Table XII. Comparison of Performance When Test Compounds Are Scrambled by Assignment to Arbitrary log P Ranges[a]

	cumulative percent actives			
	scrambled		augmented	
percentile	A	C	A	C
99	14	3	14	3
98	23	11	23	12
95	33	20	33	19
90	42	28	47	30
80	61	43	61	43
70	67	55	67	55
50	77	68	79	71
30	89	81	91	81
10	100	97	100	97
0		100		100

[a] Original from Table IX vs. augmented fragment-weight table.

compounds. The true determinants of performance in the two-component model are distinctions among the differentiated weights of fragments already common to all the log P ranges.

Remarks and Conclusions

In constructing a two-component approach to apply to a diverse set of compounds, it was necessary to radically depart from some of the concepts used in standard Hansch analyses. First, there cannot be a single optimum value of log P. Second, the use of indicator variables for fragments allowed only one weight upon the presence of a fragment. Here, the weight is dependent on the range of log P.

Other stratifications of the training set besides log P can be tried. Along these lines, an earlier experiment in separating large and small compounds was not very satisfactory.

In summary, the experiments on the disjoint test set showed a significant loss in performance when the compounds were randomized over the log P ranges. When the fragment-weight table was augmented, the results were not greatly changed. This shows that the difference in preformance was due to a difference in weights for varying log P of fragments in the original table.

The large amount of testing of the second, more definitive version of the two-component model indicates the amount of improvement in performance that can be expected when ranges of log P are introduced into the earlier model that was based on structure alone. The improvement shown in the two main tests, Tables V and VII, appears mostly in the upper two percentiles of the score. Thus, the two-component model would be especially useful for automated literature surveillance where only the top few percent of compounds are examined.

Some of the compounds appearing in Table VIII that had poor ranking under the two-component model were examined. They would have ranked much higher with a different log P assignment. Perhaps with a more discriminating log P model they would have been classified into a more appropriate log P range. This may be achieved if there will be a lot more measured log P data. That points to the weakness of this approach. Data on 4000 compounds were used to classify 100 000 more diverse compounds. The log P data were especially lacking toward the low log P end where performance was worst.

Acknowledgment. All of the programs and much of the programming were performed for NCI as part of a contract by Chemical Abstracts Service. Arthur Levitt, in charge of this work at CAS, contributed a great deal, including the essential idea to use the original method for the log P model.

Antibacterial Activity of Phosphono Dipeptides Related to Alafosfalin

Barbara Lejczak, Paweł Kafarski, Helena Sztajer, and Przemysław Mastalerz*

Institute of Organic and Physical Chemistry, Technical University of Wrocław, 50-370 Wrocław, Poland.
Received December 23, 1985

A series of dipeptides containing N-terminal alanine or leucine and a wide range of P-terminal racemic 1-aminoalkanephosphonates were prepared and tested in vitro for their ability to inhibit the growth of various bacterial species. The results demonstrate that peptides containing 4-amino-4-phosphonobutyric acid and 1-amino-1-methylethanephosphonic acid exhibit antibacterial activity comparable with that observed in the case of peptides containing P-terminal racemic 1-aminoethanephosphonic acid (analogue of alanine) used as a positive control.

For a substance to be an effective antimicrobial agent, it must be able to interfere with an essential function of the microbial cell. Target sites within the cell are often susceptible to inhibitors when tested in cell-free systems, but the intact microbe is often not susceptible to the same agents. This difference in inhibitory activity between intact and cell-free systems is commonly attributed to cell permeability, whereby elements of the cell membrane restrict the access of external molecules from the environment.

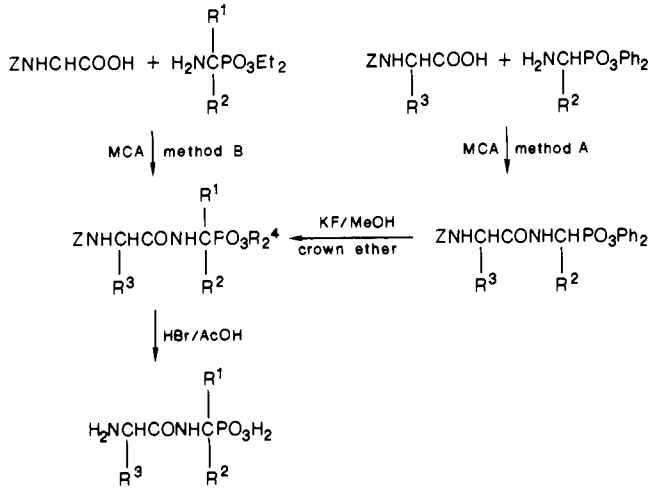
In recent years a variety of naturally occurring, as well as synthetic, antibiotics have been recognized that are analogues of small peptides and that function by entering susceptible microorganisms via peptide permeases and attacking intracellular targets. The inhibitory agent may be an intact peptide or a moiety released from it by intracellular hydrolysis.[1-3]

The most extensively studied antibiotics have been analogues of small peptides in which the C-terminal amino acid is replaced by the mimetics of alanine.[4-10] These

(1) Alper, M. D.; Ames, B. N. *J. Bacteriol.* **1978**, *133*, 149.
(2) Ringrose, P. S. In *Microorganisms and Nitrogen Source*; Payne, J. W., Ed.; Wiley: Chichester, 1980; pp 641-692 and 805-807.
(3) Ringrose, P. S. *Biochem. Soc. Trans.* **1983**, *11*, 804.
(4) Allen, J. G.; Atherton, F. R.; Hall, M. J.; Hassall, C. H.; Holmes, S. W.; Lambert, R. W.; Nisbet, L. J.; Ringrose, P. S. *Nature (London)* **1978**, *272*, 56.
(5) Atherton, F. R.; Hall, M. J.; Hassall, C. H.; Lambert, R. W.; Ringrose, P. S. *Antimicrob. Agents Chemother.* **1979**, *15*, 677.

