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ORGANOPHOSPHOROUS ANALOGUES AND DERIVATIVES OF NUCLEOTIDES. I. PHOSPHONO- AND METHYLPHOSPHINO ANALOGUES OF AMINOACYL-5'-ADENILIC ACID

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Abstract - The organophosphorous analogues 2-7 of aminoacyl-5'-adenilic acid 1 have been synthesized by substitution of the anhydride oxygen atom with a methylene group, and of the hydroxyphosphinyl group with a methyl one. A new synthetic method has been developed for obtaining organophosphorous acids by condensation of the acidic chlorides of glycine, L-alanine and L-phenylalanine (N-acetyl derivatives, containing $EtOCOCH_2PO(OEt)R$, where R=OEt, CH_3), using butyl-lithium, to esters 8-13, and by enzyme-catalyzed hydrolysis of the ethoxycarbonyl group and subsequent decarboxylation to the phosphino- and phosphono esters 14-19. Strict selectivity has been observed in the enzyme-substrate interaction with the substrates 14-19, which contain ethoxyphosphinyl groups, and the enzymes: phosphodiesterase I and alkaline phosphatase, to the acids 20-25. These acids, when condensed by the DCC method with a protected adenosine, afford 5'-phosphino, phosphono esters 26-31. The protective groups of 26-28 have been removed by mineral and selective enzyme-catalyzed hydrolysis to give 2-4, while the protective groups of 29-31 removed by treatment with phosphorus pentachloride to 32-34, followed by enzyme and mineral hydrolysis to 5-7. Upon mineral hydrolysis of 14, 17, 26, and 29, the aminoketophosphinic acid 35 and the aminoketophosphonic acid 36 have been isolated. The trialkylphosphine oxide 37 has been obtained by using a double quantity of N-acetylglycylchloride and (ethoxymethylphosphinyl)-acetic acid ethyl ester with butyl-lithium as condensing agent. The aminoketo(phosphinoxido)carboxylic acid has been realesed by mineral hydrolysis from 37. Successive enzyme-catalyzed hydrolysis of the substrates 8-13 with α-chymotrypsin and then phosphodiesterase I affords a new class of optically active β-amino-α-keto-α-(phosphino-, phosphono)carboxylic acids 39-44.

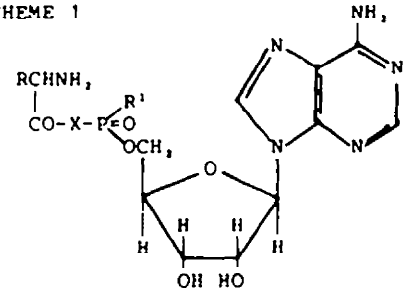
A key stage in the biosynthesis of the proteins' is the formation of a co-anhydride between the introduced amino acid and the 5'-adenilic acid. This anhydride is bonded with the active centre of an enzyme, which is specific for each amino acid. The second stage is transferring the aminoacyl group from the co-anhydride to the t-RNA: the protein is formed as a result of a peptidyltransferase reaction. It could be expected that, should the transfer of the co-anhydride to the t-RNA be prevented, favourable conditions would be created for blocking "in vivo" of the protein synthesis, i.e. a new type of anti-metabolites would be obtained.

To check the validity of this hypothesis, we investigated synthesis of the analogues of the monoacyl-5'-adenilic acid 1 by substitution of an oxygen atom from the anhydride group - $RCH(NH_2)CO[O]PO(OH)A$ with $[CH_2]$, where R is the residue of the amino acid and A is an adenosine residue. Another possibility is to substitute the hydroxyphosphinyl group OH with a CH_3 group. In this case it is assumed that,

due to the insignificant structural changes the analogues 2-7 will at some stage be included into the enzyme process, which regulates the biosynthesis of the coded protein, but the peptidyltransferase reaction will not actually take place, due to inhibition of the aminoacyl-tRNA synthetase, or peptidyltransferase, and to the impossibility to hydrolyze the $COCH_3$ and CH_3PO groups.

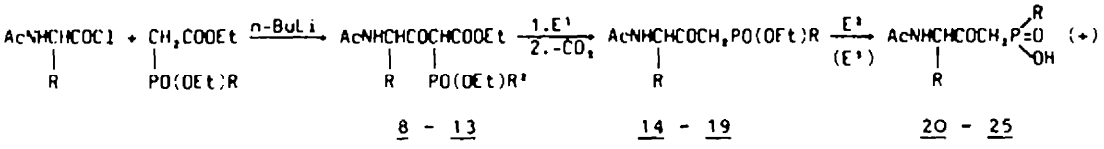
Starting products for the synthesis of 2-7 were the N-acetylated acidic chlorides of glycine[2], L-alanine - synthesized as described in refs.[2,3]: AcCl, PCl_5, 2h, 0°C, yield 72-75%, m.p. 72-74°C (from AcCl), and of L-phenylalanine[3]. Butyl-lithium is used in the condensation of these chlorides with (ethoxymethylphosphinyl)acetic acid ethyl ester[4] and triethylphosphonoacetate (commercially available). As a result the polyfunctional ketons 8-13 are isolated in a satisfactory yield. The condensation does not affect the optical activity of the acidic chlorides of L-alanine and L-phenylalanine. The relatively low yields of the derivatives 8-10 are due to partial interaction between the methyl group at the phosphorus atom and the butyl-lithium, and then the acidic chlorides of glycine, L-alanine and L-phenylalanine.

SCHEME 1

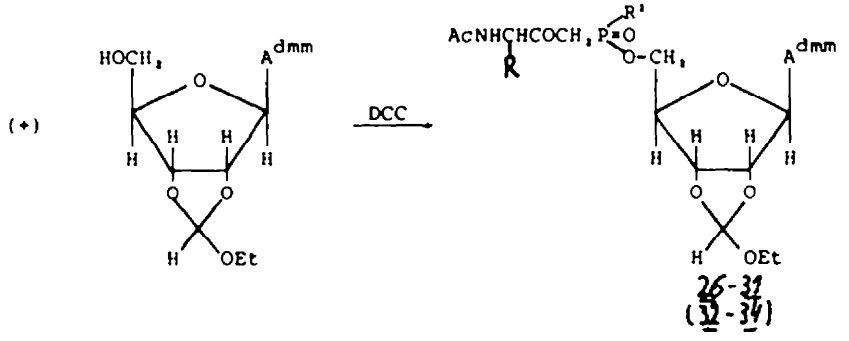


	X	R'	R
1	O	OH	amino acid residue
2	CH_2	CH_3	H(Gly)
3	CH_2	CH_3	CH_3(L-Ala)
4	CH_2	CH_3	$CH_2C_6H_5$(L-Phe
5	CH_2	OH	H
6	CH_2	OH	CH_3
7	CH_2	OH	$CH_2C_6H_5$

