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AMINOPHOSPHONIC AND AMINOPHOSPHINIC ACID ANALOGUES OF ASPARTIC ACID

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Abstract - 4-Oxoazetidin-2-ylphosphonates and phosphinates, obtained from Arbusov reactions of 4-acetoxyazetidin-2-one and 4α-acetoxy-3β-phthalimido-2-one with a variety of phosphites and phosphonites, were hydrolysed to β-phosphono- and β-phosphino β-alanine (phosphono- and phosphinoaspartic acid) derivatives. In model studies for their incorporation in peptides, conditions for the selective removal of protecting groups for carboxylic acids, phosphonic and phosphinic acids, and amines, in derived di- and tri-peptides were investigated. Alanyl and alanyl alanyl peptide incorporation into bacteria was studied.

Aminophosphonic acids are becoming increasingly important, analogues of most of the natural aminoacids having recently been synthesized.[1] Our investigation of 4-acetoxyazetidin-2-one (1)[2] and a brief study from the Hoescht group,[3] indicated that the remarkable ease of displacement of the 4-acetate could be exploited in Arbusov reactions, and that phosphorus containing analogues of aspartic acid and a range of derived peptides could be synthesized.

Nucleophilic Reactions

Initial studies rapidly established that Michaelis Becker reactions of (1) with dialkyl phosphite anions were not possible, β-lactam cleavage occurring because of the strongly basic nature of the reagents. Triethyl phosphite, however, when heated with (1) gave

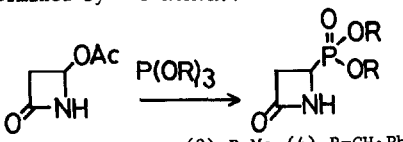
$$(RO)_2\overset{O}{\overset{\|}{P}} \xrightarrow{R'X} (RO)_2\overset{O}{\overset{\|}{P}}R' \longleftarrow (RO)_3\overset{+}{P}R' \xleftarrow{R'X} (RO)_3P$$

Michaelis Becker Arbusov

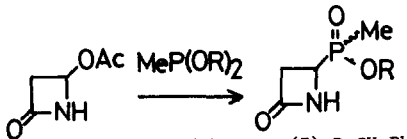
diethyl azetidin-2-ylphosphonate (3) by an Arbusov reaction, corroborating an earlier report[3] in which the product stucture was not rigorously established. One of our objectives was to deprotect the phosphono group without perturbing the labile β-lactam ring, and it was rapidly established that this was not possible with the diethyl ester. Accordingly, a range of phosphites and methylphosphonites were prepared in order to assess their participation in this atypical Arbusov reaction, and also to evaluate deprotection methods. Tribenzyl phosphite,[4] tris-(2,2,2-trichloroethyl)phosphite,[5] diethyl methylphosphonite,[6] dibenzyl methylphosphonite and bis-(2,2,2-trichloroethyl)-methylphosphite[7] were prepared from phosphorus trichloride or from methylphosphonous dichloride, the optimum processes requiring an amine base which differed appreciably from product in boiling point, and a solvent which afforded the most insoluble base hydrochloride. Phosphorus esters were purified by vacuum distillation, but residual traces of amine (<5%) had no discernible effect on yield of Arbusov product.

The Arbusov reaction was general (Table 1), only tris-(2,2,2-trichloroethyl)phosphite failing because of its reduced nucleophilicity. Interestingly, bis-(2,2,2-trichloroethyl)-methylphosphonite was sufficiently nucleophilic, affording (8). It was also noted that tribenzyl phosphite, previously reported to be

unreactive in the Arbusov process,[8] gave (4). In general, the methylphosphonites were more reactive than their phosphite counterparts. The former reagents gave diastereoisomeric products, the approximate ratios being best determined by ^{31}P N.M.R..



(1) (2) R=Me (4) R=CH_2Ph
(3) R=Et (5) R=CH_2CCl_3



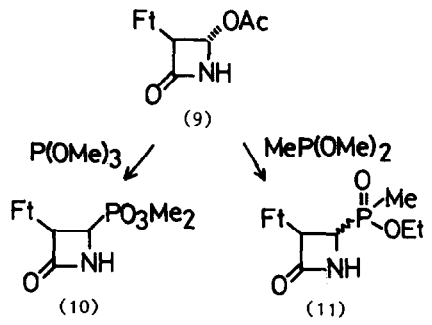
(1) (6) R=Et (7) R=CH_2Ph
(8) R=CH_2CCl_3

Yields of Phosphonate/Phosphinate

Product	(2)	(3)	(4)	(5)	(6)	(7)	(8)
Temp.°	110	110	120	110	60	90	120
Time	2h	1.5h	7h	96h	1h	5h	24h
Yield %	82	90	46	0	93	84	42

TABLE 1

Extrapolation of these studies to 3-substituted azetidin-2-ones was of further interest, and trans-4-acetoxy-3-phthalimidoazetidin-2-one (9) was therefore prepared from 6β-phthalimidopenicillanic acid.[9] Benzylation,[10] secoacetoxylative cleavage of the thiazolidine ring,[11] and oxidative removal of the 3-methylbut-2-enoate group[12] gave (9) as the purely trans-product (compared with a Sheehan procedure[13] which gave a 1:6 cis/trans mixture).



(9)

(10) (11)

Ft = phthalimido

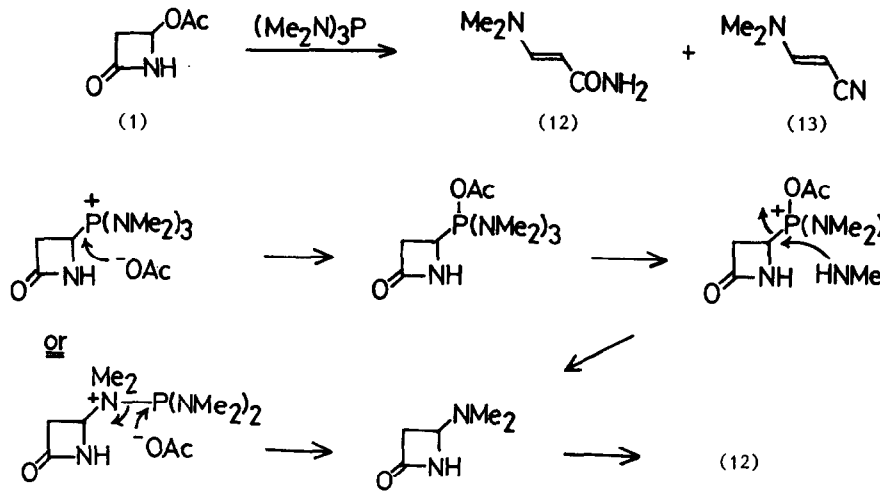
Arbusov reaction of (9) with trimethylphosphite gave (10) as a 99:5 cis/trans mixture of isomers, whereas diethyl methylphosphonite gave (11) as a 1:1 cis/trans mixture of isomers. Subtle differences in reaction mechanism were therefore indicated.

Whilst trialkyl phosphites, and to a lesser extent, phosphorotrithioites, undergo Arbusov reaction, alkylphosphorus triamides do not give similar products because of the difference in bond energies between P=O (627 $kJmol^{-1}$) and P=N (459 $kJmol^{-1}$). Nevertheless, the reaction of (1) with hexamethylphosphorus triamide was investigated because of the unexpected reactivity of (1) in the Arbusov studies. Room temperature reaction gave β-dimethylaminoacrylamide (12) (12%), and β-dimethylaminoacrylonitrile (13) (48%) as the sole non-polar products. The instability of (13) precluded rigorous structure assignment, but I.R., N.M.R. and High Resolution Mass Spectrometry supported the structure. Formation of (13) probably occurs by dehydration of (12) via an imidoyl phosphite. The reagent, hexamethylphosphorus triamide, contained no dimethylamine (N.M.R.), and two plausible mechanisms (Scheme 1) are thus apparent, depending upon reaction either as a phosphorus or as a nitrogen nucleophile.[14] Whichever mechanism was involved, it was apparent that secondary amines might react at the 4-acetoxy group, rather that at the β-lactam carbonyl, a feature already indicated in a Hoescht study.[3]

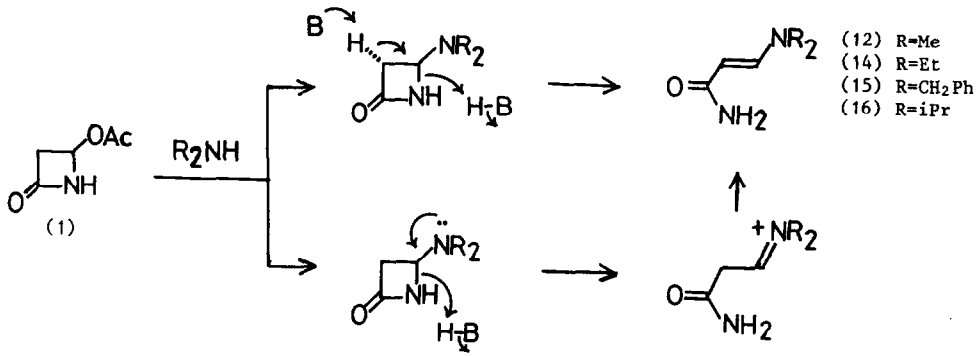
Dimethylamine, diethylamine and dibenzylamine indeed gave highly unstable trans-β-dialkylaminoacrylamides (12), (14) and (15), characterized spectroscopically. Diisopropylamine gave in 53% yield a stable product (16). This procedure therefore affords access to an unusual group of β-substituted acrylamides with considerable potential in synthesis. Mechanistic routes are presented in Scheme 2.

