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Synthesis of Constrained 4-(Phosphonomethyl)Phenylalanine Derivatives As Hydrolytically Stable Analogs of *O*-Phosphotyrosine.

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Abstract : In order to elucidate the role of protein tyrosine phosphorylation involved in various intracellular signaling pathways, peptides containing *O*-phosphotyrosine have been developed. However, in order to improve the stability of the phosphorylated amino acid, we have designed some years ago a hydrolytically stable analogue, the 4-(phosphonomethyl)phenylalanine (Pmp). Introduced in peptide sequences, this residue, which is resistant to phosphatase action, was shown also able to inhibit substrate recognition by protein targets. With the aim to design peptidomimetics endowed with improved affinity and selectivity, we report in this study the synthesis of five new sterically hindered amino acids derived from Pmp. These modifications include α-methyl, ß-methyl, ß,ß-dimethyl substitutions, α,ß-cyclization of Pmp and methyl substitution on the phosphonomethyl group of Pmp.

INTRODUCTION

Phosphorylation and dephosphorylation of proteins on tyrosine residues play essential roles in signal transduction pathways such as cellular growth differentiation and transformation. [1,2] These post-translational events allow specific recognition of proteins and are modulated by two kinds of enzymes, the phosphorylating protein tyrosine kinase (PTKase) and the dephosphorylating protein tyrosine phosphatase (PTPase). [3,4] Moreover, dysfunctioning of these pathways may induce cellular diseases like neoplastic transformation. [5,6] Therefore, in order to elucidate the molecular mechanisms involved in the recognition of the peptide or protein substrates by these enzymes and also in order to develop new pharmacological agents, competitive peptides corresponding to such tyrosine phosphorylated sequences have already been synthesized. [7-9] 4-(phosphonomethyl) phenylalanine (Pmp), [10,11] a hydrolytically stable analogue of *O*-phosphotyrosine that we have developed for this purpose was introduced in several of these peptides. [12-17] Different protecting groups and synthetic pathways have been reported to prepare the Pmp residue and its precursors. [15-19] Recently, synthesis of fluoro and hydroxy 4-(phosphonomethyl)phenylalanine were also reported, [20-24] with the aim of modifying the second ionization constant of Pmp phosphonate group ($pKa_2 = 7.1$), which is significantly higher than that of the parent phosphate ($pKa_2 = 5.7$). Thus, this decreased acidity was shown to slightly reduce the affinity of the phosphonopeptide as compared with its native *O*-phosphorylated analogue in the case of SH2 targets. [25]

On the other hand, numerous structure-activity analyses of short bioactive peptides have shown that constrained peptides are expected to bind their target more tightly, as their binding occurs with a smaller decrease in the conformational entropy than the binding of unconstrained analogues. The general approaches used consist in the replacement of L-amino acids by their D-isomers, cyclization of linear peptides or modifications of peptide side chains. [26,27] Linear, as well as cyclic small peptides containing D- or L-Pmp residue have been synthesized as conformationally constrained potential inhibitors of *O*-phosphopeptide-SH2 domain interactions. [13,28-30] Since amino acid side chains provide the major recognition sites, structural features that could influence the side chain rotations could modulate the peptide topography and subsequently its biological properties. Thus, in this paper, we report the synthesis of sterically constrained new unnatural amino acids as derivatives of Pmp. These amino acids might stabilize particular backbone conformations when

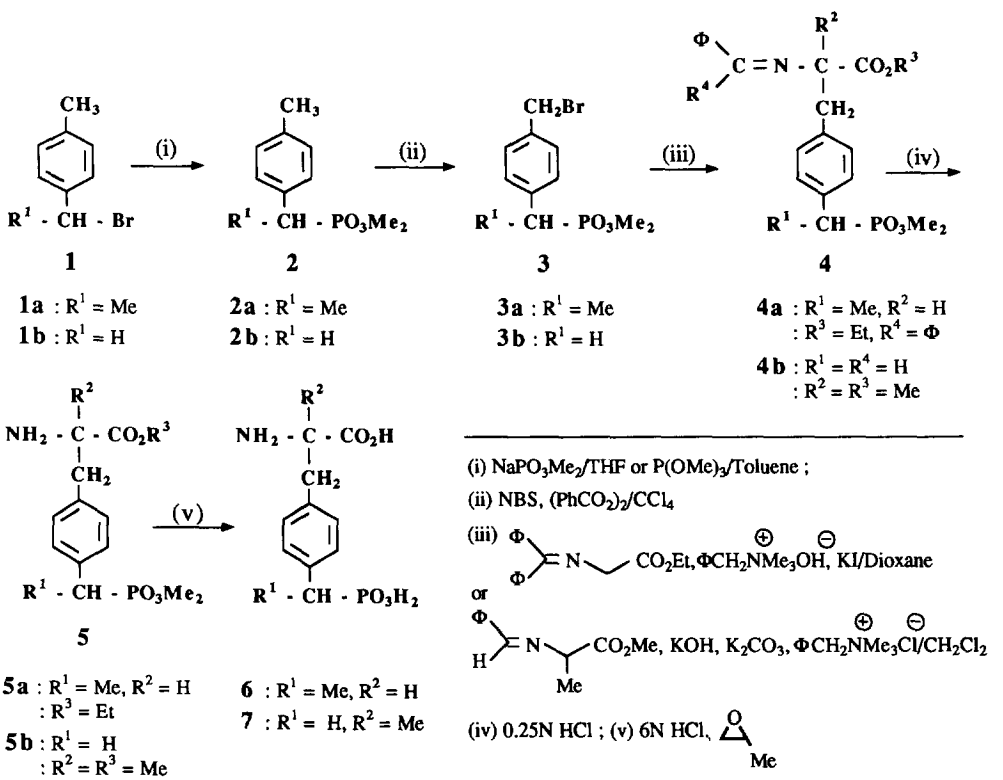
incorporated in peptide sequences. These modifications include α-methyl, ß-methyl, ß,ß-dimethyl substitutions, α,ß-cyclization of Pmp and methyl substitution on the phosphonomethyl group of Pmp.

