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ORGANOPHOSPHOROUS ANALOGUES AND DERIVATIVES OF NUCLEOTIDES. II. N-PHOSPHONO-, METHYLPHOSPHINOMETHYL-NICOTINAMIDE AND ANALOGUES

IVAN A. NATCHEV

Research Centre "Konstrukcionni Polymeri"
5-003 Gara Iskar, 1528 Sofia, BULGARIA

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Abstract. An Arbuzov analogous transformation is carried out of nicotinamide 1 and N-acetoxymethylnicotinamide 9 with triethylphosphite, diethylphosphonite, 2-ethoxy-1,3,2-dioxaphospholane as well as with phosphorus trichloride, methyldichlorophosphine and 1-chlorine-1,3,2-dioxaphospholane to the esters 2, 3, and 7, and to the acids 4 and 6. The hydrolysis of the cyclic ester 7 leads to the monophosphono ester 8. A high selectivity is established in the enzyme-substrate interaction of the substrates 2 and 3 with the enzymes phosphodiesterase I and alkaline phosphatase to the acids 4 and 6, and the monoester 5. In a similar way, from the N-acetoxymethylated analogues of nicotinamide-nipecotic acid 10, L-pipecolinic acid 11 and L-proline 12 when treated with phosphorus trichloride and methylchlorophosphine, the acids 13-18 are obtained.

Using of inhibitors is one of the often used approaches in biochemical practice for studying the structure and function of the enzymes. With set purpose are synthesized and studied as inhibitors some phosphono-, phosphino analogues and derivatives of the natural amino acids. Osipova et al.[1] have investigated valine and methionine phosphino analogues and they have established the inhibiting activity towards valyl and methionyl-t-RNA-synthetases. Yacovleva[2] have carried out a study of participation of a phosphinate analogue of peptidyladenylic acid in the donor reaction of ribosomes. Naturally occurring phosphinotricine and the tripeptide bialaphos[3] inhibit some enzymatic processes. In addition to their antibiotic properties, it has been reported that they have strong herbicidal activity.

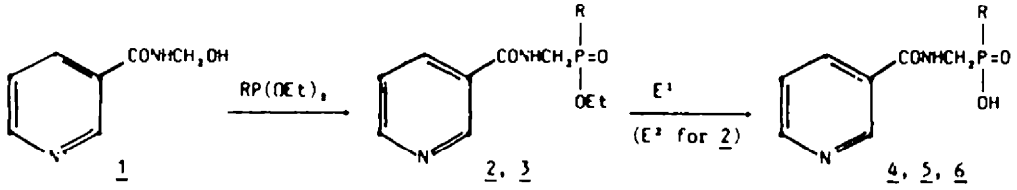
The role of β-nicotinamide adenine coenzymes as cofactors is well known. It is interesting to study the synthesis and properties of these coenzymes using derivatives of the organophosphorus acids. A very rich possibility for such modifications is offered by β-NAD.

As a first study in our project, in the present paper are the syntheses of N-phosphono-, methylphosphinomethyl nicotinamide. With view to study the relationship of chemical structure and physiological activity, analogous derivatives are obtained and from the similar structural analogues of nicotinamide: nipecotamide 10, L-proline homolog-L-pipecolinamide 11 and L-prolinamide 12.

The interaction of N-hydroxymethylnicotinamide 1[4] with triethylphosphite we have carried in conditions close to those described in ref.[5] for similar interaction with some N-hydroxymethylated cyclic amides. At stepwise heating of the components (three times excess of the phosphonite), a sudden boiling of the reaction mixture is observed at about 120°C accompanied by an intensive liberation of ethanol. After

2h heating at 120-130°C the phosphono ester 2 is isolated in a yield of about 70%.