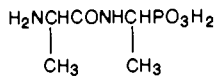
0022-2623/86/1829-2212$01.50/0

Scheme I[a]



[a] See Table I for R^1 and R^2; R^3 = CH_3, $(CH_3)_2CHCH_2$; R^4 = CH_3, CH_3CH_2.

compounds are transported into bacteria by means of peptide permeases and cleaved enzymatically within the cell to liberate the mimetic of alanine, which usually affects alanine racemase. The most promising among these antibiotics are those in which the C-terminal carboxy group is replaced by a phosphonate moiety,[4,5,11] for example, the clinically studied antibacterial agent alafosfalin (**1**).



1

As far as structure–activity relationship studies are concerned, there is a huge amount of data available on the influence of the transporting fragment of phosphono peptide (i.e., the N-terminus of the molecule) on its antibacterial activity.[5-7,12] On the other hand, little attention has been paid to the effects of the structural changes at the P-terminus of these peptides on their activity.[5,6,13] It is generally believed that, with the exception of the case of a phosphonic analogue of glycine, which yielded less interesting compounds, only alanine mimetics give rise to phosphono peptides with significant antibacterial properties.[14]

The biochemical studies, on the other hand, indicate that phosphonic analogues of amino acids often act as effective enzyme regulators.[15] For example, phosphonic analogues of valine, leucine, methionine, and phenylalanine are inhibitors of the corresponding aminoacyl-tRNA synthetases,[16-18] while analogues of glutamic acid strongly inhibit glutamine synthetase[19,20] and glutamate decarboxylase.[21]

These data encouraged us to study antibacterial properties of phosphono dipeptides containing N-terminal alanine or leucine (chosen as the most effective transporting moieties[12]) and a wide range of P-terminal 1-aminoalkanephosphonates.

Chemistry. Conventional mixed anhydride (MCA) procedure has been used in the preparation of phosphono dipeptides. The syntheses were accomplished starting from diethyl or diphenyl 1-aminoalkanephosphonates (Scheme I) as described previously.[22-24]

Phosphono dipeptides were synthesized with racemic 1-aminoalkanephosphonates, a preparation that obviously yields a mixture of diastereomers. In the 100-MHz proton magnetic resonance spectra of these compounds, pairs of signals are resolved that are consistent with diastereotopic resonances for a single proton (or set of protons). In the case, for example, of 1-(*N*-L-alanylamino)-2-acetoxyethanephosphonic acid (**11**), the alanyl methyl resonance appears as a pair of doublets (δ 1.04, 1.09), which together integrate to three protons. Usually the chemical shift differences are great enough that integration is possible and allows an estimate of the ratio of diastereomers. Thus, analysis of the NMR data for our phosphono dipeptides suggests that they are present as nearly equimolar mixtures of two diastereomers.

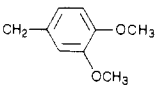
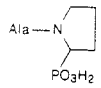
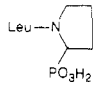
The obtained peptides containing P-terminal racemic 1-aminoalkanephosphonic acids (Table I) were used for antibacterial screening. Since L–D isomers of phosphono dipeptides are usually not transported through bacterial cell membranes,[25] we assumed that diastereomeric dipeptides would yield relevant results of preliminary biological studies.

Antibacterial Activity. The antibacterial activities of various phosphono dipeptides containing P-terminal phosphonic analogues of glycine, alanine, and β-alanine, as well as a few other aminophosphonates, have been reported by other workers.[5-7,12] New analogues have now been synthesized in our laboratory (Table I) to extent this series to include wide structural changes of the P-terminal moiety.

Incorporation of 1-aminoalkanephosphonate, inactive by itself, into a peptide chain results, in some cases, in compounds with in vitro antibacterial activity. This result confirms that warhead aminophosphonates are transported

