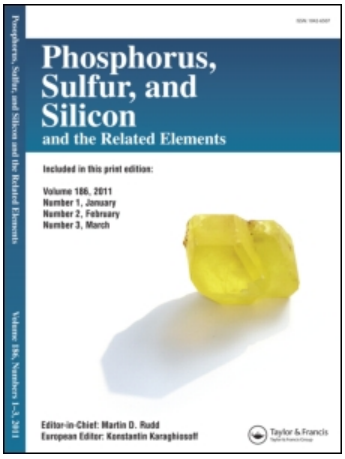

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BIOLOGICAL ACTIVITY OF AMINOPHOSPHONIC ACIDS

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BIOLOGICAL ACTIVITY OF AMINOPHOSPHONIC ACIDS

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Aminophosphonates are analogues of amino acids in which a carboxylic moiety is replaced by phosphonic acid or related groups. Acting as antagonists of amino acids, they inhibit enzymes involved in amino acid metabolism and thus affect the physiological activity of the cell. These effects may be exerted as antibacterial, plant growth regulatory or neuromodulatory. Chosen representative examples of biologically active aminophosphonates are presented in some detail.

Key words: Antibacterials; enzyme inhibitors; herbicides; neuroactivity; phosphonate; phosphono peptides.

INTRODUCTION

Aminoalkanephosphonic acids are broadly defined as analogues of amino acids in which a carboxylic group is replaced by phosphonic or related function (phosphonous, phosphinic, phosphine oxide etc.). Although they were first mentioned in the literature in 1943 as a patent describing the synthesis of aminomethanephosphonic acid,[1] for about two decades they had received only marginal attention. This situation began to change in the sixties after the discovery of 2-aminoethanephosphonic acid and related compounds in a wide range of living organisms.[2,3] However, they have become increasingly important from the early 1970's when it was revealed that the synthetic and natural aminophosphonates display diverse and useful biological properties.

Being the structural analogues of amino acids, aminophosphonic acids usually act as their antagonists and compete with their carboxylic counterparts for the active sites of enzymes or other cell receptors. As inhibitors of metabolic processes, they exert their physiological activity as antibacterial agents, neuroactive compounds, anticancer drugs or pesticides, possible application of which range from medicine to agriculture.

These are rather remarkable findings considering that carboxylic and phosphonic groups differ substantially in many respects including size (phosphonic function is considerably larger), shape (flat carboxylic versus tetrahedral phosphonic one) and

AMINO ACID AND ITS PHOSPHONIC ANALOGUE

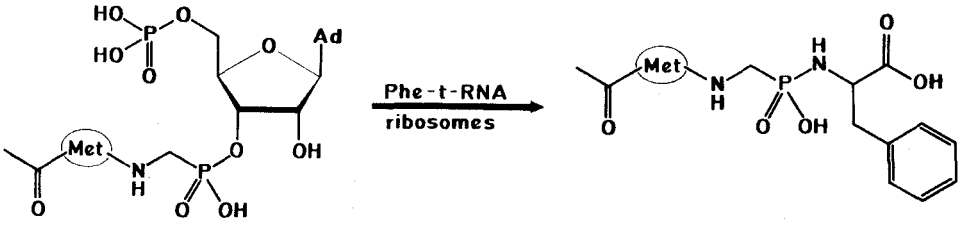
acidity (pK difference of at least three units). Thus, the simple aminophosphonic acids defy the intuitive understanding of structural analogy and the reasons of their biological activity are not apparent at present.

INHIBITION OF ENZYMES BY AMINOPHOSPHONIC ACIDS

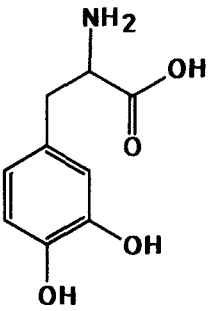
The history of aminophosphonic acids as enzyme inhibitors dates back to 1959 and begins with a report on the inhibition of avian glutamine synthetase by some analogues of glutamic acid.[4] That report went largely unnoticed, however, and the exploration of enzyme inhibition by aminophosphonates was not resumed until early 1970's.

From 1970 to the present there have been published over 100 papers dealing with the interaction of enzymes with aminophosphonic acids, and over 50 enzymes have been examined. Most of the enzymes are those involved in the metabolism of amino acids. The inhibition frequently observed indicates that there is a structural antagonism between amino acids and their phosphonic acid counterparts, and the numerous enzymes do recognize aminophosphonic acids as being more or less similar to the respective amino carboxylic acids. The most conspicuous examples are provided by strong inhibition of glutamine synthetase,[4-11] angiotensin converting enzyme,[12,13] adenylosuccinate lyase,[14] alanine racemase,[15-19] phenylalanine ammonia lyase,[20,21] tyrosinase,[22,23] and folylpolyglutamate synthetase.[24]

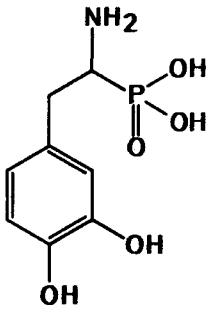
Structural differences between carboxylic and phosphonic acid groups do not prevent aminophosphonic acids from serving as substrates of some enzymes which normally utilize aminocarboxylic acids. This is not particularly remarkable if considering the fact that relatively few reported examples include only reactions without direct participation of carboxylic (or phosphonic) acid functions. Only one exception has been reported to date. Ribosomes of *Escherichia coli* catalyze the formation of a phosphonamide with direct P—N bond formation from a phosphono peptide and phenylalanine t-RNA[25]:



A good example is the interaction of the phosphonic analogues of 3,4-dihydroxyphenylalanine (dopa) with tyrosinase.[23] A simple replacement of carboxylic acid group of dopa by a phosphonic moiety leads to compound 1 which serves as a synthetic substrate for tyrosinase. This indicates that tyrosinase does not differentiate between the carboxylic and the phosphonic functions. A significant change in activity was achieved by shortening the alkyl chain of the analogue **1** by one methylene group which led to compound **2**. This analogue turned out to be one of the most powerful known inhibitors of the enzyme.



DOPA



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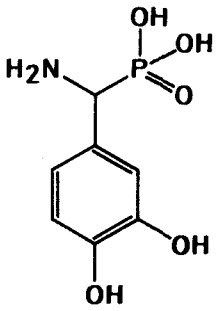

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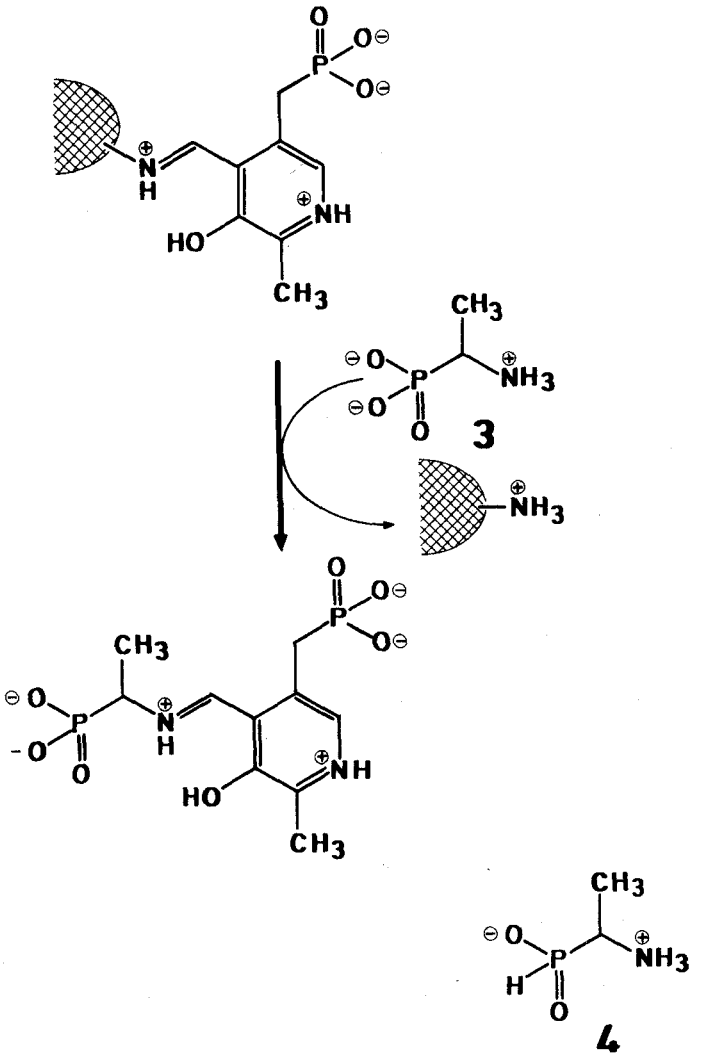
Both, phosphonic substrate **1** and inhibitor **2** form complexes with tyrosinase by a fit of the hydrophobic fragment of the molecule into the catechol-binding site and by electrostatic complexation of the negatively charged phosphonate dianion by the positively charged carboxylate-binding site of the enzyme.

Strong electrostatic binding of phosphonate dianion by the appropriate portion of enzyme accounts significantly for the inhibitory action of most of the phosphonic acid analogues of amino acids.

