Novel methodology for the solid-phase synthesis of phosphinic peptides †

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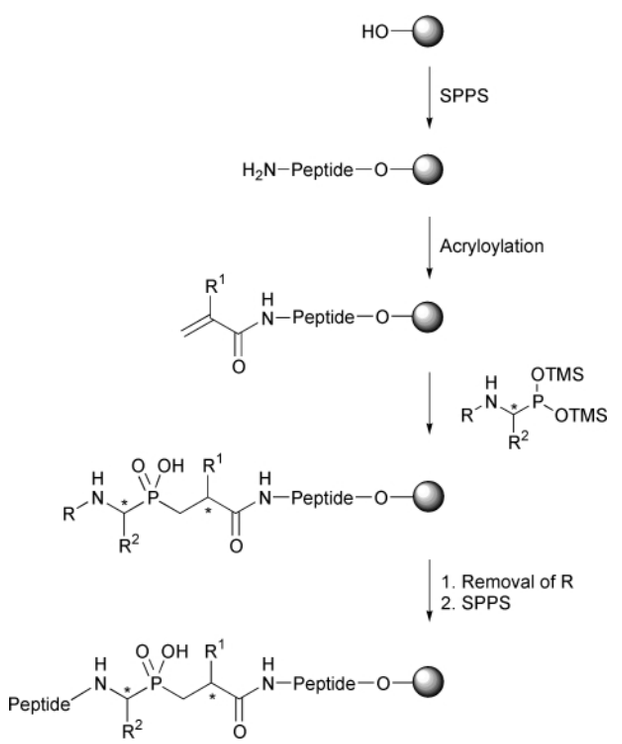
A novel, versatile strategy for the solid phase synthesis of phosphinic peptides is developed in which the phosphorus–carbon bond is formed on a polymer support during peptide synthesis. The formation of bis(trimethylsilyl) 1-(allyloxycarbonylamino)ethylphosphonite from 1-(allyloxycarbonylamino)ethylphosphinic acid is investigated, as well as the Michael addition of the former to a resin-bound acrylate. Conditions are also established for the clean, quantitative conversion of a resin-bound, N-terminus acryloylated peptide with bis(trimethylsilyl) 1-(allyloxycarbonylamino)-ethylphosphonite. Conventional peptide synthesis is then employed to obtain a phosphinic undecapeptide in high yield and purity.

Introduction

Phosphorus-backbone isosteres of peptides have received much attention as inhibitors of various proteases,[1–4] including matrix metalloproteinases (MMPs).[5–7] In phosphinic peptides, the conventional peptide bond is replaced by a phosphorus–carbon bond, namely the -P(O)OH-CH_2- moiety. As a consequence this particular kind of peptide isostere is more stable towards chemical hydrolysis than the related phosphonic [-P(O)OH-O-] and phosphonamidic [-P(O)OH-NH-] isosteres. The phosphinic moiety has been introduced into peptides in a number of different ways. Most recently phosphinic pseudo-dipeptide building blocks have been used to prepare phosphinic peptides on solid phase.[6–8] The solid-phase technology is advantageous for the preparation of peptides and peptidomimetics because of their oligomeric structure.[9] It is therefore desirable to establish reaction conditions which allow the formation of the phosphinic moiety to be conducted on solid support, thereby avoiding the need for the 400 different phosphinic dipeptide building blocks which would be necessary to represent the natural amino acid side-chains. By contrast, a direct solid-phase approach would require only 40 compounds (amino acid analogues) to give synthetic access to the complete spectrum of phosphinic peptides.

In the preparation of phosphinic peptides the P–C bond is conveniently formed by a Michael-type addition of a bis(trimethylsilyl) phosphonite to an acrylate.[10,11] This transformation has been restricted almost exclusively to solution-phase synthesis of phosphinic dipeptide building blocks,[6,8] except for one example in which phosphinic dipeptoids were prepared on a solid support.[12] The present work describes the development of a methodology for the assembly of a phosphinic peptide on a solid support (Scheme 1), by a process which comprises conventional solid-phase peptide synthesis (SPPS); acryloylation of the peptide N-terminal; addition of a protected 1-aminoalkylphosphinic acid [*via* the corresponding bis(trimethylsilyl) phosphonite] to the N-terminal acrylamide, and finally elongation of the pseudo-peptide by SPPS.



Scheme 1 Schematic presentation of the preparation of a complete phosphinic peptide by solid-phase synthesis, including the coupling of a bis(trimethylsilyl) 1-(allyloxycarbonylamino)alkylphosphonite to a resin bound acrylate.

