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Organophosphorus Analogues and Derivatives of the Natural L-Amino Carboxylic Acids and Peptides. III. Synthesis and Enzyme-Substrate Interactions of D-, DL-, and L-5-Dihydroxyphosphinyl-3,4-didehydronorvaline and Their Cyclic Analogues and Derivatives

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The unusual natural amino acid phosphorus containing didehydronorvaline (**5**) has been synthesized by consecutive treatment of the 4-bromo-2-butenal (**1**) with potassium cyanide and ammonium carbonate to the hydantoin (**2**), converted into the organophosphorus derivative **3**, followed by enzyme-catalyzed hydrolysis of the diethoxyphosphinyl group to the phosphonic acid **4**, and hydrolysis. The tetrahydro-1,2-azaphosphorine (**8**) and the protected didehydronorvaline derivatives **6**, **7**, **9**, and **10**, have been synthesized.

The tripeptide antibiotics Plumbemicin A: *N*-(*N*-L-alanyl-L-α-aspartyl)-3,4-didehydro-5-phosphono-D-norvaline, and Plumbemicin B: *N*-(N^2-L-alanyl-L-asparaginyl)-3,4-didehydro-5-phosphono-D-norvaline, have been isolated from *Streptomices plumbens strains* by Japanese researchers[1,2] and their biological activity has been studied.[3] To this moment, no information is available in the scientific and patent literature about the synthesis of the two tripeptides and of the unusual allylphosphonic acid **5**. The latter has been isolated and identified[2] by hydrolysis of the tripeptides. It is our intention, in two consecutive papers, to demonstrate the possibilities for their synthesis.

4-Bromo-2-butenal (**1**)[4] was chosen as the starting material for the synthesis of norvaline (**5**). Upon condensation with potassium cyanide/ammonium carbonate under the conditions of the Bucherer-Bergs reaction, the hydantoin (**2**) was obtained in a satisfactory yield (85%).

The next step was the Arbuzov reaction with the bromo-derivative **2** and triethyl phosphite. Despite the fact that a number of variations were introduced into the working conditions, the yield of the organophosphorus product never exceeded 50—55%. This could be explained with the rather "hard" conditions (3 h in boiling xylene) and with the sensitivity of the double bond. Varlet et al.[5] have synthesized the very similar structural analogue of the hydantoin (**3**), which has a saturated side chain, by using the Bucherer-Bergs 4-(diethoxyphosphinyl) butanal in their reaction. We employed an alternative approach, i.e. treatment of the acetal of 4-bromo-2-butenal, $BrCH_2CH{=}CHCH(OR)_2$ with triethyl phosphite, releasing the aldehyde and a subsequent treatment with potassium cyanide/ammonium carbonate. The yields were satisfactory and the approach will be discussed separately, accompanied with a demonstration of the catalytic activity of the enzyme α-chymotrypsin and urease towards the hydantoin (**3**) and its hydrolysis product.

We failed to achieve selective mineral hydrolysis of the diethoxyphosphinyl groups and the hydantoin ring. Under the markedly "hard" hydrolysis conditions, no individual products were obtained.

