Protection of the Hydroxyphosphinyl Function of Phosphinic Dipeptides by Adamantyl. Application to the Solid-Phase Synthesis of Phosphinic Peptides

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To develop solid-phase synthesis of phosphinic peptides, different FmocXaaΨ{PO(OAd)CH_2}XaaOH building blocks have been prepared, where Fmoc is (fluorenylmethoxy)carbonyl. In this respect, the protection of the hydroxyphosphinyl function in these phosphinic dipeptides by the adamantyl group turns out to be convenient. The phosphinic adamantyl esters are completely stable in basic conditions and can be removed under relatively mild acidic conditions. Using these building blocks, despite the bulkiness of the adamantyl group, no particular problem of coupling was observed during the solid-phase synthesis of phosphinic peptides by the Fmoc strategy. The developed methodology is of particular interest to facilitate the development of potent inhibitors of zinc−metalloproteases.

Introduction

In the last decade, several studies have demonstrated that the synthesis of phosphinic peptides is a very effective approach to the development of highly potent inhibitors of zinc−metalloproteases.[1−6] Recently, the resolution of the three-dimensional structure of a complex between a zinc−metalloprotease and a phosphinic peptide inhibitor has confirmed the view that these pseudopeptides are indeed good mimics of the substrate in the transition state.[7] Phosphinic peptides are more stable than the phosphonamide peptides and are therefore more suitable for the development of zinc−metalloprotease inhibitors.[5,8,9] However, in the view of pharmacological studies such inhibitors should not only be potent but also as selective as possible, in order to avoid their interactions with multiple zinc−metalloproteases. This problem could be addressed by systematically investigating the influence on selectivity of the different side-chains of phosphinic peptide inhibitors. To this end, we recently elected to develop, by combinatorial chemistry, phosphinic peptide libraries which can be screened by various zinc−metalloproteases. However, the use of such an approach requires the development of a reliable and convenient solid-phase synthesis of these phosphinic peptides.

In this paper, we demonstrate that the adamantyl is a suitable protecting group for the hydroxyphosphinyl function of phosphinic dipeptides, which allows the development of solid-phase synthesis of phosphinic peptides by the Fmoc ((fluorenylmethoxy)carbonyl) strategy.

Results

As a protecting group for the hydroxyphosphinyl function of FmocXaaΨ{PO(OH)CH_2}XaaOH phosphinic dipeptides, we looked for one which can be removed easily under classical conditions. Among different possible protecting groups, adamantyl appears promising. In fact, 1-adamantyl is a tertiary system which reacts by S_N1 mechanism, because backside attack is hindered for steric reasons. This means that 1-adamantyl ester must be labile under acidic conditions due to tertiary cation formation. On the other hand, due to its constrained structure, the adamantyl is less stable than other tertiary group cations.[10] Consequently, 1-adamantyl esters are expected to be less acid-sensitive than *tert*-butyl esters.[11]

Starting from the classical synthon **1**, adamantyl and Fmoc protecting groups can be introduced in three steps (Scheme 1). To check the potential influence that the residues framing the phosphinic bond may play on the introduction of the adamantyl group, four different phosphinic synthons were prepared (Table 1).

The first step involves the formation of a silver salt which reacts with 1-adamantyl bromide to give the corresponding ester (Scheme 2). However, it should be stressed that our first attempts to prepare these adamantyl esters resulted in poor yields. Closer examination of this reaction led to the isolation of an unexpected silver salt complex where silver and synthon **1** counterions

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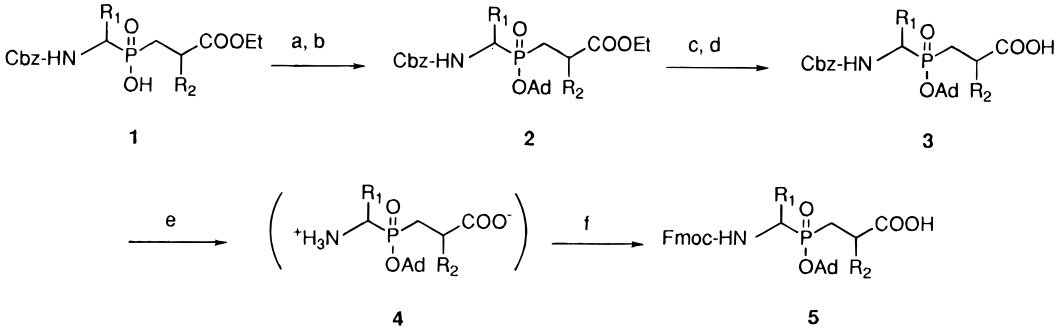
Scheme 1[a]



1 2 3 4 5

[a] (a) Ag_2O, (b) 1-Ad-Br, (c) NaOH, (d) H^+, (e) $HCOO^-NH_4^+$, 10% Pd/C, (f) Fmoc-Cl.

