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Peptides containing the novel Methylphosphinamide Transition-State Isostere

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Abstract: A convenient route towards the synthesis of peptides containing the methylphosphinamide moiety as a novel transition-state isostere of the amide bond hydrolysis is described. The key step being the coupling of a methylphosphinic chloride with an amino acid or peptide protected at the C-terminus. A proper choice of the amino protecting group appeared to be essential.

INTRODUCTION

Peptides, containing a transition-state analogue of the amide bond hydrolysis, are important as potential enzyme inhibitors[1]. They also play an important role in the development of catalytic antibodies[2]. Nowadays there is a wide variety of transition-state analogues[1-3] available. The phosphonamidate (Figure 1b) and sulfonamide (Figure 1c) moiety[4a] are among the transition-state analogues, which show the best resemblance, both from a steric and electronic point of view, to the transition-state of the amide bond hydrolysis (Figure 1a).

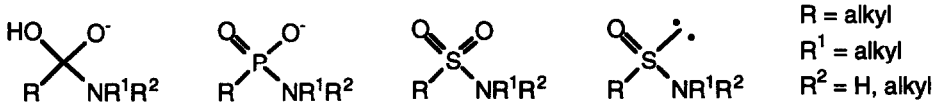


Figure 1a 1b 1c 1d

In view of our interest in the development of abzymes against the conserved sequence (312-314) i.e. Gly-Pro-Gly in HIV gp120[5] we have synthesized β-aminoethane sulfonamide (Figure 1c) and sulfinamide (Figure 1d) containing peptides mimicking the Gly-Pro-Gly sequence[4a]. By incorporation of α- as well as β-substituted sulfonamides into peptides it became possible to mimick other amino acids than glycine[4b,c]. The methodology developed for the preparation of α-substituted sulfonamide transition-state isosteres enabled us to prepare sulfonamides mimicking the amide bond hydrolysis of the p17/p24 cleavage site Phe-Pro in the gag-pol precursor protein of HIV[4c], thus giving rise to a new type of potential HIV protease inhibitors. However, preliminary test results show a very poor inhibition of HIV protease[4c]. In contrast, phosphonamidate analogues have been used with success both in the preparation of (HIV) protease inhibitors[6a,b] and in the development of abzymes against an aromatic amide[2a]. Due to their instability under acidic conditions[6d,e,g,h] the more stable phosphonamidate ester (Figure 2a)[6a,b], phosphinate (Figure 2b)[7] and phosphonate ester (Figure 2c)[8] have been synthesized.

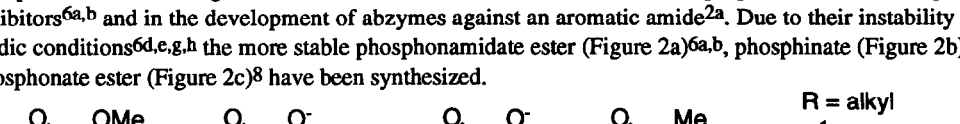


Figure 2a 2b 2c 2d

In this respect, a methylphosphinamide (Figure 2d) could also be a valuable extension of this series of phosphor based transition-state isosteres. Natchev described the synthesis of phospha^{C}peptide analogues of 'Biapholos' (Figure 3a), containing a methylphosphinamide moiety in the side chain[9]. However the synthesis of peptides containing a methylphosphinamide moiety transition state isostere in the peptide back bone have not been described as far as we know (Figure 3b).

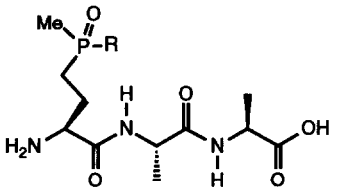


Figure 3a

The structure of 'Biapholos' (R=OH) and its phospha^{C}peptide analogue (R = N(H) peptide).

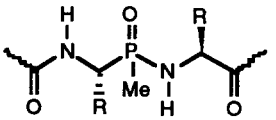


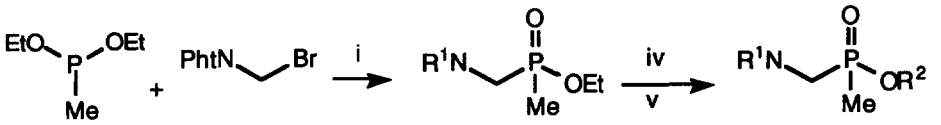
Figure 3b

Peptidebackbone with methylphosphinamide transition state isostere.

RESULTS AND DISCUSSION

As a first approach to the synthesis of peptides containing a methylphosphinamide transition-state isostere we started the preparation of the phthaloyl protected (aminomethyl)methylphosphinic ethylester **3a**, because this building block is easily prepared by an Arbuzov reaction and might be coupled with amino acids or peptides followed by deprotection of the phthaloyl group analogous to the synthesis of peptides containing a phosphonamidate transition state isostere[6e]. According to the method of Popoff *et al* [10], diethyl methylphosphonite **1**, obtained by a Grignard reaction of methylmagnesium bromide and diethyl phosphonochloridite[11], reacted with N-(bromomethyl)phthalimide **2**[12] to give protected (aminomethyl)methylphosphinic acid **3a**. Subsequently the ethyl group of **3a** was deprotected in a two step procedure. The ethylester was transesterified with trimethylsilyl bromide[13], followed by hydrolysis with MeOH/H_2O to give the phosphinic acid **4d**. Coupling of **4d** with ProN(H)Me in the presence of DCC, analogous to the synthesis of phospha^{C}peptide analogues of "Bialaphos"[9b], was very slow and gave rise to byproducts. Therefore we converted the trimethylsilylester **4a** to the more reactive phosphinic chloride **5a** under Vilsmeier-Haack conditions[14]. Coupling of **5a** with ProN(H)Me[15] using N-methylmorpholine as a base gave the desired phosphinamide **6a.** On acidic aqueous work up the phosphinamides decomposed, whereas neutral aqueous work up resulted in considerable loss of product. Therefore the reaction mixture was applied directly to a silica gel column chromatography to give the diastereomers of **6a** in a total yield of 93% (Scheme 1). Unfortunately, removal of the phthaloyl group of phosphinamide **6a** with hydrazine monohydrate[6e] in MeOH was unsuccessful as indicated by ^{31}P NMR spectroscopy. The phosphinamide moiety was apparently not stable under the prolonged basic exposition.