Results and discussion

Prior to the synthesis of a phosphinic peptide, conditions were established for addition of a protected 1-aminophosphinic acid to a resin-bound acrylate (Scheme 2). As a model system, 1-aminoethylphosphinic acid (alanine analogue) and resin-bound acrylic ester **1** (glycine analogue) were selected. The PEG-based resin POEPS-3 [13,14] was used because of its chemical inertness and was acylated with acryloyl chloride. The quantitative formation of the acrylic ester on the solid support was confirmed from a single bead Nano-probe Magic Angle

† ^{1}H NMR spectra for **9** and **11** after cleavage are available as supplementary data. For direct electronic access see http://www.rsc.org/suppdata/p1/b0/b003848m/

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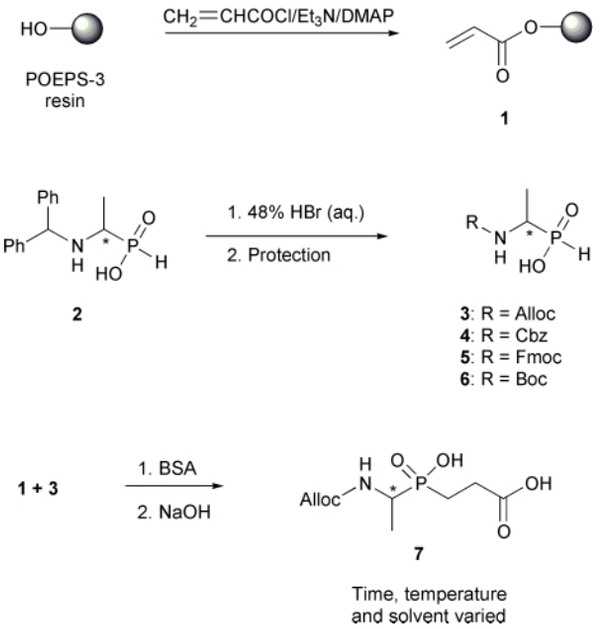
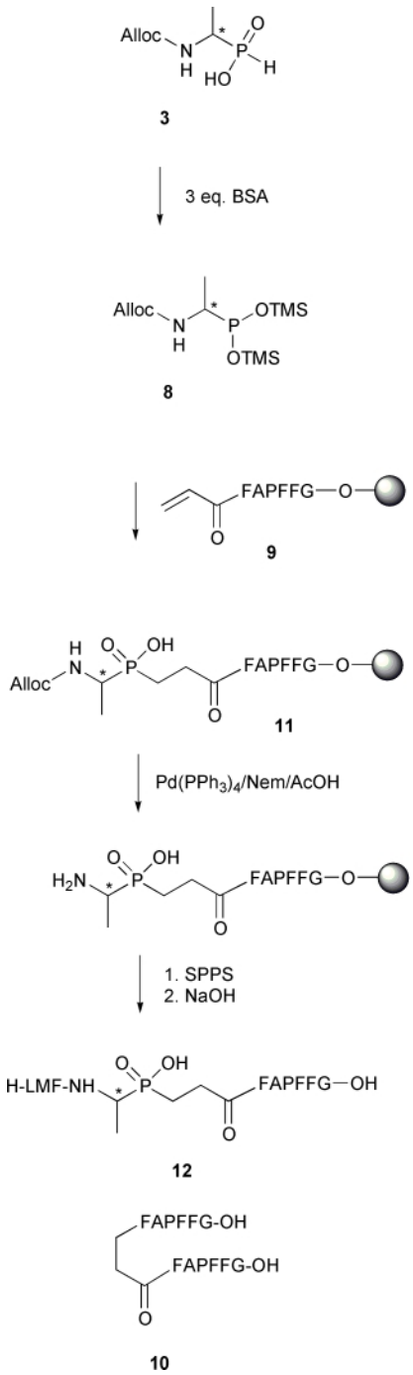


Scheme 2 Preparation of resin **1** and building blocks for the investigation of the solid-phase Michael additions. Protection of the phosphinic amino acids was performed with Alloc-Cl, Cbz-Cl, Fmoc-OSu and Boc_2O, respectively, in basic aqueous solution.



Scheme 3 Reactions performed to (i) investigate the solid-phase Michael addition of the phosphonite **8** to the resin-bound acrylic ester, and (ii) prepare a phosphinic undecapeptide using only solid-phase reactions.

Spinning (MAS) ^{1}H NMR spectrum. In contrast, attempts to couple acrylic acid to the resin using 2,4,6-mesitylsulfonyl-3-nitro-1,2,4-triazole (MSNT)[15] were unsuccessful. After reaction, the hydroxy groups of the resin were blocked. However, the acrylic ester was not formed, as determined by Nano-probe ^{1}H NMR spectroscopy.

The alanine analogue 1-aminoethylphosphinic acid is readily available[16] in the benzhydryl-protected form, **2**. It was converted into the differently protected phosphinic alanine analogues **3**–**6** by standard procedures. The Fmoc group was anticipated to be the most convenient amine protection because it can be removed under mild conditions as in conventional SPPS. Since the Boc group is usually removed with acid it would be limited to the preparation of peptides not containing acid-labile side-chain-protective groups. In contrast, Cbz and Alloc protective groups are stable to a variety of conditions. Although Alloc protection is readily removed on solid support using $Pd(PPh_3)_4$,[17] the Cbz group is cleaved either by hydrogenolysis, which is not always easily achieved on solid support, or by alternative conditions such as trimethylsilyl iodide (TMSI), which is not compatible with many peptide side-chain-protective groups.

