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Synthesis and antibacterial evaluation of phosphonic acid analogues of diaminopimelic acid

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Summary — Diaminopimelic acid is an essential amino acid in the peptidoglycan of Gram-negative bacteria, mycobacteria and some Gram-positive bacteria. It can be used as a target for the development of antibacterial agents. In this article the synthesis of a series of phosphonic acid analogues of this diamino-dicarboxylic acid is described: mono- and diphosphonic acid derivatives as well as their homologues, *N*-alkyl analogues and some peptidyl derivatives. A phenylphosphinic derivative was also prepared. All the prepared compounds were tested for their antibacterial activity against a series of Gram-positive, Gram-negative and mycobacteria. They were also tested as possible inhibitors of the UDP-MurNAc-L-Ala-γ-D-Glu: *meso*-DAP synthetase. No significant biological activity was found.

Résumé — Synthèse et évaluation antibactérienne d'acides phosphoniques analogues de l'acide diaminopiméliques. *L'acide diaminopimélique est un acide aminé essentiel dans le peptidoglycane des bactéries Gram–, mycobactéries et quelques bactéries Gram+. Il peut être utilisé comme une cible dans le développement d'agents antibactériens. Le présent travail rapporte la synthèse d'une série d'acides phosphoniques analogues de cet acide diaminodicarboxylique: dérivés de l'acide mono- et diphosphonique aussi bien que leurs homologues, analogues* N*-alkyles et quelques dérivés peptidiques. Un dérivé phénylphosphonique a été également préparé. Tous les composés décrits ont été essayés pour leur activité antibactérienne vis-à-vis d'une série de bactéries Gram+, Gram– et de mycobactéries. Ils ont été également étudiés comme possible inhibiteurs de l'UDP-MurNAc-L-Ala-γ-D-Glu: méso-DAP synthétase. Aucune activité biologique significative n'a été trouvée.*

diaminopimelic acid / phosphono- and phosphino amino acids and peptides / diamino (bisphosphonic acids) / diamino (monophosphonic) monocarboxylic acids / antibacterial evaluation

Introduction

Meso-Diaminopimelic acid (*meso*-2,6-diaminoheptanedioic acid, DAP) **1** is the cross-linking amino acid in the peptidoglycan of the cell wall of most Gram-negative bacteria, mycobacteria and some Gram-positive bacteria [1]. It is biosynthesized from aspartate in the lysine biosynthetic pathway and is built into the peptidoglycan by the UDP-MurNAc-L-Ala-γ-D-Glu: *meso*-DAP synthetase. As DAP has not been found in mammals, it is clear that interference with its biochemistry would not, at least in theory, have any toxic effects in the host. Hence it can be considered as an ideal target in the design of new antibacterial and antimycobacterial agents.

However, only a few studies concerning the target properties of this amino acid in antibacterial drug design have been published. Inhibitors were developed against several enzymes of the biosynthetic pathway, *eg*, the succinyl-CoA: tetrahydrodipicolinate *N*-succinyltransferase [2, 3], the L, L-DAP epimerase [4, 5] and the *meso*-DAP decarboxylase [6, 7]. Some compounds were tested against the peptidoglycan building enzymes [8, 9] and other compounds were prepared as analogues of DAP and their antibacterial activity was determined [10–15]. Mostly, the inhibitory effect found was very weak and was seldom associated with an antibacterial activity *in vitro*. In the

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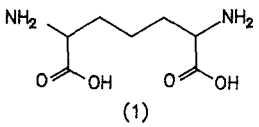
latter case, auxothrophes with a requirement for DAP were nearly always used.

Taking alafosfalin **2** [16] as an example we planned the synthesis of some phosphonic acid analogues of DAP. This dipeptide of L-Ala and the phosphonic acid analogue of L-Ala is a potent antibacterial compound. Its activity is due to the inhibitory activity of this phosphonic acid on both L-Ala racemase and UDP-MurNAc-L-Ala synthetase, which are essential for peptidoglycan biosynthesis. The dipeptide is selectively transported through the bacterial cell membrane, resulting in increased intracellular concentrations of the drug and enhanced *in vivo* antibacterial activity [17].