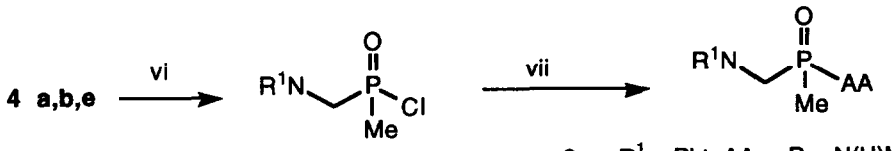
Next we tried the trifluoroacetyl protecting group as employed by Natchev[9b] for the synthesis of phospha^{C}peptides. Phthaloyl derivative **3a**, was treated with hydrazine monohydrate in EtOH to give the amino compound, which was directly treated with trifluoroacetic anhydride to give fully protected **3b**. This compound was converted to the diastereomeric phosphinamides **6b** using the same procedure as described for the synthesis of phosphinamides **6a**. Unfortunately, deprotection of the trifluoroacetyl group from the methylphosphinamides **6b** with either NH_4OH, NH_4OH/dioxane[9b], NH_3/MeOH[16] or K_2CO_3[17] was slow and resulted in a mixture of products as was indicated by ^{31}P NMR spectroscopy.



1 **2**

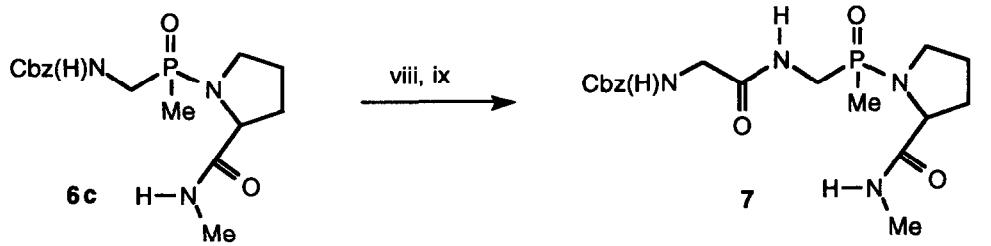
3 a: R^1 = Pht
b: R^1 = $CF_3C(=O)$
c: R^1 = Cbz

4 a: R^1 = Pht, R^2= TMS
b: R^1 = $CF_3C(=O)$, R^2= TMS
c: R^1 = Cbz, R^2= TMS
d: R^1 = Pht, R^2= H
e: R^1 = Cbz, R^2= H



5 a: R^1 = Pht
b: R^1 = $CF_3C(=O)$
c: R^1 = Cbz

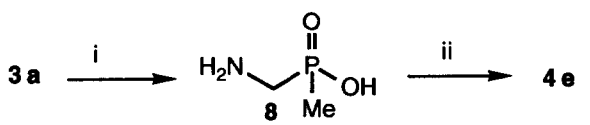
6 a: R^1 = Pht, AA = Pro-N(H)Me
b: R^1 = $CF_3C(=O)$, AA = Pro-N(H)Me
c: R^1 = Cbz, AA = Pro-N(H)Me
d: R^1 = Cbz, AA = Pro-Gly-N(H)Me
e: R^1 = Cbz, AA = Phe-OMe
f: R^1 = Cbz, AA = Gly-OEt



6c **7**

Reagents and conditions: i. Δ, ii. $NH_2NH_2.H_2O$/EtOH then $(CF_3CO)_2O$ / NMM, iii. $NH_2NH_2.H_2O$ / EtOH then Cbz_2O / NMM, iv. TMSBr, v. MeOH/H_2O, vi. $(COCl)_2$, cat. DMF, vii. AA/NMM, AA = Pro-N(H)Me, Pro-Gly-N(H)Me, HCl.Phe-OMe, HCl.Gly-OEt, viii. H_2 Pd/C, ix. CbzGly-OH / *i* Butylchloroformate / NMM

Scheme 1



3 a **8** **4 e**

Reagents and conditions: i. 6N HCl then propyleneoxide, ii. CbzCl, NaOH, $NaHCO_3$ Na_2CO_3

Scheme 2

Thus although we were able to prepare a small peptide containing the methylphosphinamide moiety, we were unable to remove the amino-protecting group without the formation of byproducts. The apparent instability under acidic and basic conditions forced us to use a protecting group, which can be removed under neutral conditions, such as the carbobenzoxy group, although Natchev[9b] remarked that 'protective groups released by hydrogenolysis are ineligible because they cause side reactions'. We had to change the route though, because treatment of the N-carbobenzoxy protected phosphinic ester **3c** with TMSBr caused partly deprotection of the carbobenzoxy group. Starting again from the phthaloyl derivative **3a**, which was deprotected with 6N HCl and treated with propyleneoxide, analogous to the synthesis of aminomethylphosphonous acid[18], the (aminomethyl)methylphosphinic acid **8** was obtained in 94% yield. The phosphinic acid **8** was transformed into **4e** with benzylchloroformate in 95% yield[19] (Scheme 2). This compound was treated with oxalyl chloride and a catalytic amount of DMF to give phosphinic chloride **5c**, followed by coupling with a C-terminus protected amino acid or peptide. The resulting peptide mimetics **6c-f** were isolated in yields ranging from 74 to 94%. The diastereomers of **6c** could be separated. The carbobenzoxy protecting group in the phosphinamides was removed by hydrogenolysis, without any side reactions. After deprotection of **6c**, coupling at the N terminus was carried out using the mixed anhydride method to give the tripeptide **7** in 87% yield (Scheme 1).

In order to use transition-state analogues as enzyme inhibitors or in the development of catalytic antibodies it is important to estimate their stability under physiological conditions. Therefore we studied the stability of the methylphosphinamides in an aqueous environment at different pH values with ^{31}P NMR. The $t_{1/2}$ for hydrolysis of **6c** in the methylphosphinic acid and the amine in buffers of 0.05 M NaOAc/HOAc (20% DMSO) with pH values ranging from 3.4, 4.0, 5.0, 6.0 to 7.6 were found to be approximately 1 d, 3.5 d, 25 d, 126 d and ∞ respectively. Thus in comparison to a phosphonamidate *e.g.* CbzGlyP(O$^-$)(O)PheOH with a $t_{1/2}$ at pH 6.2 of 4 h[6e] the phosphinmethylamide is rather stable and can be employed under physiological conditions.

This method enables us to prepare in principle every possible peptide sequence with a methylphosphinamide moiety mimicking the transition-state of hydrolysis of the amide bond at the C-terminus of a glycine. Methylphosphinamide transition-state analogues of other amino acids than glycine, important for *e.g.* the development of HIV-protease inhibitors, are in principle accessible starting from α-substituted (aminomethyl)methylphosphinic acids[20].