The first four new amino acids are able to fix primarily χ_1 and χ_2 rotation angles. (Torsional angles are defined in ref. [31]) Essentially only the two conformations with χ_2 ranging ± 90° are allowed for the aromatic ring of these amino acids. [32] Moreover, the amino acid with C^{α} methyl substitution restricts the Φ, Ψ angles of the backbone and χ_1 rotation of the side chain. [33,34] ß-methyl substitution bias χ_1 to the possible angles of -60°, ± 180° or + 60°. [35] C^{β}-dimethyl substitution is more constrained and restricts aromatic ring flipping (χ_2) as well as χ_1 rotation. [27] Compound **27**, which cyclizes C^{α} and C^{β} in a high torsionally constrained cyclopropyl ring, completely blocks χ_1 angle. [27] Amino acid **6** with methyl substitution on the phosphonomethyl group of Pmp was synthesized to verify the influence of steric hindrance and electronic effects of the methyl group near the phosphonate on the recognition with SH2 domain.

RESULTS AND DISCUSSION

The synthesis of Pmp with appropriate protections for solid-phase peptide synthesis has already been achieved in our laboratory by the alkylation of Schiff base derived from glycinate with substituted benzyl bromide, followed by hydrolysis and amino group protection. [16,30]

In this paper, amino acids **6** and **7** with methyl substitution on phosphonomethyl group or α-carbon were prepared following a similar synthetic pathway which is outlined in Scheme 1.



Scheme 1. Chemical synthetic pathway to constrained phosphonomethylphenylalanine derivatives **6** and **7**.

Substituted benzyl bromides **1a** and **1b** were converted into phosphonates **2a** and **2b** by a reaction with sodium dimethylphosphite and trimethylphosphite respectively. **1a**, which was prepared quantitatively by bromination of 1-(4-methylphenyl)ethanol with bromotrimethylsilane in mild conditions, did not react with trimethylphosphite due to the steric effect of methyl substitution and therefore the use of the more reactive reagent sodium dimethylphosphite was necessary to prepare **2a**. The *para*-methyl group of **2a** and **2b** was then brominated with N-bromosuccinimide to form **3a** and **3b**. Phase-transfer catalytic alkylation of Schiff bases derived from alaninate and glycinate with **3a** and **3b** respectively provided **4a** and **4b**. **4a** was prepared by the general method using diphenylketimine of glycinate. However, **4b** could not be obtained under these conditions, but instead was prepared using the more reactive schiff base aldimine of alaninate under anhydrous conditions as described by Jiang *et al.* [36] Due to their hydrolytic liability, **4a** and **4b** were directly hydrolyzed in 0.25N HCl to yield **5a** and **5b** and subsequently purified. Hydrolysis of **5a** and **5b** in refluxing 6N HCl provided amino acids **6** and **7** after treatment with propylene oxide.

Due to steric hindrance brought about by the α- and ß-substitutions, amino acids **15**, **21** and **27** could not be synthesized by the procedure described above. Instead, they were prepared by the synthetic pathways which are outlined in Scheme 2 using different α,ß-unsaturated formyl amino acids.

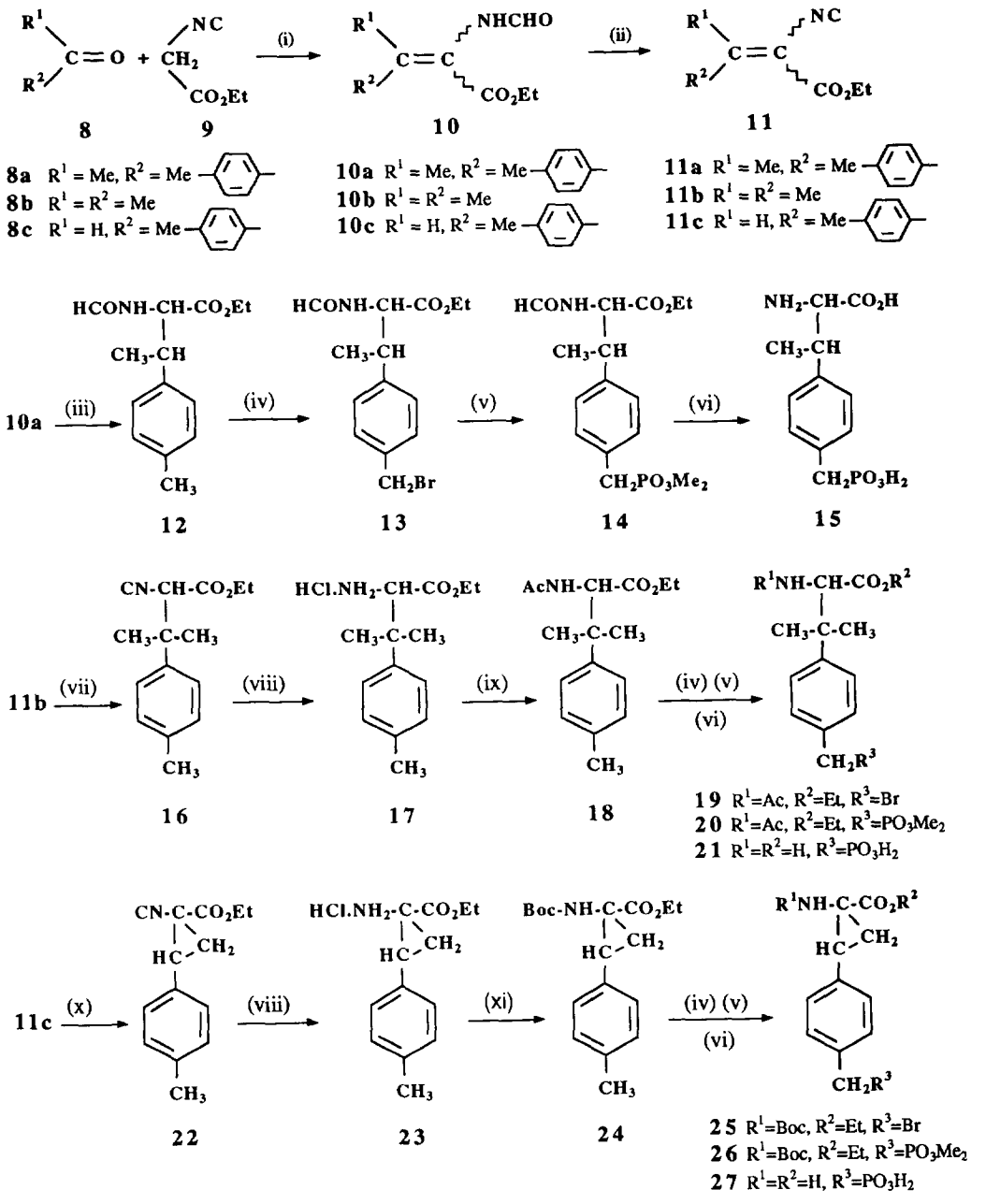
Compounds **10** (**10a**, **10b** and **10c**) were prepared by condensation of the appropriate ketones or aldehyde (**8a**, **8b** and **8c**) with ethyl isocyanoacetate **9**. [37]