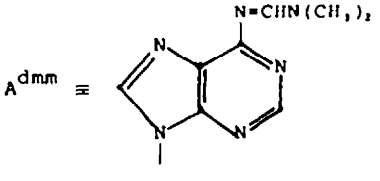
1 - 7



8 - 13 14 - 19 20 - 25



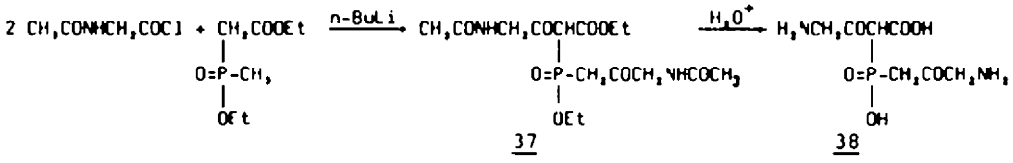
26-31 (32-34)



E^1 : Alkaline mesintericopeptidase
E^2 : Phosphodiesterase I
E^3 : Alkaline phosphatase

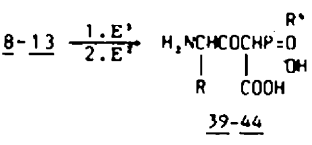
Experiments with a double quantity of N-acetylglycyl chloride afforded the ester amide 37 in a yield of about 80%. Upon acid hydrolysis, it realeses the acid 38. Studies on the intramolecular cyclization of the polyfunctional acid 38 and its physiological activity are under way and will be published separately.

SCHEME 2



Deesterification of the ethoxycarbonyl function of 8-13 was carried out by an original method for the enzyme-catalyzed hydrolysis of esters of unusual substrates.[6] It turned out that α-chymotrypsin cannot be of use for the synthetic purposes in hand. This enzyme enhances the hydrolysis of both ethoxycarbonyl and N-acetyl groups of the substrates 8-13 to free aminoketocarboxylic acids. With a view to liberating the methylphosphino- and phosphono acids as well, we used phosphodiesterase I as the phosphoterase enzyme without isolating the products from the decomposition with the enzyme α-chymotrypsin. Thus, with a yield of over 90%, the aminoketomethylphosphino(-phosphono)carboxylic acids 39-44 were isolated.

SCHEME 3



39-44

	39	40	41	42	43	44
R :	H	CH_3	$CH_2C_6H_5$	H	CH_3	$CH_2C_6H_5$
R':	CH_3	CH_3	CH_3	OH	OH	OH

Despite the fact that the synthesis of 8-13 leads to the formation of a new asymmetric centre (C*HPO), the use of the enzyme α-chymotrypsin and phosphodiesterase I does not result in the separation of the optical antipodes: both the L- and D-form are hydrolyzed, when using D,L-alanine and D,L-phenylalanine as starting materials. However, if the L-configuration of the C*-carbon atom is preserved, the compounds 40, 41, 43, and 44 are optically active.

Hydrolysis of the esters 8-13 can be carried out by treatment with mineral acids and alkali. Unfortunately, the hydrolysis (6h boiling in 22% hydrochloride acid) of the first optically active ester studied 11, resulted in a complete loss of the optically activity of the obtained acid 42 (yield 85%). Spectral data showed that the low hydrolyzability of 11 is due to the diethoxyphosphinyl group. This comparison brings out the advantages of the enzyme approach in the case when optically active substrates are involved.

The third enzyme we applied to the unusual substrates was alkaline mesintericopeptidase. At its temperature and pH optimum (25°C, pH 8.0), the enzyme enhances the hydrolysis of only the ethoxycarbonyl group of the substrates 8-13. It is not necessary to isolate the free acids in pure form. It would suffice, once the hydrolysis is over (20g substrate, 15mg enzyme, 6h, 25°C in 500ml buffer, pH 8.0) and the acidified solution has been destilled in vacuum to suspend the reaction residue in xylol containing catalytic quantities of aniline, and to heat the mixture until carbon dioxide is no longer released. Thus, the corresponding methylphosphino- and phosphono esters 14-19 are isolated in yields of 70-80%.

The chemical transformation that come next requires a protected amino group and a free methylphosphino (-phosphono) acidic function. Considerable complications oc-

cur when both hydroxyl groups of the phosphono group are free. If esterification with 5'-adenosine is carried out, this again, causes complications. The problem could to some extent be solved by treatment of the diethoxyphosphono derivative 17 with phosphorus pentachloride, followed by decomposition of the resultant chloroethoxyphosphinyl derivative with ice. However, the rather low yield (about 30%) prompted us to look after for some other ways. Analogous treatment of the methylphosphino ester 14 affords even lower yields. We attempted acidic hydrolysis of the ester group of 14, but the N-acetylgroup turned out to be the most easily hydrolyzable. The phosphono ester 17 behaves analogously. After several hours heating in hydrochloric acid satisfactory yields of the aminoketophosphinic acid 35 and the aminoketophosphonic acid 36 are obtained. It is dificult to protect the amino groups of 35 and 36 as acetyl groups due to the strong tendency to enolization: O-acetyl derivatives are obtained. All other protective groups we experimented with, proved to be also unsuitable.

The problems were all solved by the application of enzyme-catalyzed hydrolysis processes. The combination of phosphodiesterase I and the esters 14-16 as substrates afforded the free acids 20-22 in practically quantitative yields. The enzyme-substrate interaction was carried out by the general method described above: the substrate (20g), emulsified with Tween-80, is added to a tempered buffer medium (pH 8.8, 37°C), containing 10-15mg of the enzyme phosphodiesterase I on a polymer carrier. This is accompanied by continuous stirring. Stirring is kept for 6h more after which the enzyme is removed and the reaction mixture is acidified, concentrated and cooled down. The same enzyme was used for nine consecutive experiments (substrates 14-16 and 39-44) and no inhibition of its activity was noted.

The enzyme alkaline phosphatase with the phosphono esters 17-19 as substrates leads to the isolation of the monophosphono esters 23-25 in practically quantitative yields (conditions as above).

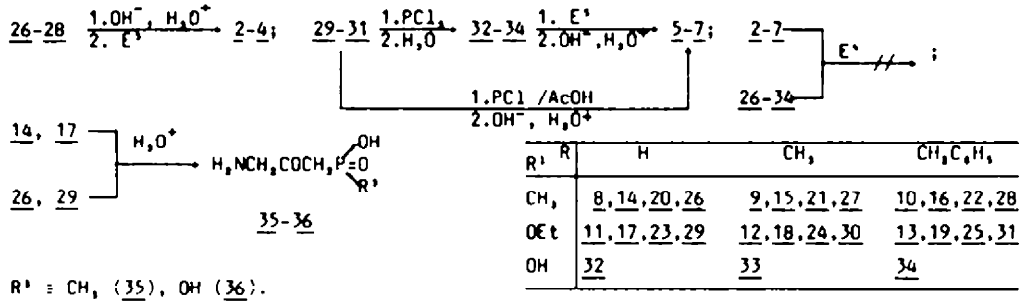
The condensation of the methylphosphino acids 20-22 and the monophosphono esters 23-25 with N-dimethylaminomethylene-2',3'-O-ethoxymethylenadenosine[5] proceeds with very good yields by the N,N'-dicyclohexylcarbodiimide (DCC) method. We tried to use the chlorides of the acids 20 and 23, but the yields were unsatisfactory and the method was abandoned.

