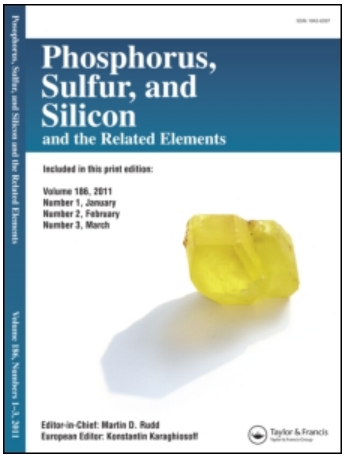

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SYNTHESIS OF N-PHOSPHORYLATED AMINOACIDS

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SYNTHESIS OF N-PHOSPHORYLATED AMINOACIDS

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Dedicated to Professor Reinhard Schmutzler on the occasion of his 60th birthday

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N-Phosphorylated α-aminoacids have been synthesized by the reaction of organophosphoryl chlorides with O,N-bis(trimethylsilylated) derivatives of the corresponding aminoacids.

Key words: N-Phosphorylated aminoacids, synthesis, O-silylated intermediates, NMR.

INTRODUCTION

One of the potential synthetic routes to prepare N-phosphorylated peptides embraces the preparation of N-phosphorylated aminoacids.[1] It may be accomplished using the Todd-Atherton reaction of dialkylphosphites with α-aminoacids (Figure 1).[2] Some limitations of this method are worthy of mention. Thus, as to dialkylphosphites, satisfactory results have been attained for diisopropylphosphite and dibutylphosphite only,[2,3] whereas under the conditions of the Todd-Atherton reaction, dimethylphosphite gives rise to a complex mixture of compounds.[2] In addition, the racemization of the aminoacid or its N-phosphorylated derivative can be provoked by the use of excess free base (3–4 moles of triethylamine per 1 mole of aminoacid).[4]

This paper reports the synthesis of N-phosphorylated α-aminoacids via their N,O-bis(trimethylsilylated) derivatives, following the method developed earlier[5,6] for the synthesis of α-acylaminocarboxylic[5] and phosphonic acids.[6] Additionally, we focused our attention on the study of NMR-spectra of these phosphoaminoacids.

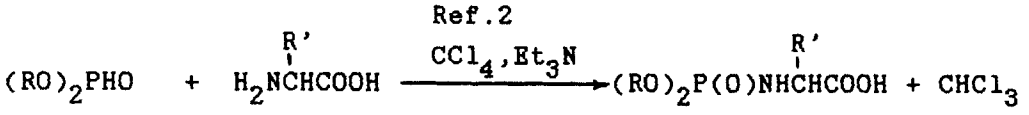
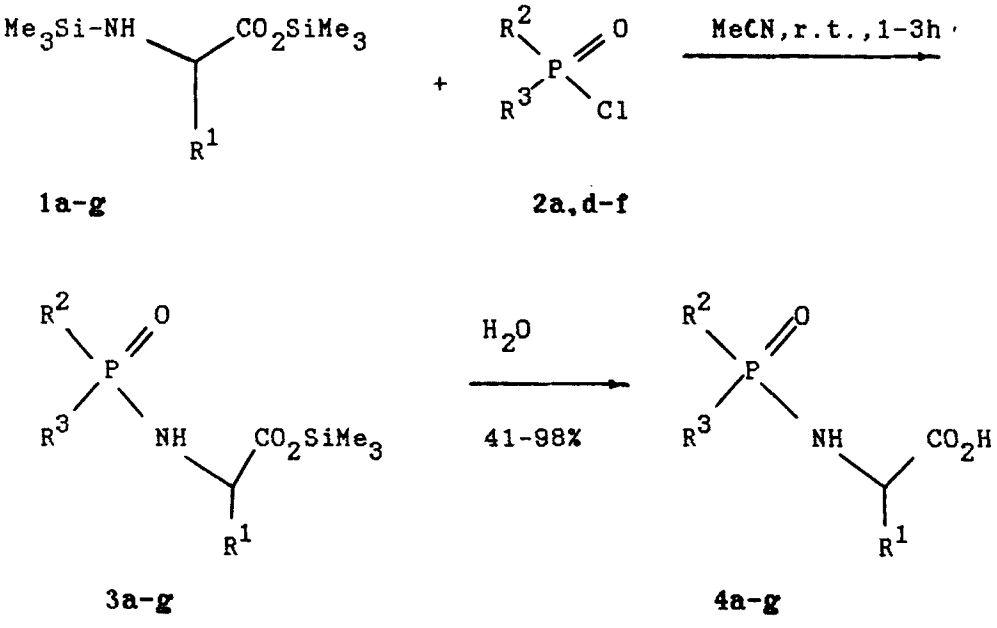


FIGURE 1

RESULTS AND DISCUSSION

N,O-Bis(trimethylsilylated) α-aminoacids **1a–c**, prepared according to the literature,[7] were reacted with dialkylphosphorochloridates **2a,d**, phosphonic chloride **2e** or diphenylphosphinic chloride **2f** at ambient temperature in acetonitrile for 1–3 hours to give trimethylsilyl esters of N-phosphorylated aminoacids (**3a–g**; Scheme 1). All compounds **3** are sensitive to atmospheric moisture. Compound **3a** has been isolated analytically pure, its structure being characterized by ^{1}H and ^{31}P-NMR