Interestingly, ^{1}H NMR spectra of compound **10a** revealed the presence of a mixture of Z and E-isomers in a 1:1 ratio. Chemical shift assignments for both isomers were obtained in a straightforward manner using NOESY experiments, since NOE crosspeaks indicate nuclei which are spatially close. Therefore, the assignment of the signals corresponding to the Z and E forms was based on the observation of strong NOE crosspeaks between 3-CH_3 (2.0 ppm) and NH (9.7 ppm) for the E-isomer and between NH (9.2 ppm) and H2',6' aromatic protons (7-7.1 ppm) for the Z-isomer. In addition, as expected NOE crosspeaks between 3-CH_3 (2-2.1 ppm) and H2',6' aromatic protons (7-7.1 ppm) and between 4'-CH_3 (2.25 ppm) and H3',5' aromatic protons (7-7.1 ppm) were observed for both isomers. Moreover, this mixture of Z and E-isomers was subsequently separated by chromatography and the ^{1}H NMR spectra of the separated isomers were in accordance with the previous assignment.

Compound **10c** was obtained as a 2:1 mixture of geometrical isomers which was not separated. The assignment of the proton resonances of both isomers was also performed using NOESY experiment. As in the case of **10a**, the crosspeak observed between H-3 (6.72 ppm) and NH (10.08 ppm) allowed to assign the E configuration to the minor isomer, while the crosspeak observed between NH (9.72 ppm) and H2',6' aromatic protons (7.50 ppm) allowed to assign the Z configuration to the major isomer. Other expected crosspeaks were also observed for both isomers.

Hydrogenation of **10a** (mixture of isomers) yielded **12**, which was then brominated with NBS to provide **13**. Compounds **12** and **13** have quite similar R_f values on TLC, and therefore unpurified **13** was submitted to Arbuzov reaction to provide **14**. After purification, **14** was hydrolyzed in refluxing 6N HCl to give amino acid **15**.

Isocyano compounds **11b** and **11c** obtained by dehydratation of **10b** and **10c** were used to prepare compounds **21** and **27**. Since the addition of the Grignard reagent methyl magnesium to **11** happened at the carboxylate group and not at the conjugated C=C double bond, [38] **11a** could not be used to prepare **21**. Therefore, **11b** was prepared and the Michael addition of (4-methyl)-phenylmagnesium bromide to this compound provided **16**. Selective hydrolysis [39] of **16** yielded **17**, which was acetylated by acetic anhydride.



(i) KOtBu/THF ; (ii) Ph_3P, NEt_3, $CCl_4/CHCl_3$; (iii) H_2/Pd - C ; (iv) NBS, $(PhCO_2)_2/CCl_4$; (v) $P(OMe)_3$/Toluene ; (vi) 6N HCl ; (vii) CH_3-⟨O⟩-$MgBr/Et_2O$; (viii) HCl/EtOH - H_2O ; (ix) Ac_2O, NEt_3 ; (x) $Me_2\overset{+}{S}(O)\overset{-}{C}H_2$/DMSO, THF ; (xi) Boc_2O, NEt_3/DMF.

Scheme 2. Chemical synthetic pathways of constrained phosphonomethylphenylalanine derivatives **15**, **21** and **27**.

The acetamide **18** was then brominated and after Arbuzov reaction afforded **20**, whose hydrolysis in 6N HCl provided the final amino acid **21**. In order to prepare compound **27**, **11c** was derived from **10c** (mixture of Z and E isomers) and crystallized as yellow crystals containing only **11c** as Z isomer. The Michael type addition of dimethylsulfoxonium methylide [39] ($(CH_3)_2S^+(O)^-CH_2$) (prepared in situ [40]) to **11c**, followed by cyclization with elimination of DMSO yielded **22**, which was then selectively hydrolyzed to form **23**. After Boc protection, compound **24** was brominated and submitted to Arbuzov reaction followed by hydrolysis in 6N HCl to provide amino acid **27**. Starting from **11c** as Z isomer, we could expect to obtain a couple of enantiomers. However, the complexity of NMR spectra of intermediate compounds indicated that each of them exists as two couples of diastereoisomers. This result suggested that the formation of the cyclopropyl ring might proceed by a two-step reaction (addition and elimination).

Preparation of these constrained new unnatural amino acids with appropriate protections in order to allow their use for solid phase peptide synthesis is now in progress in our laboratory.

EXPERIMENTAL SECTION

^1H NMR spectra were recorded on a Brüker Ac 270 spectrometer at 270 MHz. Chemical shifts were given in ppm with HMDS as internal standard. In the case of compounds **10a** and **10c**, 2D spectra were recorded at 293K with 2048 data points, 512 t_1 increments and 64 scans per t_1 increment. After zero filling and double Fourier transformation, base-line corrections were performed in both dimensions. Digital resolution in the final transformed spectra was usually 5.883 Hz/point in the ω_2 and ω_1 dimensions. NOESY spectra were recorded with mixing time of 200 msec. Mass spectra were recorded on a double focusing VG7°-250 spectrometer equipped with a FAB gun. Melting points were determined on an Electrothermal apparatus and are uncorrected. Purity of compounds and reaction progress were checked on precoated plates of silica gel (60 F_{254}, 0.2 mm thick, Merck). Flash chromatography was performed on silica gel 60A (230-400 mesh, Merck).

1-(1-bromoethyl)-4-methylbenzene (1a)

Bromotrimethylsilane (5.23 ml, 39 mmol) was added dropwise to the solution of 1-(4-methylphenyl)ethanol (3 g, 22 mmol) in $CHCl_3$ (150 ml) at -10°C under N_2. The reaction mixture was stirred at -10°C for 2 hours. Evaporation of solvent yielded **1a** as a colorless oil (4.39 g, 100%). ^1H NMR ($CDCl_3$) δ : 1.97(d, 3H, J=7Hz, CH_3-CH), 2.28(s, 3H, CH_3-Ar), 5.15(g, 1H, J=7Hz, CH_3-CH), 7.10 and 7.28(d, 4H, J_{AB}=8Hz, Ar).