Bis(trimethylsilyl)acetamide (BSA) and a mixture of TMSCl and Et_3N were both investigated for the formation of bis-(trimethylsilyl) 1-(allyloxycarbonylamino)ethylphosphonite **8** (Scheme 3). The former was found to be superior since TMSCl–Et_3N partially cleaved the carbamate protective groups, especially at elevated temperatures. Furthermore, the Fmoc group was slowly cleaved by BSA. Considering the general disadvantages of using Boc and Cbz groups in Fmoc-based SPPS it was decided to use the acryloylated resin **1** and 1-(allyloxycarbonyl)ethylphosphinic acid **3** together with BSA for further investigations.

Previous work on this type of Michael addition both in solution[10] and on polymeric support[12] has described the reaction of phosphinic acids with acrylic esters using BSA at room temperature. However, the reaction between **1**, **3** and BSA (proportions 1 : 3 : 9) for a period 20 h gave rise to less than 5% of the desired product **7** although the product obtained was of high purity. Increasing the reaction temperature to 60 °C resulted in a higher yield of **7**, but complete conversion of **1** was still not achieved and the formation of the phosphonite **8** was therefore investigated. When reaction mixtures of **3** and increasing amounts of BSA at room temperature were analysed by ^{31}P NMR ($CDCl_3$), the resonance originating from **3** (δ_P 33.4) was seen to diminish and be replaced by multiple resonances around δ_P 22.5, showing the formation of other tervalent phosphorus species. However, on heating of the solutions to 100 °C for a short period in a closed container, a single resonance at δ_P 7.6 appeared corresponding to complete conversion of **3** into **8**. When less than three molar equivalents of BSA with respect to **3** was used, the conversion at 100 °C remained incomplete and a ratio of **3** to BSA of 1 : 3 was therefore selected to generate the phosphonite **8**.

A series of experiments in 1,2-dichloroethane (DCE) showed that the Michael addition also required elevated temperatures to proceed satisfactorily. Reaction of 6 equivalents

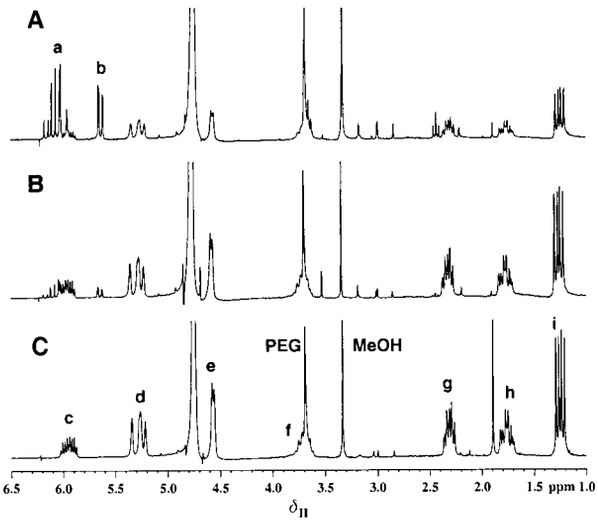


Fig. 1 ^{1}H NMR spectra of crude products formed in the reaction between **1**, **3** and BSA (1 : 6 : 18) at different periods of time and temperatures. **A**: rt, 18 h (40% conversion); **B**: 60 °C, 22 h (80% conversion); **C**: 100 °C, 1 h (100% conversion). Resonances a, b, c–e, and f–i originate from acrylic acid, the Alloc group, and the phosphinic dipeptide analogue, respectively.

of **8** with **1** at either room temperature for 18 h, or at 60 °C for 22 h, both gave incomplete conversion of **1** (Fig. 1 A + B). However, reaction at 100 °C for 1 h resulted in clean and quantitative conversion into **7**, with no detectable side-products (Fig. 1C). Shorter reaction times at 100 °C led to incomplete conversion of **1** and, moreover, a large excess of **8** (**1**:**3**:BSA 1:6:18) was necessary, since TLC analysis of the supernatant showed extensive degradation of **3** after 1 h (**8** decomposed to **3** on the TLC plate). The degradation products did, however, not appear to react with the acrylate and the crude product obtained after cleavage from the resin was of high purity.

The reaction conditions were also applied to the analogues **4**–**6**. The Fmoc group did not tolerate these reaction conditions, resulting in a complex mixture of products, whereas both the Boc and the Cbz groups were unaffected though the reaction rate was slower.

The rate of addition was investigated using a variety of solvents ranging from acetonitrile to toluene in polarity (Table 1). Although the rate was highest in DCM the vapor pressure of this solvent at 100 °C was too high for it to be useful and reactions were therefore conducted in DCE. The rate of a solid-phase reaction is known to be strongly influenced by the properties of the solid support [18] since this may be regarded as a co-solvent in the reaction.[19] Therefore, the minimum reaction time required is likely to be different if a resin other than POEPS-3 is used.