Phosphonoaspartic Acids and Model Studies for Derived Peptides

Deprotection of the β-lactam phosphonates and phosphinates (2) - (8), to provide phosphorus analogues of cycloaspartic acid, proved to be particularly difficult. Thus, a range of conventional methods including trimethylsilyl halide (Cl, Br, I) deesterification, sodium iodide in DMF, lithium iodide in pyridine,

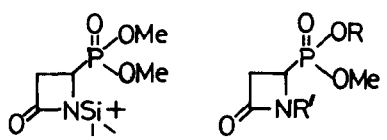


SCHEME 1



SCHEME 2

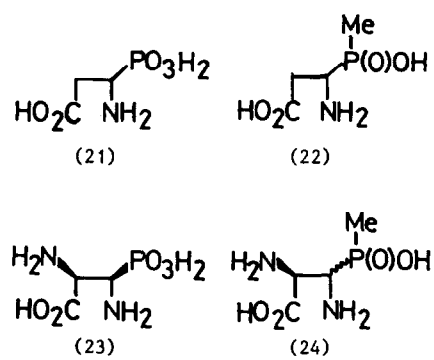
acid ion exchange resin, catalytic hydrogenation of (4), (5) and (8), and sodium hydroxide in THF, all gave β-lactam cleavage. However,



(17) (18) R=$SiMe_3$, R'=Si^tBuMe_2
(19) R=H, R'=Si^tBuMe_2
(20) R,R'=H

formation of the N-^{t}butyldimethylsilyl derivative (17), and reaction with one equivalent of trimethylsilyl bromide gave the mixed silyl derivative (18), characterized spectroscopically. Aqueous acetone gave a mixture of the monophosphonic acids (19) and (20), the former gradually deprotecting to give the latter on standing. The monophosphonic acid (20) was highly unstable, explaining the failure of the earlier reactions to give a β-lactam. The diacid could not be made.

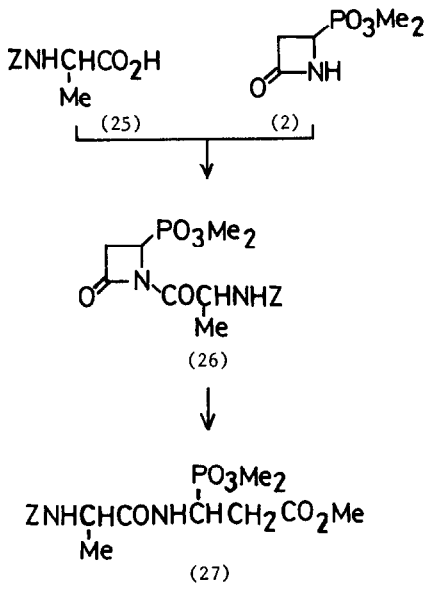
Acid hydrolysis of the 4-oxoazetidin-2-ylphosphonates and phosphinates (2), (3) and (6) gave a direct entry to aspartic acid analogues (21) and (22), complementing the more standard method of Soroka and Mastalerz.[15]



(21) (22)

(23) (24)

Similarly, phthalimidoazetidinone (10) when treated with 6M HCl gave the chiral 2,3-diamino-3-phosphonopropanoic acid (23), and its phosphino counterpart (11) gave the diastereoisomeric 2,3-diamino-3-methylphosphinopropanoic acids (24).



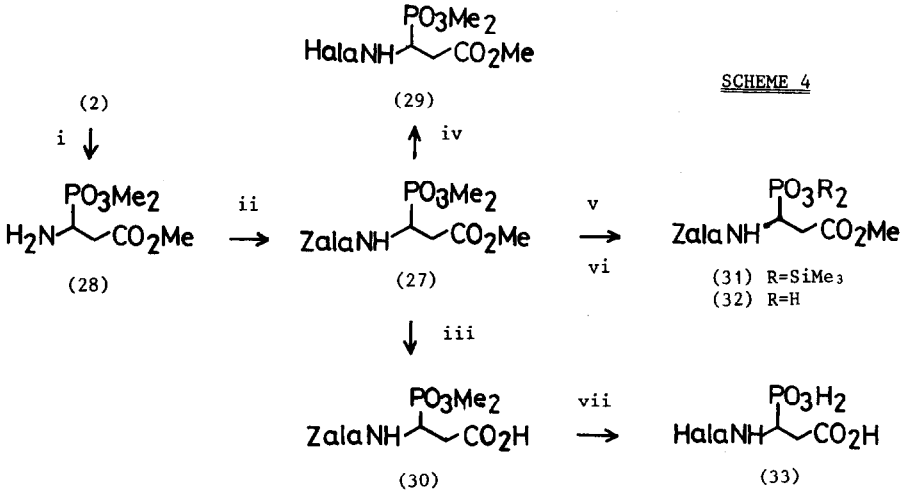
SCHEME 3

Incorporation of (20) and (21) into bacteria via dipeptide and oligopeptide transport systems[16] was an objective, and to this end alanyl and alanyl alanyl peptide derivatives were synthesized. In the first approach (Scheme 3) the β-lactam ring was regarded as an internally protected carboxylic acid. Racemic N-benzyloxycarbonyl alanine was coupled to N-1, giving (26). Removal of the terminal amino protecting group by hydrogenolysis was not possible because of competing side-reactions. β-Lactam cleavage (HCl-MeOH) gave, in low yield (15%), the desired product (27), loss of the alanyl moiety being a complication.

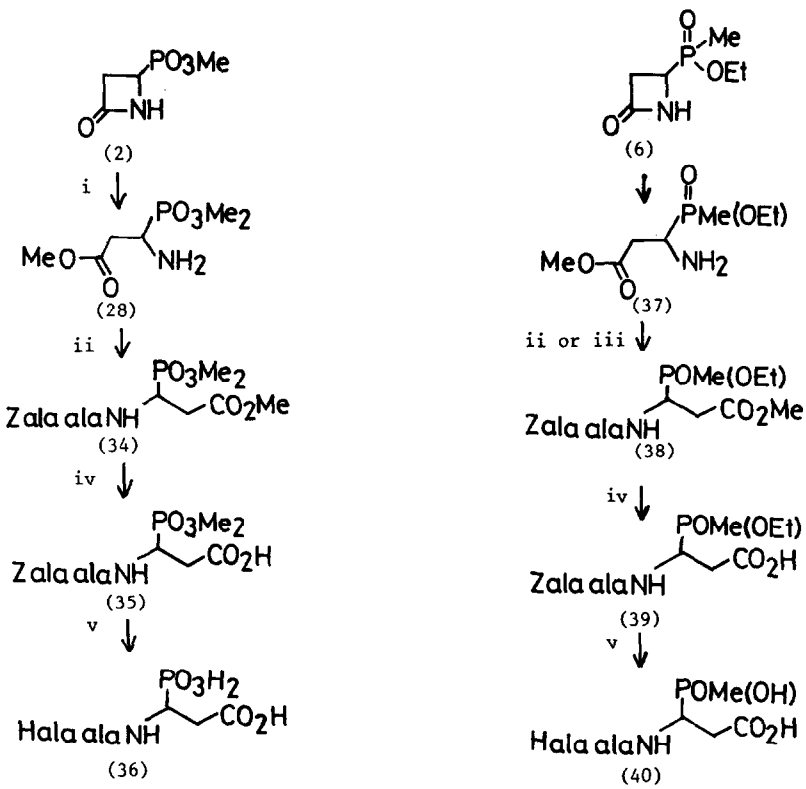
The second approach involved preliminary acidic methanolysis of (2) (Scheme 4), to give the free amine (28). (Basic hydrolysis caused polymerization.) Mixed anhydride coupling with N-benzyloxycarbonyl alanine gave the fully protected dipeptide (27) in good yield. Selective deprotection of (27) was then demonstrated, catalytic hydrogenolysis giving amine (29), sodium hydroxide giving carboxylic acid (30) and trimethylsilyl bromide giving the phosphonic acid (32) via the bistrimethylsilyl-phosphonate (31). Complete deprotection to give (33) was best achieved by HBr-CH_3CO_2H hydrolysis of (30).

For the preparation of alanyl alanyl tripeptide systems a similar stratagem was employed, and (2) and (6) (Scheme 5) were hydrolysed to (28) and (37) respectively. Amine (28) (racemic) was coupled with N-benzyloxycarbonyl-L-L-alanyl-alanine in trial reactions using the isobutylchloroformate and the DCC procedures. The former method has disadvantages is racemization-free coupling is required, and

SCHEME 4



Reagents: (i) MeOH, HCl; $NaHCO_3$; (ii) Z-ala-OH, CMA procedure; (iii) 4M NaOH-H_2O; (iv) H_2/Pd-C, MeOH-AcOH; (v) TMSBr, CH_2Cl_2; (vi) acetone, H_2O; (vii) HBr-AcOH.



Reagents: (i) HCl, MeOH; $NaHCO_3$; (ii) Z-ala-ala-OH, CMA procedure; (iii) Z-ala-ala-OH, DCC procedure; (iv) 4M NaOH, MeOH-H_2O; (v) HBr-AcOH

SCHEME 5

in this case it also gave a lower yield than the DCC method, which afforded (34) in 40% yield as a mixture of diastereoisomers at the aminophosphonic methine carbon. Within the limits of the N.M.R. techniques available, racemization at the neighbouring alanine could not be detected. Mono-deprotection gave the carboxylic acid (35), which was completely deprotected by HBr-CH_3CO_2H to the diastereoisomeric phosphonotripeptides (36). Similarly, the aminophosphinic acid (37) was transformed via (38) and (39) into the phosphinotripeptide diastereomers (40).