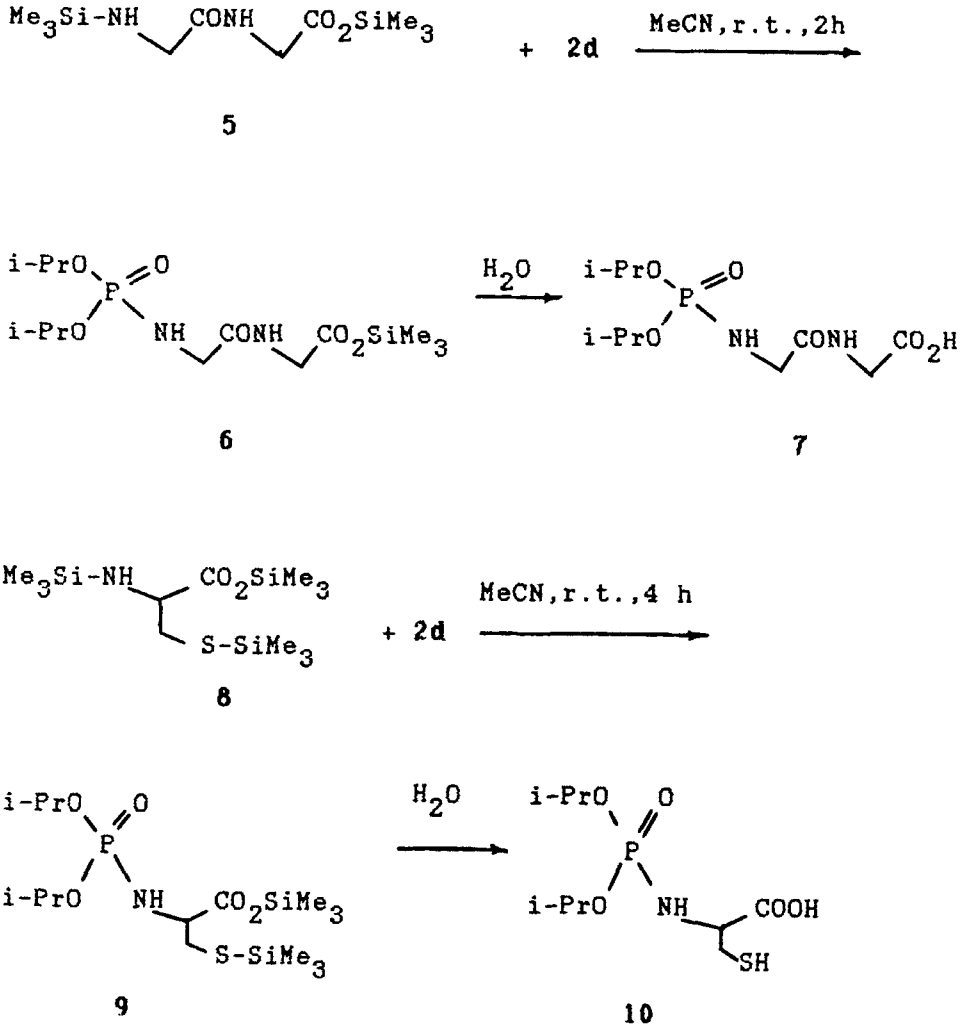


1-4	R^1	R^2	R^3
a	H	EtO	EtO
b	Me	EtO	EtO
c	i-Pr	EtO	EtO
d	Me	i-PrO	i-PrO
e	H	Me	EtO
f	Me	Ph	Ph
g	i-Pr	Ph	Ph

SCHEME 1 Synthesis of N-phosphorylated aminoacids and their intermediate silylic esters.

spectral data (see Experimental). Ester **3a** can be purified by distillation in vacuo[8]; compound **3e** was decomposed when distilled. Compounds **3b–d** and **3f,g** were not isolated from the reaction mixtures, but were hydrolyzed directly under mild conditions to give **4b–d,f,g**. Similarly, the compounds **3a,e** have been hydrolyzed, without their preliminary isolation, to provide **4a,e**. It should be pointed out that only volatile side-products are formed in both steps; thus, the purification of **4a–g** is quite easy. The yields of acids **4a–d,g** are high (81–98%), those of **4e,f** are moderate (41 and 46%). The structures of **4a–g** have been determined by NMR ^1H and ^{31}P-spectral data and microanalysis. Phosphorylamidophosphate **4d** has been prepared by the literature procedure[2]; both specimens had identical characteristics.

The phosphorylation of N-silylated derivatives is also a useful method for the



SCHEME 2 Synthesis of phosphorylated dipeptide **7** and cystein **10**.

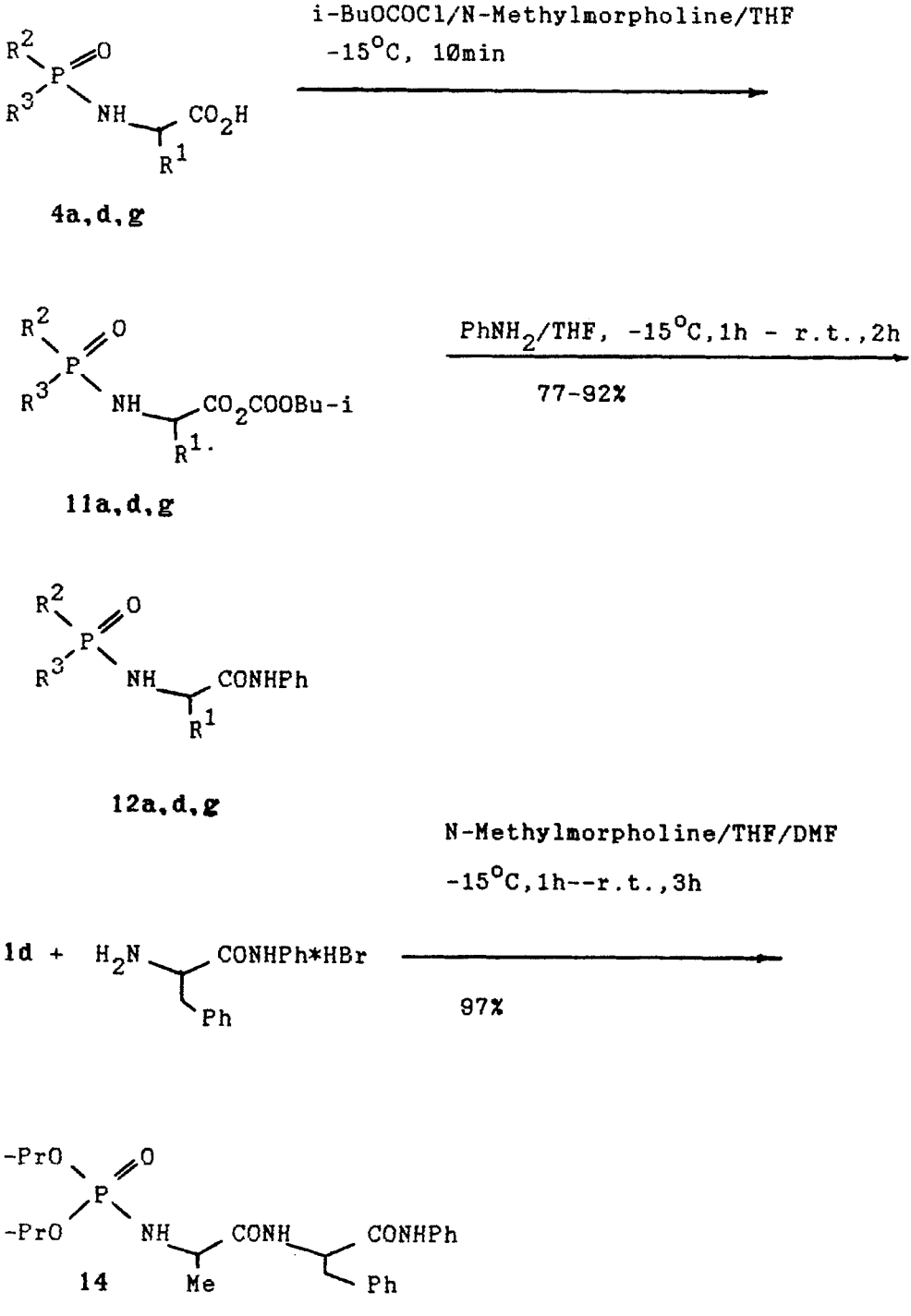
synthesis of N-phosphorylated dipeptides (**7**) or α-aminoacids, containing side-chain functional groups (**10**) (Scheme 2).