The alanine racemases are a group of bacterial enzymes that catalyze the racemization of alanine. These enzymes are operative at early stages of cell growth, thus providing *D*-alanine for inclusion into the bacterial cell wall.[26] Thus, racemases have been an attractive target for antibacterial drug development. Phosphonic analogue **3** of alanine is a potent inhibitor of a racemase from Gram-positive bacteria.[17–19] The aminophosphonate binds with pyridoxal 5′-phosphate in a manner identical to alanine. The formation of inhibitor-coenzyme aldimine is followed by a change in the enzyme conformation resulting in tight binding of the inhibitor. It is possible that a conformational change results from strong electrostatic binding of the inhibitor phosphonate dianion with a positively charged residue (e.g. lysine or histidine amino function) in the enzyme active center. Phosphonous acid analogue **4**, which contains only a monophosphate anion, does not lead to such a significant enzyme inactivation.[17]

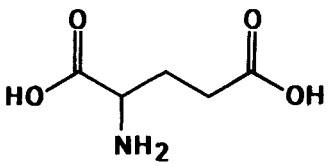
Glutamine synthetase catalyzes a reaction of central importance in nitrogen metabolism, namely the conversion of *L*-glutamate to *L*-glutamine. The amide source of glutamine is the ultimate source of nitrogen introduced into amino acids *via* transaminase-catalyzed reactions and also provides nitrogen for the urea cycle and pyrimidine biosynthesis. Phosphinothricin (**5**), a phosphonic acid mimetic of glutamic acid produced by various *Streptomycete* species,[27,28] is a potent inhibitor of this enzyme. Other examples of potent inhibitors include its α-methyl-,[6,10] and cyclic[9,10] analogues (**6** and **7** respectively), as well as γ-hydroxyphosphinothricin (**8**).[9,10] However, phosphonic acid analogue **9** of glutamic acid exhibits a much lower affinity for the enzyme. This indicates that, contrary to the two examples discussed earlier, the electrostatic binding of phosphonate dianion is not vital for inhibitory action.

Interaction of these inhibitors with glutamine synthetase occurs only under phosphorylation conditions (i.e. both magnesium chloride and ATP are required for enzyme inhibition). This strongly suggests that they undergo enzyme-mediated

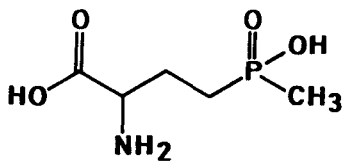


phosphorylation, generating an isostere of the presumed reaction intermediate, namely an unstable tetrahedral adduct between phosphorylated glutamic acid and ammonia.[10] Thus, the phosphorylated phosphinothricins formed mimic a putative tetrahedral transition state of the enzymatic reaction and may be considered as enzymatically formed transition state analogues.

Transition state analogues are interesting primarily as enzyme inhibitors that reflect on the probable mechanism of catalysis.[29,30] Such analogues are synthesized on the premise that binding interactions between an enzyme and its substrate are optimal at the transition state. The similarity between phosphorus compounds and tetrahedral intermediates of enzyme reactions taking place at carbonyl atoms was recognized thirty years ago.[31] However, the practical development of this concept for the design of enzyme inhibitors is of much more recent origin and was initiated by Bartlett[32] and by Khomutov.[33] Thus, the concept that phosphonic and phosphinic



GLUTAMIC ACID



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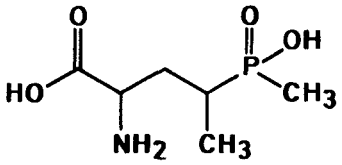

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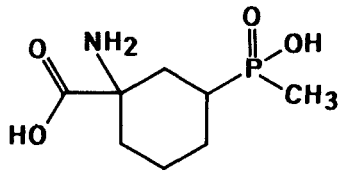

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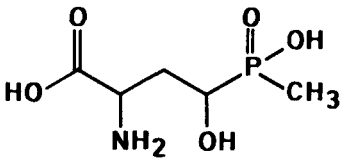

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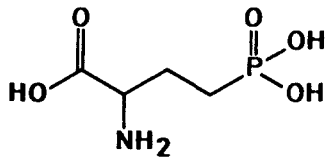

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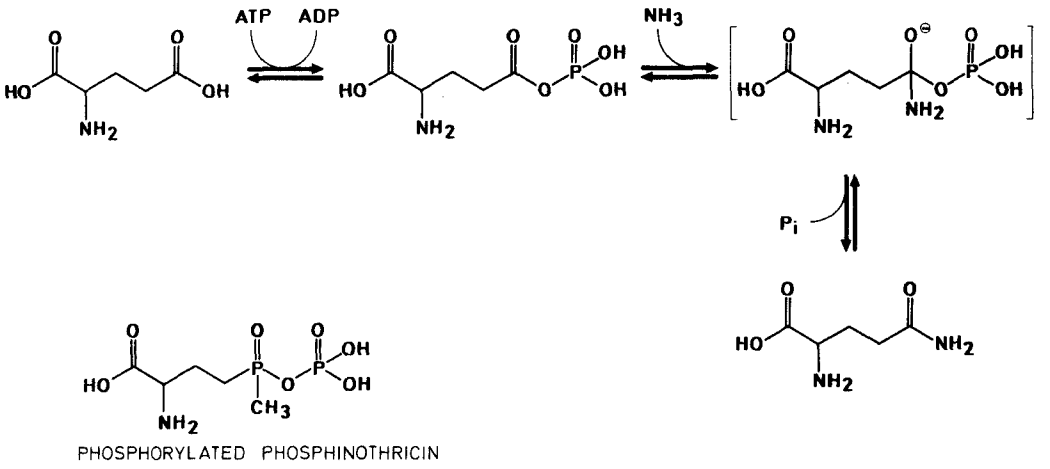
acids mimic the putative tetrahedral transition state of the catalytic hydrolysis involving direct attack of nucleophile on the amide or ester bond of the peptide or carboxylic ester substrate has proven very effective as a basis for designing potent enzyme inhibitors.[32–37]

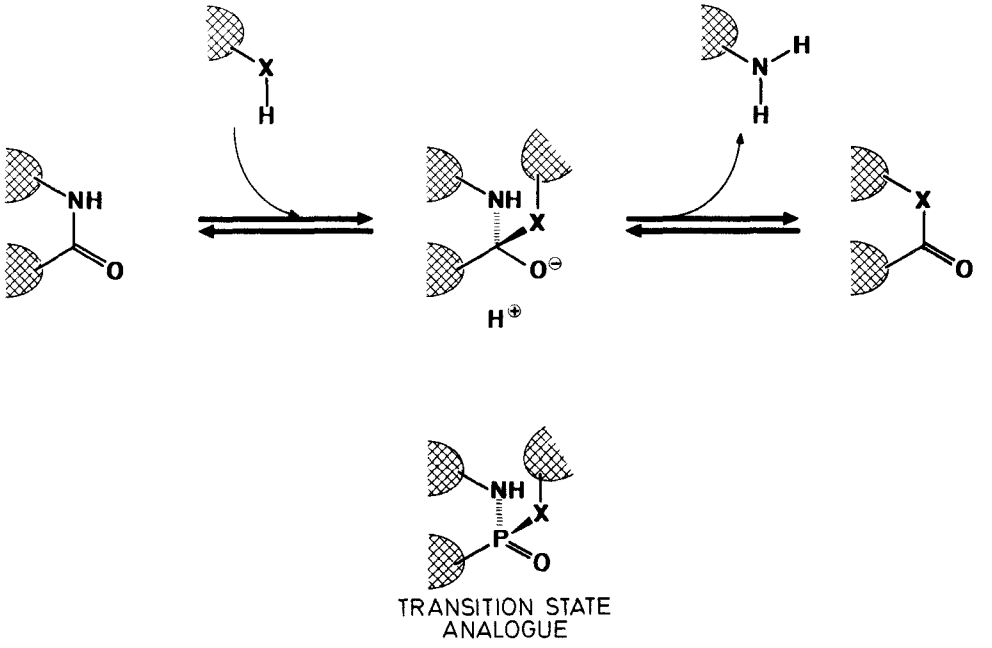
The representative examples of these inhibitors include the simple phosphonic acid analogue **10** of leucine, a potent inhibitor of leucine aminopeptidase[34,35]; phosphonamidate analogues **11** of peptides with powerful activity against carboxypeptidase A,[36–38] thermolysin,[38–42] and collagenase[43]; phosphinic acid analogues **12** of pepstatine, an inhibitor of pepsin,[44] and compound **13** which displays a powerful inhibitory activity against renin.[45]

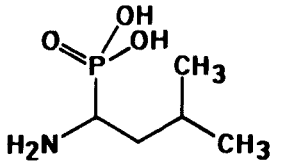
The inhibition of metalloproteinases, especially those containing active-site zinc ion, arise additionally through chelation of metal ion by the phosphonyl oxygen atom. This was unequivocally shown by X-ray crystallographic studies of stable complexes of phosphonamidate peptide inhibitors with thermolysin[46] and carboxypeptidase A.[47] A powerful inhibitor of thermolysin, analogue **14** of the substrate (N-carbobenzoxy-*L*-phenylalanyl-*L*-leucyl-*L*-alanine), binds to the enzyme in the



manner expected for the transition state displaying all the interactions that are presumed to stabilize the tetrahedral intermediate, thus supporting the postulated mechanism of enzymatic catalysis. The two oxygens of the phosphonamidate moiety are ligated to zinc to give the overall pentacoordination of the metal ion. The observed increase in the phosphorus-nitrogen bond length strongly suggests that the phosphonamidate nitrogen atom is protonated, and thus positively charged. This results in additional binding of the phosphonamidate portion of the inhibitor in the active site of thermolysin by donation of two hydrogen bonds to the protein.

The extensive work on phosphonic acid inhibitors of angiotensin converting enzyme[48,49] led to fosinopril (**15**),[48-50] the compound which represents the most successful commercial implementation of the concept of transition state analogy.





