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STUDIES ON THE SYNTHESIS OF CHEMOTHERAPEUTICS. PART XI$^{1)}$. SYNTHESIS AND ANTIBACTERIAL ACTIVITIES OF PHOSPHONOPEPTIDES$^{2)}$

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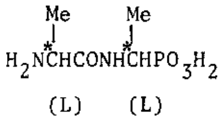
Abstract —— A variety of phosphonopeptides, shown in 2 as a general formula, containing natural and/or unnatural amino acids were synthesized, and their *in vitro* antibacterial activities were examined. N-Protected amino acids were condensed with 1-aminoethylphosphonic acid or its ester followed by deprotection and hydrolysis to give the requisite phosphonopeptides. Several compounds showed higher levels of activity against certain members of Gram-negative bacteria than those of Alafosfalin (1)$^{3)}$ as the standard phosphonopeptide. A brief discussion on structure-activity relationships is also described.

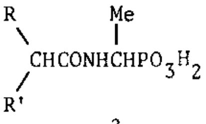
Alafosfalin 1, N-(L-alanyl)-L-1-aminoethylphosphonic acid, with potent antibacterial activity *in vitro* and *in vivo* was developed in The Roche Products, U. K.$^{3,4a)}$, as based on the idea of antimetabolite interfering with the biosynthetic pathway of bacterial cell wall. This idea was substantially confirmed by the facts that alafosfalin 1 was transported into bacteria and cleaved enzymatically to liberate

L-1-aminoethylphosphonic acid[4a,c,e] (abbreviated to L-AEPA in this paper), which inhibits alanine racemase and related processes due to structural mimetic to L-alanine.



$$H_2N\overset{\overset{\textstyle Me}{|}}{\overset{*}{C}}HCONH\overset{\overset{\textstyle Me}{|}}{\overset{*}{C}}HPO_3H_2 \quad (L)\ (L)$$

Alafosfalin **1**·



2

R,R': amino acid, dipeptide and carboxylic acid residue

Not only potent antibacterial activity of **1** but also its synergistic activity with D-cycloserine or β-lactam antibiotics could be well explained by the mechanism of action mentioned above. Dipeptide to hexapeptide variants of **1** with differences in potency and antibacterial spectrum in vitro and in vivo have been recently reported by the same institute[3-5]. A plenty of findings in these reports stimulated us to intend further development of **1** and related compounds[6], bringing our focus into synthesis of dipeptide to tripeptide variants of alafosfalin with unnatural amino acids[7]. This is the first report from our research group where chemistry, biochemistry and antibacterial activity are described on approx. a hundred novel compounds. From these results preliminary structural requirements for antibacterial activity in vitro and in vivo may be derived.

Chemistry A variety of phosphonopeptides listed in Table I - IV were synthesized in order to investigate influence upon the antibacterial activity of the chemical modification of **1** mainly applied on the N-terminus, because the AEPA moiety is considered to be a "warhead" for inhibition of the bacterial enzyme. These phosphonopeptides were classified into types 1 - 14 in a manner of the chemical modification, and their activities described in the next section.

As illustrated in Chart 1, synthetic methods for the phosphonopeptides were, classified into sixteen categories (Method A - P) according to types of their structures and/or the employed reactions. Each method was outlined in the followings.

Method A: The N-carbobenzoxyaminocarboxylic acids were coupled with diethyl 1-aminoethylphosphonate (abbreviated to DAEPA in this paper) in the presence of dicyclohexylcarbodiimide (DCC) and the resulting products were treated with 25 - 30 % HBr in AcOH at room temperature. This method was most extensively used for preparation of various types of the phosphonodipeptides (Table I and II).

Method B: Preparation of the phosphonodipeptides containing the acid-labile moiety (**27**, **35**α, and **35**β) was accomplished by the use of trimethylsilyl chloride and sodium iodide[8)] for deprotection and hydrolysis of the condensed products prepared by Method A (Table 1).

Method C: For preparation of **3**, the activated N-carbobenzoxyaminobenzoate of N-hydroxysuccinimide was condensed with AEPA and followed by removal of the protective group by catalytic hydrogenation in the presence of Pd-C (Table I).

Method D: The mixed anhydride prepared from the quinoline-3-carboxylic acid (or its congener) and ethyl chloroformate was condensed with AEPA to give the compounds of type 3 (Table I).

Method E: Condensation of N-acetyl-L-thioproline N-hydroxysuccinimide ester with AEPA afforded **19** (Table I).

Method F: Diethyl N-alanyl-1-aminoethylphosphonate was condensed with the quinoline-3-carboxylic acid (or its congener) anhydride and followed by deprotection of the product in a similar way as Method A giving the compounds of type 11 (Talbe IV).

Method G: Diethyl N-(α-bromopropionyl)-1-aminoethylphosphonate prepared from α-bromopropionic acid and DAEPA was treated with a secondary or tertiary amine, and the condensed product was deprotected as described for Method A to give **63** and **65** (Table II).

Method H: An amino group of the phosphonodipeptide was formylated with formic acid in the presence of acetic anhydride to give **66** (Table II).

Method I: N-Carbamoylation of amino group of the phosphonodipeptide was achieved by the treatment with hexamethyldisilazane, followed by a reaction with isocyanate to give **68**α and **68**β (Table II).

Method J: The N-hydroxyphosphonodipeptide (**71**) was prepared by catalytic debenzylation of the corresponding N-benzyloxy compound (Table II).

Method K: The N-(alanyl)-N-methyl-1-aminoethylphosphonic acids (**72** and **73**) were prepared from diethyl N-methyl-1-aminoethylphosphonate and the carboxylic acids through a similar sequence as Method A (Table II).

Method L: Reaction of 1-(N-carbobenzoxyalanyl)piperazine with dibenzyl phosphite and formaldehyde produced dibenzyl (4-N-carbobenzoxyalanyl-1-piperazinyl)methylphosphonate which was catalytically debenzylated to give **74** and **75** (Table III).

Method M: 2-(1-Piperazinyl)ethylphosphonic acid was condensed with the alanine N-hydroxysuccinimide ester and the product was deprotected as in the case of Method A to give **76** and **77** (Table III).

Method N: This is a modified case of Method L in which acetaldehyde in place of formaldehyde was used and a pH controlled reaction conditions were employed to give **78** and **79** (Table III).

Method O: In preparation of some of the phosphonotripeptides, the peptide chain was elongated from the N- to C-terminus. The condensation and deblocking reaction *were carried out in a similar way as Method A (Table IV).*

Antibacterial Activities The antibacterial activities against various organisms were determined as described in Experimental section, and expressed in terms of average bacteriostatic index (B. I.) for all compounds *(Table I - IV) and minimum* inhibitory concentration (MIC) for selected compounds (Table V - VI).

Discussion The investigations which are described in the present publication[3-5] provide the evidence to suggest that the action of our compounds on bacteria in vitro is essentially the same as those of **1** and that the variation in potency and antibacterial spectrum should be attributed to differences in their action on different bacteria at the stages of (i) active transport from the medium into the bacterial cell, (ii) intracellular cleavage to release the alanine mimetic (L-AEPA), and (iii) inhibition of alanine racemase as well as of uridine diphosphate-N-acetyl-muramyl-alanine synthetase.

Preliminary structure-activity relationships of our compounds will be discussed as follows:- Removal of one methylene unit and/or α-amino groups from **1** resulted in a complete loss of the activity (compare type 1 with **1** and **25**). Change of position of the amino group or introduction of an additional amino group gave a similar result (type 2).

Introducing AEPA (type 3) or **1** (type 11) into nalidixic acid or related drugs through peptide bonding resulted in loss of antibacterial activities of both compounds.

Replacement of the N-terminus of **1** by some cyclic α-amino acid e.g. thioproline gave decreased or no activity (type 4). A marked modification, replacement of amino group of AEPA by piperazine ring (the type 10 compounds) resulted in vanishing of activity of **1**.

It has been suggested that L-configuration of the left-half amino acid was essential for the activity from comparison of the activities and structures of the type 5 compounds[9].

A successful modification for enhancing activity was shown in the type 6 compounds, where the alanine part of **1** was replaced by unnatural α-amino acids as the substituted phenylalanines or 5- or 6-membered heteroaromatic ring substituted alanines.

Especially the compounds with such a heterocyclic ring as indolyl (27), thienyl (28,

28β, and **29**β), benzothienyl (**39**β) showed equivalent activity to that of **1** in spite of their racemic form as for one or both amino acid residues. The activity was reinforced by separation of a mixture of the diastereoisomers into active β-isomer as in from **28** to **28**β. For these compounds (**28**, **28**β, **29**β, and **39**β), an additional blockade of the bacterial enzymes is conceivable owing to the left-half amino acid mimetic.[10-12] The activity was moderate when the furyl or pyridyl ring was substituted but it was poor in the case of the thiazolyl rings.

The activity of the phenylalanyl derivatives of type 6 substituted by the halogen, lower alkyl, alkoxy, alkylenedioxy or amino group (**40** - **56**) was lower than that of **25**. A few exceptions were observed in **44**β, **45**, and **53**β, and their high activity might be due to resolution of the stereoisomers. A marked difference in the activities was not caused by change of the substituted position on the phenyl ring but by alternation of the substituent.

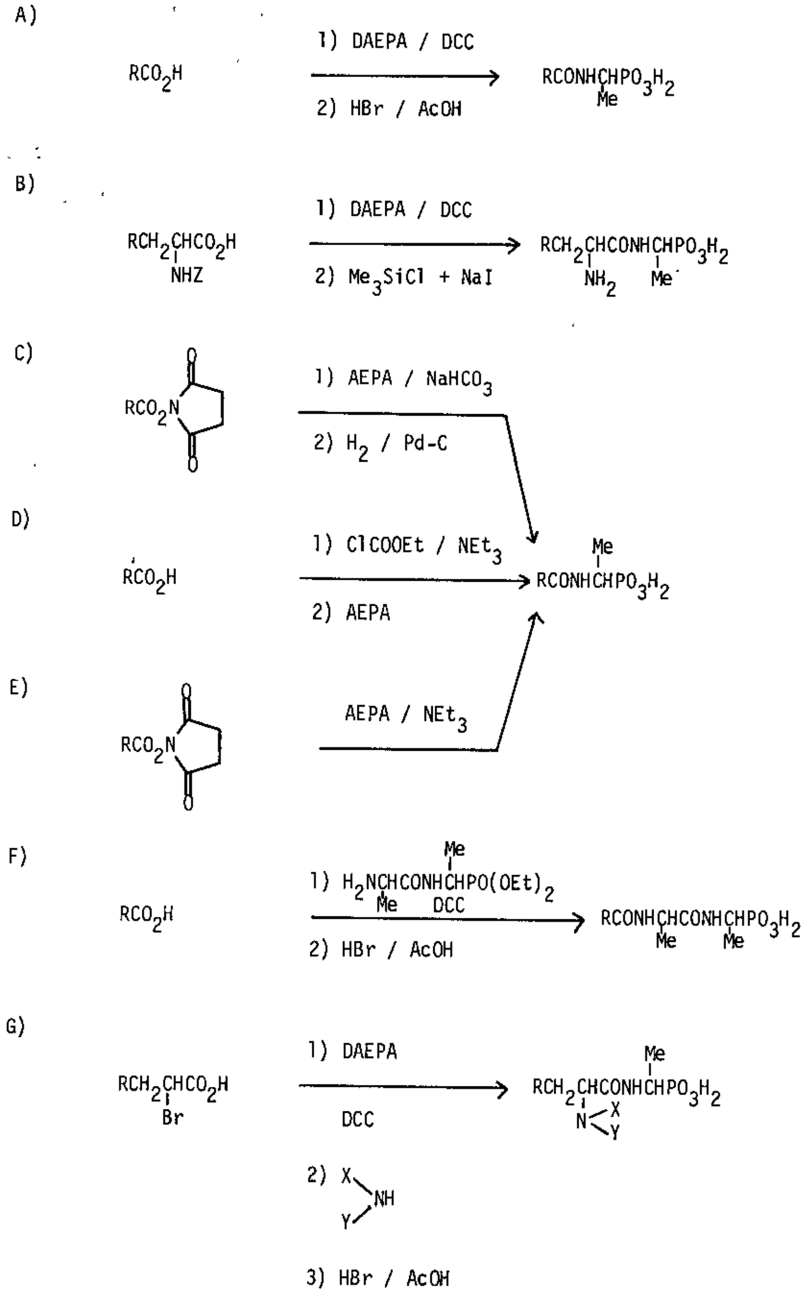
The types 5 and 6 compounds were subjected to further modifications. Increase of methylene units in the alanine residue as in type 7 possessed potent or moderate activity (e.g. compare **58** with **25**), but introduction of any substituent on amino group of the N-terminus or acid residue resulted in vanishing the activity with exception of **66**, whose activity was kept low.