However, one limitation should be mentioned. When **2f** is used as the phosphorylating agent, the rates of N- and O-phosphorylation are comparable, which is reflected by noticeable amounts of N-phosphorylated dipeptides formed by the interaction of **1b,c** with intermediate mixed anhydrides. The yields of dipeptides, characterized as anilides, are relatively high (see Experimental).

The ^{1}H-NMR spectra of compounds **4b–d** are characterized by the double magnetic nonequivalence[9,10] of the protons of alkoxy-groups at phosphorus. Two factors influence the multiplicity of methylenic protons of one CH_2O group in **4b–c** and every methyl group of isopropoxy-radicals of **4d**: the asymmetric center on the α-carbon atom of the amino-acid residue, which causes diastereotopy of alkoxy-groups, and conformational differences of groups. Compound **4b** has identical constants $^3J_{CH_3CH_2}$ and $^3J_{POCH_2}$; that result in quintet for one CH_2O-group and typical AB-system for the other one (^2J = 10 Hz). Two clear doublets (δ 1.27 and 1.24 ppm) can be observed for methyl groups of one isopropoxy radical of **4c**, as to the other radical, the diminished chemical shift difference between doublet centers can hardly be registered, the resolution of NMR-spectrometer being taken into consideration.

The magnetic non-equivalence of alkoxy-protons[9,10] in ^{1}H-NMR spectra of **4b,d** (see Experimental) being connected with the conformational flexibility of the alkoxy-group at phosphorus, the synthesis of some derivatives of **4** seemed reasonable to study the influence of such a derivatisation of structure on their spectral characteristics. Since some of the N-phosphorylated aminoacids **4** decomposed rapidly under storage, the stable anilides **12** have been synthesized via their mixed anhydrides[11] (Scheme 3). As to compound **14**, prepared under conditions similar to that used for the synthesis of **12**, the unique signal δ = 5.3 ppm in its ^{31}P-NMR spectrum clearly shows the absence of essential racemization in the course of N-phosphorylation.

The "double magnetic non-equivalence" mentioned above for **4d** disappears in ^{1}H-NMR spectrum of **12d**, non-equivalence of CH_3-groups caused by the chiral carbon atom being diminished to 0.015 ppm as compared with **4d** ($\Delta\delta$ = 0.032 ppm) at the same time. The reduced association of **12d** in solution as well as the enhanced conformational flexibility of its isopropoxy groups as compared with **4d** may be responsible for this effect. On the contrary, the second amino-acid fragment insertion results in the magnetic non-equivalence of all four methyl groups in isopropoxy-radicals of **14**. It may be associated with the more rigid conformation of **14** as compared to **12d** in view of intra- or intermolecular associative bonds. It is confirmed by the increased (0.119 ppm) chemical shift difference for methine protons of the isopropoxy groups of **14**, this value being equal to 0.046 ppm for **4d** and negligible for **12d**. The CH_3-decoupled signal of one methine proton turns out to be a doublet with $^3J_{POCH}$ = 7.6 Hz, but the components of the second methine proton doublet remain split with $^5J_{NHPOCH}$ = 1.5 Hz. The long distance coupling occurrence may be explained by the planar structure of C—O—P—N-fragment reported elsewhere[12] for the crystal structure of **4d**. Such a spatial orientation of one isopropoxy group may limit the conformational flexibility of the molecule.



SCHEME 3 Synthesis of N-phosphorylated aminoacids anilides.

CONCLUSION

The reaction of O,N-bis(trimethylsilylated) derivatives of aminoacids with organophosphorylchlorides could be used for preparation of N-phosphorylated aminoacids and their derivatives, the structure of starting materials being taken into consideration.

EXPERIMENTAL

Methods. ^{1}H-NMR and ^{31}P-NMR-spectra were recorded on a Bruker WP-200 spectrometer (200/81 MHz); chemical shifts of ^{1}H-NMR are reported in parts per million relative to internal hexamethyldisiloxane (0.00 ppm). 1% H_3PO_4 was used as external standard for ^{31}P-NMR spectra. IR spectra were measured on a UR 20 spectrometer. Observed rotations at the Na-D line were obtained using a Polamat A polarimeter. The reactions were monitored and the purity of compounds was checked by thin-layer chromatography, using analytical TLC plates (Silufol UV_{254}), purchased by Sklo Union (CSFR). Melting points were determined on a Boetius micro-mp apparatus and are not corrected. Elemental analyses were performed by the Department of Analytical Chemistry.

Preparation. All reagents were distilled freshly. Reagent grade solvents were purified before use. Acetonitrile was distilled over P_2O_5.

The preparation of silylated amino-acids **1a–c** and **8** was carried out according to the literature,[7,13] their structure being confirmed by ^{1}H-NMR spectroscopy. Starting materials **2** were synthesized as previously[14–17] described. All physical constants and spectroscopic data of compounds agree with the literature values.

Preparation of N,O-Bis(trimethylsilyl)glycyl-glycine, **5**. Modified Procedure.[13] To a vigorously stirred suspension of glycyl-glycine (0.76 g, 5.7 mmol) in acetonitrile (4 mL) was added trimethylsilyldiethylamine (2.17 g, 14.9 mmol). The mixture was stirred for 2 hrs until the dissolution is completed. The solvent was removed in vacuo to give **5** (1.61 g, 100%) as a colorless residue. ^{1}H-NMR ($CDCl_3$, HMDS): 0.07 (s, 9H, Me_3SiN); 0.29 (s, 9H, Me_3SiO); 0.91 (t, 1H, J = 9.0 Hz, NHSi); 3.36 (d, 2H, J = 9.0 Hz, CH_2CON); 4.02 (d, 2H, J = 5.4 Hz, CH_2COO); 7.50 (t, 1H, J = 5.4 Hz, CONH). The so obtained **5** was used directly for further reaction with **2d**.

