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Organophosphorus Analogues and Derivatives of the Natural L-Amino Carboxylic Acids and Peptides. I. Enzymatic Synthesis of D-, DL-, and L-Phosphinothricin and Their Cyclic Analogues

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Acetylation of D-, DL-, and L-3-amino-2-pyrrolidinone (**1**) to the 3-acetamido derivative **2** and treatment with ethyl methylphosphinate afforded the addition product **3**. Strict selectivity was observed in the enzyme-catalyzed hydrolysis with the enzyme phosphodiesterase I, acylase, glutaminase, and α-chymotrypsin. The ester **7** and 1,2-azaphospholidine (**8**) were isolated by esterification of L-phosphinothricin (**6**).

The tripeptide γ-(hydroxymethylphosphinyl)-L-α-aminobutyryl-L-alanyl-L-alanine,[1] usually called Bialaphos (SF-1293), belongs to the small group of known natural phosphino analogues of the amino carboxylic acids. The antibiotic is produced from *Streptomices viridochromogenes strains* and has been found to possess herbicidal and fungicidal activity.[2,3] Upon enzyme decomposition with *E. coli* protease with a view to its structure elucidation, the unusual amino carboxylic acid 4-(hydroxymethylphosphinyl)-L-2-aminobutanoic acid (**6**), known as L-phosphinothricin, has been isolated.[4] It is produced[5] from *Streptomices hydroscopicus strains.*

The tripeptide Bialaphos has been synthesized[6] as a phosphonoethyl ester by the participation of the DL-forms of phosphinothricin and alanine, following a multistep procedure. In this case the yields were rather low. L-Phosphinothricin (**6**) has been synthesized[7] by Michael addition of chiral glycine Schiff base to a vinylphosphorus compound, $CH_2{=}CH{-}P(=O)(CH_3)(OH)$ The racemic phosphinothricin, widely used as herbicide and fungicide,[8] is produced synthetically. The introduction of the ethoxymethylphosphinyl, $EtO{-}P(=O)(CH_3){-}$ group is achieved by the Arbuzov reaction. Thus, Wasielewski and Antczak[9] treated 4-bromobutanoic acid with diethyl methylphosphonite $CH_3P(OEt)_2$ and obtained the product after α-amination. Recently, Logusch[10] suggested that methyl DL-4-bromo-2-phtalimidobutanoate, could be used in a similar interaction, so that phosphinothricin is finally obtained by hydrolysis.

It is interesting to note that the biological activity of the natural L-phosphinothricin is twice as strong as that of the racemic mixture.[8]

The key approach in the pathway we chose to the synthesis of phosphinothricin (**6**) is a variant of the reaction of Michaelis-Becker,[11] usually applied to the alkylation hydrophosphoryl compounds, $>P(=O)H$ Despite the abundant data that have been published about this reaction, many of its aspects are still inadequately studied. Surprisingly, no one has as yet taken up McConnel and Coover[12] on their suggestion that lactams could be used in interactions of this type.

In view of the synthetic purposes in hand, the γ-lactam **1** of the D-, DL-, and L-2,4-diaminobutanoic acid was chosen as the starting material. It is very easily obtained[13] by dehydration.

In his work,[13] Adamson reports the acetylation of **1** with acetic anhydride, but his yields were very low and therefore unsatisfactory. It was found that, in the subsequent stages, any protective group, other that an acetyl group, caused considerable complications. The best acetylating agent proved to be acetic phosphoric anhydride, $AcOPO_3H_2$,[14] obtained immediately before use. The reaction proceeds at 60—70 °C for 15—20 min in the reagent medium the acetylated product **2** is isolated in a very high yield (95—98%).

The interaction of the lactam **2** with ethyl methylphosphinate takes 2.0—2.5 h of heating of the components at 120—130 °C in a closed steel cylinder and in an ambience of dry argon or nitrogen. Here, the presence of a catalyst is essential. Boron trifluoride etherate proved to be the most suitable catalyst in this case. Without it, the result is a resinous mass, from which the organophosphorus product is isolated in a very low yield (6—10%). To enhance the reaction, it is important that the temperature should be increased gradually, starting from ambient and going up to 120 °C for the duration of 1.0—1.5 h. When the process is completed, the volatile components are distilled off (100 °C/6×10^{-2} Torr (1 Torr=133.322 Pa)). The reaction product is then purified by column chromatography (silica gel and eluent chloroform : methanol=9 : 1). The product 2-acetamido-4-(ethoxymethylphosphinyl)butanamide (**3**) is obtained in a yield of 50—55%.