Table 1. Characterization of the Type 1 Synthons

synthons			yield	FAB-MS (M + H)		δ(ppm) ^{31}P	TLC (R_f)			analytical data (found/calcd)			
R_1	R_2	formula	(%)	calcd	found	($CDCl_3$)	(1)[a]	(2)[a]	(3)[a]	C	H	N	P
$C_6H_5CH_2$	H	$C_{21}H_{26}NO_6P$	90	419.41	419.5	53.8, 53.8	0.52	0.80	0.64	60.35/60.14	6.15/6.25	3.26/3.23	6.98/7.39
$C_6H_5CH_2$	CH_3	$C_{22}H_{28}NO_6P$	91	433.41	433.5	53.1, 53.2	0.56	0.96	0.61	60.69/60.96	6.32/6.51	3.18/3.23	6.73/7.15
$C_6H_5CH_2$	$(CH_3)_2CHCH_2$	$C_{25}H_{34}NO_6P$	89	475.49	475.6	53.4, 53.9	0.70	0.91	0.65	62.89/63.15	7.22/7.20	2.81/2.94	6.12/6.52
CH_3	$(CH_3)_2CHCH_2$	$C_{19}H_{30}NO_6P$	96	399.40	399.6	54.2, 54.6	0.50	0.73	0.51	57.29/57.13	7.41/7.57	3.32/3.50	7.32/7.76

[a] See Experimental Section for the definition of the solvent systems.

Table 2. Characterization of the Type 2 Synthons

synthons			yield	FAB-MS (M + H)		δ(ppm) ^{31}P	TLC (R_f)		analytical data (found/calcd)			
R_1	R_2	formula	(%)	calcd	found	($CDCl_3$)	(4)[a]	(5)[a]	C	H	N	P
$C_6H_5CH_2$	H	$C_{31}H_{40}NO_6P$	84	553.64	554.1	48.8, 48.8	0.52	0.63	67.39/67.25	7.02/7.28	2.41/2.53	5.18/5.59
$C_6H_5CH_2$	CH_3	$C_{32}H_{42}NO_6P$	79	567.64	567.8	47.7, 47.7, 48.0, 48.5	0.57	0.70	68.02/67.71	7.41/7.45	2.32/2.46	5.21/5.46
$C_6H_5CH_2$	$(CH_3)_2CHCH_2$	$C_{35}H_{48}NO_6P$	81	609.76	610	47.4, 47.8, 48.0, 48.3	0.54	0.58	69.18/68.94	7.82/7.93	2.19/2.29	4.63/5.08
CH_3	$(CH_3)_2CHCH_2$	$C_{29}H_{44}NO_6P$	80	533.67	534.5	47.3, 47.3, 48.3, 49.0	0.53	0.56	65.33/65.26	8.41/8.31	2.56/2.62	5.41/5.80

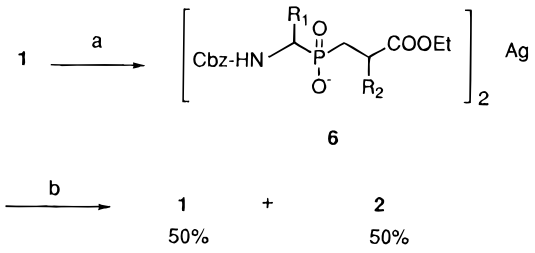
[a] See Experimental Section for the definition of the solvent systems.