N-(Diethoxyphosphoryl)-O-trimethylsilylglycine, **3a**. To the solution of **1a** (0.696 g, 3.17 mmol) in acetonitrile (1.5 mL) was added dropwise phosphorochloridate **2a** (0.546 g, 3.17 mmol) in acetonitrile (2 mL). The mixture was stirred at 20°C for 0.5 h, then allowed to stand for 2.5 hrs and evaporated at 40°C under reduced pressure to afford **3a** as colorless thick syrup. Pure **3a** (0.720 g, 80%) may be obtained by direct distillation of the crude product under protection from traces of moisture, b.p. 140–143°C/0.1 Torr. ^{1}H-NMR ($CDCl_3$/HMDS): 0.20 (s, 9H, Me_3Si), 1.21 (t, 6H, J = 7.0 Hz, $CH_2\underline{CH_3}$), 3.36 (dt, 1H, J = 10.6, 6.2 Hz, NH), 3.57 (dd, 2H, J_{PNCH_2} = 9.8 Hz, CH_2CO), 3.98 (pseudo q, 4H, J_{POCH_2} = 7.2 Hz, $\underline{CH_2}CH_3$). ^{31}P-NMR ($CDCl_3$): δ = 7.9 ppm. Anal. Calcd. for $C_9H_{22}NO_5PSi$ (mol. weight 283.4), C, 38.15; H, 7.83; P, 10.93; Si, 9.91. Found: C, 38.12; H, 7.79; P, 11.10; Si, 10.34.

N-(O-Ethylmethylphosphonyl)-O-trimethylsilylglycine, **3e**. The workup was carried out by the procedure, described for ester **3a**. The residual colorless oil was allowed to stand for 1h at 40°C/0.1 Torr to afford crude **3e** of more than 97% purity, suitable for further reaction. ^{1}H-NMR ($CDCl_3$/HMDS): 0.28 (s, 9H, Me_3Si), 1.27 (t, 3H, J = 7.0 Hz, $CH_2\underline{CH_3}$), 1.47 (d, 3H, J = 17.0 Hz, CH_3P), 3.64–3.83 (m, 2H, CH_2N), 3.96–4.10 (m, 3H, $\underline{CH_2}CH_3$, NH). ^{31}P NMR (CH_3CN): δ = 33.6 ppm. Anal. Calcd. for $C_8H_{20}NO_4PSi$ (mol. weight 252.3), C, 37.93; H, 7.96; P, 12.23; Si, 11.09. Found: C, 37.42; H, 7.82; P, 12.14; Si, 9.97.

The attempt to purify crude **3e** by distillation (123–125°C/0.1 Torr) failed because of its rapid decomposition (see also **4e**). Due to high lability of **3e** its spectra include signals of corresponding acid **4e**.

N-(Diethoxyphosphoryl)glycine, **4a**. Typical procedure for water-soluble N-phosphorylated aminoacids. The reaction of **1a** (1.59 g, 7.3 mmol) and **2a** (1.14 g, 6.6 mmol) was carried out by the procedure described for **3a**. After removal of the solvent with a rotary evaporator, crude **3a** was chromatographed on a short silica gel column, eluted sequentially with neat $CHCl_3$ and $CHCl_3$/EtOH mixtures, to give **4a** (1.37 g, 98%) as the thick colorless oil, which readily crystallized on cooling in the refrigerator, mp

36–38°C. ^{1}H NMR ($CDCl_3$/HMDS): 1.30 (t, 6H, J = 7.1 Hz, $2CH_3$), 3.64 (d, 2H, J = 4.7 Hz, $NHCH_2$), 4.05 (m, 4H, J_1 = 7.1 Hz, J_2 = 8.0 Hz, CH_2O), 5.42 (br s, 1H, NH), 9.98 (br s, 1H, COOH). ^{31}P NMR ($CDCl_3$): δ = 7.7 ppm. Anal. Calcd. for $C_6H_{14}NO_5P$ (mol. weight 211.1), C, 34.13; H, 6.68; N, 6.63; P, 14.67. Found: C, 34.41; H, 6.59; N, 6.50; P, 14.62.

N-(O-Ethylmethylphosphonyl)glycline, **4e**. The reaction of **1a** (0.46 g, 2.1 mmol) in acetonitrile (2.5 mL) and **2e** (0.30 g, 2.1 mmol) in acetonitrile (1 mL) was carried out by the procedure described for **3e**. After removal of the solvent under reduced pressure crude **3e** was flash-chromatographed immediately on a short silica gel column, eluted sequentially with neat $CHCl_3$ and $CHCl_3$/EtOH mixtures to afford **4e** (0.16 g, 41%) as a white solid. Recrystallisation from ethyl ether/ethanol gave pure **4e**, mp 100–101°C. ^{1}H NMR ($CDCl_3$/HMDS): 1.24 (t, 3H, J = 7.1 Hz, CH_3CH_2), 1.42 (d, 3H, J = 16.8 Hz, CH_3P), 3.55 (unresolved dd, 2H, CH_2), 3.82–4.07 (m, 2H, CH_3CH_2), 5.11 (br s, 1H, NH), 11.04 (br s, 1H, COOH). ^{31}P NMR ($CDCl_3$): δ = 35.5 ppm. Anal. Calcd. for $C_5H_{12}NO_4P$ (mol. weight 181.1), C, 33.15; H, 6.67; P, 17.10. Found: C, 33.27; H, 6.78; P, 17.34. Prolonged storage of non-purified acid **4e** is accompanied by its decomposition.

N-(Diethoxyphosphoryl)aminoacids, **4b,c**. General Procedure. To a solution of **1b,c** (3 mmol) in acetonitrile (3 mL) compound **2a** (3 mmol) was added and the mixture was maintained at room temperature for 1–3 hrs. After removal of the solvent by evaporation in vacuo the residue was dissolved in 5 mL of dry ether and 3–4 drops of water were added. The mixture was evaporated in vacuo, and then the residue was dissolved in 20 mL $CHCl_3$ and washed with 10 mL of 10% aqueous solution of citric acid. The aqueous layer was extracted with chloroform (2 × 20 mL), the combined organic layers were dried over anhydrous Na_2SO_4. The solvent was evaporated under reduced pressure to afford **4b** (83%) as a crystalline product, mp 74–76°C ($CHCl_3$-hexane), and **4c** (86%) as a viscous oil, their purity being confirmed by TLC (EtOH/BuOH/NH_4OH/H_2O, 4:4:4:1).