These findings indicate that the free α-amino group of the N-terminus is essential for antibacterial activity. Failure of modification for activity by removal, position change and substitution on the amino group might be due to the decrease in the phosphonodipeptide transport into the bacterial cell by permease.

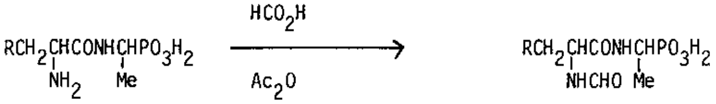
The phosphonotripeptides of type 12 - 14 showed in general lower activity and narrower spectrum as seen in against Serratia than the phosphonodipeptides (Table VI). These properties were similar to those of the phosphonotripeptides containing two natural amino acids.[3,4a] The activities of type 12 (arylalanyl-alanyl) and 13 (alanyl-arylalanyl) compounds were almost equivalent, and higher than those of the type 14 (arylalanyl-arylalanyl).

As seen in **1**, the activities of our compounds containing unnatural amino acids against Gram-negative bacteria were much higher than those against Gram-positive bacteria. This level against certain members of Gram-negative bacteria is higher than or equal to those of clinically used antibiotics as β-lactam and aminoglycoside. The antibacterial spectra of our compounds resembled also to those of phosphonopeptides containing natural amino acids.[3-5] Further investigations relating to the in vivo activity and pharmacokinetic properties will be reported separately.

Chart 1

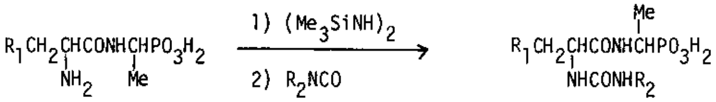

A)
RCO2H
1) DAEPA / DCC
2) HBr / AcOH
RCONHCHPO3H2
Me
B)
RCH2CHCO2H
NHZ
1) DAEPA / DCC
2) Me3SiCl + NaI
RCH2CHCONHCHPO3H2
NH2 Me
C)
RCO2N
O
O
1) AEPA / NaHCO3
2) H2 / Pd-C
D)
RCO2H
1) ClCOOEt / NEt3
2) AEPA
Me
RCONHCHPO3H2
E)
RCO2N
O
O
AEPA / NEt3
F)
RCO2H
Me
1) H2NCHCONHCHPO(OEt)2
Me DCC
2) HBr / AcOH
RCONHCHCONHCHPO3H2
Me Me
G)
RCH2CHCO2H
Br
1) DAEPA
DCC
2) X NH Y
3) HBr / AcOH
Me
RCH2CHCONHCHPO3H2
N X Y

H)



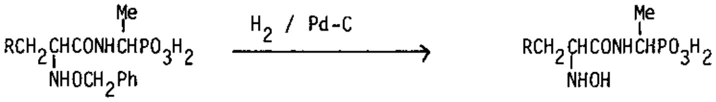
$$RCH_2\underset{NH_2}{CH}CONH\underset{Me}{CH}PO_3H_2 \xrightarrow[Ac_2O]{HCO_2H} RCH_2\underset{NHCHO}{CH}CONH\underset{Me}{CH}PO_3H_2$$

I)



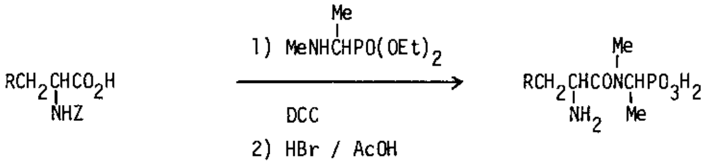
$$R_1CH_2\underset{NH_2}{CH}CONH\underset{Me}{CH}PO_3H_2 \xrightarrow[2)\ R_2NCO]{1)\ (Me_3SiNH)_2} R_1CH_2\underset{NHCONHR_2}{CH}CONH\overset{Me}{CH}PO_3H_2$$

J)



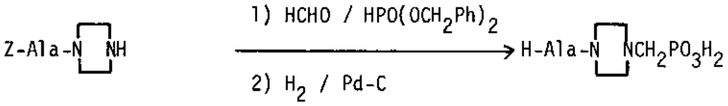
$$RCH_2\underset{NHOCH_2Ph}{CH}CONH\overset{Me}{CH}PO_3H_2 \xrightarrow{H_2\ /\ Pd\text{-}C} RCH_2\underset{NHOH}{CH}CONH\overset{Me}{CH}PO_3H_2$$

K)

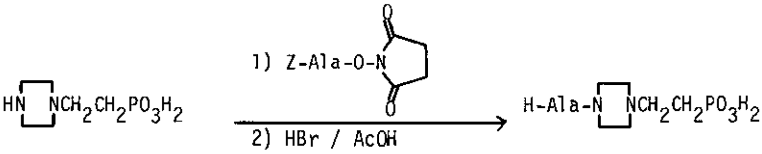


$$RCH_2\underset{NHZ}{CH}CO_2H \xrightarrow[DCC\quad 2)\ HBr\ /\ AcOH]{1)\ MeNH\overset{Me}{C}HPO(OEt)_2} RCH_2\underset{NH_2}{CH}CO\overset{Me}{N}\underset{Me}{CH}PO_3H_2$$

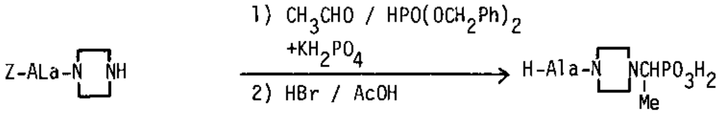
L)



M)

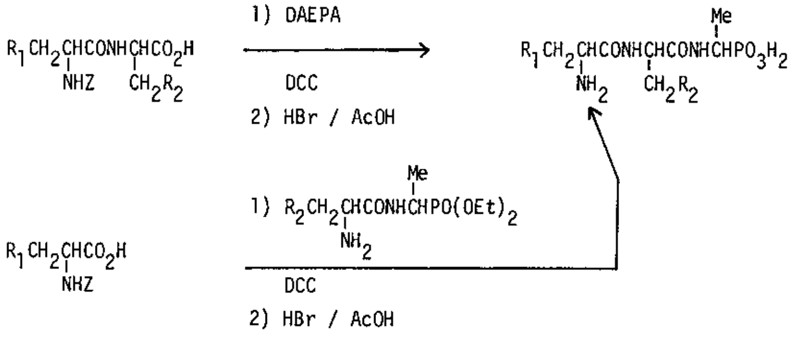


N)

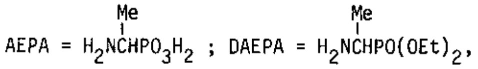


O)

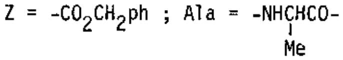
P)



R, R_1, R_2, X and Y = Appropriate substituents



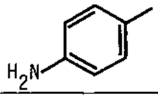
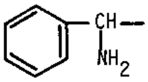
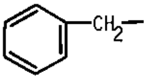
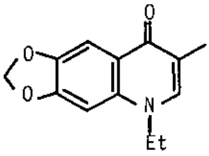
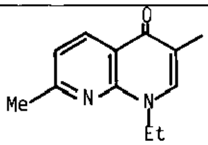
AEPA = $H_2N\overset{Me}{C}HPO_3H_2$; DAEPA = $H_2N\overset{Me}{C}HPO(OEt)_2$,

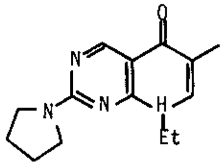
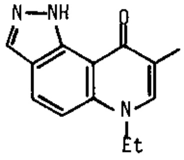
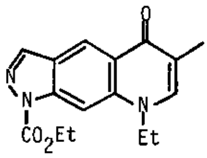
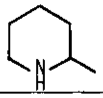
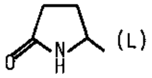
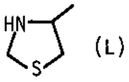
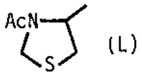


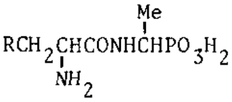
Z = $-CO_2CH_2ph$; Ala = $-NH\underset{Me}{C}HCO-$

Table I: Phosphonodipeptides and Related Compounds

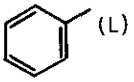
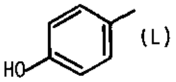
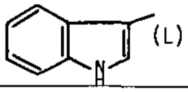
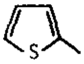
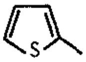
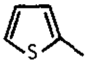
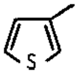
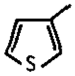
$\mathrm{RCONH\overset{Me}{C}HPO_3H_2}$