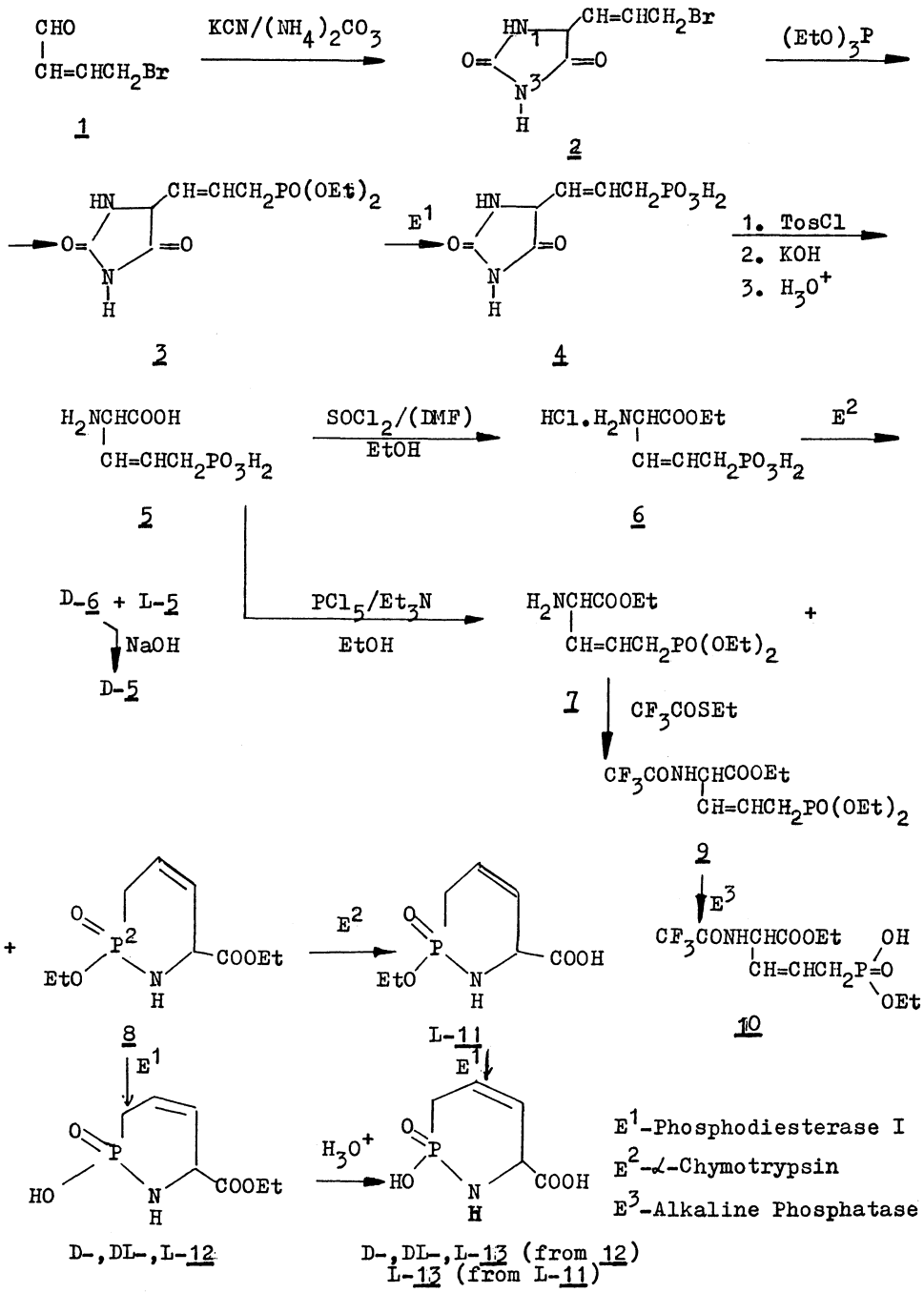
When the phosphonic diester **3** was treated with the enzyme phosphodiesterase I under conditions, described in our previous papers,[6] the free phosphonic acid **4** was isolated in a practically quantitative yield.

Patent data[7] report that 5-substituted hydantoins can be hydrolyzed by enzyme-catalyzed hydrolysis with dihydropyrimidinase to the corresponding carbamoyl derivatives. When the same enzyme was applied to the hydantoin (**3**), no enzyme-substrate interaction was observed. Furthermore, when the free phosphonic acid **4** was used as substrate, complete inhibition of the enzyme occurred.

To hydrolyze the hydantoin (**4**), we employed the method of Hiroi et al.[8] for preliminary N^3-tosylation, followed by mild mineral hydrolysis. It was found that isolation of the tosyl derivatives was unnecessary (cf. Experimental). Hydrolysis afforded the norvaline (**5**) as a racemic mixture in a yield of approx. 60%. Its spectral data were identical with those of an authentic sample.

It our laboratory, we succeeded in obtaining the same product **5**, as well as the tetrahydro-1,2-azaphosphorine analogue discussed below, by a four-component isonitrile condensation after the reaction of Ugi and a modified three-component condensation after the same reaction. This approach will be discussed separately.