(6) Kametani, T.; Suzuki, Y.; Kisagawa, K.; Hiiragi, M.; Wakisaka, K.; Sugi, H.; Tanigawa, K.; Fukawa, K.; Irino, O.; Saita, O.; Yamabe, S. *Heterocycles* **1982**, *18*, 295.
(7) Kametani, T.; Kisagawa, K.; Hiiragi, M.; Wakisaka, K.; Haga, S.; Sugi, H.; Tanigawa, K.; Suzuki, Y.; Fukawa, K.; Irino, O.; Saito, O.; Yamabe, S. *Heterocycles* **1981**, *16*, 1205.
(8) Morley, J. S.; Payne, J. W.; Hennessey, T. D. *J. Gen. Microbiol.* **1983**, *129*, 3701.
(9) Loennechen, T.; Bergan, T.; Sydnes, L. K.; Aasen, A. J. *Acta Chem. Scand., Ser. B* **1984**, *B38*, 647.
(10) Hagen, E. A.; Bergan, T.; Aasen, A. J. *Acta Chem. Scand., Ser. B* **1984**, *B38*, 5.
(11) Neuhaus, T. C.; Hammes, W. P. *Pharmacol. Ther.* **1981**, *14*, 265.
(12) Atherton, F. R.; Hall, M. J.; Hassall, C. H.; Holmes, S. W.; Lambert, R. W.; Lloyd, W. J.; Ringrose, P. S. *Animicrob. Agents Chemother.* **1980**, *18*, 897.
(13) Atherton, F. R.; Hall, M. J.; Hassall, C. H.; Lambert, R. W.; Lloyd, W. J.; Ringrose, P. S.; Wesmacott, D. *Antimicrob. Agents Chemother.* **1982**, *22*, 571.
(14) Hassall, C. H.; Atherton, F. R.; Hall, M. J.; Lambert, R. W.; Lloyd, W. J.; Ringrose, P. S. In *Peptides 82*; Blaha, K., Malon, P., Eds.; Walter de Gruyer: Berlin, 1982; pp 607–612.
(15) Hilderbrand, R. I.; Curly-Joseph, J.; Lubansky, H. J.; Henderson, T. O. In *Topics in Phosphorus Chemistry*; Grayson, M., Griffith, E. J., Eds.; Interscience: New York, 1983; pp 297–338.
(16) Anderson, J. W.; Fowden, L. *Chem.-Biol. Interact.* **1970**, *2*, 53.
(17) Neale, S. *Chem.-Biol. Interact.* **1970**, *2*, 349.
(18) Biryukov, A. J.; Osipova, T. I.; Khomutov, R. M. *FEBS Lett.* **1978**, *91*, 246.
(19) Lejczak, B.; Starzemska, H.; Mastalerz, P. *Experientia* **1981**, *37*, 461.
(20) Mastalerz, P. *Arch. Immunol. Ter. Dośw.* **1959**, *7*, 201.
(21) Lacoste, A. M.; Mansour, S.; Cassaigne, A.; Neuzil, E. *Experientia* **1985**, *41*, 643.
(22) Kafarski, P.; Lejczak, B.; Mastalerz, P.; Szewczyk, J.; Wasielewski, C. *Can. J. Chem.* **1982**, *60*, 3081.
(23) Lejczak, B.; Kafarski, P.; Szewczyk, J. *Synthesis* **1982**, 412.
(24) Lejczak, B.; Kafarski, P.; Soroka, M.; Mastalerz, P. *Synthesis* **1984**, 577.
(25) Atherton, F. R.; Hall, M. J.; Hassall, C. H.; Lambert, R. W.; Lloyd, W. J.; Lord, A. V.; Ringrose, P. S.; Wesmacott, D. *Antimicrob. Agents Chemother.* **1983**, *24*, 522.