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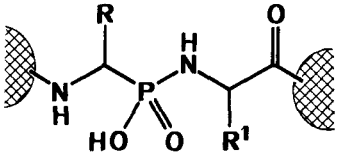

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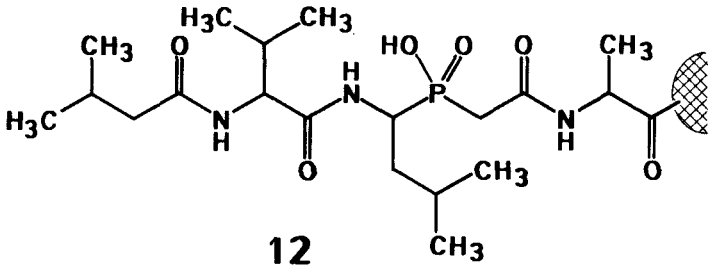

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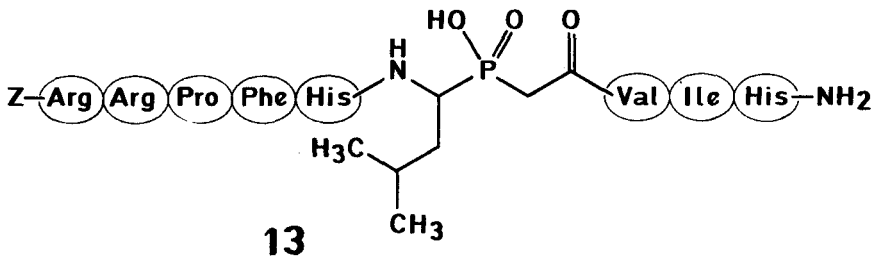

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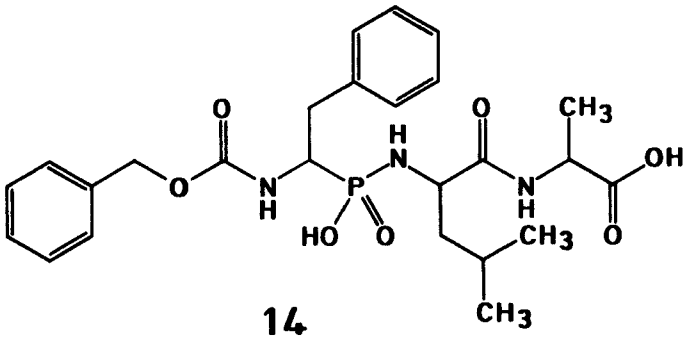
Fosinopril (**15**), inactive *per se*, is an (acyloxy) alkyl prodrug of compound **16**. It is metabolized primarily by intestinal and liver esterases, which results in the release of the phosphonic acid **16**,[51] a potent long-lasting inhibitor of angiotensin converting enzyme. Fosinopril appeared to be an effective oral antihypertensive agent.

If enzymes act as catalysts because they are more closely complementary to the transition states than to the substrates or products of the reaction, then the reverse of this relationship would suggest that proteins which are designed to complex specifically with the transition state analogue can express catalytic function. Thus, a good model of a transition state would serve as a structural template for the desired activity. This concept was encompassed by raising monoclonal antibodies to synthetic analogues of the presumed transition states of various reactions.[52–54]

The immune system is capable of producing antibodies which, like enzymes, bind both macromolecules and small synthetic molecules with high specificities and high affinities.[55,56] In other words, immunoglobulins can be rapidly and selectively generated against any molecule of interest. Consequently, the development of strategies for introducing the catalytic activity into the combining sites of antibodies



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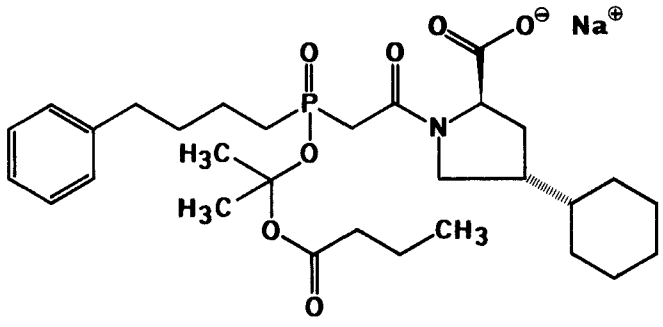
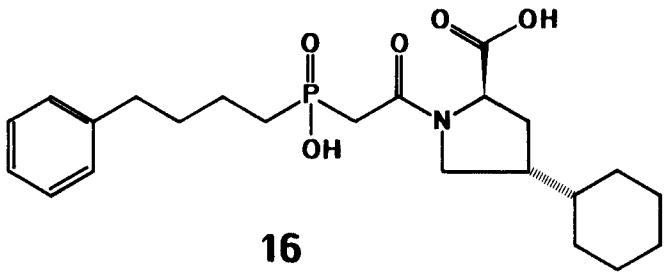
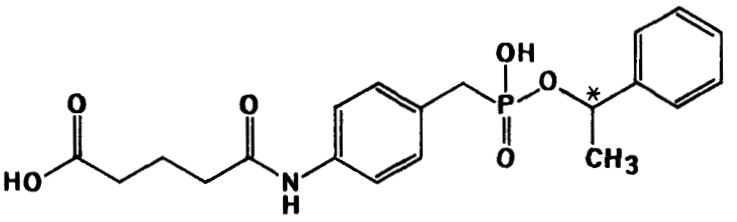


15 FOSINOPRIL



16

enables the generation of rationally designed enzyme-like catalysts with tailored specificities and catalytic properties—so called catalytic antibodies or abzymes. Being effective analogues of transition states of hydrolytic reactions phosphonates appeared to be ideal templates for obtaining abzymes of esterolytic and amidolytic activity.[52–54,57] For example, racemic phosphonate **17** was coupled to protein carrier and the conjugate was used to immunize mice for production of monoclonal antibodies. This procedure produced 11 antibodies with significant (10^3 to 10^5 reaction rate accelerations) enzyme-like activity. Nine of them were enantiospecific for the hydrolysis of (R)-**18** ester, whereas the other two were stereospecific for the hydrolysis of the antipodal ester (S)-**18**.[53]



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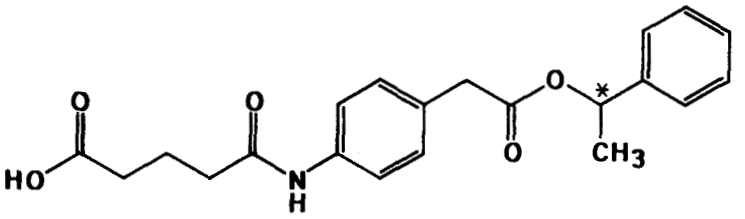

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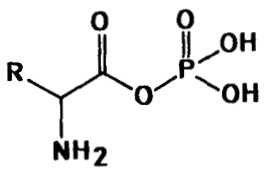
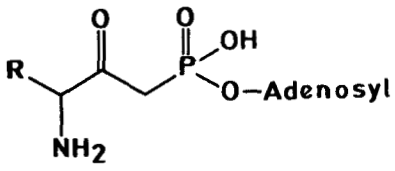
Phosphonate analogues of aminoacyl phosphates and aminoacyl adenylates constitute another group of analogues of reaction intermediates. These analogues differ from the parent compounds by having the methylene group in place of phosphate oxygen atom. Such a replacement produces very close isosteric (but not isopolar) analogues of natural phosphate esters, and the use of this approach for the design of inhibitors of amino acid metabolism was originally proposed by Göhring and Cramer.[58]

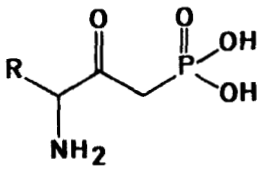
The most successful example of this approach is the inhibition of *D*-alanine:*D*-alanine ligase by analogue **19** of *D*-alanyl phosphate, the presumed intermediate of enzymatic formation of *D*-alanyl-*D*-alanine, an essential precursor of bacterial cell wall biosynthesis.[59] Similar to other acyl phosphate analogues, compound **19** exhibits only moderate inhibitory activity and thus confirms the rule that a re-

