interesting to note that dipeptidyl-vinylaminophoaphonates (**15a–g**) were more active than their corresponding tripeptidyl-vinylaminophosphonates (**15h–j**).

3. Conclusions

In summary, we have accomplished designing and synthesis of peptidyl-vinylaminophosphonates (**15a–j**) as a novel class of cysteine protease inhibitors by employing Tsuji–Trost reaction as a key step. All the synthesized compounds having phenylalanine residue in P_2 pocket (compounds **15a** and **15c**) were found to be active. Among all the synthesized compounds, compound **15a**, **15b**, and **15e** showed remarkable inhibition against papain with IC_{50} values in the range of 30, 40, and 54 μM, respectively. The increase in the length of the peptide moiety to tripeptide was found to be not favourable from the activity point of view. The in vitro protease inhibition study of our designed and synthesized peptidyl-vinylaminophosphonates (**15a–j**) suggests that these chemical entities could further be explored as an interesting template for the design of potential novel protease inhibitors.

4. Experimental section

4.1. General

The FT-IR spectra were recorded on an FT-IR-8300 Shimadzu spectrometer and microanalyses were carried out on a Carlo-Erba instrument. NMR spectra were recorded on Bruker ACF 200 and AV200 (200 MHz for ^{1}H NMR and 50 MHz for ^{13}C NMR) and AV400 (400 MHz for ^{1}H NMR and 100 MHz for ^{13}C NMR), using $CDCl_3$ as solvent. Tetramethylsilane (0.00 ppm) served as an internal standard in ^{1}H NMR and $CDCl_3$ (77.0 ppm) in ^{13}C NMR, respectively. Chemical shifts are expressed in parts per million (ppm). In case of NMR data of mixture of diastereomers, the peaks corresponding to the one isomer is given. Mass spectra were recorded on LC–MS/MS-TOF API QSTAR PULSAR spectrometer, samples introduced by infusion method using Electrospray Ionization Technique (ESI). Papain, *N*-α-benzoyl-DL-arginine-*p*-nitroanilide (BAPNA), dithiothreitol (DTT) and dimethoxy sulfoxide (DMSO) were purchased from Sigma Chem. Co. (USA). All other chemicals were of analytical grade.

4.1.1. Compound (4)

To the cooled solution of hydroxyphosphonate **3** (135 mg, 0.5 mmol) in acetonitrile (3 mL) at 0 °C was added $BF_3{\cdot}Et_2O$ (0.4 mL, 48% w/v) drop wise. The reaction mixture was allowed to stir for 24 h at rt. After completion of reaction (TLC), saturated aq $NaHCO_3$ (10 mL) was added and the product was extracted with DCM (3 × 10 mL). The combined organic layers was dried over anhydrous Na_2SO_4 and concentrated under reduced pressure to furnish the crude product which was purified by silica gel column chromatography using ethyl acetate/petroleum ether (1:1 to 2:1) as eluant to furnish pure product **4** (220 mg, 70%) as colourless syrup; (Found: C, 57.92; H, 7.24; N, 4.61. Calcd for $C_{15}H_{22}NO_4P$: C, 57.87; H, 7.12; N, 4.50%); ν_{max} ($CHCl_3$)/cm^{-1} 3439, 3019, 1673, 1495, 1395 and 1215 cm^{-1}; δ_H (200 MHz, $CDCl_3$) 1.29 (3H, t, $^3J_{PH}$ 6.7), 1.32 (3H, t, $^3J_{PH}$ 6.9), 2.04 (3H, s), 3.99–4.14 (4H, m), 5.74–5.94 (2H, m), 6.54 (1H, d, *J* 8.1), 6.77–6.98 (1H, m) and 7.24–7.38 (5H, m); δ_C (50 MHz, $CDCl_3$) 16.3 (d, $^3J_{PC}$ 6.6), 16.4 (d, $^3J_{PC}$ 6.2), 23.2, 55.1 (d, $^3J_{PC}$ 22.3), 61.9, 62.1, 117.5 (d, $^1J_{PC}$ 187.4), 127.4, 128.2, 129.0, 138.9, 150.7 (d, $^2J_{PC}$ 5.9) and 169.3; δ_P (161 MHz, $CDCl_3$) 17.67; ESI-MS: *m/z* 312.5 (M+H)$^+$.

4.1.2. Compound (5)

DMAP (12 mg, 0.1 mmol) was added to a stirred solution of compound **4** (155 mg, 0.5 mmol) in dry acetonitrile (3 mL) followed by Boc_2O (240 mg, 2.05 mmol). After stirring for 12 h at rt when all the starting material was consumed (TLC), the brownish reaction mixture was evaporated under vacuum and the oily residue partitioned between diethyl ether (25 mL) and 1 M aq $KHSO_4$ (15 mL). The organic extract was thoroughly washed successively with 1 M aq solution of $KHSO_4$ (15 mL), saturated $NaHCO_3$ (15 mL) and finally brine (15 mL) and dried over Na_2SO_4. Evaporation of the solvent furnished a light yellow oil, which was purified by silica gel column chromatography using ethyl acetate/petroleum ether (1:1) as eluant to furnish pure product **5** (170 mg, 83%) as colourless syrup; (Found: C, 58.45; H, 7.42; N, 3.36. Calcd for $C_{20}H_{30}NO_6P$: C, 58.39; H, 7.35; N, 3.40%); ν_{max} ($CHCl_3$)/cm^{-1} 2981, 2934, 1738, 1713, 1446, 1394 and 1370 cm^{-1}; δ_H (200 MHz, $CDCl_3$) 1.26 (9H, s), 1.34–1.35 (6H, m), 2.53–2.69 (2H, m), 2.60 (3H, s), 4.11–4.19 (4H, m), 6.07–6.19 (1H, m) and 7.31–7.33 (5H, m); δ_C (50 MHz, $CDCl_3$) 14.4 (d, $^3J_{PC}$ 6.2), 23.9, 24.3 (d, $^1J_{PC}$ 142.3), 25.5, 59.9 (d, $^2J_{PC}$ 6.6), 60.1 (d, $^2J_{PC}$ 6.6), 81.3, 115.5 (d, $^2J_{PC}$ 8.4), 123.0, 126.1, 126.4, 135.1 (d, $^4J_{PC}$ 2.2), 137.0 (d, $^3J_{PC}$ 16.5), 149.9 and 169.6; δ_P (161 MHz, $CDCl_3$) 26.52; ESI-MS: *m/z* 434.4 (M+Na)$^+$.