Table I. Phosphono Dipeptides: $XNHC(R^1)(R^2)PO_3H_2$

no.	X	R^1	R^2	method	yield, %	mp, °C dec	$[\alpha]^{20}_{578}$ (c 1, H_2O)	formula	anal. or lit.
1	Ala	H	CH_3	A	61	278–282	+12	$C_5H_{13}NO_4P\cdot1.5H_2O$	22, 23
2	Leu	H	CH_3	A	56	247–251	+30	$C_8H_{19}N_2O_4P\cdot2H_2O$	22, 23
				B	50				
3	Ala	H	$(CH_3)_2CH$	A	50	262–264	+12	$C_7H_{17}N_2O_4P$	23
				B	52				
4	Leu	H	$(CH_3)_2CH$	A	69	260–263	+26	$C_{10}H_{23}N_2O_4P\cdot0.5H_2O$	22, 23
				B	48				
5	Ala	H	$(CH_3)_2CH_2CH$	A	63	261–264	+18	$C_8H_{19}N_2O_4P\cdot4H_2O$	N, P
				B	47				
6	Leu	H	$(CH_3)_2CH_2CH$	A	69	266–268	+28	$C_{11}H_{25}N_2O_4P\cdot2H_2O$	22, 23
				B	50				
7	Ala	H	$C_6H_5CH_2$	A	75	261–264	+4[a]	$C_{11}H_{17}N_2O_4P\cdot2H_2O$	N, P
8	Leu	H	$C_6H_5CH_2$	A	53	268–272	+8[a]	$C_{14}H_{23}N_2O_4P\cdot1.5H_2O$	N, P
9	Ala	H		B	59	288–289	+37	$C_{13}H_{21}N_2O_6P\cdot H_2O$	N, P
10	Leu	H		B	46	265–266	+27	$C_{16}H_{27}N_2O_6P\cdot H_2O$	N, P
11	Ala	H	CH_3COOCH_2	A	39	186–189	+9	$C_7H_{15}N_2O_6P\cdot2H_2O$	24
12	Leu	H	CH_3COOCH_2	A	35	240–242.5	+21	$C_{10}H_{21}N_2O_6P\cdot3H_2O$	24
13	Ala	H	$HOOCCH_2CH_2$	B	47	230–234	+15	$C_7H_{15}N_2O_6P\cdot0.5H_2O$	N, P
14	Leu	H	$HOOCCH_2CH_2$	B	61	223–224	+28	$C_{10}H_{21}N_2O_6P\cdot1.5H_2O$	N, P
15	Ala	H	cyclopropyl	B	44	270–274	+20	$C_7H_{15}N_2O_4P\cdot1.5H_2O$	N, P
16	Leu	H	cyclopropyl	B	44	258–260	+34	$C_{10}H_{21}N_2O_4P\cdot H_2O$	N, P
17	Ala	H	cyclobutyl	B	66	280–284	+23	$C_8H_{17}N_2O_4P\cdot H_2O$	N, P
18	Leu	H	cyclobutyl	B	20	263–265	+33	$C_{11}H_{23}N_2O_4P\cdot H_2O$	N, P
19	Ala	H	cyclopentyl	B	55	245–251	+15	$C_9H_{19}N_2O_4P\cdot5H_2O$	N, P
20	Leu	H	cyclopentyl	B	55	265–267	+25	$C_{12}H_{25}N_2O_4P\cdot1.5H_2O$	N, P
21	Ala	H	cyclohexyl	B	61	278–280	+11	$C_{10}H_{21}N_2O_4P\cdot3H_2O$	N, P
22	Leu	H	cyclohexyl	B	38	367–269	+7.5[b]	$C_{13}H_{27}N_2O_4P\cdot1.5H_2O$	N, P
23	Ala	H	adamantyl	B	30.5	276–279	+12[a]	$C_{11}H_{17}N_2O_4P\cdot9H_2O$	N, P
24	Leu	H	adamantyl	B	23.5	261–263	+62[a]	$C_{14}H_{25}N_2O_4P\cdot8H_2O$	N, P
25	Ala	H	$(CH_3)_3C$	B	21	215–216	+12	$C_8H_{19}N_2O_4P\cdot5H_2O$	N, P
26	Leu	H	$(CH_3)_3C$	B	23	263–264	+23	$C_{11}H_{25}N_2O_4P$	N, P
27	Ala	CH_3	CH_3	B	81	238–240	+72	$C_6H_{15}N_2O_4P\cdot1.5H_2O$	N, P
28	Leu	CH_3	CH_3	B	71.5	249–251	+82	$C_9H_{21}N_2O_4P$	N, P
29	Ala		$-(CH_2)_5-$	B	67	246–247	+47	$C_9H_{19}N_2O_4P\cdot3H_2O$	N, P
30	Leu		$-(CH_2)_5-$	B	55	245–247	+51[c]	$C_{12}H_{25}N_2O_4P\cdot3H_2O$	N, P
31				B	15	237–240	+88	$C_7H_{15}N_2O_4P\cdot3.5H_2O$	N, P
32				B	30	266–268	+27	$C_{10}H_{21}N_2O_4P\cdot0.5H_2O$	N, P

[a] (*c* 1, 1 N NaOH). [b] (*c* 0.8, 0.4 N NaOH). [c] (*c* 0.75, 0.33 N NaOH).

Table II. Minimum Inhibitory Concentration (μg/mL) for Phosphono Peptides

	bacterial species					
peptide[a]	*Escherichia coli*, PCM[b] 2057	*Klebsiella aerogenes*, PCM 2063	*Serratia marcescens*, PCM 549	*Staphylococcus aureus*, PCM 2054	*Streptococcus faecalis*, PCM 896	*Bacillus subtilis*, PCM 1949
1[c]	8	>512	64	>512	>512	>512
2[c]	8	>512	0.5	32	>512	16
4	>512	>512	>512	>512	>512	512
5	>512	>512	>512	256	>512	>512
6	>512	>512	>512	512	>512	>512
13	64	128	>512	256	>512	16
14	128	128	>512	>512	>512	128
16	>512	>512	>512	128	>512	>512
20	>512	>512	128	>512	>512	>512
27	64	>512	>512	256	>512	>512
28	64	256	>512	256	8	256
32	128	256	512	64	>512	>512

[a] MIC values for all other peptides are above 512 μg/mL. [b] Polish Collection of Microorganisms. [c] Positive control.

through the bacterial cell wall.

Since all data are reported for diastereomeric mixtures of these peptides, the compounds **1** and **2** containing the racemic phosphonic analogue of alanine are included as a positive control.

Among the tested peptides, only those containing P-terminal analogues of glutamic acid (compounds **13** and **14**) and α-methylalanine (compounds **27** and **28**) had an activity (MIC) comparable to that of the control compounds **1** and **2** (Table II). Less active was 1-(*N*-L-alanylamino)pyrrolidinephosphonic acid (**32**) (containing the analogue of proline), while other compounds had no antibacterial activity against the tested strains. The examples in Table II are representative.

