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in collaboration with KenoGard AB (Sweden)

The Synthesis of Fungicidal Aminophosphonic Acid Derivatives

by

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For Mum and Dad

Abstract

The Synthesis of Fungicidal Aminophosphonic Acid Derivatives

J.F.Volckman 1988

A number of 1-aminoalkanephosphonic acid derivatives incorporating physiologically labile groupings (ester, amide e.g. peptide, carbamate and tosyl) were prepared as a development from earlier studies in which certain phosphonic acid derivatives were shown to have previously unrecognised activity as fungicides.[1] Such derivatisation might aid the translocation of the active phosphonic acid to the site of action.

Compounds were screened for *in vivo* fungicidal activity against *Drechslera teres*, *Drechslera graminae*, *Drechslera avenae*, *Pythium ultimum*, *Rhizoctonia solani*, *Fusarium culmorum*, *Pyricularia oryzae*, *Puccinia recondita*, *Erysiphe graminis* and *Septoria nodorum*. Results showed that all the phosphonopeptides prepared had moderate to good antifungal activity, with the L-alanine di- and tripeptides of 1-aminopropanephosphonic acid having superior activity to the commercial fungicide imazalil when tested against *D. teres* and *D. avenae*.

The proton, carbon-13 and phosphorus-31 nmr spectroscopy of the compounds prepared was examined in detail with carbon-13 proving particularly useful. The carbon-phosphorus coupling constants varied along the alkyl chain in the phosphonopeptides with J_{PC} = 145-148 Hz, J_{PCC} = 0 Hz, J_{PCCC} = 12-14 Hz and J_{PCNC} = 4-6 Hz.

The use of Fast Atom Bombardment mass spectrometry has proven to be successful for the characterisation of aminophosphonic acid derivatives, especially phosphonopeptides, with the MH^+ ion normally being the base peak. Phosphonopeptides give characteristic fragmentation patterns due to the sequential loss of α-lactam units arising from the amino acid chain.

A number of routes to the preparation of 1-aminoalkanephosphonic acids were investigated, in particular those routes utilizing 1-oxo-alkanephosphonates, and a detailed study into the hydrogenation of dialkyl 1-hydroxyiminoalkanephosphonates using Raney Nickel and, more successfully, palladium-on-carbon catalysts was undertaken.

Preliminary attempts were made to prepare phosphonopeptides *via* the previously unexamined route of solid phase synthesis and some of the difficulties involved with this method are discussed.

1. European Patent Application E.P. 153,284

Acknowledgements

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Contents

Introduction

A fungicide is an agent that kills or inhibits fungal spores or the fungal mycelium. Fungicides may be classified as protectant (prophylactic), or therapeutic.

Protectant fungicides cannot penetrate the plant tissues in effective amounts; they must act on the plant surface preventing infection and have little or no effect on established diseases within the plant.

A therapeutant is an agent which inhibits further development of an established disease; it may act topically or be systemic.

Until the late 1960's practically all fungicides had a protectant action. The first fungicides were inorganic compounds such as Bordeaux Mixture (copper sulphate and lime) and sulphur. Since the introduction of the first organic fungicides in the 1930's important advances have been made towards achieving greater selectivity of fungicidal action.

Phytotoxicity is probably the most important factor to be considered next to fungitoxicity. Most protectant fungicides are general cell poisons and are selective because they remain on the surface of the plant. If a chemical is to penetrate the plant cuticle an entirely different type of selectivity is required, one which discriminates between the living tissue of the host and that of the pathogen.

Selectivity can depend upon differences between plant and fungal cell structure and biochemistry. Also, by

altering the physical properties of candidate fungicides - for example molecular size and shape, lipophilic/ hydrophilic balance, and hydrogen-bond capacity - it is possible to obtain differential movement through plant and fungal membranes and, hence, uptake into plant and fungal cells. In this way, low phytotoxicity is achieved whilst retaining high fungitoxicity. Low phytotoxicity is all important in systemic fungicides where the chemotherapeutant is translocated within the plant and, thus, has intimate contact with the plant tissues.

In a previous study[1] a way was sought to improve the water solubility and systemic activity of guanidine compounds related to the protectant fungicide dodine (dodecylguanidinium acetate). It was thought that this could be achieved by combining them with the phosphonic acid moiety present in the herbicide glyphosate (which has good systemic activity).

As a means to this end various aminoalkanephosphonic acid intermediates were prepared and a number of these were shown to be fungitoxic.

Aminophosphonic acids may be considered to be analogues of the naturally occurring aminocarboxylic acids The recent discovery of the antibacterial activity of alafosfalin, a 1-aminoalkanephosphonic acid dipeptide (L-alanyl-L-1-aminoethanephosphonic acid) has prompted much research into the field of aminophosphonic acids.

The aim of this project is to prepare various N-substituted derivatives of 1-aminoalkanephosphonic acids (such as dipeptides) and to correlate their

structure/activity relationships by testing them against a variety of fungi.

Some aspects of historical importance are reviewed in the following sections.

Chapter 1

Historical Review

Systemic fungicides[2]

Systemic insecticides, including many organophosphorus compounds, were reported as early as 1945, in contrast antifungal compounds with systemic activity have been slower to reach a level of relative significance.

If a chemical is to be an effective systemic fungicide it must satisfy the following criteria:

i) it must itself be fungicidal, or be converted into an active fungitoxicant within the host plant;

ii) it must possess very low phytotoxicity since the chemical is brought into intimate contact with the host plant;

iii) it must be capable of being absorbed by the roots, seeds or leaves of the plant and then be translocated within the host plant.

Systemic antifungal action has been demonstrated for many compounds[3-5], for instance sulphonamides, antibiotics, phenoxyalkanecarboxylic acids, 6-azauracil and phenylthiourea, although their dicovery made little impact on the large-scale control of fungal diseases because they were either too expensive, not sufficiently active under field conditions, or caused phytotoxic damage.

The development of systemic fungicides has largely arisen from the significant advances in the systemic chemotherapy of human diseases. This is based on the

discoveries of the antibacterial action of a Penicillium mould by Fleming (1929) and of Prontosil by Domagk (1935) which led to the production of antibiotics and sulphonamide drugs respectively. Plant pathologists considered that as bacteria and fungi are closely related, these materials might also show systemic activity against plant pathogenic diseases, and accordingly a number of synthetic bactericides and antibiotics were examined as potential systemic fungicides.

Sulphonamides are the most important class of synthetic bactericides from the viewpoint of systemic antifungal properties. however, none have reached commercial exploitation. Hassabrauk showed in 1938 that wheat rust could be controlled by root treatment with p-aminobenzenesulphonamide (sulphanilamide) and the systemic activity of sulphonamides has been confirmed by several workers[4-5].

Antibiotics as systemic fungicides

Antibiotics are chemicals produced by living organisms that are toxic to certain other organisms. More than one hundred of the three hundred[6] listed antibiotics are produced by fungi. The first antibiotics examined against pathogenic fungi were those employed in human chemotherapy. Penicillin never achieved commercial significance as a systemic fungicide, however, more promise was shown by streptomycin (I), cycloheximide (II) and griseofulvin (III).

Streptomycin is used for the control of bacterial pathogens of plants and is especially effective against

bacterial diseases of stone fruits[4]. It is also used against downy mildew on hops, peach blast fungus, and the copper chelate is very effective as a foliage spray against Phytophthora infestans on tomato[3].

Streptomycin is easily taken up by plant roots but it is phytotoxic due to its inhibition of chlorophyll synthesis; this and its high cost are major obstacles to the development of streptomycin as a commercial fungicide.

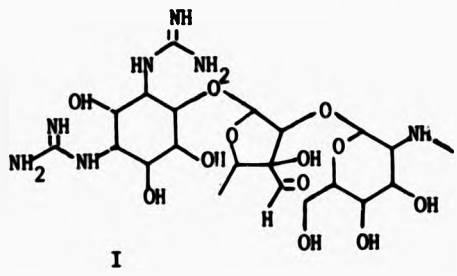
Cycloheximide or acti-dione, obtained from Streptomyces griseus[3,4], is active against cherry leaf spot and wheat stem rust but, like streptomycin, it is limited by its phytotoxicity.

Griseofulvin, isolated from Penicillium griseofulvum in 1939[7], shows a wide spectrum of antifungal activity especially against Botrytis cinerea on lettuce and Alternaria solani on tomato. It is less phytotoxic than streptomycin or cycloheximide and is readily translocated in plants[8].

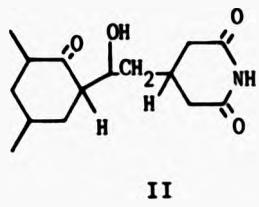
Many polypeptide antibiotics possess activity against fungi. Control of various plant diseases has been observed for antimycin (IV)[9,10] and phytoactin has proved particularly promising against needle rust of Douglas Fir[11]. The structures of many antifungal polypeptide antibiotics are unknown, however, an example of a relatively small antifungal peptide is eulicin (V) which has structural similarities to the synthetic fungicide guazatine.

Research is now being directed towards the discovery of new antibiotics which, though of no value in human chemotherapy, show specific fungicidal activity against economically important plant pathogens. The Japanese in

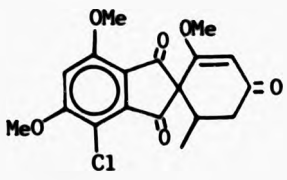
particular have made extensive use of antibiotics. Blasticidin-S (VI) a purine antibiotic discovered in Japan in 1958, has proved very effective against the rice blast pathogen (Pyricularia oryzae) and is used commercially[12].



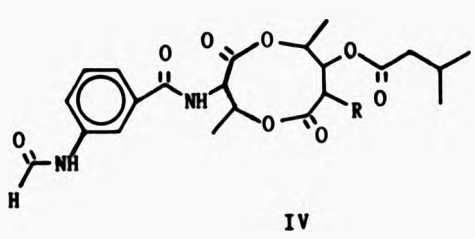
I



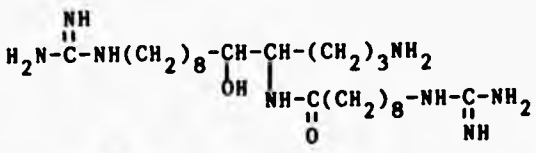
II



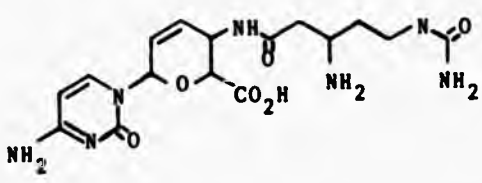
III



IV



V

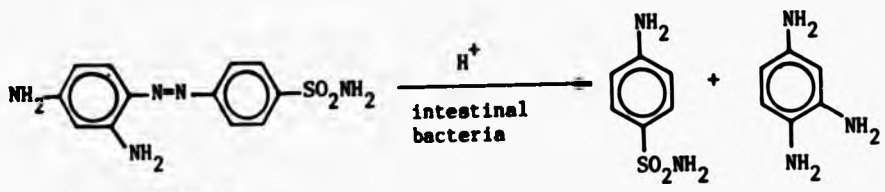


VI

Propesticides

A propesticide is a pesticidally active material or compound which in its original form is inactive. It must be transformed into an active state by a plant, animal or microorganism. In most cases, the target organism unwittingly carries out a self-destructive synthesis by chemically or biochemically converting the inactive compound into the active product.

The concept behind the development of propesticides has been known for half a century and has been exploited for many years in the design of drugs by the pharmaceutical industry. An early example of a synthetic prodrug is prontosil. Scientists at the Pasteur Institute showed that prontosil was metabolically converted into sulphanilamide (p-aminobenzenesulphonamide) and that it was sulphanilamide which was responsible for the therapeutic value of prontosil.

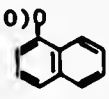


prontosil sulphanilamide

There are a large number of pesticides which fall into the same category as Prontosil i.e. they are active by virtue of their sucseptibility to metabolic or chemical modification to active intermediates. The classic example of a pesticide of this type is the insecticide parathion, a phosphorothionate

ester, which in animals or plants is oxidatively desulphurated to the potent anticholinesterase paraoxon.

The discovery of most prodrugs and propesticides has been fortuitous and not based on rational design. In recent years a number of propesticides have been developed which were designed on the basis of their anticipated activation in biological systems. In these cases, active compounds were modified by derivatization into products which reverted back to the original compound within the target organism.

The activity spectrum of a pesticidal compound is often determined by the physical properties of the compound. For example, systemic pesticides usually require both water and lipid solubility since it is necessary for them to pass through aqueous and lipid phases while moving through a plant or animal. Therefore, by attaching an appropriate functional group to a pesticide it is possible to convert a non-systemic compound into one which is systemic. Moreover, by proper selection of the derivatizing moiety, the physical properties of a compound may be manipulated to obtain products with other selected types of activity. However, it should be added that derivatization may also lead to a partial or complete loss of activity. For example, Pianka[12a] showed that condensation of the insecticidal compound carbaryl (1-naphthyl-*N*-methylcarbamate) to give diethyl S-(*N*-naphthyloxycarbonyl-*N*-methylcarbamoylmethyl)-phosphorothiolothionate [$(EtO)_2P(S)SCH_2CON(Me)C(O)O$ ] resulted in a significant loss of activity.

Derivatization can also be used to improve the toxicological properties of a pesticide. Improvement in mammalian toxicity has been attributed to the delayed factor provided by the derivatizing group, giving the animal the opportunity to metabolize the compound to non-toxic products.

The methylcarbamate insecticides are particularly suitable to derivatization due to the presence of a replaceable proton on the methylcarbamoyl nitrogen.

The number of groups represented by Y which give active compounds is very large and include the following:-

Y = $-\overset{O}{\overset{\|}{C}}-R$ $\quad -\overset{S}{\overset{\|}{P}}(OR)_2$ $\quad -SR$ $\quad -SOR$ $\quad -S-\underset{R'}{N}\overset{O}{\overset{\|}{C}}OR$

R, R' = alkyl or aryl

Group Y must be of such a structure to give a product with a moderately labile N-Y bond so that activation to the toxic methylcarbamate may occur readily within the target organism.

Mechanism of action of fungicides[13]

Many fungicidal compounds affect respiration or, more generally, energy production. Many others affect biosynthetic processes and a few compounds destroy cells by directly affecting membrane function.

With the exeption of carboxin and carboxin related compounds, fungicides affecting respiration are not systemic. In contrast, those affecting biosynthetic processes generally appear to have systemic properties.

Fungicides that inhibit energy production

Specific inhibitors

Dimethyldithiocarbamates, e.g. thiram (VII) and its salts, specifically inhibit the dehydrogenation of pyruvate at low concentrations. A 1:1 complex of copper and dimethyldithiocarbamate is formed. This probably combines with the dithiol compounds (lipoic acid or lipoic acid dehydrogenase) that are essential for the dehydrogenation of pyruvate[14].

Carboxin (VIII) interferes with respiration, causing a block in succinate dehydrogenation. The related compounds mebenil and pyracarbolid act in the same way. This may also be the case with the later developed carboxamides e.g. methfuroxam.

It is believed that triphenyltin compounds, such as fentin acetate (IX), interfere with fungal growth by

inhibiting oxidative phosphorylation. Dinocap (X) and other dinitrophenols act by uncoupling oxidative phosphorylation from respiration.

Unspecific inhibitors

The thio reagents, e.g. zineb (XI) and captan (XII), react quite generally with -SH enzymes and other -SH compounds involved in respiration.

Fungicides which inhibit biosynthetic processes

Inhibition of protein synthesis

Cycloheximide (II) inhibits growth by interference with protein biosynthesis at the stage where amino acids are transferred from tRNA into polypeptides, whilst in a complex with the ribosomes.

The probable site of action of blasticidin-S is the final step of protein synthesis, which takes place on the ribosomes. The antibiotic kasugamycin (XIII) also affects some stage of protein biosynthesis. The action of streptomycin (I) is ascribed to interference with protein synthesis, the primary target being the 30 S sub-unit of the ribosomes.

Inhibition of nucleic-acid synthesis

There is strong evidence to suggest that metalaxyl (XIV) interferes with nucleic acid synthesis in Pythium splendens: presumably RNA synthesis is the primary site

of inhibition[15]. Hydroxyisoxazole inhibits a variety of fungi. In Fusarium oxysporum, interference with DNA synthesis is its primary site of action[16].

Interference with nuclear processes

Benomyl and thiophanate-methyl are known to act after their conversion to the toxic agent carbendazim (XV). The primary process inhibited by this broad-spectrum fungicide is mitosis. Recently thiabendazole was shown to have the same mode of action[17].

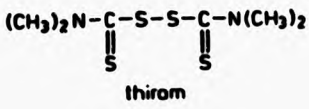
The dicarboximides [eg. vinclozolin (XVI) and iprodione] and the aromatic-hydrocarbon fungicides [eg. chloroneb (XVII)] all cause mitotic instability in Aspergillus nidulans, which points to the interference with mitotic division[18]. Inhibition of DNA synthesis has also been observed for chloroneb.

Inhibition of cell-wall synthesis

The antibiotic polyoxin D (XVIII) inhibits chitin synthesis. The fungicide shows a structural resemblance to the intermediate UDP-N-acetylglucosamine and obviously acts as an antimetabolite, resulting in inhibition of the enzyme chitin synthetase.

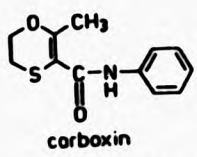
Inhibition of lipid biosynthesis

Kitazin P (XIX) inhibits the conversion of phosphatidylethanolamine to phosphatidylcholine by transmethylation of S-adenosylmethionine. Kitazin P has some structural resemblance with phosphatidyl-



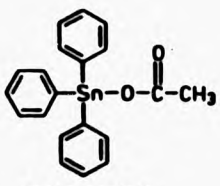
thiram

VII



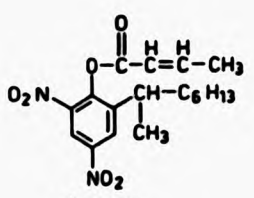
carboxin

VIII



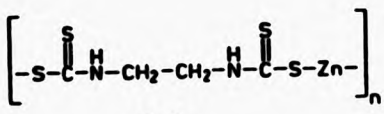
fentin acetate

IX



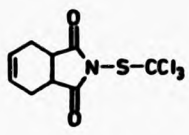
dinocap

X



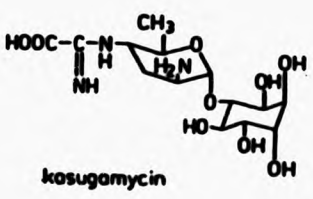
zineb

XI



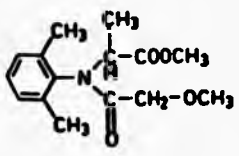
captan

XII



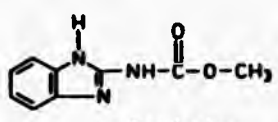
kasugamycin

XIII



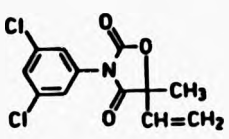
metalaxyl

XIV



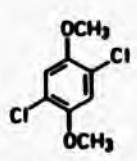
carbendazim

XV



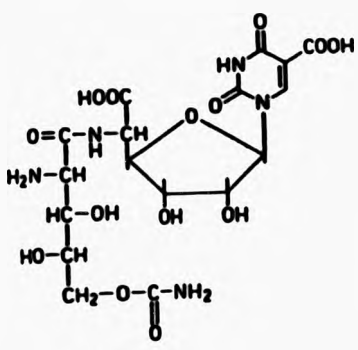
vinclozolin

XVI



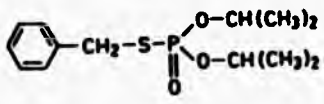
chloroneb

XVII



polyoxin D

XVIII



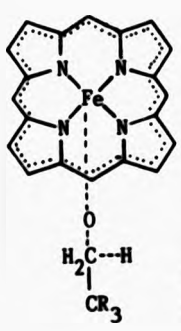
IBP 'Kitazin P'

XIX

ethanolamine and so may act as an antimetabolite of this substrate.

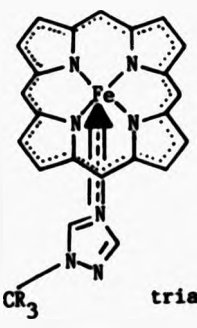
Many fungicides are now known to be sterol biosynthesis inhibitors. Ergosterol is the principal sterol in most fungi. An early precursor in the biosynthesis of ergosterol is mevalonic acid which is condensed by several steps to squalene. The cyclization of squalene leads to the formation of lanosterol and 24-methylenedihydrolanosterol. Demethylation at C-14 of this latter compound is the next step in the ergosterol biosynthetic pathway, and it is this step which many ergosterol biosynthesis inhibitors (EBI'S) inhibit.

The demethylation is catalyzed by a specific cytochrome P-450 mixed-function oxygenase enzyme which converts the methyl group at C-14 to $-CH_2OH$. This mechanism involves oxygen binding to the protoheme iron atom. In general, compounds which inhibit C-14 demethylation contain an sp^2 nitrogen with a free electron pair (eg. pyrimidine, pyridine or azole substituents) and it is thought that the binding of the nitrogen to the protoheme iron atom results in the exclusion of oxygen and, thus, the inhibition of C-14 demethylation.



natural substrate

ferric porphyrin - prosthetic group in P-450 protein



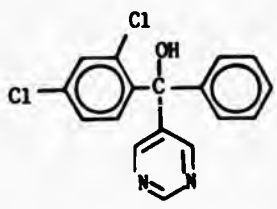
triazole

The pyrimidines [such as triarimol (XX)] were the first ergosterol biosynthesis inhibitor fungicides to be discovered. At the end of the 1960's the antifungal 1-substituted imidazoles [eg. prochloraz (XXI)] and 1,2,4-triazoles (eg. triadimefon (XXII)) were discovered. These azole fungicides act in the same way as the pyrimidines.

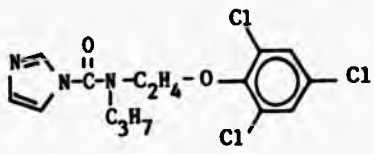
Most demethylation inhibitor fungicides (DMI's) are also plant growth regulators (PGR's) due to giberellin biosynthesis inhibition. Giberellins are plant hormones involved in the elongation growth of plants. Giberellin biosynthesis is catalyzed by a cytochrome P-450 enzyme in the same way as C-14 demethylation.

Fungicides that interfere with cell structure

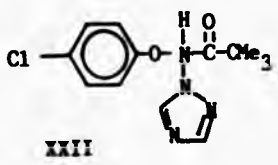
Dodine affects the permeability of the cell membrane, which results in leakage of the cell contents. The exact nature of the interference with the membranes is not yet known.



XX



XXI



XXII

Methods for the preparation of 1-aminoalkanephosphonic acids

One of the first general methods for the preparation of 1-aminoalkanephosphonic acids was described by Kabachnik and Medved[19] who condensed both aldehydes and ketones with ammonia and dialkyl phosphites. Hydrolysis of the dialkyl 1-aminoalkanephosphonates produced gave the free amino acids. Chalmers and Kosolapoff[20] used the same method for the preparation of analogues of naturally occurring amino acids, for example 1-aminoethylphosphonic acid (the ∝-alanine analogue). The overall yields of this method, however, were never greater than 41.5%.

Fields[21] used a variety of methods to prepare N-substituted esters of 1-aminophosphonic acids. He condensed aldehydes and ketones with dialkyl phosphites and primary or secondary amines, e.g.

$$CH_2O + HP(O)(OEt)_2 + Et_2NH \longrightarrow Et_2NCH_2P(O)(OEt)_2 + H_2O$$

94%

It was dicovered that primary amines gave much lower yields than secondary amines, possibly due to the following side reaction:-

$$R'CHO + HP(O)(OEt)_2 + RNH_2 \longrightarrow R'CH(NHR)P(O)(OEt)_2$$

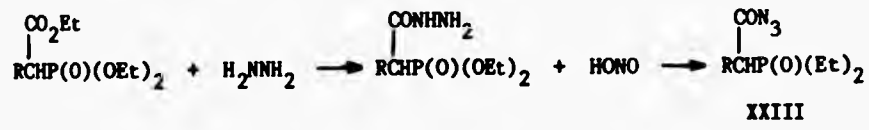
$$R'CHO / \downarrow HP(O)(OEt)_2$$

$$RN-[CH(R')P(O)(OEt)_2]_2$$

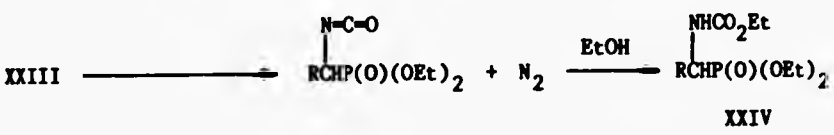
Fields also found that aldehyde and ketone imines reacted vigorously with dialkyl phosphites.

The N-substituted esters formed could be hydrolysed by heating under reflux with alcoholic potassium hydroxide solution or 48% aqueous hydrobromic acid for 1 - 5 hours to give the corresponding phosphonic acids. However, it was difficult to obtain the free acids in a pure form. Generally, non-crystallizing hygroscopic syrups resulted.

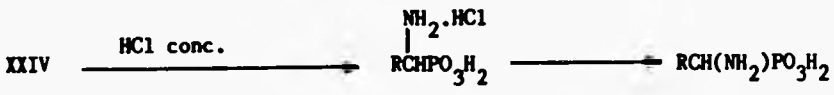
Chambers and Isbell[22] were the first workers to use the Curtius reaction for the preparation of 1-aminoalkane-phosphonic acids.



XXIII

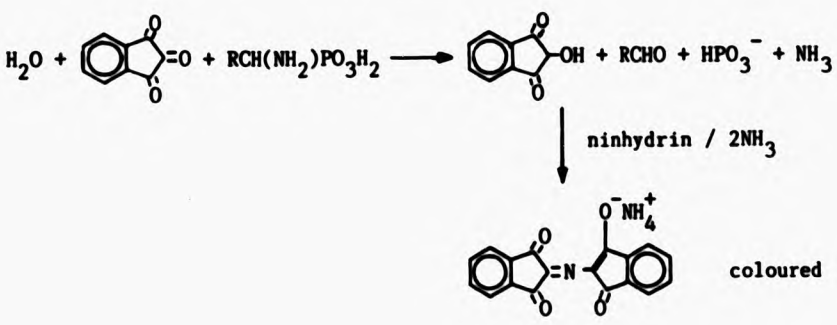


XXIV

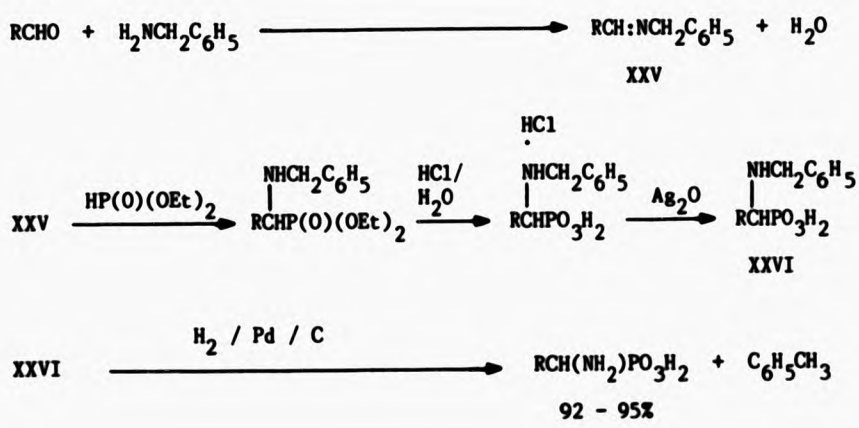


Yield when R = H 54%
Me 80%
$C_6H_5CH_2$ 56%

They also found that 1-aminophosphonic acids gave positive ninhydrin tests indicating that the C-P bond is cleaved in the reaction with ninhydrin. This was confirmed by the positive identification of phosphate ion in the reaction solution.



Tyka[23] used the method of Fields to prepare N-benzyl 1-aminophosphonic acids which were then converted to the free amines by hydrogenolysis. The required Schiff's bases were prepared from benzylamine and various aldehydes.



When ethylhydrogenalkanephosphonites (R'-P(OH)(OEt)) were substituted for dialkyl phosphites in the above reaction the corresponding 1-aminoalkanephosphinic acids $RCH(NH_2)P(O)(OH)(R)$ were obtained.

Birum[24] caused urea and many mono- and disubstituted ureas and their thio analogues to react with aldehydes and trialkyl and triphenyl phosphites to give numerous 1-ureido-phosphonates. Surprisingly, triphenyl phosphite was more readily converted into phosphonates in this process than were trialkyl phosphites - the presence of acid facilitating reaction with trialkyl phosphites.

Urylenediphosphonates as well as monophosphonates were obtained when unsubstituted urea was used, whereas mono- and disubstituted ureas gave only monophosphonates.

$$2(RO)_3P + H_2NCONH_2 + 2R'CHO \longrightarrow (RO)_2P(O)CHNHCNHCHP(O)(OR)_2 + 2ROH$$

$R = C_6H_5$ R' = alkyl, aryl

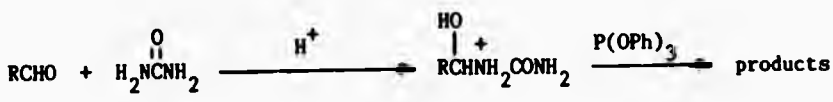
$$(RO)_3P + H_2NCONHR'' + R'CHO \longrightarrow R''NHCNHCHP(O)(OR)_2 + ROH$$

i) $R = C_6H_5$; R' = alkyl; $R'' = CHCO_2Et$

ii) R = Et; $R' = C_6H_5$; $R'' = C_6H_5$

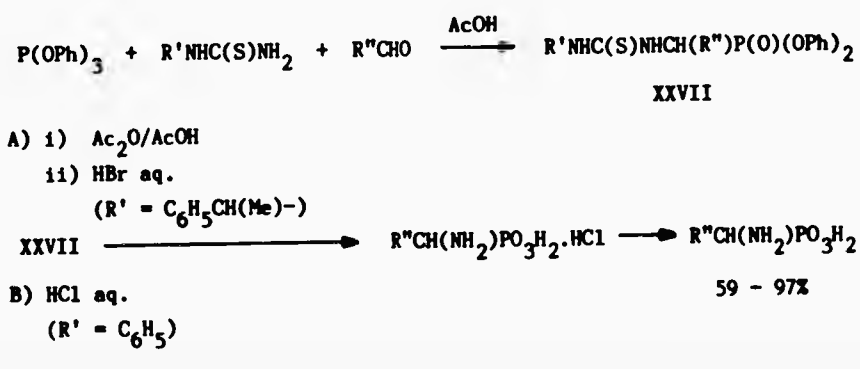
An explanation for the reversal of the normal order of reactivity of the phosphite esters was put forward as being due to differences in basicity. The urea and aldehyde were said to undergo an acid-catalysed reaction.

Trialkyl phosphites were basic enough to inhibit this first step, whereas the less basic triphenyl phosphite did not lower the acidity below the level needed for the reaction of urea and aldehyde to occur.

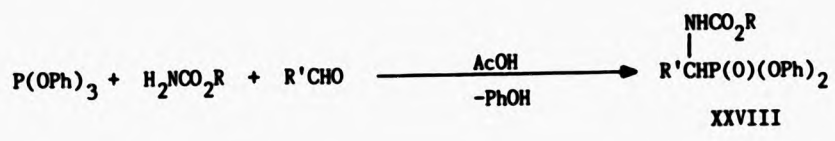


Ureaphosphonic acids were obtained by warming the diphenyl monophosphonates with distilled water. Urea-diphosphonic acids were obtained only after heating the tetraphenyl diphosphonates with dilute hydrochloric acid for several hours.

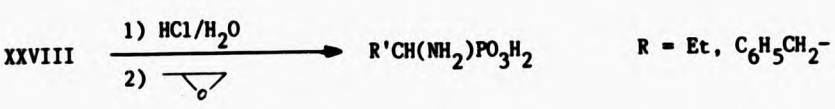
Kudzin and Stec[25] used an analogous procedure to prepare 1-aminoalkanephosphonic acids in moderate to high yields. A one-pot reaction was devised in which an N-substituted thiourea, an aldehyde and triphenyl phosphite were heated in the presence of acetic acid before being hydrolysed to give the phosphonic acid.



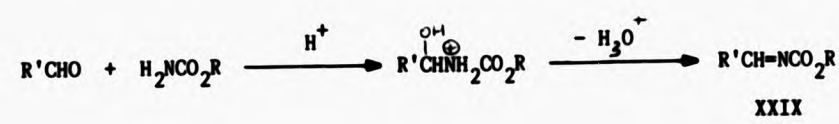
Another simple procedure for the preparation of 1-aminoalkanephosphonic acids is that of Oleksyszyn and Tyka[26] who condensed alkyl or benzyl carbamates with aldehydes and triphenyl phosphite.



XXVIII



The mechanism of this reaction (as with the others) is not clear although it is likely that the aldehyde and carbamate initially combine to form a Schiff's base which subsequently undergoes nucleophilic attack by the triphenyl phosphite.



XXIX

XXX

Oleksyszyn et al[27] isolated the N-substituted intermediate (XXX) (R = $C_6H_5CH_2$-) during their synthesis of diphenyl 1-aminoalkanephosphonates.

$$PhCH_2OC(O)NHCH(R)P(O)P(OPh)_2 \xrightarrow[2)\ NH_3/Et_2O]{1)\ HBr/AcOH} RCH(NH_2)P(O)(OPh)_2$$

Phosphorus trichloride may be used in these reactions in place of triphenyl phosphite[28].

$$R'C(O)R'' + H_2NCO_2R''' + PCl_3 \xrightarrow[3)\ \Delta]{1)\ AcOH;\ 2)\ H^+/H_2O} RCH(NH_2)PO_3H_2$$

R' = alkyl, aryl; R" = H, alkyl; R'" = Et, $C_6H_5CH_2$-

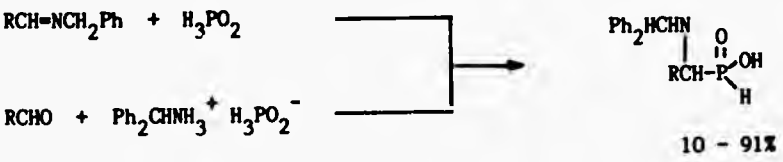
Replacement of the carbonyl compounds in this reaction with corresponding acetals or replacement of carbamates with simpler amides (eg. acetamide or benzamide) resulted in decreased yields. Amines and ammonia were unsuccessful as sources of amino groups.

Phosphorous acid has been used in the Mannich-type procedure by Moedritzer and Irani[29], however, only formaldehyde may be used as the carbonyl reactant and strongly acidic conditions are required.

$$R_2NH + HCHO + HP(O)(OH)_2 \xrightarrow{HCl} R_2NCH_2PO_3H_2$$

Redmore[30] found that heating equimolar amounts of imine and phosphorous acid in the absence of solvent produced 1-aminophosphonic acids. However, imines derived from aliphatic aldehydes or ketones gave much lower yields. In these cases it was found that reduction of the imine to the corresponding amine was a competing reaction.

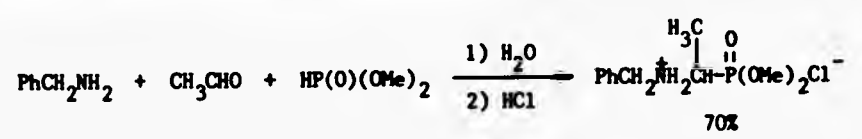
Schmidt[31] demonstrated the addition of hypophosphorous acid to imines to give N-substituted 1-aminoalkanephosphonous acids. More recently, this method has been utilized by Baylis *et al*[32] who condensed hypophosphorous acid with diphenylmethylimines. The reaction of the diphenylmethylamine salt of hypophosphorous acid with aldehydes gave the same product.



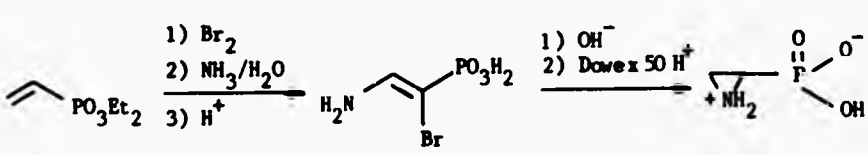
R = alkyl, aryl

Cleavage of the diphenylmethyl group was accomplished under acidic conditions. The 1-aminoalkanephosphonous acids thus obtained can be easily oxidised to the corresponding 1-aminoalkanephosphonic acids with mercuric chloride or bromine water.

Atherton *et al*[33] managed to prepare *N*-benzyl 1-aminoethanephosphonate using an aqueous mixture of benzylamine, acetaldehyde and dimethyl phosphite at 0 °C. The product was isolated as its hydrochloride and then hydrogenated to give the free aminophosphonate.

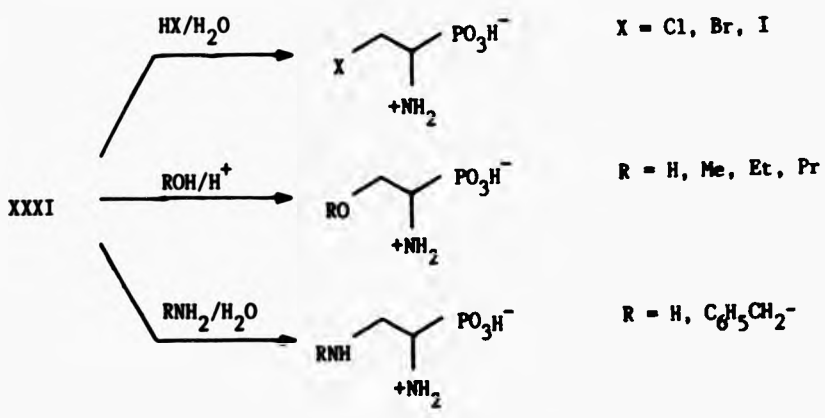


Aziridine-2-phosphonic acid (XXXI) has been found to be a very useful synthon in preparing 1-amino-2-functionalized ethanephosphonic acids. Zygmut[34] prepared aziridine-2-phosphonic acid using commercially available diethyl vinylphosphonate (XXXII) which was converted to the intermediate 1-bromovinylphosphonate (XXXIII).



XXXII XXXIII XXXI

The aziridine ring was then opened by reaction with various reagents. The nucleophilic attack was generally directed to the less substituted C-2 carbon giving the 1-amino-2-functionalized phosphonic acids.



The preparation of 1-aminoalkanephosphonic acids via O,O-dialkyl 1-oxoalkanephosphonates

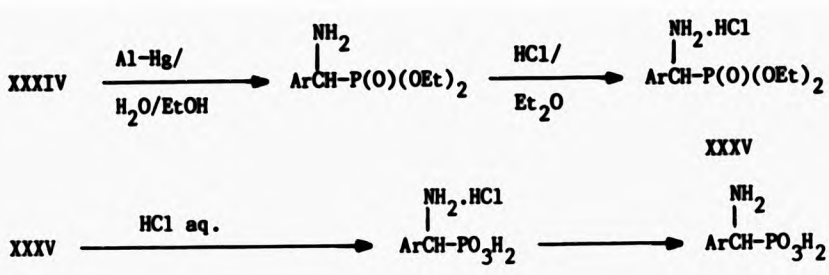
O,O-dialkyl 1-oxoalkanephosphonates were first synthesised by Kabachnik *et al*[35] from acyl chlorides and trialkyl phosphites. The reaction procedure has since been modified[36,37] and is now carried out in a nitrogen atmosphere.

$$RCOCl + P(OR')_3 \xrightarrow{N_2} RC(=O)-P(OR')_2 + R'Cl$$

The traditional qualitative reactions of ketones are also undergone by 1-oxoalkanephosphonates, which form hydrazones and oximes. Reduction of the hydrazones and oximes leads to the dialkyl 1-aminoalkanephosphonates which on hydrolysis yield the 1-aminoalkanephosphonic acids. This reaction scheme was first utilized by Kosolapoff[38] who reduced the p-nitrophenylhydrazone of diethyl 1-oxo-benzylphosphonate with 2% palladium-on-charcoal catalyst to give, on hydrolysis, the 1-aminobenzylphosphonic acid. Berlin *et al*[39] reduced the oximes of dialkyl 1-oxoaryl-phosphonates by aluminium-amalgam in water/ethanol. Hydrolysis of the aminophosphonates thus formed gave the 1-aminoarylphosphonic acids in yields of 67 - 88%.

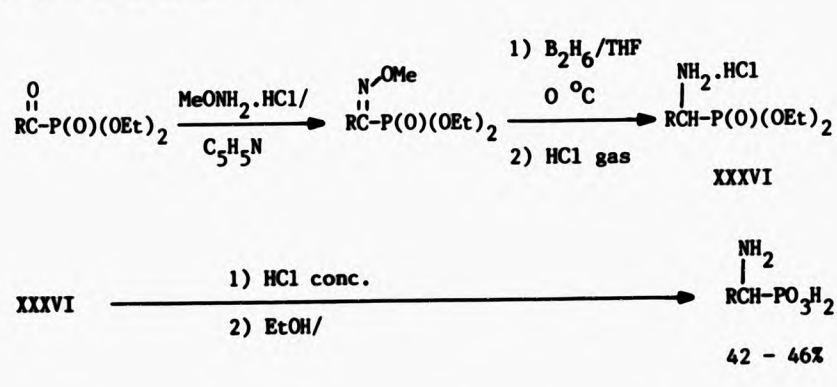
$$ArCOCl + P(OEt)_3 \longrightarrow ArC(=O)P(O)(OEt)_2 \xrightarrow[C_5H_5N]{NH_2OH.HCl/} ArC(=NOH)P(O)(OEt)_2$$

XXXIV

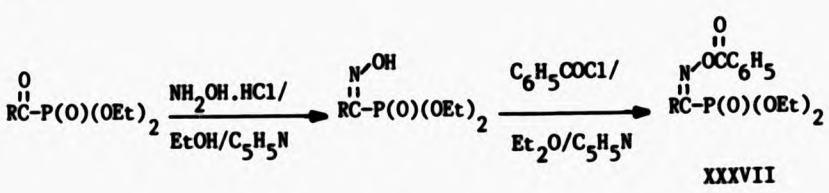


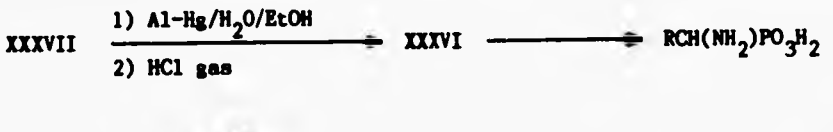
Ar = C_6H_5, p-ClC_6H_4, o-ClC_6H_4, p-$MeOC_6H_4$, o-$MeOC_6H_4$, p-$Me_3CC_6H_4$

Berlin et al[40], however, found this method inferior for the preparation of diethyl 1-aminoalkanephosphonates and reduced the O-methylated oximes of 1-oxoalkanephosphonates with diborane in THF.