When the optically active D- and L-lactams **2** were used under the above conditions, no racemization was observed.

In our laboratory, we carried out analogous interactions, not only with the lactam of 2,4-diaminobutanoic acid, but also with the lactams of L-ornithine and L-lysine, where a cobalt-complex of 5-amino-1,10-

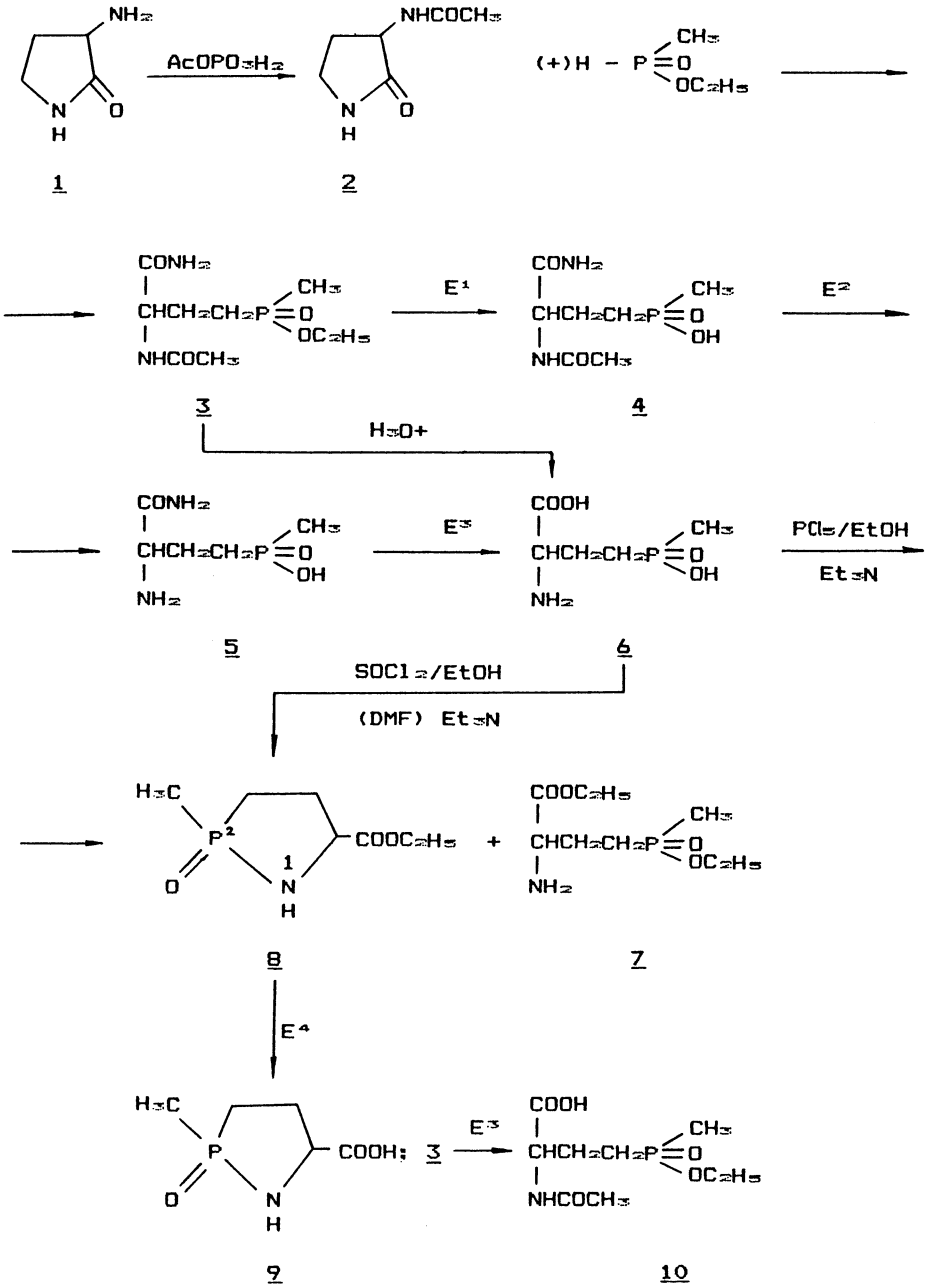
phenanthroline was used as catalyst. The results will be published separately.