4.1.3. Compound (7)

The hydroxy phosphonate **3** (387 mg, 1.4 mmol) was dissolved in a mixture of acetonitrile (10 mL) and pyridine (1 mL) and then DMAP (10 mg) was added. The resulting solution was cooled to 0 °C and methyl chloroformate (850 μL, 9.0 mmol) was added drop wise. The solution was allowed to warm up to rt and stirring was continued overnight. The solution was diluted with DCM (10 mL) and washed with water (2 × 10 mL) and saturated $CuSO_4$ solution (4 × 10 mL). The solvent was dried over Na_2SO_4, filtered, and evaporated in vacuum to give crude product which was purified by silica gel column chromatography using ethyl acetate/petroleum ether (3:7) as eluant to furnish pure product **7** (320 mg, 70%) as colourless syrup; (Found: C, 54.75; H, 6.57. Calcd for $C_{15}H_{21}O_6P$: C, 54.88; H, 6.45%); ν_{max} ($CHCl_3$)/cm^{-1} 3017, 1755, 1650, 1442, 1266, 1216 and 1027 cm^{-1}; δ_H (200 MHz, $CDCl_3$) 1.34 (6H, t, $^3J_{PH}$ 7.1), 3.84 (3H, s), 4.13–4.28 (4H, m), 5.59–5.70 (1H, m), 6.20–6.34 (1H, m), 6.75–6.85 (1H, m) and 7.28–7.44 (5H, m); δ_C (50 MHz, $CDCl_3$) 16.4 (d, $^3J_{PC}$ 5.8), 16.5 (d, $^3J_{PC}$ 5.5), 55.4, 63.4 (d, $^2J_{PC}$ 6.6), 63.4 (d, $^2J_{PC}$ 7.3), 73.4 (d, $^1J_{PC}$ 170.9), 119.6 (d, $^2J_{PC}$ 4.4), 126.9, 128.5, 128.7, 135.4 (d, $^3J_{PC}$ 12.8), 135.6 (d, $^4J_{PC}$ 2.5) and 154.8 (d, $^3J_{PC}$ 9.8); δ_P (161 MHz, $CDCl_3$) 16.64; ESI-MS: *m/z* 351.2 $(M+Na)^+$.

4.1.4. Compound (9)

To a flask purged with nitrogen, $Pd(OAc)_2$ (8 mg, 0.03 mmol), PPh_3 (19 mg, 0.07 mmol) and compound **7** (230 mg, 0.7 mmol) dissolved in DMF (5 mL) were slowly added. Then the reaction mixture was stirred at 80 °C for 3 h. After completion of reaction (TLC), diethyl ether (30 mL) was added. The mixture was washed with water (3 × 20 mL), the organic layer was dried over Na_2SO_4 and concentrated in vacuum. The residue was purified by column chromatography over silica gel by using ethyl acetate/petroleum ether (2:3) as eluant to provide pure product **9** (93 mg, 33%) as colourless syrup; (Found: C, 63.27; H, 5.63; N, 3.43. Calcd for $C_{21}H_{22}NO_5P$: C, 63.15; H, 5.55; N, 3.51%) ν_{max} ($CHCl_3$)/cm^{-1} 2986, 2932, 1771, 1714, 1634, 1613, 1469, 1355 and 1245 cm^{-1}; δ_H (200 MHz, $CDCl_3$) 1.31 (3H, t, $^3J_{PH}$ 7.1), 1.37 (3H, t, $^3J_{PH}$ 7.1), 4.05–4.21 (4H, m), 5.71–5.90 (1H, m), 6.04–6.10 (1H, m) and 7.22–7.86 (10H, m); δ_C (50 MHz, $CDCl_3$) 16.3, 16.4, 56.1 (d, $^3J_{PC}$ 24.5), 62.1 (d, $^3J_{PC}$ 5.5), 62.2 (d, $^3J_{PC}$ 5.5), 120.6 (d, $^1J_{PC}$ 186.9), 123.6, 128.4, 128.5, 128.6, 128.7, 128.9, 131.7, 132.0, 132.2, 134.3, 136.8, 147.6 (d, $^2J_{PC}$ 6.6) and 167.4; δ_P (161 MHz, $CDCl_3$): 16.94; ESI-MS: *m/z* 422.2 $(M+Na)^+$.