With a view to obtaining optically active antipodes of 3,4-didehydronorvaline (**5**) and such derivatives as would be suitable for peptide synthesis, treatment was carried out with 1 equiv thionyl chloride in a medium of ethanol and in the presence of *N*,*N*-dimethylformamide in catalytic quantities. Thionyl chloride was gradually added to a cooled suspension of didehydronorvaline (**5**) in ethanol, which was continuously stirred. An argon, or nitrogen, current was passed through the reaction mixture, in order to carry off the released gases (sulfur dioxide and hydrogen chloride). The reaction mixture was then quickly brought to the boil,



Scheme 1.

which was accompanied by dissolution and an intense release of gases, carried out of the reaction mixture by the argon (nitrogen). When a slight vacuum was applied to the reaction vessel and the released gases were collected in absorbers, it was found that sulfur dioxide and hydrogen chloride had been removed almost quantitatively, which clearly indicated that they could not have reacted with the starting material, or with the reaction product. Cooling resulted in spontaneously precipitation of the ethyl ester hydrochloride of the 3,4-didehydro-DL-norvaline (**6**), which was isolated in a yield of about 90%.

The separation of optically active antipodes was achieved by enzyme-catalyzed hydrolysis with α-chymotrypsin. When the method in reference[6] was used, the enzyme catalyzed hydrolysis of only the L-form of the racemic mixture DL-**6** to the 3,4-didehydro-L-norvaline L-**5**, while the D-form D-**6** remained unchanged. The D-form-**5**, free from its ester protection, was easily obtained by mineral hydrolysis of the ester D-**6**.

Norvaline with protected phosphono and carboxyl functions was obtained by treatment of D-, DL- and L-didehydronorvaline (**5**) with phosphorus pentachloride. Without isolating the rather unstable chlorinated products, we subjected them to treatment with

ethanol, with triethylamine as the condensation agent. As it was the case with our previous studies of phosphinothricin,[6] here, analogously, the interaction led to two products: triethyl ester **7** as the major one (yield of about 60%), and the ethyl ester of tetrahydro-1,2-azaphosphorinecarboxylic acid (**8**) (yield approx. 10%). All our attempts to increase the yield of **8** by using alternative synthetic methods were unsuccessful and even protection of the reactive double bond brought no results.

We succeeded in obtaining a completely protected norvaline by treating the triester **7** with *S*-ethyl trifluorothioacetate by the method in reference.[6] Thus, the *N*-trifluoroacetylated triester of the didehydronorvaline (**9**) was isolated in a yield of about 80%.

The enzyme alkaline phosphatase is very suitable when strict selectivity is needed in the enzyme-catalyzed hydrolysis of the diethoxyphosphinyl groups. When taken at its pH optimum (10.4) and at 37 °C, the enzyme enhances the hydrolysis of only one of the two ethoxyl groups to the phosphonic monoester **10**. The enzyme-substrate interaction was carried out under conditions which have been empirically proved effective in our laboratory: 20 g substrate, about 5 mg enzyme, 6 h. Of course, larger or smaller amounts of substrate can be used, provided that the quantitative ratio substrate/enzyme is kept within the prescribed limits. When one and the same enzyme is used repeatedly, or when larger amounts of substrate are involved, it is better to spread the enzyme on a polymer carrier, as for instance the polyacrylamide gel "Sigma", which is very good for the purpose. In this case, the quantity of the enzyme should be twice as much (10—15 mg per 20 g substrate) and it can be regenerated simply by centrifugation.

We already observed that the optical form of the substrate is quite independent of the catalytic action of phosphodiesterase I. The same was found to be also true about alkaline phosphatase. This enzyme, however, catalyzes both the D- and the L-forms of 5-(diethoxyphosphinyl)-3,4-didehydronorvaline.