Dipeptide (33) and tripeptides (36) and (40) showed no antibacterial activity *per se*. However, phosphonotripeptide (36) exhibited low-level synergy with ampicillin against two strains of *Staphylococcus* and one strain of *E. Coli*, and phosphinotripeptide (40) was a synergist with ampicillin against two strains of *Staphylococcus* and three strains of *E. Coli*. The origin of this synergy remains to be elucidated.

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EXPERIMENTAL

^{1}H N.M.R. spectra were obtained at 60MHz with a Perkin Elmer R12, at 100MHz with a JEOL JNM-MH-100 or a Varian XL-100A spectrometer. ^{13}C N.M.R. spectra were recorded at 25.2MHz on the XL-100A. Mass spectra were run on A.E.I. MS 30, A.E.I. MS 50, A.E.I. MS 902 and VG 7070F spectrometers.

Reactions were monitored by t.l.c. on Merck DC-Alufolien Kieselgel 60 F254 or Merck DC-Alufolien Aluminiumoxid 60 F254 neutral (Typ E). Column chromatography was on pressurized short-path columns with Kieselgel H. Development was with 5% ethanolic anisaldehyde with a trace of acetic acid and sulphuric, or with 5% ammonium molybdate in 1M sulphuric acid. Amino acid analysis was carried out on a JEOL 6AH amino acid analyzer.

^{n}Butyl lithium was standardized by the method of Kofron and Baclawski[19] prior to use.

Tribenzyl phosphite. Several procedures for the preparation of tribenzyl phosphite have been reported,[4] but physical data is inconsistent. The most reliable preparation is by Rydon,[5,17] and the pure compound was spectroscopically in agreement with n.m.r. and i.r. data by Ramirez.[4]

N,N-Diethylaniline (22.4g, 23.9ml, (150mmol), benzyl alcohol (16.2g, 15.5ml, 150mmol) and dry ether (50ml) were placed in a three-necked round bottom flask fitted with a reflux condenser and pressure-equalizing dropping funnel, thermometer and a mechanical stirrer, under a nitrogen stream. The solution was cooled to -10° and phosphorus trichloride (6.87g, 4.36ml, 50mmol) in dry ether (10ml) was added via the dropping funnel with stirring at such a rate that the solution temperature remained below 10°. Once all the phosphorus trichloride was added, the reaction mixture was stirred at 0° for 2h. The N,N-diethylaniline hydrochloride was filtered off under nitrogen and the residue washed with dry ether (3x40ml). The ether washings and filtrate were combined and evaporated in vacuo to yield an oil. Residual N,N-diethylaniline and benzyl alcohol were removed by vacuum distillation (1mmHg, 60°) to afford tribenzyl phosphite (14.3g, 81%), ν_{max} (thin film) 3040(arom.CH), 2900(ali.CH), 1600(C===C) and 970cm^{-1}(P-O-CH_2Ph), δ($CDCl_3$) 4.7(6H,d,J_{HP}=8Hz,PO$\underline{CH_2}$Ph) and 7.15(15H,s,Ph).

Tris-(2,2,2-trichloroethyl)phosphite. In the best procedure,[5] N,N-Diethylaniline (22.4g, 23.9ml, 150mmol), 2,2,2-trichloroethanol (22.4g, 14.5ml, 150mmol) and dry ether (50ml) were reacted as above with phosphorus trichloride (6.87g, 4.36ml, 50mmol) to yield tris-(2,2,2-trichloroethyl)phosphite (14.8g, 62%), ν_{max} (thin film) 1080(P-O-CH_2CCl_3) and 1020cm^{-1}(P-O-CH_2CCl_3), δ($CDCl_3$) 4.28 (6H,d,J_{HP}=6Hz,PO$\underline{CH_2}CCl_3$).

Diethyl methylphosphonite. Diethyl methylphosphonite was prepared initially according to Hoffman,[18] and latterly by the procedure of Miles,[6] which was published during the course of this work. The latter method offers an advantage in that the boiling point of the amine base differs markedly from the boiling point of the product and that it yields an insoluble non-hygroscopic hydrochloride. The procedure is as follows: under nitrogen, N,N-diethylaniline (335g, 379ml, 2.39mmol), ethanol (110g, 139ml, 2.39mmol) and n-pentane (1000ml) were cooled to 0° and the reaction temperature maintained at 0° as the solution was treated with methylphosphonous dichloride (127.9g, 1.09mmol). The reaction mixture was stirred at 0° for a further 30min, the base hydrochloride removed by filtration and the residue washed with n-pentane (1x250ml). The filtrate and washings were combined and the n-pentane removed by reduced pressure (water pump) distillation maintaining the temperature below 10°. The residual diethyl methylphosphonite could normally be used without further purification, but fractionally distilled at reduced pressure (b.p.47°, 50mm; 120°, 760mm). Yield, (133g, 90%), ν_{max} (thin film) 3000(ali.CH), 1250(P-CH_3) and 1035cm^{-1}(P-O-CH_2CH_3), δ($CDCl_3$) 1.2(6H,t,H=6Hz,$POCH_2\underline{CH_3}$), (resonance due to PCH_3 covered by triplet) and 3.35 (4H,m, $PO\underline{CH_2}CH_3$).

Dibenzyl methylphosphonite. Methylphosphonous dichloride (13.3g, 113.5mmol) and dry ether (100ml) were cooled to 0° and treated under nitrogen with N,N-diethylaniline (37.8g, 24ml, 227mmol) and benzyl alcohol (37.6g, 36ml, 227mmol) which were added with rapid stirring via the dropping funnel at a rate such that the reaction temperature did not exceed 10°. The reaction mixture was stirred at 0° for a further 2h. The amine hydrochloride was filtered off under nitrogen, the residue washed with dry ether (2x100ml) and the ether washings were added to the filtrate. The combined washings and filtrate were evaporated under reduced pressure to yield an oil. Residual N,N-diethylaniline and benzyl alcohol were removed by vacuum distillation (100°, 1mm) to give dibenzyl methylphosphonite (13g, 44%), ν_{max} (thin film) 3020(arom.C-H), 2980(ali.C-H), 1600, 1300(P-CH_3) and 980cm^{-1}(P-O-CH_2Ph), δ($CDCl_3$) 1.36(3H,d,J_{HP}=9Hz,P-$\underline{CH_3}$), 4.90(4H,d,J_{HP}=8Hz,P-O-$\underline{CH_2}$Ph) and 7.31(10H,s,Ph). (Found: $\underline{M}^+$,260.0981. $\underline{C}_{15}H_{17}PO_2$ requires $\underline{M}$, = 260.0966).

Bis-(2,2,2-trichloroethyl)methylphosphonite. The preparation of bis-(2,2,2-trichloroethyl) methylphosphonite has been reported[7] with only brief experimental details.

Methylphosphonous dichloride (13.62g, 116.4mmol) and dry ether (100ml) were cooled to below 0° (ice/salt) and treated with N,N-diethylaniline (34.8g, 37ml, 233mmol) together with 2,2,2-trichloroethanol (34.8g, 54ml, 233mmol) via the dropping funnel, the temperature being maintained at 0°. The reaction mixture was stirred at 0° for a further 90min. The amine hydrochloride was filtered off under nitrogen and the residue washed with ether (2x100ml). The ether washings and filtrate were combined and after fractional distillation at reduced pressure gave bis-(2,2,2-trichloroethyl)methylphosphonite (17.2g, 43%), νmax (thin film) 1285(P-CH_3), 1030(P-O-CH_2CCl_3), 830(C-Cl) and 725cm^{-1}(C-Cl), δ($CDCl_3$) 1.4 (3H,d,J_{HP}=10Hz,PCH_3) and 4.35(4H,d,J_{HP}=7Hz, $POCH_2CCl_3$). (Found: M^+, 339.8325. $C_5H_7{}^{35}Cl_6O_2P$ requires M, 339.8314).

0,0-Dimethyl 4-oxoazetin-2-ylphosphonate (2) 4-Acetoxyazetidin-2-one† (1) (4.01g, 31.1mmol) in redistilled trimethyl phosphite (25ml) was heated at reflux temperature under a slow nitrogen stream for 2h. The solvent was evaporated in vacuo to yield a clear oil.

† Accepted nomenclature for this precursor, as in reference 2.

Addition of ether afforded O,O-dimethyl 4-oxo-azetidin-2-ylphosphonate (2) (4.55g, 82%), m.p. 96-99°, ν_{max}(KBr) 3200, 1760 (β-lactam C=O), 1220(P=O) and 1030cm^{-1}(P-O-CH$_3$), δ(CDCl$_3$) 3.2 (2H,m,3-H), 3.83 (7H,d,J_{HP}=13Hz,P-OCH$_3$), (resonance due to 4-H covered by doublet), and 7.3(1H,s,NH), (addition of D$_2$O caused the signal at 7.3 to disappear). (Found: C,33.45; H,5.96; N,7.81; P,17.81%; M(mass spectrum), 179. $C_5H_{10}NPO_4$ requires C,33.5; H,5.6; N.7.82; P,17.3%; M,179).