The mineral hydrolysis of **3** is extremely difficult, due to the presence of an ethoxyphosphoryl group. Complete hydrolysis would require at least 8 h of boiling in 20—22% hydrochloric acid. Unfortunately, when the optically active starting esters **3** (D- or L-forms) were used, complete racemization of the product phosphinothricin (**6**) (approx. 90% yield) was observed.

We concentrated our efforts on such approaches as would help us to avoid the complications accompanying the hydrolysis of the esters of organophosphinic acids, at the same time preserving their optical activity.

The phosphoesterase enzymes, exo- and endonuclease, are widely employed in biochemical routine investigations of nucleic acids. To this moment, however, they have never been used to catalyzed the hydrolysis of the esters of organophosphinic acids. This approach was developed in our laboratory and will be discussed in a number of consecutive papers.

It was empirically established that the most favorable conditions for enzyme-catalyzed hydrolysis processes are as follows: 20 g substrate and 5 mg enzyme



E¹ - Phosphodiesterase I; E² - Acylase I;
E³ - Glutaminase; E⁴ - α-Chymotrypsin.

Scheme 1.

are stirred for 6 h in an aqueous buffer solution, or emulsion, at the pH and temperature optimum of the enzyme. Naturally, larger of smaller quantities of the substrate can be used, provided that the ratio of substrate/enzyme remains the same. To ensure the easier manipulation and subsequent regeneration of the enzyme, especially if it is not easily available, a polymer carrier should be used, on which to spread the enzyme. In this case, 20 g substrate would require 10—15 mg enzyme. With very few exceptions, the enzyme-catalyzed hydrolysis affords practically quantitative yields and is strictly selective. If not inhibited, one and the same enzyme can be used in at least ten reactions. It is characteristic for the enzyme approach that no racemization occurs with optically active substrates, while the traditional hydrolysis methods usually lead to a complete loss of the optical activity. Of course, the different enzymes could be kinetically studied and the above conditions additionally refined to accomodate each separate type of enzyme. Where the synthesis of organophosphorus compounds is comcerned, however, this method is quite applicable. In our laboratory, we conducted experiments, using substrates that contain esters of the types R-P(=O)(H)OR^1, R-P(=O)(CH$_3$)OR^1, R-P(=O)(OR^1)OR^1, and R-P(=O)(OR^1)NHR^2 and other hydrolyzable groups (R, R^1, and R^2), with not only the enzymes phosphodiesterase I and alkaline phosphatase, but also with α-chymotrypsin, protease, mesintericopeptidase, acylase, urease, crude bee venom, and glutaminase. The concrete applications of these will be discussed separately.

With the phosphinic ester **3** we used the enzyme phosphodiesterase I in its free state and spread over polyacrylamide. As **3** is water-insoluble, a good initial homogenization of the substrate was achieved with 2—3 ml Tween-80^R per 20 g substrate the aqueous buffer (pH 8.8, 800 ml) was gradually added to the obtained jelly mass by continuous stirring. The homogeneous mixture was then tempered at 37 °C, and 5 mg (or 10—15 mg, if spread on polyacrylamide) phosphodiesterase I were added. After stirring for 6 h at the same temperature, the enzyme was removed by dialysis (ultrafiltration), or centrifugation, and was ready to use in next experiment. The buffer was acidified to pH 5.0 and extracted with diisopropyl ether. The acid **4** was isolated in a practically quantitative yield after drying with anhydrous magnesium sulfate and distillation of the extraction agent. Surprisingly, it was found that the catalytic action of the enzyme was not affected by the type of the optically active form: both the D- and L-forms of the D-, DL- and L-substrates **3** were hydrolyzed. It is interesting to note that alkaline phosphatase, which has a very similar activity, does not catalyze the hydrolysis of the substrate in question.