Having established suitable reaction conditions for the model system, the methodology was applied to the preparation of a phosphinic peptide (Scheme 3). The peptide sequence -FAPFFG- was assembled on the POEPS-3 resin without the use of a linker. The MSNT reagent was used to attach glycine and standard Fmoc chemistry and pentafluorophenyl (Pfp) esters [20,21] were used in the subsequent couplings. The sequence was selected such that reactions could be analysed by ^{1}H NMR, RP-HPLC and matrix-assisted laser desorption/ionisation time-of-flight mass spectrometry (MALDI-TOF MS), after cleavage of the crude product from the resin with NaOH. In addition, amino acids containing side-chain-protective groups were excluded to avoid competing reactions that could impair structural analysis.

Acylation of the peptide N-terminal was carried out using acryloyl chloride, since standard activation of acrylic acid using *O*-(1*H*-benzotriazol-1-yl)-*N*,*N*,*N*′,*N*′-tetramethyluronium tetrafluoroborate (TBTU) [22] and reaction with the N-terminal resulted in a complex mixture of products (as determined by RP-HPLC and MALDI-TOF). Although the most abundant component was the desired coupling product **9**, the mass spectrum of the mixture also indicated the presence of the bis-adduct **10**.

Table 1 Conversion of resin-bound acrylate **1** with phosphinic acid **3** and BSA (1 : 6 : 18) at 100 °C for 30 min in various solvents

Solvent	Conversion (%)	Relative to DCM
DCM	57	1.00
DCE	52	0.91
MeCN	36	0.63
Ethyl acetate	30	0.53
Toluene	28	0.49
DMSO	26	0.46
$CHCl_3$	26	0.46
THF	16	0.28
Neat BSA	16	0.28

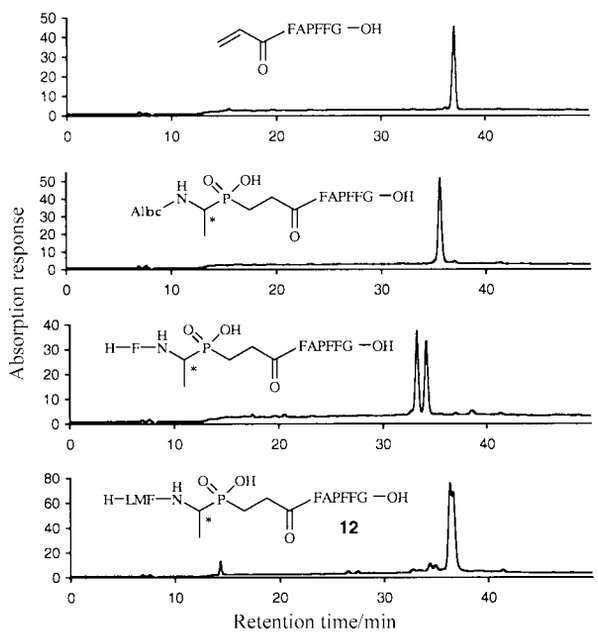


Fig. 2 RP-HPLC chromatograms showing the high purity of the crude compounds obtained at different stages of the synthesis sequence during the preparation of the phosphinic undecapeptide **12** by solid-phase reactions only.

In the next step, the Michael addition of **8** to **9**, the optimised conditions from the model study failed to give complete conversion of **9** into **11**. ^{1}H NMR spectroscopic analysis of the crude cleavage products revealed that the amounts of both **3** and BSA affected the yield of **11**. The crude products contained only varying amounts of the acryloylated peptide **9** in addition to **11**. When the addition reaction was run at 100 °C, using 12 equivalents of **8** (**1**:**3**:BSA 1:12:36), conversion was complete in 4 h (as determined by ^{1}H NMR, RP-HPLC (Fig. 2) and MALDI-TOF MS). Inconveniently long reaction times were required when lower concentrations of **8** were used and the use of a larger excess to BSA compared with **3** (1:4) increased the conversion of **9** only marginally.

Removal of the Alloc group was effected with a solution of $Pd(PPh_3)_4$ and *N*-ethylmorpholinium acetate in chloroform.[17] Subsequent amino acid couplings were performed using Fmoc-protected amino acid Pfp esters with 3,4-dihydro-3-hydroxy-4-oxo-1,2,3-benzotriazine (Dhbt-OH) as the catalyst. The coupling of Fmoc-Phe-OPfp to the *N*-terminus of the phosphinic peptide proved to be slow, but was complete within 16 h using 6 equivalents of Fmoc-Phe-OPfp, as determined by RP-HPLC

analysis (50, 64, 84 and 100% complete after periods of ¼, ½, 2 and 16 h, respectively). Subsequent couplings with Fmoc-protected amino acid pentafluorophenyl esters also required extended reaction times.