Condensation by the DCC-method is achieved by simply mixing the components in the the presence of a slight excess of DCC and then leaving the mixture for 48h. Routine work-up leads to the isolation of the 5'-esters of adenosine 26-31 (yield 60-70%) fully protected in their functional groups.

We failed to liberate the ethoxyphosphinyl protective group of the esters 29-31 by means of enzymatic hydrolysis. In contrast to all hitherto studied cases of selective enzymatic hydrolysis of phosphino and phosphono esters by using 5'- and 3'-exonucleases (phosphodiesterase I and II), endonuclease, and alkaline and acidic phosphatase, to enzyme-substrate interaction with the substrates 29-31 occured and they remained unchanged. Attempts at initial liberation of the other protective groups, followed by enzyme-catalyzed hydrolysis in the presence of the same enzymes were unsuccessful as well. Thus, we decided to treat first 29-31 with phosphorus pentachloride and then, without isolating the chlorinated products, to treat the reaction mixture with ice. Chromatographic separation resulted in the isolation of the 5'-adenosinephosphono esters 32-34 in yields between 20 and 25%.

Enzyme hydrolysis with α-chymotrypsin of 32-34, followed by removal of the adenosine protective groups by routine procedures[5], affords the entirely free analogues of aminoacyl-5'-adenilic acid 5-7, in yields of approx. 30%. Apart from the enzyme approach, still another pathway exists to the synthesis of 5-7. After treatment of the completely protected adenosine derivatives 29-31 with phosphorus pentachloride,

SCHEME 4



R¹ \ R	H	CH_3	$CH_2C_6H_5$
CH_3	8,14,20,26	9,15,21,27	10,16,22,28
OEt	11,17,23,29	12,18,24,30	13,19,25,31
OH	32	33	34

E¹ : (3'- and 5'-Exonuclease, Endonuclease, Phosphatase (alkaline and acid); E² : α-Chymotrypsin

the reaction mixture is left overnight in acetic acid. The volatile components are evaporated in vacuum and the adenosines 5-7 are isolated in a yield of 15-20% by treatment with ammonia.

The absence of an ethoxyphosphoryl group in the structure of the adenosine derivatives 26-28 considerably simplifies the problem of obtaining the free methylphosphino analogues 2-4. Mineral hydrolysis of the adenosine protective groups by the method described above[5] and enzyme-catalyzed hydrolysis of the N-acetyl groups with α-chymotrypsin would suffice to isolate the phosphino analogues 2-4 in a yield of 50-55%. Notwithstanding the type of the ester bond (phosphono or phosphino) and the presence or absence of protective groups, in none of the fifteen analogues of 5'-aminoacyladenosinic acid 2-7 and 26-34, was enzymatic decomposition of the P-5'-O-adenosine bond observed with the participation of endo- and exonuclease and alkaline and acidic phosphatase. Of course, when mineral hydrolysis is applied the products 26 and 29, much like 14 and 17, released the aminoketomethylphosphino acid 35 and the aminoketomethylphosphono acid 36.

Preliminary biological tests have shown that the phosphono analogues 5-7 possess a very well pronounced bactericidal activity. The type of the R-group of 5-7 does not have any influence on this activity. Reversely, the fungicidal activity of the phosphino analogues 2-4 is much stronger than this of the phosphono derivatives. All tested compounds were found to possess a statistically reliable anti-tumour activity, although in a varying extent, towards an experimental tumour L1210 in mice. However, towards insects 2-7 are inactive. Compounds 5-7 display a slight growth-stimulating effect on seeds from mono- and dicotyledons (wheat and cucumber), whereas the effect of the phosphino analogues 2-4 is growth-suppressing (herbicidal). This effect can most clearly be observed in the phosphino and phosphono acids 35 and 36. When two-weeks-old sprouts of sample plants, grown in a vegetation chamber, were treated with the acids 35 and 36 in doses corresponding to 150g/1000m², they all perished completely. The polyfunctional acid 38 gives every hope for very successful results in the future. When applied to an experimental tumour L1210 in mice, the T/C index was 186% at LD_{10} = 620mg/kg.

At the moment, detailed studies are going on of the rather versatile physiological activity of the newly synthesized phosphino and phosphono analogues of aminoacyl-5'-adenilic acid 1. The author is inviting the cooperation of any colleagues who might be interested to work in this field.

EXPERIMENTAL

General notes. IR-spectra, elemental analysis, $[\alpha]_D^{20}$ and HPLC - on a "Perkin-Elmer" instruments; ^1H NMR-spectra - on Jouel-100MHz and Brucker-250MHz; Mass-spectra - on LKB-900 and Varian; TLC - silica gel film, phosphomolybdenite detection; reagents and solvents - "Merck" and "Aldrich"; alkaline

mesintericopeptidase was kindly supplied by Dr. Stambolieva, Institute of Organic Chemistry, Bulgarian Academy of Sciences; and α-chymotrypsin - by "Pharmachim", Bulgaria.

The analytical data for all compounds having numbers in the text are given in the following order: chemical formula; yield; IR-spectrum (KBr cm^{-1}); ^1H NMR-spectrum: DMSO-d_6, $CDCl_3$, D_2O+NaOD, TMS, δ-scale, ppm (number of protons are calculated along the integral curve, multiplicity); mass-spectrum: M⁺/e, calcd./found, (%); elemental analysis, % C, H, N calcd./found; R_f; m.p.; $[\alpha]_D^{20}$.

1. Synthesis of the esters 8-13. A butyl-lithium solution (2.5M solution in hexane, "Aldrich"), cooled to -78°C, is added to a solution, also cooled to -100°C, of (ethoxymethylphosphinyl)acetic acid ethyl ester and triethyl phosphonoacetate (0.12M each) in dry tetrahydrofuran. The addition is accompanied by continuous stirring. After 5 min the mixture is gradually added to a dry tetrahydrofuran solution (cooled to -78°C) of the N-acetyl chlorides of glycine, L-alanine or L-phenylalanine (0.12M). All procedures are carried out in an ambience of dry argon or nitrogen. For the duration of two hours, the temperature is gradually brought back to ambient. The mixture is evaporated in vacuum to dryness, extracted with chloroform, washed with water, dried over non-aqueous magnesium sulphate, and evaporated in vacuum to an oily product, which is crystallized with some dificulty after a prolonged period in refrigerator over ethylacetate/hexane. The following products are isolated:

[4-Acetylamino-3-oxo-2-(ethoxymethylphosphinyl)]butanoic acid ethyl ester, 8: $C_{11}H_{20}NO_6P$; 18.27g (62.3%); 1760-1740 (CO), 1645 (CONH), 1310 ($P-CH_2$), 1250 (P=O), 1110-980 (P-O-C); ($CDCl_3$): 1.2-1.9 (9H,m), 2.18 (3H,d,J=16Hz), 3.83 (1H,d,J=15Hz), 4.0-4.4 (6H,m), 5.20 (1H,br.); 293.3/293 (26%); 45.05/44.83, 6.87/7.00, 4.78/4.39; 0.62 ($CHCl_3$:MeOH = 9:1); 78-80°C.

