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A rational approach to the synthesis of phosphonamidate peptides

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SUMMARY

A rational approach to the synthesis of phosphonamidate pseudopeptides is described. This strategy can be easily applied to the preparation of peptides containing phosphonic acid residues at various positions, as well as side-chain-functionalized amino acid residues. The reaction conditions are compatible with the severe lability of the P-N bond, and the absence of racemization is demonstrated by ^{31}P NMR analysis. This approach is suitable for application in solid-phase synthesis of biologically active phosphonopeptides.

INTRODUCTION

The stereoelectronic similarities between the tetrahedral phosphorus atom and the transition state resulting from the hydrolysis of a peptide bond by protease has led to the design of numerous potent phosphonopeptide inhibitors [1,2], as well as haptens which can elicit catalytic antibodies [3]. The preparation of phosphonamidate peptides has previously been dictated by the ease of preparation of phosphonic acid, rather than by methodological considerations. One of the major problems with the P-N bond is its inherent instability under acidic conditions, although this may be partly offset by protection of the phosphonic moiety [4]. The most widely used approach is the Z/OMe strategy, which has greatly contributed to the development of potent enzyme inhibitors containing mainly a Z-protected N-terminal amino-phosphonic residue [1]. However, this strategy severely restricts the choice of side-chain-protecting groups. Moreover, the highly basic conditions which are needed to deprotect the final phosphonamidate may lead to products that are contaminated with a salt excess. In the few reported examples on a nonterminal amino-phos-

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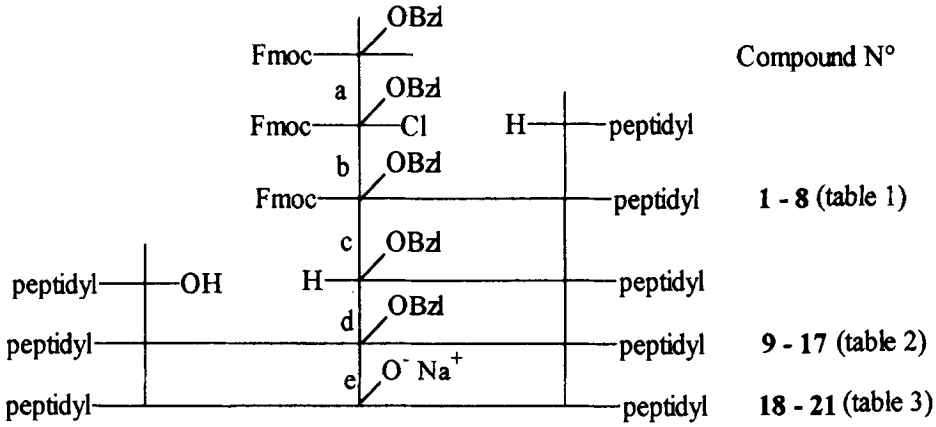

Scheme 1. Synthesis of phosphonamidate pseudopeptides. (a) $SOCl_2$ (1.4 equiv), CH_2Cl_2 dry, 30 min; (b) HCl, H-peptidyl (0.9 equiv), CH_2Cl_2 dry, Et_3N (2.2 equiv), 25 °C, 2 h; (c) Et_2NH (10 equiv), CH_2Cl_2/DMF, 25 °C; (d) BOP (1 equiv), peptidyl-OH (1 equiv), DIEA (3 equiv), 30 min; (e) Pd/C (10%), $NaHCO_3$ (2 equiv), EtOH/H_2O (1/1), 1 h at atmospheric pressure, 25 °C.

phonic residue-containing peptide, the cleavage is complicated by a partial P-N bond rupture [2], probably via P-oxazolone formation under these basic conditions.

As a continuation of our studies in the area of phosphonamide-substituted peptides, we wished to develop a more versatile approach to the synthesis of such compounds which could potentially be adapted to Solid Phase Peptide Synthesis (SPPS).

RESULTS AND DISCUSSION

We have used the same considerations as in classical peptide synthesis [5], but taken into account the lability of the phosphorus-nitrogen bond in the choice of permanent and temporary protecting groups, Pi and Ti (Fig. 1).

When an amino-phosphonic residue is introduced, the P-N bond restricts the possible combinations of protecting groups, thus the choice of protected amino-phosphonic analogue controls the synthetic strategy. For permanent protection of the phosphonic unit and amino acid side chain, we chose a hydrogenolysable benzyl-type protecting group. This may be removed under mild and neutral conditions, which are compatible with the phosphonamidate linkage. Furthermore, it allows the use of side-chain-protected amino acid residues in the synthesis, which previously has been problematic. For temporary protection of the amino terminus, the Fmoc group was chosen. This group can be cleaved by piperidine or diethylamine without affecting the permanent protection. Scheme 1 contains a schematic overview of the synthesis of the phosphonamidate pseudopeptides.

