Organophosphorus Analogues and Derivatives of the Natural L-Amino Carboxylic Acids and Peptides. V.[1] Synthesis of Analogues of Plumbemycin A

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Synopsis. Analogues of antibiotic Plumbemycin A were synthesized by an exchange of D-norvaline residue with 1,2,3,6-tetrahydro-1,2-azaphosphorine and by coupling of alanylaspartic acid with N-terminal norvaline. The protecting groups were released by highly selective enzyme hydrolysis.

With a view to studying the relationship between the structure and the physiological activity, we wish to report some of the chemical modifications of the tripeptide antibiotic Plumbemycin A.[2,3]

As a starting substance for the first modification, we have selected (*R*)-2-ethoxy-1,2,3,6-tetrahydro-1,2-azaphosphorine-6-carboxylic acid 2-oxide (**1**) [H-Phos(OEt)-OH] recently synthesized by us. The condensation with H-Ala-Asp(OEt)-OEt (**2**)[4] was carried out by the usual DCC method for peptide synthesis to give H-D-Phos(OEt)-Ala-Asp(OEt)-OEt in a yield of 72—75%.

As the tetrahydro-1,2-azaphosphorine ring is very unstable under the conditions of both base- and acid-catalyzed hydrolysis, we have chosen the method[5] of enzyme-catalyzed hydrolysis of substrates containing organophosphorus groups.

It was found that the enzyme phosphodiesterase I hydrolyzes only the ethoxyphosphinylidene group of **3** to give the corresponding free cyclic derivative **4** quantitatively.

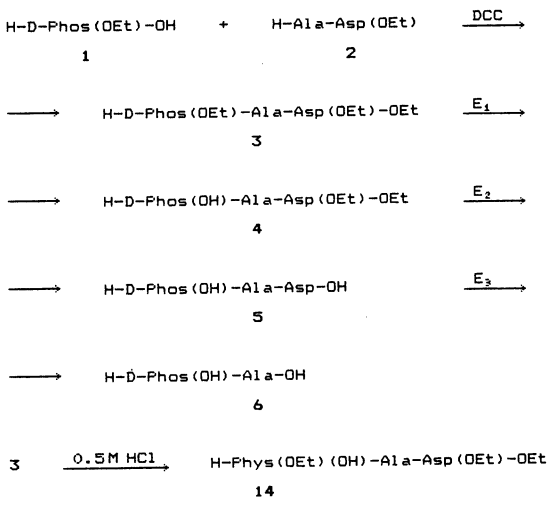
The hydrolysis of the *O*-protected groups of **4** can not be carried out by conventional methods of base- or acid-hydrolysis,because the 1,2-azaphosphorine ring is easily hydrolyzed in the bond PO-NH. The ester hydrolysis of **4** with alkaline mesintericopeptidase was applied successfully to give the corresponding free tripeptide **5**.

When the treatment of **5** with α-chymotrypsin is carried out, only the hydrolysis of the peptide bond in Ala-Asp sequence took place to give H-D-Phos(OH)-Ala-OH (**6**) (see Scheme 1) due to the D-tetrahydro-1,2-azaphosphorine ring. It is worthwhile noting that the catalytic activity of phosphodiesterase I is quite independent on the optical form of 2-ethoxy-1,2,3,6-tetrahydro-1,2-azaphosphorine 2-oxide.

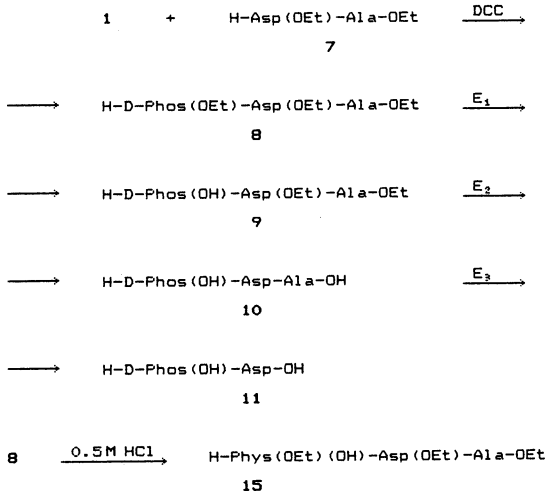
An analogous scheme was applied for the synthesis of the other possible modified peptide with C-terminal Ala residue. The condensation of **1** with H-Asp(OEt)-Ala-OEt (**7**)[6] was carried out by the DCC method described above to give the expected tripeptide **8** obtained in ca. 75% yield. The subsequent enzyme-catalyzed hydrolysis of the ethoxyphosphinylidene group of **8** with phosphodiesterase I was similarly worked up under the above conditions to give the corresponding tripeptide **9**. The two ethylesters of **9** were hydrolyzed with alkaline mesintericopeptidase to give the free tripeptide **10**.