Table III. IC_{50} Values (μg/mL) for Phosphono Dipeptides

peptide	bacterial species[a]					
	E. coli	*K. aerogenes*	*S. marcescens*	*S. aureus*	*S. faecalis*	*B. subtilis*
1[b]	0.22	64	0.6	58	30	512
2[b]	0.09	1.6	0.1	8	2	4
3	>512	>512	>512	6.8	0.09	>512
4	282	0.065	>512	32	410	179
5	>512	>512	>512	48	90	153
6	307	>512	64	104	>512	32
7	>512	>512	14	333	>512	6.8
8	>512	>512	>512	0.6	>512	0.065
9	>512	>512	>512	>512	>512	>512
10	>512	>512	>512	>512	>512	>512
11	>512	96	>512	0.065	512	>512
12	>512	>512	>512	>512	0.065	1.4
13	5.6	4.8	0.065	1.8	32	14
14	18	22	16	128	32	14
15	>512	>512	>512	>512	>512	>512
16	>512	>512	>512	83	>512	>512
17	>512	>512	>512	>512	>512	>512
18	>512	>512	>512	>512	230	>512
19	>512	>512	>512	512	16	>512
20	>512	>512	64	6	>512	435
21	>512	>512	>512	256	>512	>512
22	>512	>512	>512	>512	>512	>512
23	>512	>512	>512	>512	>512	>512
24	>512	>512	>512	>512	>512	>512
25	>512	>512	>512	>512	>512	>512
26	>512	>512	>512	>512	>512	>512
27	5.6	512	48	282	19	16
28	35	4.4	384	48	4.4	45
29	>512	>512	>512	>512	>512	>512
30	>512	>512	>512	0.065	>512	5.6
31	>512	>512	>512	>512	>512	>512
32	70	154	83	35	42	>512

[a] For full names see Table II. [b] Positive control.

Examination of the growth inhibition (IC_{50}, Table III) of Gram-positive and Gram-negative bacteria showed that peptides **13**, **14**, **27**, and **28** most significantly affect bacterial growth. It is worth noting that IC_{50} and MIC values obtained for the peptide **32** are very close to each other, suggesting bactericidal properties of this compound.

Most of the peptides had no significant influence on the growth of Gram-negative strains. On the other hand, phosphono peptides containing analogues of valine, leucine, and phenylalanine (compounds **3**–**8**), analogues of the known inhibitor of alanine racemase, *O*-acetylserine[11] (compounds **11** and **12**), and a structural fragment of herbicide Trakephon[26] (compound **30**), as well as amino(cyclopentyl)methanephosphonic acid (compounds **19** and **20**), exhibited moderate growth inhibitory properties against Gram-positive species.

Generally, the investigations described here provide evidence to suggest that replacement of phosphonic analogues of alanine in phosphono dipeptides by analogues of glutamic acid or α-methylalanine give compounds of interesting antibacterial properties.

The observed effect of the N-terminus upon the antibacterial properties is probably related to the peptide transport across the bacterial cell wall. If so, our results show that the leucine dipeptides are more easily transported than the corresponding alanine peptides. This observation is in agreement with literature data.[5,14] Unexpectedly, the compounds **13** and **14** exhibit an inversed pattern of activity, that is, alanyl dipeptide **13** is more active than its leucyl counterpart **14**.

Since phosphono dipeptides containing N-terminal leucine are much more easily hydrolyzed by aminopeptidases[6,27] (thus inactivated in human organism), the finding that 4-(*N*-L-alanylamino)-4-phosphonobutyric acid (**13**) is more effective than corresponding leucyl dipeptide **14** can facilitate its use as an in vivo agent.

Although the mechanism of action of phosphono dipeptides remains to be determined, we believe that there exists bioequivalence between carboxylic acid–phosphonic acid functions. In trying to confirm this, the competition between the amino acid and a peptide containing its mimetic was studied. Thus, in the case of peptides **13** and **14** the broth was supplemented with glutamic acid (originally absent in the broth), which drastically decreased the MIC and IC_{50} values (Table IV). This indicates that the P-terminal component of these peptides, 4-amino-4-phosphonobutyric acid, acts within the cell as glutamic acid antimetabolite. It can either act on essential bacterial enzymes (for example, on glutamine synthetase or glutamate decarboxylase) or interact with the synthesis of bacterial cell wall as a false substrate.

Similarly, the peptides **7** and **9**, containing P-terminal 1-amino-2-phenylethanephosphonic acid, exhibited higher antibacterial activity when the broth was deficient in phenylalanine (Table IV). In this case, however, the effect was less drastic.

Taken together, the observations seem to suggest that variations of P-terminus in phosphono dipeptides can lead to compounds of interesting antibacterial properties. These investigations have identified novel phosphono dipeptides, containing P-terminal mimetics of glutamic acid and α-methylalanine, with promising antibacterial activity in vitro.

(26) Kramer, H.; Günther, E.; Löttge, W.; Beck, R.; Kochmann, W. *Environ. Qual. Saf., Suppl.* **1976**, 686.

(27) Lejczak, B.; Kafarski, P.; Mastalerz, P. *Eur. J. Med. Chem.—Chim. Ther.* **1985**, *20*, 375.