SCHEME 1



1 2, 3 4, 5, 6

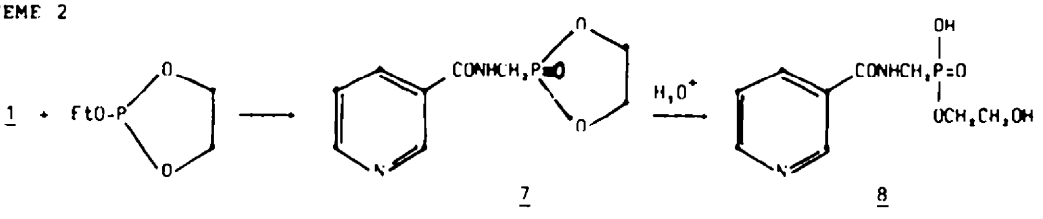
2: R=OEt; 3: R=CH3; 4: R=OH; 5: R=OEt; 6: R=CH3

1 has been treated with diethyl methylphosphonite at 3h boiling of the components (with three times excess of the phosphonite) in dry xylene medium. For the complete running of the interaction it is necessary to use freshly distilled phosphonite. In order to protect the reaction mixture from the atmospheric moisture and oxygen, a continuous current of dry argon or nitrogen is used. The methylphosphino derivative 3 is obtained with a yield of about 55%.

With a view to extend the field of application of Arbuzov-analogous transformation of N-hydroxymethylated amides, in the present work an interaction with 2-ethoxy-1,3,2-dioxaphospholane, a compound containing only one ethoxy group bonded with the phosphorus atom, is carried out. This condensation provides also a verification for the stability of the cyclic ester groups under reaction conditions.

The treatment of 1 with the phospholane has been carried out in conditions completely analogous to these for the phosphonite. In this case liberation of ethanol also has been observed during the interaction. The cyclic phosphono ester 7 is isolated with a yield of less than 40%. This lower yield, in comparison with that of 2 and 3 is due to some side reactions giving oligomeric products.

SCHEME 2



7 8

Liberation of nicotinic acid has been observed at studing the acid or alkaline hydrolysis of the esters 2, 3, and 7. Upon "mild" acid hydrolysis of 7 the opening of the dioxaphospholane cycle is reached to the monophosphono ester 8.

For selective hydrolysis of phosphono-, methylphosphino ester groups we applied the enzyme approach used for unusual substrates[5].

When the enzyme phosphodiesterase I and the substrate phosphono ester 2 are used with a practically quantitative yield the free acid 4 is isolated. The enzyme-substrate interaction is carried out by stirring the reaction mixture containing the substrate 2 (20g), beforehand homogenized in "Tween-80" and the enzyme (10mg spread on a polymer carrier) in an aqueous-buffer medium (500ml, pH 8.8) for 6h at 37°C. In an analogous way the free acid 6 is obtained from the substrate 3 and the same enzyme in a practically quantitative yield.

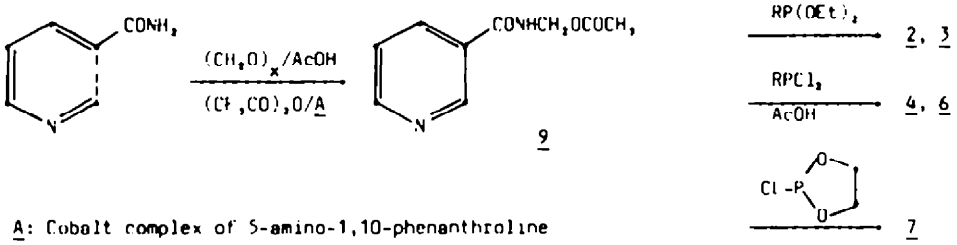
The enzyme alkaline phosphatase shows high selectivity in the enzyme-substrate interaction towards the substrate 2. Used in its optimal pH-range - 10.4 and at temperature of 37°C, the enzyme helps for the hydrolysis only of the one from the two ethoxyphosphono groups of the substrate 2 to the monoester 5 in a yield of

about 90%. The monoester 5 offers a very interesting opportunity for the synthesis of phospha^{C}-peptides in condensation with aminoacids. Such a possibility is realized and soon will be published.