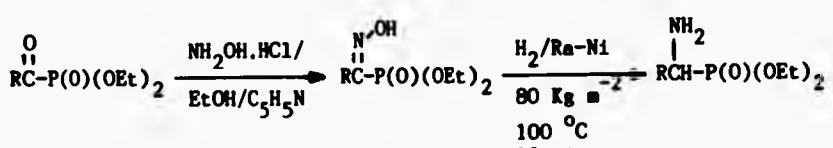


The 1-aminoalkanephosphonic acids were also prepared by the following method but the yields were lower by 10 - 20%.



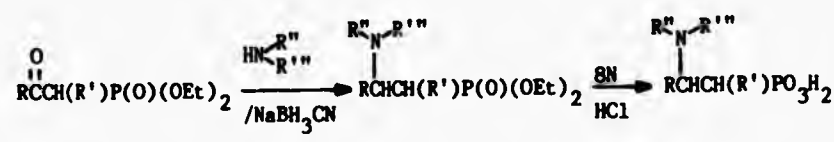


Asano *et al*[37] reduced the oximes of diethyl 1-oxoalkanephosphonates by hydrogenation over Raney-nickel catalyst giving crude yields of aminophosphonates of *ca* 70%.



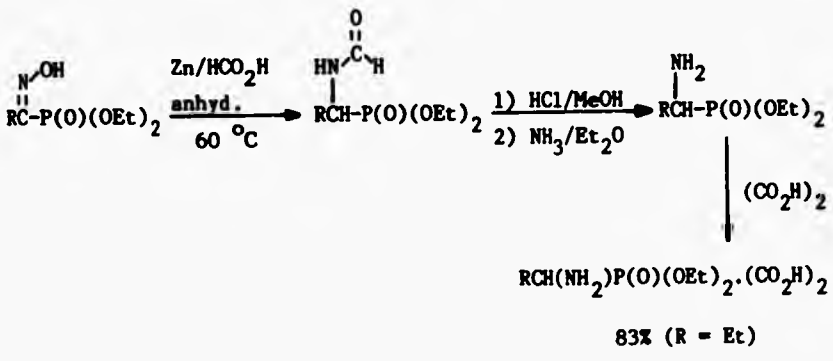
R = alkyl, aryl

2-aminoalkanephosphonic acids have been prepared by reductive amination of 2-oxoalkanephosphonates[41]. The reaction was carried out using primary or secondary aliphatic or aromatic amines. The use of ammonium acetate yielded compounds unsubstituted on nitrogen. Hydrolysis of the intermediate esters gave the 2-aminophosphonic acids in yields of 38 - 70%.

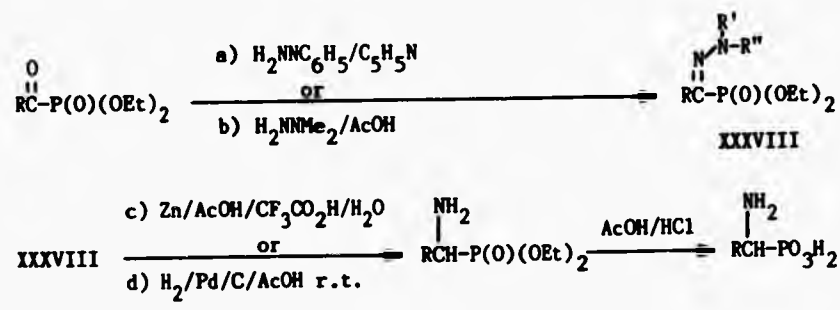


Another method described in a Polish Patent[42] refers to the reduction of the oximes of 1-oxoalkanephosphonates by zinc in anhydrous formic acid. The formyl group of the thus formed dialkyl *N*-formyl-1-aminoalkanephosphonates was

removed according to a known procedure and the crude aminophosphonate was isolated as its oxalate salt.



Kudzin and Kotynski[43] used a similar method to the above but used the phenyl or dimethyl hydrazones of 1-oxoalkanephosphonates as substrates and reduced them with zinc in a mixture of acetic and trifluoroacetic acids.

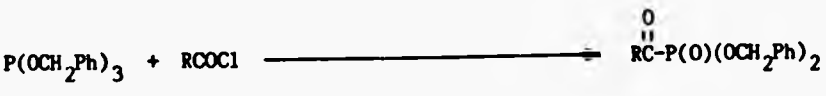


The 1-aminoalkanephosphonic acids may be prepared without isolation of the intermediate products in which case yields range from 52 - 70%.

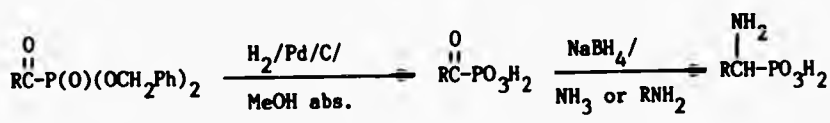
An alternative method is the hydrogenolysis of the hydrazones over palladium-on-charcoal catalyst in acetic acid (atmospheric pressure, room temperature, 48 h) which

gives the aminophosphonates in yields of 30 - 50%.

A Russian patent[44] describes the use of tribenzyl phosphite in the preparation of 1-oxoalkanephosphonates.



The 1-oxoalkanephosphonate then undergoes hydrogenolysis to produce the 1-oxoalkanephosphonic acid which is reacted with sodium borohydride in aqueous ammonia or in a primary amine at 0 - 20 °C. The aminophosphonic acids are prepared in yields of 50 - 60%.

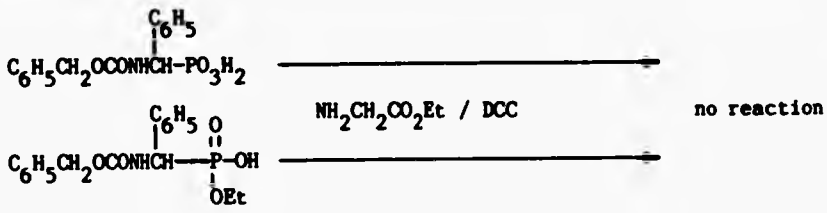


Other existing methods for the preparation of the 1-oxoalkanephosphonic acids involve the hydrolysis of disilyl 1-oxoalkanephosphonates[44-47]. Dialkyl 1-oxoalkanephosphonates cannot be hydrolysed in the normal way as acid or alkaline treatment usually leads to cleavage of the P-C bond prior to hydrolysis of the ester linkages[48,49]. Disilyl esters, however, may be hydrolysed by water or on exposure to air for several hours. Under these mild conditions the P-C bond remains intact.

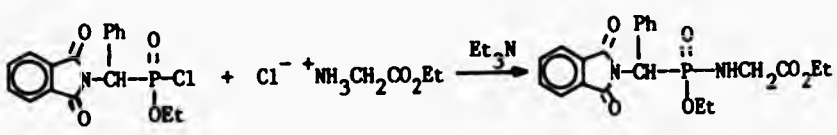
Synthesis of phosphonopeptides

Phosphorus is found in nature in combination with oxygen and nitrogen giving P-O, P=O and P-N bonds. The occurrence of the P-C bond was first demonstrated by Horiguchi and Kandatsu[50] who isolated 2-aminoethane-phosphonic acid (2AEP) from rumen protozoa. This has since been found in several other organisms[51] and in man[52] . Early publications of the natural occurrence of 2AEP suggested participation of the compound in lipid structures[51,52] but Quin[53] showed that occurrence in protein structures was also possible. Quin suggested[54] that the aminophosphonic acids could form part of polypeptide chains by amide formation through either one or both of their amino and phosphonic acid groups.

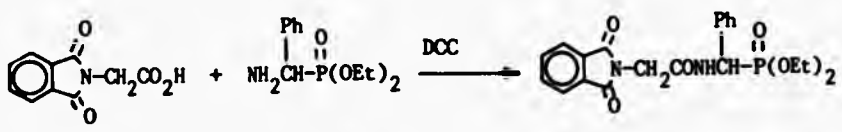
Yamauchi *et al*[55] were the first to investigate the incorporation of aminophosphonic acids into synthetic peptides. Using 1-aminobenzylphosphonic acid as a model they studied its ability to form amide and phosphonamide bonds. They found that the amino groups of aminophosphonic acids have a reactivity similar to that of amino acids and , thus, the protecting groups used in conventional peptide synthesis (eg. carbobenzoxy and phthalimido groups) could be used without any modification. However, an attempted condensation of the N-protected 1-aminobenzyl-phosphonic acid and monoester with ethylglycinate by means of the dicyclohexylcarbodiimide (DCC) method failed.

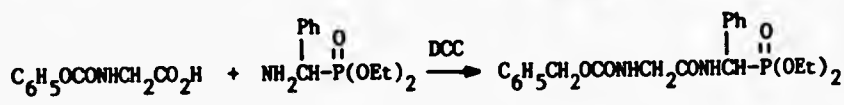


Activation of the phosphonic acid group through its conversion to the phosphonochloridate with PCl_5 enabled its reacton with ethylglycinate to form a phosphonamide bond.



Placing amino acids to the left of the aminophosphonic acid esters proved more straightforward and was accomplished with N-protected amino acids using DCC* according to methods used in conventional peptide synthesis.

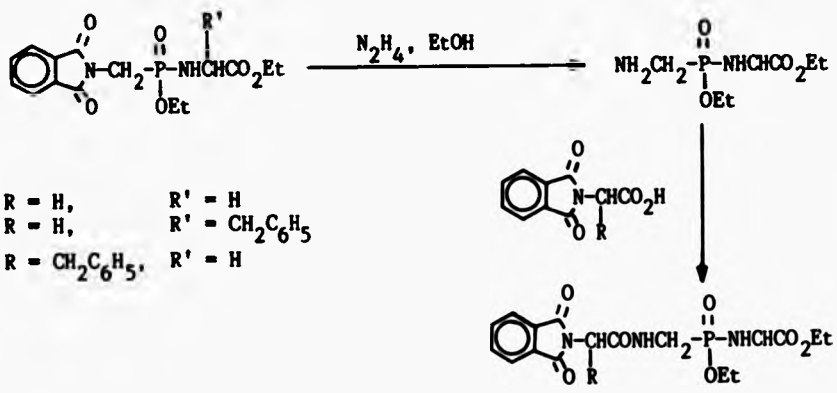




Using the above methods Yamauchi *et al*[56] synthesised a tripeptide analogue containing 1-aminomethanephosphonic

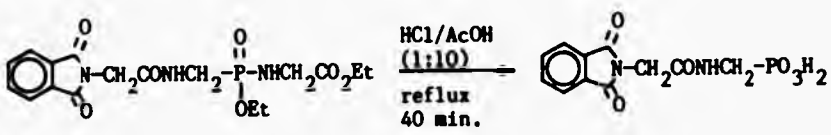
*DCC = dicyclohexylcarbodiimide

acid in the centre.

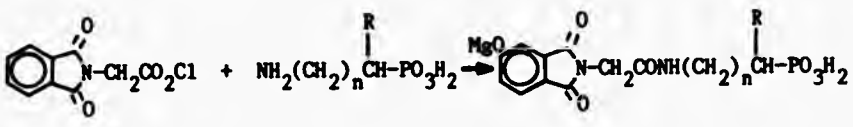


R = H, R' = H
R = H, R' = $CH_2C_6H_5$
R = $CH_2C_6H_5$, R' = H

The phthalyl groups of the above compounds could be removed by hydrazine, however, the removal of the ethyl groups from the carboxylic and phosphonic acid groups was difficult since P-N bond cleavage took place exclusively upon a mild acid treatment.

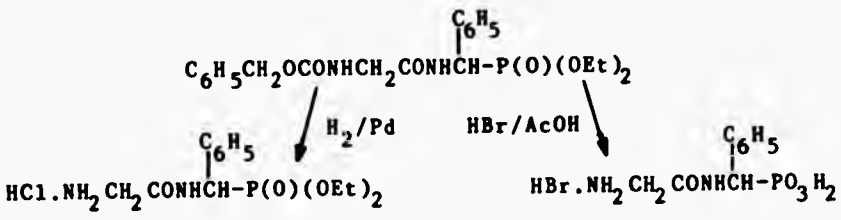


Hariharan et al[57] managed to prepare peptide analogues using free aminophosphonic acids and phthalylglycylchloride.



R = H, Me, n = 0
R = H, n = 1

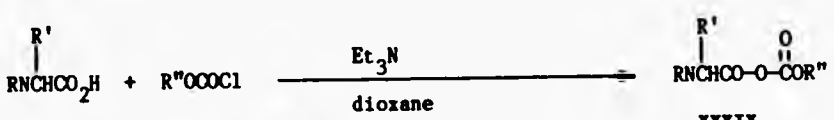
Gilmore and McBride[58] tried this reaction using N-carbobenzoxyglycylchloride, 1-aminobenzylphosphonic acid and a variety of bases and found that peptide formation only occurred at a pH of 8.5. Gilmore and McBride also found that 1-ethoxycarbonyl-2-ethoxy-1,2-dihydroquinoline was an exellent coupling agent for esters of aminophosphonic acids and carbobenzoxy amino acids. The peptides formed from such reactions could be deblocked with HBr/AcOH when the carbobenzoxy group was removed and the phosphonate hydrolysed simultaneously. In contrast, treatment of the N-carbobenzoxy 1-aminobenzylphosphonate with HBr/AcOH removed the carbobenzoxy group but the phosphonate esters remained intact. Hydrogenolysis of the dipeptide removed solely the carbobenzoxy group.



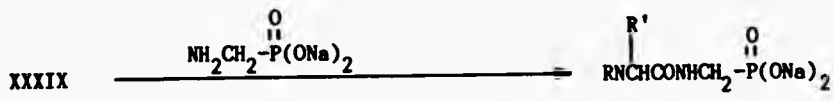
The antimicrobial properties of the N-glycyl 1-aminobenzylphosphonic acid formed above were investigated. At a concentration of 10 mg/ml this compound was inactive

against selected bacteria and fungi.

The first synthesis of phosphonopeptides by the mixed carboxylic-carbonic anhydride method was reported by Hariharan *et al*[59]. An advantage of this method is that when an optically active amino acid is used as one of the reactants, very little optical deactivation occurs in the formation of the mixed anhydride and hence the peptide.



XXXIX

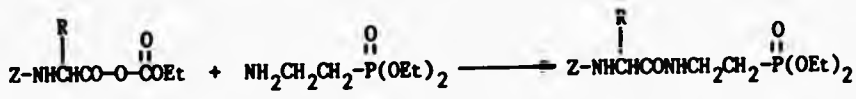


R = phthalyl or carbobenzoxy, H
R' = alkyl, aryl
R" = Et

These workers also studied the ^{1}H nmr, pKa's and stability constants of the phosphonopeptides.

Atherton *et al*[60,61] used the above method preparing the mixed anhydrides from *N*-carbobenzoxy amino acids and isobutyl chloroformate.

Kafarski and Mastalerz[62] condensed mixed anhydrides with the diethyl ester of 2-aminoethylphosphonic acid.

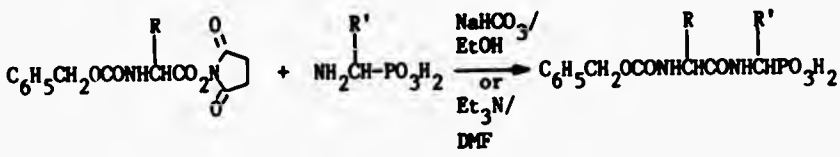


XXXX

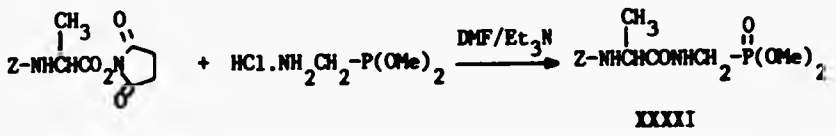
R = H, Me, $C_6H_5CH_2$, $CH(Me)_2$, prolyl-2-AEP; Z = carbobenzoxy

Kafarski et al[63] came to the conclusion that the dialkyl 1-aminoalkanephosphonates were more suitable substrates for peptide synthesis than the free acids. They found the mixed carboxylic-carbonic anhydride method preferable to the DCC method - the latter often producing the corresponding N-acylureas - the side products of the reaction - in relatively high yields (ca 30%).

Atherton et al[60,61] also prepared phosphonopeptides by the activated ester method. The N-hydroxysuccinimide esters of N-carbobenzoxy amino acids were condensed with 1-aminoalkanephosphonic acids in aqueous alcohol or dimethylformamide.



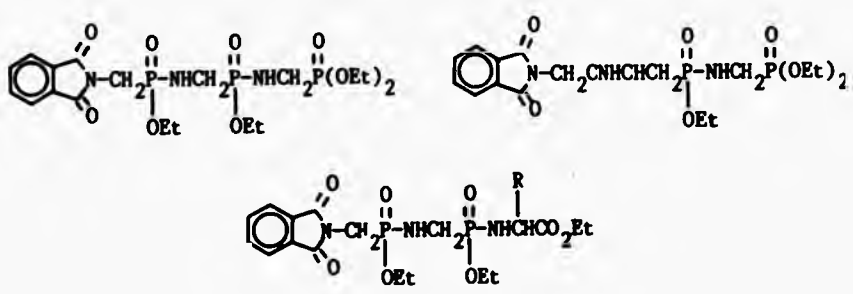
Use of the dimethyl 1-aminoalkanephosphonate hydrochlorides instead of the free acids gave more successful coupling reactions.



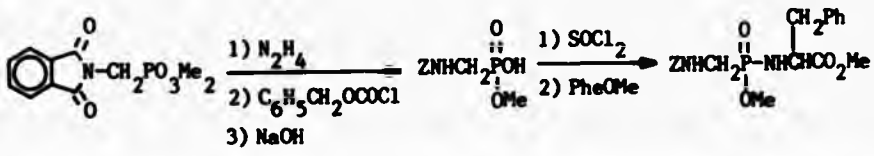
XXXI

The 2,4,5-trichlorophenyl esters of N-benzyloxycarbonyl amino acids could be used in the above reaction in place of the N-hydroxysuccinimide esters.

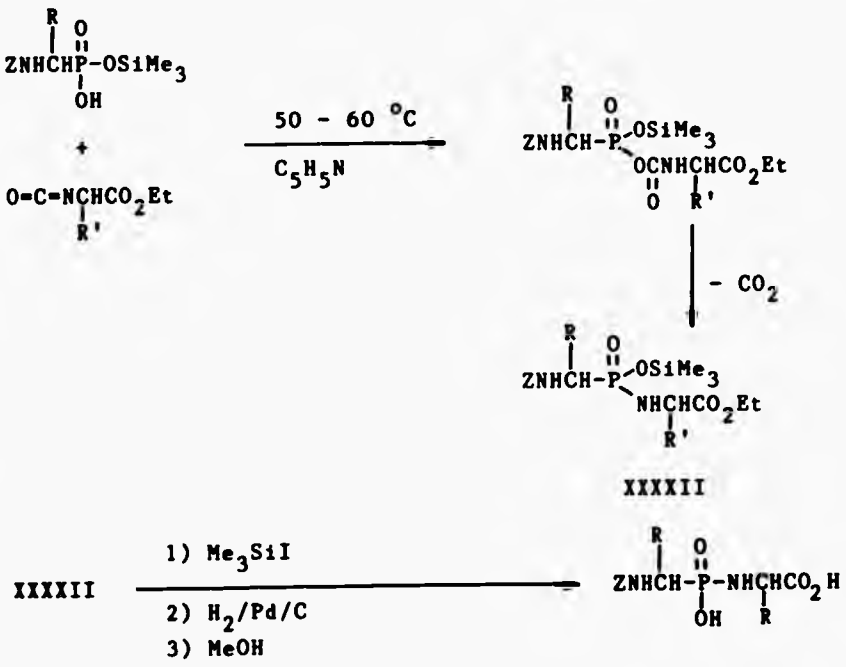
As mentioned previously, the synthesis of peptides containing phosphonamide bonds has proved more difficult than the synthesis of peptides containing amide bonds. Despite this, Yamauchi et al[64] managed to prepare compounds containing more than one aminophosphonic acid unit joined by phosphonamide bonds via the phosphonochloridate method[55].



Jacobsen and Bartlett[65] used the methods of Hariharan et al[59] and Yamauchi et al[64] to prepare N-[[[(benzyloxy-carbonyl)amino]methyl]methoxyphosphinyl]-L-phenylalanine methyl ester.

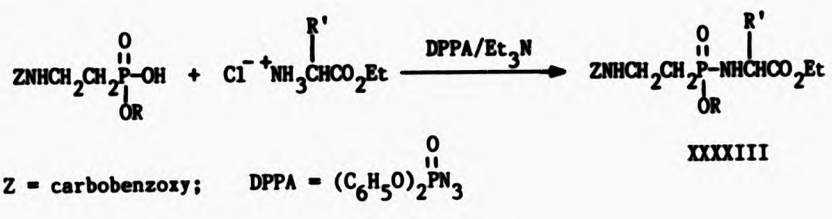


Issleib *et al*[66], however, used the isocyanato method to prepare the phosphonamide bond. The N-carbobenzoxy l-aminophosphonic acids were converted to their mono trimethylsilyl esters which were treated *in situ* with $OCN-CH(R')CO_2Et$.



Thus, a peptide is produced with a free -OH group attached to phosphorus.

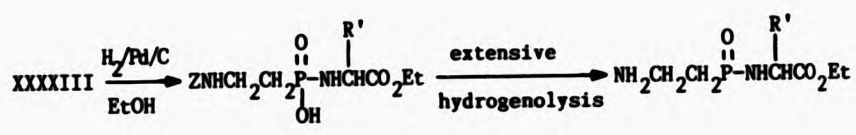
Yamauchi *et al*[67] examined various coupling agents in order to prepare the phosphonamide bond without having to convert the phosphonic acid to the phosphonomonochloridate. A siutable agent was found in diphenylphosphorylazide (DPPA).



XXXXIII

Z = carbobenzoxy; DPPA = $(C_6H_5O)_2\overset{O}{\overset{\|}{P}}N_3$

When R = benzyl, catalytic hydrogenolysis using palladium-on-charcoal catalyst preferentially deprotected the phosphorus ester group. Continued hydrogenation eventually removed the carbobenzoxy group to give the free amine.



Resolution of diastereoisomers

Diastereomeric dipeptides and their derivatives have variable physical properties and therefore can sometimes be separated by crystallization, countercurrent distribution, ion-exchange, gas chromatography, HPLC or paper chromatography. Sokolowska and Biernat[68] separated diastereomeric dipeptides by paper chromatography and found that the R_f values for L-L dipeptides were identical to those observed for D-D compounds (since L-L is the mirror image of D-D) and differed markedly from those of the L-D and D-L isomers. There was also a regular dependence of the R_f value of the dipeptides upon the relative configuration of the amino acids.

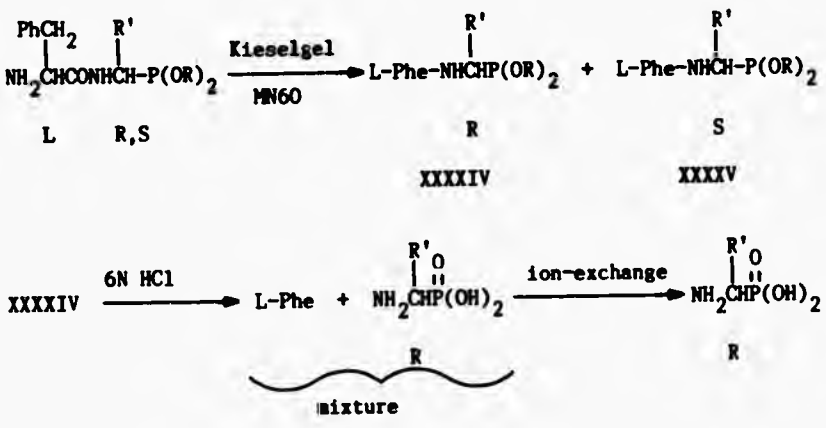
$$R_f(\text{L-L/D-D}) > R_f(\text{L-D/D-L})$$

Using this rule Kafarski *et al*[63] were able to assign tentative configurations to phosphonopeptides separated by ion-exchange column chromatography.

Dipeptides of L-amino acids and racemic 1-amino-alkanephosphonic acids were separated on a Dowex 50 W (H^+ form) 100 - 200 mesh column with water as elutant. The peptides which were eluted fastest were considered as possessing S,S (L,D) configuration.

Kupczyk-Subotkowska and Mastalerz[69] separated the dialkyl esters of diastereomeric L-phenylalanine

phosphonopeptides by column chromatography on a kieselgel MN60, 100 - 200 mesh column. The resolved dipeptides were hydrolysed by heating under reflux with 6N hydrochloric acid over 30 h. The resulting mixtures were separated by ion-exchange with water as elutant, which did not elute the phenylalanine. In this way the resolved aminophosphonic acids were obtained.



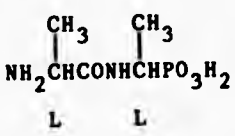
Lejczak et al[70] used a combination of the above methods, separating diastereomeric phosphonopeptides by ion-exchange column chromatography, hydrolyzing the resolved dipeptides and separating the resulting amino acid/amino-phosphonic acid mixture by ion-exchange chromatography.

Atherton et al[60,61] separated diastereomeric phosphonopeptides and racemic N-benzyloxycarbonyl 1-amino-phosphonic acids by fractional crystallization. N-benzyl-oxycarbonyl phosphonopeptides were separated into their diastereoisomers by their benzylamine salts. N-Benzyloxy-

carbonyl 1-aminophosphonic acids were resolved by their quinine and dehydroabietylamine salts.

The mode of action of antibacterial phosphonopeptides

Antibacterial phosphonopeptides such as alafosfalin (XXXXVI) owe their activity to their ability to inhibit the biosynthesis of bacterial cell wall material. All pathogenic bacteria so far investigated have peptidoglycan cell walls. The peptidoglycan layer imparts rigidity and osmotic stability to the cell wall. Rigidity is imposed at the molecular level by cross-linking of D-ala-D-ala terminal units in adjacent pentapeptide chains. The antibacterial phosphonopeptides work by inhibiting the biosynthesis of these pentapeptide chains.

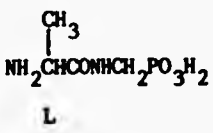


XXXXVI

Phosphonopeptides are transported into bacterial cells by L,L-stereospecific peptide permeases[71,72] using an irreversible active transport mechanism. Thus, peptides containing one or more amino acids in the D-configuration will be absorbed into the cell slowly or not at all[72]. Research by Atherton *et al*[72] has shown that for antibacterial peptides containing 1-aminoethane-phosphonic acid (L-ala(P)) the aminophosphonic acid unit is the active agent. However, the acid alone does not inhibit bacterial growth significantly. It needs to be combined in a suitable di- or higher peptide mimetic to enable its active transport. There is also evidence to suggest that L-ala-L-ala-L-ala(P) and L-ala-L-ala(P)

(alafosfalin) utilise different permease systems[72]. Therefore, L-stereochemistry is the first requirement for *in vitro* activity. Also, variation of the ala(P) moiety (eg. by extension of the alkyl chain) results in a reduction of uptake by the bacterial cell. Only close mimetics of alanine undergo active transport.

Once the peptide has passed through the cell wall it needs to be cleaved intracellularly by L-specific peptidases to yield the active aminophosphonic acid moiety. The rate and amount by which the peptide undergoes hydrolysis is a factor which determines its activity. Peptides containing different amino acid residues or amino acids and phosphonic acids of varying stereochemistry are cleaved at different rates. Alafosfalin is cleaved rapidly to release L-ala(P) as is L-ala-gly(P) (XXXXVII) (gly(P) = aminomethanephosphonic acid), however, the rates of hydrolysis of these compounds by *E.coli* were less than one-tenth of that found for their all natural L-amino acid counterparts L-ala-L-ala and L-ala-gly. Gly-gly(P) (XXXXVIII) and pro-gly(P) (XXXXIX) have reduced activity as they are particularly stable to peptide hydrolases[72].



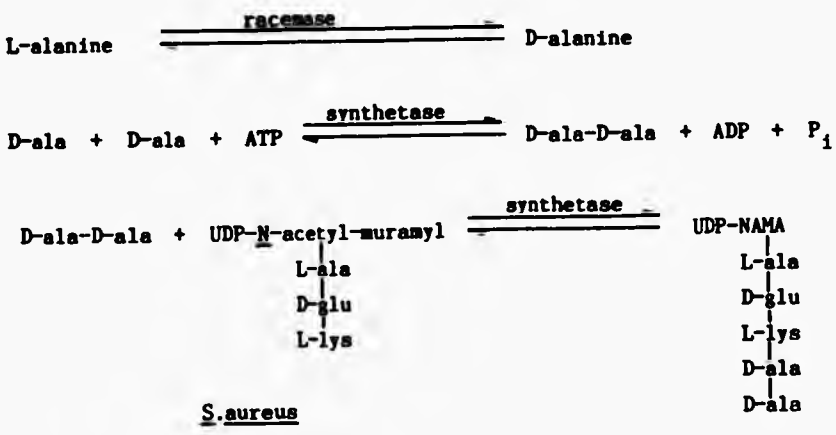
XXXXVII

XXXXVIII

XXXXIX

Early steps in bacterial cell wall biosynthesis involve the conversion of L-alanine to D-alanine and the

incorporation of D-ala-D-ala into the UDP-N-acetyl-muramic acid peptide (scheme 1).



Scheme 1: Early steps in bacterial cell wall biosynthesis

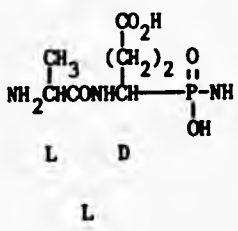
It has been found[72-74] that L-ala(P) (and D-ala(P)) inhibit alanine racemase. In this way, formation of the D-ala-D-ala unit is inhibited. In addition, gly(P) has been shown to inhibit D-ala-D-ala synthetase[72].

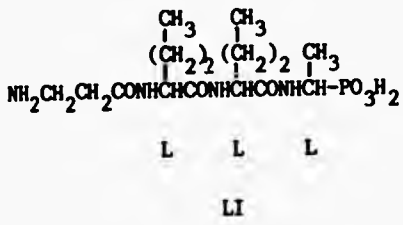
Studies involving ^{14}C labelling have proved the existence of UDP-NAMA-(^{14}C)-ala(P)[75] and UDP-NAMA-(^{14}C)-gly(P)[72] where ala(P) and gly(P) are acting as false substrates for natural L-alanine. The addition of subsequent amino acid residues to form the necessary pentapeptide chain is thus precluded by the inability of the phosphonic acid group to substitute for carboxyl in the formation of the peptide bond. L-ala(P) and gly(P)

were also seen to inhibit UDP-NAMA-L-ala synthetase[72].

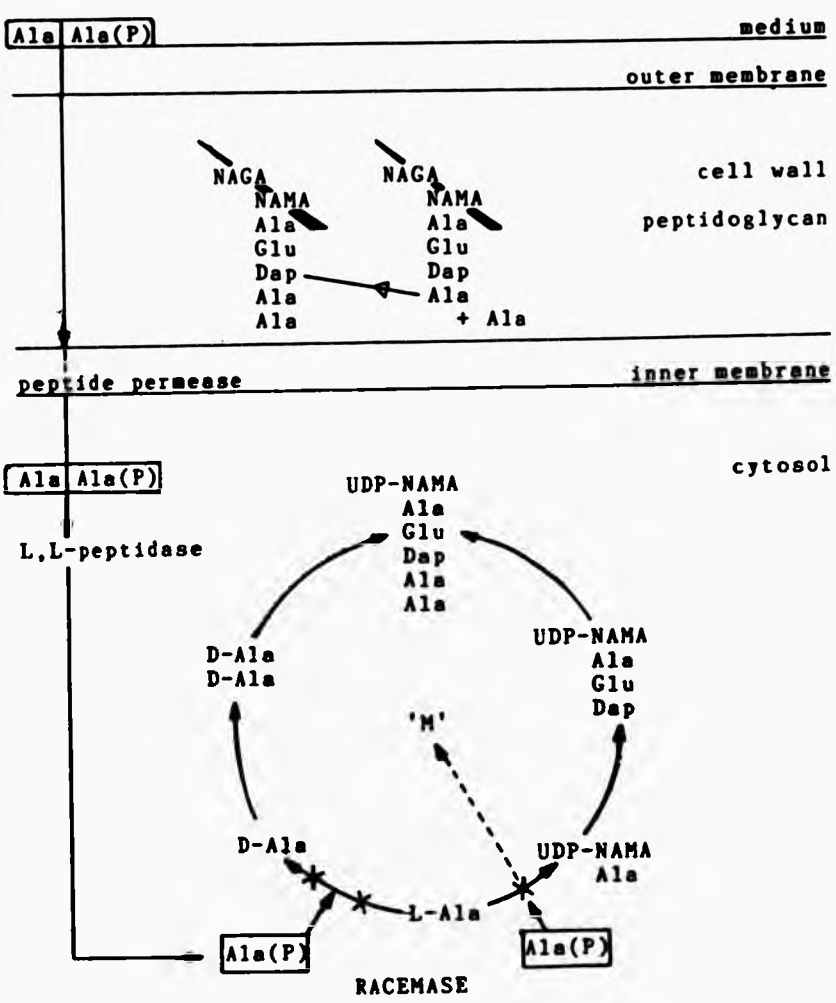
Atherton et al[33] reported weak antibacterial activity for both L-ala-L-glu(P) and L-ala-D-glu(P) (L) (the only example of an active phosphonopeptide incorporating a D-residue). These peptides could act on peptidoglycan biosynthesis in a different way by virtue of the role of glutamic acid in the bacterial cell wall[75] (NB. L-ala-D-glu is a fragment of the UDP-NAMA pentapeptide).

The mechanism of action of alafosfalin is summarised in scheme 2. Toxicological studies in rodents and baboons suggest there is a considerable margin of safety for alafosfalin at doses expected to be effective in man. It is well absorbed orally and has good tolerance[33]. Although alafosfalin is more active against Gram-negative than Gram-positive organisms, there is evidence to suggest[33] that phosphonopeptides such as sar-L-Nva-L-Nva-L-ala(P) (LI) could be used for applications which require a broader spectrum of activity.





Mechanism of action of alafosfalin in E. coli



UDP-NAMA = uridine diphospho-N-acetylmuramic acid
NAGA = N-acetylglucosamine
'M' = UDP-NAMA-Ala(P)
X = site of inhibition of Ala(P)
Δ = site of inhibition of penicillins

Scheme 2

Chapter 2

Discussion

Synthesis of 1-aminoalkanephosphonic acids

One-pot procedures

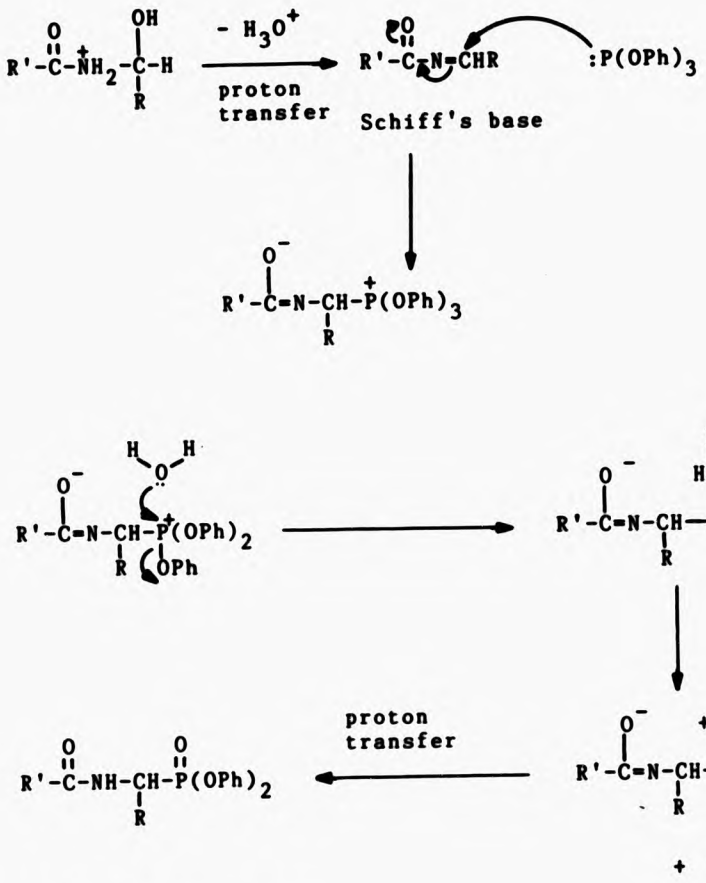
The 1-aminoalkanephosphonic acids were prepared using three different procedures[24-26]:

i) using an aldehyde with triphenyl phosphite and urea;

ii) using an aldehyde with triphenyl phosphite and ethyl carbamate;

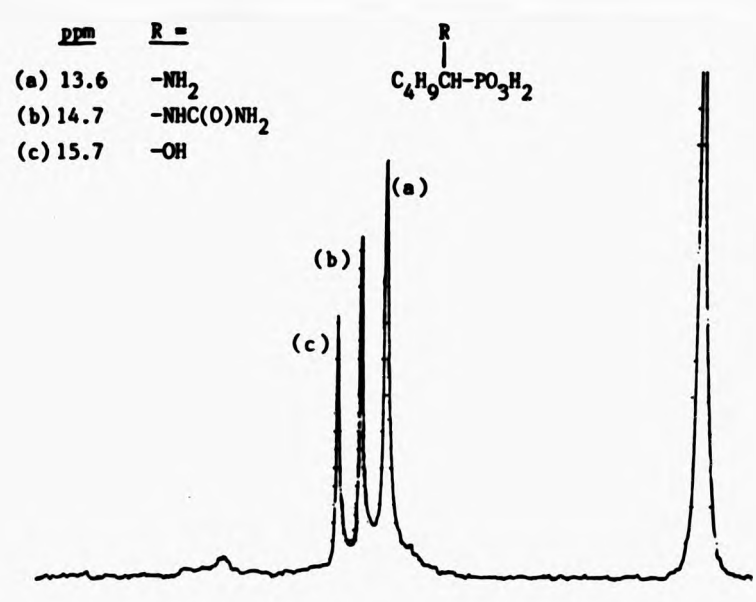
iii) using an aldehyde with triphenyl phosphite and benzyl carbamate.

All the methods involved heating the reactants under reflux for one hour in the presence of glacial acetic acid. It was assumed that the mechanism of reaction involved the initial condensation of the carbamate or urea with the aldehyde to form a Schiff's base. This then underwent nucleophilic attack by the phosphite with subsequent loss of phenol.



$R' = C_6H_5CH_2O-,\ NH_2-$

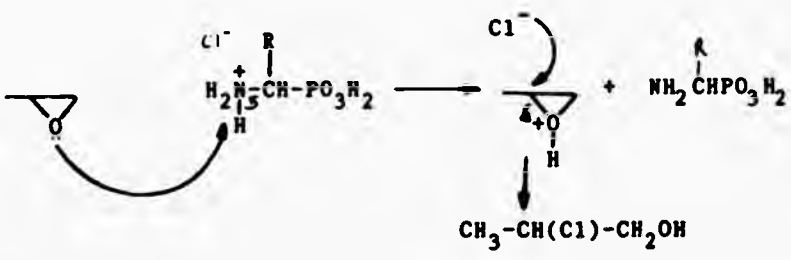
The intermediate N-carbobenzoxy- or ureidophosphonate was hydrolysed by boiling under reflux with concentrated hydrochloric acid for 6 - 10 hours. However, the ureido group was more difficult to cleave than the carbobenzoxy group and was sometimes still present after 6 hours of boiling acid treatment as shown by the following ^{31}P nmr:



^{31}P nmr of 1-aminopentanephosphonic acid prepared by method i)

Fortunately the ureido (and hydroxy) derivative could usually be removed by recrystallisation.

The above by-products were not observed with the carbamate method and using this procedure the yields of 1-aminoalkanephosphonic acids ranged from 20-40 %. Propylene oxide was used to liberate the free amine from its hydrochloride salt.



$CH_3-CH(Cl)-CH_2OH$

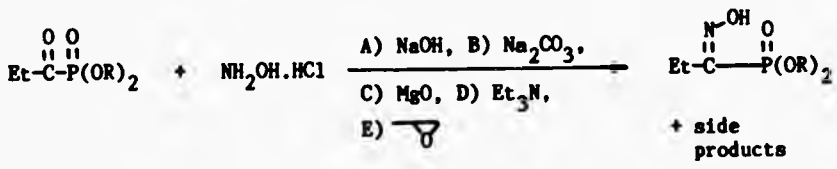
propylene chlorohydrin

This reaction was best carried out by warming the aminophosphonic acid hydrochloride solution with a slight excess of propylene oxide. In the cold a large excess of propylene oxide was required in order to isolate the aminophosphonic acid.

Synthesis via 1-oxoalkanephosphonates

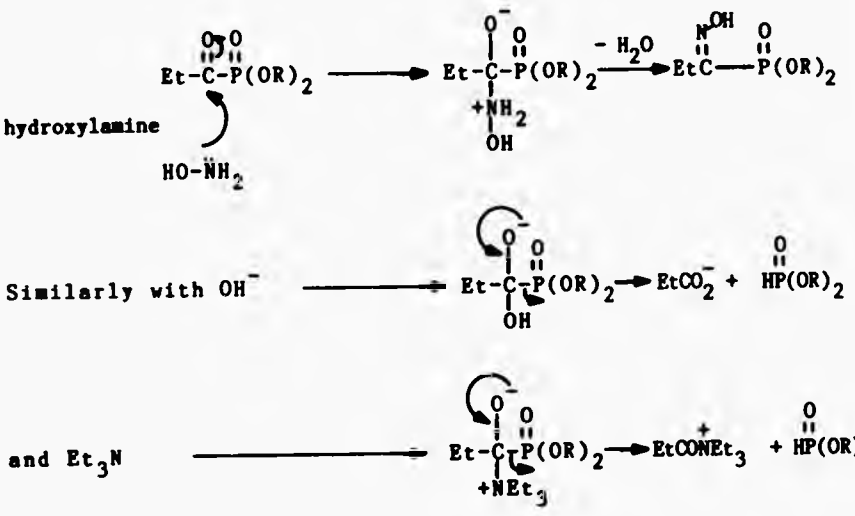
The O,O-dialkyl 1-oxoalkanephosphonates (ketophosphonates) were prepared from acyl chlorides and trialkyl phosphites in excellent yields using established procedures[36]. Some decomposition occasionally occurred on distillation of the diethyl ketophosphonates and for this reason the dimethyl ketophosphonates were preferred.