When we tried to hydrolyze the ethoxy carbonyl and ethoxyphosphinyl groups of tetrahydro-1,2-azaphosphorine (**8**) by treatment with mineral acids and bases, it turned out that the cyclic phosphinic amide group was the most easily hydrolyzable. To overcome this problem, which occurred also with the cyclic analogue of phosphinothricin,[6] again we used the enzyme approach. The ethoxycarbonyl derivative **8**, as substrate of the enzyme α-chymotrypsin, affords the free acid L-**11** in a practically quantitative yield. As expected, the D-form-D-**8** does not participate in the interaction.

We already discussed some particular characteristics of the enzyme phosphodiesterase I. It is capable of catalyzing the substartes, irrespective of the type (D- or L-) of their optical activity. Thus, with the phosphonamidic ester (**8**) as substrate, the free acids D-, DL-, and L-**12** were isolated in practically quantitative yields. The free carbonyl group of the substrate L-**11** with the enzyme phosphodiesterase I afforded the free tetrahydro-1,2-azaphosphorine L-**13**, again with a quantitative yield.

Alkaline phosphatase does not have any catalytic action on the substrates **8**, **11**, **12**, and **13**.

It seems that the presence of a free hydroxyphosphinyl group, $O=\underset{HO}{P}-$, increases, to a certain extent, the stability of the cyclic PO-NH bond. Thus, mineral hydrolysis of the esters D-, DL-, and L-**13**, although in yields of about 40%.

Studies on the physiological activity of the newly-synthesized products, as well as of the natural didehydro-D-norvaline (**5**), are under way and will be published soon.

Experimental

General Notes: IR-spectra, elemental analysis, optical activity and HPLC—on a Perkin-Elmer instrument; ^{1}H NMR-spectra—on Jouel-100 MHz; mass-spectra—on LKB-900; TLC—silica-gel film "Merck"; reagents and solvents—from "Aldrich" and "Merck"; enzymes and buffers—from "Sigma"; α-chymotrypsin—from "Pharmachim", Bulgaria. Special credit goes to Dr. N. Stambolieva of the Institute of Organic Chemistry, Bulgarian Academy of Sciences, for kindly supplying the mesintericopeptidase. The IR-spectra and mixed mp's of known compounds have been compared with those of authentic samples.

Synthesis of the Hydantoin (2). A mixture of 4-bromo-2-butenal (**1**) (14.90 g, 0.1 mol, potassium cyanide (7.81 g, 0.12 mol) and ammonium carbonate (14.41 g, 0.15 mol) is heated for 6 h at 55—60 °C in 150 ml 50% aqueous ethanol. A crystalline product is gradually released. After filtration, the filtrate is placed in a ventilation cupboard and strong draught is applied. The filtrate is then carefully acidified (the unreacted potassium cyanide releases hydrogen cyanide). After cooling, an additional quantity of the product is filtered out: 5-(3-bromo-1-propenyl)-2,4-imidazolidinedione (**2**): $C_6H_7BrN_2O_2$; 18.71 g (85.4%); IR (KBr, cm^{-1}): 3320—2860, 1750—1680, 860, 620; ^{1}H NMR ($CDCl_3$, TMS): δ=4.20 (3H, m, CHCO and CH_2Br), 6.20 and 6.76 (2H, m, C<u>H</u>=C<u>H</u>), 7.52 and 9.61 (2H, br.,NHX2); MS, *m/z* Calcd/Found: 219.04/219; elemental analysis, % C, H, N-Calcd/Found: 32.90/33.11, 3.22/3.46, 12.79/13.02; mp 183—186 °C (EtOH). (All analytical data to follow are given in the same order.)

Synthesis of the Diester 3. A mixture of the hydantoin (**2**) (21.90 g, 0.1 mol) and triethyl phosphite (19.94 g, 0.12 mol) is boiled for 3 h in 500 ml dry xylene in an ambience of dry argon, or nitrogen. The volatile components are evaporated in vacuum and the oily residue is passed first through florisil and then through a silica-gel column: 3-(2,5-dioxo-4-imidazolidinyl)-2-propenylphosphonic acid diethyl ester (**3**): $C_{10}H_{17}N_2O_5P$; 14.84 g (53.7%); IR (cm^{-1}) 3350—3200, 3030, 1750—1690, 1300, 1255, 1110—980, 865, 630; ^{1}H NMR (DMSO-d_6): δ=1.18 and 1.36 (6H, t, J=7 Hz, $OCH_2C\underline{H}_3$X2), 2.80 (2H, m, PCH_2), 3.50 and 3.62 (4H, q, $OC\underline{H}_2CH_3$X2), 4.18 (1H, d, J=8Hz, CHN), 5.62 and 6.12 (2H, m, C<u>H</u>=C<u>H</u>), 7.52 and 9.60 (2H, br., NHX2); MS, *m/z* Calcd/Found: 276.31/276; % C, H, N Calcd/Found: 43.48/43.56, 6.70/6.93,