4.1.5. Compound (10)

A solution of compound **7** (328 mg, 1 mmol) in THF (3 mL) was stirred at rt under nitrogen, and then $Pd(OAc)_2$ (11 mg, 5 mol%) and PPh_3 (27 mg, 10 mol%) were added to it. After stirring for 10 min, *p*-anisidine (246 mg, 2 mmol) was added and the reaction was further stirred at rt. After completion of the reaction (TLC), reaction mixture was evaporated to give the crude compound, which was purified by flash chromatography over silica gel using ethyl acetate/petroleum ether (2:3) as eluant to obtain pure compound **10** (355 mg, 95%) as yellow syrup; (Found: C, 63.90; H, 6.86; N, 3.65.Calcd for $C_{20}H_{26}NO_4P$: C, 63.99; H, 6.98; N, 3.73%); ν_{max} ($CHCl_3$)/cm^{-1} 3419, 2995, 1633, 1512, 1454, 1242 and 1216 cm^{-1}; (200 MHz, $CDCl_3$): δ_H 1.26 (3H, t, $^3J_{PH}$ 7.1), 1.36 (3H, t, $^3J_{PH}$ 7.1), 3.73 (3H, s), 3.96–4.11 (4H, m), 4.94–4.99 (1H, m), 5.97 (1H, ddd, *J* 19.9, 1.6, 17.1), 6.52–6.77 (4H, m), 6.93 (ddd, *J* 4.7, 17.1, 21.6) and 7.29–7.68 (5H, m); δ_C (50 MHz, $CDCl_3$) 16.3 (d, $^3J_{PC}$ 6.2), 55.7, 61.6 (d, $^3J_{PC}$ 26.4), 61.8, 61.9, 114.8, 114.9, 117.4 (d, $^1J_{PC}$ 186.6), 127.4, 128.1, 129.0, 139.8, 140.8, 152.2 (d, $^2J_{PC}$ 5.8) and 152.5; δ_P (161 MHz, $CDCl_3$): 18.40; ESI-MS: *m/z* 398.1 $(M+Na)^+$.

4.1.6. Compound (11)

To a solution of **10** (240 mg, 0.64 mmol) in acetonitrile (4 mL), aqueous cerium ammonium nitrate solution (1800 mg, 3.2 mmol, 4 mL) was added at 0 °C. After being stirred at 0 °C for 1 h, the reaction mixture was neutralised by addition of satd $NaHCO_3$ solution. The aqueous layer was extracted with diethyl ether (3 × 25 mL) and the combined organic layers were successively washed with sat. $NaHCO_3$ solution (20 mL), brine (20 mL), and dried over anhydrous Na_2SO_4. The solvent was evaporated in vacuum to afford the crude material, which was purified by flash chromatography over silica gel using ethyl acetate/petroleum ether (2:3) as eluant to furnish the pure compound **11** (16 mg, 10%) as colourless syrup; (Found: C, 58.15; H, 6.29. Calcd for $C_{13}H_{17}O_4P$: C, 58.21; H, 6.39%); ν_{max} ($CHCl_3$)/cm^{-1} 3019, 1672, 1597, 1448, 1259 and 1216 cm^{-1}; δ_H (200 MHz, $CDCl_3$) 1.37 (6H, t, $^3J_{PH}$ 7.1 Hz), 4.10–4.25 (4H, m), 6.95 (1H, dd, *J* 16.9, 19.3), 7.45–7.64 (3H, m), 7.83 (1H, dd, *J* 16.9, 21.1) and 7.99–8.04 (2H, m); δ_C (50 MHz, $CDCl_3$) 16.4, 62.5, 128.9, 129.0, 130.8 (d, $^1J_{PC}$ 184.1), 133.9, 136.3, 140.2 (d, $^2J_{PC}$ 5.9) and 188.5 (d, $^3J_{PC}$ 22.0); δ_P (161 MHz, $CDCl_3$) 15.78; ESI-MS: *m/z* 291.2 $(M+Na)^+$.

4.1.7. Compound (6) or its HCl salt (12)

To a solution of **10** (150 mg, 0.4 mmol) in MeCN/H_2O (10 mL, 1:1) were added TCCA (46 mg, 0.2 mmol) and 1 M aqueous H_2SO_4 (0.4 mL). The mixture was stirred for 12 h at rt and then washed with CH_2Cl_2 (3 × 20 mL). The resulting aqueous phase was subsequently brought to pH 10.5 through the addition of 5 M aqueous KOH and extracted with EtOAc (4 × 30 mL). The combined organic layers were brought to pH 1 by the addition of EtOAc/HCl, dried (Na_2SO_4), and concentrated to afford the HCl salt **12**. In an alternative work up procedure, the combined organic layers were evaporated in vacuum to furnish crude material, which was purified by flash chromatography over silica gel using ethyl acetate as eluant to afford pure free amine **6** (63 mg, 59%) as yellow syrup; (Found: C, 57.83; H, 7.38; N, 5.15.Calcd for $C_{13}H_{20}NO_3P$: C, 57.98; H, 7.49; N, 5.20%); ν_{max} ($CHCl_3$)/cm^{-1} 3420, 2985, 2641, 1636, 1605, 1532, 1456, 1343 and 1217 cm^{-1}; δ_H (200 MHz, $CDCl_3$) 1.31 (3H, t, $^3J_{PH}$ 7.1 Hz), 1.34 (3H, t, $^3J_{PH}$ 7.1), 4.01–4.10 (4H, m), 4.61 (2H, br s), 5.13 (1H, m), 6.08 (1H, m), 6.96 (1H, m) and 7.45 (5H, m); δ_C (50 MHz, $CDCl_3$) 15.6, (d, $^3J_{PC}$ 6.2), 56.0 (d, $^3J_{PC}$ 24.2), 62.4 (d, $^2J_{PC}$ 5.5), 120.5 (d, $^1J_{PC}$ 186.3), 127.3, 129.0, 129.3, 133.7 and 145.9 (d, $^2J_{PC}$ 5.9); δ_P (161 MHz, $CDCl_3$): 16.1; ESI-MS: *m/z* 270.7 $(M+H)^+$, 292.7 $(M+Na)^+$.