Table 3. Characterization of the Type 3 Synthons

synthons			yield	FAB-MS (M + H)		δ(ppm) ^{31}P	TLC (R_f)		analytical data (found/calcd)			
R_1	R_2	formula	(%)	calcd	found	($CDCl_3$)	(4)[a]	(5)[a]	C	H	N	P
$C_6H_5CH_2$	H	$C_{29}H_{36}NO_6P$	86	525.57	525.4	50.7−50.8	0.15	0.41	65.89/66.27	6.67/6.90	2.55/2.66	5.49/5.89
$C_6H_5CH_2$	CH_3	$C_{30}H_{38}NO_6P$	98	539.59	540.1	48.7, 50.1, 50.3, 51.0	0.16	0.44	66.38/66.77	6.91/7.09	2.34/2.59	5.39/5.74
$C_6H_5CH_2$	$(CH_3)_2CHCH_2$	$C_{33}H_{44}NO_6P$	98	581.66	582.1	47.9, 49.6, 50.2, 51.5	0.20	0.53	67.96/68.14	7.29/7.69	2.31/2.40	4.99/5.33
CH_3	$(CH_3)_2CHCH_2$	$C_{27}H_{40}NO_6P$	91	505.56	505.7	48.1, 50.0, 51.1, 51.8	0.16	0.46	63.87/64.14	7.71/7.97	2.71/2.76	5.51/6.13

[a] See Experimental Section for the definition of the solvent systems.

Scheme 2[a]



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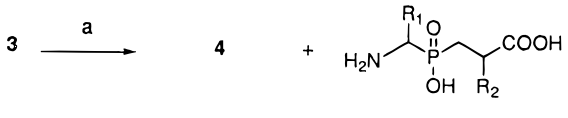
[a] (a) $AgNO_3$, (b) 1-Ad-Br.

Scheme 3[a]



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[a] (a) H_2/Pd/C.

coprecipitate in a ratio of 1:2 (compound **6**, Scheme 2). To overcome this problem, a large excess of silver nitrate was used to obtain a 1:1 molar ratio of the desired silver and synthon **1** salt (see Experimental Section). Given these precautions, the adamantylation reaction proceeds in good yield (Table 2).

In the second step, the saponification of the ethyl ester **2**, in spite of the presence of the adamantyl ester, is completely selective and allows for the preparation of synthon **3** in good yields (Table 3).

In the third step, in addition to the decarbobenzoxylation reaction, a certain degree of deadamantylation was observed during the catalytic hydrogenation (compound **7**, Scheme 3). These observations are in contrast with previous work showing that the adamantyl esters of amino acids are completely stable to catalytic hydrogenation.[12] To avoid this problem, the fully protected synthon **2** was subjected to catalytic hydrogenation followed by

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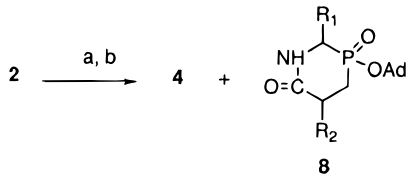
Table 4. Characterization of the Type 5 Synthons

synthons			yield	FAB-MS (M + H)		δ(ppm) ^{31}P	TLC (R_f)		analytical data (found/calcd)			
R_1	R_2	formula	(%)	calcd	found	($CDCl_3$)	(4)[a]	(5)[a]	C	H	N	P
$C_6H_5CH_2$	H	$C_{36}H_{40}NO_6P$	61	613.68	614	50.7, 51.0	0.40	0.38	70.19/70.49	6.34/6.57	2.32/2.28	4.65/5.05
$C_6H_5CH_2$	CH_3	$C_{37}H_{42}NO_6P$	65	627.74	627.7	49.0, 49.0, 50.6, 51.3	0.46	0.40	70.41/70.79	6.39/6.74	2.20/2.23	4.59/4.93
C_6H_5–CH_2	$(CH_3)_2CHCH_2$	$C_{40}H_{48}NO_6P$	67	669.77	669.6	48.2, 49.6, 50.2, 52.0	0.57	0.49	71.58/71.73	7.13/7.22	2.12/2.09	4.22/4.62
CH_3	$(CH_3)_2CHCH_2$	$C_{34}H_{44}NO_6P$	62	593.67	593.4	48.5, 50.4, 51.2, 52.2	0.54	0.50	68.37/68.78	7.51/7.47	2.21/2.35	4.89/5.22

[a] See Experimental Section for the definition of the solvent systems.