Following Scheme 1, we subjected **10** to a treatment similar to that employed for α-chymotripsin. As was expected, enzyme-substrate interaction took place to give H-D-Phos(OH)-Asp-OH (**11**) in about 90% yield (Scheme 2).

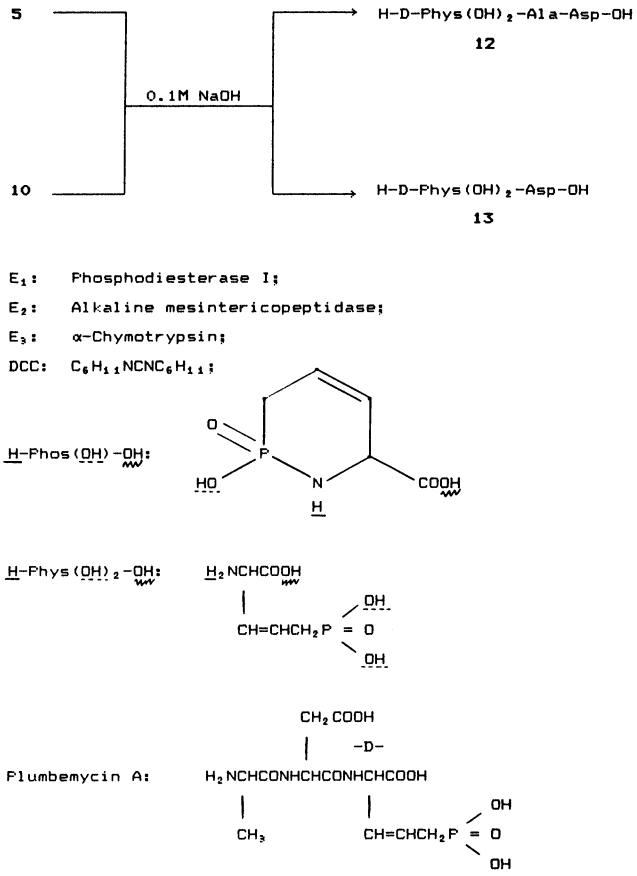
The marked difference in the case of hydrolysis of the peptide bond on the one hand and the 1,2,3,6-tetrahydro-1,2-azaphosphorine ring on the ether makes it possible to be "soft" base-catalyzed hydrolysis and to obtain Plumbemycin A analogues, which L-amino acids were arranged each other. However, heating **5** and **10** in 0.1 M NaOH (1 M=1 mol dm^{-3}), the



Scheme 1.



Scheme 2.



Scheme 3.

H-D-Phys(OH)$_2$-Ala-Asp-OH (**12**) and H-D-Phys-(OH)$_2$-Asp-OH (**13**) were obtained in about 90% yields, respectively (Scheme 3).

The facile hydrolysis of PO-NH bond also occured when compounds **3** and **8** were treated with dilute hydrochloric aicd. As a result, the protected tripeptides **14** and **15**, in which ethoxyl groups remain unchanged, were obtained in about 80% yields in both cases.

Preliminary tests for physiological activity point to some interesting problems with structure changes of the studied compounds. Thus, the modified tripeptides **5** and **10** were found to have a more pronounced bactericidal activity than Plumbemycin A, whereas that of three tripeptides **12**, **13**, and Plumbemycin A is almost the same. The modified aspertic acid **11** was proved to possess the highest bactericidal activity, while that of the modified alanine **6** is much weaker, though still better pronounced than that of Plumbemycin A. A detailed study of the discussed activities is now under way and will be published separately.