We used another approach to obtain the esters 2 and 3. First, N-acetoxymethylnicotinamide 9 was obtained by treating nicotinamide with paraformaldehyde and trifluoroacetic anhydride in acetic acid medium in the presence of cobaltic complex of 5-amino-1,10-phenanthroline[6] as catalyst.

The interaction of 9 with triethylphosphite and diethylmethylphosphonite was carried out in conditions analogous to these at which the hydroxymethyl derivative 1 had been treated. During the interaction in both cases the liberation of equivalent amount of ethylacetate was observed. The yields of the esters 2 and 3 are 10-15% higher compared with the one of the hydroxymethyl derivative 1.

SCHEME 3



A: Cobalt complex of 5-amino-1,10-phenanthroline

A second approach for the synthesis of the acids 4 and 6 was found by treating the N-acetoxymethyl derivative 9 with phosphorus trichloride and methyldichlorophosphine, respectively.

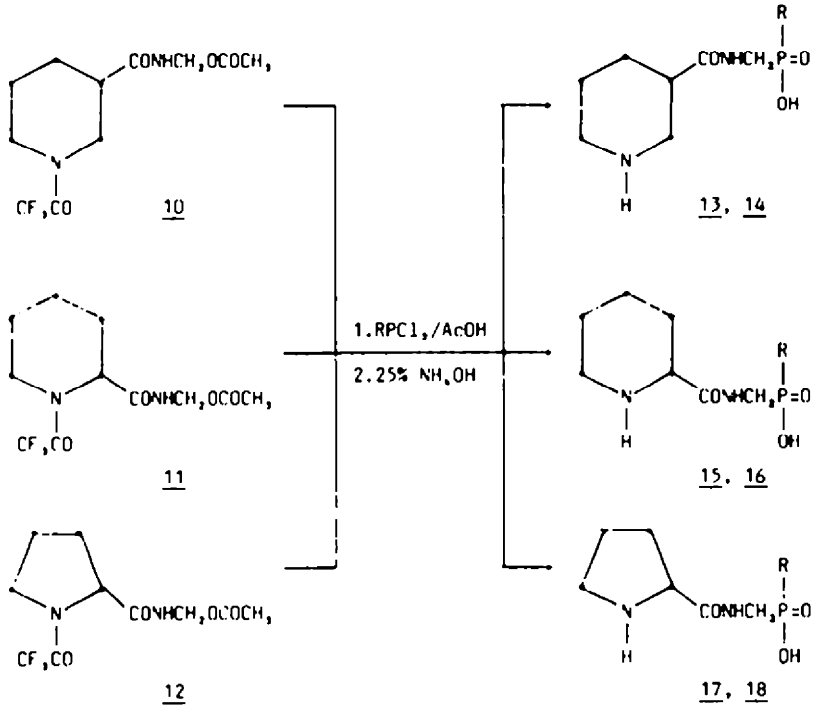
The interaction of 9 with phosphorus trichloride was carried out simply by mixmixing the components and stirring for 3h. An equivalent amount of acetylchloride was obtained as a side product. Without isolating the reaction products, after evaporation of the volatile components in vacuum, acetic acid is added to the reaction residue. Liberation of an equivalent amount of acetylchloride was also observed. The acid 2 is isolated with a yield of about 80%. The yield does not change if an inert solvent is used as a reaction medium. It is interesting to note that the hydroxymethyl derivative 1 treated at these conditions decomposes liberating formaldehyde. Similar decomposition is observed and with the N-hydroxymethylated amides of some other aromatic acids, but it is no characteristic for the amides of alkanoic acids.

The treatment of 9 with methyldichlorophosphine was carried out in an inert solvent medium at three times excess of the phosphine and heating at 40-45°C for 3h. An equivalent amount of acetylchloride was liberated. The volatile components were evaporated in vacuum like in the above interaction with phosphorus trichloride and without isolating the products the reaction mixture was treated with acetic acid. The liberation of acetylchloride is observed and the methylphosphinic acid 3 is isolated in a yield of about 70%.