EXPERIMENTAL

General methods: Dioxane and THF were dried by refluxing on $LiALH_4$ and distilled immediately prior to use. DMF was stirred with CaH_2 for 16 h and then distilled under reduced pressure. Ethanol free dichloromethane used for synthesis of the phosphinic chlorides was purchased from Baker, dried by refluxing on CaH_2 and distilled directly prior to use. N-methyl morpholine (NMM) was distilled from calcium hydride, *iso*-butylchloroformate was distilled under Ar.

Diethyl phosphonochloridite and Glycine ethylester hydrochloride were purchased from Janssen.

Melting points were determined on a Büchi Schmelzpunktbestimmungsapparat and are uncorrected. TLC analysis was performed on Merck pre-coated silicagel 60 F-254 plates. Spots were visualized with UV light and ninhydrin (after treatment with HCl). Column chromatography was carried out on Merck Kieselgel 60 (70-230 Mesh, ASTM).

^1H NMR spectra were recorded on a Jeol NM-Fx 200 (200 MHz) spectrometer or a Bruker WM-300 (300 MHz) spectrometer interfaced with an ASPECT-2000 computer, operating in the Fourier transform mode and are given in ppm (δ) relative to TMS or TSP as internal standard. ^{13}C and ^{31}P NMR spectra were recorded on a Jeol JNM-Fx 200 spectrometer on line with a JEC 980B computer at 50.1 MHz and 80.7 MHz respectively. ^{13}C-chemical shifts are given in ppm (δ) relative to $CDCl_3$ as internal standard and ^{31}P-chemical shifts in ppm (δ) to 85% H_3PO_4 as external standard. $CDCl_3$ was used as a solvent unless stated otherwise. The numbering of the carbon atoms in the amino acids is according to IUPAC recommendations[21]. Assignments of ^1H NMR

spectra were made possible by means of ^1H $\{^{31}P\}$NMR spectroscopy.
Fast Atom Bombardment (FAB) mass spectometry was carried out using V.G. Micromass ZAB-HFqQ mass spectometer, coupled to a V.G. 11/250 data system. The samples were loaded in a glycerol/thioglycerol/nitrobenzylalcohol (NBA) solution onto a stainless steel probe and bombarded with Xenon atoms with an energy of 8KeV. Glycerol was used to calibrate the mass spectrometer.

Ethyl (Phthaloylaminomethyl)methylphosphinate (**3a**)
Compound **3a** was prepared according to the procedure described by Popoff *et al*[10]. However, the product was purified by silicagel column chromatography (250 g, eluent EtOAc) and obtained as a solid in 84% yield. R_f (EtOAc/MeOH 95/5 v/v) 0.31; ^1H NMR (300 MHz) 1.32 (t, 3H, $OCH_2C\underline{H}_3$, J = 7.0 Hz), 1.58 (d, 3H, CH_3P, J_{HP} = 14.6 Hz), 4.08 (d, 2H, CH_2P, J_{HP} = 8.6 Hz), 4.18, 4.23 (two m 12 lines, $OC\underline{H}_2CH_3$, J_{AX} = 7.1 Hz, J_{BX} = 7.1 Hz, J_{AB} = 9.9 Hz, J_{HP} = 9.9 Hz), 7.73-7.80 (m, 2H, Pht arom.), 7.85-7.92 (m, 2H, Pht arom.); ^{13}C NMR 14.5 (d, CH_3P, J_{CP} = 96.7 Hz), 16.3 (d, $OCH_2\underline{C}H_3$, J_{CP} = 5.9 Hz), 36.1 (d, CH_2P, J_{CP} = 98.2 Hz), 61.0 (d, $O\underline{C}H_2CH_3$, J_{CP} = 5.9 Hz), 123.4, 131.5, 134.1 (Pht, C arom.), 167.0 (C=O); ^{31}P NMR 46.2.

N-[(N-Phthaloylaminomethyl)methyl]phosphinoyl-proline-methylamide (**6**)
Phosphinic ester **3a** (0.52 g, 1.95 mmol) was coevaporated in dioxane (3x 30 mL) and dissolved in CH_2Cl_2 (5 mL) under Ar. TMSBr (0.39 mL, 2.93 mmol) was added dropwise. The mixture was stirred and another portion of TMSBr (0.29 mL, 2.19 mmol) was added until ^{31}P NMR showed complete conversion to the TMS ester **4a** (3 h). Concentration and removal of excess of TMSBr *in vacuo* gave the TMS ester **4a**, which was used without further purification. To a solution of TMS ester **4a** in CH_2Cl_2 (10 mL) under Ar, oxalyl chloride (0.255 mL, 2.93 mmol) and a catalytic amount of DMF were added. The mixture was stirred at rt until ^{31}P NMR showed complete conversion to the phosphinic chloride (1h). Concentration *in vacuo* to remove the excess of oxalyl chloride gave the phosphinic chloride **5a**, which was used without further purification.^{31}P NMR 57.0.
The phosphinic chloride **5a** was dissolved in CH_2Cl_2 (6 mL) and stirred under Ar. A solution of Pro-N(H)Me[15] (0.275 g, 2.14 mmol) in CH_2Cl_2 (4 mL) and N-methyl morpholine (0.214 mL, 1.95 mmol) were added simultaneously. The mixture was stirred until ^{31}P NMR showed complete disappearance of the phosphinic chloride (ca. 30 min), concentrated *in vacuo* and chromatographed (40 g silica gel, eluent: EtOAc/MeOH 9/1 v/v) to give the individual diastereomers of **6a** (ratio 1/1) in a combined yield of 93%.
High running stereoisomer: R_f (CH_2Cl_2/MeOH 9/1 v/v) 0.69; Oil which solidified on standing. ^1H NMR (300 MHz) 1.61 (d, 3H, CH_3P, J_{HP} = 13.5 Hz), 1.83-2.09 (m, 3H, Pro-C^3H_a, Pro-C^4H_2), 2.16-2.25 (m, 1H, Pro-C^3H_b), 2.80 (d, 3H, N(H)$C\underline{H}_3$, J = 4.9 Hz), 3.37-3.47 (m, 1H, Pro-C^5H_a, J_{AX} =3.5 Hz, J_{AY} = 6.4 Hz, J_{AB} = 9.2 Hz, J_{HP} = 4.9 Hz), 3.42-3.50 (m, 1H, Pro-C^5H_b, J_{BX} = 3.7 Hz, J_{BY} = 6.2 Hz, J_{AB} = 9.2 Hz, J_{HP} = 2.9 Hz), 4.02 (m 7 lines, 1H, Pro-C^2H, J_{AX} = 2.3 Hz, J_{AY} = 8.2 Hz, J_{HP} = 5.8 Hz), 4.13 (d, 2H, CH_2P, J_{HP} = 7.9 Hz), 7.12 (q, 1H, N($\underline{H}$)CH_3, J = 4.9 Hz), 7.72-7.80 (m, 2H, Pht arom.), 7.85-7.92 (m, 2H, Pht arom.); ^{13}C NMR 13.8 (d, CH_3P, J_{CP} = 85.0 Hz), 24.8 (d, Pro-C^4, J_{CP} = 5.9 Hz), 26.1 (N(H)CH_3), 31.5 (d, Pro-C^3, J_{CP} = 5.9 Hz), 36.4 (d, CH_2P, J_{CP} = 93.8 Hz), 46.5 (Pro-C^5), 61.7 (Pro-C^2), 123.5, 131.5, 134.3 (Pht, C arom.), 167.3, 173.4 (C=O); ^{31}P NMR 38.9.
Low running stereoisomer: R_f (CH_2Cl_2/MeOH 9/1 v/v) 0.65; Oil; ^1H NMR (300 MHz) 1.66 (d, 3H, CH_3P, J_{HP} = 13.7 Hz), 1.81-1.92 (m, 2H, Pro-C^4H_2), 1.96-2.09, 2.36-2.44 (two m, 2H, Pro-C^3H_2), 2.69 (d, 3H, N(H)$C\underline{H}_3$, J = 4.8 Hz), 3.28 (m 9 lines, 1H, Pro-C^5H_a, J_{AX} = 7.0 Hz, J_{AB} = 8.7 Hz, J_{HP} = 7.0 Hz), 3.34-3.41 (m, 1H, Pro-C^5H_b, J_{BX} = 4.4 Hz, J_{BY} = 7.0 Hz, J_{AB} = 8.7 Hz, J_{HP} = 3.0 Hz), 4.11 (dd, 1H, CH_aP, J_{AB} = 15.5 Hz, J_{HP} = 7.0 Hz), 4.18 (dd, 1H, CH_bP, J_{AB} = 15.5 Hz, J_{HP} = 8.6 Hz), 4.21 (m 6 lines, 1H, Pro-C^2H, J_{AX} = 2.4 Hz, J_{AY} = 8.6 Hz, J_{HP} = 8.6 Hz), 7.33 (q, 1H, N($\underline{H}$)CH_3, J = 4.8 Hz), 7.74-7.80 (m, 2H, Pht arom.), 7.86-7.93 (m, 2H, Pht arom.); ^{13}C NMR 14.1 (d, CH_3P, J_{CP} = 86.5 Hz), 25.2 (d, Pro-C^4, J_{CP} = 5.9 Hz), 26.2 (N(H)CH_3), 30.6 (d, Pro-C^3, J_{CP} = 4.4 Hz), 36.5 (d, CH_2P, J_{CP} = 93.8 Hz), 47.5 (Pro-C^5), 61.0 (Pro-C^2), 123.7, 131.5, 134.5 (Pht, C arom.), 167.5, 173.1 (C=O); ^{31}P NMR 39.7.