10.14/10.16; mp 183—186 °C; R_f: 0.33 ($CHCl_3$: MeOH=9 : 1) and 0.83 (*n*-BuOH : AcOH : H_2O=9 : 1 : 1).

Enzyme Hydrolysis of the Phosphonic Diester 3. A homogeneous mixture of the substrate **3** (27.63 g, 0.1 mol) and the emulgator "Tween-80" is added to an aqueous buffer solution (500 ml, pH 8.8) of phosphodiesterase I (7 mg), tempered at 37 °C. Stirring is kept up for 6 h at the same temperature. After acidification, condensation and cooling, the following product is filtered out: 3-(2,5-dioxo-4-imidazolidinyl)-2-propenylphosphonic acid (**4**): $C_6H_7N_2O_2P$; 21.24 g (97.4%); IR (cm^{-1}) 2860—2430 (PO_3H_2); ^1H NMR (D_2O-NaOD): δ=2.98 (2H, m, PCH_2), 5.70 and 6.21 (2H, m, CH=CH-); MS *m/z* Calcd/Found: 218.11/218; % C, H, N Calcd/Found: 33.04/32.84, 3.24/3.56, 12.84/12.96; mp about 250 °C (decomp); R_f: 0.42 (*n*-BuOH : AcOH : H_2O=9 : 1 : 1).

Synthesis of Didehydronorvaline (5).[2] *p*-Toluenesulfonyl chloride (20.98 g, 0.11 mol) is gradually added for 1 h to 300 ml 80% aqueous ethanol, containing sodium hydroxide (10 mg) and the hydantoin (**4**) (21.81 g, 0.1 mol). After 30 min the reaction mixture is evaporated in vacuum to dryness, 20 g potassium hydroxide in 110 ml water are added and the mixture is heated for 30 min in a water bath. After acidification, heating for 30 min and elution through Amberlite IR-120 resin with 0.1 M (1 M=1 mol dm^{-3}) HCl, the following product is isolated: 3,4-didehydro-5-phosphono-DL-norvaline (**5**): $C_5H_{10}NO_5P$; 11.45 g (58.7%); spectral data coincided with those of an authentic sample; MS *m/z* Calcd/Found: 195.11/195; % C, H, N Calcd/Found: 30.78/31.01, 5.17/5.02, 41.00/39.78.

Synthesis of the Ethyl Ester 6. Thionyl chloride (13.09 g, 0.11 mol) is added dropwise to a continuously stirred suspension, cooled to 5 °C, of didehydronorvaline (**5**) (19.81 g, 0.1 mol) in 200 ml ethanol, containing 5 ml *N,N*-dimethylformamide. A swift current of dry argon, or nitrogen, is simultaneously passed through the reaction mixture. The cooling bath is replaced with a warmed-up heater. Gasses are removed from the system by a continuous flow of inert gas. After 5 min of boiling (highly-effective condenser), complete dissolution is observed. The product crystallizes after cooling: 3,4-didehydro-5-phosphono-DL-norvaline ethyl ester hydrochloride (**6**): $C_7H_{14}NO_5P \cdot HCl$; 20.31 g (91.0%); IR (cm^{-1}) 1750 (CO), 1550 (NH_3^+);% C, H, N Calcd/Found: 36.67/36.99, 6.32/6.11, 6.28/6.35; mp 210 °C (decomp).