Dimethyl [1-(4-methylphenyl)ethyl]phosphonate (2a)

The solution of dimethylphosphite (1.10 ml, 12 mmol) in anhydrous THF (10 ml) was added dropwise to the suspension of sodium hydride (80% in mineral oil, 0.30 g, 12.5 mmol) in anhydrous THF (10 ml) at room temperature over 15 min. Then **1a** (2.00 g, 10 mmol) in anhydrous THF (20 ml) was introduced dropwise at room temperature. The reaction mixture was then refluxed for 24 hours. After solvent evaporation, the residue was subjected to extractive work up (EtOAc/H_2O) to give crude product, which was purified by chromatography (EtOAc/n-hexane 3/1 as eluent) and provided **2a** (1.80 g, 60%) as a colorless oil. R_f(EtOAc/c-hexane 4/1)0.27. ^1H NMR ($CDCl_3$) δ : 1.47 and 1.54(d, 3H, J=8Hz, CH_3-CH), 2.28(s, 3H, CH_3-Ar), 3.08 and 3.20(q, 1H, J=8Hz, CH_3-CH), 3.47 and 3.63(d, 6H, J=9.5Hz, 2xOCH_3), 7.10 and 7.28(d, 4H, J_{AB}=8Hz, Ar), Anal. calc. for $C_{11}H_{17}O_3$: C, 57.89 ; H, 7.51. Found : C, 57.78 ; H, 7.62.

Dimethyl [(4-methylphenyl)methyl]phosphonate (2b)

4-methylbenzyl bromide (**1b**) (10 g, 54 mmol) and trimethyl phosphite (20 ml, 162 mmol) were refluxed in toluene (25 ml) overnight. After solvent evaporation, the residue was purified by chromatography (EtOAc/n-hexane 1/5 as eluent) to yield **2b** as a colorless oil (9.07 g, 78%). R_f(EtOAc/n-hexane 3/1)0.31. ^1H NMR ($CDCl_3$) δ : 2.25(s, 3H, CH_3-Ar), 3.08(d, 2H, J=21Hz, CH_2-P), 3.60(d, 6H, J=12Hz, 2xOCH_3), 7.09(m, 4H, Ar). Anal. calc. for $C_{10}H_{15}O_3P$: C, 56.07 ; H, 7.06. Found : C, 56.18 ; H, 7.27.

Dimethyl [[1-(4-bromomethyl)phenyl]ethyl]phosphonate (3a)

Dibenzoylperoxide (0.025 g, 0.10 mmol) was added in portions to the suspension of **2a** (0.5 g, 2.2 mmol) and N-bromosuccinimide (0.390 g, 2.2 mmol) in CCl_4 (5 ml). The mixture was refluxed 3 hours and then cooled to 10°C, the floating solid (succinimide) removed by filtration and the filtrate was washed with water and brine and then dried with anhydrous sodium sulfate. The residue obtained after evaporation of solvent was purified by flash chromatography (EtOAc/c-hexane 8/1 as eluent) to provide **3a** as a colorless oil (0.54 g, 80%). R_f(EtOAc/c-hexane 8/1)0.30. ^1H NMR ($CDCl_3$) δ : 1.48 and 1.55(d, 3H, J=8Hz, CH_3-CH), 3.12 and 3.19(q, 1H, J=8Hz, CH_3-CH), 3.51 and 3.65(d, 6H, J=10Hz, 2xOCH_3), 4.42(s, 2H, CH_2Br), 7.28-7.32(m, 4H, Ar). Anal. calc. for $C_{10}H_{16}BrO_3P$: C, 43.02 ; H, 5.25. Found : C, 43.17 ; H, 5.30.

Dimethyl [[(4-bromomethyl)phenyl]methyl]phosphonate (3b)

As described for **3a**, **2b** (4.00 g, 18.7 mmol) was brominated and the crude product was purified by chromatography (EtOAc/n-hexane 3/1 as eluent) to yield **3b** as a white solid (3.92 g, 72%). R_f(EtOAc/n-hexane 3/1)0.19, mp 55-7°C. ^1H NMR (DMSO-d6) δ : 3.21(d, 2H, J=22Hz, CH_2-P), 3.55(d, 6H, J=10Hz, 2xOCH_3), 4.64(s, 2H, CH_2-Br), 7.20-7.38(m, 4H, Ar). Anal. calcd for $C_{10}H_{14}BrO_3P$: C, 40.97 ; H, 4.81. Found : C, 40.91 ; H, 4.93.

Ethyl 4-(1-dimethylphosphono)ethyl-D,L-phenylalaninate (5a)

Benzyltrimethylammonium hydroxide (40% aqueous solution) (0.75 ml, 1.5 mmol) was added dropwise to a dioxane solution of **3a** (0.50 g, 1.6 mmol), KI (0.03 g, 0.18 mmol) and ethyl [N-(diphenylmethylene)]glycinate (0.40 g, 1.5 mmol) at 10°C. The mixture was then brought to ambient temperature and stirred 4 hours. The reaction mixture was diluted with water (5 ml) and extracted with EtOAc. After evaporation of solvent, the residue containing **4a** was dissolved in ethyl ether (5 ml) without purification and 6 ml 0.25N HCl aqueous solution was added. This two-phase solution was stirred at room temperature overnight. Then the aqueous phase was separated, neutralized with saturated aqueous solution of sodium bicarbonate and was subjected to extractive work up (CH_2Cl_2) to provide crude product, which was purified by chormatography (CH_2Cl_2/MeOH 20/1 as eluent) to yield **5a** as a colorless oil (0.38 g, 71%). R_f(CH_2Cl_2/MeOH 9/1)0.45. ^1H NMR ($CDCl_3$) δ : 1.18(t, 3H, J=8Hz, CH_3CH_2O), 1.45 and 1.52(d, 3H, J-7Hz, CH_3-CH), 2.90-3.20(m, 3H, CH_2-Ar, CH_3-CH-Ar), 3.48 and 3.63(d, 6H, J=12Hz, 2xOCH_3), 3.95(m, 1H, CH-NH_2), 4.16(q, 2H, J=8Hz, CH_3CH_2O), 7.03-7.28(m, 4H, Ar). Anal. calc. for $C_{15}H_{24}NO_5P$: C, 54.70 ; H, 7.34 ; N, 4.25. Found : C, 54.55 ; H, 7.32 ; N, 4.30.