In this article we report the synthesis, inhibitory activity against the UDP-MurNAc-L-Ala-γ-D-Glu: *meso*-DAP synthetase and the antibacterial evaluation of a series of phosphonic acid analogues of DAP: the monophosphonic acid analogue **3** and its lower homologue **4**, the bis(phosphonic acid) analogue **5**, as well as several *N*-alkyl **6–9** and *N*-acyl (Gly, L-Ala) derivatives **10–11** and homologues **12–15** of this bis(phosphonic) analogue. A phenylphosphinic analogue **16** was also prepared.



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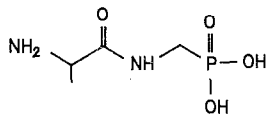

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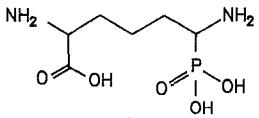

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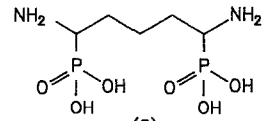

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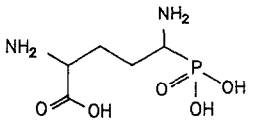

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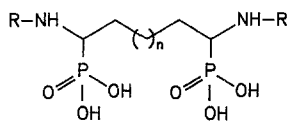

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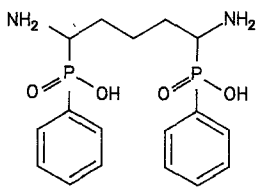


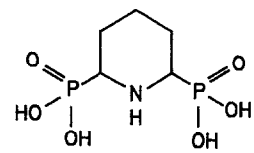
(16)

	n	R
6	1	$-CH_3$
7	1	$-CH_2CH_3$
8	1	$-CH(CH_3)_2$
9	1	$-C(CH_3)_3$
10	1	$-CO-CH_2-NH_2$
11	1	$-CO-CH(CH_3)-NH_2$
12	0	$-CH(CH_3)_2$
13	2	$-H$
14	2	$-CH(CH_3)_2$
15	2	$-C(CH_3)_3$

Chemistry

A variety of aminophosphonic acid preparations have been examined for their usefulness in the synthesis of the 1,5-diamino-1,5-pentane bis(phosphonic acid) **5**. Most of the methods used gave disappointing results; this was the case with amination of 2-ketophosphonic acids [19, 20], amidoalkylation of trivalent phosphate esters [21, 22] and the alkylation of a protected aminomethylphosphonic acid with diiodopropane [23–25]. Good results were obtained using amidoalkylation reactions and PCl_3 [26] and addition reactions of dialkyl phosphites to imines.

Using pentanedial **18** as starting aldehyde in the amidoalkylation reaction 2 compounds in ≈ 1:1 ratio were obtained: the desired 1,5-diamino-1,5-pentane bis(phosphonic acid) **5** and the cyclic analogue, 2,6-piperidine bis(phosphonic acid) **21** (scheme 1). The same reaction was used to prepare the higher homologue, 1,6-diamino-1,6-hexane bis(phosphonic acid) **13**. Hexanedial was the starting material and no cyclisation was observed. In an attempted preparation of the lower homologue using butanedial as starting material, the only compound isolated was the cyclic product, 2,5-pyrrolidine bis(phosphonic acid) **22**. In an analogous reaction 1,5-diamino-1,5-pentane bis(phenylphosphinic acid) **16** was prepared when dichlorophenylphosphine was used. In this case no cyclisation was observed.



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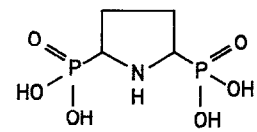
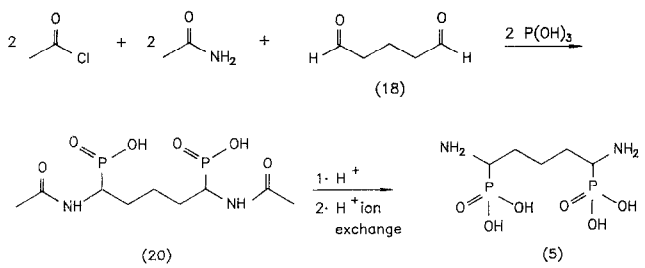


(22)

In each of the above mentioned preparations racemic mixtures were obtained. In the case of the linear compound **5** the *meso*-form and the racemate (L, L- and D, D-form) were formed in a 1:1 ratio, as seen on the P-NMR spectra. In the case of the piperidine compound **21** the ratio was 1:3 (table I).