Table IV. Influence of Liquid Peptide Medium (Broth) Composition on Antibacterial Activity of the Peptides Containing P-Terminal Phosphonic Analogues of Phenylalanine and Glutamic Acid

	7				8				13				14			
	standard[b]		deficient in Phe[b]		standard[b]		deficient in Phe[b]		standard[b]		enriched in Glu[b]		standard[b]		enriched in Glu[b]	
bacterial species[a]	IC_{50}[c]	MIC[c]	IC_{50}	MIC	IC_{50}	MIC	IC_{50}	MIC	IC_{50}	MIC	IC_{50}	MIC	IC_{50}	MIC	IC_{50}	MIC
E. coli	>512	>512	272	>512	>512	>512	>512	>512	5.6	64	>512	>512	18	128	35	>512
K. aerogenes	>512	>512	>512	>512	>512	>512	>512	>512	4.8	128	>512	>512	22	128	256	>512
S. marcescens	14	>512	>512	>512	>512	>512	2.2	>512	0.065	>512	42	>512	16	>512	64	>512
S. aureus	333	>512	10	32	0.6	>512	18	64	1.8	256	48	>512	14	>512	58	>512
S. faecalis	>512	>512	>512	>512	>512	>512	>512	>512	3.6	>512	>512	>512	32	>512	>512	>512
B. subtilis	6.8	>512	>512	>512	0.065	>512	84	512	3.8	>512	>512	>512	128	128	512	>512

[a] For full names see Table II. [b] Broth; for explanation see text. [c] In micrograms per milliliter.

Experimental Section

Synthesis. Melting points (uncorrected) were determined on a Koeffler apparatus. The structures of all compounds were supported by their IR (Perkin-Elmer 621) and NMR (Tesla BS 497) spectra.

Diphenyl 1-aminoalkanephosphonates were prepared according to Oleksyszyn et al.,[28] while diethyl esters of these acids were made by the method of Kowalik et al.[29,30]

Diphenyl 1-Amino-2-phenylethanephosphonate Hydrobromide (34). Amidoalkylation[28] of triphenyl phosphite (23.5 mL, 0.15 mol) with freshly distilled phenylacetaldehyde (31.05 g, 0.23 mol) and benzyl carbamate (23.15 g, 0.15 mol) yielded diphenyl 1-[*N*-(benzyloxycarbonyl)amino]-2-phenylethanephosphonate (**33**): yield 34.9 g (48%); mp 122–123.5 °C. Anal. ($C_{28}H_{26}NO_5P$) N, P.

With use of a commercially available mixture (50:50, v/v) of phenylacetaldehyde and diethyl phthalate, without distillation of the aldehyde, compound **33** was obtained in 29% yield.

The carbobenzoxy group of **33** was then removed by acidolysis with 45% hydrogen bromide in glacial acetic acid,[28] giving hydrobromide **34**: yield 26.4 g (85%); mp 179–180 °C. Anal. ($C_{20}H_{21}NO_3PBr$) N, P.

Diethyl 1-amino-2-(3,4-dimethoxyphenyl)ethanephosphonate oxalate (35) was prepared according to Kowalik et al.[29] starting from 98.1 g (0.5 mol) of 3,4-dimethoxyphenylacetic acid: yield 78.5 g (39%); mp 126–127 °C. Anal. ($C_{16}H_{26}NO_7P$) N, P.

Diethyl amino(adamant-1-yl)methanephosphonate oxalate (36) was prepared as described earlier,[29] starting from 24.0 g (0.12 mol) of adamantanecarboxylic acid chloride: yield 37.5 g (95%); mp >300 °C. Anal. ($C_{17}H_{30}NO_7P$) N, P.

1-(*N*-L-Alanylamino)-2-phenylethanephosphonic Acid (7). Typical Example of Method A. Carbobenzoxy-L-alanine (4.46 g, 0.02 mol) was dissolved in dry chloroform (50 mL) containing triethylamine (3.0 mL) and cooled to −5 to 0 °C. Then ethyl chloroformate (2.0 mL, 0.022 mol) was added, and the mixture was kept at −5 to 0 °C for 30 min. Then a solution of diphenyl 1-amino-2-phenylethanephosphonate (7.1 g, 0.02 mol) in dry chloroform (30 mL) was added with stirring. The mixture was slowly heated to boiling, cooled to room temperature, and washed successively with water (40 mL), 5% hydrochloric acid (2 × 40 mL), water (40 mL), saturated sodium bicarbonate solution (2 × 40 mL), water (40 mL), and brine (60 mL). The solvent was then evaporated and the oily residue dissolved in methanol (60 mL); potassium fluoride hydrate (8.0 g) and 18-crown-6 (20 mg) were added, and the mixture was heated to boiling for 5 min and allowed to stand overnight. The solvent was then removed in vacuo, and the residue was suspended in ethyl acetate (100 mL). This suspension was washed with water (40 mL) and brine (40 mL) and dried with anhydrous sodium sulfate, and the solvents were evaporated under reduced pressure. The resulting oil was dissolved in a 45% solution of hydrogen bromide in glacial acetic acid (40 mL) and left overnight. The volatile components were then removed in vacuo, and the residue was dissolved in water (80 mL). This solution was extracted with ethyl ether (2 × 40 mL) to remove benzyl bromide and decolorized with charcoal. The water was removed under reduced pressure, and the residue was dissolved in ethanol (60 mL). Free phosphono peptide was precipitated by addition of pyridine (until pH reached 5–6): yield 4.45 g (75%); mp 260–264 °C dec; ^1H NMR (D_2O + D_2SO_4, HMDS) δ 1.31 and 1.77 (d, J = 7 Hz, 1.5 H, CH_3), 2.9–3.8 (m, 2 H, CH_2), 4.23 (q, J = 7 Hz, 1 H, CHCO), 4.5–5.1 (m, 1 H, CHP), 7.5–7.8 (m, 5 H, C_6H_5).