Experimental

General. IR spectra, elemental analysis, mp, HPLC, and $[\alpha]_D^{20}$ were measured with Perkin-Elmer instruments; TLC-molybdophosphate acid or ninhydrin detection; enzymes and buffers from "Signa."

H-D-Phos(OEt)-Ala-Asp(OEt)-OEt (3) and H-D-Phos-(OEt)-Asp(OEt)-Ala-OEt (8). A mixture of **1** (20.52 g, 0.12 mol) and dipeptides **2** (26.03 g, 0.10 mol) or **7** (22.70 g, 0.11 mol) and DCC (22.70 g, 0.11 mol) in dry ethyl acetate (250 ml) was stirred at room temperature for 12 h. After filtration, the filtrate is washed consecutively with 5% aqueous sodium carbonate, water, 5% hydrochloric acid, water, and finally dried over anhydrous $MgSO_4$ and distilled. The oily residue was purified on a silica-gel column using a mixture of chloroform and methanol (9:1). After evaporation under vacuum the residue was dissolved in dry ethyl acetate (200 ml) and cooled to −5 °C and then hexane was added until the mixture became turbid. The mixture was left overnight in refrigerator and products precipitated were collected.

Compound 3: Yield, 32.71 g (73.1%); mp 73—75 °C (EtOAc/*n*-C_6H_{14}); R_f: 0.76 (DMF:$CHCl_3$:MeOH=2:8:1); $[\alpha]_D^{20}$ 78.3° (*c* 1, MeOH).

Found: C, 48.66; H, 6.52; N, 9.38%. Calcd for $C_{18}H_{30}N_3O_9P$: C, 48.31; H, 6.76; N, 9.39%.

Compound 8: Yield, 34.14 g (76.3%); mp 98—101 °C (EtOAc/*n*-C_6H_{14}); R_f: 0.80 (DMF:$CHCl_3$:MeOH=2:8:1); $[\alpha]_D^{20}$ 79.1° (*c* 1, MeOH).

Found: C, 48.11; H, 6.93; N, 9.61%. Calcd for $C_{18}H_{30}N_3O_8P$: C, 48.31; H, 6.76; N, 9.39%.

H-Phys(OEt)(OH)-Ala-Asp(OEt)-OEt (14) and H-D-Phys(OEt)(OH)-Asp(OEt)-Ala-OEt (15). A suspension of **3** or **8** (4.47 g, 0.01 mol) in 0.5 M HCl (50 ml) was heated with stirring at 45 °C for 30 min. A homogeneous solution obtained was cooled and neutralized with 1% aqueous sodium carbonate. After extraction with ethyl acetate (3×20 ml) the combined organic extracts were dried over anhydrous $MgSO_4$ and distilled under vacuum to dryness. The residue was dissolved in chilled ethyl acetate (25 ml) and hexane was added until it became turbid. After standing overnight in refrigerator, the tripeptides **14** and **15** were collected, respectively.

Compound 14: Yield, 4.13 g (88.7%); mp 102—104 °C (EtOAc/*n*-C_6H_{14}); R_f: 0.81 (DMF:$CHCl_3$:MeOH=2:1:3); $[\alpha]_D^{20}$ 69.7° (*c* 1, MeOH).

Found: C, 46.31; H, 7.11; N, 8.77%. Calcd for $C_{18}H_{32}N_3O_9P$: C, 46.45; H, 6.93; N, 9.03%.

Compound 15: Yield, 3.83 g (76.3%); mp 111—114 °C (EtOAc/*n*-C_6H_{14}); R_f: 0.80 (DMF:$CHCl_3$:MeOH=2:1:3); $[\alpha]_D^{20}$ 77.7° (*c* 1, MeOH).

Found: C, 46.66; H, 6.76; N, 9.17%. Calcd for $C_{18}H_{32}N_3O_9P$: C, 46.45; H, 6.93; N, 9.03%.