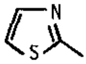
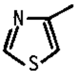
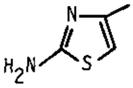
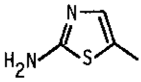
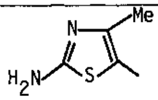
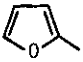
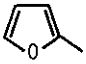
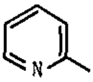
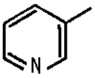
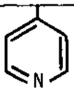
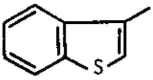
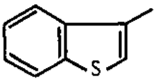
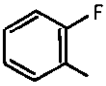
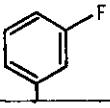
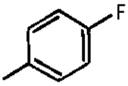
Type	Compd. No.	R[a]	Method	Yield[b] %	mp(dec.) °C	Average B. I.		
						G(+)	G(-)	G(+)&G(-)
1	3		A C	65.9 62.2	>300	0	0	0
	4		A	77.8	243 - 248	0	0	0
	5		A	86.8	287 - 292	0	0	0
2	6	$H_2N(CH_2)_2$-	A	73.3	255 - 257	0	0	0
	7	$H_2N(CH_2)_3$-	A	59.5	242 - 244	0	0	0
	8	$H_2N(CH_2)_5$-	A	83.2	256 - 257	0	0	0
	9	$\mathrm{H_2NCH_2\underset{NH_2}{C}H}$-	A	34.7	232 - 240	0	0.3	0.3
	10	$\mathrm{H_2N(CH_2)_2\underset{NH_2}{C}H}$-	A	20.2	245 - 251	0	0	0
3	11		D	41.4	282 - 284	0	0	0
	12		D	25.8	302 - 303	0	0	0

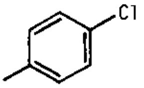
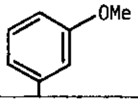
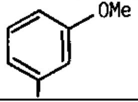
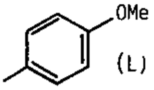
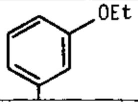
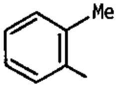
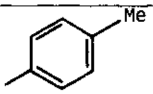
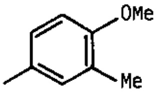
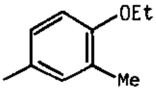
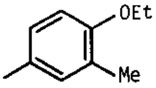
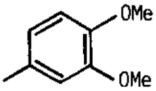
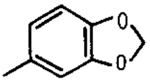
3	13		D	62.7	284 - 286	0	0	0
	14		D	19.3	285 - 287	0	0	0
	15		D	62.2	246 - 248	0	0	0
4	16		A	94.3	278 - 279	0	0.2	0.1
	17		A	63.2	175 - 182	0	2.5	2.0
	18		A	11.2	210 - 212	0	1.3	1.1
	19		E	30.5[c]	205 - 210	0	0	0

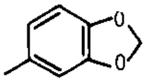
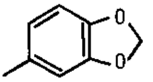
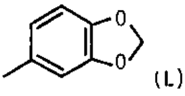
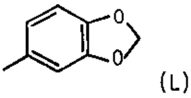
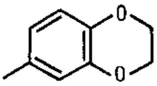
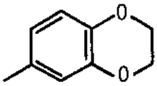
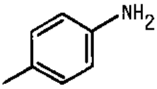
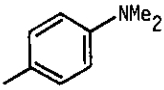
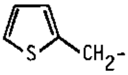


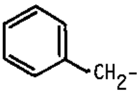
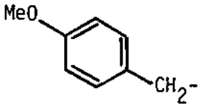
$$\mathrm{RCH_2\underset{NH_2}{C}HCONH\overset{Me}{C}HPO_3H_2}$$

5	1[g]	H (L)	h	68.3	278 - 279	0.7	5.5	4.5
	20	H	A	65.5	283 - 286	0	2.5	2.0
	21	H (L)	A	64.2	280 - 282[d]	0.7	4.3	3.6
	22	H (D)	A	75.2	279 - 282[e]	0	0	0
	23	Me	A	74.5	271 - 275	1.0	5.3	4.4
	24	$\mathrm{MeSCH_2}$-(L)	A	65.6	223 - 235	2.0	5.6	4.9
6	25	 (L)	A	92.0	278 - 280	1.3	4.6	3.9
	26	 (L)	A	83.2	275 - 290	1.0	3.8	3.2
	27	 (L)	B	24.0	268 - 273	0.3	5.3	4.3
	28		A	81.9	274 - 278	1.7	5.4	4.4
	28α		A	41.3	275 - 276	0	1.2	0.9
	28β		A	38.0	227 - 230	2.3	5.7	5.0
	29α		A	62.0	260 - 261	0	1.1	0.9
	29β		A	46.0	227 - 228	1.3	5.3	4.5

6	30		A	32.3	218 - 220	1.0	3.2	2.7
	31		A	65.4	234 - 237	0.3	3.3	2.7
	32		A	44.2	255 - 270	0	0.3	0.2
	33		A	50.2	243 - 250	0.7	1.5	1.3
	34		A	27.6	235 - 243	0	0	0
	35α		B	25.5	251 - 253	0	0.8	0.6
	35β		B	7.9	231 - 233	1.0	4.1	3.6
	36		A	21.4	220 - 223	0	3.6	2.9
	37		A	49.2	225 - 231	0.7	3.6	3.0
	38		A	48.8	218 - 223	0	2.4	1.9
	39α		A	48.0	252 - 254	0	2.0	1.6
	39β		A	78.0	247 - 250	0	4.9	3.9
	40		A	73.6	242 - 245	2.0	3.5	3.2
	41		A	65.9	254 - 257	0	2.9	2.3
	42		A	57.2	250 - 253	0.7	2.9	2.4

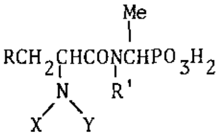
6	43		A	35.1	246 - 251	0.7	3.8	3.1
	44α		A	65.0	250 - 252	0	1.9	1.5
	44β		A	78.5	237 - 240	1.0	4.8	4.0
	45		A	55.3	251 - 254	1.0	4.2	3.5
	46		A	54.7	241 - 243	0	3.7	2.9
	47		A	59.8	244 - 248	0	2.2	1.7
	48		A	57.0	249 - 252	0	2.8	2.2
	49		A	48.2	240 - 245	0	2.1	1.7
	50α		A	47.4	251 - 255	0	0.7	0.5
	50β		A	46.7	235 - 238	0	1.9	1.5
	51		A	45.0	238 - 241	0	1.9	1.5
	52		A	85.0	264 - 268	0	2.8	2.3

6	52α		A	67.0	281 – 284	0	1.0	0.8
	52β		A	79.0	238 - 240	0.3	3.8	3.1
	53		A	44.8	220 - 224	0.3	3.2	2.6
	$53\beta^{f}$		A	60.6	239 - 241	0.7	4.1	3.4
	54		A	58.8	239 - 242	0	2.0	1.6
	$54\beta^{f}$		A	47.0	224 - 227	0.3	2.3	1.9
	55		A	61.9	287 - 292	0	2.3	1.8
	56		A	57.0	244 - 247	0	0.7	0.5
	57		A	25.4	258 - 270	0	1.5	1.2

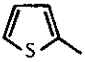
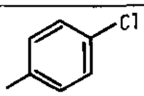
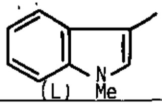
7	58		A	31.4	236 - 240	0.3	4.9	4.0
	59		A	53.4	228 - 233	0	3.7	2.9

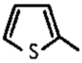
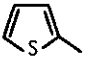
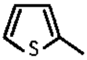
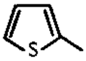
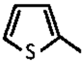
[a] Some of the N-terminal amino acids used as the starting materials are optically active as indicated in the parentheses, and the others unspecified are recemic, thus giving the diastereoisomeric mixture of racemic form. [b] Based on the starting N-protected amino acid or carboxylic acid described in an experimental section. [c] Based on AEPA. [d] Lit.[7b]: mp 260 - 265°(dec.). [e] Lit.[7b]: mp 267 - 269°(dec.) and lit.[7a]: 220 - 230°(dec.). [f] The α-isomer could not be isolated in a satisfactory pure state. [g] The AEPA moiety has L-form. [h] Prepared according to the literature[5a].

Table II: N-Substituted Phosphonodipeptides



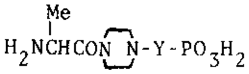
$$\mathrm{RCH_2\underset{\underset{X\ \ Y}{N}}{C}HCO\overset{Me}{N}\underset{R'}{C}HPO_3H_2}$$

Type	Compd. No.	R[a]	R'	X	Y	Method	Yield[b] %	mp(dec.) °C	Average B.I.		
									G(+)	G(-)	G(+)&G(-)
8	60		H	H	Me	A	86.5	266 - 270	0	1.5	1.2
	61		H	H	Me	A	80.4	271 - 275	0	0	0
	62		H	H	Me	A	83.4	260 - 264	0	0	0
	63	H	H	H	Me	A G	71.7 18.9	282 - 286	0	0	0

8	64	H (L)	H	H	Me	A	74.4	284 - 287	0	0	0
	65	H	H	Me	Me	G	21.0	262 - 266	0	0	0
	66		H	H	CHO	A H	20.7 57.5	185 - 195	0.7	3.0	2.5
	67		H	H	COMe	A	33.8	132 - 134[c]	0	1.0	0.8
	68α		H	H	CONMe H	I	30.4	209 - 211	0	0	0
	68β		H	H	CONMe H	I	26.6	208 - 212	0	0.1	0.1
	69	H	H	H	OMe	A	14.3	191 - 195	0	0.3	0.2
	70	H	H	H	OCH_2Ph	A	16.5	188 - 190	0	0	0
9	71	H	H	H	OH	J	97.2	228 - 235	0	0.8	0.7
	72	H	Me	H	H	K	41.4	214 - 221	0	0.4	0.3
	73		Me	H	H	K	27.8	207 - 215	0	0.4	0.3

[a] The starting amines and amino acids are racemic unless otherwise specified in the parentheses. See footnote a in Table I. [b] Based on the corresponding starting material described in an experimental section. [c] Melted without decomposition.

Table III: (Alanylpiperazinyl)alkylphosphonic Acids



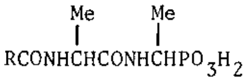
$H_2NCH(Me)CON\langle\rangle N\text{-}Y\text{-}PO_3H_2$

Type	Compd. No.	Y[b]	Method	Yield[c] %	mp (dec.) °C	Average B.I.		
						G(+)	G(-)	G(+)&G(-)
10	74	CH_2	L	8.2	184 - 195	0	0	0
	75	CH_2(L)	L	6.9	235 - 243	0	0	0
	76	CH_2CH_2	M	50.0	247 - 250	0	0	0
	77	CH_2CH_2(L)	M	36.7	238 - 245	0	0	0
	78[a]	CHMe	N	3.6	235 - 239	0	0	0
	79[a]	CHMe(L)	N	12.9	250 - 255	0	0	0

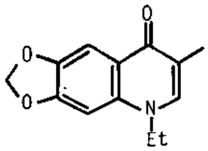
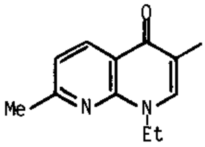
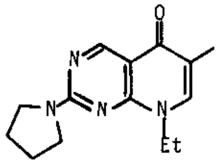
[a] A diastereoisomeric mixture. [b] The starting N-terminal alanine used are optically active as indicated in the parentheses, and the others unspecified racemic.

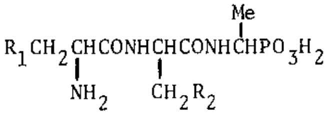
[c] Based on the starting 1-(N-carbobenzoxyalanyl)piperazine for the method L and N, and 2-(1-piperazinyl)ethylphosphonic acid for the method M.