O,O-Diethyl 4-oxoazetidin-2-ylphosphonate (3). 4-Acetoxyazetidin-2-one (1) (5.10g, 39.6mmol) in redistilled triethylphosphite (50ml) was heated at 90-120° under a slow nitrogen stream for 1.5h. The solvent was evaporated in vacuo to yield a waxy solid which was crystallised from ether to give O,O-diethyl 4-oxoazetidin-2-ylphosphonate (3) (7.4g, 90%), m.p. 49-51°, ν_{max}(KBr) 3200, 2980, 1780 (β-lactam C=O), 1230 (P=O) and 1020cm^{-1}(P-O-CH$_2$CH$_3$), δ(CDCl$_3$) 1.36 (6H,t,J=6Hz,POCH$_2$CH$_3$), 3.16(2H,m,3-H), 3.76 (4H,m,POCH$_2$CH$_3$) and 7.36(1H,s,NH), (addition of D$_2$O causes the signal at 7.36 to disappear). (Found: C,40.88; H,6.58; N,6.86; P,14.44%); M (mass spectrum), 207. $C_7H_{14}NO_4P$ requires C, 40.58; H,6.81; N,6.76; P,14.95%; M,207).

O,O-Dibenzyl 4-oxoazetidin-2-ylphosphonate (4). 4-Acetoxyazetidin-2-one (1) (0.5g, 3.9mmol) in tribenzyl phosphite (10ml) was heated at 120° under a slow nitrogen stream for 7h. Solvent was evaporated in vacuo to yield an oily solid. Chromatography afforded O,O-dibenzyl 4-oxo-azetidin-2-ylphosphonate (4) (0.6g, 46%), m.p. 86° (from methylene chloride ether), ν_{max}(KBr) 1755(β-lactam C=O), 1240(P=O) and 950 cm^{-1}(br. P-O-CH$_2$Ph), δ(CDCl$_3$) 3.08(2H,m,3-H), 3.64(1H, m,2-H), 5.04(4H,d,J_{HP}=10Hz,POCH$_2$Ph) and 7.40 (11H,s,Ph), (resonance at 7.40 covers NH as indicated by change in integral on addition of (D$_2$O). (Found: C,61.68; H,5.44; N,4.27; P,9.30. $C_{17}H_{18}NO_4P$ requires C,61.61; H,5.48; N,4.23 and P,9.35%).

O-Ethyl(4-oxoazetidin-2-yl)methylphosphinate (6). 4-Acetoxyazetidin-2-one (1) (5.15g, 40mmol) in diethyl methylphosphonite (40ml) was heated at 60° under a slow nitrogen stream for 1h. Solvent was removed in vacuo to yield a yellow oil. Chromatography gave O-ethyl(4-oxo-azetidin-2-yl)methylphosphinate (6) (6.6g, 93%) as a homogeneous oil consisting of the two diastereometric products, ν_{max}(thin film) 3400, 2950, 1770(β-lactam C=O), 1195(P=O), 1035 (P-O-CH$_2$CH$_3$) and 955cm^{-1}, δ(CDCl$_3$) 1.32(3H,t, J=8Hz,POCH$_2$CH$_3$), 1.52(3H,d,J_{HP}=14Hz,PCH$_3$), 3.12(2H,m,3-H), 3.72(1H,m,2-H), 4.12(2H,m, POCH$_2$CH$_3$) and 7.68(1H,s,NH), (addition of D$_2$O caused the signal at 7.68 to disappear). (Found: M$^+$,177.0558. $C_6H_{12}NO_3P$ requires M, 177.0555).

O-Benzyl(4-oxoazetidin-2-yl)methylphosphinate (7). 4-Acetoxyazetidin-2-one (1) (0.385g, 2.98mmol) in dibenzyl methylphosphonite (2ml) was heated at 90° under a slow nitrogen stream for 5h. Solvent was evaporated in vacuo to give a yellow oil. Chromatography afforded O-benzyl-(4-oxoazetidin-2-yl)methylphosphinate (7) (0.6g, 84%), m.p. 71° (from dichloromethane-petroleum ether), ν_{max}(KBr) 3100, 1750(β-lactam, C=O), 1200(P=O) and 1020cm^{-1}(P-O-CH$_2$Ph) δ(CDCl$_3$) 1.3(3H,d,J_{HP}=12Hz,PCH$_3$), 3.1(2H,m,3-H) 3.65(1H,m,2-H), 5.08(2H,d,J_{HP}=8Hz,POCH$_2$Ph), 7.10(1H,brs,NH) and 7.36(5H,s,Ph), (addition of D$_2$O causes the signal at 7.1 to disappear). (Found: C,55.07; H,5.95; N,5.76; P,13.27%. M(mass spectrum) 239. $C_{11}H_{14}NO_3P$ requires C,55.23; H,5.90; N,5.86; P,12.95%; M,239).

O-(2,2,2-Trichloroethyl)(4-oxoazetidin-2-yl) methylphosphinate (8). 4-Acetoxyazetidin-2-one (1) (0.65g, 5.04mmol) in toluene (2ml) and bis-(2,2,2-trichloroethyl)methyl-phosphonite (2.22g, 6.5mmol) was heated at 120° under a slow nitrogen stream for 24h. On cooling the reaction mixture crystals formed. A dichloromethane solution was washed with water (2x100ml) and the aqueous extracts evaporated in vacuo to give a white amorphous solid. Crystallisation from ethanol afforded O-(2,2,2-trichloroethyl)(4-oxoazetidin-2-yl)-methylphosphinate (8), (0.587g, 42%) as fine needles, m.p. 187-188°, ν_{max}(KBr) 3180, 1760 (β-lactam C=O), 1300(P-CH$_3$), 1200(P=O), 1090 (P-O-CH$_2$CCl$_3$) and 1020cm^{-1}(P-O-CH$_2$CCl$_3$), δ(C$_5$D$_5$N) 1.56(3H,d, J_{HP}=14Hz, PCH$_3$), 3.2(2H, m,3-H), 3.92(1H,m,2-H), 4.72(2H,m,POCH$_2$CCl$_3$) and 9.6(1H,s,NH), (addition of D$_2$O causes the signal at 9.6 to disappear). (Found: C,25.87; H,3.17; N,5.18; Cl,38.03; P,10.93%; M(mass spectrum), 280. $C_6H_9Cl_3CO_3P$ requires C,25.7; H,3.24; N,5.00; Cl,38.00 and P,11.05%; M, 280).

(2R,3R)-O,O-Dimethyl 4-oxo-3-phthalimido-azetidin-2-ylphosphonate (10). Azetidinone (9) (1.01g, 3.69mmol) in trimethylphosphite (10ml) was heated at reflux temperature under a slow nitrogen stream for 3h. The reaction mixture was allowed to cool and the solvent evaporated in vacuo to yield an oily solid. Crystallisation from dichloromethane-petroleum ether afforded (2R,3R)-O,O-dimethyl-4-oxo-3-phthalimidoazetidin-2-ylphosphonate (10) (1.07g, 89%), m.p. 160-162°, ν_{max}(KBr), 3480(amide NH), 1795 (β-lactam C=O), 1770 and 1710(phthalimido), 1230(P=O), 1050(P-O-CH$_3$) and 720cm^{-1}, δ ((CD$_3$)$_2$C=O), 3.82(6H,d,J_{HP}=12Hz,POCH$_3$), 4.08 (1H,dd,J_{HP}=10Hz,J=4Hz,2-H), 5.52(1H,dd,J_{HP}=10Hz, J=4Hz,3-H), 7.92(4H,s,phthalimido) and 8.12 (1H,brs,NH), (addition of D$_2$O causes the signal at 8.12 to disappear). (Found: C,48.46; H,4.05; N,8.57; P,9.47; M(mass spectrum) 324. $C_{13}H_{13}N_2O_6P$ requires C,48.16; H,4.04; N,8.64; P,9.55%; M,324).

(2R,3R)-O-Ethyl 4-oxo-3-phthalimidoazetidin-2-ylphosphinate (11). Azetidinone (1) (0.265g, 0.98mmol) in diethylmethylphosphonite (5ml) was heated at 60-70° for 30min under a slow nitrogen stream. Solvent was evaporated in vacuo to give a yellow oil. Chromatography afforded (2R,3R)-O-ethyl 4-oxo-3-phthalimido-azetidin-2-ylphosphinate (11) (0.262g, 83%), m.p. 194-198°, (from dichloromethane-petroleum ether), ν_{max}(KBr), 3450(amide NH), 1785(β-lactam C=O), 1720(phthalimido), 1215(P=O) and 1030cm^{-1}(P-O-CH$_2$CH$_3$), δ((CD$_3$)$_2$C=O), 1.28(3H,t, J=7Hz,POCH$_2$CH$_3$), 1.55(3H,d,J_{HP}=14Hz,PCH$_3$), 4.16(3H,m,2-H and POCH$_2$CH$_3$), 5.48(1H,m,3-H), 7.85(4H,s,phthalimido) and 8.20(1H,brs,NH), (addition of D$_2$O causes the signal at 8.2 to disappear). (Found: C,52.03; H,4.62; N,8.63; P,9.46%; M,322.0719. $C_{14}H_{15}N_2PO_5$ requires C,52.16; H,4.69; N,8.70; P,9.62; M,322.0718). ^{31}P and ^{1}H n.m.r spectra indicated the presence of both cis and trans isomers about C-3 - C-4 and the presence of two isomers about phosphorus.