The above-mentioned cyclisation prompted us to examine the addition reaction of dialkyl phosphites to imines giving rise, after hydrolysis, to the corresponding 2-aminophosphonic acids. Depending on the amine used, both free amino- and N-substituted aminophosphonic acids can be prepared (scheme 2). An analogous reaction for the preparation of our target compound has been vaguely mentioned in the literature [27]. We used the tritylimine derivative of pentanedial as starting material. This *N,N'*-ditrityl-1,5-pentanediimine **23**, prepared from the dialdehyde with



Scheme 1.

tritylamine in benzene with Na_2SO_4 and purified by crystallisation, reacted readily with dimethyl phosphite. The *N,N'*-dityrityl-1,5-diamino-1,5-pentane bis (phosphonic tetramethylester) **31** was not isolated, but immediately hydrolysed with 6 N HCl to the free aminophosphonic acid **5**.

With dilute acid the trityl groups were selectively removed [28], giving free aminophosphonic ester **38** which can be transformed into the corresponding aminoprotected tripeptides esters **39–40** after reaction with respectively Z-Gly and Z-L-Ala as mixed anhydride. Mild hydrolysis with HBr in AcOH afforded the free tripeptides **10–11**.

N,N'-dialkylsubstituted-1,5-diamino-1,5-pentane bis(phosphonic acids) **6–9** were prepared by addition reactions to *N,N'*-dialkyl substituted imines **24–26** of pentanedial. The diimine synthesis with pentanedial and simple alkylamines has already been studied by Lubig *et al* [29]. They found that with alkylamines the predominant compound was the cyclic *N*-alkyl-1,4-dihydropyridine, the *N,N'*-dialkyl-1,5-pentanediimine being formed in a 2–3% yield. We reinvestigated this reaction and found that with branched amines such as *t*-butylamine and isopropylamine no cyclisation occurred. In the case of ethylamine we found mixtures of both compounds in a 4:6 ratio as determined by ^{1}H-NMR. The linear imine can be isolated by distillation. All these *N,N'*-diimines were, in contrast to the trityl derivative, unstable and were used immediately after distillation. Due to isolation problems with the *N,N'*-dimethyldiimine, the *N,N'*-dimethyl bis(phosphonic acid) **6** was prepared from *N*-methylacetamide by the above-mentioned amidoalkylation reaction.

The above reactions were also used for the preparation of the homologues **12–14**, **15**. The imines **27–30** were prepared as described above and, after addition of dimethyl phosphite, gave the *N,N'*-dialkyl substituted esters **35–37**. Final hydrolysis afforded the aminophosphonic acids. The preparation of the lower homologue failed, possibly due to steric hindrance. The *N,N'*-dityrityldiimine from butanedial **27** was readily prepared but after addition of dimethyl phosphite only starting materials were recovered. In contrast, the *N,N'*-diisopropyl derivative **12** was easily prepared by this route. No problems occurred in the synthesis of the higher homologues prepared from hexanedial. The tetraethyl ester of 1,6-diamino-1,6-hexanediphosphonic acid has already been reported. It was prepared from the corresponding diketophosphonate by reductive amination [19].

Finally we also report the synthesis of the monophosphonic analogue **3** and homologue **4** of DAP. We prepared **3** from a protected D, L-2-aminoadipic acid **41** as outlined in scheme 3. The aldehyde in question was prepared by a reduction oxidation reaction of **43** with borane-dimethylsulfide and pyridinium chlorochromate in pyridine [30]. For the preparation of the lower homologue **4** we used ethyl 2-ethoxycarbonyl-4-formyl-2-phthalimidobutanoate **48** as starting material (scheme 4). This was prepared by a 1,4-addition of diethyl phthalimidomalonate **47** to acrolein.