Methyl α-methyl-4-(dimethylphosphonomethyl)-D,L-phenylalaninate (5b)

The solution of **3b** (0.91 g, 3.1 mmol) in anhydrous CH_2Cl_2 (2 ml) was added to a suspension of methyl (N-benzylidene)alaninate (0.50 g, 2.6 mmol), potassium hydroxide (0.22 g, 3.9 mmol), potassium carbonate

(1.07 g, 7.7 mmol) and benzyltrimethylammonium chloride (0.06 g, 0.2 mmol) in anhydrous CH_2Cl_2 (4 ml). The mixture was stirred at room temperature overnight and the solids were removed by filtration. The solvent was removed by evaporation and the residue obtained was dissolved in ethyl ether (6 ml) and 0.25N HCl aqueous solution (7 ml). This two-phase solution was stirred at room temperature overnight. The aqueous phase was then separated and neutralized by saturated aqueous solution of sodium bicarbonate and subjected to extractive work up (CH_2Cl_2) to give an oily crude product, which was purified by chromatography (CH_2Cl_2/MeOH 20/1 as eluent) to provide **5b** as a colorless oil (0.63 g, 65%). R_f(CH_2Cl_2/MEOH 9/1)0.10. ^1H NMR (DMSO-d6 + TFA) δ : 1.15(s, 3H, CH_3-C-NH_2), 2.68 and 2.81(d, 2H, J_{AB}=13Hz, CH_2-C-NH_2), 3.16(d, 2H, J=20Hz, CH_2-P), 3.25(s, 3H, CO_2CH_3), 3.52(d, 6H, J=12Hz, 2xPOCH_3), 6.93-7.20(m, 4H, Ar). Anal. calc. for $C_{14}H_{22}NO_2P$: C, 53.33 ; H, 7.03 ; N, 4.44. Found : C, 53.12 ; H, 7.17 ; N, 4.37.

Ethyl 2-(formylamino)-3-(4-methylphenyl)-2-butenoate (10a)

Ethyl isocyanoacetate (**9**) (2.4 ml, 22 mmol) in anhydrous THF (5 ml) was added dropwise to the suspension of potassium tert-butoxide (2.60 g, 23 mmol) in anhydrous THF (15 ml) at 0°C. Stirring was continued for 15 min, (4-methyl)acetophenone (**8a**) (3.0 g, 22 mmol) in anhydrous THF (5 ml) was introduced dropwise at 0°C. The mixture was then warmed up to room temperature gradually and stirred for 3 hours. After solvent evaporation, water was added and the solution was neutralized with acetic acid to pH 6 and subjected to extractive work up (CH_2Cl_2). The organic extract was evaporated to yield **8a** as a yellow solid (5.2 g, 95%). The geometrical isomers (Z/E ~ 1/1) were separated by chromatography (EtOAc/c-hexane 1/1 as eluent). MS $^m/_e$(M+1)$^+$248. Anal. calc. for $C_{14}H_{17}NO_3$: C, 68.08 ; H, 6.93 ; N, 5.67. Found : C, 68.06 ; H, 6.83 ; N, 5.51. ***E-Isomer.*** R_f(EtOAc/c-hexane 1/1)0.16, mp 215-216°C. ^1H NMR (DMSO-d6) δ : 0.76(t, 3H, J=8Hz, CH_3CH_2O), 2(s, 3H, CH_3-C=), 2.24(s, 3H, CH_3-Ar), 3.74(q, 2H, J=8Hz, CH_3CH_2O), 7.00(d, 2H, J=7Hz, 2'-6' aromatic protons), 7.10(m, 2H, 3'-5' aromatic protons), 10.05(s, 1H, CHO), 9.70(s, 1H, NH). ***Z-Isomer.*** R_f(EtOAc/c-hexane 1/1)0.34, mp 86-87°C. ^1H NMR (DMSO-d6) δ : 1.15(t, 3H, J=8Hz, CH_3CH_2O), 2.10(s, 3H, CH_3-C=), 2.25(s, 3H, CH_3-Ar), 4.10(q, 2H, J=8Hz, CH_3CH_2O), 7.1(m, 4H, Ar), 7.78(s, 1H, CHO), 9.2(s, 1H, NH).

Ethyl 2-(formylamino)-3-methyl-2-butenoate (10b) [37]

As described for **10a** and starting from acetone (3.7 ml, 50 mmol), the residue obtained from extractive work up was triturated with n-hexane to yield **10b** as a yellow solid (5.16 g, 50%). R_f(EtOAc/n-hexane 1/5)0.42, mp 77-79°C. ^1H NMR (DMSO-d6) δ : 1.15(t, 3H, J=8Hz, CH_3CH_2O), 1.70(s, 3H, $^1CH_3$-C=), 1.90(s, 3H, $^2CH_3$-C), 4.02(q, 2H, J=8Hz, CH_3CH_2O), 7.95(s, 1H, CHO), 9.29(s, 1H, NH).

Ethyl 2-(formylamino)-3-(4-methylphenyl)-2-propenoate (10c)

As described for preparation of **10a** and starting from 4-methylbenzaldehyde (5.47 g, 44.2 mmol), the residue obtained from extractive work up was purified by chromatography (EtOAc/n-hexane 1/3 as eluent) to provide **10c** as a white maxy solid (7.30 g, 69%). R_f(EtOAc/n-hexane 1/1)0.62. MS $^m/_e$(M+1)$^+$234. Anal. calc. for $C_{13}H_{15}NO_3$: C, 66.94 ; H, 6.48 ; N, 6.00. Found : C, 66.88 ; H, 6.52 ; N, 5.72. ^1H NMR (DMSO-d6) δ ***E-isomer*** : 1.05(t, 3H, J=7Hz, CH_3CH_2O), 2.23(s, 3H, CH_3-Ar), 4.05(q, 2H, J=7Hz, CH_3CH_2O), 6.72(s, HC=C), 7.10(m, 4H, Ar), 10.05(s, 1H, CHO), 10.08(s, 1H, NH) ; ***Z-isomer*** : 1.19(t, 3H, J=7Hz, CH_3CH_2O), 2.27(s, 3H, CH_3-Ar), 4.12(q, 2H, J=7Hz, CH_3CH_2O), 7.20(m, 3H, HC=C, 3'-5' aromatic protons), 7.50(d, 2H, J=9Hz, 2'-6' aromatic protons), 10.16(s, 1H, CHO), 9.72(s, 1H, NH).

Ethyl 2-isocyano-3-methyl-2-butenoate (11b) [37]

The solution of **10b** (5.16 g, 22.10 mmol), triphenylphosphine (7.20 g, 27.5 mmol), triethylamine (3.20 ml, 22.10 mmol) and tetrachlorocarbon (2.21 ml, 22.10 mmol) in anhydrous chloroform (30 ml) was refluxed 4 hours. Solvent was evaporated and a small quantity of ethyl ether was added to precipitate triphenylphosphinoxide formed in the reaction. The precipitate was filtered off and the filtrate concentrated and treated with ethyl ether. The procedure of precipitation was repeated several times and the oily residue obtained from the evaporation of the filtrate was purified by chromatography on basic Al_2O_3 (Et_2O/n-hexane 1/3 as eluent) to yield **11b** as a yellow oil (2.85 g, 60%). R_f(EtOAc/n-hexane 1/5)0.91. ^1H NMR (DMSO-d6) δ : 1.19(t, 3H, J=7Hz, CH_3CH_2O), 2.05(s, 3H, $^1CH_3$-C=), 2.19(s, 3H, $^2CH_3$-C=), 4.18(q, 2H, CH_3CH_2O).