The phosphinic undecapeptide **12** was cleaved from the resin to afford a crude product which was essentially pure. Two closely eluting peaks were visible by RP-HPLC, corresponding to the two diastereomeric peptides resulting from the introduction of the racemic Ala-Gly phosphinic dipeptide moiety (Fig. 2). Interestingly, the two diastereomers were not resolved in the RP-HPLC chromatogram until after coupling of the phenylalanine residue. Finally, purified phosphinic undecapeptide **12** was isolated by preparative RP-HPLC in 45% yield. The yield and purity of undecapeptide **12** was comparable to yields previously obtained by a more tedious building-block approach.[6]

Conclusions

An efficient protocol for the solid-phase preparation of phosphinic peptides was developed. Conditions were established for the reaction of a resin-bound acrylate or acrylamide with a bis(trimethylsilyl) phosphonite derived from 1-(allyloxycarbonylamino)ethylphosphinic acid. The synthesis of a phosphinic undecapeptide was performed in which all reactions were conducted on solid support, including the Michael addition reaction which generates the phosphinic pseudo-dipeptide moiety. The final phosphinic peptide was isolated in high yield and purity. Further studies will be directed towards applications of this method for the combinatorial synthesis of phosphinic peptides containing a large variety of both natural amino acids as well as substitued acrylates and 1-aminoalkylphosphinic acids.

Experimental

General

Most anhydrous solvents were obtained by storing analytical quality solvents over 3 or 4 Å activated molecular sieves after which the water content was verified to be below 30 ppm by Karl Fischer titration. Anhydrous DMF was obtained by fractionally distillation at reduced pressure and stored over 4 Å molecular sieves. All commercial starting materials were used without purification. Solution NMR data were acquired on a Bruker 250 MHz Avance DRX 250 spectrometer and were referenced to $CHCl_3$ (δ 7.24, ^{1}H), HDO (δ 4.75, ^{1}H) or H_3PO_4 (δ_P 0, ^{31}P, external), and MAS NMR spectra were aquired on a Varian Inova 500 MHz with a 4 mm ^{1}H-observe nano-probe and a spinning rate of 2.1 kHz. Coupling constants J are given in Hz. Analytical RP-HPLC was performed on a Waters system (490E detector, using two 510 pumps with a gradient controller and 8 mm diameter RCM C_{18} column). Preparative RP-HPLC purification of phosphinic undecapeptide **12** was carried out on a Waters system (991 photodiode array detector and 600 E system controller) connected to a Waters 25 mm diameter RCM C_{18}-column. All RP-HPLC procedures were carried out with a linear gradient. Buffers: A (0.1% TFA in water) and B (0.1% TFA in MeCN–water 9:1), 50 min gradient of 2% B min^{-1}. MALDI-TOF mass spectra were acquired on a Bruker Reflex III high-resolution mass spectrometer. Electrospray mass spectra were obtained on a Fisons VG Quattro 5098 mass spectrometer (mobile phase 50% aq. MeCN, 8 µl min^{-1}, sample: 10 µl, ≈20 pmol µl^{-1}). Light petroleum refers to the fraction with distillation range 60–80 °C.

Solid-phase synthesis procedures

Deprotection of N$^{\alpha}$ Fmoc was performed with piperidine (20% in DMF) for 2 and 10 min. Volumes of washing solvent were 1–2-times the volume necessary to swell the resin and washings were 6 × 1 min unless otherwise stated. Minimum volume was used for solid-phase reactions. Solid-phase reactions at elevated temperatures were performed in 5 ml reaction vials (Pierce). Amino (or hydroxy) group loadings were determined by spectrophotometric determination of released piperidine–dibenzofulvene adduct (290 nm)[9] after coupling with Fmoc-Gly-OPfp/DhbtOH (or Fmoc-Gly-OH/MSNT) and treatment of a known sample with 20% piperidine in DMF.

Acryloylated POEPS-3 resin 1. 4-(Dimethylamino)pyridine (DMAP) (12.2 mg, 0.1 mmol) and Et_3N (277 µl, 2.0 mmol) were dissolved in DCM (10 ml) and the solution was added to POEPS-3 resin[13] (500 mg, 0.1 mmol) which was allowed the swell for 10 min. A solution of acryloyl chloride (163 µl, 2.0 mmol) in DCM (1 ml) was added to the swelled resin while it was being stirred gently with a spatula. After a period of 2.5 h the resin was drained and washed successively with DCM × 3, MeOH × 3, DCM and lyophilised to obtain *acryloylated resin* **1**, $\delta_{H,MAS}$(500 MHz; $CDCl_3$; $CHCl_3$) 4.30 [2 H, br t, J 4.7, C(O)OCH_2CH$_2$-OPEG], 5.82 (1 H, d, J 10.4, *cis* CH_2=CHCO$_2$), 6.14 (1 H, dd, J 10.4 and 17.6, CH$_2$=CHCO$_2$), 6.41 (1 H, d, J 17.6, *trans* CH_2=CHCO$_2$). Resonances corresponding to the resin were also observed in the ^{1}H NMR MAS spectrum.