Biological activity

All compounds were tested for their antimicrobial activity *in vitro* against a series of Gram+, Gram– and Mycobacteria: *Staphylococcus aureus, Streptococcus* sp, *Bacillus cereus, Escherichia coli, Klebsiella pneumoniae, Salmonella* sp, *Mycobacterium tuberculosis, Mycobacterium avium, Mycobacterium fortuitum.* The activity against Gram-positive and Gram-negative bacteria was determined by means of the usual dilution method in tryptic soya broth. Mycobacteria were tested by the agar dilution method in Middlebrook 7H9 agar supplemented with 10% oleic acid, albumin and dextrose solution.

In addition to the *in vitro* screening for anti(myco) bacterial activity these DAP analogues were also tested as potential inhibitors of the partially purified UDP-MurNAc-L-Ala-γ-D-Glu: *meso*-DAP synthetase from *E coli* [31]. The activity of this enzyme was assayed by following the appearance of UDP-MurNAc-L-Ala-γ-D-Glu-*meso*[^{14}C]DAP by high-voltage electrophoresis [32]. Compounds were tested at 1 and 10 mM concentrations.

Analogue **3** was tested at 10 mM as a possible substrate for this enzyme. UDP-MurNAc-L-Ala-γ-D-[^{14}C]Glu [33] was used for this purpose. The formation of a radioactive UDP-MurNAc-tripeptide was checked by reverse-phase HPLC [34]. Even with large amounts of enzyme, no new radioactive peak could be detected. Therefore analogue **3** is not a substrate for the UDP-MurNAc-tripeptide synthetase from *E coli*.

Results and Discussion

All the compounds prepared gave negative results in the *in vitro* antibacterial and antimycobacterial tests

Table I. Aminophosphonic acids.

n°	*R*	*R'*	*R"*	*n*	*Molecular[a] Formula*	*Yield %*	*IR*	*^{1}H NMR (D_2O + DCl)*	*^{13}C NMR (D_2O + DCl)*	*^{31}P NMR*
3	H	COOH	OH	1	$C_6H_{15}N_2O_5P$	32	3600-2300 1720,1625,1520, 1175,1060,920	1.55-2.05(m,6H, CH_2),3.28(m,1H, CHP),3.95(t,1H, CHCOOH)	24,42/24,38 (2d,CH_2,J_{CP}=8Hz), 31.12(CH_2CHCOOH), 51.68/51.62(2d,CHP, J_{CP}=142.9Hz), 56.40/56.51(2s, CHCOOH)	13.77-13.81
4	H	COOH	OH	0	$C_5H_{13}N_2O_5P$	45	3600-2400,1720, 1620,1525,1175,	1.80-2.20(m,4H,CH_2) 3.30(m,1H,CHP), 4.00(t,1H,CHCOOH)	27.59(CH_2CHP), 30.13(CH_2CHCOOH), 51.33(d,CHP) J_{CP}= 143.6Hz, 56.56(CHCOOH), 176.19(COOH)	-
5	H	O=P(OH)$_2$	OH	1	$C_5H_{16}N_2O_6P_2$	31[b] 75[c]	3600-2400,1620, 1540,1150,1040, 900	1.70-2.05(m,6H, CH_2),3.34(m,2H,CH)	25.36/25.69(2xt, CH_2,J_{CP}=8.1Hz), 31.04/31.25(2s, $CH_2CH_2CH_2$), 51.86/51.63 (2xd,CH,J_{CP}=142Hz)	13.84-13.88
6	CH_3	O=P(OH)$_2$	OH	1	$C_7H_{20}N_2O_6P_2$	25	3600-2200,1620, 1455,1155,1050, 900	1.75(m,2H,CH_2),1.94 (m,4H,$CH_2CH_2CH_2$), 2.82(s,6H,CH_3), (m,2H,CH)	23.60(CH_2),27.33 27.57(2s,CH_2CH_2 CH_2);32.42(CH_3), 57.12-57.29 (2d,CH,J_{CP}=141)	12.23-12.26.
7	CH_2CH_3	O=P(OH)$_2$	OH	1	$C_9H_{24}N_2O_6P_2$	45	3600-2200,1610, 900	1.31(t,6H,CH_3), CH_2),1.71-2.06 (m,4H,$CH_2CH_2CH_2$), 3.20-3.34(m,6H, CH_2CH_3CH)	11.74(CH_3),23.89 (2s,$CH_2CH_2CH_2$),42.75 (CH_2CH_3),55.95-55.80(2d,CH,J_{CP}=132.9 Hz)	12.42[d]