The third studied substance in condensation with the acetoxymethyl derivative 9 was 2-chloro-1-3-2-dioxaphospholane. The aim of this investigation was to compare the activity of phosphorous compounds containing one, two, and three chlorine atoms bonded with phosphorus as well as to check the behaviour of the cyclic ester group in this interaction. It turns out that there is no essential difference in the behaviour of the three studied compounds. When 9 is treated with 2-chloro-1,3,2-dioxaphospholane in the above described conditions, the cyclic ester obtained above is isolated in a yield of about 55%.

With a view to study the relationship of chemical structure - physiological activity, analogous derivatives of nipecotamide and of the natural L-pipecolamide and L-prolinamide were synthesized. Four variants for their synthesis were studied: synthesis of N-hydroxymethyl and N-acetoxymethyl derivatives and next treatment of each of them with triethylphosphite, diethylmethyl phosphonite, phosphorus trichloride and methyldichoro phosphine. Most successful esperiments are these at which the 1-trifluoroacetylated derivatives are N-acetoxymethylated to 10, 11 and 12 (cf. our later communications) and next treatment with phosphorus trichloride or methyldichlorophosphine and acetic acid.

SCHEME 4



(13, 15, 17): R=OH; (14, 16, 18): R=CH,

The interaction was carried out in conditions completely analogous to the ones described above. The removal of the trifluoroacetyl group was done by treatment with aqueous ammonia in dioxan solution. The methylphosphine derivatives 14, 16, and 18 are hardly crystallizing oils. After their solution in chloroform and passing through hydrogen chloride, the hydrochlorides of 14, 16, and 18 are obtained. The phosphono derivatives are in the form of inner salts. The yields of the substances 13, 15, and 17 are between 65 and 72%, and these of methylphosphine derivatives 14, 16, and 18 are with 10-15% lower.

EXPERIMENTAL

General notes. Elemental analysis, IR-spectra, HPLC, GC, $[\alpha]_D^{20}$, M.w. and m.p. on a Perkin-Elmer instruments; ^1H NMR-spectra on Brucker-250MHz; Mass-spectra on Varian; TLC - silica gel film "Merck", phosphomolybdenate detection; reagents and solvents from "Merck" and "Aldrich"; α-chymotrypsin from "Pharmachim", Bulgaria; phosphodiesterase I, alkaline phosphatase and buffers from "Sigma".

1. Synthesis of the ester 2. A mixture of N-hydroxymethylnicotinamide 1 (15.22g, 0.1mol) and triethylphosphite (49.85g, 0.3mol) is slowly heated during 30min to 120°C. The temperature is kept in the range 130-140°C for 2h. After vacuum distillation of the volatile components, the oily residue is taken to a silica gel column and eluted with chloroform:methanol=9:1 to give the product: (Nicotinamido)methylphosphonic acid ethyl ester 2: $C_{11}H_{17}N_2O_5P$; yield 16.93g (72.1%); b.p., distils with decomp. at 120°C/6.10^{-3}Torr; IR (film, cm^{-1}): 1650, 1260, 1110-930, 840, 720, 650; ^1H

NMR ($CDCl_3$,TMS,δ): 1.26 (6H,t,J=6Hz,2xCH_3), 2.62 (2H,d,J=12Hz,PCH_2), 6.44 (1H,br.,NH), 4.11 (4H,q, J=5.5Hz,2xCH_2), and 7.2-8.4 ppm (4H,m,4xCH); mass-spectra: M^+/e calcd/found 272.240/272 (18%); R_f 0.62 (chloroform:methanol:dioxan=9:1:0.5);

Anal. calcd.: C 48.53, H 6.29, N 10.29%
found: C 48.68, H 5.97, N 10.33%.