1-(Allyloxycarbonylamino)ethylphosphinic acid 3. 1-(Benzhydrylamino)ethylphosphinic acid **2**[16] (5.00 g, 18.2 mmol) was mixed with 48% aq. HBr (16.7 ml, 8.2 eq.) and brought to 126 °C for 15 min. After cooling to room temperature the mixture was concentrated *in vacuo* and mixed with water (25 ml) and DCM (25 ml). Disregarding the precipitate present at this time,‡ the aqueous layer was washed with DCM (3 × 25 ml), adjusted to pH 7 with aq. NaOH (10 M; 3 ml), stirred for 30 min at 0 °C, and filtered. Solid Na_2CO_3 (3.85 g, 2 eq.) was added to the filtrate and allyl chloroformate (2.30 ml, 1.2 eq.) was added dropwise at room temperature with vigorous stirring. Reaction was complete in 30 min after which the pH was adjusted to between 0 and 1 with aq. HCl (10 M; 4 ml). The mixture was stirred 30 min at 0 °C, filtered, and NaCl (approx. 30 g) was added to supersaturate the solution with NaCl. Extraction with ethyl acetate (12 × 25 ml) was carried out while keeping the aqueous phase at pH 0–1 at all times. The combined extracts were dried with $MgSO_4$ and concentrated to dryness. The resulting partly crystallised product was re-dissolved in ethyl acetate (50 ml) and the compound was precipitated by addition of light petroleum (75 ml); cooling overnight to give *phosphinic acid* **3** (2.47 g, 70%) as a white powder, mp 74–76 °C (Found: C, 37.6; H, 6.1; N, 6.9. $C_6H_{12}NO_4P$ requires C, 37.3; H, 6.3; N, 7.3%); δ_H(250 MHz; 0.1 M NaOD in D_2O; HDO) 1.23 (3 H, dd, J 15.5 and 7.4, CH_3), 3.62 (1 H, m, NHCHCH$_3$), 4.57 (2 H, d, J 4.6, CH$_2$=CHCH_2O), 5.24 (1 H, d, J 10.6, *cis* CH_2=CHCH$_2$O), 5.31 (1 H, d, J 17.4, *trans* CH_2=CHCH$_2$O), 5.94 (1 H, m, CH$_2$=CHCH$_2$O), 6.79 (1 H, d, J 517, PH); δ_P(250 MHz; 0.1 M NaOD in D_2O; H_3PO_4) 27.0.

Reaction of acryloylated resin 1 with 1-(allyloxycarbonylamino)ethylphosphinic acid 3. BSA (45 µl, 180 µmol) was added to a suspension of **3** (11.6 mg, 60 µmol) in degassed, anhydrous solvent (1 ml, DCE) and the resulting solution was purged for 2 min with Ar. The solution was added to resin **1** (50 mg, 10 µmol) in a 5 ml reaction vial and purged with Ar for 1 min. The vial was placed in a heating block at the reaction temperature (100 °C) for the required time (1 h). After reaction the resin was washed successively DCM and MeOH and the product(s) was/were cleaved by treatment with aq. NaOH (500 µl; 0.1 M) for 1 h. The solution was lyophilised and the product was dissolved in D_2O and analysed by ^{31}P and ^{1}H NMR. The optimum reac-

‡ The precipitate was an aromatic compound not related to the 1-aminoethylphosphinic acid.

tion conditions/amounts are given in parentheses. Under these conditions the following analytical data were obtained (Fig. 1). δ_H(250 MHz; 0.1 M NaOD in D_2O; HDO) 1.24 (3 H, dd, *J* 13.2 and 7.2, CH_3), 1.76 (2 H, dd, *J* 12.2 and 6.0, PCH_2), 2.31 (2 H, dd, *J* 11.9 and 6.9, CH_2CO), 3.70 (2 H, m, C*H*MeP), 4.56 (2 H, d, *J* 4.7, CH_2=CHCH_2O), 5.24 (1 H, d, *J* 11.0, *cis* CH_2=CH-CH_2O), 5.30 (1 H, d, *J* 18.0, *trans* CH_2=CHCH_2O), 5.97 (1 H, m, CH_2=C*H*CH_2O); δ_P(250 MHz; 0.1 M NaOD in D_2O; H_3PO_4) 41.6; *m*/*z* (ES-MS) 265.9 ([M + H]$^+$. $C_9H_{17}NO_6P$ requires *m*/*z* 266.1), 287.9 ([M + Na]$^+$. $C_9H_{16}NNaO_6P$ requires *m*/*z* 288.1).