Ethyl 2-isocyano-3-(4-methylphenyl)-2-propenoate (11c)

As described for preparation of **11b**, **11c** was obtained as a yellow needle crystalline solid (3.80 g, 59%). R_f(EtOAc/n-hexane 1/3)0.61, mp 73-75°C. ^1H NMR (DMSO-d6) δ ***Z-isomer*** : 1.25(t, 3H, J=7Hz, CH_3CH_2O), 2.32(s, 3H, CH_3-Ar), 4.25(q, 2H, J=7Hz, CH_3CH_2O), 7.31 and 7.80(d, 4H, J_{AB}=8Hz, Ar), 7.70(s, 1H, HC=C). Anal. calc. for $C_{13}H_{13}NO_2$: C, 72.54 ; H, 6.09 ; N, 6.51. Found : C, 72.64 ; H, 6.17 ; N, 6.33.

Ethyl N-formyl-ß-methyl-4-methyl-D,L-phenylalaninate (12)

Palladium (10% on charcoal, 1.0 g) was added to the solution of **10a** (mixture of Z and E isomers 2.00 g, 10.1 mmol) in ethanol (10 ml). The mixture was hydrogenated at 1 atm for 3 hours. The catalyst was then filtered off and the solvent evaporated to yield crude product, which was purified by chromatography (EtOAc/n-hexane 2/1 as eluent) to provide **12** (1.80 g, 89%). R_f(EtOAc/n-hexane 3/1)0.58. ^1H NMR (DMSO-d6) δ : 0.90 and 1.15(tm, 6H, CH_3CH_2O, CH_3-CH-Ar), 2.20(s, 3H, CH_3-Ar), 3.02(m, 1H, CH_3-CH-Ar), 3.82 and 4.04(q, 2H, CH_3CH_2O), 4.42(m, 1H, CH-NH), 7.02(m, 4H, Ar), 7.02 and 7.97(s, 1H, CHO), 10.21 and 10.55(d, 1H, NH). MS $^m/_e$(M+1)$^+$250. Anal. calc. for $C_{14}H_{19}NO_3$: C, 67.47 ; H, 7.63 ; N, 5.62. Found : C, 66.98 ; H, 7.64 ; N, 5.56.

Ethyl 2-isocyano-3-methyl-3-(4-methylphenyl)-butanoate (16)

4-Bromotoluene (3.79 g, 22.2 mmol) in anhydrous ethyl ether (20 ml) was added dropwise to the refluxing suspension of magnesium turning (0.54 g, 22.2 mmol) and one crystal of iodine in 10 ml anhydrous ethyl ether. The mixture was refluxed for 2 hours and then cooled to 0°C. A solution of **11b** (2.30 g, 11.1 mmol) in anhydrous ethyl ether (10 ml) and anhydrous petroleum ether (10 ml) was then introduced dropwise to the already prepared Grignard reagent. Brown tar formed and the mixture was stirred at 0°C for 30 min. 20% aqueous acetic acid solution was then dropped in untill tar disappeared. The clear brown solution was then subjected to extractive work up (ethyl ether) and after evaporation of ethyl ether, the residue was purified by chromatography (EtOAc/n-hexane 1/10 as eluent) to yield **16** as a yellow oil (1.33 g, 49%). R_f(EtOAc/n-hexane 1/10)0.31. ^1H NMR (DMSO-d6) δ : 0.92(t, 3H, J=8Hz, CH_3CH_2O), 1.34(s, 3H, $^1CH_3$-C-Ar), 1.37(s, 3H, $^2CH_3$-C-Ar), 2.21(s, 3H, CH_3-Ar), 3.93(q, 2H, J=8Hz, CH_3CH_2O), 4.90(s, 1H, CH-NC), 7.10 and 7.26(d, 4H, J_{AB}=8Hz, Ar).

Ethyl ß,ß-dimethyl-4-methyl-D,L-phenylalaninate hydrochloride (17)

14N HCl ethanol solution (5 ml) was added water (0.085 ml, 4.70 mmol) and cooled to 0°C. The solution of **16** (0.89 g, 3.62 mmol) in ethanol (5 ml) was introduced dropwise. The reaction mixture was then brought to room temperature and stirred 2.5 hours. Solvent was removed and residue triturated with ethyl ether to provide **17** as a white solid (0.81 g, 82%), mp 109-112°C. ^{1}H NMR (DMSO-d6) δ : 0.90(t, 3H, J=8Hz, CH_3CH_2O), 1.32(s, 3H, $^1CH_3$-C-Ar), 1.35(s, 3H, $^2CH_3$-C-Ar), 2.25(s, 3H, CH_3-Ar), 3.90(q, 2H, J=8Hz, CH_3CH_2O), 4.09(s, 1H, CH-NH_2), 7.10 and 7.23(d, 4H, J_{AB}=8Hz, Ar), 10.37(bs, 3H, NH_3^+). MS $^m/_e$(M+1)$^+$236.

Ethyl N-acetyl-ß,ß-dimethyl-4-methyl-D,L-phenylalaninate (18)

A solution of **17** (0.50 g, 2.94 mmol) and triethylamine (0.61 ml, 4.41 mmol) in 10 ml acetic anhydride was stirred at room temperature for 3 hours. The reaction mixture was then concentrated in vacuo and the residue dissolved in ethyl acetate. This ethyl acetate solution was washed with saturated sodium bicarbonate aqueous solution, water, brine and dried with sodium sulfate. Evaporation of solvent gave **18** as a colorless solid (0.80 g, 97%). R_f(EtOAc/n-hexane 3/1)0.56, mp 105-107°C. ^{1}H NMR (DMSO-d6) δ : 0.88(t, 3H, J=8Hz, CH_3CH_2O), 1.22(s, 3H, $^1CH_3$-C-Ar), 1.28(s, 3H, $^2CH_3$-C-Ar), 1.75(s, 3H, CH_3C=O), 2.20(s, 3H, CH_3-Ar), 3.80(q, 2H, J=8Hz, CH_3CH_2O), 4.59(d, 1H, J=9Hz, CH-NH), 7.04 and 7.20(d, 4H, J_{AB}=8Hz, Ar), 7.93(d, 1H, J=9Hz, CH-NH). Anal. calc. for $C_{16}H_{23}NO_3$: C, 69.29 ; H, 10.36 ; N, 5.05. Found : C, 69.41 ; H, 10.32 ; N, 5.00.