Reaction of 4-acetoxyazetidin-2-one (1) with hexamethylphosphorus triamide. 4-Acetoxyazetidin-2-one (1) (0.25g, 1.9mmol) in hexamethylphosphorus triamide (2ml) was stirred overnight under a slow nitrogen stream. Evaporation of the solvent in vacuo gave a yellow oil which was chromatographed to afford firstly, (E)-3-(N,N-dimethylamino)acrylonitrile (13) (0.09g, 49%) as a homogeneous oil, ν_{max}(thin film) 2920, 2190 (nitrile) and 1635 cm^{-1} (enamine), $\delta(CDCl_3)$ 2.8(6H,s,N($\underline{CH_3}$)$_2$), 3.64(1H,d,J=14Hz, $\underline{CHCN}$) and 6.86(1H,d,J=14Hz, $\underline{CHN}(CH_3)_2$). (Found: $\underline{M}^{\oplus}$, 96.0682. $C_5H_8N_2$ requires M,96.0687). The second product eluted was unchanged (1) (0.02g, 8%) and thirdly (E)-3-(N,N-dimethylaminoprop-2-eneamide (12) (0.027g, 12%) m.p. 119-120° (from dichloromethane-petroleum ether) ν_{max}(KBr) 3380 (amide NH), 1645br(amide C=O and enamine) and 1560cm^{-1}(amide II), $\delta(CDCl_3)$ 2.84(6H,s,N($\underline{CH_3}$)$_2$), 4.54(1H,d,J=12Hz,$\underline{CHCONH_2}$), 5.50(2H,brs,$\underline{NH_2}$) and 7.38(1H,d,J=12Hz,$\underline{CHN}(CH_3)_2$), (addition of D_2O caused the signal at 5.50 to disappear). (Found: C,52.18; H,9.08; N,23.54%, $\underline{M}^{\oplus}$,114.0792. $C_5H_{10}N_2O$ requires C,52.63; H,8.77; $\underline{N}$,24.56%; $\underline{M}$, 114.0793).

The nitrile (13) decomposed to an unidentified compound; m.p. 122-123° (from dichloromethane-petroleum ether), ν_{max}(KBr) 2205s, 1640s and 1595s cm^{-1} $\delta(CDCl_3)$ 3.4(6H,s), 5.32(1H,d,J=15Hz), 7.04(1H,s) and 7.06(1H,d, J=15Hz). (Found: C,64.93; H,6.33; N,28.38%).

(E)-3-(N,N-Dimethylamino)-prop-2-enamide (12). 4-Acetoxyazetidin-2-one (1) (0.754g, 5.8mmol) in methanol (25ml) was reacted with dimethylamine (3.4g, 5ml, 75.4mmol) at 0° for 4h. Evaporation of the solvent in vacuo gave (E)-3-(N,N-dimethylamino)-prop-2-enamide (12) (0.65g, 98%).

(E)-3-(N,N-Diethylamino)-prop-2-enamide (14). Azetidin-2-one (1) (0.46g, 3.5mmol) in dry dichloromethane (6ml) was treated with diethylamine (0.71g, 1mol, 9.7mmol) and stirred overnight. Evaporation in vacuo gave (E)-3-(N,N-diethylamino)-prop)-2-enamide (14) as an oil. ^1H N.M.R. spectroscopy indicated quantitative conversion, but following aqueous bicarbonate work-up to remove acetic acid, the yield was reduced to 20%, ν_{max}(film) 3300, 2970, 1650, 1570 and 1385cm^{-1}, $\delta(CDCl_3)$ 1.12 (6H,t,J=8Hz, $NCH_2\underline{CH_3}$), 3.16(4H,q,J=8Hz,$\underline{NCH_2}CH_3$), 4.64(1H,d, J=13Hz,$\underline{CHCONH_2}$), 5.5(2H,bs,$\underline{NH_2}$) and 7.24(1H,d, J=13Hz, $\underline{CHNEt_2}$), $\underline{M}^{\oplus}$,142. This compound decomposed on standing at 0° under nitrogen.

(E)-3-(N,N-Dibenzylamino)-prop-2-enamide (15). Reaction as above gave (15) as an unstable oil, ν_{max}(film) 3300, 3020, 2960, 1655, 1570 and 1375cm^{-1}, $\delta(CDCl_3)$ 3.76(4H,s,$PhCH_2$), 4.68(1H,d, J=14Hz,$\underline{CHCONH_2}$), 5.1(2H,bs,$\underline{NH_2}$), 7.28(10H,s,Ph) and 7.78(1H,d,J=14Hz,$\underline{CHNR_2}$).

(E)-3-(N,N-Diisopropylamino)-prop-2-enamide(16). Azetidin-2-one (1) (0.45g, 3.5mmol) in dry dichloromethane was reacted with diisopropylamine (0.72g, 1ml, 7.13mmol) at room temperature overnight. Evaporation in vacuo, dissolution in dichloromethane (50ml), washing with water (50ml), saturated aqueous sodium hydrogen carbonate (50ml), drying ($MgSO_4$) and evaporation in vacuo gave (E)-3-(N,N-diisopropylamino)-prop-2-enamide (16) (0.32g, 53%) as an ammophous solid, m.p. 162-165°, ν_{max}(KBr) 3430, 3150, 2980, 1655, 1560 and 1270cm^{-1}, δ $(CDCl_3)$ 1.16(12H,d,J=6Hz,$\underline{CHMe_2}$), 3.6(2H,m,$\underline{CH}$ Me_2), 4.7(1H,d,J=16Hz,$\underline{CHCONH_2}$), 5.42(2H,bs,$\underline{NH_2}$) and 7.54(1H,d,J=16Hz,$\underline{CHNEt_2}$). (Found: C,63.60; H,10.86; N,16.54%. $C_9H_{18}N_2O$ requires C,63.47; H,10.66; N,16.46%).

4-Oxoazetidin-2-ylphosphonic acid monomethyl ester (20). O,O-Dimethyl 4-oxoazetidin-2-ylphosphonate (2) (1.79g, 10mmol) in dry tetrahydrofuran at -50° under argon was treated with n-butyllithium (10mmol), and stirred for 1h. tert-Butyldimethylsilyl chloride (1.50g, 10mmol) was added and the solution allowed to reach room temperature, then stirred overnight. The mixture was poured onto ice water - ether acetate, the organic phase washed (H_2O), dried ($MgSO_4$) and evaporated in vacuo to give a yellow oil. Flash chromatography gave (17) as an oil (1.03g, 35%), $\nu_{max}(CHCl_3)$ 1740, 1250 and 1040cm^{-1}, $\delta^1H(CDCl_3)$ 3.80(7H,d,J=12Hz$_{P-H}$, collapses to singlet upon ^{31}P irradiation, P($\underline{OMe_2}$) and H-2), 3.30(2H,m,H-3), 0.99(9H,s, Si$\underline{^tBu}$), 0.28(6H,s,$\underline{SiMe}$), $\delta^{13}C(CDCl_3)$ 171.6 (C=O), 53.36(m,PO($\underline{OMe}$)$_2$), 44.46(dd,J_{C-P}=130Hz, C-2), 39.75(t,C-3), 26.42(q,$\underline{CMe_3}$), 18.73(s, $\underline{CMe_3}$), 5.58(q,$\underline{SiMe_2}$), $\delta^{31}P(CDCl_3)$ -25(p.p.m. downfield from 88% H_3PO_4). (Found: C,45.11; H,8.12; N,4.82%. $C_{11}H_{24}NO_4PSi$ requires C, 45.05; H,8.19; N,4.78%; $\underline{M}^{\oplus}$,293).

Derivative (17) (50mg, 0.17mmol) was dissolved in dry deuteriochloroform (0.38ml) under argon at room temperature and treated with trimethylsilyl bromide (26mg, 0.17mmol). N.M.R. indicated complete reaction in 2h. Evaporation in vacuo gave (18) as a waxy yellow oil (58mg, 98%), $\nu_{max}(CDCl_3)$ 1740, 1240 and 1040cm^{-1}, $\delta^1H(CDCl_3)$ 3.72(4H,d,J=12Hz, P(O)OMe and H-2), 3.20(2H,m,H-3), 0.90(9H,s,Si$\underline{^tBu}$), 0.30(9H,d,P(O)OSiMe) and 0.15(6H,s,$\underline{SiMe_2}{}^tBu$).

Derivative (18) (58mg, 0.17mmol) was dissolved in dichloromethane and stirred with 10% aqueous acetone for 16h at room temperature. Evaporation in vacuo gave a yellow oil containing the N-silyl phosphonic acid (19), (M^+279), together with the monophosphonic acid (20), (M^+165). Treatment of this mixture with diazomethane afforded a mixture of O,O-dimethyl 4-oxoazetidin-2-ylphosphonate (2) and the N-tert-butyldimethylsilyl derivative (17), spectroscopically identical to authentic samples. Over three days at 0°, compound (19) autocatalytically deprotected to give (20), but the totally deprotected product (20) was too unstable to be isolated pure, undergoing β-lactam cleavage under all conditions investigated. Impure (20), $\nu_{max}(CDCl_3)$ 1740, 1240 and 1040cm^{-1}, $\delta^1H(CDCl_3)$ 3.70(4H,d,J=12Hz, P(O)OMe + H-2), 3.20(2H,m,H-3), 7.38(1H, brs, NH).

3-Amino-3-phosphonopropanoic acid (21). O,O-Diethyl 4-oxoazetidin-2-ylphosphonate (3) (0.25g, 1.39mmol) in 6M hydrochloric acid (10ml) was heated at 90° for 18h. Solvent was evaporated in vacuo and the residue dissolved in a minimum of ethanol-water (5:1). The solution was treated with propylene oxide until no further precipitation occurred, the precipitate was filtered and dried giving 3-amino-3-phosphonopropanoic acid (21) (0.175g, 74%), m.p. 224°(dec), ν_{max}(KBr) 3400(br NH), 3030, 2700-2500(br P-O-H and OH), 1710(C=O), 1270 (P=O), 1140 and 1090cm^{-1}. (Found: C,21.0; H, 4.77; N,8.37; P,18.32%. $C_3H_8NO_5P$ requires C,21.31; H,4.76; N,8.28; P,18.32%).