Enzyme Hydrolysis and Separation of Optical Antipodes of the Ester 6. A mixture of the ester **6** (22.31 g, 0.1 mol) and α-chymotrypsin (6 mg) is stirred for 6 h at 25 °C in an aqueous buffer medium (pH 7.8, 500 ml). After acidification, evaporation to dryness and extraction with boiling ethanol, the following products are fractionally isolated: 3,4-didehydro-5-phoshono-D-norvaline ethyl ester hydrochloride D-**6**: $C_7H_{14}NO_5P \cdot HCl$; 9.00 g (96.4%); spectral data coicide with those of DL-**6**; mp: approx. 220 °C (decomposition); $[\alpha]_D^{20}$ +55.3°, (*c* 0.1, MeOH): 3,4-didehydro-5-phosphono-L-norvaline (L-**5**): $C_5H_{10}NO_5P$; 9.47 g (97.1%); spectral data identical with those of DL-**5**; mp 188—190 °C; $[\alpha]_D^{20}$ −53.3 (*c* 1, H_2O).

Hydrolysis of the Ester D-6. The ester D-**6** (22.32 g, 0.1 mol) is heated for 30 min at 40—45 °C in aqueous NaOH (2 M, 200 ml). After acidification, evaporation in vacuum to dryness and crystallization from ethanol, the following product is isolated: 3,4-didehydro-5-phosphono-D-norvaline (D-**5**): $C_5H_{10}NO_5P$; 17.57 g (90.1%); spectral data identical with those of D- and DL-**5**; $[\alpha]_D^{20}$ −53.3° (lit,[2] +51.4°), (*c* 1, H_2O).

Synthesis of the Triester 7 and Tetrahydro-1,2-azaphosphorine (8). Portions of phosphorus pentachloride (68.72 g, 0.33 mol) in 200 ml dry tetrachloromethane are added for 1 h at 80 °C to 300 ml dry tetrachloromethane, containing D-, DL-, or L-didehydronorvaline (**5**) (19.51 g, 0.1 mol). A current of dry argon, or nitrogen, is simultaneously passed through the suspension. After 30 min, the reaction mixture is evaporated in vacuum to dryness and mixed, first with 500 ml absolute ethanol, and then with 50 ml triethylamine. It is then boiled for 30 min, filtered and evaporated in vacuum to dryness. The residue is dissolved in dry chloroform and saturated with dry hydrogen chloride. The resultant residue is filtered and recrystallized from ethanol: 3,4-didehydro-5-diethoxyphosphinyl-(D-, DL-, L-)norvaline ethyl ester hydrochloride (**7**): $C_{11}H_{22}NO_5P \cdot HCl$; 18.72 g (59.3%); IR ($cm^{-1}$) 3020, 1740, 1525, 1255, 1110—975; ^1H NMR ($CDCl_3$, free base) δ=1.2—1.9 (9H, m, CH_3×2), 2.22 (2H, s, NH_2), 2.80 (2H, m, PCH_2), 3.20—3.60 (6H, m, OCH_2×3), 4.82 (1H, d, *J*=7 Hz, CHN), 578 and 6.20 (2H, m, CH=CH); MS *m/z* Calcd/Found 279.30/279; % C, H, N Calcd/Found: 41.85/42.01, 7.34/7.12, 4.44/4.69; R_f 0.55 (DMF: $CHCl_3$: MeOH=5 : 1 : 2); mp D-, DL-, D-: 140—146 °C (decomp); $[\alpha]_D^{20}$ +59.3°, (*c* 0.1, MeOH).

The filtrate, left after the filtration of the triester **7**, is evaporated in vacuum and eluted in a silica-gel column (eluent chloroform:hexane=5:2). Fractions of 50 ml each are collected. The fourth, fifth, and sixth fractions are combined, evaporated and passed through the columun: D-, DL-, L-2-ethoxy-6-ethoxycarbonyl-1,2,3,6-tetrahydro-1,2-azaphosphorine-2-oxide (**8**): $C_9H_{16}NO_4P$; 2.61 g (11.2%); IR (cm^{-1}) 1760, 1390, 1250, 920, 840; ^1H NMR ($CDCl_3$): δ=1.18 and 1.32 (6H, t, OCH_2CH_3×2), 2.46 (2H, m, PCH_2), 3.4—3.9 (4H, m, OCH_2CH_3×2), 4.62 (1H, d, *J*=8 Hz, CHCO), 5.82 and 6.35 (2H, m, CH=CH); MS *m/z* Calcd/Found: 233.20/233; % C, H, N Calcd/Found: 46.35/46.28, 6.91/7.07, 6.01/5.79; R_f: 0.72 ($CHCl_3$: *n*-C_6H_{14}=5 : 2); mp D-, DL-, L-: 86—90 °C; $[\alpha]_D^{20}$ +78.2° and −77.6°, (*c* 0.1, MeOH).