Ethyl 1-isocyano-2-(4-methylphenyl)-cyclopropane-1-carboxylate (22)

Anhydrous dimethyl sulfoxide (18 ml) was added dropwise to the mixture of trimethyloxosulfonium iodine (3.98 g, 18.1 mmol) and sodium hydride (80% in mineral oil, 0.63 g, 20.9 mmol) under nitrogen at room temperature. Stirring was continued about 30 min untill hydrogen evolution ceased and a milky-white mixture formed. Then the mixture was cooled at 10°C and **11c** (3.00 g, 16.9 mmol) in anhydrous THF (36 ml) was added dropwise. The reaction mixture was brought to room temperature, stirred 1 hour, and then stirred at 70°C for another 1 hour. Diluted with water (70 ml), the reaction mixture was extracted with ethyl ether (50 ml x 4) and after evaporation of solvent, the residue was purified by chromatography (EtOAc/n-hexane 1/5 as eluent) to yield **22** as a colorless oil (1.67 g, 52%). R_f(EtOAc/n-hexane 1/5)0.45. ^{1}H NMR (DMSO-d6) δ : 1.20(t, 3H, J=7Hz, CH_3CH_2O), 2.00 and 2.23(q, 2H, CH_2-CH-Ar), 2.25(s, 3H, CH_3-Ar), 3.05(t, 1H, J=9Hz, CH_2CH-Ar), 4.18(q, 2H, J=7Hz, CH_3CH_2O), 7.17 and 7.20(d, 4H, J_{AB}=8Hz, Ar). MS $^m/_e$(M+1)$^+$230. Anal. calc. for $C_{14}H_{15}NO_2$: C, 73.34 ; H, 6.59 ; N, 6.11. Found : C, 73.32 ; H, 6.66 ; N, 6.06.

Ethyl 1-amino-2-(4-methylphenyl)-cyclopropane-1-carboxylate hydrochloride (23)

As described for **17** and starting from **22** (1.00 g, 4.36 mmol), **23** was yielded as a white solid (1.07 g, 96%). Mp 197°C(dec.). ^{1}H NMR (DMSO-d6) δ : 1.20(t, 3H, J=7Hz, CH_3CH_2O), 1.85(d, 2H, J=9Hz, CH_2-CH-Ar), 2.25(s, 3H, CH_3-Ar), 2.95(t, 1H, J=9Hz, CH_2-CH-Ar), 4.19(q, 2H, J=7Hz, CH_3CH_2O), 7.05 and 7.22(d, 4H, J_{AB}=8Hz, Ar), 10.55(bs, 3H, NH_3^+). MS $^m/_e$(M+2)$^+$221.

Ethyl 1-(N-*tert*-butoxylcarbonyl)amino-2-(4-methylphenyl)-cyclopropane-1-carboxylate (24)

Di-*tert*-butyl-dicarbonate (1.02 g, 4.69 mmol) was added to a DMF solution of **23** (0.60 g, 2.35 mmol) and triethylamine (0.78 ml, 5.63 mmol) at 0°C. The mixture was brought to room temperature and stirred overnight. After evaporation of DMF in vacuo the residue was dissolved in EtOAc and the solution was washed with 10% citric acid solution, water, brine and dried with Na_2SO_4. Solvent was removed by evaporation and the residue triturated with n-hexane yielded **24** as a white solid (0.62 g, 82%). R_f(EtOAc/n-hexane 1/5)0.24, mp 120-122°C. ^{1}H NMR (DMSO-d6) δ : 0.98 and 1.16(s, 9H, tBu), 1.15(m, 3H, CH_3CH_2O), 1.53(m, 2H, CH_2-CH-Ar), 2.19 and 2.20(s, 3H, CH_3-Ar), 2.83(m, 1H, CH_2-CH-Ar), 4.05(m, 2H, CH_3CH_2O), 6.95-7.05(m, 4H, Ar), 7.16(s, 1H, NH). MS $^m/_e$(M+1)$^+$320. Anal. calc. for $C_{18}H_{25}NO_4$: C, 67.69 ; H, 7.89 ; N, 4.38. Found : C, 67.61 ; H, 7.92 ; N, 4.33.

Ethyl N-formyl-ß-methyl-4-(dimethylphosphono)methyl-D,L-phenylalaninate (14)

As described for **3a** and starting from **12** (0.50 g, 2.0 mmol), the crude product ethyl N-formyl-ß-methyl-4-bromomethyl-D,L-phenylalaninate (**13**), was treated without purification following the procedure described for preparation of **2b**. The product obtained from this Arbuzov reaction was purified by chromatography (EtOAc/MeOH 98/2 as eluent) to provide **14** as a colorless oil (0.51 g, 71%). R_f(EtOAc/MeOH 98/2)0.16. ^{1}H NMR (DMSO-d6) δ : 0.90 and 1.16(tm, 6H, CH_3CH_2O, CH_3-CH-Ar), 3.15(m, 2H, CH_2-P), 3.52(d, 6H, J=10Hz, 2x$POCH_3$), 3.85 and 4.02(q, 2H, CH_3CH_2O), 4.45(m, 1H, CH-NH), 7.17(m, 4H, Ar), 7.85 and 7.98(s, 1H, CHO), 10.26 and 10.57(d, 1H, NH). MS $^m/_e$(M+1)$^+$358.

Ethyl N-acetyl-ß,ß-dimethyl-4-(dimethylphosphono)methyl-D,L-phenylalaninate (20)

As described for **14** and starting from **18** (0.77 g, 2.78 mmol). The crude product was purified by flash chromatography (EtOAc/n-hexane/MeOH 2/3/0.2 as eluent) to yield **20** as a yellow oil (0.58 g, 54%). R_f(EtOAc/n-hexane/MeOH 2/3/0.2)0.12. ^{1}H NMR (DMSO-d6) δ : 0.89(t, 3H, J=8Hz, CH_3CH_2O), 1.25(s, 3H, $^1CH_3$-C-Ar), 1.28(s, 3H, $^2CH_3$-C-Ar), 1.75(s, 3H, CH_3-C=O), 3.16(d, 2H, J=20Hz, CH_2-P), 3.53(d, 6H, J=11Hz, 2x$POCH_3$), 3.79(q, 2H, J=8Hz, CH_3CH_2O), 4.60(d, 1H, J=9Hz, CH-NH), 7.12-7.25(m, 4H, Ar), 7.98(d, 1H, J=9Hz, CH-NH). MS $^m/_e$(M+1)$^+$386. Anal. calc. for $C_{18}H_{28}NO_6P \cdot 0.75\ H_2O$: C, 54.20 ; H, 7.45 ; N, 3.51. Found : C, 54.19 ; H, 7.58 ; N, 3.48.