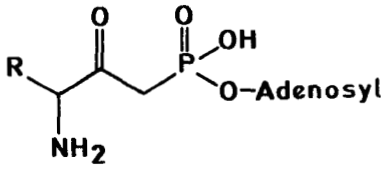


AMINOACYL PHOSPHATE

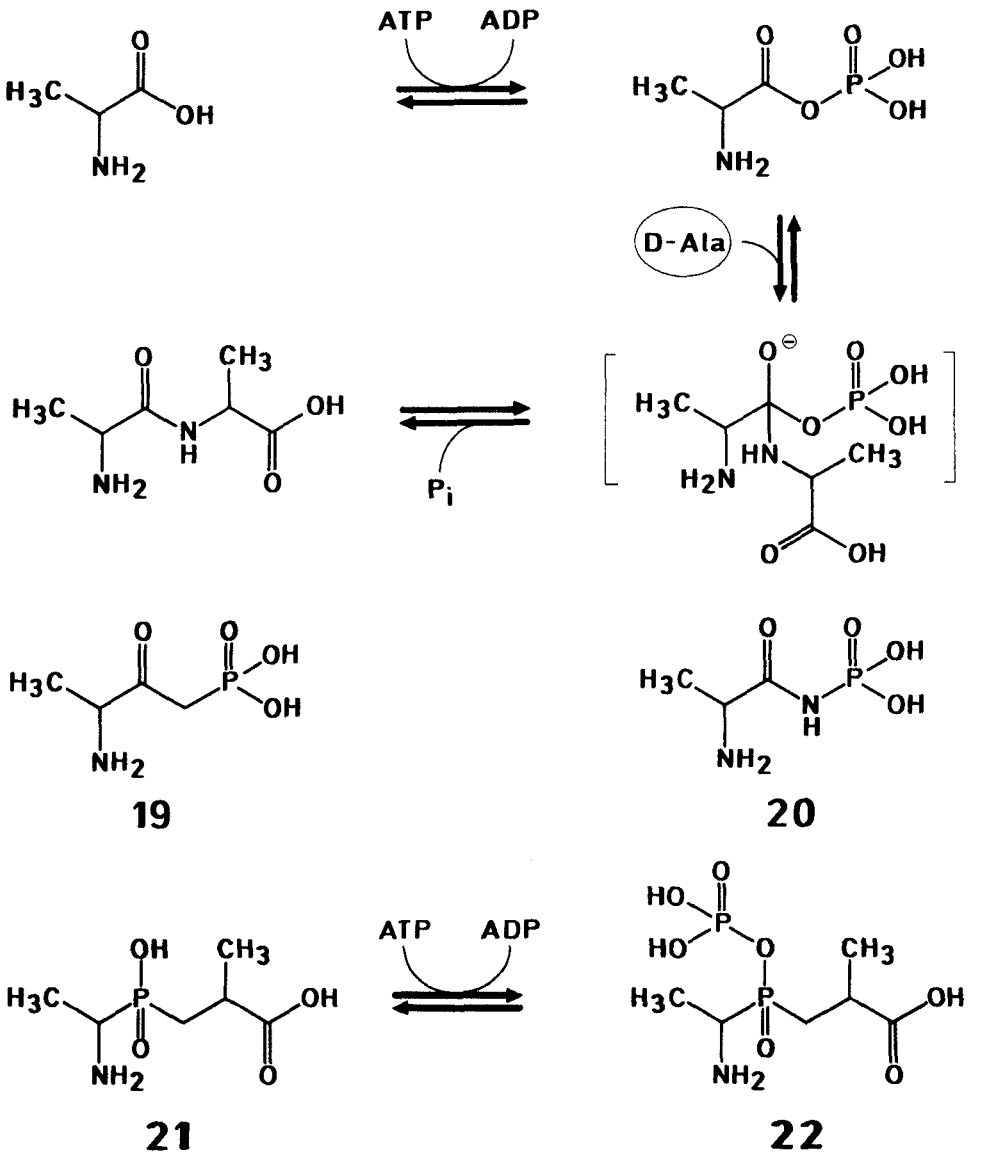


AMINOACYLADENYLATE



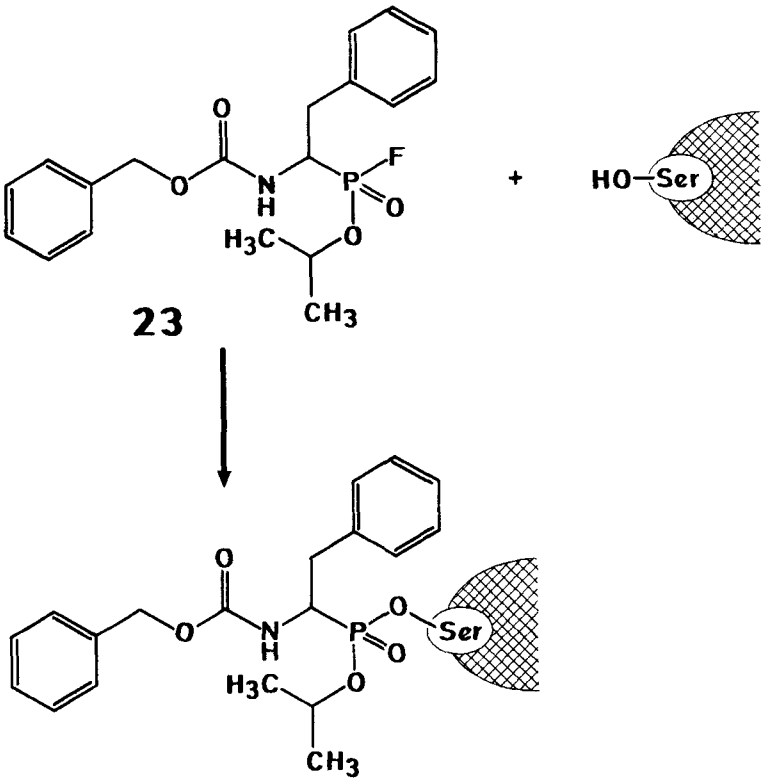


placement of the oxygen atom by the methylene group does not produce analogues with high affinities to the active centers of the respective enzymes.[60] Thus, it is not surprising that the aza-derivative **20**, the isosteric and isopolar analogue of *D*-alanyl phosphate, is a better inhibitor of *D*-alanine:*D*-alanine ligase than the compound **13**.[59] The most powerful inhibitor of this enzyme is, however, an analogue, **21**, of the substrate which contains a phosphinate moiety in a position analogous to the amide carbonyl in the dipeptide.[61] The mechanism of enzyme inactivation by this transition state analogue is in some respect similar to inactivation of glutamine synthetase by phosphinothricin (**15**) and involves the phosphorylation of the enzyme-bound inhibitor by ATP to form a phosphoryl-phosphinate adduct **22**.[62]



19 **20**

21 **22**

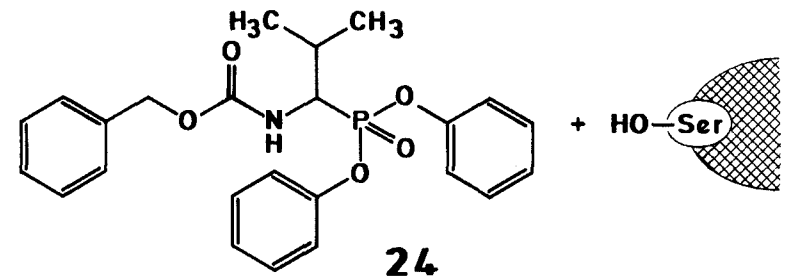


The development of active-site directed inhibitors has the goal of covalently modifying groups essential for enzyme function by making a bond between the inhibitor and the enzyme. For this purpose the substrate of the enzyme is provided with the additional, reactive chemical function. After formation of a normal enzyme-substrate complex in a reversible phase, a covalent bond joints the substrate analogue to the active center. This irreversibly inactivates the enzyme. Selective phosphorylation has been little studied with this respect. Phosphonofluoridate analogue **23** of carbobenzoxyphenylalanine was extremely effective and highly specific in the inactivation of chymotrypsin.[63] This analogue, however, also undergoes very fast nonenzymatic hydrolysis.

Peptidyl α-aminoalkylphosphonate diphenyl esters, resistant to hydrolysis, are also effective inactivators of serine proteases.[64] Extending the peptide chain from a single aminophosphonate residue in **24** to a tetrapeptide derivative **25** resulted in a 2800-fold improvement of inhibitory potency towards chymotrypsin.

ANTIBACTERIAL ACTIVITY

Within the vast array of available scientific literature, several thousands antibiotics and antibiotic-like agents have been reported. The phosphorus-containing antibiotics, especially phosphonic acids, represent an interesting group of antimicrobial



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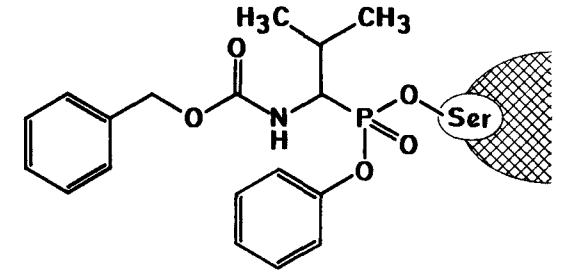

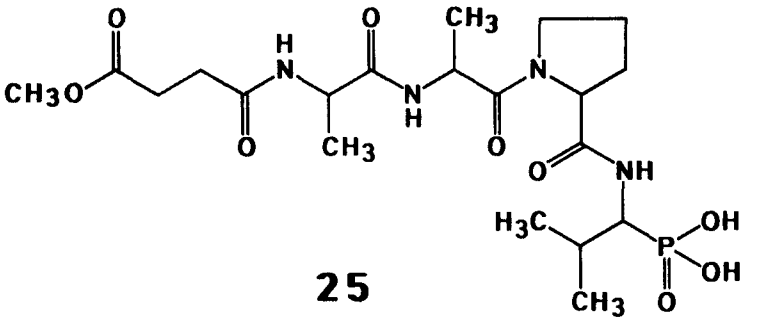

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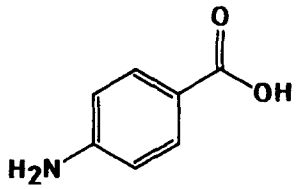
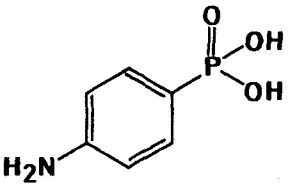
agents which is steadily increasing in number. Some of these compounds are produced by total chemical synthesis but many represent products of microbial origin.

The subject of antimicrobial aminophosphonic acids is relatively young and no major developments were recorded in the literature before 1970. The only exception is the discovery of phosphanilic acid (**26**) designed as mimetic of *p*-aminobenzoic acid and antimicrobial sulfanilic acid.[65] Low toxicity, effectiveness comparable to that of sulfonamides and interesting antibacterial properties in combination with other antibiotics account for continued, moderate interest in phosphanilic acid.