[4-Acetylamino-3-oxo-2-(ethoxymethylphosphinyl)]-L-pentanoic acid ethyl ester, 9: $C_{12}H_{22}NO_6P$; 18.25g (59.4%); 1760-1740, 1640, 1305, 1250, 1105-975; 1.2-2.0 (12H,m), 2.22 (3H,d,J=16Hz), 3.80 (1H,d,J=15Hz), 4.10 (1H,q), 4.2-4.4 (4H,m), 5.20 (1H,br.); 307.3/307 (18%); 46.92/47.11, 7.22/7.26, 4.56/4.47; 0.60; 63-65°C; +42.6°, c = 0.1, MeOH.

[5-Phenyl-4-acetamino-3-oxo-2-(ethoxymethylphosphinyl)]-L-pentanoic acid ethyl ester, 10: $C_{18}H_{26}NO_6P$; 24.67g (64.2%); 1760-1740, 1640, 1310, 1250, 1100-985, 860; 1.2-1.8 (9H,m), 2.16 (3H,d, J=16Hz), 3.84 (1H,d,J=15Hz), 4.0-4.5 (7H,br.), 7.12 (5H,s); 383.4/383 (10%); 56.39/56.72, 6.84/6.71, 3.65/4.00; 0.49; 93-95°C; +55.2°.

[4-Acetylamino-3-oxo-2-(diethoxyphosphinyl)]butanoic acid ethyl ester, 11: $C_{12}H_{22}NO_6P$; 25.00g (77.2%); 1780-1740, 1640, 1255, 1110-960; 1.2-1.9 (12H,m), 3.79 (1H,d,J=15Hz), 4.0-4.5 (8H,m), 5.20 (1H,br.); 323.3/328 (8%); 44.58/44.69, 6.86/7.01, 4.33/3.98; 0.39; 102-104°C.

[4-Acetylamino-3-oxo-2-(diethoxyphosphinyl)]-L-pentanoic acid ethyl ester, 12: $C_{13}H_{24}NO_6P$; 27.79 (82.4%); 1780-1740, 1645, 1250, 1115-960; 1.2-2.0 (15H,m), 3.82 (1H,d,J=15Hz), 4.08 (1H,q), 4.3-4.6 (6H,m), 5.20 (1H,br.); 337.3/337 (11%); 46.29/46.37, 7.17/6.96, 4.15/4.35; 0.46; 95-98°C; +55.3°.

[5-Phenyl-4-acetylamino-3-oxo-2-(diethoxyphosphinyl)]-L-pentanoic acid ethyl ester, 13: $C_{19}H_{28}NO_6P$; 31.96g (77.3%); 1780-1740, 1645, 1255, 1100-965, 860; 1.2-1.9 (12H,m), 3.84 (1H,d,J=15Hz), 3.96 (1H,t), 4.2-4.5 (8H,m), 5.20 (1H,br.), 7.14 (5H,s); 413.4/413 (8%); 55.20/55.31, 6.83/6.59, 3.39/3.63; 0.50; 112-115°C; +53.6°.

2. Enzyme-catalyzed hydrolysis and decarboxylation of the esters 8-13. Each of the substrates 8-13 (20g) and the enzyme alkaline mesintericopeptidase (15mg) are stirred for 6h in an aqueous buffer (500ml, pH 8.0). After acidification and vacuum distillation to dryness, the reaction residue is suspended in dry xylol (200ml), containing aniline (5ml), and the mixture is heated until no more carbon dioxide is released. The reaction mixture is washed with water, dried over anhydrous magnesium sulphate and distilled to an amorphous mass, which is crystallized from ethylacetate/hexane to the products:

(3-Acetylamino-2-oxopropyl)methylphosphinic acid ethyl ester, 14: $C_6H_{12}NO_5P$; 14.12g (93.6%), after decarboxylation: 11.34g (80.2%); 1740, 1645, 1300, 1255, 1110-980; 1.36 (3H,t), 1.94 (3H,s), 2.28 (3H,d,J=16Hz), 3.92 (2H,q), 4.12 (2H,s), 4.38 (2H,d,J=15Hz), 5.20 (1H,br.); 221.2/221 (23%); 43.44/43.72, 7.29/6.98, 6.33/6.28; 0.69; 124-126°C.

L-(3-Acetylamino-2-oxobutyl)methylphosphinic acid ethyl ester, 15: $C_7H_{14}NO_5P$; 14.40g (94.1%) and 10.70g (74.3%); 1740, 1640, 1305, 1250, 1115-980; 1.39 (3H,t), 1.64 (3H,d), 1.91 (3H,s), 2.32 (3H,d, J=16Hz), 3.96 (1H,q), 4.33 (2H,d,J=15Hz), 4.44 (2H,q), 5.20 (1H,br.); 235.2/235 (21%); 45.96/46.08, 7.71/7.59, 5.96/6.06; 98-100°C; 0.73; +39.6°.

L-(4-Phenyl-3-acetylamino-2-oxobutyl)methylphosphinic acid ethyl ester, 16: $C_{13}H_{18}NO_5P$; 15.03g (92.8%) and 10.70g (71.2%); 1745, 1640, 1305, 1115-980, 860; 1.35 (3H,t), 1.95 (3H,s), 2.35 (3H,d, J=16Hz), 3.91 (1H,t), 4.1-4.4 (6H,m), 5.20 (1H,br.), 7.13 (5H,s); 311.3/311 (18%); 57.87/58.03, 7.12/6.96, 4.50/4.36; 0.48; 126-129°C; +48.6°.

(3-Acetylamino-2-oxopropyl)phosphonic acid ethyl ester, 17: $C_7H_{14}NO_5P$; 14.06g (90.5%) and 10.42g (74.1%); 1765-1740, 1645, 1250-1230, 1120-960; 1.28 and 1.39 (6H,t), 1.97 (3H,s), 3.96 (2H,d), 4.18 (2H,d,J=15Hz), 4.29 and 4.37 (4H,q), 5.20 (1H,br.); 251.2/251 (12%); 43.03/42.88, 7.22/7.36, 5.58/5.63; 0.48; 136-139°C.

L-(3-Acetylamino-2-oxobutyl)phosphonic acid ethyl ester, 18: $C_8H_{16}NO_5P$; 14.04g (89.3%) and 9.43g (67.2%); 1780-1740, 1645, 1260-1240, 1115-965; 1.25 and 1.36 (6H,t), 1.71 (3H,d), 2.00 (3H,m), 3.95 (1H,q), 4.21 (2H,d,J=15Hz), 4.31 and 4.39 (4H,q), 5.20 (1H,br.); 265.2/265 (10%); 45.28/45.33, 7.60/7.71, 5.28/4.89; 0.39; 125-128°C; +39.3°.