Scheme 4[a]



[a] (a) H_2, 10% Pd/C, (b) NaOH.

saponification (Scheme 4). With this protocol, no cleavage of the adamantyl group was observed during the hydrogenation. However, in this case, depending on the substituents R_1 and R_2 in the synthon **2**, the saponification leads to the formation of two products, the expected pseudodipeptide **4** and the pseudodiketopiperazine **8** (Scheme 4). For instance, when the R_1 and R_2 substituents were benzyl and isobutyl groups, respectively, the pseudodiketopiperazine turned out to be the main product of the reaction. Finally, satisfactory results for the decarbobenzyloxylation of synthon **3** were obtained using the method described by Anwer and Spatola,[13] which utilizes ammonium formate as the hydrogen donor and palladium/carbon as the catalyst in methanol (Scheme 1). The N-deprotected synthon **4** was easily converted to the final Fmoc product, synthon **5**, in good yield (Table 4).

Using the synthons prepared in this study and the acid-sensitive 2-chlorotrityl resin[14] as a solid support, the different phosphinic pseudopeptides **9**−**12** were prepared by classical Fmoc solid-phase peptide synthesis (see Experimental Section). Satisfactory couplings were achieved using only 1.5 equiv of these synthons with HBTU, where HBTU is 2-(1*H*-benzotriazol-1-yl)-1,1,3,3-tetramethyluronium hexafluorophosphate, as an activating agent and a 60 min reaction time. In the same way, coupling of Fmoc amino acids to these synthons was found to proceed in good yield using standard conditions. As shown by the HPLC profiles of crude materials (Figure 1), this method yields relatively pure phosphinic peptides, the main peaks corresponding to the different diastereoisomeric forms of these compounds.

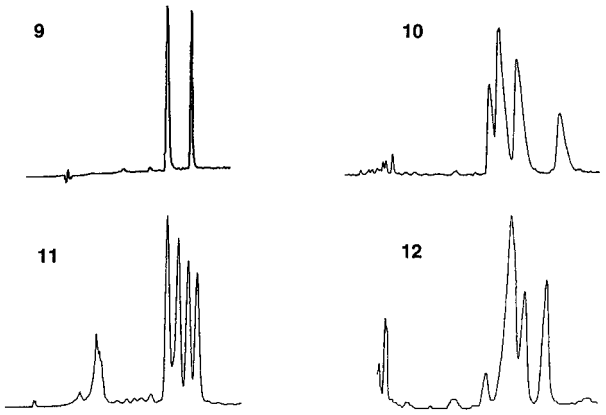


Figure 1. HPLC traces of Pro-(L,D)-PheΨ(PO_2CH_2)Gly-Phe (**9**), Cbz-Pro-Lys-(L,D)-PheΨ(PO_2CH_2)-(L,D)-Ala-Pro-Leu-Val (**10**), Cbz-Pro-Gln-(L,D)-PheΨ(PO_2CH_2)-(L,D)-Leu-Trp-Ala (**11**), and Cbz-Pro-Gln-(L,D)-AlaΨ(PO_2CH_2)-(L,D)-Leu-Trp-Ala (**12**). The profiles correspond to the crude material obtained after the cleavage of the protecting groups and the peptide from the resin (Vydac C_{18} column, 4.66 × 25 cm).

Discussion

Although a number of phosphinic peptides have been synthesized by the solution-phase method without protection of the hydroxyphosphinyl group, the coupling of a R-XaaΨ($PO(OH)CH_2$)Xaa′ unit to an amino peptidyl moiety is problematic.[15−19] The synthesis of phosphinic peptides by solution methods and the synthesis of phosphonate peptides by a solid-phase approach have been described using the methyl ester as a protecting group of the hydroxyphosphinyl function.[4,20−22] However, the final deprotection of methyl ester of phosphinic or phosphonate peptides has usually been achieved under drastic conditions (e.g., lithium propanethiolate, trimethylsilyl bromide, concentrated sodium, or lithium hydroxide solutions, or a mixture of thiophenol, triethylamine and dioxane).[4,20−22]

The need to protect the hydroxyphosphinyl function with a protecting group which can be removed easily under classical conditions led us to investigate the adamantyl group for this purpose.[23,12] Interestingly, we observed that phosphinic adamantyl esters can be removed under relatively mild acidic conditions (50% TFA/DCM (dichloromethane), 30 min). However, these esters were also found to be quite resistant to acidic conditions, since they remain stable during acidification which follows saponification. It should be noted that the benzyl esters, especially the diphenylmethyl phosphinic esters,