3-Amino-3-methylphosphinopropanoic acid (22). O-Ethyl-4-oxoazetidin-2-ylmethylphosphinate (6) (0.191g, 1.08mmol) in 6M hydrochloride acid (3ml) was heated at 90° for 18h. Solvent was evaporated in vacuo to give an oily foam which was re-dissolved in a minimum of ethanol-water (5:1). The solution was treated with propylene oxide until the pH remained constant (pH6), evaporated to a small volume (5ml) and triturated with ethanol until precipitation ceased. The precipitate was filtered off and dried giving 3-amino-3-methylphosphinopropanoic acid (22) (0.117g, 65%), m.p. 221°(dec), ν_{max} (KBr) 3220(NH), 3000-2480(br P-O-H and OH), 1695(C=O), 1300(P=O) and 1020cm^{-1}. (Found: C,28.4; H,5.94; N,8.77%. $C_4H_{10}NO_4P$ requires C,28.75; H,6.03; N,8.38%).

(2R,3R)-2,3-Diamino-3-phosphonopropanoic acid (23). (R,R)-O,O-Dimethyl 4-oxo-3-phthalimidoazetidin-2-ylphosphonate (10) (0.25g, 0.77mmol) in 6M hydrochloric acid (4ml) was heated at 100° overnight. The reaction mixture was cooled, diluted with water (25ml), the aqueous solution extracted with ether (4x25ml) and the aqueous solution evaporated in vacuo. The gummy residue obtained was dissolved in a minimum of ethanol-water (5:1) and treated with propylene oxide until there was no further change in pH. The solution was cooled overnight before filtering off a precipitate of (2R,3R)-2,3-diamino-3-phosphonopropanoic acid (23) (0.047g, 28% (corrected)), m.p. 198°(dec) ν_{max} 3400(NH), 1700(C=O), 1170 and 1070cm^{-1}. (Found: C,16.66; H,5.52; N,13.07; P,14.25%. $C_3H_9N_2O_5P(H_2O)_2$ requires C,16.4; H,5.9; N,12.7; P,14.1%).

2,3-Diamino-3-methylphosphinopropanoic acid (24) O-Ethyl 4-oxo-3-phthalimidoazetidin-2-ylmethylphosphinate (11) (0.209g, 0.65mmol) in 6M hydrochloric acid (4ml) was heated at 100° overnight. The reaction mixture was diluted with water (25ml) and extracted with ether (4x25ml) and the aqueous solution separated and evaporated in vacuo. The residue was dissolved in a minimum of ethanol-water (5:1) and treated with propylene oxide until no further change in pH occurred. The solution was cooled overnight before filtering off a precipitate of 2,3-diamino-3-methylphosphinopropanoic acid (24) (0.048g, 35%(corrected)), m.p. 184°(dec), ν_{max}(KBr) 3400-2500(br,NH,C-O-H,P-O-H), 1700 (C=O), 1220(P=O) and 1085cm^{-1}(P-O-H and P-CH_3). (Found: C,23.9; H,6.5; N,12.8; P,14.7%. $C_4H_{11}N_2O_4P(H_2O)_{1.5}$ requires C,23.0; H,6.7; N, 13.4; P,14.8%).

O,O-Dimethyl 4-oxo-1-(N-benzyloxycarbonyl-D,L-alanyl)-2-ylphosphonate (26). Azetidin-4-one (2) (0.25g, 1.4mmol) and triethylamine (0.17g, 0.234ml, 1.68mmol) in dry dichloromethane (5ml) were cooled to -20° under nitrogen. Separately N-benzyloxycarbonyl-D,L-alanine (25) (0.312g, 1.4mmol) was dissolved in dry dichloromethane (5ml), cooled to -20° under nitrogen, triethylamine (0.17g, 0.234ml, 1.68mmol) added, stirred for 5min and treated with isobutyl chloroformate (0.211g, 0.2ml, 1.54mmol). After 5min the solution of azetidin-4-one (2) was added. The combined solutions were stirred at -20° for a further 30min and then overnight at 4°. The reaction mixture was washed with 0.1M hydrochloric acid (until the washings were acidic), saturated sodium hydrogen carbonate solution (50ml), dried ($MgSO_4$) and evaporated in vacuo to yield an oil. Chromatography afforded O,O-dimethyl 4-oxo-1-(N-benzyloxycarbonyl-D,L-alanyl)-2-ylphosphonate (26) (0.28g, 52%) as a homogeneous oil, ν_{max}(thin film) 3280(NH), 1800 (β-lactam C=O), 1710(imide and urethane C=O), 1250(P=O) and 1040cm^{-1}(P-O-CH_3), δ($CDCl_3$) 1.4 (3H,d,J=6Hz,$CHCH_3$), 3.3(2H,m,3-H), 3.8(6H,d, J_{HP}=11Hz,$POCH_3$), 4.12(1H,m,2-H), 4.84(1H,m, $CHCH_3$), 5.08(2H,s,$PhCH_2$), 5.60(1H,brm,NH) and 7.12(5H,s,Ph), (addition of D_2O causes the signal at 5.6 to disappear). (Found: C,50.9; H,6.03; N,6.56%; $M^{\oplus}$,384.1074. $C_{16}H_{21}N_2O_7P$ requires C,50.0; H,5.51; N,7.3%; M,384.1086).

Methanolysis of O,O-dimethyl 4-oxo-1-(N-benzyloxycarbonyl-D,L-alanyl)-2-ylphosphonate (26). Azetidin-4-one (2) (0.653g, 1.7mmol) in dry chloride initially at 0° for 2.5h and then at room temperature for a further 11h. The solvent was evaporated in vacuo to give an oil which was re-dissolved in dry dichloromethane (25ml) and treated with sodium hydrogen carbonate (c.a. 2.5g). The slurry obtained was stirred for 30min, filtered through "Celite", the filtrate evaporated in vacuo and the oily residue chromatographed to give methyl-3,3-dimethylphosphono-3-[(N-benzyloxycarbonyl-D,L-alanyl)-amino]-propanoate (27) (0,109g, 15%) (0.069g crystallised from dichloromethane-ether, m.p. 82-85%, 0.04g as a homogeneous oil), ν_{max}(KBr) 3350(br NH amide), 1740(ester C=O), 1680(urethane C=O), 1660(amide C=O), 1530(amide II), 1220(P=O) and 1030cm^{-1} (P-O-CH_3), δ($CDCl_3$) 1.22(3H,d,J=6Hz,$CHCH_3$), 2.60(2H,m,2-H), 3.54(3H,s,CO_2CH_3), 3.58(6H,d, J_{HP}=11Hz,$POCH_3$), 4.14(1H,m)*, 4.70(1H,m)*, 4.89(2H,s,$PhCH_2$), 5.52(1H,brs,NH), 7.04(5H,s, Ph) and 7.3(1H,brs,NH), (addition of D_2O causes the signals at 5.52 and 7.3 to disappear). (Found: $M^{\oplus}$,416.1345. $C_{17}H_{25}N_2O_8P$ requires M, 416.1348).

* These signals are due to 3-H and the alanyl methine H.

Methyl 3-amino-3,3-dimethylphosphonopropanoate (28). Azetidinone (2) (0.243g, 1.34mmol) in dry methanol (10ml) was cooled to 0° and treated with dry HCl for 2.5h. Evaporation in vacuo gave an oily residue which was dissolved in methylene chloride and stirred with sodium hydrogen carbonate (2g) for 30min, filtered and evaporated in vacuo to yield methyl 3-amino-3,3-dimethylphosphonopropanoate (28) (0.284g, 99%) as a homogeneous oil, ν_{max}(film) 3400, 1740, 1030 and 830cm^{-1}, δ($CDCl_3$) 1.7(2H,bs,NH_2), 2.54(2H,m,2-H), 3.4(1H,m,3-H), 3.64(3H,s,CO_2Me) and 3.73(6H,d,J_{HP}=10Hz,POMe). (Found: M^+ 211.0609. $C_6H_{14}NO_5P$ requires M^+, 211.0610).

Methyl 3,3-dimethylphosphono-3-[(N-benzyloxycarbonyl-D,L-alanyl)amino]-propanoate (27). Phosphonopropanoate (28) (1.68g, 7.98mmol) in dry dichloromethane (50ml) was treated with triethylamine (0.967g, 1.33ml, 9.6mmol) and cooled to -20° under nitrogen. Separately N-benzyloxycarbonyl-D,L-alanine (1.78g, 7.98mmol) was dissolved in dry dichloromethane (75ml), cooled to -20° under a slow nitrogen stream, treated with triethylamine (0.967g, 1.33ml, 9.6mmol), stirred for 5min and treated with isobutyl chloroformate (1.2g, 1.14ml, 8.78mmol). The mixed anhydride solution was stirred for a further 5min and then the phosphonopropanoate solution added. The combined solutions were stirred for a further 30min at -20° and then overnight at 4°. The reaction mixture was washed with ice-cold 0.2M hydrochloric acid (2x25ml), water (3x25ml), saturated sodium hydrogen carbonate solution (2x25ml), water (4x25ml), dried ($MgSO_4$) and evaporated in vacuo to yield a crude oily product. Chromatography

afforded methyl 3,3-dimethylphosphono-3-[(N-benzyloxycarbonyl-D,L-alanyl)amino]-propanoate (27) (1.9g, 57%(. (Found: M$^{\oplus}$,416.1345. $C_{17}H_{25}N_2O_8P$ requires M, 416.1348).