(*N*-L-Alanylamino)cyclopropylmethanephosphonic Acid (15). Typical Example of Method B. Carbobenzoxy-L-alanine (4.46 g, 0.02 mol) was converted into mixed anhydride by the reaction with ethyl chloroformate (2.0 mL, 0.022 mol) as in method A. Then a solution of diethyl amino(cyclopropyl)methane-

(28) Oleksyszyn, J.; Subotkowska, L.; Mastalerz, P. *Synthesis* **1982**, 985.
(29) Kowalik, J.; Kupczyk-Subotkowska, L.; Mastalerz, P. *Synthesis* **1981**, 57.
(30) Kafarski, P.; Lejczak, B.; Mastalerz, P.; Duś, D.; Radzikowski, C. *J. Med. Chem.* **1985**, *28*, 1555.

phosphonate oxalate etherate (7.42 g, 0.02 mol) in dry chloroform (40 mL) containing triethylamine (6.0 mL) was added, and the mixture was slowly heated to boiling, cooled to room temperature, and allowed to stand overnight. Then it was washed successively as in method A, yielding crude diethyl [*N*-(benzyloxycarbonyl)-L-alanylamino]cyclopropylmethanephosphonate, which was deblocked by acidolysis with 45% hydrogen bromide in glacial acetic acid solution. Workup procedure as in method A yielded the crystalline dipeptide **15**: yield 2.2 g (44%); mp 270–274 °C dec; ^{1}H NMR (D_2O + D_2SO_4, HMDS) δ 0.2–1.25 (m, J = 7 Hz, 5 H, cyclopropyl protons), 1.50 (d, J = 7 Hz, 3 H, CH_3), 3.36 and 3.39 (dd, J = 8 Hz, J = 175 Hz, 0.5 H, CHP), 4.08 (q, J = 7 Hz, 1 H, CHCO).

Microbiology. All organisms were obtained as lyophilized preparations from the Polish Collection of Microorganisms (PCM) and are indicated by the appropriate accession numbers.

The IC_{50} values of each peptide for each strain were determined on a defined liquid peptide medium free from antagonists to small peptide mimetics (broth) as described by Atherton et al.[5]

Inocula of all strains were prepared by growing the test organisms overnight in the liquid peptide medium at 37 °C and diluting the cultures to approximately 4×10^6 cfu (colony-forming units) per milliliter.

A doubling dilution series was prepared in the liquid peptide medium for each compound. A 0.5-mL amount of each concentration was added to 9.4 mL of the peptide medium to give, after addition of 0.1 mL of inoculum, a final concentration range of from 0.065 to 512 μg/mL. The incubation was carried out overnight at 37 °C. The turbidity of each culture was then measured.

IC_{50} values were defined as the concentration required to reduce the growth of the 24-h culture in the liquid peptide medium to 50% of the control value.

The samples in which no turbidity was observed were used for MIC determination. Thus, 0.1 mL of such sample was transferred from the wells onto the plates containing solid peptide medium[5] (agar) and incubated overnight at 37 °C.

MIC was defined as the lowest concentration of the peptide that either completely inhibited growth or permitted 10 or fewer microcolonies to grow.