As well as the established method for the preparation of the 1-hydroxyiminophosphonates using hydroxylamine hydrochloride and pyridine[37], attempts were also made to prepare the oximes using other bases (A to E).



In all cases the oxime was obtained, however, the product was contaminated with impurities.

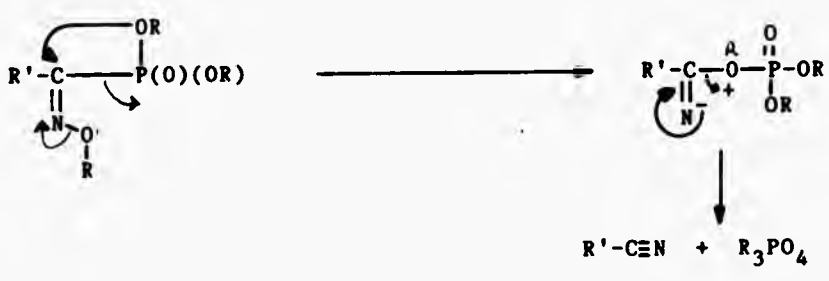
In the reactions involving sodium hydroxide, sodium carbonate and triethylamine, dialkyl phosphite was produced as a result of ketophosphonate decomposition[76] although anhydrous solvents were used. The trace amounts of water present provided OH^- which competed with hydroxylamine in nucleophilic attack at the carbonyl carbon. Triethylamine probably acted in the same way.



However, only in the first case is water lost to form the stable oxime. In the latter two cases C-P bond cleavage occurs to form the dialkyl phosphite.

When propylene oxide was employed as base the oxime was contaminated with the chlorohydrin formed as a result of the reaction of propylene oxide with hydroxylamine hydrochloride. The chlorohydrin has a relatively high

boiling point (135-4 °C) and could not be effectively removed from the product at the low temperatures required to avoid oxime decomposition. The heat sensitivity of the oximes[77] also prohibited the use of distillation as a means of purification. An analogous mechanism to that observed for the arylphosphonate oximes would give the following decomposition[77].



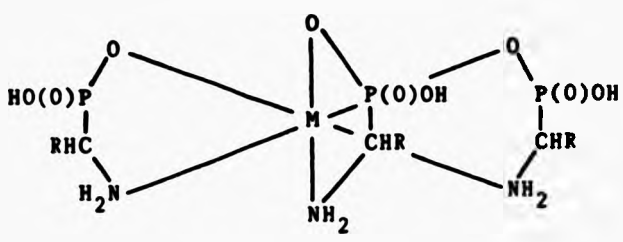
The oximes were reduced by hydrogenation and chemical methods. Using zinc in formic acid[42], diethyl 1-amino-phosphonate was obtained in 40 % yield. This method involves a laborious work-up procedure to remove the formyl group attached to nitrogen, and during the many steps losses in yield may have occurred.

Hydrogenation is a more straightforward way of reducing the oxime. There is only one report in the literature[37] that describes the reduction of phosphonate oximes using hydrogenation. This report describes the use of Raney nickel, however high temperatures and pressures were required and the aminoalkanephosphonates were obtained in

unspecified yields. A more economic procedure was sought and so hydrogenations were attempted at low temperatures and pressures, first with palladium-on-charcoal, then with Raney nickel.

At room temperature and 38 psi, palladium failed to reduce the oximes either in acetic acid or anhydrous alcohol. Raney nickel failed to produce the dialkyl aminophosphonates at room temperature and 300 psi but at the same pressure and 100 ^{o}C the oximes were reduced. Hydrolysis of the dialkyl 1-aminopropanephosphonate obtained by the above method gave crude 1-aminopropane-phosphonic acid in 60% yield.

A major problem encountered when using Raney nickel was that the intermediate dialkyl 1-aminoalkanephosphonates contained a substantial amount of dissolved nickel. When ^{1}H nmr spectra of these green oils were attempted, ambiguous broad peaks were observed which were of no use in characterising the esters. The 1-aminopropanephosphonic acid obtained from the hydrolysis of the corresponding dimethyl ester prepared by the Raney nickel method was shown to contain 0.33% nickel by atomic absorption analysis. Further crops of aminophosphonic acid obtained from the recrystallization mother liquors were shown to contain 11.2% nickel, which suggests the possibility of a 3:1 complex (see over). Analogous 3:1 complexes have been reported for divalent metal ions, including Ni(II), and aminocarboxylic acids[109]. The metals have a natural tendency to assume a coordination number of five or six.



The nickel was removed from the phosphonic acid by ion-exchange chromatography.

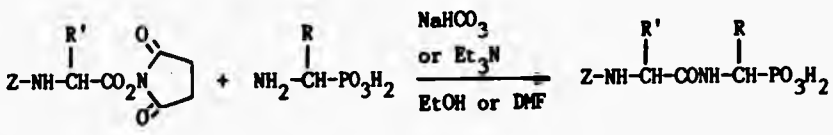
As the oxime reduction was attempted at higher reactant concentrations, it was found that the yield of aminophosphonic acid obtained was reduced. A high reactant concentration is desirable if the hydrogenations are to be carried out on an industrial scale - smaller reaction vessels are required and solvent costs are reduced. It was thought that the loss in yield might have been due to the formation of secondary amines - a common problem in oxime hydrogenations. To prevent this, liquid ammonia was added to the reactants. This increased the yield of the aminophosphonic acid from 27% without ammonia to 51% with ammonia at an oxime concentration of 20%. However, care was taken to ensure the removal of as much ammonia as possible before the hydrolysis step. Any ammonia remaining in the dialkyl aminophosphonate would have produced ammonium chloride which would have complicated the isolation of the aminophosphonic acid. The ammonia was removed by evaporating and then reevaporating the aminophosphonate

with water.

The best yields of all were obtained with 5% palladium-on-charcoal at 115 ^{o}C and 400 psi in the presence of ammonia. Under these conditions the 1-aminopropanephosphonic-acid was obtained in 61% yield at a dimethyl oxime concentration of 50%. Also with palladium the final product is no longer contaminated by the catalyst.

Synthesis of phosphonopeptides

Two methods were used to prepare the phosphonopeptides. The first, and most successful, was that of Atherton *et al*[60,61] by way of the activated ester approach which used the free 1-aminoalkanephosphonic acids and *N*-hydroxy-succinimide esters of *N*-carbobenzoxy amino acids.



The *N*-carbobenzoxy amino acids were prepared by the method of Bergmann and Zervas[79] and their *N*-hydroxy-succinimide esters by the method of Anderson *et al*[84].

After coupling, the peptides were deprotected using hydrobromic acid in glacial acetic acid according to Kafarski *et al*[63]. The free peptides were precipitated from their hydrobromide salts by using propylene oxide at room temperature.

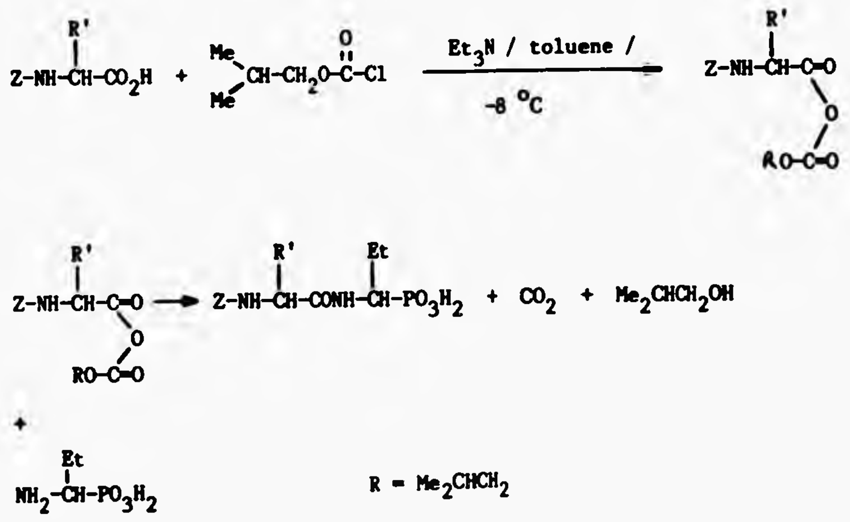
Using this procedure a total of twenty two peptides were prepared, eighteen of which are novel compounds. Yields ranged from 2.5 - 82%, but on average yields of 50 - 60% could be expected.

The peptides were usually obtained as jelly-like solids even after recrystallization and on drying in a vacuum oven at 60 ^{o}C these became white powders or crystals.

Microanalysis of the peptides was not always successful even when ^{1}H, ^{13}C and ^{31}P nmr and FAB mass spectrometry showed the samples to be correct. This may have been due to recrystallization solvents such as water remaining associated with the peptides even after extensive drying.

Sodium bicarbonate or triethylamine were used as bases in the peptide coupling step. More aqueous conditions were used with sodium bicarbonate and this procedure favoured preparations involving L-alanine. The slightly more ethanolic triethylamine method worked best with D-alanine and glycine.

The second method of peptide preparation attempted was the mixed carboxylic - carbonic anhydride method of Atherton _et al_[60,61], using isobutyl chloroformate.



The mixed anhydride was prepared *in situ* and then allowed to react with a solution of the aminophosphonic acid in triethylamine and aqueous ethanol. This procedure gave lower yields than the activated ester method as shown in table 1.

Preparation of phosphonopeptides

Phosphonopeptide	Method	Yield / %
L-ala-P	A	56
D-ala-P	B	63
DL-ala-P	A	2.5
	B	66
	C	31
gly-P	B	82
L-ala-L-ala-P	A	62
D-ala-L-ala-P	B	49
	C	38
gly-L-ala-P	C	41
L-ala-L-ala-L-ala-L-ala-P	A	67

P = -NH-CH(Et)-PO_3H_2

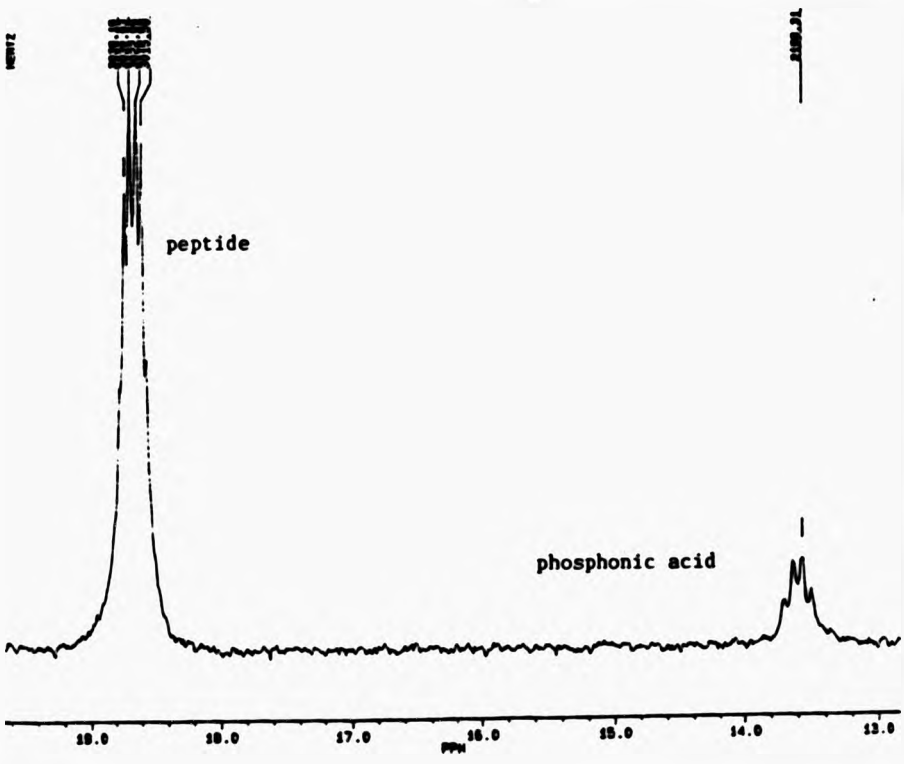
Method A - activated ester method using $NaHCO_3$

Method B - activated ester method using Et_3N

Method C - mixed carboxylic-carbonic anhydride method

Table 1

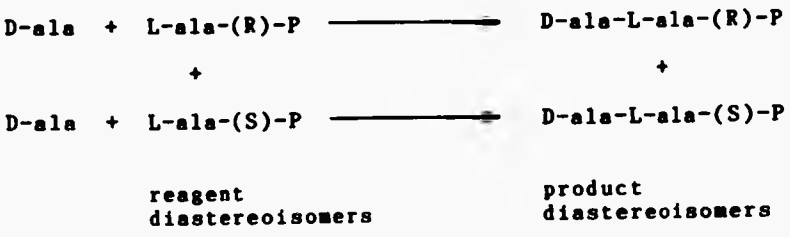
In the case of the DL-alanine dipeptide of 1-aminopropanephosphonic acid the mixed anhydride method (method C) not only gave a lower yield than the activated ester method (method B) but also the melting point of the product was lower by 15 degrees. Examination of the ^{31}P nmr of the peptide showed two signals - one due to the peptide and one due to the unreacted 1-aminopropanephosphonic acid. This explains the depression in melting point.



^{31}P nmr proton-coupled spectrum for (1R,S)-1-(DL-alanylamino)propanephosphonic acid

However, the mixed anhydride method did not always result in a loss in purity, but the physical properties of the peptides sometimes differed from those obtained by the activated ester method for other reasons.

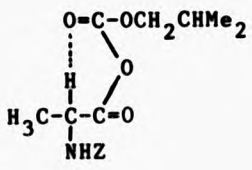
As racemic 1-aminoalkanephosphonic acids were used as substrates, the resulting phosphonopeptides were always produced as a mixture of at least two diastereoisomers. For example, in the case of the D-alanyl-L-alanyl-1-aminopropanephosphonic acid tripeptide:



It could be envisaged that the reaction of D-alanine with one diastereoisomer may be favoured over its reaction with the other diastereoisomer. The rates of reaction could be altered by changing the reaction conditions. This could be why the optical activities of the D-alanyl-L-alanyl-1-aminopropanephosphonic acid peptides obtained from methods B and C differ.

	Method	m.p./°C	$[\alpha]_{578}^{25}$
D-ala-L-ala-P	B	240-3	-11.3
D-ala-L-ala-P	C	251-3	-19.6

Another explanation for this result could be that partial racemization of the mixed anhydride occurs before its reaction with the phosphonopeptide (as reported for conventional peptides).



-proton abstraction

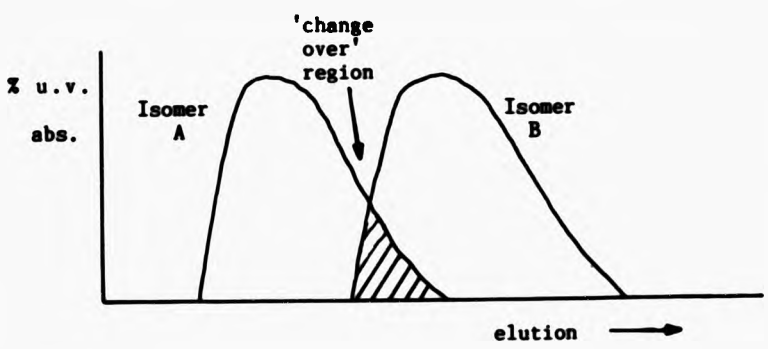
Reaction conditions were chosen so as to supress the possibility of racemization - the anhydride preparation was carried out at low temperature in a non-polar solvent and the bulky isobutyl chloroformate was used.

It can be seen from the above figures that the melting points of the peptides were highly dependent upon the relative amounts of diastereoisomers present. For this reason, melting points were of limited use in peptide characterization.

Resolution of diastereoisomers

Two peptides were resolved using the method of Kafarski *et al*[63]. The peptides were chromatographed using a 20 x 2.5 cm column packed with a fine bore cation-exchange resin. An attempt was made to separate the diastereoisomers using a 16-40 mesh resin, however, only partial resolution was obtained.

Water was used as the elutant and the fractions collected were monitored by u.v. spectroscopy. The peptides gave an absorption between 190 and 230 nm with the size of the peak indicating the concentration of peptide present. This was a useful method of determining the 'change over' region - where one diastereoisomer overlapped the other.



The reported[63] method for monitoring the elution of phosphonopeptides is by ninhydrin, however, it was found that this was too insensitive a method which gave ambiguous results.

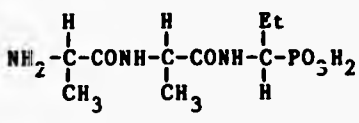
The first phosphonopeptide to be resolved was (1R,S)-1-(L-alanylamino)propanephosphonic acid (LVIII) which has been resolved in the literature[63]. An 80% recovery yield was obtained, however, the optical rotations of the diastereoisomers differed from those in the literature. The S,S-dipeptide had an optical rotation of $[\alpha]_{578}^{25}$ +88° (literature value $[\alpha]_{578}^{20}$ +75°) and the S,R-dipeptide had an optical rotation of $[\alpha]_{578}^{25}$ -46° (literature value $[\alpha]_{578}^{20}$ -53°). These discrepancies could be due to instrumental error or the S,S-dipeptide could have a greater optical purity and the S,R-dipeptide a lesser optical purity than the reported phosphonopeptides.

The tripeptide (1R,S)-1-(L-alanyl-L-alanylamino)-propanephosphonic acid was also resolved by the above method. No references to the separation of phosphonotripeptides have been found in the literature. Any assignments made to the configuration of the resolved tripeptide diastereoisomers are thus tentative.

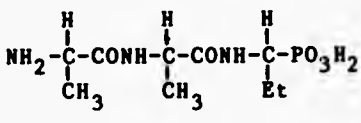
In the case of the phosphonodipeptides it is generally observed[63] that peptides of higher positive optical rotation are eluted fastest. However, in the case of the tripeptides, the first diastereoisomer collected had an optical rotation of $[\alpha]_{578}^{25}$ -52° and the second diastereoisomer had an optical rotation of $[\alpha]_{578}^{25}$ -14.3°. The peptide of highest positive rotation is thus eluted slowest. Using the general rule for dipeptides:

$$R_f \text{ (L-L/D-D)} > R_f \text{ (L-D/D-L)}$$

It could be considered that the first peptide eluted had an S,S,S configuration (L,L,D) and the second peptide had an S,S,R configuration (L,L,L) as shown:

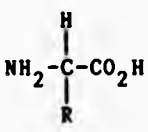


S(L) S(L) S(D)

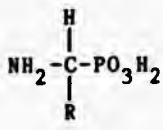


S(L) S(L) R(L)

It is important to note that the notation 'S' for an aminocarboxylic acid corresponds to the notation 'R' if the $-CO_2H$ group of the aminocarboxylic acid is replaced by the phosphonic acid group (PO_3H_2) and <u>vice versa</u>.



S (L-amino acid)



(L-aminophosphonic acid) R

On resolution the phosphonopeptide diastereoisomers had different melting points (the peptides of higher positive optical rotation having the higher melting points) and different ^{13}C nmr spectra.

Prior to the publication of Lejczak <u>et al</u>[70] which referred to the hydrolysis of resolved phosphonodipeptides as a way of preparing optically active aminophosphonic acids, an attempt was made to resolve the isomers of 1-aminopropanephosphonic acid.

The dipeptide (1S)-1-(L-alanylamino)propanephosphonic acid was hydrolysed by heating under reflux with 6N

hydrochloric acid. On determining the melting point of the product obtained, it was observed that part of the product underwent sublimation. The mixture was separated in a sublimation apparatus and the unsublimed solid was found to be the 1-aminopropanephosphonic acid. The phosphonic acid had an optical rotation of $[\alpha]_{578}^{25}$ +12.5° whereas the literature value for (S)-1-aminopropane-phosphonic acid is $[\alpha]_{578}^{20}$ +21° [70]. The melting point of the 1-aminopropanephosphonic acid obtained was higher than that of the racemic mixture (268-9 °C compared to 262-3 °C[26]) but lower than that reported for (S)-1-amino-propanephosphonic acid (273-4 °C[70]).

^{1}H, ^{13}C and ^{31}P nmr of the aminophosphonic acid showed it to be pure with no unsublimed alanine present. Aminophosphonic acids often show a variation in melting point, for example melting points of 262-3 °C[26], 264-6 °C[23], 272-4 °C[104] and 285-6 °C[105] have been reported for 1-aminopropanephosphonic acid. It thus appears that one of the features of this type of compound is variable melting point. This may be due to crystal size or unusual sensitivity to small amounts of impurities. Therefore, the variation between the observed and literature values for the melting point of (S)-1-aminopropanephosphonic acid is not significant.

However, the variation in optical rotation is more difficult to explain. The aminophosphonic acid may not have been as optically pure as that obtained in the literature.

Solid phase synthesis of phosphonopeptides

Solid phase peptide synthesis was introduced by Merrifield[85] in 1963. The classical approach to peptide synthesis (the solution method) has been perfected over the years so that small peptides may be produced in yields of over 90%. However, these procedures are not ideally suited to the synthesis of larger peptides. The technical difficulties with respect to insolubility and purification become formidable as the number of amino acid residues increases. After each coupling stage the peptide must be purified, usually by recrystallization, to remove non-coupled reagents and side products. This results in a loss in yield at each coupling step. Also, as the peptide increases in length, its solubility decreases up to a point where suitable recrystallization solvents cannot be found.

If phosphonopeptides are to be considered as commercially viable fungicides, they must be produced cheaply and effectively. If the di- or tripeptides are to be used, then the classical methods of peptide synthesis will be most suitable. However, if longer peptides are required, solid phase peptide synthesis could be a more successful method and one that could be automated.

Phosphonopeptides have not previously been synthesized by solid phase methods. By considering the procedures perfected by Merrifield *et al*[85-87], a number of possible routes to the synthesis of phosphonopeptides *via* solid supports were devised.

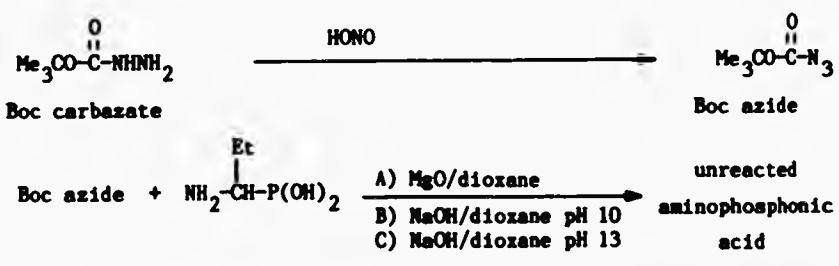
The first route considered involved the use of a

chloromethylstyrene-divinylbenzene polymer support (Merrifield's Peptide Resin) and an N-t-butyloxycarbonyl (Boc) protected 1-aminophosphonic acid or monoester as shown below:

The aminophosphonic acid was to be introduced onto the resin support as its triethylamine salt producing a benzyl ester link. The benzyl ester would, hopefully, be stable to dilute acids and bases allowing the deprotection of the amino group and its subsequent coupling with an N-protected amino acid. The protected peptide could then either undergo further deprotection and coupling steps, or be cleaved from the support and deprotected by hydrobromic acid in trifluoroacetic acid in one step.

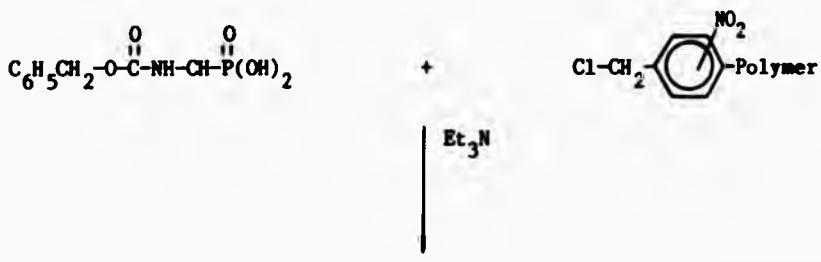
At each stage of the synthesis the peptide-polymer could be purified by simply filtering and washing the insoluble resin. In this way yield loss by recrystallization is eliminated and the insolubility of larger peptides ceases to be a problem.

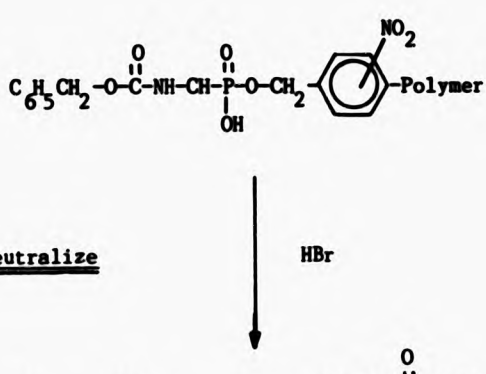
The above scheme requires the synthesis of N-t-butyloxycarbonyl 1-aminoalkanephosphonic acids. The t-butyloxycarbonyl aminocarboxylic acids are usually synthesized using t-butyloxycarbonyl azide. However, after several attempts using this procedure we were unable to isolate the Boc aminophosphonic acid, although base uptake was observed throughout the reaction.



It is known that Boc aminocarboxylic acids are stable at room temperature if pure, but any traces of acid remaining in them during storage will promote cleavage of the Boc group and accumulation of the free amino acid. As aminophosphonic acids are stronger acids than aminocarboxylic acids, it could be supposed that, once formed, the Boc aminophosphonic acids could be cleaved by any remaining free aminophosphonic acid during the work-up procedure.

The benzyloxycarbonyl (carbobenzoxy) group has also been used as an N-protecting group in solid phase synthesis[85]. However, its deprotection requires relatively harsh conditions - anhydrous hydrogen bromide in acetic acid - which normally cleaves benzyl esters, and would therefore cleave the amino acid from the resin support. To prevent this, the chloromethyl resin is nitrated. On attaching the N-carbobenzoxy amino acid to the resin a nitrobenzyl ester is formed which is stable to the action of hydrobromic acid in acetic acid. As the N-carbobenzoxy 1-aminoalkanephosphonic acids are readily available[80] the following scheme was proposed:

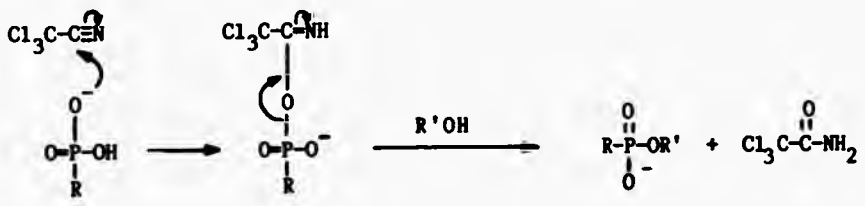




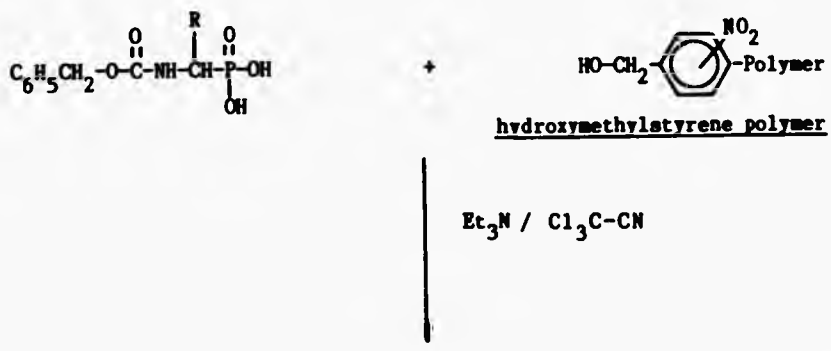
However, following established methods for the attachment of N-carbobenzoxy aminocarboxylic acids to nitrochloromethyl polymers (heating under reflux in ethyl acetate for 65 h in the presence of triethylamine)[85,86] no mentionable degree of attachment was observed with

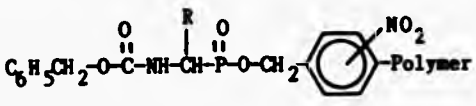
the phosphonic acid derivative. More success might have been obtained with the phosphonic acid monomethyl ester.

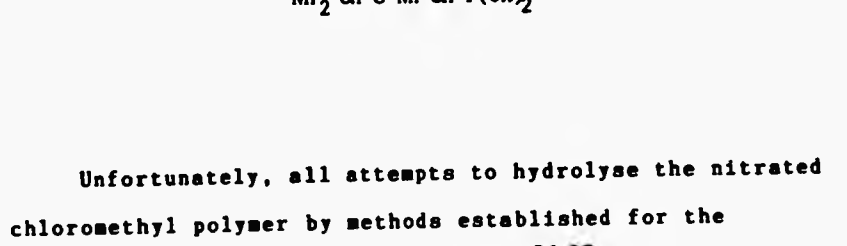
The main problem appeared to be the esterification of the phosphonic acid to produce the benzyl ester link with the resin. N- Carbobenzoxy-1-aminophosphonic acid monobenzyl esters are readily prepared when trichloroacetonitrile is employed as the condensing agent[80].

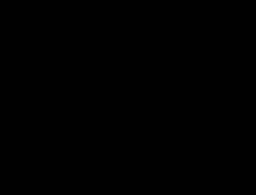


Hydroxymethylstyrene polymers have been used as support resins, usually with Boc protected amino acids. Therefore, an attempt was made to prepare a nitrated hydroxymethylstyrene polymer to take part in the following scheme:

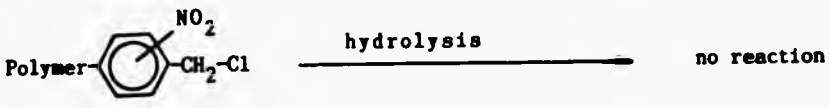




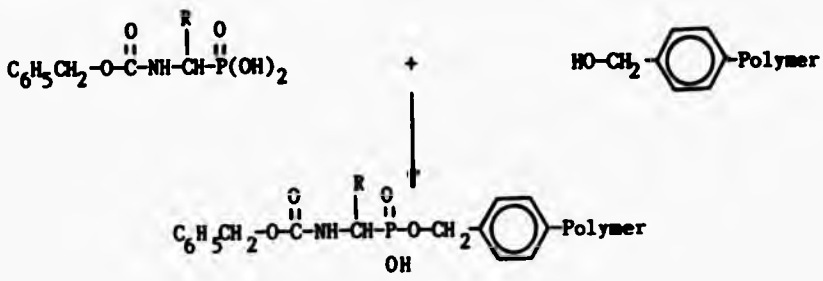




Unfortunately, all attempts to hydrolyse the nitrated chloromethyl polymer by methods established for the preparation of hydroxymethyl polymers[86,87] failed.



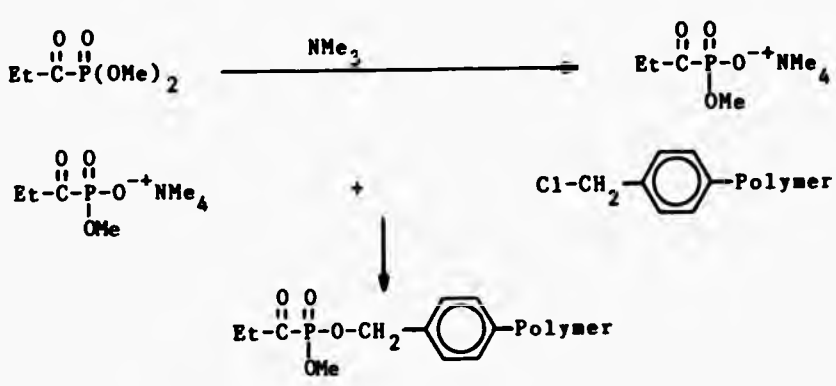
This is probably because the electron-withdrawing $-NO_2$ group reduces the rate of CH_2-Cl bond heterolysis. In an attempt to ascertain whether trichloroacetonitrile could be used as a condensing agent for attaching N-protected aminophosphonic acids to hydroxymethyl resins, the following reaction was attempted:



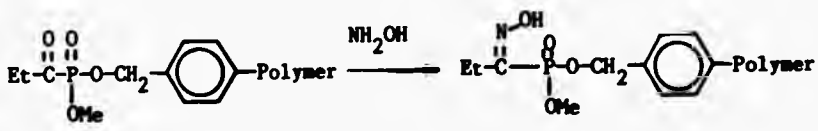
Following established methods[86,87], the preparation of the hydroxymethyl polymer proved more difficult than expected. Acetolysis using potassium acetate in benzyl alcohol[87] failed and the reported[86,87] use of infrared spectroscopy in monitoring the acetolysis and saponification steps proved of little use. Comparison of the spectra of the chloromethyl resin before and after attempted acetylation showed no change. The attempted attachment of the N-carbobenzoxy aminophosphonic acid to the hydroxymethyl resin failed.

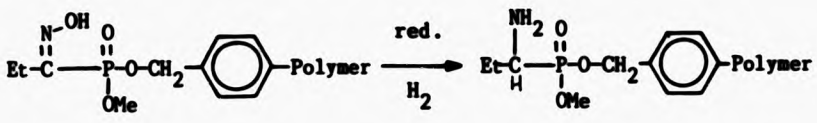
The only literature relating to the attachment of a phosphonic acid derivative to a solid support by way of a covalent P-O-C bond refers to β-ketophosphonates (2-oxo-alkane phosphonates)[88]. A well-known property of methylic esters of phosphonic acids is their ability to give tetramethylammonium (TMA) salts when caused to react with trimethylamine[89]. The TMA salts of various β-keto-phosphonates were attached to a chloromethyl resin support by heating under reflux in dimethylformamide. Therefore, the tetramethylammonium salt of dimethyl 1-oxopropane-phosphonate was prepared and caused to react with

chloromethylstyrene polymer:



Microanalysis of the product gave a phosphorus content of 0.68% and a chlorine content of 0.26%. The original chlorine content of the resin was stated to be 7.1%, therefore, chlorine had been removed from the resin but it had not been replaced by phosphorus. The small amount of phosphorus contained in the resin may have been due to covalently bonded phosphorus, or it may have been due to impurities. If the conditions for this reaction could be optimised, the ketophosphonate could be transformed to the aminophosphonic acid derivative whilst still attached to the resin:





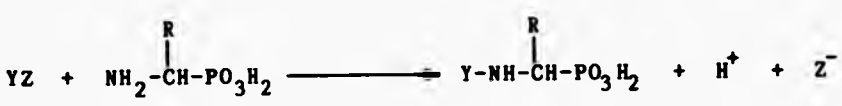
The aminophosphonic acid derivative could then undergo coupling reactions with N-protected amino acids.

More research is needed in this area and reliable methods of analysis need to be developed. No form of nmr spectroscopy (apart from solid state) can be used to follow reactions qualitatively and infra red spectroscopy is not reliable. There are no obvious reasons why phosphonopeptides should not be prepared by solid phase methods.

Synthesis of N-protected 1-aminoalkanephosphonic acids

Replacing a proton of an $-NH_2$ group with another derivatising group is a relatively simple process. As mentioned earlier (see introduction), derivatisation can increase the systemic activity of a pesticide and improve its toxicological properties. However, derivatisation can also result in a complete loss of activity.

As in the case of the methylcarbamate insecticides (see introduction), the 1-aminoalkanephosphonic acid derivatising group, Y, must be selected so that the bond N-Y is moderately labile. The N-protected product may



then be activated within the target organism to produce the toxic parent compound.

Protection using carbamate groups

Many labile N-protecting groups are available due to their importance in peptide chemistry. One of the most common protecting groups is the carbobenzoxy group.

Carbamate protecting groups are useful for the folowing reasons:

i) they are easily introduced using chloroformates;

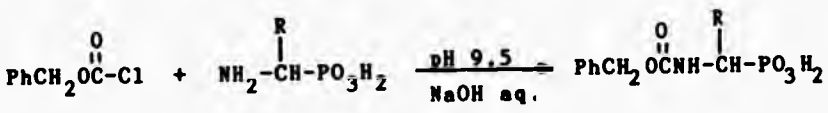
ii) crystalline derivatives are usually obtained;

iii)the liposolubility of the parent compound is increased;

iv) the C-N bond of the -OC(O)-N- group is acid labile and also undergoes hydrogenolysis.

For the above reasons, introducing the carbamate protecting group appeared an excellent way of possibly increasing the systemic activity of the 1-aminoalkane-phosphonic acids.

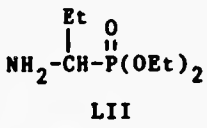
One method has been used in the literature to prepare N-carbobenzoxy-1-aminoalkanephosphonic acids[78]. This involves maintaining a pH of 9.5 throughout the reaction by the intermittent addition of 4N sodium hydroxide solution.



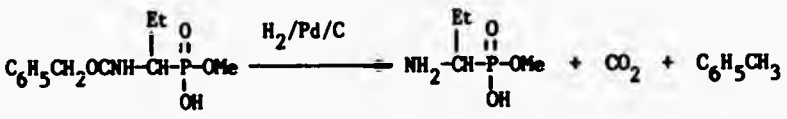
Using this method, N-carbobenzoxy-1-aminopropane-phosphonic acid was prepared in 62% yield. A method analogous to that used by Bergmann and Zervas[79] for the preparation of N-carbobenzoxy amino acids was also attempted. However, this method gave yields of only 10%.

The N-carbobenzoxy-1-aminopropanephosphonic acid obtained had a melting point and proton nmr properties as described in the literature by Wasielewski et al[80]. These authors also reported the preparation of aminophosphonic acid monoesters using trichloroacetonitrile as the condensing agent. Knowing that diethyl 1-aminopropane-phosphonate (LII) exhibited a very low antifungal activity[1a] the N-carbobenzoxy-1-aminopropanephosphonic acid monomethyl ester (LIII) was prepared as a route to obtaining the 1-aminopropanephosphonic acid monomethyl ester.

It was thought that the introduction of one ester group into the aminophosphonic acid molecule might have increased its systemic activity without reducing its fungicidal activity by too great an extent. The free 1-aminopropanephosphonic acid monoester was obtained by hydrogenolysis of the carbobenzoxy group and was isolated as an oil in 80% yield.



LII

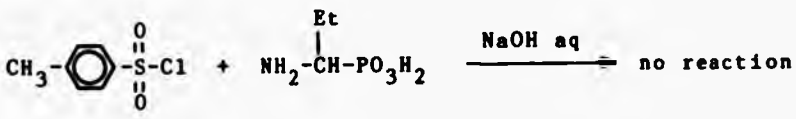


LIII

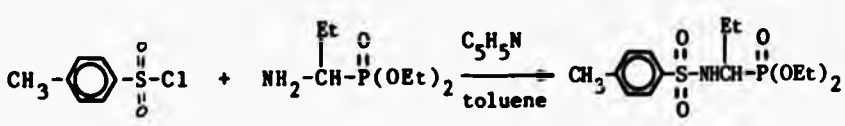
Other readily available chloroformates were condensed with 1-aminopropanephosphonic acid to prepare N-protected derivatives. The reaction with ethyl chloroformate failed, however, 2,2,2-trichloroethyl chloroformate reacted to give the N-protected product in 32% yield.

Protection using p-toluenesulphonyl group

Amino acids are reported to react with p-toluenesulphonyl-chloride in hot aqueous sodium hydroxide solution[81]. However, this reaction failed when repeated with 1-amino-propanephosphonic acid.

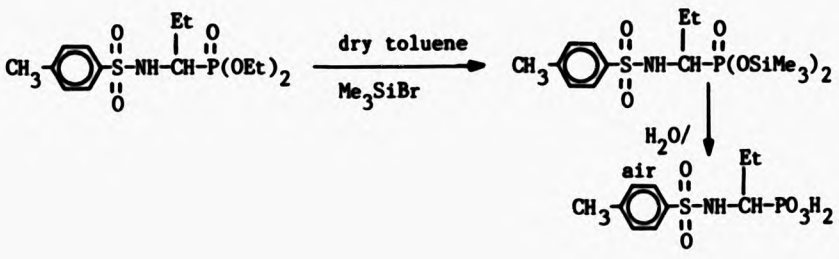


The same reaction was attempted with the diethyl ester of 1-aminopropanephosphonic acid. Using pyridine as base, the crystalline N-p-toluenesulphonyl derivative (LIV) was obtained in 36% yield.



LIV

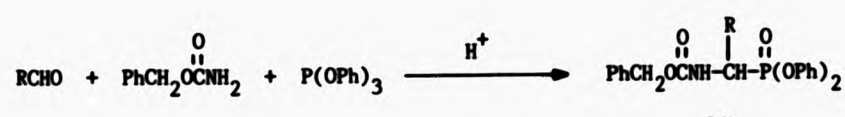
The ester was converted to the phosphonic acid using trimethylsilylbromide[45].



Under these mild conditions the acid-labile tosyl group remained intact.

Diphenyl N-carbobenzoxy-1-aminoalkanephosphonates

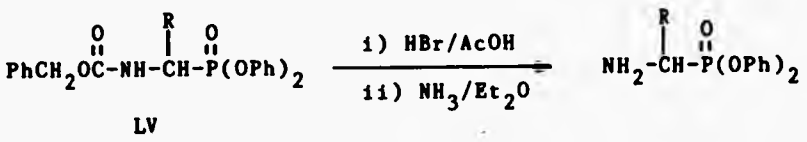
Although it is known that the dialkyl 1-aminoalkanephosphonates have low fungicidal activity, the diaryl 1-aminoalkanephosphonates have not been screened. Diphenyl N-carbobenzoxy-1-aminoalkanephosphonates have been isolated as intermediates in the preparation of aminophosphonic acids[27].



LV

R = CH_3, $(CH_3)_2CH$, $(CH_3)_2CHCH_2$, C_6H_5, $C_6H_5CH_2$

Oleksyszyn et al[27] also reported the deprotection of the above compounds using hydrobromic acid in glacial acetic acid. Surprisingly, the phenyl ester groups are not hydrolysed during this step.