3,3-Dimethylphosphono-3-[(N-benzyloxycarbonyl-D,L-alanyl)amino]-propanoic acid (30). Dipeptide (27) (0.597g, 1.55mmol) in 30% aqueous methanol (25ml) at 0° was treated with sodium hydroxide (4M, 1.1 equivalents) and stirred for a further 3h at 0°. Evaporation of the solvent in vacuo gave a crude oil which was re-dissolved in water (25ml) and extracted with ether (2x25ml). The aqueous layer separated and was acidified (Congo Red) by addition of 2M hydrochloric acid and extracted with n-butanol (5x20ml). The butanol washings were combined, dried ($MgSO_4$) and evaporated in vacuo to afford 3,3-dimethylphosphono-3-[(N-benzyloxycarbonyl-D,L-alanyl)amino]-propanoic acid (30) (0.45g, 78%)*, m.p. 175°, ν_{max}(KBr) 3300 (amide NH), 1700(br 3 bands, acid C=O, urethane C=O and amide C=O), 1530(amide II), 1200(P=O) and 1050cm^{-1} (P-O-CH_3), δ(CD_3OD), 1.24(3H,d,J=6Hz, $CHCH_3$), 2.56(2H,m,$CH_2CO_2CH_3$), 3.58(6H,d,J_{HP}=10Hz,$POCH_3$), 3.96(1H,m), 4.84(2H,s,$PhCH_2$) and 7.0(5H,s,Ph). (Found; M$^{\oplus}$,402.1192. $C_{16}H_{23}N_2O_8P$ requires M,402.1192).

* Greater yields could be obtained by acidifying the reaction mixture, evaporating in vacuo and removing any residual sodium chloride by ion exchange chromatography of the final product.

Methyl-3,3-dimethylphosphono-3-[(D,L-alanyl)-amino]-propanoate (29). Dipeptide (27) (0.155g, 0.37mmol) in methanol (9ml) and acetic acid (ml) containing palladium on charcoal (10%) (0.1g, 5%) was stirred under hydrogen at atmospheric pressure for 2h. Solvent was evaporated in vacuo to yield an oil residue contaminated with acetic acid. The residue was dissolved in dry dichloromethane (15ml) and treated with sodium hydrogen carbonate (0.5g). The slurry obtained was stirred for 2.5h and the solution filtered through "Celite". Evaporation of the filtrate in vacuo gave methyl-3,3-dimethylphosphono-3-[(D,L-alanyl)amino]-propanoate (29) (0.104g, quantitative), m.p. 132-133° (from dichloromethane-ether), ν_{max}(KBr) 3300(NH), 1750(ester C=O), 1554(amide C=O), 1540(amide II), 1235(P=O) and 1030cm^{-1} (P-O-CH_3), δ($CDCl_3$), 1.27(3H,d, J=6Hz, $CHCH_3$), 2.68(2H,m,$CH_2CO_2CH_3$), 3.60(3H,s, CO_2CH_3), 3.71(6H,d,J_{HP}=11Hz,$POCH_3$), 4.50(1H,m, CHP), 4.75(1H,m,$CHCH_3$), 6.88(1H,d,J=9Hz,NH), 7.81(1H,d,J=8Hz,NH), (addition of D_2O causes the signals at 6.88 and 7.81 to disappear). (Found: M$^{\oplus}$,282.1008. $C_9H_{19}N_2O_6P$ requires M,282.0980).

Methyl-3,3-bis(trimethylsilyl)phosphono-3-[(N-benzyloxycarbonyl-D,L-alanyl)amino]-propanoate (31). Dipeptide (27) (0.2g, (0.48mmol) in dry dichloromethane (12ml) under nitrogen was treated with trimethylsilyl bromide (0.164g, 0.14ml, 1.06mmol) dropwise over 3min. The solution was stirred for 3h and then all solvent and alkyl bromide evaporated in vacuo to give methyl-3,3-bis(trimethylsilyl)-phosphono-3-[(N-benzyloxycarbonyl-D,L-alanyl)-amino]-propanoate (31) (0.24g, 94%) as a homogeneous oil, δ($CDCl_3$), 0.24(18H,s,$OSi(CH_3)_3$) 1.32(3H,d,J=8Hz,$CHCH_3$), 2.68(6H,m,$CH_2CO_2CH_3$), 4.26(1H,m), 4.70(1H,m), 4.96(2H,s,$PhCH_2$), 6.00 (1H,brs,NH), 7.18(5H,s,Ph) and 7.94(1H,brs,NH). (Found: M$^{\oplus}$,532.1818. $C_{21}H_{37}N_2O_8PSi_2$ requires M,532.1826).

Methyl-3-[(N-benzyloxycarbonyl-D,L-alanyl)-amino]-3-phosphonopropanoate (32). Dipeptide (31) (0.094g, 0.18mmol) in acetone (10ml) was stirred with water (0.1g, 5.6mmol) for 3h. Solvent was evaporated in vacuo to yield a yellow gum, crystallisation from acetone-petroleum ether affording methyl-3-[(N-benzyloxycarbonyl-D,L-alanyl)amino]-3-phosphonopropanoate (32) (0.055g, 80%), m.p. 93-96°, ν_{max} (KBr) 3320(NH), 1710(ester C=O, urethane C=O), 1655(amide C=O), 1530(amide II) and 1240cm^{-1} (P=O), δ($(CD_3)_2C$=O), 1.34(3H,d,J=6Hz,$CHCH_3$), 2.76(2H,m,CH_2CO_2Me), 3.61(3H,s,CO_2CH_3), 4.40 (2H,brm,$CHCH_3$ and CHP), 6.63(1H,brs,NH), 7.34 (5H,s,Ph) and 7.82(1H,brs,NH). (Found: M$^{\oplus}$, 388.1036. $C_{15}H_{21}N_2O_8P$ requires M,388.1035).

3-[(D,L-Alanyl)amino]-3-phosphonopropanoic acid (33). Dipeptide (30) (0.411g, 1.02mmol) was stirred with 45% hydrogen bromide in acetic acid (2.5ml) for 2h. Ether (10ml) was added dropwise with rapid stirring and then decanted off, this procedure being repeated a further two times. The residual gum was dissolved in methanol (10ml) and treated with propylene oxide until the pH remained constant at which point the clear solution rapidly became cloudy and a white precipitate formed. The solution was placed in the cooling compartment of a refrigerator overnight. The precipitate was removed by filtration and washed with dry methanol (2x3ml). The precipitate was dried in vacuo to give 3-[(D,L-alanyl)amino]-3-phosphonopropanoic acid (33) (0.156g, 59% (corrected)), m.p. 237°(dec), ν_{max}(KBr) 3320 (NH), 3180(OH), 1725(C=O), 1600, 1200(P=O) and 960cm^{-1}. (Found: C,28.6; H,5.4; N,11.5%. $C_6H_{15}N_2O_6P(H_2O)$ requires C,27.9; H,5.8; N, 10.9; P,12.0%). This product was shown to be free of amino acid contaminants by amino acid analysis. Hydrolysis afforded alanine and 3-amino-3-phosphonopropanoic acid as the sole products.

Methyl-3-amino-3-[(O-ethyl)-methylphosphino]-propanoate (37). O-Ethyl-4-methylphosphino-azetidin-2-one (6) (1.53g, 8.66mmol) in dry methanol (20ml) was cooled to 0° and treated with dry hydrogen chloride for 3.5h. Solvent was evaporated in vacuo to give an oily residue which was dissolved in dry dichloromethane (25ml), treated with sodium hydrogen carbonate (c.a. 5g) and the resulting slurry stirred for 20min. The solution was filtered through "Celite" and the filtrate evaporated in vacuo to afford methyl-3-amino-3-[(O-ethyl)-methylphosphino]propanoate (37) (1.3g, 72%) as a homogeneous oil, ν_{max}(thin film) 3400(amine NH), 1735(ester C=O), 1200(P=O), 1030(P-O-CH_2CH_3) and 955cm^{-1}(P-O-CH_2CH_3), δ($CDCl_3$), 1.33(3H,t, J=7Hz,$POCH_2CH_3$), 1.55(3H,d,J_{HP}=14Hz, PCH_3), 2.04(1H,brs,NH)*, 2.60(2H,brm,$CH_2CO_2CH_3$), 3.73 (3H,s,CO_2CH_3) and 4.10(2H,m,$POCH_2CH_3$), (addition of D_2O causes the signal at 2.04 to disappear). (Found: M+1$^{\oplus}$,210.0892. $C_7H_{17}NO_4P$ requires M+1, 210.0895).

* The second NH proton gave a broad signal over the region 2 to 4 and did not exchange with D_2O.