The slow progress before 1970 is contrasted by a very rapid development over last decades which witnessed the identification of several natural antibiotic ami-



p-AMINOBENZOIC ACID



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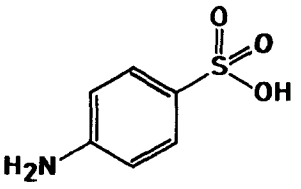
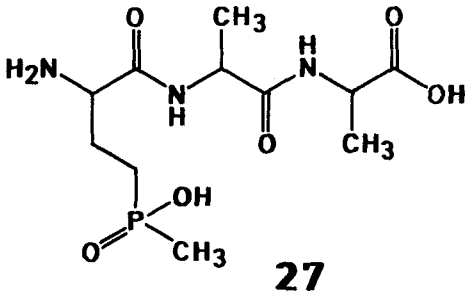


SULFANILIC ACID

nophosphonates: bialaphos (**27**) (produced by *Streptomyces viridochromogenes*)[27,28]; phosalacine (**28**) (*Kitasasporia phosalacinea*)[66]; plumbeomycins (**29**) (*S. plumbeus*)[67] and structurally related rhizotocins (**30**) (*Bacillus subtilis*)[68]; and fosmidomycin (**31**), as well as its derivatives (*S. lavenulae* and *S. rubellomurins*).[69]



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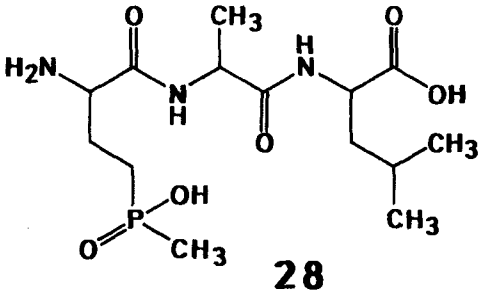

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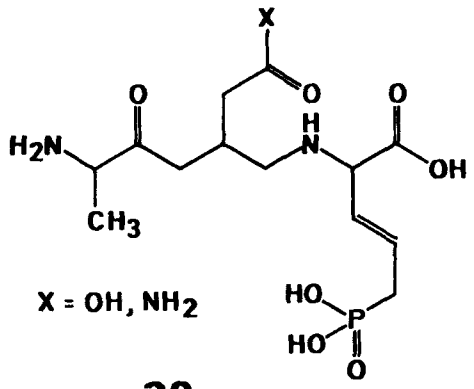

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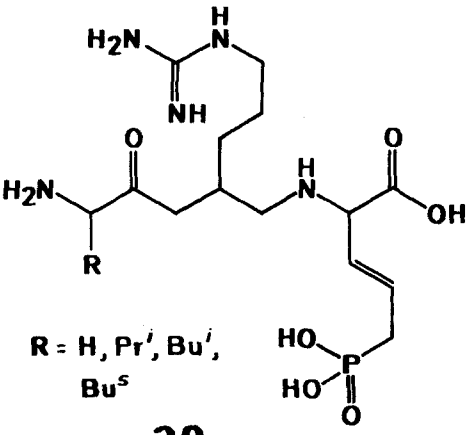

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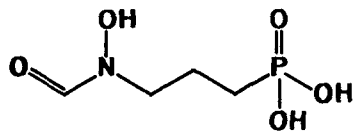

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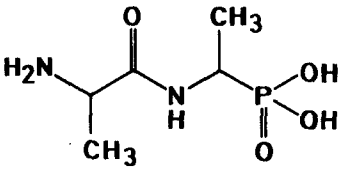
Fosmidomycin (**31**), chosen for development among four structurally related phosphonic acids isolated from *Streptomyces*, is the most interesting one among natural phosphonate antibiotics for use in human medicine. Consequently, fosmidomycin has been the subject of extensive pharmacological studies and appears to be highly promising. It has already passed phase I tolerance studies and has been recommended for further clinical evaluation.[70] The enzymatic details of the mechanism of action of fosmidomycin is not clear. It inhibits the biosynthesis of both carotenoids and menaquinones in *Micrococcus luteus*, suggesting that inhibition of the biosynthesis of a common precursor of these isoprenoids (possibly farnesyl pyrophosphate) might be the main site of its antibacterial action.[71]

The discovery of natural antibacterial phosphono peptides was followed by the discovery of strong activity of alafosfalin (**32**).[72] This compound is perhaps the most intensively studied member of the phosphorus-containing antibiotics. Alafosfalin is an effective inhibitor of bacterial cell wall biosynthesis and a useful antibiotic resulted from an extensive effort at Hoffman La Roche Company initiated in 1971 aimed at the synthesis and antibacterial evaluation of peptide mimetics of *D*-alanyl-*D*-alanine,[73] a peptide essential for the formation of bacterial cell wall. Alafosfalin was selected from a range of related peptides on the basis of its activity, pharmacokinetic properties and stability to peptidases. Extensive studies established its usefulness in human medicine for treatment of urinary tract bacterial infections.

The discovery of alafosfalin stimulated studies on the preparation of other potential antibacterial compounds of similar structures. These investigations have identified novel phosphono peptides **33**, **34** and **35** containing P-terminal mimetics of glycine,[74] α-methylalanine,[75,76] and glutamic acid[75,77] with promising *in vitro* activity.



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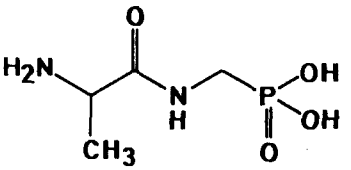

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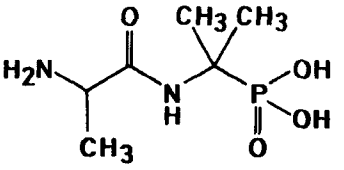

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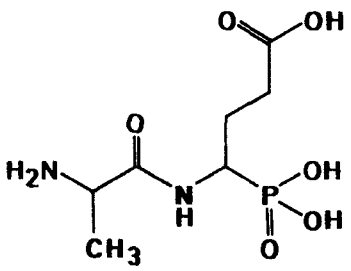

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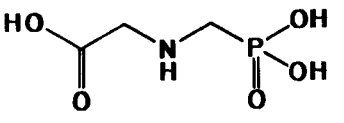
The mechanism of action of synthetic and naturally occurring phosphono peptides involves active transport through the bacterial cell by means of peptide permeases and enzymatic cleavage of the peptide bond within the cell to liberate the aminophosphonic acid.[79,80] The released P-terminal aminophosphonic acid acts on essential bacterial enzymes (e.g. the phosphonic analogue of alanine liberated from alafosfalin inhibits alanine racemase or phosphinothricin, a powerful inhibitor of glutamine synthetase, which is released from bialaphos), exerting its toxic effect.

PLANT GROWTH REGULATORY AMINOPHOSPHONIC ACIDS

The disclosure of herbicidal activity of glyphosate (**36**), in 1971, instituted a milestone in biochemistry of aminophosphonic acids.[81] This discovery initiated the extensive research concerned with the design, synthesis and evaluation of biological properties of new aminophosphonates.

Glyphosate, (N-phosphonomethyl)glycine (**36**),[56] is an extremely effective, nonselective, postemergence herbicide with an increasing number of international applications. It is the active ingredient of *Roundup*, Monsanto's herbicide formulation which is now marketed in more than 100 countries. Glyphosate has a relatively low molecular weight and high water solubility, factors which aid in its rapid absorption and translocation by plant tissues. Once inside the plant, the herbicide does not break down, nor is it metabolized to a significant degree. In soils, however, the compound is strongly absorbed (preventing leaching) and is rapidly degraded by microorganisms to the non-toxic organic products which are then broken down to ammonia, water and carbon dioxide. Moreover, glyphosate is non-toxic to insects and vertebrates and does not accumulate in animal tissues.

Glyphosate has been shown to elicit its herbicidal action by the inhibition of 5-enolpyruvylshikimate-3-phosphate synthase, thus blocking the shikimate pathway.[83] This blockage causes a reduction in synthesis of aromatic amino acids, cinnamate, and all cinnamate-derived phenolic compounds. Pools of aromatic amino acids are apparently further reduced by increased phenylalanine ammonia-lyase activity in glyphosate-treated plant tissues. Blockage of the shikimate pathway also results in the accumulation of high level of shikimate. This accumulation is exacerbated by the loss of feedback control of the pathway and unregulated carbon flow into this pathway. Certain benzoic acids that are derived primarily from shikimate rather than cinnamate also accumulate in glyphosate-treated tissues. Thus, the mechanism of development of phytotoxicity is complicated,[82] due to depletion of aromatic amino acids, unregulated drain of carbon into the shikimate pathway, and greatly disrupted secondary metabolism.



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The success of glyphosate (**36**) almost completely eclipsed the simultaneous discovery of herbicidal activity of *Trakephon* (**37**),[84] which is now manufactured and used in Germany. The molecular basis of the action of this effective herbicide is quite unclear. Recent studies, however, have shown that it disrupts the integrity of plant cell membranes.[85]

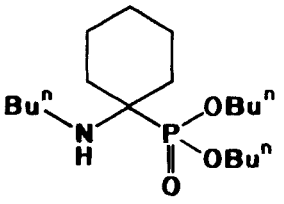
The lead provided by the structure of (N-phosphonomethyl)glycine (**36**) has been explored exhaustively by industrial and academic laboratories where hundreds, or perhaps thousands of glyphosate derivatives, homologues and various possible analogues have been synthesized and screened for the activity. However, information on the chemical structures of these analogues and their interference with plant and fungal growth is quite fragmentary and is presented, almost exclusively in the patent literature. Thus, it is not possible at present to draw any meaningful conclusion on the structure-activity relationship encompassing all glyphosate derivatives, homologues and analogues. Obviously, it is difficult to improve on a compound which is as simple as glyphosate (**36**) and exhibits such a powerful activity. Indeed most of the analogues are less active than (N-phosphonomethyl)glycine itself.

However, these efforts were not totally unsuccessful and resulted in discoveries of numerous highly active compounds. These include, at least, herbicidal phosphinothricin (**6**, Scheme 5), which is now been introduced in Germany and Japan,[86] N-pyridyl derivatives **38** of aminomethanephosphonic acid being developed in Japan,[87] as well as plant growth regulatory phosphonic analogues of morphactines (for example, compounds **39** and **40**),[88–91] and the phosphonic analogue **40** of dopa[90] first synthesized in our laboratories.