LV

Using the above procedure an attempt was made to prepare the N-protected diphenyl ester with propanal as the reagent aldehyde (R = Et). The reaction was repeated several times, but the expected diphenyl N-carbobenzoxy-1-aminopropanephosphonate (LV)(R = Et) was not obtained. ^{31}P nmr of the reaction mixture[82] suggested that the product (LV) had been formed along with many other phosphorus-containing compounds.

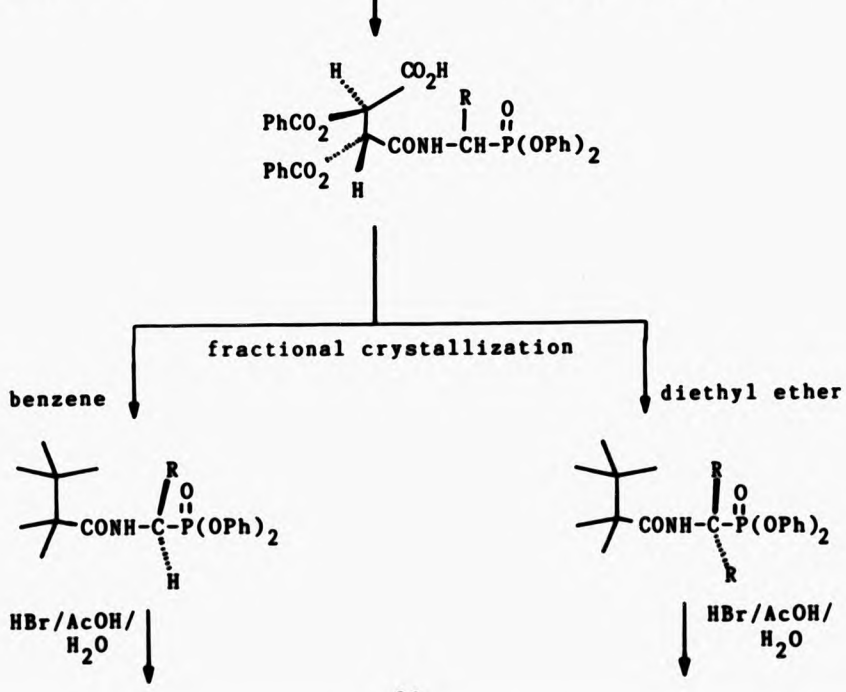
The reaction was repeated using acetaldehyde as the reagent aldehyde (R = Me). In this case the reported[27] diphenyl N-carbobenzoxy-1-aminoethanephosphonate was obtained in a 53% yield. The reaction was also repeated using butanal (R = n-Pr) when the corresponding N-protected diphenyl phosphonate was obtained in a 36% yield.

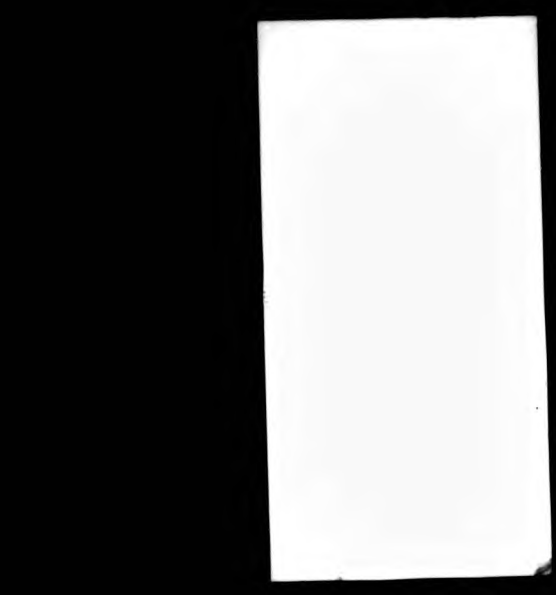
It thus appears that separation/solubility problems were responsible for the failure of the reaction with propanal. Column chromatography of the reaction products

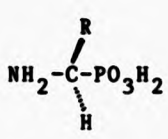
of this reaction was unsuccessful.

Attempts were made to deprotect the N-protected diphenyl phosphonates obtained (R = Me, n-Pr) by stirring them at room temperature with hydrobromic acid as reported[27]. However, after one hour, no deprotection was observed and the original N-protected diphenyl phosphonates were recovered.

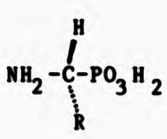
The free diphenyl 1-aminoalkanephosphonates have been used to resolve the 1-aminoalkanephosphonic acids[83].







S-isomer



R-isomer

This may, thus, be a useful method for the preparation of the R and S isomers of 1-aminopropanephosphonic acid.

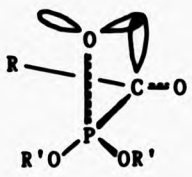
Infrared spectroscopy

Infrared spectra of phosphonic acids are characterized by broad diffuse bands from 3630-1800 cm^{-1} making it impossible in the majority of cases to make definite assignments in this region. The broadness of the bands can be attributed to the extensive hydrogen-bonding present[1]. Therefore, infrared spectroscopy is of little use when characterizing aminophosphonic acids. However, where derivatives containing amide groups have been prepared, infrared has been used to confirm the presence of carbonyl bonds.

In the case of the phosphonopeptides, the carbonyl absorption occurs around 1680-1640 cm^{-1}, N-H stretching vibrations are observed at 3300-3280 cm^{-1}, with an $^{+}NH_3$ symmetrical bending band near 1550-1500 cm^{-1}.

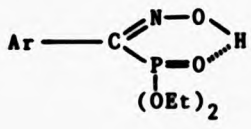
The alkoxycarbonyl aminophosphonic acids (eg. N-carbobenzoxy- and N-2,2,2-trichlorocarboethoxy-1-amino-propanephosphonic acid) show peaks in the region of 1720-1685 cm^{-1}. These are due to the urethane carbonyl function.

Infrared spectra of the dialkyl 1-oxoalkanephosphonates and their oximes are quite well documented[36,37,39,50,76]. The surprisingly low (1695-1670 cm^{-1}) frequency of absorption for the carbonyl function in the 1-oxoalkanephosphonates has been attributed to one of the non-bonding orbitals on the oxygen atom of the phosphoryl group orientating so that overlap with the p-orbital on the carbonyl carbon atom occurs. This could result in alteration of the force constant of the carbon oxygen bond[50].



The phosphoryl peaks (1266-1250 cm^{-1}) and the P-O-C linkage (1040-1020 cm^{-1}) are also useful in identifying these compounds.

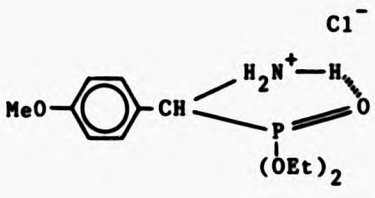
The dialkyl 1-hydroxyiminopropanephosphonates show phosphoryl absorptions of 1240-1230 cm^{-1}. It is widely believed that phosphoryl absorptions between 1250 and 1200 cm^{-1} indicate hydrogen-bonded phosphoryl groups, whereas P=O frequencies from 1300 to 1250 cm^{-1} represent free phosphoryl functions[39,50]. Dilution studies of diethyl 1-oxoarylmethylphosphonate oximes have suggested the presence of intramolecular hydrogen-bonding.



It is thus reasonable to assume that the dialkyl 1-hydroxyiminopropanephosphonates are hydrogen bonded in the same way. These oximes exhibit a broad OH-stretch (3300-3125 cm^{-1}) and P-O-C absorptions around 1040-1020 cm^{-1}.

The dialkyl 1-aminoarylmethylphosphonate hydrochlorides are believed to be intramolecularly hydrogen-bonded as shown by their P=O frequency and dilution

studies.



Diethyl 1-aminopropanephosphonate has a P=O frequency of 1240 cm^{-1}, therefore, it may be hydrogen-bonded. This compound has few other prominent peaks in its infrared spectrum save its P-O-C absorption at 1030 cm^{-1}.

^{1}H nmr spectroscopy

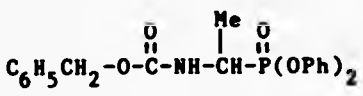
1-Aminoalkanephosphonic acids

The proton nmr spectra of 1-aminoalkanephosphonic acids have been reported[1,25,37,90-93]. The spectrum of 1-aminopropanephosphonic acid in D_2O shows δ 1.05 (3H, t, $C\underline{H}_3$, $^3J_{HCCH}$ 7.0 Hz), 1.45-2.15 (2H, m, $C\underline{H}_2$), 2.85-3.45 (1H, m, C$\underline{H}$). The multiplet due to $-CH_2$ has been shown to consist of around 20 separate peaks at 220 MHz[1] instead of the sixteen expected as a result of coupling to phosphorus, the -CH and $-CH_3$ protons (i.e. 2 x 2 x 4 = 16). Similarly the -CH signal consisted of 7 peaks whereas only 6 peaks would be expected due to splitting by phosphorus and the $-CH_2$ group.

The spectrum can be explained by the fact that the protons of the $-CH_2$ group are non-equivalent and as such give an AB coupling pattern. This non-equivalence is due to the methylene group being next to a chiral centre, although there is free rotation about the carbon-carbon bond.

Such non-equivalent protons are described as diastereotopic, since replacement of one of the two protons by a group X would produce a pair of diastereoisomers. This effect is absent in the spectrum of diphenyl N-carbobenzoxy 1-aminoethane-phosphonate (LVI) where the methyl group appears as a simple doublet of doublets due to coupling with the -CH proton and phosphorus, although slight shouldering is

evident.

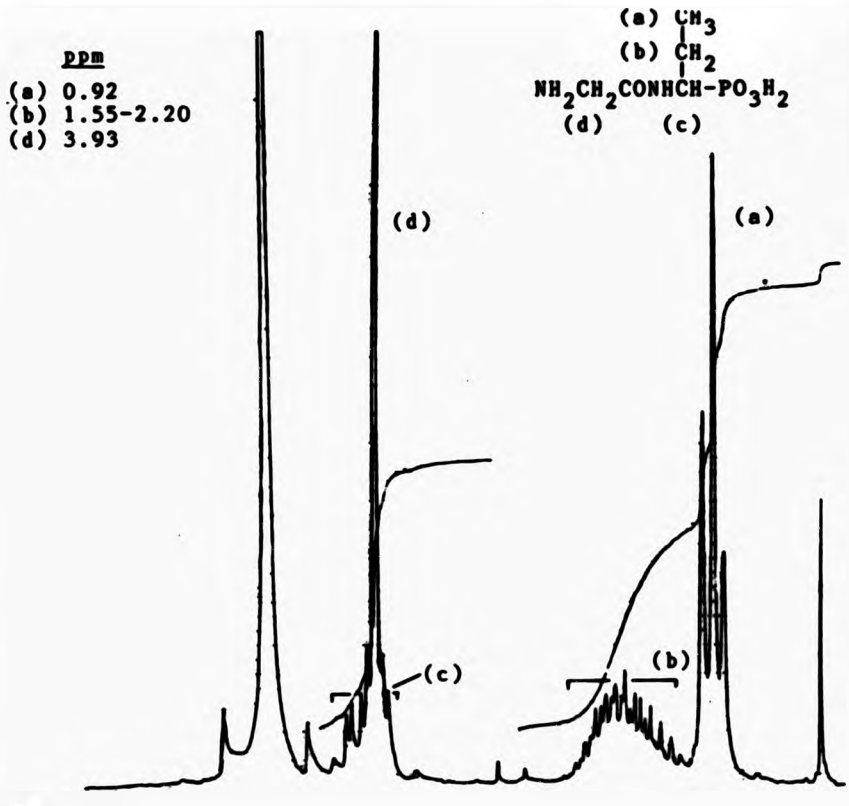


LVI

As the length of the alkyl chain increases, ^1H nmr becomes more second order. The $-CH_3$ triplet and -CH multiplet may still be observed, however, the $-CH_2$ protons of the alkyl chain overlap to produce a broad multiplet.

Phosphonopeptides

Analysis of the proton nmr spectra of phosphonopeptides is facilitated by a comparison of the spectra with those of the corresponding amino and aminophosphonic acids. The phosphonopeptide spectra are essentially a combination of these two. The $-CH_3$ triplet of the aminoalkane phosphonic acid is shifted slightly upfield in the peptide to ca 0.90 ppm. Methyl protons of alanine residues occur as doublets around 1.50 ppm. When more than one alanine residue is included in the phosphonopeptide, the $-CH_3$ doublets may overlap to form a pseudo triplet or a more complicated multiplet. Half of the $-CH_2$ multiplet of the 1-aminoalkanephosphonic acid is hidden beneath the alanine methyl signals, however, when ^1H nmr spectra of peptides containing other amino acids (eg. glycine) are considered, the $-CH_2$ multiplet can be seen to consist of around 20 peaks as mentioned above.



^{1}H nmr spectrum of (1R,S)-1-(glycylamino)propanephosphonic acid (80 MHz)

Spectrum 1

The -CH multiplets of the 1-aminoalkanephosphonic acid and the amino acid residues overlap to give a broad, complicated multiplet at ca 3.60 - 4.50 ppm. In glycine-containing peptides the broad singlet due to $-CH_2$ of glycine is superimposed upon this multiplet.

Proton nmr does not distinguish between phosphono-peptide diastereoisomers and, on the whole, is less useful in the characterization of phosphonopeptides than ^{13}C nmr.

Peptide	CH_3(P) δ/ppm	mult.	$^3J_{HCCH}$ /Hz	CH_3(ala) δ/ppm	mult.	$^3J_{HCCH}$ /Hz	CH_2(P) δ/ppm	CH(P + ala) δ/ppm
L-ala(P)	0.92	t	7.06	1.56	d	7.30	1.70-2.16	3.66-4.22
L-ala(R)(P)	0.92	t	7.32	1.56	d	6.84	1.63-2.25	3.72-4.27
D-ala(P)	0.92	t	7.08	1.56	d	6.84	1.88-2.00	3.62-4.25
$(DL\text{-ala})_2$-(P)	0.90	t	6.84	1.40-1.60	't'	-	1.75-2.25	3.61-4.46
$(L\text{-ala})_3$-(P)	0.63	t	6.80	1.13-1.34	m	-	1.38-3.50	3.78-4.25
$(L\text{-ala})_4$-(P)	0.90	t	6.84	1.42,1.55	d,d	6.84	1.75-2.25	3.50-4.50

P = CH_3CH_2-CH(NH)PO_3H_2 ala = CH_3-CH(NH)-CO

d = doublet, t = triplet, m = multiplet

^1H nmr of phosphonopeptides in D_2O

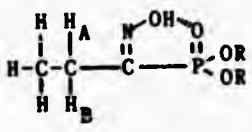
Table 2

Dialkyl esters of substituted alkanephosphonic acids

The diethyl 1-oxopropanephosphonate exhibits two triplets - one centered at 1.04 ppm due to the $-CH_3$ of the alkyl chain, the other at 1.39 ppm due to the $-CH_3$ of the ethyl ester. The $-CH_2$ adjacent to the carbonyl group is shown as a quartet due to coupling with $-CH_3$, and is sometimes seen to be split into a doublet of quartets due to HCCP coupling with phosphorus. The $-CH_2$ of the ethyl ester group is observed as an imperfect quintet, presumably due to overlapping H-H and P-H splitting patterns.

The dimethyl 1-oxopropanephosphonate differs from the ethyl ester only in the position of the ester protons. The methyl ester protons appear as a doublet at 3.89 ppm due to coupling with phosphorus.

The spectra of the 1-oxoalkanephosphonate oximes differ little from those of the 1-oxoalkanephosphonates. The $-CH_2$ of the alkyl chain, however, appears as a multiplet with 6 peaks - this may be due to overlapping quartets arising from non-equivalent methylene protons[30]. This non-equivalence could be a result of hydrogen bonding as illustrated below:



This theory assumes $J_{AB} \triangleq 0$.

The diethyl 1-aminopropanephosphonate has multiplets similar to those observed in the 1-aminopropanephosphonic

acid spectrum. Half of the complex multiplet due to $-CH_2$ of the alkyl chain is hidden beneath the methyl triplet of the ethyl ester. The methine proton multiplet shows 8 peaks which are the result of coupling with the diastereotopic protons of the $-CH_2$ group as well as coupling to phosphorus.

Compound	Ester Protons							Alkyl chain Protons							
	δ-CH_3 /ppm	m	$^3J_{HCCH}$ /Hz	$^3J_{POCH}$ /Hz	δ-CH_2 /ppm	m	$^3J_{HCCH}$ /Hz	δ-CH_3 /ppm	m	$^3J_{HCCH}$ /Hz	δ-CH_2 /ppm	m	J/Hz	δ-CH /ppm	δ-OH /ppm
$EtCP(OMe)_2$	3.89	d	-	10.74	-	-	-	1.12	t	7.10	2.88	q	7.10	-	-
$EtCP(OEt)_2$	1.39	t	7.50	-	4.16	qt	7.00	1.04	t	7.10	2.88	q	7.08	-	-
$EtC(=N-OH)-P(OMe)_2$	3.80	d	-	11.23	-	-	-	1.14	t	7.32	2.54	h	7.32	-	10.95
$EtC(=N-OH)-P(OEt)_2$	1.34	t	7.08	-	4.17	qt	7.57	1.15	t	7.81	2.54	h	7.08	-	10.87
$EtCH(NH_2)-P(OEt)_2$	1.34	t	7.04	-	4.15	qt	7.04	1.06	t	7.19	1.63-2.25	m	-	2.72-3.03	-

m = multiplicity; d = doublet, t = triplet, q = quartet, qt = quintet, h = hextet, m = multiplet

^{1}H nmr of dialkyl esters of substituted alkanephosphonic acids in $CDCl_3$

Table 3

^{13}C nmr spectroscopy

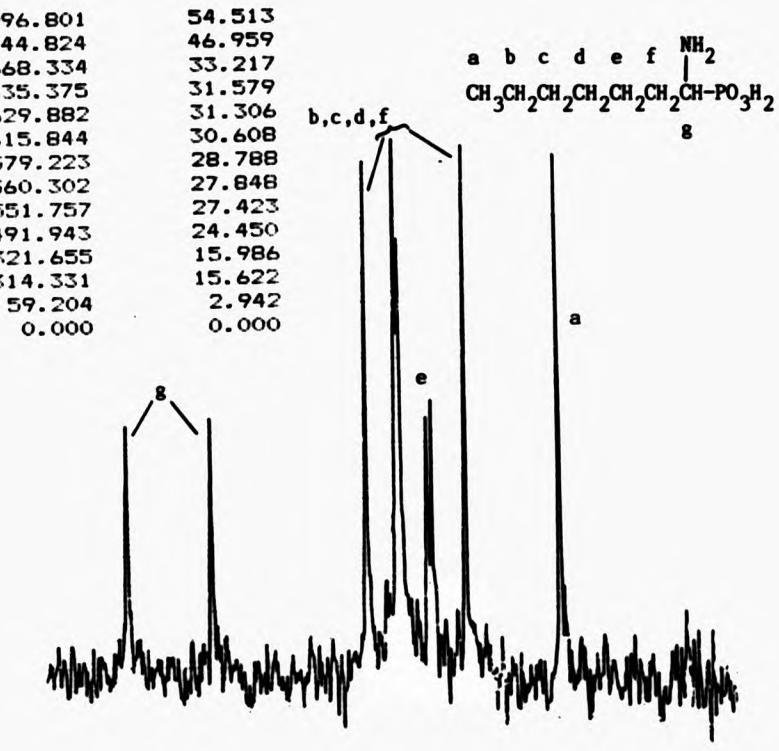
The ^{13}C nmr spectra of phosphonic acid derivatives are made more interesting by the presence of P-C coupling. This facilitates the identification of spectral peaks and gives added information about the compound being characterised. Parallel to H-H and C-H interactions, P-C coupling constants are dependent upon the configuration of the bonds within the coupling path and also the presence of electronegative substituents.

1-Aminoalkanephosphonic acids

The ^{13}C nmr chemical shifts and coupling constants for 1-aminoalkanephosphonic acids are shown in tables 4 and 5. Signals for the methyl carbons usually occur around 16 ppm. These are either seen as a doublet or singlet depending on their distance from phosphorus. The same applies to the signals for the methylene carbons which occur from 22-34 ppm. Unlike ^{1}H nmr, where in longer chain 1-aminoalkanephosphonic acids the methylene proton signals overlap to form broad multiplets, ^{13}C nmr can be used to identify each $-CH_2$ group in the alkyl chain (see spectrum 2). The CH-P methine carbon signals are always seen as a distinct doublet with $^{1}J_{PC}$ around 150 ppm.

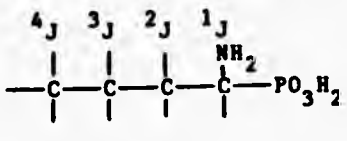
In general, the coupling constants along the alkyl chain vary as follows:

FREQUENCY	PPM
1096.801	54.513
944.824	46.959
668.334	33.217
635.375	31.579
629.882	31.306
615.844	30.608
579.223	28.788
560.302	27.848
551.757	27.423
491.943	24.450
321.655	15.986
314.331	15.622
59.204	2.942
0.000	0.000



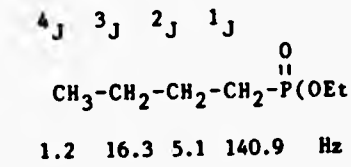
^{13}C nmr of 1-aminoheptanephosphonic acid at 80 MHz

Spectrum 2



0 9 0 150 Hz

This can be compared with an unsubstituted alkyl chain[94]:



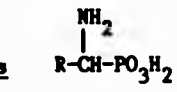
1.2 16.3 5.1 140.9 Hz

This illustrates that $^{1}J_{PC}$ increases with the growing number of electronegative substituents attached to phosphorus or C-1. It also shows that electronegative substituents within the coupling path decrease ^{2}J, ^{3}J and ^{4}J.

The phosphorus-carbon coupling constants for 1-aminoalkanephosphonic acids are also dependent upon the acidity of the nmr sample solution as can be seen from Table 5. The addition of D_2SO_4 significantly increases the value of $^{1}J_{PC}$ to that obtained in aqueous solution. This effect has also been observed for ω-guanidinoalkanephosphonic acids[1] and can be explained by considering the zwitterionic nature of these types of compound.

The structure of the 1-aminoalkanephosphonic acids in aqueous and acidic solutions would be expected to be

^{13}C nmr chemical shifts for 1-aminoalkanephosphonic acids

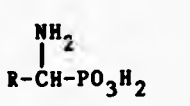


R =	Methyl $-\underline{C}H_3$		Methylene $-\underline{C}H_2$		Methine $-\underline{C}H$		Solvent
	δ/ppm	m	δ/ppm	m	δ/ppm	m	
C_2H_5	12.6	d	24.0	s	52.6	d	D_2O/H_2SO_4
C_3H_7	15.7	s	21.6	d	51.4	d	D_2O
			33.3				
C_4H_9	15.8	s	24.5	s	51.1	d	D_2O
			30.6	d			
			30.9	s			
C_5H_{11}	13.5	s	21.7	s	48.5	d	D_2O/D_2SO_4
			24.8	d			
			27.7	s			
			30.6	s			
C_6H_{13}	16.0	s	24.5	s	50.7	d	D_2O/D_2SO_4
			27.6	d			
			30.3	s			
			30.6	s			
			33.2	s			
C_7H_{15}	14.1	s	22.5	s	49.2	d	D_2O/H_2SO_4
			25.5	d			
			28.6	s			
			28.9	s			
			29.3	s			
			31.6	s			

m = multiplicity

Table 4

^{13}C nmr coupling constants for 1-aminoalkanephosphonic acids

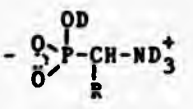


R =	coupling constant J/ Hz			Solvent
	$^{1}J_{PC}$	$^{2}J_{PCC}$	$^{3}J_{PCCC}$	
C_2H_5	154.4	0	9.0	D_2O/H_2SO_4
C_3H_7	150.8	0	9.8	D_2O
C_4H_9	145.9	0	9.0	D_2O
C_5H_{11}	152.6	0	8.6	D_2O/D_2SO_4
C_6H_{13}	152.0	0	8.6	D_2O/D_2SO_4
C_7H_{15}	153.2	0	9.0	D_2O/H_2SO_4

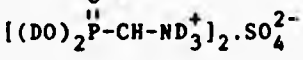
Table 5

as follows:-

in aqueous solution



in acid solution



Thus, in acid solution, the phosphorus atom is de-shielded. This provides an explanation why ^{31}P chemical shifts are extremely sensitive to pH. We would expect the phosphorus atom to affect its neighbouring groups in different ways in the above two forms and, hence, the P-C coupling constants would be alterred. We could predict that a change in the chemical environment at phosphorus would have a greater effect on $^{1}J_{PC}$ coupling constants than $^{3}J_{PCCC}$ coupling constants and this, in fact, is what is observed.

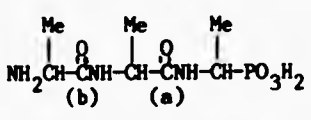
Phosphonopeptides

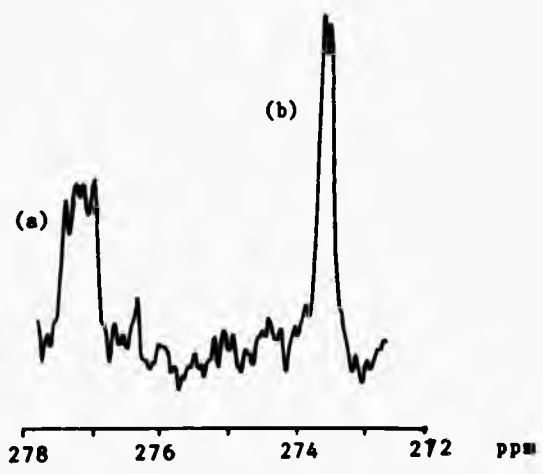
^{13}C nmr chemical shifts and coupling constants have been reported for aminoalkanephosphonic acids[1,93], but no references have been found with respect to ^{13}C nmr of phosphonopeptides. However, these spectra can be interpreted by comparing them with those of amino and aminophosphonic acids.

The chemical shifts of the aminophosphonic acid

substituents are shifted slightly downfield in the phosphonopeptides, also, $^{1}J_{PC}$ is reduced.

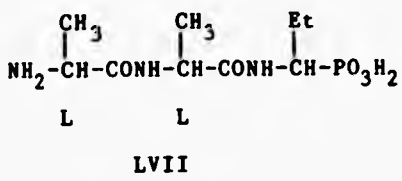
When ^{13}C nmr spectra of diastereomeric mixtures are obtained, the phosphonopeptide spectra may be complicated by overlapping signals due to the different diastereoisomers. This is especially true for alanine methyl and methine carbons, and amide carbonyl carbon signals. Carbonyl carbons often appear as broad singlets or multiplets (see below).





^{13}C nmr of (1R,S)-1-(L-alanyl-L-alanylamino)propane phosphonic acid

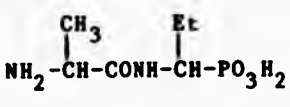
On resolution the phosphonopeptides give much simpler spectra. For example, if we consider the spectra of the resolved and unresolved tripeptide L-alanyl-L-alanylamino-propanephosphonic acid (LVII), we can see that combination of the two resolved peptide spectra gives that of the unresolved peptide.



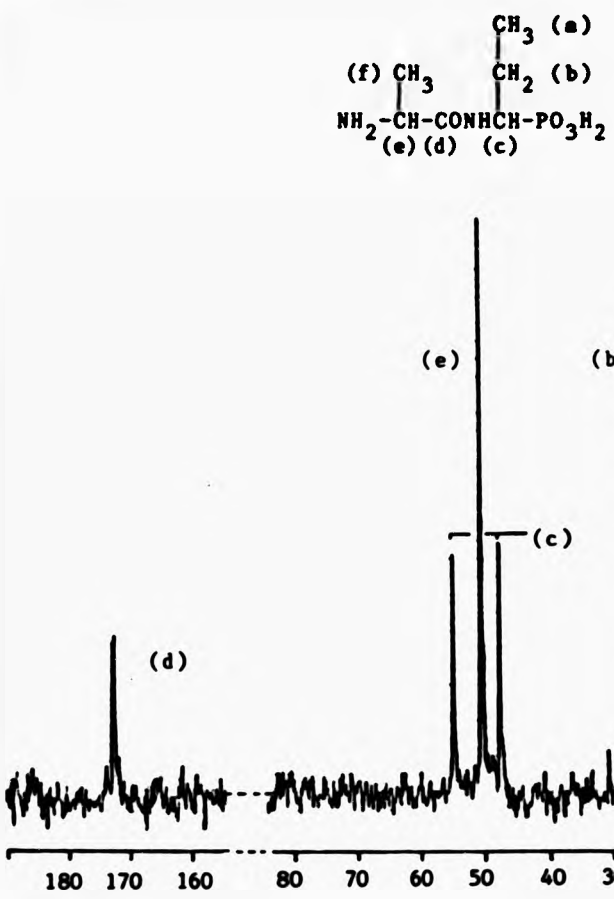
LVII

Peptide	Chemical shift, δ, in ppm	
	-CH (alanine)	-C=O
L-ala-L-ala-(R,S)(P)	51.8, 52.8, 53.1	173.4, 173.5, 177.2, 177.4
L-ala-L-ala-(S)(P)	51.8 53.1	173.6 177.4
L-ala-L-ala-(R)(P)	51.8, 52.8	173.5 177.3

The same phenomenon is observed for the methine carbons of the dipeptide (LVIII).



LVIII



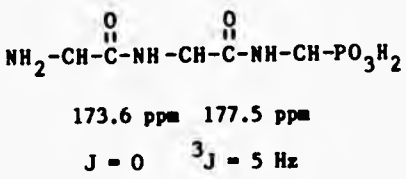
^{13}C nmr spectrum of (1S)-1-(L-alanylamino)propanephosphonic acid in D_2O (20.12 MHz)

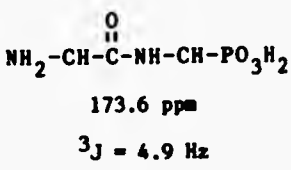
Spectrum 3

Peptide	Chemical shift, δ, in ppm -CH (alanine)
L-ala-(R,S)(P)	52.2, 52.5
L-ala-(S)(P)	52.5
L-ala-(R)(P)	52.2

A comparison of the above sets of data indicates that signals due to diastereoisomers with S stereochemistry at the phosphorus moiety occur slightly downfield to those with R stereochemistry, though this may be a coincidence.

The carbonyl carbon signals appear reduced in size, as expected, due to the absence of the nuclear overhauser effect. They appear in two distinct regions - 173 and 177 ppm.





^{13}C nmr chemical shift data for phosphonopeptides

Peptide	δ / ppm			
	Methyl $-\underline{C}H_3$	Methylene $-CH_2$	Methine $-\underline{C}H$	Carbonyl $-C(O)-$
L-ala-P	13.3(d), 19.7	25.7	52.2, 52.5, 53.4	173.6
D-ala-P	13.4(d), 19.7	25.8	52.3, 52.6	173.6
DL-ala-P	13.3(d), 19.7	25.7	52.2, 52.5, 53.3(d)	173.6
gly-P	13.4(d)	25.8, 43.6	53.4(d)	169.8
DL-but-P	11.4, 13.6(d)	25.7, 27.2	53.5(d), 57.9	172.8
$(L\text{-ala})_2$-P	13.3(d), 19.4, 19.8	26.0	51.8, 52.8, 53.0(d), 53.1	173.4,173.5, 177.2,177.4
L-ala-D-ala-P	13.3(d), 19.4, 19.9	26.0	52.1, 53.0, 53.1(d)	173.7, 177.3
$(DL\text{-ala})_2$-P	13.3(d), 19.4, 19.7, 19.9	26.0	51.9, 52.0, 52.5, 52.9, 53.0(d), 53.1	173.6, 177.4
gly-L-ala-P	13.3(d), 20.0	26.0, 49.6	52.9, 53.2(d)	169.9, 177.5
$(L\text{-ala})_3$-P	13.4(d), 19.5	25.9	51.9, 52.7, 53.0, 53.0(d)	177.4
D-ala-$(L\text{-ala})_2$-P	13.3(d), 19.1, 19.7, 20.0	25.8	52.2, 52.5, 53.4(d)	173.6, 178.8
$(L\text{-ala})_4$-P	13.3, 19.4, 19.7, 19.8	25.9	51.9, 52.5, 52.8, 53.0(d)	173.6, 177.4

all spectra run in D_2O

ala = $-HN-CH(CH_3)CO_2-$

gly = $-HN-CH_2CO_2-$

but = $-HN-CH(Et)CO_2-$

P = $-HN-CH(Et)PO_3H_2$

Table 6

P-C coupling constants for phosphonopeptides

Peptide	Coupling Constant / Hz		
	$^{1}J_{PC}$	$^{3}J_{PCCC}$	$^{3}J_{PCNC}$
L-ala-P	147.7	13.4	
D-ala-P	147.7	13.4	
DL-ala-P	147.7	13.4	
L-ala-(S)P	147.7	13.4	4.9
L-ala-(R)P	148.9	13.4	4.3
L-ala-(hex)P	147.7	12.2	6.0
gly-P	147.7	13.4	
DL-but-P	147.7	13.4	
$(L\text{-ala})_2$-P	147.7	13.4	5.0
$(L\text{-ala})_2$-(S)P	147.7	12.2	
$(L\text{-ala})_2$-(R)P	147.7	13.4	
L-ala-D-ala-P	148.3	13.4	6.1
gly-L-ala-P	144.7	12.8	
$(L\text{-ala})_3$-P	146.5	12.8	
D-ala-$(L\text{-ala})_2$-P	148.9	14.0	
$(L\text{-ala})_4$-P	147.7	13.4	

(hex)P = 1-aminohexanephosphonic acid

Table 7

$^{3}J_{PCNC}$ is of the expected value (ca. 5 Hz) for these compounds[1].

A consideration of the P-C coupling constants for phosphonopeptides and 1-aminoalkanephosphonic acids leads to the following conclusions:-

i) $^{1}J_{PC}$ has the largest value;

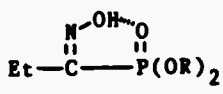
ii) $^{3}J_{PCCC}$ is greater than $^{2}J_{PCC}$;

iii) $^{2}J_{PCC}$ is usually too small to detect and is essentially zero;

iv) $^{3}J_{PCNC}$ has a value of ca. 5 Hz.

Dialkyl esters of substituted alkanephosphonic acids

The ^{13}C nmr chemical shift data for a variety of alkanephosphonic acid dialkyl esters is included in table 8. The chemical shifts of the carbonyl and oxime carbons are of the same order as reported values for these functional groups[95]. The fact that only one oxime doublet is observed lends weight to the theory of a hydrogen-bonded structure as predicted by infrared spectra. Syn- and anti- oxime isomers show separate chemical shifts in proton and ^{13}C nmr. Both ^{1}H and ^{13}C nmr indicate that only one isomer is present.



As with the aminoalkanephosphonic acids, it can be seen that $^{1}J_{PC}$ is dependent upon the electronegativity of the groups attached to C-1.

	$^{1}J_{PC}$	$^{2}J_{PCC}$	$^{3}J_{PCCC}$
$Et-\overset{O}{\overset{\|}{C}}-P(OEt)_2$	166.6	56.2	3.7
$Et-\overset{N-OH}{\overset{\|}{C}}-\overset{O}{\overset{\|}{P}}(OEt)_2$	212.4	16.5	0.0

The more electron-withdrawing groups cause an increase in $^{1}J_{PC}$. As mentioned earlier, increasing the electonegativity of substituents within the coupling path reduces both $^{2}J_{PCC}$ and $^{3}J_{PCCC}$. It should be noted, however, that in the case of the ketophosphonates and oximes, $^{2}J_{PCC}$ is much larger than $^{3}J_{PCCC}$, whereas in saturated systems such as $EtCH(NH_2)P(O)(OEt)_2$ the situation is reversed, $^{3}J_{PCCC}$ becoming much larger than $^{2}J_{PCCC}$.

The following conclusions can be made regarding the ketophosphonates and their oximes:-

i) $^{1}J_{PC}$ has the largest value and is dependent upon the electronegativity of the groups attached to C-1;

ii) $^{2}J_{POC}$ is greater than $^{3}J_{POCC}$ and has a value of <u>ca.</u> 6.5 Hz;

iii) $^{3}J_{POCC}$ has a value of <u>ca.</u> 6.0 Hz;

iv) $^{2}J_{PCC}$ is greater than $^{3}J_{PCCC}$ and both are dependent upon the electronegativity of substituents within the coupling path.

^{13}C nmr chemical shift data for dialkyl esters of substituted alkanephosphonic acids

Compound	δ /ppm in $CDCl_3$						
	Ester carbons		Alkyl chain carbons				
	$-CH_3$	$-CH_2$	$-CH_3$	$-CH_2$	C=O	C=NOH	-CH
$EtC(O)P(OMe)_2$	54.0	-	6.4	37.2	211.0	-	-
$EtC(O)P(OEt)_2$	16.4	63.8	6.4	36.9	211.5	-	-
$EtC(NOH)P(OMe)_2$	53.5	-	10.2	19.9	-	154.7	-
$EtC(NOH)P(OEt)_2$	16.3	63.0	10.3	20.0	-	155.3	-
$EtCH(NH_2)P(OEt)_2$	16.6	62.1	10.9	24.7	-	-	50.5

Table 8

P-C coupling constants for dialkyl esters of substituted alkanephosphonic acids

Compound	Coupling constant / Hz				
	Ester carbons		Alkyl chain carbons		
	$^2J_{POC}$	$^3J_{POCC}$	$^3J_{PCCC}$	$^2J_{PCC}$	$^1J_{PC}$
$EtC(O)P(O)(OMe)_2$	7.3	-	4.3	56.8	166.0
$EtC(O)P(O)(OEt)_2$	7.3	5.5	3.7	56.2	166.6
$EtC(:NOH)P(OMe)_2$	6.1	-	0	16.5	213.0
$EtC(:NOH)P(OEt)_2$	6.1	6.1	0	16.5	212.4
$EtCH(NH_2)P(OEt)_2$	7.3	5.5	12.8	0	148.9
$CH_3CH_2P(O)(OEt)_2$	6.9	6.2	-	7.1	143.0 *

*Lit.[96] value

Table 9

^{31}P nmr spectroscopy

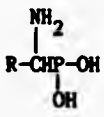
Phosphorus nmr has proved very useful in determining the purity of the compounds prepared. Proton decoupled ^{31}P nmr gives a single peak for each phosphorus containing compound present. This is most important in the case of phosphonopeptides where ^{31}P nmr can detect the presence of any free unreacted aminophosphonic acid.

Literature pertaining to phosphorus nmr of aminophosphonic acids is rare, however, Appleton *et al*[93] have made a study into the pH dependence of ^{31}P chemical shifts of these compounds. Changes in pH produce large differences in the observed chemical shift. Aminophosphonic acid and phosphonopeptide spectra are usually obtained in D_2O, however, less soluble compounds are dissolved in D_2O/D_2SO_4 when the above factor must be taken into consideration.

^{31}P Chemical shift data for the aminophosphonic acids and phosphonopeptides are listed in tables 10 and 11. All values obtained have been within the expected range for phosphonic acid derivatives (between 14 and 27 ppm downfield from 85% H_3PO_4). The only reference to ^{31}P nmr of phosphonopeptides found referred to those containing phosphonamide bonds - these had chemical shifts of 20-25 ppm.

Phosphorus-proton coupled spectra at 161.967 MHz of 1-aminopropanephosphonic acid (LVIX) and its L-alanine dipeptide (LVIII) both appear as sextets with an approximately equal spacing of 12 and 7.5 Hz respectively. Assuming that $^3J_{PCCH}$ is less than $^4J_{PCCCH}$ (as is usually the case

^{31}P chemical shift data for 1-aminoalkanephosphonic acids



R =	δ /ppm	solvent
C_2H_5	19.5	D_2O
C_3H_7	22.6	D_2O
C_4H_9	13.8	D_2O
C_5H_{11}	17.4	D_2O/D_2SO_4
C_6H_{13}	14.4	D_2O/D_2SO_4
C_7H_{15}	18.2	D_2O/H_2SO_4

Table 10

^{31}P chemical shift data for phosphonopeptides

Compound	δ /ppm
L-ala(RS)(P)	18.2
L-ala(S)(P)	18.0
D-ala(RS)(P)	18.5
gly(RS)(P)	18.3
L-ala-L-ala(S)(P)	18.3
L-ala-L-ala(R)(P)	18.3
L-ala(RS)(P')	18.2

all spectra run in D_2O

ala = alanine

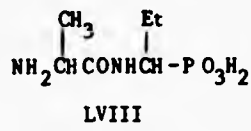
gly = glycine

P = 1-aminopropanephosphonic acid

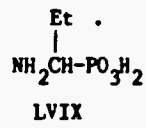
P' = 1-aminohexanephosphonic acid

Table 11

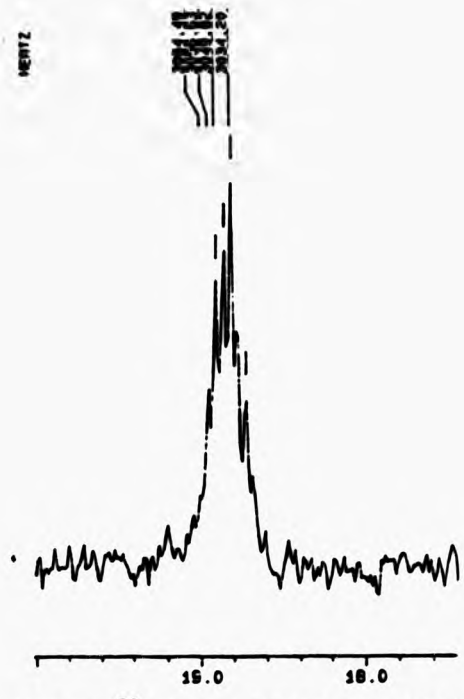
for this class of compound) and $^{4}J_{PCCCH}$ is approximately half the value of $^{2}J_{PCH}$ (reported value 7 - 30 Hz)[96] the sextets may be a result of overlapping quartets due to splitting by the CH and CH_3 protons.