Synthesis of *N*-Trifluoroacetyl-(D-, DL-, L-)didehydronorvaline (9). The triester **5** (D-, DL-, or L-) (19.51 g, 0.1 mol) and *S*-ethyl trifluorothioacetate (24.72 g, 0.16 mol) are shaken for 8 h at room temperature in 100 ml 2 M NaOH, 600 ml borate buffer and 500 ml chloroform. The layers are separated and the aqueous layer acidified and extracted with chloroform (3×20 ml) and the combined organic extracts are dried over anhydrous magnesium sulfate and distilled in vacuum to dryness. The product is isolated as a noncrystallizing oil. For analytical purposes, purification is carried out by passing through a silica-gel column (eluent dioxane: chloroform=5 : 3): *N*-trifluoroacetyl-3,4-didehydro-5-diethoxyphosphinyl-(D-, DL-, L-)norvaline ethyl ester (**9**): $C_{13}H_{21}F_3NO_6P$; 28.87 g (average for the three forms, 79.6%); IR (film, cm^{-1}) 1760, 1520, 1210, 1110—860; % C, H, N Calcd/Found: 41.61/41.33, 5.64/5.81, 3.73/4.12; R_f: 0.65 (dioxane : chloroform=6 : 1); mp decomposes upon attempts at distillation; $[\alpha]_D^{20}$ +72.3° and −70.6°, (*c* 0.1, MeOH).

Enzyme Hydrolysis of the Didehydronorvaline (9). A homogeneous mixture of the ester **9** (18.77 g, 0.05 mol) and emulgator "Tween-80" is added to a buffer medium (pH 10.4, 800 ml), containing alkaline phosphatase (10 mg on agarose) and tempered at 37 °C. The mixture is stirred for 6 h and the enzyme is removed. The mixture is acidified, evaporated in vacuum to dryness and extracted with chloroform. The organic extract is dried over anhydrous magne-

sium sulfate and distilled in vacuum to dryness. The oily residue slowly crystallizes after a prolonged period in a refrigerator on hexane: *N*-trifluoroacetyl-3,4-didehydro-5-ethoxyhydroxyphosphinyl-(D-, DL-, L-)norvaline ethyl ester (**10**): $C_{11}H_{17}F_3NO_6P$; 16.86 g, (97.1%); IR (cm^{-1}) 2860—2420, 1760, 1540, 1260, 1110—840; ^1H NMR ($CDCl_3$): δ=1.13 and 1.30 (6H, t, $OCH_2C\underline{H}_3\times2$), 2.95 (2H, m, PCH_2), 4.1—4.4 (5H, m, $OC\underline{H}_2CH_3\times2$, C<u>H</u>NH), 5.38 (1H, m, CH=CHCH$_2$), 6.30 (2H, m, CH=C<u>H</u>CH$_2$, N<u>H</u>), 10.2—10.6 (1H, br., POOH); MS *m/z* Calcd/Found: 247.23/247; % C, H, N Calcd/Found: 38.05/34.44, 4.93/4.88, 4.03/3.96; R_f: 0.46 (dioxane : chloroform=6 : 1); $[\alpha]_D^{20}$ +76.2° and −73.1°, (*c* 0.1, MeOH); mp D-, DL-, L-: 95—96 °C (decomp).