2. Synthesis of the ester 3. A mixture of the amide 1 (15.22g, 0.1mol) and diethylmethylphosphonite (40.84g, 0.3mol) is boiled in an atmosphere of dry argon or nitrogen for 3h. After distillation in vacuum of the volatile components, the oily residue is taken to a silica gel column and eluted with chloroform:methanol=9:1 to give:

[(Nicotinamido)methyl]methylphosphinic acid ethyl ester 3: $C_{10}H_{13}N_2O_3P$; yield 13.42g (55.4%); oil, which distils with decomp. at 120°C/6.10^{-3}Torr; IR (film, cm^{-1}): 1650, 1355, 1280, 1110-920; ^1H NMR ($CDCl_3$): 1.44 (3H,t,J=6Hz,OCH_2CH_3), 1.94 (3H,d,J=18Hz,PCH_3), 3.42 (2H,d,J=12Hz,PCH_2), 4.12 (2H,q,J=5.5Hz,OCH_2CH_3), 6.11 (1H,br.,NH), and 7.2-8.4 ppm (4H,m,4xCH); R_f 0.70 (chloroform:methanol:dioxan=9:1:0.5);

Anal. calcd.: C 49.59, H 6.24, N 11.57%
found: C 49.33, H 6.51, N 11.62%
M.w.: calcd./found, 242.214/240.

3. Synthesis of the ester 7. The method described in Item 2 is followed, but the reaction component is 2-ethoxy-1,3,2-dioxaphospholane (40.83g, 0.3mol) and the product is:

2-[(Nicotinamido)methyl]-1,3,2-dioxaphospholane 2-oxide 7: $C_9H_{11}N_2O_4P$; yield 9.49g (39.2%); m.p. 126-128°C; IR (KBr, cm^{-1}): 1650, 1540, 1285, 1200, 1100-900; ^1H NMR ($CDCl_3$): 1.3-1.6 (4H,m,2xOCH_2), 3.48 (2H,d,J=15Hz,PCH_2), 6.18 (1H,br.,NH), and 7.2-8.4 ppm (4H,m,4xCH); R_f 0.62 (chloroform:methanol:dioxan=9:1:0.5);

Anal. calcd.: C 44.64, H 4.58, 11.57%
found: C 44.71, H 4.30, N 11.71%.

4. Enzyme-catalyzed hydrolysis of the esters 2 and 3. To an aquea-buffer medium (500ml, pH 8.8) containing phosphodiesterase I (10mg, spread on a polymer carrier), the ester 2, beforehand homogenized in Tween-80® (2-3ml) is added. The mixture is stirred at 37°C for 6h. After removing of the enzyme, the reaction mixture is acidified and evaporated in vacuum to dryness, then extracted with boiling ethanol (200ml). After cooling the following product is filtered:

(Nicotinamido)methylphosphonic acid 4: $C_7H_9N_2O_4P$; yield 21.09g (97.2%); m.p. about 280°C (decomp.); IR (KBr,cm^{-1}): 2840-2450, 1625, 1555, 1100-920; ^1H NMR (D_2O+Na_2CO_3): 3.71 (2H,d,J=12Hz,PCH_2), 7.2-8.4 ppm (4H,m,4xCH); and three exchangeable protons NH, PO_3H_2; mass-spectra M^+/e, calcd./found 216.133/216 (28%); R_f - 0.33 (dioxan:25%NH_4OH=4:0.5);

Anal. calcd.: C 38.90, H 4.20, N 12.96%
found: C 38.75, H 4.00, N 13.09%.