Enzyme-Catalyzed Hydrolysis of 3 and 8. A mixture of the corresponding substrates **3** and **8** (each 20 g), phosphodiesterase I (5 mg or 10—15 mg in case that it is spread on a polymer carrier) in aqueous buffer (500 ml, pH 8.8) was stirred at 37 °C for 6 h. After removal of the enzyme acidification and concentration, the reaction mixture is extracted with diisopropyl ether (3×50 ml). The organic extracts collected are dried over anhydrous $MgSO_4$ and distilled to dryness. The residue was dissolved in dry ethyl acetate (200 ml) and hexane was added until the solution became turbid. After overnight in refrigerator, the products **4** and **9** were filtered.

***N*-[(*R*)-(2-Hydroxy-1,2,3,6-tetrahydro-1,2-azaphosphorin-6-yl)carbonyl]alanylaspartic Acid α,β-Diethyl Ester (4):** Yield, 15.27 g (81.6%); mp 92—94 °C (EtOAc/*n*-C_6H_{14}); IR (KBr) cm^{-1}: 2840—2320, 1760—1735, 1640, 1550, 1250, 1100, 930, 810, 720, 630; R_f: 0.61 (DMF:$CHCl_3$:MeOH=2:8:1); $[\alpha]_D^{20}$ 80.4° (*c* 1, MeOH).

Found: C, 46.00; H, 6.18; N, 9.73%. Calcd for $C_{16}H_{26}N_3O_8P$: C, 45.82; H, 6.25; N, 10.02%.

***N*-[(*R*)-(2-Hydroxy-1,2,3,6-tetrahydro-1,2-azaphosphorin-6-yl)carbonyl]aspartylalanine Asp$^{\beta}$, Ala-Diethyl Ester (9):** Yield, 14.95 g (79.9%); mp approx. 100 °C (decomp); IR (KBr) cm^{-1}: 2840—2350, 1765—1725, 1645, 1250, 1100—930, 815, 770, 635; R_f: 0.66 (DMF:$CHCl_3$:MeOH=2:8:1); $[\alpha]_D^{20}$ 84.2° (*c* 1, MeOH).

Found: C, 45.62; H, 6.33; N, 9.90%. Calcd for $C_{16}H_{26}N_3O_8P$: C, 45.82; H, 6.25; N, 10.02%.

Enzyme-Catalyzed Hydrolysis of 4 and 9. A mixture of the corresponding substrates **4** and **9** (each 20 g), the enzyme alkaline mesintericopeptidase (8 mg) in aqueous buffer (500 ml, pH 7.8) was stirred at 27 °C for 4 h until a ninhydrin-positive detection was observed. After acidification and evaporation of the reaction mixture to dryness, the amorphous residue was extracted with boiling ethanol. After cooling, the corresponding **5** and **10** were filtered.

***N*-[(*R*)-(2-Hydroxy-1,2,3,6-tetrahydro-1,2-azaphosphorin-6-yl)carbonyl]alanylaspartic Acid (5):** Yield, 13.91 g (80.3%); mp 182—186 °C (EtOH); IR (KBr) cm^{-1}: 3855—3200, 2850—2430, 1750, 1640, 1520, 1305, 1255, 985, 840, 720, 630; R_f: 0.61 (dioxane: 25% aqueous ammonia=4:1); $[\alpha]_D^{20}$ 72.6° (*c* 1, NaOH).

Found: C, 39.81; H, 4.71; N, 11.44%. Calcd for $C_{12}H_{18}N_3O_8P$: C, 39.67; H, 11.57; N, 11.57%.

***N*-[(*R*)-(2-Hydroxy-1,2,3,6-tetrahydro-1,2-azaphosphorin-6-yl)carbonyl]aspartylalanine (10):** Yield, 13.20 g (76.2%); mp about 186 °C (decomp) (EtOH); IR (KBr) cm^{-4}: 3620—3200, 2855—2430, 1755, 1640, 1525, 1300, 1250, 980, 845, 715, 630; R_f: 0.61 (dioxane: 25% aqueous ammonia=4:1); $[\alpha]_D^{20}$ 82.3° (*c* 1, 0.1 M NaOH).

Found: C, 39.91; H, 4.76; N, 11.52%. Calcd for $C_{12}H_{18}N_3O_8P$: C, 39.67; H, 11.57; N, 11.57%.