Ethyl (N-Trifluoroacetylaminomethyl)methylphosphinate (**3b**)

To a solution of phthaloyl derivative **3a** (2.67 g, 9.98 mmol) in absolute ethanol (40 mL) was added a solution of hydrazine monohydrate (0.53 mL, 11.0 mmol) in ethanol (3 mL). After stirring overnight at rt, the mixture was refluxed for 4 h and subsequently cooled to 0°C. The precipitate was removed by filtration and washed with CH_2Cl_2. The combined filtrates were evaporated *in vacuo* and directly used in the next step. ^{13}C NMR 11.4 (CH_3P, J_{CP}= 89.4 Hz), 16.6 ($OCH_2\underline{C}H_3$, J = 4.4 Hz), 40.7 (CH_2P, J_{CP}= 90.9 Hz), 59.9 ($O\underline{C}H_2CH_3$); ^{31}P NMR 57.3

Trifluoroacetic anhydride (0.99 mL, 7.02 mmol) and N-methyl morpholine (0.77 mL, 7.02 mmol) were added simultaneously to a solution of the crude amino compound (0.95 g, 6.94 mmol) in THF (30 mL). The mixture was stirred for 2 h at rt, evaporated, dissolved in EtOAc (75 mL) and washed with 1N $KHSO_4$ (4 x 10 mL), 5% $NaHCO_3$ (2x 10 mL) and brine (1x 10 mL). After drying ($MgSO_4$) and concentration *in vacuo* **3b** was chromatographed (40 g silica gel, eluent: EtOAc/MeOH 95/5 v/v) and isolated in 95% yield. R_f (CH_2Cl_2/MeOH 9/1 v/v) 0.44; ^1H NMR (200 MHz) 1.33 (t, 3H, $OCH_2\underline{C}H_3$, J = 7.1 Hz), 1.55 (d, 3H, CH_3P, J_{HP} = 14.1 Hz), 3.52-3.96 (m, 2H, CH_2P), 4.04-4.22 (m, 2H, $O\underline{C}H_2CH_3$), 9.66 (t, 1H, NH, J = 6.0 Hz); ^{13}C NMR 12.4 (d, CH_3P, J_{CP} = 93.8 Hz), 15.8 (d, $OCH_2\underline{C}H_3$, J_{CP} = 5.9 Hz), 38.0 (d, CH_2P, J_{CP} = 102.6 Hz), 61.0 (d, $O\underline{C}H_2CH_3$, J_{CP} = 7.3 Hz), 115.6 (q, CF_3, J_{CF} = 286.7 Hz), 167.0 (q, C=O, J_{CF} = 37.4 Hz); ^{31}P NMR 49.2.

N-[(N-Trifluoroacetylaminomethyl)methyl]phosphinoyl-proline-methylamide (**6b**)

The trifluoroacetyl phosphinic chloride **5b** was prepared from the phosphinic ester **3b** (0.197 g, 0.85 mmol) *via* the TMS ester **4b**, using the same procedure as described for the synthesis of the phthaloyl phosphinic chloride **5a**. ^{31}P NMR 61.2.