From substrate 3 (24.22g, 0.1mol) in an analogous way the following product is isolated:

[(Nicotinamido)methyl]methylphosphinic acid 6: $C_8H_{11}N_2O_3P$; yield 20.86g (97.4%); m.p. 265-268°C (decomp.): IR (KBr,cm^{-1}): 2900-2400, 1625, 1550, 1350, 1200, 940, 870, 720, 640; ^1H NMR (D_2O+Na_2CO_3) 1.93 (3H,d,J=18Hz,PCH_3), 3.40 (2H,d,J=12Hz,PCH_2), and 7.2-8.4 ppm (4H,m,4xCH); mass-spectra calcd./found 214.160/214 (20%); R_f - 0.42 (dioxan:25%NH_4OH=4:0.5);

Anal. calcd.: C 44.87, H 5.18, N 13.08%
found: C 44.51, H 5.26, N 13.11%.

Analogously from substrate 2 (27.22g, 0.1mol) and the enzyme alkaline phosphatase (pH 10.4) the monoester is obtained:

(Nicotinamido)methylphosphonic acid monoethyl ester 5: $C_9H_{13}N_2O_4P$; yield 23.98g (98.2%); m.p. about 260°C (decomp.); IR)KBr,cm^{-1}): 2840-2400, 1645, 1555, 1245, 1110-900, 870, 780, 640; ^1H NMR (D_2O+Na_2CO_3): 1.40 (3H,t,J=6Hz,CH_3), 3.42 (2H,d,J=12Hz,PCH_2), 4.12 (2H,q,J=5.5Hz, OCH_2CH_3), and 7.2-8.4 ppm (4H,m,4xCH); mass-spectra M^+/e, calcd./found 244.186/244; R_f - 0.56 (dioxan:methanol: 25%NH_4OH=5:1:0.5);

Anal. calcd.: C 44.24, H 5.37, N 11.47%
found: C 43.96, H 5.63, N 11.21%.

5. Hydrolysis of 7. The ester 7 is added to hydrochloric acid (2N, 120ml), (24.22g, 0.1mol). The mixture is heated in a water bath for 30min. After distillation in vacuum to dryness water is added (100ml) and the mixture is again evaporated in vacuum. Ethanol (120ml) is added to the oily residue. The mixture is boiled to solution. After cooling, the following product is filtered:

(Nicotinamido)methylphosphonic acid mono(2-hydroxyethyl) ester 8: $C_9H_{13}N_2O_5P$; yield 21.36g (82.1%); m.p. 218-221°C (decomp.); IR (KBr,cm^{-1}): 3280-2940, 2860-2400, 1650, 1150, 1255, 1110-900, 870, 740, 630; ^1H NMR (D_2O+Na_2CO_3): 3.40 (2H,d,J=12Hz,PCH_2), 3.8-4.2 (4H,m,2xCH_2) and 7.2-8.4 ppm (4H,m,4xCH); mass-spectra M^+/e, calcd./found 260.183/260 (12%); R_f - 0.62 (DMF:MeOH:dioxan=6:1:1);

Anal. calcd.: C 41.55, H 5.04, N 10.77%
found: C 41.32, H 5.22, N 10.83%.

6. Synthesis of N-acetoxymethyl derivative 9. Nicotinamide (12.21g, 0.1mol), paraformaldehyde (4.50g 0.15mol), trifluoroacetic anhydride (31.50g, 0.15mol) and cobaltic complex of 5-amino-1,10-phenanthroline (0.5g) in acetic acid are heated in a water bath for 1h. After vacuum distillation of the volatile components, the oily residue is passed through Florisil R 100-200 mesh and silica gel column to:

N-Acetoxymethylnicotinamide $C_9H_{10}N_2O_3$; yield 15.26g (78.6%); m.p. 121-124°C; IR (KBr,cm^{-1}): 1740-1650, 980, 740; ^1H NMR ($CDCl_3$): 1.87 (3H,s,CH_3), 2.12 (2H,s,CH_2), 6.15 (1H,br.,NH) and 7.2-8.4 ppm (4H,m,4xCH); mass-spectra M^+/e, calcd./found 194.186/194 (22%); R_f - 0.42 ($CHCl_3$:MeOH:dioxan= 9:1:1);

Anal. calcd.: C 55.67, H 5.19, N 14.43%
found: C 55.42, H 5.38, N 14.56%.