Table I. *Continued.*

n°	R	R'	R''	n	Molecular[a] Formula	Yield %	IR	^{1}H NMR (D_2O + DCl)	^{13}C NMR (D_2O + DCl)	^{31}P NMR
8	$CH(CH_3)_2$	$O=P(OH)_2$	OH	1	$C_{11}H_{28}N_2O_6P_2$	55	3600-2400,1615, 1460,1390,1160, 1055,900	1.35(2d,12H,CH_3), 1.70-2.05(m,6H,CH_2), 3.30(m,2H,CHP),3.72 (m,2H,CHN)	19.28-19.51(CH_3), 24.01(CH_2),29.09-28.91(2s,$\underline{CH_2}CH_2\underline{CH_2}$), 51.69(CHN),54.26 (d,CHP,J_{CP}=140.7Hz)	12.65[d]
9	$C(CH_3)_3$	$O=P(OH)_2$	OH	1	$C_{13}H_{32}N_2O_6P_2$	40	3700-2200,1610, 1475,1400,1380, 1160,1050,900	1.43(s,18H,CH_3), 1.70-2.10(m,6H,CH_2), 3.28(m,2H,CH)	25.12(CH_2),26.22 (CH_3),31.25($\underline{CH_2}$ $CH_2\underline{CH_2}$),52.59(d,CH, J_{CP}=137.7Hz), 61.03(C)	13.50-13.55
10	GLY[e]	$O=P(OH)_2$	OH	1	$C_9H_{22}N_4O_8P_2$	40	3600-2400,1675, 1550,1170,1060, 930	1.30-1.90(m,6H,CH_2), CH_2CH_2),3.85(s,4H, CH_2);4.02(m,2H,CH)	23.54(CH_2),29.56-30.14(2s,$\underline{CH_2}CH$), 41.47(CH_2),48.92-49.27(2d,CH,J_{CP}= 149.5Hz),167.32 (CO)	19.03-19.17
11	ALA[f]	$O=P(OH)_2$	OH	1	$C_{11}H_{26}N_4O_8P_2$	45	3600-2200,1660, 1540,1165,1050, 915	1.30-1.90(m,6H,CH_2), CH_2CH_2),1.55(d,6H, CH_3),3.90-4.10(m,4H, CH)	17.53(CH_3),23.54 (CH_2),29.21-29.85 (2s,$\underline{CH_2}CH$),48.87-49.23(2d,CHP,J_{CP} = 149.5Hz),50.14-171.17(CO)	18.77-18.89
12	$CH(CH_3)_2$	$O=P(OH)_2$	OH	0	$C_{10}H_{26}N_2O_6P_2$	45	3500-2200,1600, 1575,1400,1210, 1160,1055,915	1.38(2d,12H,CH_3), 2.05-2.30(m,4H, CH_2),3.49(m,2H,CHP), 3.74(m,2H,CHN)	19.22-19.40(CH_3), 25.65(CH_2CH_2),52.57 (CHN),53.15(d,CHP, J_{CP}=146.5Hz)	14.22-14.31
13	H	$O=P(OH)_2$	OH	2	$C_6H_{18}N_2O_6P_2$	36	3600-2200,1640, 1580,1525,1250, 1190,1065,950	1.58(m,4H,CH_2), 1.73-2.06(m,4H,$\underline{CH_2}$ CH),3.46(m,2H,CHP)	25.41(CH_2),28.22 ($\underline{CH_2}CH$),48.95(d,CHP, J_{CP}=152.4Hz)	16.84[g]