4.1.8. Compound (14)

HCl salt **12** (152 mg, 0.5 mmol), HOBt (77 mg, 0.5 mmol), acid **13** (0.5 mmol) and Et_3N (50 mg, 0.5 mmol) were dissolved in dry THF (2 mL) and the solution was stirred in an ice water bath and then DCC (123 mg, 0.6 mmol) was added to it. Stirring was continued for 1 h at 0 °C and an additional 1 h at rt. The solid which separated was removed by filtration and the solvent evaporated in vacuum. A mixture of ethyl acetate (5 mL) and saturated solution of $NaHCO_3$ in water (2.5 mL) were added to the residue and the organic phase was washed successively with a solution of aq citric acid (10%, 5 mL), saturated $NaHCO_3$ (5 mL) and finally with water (5 mL). The organic solution was dried over anhydrous Na_2SO_4, filtered and evaporated to dryness in vacuum. The crude dipeptide derivative was purified by chromatography on a silica gel column with ethyl acetate/petroleum ether (4:1) as eluant to furnish **14** (140 mg, 52%) as colourless syrup; $[\alpha]_D^{20}$ −11.0 (*c* 1.00, $CHCl_3$); (Found: C, 65.54; H, 6.35; N, 5.18. Calcd for $C_{30}H_{35}N_2O_6P$: C, 65.44; H, 6.41; N, 5.09%); ν_{max} ($CHCl_3$)/cm^{-1} 3423, 3300, 3018, 1715, 1685, 1496, 1216, 1053 and 759 cm^{-1}; δ_H (200 MHz, $CDCl_3$) 1.26 (6H, t, $^3J_{PH}$ 7.1), 2.35–2.72 (2H, m), 3.00–3.15 (2H, m), 3.94–4.10 (4H, m), 4.49–4.64 (1H, m), 5.08 (2H, s), 5.53–5.63 (1H, m), 5.72 (1H, br s), 7.05 (1H, br s) and 7.13–7.31 (15H, m); δ_C (50 MHz, $CDCl_3$) 16.4 (d, $^3J_{PC}$ 5.9), 16.5 (d, $^3J_{PC}$ 5.9), 25.7 (d, $^1J_{PC}$ 138.7), 38.2, 56.6, 62.4 (d, $^2J_{PC}$ 7.0), 62.6 (d, $^2J_{PC}$ 7.0), 66.9, 111.2 (d, $^2J_{PC}$ 6.6), 125.9, 126.0, 127.1, 127.3, 127.4, 128.0, 128.1, 128.3,

128.3, 128.5, 128.8, 128.9, 129.0, 129.4, 129.6, 136.4, 136.8 (d, $^4J_{PC}$ 4.0), 136.3, 139.3 (d, $^3J_{PC}$ 13.5), 156.1 and 170.2; δ_P (161 MHz, $CDCl_3$) 28.34; ESI-MS: *m/z* 573.7 $(M+Na)^+$.

4.2. General procedure for peptide coupling reaction

Compound **6** (135 mg, 0.5 mmol), HOBt (77 mg, 0.5 mmol) and protected amino acid (0.5 mmol) were dissolved in dry THF (2 mL) and the resulting solution was stirred in an ice-cooled water bath. DCC (123 mg, 0.6 mmol) was added to it. Stirring was continued for 1 h at 0 °C and an additional 1 h at rt. The solid which separated was removed by filtration and the solvent evaporated in vacuum. The crude dipeptide derivative was purified by chromatography on a silica gel column with ethyl acetate/petroleum ether (3:2 to 4:1) as eluant to furnish the corresponding peptide **15a–j**.

4.2.1. Compound (15a)

Yield: 53%; colourless syrup; (Found: C, 65.55; H, 6.33; N, 5.23. Calcd for $C_{30}H_{35}N_2O_6P$: C, 65.44; H, 6.41; N, 5.09%); ν_{max} $(CHCl_3)$/cm^{-1} 3424, 3019, 1713, 1676, 1497, 1395 and 1215 cm^{-1}; δ_H (200 MHz, $CDCl_3$) 1.17–1.33 (6H, m), 3.04 (2H, dd, *J* 6.4, 5.4), 3.90–4.09 (4H m), 4.52–4.54 (1H, m), 4.94–5.00 (2H, m), 5.53–5.95 (2H, m), 5.71–5.72 (1H, br s), 6.65–7.00 (1H m) and 7.07–7.42 (15H, m); δ_C (50 MHz, $CDCl_3$) 16.3, 16.4, 38.9, 55.0 (d, $^3J_{PC}$ 21.6), 56.2, 61.9 (d, $^2J_{PC}$ 5.8), 62.1 (d, $^2J_{PC}$ 5.8), 66.9, 117.6 (d, $^1J_{PC}$ 187.4), 126.9, 127.1, 127.3, 127.4, 128.0, 128.1, 128.2, 128.5, 128.7, 128.9, 129.4, 136.2, 136.3, 138.7, 150.4 (d, $^2J_{PC}$ 4.7), 155.9 and 170.4; δ_P (161 MHz, $CDCl_3$) 17.40; ESI-MS: *m/z* 573.4 $(M+Na)^+$.

4.2.2. Compound (15b)

Yield: 76%; colourless syrup; (Found: C, 62.68; H, 7.32; N, 5.35. Calcd for $C_{27}H_{35}N_2O_6P$: C, 62.78; H, 7.22; N, 5.42%); ν_{max} $(CHCl_3)$/cm^{-1} 3423, 3264, 3017, 2960, 2933, 1718, 1685, 1499, 1509, 1449, 1217 and 1028 cm^{-1}; δ_H (200 MHz, $CDCl_3$) 0.85–0.88 (6H, m), 1.15–1.34 (6H, m),1.52–1.64 (3H, m), 3.88–4.12 (4H, m), 4.33–4.37 (1H, m), 4.98–5.06 (2H, m), 5.73–5.79 (1H, m), 5.85–6.05 (1H, m), 6.72–6.98 (1H, m) and 7.24–7.30 (10H, m); δ_C (50 MHz, $CDCl_3$): 16.2 (d, $^3J_{PC}$ 6.6), 16.3 (d, $^3J_{PC}$ 6.6), 22.9, 23.0, 24.7, 41.8, 53.7, 54.9 (d, $^3J_{PC}$ 22.7), 62.1 (d, $^2J_{PC}$ 9.9), 62.0 (d, $^2J_{PC}$ 9.9), 66.9, 117.2 (d, $^1J_{PC}$ 187.4), 127.3, 127.9, 128.1, 128.5, 128.7, 128.9, 136.2, 138.8, 150.8 (d, $^2J_{PC}$ 5.5), 156.2 and 172.0; δ_P (161 MHz, $CDCl_3$) 17.64; ESI-MS: *m/z* 517.4 $(M+H)^+$.