In the racemic series, the fully protected trifunctional phosphonic derivative was obtained

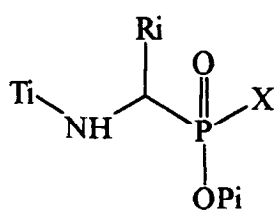

Fig. 1. The α-amino-phosphonic acid precursor. X: leaving group; Ti: temporary protection; Pi: permanent protection.

TABLE 1
P-N BOND FORMATION IN PHOSPHONAMIDE PSEUDOPEPTIDES

	Phosphonamide pseudopeptide	Yield (%)	^{31}P NMR (ppm)
1a	Fmoc-Ala(P)(OBzl)-Ala-OBzl	42	30.5; 29.9; 29.2 and 28.8
1b	Fmoc-(R)Ala(P)(OBzl)-Ala-OBzl	63	30.0; 29.5
1c	Fmoc-(S)Ala(P)(OBzl)-Ala-OBzl	52	30.5 28.7
2	Fmoc-Ala(P)(OBzl)-Gly-OMe	62	31.6 and 31.5
3	Fmoc-Val(P)(OBzl)-Gly-OMe	57	29.7 and 29.4
4a	Fmoc-Val(P)(OBzl)-Ala-OMe	65	29.9; 29.4; 29.3 and 29.3
4c	Fmoc-(S)Val(P)(OBzl)-Ala-OMe	50	29.9 29.3
5a	Fmoc-Phe(P)(OBzl)-Ala-OMe	63	28.8; 28.6; 28.5 and 28.4
5b	Fmoc-(R)Phe(P)(OBzl)-Ala-OMe	55	28.6 28.4
5c	Fmoc-(S)Phe(P)(OBzl)-Ala-OMe	50	28.8 28.5
6a	Fmoc-Phe(P)(OBzl)-Gly-OMe	65	30.64 and 30.60
6b	Fmoc-(R)Phe(P)(OBzl)-Gly-OMe	50	30.50 and 30.46
6c	Fmoc-(S)Phe(P)(OBzl)-Gly-OMe	51	30.51 and 30.47
7	Fmoc-Val(P)(OBzl)-Gly-Val-Lys(Z)-OBzl	64	30.3; 30.2; 29.9 and 29.8
8	Fmoc-Phe(P)(OBzl)-Gly-Val-Val-Ahx-OBzl	40	30.4; 30.3; 29.6 and 29.5

from the α-amino-phosphonic acid, as previously described [6]. For chiral synthesis, the optically pure α-amino-phosphonic acid was obtained by racemic resolution [7], and was monoesterified according to the Hoffman procedure [8].

The phosphonic residue was introduced via the acid chloride, prepared conveniently by reaction with $SOCl_2$, and an amino acid or peptide ester hydrochloride. This gave the desired phosphonopeptides in moderate to good yields (40–65%) after chromatographic purification (Table 1). However, when trifluoroacetate salts were used, the phosphonopeptides were obtained only in very low yield (5–15%) due to competing trifluoroacetylation of the amino component, probably via a mixed anhydride intermediate.

The phosphonopeptides in the racemic series are characterized by four signals in the ^{31}P NMR spectrum at 28.4 to 31.6 ppm, due to the four diastereoisomers which are epimeric at phosphorus and the C^{α} atom. Comparison of these results with those obtained using chiral derivatives demonstrated that no racemization occurred during the monoesterification of the latter, or in the