Table IV: Phosphonotripeptides and the Related Compounds

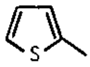
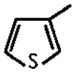
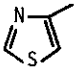
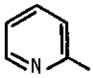
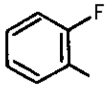
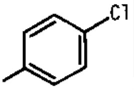
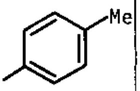
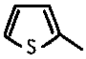
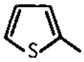
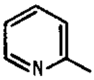
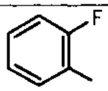
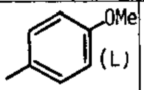
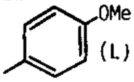


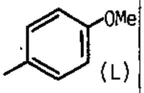
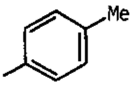
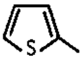
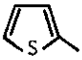
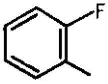
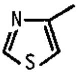
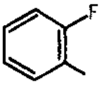
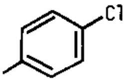
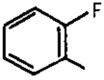
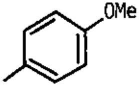
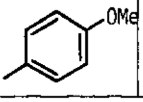
$$\mathrm{RCONH}\overset{\mathrm{Me}}{\overset{|}{\mathrm{C}}}\mathrm{HCONH}\overset{\mathrm{Me}}{\overset{|}{\mathrm{C}}}\mathrm{HPO_3H_2}$$

Type	Compd. No.	R	Method	Yield [a] %	mp (dec.) °C	Average B.I.		
						G(+)	G(-)	G(+)&G(-)
11	**80**		F	16.0	239 - 241	0	0	0
	81		F	56.8	235 - 243	0	0	0
	82		F	47.2	263 - 267	0	0	0



$$R_1CH_2\underset{NH_2}{CH}CONH\underset{CH_2R_2}{CH}CONH\overset{Me}{C}HPO_3H_2$$

Type	Compd. No.	R_1 [b]	R_2 [c]	Method	Yield [d] %	mp (dec.) °C	Average B.I.		
							G(+)	G(-)	G(+)&G(-)
12	83		H (L)	O P	69.1 76.7	258 - 261	0	2.1	1.8
	84		H	P	72.7	248 - 254	1.0	1.1	1.1
	85		H	P	55.9	236 - 243	0.3	1.0	0.9
	86		H (L)	P	6.2	223 - 243	0	1.3	1.0
	87		H (L)	P	29.5	210 - 218	1.7	0.9	1.1
	88		H (L)	P	40.0	210 - 217	0.3	1.9	1.6
	89		H (L)	P	27.8	250 - 254	0	0.8	0.6
13	90	H (L)		O	43.2	259 - 261	0.3	2.9	2.4
	91	H (D)		O	43.3	258 - 261	0	0	0
	92	H (L)		P	34.0	229 - 237	0	1.3	1.0
	93	H (L)		P	62.6	259 - 263	1.7	0.9	1.1
	94	H (L)	 (L)	P	15.3	250 - 256	0.3	1.9	1.6
	95α	H (L)	 (L)	P	17.6	250 - 253	0	0	0

13	95$^{\beta}$	H (L)	 (L)	P	11.0	233 - 236	0	0.9	0.7
	96	H (L)		P	57.5	255 - 262	0	0.3	0.3
	97	NH_2 $(CH_2)_3$ (L)		O	65.7	232 - 236	0	2.6	2.2
14	98			P	58.5	241 - 258	0	0.2	0.1
	99			P	77.6	240 - 245	0	0.2	0.1
	100			P	59.6	245 - 253	0	0.3	0.3
	101			P	60.6	240 - 245	0	0	0

[a] Based on the starting carboxylic acids. [b] See footnote a in Table I. [c] The starting amino acids for the middle amino acid moiety are racemic unless otherwise specifield in the parentheses. [d] Based on the corresponding dipetides shown in an experimental section.

Tabel V: Antibacterial Activities (MIC, μg/ml) of Phosphonodipeptides

	25	28β	29β	35β	39β	43	44β	48	52β	55	Alafosfalin
Escherichia coli NIHJ	0.39	0.39	0.20	0.78	0.20	1.56	0.78	6.25	1.56	100	0.39
E. coli IAM 1182	0.39	0.10	0.20	0.78	0.78	3.13	0.78	3.13	1.56	3.13	0.10
Shigella flexneri 2a EW 10	3.13	0.78	1.56	6.25	1.56	0.78	0.78	12.5	6.25	25	0.78
Shi. sonnei EW 33	0.20	<0.05	<0.05	0.20	0.10	0.39	0.20	1.56	0.39	6.25	≤0.05
Salmonella typhosa 0901	<0.05	<0.05	<0.05	<0.10	0.05	0.39	<0.05	0.39	0.10	3.13	≤0.05
Sal. enteritidis G 14	25	25	25	100	25	100	25	50	50	>100	50
Klebsiella pneumoniae Kasuya	6.25	1.56	3.13	12.5	3.13	6.25	3.13	25	6.25	>100	1.56
Proteus vulgaris OX 19 US	>100	100	>100	>100	>100	>100	>100	>100	>100	>100	>100
Pseudomonas aeruginosa P 1	>100	>100	>100	>100	>100	>100	>100	>100	>100	>100	>100
Pse. aeruginosa P 31	>100	>100	>100	>100	>100	>100	>100	>100	>100	>100	>100
Serratia marcescens IID 618	3.13	0.78	1.56	6.25	1.56	6.25	1.56	12.5	3.13	100	0.78
Ser. marcescens IID 619	6.25	0.78	1.56	3.13	6.25	12.5	6.25	50	50	>100	0.78
Staphylococcus aureus ATCC 6538P	>100	100	>100	100	>100	>100	100	>100	>100	>100	100
S. aureus Terajima	50	25	25	25	>100	25	50	100	50	>100	12.5
Bacillus subtilis PCI 219	12.5	3.13	25	25	>100	>100	25	>100	>100	100	100

Table VI: Antibacterial Activities (MIC, μg/ml) of Phosphonotripeptides

	83	**90**	**98**
Escherichia coli NIHJ	6.25	50	100
E. coli IAM 1182	12.5	0.78	100
Shigella flexneri 2a EW 10	25	0.39	100
Shi. sonnei EW 33	1.56	<0.10	25
Salmonella typhosa 0901	3.13	12.5	100
Sal. enteritidis G 14	>100	>100	>100
Klebsiella pneumoniae Kasuya	3.13	1.56	>100
Proteus vulgaris OX 19 US	>100	>100	>100
Pseudomonas aeruginosa P 1	>100	>100	>100
Pse. aeruginosa P 31	>100	>100	>100
Serratia marcescens IID 618	100	>100	>100
Ser. marcescens IID 619	>100	>100	>100
Staphylococcus aureus ATCC 6538P	>100	>100	>100
S. aureus Terajima	>100	>100	>100
Bacillus subtilis PCI 219	>100	50	100

EXPERIMENTAL

All melting points are not corrected. IR spectra were determined on a Hitachi 215 recording spectrophotometer and NMR spectra on a JNM PMX-60 spectrometer using tetramethylsilane or sodium trimethylsilylpropanesulfonate as an internal standard.

Starting Amino Acids and Their N-Substituted Derivatives

a) The following amino acids were prepared according to the literature shown: β-2-thienyl-DL-alanine[10], N-acetyl-β-2-thienyl-DL-alanine[10a], N-formyl-β-2 thienyl-DL-alanine[11], β-3-thienyl-DL-alanine[12], β-4-thiazolyl-DL-alanine[13] β-(2-amino-4-thiazolyl)-DL-alanine[14], β-(2-amino-5-thiazolyl)-DL-alanine[15], β-(2-amino-4-methyl-5-thiazolyl)-DL-alanine[15], β-2-furyl-DL-alanine[16], β-2- and 3-pyridyl-DL-alanine[17], β-4-pyridyl-DL-alanine[18], β-3-benzo[b]thienyl-DL-alanine[19], 3-methoxy-DL-phenylalanine[20], 4-methoxyl-DL- and L-phenylalanine[21], 2- and 4-methyl-DL-phenylalanine[22], 3,4-dimethoxy-DL-phenylalanine[23], 3,4-methylenedioxy-DL- and L-phenylalanine[24], 4-(N,N-dimethylamino)-DL-phenylalanine[25], N-acetyl-L-thioproline[26], N-methyl-DL- and L-alanine[27], and N-methoxy- and benzyloxy-DL-alanine[28].

b) Since the following aryl-substituted amino acids were unknown, they were prepared by the usual method for α-amino acid[29]; i.e. condensation of diethyl acylamino-malonate with appropriate aralkyl halide in the presence of sodium ethoxide followed by hydrolysis with hydrochloric acid: 4-methoxy-3-methyl-DL-phenylalanine; mp 217 - 218° (dec.), 4-ethoxy-3-methyl-DL-phenylalanine, mp 206 - 207° (dec.); 3,4-ethylenedioxy-DL-phenylalanine, mp 226 - 229° (dec.); DL-2-amino-4-phenylbutyric acid hydrochloride, mp 267 - 270° (dec.); and DL-2-amino-4-(2-thienyl)butyric acid hydrochloride, mp 241 - 245° (dec.).

c) The N-carbobenzoxyamino acids were prepared by usual procedure for carbobenzoxylation of α-amino acid; the free amino acid was treated with a slight excess of carbobenzoxy chloride in the presence of sodium hydroxide to obtain an objective product after usual work-up.

d) Three N-carbobenzoxy-N-methyl derivatives of β-2-thienyl-DL-alanine, β-4-chloro-DL-phenylalanine, and 1-methyl-L-tryptophan were respectively obtained by the reaction of methyl iodide with the corresponding N-carbobenzoxyamino acids in the presence of silver oxide[27].

e) N-Carbobenzoxy-3-ethoxy-DL-phenylalanine was prepared by O-ethylation of N-carbobenzoxy-DL-m-tyrosine by diethyl sulfate.

f) The other amino acids including their N- or O-protected derivatives were commercially available.

Method A. N-(β-3-Thienyl-DL-alanyl)-1-aminoethylphosphonic Acid (29α and 29β). A mixture of N-carbobenzoxy-β-3-thienyl-DL-alanine (1 g), DAEPA (600 mg), DCC (690 mg), and CH_2Cl_2 (10 ml) was stirred under ice-cooling for 1.5 hr and at room temperature for 5 hr, and the mixture was filtered to remove a precipitated dicyclohexylurea. The filtrate was subjected to column chromatography on silica gel. Elution with $CHCl_3$ gave an isomer of diastereomeric diethyl N-carbobenzoxy-β-3-thienyl-DL-alanyl-1-aminoethylphosphonate (α-isomer) (650 mg; 42.5 %) as colorless prisms (from ether), mp 119 - 120°: IR (KBr) cm^{-1}: 3240, 1710, 1655; NMR δ($CDCl_3$): 1.22 (3H, dd, J = 16.5, 7.5 Hz, P-$CH\underline{H}_3$), 1.25 and 1.28 (each 3H, t, J = 7.0 Hz, O-$CH_2C\underline{H}_3$ × 2), 3.11 (2H, d, J = 7.0 Hz, thienyl-CH_2), 4.08 (4H, q, J = 7.0 Hz, O-$C\underline{H}_2CH_3$ × 2), 5.08 (2H, s, $C\underline{H}_2C_6H_5$), 5.50 - 5.57 and 6.50 - 6.85 (each 1H, m, CONH × 2), 6.92 - 7.50 (3H, m, thienyl-H), 7.32 (5H, s, C_6H_5).