Coupling of **5b** with Pro-N(H)Me (0.114 g, 0.88 mmol) was preformed under the same conditions as described for the synthesis of methylphosphinamide **6a**. Silica gel column chromatography (20 g silica gel, eluent: CH_2Cl_2/MeOH 95/5 v/v) gave the individual diastereomers of **6b** (ratio 1/1) as an oil in a combined yield of 95%. High running stereoisomer: R_f (CH_2Cl_2/MeOH 9/1 v/v) 0.33; ^1H NMR (300 MHz) 1.58 (d, 3H, CH_3P, J_{HP} = 13.5 Hz), 1.89-2.12 (m, 3H, Pro-C^4H_2, Pro-C^3H_a), 2.13-2.23 (m, 1H, Pro-C^3H_b), 2.79 (d, 3H, N(H)$C\underline{H}_3$, J = 4.8 Hz), 3.24-3.37 (m, 2H, Pro-C^5H_2), 3.37 (m 8 lines, 1H, CH_aP, J_{AX} = 4.6 Hz, J_{AB} = 15.6 Hz, J_{HP} = 11.5 Hz), 4.01 (m, 8 lines, 1H, CH_bP, J_{AX} = 5.7 Hz, J_{AB} = 15.6 Hz, J_{HP} = 6.9 Hz), 4.28 (m 8 lines, 1H, Pro-C^2H, J_{AX} = 3.1 Hz, J_{AY} = 8.0 Hz, J_{HP} = 3.9 Hz), 7.18 (b, 1H, NH), 9.08 (b, 1H, NH); ^{13}C NMR 13.4 (d, CH_3P, J_{CP} = 85.0 Hz), 25.4 (d, Pro-C^4, J_{CP} = 5.9 Hz), 26.3 (N(H)CH_3), 32.5 (d, Pro-C^3, J_{CP} = 7.3 Hz), 39.4 (d, CH_2P, J_{CP} = 93.8 Hz), 48.8 (Pro-C^5), 59.4 (d, Pro-C^2, J = 2.9 Hz), 115.9 (q, CF_3, J_{CF} = 281.5 Hz), 157.9 (q, $CF_3\underline{C}$=O, J = 37.8 Hz), 175.5 (C=O); ^{31}P NMR 39.2.

Low running stereoisomer: R_f (CH_2Cl_2/MeOH 9/1 v/v) 0.25; ^1H NMR (MeOD, 300 MHz) 1.61 (d, 3H, CH_3P, J_{HP} = 13.9 Hz), 1.85-1.93 (m, 2H, Pro-C^4H_2), 1.97-2.06, 2.11-2.24 (two m, 2H, Pro-C^3H_2), 2.74 (s, 3H, N(H)$C\underline{H}_3$), 3.28-3.42 (m, 2H, Pro-C^5H_2, J_{AX} = 5.1 Hz, J_{AY} = 6.9 Hz, J_{AB} = 9.1 Hz, J_{HP} = covered), 3.81 (d, 2H, CH_2P, J_{HP} = 8.4 Hz), 4.16 (m 8 lines, 1H, Pro-C^2H, J_{AX} = 3.2 Hz, J_{AY} = 8.5 Hz, J_{HP} = 7.2 Hz); ^{13}C NMR (MeOD) 12.8 (d, CH_3P, J_{CP} = 87.9 Hz), 25.8 (d, Pro-C^4, J_{CP} = 5.9 Hz), 26.5 (N(H)CH_3), 33.2 (d, Pro-C^3, J_{CP} = 5.9 Hz), 38.8 (d, CH_2P, J_{CP} = 96.7 Hz), 54.4 (Pro-C^5), 61.9 (Pro-C^2), 117.1 (q, CF_3, J_{CF} = 286.7 Hz), 159.6 (q, $CF_3\underline{C}$=O, J = 38.0 Hz), 176.3 (C=O); ^{31}P NMR (MeOD) 43.9.

(Aminomethyl)methylphosphinic acid(**8**)

Compound **8** was prepared analogous to the synthesis of aminomethylphosphonous acid described in the literature[18]. Phthaloyl derivative **3a** (1.34 g, 5.0 mmol) was dissolved in 6N HCl (8 mL) and refluxed for 8 h at 100-120°C. After cooling to 4°C overnight, the precipitate of o-phthalic acid was filtered and washed with cold water. The filtrate was evaporated and dissolved in methanol (20 mL). Propylene oxide was added until a precipitate appeared. After standing overnight at 4°C, the mixture was concentrated to a small volume. The

precipitate was filtered, washed with ether (10 mL), dried over P_2O_5 and isolated in 96% yield. Mp. 292-294°C; ^1H NMR (D_2O, 200 MHz) 1.38 (d, 3H, CH_3P, J_{HP} = 14.1 Hz), 3.05 (d, 2H, CH_2P, J_{HP} = 10.0 Hz); ^{13}C NMR (D_2O) 15.5 (d, CH_3P, J_{CP} = 98.2 Hz), 39.2 (d, CH_2P, J_{CP} = 90.9 Hz); ^{31}P NMR (D_2O) 32.1.

(N-Carbobenzoxyaminomethyl)methylphosphinic acid (**4e**)
(Aminomethyl)methylphosphinic acid **8** (0.265 g, 2.43 mmol) was dissolved in 1N NaOH (2.4 mL) and $NaHCO_3$ (0.408 g, 4.86 mmol) and Na_2CO_3 (0.515 g, 4.86 mmol) were added. The mixture was cooled to 0°C and benzylchloroformate (0.62 mL, 4.34 mmol) was added. The temperature was allowed to raise to rt, after 1hr another portion of benzylchloroformate (0.62 mL, 4.34 mmol) was added. After stirring overnight at rt, the mixture was diluted with 0.5N NaOH (2.34 mL, 1.2 mmol) and washed with ether (2x 20 mL). The water layer was acidified with conc. HCl to pH 2 and extracted with EtOAc (3x 30 mL). After drying (Na_2SO_4) and evaporation, the phosphinic acid **4e** was crystallized from EtOAc/Petroleum ether 40-60 and obtained in a yield of 92%. Mp.119-121°C; ^1H NMR (MeOD, 200 MHz) 1.43 (d, 3H, CH_3P, J_{HP} = 14.1 Hz), 3.48 (d, 2H, CH_2P, J_{HP} = 8.7 Hz), 5.10 (s, 2H, CH_2 Cbz), 7.28-7.42 (m, 5H, arom. Cbz); ^{13}C NMR (MeOD) 13.3 (d, CH_3P, J_{CP} = 90.9 Hz), 41.5 (d, CH_2P, J_{CP} = 107.0 Hz), 67.7 (CH_2 Cbz), 128.7, 128.8, 129.2, 137.9 (arom. Cbz), 158.4 (C=O); ^{31}P NMR (MeOD) 48.7.