L-(4-Phenyl-3-acetylamino-2-oxobutyl)phosphonic acid ethyl ester, 19: $C_{14}H_{20}NO_5P$; 15.57g (94.3%) and 10.81g (69.4%); 1760-1740, 1640, 1265-1240, 1110-960, 860; 1.21 and 1.36 (6H,t), 2.00 (3H,s), 3.90 (1H,t), 4.1-4.4 (8H,m), 5.20 (1H,br.), 7.12 (5H,s); 341.3/341 (10%); 56.30/56.22, 7.09/6.79, 4.10/4.12; 0.42; 150-153°C; +55.3°.

3. Enzyme-catalyzed hydrolysis of the esters 14-16. Each of the substrates 14-16 (20g) is added to a buffer medium (600ml, pH 8.8) containing phosphodiesterase I (15mg on a polymer carrier). The mixture is stirred for 6h at 37°C and the enzyme is removed. The mixture is acidified and evaporated in vacuum to dryness. The organic mass is extracted with dioxane to give following products:

(3-Acetylamino-2-oxopropyl)methylphosphinic acid, 20: $C_6H_{12}NO_5P$; 17.04g (97.6%); 2965-2480 and 2450-2320 (P-OH), 1740, 1650, 1305, 1280-1160; (DMSO-d_6): 1.92 (3H,s), 2.33 (3H,d,J=16Hz), 3.86 (2H,s), 3.98 (2H,d,J=15Hz), 5.20 (1H,br.), 10.1-10.3 (1H,br.); 193.1/193 (62%); 37.31/36.92, 6.26/

6.38, 7.25/7.33; 0.42 (in n-BuOH:EtOH:H_2O=4:1:1); the product melts with decomposition at 170°C.

L-(3-Acetylamino-2-oxobutyl)methylphosphinic acid, 21: $C_7H_{14}NO_4P$; 16.97g (96.3%); 2960-2840, 2455-2320, 1740, 1640, 1300, 1280-1160; 1.68 (3H,d), 1.94 (3H,s), 2.36 (3H,d,J=16Hz), 4.02 (2H,d,J=15Hz), 4.18 (1H,q), 5.20 (1H,br.), 10.1-10.3 (1H,br.); 207.2/207 (42%); 40.58/40.33, 6.81/7.03, 6.76/6.66; 0.50; 135-140°C (decomp.); +44.3°.

L-(4-Phenyl-3-acetylamino-2-oxobutyl)methylphosphinic acid 22: $C_{13}H_{18}NO_4P$; 17.53g (96.3%); 2955-2830, 2430-2315, 1740, 1650, 1300, 1285-1155, 865; 1.90 (3H,s), 2.14 (2H,d), 2.30 (3H,d,J=16Hz), 3.95 (2H,d,J=15Hz), 4.21 (1H,t), 5.20 (1H,br.), 7.14 (5H,s), 10.1-10.4 (1H,br.); 283.3/283 (14%); 55.12/55.31, 6.40/6.22, 4.94/5.09; 160°C (decomp.); +52.3°.

4. Enzyme-catalyzed hydrolysis of the esters 17-19. Each of the substrates 17-19 (20g) is added to a buffer medium (800ml, pH 10.4) containing alkaline phosphatase (15mg on a polymer carrier). The mixture is stirred for 6h at 37°C. The enzyme is removed and after acidification and distillation in vacuum to dryness the organic mass is extracted with N,N-dimethylformamide to the acids:

(3-Acetylamino-2-oxopropyl)phosphonic acid monoethyl ester, 23: $C_7H_{14}NO_6P$; 17.22g (96.9%); 3000-2300, 1745, 1640, 1255, 1110-975; 1.43 (3H,t), 1.92 (3H,s), 2.47 (2H,q), 3.79 (2H,s), 3.96 (2H,d,J=15Hz), 5.20 (1H,br.), 10.0-10.3 (1H,br.); 223.2/223 (80%); 37.67/38.08, 6.32/6.18, 6.28/6.31; 0.39; decomposition at about 160°C.

L-(3-Acetylamino-2-oxobutyl)phosphonic acid monoethyl ester, 24: $C_8H_{16}NO_6P$; 17.40g (97.2%); 3000-2300, 1740, 1645, 1250, 1115-980; 1.38 (3H,t), 1.67 (3H,d), 1.89 (3H,s), 2.46 (2H,q), 3.98 (2H,d), 4.21 (1H,t), 5.20 (1H,br.), 10.0-10.3 (1H,br.); 237.2/237 (12%); 40.51/40.69, 6.80/7.01, 5.91/5.77; 0.47; decomp. ; +38.6°.

L-(4-Phenyl-3-acetylamino-2-oxobutyl)phosphonic acid monoethyl ester, 25: $C_{14}H_{20}NO_6P$; 17.77g (96.8%); 3000-2310, 1740, 1250, 1110-980, 860; 1.42 (3H,t), 1.59 (3H,s), 2.4-2.6 (4H,m), 4.03 (2H,d,J=15Hz), 4.18 (1H,t), 5.20 (1H,br.), 7.2-7.4 (5H,m), 10.0-10.3 (1H,br.); 313.3/313 (9%); 53.67/53.44 6.43/6.28, 4.47/4.52; 0.52; decomposition; +49.3°.

5. Synthesis of the adenosines 26-31. A solution of each of the acids 20-25 (0.05M), N-dimethylaminomethylene-2',3'-O-ethoxymethylenadenosine (18.92g, 0.05M) and N,N'-dicyclohexylcarbodiimide (12.38 g, 0.06M) is left in ethylacetate for 48h at room temperature in an Erlenmeyer flask. The N,N'-dicyclohexylcarbamide is filtered out and the reaction mixture is successively washed with water, 5% sodium carbonate, water, 5% hydrochloric acid, water. The solution is dried over anhydrous magnesium sulphate and distilled to dryness. All compounds obtained are amorphous. As 26-31 decompose under mass-spectral conditions, their molecular weight (m.w.) was measured using a Perkin-Elmer apparatus.

N^6-Dimethylaminomethylene-5'-O-[methyl(3-acetylamino-2-oxopropyl)phosphinyl]-2',3'-O-ethoxymethylenadenosine, 26: $C_{22}H_{33}N_7O_8P$; 21.11g (76.3%); 1775-1740 (CO and CH=N), 1640 (CONH), 1310 (P-CH_3), 1250 (P=O), 1160-960 (O-C-O and P-O-C), 650-550 (Ar); (DMSO-d_6): 1.1-1.9 and 2.0-2.3 (15H,m), 2.83 (1H,s), 4.0-4.5 (9H,m), 4.83 (1H,s), 5.00 (1H,d), 5.20 (1H,br.), 5.45 (1H,d), 5.75 (2H,m), 8.12 and 8.16 (2H,s); m.w. 553.5/550; 47.74/47.83, 5.83/6.11, 17.71/17.39; 0.65 in Ph:MeOH:Me_2CO=6:1:3; --72.3°, c=0.1, MeOH; UV-spectra registered on a Perkin-Elmer apparatus (MeOH): 286(17200),215(19300).