Ethyl 1-(N-*tert*-butoxylcarbonyl)amino-2-(4-bromomethyl)phenyl-cyclopropane-1-carboxylate (25)

As described for **3a** and starting from **24** (0.49 g, 1.53 mmol), the crude product was purified by chromatography (EtOAc/n-hexane 1/3 as eluent) to give **25** as a white solid (0.72 g, 72%). R_f(EtOAc/n-hexane 1/3)0.23, mp 142-144°C. ^{1}H NMR (DMSO-d6) δ : 0.97 and 1.10(s, 9H, tBu), 1.15(m, 3H, CH_3CH_2O), 1.59(m, 2H, CH_2-CH-Ar), 2.90(q, 1H, CH_2-CH-Ar), 4.05(m, 2H, CH_3CH_2O), 4.50 and 4.51(s, 2H, CH_2Br), 7.09 and 7.29(m, 4H, Ar). MS $^m/_e$(M)$^+$398.

Ethyl 1-(N-*tert*-butoxylcarbonyl)amino-2-[4-(dimethylphosphono)methyl]phenyl-cyclopropane-1-carboxylate (26)

As described for **2b** and starting from **25** (0.20 g, 0.50 mmol), the crude product was purified by chromatography (EtOAc/n-hexane/MeOH 2/3/0.2 as eluent) and triturated with n-hexane to provide **25** as a

white solid (0.15 g, 68%). R_f(EtOAc/n-hexane/MeOH 2/3/0.2)0.20, mp 110-113°C. ^{1}H NMR (DMSO-d6) δ : 0.90 and 1.16(s, 9H, tBu), 1.15(m, 3H, CH_3CH_2O), 1.55(m, 2H, CH_2-CH-Ar), 2.85(m, 1H, CH_2-CH-Ar), 3.20 and 3.18(d, 2H, J=22Hz, CH_2-P), 3.51 and 3.52(d, 6H, J=9Hz, 2x$POCH_3$), 4.07(m, 2H, CH_3CH_2O), 7.10(m, 4H, Ar), 7.21(s, 1H, NH). MS $^m/_e$(M+1)$^+$428.

4-(1-Phosphono)ethyl-D,L-phenylalanine hydrochloride (6)

5a (0.20 g, 0.6 mmol) was dissolved in 5 ml 6N HCl and the mixture was refluxed for 6 hours. Water was removed by vacum pump and the residue was dissolved in ethanol (5 ml). Propylene oxide was progressively added to the ethanolic solution untill complete precipitation. The precipitate was collected by filtration and washed with ethyl ether to provide **6** as a white solid (0.10 g). Mp 209°C(dec.). ^{1}H NMR (DMSO-d6 + TFA) δ : 1.37-1.42(d, 3H, J=8Hz, CH_3-CH-P), 2.97-3.16(m, 3H, CH_3-CH-P, CH_2-Ar), 4.15(m, 1H, CH-NH_2), 7.12-7.16(m, 4H, Ar), 10.28(bs, 3H, NH_3^+). MS $^m/_e$(M+1)$^+$274.

α-methyl-4-phosphonomethyl-D,L-phenylalanine hydrochloride (7)

Following the procedure described for preparation of **6**, starting from **5b** (0.20 g, 0.6 mmol), **7** was obtained as a white solid (0.07 g). Mp 255°C(dec.). ^{1}H NMR (DMSO-d6 + TFA) δ : 1.42(s, 3H, CH_3-C-NH_2), 2.98(d, 2H, J=22Hz, CH_2-P), 2.95 and 3.08(m, 2H, CH_2-Ar), 7.10 and 7.22(d, 4H, Ar), 10.24(bs, 3H, NH_3^+). MS $^m/_e$(M+1)$^+$274.

ß-methyl-4-phosphonomethyl-D,L-phenylalanine hydrochloride (15)

Following the procedure described for preparation of **6**, starting from **14** (0.20 g, 0.66 mmol), **15** was obtained as a white solid (0.10 g). Mp 236°C(dec.). ^{1}H NMR (DMSO-d6 + TFA) δ : 1.31(d, 3H, J=8Hz, CH_3-CH-Ar), 2.97(d, 2H, J=23Hz, CH_2-P), 3.23(m, 1H, CH_3-CH-Ar), 4.00(m, 1H, CH-NH_3^+), 7.11-7.22(m, 4H, Ar), 10.28(bs, 3H, NH_3^+). MS $^m/_e$(M+1)$^+$274.

ß,ß-dimethyl-4-phosphonomethyl-D,L-phenylalanine hydrochloride (21)

Following the procedure described for preparation of **6**, starting from **20** (0.55 g, 1.4 mmol), **21** was obtained as a white solid (0.36 g). Mp 219°C(dec.). ^{1}H NMR (DMSO-d6 + TFA) δ : 1.38(s, 3H, $^1CH_3$-C-Ar), 1.40(s, 3H, $^2CH_3$-C-Ar), 3.02(d, 2H, J=22Hz, CH_2-P), 4.16(d, 1H, J=5Hz, CH-NH_3^+), 7.29 and 7.36(d, 4H, J_{AB}=7Hz, Ar), 10.03(s, 3H, NH_3^+). MS $^m/_e$(M+1)$^+$288.

1-amino-2-(4-phosphonomethyl)phenyl-cyclopropane-1-carboxylic acid hydrochloride (27)

Following the procedure described for preparation of **6**, starting from **26** (0.10 g, 0.25 mmol). **27** was obtained as a white solid (0.046 g). Mp 209°C(dec.). ^{1}H NMR (D_2O + TFA) δ : 1.72(m, 1H, $^1CH_2$-CH-Ar), 1.88(m, 1H, $^2CH_2$-CH-Ar), 3.00(d, 2H, J=21Hz, CH_2-P), 3.05(m, 1H, CH_2-CH-Ar), 7.12(m, 4H, Ar). MS $^m/_e$(M+1)$^+$272.

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