(N-Carbobenzoxyaminomethyl)methylphosphinic chloride (**5c**)
The phosphinic acid **4e** (0.119 g, 0.49 mmol) was coevaporated in dioxane (2x 20 mL) and suspended in CH_2Cl_2 (3 mL) under Ar. To the cooled mixture (0°C) oxalyl chloride (64 µL, 0.73 mmol) and a catalytic amount of DMF were added and the mixture was stirred until ^{31}P NMR showed complete conversion into the phosphinic chloride **5c** (1h). Concentration and removal of excess oxalyl chloride *in vacuo* gave the phosphinic chloride **5c** as an oil which was used without further purification.^1H NMR (200 MHz) 1.97 (d, 3H, CH_3P, J_{HP} = 13.4 Hz), 3.91 (b, 2H, CH_2P), 5.13 (s, 2H, CH_2 Cbz), 6.06 (b, 1H, NH), 7.28-7.44 (m, 5H, arom. Cbz); ^{13}C NMR 20.3 (d, CH_3P, J_{CP} = 73.3 Hz), 45.8 (d, CH_2P, J_{CP} = 90.9 Hz), 67.1 (CH_2 Cbz), 127.8, 128.0, 128.2, 135.7 (arom. Cbz), 156.1 (C=O); ^{31}P NMR 62.6.

N-[(N-Carbobenzoxyaminomethyl)methyl]phosphinoyl-proline-methylamide (**6c**)
The phosphinic chloride **6c** was dissolved in CH_2Cl_2 (2 mL) under Ar and cooled to 0°C, a solution of ProN(H)Me (66 mg, 0.51 mmol) in CH_2Cl_2 (2 mL) and N-methyl morpholine (57 µL, 0.51 mmol) were added simultaneously. The mixture was stirred for 1 hr at rt, concentrated *in vacuo* and chromatographed (15 g silica gel, eluent: CH_2Cl_2/MeOH 97/3 v/v). The two diastereomers were isolated (ratio 1/1) in a total yield of 94%. High running stereoisomer (solid): R_f (CH_2Cl_2/MeOH 9/1 v/v) 0.43; ^1H NMR (300 MHz) 1.49 (d, 3H, CH_3P, J_{HP} = 13.2 Hz), 1.83-2.13 (m, 4H, Pro-C^4H_2, Pro-C^3H_2), 2.75 (d, 3H, N(H)C$\underline{H}_3$, J = 4.8 Hz), 3.19 (m 10 lines, 1H, Pro-C^5H_a, J_{AX} = 7.8 Hz, J_{HP} = 5.1 Hz), 3.33 (m 11 lines, 1H, Pro-C^5H_b, J_{BX} = 4.4 Hz, J_{BY} = 7.2 Hz, J_{AB} = 8.8 Hz, J_{HP} = covered), 3.42 (m 8 lines, CH_aP, 1H, J_{AX} = 5.6 Hz, J_{AB} = 15.7 Hz, J_{HP} = 8.3 Hz), 3.69 (m 6 lines, CH_bP, 1H, J_{AX} = 6.6 Hz, J_{AB} = 15.7 Hz, J_{HP} = 6.6 Hz), 4.17 (m 7 lines, 1H, Pro-C^2H, J_{AX} = 2.9 Hz, J_{AY} = 8.4 Hz, J_{HP} = 5.5 Hz), 5.12 (s, 2H, CH_2 Cbz), 6.24 (b, 1H, NH), 7.19 (b, 1H, NH), 7.30-7.38 (m, 5H, arom. Cbz); ^{13}C NMR 12.3 (d, CH_3P, J_{CP} = 83.5 Hz), 24.7 (d, Pro-C^4, J_{CP} = 5.9 Hz), 26.1 (N(H)CH_3), 31.9 (d, Pro-C^3, J_{CP} = 5.9 Hz), 40.4 (d, CH_2P, J_{CP} = 98.2 Hz), 47.9 (Pro-C^5), 59.9 (Pro-C^2), 67.0 (CH_2 Cbz), 128.1, 128.4 , 136.3 (arom. Cbz), 156.6, 174.6 (C=O); ^{31}P NMR 41.8.
Low running stereoisomer (oil): R_f (CH_2Cl_2/MeOH 9/1 v/v) 0.38; ^1H NMR (300 MHz) 1.50 (d, 3H, CH_3P, J_{HP} = 13.2 Hz), 1.65-1.85 (m, 2H, Pro-C^4H_2), 1.90-2.10, 2.15-2.33 (two m, 2H, Pro-C^3H_2), 2.77 (d, 3H, N(H)C$\underline{H}_3$, J = 4.8 Hz), 3.09-3.16 (m, 1H, Pro-C^5H_a, J_{AX} =4.5 Hz, J_{AY} =7.2, J_{AB} = 8.8 Hz, J_{HP} = covered), 3.23-3.31 (m, 1H, Pro-C^5H_b), 3.55 (m 6 lines, CH_aP, 1H, J_{AX}= 5.8 Hz, J_{AB} = 15.6 Hz, J_{HP} = 5.8 Hz), 3.67 (m 6 lines, CH_bP, 1H, J_{BX} = 6.6 Hz, J_{AB} = 15.6 Hz, J_{HP} = 6.6 Hz), 4.12 (m 6 lines, 1H, Pro-C^2H, J_{AX} = 2.8 Hz, J_{AY} = 8.2 Hz, J_{HP} = 8.2 Hz), 5.09, 5.15 (two d, 2H, CH_2 Cbz, J_{AB} = 12.2 Hz),

6.28 (b, 1H, N(H)CH_3), 7.20-7.40 (m, 6H, arom. Cbz, NH); ^{13}C NMR 12.2 (d, CH_3P, J_{CP} = 85.0 Hz), 25.2 (d, Pro-C^4, J_{CP} = 5.9 Hz), 26.1 (N(H)CH_3), 31.1 (d, Pro-C^3, J_{CP} = 4.4 Hz), 39.9 (d, CH_2P, J_{CP} = 96.7 Hz), 46.9 (Pro-C^5), 61.1 (Pro-C^2), 67.1 (CH_2 Cbz), 128.1, 128.2, 128.4, 136.1 (arom. Cbz), 156.7, 173.6, (C=O); ^{31}P NMR 42.0; FAB MS 354 $(M + H)^+$.