Registry No. (L,L)-1, 60668-24-8; (L,D)-1, 66023-94-7; (L,L)-**2**, 60668-50-0; (L,D)-**2**, 84340-65-8; (L,L)-**3**, 97993-89-0; (L,D)-**3**, 97993-87-8; (L,L)-4, 84340-73-8; (L,D)-4, 84340-74-9; (L,L)-**5**, 104155-45-5; (L,D)-**5**, 104131-05-7; (L,L)-**6**, 84340-79-4; (L,D)-**6**, 84340-80-7; (L,L)-**7**, 60668-65-7; (L,D)-**7**, 66024-04-2; (L,L)-**8**, 104130-76-9; (L,D)-**8**, 104131-06-8; (L,L)-**9**, 104130-77-0; (L,D)-**9**, 104131-07-9; (L,L)-**10**, 104130-78-1; (L,D)-**10**, 104131-08-0; (L,L)-11, 104130-79-2; (L,D)-11, 104155-03-5; (L,L)-**12**, 104130-80-5; (L,D)-**12**, 104131-09-1; (L,L)-**13**, 98820-97-4; (L,D)-**13**, 98820-98-5; (L,L)-14, 97993-91-4; (L,D)-**14**, 97993-88-9; (L,L)-**15**, 104130-81-6; (L,D)-**15**, 104131-10-4; (L,L)-**16**, 104130-82-7; (L,D)-**16**, 104131-11-5; (L,L)-**17**, 104130-83-8; (L,D)-**17**, 104131-12-6; (L,L)-**18**, 104130-84-9; (L,D)-**18**, 104131-13-7; (L,L)-**19**, 104130-85-0; (L,D)-**19**, 104131-14-8; (L,L)-**20**, 104130-86-1; (L,D)-**20**, 104131-15-9; (L,L)-**21**, 104130-87-2; (L,D)-**21**, 104131-16-0; (L,L)-**22**, 104130-88-3; (L,D)-**22**, 104131-17-1; (L,L)-**23**, 104130-89-4; (L,D)-**23**, 104131-18-2; (L,L)-**24**, 104130-90-7; (L,D)-**24**, 104131-19-3; (L,L)-**25**, 104130-91-8; (L,D)-**25**, 104131-20-6; (L,L)-**26**, 104130-92-9; (L,D)-**26**, 104131-21-7; **27**, 104130-93-0; **28**, 104130-94-1; **29**, 84139-32-2; **30**, 98188-76-2; (L,L)-**31**, 104130-95-2; (L,D)-**31**, 104131-22-8; (L,L)-**32**, 97993-96-9; (L,D)-**32**, 97993-95-8; **33**, 104130-96-3; **34**, 88024-19-5; **35**, 104130-97-4; **36**, 104130-99-6; (L,L)-Z-Ala-NHCH(CH_2Ph)P(O)(OPh)$_2$, 104131-00-2; (L,D)-Z-Ala-NHCH(CH_2Ph)P(O)(OPh)$_2$, 104131-23-9; (L,L)-Z-Ala-NHCH(CH_2Ph)P(O)(OMe)$_2$, 104131-01-3; (L,D)-Z-Ala-NHCH(CH_2Ph)P(O)(OMe)$_2$, 104131-24-0; triphenyl phosphite, 101-02-0; phenylacetaldehyde, 122-78-1; benzyl carbamate, 621-84-1; diethyl phthalate, 84-66-2; carbobenzoxy-L-alanine, 1142-20-7; diethyl amino(cyclopropyl)methanephosphonate oxalate, 104131-03-5; (L,L)-diethyl [*N*-(benzyloxycarbonyl)alanylamino]cyclopropylmethanephosphonate, 104131-04-6; (L,D)-diethyl [*N*-(benzyloxycarbonyl)alanylamino]cyclopropylmethanephosphonate, 104131-25-1.

Synthesis and Radioprotective Activity of New Cysteamine and Cystamine Derivatives

J. Oiry,[†] J. Y. Pue,[†] J. L. Imbach,*[†] M. Fatome,[‡] H. Sentenac-Roumanou,[§] and C. Lion[⊥]

Laboratoire de Chimie Bio-Organique, U.A. 488 du CNRS, Université des Sciences et Techniques du Languedoc, 34060 Montpellier-Cédex, France, Centre de Recherche du Service de Santé des Armées, 92141 Clamart, France, Direction des Recherches, Etudes et Techniques, 75996 Paris-Armées, France, and Institut de Topologie et de Dynamique des Systèmes, Université de Paris VII, Associé au CNRS, 75005 Paris, France. *Received February 20, 1986*

A variety of *N*-(aminoalkanoyl)-*S*-acylcysteamine and *N,N'*-bis(aminoalkanoyl)cystamine salt derivatives were synthesized. Toxicity and radioprotective activity (as the dose reduction factor DRF) were determined in vivo on mice and compared to WR 2721 and *S*-acetylcysteamine hydrochloride. One of the most interesting compounds of this series was *N*-glycyl-*S*-acetylcysteamine trifluoroacetate (**16**, I 102). Structure–activity relationships are discussed.

Since the 1950s, numerous radioprotectors have been described. However, there has been renewed interest in the area since it has been shown that the most promising radioprotector WR 2721 (**1**)[1] appears to afford preferen-

$$H_2N(CH_2)_3NH(CH_2)_2SPO_3H_2$$

1

tially radioprotection for certain normal tissues[2–4] as opposed to tumors.[4–7] The origin of such differentiation has not been firmly established although some correlations between the activity and the hydrophilicity of the drug have been presented.[8]

Regarding the mechanisms of action of such phosphorothioates, it is postulated that they act through their

[†] Université des Sciences et Techniques du Languedoc.
[‡] Centre de Recherche du Service de Santé des Armées.
[§] Direction des Recherches, Etudes et Techniques.
[⊥] Université de Paris VII.

(1) Piper, J. R.; Stringfellow, C. R.; Elliot, R. D.; Johnston, T. P. *J. Med. Chem.* **1969**, *12*, 236.
(2) Yuhas, J. M.; Storer, J. B. *Int. J. Radiat. Biol.* **1969**, *15*, 233.
(3) Phillips, T. L. *Cancer Clin. Trials* **1980**, *3*, 165.
(4) Yuhas, J. M.; Spellman, J. M.; Culo, F. *Radiation Sensitizers*; Brady, L. W., Ed.; Masson: New York, 1980; pp 303–308.
(5) Kollman, G.; Shapiro, B. *Radiat. Res.* **1966**, *27*, 474.
(6) Phillips, T. L.; Kane, L.; Utley, J. F. *Cancer* **1973**, *32*, 528.
(7) Yuhas, J. M.; Spellman, J. M.; Culo, F. *Cancer Clin. Trials* **1980**, *3*, 211.
(8) Yuhas, J. M.; Davis, M. E.; Glover, D.; Brown, D. Q.; Ritter, M. *Int. J. Radiat. Oncol. Biol. Phys.* **1982**, *8*, 519.

0022-2623/86/1829-2217$01.50/0