Evaporation of the above mother liquor of recrystallization, after removal of the α-isomer, gave an another isomer of the above phosphonate (β-isomer) (740 mg; 48.3 %) as a colorless oil: NMR δ($CDCl_3$): 1.32 (3H, dd, J = 17.0, 7.0 Hz, P-$CHC\underline{H}_3$), 1.28 and 1.32 (each 3H, t, J = 7.0 Hz, O-$C\underline{H}_2CH_3$ × 2), 3.14 (2H, d, J = 7.0 Hz, thienyl-CH_2), 4.07 and 4.13 (each 2H, q, J = 7.0 Hz, O-$CH_2C\underline{H}_3$ × 2), 5.11 (2H, s, $C\underline{H}_2C_6H_5$), 5.50 - 6.75 (2H, m, CONH × 2), 6.90 - 7.45 (3H, m, thienyl-H), 7.36 (5H, s, C_6H_5).

A mixture of the above α-isomer (300 mg) and 25 % HBr in AcOH (3 ml) was stirred at room temperature while 15 hr and poured into ether. A precipitated gum separated by decantation was taken up in a small amount of MeOH. To this solution was added an excess of propylene oxide in order to cause a solid precipitate. It was collected by filtration and washed thoroughly with MeOH to afford 29α (130 mg; 73.0 %) as colorless crystals: IR (KBr) cm^{-1}: 3260, 1650; NMR δ(D_2O + NaOD): 1.00 (3H, dd, J = 14.0, 7.0 Hz, P-$CHC\underline{H}_3$), 2.94 (2H, d, J = 7.0 Hz, thienyl-CH_2), 7.05 (1H, dd, J = 5.0, 1.5 Hz, thienyl-4-H), 7.10 - 7.25 (1H, m, thienyl-2-H), 7.43 (1H, dd, J = 5.0, 3.0 Hz, thienyl-5-H).

Similarly, the foregoing β-isomer (400 mg) was treated with 25 % HBr in AcOH (4 ml) and worked-up to give 29β (130 mg; 54.5 %) as colorless crystals: IR (KBr) cm^{-1}: 3250, 1650; NMR δ(D_2O + NaOD): 1.16 (3H, dd, J = 14.0, 7.0 Hz, P-$CHC\underline{H}_3$). 3.03 (2H, d, J = 7.0 Hz, thienyl-CH_2), 7.08 (1H, dd, J = 5.0, 1.5 Hz, thienyl-4-H), 7.20 - 7.40 (1H, m, thienyl-2-H), 7.50 (1H, dd, J = 5.0, 3.0 Hz, thienyl-5-H).

Starting with the corresponding N-carbobenzoxyamino acids and DAEPA, this method was employed to prepare **3** - **10**, **16** - **18**, **20** - **26**, **28** - **34**, **36** - **59**, **60** - **64**, **66** - **67**, and **69** - **70**.

Method B. N-L-Tryptophyl-1-aminoethylphosphonic Acid (27)

N-Carbobenzoxy-L-tryptophan (1.9 g) was reacted with DAEPA (1.0 g) and worked-up as described for method A to give a *diastereoisomeric mixture of diethyl N-(N-*carbobenzoxy-L-tryptophyl)-1-aminoethylphosphonate (2.06 g; 74.0 %) as an oil: NMR δ($CDCl_3$): 0.80 - 1.45 (9H, m, P-CHC$\underline{H}_3$ and O-$CH_2C\underline{H}_3$ × 2), 3.26 (2H, d, J = 7.0 Hz, tryptophyl-CH_2), 3.75 - 4.85 (6H, m, O-$C\underline{H}_2CH_3$ x 2 and N-CH × 2), 5.06 and 5.12 (total 2H, s, $C\underline{H}_2C_6H_5$), 5.55 - 5.85 and 6.20 - 6.80 (each 1H, m, CONH × 2), 6.95 - 7.85 (9H, m, Ar-H), 8.52 (1H, s, tryptophyl-2-H).

A mixture of the above phosphonate (500 mg), NaI (680 mg), chlorotrimethylsilane (500 mg) and CH_3CN (3 ml) was stirred at room temperature for 2 hr, and then filtered. The filtrate was evaporated and the resulting residue was extracted with water. Evaporation of the extract, after washing with ether, left an oily residue, which was chromatographed on Mitsubishi Diaion HP-20. Elution with 50 % MeOH afforded **27** (100 mg; 32.0 %) as a colorless powder (from MeOH-EtOH): IR (KBr) cm^{-1}: 1660; NMR δ(D_2O + NaOD): 0.86 and 1.04 (3H, dd, J = 7.0, 15.0 Hz, P-CHC$\underline{H}_3$), 2.86 - 3.45 (2H, m, CH_2) 3.55 - 4.05 (2H, m, CH × 2), 7.16 - 7.90 (5H, m, Ar-H).

This method was employed for the preparation of **35**α and **35**β

Method C. N-(4-Aminobenzoyl)-1-aminoethylphosphonic Acid (3).

To a mixture of 4-(carbobenzoxy)aminobenzoic acid N-hydroxysuccinimide ester (660 mg), DMF (6 ml) and EtOH (3 ml) was added a solution of AEPA (150 mg) in 5 % $NaHCO_3$ (6 ml). The mixture was vigorously stirred at room temperature for 1 hr and at 40° for 20 hr, and then evaporated *in vacuo* to dryness below 55°. Extraction of the resulting residue with water followed by acidification of the extract with conc. HCl precipitated a solid. This was collected by filtration and washed with water and ether furnishing N-[4-(carbobenzoxy)aminobenzoyl]-1-aminoethylphosphonic acid (680 mg, 74.0 %) as colorless crystals, mp 202 - 205° (dec.): IR (KBr) cm^{-1}: 3320, 1695, 1630.

This compound (350 mg) was dissolved in MeOH (10 ml) and hydrogenated in the presence of 14 % Pd-C (50 mg) within 2 hr. After removal of the catalyst by filtration, the filtrate was evaporated to leave a solid which on recrystallization from aq. EtOH gave **3** as colorless crystals (190 mg; 84.1 %): NMR δ(DMSO-d_6): 1.42 (3H, dd, J = 16.0, 6.5 Hz, P-CHC$\underline{H}_3$), 7.47 and 7.71 (each 2 H, d, J = 8.0 Hz, C_6H_4).

Method D. N-(1-Ethyl-1,4-dihydro-6,7-methylenedioxy-4-oxoquinoline-3-carbonyl)-1-aminoethylphosphonic Acid (11).

To an ice-water cooled mixture of oxolinic acid[21)] (60 mg), DMF (2 ml) and triethylamine (100 mg) was slowly added, with stirring, a solution of ethyl chlorocarbonate (50 mg) in DMF (6 ml). The mixture was stirred for 3 hr, and a mixture of AEPA (70 mg), triethylamine (0.4 ml), DMF (5 ml), and water (1 ml) was added. After the stirring was continued under cooling for an additional 3 hr and at room temperature for 18 hr, the mixture being basified with aq. $NaHCO_3$ was washed with AcOEt. On acidification of the aqueous layer with conc. HCl a colorless solid precipitated. This was recrystallized from MeOH to give **11** (35 mg; 41.4 %) as colorless crystals: IR (KBr) cm^{-1}: 1650; NMR δ(CF_3COOH): 1.70 (3H, dd, J = 7.0, 16.0 Hz, P-CHC$\underline{H}_3$), 1.76 (3H, t, J = 7.0 Hz, N-CH_2C$\underline{H}_3$), 4.77 (2H, q, J = 7.0 Hz, N-C$\underline{H}_2$$CH_3$), 6.35 (2H, s, O-$CH_2$-O).

Using the corresponding carboxylic acids, nalidixic acid[31)], pyromidic acid[32)], 6-ethyl-6,9-dihydro-9-oxopyrazolo[3,4-*f*]quinoline-3-carboxylic acid[33)], and 8-ethyl-5,8-dihydro-5-oxopyrazolo[4,3-*g*]quinoline-3-carboxylic acid[33)], and 8-ethyl-5,8-dihydro-5-oxopyrazolo[4,3-*g*]quinoline-6-carboxylic acid[34)], **12**, **13**, **14**, and **15** were obtained by this method, respectively.

Method E. N-(N-Acetyl-L-thioprolyl)-1-aminoethylphosphonic Acid (19).

To an ice-water cooled and stirred mixture of AEPA (150 mg), triethylamine (270 mg), DMF (1 ml) and water (1.5 ml) was added a solution of N-acetyl-L-thioproline[17)] N-hydroxysuccinimide ester (380 mg) in DMF (1 ml). After the stirring under the cooling for 1 hr and at room temperature for 15 hr, the mixture was filtered. Evaporation of the filtrate left a gummy residue, which was extracted with 3 % HCl and the extract washed with ether was evaporated. The residual oil was subjected to a preparative thin layer chromatography using silica gel[35)] and *n*-BuOH-AcOH-water (5 : 1 : 2) as the solvent. Extraction with MeOH of a band sensitive to iodine vapor followed by evaporation of the solvent furnished a colorless solid, which was recrystallized from MeOH-acetone to give **19** (120 mg; 30.5 %) as colorless crystals: IR (KBr) cm^{-1}: 3250, 1640, 1630; NMR δ(CF_3COOH): 1.58 (3H, dd, J = 7.0, 18.0 Hz, P-CHC$\underline{H}_3$), 2.43 (3H, s, $COCH_3$), 3.3 - 3.55 and 4.6 - 5.1 (6H, m, CH_2 × 2 and CH × 2).

Diethyl N-(DL- and L-Alanyl)-1-aminoethylphosphonate.

A mixture of N-carbobenzoxy-DL-alanine (6.2 g), DAEPA (5 g), DCC (6 g), and CH_2Cl_2 (50 ml) was stirred under cooling with ice for 1.5 hr and at room temperature for 18 hr, and then filtered. The filtrate was successively washed with 3 % NaOH, water, 3 % HCl, and water, dried (Na_2SO_4), and evaporated to give diethyl N-(N-carbobenzoxy-DL-alanyl)-1-aminoethylphosphonate (9.2 g; 86.0 %) as a colorless oil. A solution of this compound (8 g) in MeOH (200 ml) was hydrogenated in the presence of 15 % Pd-C (850 mg). Usual work-up of the reaction mixture gave diethyl N-(DL-alanyl)-1-aminoethylphosphonate (4.9 g; 93.8 %) as a colorless oil: NMR δ($CDCl_3$): 1.15 - 1.56 (9H, m, $P\text{-}CH_2C\underline{H}_3$, and $O\text{-}CH_2C\underline{H}_3$ × 2), 4.05 and 4.14 (each 2H, q, J = 7.0 Hz, $O\text{-}C\underline{H}_2CH_3$ × 2). An L-isomer of the above compound was similarly prepared from N-carbobenzoxy-L-alanine and DAEPA.