N-[(N-Carbobenzoxyaminomethyl)methyl]phosphinoyl-prolyl-glycine-methylamide (**6d**)

The phosphinamide **6d** was prepared analogous to the synthesis of **6c** from phosphinic acid **4e** (0.184 g, 0.755 mmol) and Pro-Gly-N(H)Me[22] (0.134 g, 0.72 mmol). After stirring for 1h the mixture was evaporated and chromatographed (15 g silica gel, eluent: gradient of EtOAc/MeOH 9/1 to 85/15 v/v) to give a mixture of diastereomers as an oil (ratio 1/1 by NMR) in a total yield of 74%. R_f (CH_2Cl_2/MeOH 9/1 v/v) 0.23; The chemical shifts could not be assigned to the individual diastereomers; ^1H NMR (300 MHz) 1.50 (d, 3H, CH_3P, J_{HP} = 13.0 Hz), 1.58 (d, 3H, CH_3P, J_{HP} = 13.3 Hz), 1.78-1.98 (m, 4H, Pro-C^4H_2), 2.00-2.17 (m, 4H, Pro-C^3H_2), 2.70 (d, 6H, N(H)$C\underline{H}_3$, J = 4.7 Hz), 3.02-3.13, 3.18-3.39 (m (1H), m (3H), Pro-C^5H_2, J_{AX} = 7.4 Hz, J_{AB} = 8.6 Hz, J_{HP} = covered), 3.52, 3.55-3.73 (m 6 lines (1H), m (3H), CH_2P, J_{AX} = 6.1 Hz, J_{AB} = 15.8 Hz, J_{HP} = 6.1 Hz), 3.70, 4.03 (dd (H_a), dd (H_b), 2H, Gly-C^2H_2, J_{AX} = 7.1 Hz, J_{BX} = 7.3 Hz, J_{AB} = 17.6 Hz), 3.79, 3.95 (dd (H_a), dd (H_b), 2H, Gly-C^2H_2, J_{AX} = 5.8 Hz, J_{BX} = 6.6 Hz, J_{AB} = 16.8 Hz), 4.09-4.18 (m, 1H, Pro-C^2H, J_{AX} = 4.5 Hz, J_{AY} = 6.7 Hz, J_{HP} = covered), 4.14-4.22 (m, 1H, Pro-C^2H, J_{AX} = 4.3 Hz, J_{AY} = 7.7 Hz, J_{HP} = covered), 5.09, 5.10 (two s, 4H, CH_2 Cbz), 6.50 (m, 2H, CbzN(H)), 7.20-7.36 (m, 5H, arom. Cbz), 7.50 (q, 1H, N(H)CH_3, J = 4.7 Hz), 7.58-7.61 (m, 2H, (N(H)CH_3, N(H)Gly), 7.74 (t, 1H, N(H)Gly, J = 6.1 Hz); ^{13}C NMR 11.4 (d, CH_3P, J_{CP} = 80.6 Hz), 12.5 (d, CH_3P, J_{CP} = 83.5 Hz), 25.3, 25.5 (d, Pro-C^4, J_{CP} = 7.3 Hz), 25.8 (N(H)CH_3), 31.2 (d, Pro-C^3, J_{CP} = 4.4 Hz), 39.0 (d, CH_2P, J_{CP} = 96.7 Hz), 40.2 (d, CH_2P, J_{CP} = 95.2 Hz), 42.5 (Gly-C^2), 47.5, 47.7 (Pro-C^5), 60.2, 60.7 (Pro-C^2), 66.9 (CH_2 Cbz), 127.8, 128.2, 136.0, 136.1 (arom. Cbz), 156.5, 156.7, 169.8, 169.9, 174.1, 174.4 (C=O); ^{31}P NMR 43.5, 44.3.

N-[(N-Carbobenzoxyaminomethyl)methyl]phosphinoyl-phenylalanine-methylester (**6e**)

The phosphinamide **6e** was prepared analogous to the synthesis of **6c** from phosphinic acid **4e** (0.184 g, 0.755 mmol) and HCl.Phe-OMe (0.171 g, 0.792 mmol). A mixture of HCl.Phe-OMe and Et_3N (0.11 ml, 0.792 mmol) was coevaporated in dioxane (2x 30 mL). This mixture was suspended in CH_2Cl_2 (2 mL) and another portion of Et_3N (0.105 ml, 0.755 mmol) was added under Ar. A solution of the phosphinic chloride **5c** in CH_2Cl_2 (4 mL) was injected. When ^{31}P NMR showed complete conversion to the phosphinamide (after 30 min.), the mixture was evaporated and chromatographed (25 g silica gel, eluent: CH_2Cl_2/MeOH 98/2 v/v) to give a mixture of diasteromers as an oil (ratio 1/1) in a total yield of 85%. R_f (CH_2Cl_2/MeOH 9/1 v/v) 0.63; The chemical shifts could not be assigned to the individual diastereomers; ^1H NMR (MeOD, 300 MHz) 1.18, 1.36 (two d, 6H, CH_3P, J_{HP} = 13.9 Hz), 2.87 (m 6 lines, 2H, Bzl CH_2, J_{AX} = 8.1 Hz, J_{AB} = 13.7 Hz), 3.06 (m 6 lines, 2H, Bzl CH_2, J_{AX} = 5.9 Hz, J_{AB} = 13.9 Hz), 3.20 (m 7 lines, 2H, CH_2P, J_{AB} = 15.8 Hz, J_{HP} = 8.1 Hz), 3.45 (d, 2H, CH_2P, J_{HP} = 8.3 Hz), 3.65, 3.68 (two s, 6H, OMe), 4.04 (m 6 lines, 1H, Phe-C^2H, J_{AX} = 8.9 Hz, J_{HP} = 6.4 Hz), 4.11 (m 8 lines, 1H, Phe-C^2H, J_{AX} = 8.2 Hz, J_{AY} = 5.8 Hz, J_{HP} = 9.5 Hz), 5.08 (s, 2H, CH_2 Cbz), 5.04, 5.09 (two d, 2H, CH_2 Cbz, J_{AB} = 12.5 Hz), 7.18-7.36 (m, 20H, arom. Cbz , arom. Bzl); ^{13}C NMR 13.1 (d, CH_3P, J_{CP} = 85.0 Hz), 40.4. 40.5 (Phe-C^3), 40.5 (d, CH_2P, J_{CP} = 95.3 Hz), 40.8 (d, CH_2P, J_{CP} = 98.1 Hz), 51.9, 53.4, 53.9 (OMe, Phe-C^2), 66.6 (CH_2 Cbz), 126.6, 126.8, 127.7, 128.1, 128.2, 129.1, 129.2, 136.1, 136.3 (arom. Cbz, Bzl), 156.3, 173.4, 173.6 (C=O); ^{31}P NMR 40.6.