The most important among these compounds is phosphinothricin. Its monoammonium salt, glufosinate-ammonium, is an active ingredient of *Basta*, Hoechst's herbicide formulation which is now marketed in Europe and Japan. Physiological and biochemical effects of phosphinothricin in plants have not been studied as extensively as the effects of glyphosate but it appears that glufosinate destroys plants by inhibiting glutamine synthetase.[93] This impairs ammonia assimilation and the plants treated with phosphinothricin rapidly accumulate toxic levels of ammonia.[11,94]

Also phosphinothricylalanylalanine, bialaphos (**27**, Scheme 17), an antibacterial metabolite produced by *Streptomyces hygroscopicus*, exhibits strong herbicidal activity.[95] It is, perhaps, the first herbicide manufactured by fermentation.

The mechanism of action of bialaphos is similar to that of its antibacterial activity. Thus, it consists of uptake and translocation of the peptide into plant tissues, enzymatic release of phosphinothricin which exerts its activity by inhibiting glutamine synthetase. As indicated in the preceding Section the attachment of an additional amino acid to a substance of weak activity is a well established method for making possible its transportation through cell membranes[72–80] or into tissues.[96] Similarly, the attachment of various amino acids to aminoalkylphosphonic acids resulted in a series of compounds **42** with promising plant growth regulatory properties.[97–99] Their activities depend on the chemical structure of both N-terminal and C-terminal components of the peptide **42**. For example, 1-(N-*L*-valylamino)-2-methylpropylphosphonic acid (**42**, R=R^1=*iso*-propyl, phosphonic acid analogue of valylvaline), exhibits herbicidal, while 1-(N-*L*-alanylamino)-2-methylpropyl-



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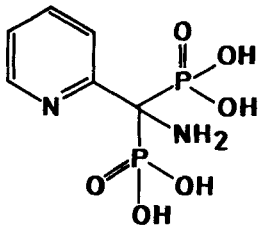

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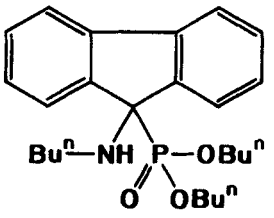

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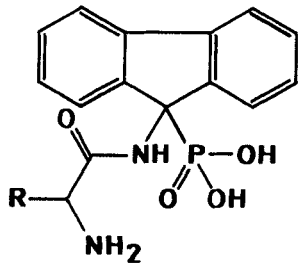

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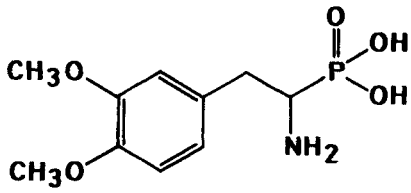

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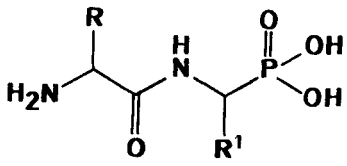
phosphonic acid (**42**, R=methyl, R^1=*iso*-propyl, analogue of alanylvaline) acts as typical plant growth regulator causing elongation of the plant roots with a weak effect on the growth of their stems.[98,99] The mechanism of action of plant growth regulatory phosphono peptides **42** is probably similar to that found for their antibacterial action and involves active uptake and transportation into plant tissues followed by enzymatic release of toxic aminoalkylphosphonic acids.



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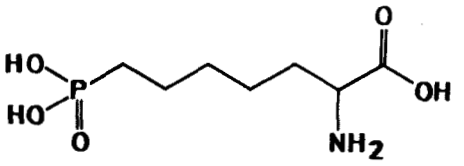
NEUROACTIVE AMINOPHOSPHONIC ACIDS

Because of the well recognized involvement of various amino acids in neurotransmission processes,[100] it appears as a natural development that aminophosphonates



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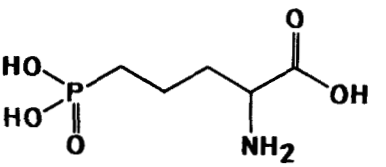

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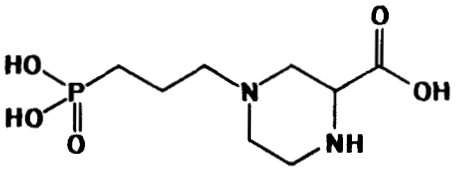

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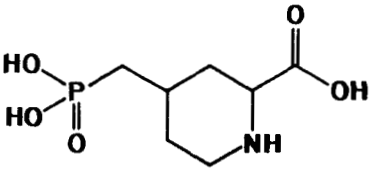

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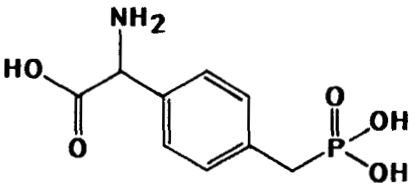
have been included among various amino acid analogues examined for neurophysiological effects. However, the recognition of the importance of aminophosphonic acids for neurotransmission research began relatively late and rather little was published before 1980.

Among all the neuromodulators, *L*-glutamic acid a common transmitter in central nervous system, is of special importance. It plays a crucial role in the development of long term potentiation and certain forms of memory.[101] Thus, it is not surprising that preliminary studies were focused on the neuroactivity of the simple phosphonic acid analogue **9** (Scheme 5) of glutamic acid. This analogue appears to be remarkable for its manifold and potent effects in various kinds of nervous tissue.[102–105]

Glutamate exerts its effects *via* several distinct receptor subtypes which are named after their preferred agonist, i.e. N-methyl-*D*-aspartate (NMDA), kainate and quisqualate. NMDA receptors are the best characterized. Such work has been made possible by the availability of selective NMDA antagonists such as: *D*-2-amino-5-phosphonoheptanoic acid (**43**, APH),[105] *D*-2-amino-3-phosphonovaleric acid (**44**, APV),[106] and 4-(3-phosphonopropyl)-2-piperazinecarboxylic acid (**45**,



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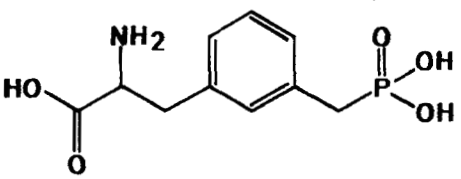

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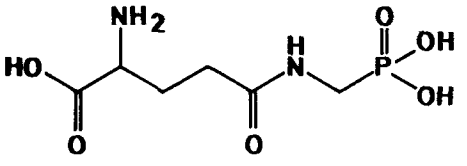

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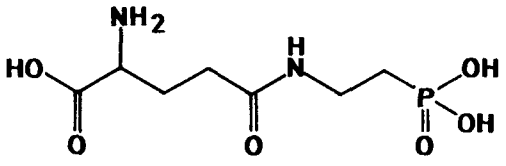

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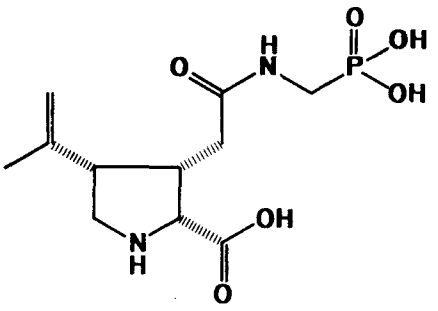

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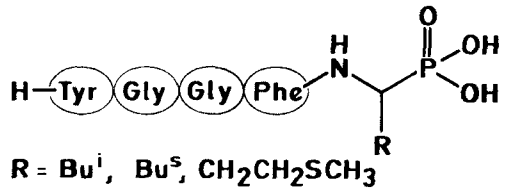

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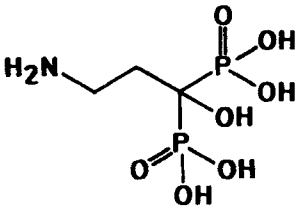
CPP),[107] and more recently *cis*-4-(phosphonopmethyl)-2-piperazinecarboxylic acid (**46**, CGS 19755).[108] Consequently, these aminophosphonic acids are now being used as tools in neurophysiological studies.

However, the generation of an equivalent antagonist structure-activity relationship is still in its infancy. In large part this situation arises from the limited structural variation of available antagonists. This stimulates intensive investigations for new aminophosphonates, which frequently result in the discovery of new compounds (for example **47** and **48**[109]) equipotent with APV or APH.

Some phosphono peptides display significant neurophysiological effects. Dipeptides **49**, **50** and **51** containing phosphonic acid analogues of glycine and β-alanine are strongly antagonistic to NMDA, inhibiting NMDA-evoked responses in the rat.[110,111] Pentapeptides **52**, phosphonic analogues of enkephalins, exhibit analgesic activity comparable with, or stronger than, that of their opiate counterparts.[112,113]

OTHER ACTIVITIES

There is a growing recognition that aminophosphonic acids affect living organisms in many ways. In addition to antibacterial, herbicidal and neurophysiological activities, there have been described a wide assortment of biological effects associated with aminophosphonates belonging to diverse structural classes. 3-Amino-1-hydroxypropane-1,1-bisphosphonic acid **53** is the most important example. It has attained a considerable practical importance in human medicine for treatment of bone disorders by regulating calcium metabolism.[114]



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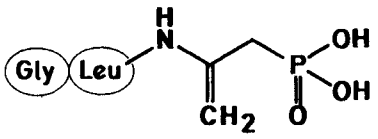

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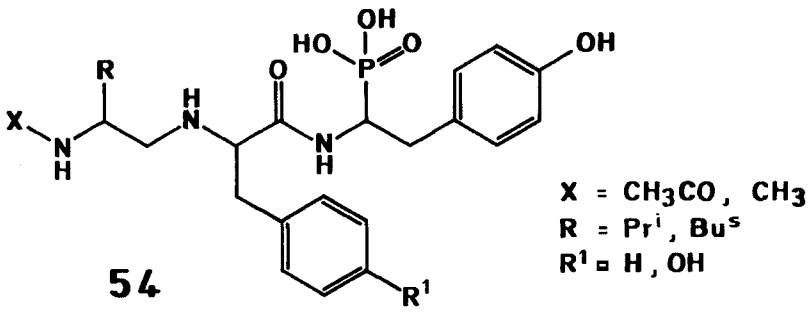

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Worth of note is also the hypertensive activity of phosphono peptide **44**, a metabolite of *Actinomycetes* and *Actinomadura*,[115,116] and immunosuppressive activity of peptide **55** produced by *Streptomyces lurides*.[117]

CONCLUSIONS

Aminoalkylphosphonic acids constitute an unique class of simple mimetics of amino acids. In most of their biological effects they compete with their carboxylic acid counterparts for the active sites of enzymes and other cell receptors. Such a competition frequently results in diverse and interesting biological and biochemical properties. Thus, aminophosphonic acids display their activity as antibacterial agents, neuromodulators, anticancer and antihypertensive drugs or plant growth regulators.