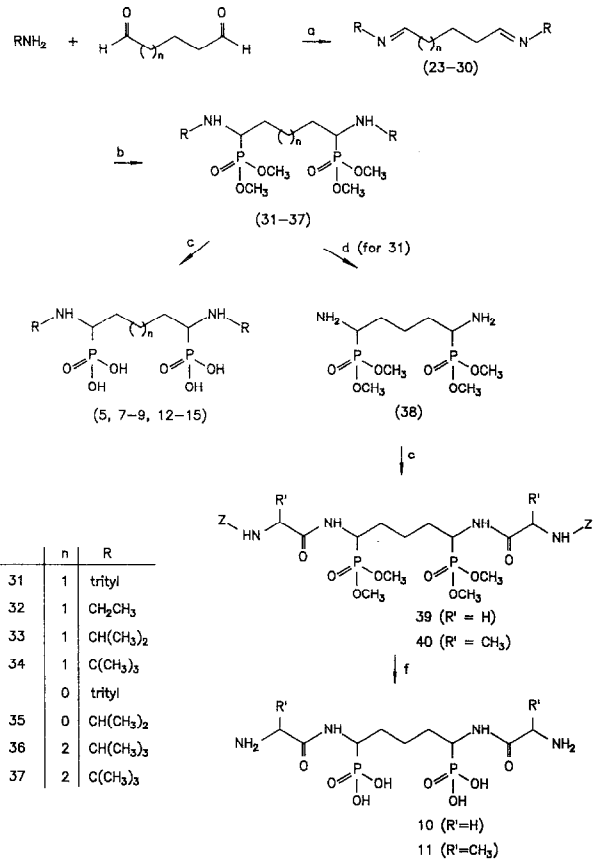
Table I. *Continued.*

n°	R	R'	R''	n	Molecular[a] Formula	Yield %	IR	^1H NMR (D_2O + DCl)	^{13}C NMR (D_2O + DCl)	^{31}P NMR
14	$CH(CH_3)_2$	$O=P(OH)_2$	OH	2	$C_{12}H_{30}N_2O_6P_2$	50	3600-2200,1600, 1472,1405,1380, 1170,1065,910	1.35(2d,12H,CH_3), 1.58(m,4H,CH_2CH_2), 1.75-2.20(m,4H,CH_2), 3.26(m,2H,CHP),3.71 (m,2H,CHN)	19.28-19.46(CH_3), 26.06(CH_2CH_2), 28.04(CH_2),52.80 (CHN),53.38(d,CHP, J_{CP}=145.2Hz)	13.03[d]
15	$C(CH_3)_2$	$O=P(OH)_2$	OH	2	$C_{14}H_{34}N_2O_6P_2$	40	3600-2200,1600, 1475,1405,1380, 1170,1065,910	1.14(s,18H,CH_3), 1.50-2.10(m,8H,CH_2), 3.24(m,2H,CHP)	26.17(CH_3),27.17 (CH_2CH_2),31.08 (CH_2),52.77(d,CH, J_{CP}=137.7Hz), 60.86(C)	13.83-13.87
16	H	$O=P(C_6H_5)(OH)$	C_6H_5	1	$C_{17}H_{24}N_2O_4P_2$	30	3600-2400,1625, 1540,1440,1180, 1130,1040,700	1.70-2.30(m,6H,CH_2), 3.35(m,2H,CH),7.61 (m,10H,ArH)	23.49(CH_2),28.33 ($\underline{CH_2}CH_2\underline{CH_2}$),51.84 (d,CH,$J_{CP}$=97Hz), 129.55-132.35- 132.81-134.73 (ArC)	25.12-25.20
21[g]	-	-	-	-	$C_5H_{13}NO_6P_2$	33	3600-2500,1600, 1160,1000,935	1.60-2.20(m,6H,CH_2), 3.33(m,2H,CH)	23.25(CH_2);24.36 (CH_2C);56.68(d,CH, J =143.6Hz)	12.08-12.90
22[h]	-	-	-	-	$C_4H_{11}NO_6P_2$	39	3200-2100,1600, 1470,1180,1080, 920	2.05-2.45(m,4H,CH_2), 3.70(m,2H,CH)	27.57(CH_2),57.15 (d,CH,J =149.5Hz)	12.58[d]

[a]Analysis C, H, N, P; [b]amidoalkylation; [c]phosphite addition; [d]insufficient resolution; [e]GLY = Glycyl; [f]ALA = L-alanyl; [g]2,6-piperidine bis(phosphonic acid); [h]2,5-pyrrolidine bis(phosphonic acid).