Method F. N-[(1-Ethyl-1,4-dihydro-7-methyl-4-oxo-1,8-naphthyridine-3-carbonyl)-L-alanyl]-1-aminoethylphosphonic Acid (81).
To an ice-cooled mixture of nalidixic acid[31)] (1.16 g), triethylamine (1.1 g), and DMF (30 ml) was added, with stirring, ethyl chlorocarbonate (0.65 g) during 10 min. After stirring of the mixture for 15 min, a solution of diethyl N-(L-alanyl)-1-aminoethylphosphonate (1.51 g) in THF (6 ml) was added. The mixture was stirred under the cooling for an additional 3.5 hr and at room temperature for 2 hr, and then evaporated to dryness. The residue was admixed with water and extracted with benzene. The extract was washed with 3 % NaOH and water. Evaporation of the dried (Na_2SO_4) extract gave diethyl N-[(1-ethyl-1,4-dihydro-7-methyl-4-oxo-1,8-naphthyridine-3-carbonyl)-L-alanyl]-1-aminoethylphosphonate (1.85 g; 79.4 %) as a pale yellow powder: IR (KBr) cm^{-1}: 3250, 1650. This compound (1.4 g) was dissolved in 30 % HBr in AcOH (15 ml) and stirred at room temperature for 6 hr. The reaction mixture was worked-up as in the case of the preceding method A to give **81** (880 mg; 71.5 %) as a colorless powder: IR (KBr) cm^{-1}: 3250, 1660, 1645; NMR δ(CF_3COOH): 1.42 - 1.85 (9H, m, $P\text{-}CHC\underline{H}_3$, $C\text{-}CHC\underline{H}_3$, and $N\text{-}CH_2C\underline{H}_3$), 3.00 (3H, s, $Ar\text{-}CH_3$), 5.07 (2H, q, J = 7.0 Hz, $N\text{-}C\underline{H}_2CH_3$), 7.88 and 8.95 (each 1H, d, J = 8.0 Hz, Ar-H × 2), 9.54 (1H, s, N-CH=). Using oxolinic acid[30)] and pyromidic acid[32)], this method was carried out for the preparation of **80** and **82**.

Method G. N-(N,N-Dimethyl-DL-alanyl)-1-aminoethylphosphonic Acid (65). A mixture of α-bromopropionic acid (2.5 g), DAEPA (2.37 g), DCC (3.53 g), and CH_2Cl_2 (70 ml) was stirred under cooling with ice-water for 3.5 hr and at room temperature for 51 hr, and then filtered. The filtrate was evaporated and the residue was extacted with AcOEt. The extract was successively washed with 3 % HCl, water, 3 % NaOH, and water, dried (Na_2SO_4), and evaporated to give diethyl N-(α-bromopropionyl)-1-aminoethylphosphonate (2.85 g; 68.1 %) as a pale yellow oil: NMR δ($CDCl_3$): 1.14 - 1.55 (9H, m, $P\text{-}CHC\underline{H}_3$ and $O\text{-}CH_2C\underline{H}_3$ × 2), 1.76 (3H, d, J = 7 Hz, $Br\text{-}CHC\underline{H}_3$), 4.08 and 4.10 (each 2H, q, J =

7.0 Hz, $\text{O-C}\underline{\text{H}}_2\text{CH}_3$ × 2).

This bromo compound was added into a mixture of dimethylamine hydrochloride (390 mg), DMF (4 ml) and K_2CO_3 (530 mg). The mixture was stirred for 24 hr and evaporated. To the residue was added water and the mixture was extracted with AcOEt. Evaporation of the extract gave an oil which was again extracted with $CHCl_3$. Removal of $CHCl_3$ from the extract, after filtration, yielded diethyl N-(N,N-dimethyl-DL-alanyl)-1-aminoethylphosphonate (360 mg; 90.3 %) as a pale yellow oil: NMR $\delta(CDCl_3)$: 1.32 and 1.61 (each 3H, d, J = 7.0 Hz, $\text{CHC}\underline{\text{H}}_3$ × 2), 1.35 (6H, t, J = 7.0 Hz, $\text{O-CH}_2\text{C}\underline{\text{H}}_3$ × 2), 1.46 (3H, dd, J = 7.0, 16.5 Hz, $\text{P-CHC}\underline{\text{H}}_3$), 4.16 and 4.45 (each 2H, q, J = 7.0 Hz, $\text{O-C}\underline{\text{H}}_2\text{CH}_3$ × 2).

A mixture of this phosphonate (330 mg) and 25 % HBr in AcOH (5 ml) was stirred for 15 hr at room temperature. Work-up as described for method A furnished **65** (90 mg; 34.1 %) as a colorless powder on recrystallization from EtOH-acetone: IR (KBr) cm^{-1}: 1670; NMR $\delta(CF_3COOH)$: 1.58 (3H, dd, J = 6.5, 18.0 Hz, $\text{P-CHC}\underline{\text{H}}_3$), 1.72 (3H, d, J = 6.5 Hz, $\text{CO-CHC}\underline{\text{H}}_3$), 3.07 and 3.16 (each 3H, s, N-CH_3 × 2).

The above reactions using 40 % aq. methylamine instead of dimethylamine hydrochloride were carried out to give **63**.

Method H. N-(N-Formyl-β-2-thienyl-DL-alanyl)-1-aminoethylphosphonic Acid (66).

A mixture of **28**β (150 mg), Ac_2O (0.7 ml), and formic acid (2 ml) was stirred at room temperature for 6 hr. The mixture was evaporated and the residue was extracted with hot EtOH. Evaporation of the extract left a pale brown solid which on treatment with aq. acetone, followed by acetone, gave a colorless powder (**66**) (95 mg; 57.5 %): IR (KBr) cm^{-1}: 3260, 1680 - 1640; NMR $\delta(CF_3COOH)$: 1.57 - 1.83 (3H, m, $\text{P-CHC}\underline{\text{H}}_3$). 8.29 (1H, s, N-CHO).

Method I. N-[(N-Methylcarbamoyl)-β-2-thienyl-DL-alanyl]-1-aminoethylphosphonic Acids (68α and 68β).

A mixture of **28** (150 mg), hexamethyldisilazane (0.8 ml) and CH_2Cl_2 (4 ml) was stirred over 2.5 hr until a clear solution was formed, and then methyl isocyanate(0.5 ml) was added. The resulting mixture was stirred under cooling with ice for 1 hr and at room temperature for 0.5 hr, and then evaporated. Recrystallization of the solid residue from EtOH furnished **68**α (55 mg; 30.4 %) as a colorless powder: IR (KBr) cm^{-1}: 3335, 3250, 1630, 1605; NMR $\delta(D_2O)$: 1.23 (3H, dd, J = 7.5, 15.5 Hz, $\text{P-CHC}\underline{\text{H}}_3$), 2.70 (3H, s, $COCH_3$)

Concentration of the above mother liquor of recrystallization gave **68**β (48 mg; 26.6 %) as a colorless solid: IR (KBr) cm^{-1}: 3380, 3250, 1650, 1605; NMR $\delta(D_2O)$:

1.27 (3H, dd, J = 7.5, 15.5 Hz, P-CHC$\underline{H}_3$), 2.72 (3H, s, COCH$_3$).

Compounds **68**α and **68**β were also obtained from **28**α and **28**β by the same reaction, respectively.

Method J. N-(N-Hydroxy-DL-alanyl)-1-aminoethylphosphonic Acid (71).

A solution of **70** (85 mg) in MeOH (15 ml) containing 25 % HBr in AcOH (0.3 ml) was hydrogenated in the presence of 15 % Pd-C (100 mg). After removal of the catalyst by filtration, the filtrate was concentrated and treated with propylene oxide to precipitate a solid, which was washed with MeOH to give **71** (58 mg; 97.2 %) as a colorless powder: IR (KBr) cm^{-1}: 3470, 3270, 1655; NMR δ(CF_3COOH): 1.63 (3H, dd, J = 18.0, 7.0 Hz, p-CHC$\underline{H}_3$), 1.78 (3H, d, J = 7.0 Hz, C-CHC$\underline{H}_3$)

Diethyl N-Methyl-1-aminoethylphosphonate.

A mixture of diethyl N-carbobenzoxy-1-aminoethylphosphonate$^{6)}$ (3 g), dry benzene (50 ml), and 60 % NaH (1.5 g) (suspension in oil) was stirred for 2.5 hr, and methyl iodide (10 ml) was added. The mixture, after the stirring for an additional 2.5 hr, was quenched by cold water and extracted with benzene. The extract was treated by column chromatography with silica gel and eluting with benzene-CHCl$_3$ (1 : 4) to give diethyl N-carbobenzoxy-N-methyl-1-aminoethylphosphonate (2.36 g; 75.3 %) as a colorless oil after evaporation of the solvent: NMR δ($CDCl_3$): 2.37 (3H, s, N-CH$_3$). Hydrogenation of this compound (806 mg) in MeOH (50 ml) in the presence of 15 % Pd-C (150 mg) followed by usual work-up of the reaction mixture afforded the title compound (450 mg; 97.0 %) as a colorless oil: NMR δ($CDCl_3$): 1.42 (6H, t, J = 7.0 Hz, O-CH$_2$C$\underline{H}_3$ × 2), 1.60 (3H, dd, J = 17.0, 7.0 Hz, P-CHC$\underline{H}_3$), 2.80 (3H, s, N-CH$_3$), 4.25 and 4.37 (each 2H, d, J = 7.0 Hz, O-C$\underline{H}_2$CH$_3$ × 2), 7.30 (1H, broad s, NH).

Method K. N-(L-Alanyl)-N-methyl-1-aminoethylphosphonic Acid (72).

A mixture of N-carbobenzoxy-L-alanine (150 mg), diethyl N-methyl-1-aminoethylphosphonate (120 mg), DCC (160 mg), and CH$_2$Cl$_2$ (5 ml) was stirred under cooling with ice-water for 2 hr and at room temperature for 16 hr. The mixture was filtered and the filtrate was chromatographed on silica gel. Evaporation of the first acetone eluate gave diethyl N-(L-alanyl)-N-methyl-1-aminoethylphosphonate (190 mg; 77.2 %) as a pale yellow oil: NMR δ($CDCl_3$): 3.12 (3H, s, N-CH$_3$), 5.11 (2H, s, O-CH$_2$), 7.34 (5H, s, C$_6$H$_5$). This compound (200 mg) was dissolved in 25 % HBr in AcOH (3 ml) and the resulting solution was kept at room temperature for 10 hr. Work-up of the reaction mixture as described for method A gave **72** (56 mg; 53.3 %) as a colorless powder: IR (KBr) cm^{-1}: 1640; NMR δ(CF_3COOH): 1.61 (3H, dd, J = 7.0, 15.0 Hz, P-$CHC\underline{H}_3$), 1.73 (3H, d, J = 7 Hz, C-$CHC\underline{H}_3$), 3.30 (3H, s, N-CH_3). The same treatment of N-carbobenzoxy-β-2-thienyl-DL-alanine provided 73.