LVIII

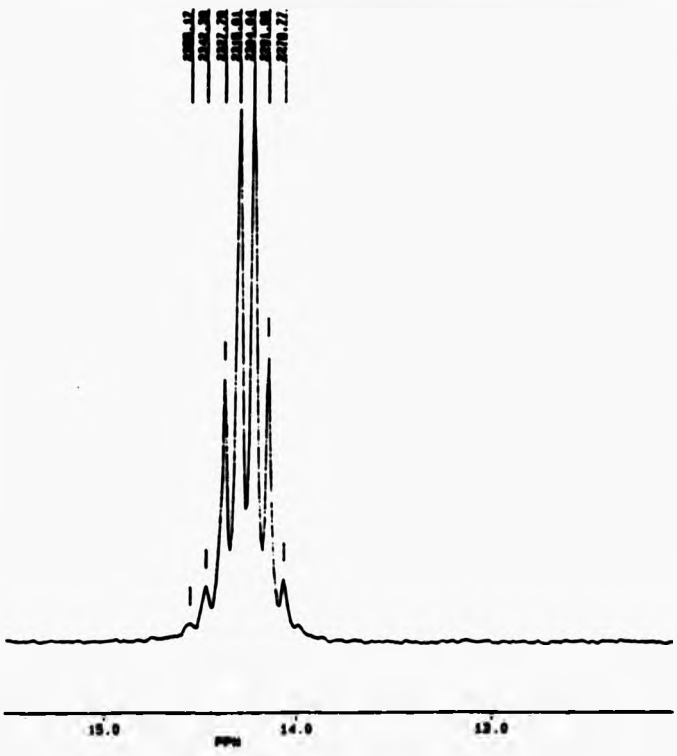


LVIX



P-H coupled ^{31}P nmr of LVIII at 161.967 MHz

Spectrum 4

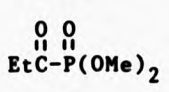
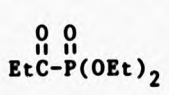
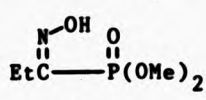
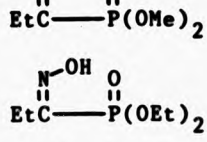
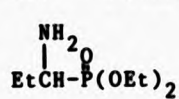


P-H coupled ^{31}P nmr of LVIX at 161.967 MHz

Spectrum 5

^{31}P chemical shifts for dimethyl and diethyl 1-oxoalkanephosphonates have been reported to occur between 0 to -2.0 ppm[97] and 0 to -3.0 ppm[98] respectively. Observed chemical shifts for dialkyl alkanephosphonates are reported in table 12.

^{31}P chemical shift data for substituted dialkyl alkanephosphonates

compound	δ/ppm
	-1.1
	-2.9
	13.6
	11.0
	29.1

all spectra run in $CDCl_3$

Table 12

Fast atom bombardment mass spectrometry of derivatives of 1-aminoalkanephosphonic acids

Fast atom bombardment (FAB) ionisation, first introduced in 1981, can provide stable, relatively long-lived intense signals that are representative of the molecular weights of the species being investigated.

When a chemical is exposed to a beam of relatively high energy neutrals (the fast atom), ions from that chemical may be observed and if the chemical is introduced in a solvent (eg. glycerol) the observed signal can be maintained for extended periods.

The real advantages of FAB follow from the fact that it yields ions from involatile species without the need to supply heat to the sample. Also the ionisation process is one in which rapid absorption of energy occurs so that translational energy is transferred before enough vibrational energy can be taken up to cause decomposition.

Three types of ionisation processes are observed in FAB. The first process results in the formation of ion radical species $M^{\dagger}$. It is generally observed in the case of non-polar compounds and is assumed to arise from ion molecule interaction from solvent ion radicals.

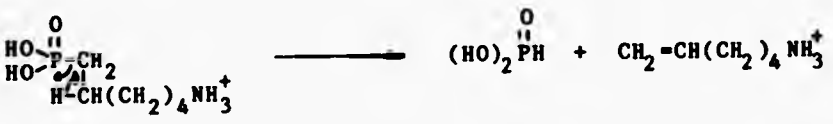
The second process involves the formation of protonated species to yield (MH^{+}). This process is the most common in organic FAB. The third process is one in which preformed ions are directly ejected from solution. The favoured ionisation process not only depends on the nature of the compound under investigation but also on

the solvent from which it is run.

Many solvents have been employed as FAB matrices. In principle the choice of solvent should be made such that its 'polarity' approximates to that of the sample under investigation.

The ionic and zwitterionic nature of many organophosphorus compounds makes them unsuitable for mass spectrometric analysis using conventional ionisation techniques. Under FAB ionisation, however, they give intense ions at M+H (normally the base peak) in the positive ion spectrum and undergo characteristic fragmentation.

The FAB spectra of α- and ω-aminophosphonic acids have been reported previously[99]. They give strong (MH^+) ions and show prominent fragments arising from cleavage of the P-C bond, with proton transfer, to yield the ions ($MH^+ - H_3PO_3$) (scheme 3) and ($MH^+ - HPO_3$) (scheme 4).



Scheme 3

R = H, CH_3 or $n\text{-}C_7H_{15}$

Scheme 4

The FAB spectra of the phosphonopeptides prepared were obtained in glycerol and also show intense (MH^+) ions which are often the base peak. Exact mass measurements of the (MH^+) ions were used to characterise the peptides, especially in the cases where microanalysis had proved unsatisfactory.

FAB Exact mass measurements for phosphonopeptides

Peptide	(MH^+) C	H	N	O	P	δ / mmu	Observed mass
gly-P	5	14	2	4	1	-0.4	197.0695000
DL-ala-P	6	16	2	4	1	-0.7	211.0855000
L-ala-P'	9	22	2	4	1	0.2	253.1315000
L-ala-gly-P	8	18	3	5	1	-0.5	268.1067000
L-ala-D-ala-P	9	21	3	5	1	0.3	282.1215600
(L-ala) -P	12	26	4	6	1	-0.8	353.1598100
(L-ala) -P	15	31	7	5	1	-1.0	424.1971000

δ = difference between calculated mass and observed mass

P = $-NH-CH(Et)PO_3H_2$

P' = $-NH-CH(n-C_5H_{11})PO_3H_2$

Table 13

Prominent ($2MH^+$) ions and other adducts, for example those with glycerol ($MH^+ + G$) are also observed in the phosphonopeptide FAB spectra. Major fragments in the spectra arise from the loss of the α-lactam $R\overline{CHNHCO}$ and of phosphorous acid (H_3PO_3), both *via* proton transfer rearrangement processes. In the polypeptides the sequential loss of amino acid units provides structural information, and is analogous to the widely reported fragmentation of non-phosphorus containing peptides[100-102].

Relative abundances of prominent fragments in phosphonopeptide spectra

Peptide	mwt	X	Relative Abundance / %						
			(MH^+)	(MH^+-X)	(MH^+-2X)	$(MH^+-H_3PO_3)$	(MH^+G)	$(2MH^+)$	$(2MH^+-X)$
gly-P	198	CH_2CO (NH)	100	23	-	17	12	16	4
L-ala-P	210	CH_3CHCO (NH)	100	20	-	13	4	19	5
$(L\text{-ala})_2$-P	281	CH_3CHCO (NH)	100	21	11	5	4	-	-
$(L\text{-ala})_3$-P	352	CH_3CHCO (NH)	100	17	36	17	-	5	-

Table 14

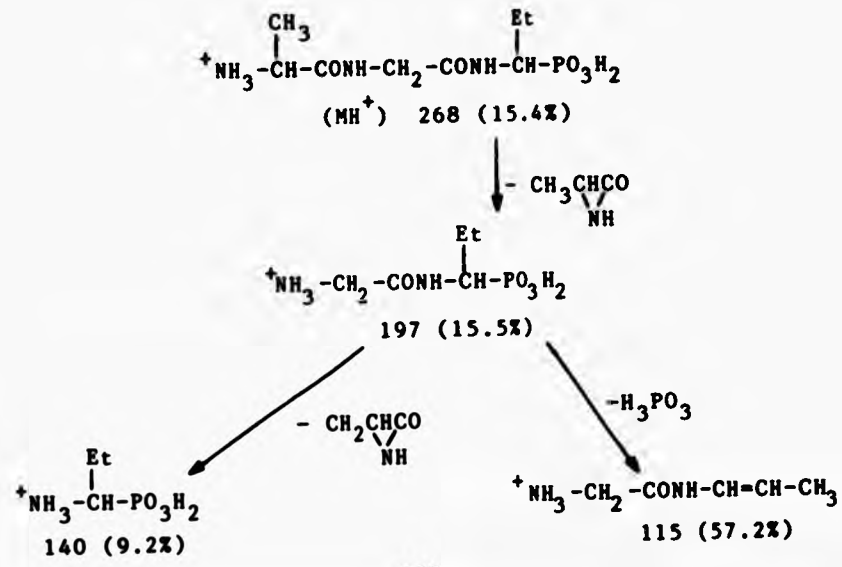
Fragmentation patterns were confirmed by exact mass measurement and linked scan techniques.

FAB Exact mass measurements for phosphonopeptide fragments

Parent Peptide	Fragment m/z					
	353	282	211	143	140	115
gly-P					$C_3H_{11}NO_3P$	$C_5H_{11}N_2O$
L-ala-gly-P						$C_5H_{11}N_2O$
L-ala-D-ala-P			$C_6H_{16}N_2O_4P$		$C_3H_{11}NO_3P$	
$(L\text{-ala})_3$-P		$C_9H_{21}N_3O_5P$	$C_6H_{16}N_2O_4P$	$C_6H_{11}N_2O_2$		$C_5H_{11}N_2O$
$(L\text{-ala})_4$-P	$C_{12}H_{26}N_4O_6P$					

Table 15

The exact mass measurements for the L-ala-gly-P and gly-P peptide fragments show clearly the loss of the α-lactam units and loss of H_3PO_3:



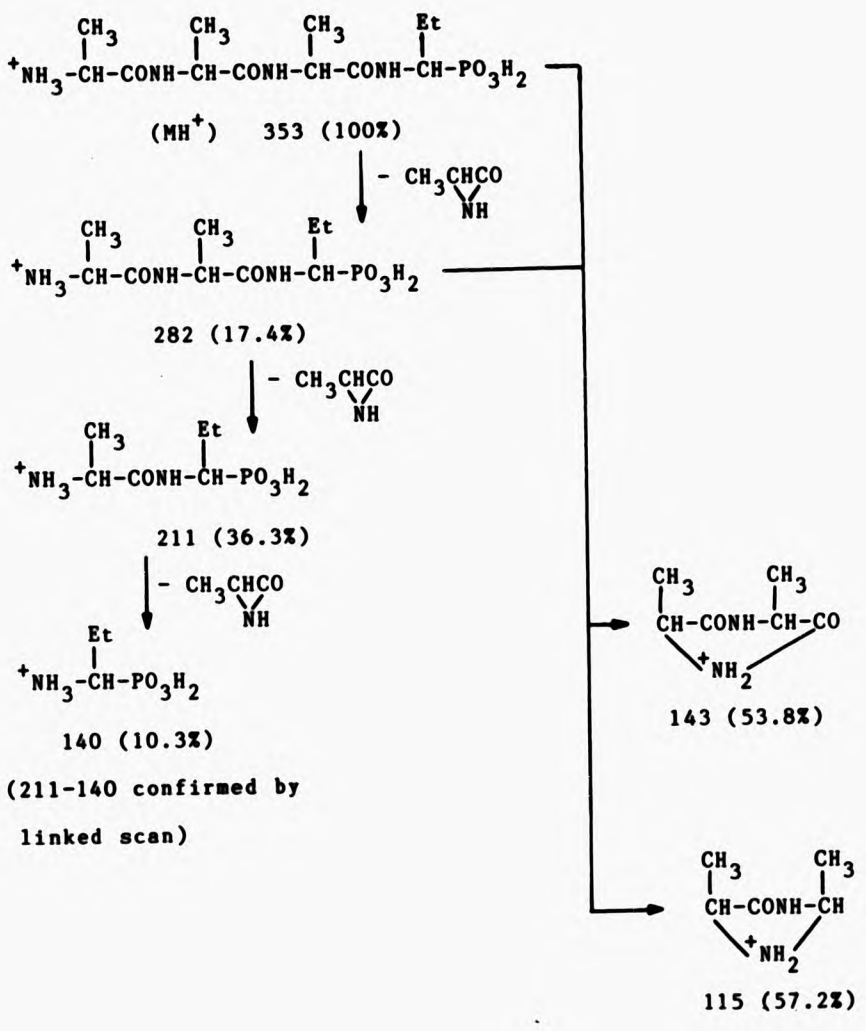
However, the structure of the aminophosphonic acid unit was not confirmed by exact mass measurements. The calculated mass of the fragment had a difference of 27.4 mmu from the observed mass of 140.0479000 mu. This can can be attributed to the unfortunate inaccuracy of the method of exact mass determination using FAB. Unlike electron impact mass measurements, the FAB exact mass is calculated by using reference compounds (for example CsI, RbI). The measured mass is expressed as a ratio of the reference mass, eg.

Reference mass	Found ratio	Measured mass
132.90543	1.053741	140.0479000

The same problem was observed when examining the pentapeptide fragment at 353 mu, supposedly formed by the loss of an α-lactam unit from (MH^+). The $C_{12}H_{26}N_4O_6P$ fragment had a calculated mass which differed from the observed mass of 353.1681000 by 9.1 mmu. However, there is no other possible structure that could give a mass of this value.

Some other fragments have been identified by exact mass measurements of the tetrapeptide spectrum. The usual losses of the α-lactam units were observed as well as fragments due to non-phosphorus containing dipeptides. Obviously, the fragment at 115 cannot be formed in the same way as the 115 fragment obtained with the glycine peptides. Here it corresponds to a peptide segment of

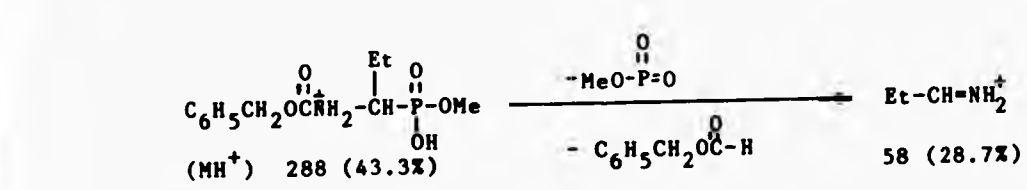
two alanine units:



FAB mass spectrometry has also been useful in characterising other non-volatile aminophosphonic acid derivatives. In the case of N-carbobenzoxy 1-aminopropane-phosphonic acid the positive ion spectrum shows the

presence of Na^+ - probably due to the sodium hydroxide used in its preparation. The ($M + Na^+$) ion is observed as well as the (MH^+) ion. The base peak is the stable tropylium ion ($C_7H_7^+$).

The N-carbobenzoxy-1-aminopropanephosphonic acid monomethyl ester exhibits a ($2MH^+$) ion and also a fragment due to the loss of $MeO\text{-}P(O)_2$ and the carbobenzoxy group.



Chapter 3

Fungicidal Screening

Results and Discussion

Fungicidal screening

In vivo screens for fungicidal activity were carried out for a number of compounds. The peptides showed the most promising antifungal activity, and within this class it appeared that stereochemistry played an important part in structure-activity relationships.

Seed treatment tests

Seeds infected with *Drechslera teres*, *Drechslera graminae* and *Drechslera avenae* were used in preliminary seed treatment experiments. The seeds were treated (10 min. in a laboratory seed treatment machine) with formulations (usually aqueous solutions) containing 20% (w/w or w/v) of test compound. The dosage rate was 2 g of solution per kilo of seed. Two hundred seeds of each treatment were placed on a moistened filter paper and incubated at 10 °C (3 days) and then at 20 °C (4 days). The coleoptile and roots of the seeds were then examined for disease symptoms and compared with untreated (control) seeds. The results for seed treatment tests are reported as percentage inhibition of disease.

Greenhouse tests

A number of peptides were evaluated in greenhouse tests using plants infected with various fungal diseases. In these evaluations the test compounds (as aqueous solutions) were applied as foliar sprays or soil drenches and their systemic activity was measured in a separate test. These tests took place over a longer time span than the seed treatment tests and the greenhouse tests are to be considered more accurate, giving a greater indication of the actual activity the test compounds may show in the field.

Greenhouse test results are given on a scale of 0 - 2 where:

0 = 0 - 49% control

1 = 50 - 74% control

2 = 75 - 100% control

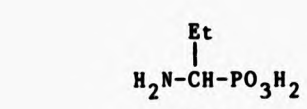
The following abbreviations are used in the tables:

D.t	Drechslera teres
D.g	Drechslera graminae
D.a	Drechslera avenae
P.u.	Pythium ultimum
R.s	Rhizoctonia solani
F.c	Fusarium culmorum
P.	Pyricularia oryzae
P.r	Puccinia recondita

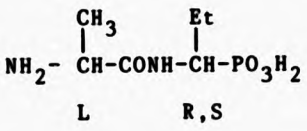
E.g Erysiphe graminis

S.n Septoria nodorum

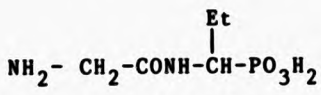
The following compounds were tested:



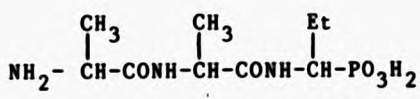
		Compound
R,S		A



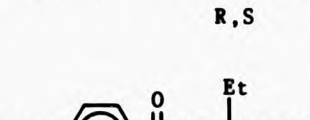
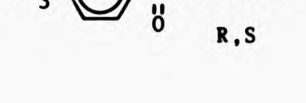
L	R,S	B
L	R	C
L	S	D
D	R,S	E
D,L	R,S	F



	R,S	G



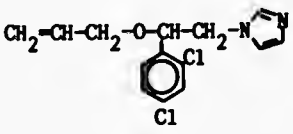
L	L	R,S	H
D	D	R,S	I

	Compound
 D,L R,S	J
 D,L R,S	K
R,S	L
R,S	M

Seed treatment tests

Compound	Configuration at			Test Organism Inhibition /%		
	P	ala I	ala II	D.t	D.g	D.a
A	R,S	-	-	98	72	84
B	R,S	L	-	98	61	93
C	R	L	-	100	84	91
D	S	L	-	88	28	84
E	R,S	D	-	7	0	0
F	R,S	D,L	-	92	75	82
G	R,S	-	-	80	20	4
H	R,S	L	L	100	-	98*
L	R,S	-	-	4	-	-
M	R,S	-	-	7	-	-
Imazalil**				41	-	87

* concentration of test compound very low

** commercial fungicide 

Results of preliminary seed treatment tests

Greenhouse tests

Compound	Foliar, 1000 ppm			Systemic Test 500 ppm	Soil Drench 500 ppm		
	P.r	S.n	P.o	E.g	F.c	R.s	P.u
B					2	2	
C	1						1
D	1	1			2		
E	1	1		1			
F	1				2	1	
G	1		1				
H	1	2			2*		
I	1						
J	1		1				
K	1		1				

*concentration of test compound less than 500 ppm

NB blank represents no test

Results of greenhouse tests

Preliminary seed treatment tests - structure activity relationships

The results of these tests were very encouraging. The most obvious conclusions to be made on examining the results were:-

i) the majority of the peptides had good antifungal activity compared with the fungicide Imazalil;

ii) the peptides and the free aminophosphonic acid were more active than the N-protected compounds;

iii) L-alanine residues appeared superior to D-alanine and glycine residues.

The poor results obtained with the N-linked compounds L and M show that N-protection alone does not increase the fungicidal activity of the aminophosphonic acid, A, but the results with the peptides show that the shape and size of the group attached to the nitrogen atom affects fungicidal activity.

Certain amino acid residues enhanced the activity of the free aminophosphonic acid, A, and others decreased its activity. The compound, E, with a D-alanine residue had a very low activity whereas, B, the same compound with an L-alanine residue had comparable activity to the free aminophosphonic acid. The incorporation of a glycine residue also decreased the activity of the free aminophosphonic acid.

From these results it appears that the configuration

of the peptide chain at the position of the first amino acid residue affects the activity of the molecule.

Further consideration of the phosphonopeptide stereochemistry with respect to the phosphonic acid moiety leads to the conclusion that this also affected fungicidal activity.

As expected, the diastereoisomers of the compound B had different activities. Compound C, the R isomer, had very good activity whereas D, the S isomer, had lower activity than the free acid. Compound C alone, therefore, was probably responsible for the good activity of compound B.

The tripeptide, H, was the most active compound of all. This prompted the synthesis of tripeptides with residues other than L-alanine and also the synthesis of tetra- and pentapeptides.

Greenhouse tests - conclusions

Comparisons between compounds are not possible on the basis of the available results as at the concentrations used the test compounds gave similar results. A further study of response to different dosages of the test compounds is necessary for a determination of structure-activity relationships.

As can be seen from the table, the majority of the peptides exhibited moderate control of P. recondita when applied as a foliar spray. Stereochemistry did not alter the compounds' activities in this particular test, nor did the nature of the amino acid residues (e.g. compound J contained a phenylalanine residue) have any significant effect.

The peptides performed well against F. culmorum when applied as a soil drench. Again stereochemistry was of limited importance - the various alanine dipeptide isomers all showing good activity.

The most promising peptide to arise as a result of these tests was the tripeptide H. In these tests (as in the seed treatment tests) only a small amount of compound was available and the experiments were carried out using very low concentrations of test compound. Taking this into account, the tripeptide H shows a very promising level of activity.

It is interesting to compare the fungicidal activity of the aminophosphonic acids and phosphonopeptides with their antibacterial activity (see chapter 1). The aminophosphonic acids themselves are fungitoxic; the length of the alkyl chain having a limited effect on activity[1]. In contrast, 1-aminoethanephosphonic acid needs to be incorporated into a peptide chain before it inhibits bacterial growth significantly and only close analogues of alanine undergo active transport into the bacterial cell.

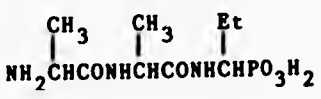
Aminophosphonic acids therefore appear to be more able to penetrate fungal membranes than bacterial membranes. The fact that certain phosphonopeptides appear to be more active than the free aminophosphonic acid may be due to increased penetration of the phosphonopeptides into the fungal cell permitting the build-up of a higher concentration of the toxicant within the cell. From the seed treatment tests it appears that stereochemistry plays an important part in determining fungicidal activity (as it does with antibacterial activity). In both cases L-stereochemistry is preferred for the amino acid residue and R- (or "L") stereochemistry is preferred for the aminophosphonic acid moiety. It has been found[71,72] that these stereochemical requirements are needed for antibacterial activity as the phosphonopeptides are transported into the bacterial cells by L,L-stereospecific peptide permeases using an active transport mechanism. It may be possible that stereospecific enzymes are involved also in the transport of phosphonopeptides across fungal membranes.

In bacterial systems the phosphonopeptides are cleaved intracellularly by L-stereospecific peptidases[72] to give the active aminophosphonic acids. Phosphonopeptides may also be cleaved intracellularly in fungal systems. If the cleavage is controlled by stereospecific enzymes, this would be another step where sterochemistry would affect activity.

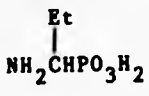
1-Aminoethanephosphonic acid and aminomethanephosphonic acid inhibit bacterial cell wall synthesis as a

result of their acting as false substrates for natural L-alanine. It is possible that the aminophosphonic acids' antifungal activity may be due to their acting as false substrates for natural amino acids in biosynthetic pathways.

The phosphonopeptides (especially the L-alanine tripeptide, H) show great promise as candidate fungicides. Tests with alafosfalin (see chapter 1) have shown the phosphonopeptides to be highly non-toxic. If we assume that only the aminophosphonic acid portion of the phosphonopeptides is fungicidal, then its incorporation into the peptides would result in a reduction in the amount of active component being released into the environment. On the assumption that the L-alanine tripeptide is of equal activity (w/w) to its free aminophosphonic acid component:



mwt 281



mwt 139

therefore, w/w the peptide contains ca. 50% aminophosphonic acid

Thus, 1g of aminophosphonic acid incorporated into the peptide has an equivalent activity to 2g of free aminophosphonic acid. The tripeptide could therefore be

considered to be twice as efficient as the free amino-phosphonic acid.

Chapter 4

Experimental

Instrumental analysis

Nuclear magnetic resonance

Routine proton magnetic resonance (^{1}H nmr) spectra were obtained using a Perkin Elmer R12B continuous wave spectrometer at a field of 60 MHz.

Higher field ^{1}H nmr spectra were recorded at 80.018 MHz on a Brucker WP-80 Fourier Transform spectrometer equipped with a BNC 28 computer, B-VT-1000 variable temperature unit and a 10 mm multinuclear probe head which also enabled carbon (^{13}C) nmr spectra to be recorded at 20.12 MHz and phosphorus (^{31}P) nmr spectra to be recorded at 32.395 MHz.

High field ^{31}P nmr spectra were recorded at 161.967 MHz using a Brucker 400 MHz spectrometer at the University of Bonn.

Sodium 3-trimethylsilylpropionate (tsp) was used as internal standard for the ^{1}H and ^{13}C nmr spectra when they were recorded in aqueous solution. For non-aqueous solutions, tetramethylsilane (tms) was used as internal standard.

The ^{31}P chemical shifts were recorded relative to an 85% solution of phosphoric acid contained in an external concentric tube.

^{1}H, ^{13}C and ^{31}P nmr chemical shifts are reported positive downfield from the standard in ppm.

Infrared spectroscopy

Infrared spectra over the region 700 - 4000 cm^{-1} were recorded as potassium bromide discs or as films between sodium chloride plates on Pye Unicam SP2000 and SP3-200 spectrophotometers.

Mass spectrometry

Routine mass spectra of volatile organic compounds were recorded on an A.E.I. MS9 double focussing spectrometer.

Positive ion fast atom bombardment (FAB) mass spectra of involatile compounds were obtained using a glycerol matrix. Spectra were recorded at the University of East Anglia using Kratos MS25 and VG Analytical ZAB-IF spectrometers. The ZAB-IF was fitted with a saddle-field ion source (Ion Tech Ltd.) operated at 8 kV and 1 mA with xenon. Spectra were recorded on the MS25 using a direct insertion probe with a capillaritron ion gun (Phrasor Scientific) operated at 8 kV and 30 mA with argon or xenon. FAB mass spectra were also recorded at the Physico-Chemical Measurements Unit (PCMU), Harwell using a ZAB-IF double focussing mass spectrometer fitted with an ion gun (Ion Tech Ltd.) operating at 8kV and providing a primary beam of xenon atoms. Linked-scan spectra were also recorded at PCMU.

Routine and high resolution FAB mass spectra were recorded at the SERC Mass Spectrometry Centre (Swansea) using a VG Analytical ZAB-E spectrometer. NaI, CsI and RbI were the reference compounds used for FAB exact mass measurements.

In reporting the spectra the abbreviation G is used to denote glycerol.

Carbon, hydrogen, nitrogen and phosphorus analysis

Microanalysis of carbon, hydrogen and nitrogen was carried out on Perkin Elmer 240B elemental analysers at the Polytechnic of North London and at Butterworths Laboratories Ltd. Phosphorus was determined by Butterworths Laboratories.

List of experiments

I.1 Preparation of N-hydroxysuccinimide[107]

Succinic anhydride (40.0 g, 0.40 mol) was added portionwise with stirring to hydroxylamine (from hydroxylamine hydrochloride (27.8 g, 0.40 mol) in water (40 ml), dioxane (20 ml) and 5N sodium hydroxide solution (80 ml)) at 20 °C. The clear solution was stirred at 60 °C for 2 h before removing the water-dioxane on a rotary evaporator. The residue was extracted exhaustively with boiling ethyl acetate (ca. 500 ml). The combined extracts were dried ($MgSO_4$), filtered and evaporated to give a white solid. The product was recrystallized from ethyl acetate to give N-hydroxysuccinimide (32.5 g, 0.28 mol, 71%) as a white crystalline solid, m.p. 98-99 °C (Lit.[107] 97-98 °C), ν_{max} (KBr) cm^{-1} 1693 (C=O), 2400-3200 (-OH); ^1H (D_2O) δ 2.30 (4H, s, CH_2), 4.75 (1H, s, OH).

I.2 Preparation of N-carbobenzoxyglycine[108]

A solution of glycine (10.0 g, 0.133 mol) in 2N sodium hydroxide solution (66.5 ml, 0.133 mol) was stirred in a flask fitted with two dropping funnels at 0 °C. Benzyl chloroformate (22.7 g, 18.9 ml, 0.133 mol) in toluene (12 ml) and 4N sodium hydroxide solution (33.3 ml, 0.133 mol) were added simultaneously with stirring over a period of 0.5 h. On completing the addition, the mixture was stirred for a further 15 min before extracting the solution once with ether to remove unreacted benzyl chloroformate. The aqueous solution was cooled in ice and acidified to pH 2 with concentrated hydrochloric acid when a white oil was precipitated. The oil crystallized on standing and the solid was filtered off and washed with iced water before drying (room temperature, vacuum oven) to give N-carbobenzoxy glycine which was used without further purification (26.5 g 0.127 mol, 95%), m.p. 116-8 °C (Lit.[108] 120 °C), ^{1}H ($CDCl_3$) δ 3.50 (2H, s, $CH_2C{=}O$), 5.10 (2H, s, CH_2arom), 7.30 (5H, s, arom).

I.3 Preparation of the N-hydroxysuccinimide ester of N-carbobenzoxyglycine[84]

N,N'-Dicyclohexylcarbodiimide (26.2 g, 0.127 mol) was added in portions to a stirred solution of N-carbobenzoxy-glycine (26.5 g, 0.127 mol) and N-hydroxysuccinimide

(14.6 g, 0.127 mol) in dioxane (245 ml) at 0 °C. The mixture was left in a refrigerator for 48 h before filtering off the precipitated dicyclohexylurea. The solid was washed with dioxane and the combined filtrate and washings evaporated to give a yellow oil. The oil was shaken with diethyl ether and left in a refrigerator for 48 h to crystallize. The resulting white crystals were filtered and dried under vacuum to give the crude N-hydroxysuccinimide ester of N-carbobenzoxyglycine (34.6 g, 0.113 mol, 89%) which was used without further purification, m.p. 110 °C (Lit.[84] 113-4 °C), ^{1}H ($CDCl_3$) δ 2.75 (4H, s, CH_2CH_2), 3.55 (2H, s, $CH_2C{=}O$), 5.15 (2H, s, CH_2arom), 7.35 (5H, s, arom).

I.4 Preparation of N-carbobenzoxy-D-alanine[108]

A solution of D-alanine (5.00 g, 0.056 mol) in 2N sodium hydroxide solution (28 ml, 0.056 mol) was stirred in a flask fitted with two dropping funnels at 0 °C. Benzyl chloroformate (9.56 g, 0.056 mol) in toluene (5 ml) and 4N sodium hydroxide solution (14 ml, 0.056 mol) were added dropwise simultaneously over a period of 0.5 h. After the addition was complete the mixture was stirred for a further 0.5 h at 0 °C. The mixture was extracted once with diethyl ether to remove unreacted benzyl chloroformate. The aqueous layer was cooled in ice and

acidified to pH 2 with concentrated hydrochloric acid when a white oil was precipitated. The oil crystallized on standing and the solid was filtered, washed with iced water and dried under vacuum to give the crude N-carbobenzoxy-D-alanine (11.5 g, 0.052 mol, 92%) which was used without further purification, m.p. 80 °C (Lit.[81] 87 °C), ^{1}H ($CDCl_3$) δ 1.45 (3H, d, CH_3, $^{3}J_{HCCH}$ 7.60 Hz), 4.25-4.50 (1H, m, CH), 5.15 (2H, s, CH_2arom), 7.35 (5H, s, arom), 9.40 (1H, br s, NH).

I.5 Preparation of the N-hydroxysuccinimide ester of N-carbobenzoxy-D-alanine[84]

N,N'-Dicyclohexylcarbodiimide (10.6g, 0.052 mol) was added in portions to a stirred solution of N-carbobenzoxy-D-alanine (11.5 g, 0.052 mol) and N-hydroxysuccinimide (5.96 g, 0.052 mol) in dioxane (100 ml) at 0 °C. The mixture was left in a refrigerator overnight before filtering off the precipitated dicyclohexylurea. The solid was washed with dioxane and the combined filtrate and washings evaporated to give a yellow oil. The oil was shaken with ether and left in a refrigerator until crystallization was complete (ca. 1 week). The crystalline white solid was filtered and dried under vacuum to give the crude N-hydroxysuccinimide ester of N-carbobenzoxy-D-alanine (14.2 g, 0.045 mol, 86%) which

was used without further purification, m.p.120-1 °C, (Lit.[84] 123-123.5 °C), ^{1}H ($CDCl_3$) δ 1.56 (3H, d, $C\underline{H}_3$, $^{3}J_{HCCH}$ 8.30 Hz), 2.80 (2H, s, $C\underline{H}_2C{=}O$), 5.12 (2H, s, $C\underline{H}_2$arom), 7.35 (5H, s, arom).

I.6 Preparation of N-carbobenzoxy-L-alanine[108]

A solution of L-alanine (5.00 g, 0.056 mol) in 2N sodium hydroxide solution (28.0 ml, 0.056 mol) was stirred in a flask fitted with two dropping funnels at 0 °C. Benzyl chloroformate (9.56 g, 0.056 mol) in toluene (5 ml) and 4N sodium hydroxide solution (14.0 ml, 0.056 mol) were added simultaneously with stirring over a period of 25 min. The mixture was stirred for a further 10 min. before extracting once with diethyl ether. The aqueous layer was cooled in ice and acidified to pH 2 with concentrated hydrochloric acid when a white oil was precipitated. The oil crystallized on standing and the solid was filtered and washed with iced water before drying (room temperature, vacuum oven) to give crude N-carbobenzoxy-L-alanine (9.36 g, 0.042 mol, 75%), m.p. 84-5 °C (Lit.[81] 87 °C) which was used without further purification, ^{1}H ($CDCl_3$) δ 1.45 (3H, d, $C\underline{H}_3$, $^{3}J_{HCCH}$ 7.0 Hz), 4.28-4.55 (1H, m, C<u>H</u>), 5.13 (2H, s, $C\underline{H}_2$arom), 7.36 (5H, s, arom), 9.38 (1H, br s, N<u>H</u>).

I.7 Preparation of the N-hydroxysuccinimide ester of N-carbobenzoxy-L-alanine[84]

N,N'-Dicyclohexylcarbodiimide (1.48 g, 7.17 mmol) was added to a stirred solution of N-carbobenzoxy-L-alanine (1.6 g, 7.17 mmol) and N-hydroxysuccinimide (0.83 g, 7.17 mmol) in dioxane (14 ml) at 0 °C. The mixture was left in a refrigerator overnight before filtering off the precipitated dicyclohexylurea. The solid was washed with dioxane and the combined filtrate and washings evaporated to give a crystalline white solid. The solid was triturated with diethyl ether and filtered to give the crude N-hydroxysuccinimide ester of N-carbobenzoxy-L-alanine (2.16 g). The product was recrytallized from isopropanol (1.95 g, 6.09 mmol, 85%), m.p. 120-2 °C (Lit.[84] 123-123.5 °C), ^{1}H ($CDCl_3$) δ 1.55 (3H, d, CH_3, $^{3}J_{HCCH}$ 7.0 Hz), 2.80 (2H, s, $CH_2C{=}O$), 5.10 (2H, s, CH_2arom), 7.35 (5H, s, arom).

I.8 Preparation of N-carbobenzoxy-DL-alanine[108]

A solution of DL-alanine (10.0 g, 0.112 mol) in 2N sodium hydroxide solution (56 ml, 0.112 mol) was stirred in a flask fitted with two dropping funnels at 0 °C. Benzyl chloroformate (19.1 g, 0.112 mol) in toluene (10 ml) and 4N sodium hydroxide solution (28 ml, 0.112 mol) were added simultaneously with stirring over a period of 0.5 h.

The mixture was stirred for a further 15 min. before extracting once with diethyl ether. The aqueous layer was cooled in ice and acidified to pH 2 with concentrated hydrochloric acid when a white oil was precipitated. The oil crystallized on standing and the solid was filtered and washed with iced water before drying (room temperature vacuum oven) to give crude N-carbobenzoxy-DL-alanine (22.8 g, 0.102 mol, 91%), m.p. 110-12 °C (Lit.[108] 114-5 °C) which was used without further purification, ^{1}H ($CDCl_3$) δ 1.45 (3H, d, CH_3, $^{3}J_{HCCH}$ 7.5 Hz), 4.25-4.50 (1H, br m, CH), 5.12 (2H, s, CH_2arom), 7.33 (5H, s, arom).

I.9 Preparation of the N-hydroxysuccinimide ester of N-carbobenzoxy-DL-alanine[84]

N,N'-Dicyclohexylcarbodiimide (10.6 g, 0.052 mol) was added to a stirred solution of N-carbobenzoxy-DL-alanine (11.5 g, 0.052 mol) and N-hydroxysuccinimide (5.96 g, 0.052 mol) in dioxane (100 ml) at 0 °C. The mixture was left in a refrigerator overnight before filtering off the precipitated dicyclohexylurea. The solid was washed with dioxane and the combined filtrate and washings evaporated to give a yellow oil. The oil was shaken with diethyl ether and left to crystallize. The product was filtered and dried under vacuum to give the crude N-hydroxysuccinimide ester of N-carbobenzoxy-DL-alanine (15.8 g, 0.049 mol, 95%), m.p. 81-83 °C, ^{1}H ($CDCl_3$) δ 1.56 (3H, d, CH_3, $^{3}J_{HCCH}$ 7.3 Hz), 2.80 (2H, s, $CH_2C{=}O$), 5.10 (2H, s, CH_2arom), 7.32 (5H, s, arom).

I.10 Preparation of N-carbobenzoxy-DL-2-aminobutanoic acid[108]

A solution of DL-2-aminobutanoic acid (6.00 g, 0.058 mol) in 2N sodium hydroxide solution (29.0 ml, 0.058 mol) was stirred in a flask fitted with two dropping funnels at 0 °C Benzyl chloroformate (9.90 g, 8.3 ml, 0.058 mol) in toluene (6 ml) and 4N sodium hydroxide solution (14.6 ml, 0.058 mol) were added simultaneously with stirring over a period of 0.5 h. On completion of the addition the mixture was stirred for a further 15 min before extracting once with ether to remove unreacted benzyl chloroformate. The aqueous solution was cooled in ice and acidified to pH 2 with concentrated hydrochloric acid. The precipitated oil crystallized on standing and the white solid was filtered off, washed with iced water and dried (room temperature, vacuum oven) to give N-carbobenzoxy-DL-2-aminobutanoic acid (11.6 g, 0.049 mol, 84%), m.p. 68-70 °C, ^{1}H ($CDCl_3$) δ 0.95 (3H, t, CH_3CH_2, $^{3}J_{HCCH}$ 6.50 Hz), 1.60-2.10 (2H, m, CH_3CH_2), 4.15-4.55 (1H, m, $CHCH_2$), 5.15 (2H, s, CH_2arom), 7.33 (5H, s, arom). 8.15 (1H, br s, NH).

I.11 Preparation of the N-hydroxysuccinimide ester of N-carbobenzoxy-DL-2-aminobutanoic acid[84]

N,N'-dicyclohexylcarbodiimide (10.1 g, 0.05 mol) was added in portions to a stirred solution of N-carbobenzoxy-DL-2-aminobutanoic acid (11.6 g, 0.05 mol) and N-hydroxy-

succinimide (5.64 g, 0.05 mol) in dioxane (95 ml) at 0 °C. The mixture was left overnight in a refrigerator before filtering off the precipitated dicyclohexylurea. The solid was washed with dioxane and the combined filtrate and washings evaporated to give a yellow oil. The oil was shaken with diethyl ether and left to stand in a refrigerator for several weeks, however, crystallization did not occur. The oil was evaporated (oil pump) to give the crude N-hydroxysuccinimide ester of N-carbobenzoxy-DL-2-aminobutanoic acid (16.0 g, 4.80 mmol, 98%) which was used without further purification, ν_{max}(film) cm^{-1} 1520 (N-H), 1695-1750 (C=O), 1780, 1810, 3320 (N-H); ^1H (CD_3OD) δ 1.05 (3H, t, CH_3, $^3J_{HCCH}$ 7.88 Hz), 1.65-2.00 (2H, br m, CH_2CH_3), 2.72 (4H, s, $CH_2C{=}O$), 4.30-4.65 (1H, m, CH), 5.05 (2H, s, CH_2arom), 7.30 (5H, s, arom).

I.12 Preparation of N-carbobenzoxy-DL-phenylalanine[108]

A solution of DL-phenylalanine (5.00 g, 0.030 mol) in 2N sodium hydroxide solution (15.0 ml, 0.030 mol) was stirred in a flask fitted with two dropping funnels at 0 °C. Benzyl chloroformate (5.12 g, 0.030 mol) in toluene (5 ml) and 4N sodium hydroxide solution (7.5 ml, 0.030 mol) were added simultaneously with stirring over a period of 0.5 h. The mixture was stirred for a further 0.5 h before extracting once with diethyl ether. The aqueous layer was cooled in ice and acidified to pH 2 with concentrated hydrochloric acid when a white oil was precipitated. The oil crystallized on standing and the solid was filtered and washed with iced water before drying (room temperature, vacuum oven) to give crude N-carbobenzoxy-DL-phenylalanine (8.73 g, 0.029 mol, 97%), m.p. 98-100 °C (Lit.[81] 103 °C) which was used without further purification, ^{1}H ($CDCl_3$) δ 3.15 (2H, d, CH_2CH, $^{3}J_{HCCH}$ 6.0 Hz), 4.60-4.85 (1H, br m, CH), 5.10 (2H, s, CH_2arom), 7.25 (5H, br s, $CHCH_2$arom). 7.35 (5H, s, OCH_2arom), 9.95 (1H, br s, NH).

I.13 Preparation of the N-hydroxysuccinimide ester of N-carbobenzoxy-DL-phenylalanine[84]

N,N'-Dicyclohexylcarbodiimide (6.19 g, 0.030 mol) was added to a stirred solution of N-carbobenzoxy-DL-phenylalanine (8.97 g, 0.030 mol) and N-hydroxysuccinimide

(3.45 g, 0.030 mol) in dioxane (60 ml) at 0 °C. The mixture was left in a refrigerator overnight before filtering off the precipitated dicyclohexylurea. The solid was washed with dioxane and the combined filtrate and washings evaporated to give a yellow oil. The oil was shaken with diethyl ether and left to crystallize. The product was filtered and dried under vacuum to give the crude N-hydroxysuccinimide ester of N-carbobenzoxy-DL-phenylalanine (10.0 g, 0.025 mol, 83%), m.p. 110-12 °C, ν_{max} (KBr) cm^{-1} 1520 (N-H), 1695-1735 (C=O), 1780, 1810, 3450 (N-H); ^1H (CD_3OD) δ 2.80 (4H, $CH_2C=O$), 3.25 (2H, d, $CHCH_2arom$, $^3J_{HCCH}$ 6.0 Hz), 5.10 (2H, s, CH_2arom), 7.25 (5H, s, $CHCH_2\underline{arom}$), 7.30 (5H, s, $CH_2\underline{arom}$).