4.2.3. Compound (15c)

Yield: 77%; colourless syrup; (Found: C, 62.88; H, 7.36; N, 5.35. Calcd for $C_{27}H_{37}N_2O_6P$: C, 62.78; H, 7.22; N, 5.42%); ν_{max} $(CHCl_3)$/cm^{-1} 3427, 3018, 1680, 1495, 1393, 1368, 1216, 1165, 1052 and 1029 cm^{-1}; δ_H (200 MHz, $CDCl_3$): 1.22–1.34 (6H, m), 1.35 (9H, s), 3.04–3.08 (2H, m), 3.96–4.11 (4H, m), 4.40–4.44 (1H, m), 5.22–5.42 (1H, m), 5.62–5.92 (1H, m), 6.67–6.93 (1H, m) and 7.10–7.29 (10H, m); δ_C (50 MHz, $CDCl_3$) 16.3, 16.4, 28.2, 38.3, 54.9 (d, $^3J_{PC}$ 22.7), 56.2, 62.0, 62.1, 80.1, 116.9 (d, $^1J_{PC}$ 187.7), 125.2, 126.8, 126.9, 127.3, 128.1, 128.6, 128.7, 128.9, 128.9, 129.3, 136.4, 138.5, 150.6 (d, $^2J_{PC}$ 5.9), 155.5 and 170.8; δ_P (161 MHz, $CDCl_3$) 17.70; ESI-MS: *m/z* 539.4 $(M+Na)^+$.

4.2.4. Compound (15d)

Yield: 62%; colourless syrup; (Found: C, 59.81; H, 8.02; N, 5.93.Calcd for $C_{24}H_{39}N_2O_6P$: C, 59.74; H, 8.15; N, 5.81%) ; ν_{max} $(CHCl_3)$/cm^{-1} 3432, 3019, 1688, 1496, 1392, 1369, 1216 and 1028 cm^{-1}; δ_H (200 MHz, $CDCl_3$) 0.88–0.94 (6H, m), 1.23–1.34 (6H, m), 1.38 (9H, s), 1.48–1.69 (3H, m),3.97–4.15 (4H, m), 5.06–5.31 (1H, m), 5.72–5.92 (2H, m), 6.74–6.99 (1H, m) and 7.22–7.39 (5H, m); δ_C (50 MHz, $CDCl_3$) 16.2, 16.3, 21.9, 22.9, 24.7, 28.2, 40.7, 53.2, 54.9 (d, $^3J_{PC}$ 22.3), 62.0, 62.1, 80.1, 117.1 (d, $^1J_{PC}$ 187.0), 127.3, 128.1, 128.9, 138.6, 150.8 (d, $^2J_{PC}$ 5.9), 155.9 and 172.0; δ_P (161 MHz, $CDCl_3$) 17.80; ESI-MS: *m/z* 505.4 $(M+Na)^+$.

4.2.5. Compound (15e)

Yield: 60%; colourless syrup; (Found: C, 63.72; H, 6.32; N, 5.10.Calcd for $C_{30}H_{35}N_2O_6P$: C, 63.60; H, 6.23; N, 4.94%); ν_{max} $(CHCl_3)$/cm^{-1} 3422, 3287, 3018, 1712, 1672, 1515, 1454, and 1216 cm^{-1}; δ_H (200 MHz, $CDCl_3$) 1.20 (6H, t, $^3J_{PH}$ 6.9), 1.26 (3H, t, $^3J_{PH}$ 6.9), 2.83–3.05 (2H, m), 3.91–4.12 (4H, m), 4.39–4.55 (1H, m), 4.93 (2H, s), 5.59–5.88 (2H, m) and 6.60–7.49 (15H, m); δ_C (50 MHz, $CDCl_3$) 16.2, 16.3, 37.9, 55.2 (d, $^3J_{PC}$ 22.7), 56.5, 62.4, 67.1, 117.5 (d, $^1J_{PC}$ 187.4), 126.0, 126.7, 127.3, 127.9, 128.2, 128.5, 128.7, 128.9, 129.0, 130.3, 130.4, 134.1, 136.0, 138.2, 150.7 (d, $^2J_{PC}$ 5.1), 155.9 and 171.0; δ_P (161 MHz, $CDCl_3$) 17.71; ESI-MS: *m/z* 589.0 $(M+Na)^+$.