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are much more sensitive under similar acidic conditions. This study also demonstrates the stability of the adamantyl esters under basic conditions. These esters remain intact even after 24 h under strong alkaline conditions. In comparison, the saponification of ethyl or methyl esters is usually completed in 2 h under the same conditions. Despite the bulkiness of the adamantyl group, its presence does not create particular problems of coupling during the solid-phase synthesis of phosphinic peptides, even with the synthon containing the benzyl and isobutyl side chains. This solid-phase strategy, the first reported to date for phosphinic peptides, now enables the development of libraries of these pseudopeptides without restrictions on peptide size. The efficiency of this approach was recently demonstrated by the discovery of the most potent and selective inhibitor of the mammalian zinc-endopeptidase 24-15 by screening a library of phosphinic peptides.[24]

Experimental Section

General. All of the compounds, for which analytical and spectroscopic data are quoted, were homogeneous by TLC. TLC analyses were performed using silica gel plates (E. Merck silica gel 60 F-254), and components were visualized by the following methods: ultraviolet light absorbance, iodine vapor, and charring after spraying with a solution of (NH_4) HSO_4 and ninhydrin spray. The solvent systems used for TLC developments were (1) 1-butanol−acetic acid−water (4:1:1), (2) chloroform−methanol−acetic acid (7:2:1), (3) chloroform−methanol−acetic acid (7:0.5:0.5), (4) chloroform−methanol (9.5:0.5), (5) hexane−ethyl acetate−acetic acid (3:3:0.2), and (6) chloroform−methanol (9:1). In most solvent systems close, but different, R_f values have been observed for the various stereoisomers of these compounds, due to the presence of asymmetric centers in these compounds. Thus, the R_f values quoted in Tables 1, 2, 3 and 4 correspond to an average value. The presence of these asymmetric centers complicates the use of ^{1}H-NMR spectroscopy to characterize these compounds, especially when the hydroxyphosphinyl function is protected by the adamantyl group. For these reasons, the compounds were characterized only by ^{31}P NMR spectroscopy, elemental analyses, and mass spectrometry. (^{31}P, ^{1}H)-decoupled spectra of the compounds dissolved in $CDCl_3$ were recorded on a WM 250 Bruker spectrometer (^{31}P, 101 MHz). ^{31}P chemical shifts are reported on δ scale (in ppm) downfield from 85% H_3PO_4. Before microanalyses, samples were dried under high vacuum at 35 °C for 12 h in a dry pistol. These analyses were obtained from Service de Microanalyses, ICSN CNRS, 91198 Gif/Yvette, France. Fast atom bombardment mass spectrometry (FAB-MS) was performed on a Nermay R3010 3-fold quadrupole instrument by Dr. Virelizier (DCC/DPE/SPEA/SAIS/CEA, CE-Saclay, 91191 Gif/Yvette, France).

General Procedures. (*R,S*)-(1-((Benzyloxycarbonyl)amino)ethyl)phosphinic acid and (*R,S*)-(1-((benzyloxycarbonyl)-amino)-2-phenylethyl)phosphinic acid were synthesized as described by Baylis et al.[25] 2-Isobutylpropenoic acid ethyl ester was prepared according to the method reported by Stetter and Kuhma.[26] The propenoic and 2-methylpropenoic acid ethyl esters were purchased from Aldrich.

In order to increase the yield of the Michael addition to obtain the synthons **1**, the method described in the literature[4,5] was modified as follows: hexamethyldisilazane was used in large excess (5 equiv excess, instead of 1 equiv) and the reaction time of the Michael addition was increased from 25 min to 4 h.

(A) General Procedure for the Synthesis of Synthons 1, Cbz-HN-(*R,S*)-CH(R_1)PO_2HCH_2-(*R,S*)-CH(R_2)COOEt. These compounds were synthesized using a procedure previously described,[4,5] with some modifications. A suspension of the corresponding *N*-benzyloxycarbonyl phosphinic acid [Cbz-HN-(*R,S*)-CH(R_1)PO(OH)H][25] (1 mmol) and hexamethyldisilazane (5 mmol) was heated at ca. 110 °C for 1 h under an argon atmosphere. After cooling to 90 °C, the appropriate ethyl alkylacrylate [CH_2=C(R_2)COOEt][26] (1.3 mmol) was added dropwise over 30 min, and the reaction mixture was stirred for an additional 3.5 h at 90 °C. The resulting mixture was allowed to cool to ca. 70 °C, and absolute ethanol (3 mL) was added dropwise. After cooling to rt, the volatile products were removed under vacuo and the residue was dissolved in 10% $NaHCO_3$. The aqueous phase was rinsed with diethyl ether, acidified to pH 1.5 with 1N HCl, and the precipitated product was extracted with ethyl acetate. The organic layer was rinsed with water, dried over Na_2SO_4 and evaporated to dryness to give the synthons 1 in very good yields.