N^6-Dimethylaminomethylene-5'-O-[methyl(4-phenyl-3-acetylamino-2-oxobutyl)phosphinyl]-2',3'-O-ethoxymethylenadenosine, 28: $C_{28}H_{35}N_7O_8P$; 23.20g (72.1%); 1770-1740, 1640, 1305, 1250, 1160-980, 860, 780, 650-550; 1.1-1.9 and 2.0-2.3 (15H,m), 2.84 (1H,s), 4.0-4.5 (9H,m), 4.83 (1H,s), 5.00 (1H,d), 5.20 (1H,br.), 5.42 (1H,d), 5.76 (2H,m), 7.20 (5H,s), 8.12 and 8.17 (2H,s); 643.6/640; 54.12/54.32, 5.95/5.63, 15.23/15.08; 0.58; -40.3°; 290(17500), 215(19000).

N^6-Dimethylaminomethylene-5'-O-[methyl(3-acetylamino-2-oxoxbutyl)phosphinyl]-2',3'-O-ethoxymethylenadenosine, 27: $C_{23}H_{35}N_7O_8P$; 22.19g (78.2%); 1770-1740, 1645, 1310, 1255, 1155-960, 650-550; 1.1-1.9 and 2.0-2.3 (18H,m), 2.82 (1H,s), 4.0-4.4 (7H,m), 4.81 (1H,s), 5.02 (1H,d), 5.20 (1H,br.), 5.44 (1H,d), 5.75 (2H,m), 8.12 and 8.17 (2H,s); 567.5/570; 48.68/48.73, 6.04/6.17, 17.28/17.01; 0.55; -44.3°; 285(17240), 219(19350).

N^6-Dimethylaminomethylene-5'-O-[ethoxy(3-acetylamino-2-oxopropyl)phosphinyl]-2',3'-ethoxymethylenadenosine, 29: $C_{23}H_{35}N_7O_9P$; 17.94g (61.5%); 1770-1745, 1645, 1255, 1160-960, 650-600; 1.1-1.9 (15H,m), 2.86 (1H,s), 4.0-4.5 (10H,m), 4.85 (1H,s), 5.03 (1H,d), 5.20 (1H,br.), 5.40 (1H,d), 5.73 (2H,m), 8.12 and 8.16 (2H,s); 583.5/580; 47.34/47.65, 5.87/5.77, 16.80/16.13; 0.42; -82.6°; 285 (17500), 215(19300).

N^6-Dimethylaminomethylene-5'-O-[ethoxy(3-acetylamino-2-oxobutyl)phosphinyl]-2',3'-O-ethoxymethylenadenosine, 30: $C_{24}H_{37}N_7O_9P$; 19.81g (66.3%); 1770-1740, 1645, 1255, 1100-965, 785, 650-550; 1.1-2.0 (18H,m), 2.82 (1H,s), 4.0-4.3 (8H,m), 4.44 (1H,q), 4.84 (1H,s), 5.03 (1H,d), 5.20 (1H,br.), 5.42 (1H,d), 5.74 (2H,m), 8.12 and 8.17 (2H,s); 597.5/593; 48.24/48.62, 6.07/6.12, 16.41/16.56; 0.39; - 39.9°; 280(17500), 210(19200).

N^6-Dimethylaminomethylene-5'-O-[etoxy(4-phenyl-3-acetylamino-2-oxobutyl)phosphinyl]-2',3'-O-ethoxymethylenadenosine, 31: $C_{30}H_{39}N_7O_9P$; 19.98g (59.3%); 1770-1740, 1645, 1250, 1160-960, 860, 785, 650-550; 1.1-1.9 (15H,m), 2.82 (1H,s), 4.0-4.5 (11H,m), 4.82 (1H,s), 5.01 (1H,br.), 5.20 (1H,d), 7.14 (5H,s), 8.12 and 8.17 (2H,s); 673.7/669; 53.49/53.68, 5.98/6.12, 14.55/14.39; 0.53; -48.7°; 285(17650), 220(19800).

6. Synthesis of the methylphosphino analogues 2-4. Each of the protected adenosines 26-28 (0.05M) is left overnight at room temperature in a mixture of dioxan/NH_4OH. After evaporation in vacuum to dryness the mixture is treated with 80% acetic acid and then again evaporated to dryness. The reaction residue is emulsified with "Tween-80" and mixed with an aqueous buffer (800ml, pH 8.0) containing α-chymotrypsin (25mg). The mixture is stirred for 8h at 25°C, neutralized, extracted with isopropyl ether, dried over anhydrous magnesium sulphate, and distilled in vacuum. The residue is put in a preparative silica gel column and eluted with dioxan:chloroform:methanol = 4:2:1 to the following products:

5'-O-[Methyl(3-amino-2-oxopropyl)phosphinyl]adenosine, 2: $C_{14}H_{21}N_6O_6P$; 10.64g (53.2%); 3350-3150 1740, 1700, 1605, 1585, 1545, 1510, 1465, 1440, 1315-1280, 1250, 1105-980, 795, 650-550; (D_2O): 2.38 (3H,d,J=16Hz), 3.89 (2H,d,J=15Hz), 4.0-4.3 (6H,m), 4.76 (1H,t), 6.35 (1H,d), 8.72 and 8.82 (2H,s); 135 (100%), 164 (77%), M+/e: 400 (12%); 42.00/42.18, 5.29/4.98, 20.99/20.13; 0.42; 293(14800), 218 (18450).

5'-O-[Methyl(3-amino-2-oxobutyl)phosphinyl]adenosine, 3: $C_{15}H_{23}N_6O_6P$; 11.65g (56.3%); 3355-3150, 2840, 1745, 1710, 1600, 1585, 1540, 1500, 1460-1440, 1315-1280, 1100-980, 790, 650-550; 1.45 (3H,d), 2.33 (3H,d,J=16Hz), 3.79 (2H,d,J=15Hz), 4.0-4.5 (5H,m), 4.82 (1H,t), 6.38 (1H,d), 8.73 and 8.84 (2H, s); 135 (100%), 164 (73%), M^+/e: 414 (15%); 43.48/43.96, 5.59/5.63, 20.28/20.19; 0.50; 164-167°C (decomp.); -335°; 286(14500), 210(19100).

5'-O-[Methyl(4-phenyl-3-amino-2-oxobutyl)phosphinyl]adenosine, 4: $C_{21}H_{25}N_6O_6P$; 12.10g (49.3%); 3350-3150, 2850, 1740, 1725, 1605, 1580, 1540, 1505, 1440, 1310-1285, 1100-980, 860, 795, 650-550; 2.34 (3H,d,J=16Hz), 3.82 (2H,d,J=15Hz), 4.0-4.4 (6H,m), 4.53 (1H,t), 4.79 (1H,t), 6.39 (1H,d), 7.14 (5H,s), 8.74 and 8.85 (2H,s); 135 (100%), 164 (75%); M^+/e: 491 (12%); 51.43/51.55, 5.55/5.26, 17.14/16.98; 0.44; decomp. at over 200°C; -39.6°; 293(14800), 219(18450).