1-(N-Carbobenzoxy-DL- and L-alanyl)piperazine.

To a stirred mixture of piperazine (8 g) and $CHCl_3$ (100 ml) was added a solution of N-carbobenzoxy-DL-alanine N-hydroxysuccinimide ester (10 g) in $CHCl_3$ (100 ml) over 10 min. The mixture was stirred at room temperature for 30 min and at 60° for a further 30 min. After cooling, the mixture was washed with water, dried (Na_2SO_4), and evaporated to give 1-(N-carbobenzoxy-DL-alanyl)piperazine (8 g; 86 %) as colorless prisms (from ether), mp 85 - 86°: IR (KBr) cm^{-1}: 3310, 3270, 1710; NMR δ($CDCl_3$): 1.35 (3H, d, J = 7.0 Hz, $CHC\underline{H}_3$), 2.76 - 3.05 and 3.35 - 3.76 (each 4H, m, N-CH_2 × 2), 5.15 (2H, s, O-CH_2), 7.37 (5H, s, C_6H_5).

Similarly, an L-analogue of this compound was prepared from N-carbobenzoxy-L-alanine N-hydroxysuccinimide ester as colorless crystals, mp 63 - 64°.

2-(1-Piperazinyl)ethylphosphonic Acid Dihydrobromide.

A mixture of 1-carbobenzoxypiperazine (4.2 g), diethyl 2-bromoethylphosphonate (4.8 g), triethylamine (2.2 g) and benzene (100 ml) was heated at 50 - 55° for 15 hr. After cooling, the reaction mixture was filtered and the filtrate was evaporated. The residue was chromatographed on silica gel and evaporation of the $CHCl_3$ eluate gave diethyl 2-(4-carbobenzoxy-1-piperazinyl)ethylphosphonate (3.5 g; 50 %) as a colorless oil: NMR δ($CDCl_3$): 1.35 (6H, t, J = 7.5 Hz, $CH_2C\underline{H}_3$ × 2), 1.65 - 2.96 and 3.42 - 3.70 (each 4H, m, N-CH_2CH_2-N × 2), 5.12 (2H, s, OCH_2), 7.35 (5H, s, C_6H_5). A mixture of this compound (2 g) and 47 % HBr (20 ml) was stirred at room temperature for 3 hr and evaporated to give a colorless powder (1.15 g; 62 %), mp 166 - 169° (dec.): NMR δ(D_2O + NaOD): 1.95 - 2.65 (2H, m, p-CH_2), 3.20 - 3.95 [10H, m, CH_2-N$(CH_2CH_2)_2$N].

Method L. (4-DL-Alanyl-1-piperazinyl)methylphosphonic Acid (74).

A mixture of 1-(N-carbobenzoxy-DL-alanyl)piperazine (1.6 g), dibenzyl phosphite (1.6 g), 37 % formaldehyde (0.5 ml) and benzene (80 ml) was heated at 80° for 1 hr and evaporated. The residue was chromatographed on silica gel using $CHCl_3$ as an eluant to yield dibenzyl (4-N-carbobenzoxy-DL-alanyl-1-piperazinyl)methylphosphonate (1.3 g; 41 %) as a colorless oil after evaporation of the solvent: NMR δ($CDCl_3$): 1.32 (3H, d, J = 7.0 Hz, $CHC\underline{H}_3$), 2.80 (2H, d, J = 12.0 Hz, N-CH_2-P). A mixture of this compound (1.2 g), 15 % Pd-C (200 mg) and MeOH (80 ml) was shaken in a current of hydrogen at room temperature. When the reaction had been completed the catalyst was filtered off and the solvent was evaporated at 40 - 45°. A methanolic solution of the residue was treated with propylene oxide to give 74 (100 mg; 20 %) as colorless prisms: IR (KBr) cm^{-1}: 1660; NMR δ(D_2O + NaOD): 1.20 (3H, d, J = 7.0 Hz, $CHC\underline{H}_3$), 2.55 (2H, d. J = 12.0 Hz, N-CH_2-P), 2.60 - 2.95 and 3.50 - 3.75 (each 4H, m, N-CH_2 × 4), 3.96 (1H, q, J = 7 Hz, $C\underline{H}CH_3$).

An L-isomer (75) of the title compound was similarly prepared from 1-(N-carbobenzoxy-L-alanyl)piperazine.

Method M. 2-(4-DL-Alanyl-1-piperazinyl)ethylphosphonic Acid (76).

A mixture of 2-(1-piperazinyl)ethylphosphonic acid dihydrobromide (400 mg), triethylamine (200 mg), N-carbobenzoxy-DL-alanine N-hydroxysuccinimide ester (380 mg), and aq. EtOH (50 ml) was heated at 80° for 1 hr. The reaction mixture was evaporated and the residue was triturated with EtOH to yield 2-(4-N-carbobenzoxy-DL-alanyl-1-piperazinyl)ethylphosphonic acid (300 mg; 66 %) as a colorless powder, mp 209 - 211° (dec.): NMR δ(CD_3OD): 1.30 (3H, d, J = 7.0 Hz, $CHC\underline{H}_3$), 5.10 (2H, s, O-CH_2), 7.40 (5H, s, C_6H_5).

A solution of this compound (200 mg) in 15 % HBr in AcOH (4 ml) was kept to stand at room temperature for 7 hr, and then poured into ether (100 ml). The precipitated mass which was collected by decantation was dissolved in a small amount of MeOH. Treatment of the solution by propylene oxide gave 76 (100 mg; 76 %) as a colorless powder: IR (KBr) cm^{-1}: 1650; NMR δ(D_2O + NaOD): 1.20 (3H, d, J = 7.0 Hz, $CHC\underline{H}_3$), 1.20 - 2.00 (2H, m, P-CH_2), 2.30 - 3.10 [6H, m, CH_2-N$(CH_2)_2$], 3.50 - 3.85 [4H, m, CON$(CH_2)_2$].

Similarly, 77 was prepared from N-carbobenzoxy-L-alanine N-hydroxysuccinimide ester.

Method N. 1-(4-DL-Alanyl-1-piperazinyl)ethylphosphonic Acid (78).

A mixture of 1-(N-carbobenzoxy-DL-alanyl)piperazine (2.0 g), dibenzyl phosphite (2.5 g), 90 % acetaldehyde (2 ml), and EtOH (50 ml), adjusted to pH = 6.5 - 7.0 by addition of 0.1 M potassium phosphate (monobasic), was heated at 80 - 85° for 8 hr. The cooled mixture was basified with ammonia and extracted with $CHCl_3$. The extract was washed with water, dried (Na_2SO_4), and evaporated to leave a yellowish caramel, which was chromatographed on silica gel. Evaporation of the benzene-AcOEt (4 : 1) eluate, followed by recrystallization of the residue from ether gave dibenzyl 1-(4-DL-alanyl-1-piperazinyl)ethylphosphonate (1.43 g; 35.5 %) as colorless prisms, mp 109 - 120°: NMR δ($CDCl_3$): 1.30 (3H, dd, J = 17.5, 8.0 Hz, P-$CHC\underline{H}_3$), 1.35 (3H, d, J = 8.0 Hz, N-$CHC\underline{H}_3$), 5.18 (2H, s, O-CH_2), 7.38 (15 H, s, C_6H_5 × 3).

This compound (1.2 g) was hydrogenated, as in the case of method M, to afford 78 (50 mg; 10 %) as an off-white powder: IR (KBr) cm^{-1}: 1650; NMR δ(D_2O + NaOD):

1.22 (3H, d, J = 7.5 Hz, N-$CHC\underline{H}_3$), 1.25 (3H, dd, J = 14.0, 7.5 Hz, P-$CHC\underline{H}_3$), 2.40 - 3.15 [5H, m, $CHN(CH_2)_2$], 3.45 - 3.80 [4H, m, $CON(CH_2)_2$], 3.95 (1H, q, J = 7.5 Hz, $C\underline{H}CH_3$).

By the same procedure, **79** was prepared from 1-(N-carbobenzoxy-L-alanyl)piperazine.

N-Carbobenzoxy-β-2-thienyl-DL-alanyl-L-alanine.

A mixture of L-alanine ethyl ester hydrochloride (271 mg), triethylamine (131 mg), N-carbobenzoxy-β-2-thienylalanine (450 mg), DCC (365 mg) and CH_2Cl_2 (20 ml) was stirred under cooling with ice-water for 1.5 hr and at room temperature for 18 hr. The mixture was filtered and the filtrate was concentrated to dryness. The residue was redissolved in AcOEt and filtered. The filtrate was washed with 3 % HCl, water, and brine, dried (Na_2SO_4), and evaporated to give N-carbobenzoxy-β-2-thienyl-DL-alanine ethyl ester (490 mg; 88.2 %) as colorless crystals (from AcOEt-petr. benzine), mp 94 - 97°. IR (KBr) cm^{-1}: 3360, 1730, 1700, 1650; NMR $\delta(CDCl_3)$: 1.26 (3H, t, J = 7.0 Hz, O-$CH_2C\underline{H}_3$), 1.34 (3H, d, J = 7.5 Hz, $CHC\underline{H}_3$), 3.30 (2H, d, J = 6.5 Hz, Ar-CH_2), 4.18 (2H, q, J = 7.0 Hz, O-$C\underline{H}_2CH_3$), 5.12 (2H, s, O-CH_2), 7.35 (5H, s, C_6H_5). To an ice-cooled solution of the above ethyl ester (250 ml) in EtOH (8 ml) was dropwise added with stirring $1\underline{N}$ NaOH (1.2 ml) over a period of 30 min, and the mixture was then stirred at room temperature for an additional 45 min. The mixture was neutralized by the addition of $1\underline{N}$ HCl and then evaporated to yield a colorless solid, to which was added 3 % HCl, and the resulting precipitate was extracted with AcOEt. The extract was washed with water, dried (Na_2SO_4), and evaporated to give a colorless powder (185 mg; 76.5 %), mp 125 - 128°: IR (KBr) cm^{-1}: 3280, 1700, 1680, 1640; NMR $\delta(CD_3OD + CDCl_3)$: 1.33 and 1.38 (each 1.5H, d, J = 7.0 Hz, $CHC\underline{H}_3$), 5.11 (2H, s, O-CH_2), 7.37 (5H, s, C_6H_5).

N-Carbobenzoxy-D- and L-alanyl-β-2-thienyl-DL-alanine.