Phosphinic undecapeptide 12. POEPS-3 resin[13] (1.5 g, 0.3 mmol) was swelled in anhydrous DCM (50 ml) and drained after a period of 10 min. A solution of Fmoc-Gly-OH (267 mg, 0.9 mmol) and *N*-methylimidazole (54 µl, 0.675 mmol) in anhydrous DCM (30 ml) was mixed with MSNT (267 mg, 0.9 mmol) and added to the resin. After 60 min the resin was drained, washed with anhydrous DCM × 3, and another coupling of Fmoc-Gly-OH/MSNT/*N*-methylimidazole was performed for 60 min. The resin was drained and washed successively with DCM, DMF × 3, (5% diisopropylethylamine in DMF) × 2, DMF × 3, and DCM, and lyophilised. Loading: 0.20 mmol g^{-1}. A fraction of the resin (840 mg, 0.167 mmol) was treated with piperidine to remove Fmoc, washed with DMF, and treated with a solution of Fmoc-Phe-OPfp (278 mg, 0.501 mmol) and Dhbt-OH (27 mg, 0.167 mmol) in DMF for 1 h. Similarly, Fmoc-protected amino acid Pfp esters of Phe, Pro, Ala, and Phe were coupled using Dhbt-OH until a negative Kaiser ninhydrin test was observed (amounts Fmoc-Pro-OPfp: 253 mg, Fmoc-Ala-OPfp: 240 mg). Loading: 0.10 mmol g^{-1}. A small aliquot of the resin was treated with piperidine, washed with DMF × 3 and with MeOH × 3 and treated with NaOH (0.1 M) to give *hexapeptide FAPFFG*. RP-HPLC (1 peak, 29.3 min); *m*/*z* (MALDI-TOF) 685.7 ([M + H]$^+$. $C_{37}H_{45}N_6O_7$ requires *m*/*z*, 685.3), 707.8 ([M + Na]$^+$. $C_{37}H_{44}N_6NaO_7$ requires *m*/*z*, 708.3).

A fraction of the resin (200 mg, 40 µmol) was treated with piperidine and washed successively with DMF and DCM. Neat Et_3N (111 µl, 800 µmol) was added to the resin followed by a solution of acryloyl chloride (33 µl, 400 µmol) in DCM. The reaction was complete within 5 min according to Kaiser ninhydrin test. The resin was drained, washed successively with DCM and MeOH × 3, and lyophilised. A small aliquot of the resin was treated with NaOH (0.1 M) to give *acryloylated hexapeptide Acr-FAPFFG* **9** RP-HPLC (1 peak, 39.0 min); *m*/*z* (MALDI-TOF) 761.8 ([M + Na]$^+$. $C_{40}H_{46}N_6NaO_8$ requires *m*/*z*, 761.3).

A fraction of the resin (50 mg, 10 µmol) was placed in a 5 ml reaction vial and a solution of **3** (23.2 mg, 120 µmol) and BSA (90 µl, 360 µmol) in degassed DCE (1 ml) was added to the resin. The resin slurry was purged with Ar for 1 min, and the vial was closed and heated to 100 °C for 4 h, after which it was cooled to rt and washed with DCM and lyophilised. A small aliquot of the resin was washed with MeOH and treated with NaOH (0.1 M) to give *phosphinic octapeptide Alloc-AlaΨ{PO$_2$H-CH$_2$}Gly-FAPFFG* **11**. RP-HPLC (1 peak, 35.6 min); *m*/*z* (MALDI-TOF) 932.7 ([M + H]$^+$. $C_{46}H_{59}N_7O_{12}P$ requires *m*/*z*, 932.4), 954.8 ([M + Na]$^+$. $C_{46}H_{58}N_7NaO_{12}P$ requires *m*/*z*, 954.4), 970.7 ([M + K]$^+$. $C_{46}H_{58}KN_7O_{12}P$ requires *m*/*z*, 970.4).

A solution of $Pd(PPh_3)_4$ (32 mg, 30 µmol) in $CHCl_3$–AcOH–*N*-ethylmorpholine 92.5 : 5 : 2.5 (800 µl) was added to the resin and left for 15 min. The resin was washed successively with $CHCl_3$, DMF × 3, (0.5% Et_2NCS_2Na in DMF) × 3 and DMF. A solution of Fmoc-Phe-OPfp (33 mg, 60 mmol) and Dhbt-OH (0.6 mg, 10 µmol) in DMF was added to the resin and allowed to react for a period of 16 h. Similarly, Fmoc-protected amino acid Pfp esters of Met and Leu were coupled using Dhbt-OH (amounts Met: 16 mg, 30 µmol and Leu: 16 mg, 30 µmol). After the final coupling the resin was washed with DMF, treated with piperidine to remove Fmoc, and washed successively with DMF and MeOH. The product was cleaved from the resin by treatment with NaOH (400 µl; 0.1 M), and the resin was washed successively with water and MeOH. Aq. HCl (400 µl, 0.1 M) was added to the combined filtrate and the solvents were removed *in vacuo*. The solid residue (10 mg) was re-dissolved in 50% aq. MeCN (2 ml) and purified by preparative RP-HPLC. Fractions containing the product were collected, and concentrated to dryness *in vacuo* to obtain *phosphinic undecapeptide LMF-AlaΨ{PO$_2$H-CH$_2$}Gly-FAPFFG* **12** as a solid mixture of two diastereomers (5 mg, 45% based on the initial resin loading or 90% based on loading of Fmoc-FAPFFG-O-POEPS-3); RP-HPLC (2 peaks, 36.7 and 37.0 min). δ_P(250 MHz; 0.1 M NaOD in D_2O; H_3PO_4) 39.2 and 39.7; *m*/*z* (MALDI-TOF) 1240.0 ([M + H]$^+$. $C_{62}H_{84}N_{10}O_{13}PS$ requires *m*/*z*, 1239.6), 1262.0 ([M + Na]$^+$. $C_{62}H_{83}N_{10}NaO_{13}PS$ requires *m*/*z*, 1261.6), 1278.0 ([M + K]$^+$. $C_{62}H_{83}KN_{10}O_{13}PS$ requires *m*/*z*, 1277.6). Amino acid analysis: Gly 1.04, Ala 1.02, Met 0.93, Leu 0.95, Phe 4.10, Pro 0.95 (LMF-AlaΨ{PO$_2$H-CH$_2$}Gly-FAPFFG requires Gly 1, Ala 1, Met 1, Leu 1, Phe 4, Pro 1).