The lack of their physiological activity does not necessarily mean that there is a lack of aminophosphonic acid-receptor interaction. It is well documented that this phenomenon may be due to the existence of transportation barriers against these acids in living systems. The attachment of an amino acid or a short peptide to an aminophosphonic acid of negligible physiological activity usually facilitates its delivery into the appropriate site of its action.

A specific and separate class of aminophosphonic acid derivatives are powerful inhibitors of certain enzymes in which the tetrahedral configuration around the phosphonate phosphorus mimics the transitions state of the carbon atom, which undergoes a transient conversion from a trigonal to a tetrahedral geometry during enzyme catalyzed hydrolysis or amidolysis. Discovery of the effective antihypertensive agent fosinopril (**15**) is the most successful commercial implementation of this concept. Fosinopril, however, is a prodrug in which a chemical structure of

angiotensin converting enzyme **16** was modified in order to facilitate its delivery to the site of action.

ACKNOWLEDGEMENTS

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REFERENCES

1. J. Pikl, *US Patent* 2.328.358 (1943); *Chem. Abstr.*, **38,** 4587 (1943).
2. M. Horiguchi and M. Kandatsu, *Nature,* **184,** 901 (1959).
3. J. S. Kittredge, E. Roberts and D. G. Simonsen, *Biochemistry,* **1,** 624 (1962).
4. P. Mastalerz, *Arch. Immun. Ter. Dosw.,* **7,** 201 (1959).
5. T. D. Meek and J. J. Villafranca, *Biochemistry,* **19,** 5513 (1980).
6. B. Lejczak, H. Starzemska and P. Mastalerz, *Experientia,* **37,** 462 (1981).
7. M. Leason, D. Cunliffe, D. Parkin, P. J. Lea and B. J. Miflin, *Phytochemistry,* **21,** 855 (1982).
8. E. W. Logusch, D. M. Walker, J. F. McDonald, G. C. Leo and J. E. Franz, *J. Org. Chem.,* **53,** 4069 (1988).
9. E. W. Logusch, D. M. Walker, J. F. McDonald and J. E. Franz, *Biochemistry,* **28,** 3043 (1989).
10. E. W. Logusch, D. M. Walker, J. F. McDonald, J. E. Franz, J. J. Villafranca, C. L. DiJanni, J. A. Colanduoni, B. Li and J. B. Schineller, *Biochemistry,* **29,** 366 (1990).
11. P. J. Lea, K. W. Joy, J. L. Ramos and M. G. Guerro, *Phytochemistry,* **23,** 1 (1984).
12. E. W. Petrillo Jr. and E. R. Spitzmiller, *Tetrahedron Lett.,* **21,** 4929 (1979).
13. G. A. Flynn and E. L. Giroux, *Tetrahedron Lett.,* **27,** 1757 (1986).
14. L. D. Brand and J. M. Lowenstein, *Biochemistry,* **17,** 1365 (1978).
15. E. Adams, K. A. Mukharjee and H. C. Dunathan, *Arch. Biochem. Biophys.,* **165,** 126 (1974).
16. A.-M. Lacoste, S. Mansour, A. Cassaigne and E. Neuzil, *Experientia,* **41,** 643 (1985).
17. B. Badet and C. T. Walsh, *Biochemistry,* **24,** 1333 (1985).
18. B. Badet, K. Inagaki, D. Soda and C. T. Walsh, *Biochemistry,* **25,** 3275 (1986).
19. V. Cope, S. Faraci, C. T. Walsh and R. G. Griffin, *Biochemistry,* **27,** 4966 (1988).
20. B. Laber, H.-H. Kiltz and N. Amrhein, *Z. Naturforsch.,* **41c,** 49 (1985).
21. K. Janas, A. Filipiak, J. Kowalik, P. Mastalerz and J. S. Knypl, *Acta Biochim. Polonica,* **32,** 131 (1985).
22. A. Iron, R. Monique, J.-P. Duboy, M. Beranger, A. Cassaigne and E. Neuzil, *Biochem. Soc. Trans.,* **9,** 246 (1981).
23. B. Lejczak, P. Kafarski and E. Makowiecka, *Biochem. J.,* **242,** 81 (1987).
24. A. Rosowsky, R. A. Forsch, R. G. Morgan, W. Kohler and J. H. Freisheim, *J. Med. Chem.,* **31,** 1326 (1988).
25. N. B. Tarussova, G. M. Yakovleva, L. S. Victorova, M. K. Kukhanova and R. M. Khomutov, *FEBS Lett.,* **130,** 85 (1981).
26. C. T. Walsh, *J. Biol. Chem.,* **264,** 2393 (1989).
27. E. Bayer, K. H. Gugel, K. Hägele, H. Hagenmeier, S. Jessipow, W. A. König and H. Zähner, *Helv. Chim. Acta,* **55,** 224 (1972).
28. Y. Ogawa, T. Tsuruoka, S. Inoue and T. Niida, *Meiji Seika Kenkyu Nempo,* **13,** 42 (1973).
29. L. Pauling, *Chem. Eng. News,* **24,** 1375 (1946).
30. R. Wolfenden, *Acc. Chem. Res.,* **5,** 10 (1972).
31. S. A. Bernhard and L. E. Orgel, *Science,* **130,** 625 (1959).
32. P. A. Bartlett, J. T. Hunt, J. L. Adams and J. C. E. Gehert, *Bioorg. Chem.,* **7,** 421 (1978).
33. A. I. Biryukov, B. K. Ishmuratov and R. M. Khomutov, *FEBS Lett.,* **91,** 249 (1978).
34. P. P. Giannousis and P. A. Bartlett, *J. Med. Chem.,* **30,** 1603 (1987).
35. B. Lejczak, P. Kafarski and J. Zygmunt, *Biochemistry,* **28,** 3549 (1989).
36. N. E. Jacobsen and P. A. Bartlett, *J. Am. Chem. Soc.,* **105,** 1613 (1981).
37. J. E. Hanson, A. P. Kaplan and P. A. Bartlett, *Biochemistry,* **28,** 6294 (1989).
38. P. A. Bartlett, C. K. Marlowe, P. P. Giannousis and J. E. Hanson, *E. Cold Spring Harbor Symp. Quant. Biol.,* **52,** 83 (1987).
39. P. A. Bartlett and C. K. Marlowe, *Biochemistry,* **22,** 4618 (1983).
40. P. A. Bartlett and C. K. Marlowe, *Science,* **235,** 569 (1987).
41. P. A. Bartlett and C. K. Marlowe, *Biochemistry,* **26,** 8553 (1987).
42. D. Grobelny, U. B. Goli and R. E. Galardy, *Biochemistry,* **28,** 4948 (1989).
43. K. A. Mookhtiar, C. K. Marlowe, P. A. Bartlett and H. E. van Wart, *Biochemistry,* **26,** 1962 (1987).