TABLE 2
DEPROTECTION AND ELONGATION OF PHOSPHONAMIDE PSEUDOPEPTIDES

	Phosphonamide pseudopeptide	Yield (%)	^{31}P NMR (ppm)
9	Boc-Phe-Phe(P)(OBzl)-Gly-OMe	50	30.44 and 30.25
10	Boc-Ala-Phe(P)(OBzl)-Ala-OMe	50	29.19; 29.05 and 28.9
11	Boc-Gly-Ala(P)(OBzl)-Gly-OMe	53	31.23 and 31.06
12a	Boc-Ala-Phe(P)(OBzl)-Gly-OMe	65	30.47; 30.35; 30.24 and 29.95
12b	Boc-(R)Ala-Phe(P)(OBzl)-Gly-OMe	66	30.48 and 30.36
12c	Boc-(S)Ala-Phe(P)(OBzl)-Gly-OMe	61	30.51 and 30.2
13	Ac-Gly-Ala(P)(OBzl)-Lys(Z)-OBzl[a]	68	30.9; 30.5; 29.6 and 28.1
14	SucO-CO-$(CH_2)_3$-CO-Val(P)(OBzl)-Gly-Val-Lys(Z)-OBzl	65	30.2; 29.9; 29.6 and 29.5
15	Fmoc-Ala-Gly-Val(P)(OBzl)-Gly-Val-Lys(Z)-OBzl	[b]	30.3 and 29.1; nonresolved
16	Ac-Ser(OBzl)-Ala-Ala-Phe(P)(OBzl)-Gly-Val-Val-Ahx-OBzl	[c]	–

[a] Fmoc-Ala(P)(OBzl)-Lys(Z)-OBzl has been described in Ref. 6.
[b] Nonisolated characterised product.
[c] Insoluble gel, characterised only by mass spectrometry.

TABLE 3
DEPROTECTION BY HYDROGENATION OF PHOSPHONAMIDE PSEUDOPEPTIDES

	Phosphonamide pseudopeptide	^{31}P NMR (ppm)	F.A.B.
17	Boc-Phe-Phe(P)(O^- Na^+)-Gly-OMe	21.1 and 20.7	541
18	Boc-Ala-Phe(P)(O^- Na^+)-Ala-OMe	19.7 and 19.3	479
19	Boc-Gly-Ala(P)(O^- Na^+)-Gly-OMe	20.6 and 19.7	375
20	Boc-Ala-Phe(P)(O^- Na^+)-Gly-OMe	21.01 and 20.71	465
21	Ac-Gly-Ala(P)(O^- Na^+)-Lys(Z)-O-Na^+	21–20; nonresolved	352

subsequent coupling step (Table 1, **1a** with **1b** and **1c**; **4a** with **4c**; **5a** with **5b** and **5c**).

Removal of the temporary *N*-Fmoc protection was achieved using an excess (10 equiv) of diethylamine in DCM/DMF at room temperature. The crude product was then coupled directly using BOP, giving the desired elongated peptides in 40–70% yield after chromatography (Table 2).

The reaction proceeded very rapidly and completion was observed in less than 30 min. Comparison of the ^{31}P NMR spectra of **12a**, **12b** and **12c** confirmed that the N-protection and acylation steps also proceeded without racemization of the phosphonic acid residue. However, problems encountered with solubility and polarity of more elaborated structures like **15** and **16** show the limitations of classical methods for peptides of this kind and emphasize the desirability of employing a solid-phase approach. The current Fmoc-benzyl strategy is ideally suited for such an approach.

Removal of benzyl-type protection was achieved by catalytic hydrogenation with palladium on charcoal in water/ethanol [2]. The products were lyophilized as the sodium salt and were characterized by MS and NMR (Table 3), complete deprotection being demonstrated by the absence of signals in the ^{31}P NMR spectrum at around 30 ppm.

The structures of the products were established by 2D ^{1}H-^{1}H COSY experiments, and the phosphonamidate linkage was confirmed unequivocally by long-range correlation COLOC P-H [9] between the phosphorus atom and the α-proton of the i+1 residue.

After deprotection, hydrolysis of the phosphonamidate linkage in D_2O (pH = 7.8) could be observed by ^{31}P NMR through the appearance of signals at about 15 ppm. The formation of a terminal phosphonic acid structure was also ascertained by the disappearance of the correlation between phosphorus and the i+1 α-proton in the COLOC spectrum, and it was further confirmed by ^{1}H-^{1}H COSY. The rate of hydrolysis differed according to the related peptide and seemed to indicate that the stability of these compounds in neutral media is structure dependent. Further studies are now in progress to confirm this hypothesis.

CONCLUSIONS

The use of an Fmoc/benzyl protection strategy allowed the incorporation of an α-amino-phosphonic residue at various positions in a peptide chain, using the corresponding phosphonyl chloride. This methodology allowed efficient access to protected phosphonamido pseudopeptides, including those with side-chain functionality. By conducting the final deprotection under neutral conditions, the problem of the instability of the phosphonamidate bond may be overcome. Since the Fmoc/benzyl strategy is suitable for the preparation of these peptides by a solid-phase approach [10], they should become readily available for applications as enzyme inhibitors or in immunology.

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