7. Interaction of 9 with triethylphosphite and diethylmethylphosphonite. The methods described in Items 1 and 2 are followed, respectively, with 9 (19.41g, 0.1mol) and triethylphosphite (49.85g, 0.3mol) and diethylmethylphosphonite (40.84g, 0.3mol) to the esters 2 and 3, which have identical IR-spectra and R_f values with the ones obtained above. The yields are: for 2: 22.75g (84.3%), and for 3: 17.63g (71.2%).

8. Interaction of 9 with phosphorus trichloride, methyldichlorophosphine and 2-chlorine-1,3,2-dioxaphospholane. N-acetoxymethylnicotinamide 9 (19.42g, 0.1mol) and the corresponding chlorine derivative (0.3mol of each) in dry carbon tetrachloride (150ml) and continuous current of dry argon or nitrogen are heated in a water bath at 40-45°C for 2-3h. After evaporation of the volatile components in vacuum, acetic acid is added. The reaction mixture is left overnight at room temperature, then evaporated in vacuum to dryness and the amber-coloured oil is treated with hot ethanol to solution. After cooling the corresponding products 4, 6, and 7 are filtered. They have identical IR data, R_f values and no melting temperature depression when mixed with the same substances obtained by enzyme-catalyzed hydrolysis of the esters 2 and 3 and by interaction of 1 and 2-ethoxyphospholane. The yields are: for 4: 17.53g (81.1%), for 6: 14.88g (69.5%), for 7: 13.58g (56.1%).

9. Synthesis of the acids 13-18. N-Acetoxymethylated amides 10, 11, 12 (0.1mol of each) and respectively, phosphorus trichloride and methyldichlorophosphine (0.3mol each) in dry carbon tetrachloride (150ml) are heated in a water bath at 50°C for 2h. After evaporation of the volatile components in vacuum, the oily residue is solved in dry carbon tetrachloride (120ml). Acetic acid is added (30ml). The reaction mixture is left overnight at room temperature and then distilled to dryness in vacuum. The residue is solved in dioxan (150ml) and 25%NH_4OH (50ml) is added. The mixture is heated in a water bath and distilled in vacuum to dryness. Water is added to the residue and again is evaporated in vacuum. The phosphono acids 13, 15 and 17 crystallyzes after treating with boiling ethanol and next cooling. The methylphosphino acids 14, 16 and 18 are dissolved in dry chloroform (100ml) and current of dry hydrogen chloride is passed till the formation of the product is stopped. From the amides 10, 11 and 12 and phosphorus trichloride the substances 13, 15 and 17 are obtained, and respectively, with methyldichlorophosphine, the products 14, 16 and 18.

(±)-(Nipecotamido)methylphosphonic acid 13: $C_7H_{15}N_2O_4P$; yield 15.82g (72.1%); m.p. 280-284°C (decomp.); IR (KBr,cm^{-1}): 2840-2400, 1650, 1550, 1245, 980, 840, 720, 630; ^1H NMR (D_2O+Na_2CO_3): 1.50 (4H,m,2xCH_2), 2.36 (2H,d,J=7Hz,$CHCH_2$), 2.71 (2H,t,J=6Hz,$NHCH_2CH_2$), 3.13 (2H,d,J=12Hz,PCH_2) and 4.11 ppm (1H,m,CHCO) and four exchangeable protons 2xNH, PO_3H_2; mass-spectra M^+/e calcd./found 222.181/222 (10%); R_f - 0.52 (DMF:dioxan:25%NH_4OH=8:2:0.5);

Anal. calcd.: C 37.84, H 6.80, N 12.61%
found: C 38.12, H 6.55, N 12.58%.