4.2.6. Compound (15f)

Yield: 60%; colourless syrup; (Found: C, 65.24; H, 6.21; N, 7.22.Calcd for $C_{32}H_{36}N_3O_6P$: C, 65.18; H, 6.15; N, 7.13%); ν_{max} $(CHCl_3)$/cm^{-1} 3419, 3297, 3019, 2933, 1712, 1675, 1500, 1216 and 1051 cm^{-1}; δ_H (200 MHz, $CDCl_3$) 1.21–1.36 (6H, m), 3.04–3.39 (2H, m), 3.91–4.14 (4H, m), 4.52–4.69 (1H, m), 5.06 (2H, s), 5.23–5.70 (2H, m), 6.27–6.89 (1H, m) and 6.84–7.72 (15H, m); δ_C (50 MHz, $CDCl_3$) 16.3 (d, $^3J_{PC}$ 6.4), 16.4 (d, $^3J_{PC}$ 6.4), 28.2, 54.9 (d, $^3J_{PC}$ 23.6), 55.6, 62.1, 62.2 (d, $^2J_{PC}$ 5.5), 66.9, 109.9, 111.9, 117.6 (d, $^1J_{PC}$ 187.1), 118.8, 119.8, 122.1, 122.2, 124.1, 124.3, 127.1, 127.3, 128.1, 128.2, 128.2, 128.6, 128.9, 129.0, 136.2, 136.6, 137.8, 138.1, 150.1 (d, $^2J_{PC}$ 4.5), 155.8 and 170.7; δ_P (161 MHz, $CDCl_3$) 17.90; ESI-MS: *m/z* 612.3 $(M+Na)^+$.

4.2.7. Compound (15g)

Yield: 85%; colourless syrup; (Found: C, 67.64; H, 6.91; N, 4.70. Calcd for $C_{34}H_{41}N_2O_6P$: C, 67.53; H, 6.83; N, 4.63%); ν_{max} $(CHCl_3)$/cm^{-1} 3274, 3065, 2958, 1716, 1670, 1541, 1450 and 1239 cm^{-1}; δ_H (200 MHz, $CDCl_3$) 0.89–0.92 (6H m,), 1.17–1.29 (6H, m), 1.60–1.69 (3H, m), 3.90–4.20 (5H, m), 4.32–4.39 (3H, m), 5.48–6.28 (2H, m), 6.74–6.98 (1H, m) and 7.22–7.84 (13H, m); δ_C (50 MHz, $CDCl_3$) 16.2 (d, $^3J_{PC}$ 6.2 Hz), 16.3 (d, $^3J_{PC}$ 6.2 Hz), 21.8, 23.1, 24.8, 41.3, 47.1, 53.9, 54.9, 62.3, 62.4, 67.1, 117.7 (d, $^1J_{PC}$ 186.9 Hz), 119.9, 125.0, 125.1, 125.8, 126.8, 127.1, 127.3, 127.7, 128.2, 129.0, 129.0, 138.3, 141.3, 141.4, 143.7, 143.9, 151.0, 156.5 and 171.8; δ_P (161 MHz, $CDCl_3$): 17.95; ESI-MS: *m/z* 627.8 $(M+Na)^+$.

4.2.8. Compound (15h)

Yield: 54%; colourless syrup; (Calcd for $C_{33}H_{48}N_3O_7P$: C, 62.94; H, 7.68; N, 6.67%); ν_{max} $(CHCl_3)$/cm^{-1} 3633, 3424, 3019, 1698, 1497, 1392, 1369, 1216 and 1028 cm^{-1}; δ_H (200 MHz, $CDCl_3$) 0.83–0.90 (6H, m), 1.22–1.37 (15H, m), 1.49–1.69 (3H, m), 2.97–3.06 (2H, m), 4.01–4.11 (4H, m), 4.25–4.35 (1H, m), 4.44–4.55 (1H, m), 5.03–5.07 (1H, m), 5.74–5.98 (2H, m), 6.70–7.00 (1H, m) and 7.13–7.34 (10H, m); δ_C (50 MHz, $CDCl_3$) 16.3, 16.4, 21.8, 22.9, 24.7, 28.1, 37.8, 40.7, 52.0, 52.1, 55.2 (d, $^3J_{PC}$ 27.8), 61.9, 62.1, 80.6, 117.4 (d, $^2J_{PC}$ 186.6), 127.0, 127.1, 127.4, 127.6, 128.1, 128.7, 128.8, 128.9, 129.2, 129,2, 136.2, 138.6, 150.6 (d, $^1J_{PC}$ 5.8), 155.7, 170.9 and 171.6; δ_P (161 MHz, $CDCl_3$) 17.72; ESI-MS: *m/z* 652.8 $(M+Na)^+$.

4.2.9. Compound (15i)

Yield: 50%; colourless syrup; (Found: C, 65.27; H, 6.86; N, 6.42. Calcd for $C_{36}H_{46}N_3O_7P$: C, 65.14; H, 6.99; N, 6.33%); ν_{max} $(CHCl_3)$/cm^{-1} 3421, 3286, 3019, 2932, 1685, 1497, 1369, 1216 and

1029 cm^{-1}; δ_H (200 MHz, $CDCl_3$) 1.15–1.36 (15H, m), 2.82–3.31 (4H, m), 3.99–4.12 (4H, m), 4.67–4.97 (2H, m), 5.65–5.83 (2H, m), 6.36–6.79 (1H, m) and 6.90–7.30 (15H, m); δ_C (50 MHz, $CDCl_3$): 16.3 (d, $^3J_{PC}$ 6.2), 16.4, (d, $^3J_{PC}$ 6.2), 28.2, 37.5, 37.7, 53.9, 55.0 (d, $^3J_{PC}$ 22.7), 56.1, 61.9, 62.0 (d, $^2J_{PC}$ 5.5), 80.6, 117.4 (d, $^1J_{PC}$ 185.5), 127.1, 127.2, 127.3, 127.4, 127.7, 128.1, 128.7, 128.8, 128.9, 129.2, 129.3, 135.9, 136.2, 138.2, 150.3, 155.6, 169.8 and 171.1; δ_P (161 MHz, $CDCl_3$) 17.72; ESI-MS: *m/z* 686.7 $(M+Na)^+$.