Acylase I was the next enzyme we applied to a rather unusual substrate, containing a phosphinic-acidic function. Under the above-mentioned conditions, at pH 7.0 and 25 °C, the enzyme catalyzed the hydrolysis of the *N*-acetyl group of the substrate L-**4** to the free amino phosphinic acid L-**5**, which was isolated in an approx. 80% yield after acidification, concentration and cooling. As a raw enzyme ("Sigma") was used, 25 mg of it were necessary for 25 g substrate **4**.

When trifluoroacetyl was used to protect the amino group in the starting lactam **1**, and when methyl methylphosphinate was employed in the reaction described above, the resultant trifluoroacetyl derivative (not given in the Scheme 1) was easily hydrolyzed in a practically quantitative yield after treatment with aqueous ammonia at pH 10.0. This is one of the rare cases (cf. also the case with acylase I above) when the classical methods for liberating the protective groups are to be preferred to the enzyme approach. However, when optically inactive (racemic) mixtures are used, the enzyme acylase I is quite suitable for the separation of optically active antipodes. The substrate DL-**4** leads to the isolation of the unchanged D-form D-**4**, and the hydrolysis product L-**5**.

The third enzyme investigated was glutaminase. Apart from the fact that this enzyme hydrolyzes the γ-amide function of glutamine to glutamic acid, it was found that using the α-amide of the modified glutamic acid, i.e. the substrate L-**5**, afforded the phosphinothricin L-**6** in a 95% yield. This is not very surprising, as in a separate experiment we used the diamide of the L-glutamic acid and obtained L-glutamic acid under the same conditions as for the enzyme-catalyzed hydrolysis of L-**5**. We discovered still another possible application of glutaminase, when a number of mono- and diamides of saturated and unsaturated dicarbonylic acids (malonoamide, succinamide, adipamide, pimelamide, fumaramide, citric 1,5-diamide, mesaconamide, ect.) were subjected to the catalytic action of glutaminase and gave the corresponding carboxylic acids. Sulfonamides R-SO_2NH_2 and amides of the type R-P(=O)(OR)NHR^2, as also aromatic mono- and diamides and cyclic imides, are not affected by the enzyme glutaminase.

In contrast to phosphodiesterase I, glutaminase is inactive towards the D-form of phosphinothricinamide **5**. It is interesting to note that, when the methylphosphinyl group of the substrate **5** is replaced with a dihydroxyphosphinyl group, the substrate 2-amino-4-phosphono-L-butanamide inhibits the enzyme completely. We have come to the conclusion that all experiments, involving glutaminase-catalyzed hydrolysis of carboxamides containing PO_3H_2 or P(O)(OH)H groups, should be approached with caution. Protection of the phosphono and phosphino groups as esters, however, does not result in the enzyme inhibition and a subsequent separation of the ester functions

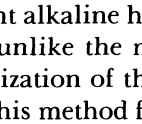
with phosphonodiesterase I is easily possible.

In our case, the inevitable contamination of the enzyme does not seriously affect the enzyme-catalyzed hydrolysis process and glutaminase from *E. coli* can therefore be used, with an enzyme-content of about 50%.

In our laboratory, we developed a variant of the above method, where D-, DL-, and L-phosphinothricin are obtained by enzyme-catalyzed hydrolysis of the phosphinic ester **3** and a subsequent alkaline hydrolysis of the amide **4**. In this case, unlike the mineral hydrolysis of the ester **3**, no racemization of the optically active forms was observed. This method for production of phosphinothricin will be discussed separately.

Esterification was carried out with a view to obtaining derivatives of L-phosphinothricin, suitable for peptide synthesis. Treatment of the acid **6** with phosphorus pentachloride afforded a chloro-containing compound, which proved extremely unstable upon air-exposure and decomposed quickly releasing hydrogen chloride. For that reason, chlorination and esterification were conducted simultaneously in the presence of triethylamine as acceptor for the released hydrogen chloride. After the usual work-up for separation of the reaction products, we isolated the main product L-phosphinothricin *O,P*-diethyl ester (**7**) (yield approx. 65%) and 1,2-azaphospholidine (**8**) (yield approx. 20%). When a 1 equivalent phosphorus pentachloride was used, the yield of the 1,2-azaphospholidine (**8**) went up to 50%, whereas the diester of L-phosphinothricin (**7**) was found only in traces. Most probably, the improved experimental conditions have caused the initial chlorination, after which the carboxyl group is esterified and the phosphinyl and amino groups of phosphinothricin form an azaphospholidine cycle. If the *P*-ester of phosphinothricin is placed under the same conditions, no such cyclization occurs. An indirect was to proving the possible mechanism of this ring-formation is the isolation of 1,2-azaphospholidine (**8**) by treatment of L-phosphonothricin (**6**) with thionyl chloride and a catalytic quantity of *N,N*-dimethylformamide, followed by treatment with absolute ethanol. The product is obtained in a yield of about 60%.