Enzyme-Catalyzed Hydrolysis of 5 and 10. A mixture of the corresponding substrates **5** and **10** (each 20 g), and α-chymotrypsin (5 ml) in aqueous buffer (500 ml, pH 7.8) was stirred at 25 °C for 6 h. After acidification and evaporation under vacuum to dryness, the amorphous residue was extracted with boiling ethanol. After cooling, the corresponding products **6** and **11** were filtered.

***N*-[(*R*)-(2-Hydroxy-1,2,3,6-tetrahydro-1,2-azaphosphorin-6-yl)carbonyl]alanine (6):** Yield, 12.12 g (88.7%); mp about 200 °C (decomp) (EtOH); IR (KBr) cm^{-1}: 3540—3200, 2840—2460, 1755, 1640, 1525, 1300, 1250, 970; ^1H NMR (D_2O, TMS, δ, ppm) 1.38 (3H, d, *J*=8 Hz, $CHC\underline{H}_3$), 2.76 (2H, m, CH_2), 4.2—4.4 (2H, m, 2×CH), 5.55 and 6.28 (2H, m, $C\underline{H}$=$C\underline{H}$) and four exchangeable protons; MS, *m/z*: Found/Calcd, 248/248.1; R_f: 0.66 (*n*-BuOH : AcOH : H_2O=9:1:1); $[\alpha]_D^{20}$ 69.3° (*c* 0.1, 0.1 M NaOH).

Found: C, 38.66; H, 5.35; N, 11.41%. Calcd for $C_8H_{13}N_2O_5P$: C, 38.72; H, 5.28; N, 11.29%.

***N*-[(*R*)-(2-Hydroxy-1,2,3,6-tetrahydro-1,2-azaphosphorin-6-yl)carbonyl]aspartic Acid (11):** Yield, 14.85 g (92.3%); mp, not defined, the substance is gradually decomposing with the melting; IR (KBr) cm^{-1}; 3845—3200, 2850—2420, 1750, 1640, 1525, 1300, 1250, 975, 840, 630; MS, *m/z*: Found/Calcd, 292/292.186; R_f: 0.72 (dioxane: 25% aqueous ammonia=9:1); $[\alpha]_D^{20}$ 83.4° (*c* 1, 0.1 M NaOH).

Found: C, 37.11; H, 4.21; N, 9.66%. Calcd for $C_9H_{13}N_2O_7P$: C, 37.00; H, 4.48; N, 9.56%.

Base-Catalyzed Hydrolysis of 5 and 10. A solution of **5** or **10** (0.1 mol each) in 0.1 M NaOH (100 ml) is heated to 50—60 °C for 30 min. After acidification and vacuum distillation to dryness, the amorphous residue was extracted with boiling ethanol. After cooling, the following products **12** and **13** are filtered:

***N*-(3,4-Didehydro-5-phosphono-D-norvalyl)alanylaspartic Acid (12):** Yield, 35.19 g (92.3%); mp about 220 °C (decomp); IR (KBr) cm^{-1}: 3845—3200, 2840—2395, 1750, 1640, 1520, 1255, 970, 720, 630; R_f: 0.50 (dioxane: 25% aqueous ammonia=4:1); $[\alpha]_D^{20}$ 79.3° (*c* 0.1, 0.1 M NaOH).

Found: C, 37.62; H, 5.44; N, 11.11%. Calcd for $C_{12}H_{20}N_3O_9P$: C, 37.80; 5.29; N, 11.02%.

***N*-(3,4-Didehydro-5-phosphono-D-norvalyl)aspartic Acid (13):** Yield, 33.97 g (89.1%); mp about 200 °C (decomp); IR (KBr) cm^{-1}: 3850—3200, 2850—2400, 1750, 1650, 1520, 1300, 1250; R_f: 0.50 (dioxane: 25% aqueous ammonia=4:1); $[\alpha]_D^{20}$ 72.4° (*c* 0.1, 0.1 M NaOH).

Found: C, 38.09; H, 5.00; N, 10.92%. Calcd for $C_{12}H_{20}N_3O_9P$: C, 37.80; H, 5.29; N, 11.02%.

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