4.2.10. Compound (15j)

Yield: 43%; colourless syrup; (Found: C, 67.27; H, 6.43; N, 6.12. Calcd for $C_{39}H_{44}N_3O_7P$: C, 67.13; H, 6.36; N, 6.02%); ν_{max} ($CHCl_3$)/ cm^{-1} 3414, 3295, 3066, 3019, 1713, 1659, 1651, 1497, 1455 and 1216 cm^{-1}; δ_H (200 MHz, $CDCl_3$): 1.18–1.34 (6H, m), 2.87–3.37 (4H, m), 3.94–4.08 (4H, m), 4.29–4.40 (1H, m), 4.64–5.04 (3H, m), 5.41–5.88 (2H, m), 6.57–6.80 (1H, m) and 6.84–7.42 (20H, m); δ_C (50 MHz, $CDCl_3$) 16.3, 16.4, 37.8, 38.0, 54.0, 54.9 (d, $^3J_{PC}$ 22.7), 56.5, 61.9, 62.1, 67.2, 117.3 (d, $^1J_{PC}$ 186.3), 126.9, 127.1, 127.2, 127.4, 127.5, 128.1, 128.2, 128.3, 128.6, 128.7, 128.8, 128.9, 129.2, 129.2, 129.3, 135.8, 135.9, 136.0, 138.5, 150.6, 156.2, 169.7 and 170.8; δ_P (161 MHz, $CDCl_3$): 17.84; ESI-MS: *m/z* 720.5 $(M+Na)^+$.

4.3. Bioassay of synthesized peptidyl-vinylaminophosphonates (15a–j)

The incubation method was used to measure the inhibition of papain.[6f] The inhibition incubation buffer for papain was 100 mM Tris–HCl buffer at pH 6.5 containing 0.5 mM DTT (dithiothreitol) and 0.4 mM EDTA. The assay uses the substrate BAPNA (0.75 mM) in the same buffer. The approximate final concentration of papain in enzymatic reaction was 0.04 mg/mL. The release of *p*-nitroanilide was monitored spectrophotometrically at 410 nm in the absence and presence of inhibitor. All assays were run in duplicate.

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Supplementary data

Supplementary data (full experimental procedures and copies of NMR spectra of all the compounds) associated with this article can be found, in the online version, at doi:10.1016/j.bmc.2011.09.058.