N-(Diethoxyphosphoryl)-L-alanine, **4b**. ^{1}H NMR ($CDCl_3$/HMDS): 1.29 (t, 3H, J = 7 Hz, CH_3CH_2), 1.32 (t, 3H, J = 7 Hz, CH_3CH_2), 1.43 (d, 3H, J = 7 Hz, CH_3CH), 3.83 (m, 1H, J_1 = 7 Hz, J_2 = 7.7 Hz, J_3 = 7.2 Hz, NHCH), 4.03 (m, 1H, J_1 = 7 Hz, J_2 = 7.1 Hz, J_3 = 10.1 Hz, CHO), 4.10 (m, 1H, J_1 = 7 Hz, J_2 = 7.1 Hz, J_3 = 10.1 Hz, CHO), 4.04 (dq, 2H, J_1 = 7 Hz, J_2 = 7.1 Hz, CH_3CH_2O), 4.98 (dd, 1H, J_1 = 7.7 Hz, J_2 = 12.5 Hz, NH), 9.57 (br s, 1H, COOH). ^{31}P NMR ($CDCl_3$): δ = 7.9 ppm. Anal. Calcd. for $C_7H_{16}NO_5P \times 0.5\ H_2O$ (mol. weight 225.2), C, 35.90; H, 7.32; N, 5.98; P, 13.23. Found: C, 36.09, H, 7.17; N, 6.13; P, 13.46.

N-(Diethoxyphosphoryl)-D-valine, **4c**. ^{1}H NMR ($CDCl_3$/HMDS): 0.81 (d, 3H, J = 6.8 Hz, CH_3CH), 0.89 (d, 3H, J = 6.8 Hz, CH_3CH), 1.18 (t, 3H, J = 7 Hz, CH_3CH_2), 1.20 (t, 3H, J = 7 Hz, CH_3CH_2), 2.00 (m, 1H, J_1 = 6.8 Hz, J_2 = 4.6 Hz, CH_3CHCH_3), 3.51 (m, 1H, J_1 = 4.6 Hz, J_2 = 10.4 Hz, J_3 = 10.4 Hz, NHCH), 3.81 (dd, 1H, J_1 = 10.4 Hz, J_2 = 10.4 Hz, NH), 3.95 (m, 1H, J_1 = 7 Hz, J_2 = 7 Hz, J_3 = 10.2 Hz, CHO), 3.98 (m, 1H, J_1 = 7 Hz, J_2 = 7 Hz, J_3 = 10.2 Hz, CHO), 3.96 (m, 2H, J_1 = 7 Hz, J_2 = 7 Hz, CH_2O), 11.47 (br s, 1H, COOH). ^{31}P NMR ($CDCl_3$): δ = 8.5 ppm. Anal. Calcd. for $C_9H_{20}NO_5P$ (mol. weight 253.2), C, 42.69; H, 7.96; P, 12.23. Found: C, 42.20; H, 7.88; P, 11.85.

N-(Diisopropoxyphosphoryl)-L-alanine, **4d**. *Method a*: To a solution of **2d** (1.04 g, 5.2 mmol) in acetonitrile (6 mL) was added **1b** (1.61 g, 6.9 mmol), and the mixture was maintained at room temperature for 1 h. After removal of the solvent under reduced pressure, the residue was treated as described for **4b,c** to afford crude **4d**. Recrystallisation from chloroform/hexane gave pure **4d** (1.16 g, 88%), mp 118–119°C, $[\alpha]_D^{25}$ −6° ± 0.2° (c = 5.0, C_2H_5OH) (lit.[2] mp 110–2°C, $[\alpha]_D^{14}$ −7° (c = 1, C_2H_5OH)). ^{1}H NMR ($CDCl_3$/HMDS): 1.24 (d, 3H, J = 6.5 Hz, CH_3CHCH_3), 1.27 (d, 3H, J = 6.4 Hz, CH_3CHCH_3), 1.29 (d, 6H, J = 6.2 Hz, CH_3CHCH_3), 1.40 (d, 3H, J = 7 Hz, CH_3CHNH), 3.80 (m, 1H, J_1 = 7 Hz, J_2 = 8 Hz, J_3 = 7.5 Hz, NHCH), 4.54 (m, 1H, J_1 = 6.5 Hz, J_2 = 7.5 Hz, CHO), 4.58 (m, 1H, J_1 = 6.2 Hz, J_2 = 7.5 Hz, CHO), 4.73 (dd, 1H, J_1 = 8 Hz, J_2 = 12.2 Hz, NH), 11.31 (br s, 1H, COOH). ^{31}P NMR ($CDCl_3$): δ = 6.1 ppm. Anal. Calcd. for $C_9H_{20}NO_5P$ (mol. weight 253.2), C, 42.69; H, 7.96; N, 5.53; P, 12.23. Found: C, 42.58; H, 8.05; N, 5.50; P, 12.38.

Method b: The reaction of diisopropylphosphite (1.66 g, 10 mmol) and **1b** (0.89 g, 10 mmol) was accomplished as described earlier[2] to afford crude **4d** (2.29 g, 90%). The crude **4d** was purified by crystallisation ($CHCl_3$-hexane) to give authentic **4d**, mp 104–107°C.

N-(Diphenylphosphinyl)-D-alanine, **4f**. To solution of **1b** (D-isomer, 1.21 g, 5.2 mmol) in acetonitrile (3.0 mL) was added a solution of **2f** (1.12 g, 4.7 mmol) in acetonitrile (2.0 mL). The solution was immediately evaporated, the residual light-yellow oil was dissolved in 40 mL of EtOAc, washed with 20 mL of water, 10% aqueous solution of citric acid (2 × 20 mL), 20 ml of water and 20 mL of brine.

The organic layer was dried over anhydrous Na_2SO_4, filtered and evaporated under reduced pressure. The residual white glass was purified by chromatography on a silica gel column, eluted with mixture of hexane/$CHCl_3$/EtOH (50:50:4), to give white solid. Recrystallisation from chloroform/hexane gave pure **4f** (0.57 g, 46%), mp 141–143°C, $[\alpha]_D^{21}$ +20.3 ± 1.5° (c = 1.03, MeOH) (lit.[18] for L-isomer of **4f** $[\alpha]_D^{25}$ −21.4° (c = 1.0, MeOH). ^{1}H NMR ($CDCl_3$/HMDS): 1.37 (d, 3H, J = 6.8 Hz, CH_3), 3.96 (dd, 1H, J_1 = 5.6 Hz, J_2 = 10.4 Hz, NH), 3.81 (m, 1H, J_1 = 6.8 Hz, J_2 = 10.4 Hz, J_3 = 10.3 Hz, NHC<u>H</u>), 7.35–7.96 (m, 10 H_{arom}), 12.61 (br s, 1H, COOH). ^{31}P NMR ($CDCl_3$): δ = 26.5 ppm. Anal. Calcd. for $C_8H_{16}NO_3P$ (mol. weight 289.3), C, 62.28; H, 5.57; N, 4.84; P, 10.71. Found: C, 62.01; H, 5.64; N, 4.61; P, 10.58.