I.14 Preparation of tetramethylammonium salt of monomethyl propionyl phosphonate

Dimethyl propionylphosphonate (16.6 g, 0.10 mol) was placed in a stainless steel bomb and cooled to -78 °C whilst cooled (-78 °C) trimethylamine (11.16 ml, 0.12 mol) and cooled (-78 °C) dry acetone (40 ml) were added. The bomb was sealed and heated at 80 °C for 3 h with stirring. The resulting solid obtained on cooling was recrystallised from dichloromethane, filtered under nitrogen and washed with dry acetone to give the tetramethylammonium salt of monomethyl propionyl phosphonate (20.0 g, 0.09 mol, 89%) as a hygroscopic crystalline solid, m.p. 139-140 °C, ν_{max} (KBr) cm^{-1} 1680 (C=O), 1230 (P=O), 780 (P-O)

1. Preparation of 1-aminopropanephosphonic acid

Ethyl carbamate (17.8 g, 0.20 mol), triphenyl phosphite (62.0 g, 0.20 mol) and propanal (16.2 g, 0.28 mol) were stirred and heated under reflux with glacial acetic acid (40 ml) for 1 h. The mixture was allowed to cool, concentrated hydrochloric acid (200 ml) was added and the mixture stirred and heated under reflux for a further 8 h. After cooling, the aqueous layer was separated and extracted with toluene (3 x 25 ml) to remove phenol. The aqueous layer was evaporated and the residue dissolved in warm methanol (50 ml) and warmed gently whilst treating with propylene oxide to give a fine white precipitate. The solid was filtered and dried before recrystallizing from water/ethanol to give 1-aminopropanephosphonic acid (6.00 g, 43 mmol, 22%), m.p. 261-3 °C, (Lit.[23] 264-6 °C), ^{1}H (D_2O/D_2SO_4) δ 1.11 (3H, t, $C\underline{H}_3$, $^{3}J_{HCCH}$ 7.4 Hz), 1.25-1.65 (2H, br m, $C\underline{H}_2$), 3.54 (1H, m, C$\underline{H}$); ^{13}C (D_2O/H_2SO_4) δ 12.6 (d, $\underline{C}H_3$, $^{3}J_{PCCC}$ 9.0 Hz), 24.0 (s, $\underline{C}H_2$), 52.6 (d, $\underline{C}H$, $^{1}J_{PC}$ 154.4 Hz); ^{31}P (D_2O) δ 19.5.

2. Preparation of 1-aminobutanephosphonic acid

Ethyl carbamate (3.21 g, 0.036 mol), triphenyl phosphite (11.2 g, 0.036 mol) and butanal (3.63 g, 0.050 mol) were stirred and heated under reflux with glacial acetic acid (7 ml) for 1.5 h. The mixture was

allowed to cool and concentrated hydrochloric acid was added (36 ml). The solution was heated under reflux for a further 12 h. After cooling, the aqueous layer was separated and extracted with dichloromethane (3 x 20 ml) to remove phenol. The aqueous layer was evaporated and the residue dissolved in warm ethanol and the solution warmed gently whilst treating with propylene oxide (5 ml) to give a fine white precipitate. The solid was filtered and dried (1.21 g) before recrystallizing from water/ethanol to give 1-aminobutanephosphonic acid (1.06 g, 6.93 mmol, 19%), m.p. 258-60 °C (Lit.[106] 263-5 °C), ^{1}H (D_2O) δ 0.96 (3H, t, $\underline{CH}_3$, $^{3}J_{HCCH}$ 6.6 Hz), 1.25-2.00 (4H, br m, $\underline{CH}_2$), 3.25-3.62 (1H, m, $\underline{CH}$); ^{13}C (D_2O) δ 15.7 (s, $\underline{C}H_3$), 21.6 (d, $\underline{C}H_2CH_3$, $^{3}J_{PCCC}$ 9.8 Hz), 33.3 (s, $\underline{C}H_2CH$), 51.4 (d, $\underline{C}HP$, $^{1}J_{PC}$ 150.8 Hz); ^{31}P (D_2O) δ 22.2

3. Preparation of 1-aminobutanephosphonic acid using urea

Urea (1.08 g, 0.018 mol), triphenyl phosphite (11.2 g, 0.036 mol) and butanal (2.60 g, 0.036 mol) were stirred and heated under reflux with glacial acetic acid (7 ml) for 2.5 h. The mixture was allowed to cool and concentrated hydrochloric acid was added (36 ml). The solution was heated under reflux overnight. After cooling, the aqueous layer was separated and extracted

with toluene (3 x 20 ml) to remove phenol. The aqueous layer was evaporated and the residue dissolved in warm ethanol and the solution warmed gently whilst treating with propylene oxide (5 ml) to give a brown oil. The oil was dissolved in water and boiled with decolourizing charcoal. After filtering, the solution was evaporated and the residual oil dissolved in methanol. The solution was treated with diethyl ether until cloudy and left to stand. The precipitated solid was filtered and dried (1.60 g) before recrystallizing from water/ethanol to give 1-aminobutanephosphonic acid (1.30 g, 8.50 mmol, 24%), m.p. 260-3 °C (Lit.[106] 263-5 °C), ^{1}H (D_2O) δ 0.96 (3H, t, $\underline{CH}_3$, $^{3}J_{HCCH}$ 6.5 Hz), 1.12-1.80 (4H, br m, $\underline{CH}$), 3.62-4.20 (1H, m, $\underline{CH}$); ^{13}C (D_2O) δ 15.6 (s, $\underline{C}H_3$), 21.4 (d, $\underline{C}H_2CH_3$, $^{3}J_{PCCC}$ 13.4 Hz), 34.5 (s, $\underline{C}H_2CH$), 50.4 (d, $\underline{C}HP$, $^{1}J_{PC}$ 152.0 Hz); ^{31}P (D_2O) δ 22.6.

4. Preparation of 1-aminopentanephosphonic acid

Urea (1.08 g, 0.018 mol), triphenyl phosphite (11.2 g, 0.036 mol) and pentanal (3.10 g, 0.036 mol) were stirred and heated under reflux with glacial acetic acid (7 ml) for 2 h. The mixture was allowed to cool and concentrated hydrochloric acid was added (36 ml). The solution was heated under reflux for 12 h. After cooling, the aqueous layer was separated and extracted with toluene (3 x 20 ml)

to remove phenol. The aqueous layer was evaporated and the residue dissolved in warm ethanol and the solution warmed gently whilst treating with propylene oxide (5 ml) to give an oil which crystallized on standing. The solid was filtered and dried (2.35 g) before recrystallizing from water/ethanol to give 1-aminopentanephosphonic acid (1.90 g, 0.011 mol, 32%), m.p. 265-6 °C (Lit.[23] 267-9 °C), ^{1}H (D_2O) δ 0.90 (3H, t, $\underline{CH}_3$, $^{3}J_{HCCH}$ 6.4 Hz), 1.25-2.25 (6H, br m, $\underline{CH}_2$), 3.07-3.40 (1H, m, $\underline{CH}$); ^{13}C (D_2O) δ 15.9 (s, $\underline{CH}_3$), 24.5 (s, $\underline{CH}_2CH_3$), 30.6 (d, $\underline{CH}_2$, $^{3}J_{PCCC}$ 9.8 Hz), 31.0 (s, $\underline{CH}_2CH$), 52.5 (d, $\underline{CHP}$, $^{1}J_{PC}$ 142.8 Hz); ^{31}P (D_2O) δ 13.8.

5. Preparation of 1-aminopentanephosphonic acid using ethyl carbamate

Ethyl carbamate (3.21 g, 0.036 mol), triphenyl phosphite (11.2 g, 0.036 mol) and pentanal (4.34 g, 0.051 mol) were stirred and heated under reflux with glacial acetic acid (7 ml) for 1.5 h. The mixture was allowed to cool and concentrated hydrochloric acid was added (36 ml). The solution was heated under reflux for 6 h. After cooling, the aqueous layer was separated and extracted with dichloromethane (3 x 20 ml) to remove phenol. The aqueous layer was evaporated and the residue dissolved in warm ethanol and the solution warmed gently whilst treating with propylene oxide (5 ml) to give a

white solid. The solid was filtered and dried (1.57 g) before recrystallizing from water to give 1-aminopentanephosphonic acid (1.20 g, 7.19 mmol, 20%), m.p. 268-70 °C (Lit.[23] 267-9 °C), ν_{max} (KBr) cm^{-1} 1180 (P=O), 1540 (N-H), 2100-3400 (P-OH); ^{13}C (D_2O) δ 15.8 (s, $\underline{C}H_3$), 24.5 (s, $\underline{C}H_2CH_3$), 30.6 (d, $\underline{C}H_2$, $^3J_{PCCC}$ 9.0 Hz), 30.9 (s, $\underline{C}H_2CH$), 51.1 (d, $\underline{C}HP$, $^1J_{PC}$ 145.9 Hz).

6. Preparation of 1-aminohexanephosphonic acid

Benzyl carbamate (5.40 g, 0.036 mol), triphenyl phosphite (11.2 g, 0.036 mol) and hexanal (5.01 g, 0.050 mol) were stirred and heated under reflux with glacial acetic acid (7 ml) for 2 h. The mixture was allowed to cool and concentrated hydrochloric acid was added (36 ml). The solution was heated under reflux for 8 h. After cooling, the aqueous layer was separated and extracted with dichloromethane (3 x 20 ml) to remove phenol. The aqueous layer was evaporated and the residue dissolved in warm methanol/water and the solution warmed gently whilst treating with propylene oxide (5 ml) to give a white solid. The solid was filtered and dried to give the crude 1-aminohexanephosphonic acid (2.70 g, 0.015 mol, 41%), m.p. 260 °C. A portion of the solid was recrystallized from water to give m.p. 260-60.5 °C, (Found: C, 38.4; H, 8.8; N, 8.7. $C_6H_{16}NO_3P$ requires: C, 39.9; H, 8.8; N, 7.7%); ^1H (D_2O/D_2SO_4) δ 0.88 (3H, t, $\underline{CH_3}CH_2$, $^3J_{HCCH}$ 7.0 Hz),

1.37 (6H, br s, $C\underline{H}_2$), 1.63-2.20 (2H, br m, $C\underline{H}_2CH$), 3.42-3.80 (1H, m, $C\underline{H}$); ^{13}C (D_2O/D_2SO_4) δ 13.5 (s, $\underline{C}H_3$), 21.7 (s, $\underline{C}H_2$), 24.8 (d, $\underline{C}H_2CH_2CHP$, $^3J_{PCCC}$ 8.6 Hz), 27.7 (s, $\underline{C}H_2$), 30.6 (s, $\underline{C}H_2$), 48.5 (d, $\underline{C}HP$, $^1J_{PC}$ 152.6 Hz); ^{31}P (D_2O/D_2SO_4) δ 17.4.

7. Preparation of 1-aminoheptanephosphonic acid

Benzyl carbamate (5.40 g, 0.036 mol), triphenyl phosphite (11.2 g, 0.036 mol) and heptanal (5.71 g, 0.050 mol) were stirred and heated under reflux with glacial acetic acid (7 ml) for 2 h. The mixture was allowed to cool and concentrated hydrochloric acid was added (36 ml). The solution was heated under reflux for 8 h. After cooling, the aqueous layer was separated and extracted with dichloromethane (3 x 20 ml) to remove phenol. The aqueous layer was evaporated and the residue dissolved in warm methanol/water and the solution warmed gently whilst treating with propylene oxide (5 ml) to give a white solid. The solid was filtered and dried to give the crude 1-aminoheptanephosphonic acid (3.07 g, 0.016 mol, 44%), m.p. 268-70 °C. A portion of the product was recrysrallized from water to give m.p. 272-3 °C, ^1H (D_2O/H_2SO_4) δ 0.86 (3H, t, $C\underline{H}_3$, $^3J_{HCCH}$ 6.00 Hz), 1.33 (8H, br s, $C\underline{H}_2$), 1.62-2.12 (2H, br m, $C\underline{H}_2CH$), 3.20-3.53 (1H, m, $C\underline{H}$); ^{13}C (D_2O/D_2SO_4) δ 16.0 (s, $\underline{C}H_3$), 24.5 (s, $\underline{C}H_2$), 27.6 (d, $\underline{C}H_2CH_2CHP$, $^3J_{PCCC}$ 8.6 Hz), 30.3 (s, $\underline{C}H_2$), 30.6 (s, $\underline{C}H_2$), 33.2 (s, $\underline{C}H_2$), 50.7 (d, $\underline{C}HP$, $^1J_{PC}$ 152.0 Hz); ^{31}P (D_2O/D_2SO_4) δ 14.4.

8. Preparation of diethyl propionylphosphonate[36]

Distilled propionyl chloride (9.62 g, 0.104 mol) was added dropwise to distilled triethyl phosphite (17.3 g, 0.104 mol) stirred under nitrogen whilst cooling in an ice bath. An exothermic reaction resulted in which a colourless gas was evolved. The solution was stirred under nitrogen overhight before distilling to give diethyl propionylphosphonate (12.1 g, 0.062 mol, 60%) as a clear liquid, b.p. 74-78 °C, 1.0 mmHg (Lit.[36] 105 °C, 7 mmHg), ν_{max} (film) cm^{-1} 1020 (P-O-C), 1265 (P=O), 1695 (C=O); ^{1}H ($CDCl_3$) δ 1.04 (3H, t, $\underline{CH}_3$, $^{3}J_{HCCH}$ 7.10 Hz), 1.35 (6H, t, $OCH_2\underline{CH}_3$, $^{3}J_{HCCH}$ 7.50 Hz), 2.88 (2H,q, $\underline{CH}_2C(O)$, $^{3}J_{HCCH}$ 7.08 Hz), 4.16 (4H, qt, $O\underline{CH}_2$, $^{3}J_{HCCH}$ 7.00 Hz); ^{13}C ($CDCl_3$) δ 6.4 (d, $\underline{CH}_3$, $^{3}J_{PCCC}$ 3.7 Hz), 16.4 (d, $OCH_2\underline{CH}_3$, $^{3}J_{POCC}$ 5.5 Hz), 36.9 (d, $\underline{CH}_2C(O)$, $^{2}J_{PCC}$ 56.2 Hz), 63.8 (d, $O\underline{CH}_2$, $^{2}J_{POC}$ 7.3 Hz), 211.5 (d, $\underline{C}(O)$, $^{1}J_{PC}$ 166.6 Hz); ^{31}P ($CDCl_3$) δ -2.9.

9. Preparation of diethyl 1-hydroxyiminopropanephosphonate[37]

Distilled diethyl propionylphosphonate (28.0 g, 0.144 mol) was added to hydroxylamine hydrochloride (12.4 g, 0.190 mol) in pyridine (16.3 ml, 16.0 g, 0.202 mol) and dry ethanol (41 ml) whilst stirring at 0 °C. The resulting clear solution was stirred for 24 h at room temperature. The solution was evaporated and the residue added to hydrochloric acid (70 ml, 10%) before extracting with dichloromethane (5 x 20 ml). The organic layer was washed with hydrochloric acid (10%) several times and then with water, sodium bicarbonate solution (10%) and water successively.

Concentration of the extract gave the crude diethyl 1-hydroxyiminopropanephosphonate (25.2 g, 0.120 mol, 84%) as a colourless oil, ν_{max} (film) cm^{-1} 1020 (P-O-C), 1230 (P=O), 3190 (OH); ^{1}H ($CDCl_3$) δ 1.15 (3H, t, $\underline{CH}_3$, $^{3}J_{HCCH}$ 7.81 Hz), 1.34 (6H, t, $OCH_2\underline{CH}_3$, $^{3}J_{HCCH}$ 7.08 Hz), 2.54 (2H, h, $\underline{CH}_2C(NOH)$, $^{3}J_{HCCH}$ 7.08 Hz), 4.17 (4H, qt, $O\underline{CH}_2$, $^{3}J_{HCCH}$ 7.57 Hz), 10.87 (1H, br s, $\underline{OH}$); ^{13}C ($CDCl_3$) δ 10.3 (s, $\underline{CH}_3$), 16.3 (d, $OCH_2\underline{CH}_3$, $^{3}J_{POCC}$ 6.1 Hz), 20.0 (d, $\underline{CH}_2C(NOH)$, $^{2}J_{PCC}$ 16.5 Hz), 63.0 (d, $O\underline{CH}_2$, $^{2}J_{POC}$ 7.3 Hz), 155.3 (d, $\underline{C}(NOH)$, $^{1}J_{PC}$ 212.4 Hz); ^{31}P ($CDCl_3$) δ 11.0 Hz.

10. Preparation of diethyl 1-aminopropanephosphonate[42]

Crude diethyl 1-hydroxyiminopropanephosphonate (25.2 g, 0.12 mol) was placed in a three-necked flask fitted with a condenser, mechanical stirrer and thermometer, and powdered zinc (31.4 g, 0.48 mol) and formic acid (98-100%, 120 ml) were added. An exothermic reaction resulted and the temperature of the reaction was maintained below 65 °C by external cooling. After 1 h the reaction was stirred and heated under reflux at 65 °C for ca. 80 h. The reaction was allowed to cool and the precipitated zinc formate was filtered off and washed with dichloromethane. The filtrate and washings were combined and evaporated and the residual oil dissolved in dichloromethane (120 ml). The solution was neutralized with sodium carbonate solution (10%, 50 ml) and the organic

layer dried ($MgSO_4$) and evaporated. The residue was dissolved in methanol (240 ml) and the solution saturated with hydrogen chloride at 0 °C. The solution was left to stand at room temperature for 24 h before evaporating off the solvent and suspending the residue in anhydrous diethyl ether (240 ml). Ammonia gas was passed into the mixture whilst stirring at 0 °C. The precipitated ammonium chloride was filtered off and the filtrate evaporated to give the crude diethyl 1-aminopropane-phosphonate (8.98 g, 0.046 mol, 38%) as a pale yellow oil. A portion of the product was distilled to give b.p. 68-70 °C, 5.0 mmHg (Lit.[20] 45-7 °C, 2.6 mmHg), ν_{max} (film) cm^{-1} 1030 (P-O-C), 1240 (P=O), 3300-3500 (N-H); ^1H ($CDCl_3$) δ 1.06 (3H, t, $\underline{CH}_3$, $^3J_{HCCH}$ 7.19 Hz), 1.34 (6H, t, $\underline{CH}_3CH_2O$, $^3J_{HCCH}$ 7.04 Hz), 1.63-2.25 (2H, m, $C\underline{H}_2$), 2.72-3.03 (1H, m, $\underline{CH}$), 4.15 (4H, qt, $\underline{CH}_2O$, $^3J_{HCCH}$ 7.04 Hz); ^{13}C ($CDCl_3$) δ 10.9 (d, $\underline{CH}_3$, $^3J_{POCC}$ 12.8 Hz), 16.6 (d, $\underline{CH}_3CH_2O$, $^3J_{POCC}$ 5.5 Hz), 24.7 (s, $\underline{CH}_2$), 62.1 (d, $\underline{CH}_2O$, $^2J_{POC}$ 7.3 Hz), 50.5 (d, $\underline{CH}P$, $^1J_{PC}$ 148.9 Hz); ^{31}P ($CDCl_3$) δ 29.1; m/e (%) 195 (23.3, M^+).

11. Hydrolysis of diethyl 1-aminopropanephosphonate

Diethyl 1-aminopropanephosphonate (3.20 g, 0.016 mol) was heated under reflux with glacial acetic acid (10 ml) and concentrated hydrochloric acid (40 ml) for 8 h. The mixture was evaporated and the residue dissolved in water and treated with decolourizing charcoal. The solution was filtered and evaporated and the residue

dissolved in methanol and warmed gently whilst treating with propylene oxide. The resulting fine white precipitate was filtered and dried before recrystallizing from water/methanol to give 1-aminopropanephosphonic acid (1.67 g, 0.012 mol, 73%), m.p. 262-4 °C (Lit.[23] 264-6 °C), ^{1}H (D_2O/H_2SO_4) δ 1.10 (3H, t, $\underline{CH}_3$, $^{3}J_{HCCH}$ 7.5 Hz), 1.30-1.70 (2H, br m, $\underline{CH}_2$), 3.50 (1H, m, $\underline{CH}$).

12. Preparation of dimethyl propionylphosphonate

Distilled propionyl chloride (323 g, 3.49 mol), was added dropwise to trimethyl phosphite (383 g, 3.08 mol) stirred under nitrogen and the reaction mixture maintained at a temperature below 25 °C by cooling in ice. The solution was stirred at room temperature overnight under nitrogen before distilling to give dimethyl propionyl-phosphonate (446 g, 2.69 mol, 87%) as a clear liquid, b.p. 89-90 °C, 3.5-4.0 mmHg (Lit.[103] 60-62 °C, 1 mmHg), ν_{max} (Film) cm^{-1} 1040 (P-O-C), 1265 (P=O), 1695 (C=O); ^{1}H ($CDCl_3$) δ 1.12 (3H, t, $\underline{CH}_3CH_2$, $^{3}J_{HCCH}$ 7.10 Hz), 2.88 (2H, q, $\underline{CH}_2$, 7.10 Hz), 3.89 (6H, d, $\underline{CH}_3O$, $^{3}J_{HCOP}$ 10.74 Hz); ^{13}C ($CDCl_3$) δ 6.4 (d, $\underline{CH}_3CH_2$, $^{3}J_{PCCC}$ 4.3 Hz), 37.2 (d, $\underline{CH}_2$, $^{2}J_{PCC}$ 56.8 Hz), 54.0 (d, $\underline{CH}_3O$, $^{2}J_{POC}$ 7.3 Hz), 211.0 (d, $\underline{C}=O$, $^{1}J_{PC}$ 166.0 Hz); ^{31}P ($CDCl_3$) δ -1.1.

13. Preparation of dimethyl 1-hydroxyiminopropane-phosphonate[37]

Distilled dimethyl propionylphosphonate (171 g, 1.03 mol) was added dropwise to a mixture of hydroxylamine hydrochloride (82.43 g, 1.19 mol), pyridine (100 g, 1.26 mol) and methanol (250 ml) whilst keeping the reaction mixture below 10 °C. The mixture was stirred overnight at room temperature and then evaporated (rotary evaporator, water bath 40 °C). Hydrochloric acid (10%, 100 ml) was added to the residue until pH 2 was reached and the solution was extracted exhaustively with dichloromethane The organic layer was washed with sodium bicarbonate solution (10%, 20 ml) and dried ($MgSO_4$) before evaporating (oil pump, room temperature) to give dimethyl 1-hydroxy-iminopropanephosphonate (163 g, 0.90 mol, 87%) as a waxy low melting solid. The oxime was used without further purification, ν_{max} (film) cm^{-1} 1040 (P-O-C), 1240 (P=O), 3190 (OH); ^1H ($CDCl_3$) δ 1.14 (3H, t, $\underline{CH_3}CH_2$, $^3J_{HCCH}$ 7.32 Hz), 2.54 (2H, h, $\underline{CH_2}$, J 7.32 Hz), 3.80 (6H, d, $\underline{CH_3}O$, $^3J_{POCH}$ 11.23 Hz), 10.95 (1H, br s, O$\underline{H}$); ^{13}C ($CDCl_3$) δ 10.2 (s, $\underline{CH_3}CH_2$), 19.9 (d, $\underline{CH_2}$, $^2J_{PCC}$ 16.5 Hz), 53.5 (d, $\underline{CH_3}O$, $^2J_{POC}$ 6.1 Hz), 154.7 (d, $\underline{C}$=NOH, $^1J_{PC}$ 213.0 Hz); ^{31}P ($CDCl_3$) δ 13.6.

14. Preparation of diethyl octanoylphosphonate

Distilled octanoyl chloride (76.5 g, 0.47 mol) was added dropwise to triethyl phosphite (77.2 g, 0.47 mol) stirred under nitrogen at 0 °C. An exothermic reaction resulted in which a colourless gas was evolved. The solution was stirred under nitrogen for 24 h before distilling to give diethyl octanoylphosphonate (64.5 g, 0.24 mol, 52%) as a clear liquid, b.p. 110-12 °C, 0.5 mmHg, ν_{max} (film) cm^{-1} 1025 (P-O-C), 1255 (P=O), 1695 (C=O); ^{1}H ($CDCl_3$) δ 0.88 (3H, t, $\underline{CH}_3$, $^{3}J_{HCCH}$ 5.0 Hz), 1.29 (8H, br s, $\underline{CH}_2$), 1.38 (6H, d of t, $\underline{CH}_3CH_2OP$, $^{3}J_{HCCH}$ 7.32 Hz, $^{4}J_{POCCH}$ 2.44 Hz), 1.87-2.55 (2H, m, $\underline{CH}_2CH_2C=O$), 2.84 (2H, t, $\underline{CH}_2C=O$, $^{3}J_{HCCH}$ 6.84 Hz), 4.23 (4H, qt, $CH_3\underline{CH}_2OP$, J 7.32 Hz); ^{13}C ($CDCl_3$) δ 14.1 (s, $\underline{CH}_3$), 16.5 (d, $\underline{CH}_3CH_2OP$, $^{3}J_{POCC}$ 6.3 Hz), 22.7 (s, $\underline{CH}_2$), 29.1 (s, $\underline{CH}_2$), 34.3 (s, $\underline{CH}_2CH_2C=O$), 43.4 (d, $\underline{CH}_2C=O$, $^{2}J_{PCC}$ 54.9 Hz), 63.8 (d, $\underline{CH}_2OP$, $^{2}J_{POC}$ 7.32 Hz), 211 (d, $\underline{C}=O$, $^{1}J_{PC}$ 165.4 Hz); ^{31}P ($CDCl_3$) δ -3.0.

15. Hydrogenation of diethyl 1-hydroxyiminopropane-phosphonate at low temperature and pressure

Diethyl 1-hydroxyiminopropanephosphonate (1.05 g, 5.00 mmol) was placed in a 500 ml glass vessel together with 5% palladium-on-charcoal catalyst (1.00 g) and glacial acetic acid (75 ml). The mixture was hydrogenated at 38 psi at room temperature for 3 h. The catalyst was filtered and washed with water and the filtrate and washings evaporated. The residue was neutralized with aqueous sodium carbonate and extracted with dichloromethane The organic extracts were dried ($MgSO_4$) and evaporated to give the unreacted diethyl 1-hydroxyiminopropanephosphonate (0.95 g), ^1H ($CDCl_3$) δ 1.15 (3H, t, $\underline{CH}_3$, $^3J_{HCCH}$ 7.8 Hz), 1.35 (6H, t, $OCH_2\underline{CH}_3$, $^3J_{HCCH}$ 7.1 Hz), 2.55 (2H, h, $\underline{CH}_2C(NOH)$, $^3J_{HCCH}$ 7.1 Hz), 4.20 (4H, qt, $O\underline{CH}_2$, $^3J_{HCCH}$ 7.5 Hz), 10.90 (1H, br s, $O\underline{H}$).

16. Hydrogenation of dimethyl 1-hydroxyiminopropane-phosphonate[37]

Dimethyl 1-hydroxyiminopropanephosphonate (2.10 g, 10.0 mmol) was placed in a stainless steel autoclave together with Raney nickel catalyst (2 ml of slurry) and methanol (100 ml). The mixture was hydrogenated at 450 psi at 105 °C for 3 h. The mixture was cooled and filtered and the catalyst washed with methanol. The combined

filtrate and washings were evaporated to give the crude diethyl 1-aminopropanephosphonate (1.55 g) as a pale green oil. The oil was heated under reflux with glacial acetic acid (10 ml) and concentrated hydrochloric acid (32 ml) for 6.5 h. The solution was evaporated and the residue dissolved in ethanol and warmed gently whilst treating with propylene oxide (5 ml). The precipitated solid was filtered and dried (vacuum oven, 60 °C) to give the crude 1-aminopropanephosphonic acid (1.04 g, 7.48 mmol, 75%), m.p. 252-4 °C. The solid was recrystallized from water/methanol (0.97 g), m.p. 262-4 °C (Lit.[23] 264-6 °C), ^{1}H (D_2O) δ 1.05 (3H, t, CH_3, $^{3}J_{HCCH}$ 7.0 Hz), 1.45-2.15 (2H, br m, CH_2), 2.85-3.45 (1H, br m, CH).

17. Hydrogenation of dimethyl 1-hydroxyiminopropane-phosphonate in the presence of ammonia

Dimethyl 1-hydroxyiminopropanephosphonate (21.0 g, 0.116 mol) was placed in a stainless steel autoclave together with 5% palladium-on-charcoal catalyst (7.40 g) and methanol (42 ml). The autoclave was cooled to ca. -60 °C and liquid ammonia (21 ml) added. The mixture was hydrogenated at 550 psi at 117 °C for 3 h. The catalyst was filtered and washed with methanol and the combined filtrate and washings evaporated to give the crude dimethyl 1-aminopropanephosphonate as a pale green oil

(22 g). The oil was heated under reflux with concentrated hydrochloric acid (100 ml) and glacial acetic acid (30 ml) for 8 h. The solution was evaporated and the residue dissolved in methanol and warmed gently whilst treating with propylene oxide. The resulting white solid was filtered and dried (vacuum oven, 60 °C) to give crude 1-aminopropanephosphonic acid (9.84 g, 0.071 mol, 61%), m.p. 262-4 °C, ^{1}H (D_2O) δ 1.05 (3H, t, $C\underline{H}_3$, $^{3}J_{HCCH}$ 7.0 Hz), 1.50-2.20 (2H, br m, $C\underline{H}_2$), 2.80-3.50 (1H, br m, $C\underline{H}$).

A number of hydrogenation experiments have been carried out and their conditions and results are summarised in Table 16.

Hydrogenation of dialkyl 1-hydroxyiminopropanephosphonate

$$A = CH_3CH_2\overset{HO-N}{\overset{\|}{C}}-\overset{O}{\overset{\|}{P}}(OR)_2$$

R =	Mass of A /g	Catalyst		Solvent /ml		P*/psi	T /°C	rxn. time	Yield** /%
Et	1.10	Pd	1.0 g	AcOH	75	38	20	3 h	-
Et	1.00	Pd	0.2 g	EtOH	20	38	20	3 h	-
Et	5.00	Ni	4 ml	EtOH	150	300	100	3 h	60
Me	2.00	Ni	2 ml	MeOH	100	300	100	3 h	72
Me	6.90	Ni	2 ml	MeOH	100	350	100	3 h	41
Me	18.50	Ni	5 ml	MeOH	90	300	100	3 h	27
Me	6.90	Ni	2 ml	MeOH /NH_3	100	400	100	3 h	51
Me	20.00	Ni	5 ml	MeOH NH_3	100 30	400	100	3 h	52
Me	21.00	Pd	7.4 g	MeOH NH_3	42 21	400	115	3 h	61

*Initial pressure at room temperature

**Yield of crude 1-aminopropanephosphonic acid

Table 16

18. Preparation of (1R,S)-1-(L-alanylamino)propane-phosphonic acid

Solid sodium bicarbonate (7.90 g, 0.094 mol) was added to a stirred solution of 1-aminopropanephosphonic acid (4.32 g, 0.031 mol) in water (157 ml) and ethanol (80 ml) at 0 °C. The N-hydroxysuccinimide ester of N-carbobenzoxy-L-alanine (10.0 g, 0.031 mol) in warm ethanol (80 ml) was added dropwise and washed in with ethanol (40 ml). The mixture was stirred at 0 °C for 1 h and then stirred at room temperature overnight after which a homogeneous solution was observed. The solution was evaporated and the residue dissolved in water (280 ml) and extracted with dichloromethane. The aqueous layer was acidified to pH 2 with dilute hydrochloric acid and extracted further with dichloromethane. The aqueous layer was concentrated before passing down a column of cation-exchange resin (Dowex 50 W - X 8(H^+) 16-40 mesh, 52 x 2.5 cm, freshly regenerated in the acid cycle) with water as elutant. The acid fractions were combined and evaporated to give a yellow oil which was stirred at room temperature overnight with hydrobromic acid (45% in glacial acetic acid, 20 ml). The solution obtained was evaporated to give a brown oil which was dissolved in water (150 ml) and extracted with diethyl ether to remove benzyl bromide. The aqueous layer was evaporated and the residual oil dissolved in ethanol (20 ml) before treating with propylene oxide until the maximum amount of a fine white solid had been precipitated. The solid

was dried and recrystallized from water/acetone to give (1R,S)-1-(L-alanylamino)propanephosphonic acid (3.66 g, 0.017 mol, 56%), m.p. 270-4 °C (Lit.[63] 271-5 °C), $[\alpha]_{578}^{30}$ +14(±0.5)° (c = 1% in water) (Lit.[63] +18°), (Calc. for: C, 32.1; H, 7.1; N, 12.2. $C_6H_{15}N_2O_4P.H_2O$ requires: C, 31.6; H, 7.5; N, 12.3%); ^1H (D_2O) δ 0.92 (3H, t, $\underline{CH}_3CH_2$, $^3J_{HCCH}$ 7.31 Hz), 1.57 (3H, d, $\underline{CH}_3CH$, $^3J_{HCCH}$ 6.99 Hz), 1.18-2.13 (2H, br m, $\underline{CH}_2$), 3.72-4.25 (2H, br m, $\underline{CH}$); ^{13}C (D_2O) δ 13.3 (d, $\underline{C}H_3CH_2$, $^3J_{PCCC}$ 13.4 Hz), 19.7 (s, $\underline{C}H_3CH$), 25.7 (s, $\underline{C}H_2$), 52.2 (s, $\underline{C}HCH_3$), 52.5 (s, $\underline{C}HCH_3$), 53.4 (d, $\underline{C}HP$, $^1J_{PC}$ 147.7 Hz), 173.6 (m, $\underline{C}$=O); ^{31}P (D_2O) δ 18.2; m/z (FAB, %) 421 (18.6, $2MH^+$), 350 (4.8, $2MH^+ - C_3H_5NO$), 303 (3.8, MH^+ + G), 232 (2.3, $MH^+ - C_3H_5NO$ + G), 211 (100, MH^+), 140 (20.1, $MH^+ - C_3H_5NO$), 129 (12.8, $MH^+ - H_3PO_3$). Linked scan showed m/z 140 to be a daughter ion of m/z 211.

19. Separation of the diastereoisomers of (1R,S)-1-(L-alanylamino)propanephosphonic acid

Crude (without recrystallization) (1R,S)-1-(L-alanylamino)propanephosphonic acid (1.00 g, 4.76 mmol) was dissolved in the minimum amount of water and passed down a column of cation-exchange resin (Dowex 50 W - X 8(H^+) 100-200 mesh, 20 x 2.5 cm, freshly regenerated in the acid cycle) with water as elutant. Elution was monitored by u.v. absorption (190-230 nm). Fractions of 16 cm were collected. The S,S-dipeptide was obtained from fractions 45-70 (0.45 g), m.p. 281 °C, $[\alpha]_{578}^{25}$ +88(±1)° (c = 1% in water) (Lit.[63] +75°), ^{1}H (D_2O) δ 0.92 (3H, t, $\underline{CH_3}CH_2$, $^3J_{HCCH}$ 7.10 Hz), 1.40-2.20 (2H, br m, $\underline{CH_2}$), 1.60 (3H, d, $\underline{CH_3}CH$, $^3J_{HCCH}$ 7.02 Hz), 3.73-4.10 (1H, m, $\underline{CH}CH_3$), 3.98-4.10 (1H, q, $\underline{CH}CH_3$, $^3J_{HCCH}$ 7.02 Hz); ^{13}C (D_2O) δ 13.3 (d, $\underline{CH_3}CH_2$, $^3J_{PCCC}$ 13.4 Hz), 19.7 (s, $\underline{CH_3}CH$), 25.8 (s, $\underline{CH_2}$), 52.5 (s, $\underline{CH}CH_3$), 53.3 (d, $\underline{CH}P$, $^1J_{PC}$ 147.7 Hz), 173.7 (d, $\underline{C}(O)$, $^3J_{PCNC}$ 4.9 Hz); ^{31}P (D_2O) δ 18.0; m/z (FAB, %) 421 (8.4, $2MH^+$), 233 (12.0), 211 (100, MH^+), 196 (10.6), 140 (21.6, $MH^+ - C_3H_5NO$), 133 (29.2), 131 (30.4), 129 (29.9, $MH^+ - H_3PO_3$), 115 (24.6, $C_5H_{11}N_2O^+$), 113 (20.0), 110 (10.8), 102 (12.3), 100 (14.1), 91 (99.3). The S,R-dipeptide was obtained from fractions 70-100 (0.35 g) m.p. 265 °C, $[\alpha]_{578}^{25}$ -46(±1)° (c = 1% in water) (Lit.[63] -53°), ^{1}H (D_2O) δ 0.92 (3H, t, $\underline{CH_3}CH_2$, $^3J_{PCCC}$ 7.06 Hz), 1.56 (3H, d, $\underline{CH_3}CH$, J_{HCCH} 6.84 Hz), 1.63-2.25 (2H, br m, $\underline{CH_2}CH_3$), 3.72-4.27 (2H, br m, 2 x $\underline{CH}$); ^{13}C (D_2O) δ 13.4 (d, $\underline{CH_3}CH_2$, $^3J_{PCCC}$ 13.4 Hz), 19.6 (s, $\underline{CH_3}CH$), 25.8 (s, $\underline{CH_2}$), 52.2 (s, $\underline{CH}CH_3$), 53.4 (d, $\underline{CH}P$, $^1J_{PC}$ 148.9 Hz), 173.5 (d, $\underline{C}(O)$, $^3J_{PCNC}$ 4.3 Hz); ^{31}P (D_2O) δ 18.8.

20. Preparation of (S)-1-aminopropanephosphonic acid

(1S)-1-(L-alanylamino)propanephosphonic acid ($[\alpha]_{578}^{20}$ +84°, 0.35 g, 1.67 mmol) was dissolved in 6N hydrochloric acid (15 ml) and the solution heated under reflux for 20 h. The solution was evaporated and the residual oil dissolved in methanol and treated with propylene oxide to give a white solid. The solid was filtered and dried under vacuum. The solid was placed in a sublimation apparatus and sublimation carried out at 200 °C, 3.0 mmHg. The non-sublimed solid was collected and recrystallized from water/methanol to give (S)-1-aminopropanephosphonic acid (0.21 g, 1.51 mmol, 91%), m.p. 268-9 °C (Lit.[70] 273-4 °C), $[\alpha]_{578}^{20}$ +12.5(±2)° c = 1% in water (Lit.[70] +21(±1)°, c = 1% in 1N NaOH), ^{1}H (D_2O) δ 1.07 (3H, t, $\underline{CH}_3$, $^{3}J_{HCCH}$ 7.07 Hz), 1.33-2.00 (2H, br m, $\underline{CH}_2$), 2.87-3.37 (1H, m, $\underline{CH}$); ^{13}C (D_2O) δ 13.2 (d, $\underline{C}H_3$, $^{3}J_{PCCC}$ 9.8 Hz), 24.9 (s, $\underline{C}H_2$), 54.1 (d, $\underline{C}HP$, $^{1}J_{PC}$ 141.6 Hz); ^{31}P (D_2O) δ 13.3.