Enzyme-Substrate Hydrolysis of the Ester 8: Each of the D-, DL-, and L-**8** substrates (23.32 g, 0.1 mol) is subjected to hydrolysis by the above method and using the enzymes α-chymotrypsin and phosphodiesterase I. The substrate L-**8** and α-chymotrypsin (6 mg) afford the product L-**1**, whereas D-, DL-, and L-**8** plus phosphodiesterase I (5 mg) afford the products D-, DL-, and L-**12**: L-2-ethoxy-1,2,3,6-tetrahydro-1,2-azaphosphorine-6-carboxylic acid 2-oxide L-**11**: $C_7H_{12}NO_4P$; 18.94 g (92.3%); IR (cm^{-1}) 3480—3200, 3025, 1760, 1390, 1250, 1110—960, 870, 640; ^1H NMR (DMSO-d_6) δ=1.21 (3H, t, *J*=7 Hz, $OCH_2C\underline{H}_3$), 2.36 (2H, m, PCH_2), 3.43 (2H, q, $OC\underline{H}_2CH_3$), 4.51 (1H, d, *J*=8 Hz, CHCO), 5.68 and 6.33 (2H, m, CH=CH), 10.4—11.0 (1H, br., COOH); MS *m/z* Calcd/Found: 205.15/205; % C, H, N Calcd/Found: 40.98/41.16, 5.90/5.71, 6.83/6.92; R_f: 0.72 (*n*-BuOH : 25%NH_3aq=8 : 1); mp 183—185 °C (decomp); $[\alpha]_D^{20}$ −82.6°, (*c* 0.1, MeOH), D-, DL-, L-6-ethoxycarbonyl-hydroxy-1,2,3,6-tetrahydro-1,2-azaphosphorine 2-oxide-D-, DL-, L-**12**: $C_7H_{12}NO_4P$; 19.92 g (97.1%); 2840—2420 (P-OH); MS *m/z* Calcd/Found: 205.15/205; % C, H, N Calcd/Found: 40.98/40.73, 5.90/6.11, 6.83/6.66; R_f: 0.52 (dioxane : chloroform=5 : 2); mp D-, DL-, L-: 118—121 °C (decomp); $[\alpha]_D^{20}$ +80.3° and −81.6°, (*c* 0.1, MeOH).

Enzyme-Substrate Hydrolysis of the Ester L-11. The above method for enzyme-catalyzed hydrolysis is used with phosphodiesterase I and the ester L-**11** (20.52 g, 0.1 mol) as substrate. The following product is isolated: L-2-hydroxy-1,2,3,6-tetrahydro-1,2-azaphosphorine-6-carboxylic acid 2-oxide L-**13**: $C_5H_8NO_4P$; 17.39 g (98.2%); IR (cm^{-1}) 3530—3200, 2840—2470, 1760, 1400, 1255, 870, 630; ^1H NMR (D_2O-NaOD): δ=2.31 (2H, m, PCH_2), 4.52 (1H, d, *J*=8 Hz, CHCO), 5.76 and 6.38 (2H, m, C<u>H</u>=C<u>H</u>), and three exchangeable protons — N<u>H</u>, PO<u>H</u>, and COOH; MS *m/z* Calcd/Found: 171.11/171; % C, H, N Calcd/Found: 33.91/34.11, 4.56/4.38, 7.91/8.06; R_f: 0.75 (*n*-BuOH : AcOH : H_2O= 9 : 1 : 1); mp approx. 230 °C (decomp); $[\alpha]_D^{20}$ −61.2° (*c* 0.1, 1 M NaOH).

Alkaline Hydrolysis of the Ester D-, DL-, and L-12. Each of the esters D-, DL-, and L-**12** (20.51 g, 0.1 mol) is stirred for 30 min at 40—45 °C in 2 M MaOH (150 ml). The reaction mixture is acidified, evaporated in vaccum to dryness and extracted with boiling ethanol. The esters D-, DL-, and L-**6** and D-, DL-, and L-**13**, respectively, are fractionally isolated in an average yield of 6.60 g (38.6%). Their spectral data are identical with those of the product L-**13**: mp D-, DL-, and L-: approx. 230 °C (decomp) $[\alpha]_D^{20}$ +59.8°, (*c* 0.1, 1 M NaOH).

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