When the fourth enzyme α-chymotrypsin, was used in an enzyme-catalyzed hydrolysis, the free acid **9** was isolated in a practically quantitative yield. The same product (cf. our subsequent publications) has been obtained by a variant of the reaction of Ugi: from 3-(hydroxymethylphosphinyl)propanal, ammonium, and cyclohexyl isocyanide, followed by enzyme hydrolysis. That enzyme-catalyzed hydrolysis should be preferred to mineral hydrolysis was due to the fact that the cyclic PONH-bond of 1,2-azaphospholidine (**8**) turned out to be highly sensitive to all interactions with acids or salts under the conditions accompanying the hydrolysis of the ethoxycarbonyl group. The enzyme-catalyzed hydrolysis is conducted by the method described above: 20 g substrate **8**, 15 mg raw α-chymotrypsin, stirring for 6 h at 25 °C in 500 ml buffer (pH 7.8). The enzyme could, of course, be spread over a polymer carrier, but as it is readily available, this was not actually necessary. An analogous deesterification occurred also when the enzyme alkaline mesintericopeptidase and protease were used.

The enzyme approach was again employed to obtain L-phosphonothricin with a free carboxyl function and protected  and amino groups. When the amide **3** was used as substrate of the enzyme glutaminase under the above conditions, the free carboxylic acid **10** was isolated in a practically quantitative yield.

Thus, by the slightly modified reaction of Michaelis-Becker and the new enzyme approach to the hydrolysis of alkoxymethylphosphinyl groups, carboxylic esters and amides, we succeeded in isolating L-phosphinothricin (**6**) in satisfactory yields. This product is rather valuable for its biological activity.

Experimental

General Notes: IR spectra, elemental analysis, HPLC, and determination of optical activity—on a Perkin-Elmer instrument; ^{1}H NMR spectra—on Jouel-100 MHz; mass-spectra—on LKB-900; melting point—measured on a Köfler apparatus; TLC—silica-gel film "Merck"; reagents and solvents— "Aldrich", "Merck"; enzymes and buffers—"Sigma"; α-chymotrypsin— "Pharmachim", Bulgaria. Special credit goes to Dr. N. Stambolieva, who kindly supplied the author with alkaline mesintericopeptidase.

Analytical data of all compounds, with their names, numbers in the text, schemes, and formulae, are given in the following order: yield; IR spectrum (*a.* KBr tablet, *b.* film, cm^{-1}); ^{1}H NMR spectrum taken in *c.* DMSO-d_6, *d.* $CDCl_3$, *e.* D_2O+NaOD, TMS standard, δ-scale, ppm (number of protons, calculated by the integral curve, multiplicity, position of proton in the molecule); mass-spectrum—*m/z*, Calcd/Found, %; elemental analysis for % C, H, N-Calcd/Found; mp; $[\alpha]_D^{20}$; R_f—phosphomolybdic acid ($12MoO_3 \cdot H_3PO_4 x H_2O$)—"Aldrich" or ninhydrin detection (for compounds containing an amino group), developed in systems:

A : *n*-BuOH: AcOH: H_2O=9 : 1 : 1;
B : *n*-BuOH: 25% aq. NH_3=4 : 1;
C : DMF: $CHCl_3$: MeOH=5 : 1 : 2;
D : $CHCl_3$: MeOH=9 : 1

The IR-spectra of the known natural compounds were compared with those of the compounds, obtained by the original methods described in the text, and elemental analysis was carried out. Full coincidence was observed in the range 4000—400 cm^{-1} and elemental analysis was satisfactory. For the optically active forms, which lack descriptions in the literature, mp's and $[\alpha]_D^{20}$ are also given.