21. Preparation of (1R,S)-1-(L-alanylamino)hexane-phosphonic acid

1-Aminohexanephosphonic acid (1.50 g, 8.29 mmol) was dissolved in water (42 ml) and stirred at 0 °C whilst triethylamine (1.72 g, 0.017 mol) and ethanol (42 ml) were added. The solid N-hydroxysuccinimide ester of N-carbobenzoxy-L-alanine (2.70 g, 8.44 mmol) was added and washed in with ethanol (21 ml). The mixture was stirred at 0 °C for 2 h then at room temperature overnight. The solution was evaporated, the residue dissolved in water (100 ml) and extracted with dichloromethane. The aqueous layer was acididfied to pH 2 with dilute hydrochloric acid and extracted again with dichloromethane. The organic extract was evaporated and the residue stirred overnight with hydrobromic acid (45% in glacial acetic acid, 15 ml) at room temperature. The solution was evaporated and the residue dissolved in water before extracting with diethyl ether to remove benzyl bromide. The aqueous layer was evaporated and the residue dissolved in methanol and treated with propylene oxide to give a white solid. The solid was dried (1.00 g) and recrystallized from water/acetone to give (1R,S)-1-(L-alanylamino)hexanephosphonic acid (0.60 g, 2.38 mmol, 29%), m.p. 228-30 °C, $[\alpha]_{578}^{25}$ 0° (c = 4% in water), m/z (FAB) Found: 253.1315000, $C_9H_{22}N_2O_4P$ requires: 253.1313000; ^1H (D_2O) δ 0.87 (3H, t, CH_3CH_2, $^3J_{HCCH}$ 4.40 Hz), 1.28 (8H, br s, CH_2), 1.48 (3H, d, CH_3CH, $^3J_{HCCH}$ 6.84 Hz), 3.65–4.23 (2H, br m, CH); ^{13}C (D_2O) δ 16.2 (s, CH_3CH_2), 19.1 (s, CH_3CH),

22. Preparation of N-carbobenzoxy-(1R,S)-1-(L-alanylamino-heptanephosphonic acid

1-Aminoheptanephosphonic acid (1.00 g, 5.13 mmol) was suspended in water (26 ml) and ethanol (23 ml) and cooled in ice whilst solid sodium bicarbonate (1.29 g, 15.39 mmol) was added with stirring. A solution of the N-hydroxysuccinimide ester of N-carbobenzoxy-L-alanine (1.66 g, 5.20 mmol) in warm ethanol (10 ml) was added and washed in with ethanol (5 ml). The resulting mixture was stirred at 0 ^{o}C for 2 h and then at room temperature overnight. The clear solution was evaporated and the residue dissolved in water (110 ml) before extracting with dichloromethane . The aqueous layer was acidified to pH 2 with dilute hydrochloric acid and extracted again with dichloromethane. The organic extract was evaporated to give an oily white solid which was recrystallized from dichloromethane to give N-carbobenzoxy-(1R,S)-1-(L-alanyl-amino)heptanephosphonic acid (1.20 g, 3.19 mmol, 62%), m.p. 190-3 ^{o}C, $[\alpha]_{578}^{30}$ -53.0(±1)^{o} (c = 4% in 0.5M NaOH), ν_{max} (KBr) cm^{-1} 1530-1550 (N-H), 1660 (C=O), 1685 (OC=O), 3260 (N-H), 3300 (N-H); ^{1}H (CD_3OD) δ 0.88 (3H, t, CH_3CH_2, $^{3}J_{HCCH}$ 7.00 Hz), 1.30 (8H, br s, CH_2), 1.35 (3H, d, CH_3CH, $^{3}J_{HCCH}$ 7.33 Hz), 1.48-2.07 (2H, br m, CH_2CH_3), 3.27-3.33 (1H, m, $CHCH_2$), 4.01-4.33 (1H, br m, $CHCH_3$), 5.09 (2H, s, CH_2arom), 7.34 (5H, s, arom); ^{13}C (CD_3OD) δ 14.5 (s, CH_3CH_2), 18.4 (s, CH_3CH), 23.7 (s, CH_2), 27.1 (d, CH_2CH_2CH, $^{3}J_{PCCC}$ 13.4 Hz), 29.9 (s, CH_2), 30.5 (s, CH_2), 32.8 (s, CH_2), 67.8 (s, CH_2arom), 128.9 (s, arom), 129.1 (s, arom), 129.6 (s, arom), 138.2 (s, C1-arom), 175.7 (d, C=O, $^{3}J_{PCNC}$

4.27 Hz); ^{31}P (CD_3OD) δ 22.0.

23. Preparation of (1R,S)-1-(L-alanylamino)heptane-phosphonic acid

1-Aminoheptanephosphonic acid (0.52 g, 2.67 mmol) was suspended in water (14 ml) and ethanol (7 ml) and cooled in ice whilst solid sodium bicarbonate (0.67 g, 8.00 mmol) was added with stirring. A solution of the N-hydroxysuccinimide ester of N-carbobenzoxy-L-alanine (0.86 g, 2.70 mmol) in warm ethanol (10 ml) was added and washed in with ethanol (5 ml). The resulting mixture was stirred at 0 °C for 2 h and then at room temperature overnight. The clear solution was evaporated and the residue dissolved in water (60 ml) before extracting with dichloromethane (3 x 25 ml). The aqueous layer was acidified to pH 2 with dilute hydrochloric acid and extracted again with dichloromethane. The organic extract was evaporated to give an oily solid which was stirred overnight with hydrobromic acid (45% in glacial acetic acid, 10 ml) at room temperature. The solution was evaporated and the residue dissolved in water and extracted with diethyl ether. The aqueous layer was evaporated and the residue dissolved in methanol before treating with propylene oxide to give a white solid. The solid was filtered and dried (0.62 g) before recrystallizing from

water to give <u>(1R,S)-1-(L-alanylamino)heptanephosphonic acid</u> (0.57 g, 2.14 mmol, 80.3%), m.p. 278-9 °C, $[\alpha]_{578}^{30}$ +2.6(±0.2)° (c = 2% in water), m/z (FAB) Found: 267.1479000, $C_{10}H_{24}N_2O_4P$ requires: 267.1474000; ^{1}H (D_2O) δ 0.92 (3H, br t, $C\underline{H}_3CH_2$), 1.30 (8H, br s, $C\underline{H}_2$), 1.48 (3H, d, $C\underline{H}_3CH$, $^{3}J_{HCCH}$ 7.32 Hz), 1.48-2.25 (2H, br m, $C\underline{H}_2CH_3$), 3.83-4.25 (2H, br m, $C\underline{H}$); ^{13}C (D_2O) δ 16.3 (s, $\underline{C}H_3CH_2$), 19.2 (s, $\underline{C}H_3CH$), 19.7 (s, $\underline{C}H_3CH$), 24.9 (s, $\underline{C}H_2$), 28.6 (d, $\underline{C}H_2CH_2CH$, $^{3}J_{PCCC}$ 12.2 Hz), 29.2 (s, $\underline{C}H_2$), 30.7 (s, $\underline{C}H_2$), 33.9 (d, $\underline{C}H_2$), 51.8 (d, $\underline{C}HP$, $^{1}J_{PC}$ 147.1 Hz), 52.3 (s, $\underline{C}H_3CH$), 53.7 (s, $\underline{C}H_3CH$), 173.2 (m, $\underline{C}$=O); ^{31}P (D_2O) δ 18.2; m/z (FAB, %) 533 (15.5, $2MH^+$), 267 (79.8, MH^+), 196 (17.6, $MH^+ - C_3H_5NO$), 185 (16.2, $MH^+ - H_3PO_3$), 114 (100, $MH^+ - C_3H_5NO - H_3PO_3$).

24. Preparation of (1R,S)-1-(D-alanylamino)propanephosphonic acid

1-Aminopropanephosphonic acid (2.76 g, 0.020 mol) was stirred in water (100 ml) at 5 °C whilst triethylamine (4 g, 0.040 mol) and ethanol (100 ml) were added. The solution was cooled to 0 °C and the solid N-hydroxysuccinimide ester of N-carbobenzoxy-D-alanine (7.60 g, 0.024 mol) was added and washed in with ethanol (50 ml). The solution was stirred at 0 °C for 2 h and then brought to room temperature and stirred overnight. The solution was evaporated and the residue dissolved in water (200 ml) before extracting with dichloromethane (1 x 60 ml, 2 x 40 ml). The aqueous layer was acidified to pH 2 with dilute hydrochloric acid before extracting again with dichloromethane (3 x 40 ml). The aqueous layer was evaporated, dissolved in water (20 ml) and ethanol (20 ml) and passed down a column of cation-exchange resin (Dowex 50 W - X 8(H^+) 16-40 mesh, 52 x 2.5 cm, freshly regenerated in the acid cycle) with water/ethanol (50:50) as elutant. The acid fractions were combined and evaporated and the residue stirred with hydrobromic acid (45% in glacial acetic acid, 35 ml) at room temperature overnight. The solution was evaporated and dissolved in water before extracting with ether. The aqueous layer was evaporated and dissolved in methanol before treating with propylene oxide to give a white solid (3.24 g). The solid was recrystallized from water/ethanol to give (1R,S)-1-(D-alanylamino)propanephosphonic acid dihydrate (2.66 g, 0.013 mol, 63%), m.p. 247-8 °C,

$[\alpha]_{578}^{25}$ -16(±1)° (c = 1% in water), (Found: C, 30.0; H, 6.9; N, 11.4. $C_6H_{15}N_2O_4P.2H_2O$ requires: C, 29.3; H, 7.7; N, 11.4%); ^1H (D_2O) δ 0.92 (3H, t, $\underline{CH}_3CH_2$, $^3J_{HCCH}$ 7.10 Hz), 1.56 (3H, d, $\underline{CH}_3CH$, $^3J_{HCCH}$ 6.80 Hz), 1.70-2.20 (2H, br m, $\underline{CH}_2$), 3.73-4.15 (2H, br m, $\underline{CH}$); ^{13}C (D_2O) δ 13.4 (d, $\underline{C}H_3CH_2$, $^3J_{PCCC}$ 13.4 Hz), 19.7 (s, $\underline{C}H_3$), 25.8 (s, $\underline{C}H_2$), 52.3 (s, $\underline{C}HCH_3$), 52.6 (s, $\underline{C}HCH_3$), 53.3 (d, $\underline{C}HP$, $^1J_{PC}$ 147.7 Hz), 173.6 (s, $\underline{C}$=O); ^{31}P (D_2O) δ 18.5; m/z (FAB, %) 421 (26.6, $2MH^+$), 350 (6.1, $2MH^+ - C_3H_5NO$), 211 (100, MH^+), 140 (26.8, $MH^+ - C_3H_5NO$), 129 (13.2).

25. Preparation of (1R,S)-1-(DL-alanylamino)propane-phosphonic acid

1-Aminopropanephosphonic acid (2.67 g, 0.019 mol) was dissolved in water (100 ml) and ethanol (50 ml) and solid sodium bicarbonate (4.88 g, 0.058 mol) was added whilst stirring the solution at 0 °C. The N-hydroxysuccinimide ester of N-carbobenzoxy-DL-alanine (6.20 g, 0.019 mol) was dissolved in the minimum amount of warm ethanol, added to the above solution and washed in with ethanol (10 ml). mixture was stirred at 0 °C for 2 h and then brought to room temperature and stirred overnight. The solution was evaporated and re-evaporated with water and the residue dissolved in water (175 ml) and extracted with dichloromethane (3 x 50 ml). The aqueous layer was acidified to pH 2 with 1N hydrochloric acid and extracted again with dichloromethane (2 x 50 ml). The aqueous layer was concentrated and passed down a column of cation-exchange resin (Dowex 50 W - X 8(H^+) 16-40 mesh, 52 x 2.5 cm, freshly regenerated in the acid cycle) with water as elutant. The acid fractions (250 ml) were combined and evaporated and the residue stirred with hydrobromic acid (45% in glacial acetic acid, 20 ml) at room temperature overnight. The solution was evaporated, dissolved in water (50 ml) and extracted with ether to remove benzyl bromide. The aqueous layer was evaporated and the residual oil dissolved in the minimum amount of ethanol and treated with propylene oxide to give a white solid. The solid was filtered and dried to give the crude (1R,S)-1-(DL-alanylamino)propanephosphonic acid (0.10 g,

0.48 mmol, 2.5%), m.p. 257-8 °C, $[\alpha]_{578}^{25}$ 0° (c = 1% in water), ν_{max} (KBr) cm^{-1} 1555 ($^{+}NH_3$), 1670 (C=O), 3290 (N-H); ^1H (D_2O) δ (3H, t, CH_3CH_2, $^3J_{HCCH}$ 7.32 Hz), 1.56 (3H, d, CH_3CH, $^3J_{HCCH}$ 7.32 Hz), 1.65-2.19 (2H, br m, CH_2), 3.70-4.24 (2H, br m, CH).

26. Preparation of (1R.S)-1-(DL-alanylamino)propanephosphonic acid

Triethylamine (1.92 g, 0.019 mol) was added to a stirred solution of N-carbobenzoxy-DL-alanine (4.32 g, 0.019 mol) in dry toluene (160 ml) at room temperature. The solution was cooled to -8 °C and isobutyl chloroformate (2.60 g, 0.019 mol) was added dropwise. The mixture was stirred at -5 to -8 °C for 0.5 h before adding a cooled solution of 1-aminopropanephosphonic acid (2.40 g, 0.017 mol) in triethylamine (3.44 g, 0.034 mol) and aqueous ethanol (10 ml) dropwise. The mixture was stirred at -8 °C for 1 h, at 0 °C for 1 h and was then brought to room temperature and stirred overnight. The mixture was evaporated and the residue dissolved in water (150 ml) before being extracted with dichloromethane. The aqueous layer was acidified to pH 2 with dilute hydrochloric acid and extracted again with dichloromethane. The aqueous layer was evaporated and the residue stirred overnight with hydrobromic acid

(45% in glacial acetic acid, 15 ml) at room temperature. The solution was evaporated and the residue dissolved in water before extracting with diethyl ether. The aqueous layer was evaporated and the residue dissolved in warm methanol. The solution was warmed gently whilst treating with propylene oxide to give a white solid. The solid was filtered and dried before treating with decolourizing charcoal and recrystallizing from water/ethanol to give <u>(1R,S)-1-(DL-alanylamino)propane-phosphonic acid</u> (1.10 g, 5.24 mmol, 31%), m.p. 245-6 ^{o}C, $[\alpha]_{578}^{25}$ 0^{o} (c = 1% in water), ^{1}H (D_2O) δ 0.93 (3H, t, $C\underline{H}_3CH_2$, $^{3}J_{HCCH}$ 7.32 Hz), 1.55 (3H, d, $C\underline{H}_3CH$, $^{3}J_{HCCH}$ 7.32 Hz), 1.64-2.20 (2H, br m, $C\underline{H}_2$), 3.67-4.25 (2H, br m, $C\underline{H}$); ^{31}P (D_2O) δ 13.5, 18.4.

27. Preparation of (1R.S)-1-(DL-alanylamino)propane-phosphonic acid

1-Aminopropanephosphonic acid (2.76 g, 0.020 mol) was dissolved in water (100 ml) and the solution stirred at 0 °C whilst triethylamine (4.00 g,0.04 mol) and ethanol (100 ml) were added. The solid N-hydroxysuccinimide ester of N-carbobenzoxy-DL-alanine (7.60 g, 0.024 mol) was added and washed in with ethanol (50 ml). The solution was stirred at 0 °C for 2 h and then brought to room temperature and stirred overnight. The solution was evaporated and the residue dissolved in water (200 ml) before extracting with dichloromethane (1 x 60 ml, 2 x 40 ml). The aqueous layer was acidified to pH 2 with 1N hydrochloric acid and extracted again with dichloromethane (2 x 50 ml). The aqueous layer was evaporated and the residue dissolved in water (20 ml) and methanol (20 ml) before passing down a column of cation -exchange resin (Dowex 50 W - X 8(H^+) 16-40 mesh, 52 x 2.5 cm, freshly regenerated in the acid cycle) with water/methanol (50:50) as elutant. The acid fractions were combined and evaporated and the residual oil stirred overnight with hydrobromic acid (45% in glacial acetic acid, 35 ml) at room temperature. The solution was evaporated and the residue dissolved in water (75 ml) before extracting with ether. The aqueous layer was evaporated and the residue dissolved in the minimum amount of methanol before treating with propylene oxide to give a white solid. The solid was filtered and dried (3.21 g) and recrystallized from water/ acetone to give (1R.S)-1-(DL-alanylamino)propanephosphonic-

acid (1.85 g, 8.81 mmol, 66%), m.p. 260-2 °C, $[\alpha]_{578}^{22}$ 0° (c = 1% in water), m/z (FAB) Found: 211.0855000, $C_6H_{16}N_2O_4P$ requires: 211.0848000; ^1H (D_2O) δ 0.92 (3H, t, $\underline{CH}_3CH_2$, $^3J_{HCCH}$ 7.32 Hz), 1.56 (3H, d, $\underline{CH}_3CH$, $^3J_{HCCH}$ 6.84 Hz), 1.63-2.25 (2H, br m, $\underline{CH}_2CH$), 3.72-4.27 (2H, m, $\underline{CH}$); ^{13}C (D_2O) δ 13.3 (d, $\underline{C}H_3CH_2$, $^3J_{PCCC}$ 13.4 Hz), 19.7 (s, $\underline{C}H_3CH$), 25.7 (s, $\underline{C}H_2CH$), 52.2 (s, $\underline{C}HCH_3$), 52.5 (s, $\underline{C}HCH_3$), 53.3 (d, $\underline{C}HP$, $^1J_{PC}$ 147.7 Hz), 173.7 (m, $\underline{C}$=O); ^{31}P (D_2O) δ 18.4.

28. Preparation of (1R,S)-1-(glycylamino)propanephosphonic acid

1-Aminopropanephosphonic acid (2.76 g, 0.020 mol) was stirred in water (5 ml) at 0 °C whilst triethylamine (4.00 0.040 mol) and dimethylformamide (10 ml) were added. The solid N-hydroxysuccinimide ester of N-carbobenzoxyglycine (7.65 g, 0.025 mol) was added in a single portion. The mixture was stirred for 3 h at 0 °C then brought to room temperature and stirred for 16 h. The resulting solution was evaporated and the residue dissolved in water (50 ml) before extracting with dichloromethane (3 x 25 ml). The aqueous layer was acidified to pH 2 with 5N hydrochloric acid and extracted again with dichloromethane (3 x 25 ml). The combined dichloromethane extracts were back-extracted with water and the combined aqueous layers were concentrated before passing down a column of cation-exchange resin (Dowex 50 W - X 8(H^+) 16-40 mesh, 52 x 2.5 cm, freshly regenerated in the acid cycle) with water as elutant. The acid fractions were combined and evaporated and the residue stirred overnight with hydrobromic acid (45% in glacial acetic acid, 20 ml) at room temperature. The solution was evaporated and the residue dissolved in water before extracting with ether. The aqueous layer was concentrated and dissolved in methanol before treating with propylene oxide to give a white solid. The solid was filtered and dried (3.39 g) before recrystallizing from water to give (1R,S)-1-(glycylamino)propanephosphonic acid (3.20 g, 0.016 mol, 81.6%), m.p. 254-5 °C, (Lit.[63] 269-72 °C),

as its monohydrate, $[\alpha]_{578}^{20}$ 0° (c = 1% in water), (Found: C, 28.7; H, 6.6; N, 13.3. $C_5H_{15}N_2O_5P$ requires: C, 28.4; H, 7.0; N, 13.1%); m/z (FAB) Found: 197.0695000, $C_5H_{13}N_2O_4P$ requires: 197.0691000; ^1H (D_2O) δ 0.90 (3H, t, $\underline{CH}_3CH_2$, $^3J_{PCCC}$ 7.0 Hz), 1.55-2.20 (2H, br m, $\underline{CH}_2CH_3$), 3.93 (2H, s, $\underline{CH}_2NH_2$); ^{13}C (D_2O) δ 13.4 (d, $\underline{CH}_3CH_2$, $^3J_{PCCC}$ 13.4 Hz), 25.8 (s, $\underline{CH}_2CH_3$), 43.6 (s, $\underline{CH}_2NH_2$), 53.4 (d, $\underline{C}HP$, $^1J_{PC}$ 147.7 Hz), 169.8 (d, $\underline{C}$=O, $^3J_{PCNC}$ 4.9 Hz); ^{31}P (D_2O) δ 18.28; m/z (FAB, %) 393 (15.8, $2MH^+$), 336 (4.0, $2MH^+$ - C_2H_3NO), 289 (12.3, MH^+ + G), 197 (100, MH^+), 140 (22.8, MH^+ - C_2H_3NO), 115 (17.4, MH^+ - H_3PO_3).

29. Preparation of (1R,S)-1-(DL-butyrylamino)propane-phosphonic acid

Triethylamine (3.67 g, 0.037 mol) was added to a stirred solution of 1-aminopropanephosphonic acid (2.53 g, 0.018 mol) in water (92 ml) at 0 °C. Ethanol (92 ml) was added together with the N-hydroxysuccinimide ester of N-carbobenzoxy-DL-butyrine (7.22 g, 0.022 mol) as an oil which was washed in with ethanol (46 ml). The mixture was stirred at 0 °C for 2 h then brought to room temperature and stirred overnight. The homogeneous solution was evaporated and the residue dissolved in water (150 ml) and extracted with dichloromethane. The aqueous layer was acidified to pH 2 with dilute hydrochloric acid and extracted again with dichloromethane. The organic layer was back-washed with water and the combined aqueous layers evaporated to give a yellow oil. The oil was stirred overnight with hydrobromic acid (45% in glacial acetic acid, 20 ml) at room temperature. The resulting solution was evaporated and the residue dissolved in water before extracting with ether. The aqueous layer was evaporated and the residue dissolved in ethanol (20 ml) and treated with propylene oxide to give a fine white solid. The solid was filtered and dried before recrystallizing from water/ethanol to give (1R,S)-1-(DL-butyrylamino)propane-phosphonic acid (1.2 g, 5.36 mmol, 30%), m.p. 260-2 °C, $[\alpha]_{578}^{28}$ 0° (c = 1% in water), (Found: C, 37.3; H, 7.5; N, 13.4. $C_7H_{17}N_2O_4P$ requires: C, 37.5; H, 7.6; N, 12.5%);

^{1}H (D_2O) δ 0.93 (3H, t, $\underline{CH_3}CH_2$, $^{3}J_{HCCH}$ 7.21 Hz), 1.03 (3H, t, $\underline{CH_3}CH_2CHP$, $^{3}J_{HCCH}$ 6.84 Hz), 1.41-2.16 (2H, br m, $\underline{CH_2}CHP$), 1.90 (2H, q, $\underline{CH_2}CH_3$, $^{3}J_{HCCH}$ 6.92 Hz), 3.67-4.09 (2H, br m, $\underline{CH}$); ^{13}C (D_2O) δ 11.4 (s, $\underline{CH_3}$), 13.6 (d, $\underline{CH_3}$, $^{3}J_{PCCC}$ 13.4 Hz), 25.7 (s, $\underline{CH_2}CHP$), 27.2 (s, $\underline{CH_2}$), 53.5 (d, $\underline{CHP}$, $^{1}J_{PC}$ 147.7 Hz), 57.9 (s, $\underline{CH}$), 172.8 (br s, $\underline{C}$=O); ^{31}P (D_2O) δ 17.9; m/z (FAB, %) 897 (0.7, $4MH^+$), 673 (2.3, $3MH^+$), 449 (22.0, $2MH^+$), 225 (86.0, MH^+), 140 (6.7, $MH^+ - C_4H_7NO$), 58 (100, $MH^+ - C_4H_7NO - H_3PO_3$).

30. Preparation of (1R,S)-1-(DL-phenylalanylamino)propane-phosphonic acid

1-Aminopropanephosphonic acid (1.57 g, 0.011 mol) was stirred in water (3 ml) at 0 °C whilst triethylamine (2.30 g, 0.023 mol) and dimethylformamide (5.5 ml) were added. The solid N-hydroxysuccinimide ester of N-carbobenzoxy-DL-phenylalanine (4.50 g, 0.011 mol) was added and the mixture stirred at 0 °C for 3 h and then brought to room temperature and stirred overnight. The solution was evaporated and the residue dissolved in water (75 ml) before extracting with dichloromethane. The aqueous layer was acidified to pH 2 with dilute hydrochloric acid and extracted with dichloromethane. The organic layer was evaporated and the resulting oil stirred overnight with hydrobromic acid (45% in glacial acetic acid, 15 ml) at room temperature. The solution was evaporated and the residue dissolved in water (30 ml) before extracting with ether. The aqueous layer was evaporated to give an oil. The oil was dissolved in ethanol and treated with propylene oxide when a white solid was precipitated. The solid was filtered and dried before recrystallizing from water/acetone to give (1R,S)-1-(DL-phenylalanylamino)-propanephosphonic acid (1.44 g, 5.04 mmol, 46%), m.p. 243-4 °C, $[\alpha]_{578}^{20}$ 0° (c = 1% in water), as its monohydrate, (Found: C, 47.1; H, 6.6; N, 9.6. $C_{12}H_{19}N_2O_4P.H_2O$ requires: C, 47.4; H, 6.9; N, 9.2%); ^{13}C (D_2O/H_2SO_4) δ 13.0 (d, $\underline{C}H_3CH_2$, $^3J_{PCCC}$ 17.7 Hz), 24.5 (s, $\underline{C}H_2$), 39.7 (s, $\underline{C}H_2$arom), 52.0 (d, $\underline{C}HP$, $^1J_{PC}$ 152.6 Hz), 57.6 (s, $\underline{C}HCH_2$arom), 58.0 (s, $\underline{C}HCH_2$arom), 131.1 (s, $\underline{C}$4-arom), 132.3-132.6 (m, $\underline{C}$2, $\underline{C}$3, $\underline{C}$5, $\underline{C}$6-arom), 136.2 (s, $\underline{C}$1-arom), 136.4 (s, $\underline{C}$1-arom),

171.9-172.5 (m, C=O); ^{31}P (D_2O) δ 17.9 ; m/z (FAB, %) 573 (10.3, $2MH^+$), 287 (58.3, MH^+), 205 (6.5, $MH^+-H_3PO_3$), 120 (100, $MH^+-C_4H_9NO_4P$), 91 (13.0, $C_7H_7^+$).

31. Preparation of (1R,S)-1-(L-alanyl-L-alanylamino)propane phosphonic acid

(1R,S)-1-(L-alanylamino)propanephosphonic acid (1.05 g, 0.005 mol), was dissolved in water (25 ml) and ethanol (12.5 ml) and treated with solid sodium bicarbonate (1.26 g, 0.015 mol) whilst stirring at 0 °C. A solution of the N-hydroxysuccinimide ester of N-carbobenzoxy-L-alanine (1.60 g, 0.005 mol) in warm ethanol (16 ml) was added dropwise and washed in with ethanol (2 x 5 ml). The mixture was stirred at 0 °C for 2 h and then brought to room temperature and stirred for 16 h. The solution was evaporated and then re-evaporated with water (10 ml). The residue was dissolved in water (100 ml) and extracted with dichloromethane (1 x 100 ml, 2 x 50 ml). The aqueous layer was acidified to pH 2 with 2N hydrochloric acid before extracting again with dichloromethane (1 x 100 ml, 2 x 50 ml). The aqueous layer was evaporated and the residue dissolved in the minimum amount of 2N ammonium hydroxide solution. The solution was passed down a column of cation-exchange resin (Dowex 50 W - X 8(H^+) 16-40 mesh, 52 x 2.5 cm, freshly regenerated in the acid cycle) with water as elutant. The acid fractions (300 ml) were combined and evaporated and re-evaporated with water. The resulting oil was stirred with hydrogen bromide (45% in glacial acetic acid, 20 ml) at room temperature overnight. The solution was evaporated and the residue dissolved in water before extracting with ether. The aqueous layer was evaporated and the residue dissolved

in methanol (10 ml) and treated with propylene oxide to give a white solid which turned to a colourless jelly on standing. The precipitate was filtered and dried to give a white solid (1.10 g) which was recrystallized from water/acetone to give (1R,S)-1-(L-alanyl-L-alanylamino)-propanephosphonic acid (0.95 g, 3.38 mmol, 67.5%), m.p. 245 °C, $[\alpha]_{578}^{30}$ -31.0(±1)° (c = 1% in water), as its dihydrate, (Found: C, 33.2; H, 6.9; N, 11.9; $C_9H_{20}N_3O_5P.2H_2O$ requires: C, 34.1; H, 7.6; N, 13.3%); ^1H (D_2O) δ 0.89 (3H, t, $\underline{CH}_3CH_2$, $^3J_{HCCH}$ 7.00 Hz), 1.49 (6H, m, $\underline{CH}_3CH$), 1.75-2.10 (2H, br m, $\underline{CH}_2$), 3.68-4.14 (1H, br m, $\underline{CH}CH_2$), 4.39 (2H, q, $\underline{CH}CH_3$, $^3J_{HCCH}$ 8.06 Hz); ^{13}C (D_2O) δ 13.3 (d, $\underline{CH}_3CH_2$, $^3J_{PCCC}$ 13.4 Hz), 19.4 (s, $\underline{CH}_3CH$), 19.8 (s, $\underline{CH}_3CH$), 26.0 (s, $\underline{CH}_2$), 51.8 (s, $\underline{CH}CH_3$), 52.8 (s, $\underline{CH}CH_3$), 53.0 (d, $\underline{CH}P$, $^1J_{PC}$ 147.7 Hz), 53.1 (s, $\underline{CH}CH_3$), 173.4 (s, $\underline{C}=O$), 173.5 (s, $\underline{C}=O$), 177.2 (d, $\underline{C}=O$, $^3J_{PCNC}$ 5.5 Hz), 177.4 (d, $\underline{C}=O$, $^3J_{PCNC}$ 4.9 Hz); ^{31}P (D_2O) δ 18.3; m/z (FAB, %) 374 (4.1, MH^+ + G), 282 (100, MH^+), 211 (21.4, $MH^+ - C_3H_5NO$), 143 (12.8, $C_6H_{11}N_2O_2^+$), 140 (10.7, $MH^+ - 2.C_3H_5NO$), 115 (58.4, $C_5H_{11}N_2O^+$).

32. Separation of the diastereoisomers of (1R,S)-1-(L-alanyl-L-alanylamino)propanephosphonic acid

Crude (without recrystallization) (1R,S)-1-(L-alanyl-L-alanylamino)propanephosphonic acid (0.80 g, 2.85 mmol) was dissolved in the minimum amount of water and passed down a column of cation-exchange resin (Dowex 50 W - 8(H^+) 100-200 mesh, 20 x 2.5 cm, freshly regenerated in the acid cycle) with water as elutant. Elution was monitored by u.v. absorption (190-230 nm). Fractions of 16 cm^3 were collected. The first diastereoisomer was collected from fractions 35-55 (0.18 g), m.p. 258-60 °C,, $[\alpha]_{578}^{25}$ -52.0(±1)° (c = 4% in water); ^1H (D_2O) δ 0.92 (3H, t, $\underline{CH_3}CH_2$, $^3J_{HCCH}$ 7.32 Hz), 1.43 (3H, d, $\underline{CH_3}CH$, $^3J_{HCCH}$ 6.84 Hz), 1.56 (3H, d, $\underline{CH_3}CH(NH_2)$, $^3J_{HCCH}$ 6.84 Hz), 1.60-2.25 (2H, m, $\underline{CH_2}$), 3.67-4.57 (3H, br m, $\underline{CH}$); ^{13}C (D_2O) δ 13.3 (d, $\underline{CH_3}CH_2$, $^3J_{PCCC}$ 13.4 Hz), 19.4 (s, $\underline{CH_3}CH$), 25.9 (s, $\underline{CH_2}$), 51.8 (s, $\underline{CHCH_3}(NH_2)$), 52.8 (s, $\underline{CHCH_3}$) 53.0 (d, $\underline{CHP}$, $^1J_{PC}$ 147.7 Hz), 173.5 (s, $\underline{C}$=O), 177.3 (br s, $\underline{CONHCHP}$); ^{31}P (D_2O) δ 18.3. The second diastereoisomer was obtained from fractions 60-70 (0.16 g), m.p. 261-2 °C, $[\alpha]_{578}^{25}$ -14.3(±1)° (c = 4% in water); ^{13}C (D_2O) δ 13.3 (d, $\underline{CH_3}CH_2$, $^3J_{PCCC}$ 12.2 Hz), 19.4 (s, $\underline{CH_3}CH$), 26.1 (s, $\underline{CH_2}$), 52.2 (s, $\underline{CHCH_3}(NH_2)$), 53.0 (d, $\underline{CHP}$, $^1J_{PC}$ 147.1 Hz), 53.1 (s, $\underline{CHCH_3}$), 173.6 (s, $\underline{C}$=O), 177.4 (br s, CONHCHP) ^{31}P (D_2O) δ 18.3. Fractions 56-59 contained a mixture of isomers (0.06 g).

33. Preparation of (1R,S)-1-(D-alanyl-D-alanylamino)-propanephosphonic acid

(1R,S)-1-(D-alanylamino)propanephosphonic acid (0.60 g, 2.86 mmol) was dissolved in water (14 ml) and stirred at 0 °C whilst triethylamine (0.58 g, 5.76 mmol) and ethanol (14 ml) were added. The solid N-hydroxy-succinimide ester of N-carbobenzoxy-D-alanine (1.10 g, 3.44 mmol) was added and washed in with ethanol (7 ml). The mixture was stirred at 0 °C for 2 h then at room temperature overnight. The solution was evaporated and the residue dissolved in water (62 ml) and extracted with dichloromethane. The aqueous layer was acididfied to pH 2 with dilute hydrochloric acid and extracted again with dichloromethane. The aqueous layer was evaporated and the residue stirred overnight with hydrobromic acid (45% in glacial acetic acid, 10 ml) at room temperature. The solution was evaporated and the residue dissolved in water before extracting with diethyl ether to remove benzyl bromide. The aqueous layer was evaporated and the residue dissolved in methanol and treated with propylene oxide to give a white solid. The solid was dried and recrystallized from water/ethanol to give (1R,S)-1-(D-alanyl-D-alanylamino)propanephosphonic acid (0.30 g, 1.07 mmol, 37%), m.p. 242-4 °C, $[\alpha]_{578}^{30}$ +18.2(±0.3)° (c = 12.5% in water), ν_{max} (KBr) cm^{-1} 1560 ($^{+}NH_3$), 1650 (C=O), 3300 (N-H); ^{1}H (D_2O) δ 0.92 (3H, t, CH_3CH_2, $^{3}J_{HCCH}$ 6.30 Hz), 1.37-1.60 (6H, m, CH_3CH), 1.65-2.20 (2H, br m, CH_2), 3.63-4.25 (3H, br m, CH); ^{13}C (D_2O) δ 13.3 (d, CH_3CH_2, $^{3}J_{PCCC}$ 13.4 Hz), 19.4

(s, $\underline{C}H_3CH$), 19.8 (s, $\underline{C}H_3CH$), 26.0 (s, $\underline{C}H_2$), 51.9 (s, $\underline{C}HCH_3$), 53.0 (s, $\underline{C}HCH_3$), 53.0 (d, $\underline{C}HP$, $^1J_{PC}$ 147.7 Hz), 173.5 (s, $\underline{C}$=O), 177.3 (d, $\underline{C}$=O, $^3J_{PCNC}$ 3.7 Hz); ^{31}P (D_2O) δ 18.3.

34. Preparation of (1R,S)-1-(D-alanyl-L-alanylamino)propanephosphonic acid

A solution of (1R,S)-1-(L-alanylamino)propanephosphonic acid (0.83 g, 3.95 mmol) in water (20 ml) was stirred at 0 °C whilst triethylamine (0.80 g, 7.90 mmol) and ethanol were added. The solid N-hydroxysuccinimide ester of N-carbobenzoxy-D-alanine (1.52 g, 4.75 mmol) was added and washed in with ethanol (10 ml). The mixture was stirred at 0 °C for 2 h and then brought to room temperature and stirred overnight. The solution was evaporated and the residue dissolved in water (85 ml) before extracting with dichloromethane. The aqueous layer was acidified to pH 2 with dilute hydrochloric acid and extracted again with dichloromethane. The aqueous layer was evaporated and the residue stirred overnight with hydrobromic acid (45% in glacial acetic acid 10 ml) at room temperature. The resulting solution was evaporated and the residue dissolved in water (30 ml) before extracting with diethyl ether. The aqueous layer was evaporated and the residue dissolved in methanol (10 ml) and treated with propylene oxide to give a white solid which was filtered and dried (1.11 g). The solid was treated with decolourising charcoal before recrystallizing from water/acetone to give (1R,S)-1-(D-alanyl-L-alanylamino)propanephosphonic acid (0.54 g, 1.92 mmol, 49%) as a crystalline, white dihydrate, m.p. 240-3 °C, $[\alpha]_{578}^{25}$ -11.3(±1)° (c = 4% in water), (Found: C, 34.2; H, 7.0; N, 13.0; $C_9H_{20}N_3O_5P.2H_2O$ requires: C, 34.1; H, 7.6; N, 13.3%); ν_{max} (KBr) cm^{-1} 1550 ($^+NH_3$), 1660-1640 (C=O), 3280 (N-H); ^1H (D_2O) δ 0.91 (3H, t, CH_3CH_2,

$^{3}J_{HCCH}$ 6.90 Hz), 1.43 (3H, d, $C\underline{H}_3CH$, J_{HCCH} 7.30 Hz), 1.55 (3H, d, $C\underline{H}_3CH$, $^{3}J_{HCCH}$ 7.32 Hz), 1.63-2.22 (2H, br m, $C\underline{H}_2CH_3$), 3.59-4.45 (3H, br m, C<u>H</u>); ^{13}C (D_2O) δ 13.4 (d, $\underline{C}H_3CH_2$, $^{3}J_{PCCC}$ 12.8 Hz), 19.5 (s, $\underline{C}H_3CH$), 19.9 (s, $\underline{C}H_3CH$), 26.0 (s, $\underline{C}H_2$), 52.0 (s, $\underline{C}HCH_3$), 52.9 (s, $\underline{C}HCH_3$), 53.0 (d, $\underline{C}HP$, $^{1}J_{PC}$ 147.1 Hz), 53.2 (s, $\underline{C}HCH_3$), 173.5 (s, $\underline{C}$=O), 177.3 (d, $\underline{C}$=O, $^{3}J_{PCNC}$ 6.1 Hz); ^{31}P (D_2O) δ 18.4.

35. <u>Preparation of (1R,S)-1-(D-alanyl-L-alanylamino)-propanephosphonic acid</u>

Triethylamine (0.23 g, 2.24 mmol) was added to a stirred solution of <u>N</u>-carbobenzoxy-D-alanine (0.50 g, 2.24 mmol) in dry toluene (30 ml) at room temperature. The solution was cooled to -8 °C and isobutyl chloroformate (0.31 g, 2.24 mmol) was added dropwise. The mixture was stirred at -5 - -8 °C for 0.5 h before adding a cooled solution of (1R,S)-1-(L-alanylamino)-propanephosphonic acid (0.40 g, 1.87 mmol) in triethylamine (0.38 g, 3.74 mmol) and aqueous ethanol (10 ml) dropwise. The mixture was stirred at -8 °C for 1 h, at 0 °C for 1 h and was then brought to room temperature and stirred overnight. The mixture was evaporated and the residue dissolved in water (45 ml) before being extracted with dichloromethane. The aqueous layer was acidified to pH 2 with dilute hydrochloric acid and extracted again with dichloromethane. The

aqueous layer was evaporated and the residue stirred overnight with hydrobromic acid (45% in glacial acetic acid, 10 ml) at room temperature. The solution was evaporated and the residue dissolved in water before extracting with diethyl ether. The aqueous layer was evaporated and the residue dissolved in warm methanol. The solution was warmed gently whilst treating with propylene oxide to give a white solid. The solid was filtered and dried before treating with decolourizing charcoal and recrystallizing from water/ethanol to give

<u>(1R,S)-1-(D-alanyl-L-alanylamino)propanephosphonic acid</u>

(0.20 g, 7.12 mmol, 38%), m.p. 251-3 °C, $[\alpha]_{578}^{25}$ -19.6(+1) (c = 4% in water), ^{1}H (D_2O) δ 0.91 (3H, t, $C\underline{H}_3CH_2$, $^{3}J_{HCCH}$ 7.32 Hz), 1.42 (3H, d, $C\underline{H}_3CH$, $^{3}J_{HCCH}$ 7.31 Hz), 1.56 (3H, d, $C\underline{H}_3CH$, $^{3}J_{HCCH}$ 7.32 Hz), 1.65-2.22 (2H, br m, $C\underline{H}_2$), 3.55-4.50 (3H, br m, $C\underline{H}$); ^{31}P (D_2O) δ 18.4.

36. Preparation of (1R,S)-1-(L-alanyl-D-alanylamino)propanephosphonic acid

A solution of (1R,S)-1-(D-alanylamino)propanephosphonic acid (1.93 g, 9.17 mmol) in water (46 ml) was stirred at 0 °C whilst triethylamine (1.84 g, 0.018 mol) and ethanol were added. The solid N-hydroxysuccinimide ester of N-carbobenzoxy-L-alanine (3.50 g, 0.011 mol) was added and washed in with ethanol (23 ml). The mixture was stirred at 0 °C for 2 h then brought to room temperature and stirred overnight. The solution was evaporated and the residue dissolved in water (200 ml) and extracted with dichloromethane. The aqueous layer was acidified to pH 2 with dilute hydrochloric acid and extracted again with dichloromethane. The aqueous layer was concentrated to precipitate a small amount of white solid which was removed by filtration. The filtrate was evaporated to give a yellow oil which was stirred overnight with hydrobromic acid (45% in glacial acetic acid, 15 ml) at room temperature. The resulting solution was evaporated and the residue dissolved in water (50 ml) before extracting with ether to remove benzyl bromide. The aqueous layer was evaporated and the residue dissolved in ethanol (15 ml) before treating with propylene oxide to give a white gelatinous solid. The solid was filtered and dried before recrystallizing from water to give (1R,S)-1-(L-alanyl-D-alanylamino)propanephosphonic acid (0.84 g, 2.99 mmol, 33%) as colourless needles, m.p. 264-5 °C, $[\alpha]_{578}^{25}$ +37.5(±2)° (c = 1% in water), m/z (FAB) Found: 282.1215600, $C_9H_{21}N_3O_5P$ requires: 282.1218600; ^1H (D_2O) δ 0.94 (3H, t, CH_3CH , $^3J_{HCCH}$ 7.08 Hz), 1.53 (6H, m, CH_3CH),

1.71-2.05 (2H, m, $\underline{CH}_2$), 3.75-4.60 (3H, br m, $\underline{CH}$); ^{13}C (D_2O) δ 13.3 (d, $\underline{CH}_3CH_2$, $^3J_{HCCH}$ 13.4 Hz), 19.4 (s, $\underline{CH}_3CH$), 19.9 (s, $\underline{CH}_3CH$), 26.0 (s, $\underline{CH}_2$), 52.1 (s, $\underline{CH}CH_3$), 53.0 (s, $\underline{CH}CH_3$), 53.1 (d, $\underline{CHP}$, $^1J_{PC}$ 148.3 Hz), 173.7 (s, $\underline{C}(O)CHNH_2$), 177.3 (d, $\underline{C}(O)NHCHP$, $^3J_{PCNC}$ 6.1 Hz); ^{31}P (D_2O) δ 18.3; m/z (FAB, %) 563 (11.8, $2MH^+$), 282 (100, MH^+), 211 (26.4, $MH^+ - C_3H_5NO$), 143 (18.3, $C_6H_{11}N_2O_2^+$), 140 (7.4, $MH^+ - 2.C_3H_5NO$), 115 (16.7, $C_5H_{11}N_2O^+$).

37. Preparation of (1R,S)-1-(DL-alanyl-DL-alanylamino)-propanephosphonic acid

(1R,S)-1-(DL-alanylamino)propanephosphonic acid (0.56 g, 2.67 mmol) was dissolved in water (16 ml) and stirred at 0 °C whilst triethylamine (0.54 g, 5.34 mmol) and ethanol (16 ml) were added. The solid N-hydroxy-succinimide ester of N-carbobenzoxy-DL-alanine (1.03 g, 3.20 mmol) was added and washed in with ethanol (8 ml). The mixture was stirred at 0 °C for 2 h then at room temperature overnight. The solution was evaporated and the residue dissolved in water (60 ml) and extracted with dichloromethane. The aqueous layer was acididfied to pH 2 with dilute hydrochloric acid and extracted again with dichloromethane. The aqueous layer was evaporated and the residue stirred overnight with hydrobromic acid (45% in glacial acetic acid, 15 ml) at room temperature. The solution was evaporated and the residue dissolved in water before extracting with diethyl ether to remove benzyl bromide. The aqueous layer was evaporated and the residue dissolved in methanol and treated with propylene oxide to give a white solid. The solid was dried (0.70 g), treated with decolourizing charcoal and recrystallized from water/ethanol to give (1R,S)-1-(DL-alanyl-DL-alanylamino)propanephosphonic acid (0.60 g, 2.14 mmol, 80%), m.p. 239-42 °C, $[\alpha]_{578}^{25}$ 0° (c = 4% in water), ^{1}H (D_2O) δ 0.90 (3H, t, CH_3CH_2, $^{3}J_{HCCH}$ 6.84 Hz), 1.40-1.60 (6H, m, CH_3CH), 1.71-2.23 (2H, br m, CH_2), 3.61-4.46 (3H, br m, CH); ^{13}C (D_2O) δ 13.3 (d, CH_3CH_2, $^{3}J_{PCCC}$ 13.4 Hz), 19.4 (s, CH_3CH),

19.9 (s, $\underline{C}H_3CH$), 26.0 (s, $\underline{C}H_2$), 52.0 (s, $\underline{C}HCH_3$), 52.5 (s, $\underline{C}HCH_3$), 52.9 (s, $\underline{C}HCH_3$), 53.0 (d, $\underline{C}HP$, $^1J_{PC}$ 147.1 Hz), 53.1 (s, $\underline{C}HCH_3$), 173.6 (s, $\underline{C}$=O), 177.4 (s, $\underline{C}$=O); ^{31}P (D_2O) δ 18.3.