(B) General Procedure for the Synthesis of Synthons 2, Cbz-HN-(*R,S*)-CH(R_1)PO(OAd)-CH_2-(*R,S*)-CH(R_2)COOEt. Method 1. The type **1** synthon (1 mmol) was dissolved in 95% ethanol (25 mL). This solution was added dropwise over 10 min to a stirred 0.5 M aqueous solution of silver nitrate (10 mL) (precautions should be taken to avoid exposure of silver synthons to the light). After 10 min, water (15 mL) was added to this reaction mixture and the ethanol was removed under vacuum. The remaining aqueous phase, containing the precipitated silver salt of the synthon, was cooled in an ice-water bath for 1 h. The product was filtered, washed with cold water, and dried over P_2O_5 to give the silver synthon salt in about 90% yield. This silver synthon salt (1 mmol) was added to a solution of 1-adamantyl bromide (1.2 mmol) in chloroform (10 mL). This reaction mixture was refluxed for 30 min. The silver bromide, which precipitated, was removed by filtration or centrifugation, and the filtrate was concentrated to dryness. This residue was purified by silica gel column chromatography (eluent: chloroform/2-propanol, 97:3) to give the pure products in about 80% yields as oils.

Method 2. The type **1** synthon (1 mmol) was dissolved in 0.1 M NaOH (10 mL) (solution 1). Silver nitrate (1 mmol) was dissolved in water (10 mL) (solution 2). Solutions 1 and 2 were added simultaneously to a reaction flask containing water (5 mL). This mixture was cooled in an ice-water bath, and after 1 h, the precipitated silver salt was filtered, washed with cold water, and dried in a desiccator over P_2O_5 to give the silver synthon salt in about 90% yield. The adamantylation and its yield were similar to those of the procedure described in method 1.

Method 3. The type **1** synthon (1 mmol) and 1-adamantyl bromide (1 mmol) were dissolved in chloroform (10 mL), and the reaction mixture was refluxed. To this refluxing mixture, silver oxide (2 mmol) was added in five equal portions over 50 min. After the solution was refluxed for an additional 30 min, the solvents were removed in vacuo and the residue was treated with diethyl ether. The silver bromide and the excess silver oxide, as well as the traces of unreacted synthon, were removed by filtration through a mixture of celite and alumina. The filtrates were concentrated to dryness to give the pure products in about 85% yield after column chromatography.

(C) General Procedure for the Synthesis of Synthons 3, Cbz-HN-(*R,S*)-CH(R_1)PO(OAd)CH_2-(*R,S*)-CH(R_2)COOH. The type **2** synthon (1 mmol) was dissolved in ethanol (10 mL), and 4 N NaOH (1 mL) was added dropwise to the reaction mixture. After 2 h (for the synthon containing the isobutyl group, the completed saponification was only achieved after 12 h), the ethanol was removed in vacuo and the residue was diluted with water (20 mL). This reaction mixture was then cooled in an ice-water bath and acidified to pH 2 with 0.5 N HCl. The precipitated product was taken up in diethyl ether and the organic phase was rinsed with water, dried over Na_2SO_4, and concentrated to dryness to give the pure saponified solid products (90−98% yields).

(D) General Procedure for the Synthesis of Synthons 5, Fmoc-HN-(*R,S*)-CH(R_1)PO(OAd)CH_2-(*R,S*)-CH(R_2)COOH. To a solution of methanol (5 mL), containing type **3** synthon (1 mmol) and ammonium formate (4 mmol), 0.25 g of 10% Pd/C was added. After 12 min at rt, the catalyst was removed by filtration through celite, and the filtrate was evaporated to