7. Synthesis of the phosphono analogues 5-7.

Method A. A solution of phosphorus pentachloride (12.49g, 0.06M) and each of the phosphono esters 29-31 (0.05M) is heated in dry tetrachlormethane on a water bath. The volatile components are evaporated in vacuum and ice is added to the amorphous residue. After 2h, the mixture is extracted with isopropyl ether, dried over anhydrous magnesium sulphate and distilled in vacuum to dryness. The residue is passed through a silica gel column and the following products are isolated:

N^6-Dimethylaminomethylene-5'-O-[hydroxy(3-acetylamino-2-oxopropyl)phosphinyl]-2',3'-O-ethoxymethylenadenosine, 32: $C_{21}H_{28}N_7O_7P$; 5.50g (19.8%); 2950-2840 and 2450-2320 (P-OH), 1775-1740, 1645, 1250, 1110-945, 785, 650-550; 1.39 (6H,s), 1.84 (3H,s), 2.84 (1H,s), 4.1-4.4 (8H,m), 4.86 (1H,s), 5.00 (1H,d), 5.20 (1H,br.), 5.46 (1H,d), 5.79 (2H,m), 8.12 and 8.16 (2H,s), 10.2-10.5 (1H,br.), in D_2O+DCl+TSP, 40°C - the protons at 5.20 and 10.2-10.5 ppm do not appear; m.w. 555.5/552; 45.41/45.52, 5.44/5.48, 17.65/17.33; 0.62; amorphous product.

N^6-Dimethylaminomethylene-5'-O-[hydroxy(3-acetylamino-2-oxobutyl)phosphinyl]-2',3'-O-ethoxymethylenadenosine, 33: $C_{22}H_{30}N_7O_7P$; 6.75g (23.7%); 2955-2840, 2455-2320, 1775-1740, 1645, 1255, 1115-950, 790, 650-550; 1.2-1.9 (1H,m), 2.85 (1H,s), 4.1-4.3 (6H,m), 4.52 (1H,q), 4.48 (1H,s), 5.03 (1H, d), 5.20 (1H,br.), 5.41 (1H,d), 5.77 (2H,m), 8.12 and 8.17 (2H,s), 10.2-10.4 (1H,br.); m.w. 569.5/571; 46.40/46.31, 5.66/5.78, 17.22/17.36; 0.56; amorphous.

N^6-Dimethylaminomethylene-5'-O-[hydroxy(4-phenyl-3-acetylamino-2-oxobutyl)phosphinyl]-2',3'-O-ethoxymethylenadenosine, 34: $C_{28}H_{34}N_7O_7P$; 7.23g (22.4%); 2950-2840, 2455-2320, 1770-1740, 1645, 1255, 1115-955, 860, 790, 650-550; 1.2-1.8 (12H,m), 2.84 (1H,s), 4.0-4.4 (9H,m), 4.83 (1H,s), 5.00 (1H,br.), 5.42 (1H,d), 5.76 (2H,m), 7.12 (5H,s), 8.12 and 8.17 (2H,s); m.w. 645.8/640; 52.09/52.36, 5.62/5.31, 15.19/15.33; amorphous.

α-Chymotrypsin (10mg) is added to each of the substituted adenosines 32-34 (0.02M) in a buffer medium (600ml, pH 7.8). The mixture is stirred for 6h at 25°C. After neutralization, extraction and vacuum distillation, the reaction residue is placed in a silica gel column and eluted with chloroform:methanol = 9:1. The ninhydrine-positive fraction is collected, evaporated in vacuum and successively treated, as in Item 6, with ammonia and acetic acid. The following products are afforded:

5'-O-[Hydroxy(3-amino-2-oxopropyl)phosphinyl]adenosine, 5: $C_{13}H_{19}N_6O_7P$; 2.78g (34.6%); 3410-3115, 2950-2840, 2455-2320, 1740-1720, 1595, 1570, 1535, 1495, 1440, 1315-1280, 1120-965, 790, 650-550; (D_2O+DCl+TSP): 3.83 (2H,d,J=15Hz), 4.0-4.3 (6H,m), 4.76 (1H,t), 6.33 (1H,d), 8.70 and 8.83 (2H, s); 135 (100%), 164 (77%), M^+/e: 402 (12%); 38.81/39.18, 4.76/4.69, 27.84/27.66; 0.36 in $CHCl_3$:THF: DMF = 4:1:1; decomp.: -45.3°, c=0.1, 1N HCl; 292(14800), 212(19300).

5'-O-[Hydroxy(3-amino-2-oxobutyl)phosphinyl]adenosine, 6: $C_{14}H_{21}N_6O_7P$; 2.52g (30.3%); 3410-3120, 2985-2845, 2450-2320, 1740-1720, 1590-1570, 1530, 1490, 1445, 1315-1280, 1120-965, 785, 650-550; 1.69 (3H,d), 3.76 (2H,d,J=15Hz), 4.0-4.4 (5H,m), 4.78 (1H,t), 6.34 (1H,d), 8.70 and 8.82 (2H,s); 135 (100%), 164 (75%), M^+/e: 416 (12%); 40.39/40.58, 5.09/4.88, 20.19/19.93; 0.40; decomp.: -28.3°; 215(18960).

5'-O-[Hydroxy(4-phenyl-3-amino-2-oxobutyl)phosphinyl]adenosine, 7: $C_{20}H_{23}N_6O_7P$; 3.52g (35.7%); 3415-3120, 2980-2840, 2455-2315, 1740-1720, 1590-1570, 1535, 1490, 1445, 1320-1285, 1120-970, 860, 785, 650-550; 3.82 (2H,d,J=15Hz), 4.0-4.4 (6H,m), 4.58 (1H,d), 4.80 (1H,t), 6.35 (1H,d), 7.21 (5H,s s), 8.70 and 8.82 (2H, s); 135 (100%), 164 (70%), M^+/e: 492 (10%); 48.78/48.83, 5.12/5.01, 17.07/16.93; 0.32; decomp.: -39.6°; 290(14840), 210(19300).

Method B. A mixture of each of the phosphono esters 29-31 (0.05M) and phosphorus pentachloride (14.58g, 0.07M) is added to dry tetrachlormethane (150ml). The mixture is heated in water bath for 1h and the volatile components are evaporated in vacuum (60°C/6.10^{-1}torrs). Iced acetic acid (6.01g, 0.1M) is added to the oily residue and the mixture is heated in a water bath for 1h. Water (5ml) is then added and heating kept up for another hour. After vacuum distillation, 25% ammonium hydroxide is added to the amorphous residue and after 6h the solution is evaporated again. The components of the reaction mixture are chromatographically separated and thus the aminoketophosphonoadenosines 5-7 are isolated in yields of 17.6% (3.54g), 20.3% (4.23g) and 16.2% (3.99g), respectively.