38. Preparation of (1R,S)-1-(L-alanylglycylamino)propane-phosphonic acid

(1R,S)-1-(glycylamino)propanephosphonic acid (1.30 g, 6.63 mmol) was dissolved in water (33 ml) and stirred at 0 °C whilst triethylamine (1.34 g, 13.30 mmol) and ethanol (33 ml) were added. The solid N-hydroxysuccinimide ester of N-carbobenzoxy-L-alanine (2.55 g, 7.96 mmol) was added and washed in with ethanol (17 ml). The mixture was stirred at 0 °C for 2 h then at room temperature overnight. The solution was evaporated and the residue dissolved in water (200 ml) and extracted with dichloromethane. The aqueous layer was acidified to pH 2 with dilute hydrochloric acid and extracted again with dichloromethane. The aqueous layer was evaporated and the residue stirred overnight with hydrobromic acid (45% in glacial acetic acid, 20 ml) at room temperature. The solution was evaporated and the residue dissolved in water before extracting with diethyl ether to remove benzyl bromide. The aqueous layer was evaporated and the residue dissolved in methanol and treated with propylene oxide to give a white, jelly-like solid. The solid was dried and recrystallized from water/acetone to give (1R,S)-1-(L-alanylglycylamino)propanephosphonic acid (1.28 g, 4.79 mmol, 72%), m.p. 255-6 °C, $[\alpha]_{578}^{30}$ +16(±0.5)° (c = 1% in water), m/z (FAB) Found: 268.1067000, $C_8H_{19}N_3O_5P$ requires: 268.1062000; ^1H (D_2O) δ 0.91 (3H, t, $C\underline{H}_3CH_3$, $^3J_{HCCH}$ 7.33 Hz), 1.57 (3H, d, $C\underline{H}_3CH$, $^3J_{HCCH}$ 7.32 Hz), 1.67-2.06 (2H, br m, $C\underline{H}_2CH_3$), 3.65-4.22 (2H, br m, $C\underline{H}$), 4.03 (2H, s, $C\underline{H}_2$); ^{13}C (D_2O) δ 13.3 (d, $\underline{C}H_3CH_2$, $^3J_{PCCC}$ 14.0 Hz),

19.3 (s, $\underline{C}H_3CH$), 25.9 (s, $\underline{C}H_2CH_3$), 45.5 (s, $\underline{C}H_2$), 52.2 (s, $\underline{C}HCH_3$), 53.2 (d, $\underline{C}HP$, $^1J_{PC}$ 145.3 Hz), 171.5-174.4 (m, $\underline{C}$=O); ^{31}P (D_2O) δ 18.4; m/z (FAB, %) 390 (11.8), 290 (16.8), 268 (15.4, MH^+), 197 (15.5, $MH^+ - C_3H_5NO$), 140 (9.2, $MH^+ - C_5H_9N_2O_2$), 115 (57.2, $C_5H_{11}N_2O^+$), 102 (100).

39. Preparation of (1R,S)-1-(glycyl-L-alanylamino)propane-phosphonic acid

Triethylamine (0.82 g, 8.12 mmol) was added to a stirred solution of N-carbobenzoxyglycine (1.70 g, 8.12 mmol) in toluene (115 ml) and the solution cooled to -10 °C. Isobutyl chloroformate (1.11 g, 8.12 mmol) was added dropwise with stirring and was washed in with toluene (2 ml). The solution was stirred at -10 °C for 0.5 h before adding a solution of 1(R,S)-1-(L-alanylamino)propane phosphonic acid (1.55 g, 7.38 mmol) in triethylamine (1.49 g, 14.8 mmol) and aqueous ethanol (10 ml) dropwise. The mixture was stirred at -10 °C for 1 h, at 0 °C for 1 h, and then at room temperature overnight. The solution was evaporated to give an oily white solid which was dissolved in water (150 ml) and extracted with dichloromethane. The aqueous layer was acidified to pH 2 with dilute hydrochloric acid and extracted again with dichloromethane. The aqueous layer was evaporated to give a white solid which was stirred overnight with hydrobromic acid (45% in glacial acetic acid, 15 ml). The solution was evaporated and the residue dissolved in water before extracting with diethyl ether to remove benzyl bromide. The aqueous layer was evaporated and the residue dissolved in ethanol before treating with propylene oxide to give a white solid. The solid was filtered and dried (1.61 g) before treating with decolourizing charcoal and recrystallizing from water /ethanol to give (1R,S)-1-(glycyl-L-alanylamino)propane-phosphonic acid (0.81 g, 3.03 mmol, 41%), m.p. 253-5 °C,

$[\alpha]_{578}^{25}$ + 6.6(±2)° (c = 2% in water),
ν_{max} (KBr) cm^{-1} 1560 ($^{+}NH_3$), 1680-1650 (C=O); ^{1}H (D_2O) δ 0.90 (3H, t, $\underline{CH}_3CH_2$, $^{3}J_{HCCH}$ 7.32 Hz), 1.43 (3H, d, $\underline{CH}_3CH$, $^{3}J_{HCCH}$ 7.81 Hz), 1.52-2.00 (2H, br m, $\underline{CH}_2$), 3.70-4.50 (2H, br m, $\underline{CH}$), 3.87 (2H, s, $\underline{CH}_2C=O$); ^{13}C (D_2O) δ 13.3 (d, $\underline{CH}_3CH_2$, $^{3}J_{PCCC}$ 13.4 Hz), 20.0 (s, $\underline{CH}_3CH$), 26.0 (s, $\underline{CH}_2$), 49.6 (s, $\underline{CH}_2C=O$), 52.2 (s, $\underline{CHCH}_3$), 53.2 (d, $\underline{CHP}$, $^{1}J_{PC}$ 144.7 Hz), 169.9 (s, $\underline{C}=O$), 177.5 (s, $\underline{C}=O$); ^{31}P (D_2O) δ 18.3; m/z (FAB, %) 631 (1.7, $3.MH^{+} - C_2H_3NO$), 421 (18.0, $2MH^{+}$), 350 (23.8, $2MH^{+} - C_3H_5NO$), 279 (17.0, $2MH^{+} - 2.C_3H_5NO$), 268 (7.7, MH^{+}), 211 (100, $MH^{+} - C_2H_3NO$), 140 (50.7, $MH^{+} - C_4H_9N_2O$), 129 (9.0, $MH^{+} - H_3PO_3$), 58 (70.0, $MH^{+} - C_4H_9N_2O - H_3PO_3$).

40. Preparation of (1R,S)-1-(L-alanyl-L-alanyl-L-alanyl-amino)propanephosphonic acid

Solid sodium bicarbonate (0.90 g, 10.68 mmol) was added to a stirred solution of (1R,S)-1-(L-alanyl-L-alanyl-amino)propanephosphonic acid (1.00 g, 3.56 mmol) in water (18 ml) and ethanol (9 ml) at 0 °C. The N-hydroxysuccinimide ester of carbobenzoxy-L-alanine (1.14 g, 3.56 mmol) in warm ethanol (10 ml) was added dropwise to the stirred solution and was washed in with ethanol (4 ml). The mixture was stirred at 0 °C for 1 h then at room temperature overnight. The resulting solution was evaporated and the residue dissolved in water (75 ml) and extracted with dichloromethane. The aqueous layer was acidified to pH 2 with dilute hydrochloric acid and extracted again with dichloromethane. The aqueous layer was concentrated before passing down a column of cation-exchange resin (Dowex 50 W - X 8(H^+) 16-40 mesh, 52 x 2.5 cm freshly regenerated in the acid cycle) with water as elutant. The acid fractions (200 ml) were combined and evaporated to give a colourless jelly which was stirred at room temperature overnight with hydrobromic acid (45% in glacial acetic acid, 15 ml). The solution obtained was evaporated to give a yellow oil which was dissolved in water (30 ml) and extracted with diethyl ether to remove benzyl bromide. The aqueous layer was evaporated and the residue dissolved in methanol (10 ml) before treating with propylene oxide to give a colourless jelly-like precipitate which was filtered and dried (0.90 g) before recrystallizing

from water/acetone to give (1R,S)-1-(L-alanyl-L-alanyl-L-alanylamino)propanephosphonic acid (0.60 g, 1.70 mmol, 48%) as a white crystalline solid (wide melting range). Further treatment with decolourizing charcoal and recrystallization gave white crystals, m.p. 239-41 °C, $[\alpha]_{578}^{25}$ -54.0(±2)° c = 0.5% in water, m/z (FAB) Found: 353.1598100, $C_{12}H_{26}N_4O_6P$ requires: 353.1590100; ^1H (D_2O/D_2SO_4) δ 0.63 (3H, t, CH_3CH_2, $^3J_{HCCH}$ 6.8 Hz), 1.13-1.34 (9H, m, CH_3CH), 1.34-1.75 (2H, m, CH_2), 3.78-4.25 (4H, br m, CH); ^{13}C (D_2O) δ 13.4 (d, CH_3CH_2, $^3J_{PCCC}$ 12.8 Hz), 19.5 (br s, CH_3CH), 25.9 (s, CH_2), 51.9 (s, $CHCH_3$), 52.7 (s, $CHCH_3$), 53.0 (s, $CHCH_3$), 53.0 (d, CHP, $^1J_{PC}$ 146.5 Hz), 177.4 (m, C=O); ^{31}P (D_2O) 18.4; m/z (FAB, %) 705 (4.9, $2MH^+$), 353 (100, MH^+), 282 (17.4, $MH^+ - C_3H_5NO$), 211 (36.3, $MH^+ - 2.C_3H_5NO$), 143 (53.8, $C_6H_{11}N_2O_2^+$), 115 (36.5, $C_5H_{11}N_2O^+$), 99 (15.3).

41. Preparation of (1R,S)-1-(D-alanyl-L-alanyl-L-alanylamino)propanephosphonic acid

(1R,S)-1-(L-alanyl-L-alanylamino)propanephosphonic acid (0.62 g, 2.19 mmol) was dissolved in water (11 ml) and stirred at 0 °C whilst triethylamine (0.44 g, 4.38 mmol) and ethanol (11 ml) were added. The solid N-hydroxysuccinimide ester of N-carbobenzoxy-D-alanine (0.70 g, 2.19 mmol) was added and washed in with ethanol (5 ml). The mixture was stirred at 0 °C for 2 h then at room temperature overnight. The solution was evaporated and the residue dissolved in water (40 ml) and extracted with dichloromethane. The aqueous layer was acididfied to pH 2 with dilute hydrochloric acid and extracted again with dichloromethane. The aqueous layer was evaporated and the residue stirred overnight with hydrobromic acid (45% in glacial acetic acid, 10 ml) at room temperature. The solution was evaporated and the residue dissolved in water before extracting with diethyl ether to remove benzyl bromide. The aqueous layer was evaporated and the residue dissolved in methanol and treated with propylene oxide to give a white solid. The solid was dried (0.60 g), treated with decolourizing charcoal and recrystallized from water/ethanol to give (1R,S)-1-(D-alanyl-L-alanyl-L-alanylamino)propanephosphonic acid (0.42 g, 1.19 mmol, 54.5%), m.p. 230-4 °C, $[\alpha]_{578}^{25}$ +7.2(±1)° (c = 4% in water), ^{1}H (D_2O) δ 0.92 (3H, t, CH_3CH_2, $^{3}J_{HCCH}$ 6.84 Hz), 1.41-1.60 (9H, m, CH_3CH), 1.65-2.25 (2H, m, CH_2), 3.61-4.22 (4H, br m, CH); ^{13}C (D_2O) δ 13.3 (d, CH_3CH_2,

$^{3}J_{PCCC}$ 14.0 Hz), 19.1 (s, $\underline{C}H_3CH$), 19.7 (s, $\underline{C}H_3CH$), 20.0 (s, $\underline{C}H_3CH$), 25.8 (s, $\underline{C}H_2$), 52.2 (s, $\underline{C}HCH_3$), 52.5 (s, $\underline{C}HCH_3$), 53.4 (d, $\underline{C}HP$, $^{1}J_{PC}$ 148.9 Hz), 53.5 (s, $\underline{C}HCH_3$), 173.6 (m, $\underline{C}$=O), 178.8 (s, $\underline{C}$=O);
^{31}P (D_2O) δ 18.2.

42. Preparation of (1R,S)-1-(L-alanyl-L-alanyl-L-alanyl-L-alanylamino)propanephosphonic acid

(1R,S)-1-(L-alanyl-L-alanyl-L-alanylaminopropane-phosphonic acid (1.25 g, 3.55 mmol) was dissolved in water (18 ml) and ethanol (9 ml) and stirred and cooled in ice whilst solid sodium bicarbonate (0.89 g, 10.65 mmol) was added. A solution of the N-hydroxysuccinimide ester of N-carbobenzoxy-L-alanine (1.20 g, 3.75 mmol) in ethanol (10 ml) was added and washed in with ethanol (4 ml). The mixture was stirred at 0 °C for 2 h and then at room temperature overnight. The solution was evaporated and the residue dissolved in water (75 ml) and extracted with dichloromethane. The aqueous layer was acididfied to pH 2 with dilute hydrochloric acid and extracted again with dichloromethane. The aqueous layer was concentrated before passing down a column of cation-exchange resin (Dowex 50 W - X 8(H^+) 16-40 mesh, 52 x 2.5 cm, freshly regenerated in the acid cycle) with water as elutant. The acid fractions were combined and evaporated to give a white solid which was stirred overnight with hydrobromic acid (45% in glacial acetic acid, 15 ml) at room temperature. The solution was evaporated and the residue dissolved in water before extracting with diethyl ether. The aqueous layer was evaporated and the residue dissolved in ethanol and treated with propylene oxide to give a white solid. The solid was filtered and dried (1.27 g) before treating with decolourizing charcoal and recrystallizing from water/ethanol to give (1R,S)-1-(L-alanyl-L-alanyl-L-alanyl-L-alanylamino)propanephosphonic

acid (1.00 g, 2.36 mmol, 67%) m.p. 251-4 °C. Further recrystallization from water/dioxane gave m.p. 258-60 °C, $[\alpha]_{578}^{25}$ -35.6(±1)° (c = 4% in 0.5M NaOH), m/z (FAB) Found: 424.1971000, $C_{15}H_{31}N_5O_7P$ requires: 424.1961000; ^1H (D_2O) δ 0.90 (3H, t, $C\underline{H}_3CH_2$, $^3J_{HCCH}$ 6.84 Hz), 1.42 (6H, d, $C\underline{H}_3CH$, $^3J_{HCCH}$ 6.84 Hz), 1.55 (6H, d, $C\underline{H}_3CH$, $^3J_{HCCH}$ 6.84 Hz), 1.65-2.25 (2H, br m, $C\underline{H}_2$), 3.52-4.50 (5H, br m, C<u>H</u>); ^{13}C (D_2O) δ 13.3 (d, $\underline{C}H_3CH_2$, $^3J_{PCCC}$ 13.4 Hz), 19.4 (s, $\underline{C}H_3CH$), 25.9 (s, $\underline{C}H_2$), 51.9 (s, $\underline{C}HCH_3$), 52.5 (s, $\underline{C}HCH_3$), 52.8 (s, $\underline{C}HCH_3$), 53.0 (d, $\underline{C}HP$, $^1J_{PC}$ 147.7 Hz), 173.6 (s, $\underline{C}$=O), 177.4 (br s, $\underline{C}$=O); ^{31}P (D_2O) δ 18.3; m/z (FAB, %) 424 (12.0, MH^+), 375 (15.0), 353 (86.7, $MH^+ - C_3H_5NO$), 282 (100, $MH^+ - 2.C_3H_5NO$), 211 (68.0, $MH^+ - 3.C_3H_5NO$), 140 (11.5, $MH^+ - 4.C_3H_5NO$), 115 (41.0, $C_5H_{11}N_2O^+$), 58 (90.0, $MH^+ - 4.C_3H_5NO - H_3PO_3$).

43. The preparation of the nitrochloromethylstyrene-divinylbenzenepolymer[85]

The dry chloromethylstyrene-divinylbenzenepolymer (5.00 g) was stirred into fuming nitric acid (90% HNO_3, sp. gr. 1.5, 50 ml) at 0 °C. The mixture was stirred for 1 h at 0 °C and then poured onto crushed ice. The light tan beads were filtered and washed with water, dioxane and methanol, the washing solvent changing composition gradually. The resin was dried under vacuum at 80 °C to give the nitrochloromethylstyrene-divinylbenzenepolymer (7.15 g).

44. Attempted preparation of the N-carbobenzoxy-1-amino-propanephosphonic acid nitropolymer[85]

A solution of N-carbobenzoxy-1-aminopropanephosphonic acid (1.26 g, 4.63 mmol) and triethylamine (0.47 g, 4.63 mmol) in ethyl acetate (27 ml) was added to the nitrochloromethylstyrene-divinylbenzenepolymer (3.00 g) and the mixture stirred and heated under reflux for 60 h. The mixture was allowed to cool and a solution of glacial acetic acid (0.90 ml) and triethylamine (1.54 g) in ethyl acetate (7.5 ml) was added and the mixture stirred and heated under reflux for a further 4 h. The resin was filtered and washed thoroughly with ethyl acetate, ethanol,

water and methanol by gradual change of solvent composition and dried under vacuum at 40 °C to give a pale yellow resin (3.15 g). The combined washings were evaporated to give a yellow oil (2.30 g) which gave a faint white precipitate when tested for chloride ions whith silver nitrate. The oil was dissolved in water and acidified to pH 1 with concentrated hydrochloric acid. The solution was extracted exhaustively with diethyl ether. The organic layer was dried ($MgSO_4$) and evaporated to give unreacted crude N-carbobenzoxy-1-amino-propanephosphonic acid (1.17 g), m.p. 139-42 °C, ^1H (acetone-D_6) δ 0.95 (3H, t, CH_3, $^3J_{HCCH}$ 7.00 Hz), 1.25-2.20 (2H, br m, CH_2CH), 3.50-4.10 (1H, br m, CH), 5.10 (2H, s, CH_2arom), 7.35 (5H, br s, arom). 8.70 (1H, br s, NH).

45. Attempted acetolysis of nitrochloromethylstyrene-divinylbenzenepolymer[87]

A mixture of the nitrochloromethylstyrene-divinylbenzenepolymer (2.00 g) and potassium acetate (1.10 g, 0.011 mol) in benzyl alcohol (17 ml) was stirred and heated under reflux at 80 °C for 12 h. The resin was filtered and washed thoroughly with water, dimethylformamide and methanol by gradual change of solvent composition. The resin was dried under vacuum at 40 °C to give a pale yellow resin (2.03 g). The combined

washings were concentrated and tested for chloride ions with silver nitrate when a negative test was observed.

46. Attempted hydrolysis of nitrochloromethylstyrene-divinylbenzenepolymer[87]

A mixture of the nitrochloromethylstyrene-divinylbenzenepolymer (1.00 g) and 2N ethanolic sodium hydroxide solution (10 ml) was stirred and heated under reflux at 185 °C for 48 h. The resin was filtered and washed with water and ethanol by gradual change of solvent composition. The resin was dried under vacuum to give a brown resin (1.01 g). The combined washings were tested for chloride ions with silver nitrate when a negative test was observed.

47. Attempted acetolysis of chloromethylstyrene-divinylbenzenepolymer[87]

A mixture of the chloromethylstyrene-divinylbenzenepolymer (1 mmol Cl/g; 2.00 g, 2.00 mmol) and potassium acetate (0.49 g, 5.00 mmol) in benzyl alcohol (17 ml) was stirred and heated under reflux for 12 h. The resin was filtered off and washed with water, dimethylformamide

and methanol by gradual change of solvent composition. The resin was dried under vacuum to give a white resin (1.98 g). The combined washings were concentrated and tested for chloride ions with silver nitrate when a negative test was observed.

48. Acetolysis of chloromethylstyrene-divinylbenzene-polymer[86]

A mixture of the chloromethylstyrene-divinylbenzene-polymer (1 mmol Cl/g; 2.00 g, 2.00 mmol) and potassium acetate (0.49 g, 5.00 mmol) in 2-methoxyethanol (12 ml) was stirred and refluxed at 150 °C for 24 h protected by a drying tube ($CaCl_2$). The resin was filtered and washed with water and methanol by gradual change of solvent composition. The resin was dried (2.05 g) and the combined washings tested for chloride ions with silver nitrate when a positive test was observed.

49. Attempted hydrolysis of acetylated hydroxymethylstyrene-divinylbenzenepolymer[88]

The acetylated hydroxymethylstyrene-divinylbenzene-polymer (2.00 g) was stirred for 48 h at room temperature with a solution of 0.5 N sodium hydroxide (17 ml) in dioxane (33 ml). The resin was filtered and washed with water and methanol and dried (2.12 g). The degree of saponification to the hydroxymethyl resin was estimated by back-titration of the aqueous wash with standard hydrochloric acid. The original 0.5 N sodium hydroxide solution required 16.7 ml of 0.5113 N hydrochloric acid for neutralization. The aqueous wash also required 16.7 ml of 0.5113 N hydrochloric acid, therefore, no reaction was assumed.

50. Hydrolysis of acetylated hydroxymethylstyrene-divinylbenzenepolymer

The acetylated hydroxymethylstyrene-divinylbenzene-polymer (1.00 g) was heated under reflux with ca 1.5 N ethanolic potassium hydroxide solution (10 ml) for 10 h. The resin was filtered, washed with water and methanol and dried (0.90 g). The degree of saponification to the hydroxymethyl resin was estimated by back-titration of

the aqueous washings with standard hydrochloric acid. The original 1.5 N ethanolic potassium hydroxide solution required 30.2 ml of 0.5113 N hydrochloric acid for neutralization. The aqueous washings required 29.5 ml of 0.5113 N hydrochloric acid, indicating that 0.358 mmol of potassium hydroxide had been used. Thus, it was estimated that the hydroxyl content of the resin was ca. 0.40 mmol/g.

51. Attachment of tetramethylammonium salt of monomethyl propionylphosphonate to chloromethylstyrene-divinylbenzenepolymer[88]

The chloromethylstyrene-divinylbenzenepolymer (1 mmol Cl/g; 2.00 g, 2.00 mmol) was suspended in dimethylformamide (90 ml) and the tetramethylammonium salt of monomethyl propionylphosphonate (2.25 g, 0.01 mol) added. The mixture was stirred at 90 ^{o}C for 2 h. After cooling, water (130 ml) was added and the mixture stirred before filtering the resin. The resin was washed with water and dried under vacuum (2.07 g), (Found: Cl, 0.26%; P, 0.68%), ν_{max} (KBr) cm^{-1} 1680 (C=O). The washings gave a positive chloride test when tested with silver nitrate.

52. Preparation of N-carbobenzoxy-1-aminopropanephosphonic acid

A solution of 1-aminopropanephosphonic acid (6.90 g, 0.050 mol) in sodium hydroxide solution (2.00 g, 0.050 mol, in 25 ml water) was placed in a flask fitted with two dropping funnels. The solution was stirred in ice whilst benzyl chloroformate (8.53 g, 7.14 ml, 0.050 mol) in toluene (3 ml) and sodium hydroxide solution (2.00 g, 0.050 mol, in 12.5 ml water) were added dropwise simultaneously over a period of 45 min. After the addition was complete the mixture was stirred for a further 10 min before transferring to a separating funnel. The toluene layer was removed and the aqueous layer was extracted with ether before acidifying to ca. pH 2 with concentrated hydrochloric acid whilst cooling in ice. A white oil was precipitated which gave a waxy white solid on standing. The solid was filtered and dried (vacuum oven) before recrystallizing from chloroform/tetrachloromethane) to give N-carbobenzoxy-1-aminopropanephosphonic acid (1.40 g, 5.13 mmol, 10.3%) as a white crystalline solid, m.p. 135-7 °C, (Lit.[80] 142-4 °C), (Found: C, 48.3; H, 5.9; N, 4.6. Calc. for $C_{11}H_{16}NO_5P$: C, 48.4; H,5.9; N, 5.1%); ^1H (acetone-D_6) δ 0.96 (3H, t, CH_3, $^3J_{HCCH}$ 7.04 Hz), 1.28-2.18 (2H, br m, CH_2CH), 3.60-4.09 (1H, br m, CH), 5.11 (2H, s, CH_2arom), 7.35 (5H, br s, arom), 8.58 (1H, br s, NH); ^{13}C (CD_3OD) δ 11.2 (d, CH_3, $^3J_{PCCC}$ 13.4 Hz), 24.2 (s, CH_2CH), 55.8 (CH, plus solvent peaks), 67.8 (s, CH_2arom), 128.9 (s, C-2, C-6 arom), 129.1 (s, C-4 arom), 129.6 (s, C-3, C-5 arom), 138.4 (s, C-1 arom), 159.2 (br s, C=O); ^{31}P (CD_3OD) δ 24.0; m/z (FAB, %), 296 (9.2, M + Na^+), 274 (16.5, MH^+), 148 (5.7, M + Na^+/2), 91 (100, $C_7H_7^+$).

53. Preparation of N-carbobenzoxy-1-aminopropanephosphonic acid[80]

A solution of 1-aminopropanephosphonic acid (3.00 g, 0.022 mol) in water (16 ml) was stirred at 0 °C and adjusted to pH 9.5 with 4N sodium hydroxide solution. Diethyl ether (8 ml) was added and benzyl chloroformate (5.87 g, 0.033 mol) was added dropwise. The mixture was stirred at room temperature for 36 h with the intermittent addition of 4N sodium hydroxide solution to maintain a pH of 9.5. The mixture was extracted with ether and the aqueous layer poured slowly on to a mixture of water (16 ml), concentrated hydrochloric acid (16 ml) and ice (54 g). The resulting solution was extracted with ether and the extracts dried ($MgSO_4$) before concentrating to give a white solid which was filtered and recrystallized from ethyl acetate/n-hexane to give N-carbobenzoxy-1-aminopropanephosphonic acid (3.73 g, 0.014 mol, 62.1%) as colourless crystals, m.p. 141-3 °C, (Lit.[80] 142-4 °C), ν_{max} (KBr) cm^{-1} 1010 (P-O), 1190 (P=O), 1540 (NH), 1690 (C=O), 3290 (NH); ^1H (D_2O/NaOH) δ 0.91 (3H, t, CH_3, $^3J_{HCCH}$ 6.84 Hz), 1.13-2.07 (2H, br m, CH_2CH), 3.30-3.80 (1H, m, CH), 5.14 (2H, s, CH_2arom), 7.44 (5H, s, arom); ^{13}C (D_2O/NaOH) δ 13.5 (d, CH_3, $^3J_{PCCC}$ 12.2 Hz), 27.3 (s, CH_2CH), 55.4 (d, CH, $^1J_{PC}$ 142.2 Hz), 69.5 (s, CH_2arom), 130.4 (s, C-2, C-6 arom), 131.1 (s, C-4 arom), 131.6 (s, C-3, C-5 arom), 139.4 (s, C-1 arom), 161.2 (d, C=O, $^3J_{PCNC}$ 7.3 Hz); ^{31}P (D_2O/NaOH) δ 20.1.

54. Preparation of the monomethyl ester of N-carbobenzoxy-1-aminopropanephosphonic acid[80]

N-Carbobenzoxy-1-aminopropanephosphonic acid (1.37 g, 0.005 mol), anhydrous methanol (0.32 g, 0.010 mol), trichloroacetonitrile (0.72 g, 0.005 mol) and anhydrous pyridine (10 ml) were stirred and heated under reflux for 3.5 h with the exclusion of moisture. The mixture was left to stand overnight before removing the volatile components (oil pump). A cold, saturated solution of sodium bicarbonate (20 ml) was added to the residual oil and the mixture left until effervescence had ceased. The solid trichloroacetamide was filtered off and the filtrate brought to pH 1 with 6N hydrochloric acid. A colourless oil was precipitated which was extracted with ethyl acetate. The organic layer was evaporated to give a white crystalline solid which recrystallized from ethyl acetate/petroleum ether to give the monomethyl ester of N-carbobenzoxy-1-aminopropanephosphonic acid (1.20 g, 4.18 mmol, 84%) as colourless crystals, m.p. 111-2 °C, (Lit.[80] 113-5 °C), ν_{max} (KBr) cm^{-1} 980 (P-O), 1060 (P-O-C), 1220 (P=O), 1550 (N-H), 1690 (C=O), 3280 (N-H); ^{1}H ($CDCl_3$) δ 0.95 (3H, t, $\underline{CH}_3$, $^{3}J_{HCCH}$ 6.40 Hz), 1.13-2.03 (2H, br m, $\underline{CH}_2CH$), 3.67 (3H, d, $O\underline{CH}_3$, $^{3}J_{POCH}$ 10.74 Hz), 3.75-4.24 (1H, br m, $\underline{CH}$), 5.11 (2H, s, $\underline{CH}_2$arom), 7.29 (5H, br s, arom), 9.70 (1H, brs, $\underline{NH}$); ^{13}C ($CDCl_3$) δ 10.5 (d, $\underline{CH}_3$, $^{3}J_{PCCC}$ 12.8 Hz), 23.1 (s, $\underline{CH}_2$-CH), 48.8 (d, $\underline{CH}$, $^{1}J_{PC}$ 148.9 Hz), 52.9 (s, $O\underline{CH}_3$), 67.3 (s, $\underline{CH}_2$arom), 128.1 (s, $\underline{C}$-2, $\underline{C}$-6 arom), 128.3 (s, $\underline{C}$-4 arom), 128.6 (s, $\underline{C}$-3, $\underline{C}$-5 arom), 136.4 (s, $\underline{C}$-1 arom), 157.2 (s, $\underline{C}$=O); ^{31}P ($CDCl_3$) 27.1; m/z (FAB, %) 575 (3.7, $2MH^+$), 561 (2.3), 420 (6.3), 372 (12.0), 310

(23.0), 288 (43.3, MH^+), 274 (5.0), 148 (16.7), 133 (12.7), 115 (6.3), 91 (100, $C_7H_7^+$), 58 (28.7, $MH^+ - C_3H_5NO - H_3PO_3$).

55. <u>The hydrogenolysis of the monomethyl ester of N-carbobenzoxy-1-aminopropanephosphonic acid</u>[80]

A solution of the monomethyl ester of N-carbobenzoxy-1-aminopropanephosphonic acid (1.57 g, 5.47 mmol) and 5% palladium-on-charcoal catalyst (1.0 g) in methanol (50 ml) was hydrogenated in a stainless steel autoclave at a pressure of 450 psi at 56 °C for 3 h. The catalyst was filtered off and washed with methanol and the combined filtrate and washings evaporated to give a pale yellow oil. The oil was left in a refrigerator for several months but did not crystallize. Crystallization also failed to occur when the oil was treated with various solvent systems. The oil was evaporated (oil pump) to give the crude <u>monomethyl ester of 1-aminopropane-phosphonic acid</u> (o.67 g, 4.38 mmol, 80%), ^1H (D_2O) δ 1.07 (3H, t, $C\underline{H}_3$, $^3J_{HCCH}$ 7.81 Hz), 1.63-2.13 (2H, m, $C\underline{H}_2$), 3.18-3.82 (1H, br m, $C\underline{H}$), 3.62 (3H, d, $C\underline{H}_3OP$, $^3J_{POCH}$ 9.77 Hz); ^{13}C (D_2O) δ 13.1 (d, $\underline{C}H_3$, $^3J_{PCCC}$ 6.1 Hz), 24.5 (s, $\underline{C}H_2$), 52.1 (d, $\underline{C}HP$, $^1J_{PC}$ 147.1 Hz), 54.9 (s, $\underline{C}H_3OP$); ^{31}P (D_2O) δ 16.6 Hz.

56. Preparation of diphenyl N-carbobenzoxy-1-aminoethanephosphonate[27]

Ethanal (1.98 g, 0.045 mol) was added to a stirred solution of benzyl carbamate (4.54 g, 0.030 mol) and triphenyl phosphite (9.31 g, 0.030 mol) in glacial acetic acid (5 ml) and the mixture stirred at room temperature for 1 h. The solution was then heated under reflux at 85-90 °C for 1 h before removing the volatile components (oil pump). Methanol (40 ml) was added to the residue and the solution stored in a refrigerator overnight. The resulting needle-like crystals were filtered off, washed with petroleum ether (b.p. 40-60 °C) and recrystallized from dichloromethane/petroleum ether (b.p. 40-60 °C) to give diphenyl N-carbobenzoxy-1-aminoethanephosphonate (6.30 g, 0.016 mol, 53%), m.p. 115-8 °C (Lit.[27] 115-7 °C), ^{1}H ($CDCl_3$) δ 1.51 (3H, q, CH_3, $^3J_{HCCH}$ 7.32 Hz), 4.22-4.82 (1H, br m, CH), 5.11 (2H, s, CH_2), 7.04-7.24 (10H, m, P-O-arom), 7.31 (5H, s, CH_2arom); ^{13}C ($CDCl_3$) δ 16.3 (s, CH_3), 44.2 (d, CHP, $^1J_{CP}$ 160.5 Hz), 67.3 (s, CH_2arom), 120.4-120.8 (m, P-O-arom), 125.5 (s, arom), 128.2 (s, C2, C6-arom), 128.6 (s, C4-arom), 129.8 (s, C3, C5-arom), 155.7 (d, C=O, $^3J_{PCNC}$ 7.9 Hz); ^{31}P ($CDCl_3$) δ 18.4.

57. Preparation of diphenyl N-carbobenzoxy-1-aminobutanephosphonate[27]

Butanal (3.24 g, 0.045 mol) was added to a stirred solution of benzyl carbamate (4.54 g, 0.030 mol) and triphenyl phosphite (9.31 g, 0.030 mol) in glacial acetic acid (5 ml) and the mixture stirred at room temperature for 1 h. The solution was then heated under reflux at 85-90 °C for 1 h before removing the volatile components (oil pump). Methanol (55 ml) was added to the residue and the solution stored in a refrigerator overnight. The resulting needle-like crystals were filtered off, washed with petroleum ether (b.p. 40-60 °C) and recrystallized from dichloromethane/petroleum ether (b.p. 40-60°C) to give diphenyl N-carbobenzoxy-1-aminobutanephosphonate (5.12 g, 0.011 mol, 36%), m.p. 104-6 °C, (Found: C, 63.5; H, 5.9; N, 2.8. $C_{24}H_{26}NO_5P$ requires: C, 65.6; H, 5.9; N, 3.2%); ^1H ($CDCl_3$) δ 0.94 (3H, t, CH_3, $^3J_{HCCH}$ 6.35 Hz), 1.30-2.25 (4H, br m, CH_2CH_2), 4.12-4.82 (1H, m, CH), 5.12 (2H, s, CH_2arom), 7.19 (10H, br s, O-arom), 7.33 (5H, s, CH_2arom): ^{13}C ($CDCl_3$) δ 13.5 (s, CH_3), 18.9 (d, CH_2CH_2CH, $^3J_{PCCC}$ 14.0 Hz), 31.7 (d, CH_2CH, $^2J_{PCC}$ 3.7 Hz), 48.3 (d, CHP, $^1J_{PC}$ 158.1 Hz), 67.1 (s, CH_2arom), 120.6 (d, PO-arom, J_{PC} 7.3 Hz), 120.6 (s, PO-arom). 125.2 (s, C1-arom), 125.3 (s, C1-arom), 128.1 (s, arom). 128.2 (s, arom). 128.6 (s, C4-arom), 129.7 (s, C3-arom, C5-arom), 156.4 (d, C=O, $^3J_{PCNC}$ 6.7 Hz); ^{31}P ($CDCl_3$) δ 17.8.

58. Preparation of N-2,2,2-trichlorocarboethoxy-1-amino-propanephosphonic acid

1-Aminopropanephosphonic acid (3.05 g, 0.022 mol) was dissolved in water (16 ml) and the solution adjusted to pH 9.5 using 4N sodium hydroxide solution. The solution was stirred in ice whilst diethyl ether (10 ml) and 2,2,2-trichloroethyl chloroformate (6.57 g, 0.031 mol) were added. The mixture was stirred at room temperature for 12 h with the intermittent addition of 4N sodium hydroxide to maintain a pH of 9.5. The mixture was extracted with diethyl ether and the aqueous layer poured slowly onto a mixture of ice (55 g), water (16 ml) and concentrated hydrochloric acid (16 ml). The solution extracted with ether and the extracts dried ($MgSO_4$) before evaporating to give an oil. The oil was dissolved in ethyl acetate and treated with pet. ether (b.p. 60-80 ^{o}C) until cloudy. The precipitated crystalline solid was filtered off to give N-2,2,2-trichlorocarboethoxy-1-amino-propanephosphonic acid (2.22 g, 7.06 mmol, 32%), m.p. 125-7 ^{o}C, (Found: C, 21.7; H, 3.9; N, 4.2; $C_6H_{11}NO_5PCl_3.H_2O$ requires: C, 21.7; H, 3.9; N, 4.2%); ν_{max} (KBr) cm^{-1} 730 (C-Cl), 1160 (C-Cl), 1540 (N-H), 1720 (C=O); ^{1}H (CD_3OD) δ 0.98 (3H, t, $\underline{CH}_3CH_2$, $^{3}J_{HCCH}$ 7.35 Hz), 1.28-2.25 (2H, br m, $\underline{CH}_2$), 3.40-3.85 (1H, m, $\underline{CH}$), 4.69 (2H, s, $\underline{CH}_2O$); ^{13} (CD_3OD) δ 11.6 (d, $\underline{C}H_3CH_2$, $^{3}J_{PCCC}$ 12.8 Hz), 25.1 (d, $\underline{C}H_2CH_3$, $^{2}J_{PCC}$ 2.4 Hz), 47.0-57.3 (d, $\underline{C}HP$), 75.8 (s, $\underline{C}H_2O$), 97.4 (s, $\underline{C}Cl_3$), 157.2 (d, $\underline{C}$=O, $^{3}J_{PCNC}$ 7.9 Hz); ^{31}P (CD_3OD) δ 17.6.

59. Attempted reaction of p-toluenesulphonyl chloride with 1-aminopropanephosphonic acid[81]

1-Aminopropanephosphonic acid (2.00 g, 0.0145 mol) was dissolved in sodium hydroxide solution (10%, 40 ml) and the solution heated to 80 °C whilst p-toluenesulphonyl chloride (2.76 g, 0.0145 mol) was added in portions with stirring. After the addition was complete the mixture was stirred at 80 °C for 0.5 h before cooling to room temperature. The solution was extracted with ether to remove unreacted p-toluenesulphonyl chloride and the aqueous layer cooled and neutralised with concentrated hydrochloric acid. The aqueous solution was concentrated when a white crystalline solid was precipitated. The solid was filtered and dried to give unreacted 1-amino-propanephosphonic acid (1.70 g), m.p. 256-8 °C.

60. Preparation of diethyl N-p-toluenesulphonyl-1-amino-propanephosphonate

Diethyl 1-aminopropanephosphonate (1.00 g, 5.13 mmol) was stirred in pyridine (0.6 ml) whilst p-toluenesulphonyl chloride (0.98 g, 5.13 mmol) in toluene (3 ml) was added. The solution was refluxed for 1 h when a brown oil was precipitated. The mixture was cooled and the upper toluene layer decanted from the oil. The toluene layer was washed with water and then evaporated to give an oil which crystallized on standing. The solid was dried (1.29 g)

before recrystallizing from acetone/water to give <u>diethyl N-p-toluenesulphonyl-1-aminopropanephosphonate</u> (0.64 g, 1.83 mmol,36%) as a shiny, white solid, m.p. 104-5 °C, (Found: C, 46.5; H, 6.8; N, 5.2. $C_{14}H_{24}NO_5PS.H_2O$ requires: C,46.8; H, 7.1; N, 3.8%); ν_{max} 1030 (P-O-C), 1170 (SO_2NH), 1220 (P=O), 1330 (SO_2NH), 1590 (NH) cm^{-1}; ^1H ($CDCl_3$) δ 0.84 (3H, t, $\underline{CH}_3CH_2$, $^3J_{HCCH}$ 7.00 Hz), 1.16-1.36 (6H, d of t, $\underline{CH}_3CH_2O$), 1.45-1.80 (2H, br m, $\underline{CH}_2CH$), 2.41 (3H, s, $\underline{CH}_3$arom), 3.35-3.75 (1H, br m, $\underline{CH}$), 3.88-4.25 (4H, q, $\underline{CH}_2O$, $^3J_{HCCH}$ 7.04 Hz), 5.94-6.10 (1H, br m, $\underline{NH}$), 7.28 (2H, d, <u>H</u>-3, <u>H</u>-5 arom), 7.80 (2H, d, <u>H</u>-2, <u>H</u>-6 arom, $^3J_{HCCH}$ 8.36 ^{13}C ($CDCl_3$) δ 10.2 (d, $\underline{CH}_3CH_2$, $^3J_{POCC}$ 9.2 Hz), 16.4 (d, $\underline{CH}_3CH_2O$, $^3J_{POCC}$ 5.5 Hz), 21.5 (s, $\underline{CH}_3$arom), 24.0 (s, $\underline{CH}_2CH_3$), 51.7 (d, $\underline{CH}$, $^1J_{PC}$ 156.9 Hz), 62.3-63.2 (m, $\underline{CH}_2O$), 127.2 (s, <u>C</u>-3, <u>C</u>-5 - arom), 129.6 (s, <u>C</u>-2, <u>C</u>-6 arom), 138.7 (s, <u>C</u>-4 arom), 143.4 (s, <u>C</u>-1 arom); ^{31}P ($CDCl_3$) δ 23.6; m/z (%) 212 (48.6), 155 (67.5, $CH_3PhSO_2^+$), 111 (13.4), 92 (9.9), 91 (100, $C_7H_7^+$), 83 (11.8), 65 (23.4).

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