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dryness. Methylene chloride was added to the residue, and the solution was evaporated to dryness. This procedure was repeated twice. The residue was treated with chloroform (50 mL), and excess insoluble unreacted ammonium formate was removed by filtration. The filtrate was evaporated to dryness, and the residue, synthon type **4**, was dissolved in 10% Na_2CO_3 (3 mL). The reaction mixture was concentrated in vacuo until half of the volume was removed, and then water (1.5 mL) and dioxane (2 mL) were added. A solution of Fmoc-Cl (1.2 mmol) in dioxane (2 mL) was added dropwise to this cold mixture (ice-water bath). After the solution was stirred for 2 h at 4 °C and 4 h at rt, the reaction mixture was diluted with water (20 mL), cooled in an ice-water bath, and acidified to pH 2.5 with 2 N HCl. The solid product, which precipitated, was quickly taken up in diethyl ether, and the organic layer was rinsed with water, dried over Na_2SO_4, and evaporated to dryness to give the crude product type **5** synthon, which was purified on a silica gel column (eluent: chloroform/methanol, 9.5:0.5) to give the synthons **5** (65% overall yields).

Synthesis of [Cbz-(*R*,*S*)-PheΨ(PO_2^--CH_2)-(*R*,*S*)-LeuOEt]$_2$Ag Complex. The synthon Cbz-(*R*,*S*)-PheΨ(PO(OH)-CH_2)-(*R*,*S*)-LeuOEt (0.951 g, 2 mmol) was dissolved in ethanol (25 mL) and water (5 mL), and 1 M $AgNO_3$ (2.5 mL, 2.5 mmol) was added dropwise with stirring. After 15 min, most of the ethanol was removed in vacuo and water (30 mL) was added to the reaction mixture. After 1 h of cooling in an ice-water bath, the solid was filtered, washed with cold water, and dried to give 0.95 g of the title complex (90% yield): FAB-MS (M + H) calcd for $C_{50}H_{67}O_{12}N_2P_2Ag$ 1057.87, found 1057.4. Anal. Calcd: C, 56.82; H, 6.29; N, 2.64; P, 5.86; Ag, 10.20. Found: C, 56.41; H, 5.94; N, 2.52; P, 4.92; Ag, 9.69.

Determination of the Silver Synthon Complex Molar Ratio. The above silver synthon complex [Cbz-(*R*,*S*)-PheΨ-(PO_2^--CH_2)-(*R*,*S*)-LeuOEt]$_2$Ag (1.055 g, 1 mmol) was suspended in chloroform (50 mL), and 4 N HCl in dioxane was added (4 mL). The resulting solid precipitate was filtered, washed with chloroform, and dried to give 0.131 g of AgCl (0.91 mmol, 91% yield). The filtrate was evaporated in vacuo, and the residue was dried over P_2O_5 and NaOH to give 0.913 g (1.92 mmol, 96% yield) of the Cbz-(*R*,*S*)-PheΨ(PO(OH)CH_2)-(*R*,*S*)-LeuOEt compound.

Formation and Isolation of the Pseudodiketopiperazine. (A) Cyclo[(*R*,*S*)-PheΨ(PO(OAd)CH_2)-(*R*,*S*)-Leu]. The synthon Cbz-(*R*,*S*)-PheΨ(PO(OAd)CH_2)-(*R*,*S*)-LeuOEt (0.61 g, 1 mmol) was dissolved in 95% ethanol (10 mL) and was hydrogenated for 5 h in the presence of 10% Pd/C catalyst (0.25 g). The catalyst was removed by filtration, and the filtrate was concentrated to dryness to give the synthon H_2N-(*R*,*S*)-PheΨ(PO(OAd)CH_2)-(*R*,*S*)-LeuOEt in 96% yield (R_f(4), 0.43). This product was dissolved in 95% ethanol (5 mL), and 4 N NaOH (1 mL) was added to the reaction mixture. After 5 h of stirring, the solvents were removed in vacuo and the residue was treated with diethyl ether. The organic layer was rinsed successively with 0.1 N HCl and 10% $NaHCO_3$, dried over Na_2SO_4, and concentrated to dryness to give the pseudodiketopiperazine (40% yield): R_f(5), 0.54; R_f(4), 0.48; ^{31}P NMR ($CDCl_3$): 45.59, 44.08, 41.45, 37.31; FAB-MS (M + H) calcd for $C_{25}H_{37}NO_3P$ 430.56, found 430.5. Anal. Calcd: C, 69.90; H, 8.44; N, 3.25; P, 7.21. Found: C, 69.64; H, 8.31; N, 3.42; P, 6.87.