N-[(N-Carbobenzoxyaminomethyl)methyl]phosphinoyl-glycine-ethylester (**6f**)

The phosphinamide **6f** was prepared analogous to the synthesis of **6e** from phosphinic acid **4e** (0.131 g, 0.54 mmol) and HCl.Gly-OEt (0.078 g, 0.56 mmol). Purification by silica gel column chromatography (15 g, eluent: CH_2Cl_2/MeOH 98/2 v/v) afforded **6f** as an oil in 78% yield. R_f (CH_2Cl_2/MeOH 9/1 v/v) 0.62; ^1H NMR (300 MHz) 1.26 (t, 3H, $OCH_2C\underline{H}_3$, J = 7.2 Hz), 1.47 (d, 3H, CH_3P, J_{HP} = 13.7 Hz), 3.38, 3.62-

3.88 (m 8 lines (1H), m (3H), CH_aP, CH_bP and Gly-C^2H_2, J_{AX} = 5.3 Hz, J_{AB} = 15.4 Hz, J_{HP} = 9.0 Hz), 3.45-3.49 (b, 1H, NH), 4.18 (q, 2H, $OCH_2C\underline{H}_3$, J = 7.2 Hz), 5.12 (s, 2H, CH_2 Cbz), 6.16 (b, 1H, NH), 7.28-7.37 (m, 5H, arom. Cbz); ^{13}C NMR 13.1 (d, CH_3P, J_{CP} = 86.5 Hz), 14.0 ($OCH_2\underline{C}H_3$), 40.6 (d, CH_2P, J_{CP} = 98.2 Hz), 40.9 (Gly-C^2), 61.4 ($O\underline{C}H_2CH_3$), 66.9 (CH_2 Cbz), 127.9, 128.0, 128.3, 136.2 (arom. Cbz), 156.5, 172.0 (C=O); ^{31}P NMR 40.8.

N-[(N-Carbobenzoxyglycylaminomethyl)methyl]phosphinoyl-proline-methylamide (**7**)

To a solution of phosphinamide **6c** (R_f 0.38) (0.101 g, 0.284 mmol) in MeOH (10 mL) a catalytic amount of Pd/C (10%) was added. The mixture was stirred under H_2 atmosphere (balloon) at rt until ^{31}P NMR showed complete removal of the carbobenzoxy group (2h). After filtering the mixture over Hyflo, the filtrate was concentrated *in vacuo* to give $NH_2CH_2P(O)MeProN(H)Me$ in quantitative yield. ^1H (MeOD, 200 MHz) 1.54 (d, 3H, CH_3P, J_{HP} = 13.4 Hz), 1.80-2.24 (m, 4H, Pro-C^4H_2, Pro-C^3H_2), 2.74 (s, 3H, N(H)$C\underline{H}_3$), 2.96-3.20 (m, 4H, CH_2P, Pro-C^5H_2), 4.14 (m, 1H, Pro-C^2H); ^{13}C (MeOD) 10.9 (d, CH_3P, J_{CP} = 86.5 Hz), 26.0 (d, Pro-C^4, J_{CP} = 5.9 Hz), 26.1 (N(H)CH_3), 33.1 (d, Pro-C^3, J_{CP} = 4.4 Hz), 39.7 (d, CH_2P, J_{CP} = 98.2 Hz), 47.2 (Pro-C^5), 61.8 (Pro-C^2), 176.3 (C=O); ^{31}P (MeOD) 48.6.

Cbz-Gly-OH (62.5 mg, 0.299 mmol) was coevaporated in dioxane (2x 10 mL) and dissolved in THF (2 mL) under Ar. To the cooled solution (-10°C), N-methyl morpholine (33 µL, 0.30 mmol) and *iso*butylchloroformate (39 µL, 0.298 mmol) were added. After stirring for 5 min, a solution of $NH_2CH_2P(O)MeProN(H)Me$ in CH_2Cl_2 (6 mL) was added. When ^{31}P NMR showed complete disappearance of the amino compound (1 hr), the mixture was concentrated *in vacuo* and chromatographed (10 g silica gel, eluent: CH_2Cl_2/MeOH 9/1 v/v) to give **7** as an oil in 87% yield.

R_f (CH_2Cl_2/MeOH 9/1 v/v) 0.22; ^1H NMR (300 MHz) 1.47 (d, 3H, CH_3P, J_{HP} = 13.2 Hz), 1.78-1.89 (m, 2H, Pro-C^4H_2), 1.98-2.19 (m, 2H, Pro-C^3H_2), 2.77 (d, 3H, N(H)$C\underline{H}_3$, J = 4.8 Hz), 3.12-3.21 (m, 1H, Pro-C^5H_a), 3.24-3.32 (m, 1H, Pro-C^5H_b), 3.57 (m 6 lines, CH_aP, J_{AX} = 5.7 Hz, J_{AB} = 15.4 Hz, J_{HP} = 5.7 Hz), 3.74 (m 5 lines, CH_bP, J_{AX}= 7.4 Hz, J_{AB} = 15.4 Hz, J_{HP} = 7.4 Hz), 3.88, 3.95 (two dd, Gly-C^2H_2, J_{AX} = 5.7 Hz, J_{BX} = 5.7 Hz, J_{AB}= 16.9 Hz), 4.15 (m 6 lines, 1H, Pro-C^2H, J_{AX} = 3.1 Hz, J_{AY} = 7.9 Hz, J_{HP} = 7.9 Hz), 5.10 (s, 2H, CH_2 Cbz), 6.22 (t, 1H, N($\underline{H}$)CH_2P, J = 5.3 Hz), 7.25-7.34 (m, 6H, arom. Cbz, N($\underline{H}$)CH_3), 8.15 (t, 1H, Gly-N(H), J = 5.7 Hz); ^{13}C NMR 12.2 (d, CH_3P, J_{CP} = 85.0 Hz), 25.2 (d, Pro-C^4, J_{CP} = 5.9 Hz), 26.1 (N(H)CH_3), 31.6 (d, Pro-C^3, J_{CP} = 4.4 Hz), 38.1 (d, CH_2P, J_{CP} = 95.3 Hz), 44.2 (Gly-C^2), 46.9 (Pro-C^5), 61.0 (Pro-C^2), 66.9 (CH_2 Cbz), 128.0, 128.1, 128.4, 136.1 (C arom. Cbz), 156.6, 170.1, 173.9, (C=O); ^{31}P NMR 42.3; FABMS m/z 411 $(M+H)^+$.

Hydrolytic stability of phosphinamide (**6c**)

Phosphinamide **6c** (approximately 20 mg) was dissolved in ca. 1 mL aqueous 0.05 M NaOAc/HOAc buffer containing 20% DMSO with pH values of 3.4, 4.0, 5.0, 6.0 and 7.6. Initially, every other hour a ^{31}P NMR was recorded, however during the experiment the interval between a ^{31}P NMR measurement was adjusted to approximately 1/10 of $t_{1/2}$. The ratio of the phosphinamide, resonating at 48.1 and 48.6 ppm to the formed phosphinic acid, resonating at 38.1 ppm was determined by integration. The $t_{1/2}$ values were obtained by extrapolation and were found to be respectively 1d, 3.5 d, 25 d, 126 d and ∞.

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