44. P. A. Bartlett and W. B. Kezer, *J. Am. Chem. Soc.*, **106,** 4282 (1984).
45. M. C. Allen, W. Fuhrer, B. Tuck, R. Wade and J. M. Wood, *J. Med. Chem.*, **32,** 1652 (1989).
46. H. M. Holden, D. E. Tonrud, A. F. Monzingo, L. H. Weaver and B. W. Matthews, *Biochemistry,* **26,** 8542 (1987).
47. D. W. Christianson and W. N. Lipscomb, *J. Am. Chem. Soc.*, **110,** 5560 (1988).
48. D. S. Karanewsky, M. C. Badia, D. W. Cushman, J. M. De Forrest, T. Dejneka, M. J. Loots, M. G. Pern, E. D. Petrillo Jr. and J. R. Powell, *J. Med. Chem.*, **31,** 204 (1988).
49. J. Krapcho, C. Turk, D. W. Cushman, J. R. Powell, J. M. De Forrest, E. R. Spitzmiller, D. S. Karanewsky, M. Duggan, G. Rovnyak, J. Schwartz, S. Natarayan, J. D. Godfrey, D. E. Ryono, R. Neubeck, K. S. Atwal and E. W. Petrillo Jr., *J. Med. Chem.*, **31,** 1148 (1988).
50. J. M. De Forrest, T. L. Waldron, C. Harvey, B. Scalese, B. Rubin, J. R. Powell, E. W. Petrillo and D. W. Cushman, *J. Cardiovasc. Pharmacol.*, **14,** 730 (1989).
51. R. A. Morrison, S. M. Sighvi, D. A. Pocetti, A. E. Peterson and B. H. Migdalof, *Pharmacologist,* **29,** 230 (1987).
52. R. A. Lerner and A. Tramontano, *Trends Biochem. Sci.*, **12,** 427 (1987).
53. K. D. Janda, S. J. Benkovic and R. A. Lerner, *Science,* **244,** 437 (1989).
54. P. G. Schulz, *Angew. Chem. Int. Ed.*, **28,** 1283 (1989).
55. A. Nisonoff, *Antibody Molecule,* J. Hopper & S. Spring, Eds., Academic Press, New York 1975.
56. J. W. Goding, *Monoclonal Antibodies. Principles and Practice,* Academic Press, New York 1986.
57. S. J. Pollack and P. G. Schulz, *Cold Harbor Symp. Quant. Biol.*, **52,** 97 (1987).
58. G. Göhring and F. Cramer, *Chem. Ber.*, **106,** 2460 (1973).
59. P. K. Chakravarty, W. J. Greenlee, W. H. Parsons, A. A. Patchett, P. Combs, A. Roth, R. D. Busch and T. N. Mellin, *J. Med. Chem.*, **32,** 1886 (1989).
60. G. M. Blackburn, *Chem. Ind.*, 134 (1981).
61. W. H. Parsons, A. A. Patchett, H. B. Bull, W. R. Schoen, D. Taub, J. Davidson, P. L. Combs, J. P. Springer, H. Gadebusch, M. E. Weissberger, M. E. Valiant, T. N. Mellin and R. D. Busch, *J. Med. Chem.*, **31,** 1772 (1988).
62. A. E. McDermott, F. Creuzet, R. G. Griffin, L. E. Zawadzke, Q.-Z. Ye and C. T. Walsh, *Biochemistry,* **29,** 5767 (1990).
63. L. A. Lamden and P. A. Bartlett, *Biochem. Biophys. Res. Commun.*, **112,** 1085 (1983).
64. J. Oleksyszyn and J. C. Powers, *Biochem. Biophys. Res. Commun.*, **161,** 143 (1989).
65. H. J. Baurer, *J. Am. Chem. Soc.*, **63,** 2137 (1941).
66. S. Omura, K. Hinotazawa, N. Imamura and M. Murata, *J. Antibiot.*, **32,** 939 (1984).
67. B. K. Park, A. Hirota and H. Sakai, *Agr. Biol. Chem.*, **40,** 1905 (1976).
68. C. Rapp, G. Jung, M. Kugler and W. Loeffler, *W. Liebigs Ann. Chem.*, 655 (1988).
69. M. Okuhara and T. Goto, *Drugs Expl. Res.*, **7,** 559 (1981).
70. H.-P. Kuemmerle, T. Murakawa, H. Sakamoto, N. Sato, T. Konishi and F. De Santis, *Internat. J. Clin. Pharmacol. Therapy Toxicol.*, **23,** 515 (1985).
71. Y. Shigi, *J. Antimicrob. Chemother.*, **24,** 131 (1989).
72. J. G. Allen, F. R. Atherton, M. J. Hall, C. H. Hassall, S. W. Holmes, R. W. Lambert, L. J. Nisbet and P. S. Ringrose, *Nature,* **272,** 56 (1978).
73. C. H. Hassall, *Actual. Chim. Ther.*, **12,** 193 (1985).
74. F. R. Atherton, M. J. Hall, C. H. Hassall, R. W. Lambert, W. J. Lloyd, P. S. Ringrose and D. Westmacott, *Antimicrob. Agents Chemother.*, **22,** 571 (1982).
75. B. Lejczak, P. Kafarski, H. Sztajer and P. Mastalerz, *J. Med. Chem.*, **29,** 2212 (1986).
76. E. Zboińska, H. Sztajer, B. Lejczak and P. Kafarski, *FEMS Microbiol. Lett.*, **70,** 23 (1990).
77. F. R. Atherton, C. H. Hassall and R. W. Lambert, *J. Med. Chem.*, **29,** 29 (1986).
78. P. S. Ringrose in: *Microorganisms and Nitrogen Sources,* J. W. Payne Ed., John Wiley, New York 1980, pp. 641–692.
79. H. Diddens, H. Zähner, E. Kraas, W. Gohring and G. Jung, *Eur. J. Biochem.*, **66,** 11 (1976).
80. H. Diddens, M. Dagerloh and H. Zähner, *J. Antibiot.*, **32,** 87 (1979).
81. D. D. Baird, R. P. Upchurch and W. B. Homesley, *Proc. North. Central Weed Control Conf.*, **26,** 64 (1971).
82. *The Herbicide Glyphosate,* E. Grossbard and D. Atkinson (Eds.), Butterworth, London 1985.
83. H. C. Steirücken and N. Amrhein, *Biochem. Biophys. Res. Commun.*, **94,** 1207 (1980).
84. E. Günther and W. Löttge, *Ger. Offen.*, 2.022.228 (1971); *Chem. Abstr.*, **74,** 100219 (1971).
85. G. Linsel, I. Dahse and K. Müller, *Phys. Plant.*, **73,** 77 (1988).
86. P. Langelüddeke, D. Krausse, E. Rose, F. Wallmüller and K. H. Walther, *Bundestalt Land Forst. Wirtsch.*, **203,** 256 (1981).
87. Y. Okamoto, *11th Int. Congr. Phosphorus Compounds,* Rabat 1977, p. 649.
88. W. Czerwiński, R. Gancarz, E. Przybylka and J. S. Wieczorek, *Acta Agrobot.*, **34,** 253 (1982).
89. B. Lejczak, P. Kafarski, R. Gancarz, E. Jaskulska, P. Mastalerz, J. S. Wieczorek and M. Król, *Pestic. Sci.*, **16,** 227 (1985).

90. R. Gancarz, W. Wielkopolski, E. Jaskulska, P. Kafarski, B. Lejczak, P. Mastalerz and J. S. Wieczorek, *Pestic. Sci.*, **16,** 234 (1985).
91. P. Kafarski, B. Lejczak, R. Gancarz, E. Jaskulska, P. Mastalerz, J. S. Wieczorek and J. Zbyryt, *Pestic. Sci.*, **16,** 239 (1985).
92. P. Kafarski, B. Lejczak, E. Ślesak and J. Przetocki, *Pestic. Sci.*, **25,** 137 (1989).
93. L. Maier and P. J. Lea, *Phosphorus and Sulfur,* **17,** 1 (1983).
94. M. Lacuesta, B. Gonzalez-Moro, C. Gonzalez-Muna, P. Aparicio-Tejo and A. Munoz-Rueda, *J. Plant. Physiol.*, **134,** 304 (1989).
95. K. Tachibana In: *Pesticide Science and Biology,* R. Greenlagh & T. R. Roberts (Eds.), Blakwell Sci. Publ., London 1987.
96. M. Sheikh, B. Gotlinsky, B. E. Tropp, R. Engel and T. Parker, *Am Chem. Soc. Symp. Ser.*, **171,** 225 (1981).
97. B. Lejczak and M. Kijas-Kaczanowska In: *Peptide Chemistry 1987,* T. Shiba and S. Sakakibara (Eds.), Protein Research Foundation, Osaka 1988, p. 315.
98. B. Lejczak, P. Kafarski and R. Gancarz, *Pestic. Sci.*, **22,** 263 (1988).
99. P. Wieczorek, M. Kaczanowska, B. Lejczak and P. Kafarski, *Pestic. Sci.*, **30,** 43 (1990).
100. J. C. Watkins, *Trends Pharmacol. Sci.*, **5,** 373 (1984).
101. F. Fonnum, *J. Neurochem.*, **42,** 1 (1984).
102. B. Bioulac, E. de Tinguy-Moreaud, J. D. Vincent and E. Neuzil, *Gen. Pharmacol.*, **10,** 121 (1979).
103. R. H. Evans and J. C. Watkins, *Ann. Rev. Pharmacol. Toxicol.*, **21,** 165 (1981).
104. R. L. Gannon, L. T. Batty and D. M. Terrian, *Brain Res.*, **495,** 151 (1989).
105. M. N. Perkins, T. W. Stone, J. F. Collins and K. Curry, *Neurosci. Lett.*, **23,** 333 (1981).
106. J. Davies, A. A. Francis, A. W. Jones and J. C. Watkins, *Neurosci. Lett.*, **21,** 771 (1981).
107. J. Davies, R. H. Evans, A. W. Herrling, A. W. Jones, M. J. Olvermann, P. Pook and J. C. Watkins, *Brain Res.*, **382,** 169 (1986).
108. C. A. Boast, S. C. Gerhardt, G. Pastor, J. Lehmann, P. E. Etienne and J. M. Liebman, *Brain. Res.*, **442,** 345 (1988).
109. C. F. Bigge, J. T. Drummond, G. Johnson, T. Malone, A. W. Probert Jr., F. W. Marcoux, L. L. Coughenour and L. J. Brahce, *J. Med. Chem.*, **32,** 1580 (1989).
110. R. H. Evans and J. C. Watkins, *Life Sci.*, **28,** 1303 (1981).
111. J. F. Collins, A. J. Dixon, G. Badman, G. de Sarro, A. G. Chapman, G. P. Hart and B. S. Meldrum, *Neurosci. Lett.*, **51,** 371 (1984).
112. P. Mastalerz, L. Kupczyk-Subotkowska, Z. S. Herman and G. Laskawiec, *Naturwissenschaften,* **69,** 46 (1982).
113. S. Bajusz, A. Ronai, J. I. Szekely, A. Turan, A. Juhasz, A. Pathy, E. Miglecz and I. Bezetei, *FEBS Lett.*, **117,** 308 (1980).
114. H. Fleisch In: *Bone and Mineral Research,* Annual 1, W. A. Peck (Ed.) Excerpta Medica, Amsterdam 1983, p. 319.
115. H. Yamamoto, T. Koguchi, R. Okachi, K. Yamada, K. Nakayama, M. Kase, A. Kurosawa and K. Shuto, *J. Antibiot.*, **39,** 44 (1986).
116. Y. Kido, T. Hamakado, M. Anno, E. Miyogawa, Y. Motoki, T. Wakamiya and T. Shiba, *J. Antibiot.*, **37,** 965 (1984).
117. R. D. Johnson, R. S. Gordee, R. E. Kastner and E. E. Ose, *UK Patent Appl.*, 2.127.413A (1984); *Chem. Abstr.*, **101,** 88837 (1984).