(B) Cyclo[(*R*,*S*)-PheΨ(PO(OH)CH_2)-(*R*,*S*)-Leu]. The above compound (0.086 g, 0.2 mmol) was dissolved in a mixture of CH_2Cl_2−TFA 1:1 (5 mL), and after 1 h of stirring, the solvents were removed in vacuo. The residue was dissolved in 10% Na_2CO_3 (10 mL). The aqueous phase was rinsed with diethyl ether and acidified to pH 1.5 with 4 N HCl, and the product was taken up in ethyl acetate. The organic layer was dried over Na_2SO_4 and concentrated to dryness to give the unprotected pseudodiketopiperazine (0.035 g, 60% yield): R_f-(2), 0.47; ^{31}P NMR ($CDCl_3$) 49.38, 43.74; FAB-MS (M + H) calcd for $C_{15}H_{22}NO_3P$ 296.33, found 296.3. Anal. Calcd: C, 61; H, 7.50; N, 4.74; P, 10.49. Found: C, 60.76; H, 5.58; N, 4.81; P, 9.97.

Solid-Phase Synthesis of Phosphinic Peptides. These syntheses were realized using the 2-chlorotrityl resin. The first amino acids were attached to the 2-chlorotrityl resin according to the procedure of Barlos et al.[14] The degree of substitution of each resin sample was determined according to the procedure of Meienhofer et al.,[27] using ϵ 7040 M^{-1} cm^{-1} at 300 nm.[28] The Fmoc groups were removed with 30% piperidine in *N*-methylpyrrolidone. Coupling of the next residue was achieved using the 2-(1*H*-benzotriazol-1-yl)-1,1,3,3-tetramethyluronium hexafluorophosphate/diisopropylethylamine in situ strategy. Typically, 3 equiv of Fmoc amino acid, 3 equiv of 2-(1*H*-benzotriazol-1-yl)-1,1,3,3-tetramethyluronium hexafluorophosphate, and 4 equiv of diisopropylethylamine as solutions in *N*-methylpyrrolidone were added to the resin, and the reaction was allowed to proceed for 30 min. The coupling of the phosphinic dipeptide synthon **5** was performed using the coupling conditions described above, except that only 1.5 equiv of the synthon was used and that the reaction was allowed to proceed for 60 min. Under these conditions for coupling, a negative Kaiser test was observed for the different phosphinic building blocks. Fully protected peptides were cleaved from the 2-chlorotrityl chloride resin with a mixture of glacial acetic acid, trifluoroethanol, and dichloromethane (2:2:6) over 2 h. Solutions of protected peptides were dried in vacuo. Protective groups were removed by the action of trifluoroacetic acid containing 5% H_20, 5% thioanisole, 5% phenol, 2.5% ethanedithiol, 2.5% triisopropylsilane, and 20% dichloromethane. Solutions of deprotected peptides were concentrated in vacuo, and peptides were treated with 2.5 equiv of $NaHCO_3$ in water. Aqueous solutions were repeatedly extracted with cold diethyl ether and lyophilized. Purification and separation of the diastereoisomeric forms of these phosphinic peptides were performed on Gilson gradient system equipped with a variable-wavelength detector. Compounds were detected at 254 and 230 nm. The following conditions were used: Vydac C18 (4.66 × 25 cm) column; mobile phase A = 0.1% TFA and 10% acetonitrile in water, B = 0.1% TFA and 10% water in acetonitrile; flow rate of 8 mL/min. The gradients used for the peptide **9** (Pro-(L,D)-PheΨ(PO_2CH_2)Gly-Phe) was t = 0 min (0% B), t = 40 min (50% B), t = 60 min (100% B), and those used for the peptides **10** (Cbz-Pro-Lys-(L,D)-PheΨ(PO_2CH_2)Ala-Pro-Leu-Val), **11** (Cbz-Pro-Gln-(L,D)-PheΨ(PO_2CH_2)-(L,D)-Leu-Trp-Ala), and **12** (Cbz-Pro-Gln-(L,D)-AlaΨ(PO_2CH_2)-(L,D)-Leu-Trp-Ala) were t = 0 min (0% B), t = 10 min (25% B), t = 45 min (42% B), t = 60 min (100% B), respectively. Mass spectroscopy analysis of the main HPLC peaks observed for the different phosphinic peptides shows that these correspond to the different diastereoisomeric forms of these peptides: FAB-MS (M + H) calcd for peptide **9** 501.46, found 501.5; calcd for peptide **10** 930.23, found 930; calcd for peptide **11** 929.3, found 929; calcd for peptide **12** 853.14, found 853.

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