L-(Pipecolamido)methylphosphonic acid 15: $C_7H_{15}N_2O_4P$; yield 14.63g (66.7%); m.p. about 280°C (decomp.); IR (KBr,cm^{-1}): 2860-2400, 1645, 1555, 1250, 980, 850, 640; ^1H NMR (D_2O+Na_2CO_3): 1.4-1.6 (6H,m,3xCH_2), 3.12 (2H,d,J=12Hz,PCH_2), 2.75(2H,t,J=6Hz,$NHCH_2CH_2$) and 3.84 ppm (1H,t,J=7Hz,CHCO) and four exchangeable protons 2xNH and PO_3H_2; mass-spectra M^+/e calcd./found 222.181/222 (12%); R_f - 0.48 (DMF:dioxan:25%NH_4OH=8:2:0.5); $[\alpha]_D^{20}$ +48.3°, c=1, 0.1N NaOH;

Anal. calcd. C 37.84, H 6.80, N 12.61%
found C 37.62, H 7.03, N 12.80%.

L-(Prolinamido)methylphosphonic acid 17: $C_6H_{13}N_2O_4P$; yield 17.74g (75.6%); m.p. 258-262°C (decomp.); IR (KBr,cm^{-1}): 1650, 1550, 1250, 940, 870, 750, 630; ^1H NMR (D_2O+Na_2CO_3): 1.66 (4H,m,2xCH_2), 3.33 (2H,d,J=12Hz,PCH_2), 2.70 (2H,t,J=7Hz,$NHCH_2CH_2$) and 4.18 ppm (1H,t,J=6Hz,CHCO) and four exchangeable protons 2xNH and PO_3H_2; mass-spectra M^+/e calcd./found 208.154/208; R_f - 0.60 (DMF:dioxan:25%NH_4OH=8:2:0.5); $[\alpha]_D^{20}$ +55.5°, c=1, 0.1N NaOH;

Anal. calcd.: C 34.62, H 6.29, N 13. 46%
found: C 34.85, H 6.12, N 13.58%.

(±)-[(Nipecotamido)methyl]methylphosphinic acid hydrochloride 14: $C_7H_{15}N_2O_3P.HCl$; yield 15.94 (62.1%); m.p. about 230°C (decomp.); IR (KBr,cm^{-1}): 3000-2400, 1640, 1570, 1350, 1000, 930, 840; ^1H NMR (D_2O+NaOD): 1.52 (6H,m,3xCH_2), 1.92 (3H,q,J=18Hz,PCH_3), 2.80 (2H,t,J=7Hz,$NHCH_2CH_2$), 3.16 (2H,d, J=12Hz,PCH_2), and 4.07 ppm (1H,t,J=7Hz,CHCO) and three exchangeable protons 2xNH and POH; mass-spectra M^+/e, free base, calcd./found 220.207/220; R_f 0.60 (dioxan:25%NH_4OH=9:1); $[\alpha]_D^{20}$ +52.3°, c=1, 0.1N NaOH;

Anal. calcd.: C 37.44, H 7.07, N 10.91%
found: C 37.62, H 7.00, N 11.12%.

L-[(Prolinamido)methyl]methylphosphinic acid hydrochloride 18: C H N O P.HCl; yield 17.01g (7 (70.1%); m.p. 262-265°C (decomp.); IR (KBr,cm^{-1}): 3020-2410, 1650, 1555, 1355, 1010, 900, 875, 720, 635; ^1H NMR (D_2O+NaOD): 1.50 (4H,m,2xCH_2), 1.94 (3H,d,J=18Hz,PCH_3), 3.17 (2H,d,J=12Hz,PCH_2), 2.75 (2H,t,J=7Hz,$NHCH_2CH_2$) and 4.16 ppm (1H,t,J=6Hz,CHCO) and three exchangeable protons 2xNH and POH; mass-spectra M^+/e, free base, calcd./found 206.198/206; R_f - 0.72 (DMF:dioxan:25%NH_4OH=8:2:0.5); $[\alpha]_D^{20}$ +61.2°, c=1, 0.1N NaOH;

Anal. calcd.: C 34.65, H 6.65, N 11.54%
found: C 34.78, H 6.38, N 11.62%.

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