To a stirred mixture of β-2-thienyl-DL-alanine ethyl ester (prepared by esterification of β-2-thienyl-DL-alanine in EtOH saturated with dry HCl gas) (760 mg), triethyl amine (392 mg), and CH_2Cl_2 (40 ml) was added under an ice-cooling, N-carbobenzoxy-L-alanine (703 mg), N-hydroxy-5-norbornene-2,3-dicarboxyimide (677 mg), and DCC (715 mg). The mixture was stirred under cooling for 2 hr and at room temperature for 16 hr, and then filtered. The filtrate was evaporated to dryness and an oily residue was extracted with AcOEt. The extract was washed with 3 % HCl, water, and brine. Evaporation of the dried (Na_2SO_4) extract gave a pale yellow oil, which was chromatographed on silica gel (Merck Art. 9385, 230 - 400 mesh). Elution with $CHCl_3$ followed by evaporation of the solvent afforded N-carbobenzoxy-L-alanyl-β-

2-thienyl-DL-alanine ethyl ester (1.161 g; 95.7 %) as colorless crystals (from AcOEt-petr.benzine), mp 85 - 105°: IR (KBr) cm^{-1}: 3295, 1725, 1680, 1635, 1520; NMR $\delta(CDCl_3)$: 1.27 (3H, t, J = 7.5 Hz, $O\text{-}CH_2C\underline{H}_3$), 1.38 (3H, d, J = 7.5 Hz, $CHC\underline{H}_3$), 3.36 (2H, d, J = 5.5 Hz, $Ar\text{-}CH_2$), 4.20 (2H, q, J = 7.5 Hz, $OC\underline{H}_2CH_3$), 5.11 (2H, s, $OC\underline{H}_2C_6H_5$), 7.36 (5H, s, C_6H_5).

The above ethyl ester (1.135 g) was hydrolyzed as described for the preparation of the preceding N-carbobenzoxy-β-2-thienyl-DL-alanyl-L-alanine to yield N-carbobenzoxy-L-alanyl-β-2-thienyl-DL-alanine (794 mg; 75.1 %) as colorless crystals (from AcOEt-petr. benzine), mp 127.5 - 130°: IR (KBr) cm^{-1}: 1725, 1705, 1680, 1645, 1620; NMR $\delta(CDCl_3 + DMSO\text{-}d_6)$: 1.24 and 1.29 (each 1.5 H, d, J = 7.5 Hz, $CHC\underline{H}_3$), 3.29 (2H, d, J = 5.2 Hz, $Ar\text{-}CH_2$), 5.07 (2H, s, OCH_2), 7.36 (5H, s, C_6H_5).

A D-isomer of this compound was similarly prepared from N-carbobenzoxy-D-alanine.

N^{α},N^{ε}-Dicarbobenzoxy-L-lysyl-β-2-thienyl-DL-alanine.

By the same procedure for the preparation of N-carbobenzoxy-L-alanyl-β-2-thienyl-DL-alanine, this compound as a colorless powder was prepared by the reaction with N^{α},N^{ε}-dicarbobenzoxy-L-lysine: IR (KBr) cm^{-1}: 3320, 1715, 1680, 1630; NMR $\delta(CD_3OD)$: 5.10 (4H, s, OCH_2 × 2), 7.34 (10H, s, C_6H_5 × 2).

Diethyl N-(β-2-Pyridyl-, o-Fluorophenyl-, p-Chlorophenyl-, p-Methoxyphenyl- and p-Methylphenyl-DL-alanyl)-1-aminoethylphosphonates.

A mixture of N-carbobenzoxy-β-2-pyridyl-DL-alanine (200 mg), DAEPA (150 mg), CH_2Cl_2 (6 ml) and DMF (2 ml) was stirred under cooling with ice for 2 hr and at room temperature for 4 hr. Filtration of the mixture followed by evaporation gave an oily residue, which was chromatographed on silica gel using AcOEt-MeOH (20 : 1) as an eluent. Removal of the solvent from the eluate afforded diethyl N-(N-carbobenzoxy-β-2-pyridyl-DL-alanyl)-1-aminoethylphosphonate (220 mg; 71.2 %) as an orange oil: NMR $\delta(CCl_4)$: 0.93 - 1.52 (9H, m, $O\text{-}CH_2C\underline{H}_3$ × 2 and $CHC\underline{H}_3$), 4.95 (2H, s, $C\underline{H}_2C_6H_5$), 7.20 (5H, s, C_6H_5).

A mixture of the above phosphonate (200 mg), 15 % Pd-C (50 mg) and MeOH (30 ml) was shaken under a current of hydrogen while 5 hr at room temperature. Usual work-up of the reaction mixture gave diethyl N-(β-2-pyridyl-DL-alanyl)-1-aminoethylphosphonate (145 mg; 99.0 %) as a pale yellow oil: NMR $\delta(CDCl_3)$: 0.97 - 1.60 (9H, m, $O\text{-}CH_2C\underline{H}_3$ × 2 and $P\text{-}CHC\underline{H}_3$), 2.90 (2H, s, NH_2), 3.83 - 4.33 (4H, m, $OC\underline{H}_2CH_3$ × 2), 7.05 - 7.83 (4H, m, Ar-H), 8.43 - 8.60 (1H, m, NH).

Similarly, the other title compounds were prepared from the corresponding N-carbobenzoxy-β-substituted alanines, respectively.

Method O. N-(L-Alanyl-β-2-thienyl-DL-alanyl)-1-aminoethylphosphonic Acid (90).

A mixture of N-carbobenzoxy-L-alanyl-β-2-thienyl-DL-alanine (414 mg), DAEPA (217 mg). DCC (258 mg), CH_2Cl_2 (20 ml) and DMF (1.5 ml) was stirred under cooling with ice for 2 hr and then at room temperature for 15 hr. Removal of the solvent from the filtered mixture gave a pale yellow oil, which was dissolved in AcOEt, and the resulting solution was washed in a usual manner, dried (Na_2SO_4), and evaporated. The residue was crystallized from AcOEt-petr. benzine to give diethyl N-(N-carbobenzoxy-L-alanyl-β-2-thienyl-DL-alanyl)-1-aminoethylphosphonate (415 mg; 69.9 %) as colorless crystals: IR (KBr) cm^{-1}: 3350, 1715, 1680; NMR δ($CDCl_3$): 3.28 (2H, d, J = 6.5 Hz, thienyl-CH_2), 5.11 (2H, s, OCH_2), 7.36 (5H, s, C_6H_5).

The above phosphonate (350 mg) was treated with 25 % HBr in AcOH as described for method A and worked-up to give a colorless powder (140 mg; 61.9 %): IR (KBr) cm^{-1} 3300, 3250, 1640; NMR δ(D_2O + NaOD): 1.01 - 1.41 (6H, m, P-$CHC\underline{H}_3$ and $CHC\underline{H}_3$), 7.02 - 7.43 (3H, m, thienyl-H).

The same reaction of the corresponding N-carbobenzoxydipeptides with DAEPA gave the compounds **83**, **91**, and **97**.

Method P. N-(L-Alanyl-β-p-methoxy-L-phenylalanyl)-1-aminoethylphosphonic Acid (95α and 95β).

A mixture of diethyl N-(β-p-methoxy-L-phenylalanyl)-1-aminoethylphosphonate (220 mg), N-carbobenzoxy-L-alanine (140 mg), DCC (140 mg) and CH_2Cl_2 (20 ml) was stirred under cooling with ice for 2 hr and at room temperature for 14 hr. The mixture was filtered and the filtrate was chromatographed on silica gel. Evaporation of the first AcOEt eluate gave an isomer of diastereomeric diethyl N-(N-carbobenzoxy-L-alanyl-β-p-methoxy-L-phenylalanyl)-1-aminoethylphosphonate (α-isomer) (100 mg; 29.1 %) as a colorless caramel: IR ($CHCl_3$) cm^{-1}: 3405, 1700, 1660; NMR δ($CDCl_3$): 0.95 - 1.60 (12H, m, O-$CH_2C\underline{H}_3$ × 2, P-$CHC\underline{H}_3$, and C-$CHC\underline{H}_3$), 3.00 (2H, d, J = 7.0 Hz, Ar-CH_2), 3.70 (3H, s, OCH_3), 5.12 (2H, s, $OC\underline{H}_2C_6H_5$), 6.10 (1H, d, J = 8.0 Hz, NH), 6.78 and 7.12 (each 2H, d, J = 9.0 Hz, Ar-H), 7.35 (5H, s, C_6H_5).

Evaporation of the second AcOEt eluate afforded an another isomer of the above phosphonate (β-isomer) (90 mg; 26.2 %) as a colorless oil: IR ($CHCl_3$) cm^{-1}: 3405, 1700, 1660; NMR δ($CDCl_3$): 1.03 - 1.53 (12H, m, O-$CH_2C\underline{H}_3$ × 2, P-$CHC\underline{H}_3$, and C-$CHC\underline{H}_3$), 2.98 (2H, d, J = 7.0 Hz, Ar-CH_2), 3.70 (3H, s, OCH_3), 5.10 (2H, s, O-$C\underline{H}_2C_6H_5$), 5.80 (1H, d, J = 8.0 Hz, NH), 6.76 and 7.10 (each 2H, d, J = 9.0 Hz, Ar-H), 7.37 (5H, s, C_6H_5).

A solution of the preceding α-isomer (100 mg) in 15 % HBr in AcOH (3 ml) was stirred for 15 hr at room temperature, and the ether (50 ml) was added to the reaction mixture. A precipitated solid was collected by decantation and dissolved in MeOH. An addition of propylene oxide into methanol solution precipitated a solid, which was washed with MeOH to give **95**α (40 mg; 60.3 %) as a colorless powder: IR (KBr) cm^{-1}: 3260, 1635; NMR δ(CF_3COOH): 1.04 - 1.90 (6H, m, P-$CHC\underline{H}_3$ and C-$CHC\underline{H}_3$), 3.20 (2H, d, J = 7.0 Hz, Ar-CH_2), 4.03 (3H, s, OCH_3). The same treatment of the foregoing β-isomer (90 mg) with 15 % HBr in AcOH followed by work-up of the product furnished **95**β (15 mg; 25.2 %) as colorless crystals: IR (KBr) cm^{-1}: 3260, 1635; NMR δ(CF_3COOH): 1.30 - 1.93 (6H, m, P-$CHC\underline{H}_3$ and C-$CHC\underline{H}_3$), 3.24 (2H, d, Ar-CH_2), 4.04 (3H, s, OCH_3).

Antibacterial Activity

Minimum inhibitory concentration (MIC) of the synthesized phosphonopeptides against various organisms was determined by the two fold serial agar dilution method recommended by the Japan Society of Chemotherapy on a difined antagonist-free medium.4a,4e) Fifteen test organisms commonly employed in the experiment were as follows; *Escherichia coli* NIHJ, *Escherichia coli* IAM 1182, *Shigella flexneri* 2aEW10, *Shigella sonnei* EW33, *Salmonella typhosa* 0901, *Salmonella enteritidis* G14, *Klebsiella pneumoniae* Kasuya, *Proteus vulgaris* OX 19 US, *Pseudomonas aeruginosa* P1, *Pseudomonas aeruginosa* P31, *Serratia marcescens* IID618, *Serratia marsescens* IID619, *Staphylococcus aureus* ATCC6538P, *Staphylococcus aureus* Terajima, and *Bacillus subtilis* PC1219.

MICs of the compounds were converted into a bacteriostatic index (B.I.) calculated by the following equation, $\log_2[100/MIC\ (\mu g/ml)]$ presented in the literature,36) and the average of B.I.s for Gram-positive and -negative strains was listed in the preceding Tables I - IV.

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