Preparation of D-, DL-, and L-3-Acetamido-2-pyrrolidinone (2). Phosphorus pentaoxide (113 g) is dissolved at 60—70 °C in orthophosphoric acid (150 g). The obtained phosphoric acid (H_3PO_4) is stored in a refrigerator at −10 °C and is quickly melted in a hot oil bath immediately before use.

Phosphoric acid (8 ml) is dissolved in acetic anhydride (32 ml) (mole ratio 1.0:1.7) and D-, DL-, or L-3-amino-2-pyrrolidinone (10.01 g, 0.1 mol), is added. The mixture is stirred for 15—20 min at 80—85 °C and is then poured over crushed ice (approx. 50 g). Rapid cooling of the liquid phase occurs and the product 3-acetamido-2-pyrrolidinone (**2**) crystallizes: $C_6H_{10}N_2O_2$; 13.96 g (98.2%); D-form, 182—184 °C, L-form, 176—178 °C, DL-form, 175—177 °C (lit,[11] 176 °C); D-: =49.2°, L-: +43.5°, (*c* 0.1, MeOH).

Synthesis of Butanamide 3. A mixture of the lactam **2** (14.22 g, 0.1 mol), ethyl methylphosphinate (12.97 g, 0.12 mol), and boron trifluoride etherate (1 ml) is gradually heated for 1—1.5 h to 120 °C in a closed steel cylindrical container in an ambience of dry argon or nitrogen. Heating is kept up for another 2—2.5 h at 125—130 °C. After cooling, the reaction mixture is extracted with boiling benzene and the undissolved portion is decanted off. Cooling affords an oil, which crystallizes after being kept for a prolonged period at 0 °C over ethyl acetate/cyclohexane. L-3-Acetamido-4-(ethoxymethylphosphinyl)butanamide (**3**): $C_9H_{19}N_2O_4P$; 14.26 g (56.3%); *a*: 3350—3300, 3170—3010, 1650, 1640, 1320 (P-CH_3), 1280 (P=O), 1110—980 (P-O-C); *c*: 1.28 (3H, s, $COCH_3$), 1.38 (3H, t, *J*=7 Hz, $OC\underline{H}_2CH_3$), 2.03 (3H, d, *J*=17 Hz, PCH_3), 2.1—2.7 (4H, m, $PC\underline{H}_2C\underline{H}_2$), 4.12 (2H, q, $OC\underline{H}_2CH_3$), 4.52 (1H, t, *J*=8 Hz, $C\underline{H}CH_2$), 6.28 (1H, s, NH), 6.46 (2H, s, NH_2); after D_2O-exchange the protons N$\underline{H}$ and NH_2 do not appear; 250.23/250 (19%); 43.20/43.26, 7.65/7.39, 11.19/10.99; +43.9°, (*c* 0.1, MeOH); 162—164 °C (decomp); 0.43-A.

D-**3**: 11.96 g (50.8%); −40.2°, (*c* 0.1, MeOH); 162—164 °C (decomp).

DL-**3**: 12.54 g (53.3%); 149—152 °C (decomp).

Preparation of Phosphinothricin (6). Method A: The phosphinyl ester DL-**3** (25.02 g, 0.1 mol) is boiled for 8 hours in hydrochloric acid (150 ml, 22%). It is then evaporated in vacuum (80 °C/6×10^{-2} Torr) to dryness and placed in a column Dowex 50W-X8 H^+ resin. The column is washed with water, the first strongly acidic eluate is thrown away and washing continues with 0.5 M HCl (1 M=1 mol dm^{-3}). The ninhydrin-positive fraction is evaporated in vacuum to dryness and the residue is crystallized from ethanol/diethyl ether; DL-2-amino-4-(hydroxymethylphosphinyl)butanoic acid hydrochloride (**6**): $C_5H_{12}NO_4P \cdot HCl$; 20.02 g (92.1%); mp 203—205 °C (lit,[9] 202—204 °C).