Methyl-3,3-dimethylphosphono-3-[(N-benzyloxycarbonyl-L-alanyl-L-alanyl)amino]-propanoate (34). Phosphonopropanoate (28) (0.156g, 0.74mmol) in dry dichloromethane (5ml) was reacted with benzyloxycarbonyl-L-alanyl-L-alanine (0.217g, 0.74mmol) as in the preparation of (27). Chromatography afforded methyl-3,3-dimethylphosphono-3-[(N-benzyloxycarbonyl-L-alanyl-L-alanyl)amino]-propanoate (34) (0.187g,

52%), m.p. 131°* (from dichloromethane-ether), ν_{max}(KBr) 3300(NH), 1745(ester C=O), 1690 (urethane C=O), 1645(amide C=O), 1530(amide II), 1250(P=O) and 1040cm^{-1}(P-O-CH_3), δ($CDCl_3$), 1.31 (3H,d,J=6Hz,$CHCH_3$), 1.33(3H,d,J=7Hz,$CHCH_3$), 2.70(2H,m,$CH_2CO_2CH_3$), 3.62(3H,s,CO_2CH_3), 3.68 (6H,d,J_{HP}=11Hz,$POCH_3$), 4.28(1H,m,CHP), 4.73(2H, brm,$CHCH_3$), 5.07(2H,s,$PhCH_2$), 5.97(1H,t,J=8Hz, NH), 7.12(1H,t,J=8Hz,NH), 7.29(5H,s,Ph) and 7.64(1H,brm,NH), (addition of D_2O causes the signals at 5.97, 7.12 and 7.64 to disappear after prolonged contact (3h)). (Found: M$^{\oplus}$, 487.1718. $C_{20}H_{30}N_3O_9P$ requires M,487.1719).
* M.p. dependent on rate of heating.

Methyl-3-[(N-benzyloxycarbonyl-L-alanyl-L-alanyl)amino]-3-[(O-ethyl)methylphosphino]-propanoate (38). (a) By the carbonic mixture anhydride (CMA) procedure.
Methylphosphinopropanoate (37) (0.35g, 1.68mmol) in dry dichloromethane (10ml) was cooled to -20° under a slow nitrogen stream. Separately, N-benzyloxycarbonyl-L-alanyl-L-alanine (0.494g, 1.68mmol) in dry dichloromethane (20ml) was cooled to -20° under nitrogen, treated with triethylamine (0.212g, 0.29ml, 2.1mmol), stirred for 5min, treated with isobutyl chloroformate (0.252g, 0.24ml, 1.85mmol) and stirred for a further 5min. The phosphinate solution was added to the solution of the mixed anhydride and the combined solutions stirred for a further 30min at -20° and overnight at 4°. The reaction mixture was washed with water (50ml), with 0.1M hydrochloric acid (50ml), with saturated sodium hydrogen carbonate solution (50ml), with water (50ml), dried ($MgSO_4$) and evaporated in vacuo to give an oil. Chromatography afforded methyl-3-[(N-benzyloxycarbonyl-L-alanyl-L-alanyl)-amino]-3-[(O-ethyl)methylphosphino]-propanoate (38) (0.327g, 40%) as a homogeneous foam, ν_{max} (KBr) 3400(NH), 1730(ester C=O), 1690(urethane C=O), 1640(amide C=O), 1540(amide II), 1210 (P=O), 1040(P-O-CH_2CH_3) and 965cm^{-1}(P-O-CH_2CH_3), δ($CDCl_3$), 1.4(12H,m,$POCH_2CH_3$,PCH_3 and $CHCH_3$), 2.82(2H,m,$CH_2CO_2CH_3$), 3.7(3H,s,CO_2CH_3), 4.10(2H, m,$POCH_2CH_3$), 4.36(1H,m,CHP), 4.76(2H,m,$CHCH_3$), 5.16(2H,s,$PhCH_2$), 6.32(1H,brs,NH), 7.44(5H,s, Ph) and 7.88(2H,brs,NH and NH), (addition of D_2O causes the signals at 6.32 and 7.88 to disappear after prolonged contact (6h)). (Found: M$^{\oplus}$,485.1937. $C_{21}H_{32}N_3O_8P$ requires M,485.1927).
(b) By the dicyclohexylcarbodiimide (DCC) procedure.
Methylphosphinopropanoate (37) (0.628g, 3mmol) and N-benzyloxycarbonyl-L-alanyl-L-alanine (0.883g, 3mmol) in dry dichloromethane (20ml) and THF (8ml) were treated with DCC (0.681g, 3.3mmol) in a single portion. The reaction mixture was stirred for 8h, treated with acetic acid (1ml) and stirred for a further 5min. The solution was filtered and the filtrate evaporated in vacuo to yield an oil. The oil was dissolved in dichloromethane (50ml) and washed with 0.1M hydrochloric acid (50ml), saturated sodium hydrogen carbonate solution (50ml) and water (50ml). The organic layer was separated, dried ($MgSO_4$) and evaporated in vacuo to yield a solid residue contaminated with dicyclohexyl urea (DCU). The DCU was removed by adding acetone to the residue and filtering off the insoluble DCU, this procedure being repeated with smaller volumes of acetone until no further DCU was obtained, giving methyl-3-[(N-benzyloxycarbonyl-L-alanyl-L-alanyl)amino]-3-[(O-ethyl)-methylphosphino]-propanoate (38) (1.0g, 69%).

3,3-Dimethylphosphono-3-[(N-benzyloxycarbonyl-L-alanyl-L-alanyl)amino]-propanoic acid (35). Tripeptide (34) (0.499g, 1.03mmol) in 30% aqueous methanol (25ml) was treated at 0° with 4M sodium hydroxide (1.1 equivalents) and stirred overnight at 4°. The reaction mixture was extracted with dichloromethane (25ml), acidified (Congo Red) and evaporated in vacuo to yield 3,3-dimethylphosphono-3-[(N-benzyloxycarbonyl-L-alanyl-L-alanyl)amino]-propanoic acid (35)* (0.578g, contaminated with sodium chloride)*, ν_{max}(KBr) 3300(br,NH and OH), 1720 (br acid C=O and urethane (C=O), 1660(amide C=O), 1590(amine II), 1240(P=O) and 1045cm^{-1} (P-O-CH_3), δ($CDCl_3$), 1.36(6H,d,J=7Hz, $CHCH_3$), 2.82(2H,brm,CH_2CO_2H), 3.84(6H,d,J_{HP}=14Hz,$POCH_3$), 4.5(3H,brm,$CHCH_3$ and CHP), 5.16(2H,s,PhCH) and 7.44(5H,s,Ph).
* For best overall yields of the free tripeptide (36) residual sodium chloride was not removed and (35) was used without further purification. Sodium chloride could be removed by ion-exchange chromatography using Amberlite IRC-120. The hygroscopic nature of (35) meant that good microanalytical data could not readily be obtained.

3-[(N-Benzyloxycarbonyl-L-alanyl-L-alanyl)-amino]-3-[(O-ethyl)methylphosphino]-propanoic acid (39). Tripeptide (38) (0.488g, 1.01mmol) in 30% aqueous methanol (10ml) was treated with 4M sodium hydroxide (1.1 equivalents) at 0° and stirred at 4° overnight. Solvent was evaporated to give a small volume (c.a. 2ml) and diluted with water (50ml). The aqueous solution was extracted with dichloromethane (2x30ml) and the aqueous portion concentrated to a small volume (c.a. 5ml) and passed through a column of Amberlite IRC-120 (c.a. 20ml). The column was eluted with methanol until the washings were neutral and the combined washings evaporated in vacuo giving 3-[(N-benzyloxycarbonyl-L-alanyl-L-alanyl)amino]-3-[(O-ethyl)-methylphosphino]-propanoic acid (39) (0.449g, 94%), m.p. 140-144°, ν_{max}, 3380(amide NH), 1715(acid C=O), 1685(urethane C=O), 1640(amide C=O), 1530(amide II), 1260(P=O) and 1040cm^{-1}(P-O-CH_2CH_3), δ(CD_3OD), 1.34(12H,m,$POCH_2CH_3$,PCH_3 and $CHCH_3$), 2.72(2H,m,CH_2CO_2H), 4.32(5H,m,$POCH_2CH_3$,$CHCH_3$ and CHP), 5.2(2H,s,$PhCH_2$) and 7.48(5H,s,Ph). The hygroscopic nature of (39) did not allow good microanalytical data to be obtained.

3-[(L-Alanyl-L-alanyl)amino]-3-phosphono-propanoic acid (36). Tripeptide (35) (0.47g, 0.97mmol) in acetic acid (4ml) was treated with 45% hydrogen bromide in acetic acid (2ml) and stirred for 2h. Ether (10ml) was added to the solution with rapid stirring and the etheral layer decanted off. This procedrue was repeated a further two times and the oily residue obtained dissolved in methanol (20ml) and treated with propylene oxide until the pH of the solution remained constant. Solvent was evaporated in vacuo to give an oily solid which was dissolved in aqueous ethanol and passed through a column of Bia-Red AG 50W-X4 (c.a. 2ml). The Ninhydrin positive fractions were retained and evaporated in vacuo to afford 3-[(L-alanyl-L-alanyl)amino]-3-phosphonopropanoic acid (36) (0.26g, 86%) as a powder*. ν_{max}(KBr) 3250(br, OH), 3240(NH), 3070(amide NH), 2980, 1680(amide C=O), 1550(amide II), 1160(P=O) and 1060cm^{-1}. This product was shown to be free of contaminants by chromatographic analysis. Hydrolysis afforded alanine and 3-amino-3-phosphonopropanoic acid as the sole products.
* An accurate melting point was not obtained

due to the hygroscopic nature of (36).

3-[(L-Alanyl-L-alanyl)amino]-3-methylphosphinopropanoic acid (40). Tripeptide (39) (0.467g, 0.99mmol) was treated as was (35), yielding 3-[(L-alanyl-L-alanyl)amino]-3-methylphosphinopropanoic acid (0.247g, 87%), m.p. 250°(dec), ν_{max}(KBr), 3250(br,OH), 3070(amide NH), 2980, 1670(amide I), 1550(amide II), 1150(P=O) and 1040cm^{-1}. The product was shown to be free of contaminants by chromatographic analysis. Hydrolysis afforded alanine and 3-amino-3-methylphosphinopropanoic acid as the sole products.

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