N-(Diphenylphosphinyl)-D-valine, **4g**. The reaction of **1c** (1.69 g, 6.5 mmol) and **2f** (1.44 g, 6.1 mmol) was carried out by the procedure described for **4f**, to afford **4g** (1.54 g, 81%), as a white glass, $[\alpha]_D^{20}$ +22.8 ± 1.4° (c = 1.14, MeOH) (lit.[18] for L-isomer of **4g** $[\alpha]_D^{25}$ −15.2 (c = 1.0, MeOH)). ^{1}H NMR ($CDCl_3$/HMDS): 0.89 (d, 3H, J = 6.8 Hz, CH_3), 0.92 (d, 3H, J = 6.8 Hz, CH_3), 2.06 (m, 1H, J = 6.8 Hz, CH), 3.47–3.81 (m, 2H, NHCH), 7.43–7.95 (m, 10H_{arom}), 11.16 (br s, 1H, COOH). ^{31}P NMR ($CDCl_3$): δ = 27.1 ppm. Anal. Calcd. for $C_{17}H_{20}NO_3P$ (mol. weight 317.3), C, 64.35; H, 6.35; N, 4.41; P, 9.76. Found: C, 64.39; H, 6.38; N, 4.18; P, 9.39.

N-(Diisopropoxyphosphoryl)glycyl-glycine, **7** *and N-(diisopropoxyphosphoryl)-L-cysteine*, **10**. The reaction of **5** (1.61 g, 5.7 mmol) and **2d** (1.08 g, 5.4 mmol), or **8** (1.37 g, 4.1 mmol) and **2d** (0.77 g, 3.8 mmol), was carried out by the procedure described for **4a**, to give **7** (97%) and **10** (87%).

Compound **7**: mp 111–113°C ($CHCl_3$/hexane). ^{1}H NMR ($CDCl_3$/HMDS): 1.29 (d, 6H, J = 5.9 Hz, 2CH_3), 1.31 (d, 6H, J = 5.9 Hz, 2 CH_3), 3.62 (d, 2H, J = 11.1 Hz, CH_2NP), 4.12 (d, 2H, J = 4.9 Hz, CH_2NCO), 4.35 (br s, 1H, NHP), 4.58 (m, 2H, J_1 = 5.9 Hz, J_2 = 8.2 Hz, CHO), 7.30 (br t, 1H, J = 4.9 Hz, CONHC<u>H</u>$_2$), 10.43 (br s, 1H, COOH). ^{31}P NMR ($CDCl_3$): δ = 6.2 ppm. Anal. Calcd. for $C_{10}H_{21}N_2O_6P$ (mol. weight 296.3), C, 40.54; H, 7.14; N, 9.46; P, 10.46. Found: C, 40.40; H, 7.34; N, 9.30; P, 10.43.

Metastable form of **7**, mp 81–83°C, was also isolated (lit[19] mp 73.5–74.5°C).

Compound **10**: oil (lit.[20] oil). ^{1}H NMR ($CDCl_3$/HMDS): 1.28 (d, 3H, J = 6.2 Hz, CH_3), 1.31 (d, 3H, J = 6.2 Hz, CH_3), 1.32 (d, 3H, J = 6.2 Hz, CH_3), 1.33 (d, 3H, J = 6.2 Hz, CH_3), 1.63 (m, 1H, J_1 = 7.7 Hz, J_2 = 9.7 Hz, SH), 2.89 (m, 1H, J_1 = 7.7 Hz, J_2 = 5 Hz, J_3 = 13.6 Hz, CHS), 2.95 (m, 1H, J_1 = 9.7 Hz, J_2 = 3.5 Hz, J_3 = 13.6 Hz, CHS), 4.09 (m, 1H, J_1 = 5 Hz, J_2 = 3.5 Hz, J_3 = 8.6 Hz, J_4 = 8.2 Hz, NHC<u>H</u>), 4.54 (dd, 1H, J_1 = 8.6 Hz, J_2 = 11.8 Hz, NH), 4.60 (m, 1H, J_1 = 6.2 Hz, J_2 = 7 Hz, CHO), 4.64 (m, 1H, J_1 = 6.2 Hz, J_2 = 7 Hz, CHO), 10.27 (br s, 1H, COOH). ^{31}P NMR ($CDCl_3$): δ = 5.8 ppm.

The compound **7** was obtained also by the literature[2] method, yield 76%.

N-(Diisopropoxyphosphoryl)glycyl-glycine anilide. To a solution of **7** (1.20 g, 4.05 mmol) and aniline (0.38 g, 4.05 mmol) in dry methylene chloride (20 mL) was added a 1.02 M solution of DCC in methylene chloride (4.05 mL, 4.13 mmol) at −20°C. The mixture was placed immediately in the refrigerator (0°C) and maintained at this temperature for 2 hrs. Then the reaction mixture was stirred at room temperature for 4 hrs. Upon addition of 5 drops of acetic acid, the precipitated dicyclohexylurea was removed by filtration and the solution was evaporated in vacuo. The residue was dissolved in EtOAc (20 mL), washed with 10 mL of 10% aqueous solution of citric acid, 10 mL of water, 10 mL of 10% aqueous $NaHCO_3$ and 10 mL of brine. The organic layer was dried over anhydrous Na_2SO_4, filtered and evaporated in vacuo to afford crude anilide. Recrystallisation from chloroform/hexane gave N-(diisopropoxyphosphoryl)glycyl-glycine anilide (1.08 g, 72%), as colorless solid, mp 118–120°C. ^{1}H-NMR ($CDCl_3$/HMDS): 1.24 (d, 6H, J = 6.1 Hz, C<u>H</u>$_3$CHCH$_3$), 1.26 (d, 6H, J = 6.1 Hz, CH_3CHCH_3), 3.63 (d, 2H, J = 12.4 Hz, CH_2NP), 4.10 (d, 2H, J = 5.5 Hz, CH_2NC), 4.24 (br s, 1H, NHP), 4.56 (m, 2H, J_1 = 6.1 Hz, J_2 = 7.4 Hz, CHO), 7.03 (t, 1H$_{arom}$, J = 7.3 Hz), 7.23 (dd, 2H$_{arom}$, J = 7.3 Hz, J_2 = 7.9 Hz), 7.53 (d, 2H$_{arom}$, J = 7.9 Hz), 7.90 (t, 1H, J = 5.5 Hz, CONHCH$_2$), 9.29 (s, 1H, NHPh). ^{31}P-NMR ($CDCl_3$): δ = 6.5 ppm. Anal. Calcd. for $C_{16}H_{26}N_3O_5P$ (mol. weight 371.4), C, 51.75; H, 7.06; N, 11.31; P, 8.34. Found: C, 51.49; H, 7.02; N, 11.23; P, 8.21.