Method B: Enzyme-Catalyzed Hydrolysis of D-, DL-, and L-3. 800 ml buffer (pH 8.8) are added to a well-homogenized mixture of D-, DL-, or L-**3** (20 g), and "Tween-80" (2 ml). After tempering at 37 °C, about 5 mg (or 10—15 mg, of spread on polyacrylamide) of the enzyme phosphodiesterase I are added. The mixture is stirred at the same temperature for 6 hours and the enzyme is then removed. Concentration and extraction with diisopropyl ether result in the isolation of: L-2-acetamido-4-(hydroxymethylphosphinyl)butanamide (L-**4**): $C_7H_{15}N_2O_4P$; 17.29 g (97.4%); *a*: 3350—3300, 3180—3025, 2845—2435, 1655, 1640, 1300, 1250, 765, 635; *c*: 1.21 (3H, s, $COCH_3$), 1.96 (3H, d, *J*=18 Hz, PCH_3), 2.2—2.7 (4H, m, PCH_2CH_2), 4.50 (1H, t, *J*=8 HZ, $CHCH_2$), 6.06 (1H, s, NH), 6.52 (2H, s, NH_2), 10.2—10.6 (1H, br., POOH); after D_2O-exchange no signals for the N$\underline{H}$, $N\underline{H}_2$, and POOH protons appear; 222.18/222 (36%); 37.84/37.55, 6.80/7.01, 12.61/12.55; +64.8°, (*c* 0.1, MeOH); 185—189 °C (decomp).

D-**4**: 17.07 g (96.3%); −63.7 °, (*c* 0.1, MeOH); 192—194 °C (decomp).

DL-**4**: 17.39g (98.1%); 180—183 °C (decomp).

Enzyme-Catalyzed Hydrolysis of L-4. A mixture of the substrate L-**4** (20 g), "Tween-80" (2 ml) and raw acylase (25 mg, 50% enzyme content) is stirred for 6 h at 25 °C in 500 ml buffer (pH 70). After acidification, passing through an ion-exchange resin (eluent 0.5 M HCl) and evaporation in vacuum to dryness, the reaction residue is crystallized with ethanol/diethylether: L-2-amino-4-(hydroxymethylphosphinyl)butanamide hydrochloride (L-phosphinothricinamide hydrochloride) (L-**5**: $C_5H_{12}N_2O_3P \cdot HCl$: 15.52 g, (79.6%); *a*: 1550 (NH_3^+); *e*: 1.95 (3H, d, *J*=18Hz, PCH_3), 2.2—2.7 (4H, m, PCH_2CH_2), 4.33 (1H, t, $C\underline{H}CH_2$); 180.14/180 (25%); as hydrochloride — 27.73/27.65, 6.51/6.48, 12.93/13.11; +52.3°, (*c* 0.1, H_2O); 250 °C (decomp); 0.43-B.

Enzyme-Catalyzed Hydrolysis of 5. A mixture of the substrate L-**5** (21.65 g, 0.1 mol) and the enzyme glutaminase (5 mg) is stirred for 6 h at 25 °C in 500 ml buffer (pH 4.9). The enzyme is then removed and the reaction mixture is worked up as per Item 4, Method A. Yield of L-phosphinothricin hydrochloride: 21.24 g (97.6%).

Synthesis of Phosphinothricin *O,P*-Diethyl Ester- (7) and 1,2-Azaphospholidine (8). A suspension of D-, DL-, or L-phosphinothricin hydrochloride (**6**) (21.76 g, 0.1 mol), in 200 ml dry dioxane is continuously stirred in a humidity proof vessel, while phosphorus pentachloride (45.81 g, 0.22 mol) in dry dioxane and triethylamine (33.38 g, 0.33 mol) in 200 ml absolute ethanol are simultaneously and gradually added for 120 min at 40—45 °C. The mixture is boiled for 1 h and filtered. The filtrate is evaporated in vacuum to dryness. The reaction residue is dissolved in chloroform and a hydrogenchloride current is passed through it until no more oil is released from the D-, DL-, or L-phosphinothricin *O,P*-diethyl ester (**7**), which is then decanted out and, with some difficulty, crystallized. Yield 62—65%.