8. Synthesis of the ester 37. The method in Item 1 is followed with the participation of N-acetylglycyl chloride (32.53g, 0.24M) and (ethoxymethylphosphinyl)acetic acid ethyl ester (19.42g, 0.1M). The following product is isolated:

4-Acetylamino-3-oxo-2-[ethoxy(3-acetylamino-2-oxopropyl)phosphinyl]-butanoic acid ethyl ester, 37: $C_{17}H_{29}N_2O_9P$; 31.11g (79.3%); 1755, 1740, 1645, 1315, 1240, 1110-980; ($CDCl_3$): 1.18 and 1.34 (6H, t), 1.96 and 2.12 (6H,s), 2.72 (2H,d,J=17Hz), 3.70 (1H,d,J=15Hz), 4.1-4.5 (8H,m), 5.00 (2H,br.); 39 392.345/392 (12%); 45.92/46.11, 6.42/6.30, 7.14/7.26; 183-186°C.

9. Synthesis of the acid 38. The ester 37 (39.23g, 0.1M) is boiled in 22% HCl (200ml) for 6h. After evaporation in vacuum to dryness, the organic mass is extracted with dioxan and saturated with hydrogen chloride. The following product is filtered out:

4-Amino-3-oxo-2-[hydroxy(3-amino-2-oxopropyl)phosphinyl]-butanoic acid, 38: $C_7H_{13}N_2O_8P$; 19.49g (77.3%); 3280-2840, 2650, 1765, 1720, 1680, 1550, 1520, 1310, 1210, 1010-860, 710, 620; (D_2O+NaOD): 2.71 (2H,d,J=18Hz), 3.76 (1H,d,J=15Hz), 3.99 and 4.12 (4H,s) and six exchangeable protons - NH_2x2, COOH, POOH; 252.163/252 (26%): 33.34/33.56, 5.20/5.11, 11.11/11.24; decomp. about 280°C.

10. Synthesis of the acids 35 and 36. Each of the esters 14 and 17 (0.1M), and 26 and 29 (0.01M) is

boiled for 8h in hydrochloric acid (22%, 200ml). After evaporation in vacuum to dryness, the residue is crystallized from water to the following products, respectively:

Methyl(3-amino-3-oxopropyl)phosphinic acid, 35: $C_4H_{10}NO_3P$; 12.89g (85.3%); 3280-3125, 2985-2860, 2450-2320, 1740, 1555, 1305, 1255, 1205; (D_2O+DCl+TMS): 2.38 (3H,d,J=16Hz), 3.80 (2H,d,J=15Hz), 4.02 (2H,s); 151.1/151 (29%); 31.80/32.18, 6.67/6.63, 9.27/9.19; 0.43 in n-BuOH:AcOH:H_2O = 4:1:1 - ninhydrine detection; decomp. at over 250°C.

3-Amino-2-oxopropylphosphonic acid, 36: $C_3H_8NO_4P$; 13.52g (88.3%); 3270-3220, 3000-2800, 2490-2345, 1740, 1260; 3.72 (2H,d,J=15Hz), 4.12 (2H,s); 153.1/153 (18%); 23.54/23.03, 5.27/5.19, 9.15/9.23; 0.32; decomp.

11. Enzyme-catalyzed hydrolysis of the esters 8-13. α-Chymotrypsin (15mg, spread on carboxymethyl cellulose) and each of the esters 8-13 (20g) are added to a buffer medium (800ml, pH 7.8), tempered at 37°C. The mixture is stirred for 6h and the enzyme is removed. The reaction mixture is neutralized and concentrated. Upon cooling, an amorphous mass is separated. After treatment with α-chymotrypsin and with no additional work-up, the residue is dissolved in aqueous buffer (800ml, pH 8.8) containing phosphodiesterase I (5mg, the same enzyme is used in all experiments). The enzyme is removed and the reaction mixture is worked up as above. The following acids are isolated, respectively:

4-Amino-3-oxo-2-methylphosphino-butanoic acid, 39: $C_5H_{12}NO_5P$; 12.17g (91.5%); 3100-2860, 2320-2200, 1740, 1550, 1300, 1220; (D_2O+Na_2CO_3): 1.98 (3H,d,J=18Hz), 3.62 (1H,d,J=15Hz), 3.81 (2H,s); 195.1/195 (19%); 30.78/30.96, 5.17/5.01, 7.18/7.29; 0.63 (n-BuOH:25%NH OH = 4:1); decomp. at 260°C.

4-Amino-3-oxo-2-methylphosphino-L-pentanoic acid, 40: $C_6H_{12}NO_5P$; 11.92g (87.6%); 3100-2860, 2300-2200, 1745, 1555, 1300, 1220; 1.39 (3H,d), 2.03 (3H,d,J=18Hz), 3.66 (1H,d,J=15Hz), 4.12 (1H,q); 209.1/209 (24%); 34.46/34.78, 5.78/5.44, 6.70/6.81; 0.70; decomp.; +66.3°, c=0.1, 1N NaOH.

5-Phenyl-4-amino-3-oxo-2-methylphosphino-L-pentanoic acid, 41: $C_{12}H_{16}NO_5P$; 14.05g (94.4%); 3100-2850, 2330-2210, 1750, 1545, 1305, 1225, 865; 2.08 (3H,d,J=18Hz), 2.26 (2H,d), 3.61 (1H,d,J=15Hz), 4.08 (1H,t), 7.18 (5H,s); 285.2/285 (9%); 50.53/50.68, 5.65/5.33, 4.91/5.10; 0.62; decomp.; +58.3°.

4-Amino-3-oxo-2-phosphono-butanoic acid, 42: $C_4H_8NO_6P$; 11.23g (92.1%); 3200-2640, 1745, 1520, 1250, 1000, 970, 735, 640; 3.36 (1H,d,J=12Hz), 3.75 (2H,s), plus five exchangeable protons: COOH, PO_3H_2, NH_2; the product decomposes under mass-spectral conditions; m.w. 197.1/194; 24.38/24.81, 4.10/3.96, 7.11/7.23; 0.33.

4-Amino-3-oxo-2-phosphono-L-pentanoic acid, 43: $C_5H_{10}NO_6P$; 10.92g (87.2%); 3200-2640, 1750, 1525, 1250, 1000, 970, 730, 635; 1.43 (3H,d), 3.32 (1H,d,J=12Hz), 4.08 (1H,q); 337.3/340; 28.45/28.83, 4.77/4.62, 6.63/6.71; 0.46; decomp.; +78.3°.

5-Phenyl-4-amino-3-oxo-2-phosphono-L-pentanoic acid, 44: $C_{11}H_{14}NO_6P$; 12.96g (93.3%); 3200-2640, 1750, 1530, 1255, 1000, 975, 740, 640; 2.30 (2H,d), 3.30 (1H,d,J=12Hz), 4.12 (1H,t); 287.2/290; 46.00/46.21, 4.91/4.67, 4.88/4.93; 0.38; decomp.; +55.7°.

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