N-(Diisopropoxyphosphoryl)-L-alanine anilide, **12d**. A solution of isobutylchloroformate (0.27 g, 2 mmol) in tetrahydrofurane (6 mL) was added dropwise to a solution of **4d** (0.51 g, 2 mmol) and N-methylmorpholine (0.20 g, 2 mmol) in tetrahydrofurane (11 mL) at −15°C under stirring. The mixture was stirred for 10 min at the same temperature, then the solution of aniline (0.19 g, 2 mmol) in tetrahydrofurane (3 mL) was added. Stirring was continued for 1 h at −15°C and 2 hrs at room temperature. An equal volume of water was added, the resulting mixture was concentrated, the precipitate was collected by filtration, then dissolved in $CHCl_3$, washed with aqueous citric acid, saturated aqueous $NaHCO_3$, water and dried over anhydrous Na_2SO_4. Removal of the solvent afforded solid crude **12d**; recrystallisation from chloroform/hexane gave pure **12d** (0.5 g, 77%), mp 151–152.5°C,

$[\alpha]_D^{20.5}$ −61,4 ± 0,7° (c = 1.0, $CHCl_3$). ^{1}H NMR ($CDCl_3$/HMDS): 1.28 (d, 9H, J = 6 Hz, $C\underline{H}_3CHC\underline{H}_3$), 1.30 (d, 3H, J = 6 Hz, $C\underline{H}_3CHCH_3$), 1.47 (d, 3H, J = 6.5 Hz, $C\underline{H}_3CHNH$), 3.84 (m, 1H, J_1 = 9.7 Hz,[21] J_2 = 10 Hz,[21] $N\underline{H}CH$), 4.02 (m, J_1 = 6.5 Hz, J_2 = 10 Hz,[21] J_3 = 10.8 Hz,[21] $NHC\underline{H}$), 4.60 (m, 2H, J_1 = 6 Hz, J_2 = 7.5 Hz,[21] CHO), 7.06 (t, $1H_{arom}$, J = 7.4 Hz), 7.28 (dd, $2H_{arom}$, J_1 = 7.4 Hz, J_2 = 7.8 Hz), 7.61 (d, $2H_{arom}$, J = 7.8 Hz), 9.27 (br s, 1H, $N\underline{H}Ph$). ^{31}P NMR ($CDCl_3$): δ = 5.5 ppm. Anal. Calcd. for $C_{15}H_{25}N_2O_4P$ (mol. weight 328.4), C 54.87; H, 7.67; N, 8.53; P, 9.43. Found: C, 54.63; H, 7.46; N, 8.44; P, 9.39.

N-(Diethoxyphosphoryl)glycine anilide, **12a**. The reaction of **4a** (0.94 g, 4.46 mmol) and isobutylchloroformate (0.58 g, 4.24 mmol) in the presence of N-methylmorpholine (0.45 g, 4.46 mmol), and with aniline (0.39 g, 4.24 mmol) was carried out in the same manner as for **12d**. Recrystallisation from chloroform/hexane gave **12a** (1.11 g, 92%), mp 93–94°C. ^{1}H NMR ($CDCl_3$/HMDS): 1.31 (t, 6H, J = 7.1 Hz, CH_3), 3.78 (d, 2H, J = 12.1 Hz, $NHC\underline{H}_2$), 4.00 (br s, 1H, NHP), 4.09 (m, 4H, J_1 = 7.1 Hz, J_2 = 7.8 Hz, CH_2O), 7.09 (t, $1H_{arom}$, J = 7.4 Hz), 7.30 (dd, $2H_{arom}$, J_1 = 7.4 Hz, J_2 = 8.1 Hz), 7.57 (d, $2H_{arom}$, J = 8.1 Hz), 9.05 (br s, 1H, $N\underline{H}Ph$). ^{31}P NMR ($CDCl_3$): δ = 8.3 ppm. Anal. Calcd. for $C_{12}H_{19}N_2O_4P$ (mol. weight 286.3), C, 50.35; H, 6.69; N, 9.79; P, 10.82. Found: C, 50.13; H, 6.61; N, 10.02; P, 10.62.

N-(Diphenylphosphinyl)-D-valine anilide, **12g**, was obtained, following the same procedure; recrystallisation from chloroform/hexane gave **12g** (75%), mp 194–197°C, $[\alpha]_D^{21}$ +157.9° ± 0.3° (c = 1.0, $CHCl_3$). ^{1}H NMR ($CDCl_3$/HMDS): 0.95 (d, 3H, J = 6.5 Hz, CH_3), 0.98 (d, 3H, J = 6.3 Hz, CH_3), 2.40 (m, 1H, $CH_3C\underline{H}CH_3$), 3.56–3.85 (m, 2H, NHCH), 7.04 (t, $1H_{arom}$, J = 7.3 Hz), 7.25 (dd, $2H_{arom}$, J_1 = 7.3 Hz, J_2 = 7.9 Hz), 7.58 (d, $2H_{arom}$, J = 7.9 Hz), 7.37–7.51 (m, 10H, Ph_2P), 9.96 (br s, 1H, $N\underline{H}Ph$). ^{31}P NMR ($CDCl_3$): δ = 25.9 ppm. Anal. Calcd. for $C_{23}H_{25}N_2O_2P$ (mol. weight 392.4), C, 70.39; H, 6.42; N, 7.14; P, 7.89. Found: C, 70.50; H, 6.48; N, 6.95; P, 7.83.