References and notes

1. (a) Leung, D.; Abbenante, G.; Fairlie, D. P. *J. Med. Chem.* **2000**, *43*, 305; (b) Otto, H. H.; Schirmeister, T. *Chem. Rev.* **1997**, *97*, 133; (c) Santos, M. M. M.; Moreira, R. *Mini-Rev. Med. Chem.* **2007**, *7*, 1040; (d) Abbenante, G.; Fairlie, D. P. *Med. Chem.* **2005**, *1*, 71; (e) Schirmeister, T.; Klockow, A. *Mini. Rev. Med. Chem.* **2003**, *3*, 585; (f) Schirmeister, T.; Kaeppler, U. *Mini-Rev. Med. Chem.* **2003**, *3*, 361.
2. (a) Jedeszko, C.; Sloane, B. F. *Biol. Chem.* **2004**, *385*, 1017; (b) Yan, S.; Sloane, B. F. *Biol. Chem.* **2003**, *384*, 845; (c) Berdowska, I. *Clin. Chim. Acta* **2004**, *342*, 41; (d) Blomgren, K.; McRae, A.; Bona, E.; Saido, T. C.; Karlsson, J. O. *Brain Res.* **1995**, *684*, 136; (e) Huang, Y.; Wang, K. K. *Trends Mol. Med.* **2001**, *7*, 355; (f) Ray, S. K.; Banik, N. L. *Curr. Drug. Targets CNS Neurol. Disord.* **2003**, *2*, 173; (g) Sedarous, M.; Keramaris, E.; O'Hare, M.; Melloni, E.; Slack, R. S., et al *J. Biol. Chem.* **2003**, *278*, 26031; (h) Shields, D. C.; Banik, N. L. *J. Neurosci. Res.* **1999**, *55*, 533; (i) Suzuki, K.; Hata, S.; Kawabata, Y.; Sorimachi, H. *Diabetes* **2004**, *53*, 12; (j) Johansson, A.; Kalfas, S. *Eur. J. Oral. Sci.* **1998**, *106*, 863; (k) Uhlmann, F. *Exp. Cell. Res.* **2004**, *296*, 80; (l) Brindley, P. J.; Kalinna, B. H.; Dalton, J. P.; Day, S. R.; Wong, J. Y. *Mol. Biochem. Parasitol.* **1997**, *89*, 1; (m) Turk, B.; Stoka, V.; Rozman-Pungercar, J.; Cirman, T.; Droga-Mazovec, G. *Biol. Chem.* **2002**, *383*, 1035.
3. (a) Keillor, J. W.; Brown, R. S. *J. Am. Chem. Soc.* **1991**, *113*, 5114; (b) Keillor, J. W.; Brown, R. S. *J. Am. Chem. Soc.* **1992**, *114*, 7983.
4. (a) Waag, T.; Gelhaus, C.; Rath, J.; Stich, A.; Leippe, M.; Schirmeister, T. *Bioorg. Med. Chem. Lett.* **2010**, *20*, 5541; (b) Loeser, R.; Abbenante, G.; Madala, P. K.; Halili, M.; Le, G. T.; Fairlie, D. P. *J. Med. Chem.* **2010**, *53*, 2651; (c) Breuning, A.; Degel, B.; Schulz, F.; Buchold, C.; Stempka, M.; Machon, U.; Heppner, S.; Gelhaus, C.; Leippe, M.; Leyh, M.; Kisker, C.; Rath, J.; Stich, A.; Gut, J.; Rosenthal, P. J.; Schmuck, C.; Schirmeister, T. *J. Med. Chem.* **2010**, *53*, 1951; (d) Loeser, R.; Gut, J.; Rosenthal, P. J.; Frizler, M.; Guetschow, M.; Andrews, K. T. *Bioorg. Med. Chem. Lett.* **2010**, *20*, 252; (e) Mallari, J. P.; Guiguemde, W. A.; Guy, R. K. *Bioorg. Med. Chem. Lett.* **2009**, *19*, 3546.
5. (a) Perlman, N.; Hazan, M.; Shokhen, M.; Albeck, A. *Bioorg. Med. Chem.* **2008**, *16*, 9032; (b) Ettari, R.; Nizi, E.; Di Francesco, M. E.; Dude, M. A.; Pradel, G.; Vicik, R.; Schirmeister, T.; Micale, N.; Grasso, S.; Zappala, M. *J. Med. Chem.* **2008**, *51*, 988; (c) Valente, C.; Moreira, R.; Guedes, R. C.; Iley, J.; Jaffar, M.; Douglas, K. T. *Bioorg. Med. Chem.* **2007**, *15*, 5340; (d) Vicik, R.; Helten, H.; Schirmeister, T.; Engels, B. *ChemMedChem* **2006**, *1*, 1021; (e) Kaeppler, U.; Schirmeister, T. *Med. Chem.* **2005**, *1*, 361.
6. (a) Altmann, E.; Cowan-Jacob, S. W.; Missbach, M. *J. Med. Chem.* **2004**, *47*, 5833; (b) Wang, G.; Yao, S. Q. *Org. Lett.* **2003**, *5*, 4437; (c) Zhou, N. E.; Guo, D.; Thomas, G.; Reddy, A. V. N.; Kaleta, J.; Purisima, E.; Menard, R.; Micetich, R. G.; Singh, R. *Bioorg. Med. Chem. Lett.* **2003**, *13*, 139; (d) Shenai, B. R.; Semenov, A. V.; Rosenthal, P. J. *Biol. Chem.* **2002**, *383*, 843; (e) Schirmeister, T. *Bioorg. Med. Chem. Lett.* **2000**, *10*, 2647; (f) Got, M. G.; Caffrey, C. R.; Hansell, E.; McKerrowb, J. H.; Powers, J. C. *Bioorg. Med. Chem.* **2004**, *12*, 5203.
7. (a) Frommer, W.; Junge, B.; Mueller, L.; Schmidt, D.; Truscheit, E. *Planta Med.* **1979**, *35*, 195; (b) Rich, D. H. In *Comprehensive Medicinal Chemistry*; Sammes, P. G., Ed.; Pergamon Press, Oxford, 1990; Vol. 2, p 391; (c) Hu, L. Y.; Abeles, R. *Arch. Biochem. Biophys.* **1990**, *281*, 271; (d) Barrett, A. J.; Rawlings, N. D.; Davies, M. E.; Machleidt, W.; Salvesen, G.; Turk, V. In *Proteinase Inhibitors*; Barrett, A. J., Salvesen, G., Eds.; Elsevier, Amsterdam, 1986; p 515; (e) Lowe, G.; Yuthavong, Y. *Biochem. J.* **1971**, *124*, 107; (f) Shaw, E. In *Advances in Enzymology and Related Areas of Molecular Biology*; Meister, A., Ed.; John Wiley & Sons, Inc., Hoboken, NJ, USA, 1990; Vol. 63, doi:10.1002/9780470123096.ch5; (g) Matsumoto, K.; Yamamoto, D.; Ohishi, H.; Tomoo, K.; Ishida, T.; Inoue, M.; Sadatome, T.; Kitamura, K.; Mizuno, H. *FEBS Lett.* **1989**, *245*, 177.
8. (a) Bhattacharya, A. K.; Rana, K. C.; Raut, D. S.; Mhaindarkar, V. P.; Khan, M. I. *Org. Biomol. Chem.* **2011**, *9*, 5407; (b) Bhattacharya, A. K.; Rana, K. C. *Tetrahedron Lett.* **2008**, *49*, 2598; (c) Bhattacharya, A. K.; Kaur, T. *Synlett* **2007**, 745.
9. (a) Lebedev, M. Y.; Erman, M. B. *Tetrahedron Lett.* **2002**, *43*, 1397; (b) Krimen, L. I.; Cota, D. J. *Org. React.* **1969**, *17*, 213.
10. (a) Zhu, J.; Lu, X. *J. Chem. Soc., Chem. Commun.* **1987**, 1318; (b) Ohler, E.; Kanzler, S. *Synthesis* **1985**, 539; (c) Lu, X.; Sun, J.; Zhu, J. *Heteroat. Chem.* **1992**, *3*, 551; (d) Ghosh, A. K.; Lei, H. *Synthesis* **2002**, 371; (e) Baeza, A.; Casas, J.; Najera, C.; Sansano, J. M. *J. Org. Chem.* **2006**, *71*, 3837; (f) Trost, B. M.; Sorum, M. T. *Org. Process Res. Dev.* **2003**, *7*, 432.
11. (a) Grehn, L.; Gunnarsson, K.; Ragnarsson, U. *J. Chem. Soc., Chem. Commun.* **1985**, 1317; (b) Kempf, D. J. *Tetrahedron Lett.* **1989**, *30*, 2029.
12. Wuts, P. G. M.; Greene, T. W. *Protective Group in Organic Synthesis*; Wiley-Interscience, 2007.
13. Takaya, J.; Kagoshima, H.; Akiyama, T. *Org. Lett.* **2000**, *2*, 1577.
14. Verkade, J. M. M.; Hemert, L. J. C.; Quaedflieg, P. J. L. M.; Alsters, P. L.; Delfta, F. L.; Rutjes, F. P. J. T. *Tetrahedron Lett.* **2006**, *47*, 8109.
15. Koenig, W.; Geiger, R. *Chem. Ber.* **1970**, *10*, 2024.