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References

1 M. C. Allen, W. Fuhrer, B. Tuck, R. Wade and J. M. Wood, *J. Med. Chem.*, 1989, **32**, 1652.
2 P. A. Bartlett, J. E. Hanson and P. P. Giannousis, *J. Org. Chem.*, 1990, **55**, 6268.
3 C. Verbruggen, S. De Craecker, P. Rajan, X.-Y. Jiao, M. Borloo, K. Smith, A. H. Fairlamb and A. Haemers, *Bioorg. Med. Chem. Lett.*, 1996, **6**, 253.
4 I. Yiallouros, S. Vassiliou, A. Yiotakis, R. Zwilling, W. Stöcker and V. Dive, *Biochem. J.*, 1998, **331**, 375.
5 M. Whittaker, C. D. Floyd, P. Brown and A. J. H. Gearing, *Chem. Rev.*, 1999, **99** , 2735.
6 J. Buchardt, M. Ferreras, C. Krog-Jensen, J.-M. Delaissé, N. T. Foged and M. Meldal, *Chem. Eur. J.*, 1999, **5**, 2877.
7 S. Vassiliou, A. Mucha, P. Cuniasse, D. Georgiadis, K. Lucet-Levannier, F. Beau, R. Kannan, G. Murphy, V. Knäuper, M.-C. Rio, P. Basset, A. Yiotakis and V. Dive, *J. Med. Chem.*, 1999, **42**, 2610.
8 A. Yiotakis, S. Vassiliou, J. Jirácek and V. Dive, *J. Org. Chem.*, 1996, **61** , 6601.
9 G. Fields, Z. Tian and G. Barany, in *Synthetic Peptides: A Users Guide*, ed. G. A. Grant, W. H. Freeman and Company, New York, 1992, p. 77.
10 J. K. Thottathil, D. E. Ryono, C. A. Przybyla, J. L. Moniot and R. Neubeck, *Tetrahedron Lett.*, 1984, **25**, 4741.
11 E. A. Boyd, M. Corless, K. James and A. C. Regan, *Tetrahedron Lett.*, 1990, **31**, 2933.
12 P. H. Dorff, G. Chiu, S. W. Goldstein and B. P. Morgan, *Tetrahedron Lett.*, 1998, **39**, 3375.
13 J. Buchardt and M. Meldal, *Tetrahedron Lett.*, 1998, **39**, 8695.
14 M. Grötli, C. H. Gotfredsen, J. Rademann, J. Buchardt, A. J. Clark, J. Duus and M. Meldal, *J. Comb. Chem.*, 2000, **2**, 108.
15 B. Blankemeyer-Menge, M. Nimtz and R. Frank, *Tetrahedron Lett.*, 1990, **31**, 1701.
16 E. K. Baylis, C. D. Campbell and J. G. Dingwall, *J. Chem. Soc., Perkin Trans. 1*, 1984, 2845.
17 S. A. Kates, S. B. Daniels and F. Albericio, *Anal. Biochem.*, 1993, **212**, 303.
18 M. Meldal, in *Solid Phase Peptide Synthesis* (*Methods in Enzymology Vol 289*), ed. G. Fields, Academic Press, New York, 1997, p. 83.
19 W. Li and B. Yan, *J. Org. Chem.*, 1998, **63**, 4092.
20 E. Atherton and R. C. Sheppard, *J. Chem. Soc., Chem. Commun.*, 1985, 165.
21 M. Bodansky and M. A. Bednarek, *J. Protein Chem.*, 1989, **8**, 461.
22 R. Knorr, A. Trzeciak, W. Bannwarth and D. Gillesen, *Tetrahedron Lett.*, 1989, **30**, 1927.