The mother layers, obtained after the isolation of the diester **7**, are evaporated in vacuum to dryness and the residue is placed in a silica-gel column and eluted with chloroform: methanol 9:1. The eluent is removed by evaporation and the product is crystallized, with some difficulty, from ethyl acetate/hexane. Ethyl (5*S*)-2-methyl-1,2-azaphospholidine-5-carboxylate 2-oxide (L-**8**): $C_7H_{14}NO_3P$; 3.69 g (19.3%); *a*: 1730, 1385 (P-N), 1320 (P-CH_3), 1270—1100 (P=O, C-O-C); *c*: 1.18 (3H, t, $OOH_2C\underline{H}_3$), 1.73 (3H, d, *J*=17 Hz, PCH_3), 2.2—2.7 (4H, m, PCH_2CH_2), 4.1—4.5 (3H, m, $CHCH_2OCH_2CH_3$), 7.03 (1H, s, NH); 191.17/191 (33%); 43.28/43.18, 7.38/7.03; 7.33/7.69; +74.3°; (*c* 0.1, MeOH); 93—95 °C; 0.72-D.

D-**8**: 3.85 g (21.5%); −70.2°; 90—92 °C.

DL-**8**: 3.65 g (20.4%); 89—91 °C.

Enzyme-Catalyzed Hydrolysis of the Ester 8. A well-homogenized mixture of the substrate L-**8** (19.71 g, 0.1 mol) and 2 ml "Tween-80" is added to a solution (thermostabilized at 25 °C) of the enzyme α-chymotrypsin (10 mg, raw, pancreatic), or 10 mg alkaline mesintericopeptidase, in 500 ml buffer (pH 7.8 and 8.2). The mixture is stirred for 6 h at the same temperature until pH 6.0 is reached and is then condensed. The product is filtered out after prolonged strage in a refrigirator: (5*S*)-2-methyl-1,2-azaphospholidine-5-carboxylic acid 2-oxide-(L-pyrophosphinothricin)-(L-**9**): $C_5H_{10}NO_3P$; 15.19 g (94.2%); *a*: 3320—2800, 1385, 1320, 1255; *e*: 1.71 (3H, d, *J*=17Hz. PCH_3), 2.2—2.7 (4H, m, PCH_2CH_2), 4.48 (1H, t, *J*=6 Hz,$CHCH_3$); 163.1/163 (26%); 36.82/37.05,

6.18/6.01, 859/8.63; +69.7°, (*c* 0.1, H_2O); 186—190 °C (decomp); 0.63-C.

Enzyme-Substrate Interaction of the Amide L-3 with Glutaminase. The method in Item 4.3 is employed. The following product is isolated: L-2-acetamido-4-(ethoxymethyl)-butanoic acid (**10**): $C_9H_{18}NO_5P$; 96.4%; *a*: 3510—3050, 1745, 1645, 1305, 1255, 1110—980; *c*: 1.18 (3H, t, *J*=7 Hz, OCH_2CH_3), 1.38 (3H, s, $COCH_3$), 2.18 (3H, d, *J*=18 Hz, PCH_3), 2.2—2.7 (4H, m, PCH_2CH_2), 4.18 (2H, q, OCH_2CH_3), 4.55 (1H, t, *J*=6 Hz, $CHCH_2$), 6.33 (1H, s, NH), 10.9—11.3 (1H, br., COOH); 251.22/251 (18%); 43.03/42.78, 7.22/7.36, 5.58/5.71; 46.7°, (*c* 0.1, MeOH); 132—135 °C; 0.33-A.

Synthesis of 1,2-Azaphospholidine L-8. Method-A: The method in Item 5 is employed, only now phosphorus pentachloride (22.91 g, 0.11 mol) and triethylamine (22.25 g, 0.22 mol) are used. L-**8** is obtained with a 50.6% (9.67 g) yield and its spectral data are identical with those of the same product obtained earlier.

Method B: A mixture of L-phosphinothricin hydrochloride (L-**6**) (21.76 g, 0.1 mol) and 2 ml *N,N*-dimethylformamide is stirred in dry benzene (200 ml) at room temperature. Thionyl chloride (13.09 g, 0.11 mol) is added in portions for the duration of 1 h, accompanied by a continuous current of dry nitrgen through the reaction mixture. The temperature is then increased to 40—45 °C and, while stirring is kept up, triethylamine (15.15 g, 0.15 mol) in 150 ml absolute ethanol is gradually added. The mixture is boiled for 1 h, filtered and worked up as above. Yield of L-**8**; 11.71 g (61.2%).

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