N-(Diphenylphosphinyl)-L-Valine anilide and N-(diphenylphosphinyl)-L-valyl-L-valine anilide. The reaction of O,N-bis(trimethylsilyl)-L-valine (1.66 g, 6.3 mmol) and **2f** (1.42 g, 6.0 mmol) and the following workup were carried out according to procedure, described for **4f**; 1.82 g of N-diphenylphosphinyl-L-valine (mixture with N-(diphenylphosphinyl)-L-valyl-L-valine) as white glass was obtained. This product was used on the second step without additional workup. To the stirred solution of glass obtained (0.62 g) and N-methyl-morpholine (0.20 g, 2.0 mmol) in THF (15 mL) a solution of isobutylchloroformate (0.27 g, 2.0 mmol) in THF (3.0 mL) was added at −10 ÷ −15°C. After 10 min the solution of aniline (0.18 g, 2.0 mmol) in THF (2 mL) was added and the mixture was stirred at −10°C for 1 h and at +20°C for 2 hrs. Then the reaction mixture was evaporated to dryness and the residue was distributed between water (20 mL) and EtOAc (40 mL). The organic layer was washed by water (2 × 15 mL), 10% aqueous citric acid (2 × 15 mL), 1 M solution of $NaHCO_3$ (2 × 15 mL) and brine (2 × 15 mL), dried over anhydrous Na_2SO_4 and evaporated. The residue (0.75 g) was crystallized fractionally from chloroform/hexane to give 0.327 g of N-(diphenylphosphinyl)-L-valine anilide, mp 194–197°C, $[\alpha]_D^{17}$ −163.0° ± 0.4° (c = 1.0, $CHCl_3$), and 0.045 g of N-(diphenylphosphinyl)-L-valyl-L-valine anilide, mp 284–286°C (dec). N-(Diphenylphosphinyl)-L-valine anilide. ^{1}H-NMR ($CDCl_3$/HMDS): 0.95 (d, 3H, J = 7.1 Hz, CH_3), 0.99 (d, 3H, J = 7.2 Hz, CH_3), 2.43 (m, 1H, $CH_3C\underline{H}CH_3$), 3.53–3.75 (m, 2H, NHCH), 7.05–7.89 (m, $15H_{arom}$), 9.89 (br s, 1H, $PhN\underline{H}$). ^{31}P-NMR ($CDCl_3$): δ = 26.7 ppm. Anal. Calcd. for $C_{23}H_{25}N_2O_2P$ (mol. weight 392.4), C, 70.39; H, 6.42; N, 7.14; P, 7.89. Found: C, 70.35; H, 6.52; N, 6.99; P, 7.79. N-(Diphenylphosphinyl)-L-valyl-L-valine anilide. ^{1}H-NMR ($CDCl_3$/HMDS): = 0.93 (d, 3H, J = 6.9 Hz, $C\underline{H}_3CHCH_3$), 1.02 (d, 6H, J = 6.9 Hz, $C\underline{H}_3CHC\underline{H}_3$), 1.09 (d, 3H, J = 6.9 Hz, $CH_3CHC\underline{H}_3$), 2.5 (m, 2H, $C\underline{H}(CH_3)_2$) 3.25 (dd, 1H, J_1 = 9.6 Hz, J_2 = 9.7 Hz, NHP), 3.53 (m, 1H, J_1 = 9.6 Hz, J_2 = 6.8 Hz, J_3 = 3.5 Hz, PNCH), 4.60 (dd, 1H, J_1 = 9.8 Hz, J_2 = 4.3 Hz, CONCH), 7.08–8.00 (m, $15H_{arom}$), 7.88 (d, 1H, J = 9.8 Hz, CONH), 9.86 (br s, 1H, $PhN\underline{H}$). ^{31}P-NMR ($CDCl_3$): δ = 25.99 ppm. Anal. Calcd. for $C_{28}H_{34}N_3O_3Px$ 0.25H_2O (mol. weight 491.6), C, 67.79; H, 7.0; P, 6.24. Found: C, 67.87; H, 7.09; P, 6.38.

L-Phenylalanine anilide hydrobromide, **13**. N-Carbobenzoxy-L-phenylalanine anilide[22] was treated with 4 N HBr to give **13** as colorless solid; yield 95%; mp 228–231°C, $[\alpha]_D^{22}$ +95 ± 3° (c = 1.4, C_2H_5OH). Anal. Calcd. for $C_{15}H_{16}N_2O \cdot HBr$ (mol. weight 321.2), C, 56.09; H, 5.33; N, 8.72. Found: C, 55.89; H, 5.15; N, 8.66.

N-(Diisopropoxyphosphoryl)-L-alanyl-L-phenylalanine anilide, **14**. A solution of isobutylchloroformate (0.27 g, 2 mmol) in THF (5 mL) was added dropwise to a solution of **4d** (0.51 g, 2 mmol) and N-methylmorpholine (0.2 g, 2 mmol) in THF (15 mL) at −15°C with stirring. After stirring this mixture at −15°C for 10 min, a solution of **13** (0.64 g, 2 mmol) and N-methylmorpholine (0.2 g, 2 mmol) in DMF (7 mL) cooled to 0°C was added dropwise. The mixture was stirred at −15°C for 1 h and at room

temperature for 3 hrs. To obtain pure **14**, the same procedure was applied as for the preparation of **12d**. Recrystallisation from chloroform/hexane gave pure **14** (0.91 g, 97%), mp 230°C (dec.). ^{1}H NMR ($CDCl_3$/HMDS): 1.23 (d, 3H, J = 6.7 Hz, $C\underline{H}_3CHCH_3$), 1.24 (d, 3H, J = 6.4 Hz, $CH_3CHC\underline{H}_3$), 1.26 (d, 3H, J = 6.5 Hz, CH_3CHCH_3), 1.27 (d, 3H, J = 6.5 Hz, $CH_3CHC\underline{H}_3$), 1.31 (d, 3H, J = 7.1 Hz,[23] CH_3CHNH), 3.18 (m, 1H, J_1 = 7.5 Hz, J_2 = 14 Hz, CHPh), 3.24 (m, 1H, J_1 = 6.1 Hz, J_2 = 14 Hz, CHPh), 3.54 (dd, 1H, J_1 = J_2 = 7.5 Hz, N$\underline{H}$P), 3.88 (m, 1H, J_1 = 7.1 Hz,[23] J_2 = 7.5 Hz, J_3 = 9.7 Hz,[23] CHNP), 4.46 (m, 1H, J_1 = 6.5 Hz, J_2 = 7.5 Hz, CHO), 4.58 (m, 1H, J_1 = 6.5 Hz, J_2 = 7.5 Hz, CHO), 4.94 (m, 1H, J_1 = 6.1 Hz, J_2 = 7.5 Hz, J_3 = 9.1 Hz, C$\underline{H}$CH$_2$Ph), 7.07 (t, 1H$_{arom}$, J = 7.4 Hz), 7.27 (dd, 2H$_{arom}$, J_1 = 7.4 Hz, J_2 = 7.7 Hz), 7.59 ((d, 2H$_{arom}$, J = 7.7 Hz), 7.25 (m, 5H$_{arom}$(Phe)), 7.63 (d, 1H, J = 9.1 Hz, CON$\underline{H}$CH), 9.12 (s, 1H, NHPh). ^{31}P NMR ($CDCl_3$): δ = 5.3 ppm. Anal. Calcd. for $C_{33}H_{34}N_3O_5P$ (mol. weight 475.5), C, 60.62; H, 7.21; N, 8.84; P, 6.51. Found: C, 60.83; H, 7.38; N, 8.68; P, 6.40. IR (KBr): 3288 (N—H), 1695, 1654, 1628, 1610, 1563 (CO—N—H), 1230 (P=O), 1029, 998 (P—O) cm^{-1}.

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