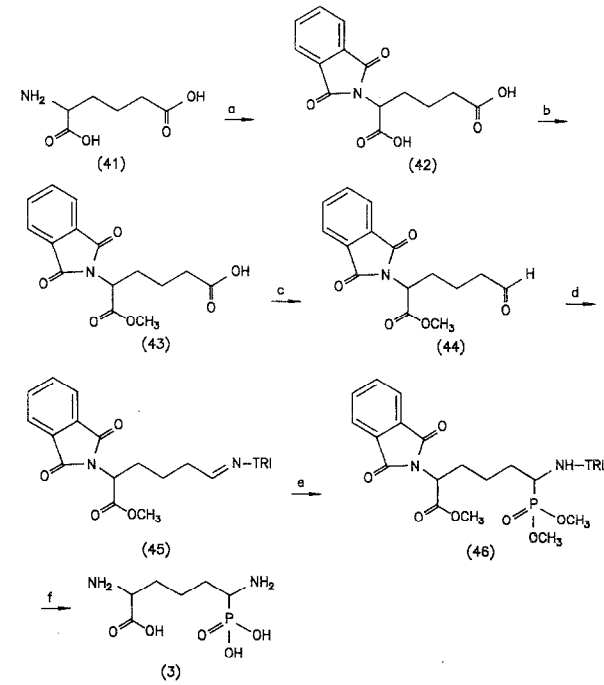
with all MIC-values higher than 100 µg/ml. Moreover, they did not show any inhibitory effect in the enzyme assay at 1 mM concentration. Even in a 10 mM solution only 50% inhibition was found with the monophosphonomonocarboxy DAP analogue **3**.

It can be concluded that the replacement of a carboxylic acid function in DAP with a phosphonic acid moiety does not result in an antimetabolite of the parent compound; this is in contrast to other amino acids such as alanine [16], glutamic acid [35] and ornithine [36] (for a review see [37]). The very low enzyme inhibitory effect of **3** can hardly be considered as important, particularly as this compound does not show any antibacterial activity *in vitro*. The *in vitro* inactivity of the peptides may indicate that at least for the diphosphonic analogues the transport through the cell membrane barrier is possibly not the main reason although transport through the cell membrane has not been studied directly in this work. Moreover, peptides corresponding to the monophosphonic acid analogue were not prepared.



		n	R
23	31	1	trityl
24	32	1	CH_2CH_3
25	33	1	$CH(CH_3)_2$
26	34	1	$C(CH_3)_3$
27		0	trityl
28	35	0	$CH(CH_3)_2$
29	36	2	$CH(CH_3)_2$
30	37	2	$C(CH_3)_3$

Scheme 2. (a) Na_2SO_4, ether or benzene; (b) dimethylphosphite; (c) 12 N HCl, H^+ ion exchanger; (d) 1 N HCl in CH_3OH; Z-Gly or Z-L-Ala, $ClCOOC_2H_5$, triethylamine; (e) HBr-CH_3COOH, methyloxirane.



Scheme 3. (a) *N*-carbethoxyphthalimide, Na_2CO_3, H_2O; (b) $(CH_3)SO_2$, triethylamine, $CHCl_3$; (c) BH_3-$(CH_3)_2S$, THF, pyridinium chlorochromate, CH_2Cl_2; (d) tritylamine, Na_2SO_4, CH_2Cl_2; (e) dimethylphosphite; (f) HCl, methyloxirane.

Conclusion

We present the synthesis of some mono- and diphosphonic acid analogues and homologues of diaminopimelic acid. The preparation of some *N*-alkyl and *N*-acyl derivatives and a phosphinic acid analogue is also presented. These compounds do not show any antibacterial activity *in vitro*. Moreover, no significant inhibitory effect was found against the enzyme, responsible for the incorporation of DAP into the cell wall peptidoglycan.

Experimental protocols

General procedures

A Varian EM 360-A (60 MHz), a Jeol JNF-FX (200 MHz) and a Varian XL (300 MHz) were used for recording ^1H, ^{13}C and ^{31}P-NMR spectra. Chemical shifts are given in ppm (δ), relative to tetramethylsilane (TMS), sodium 3-(trimethylsilyl) propionate (TSP) or 85% orthophosphoric acid as reference. The IR spectra were obtained for KBr discs using a Beckmann-Acculab-4 spectrophotometer (ν_{max} are given in cm^{-1}). Mass spectra were determined with a VG 70-SEQ hydrid mass spectrometer, equiped with an Ion Tech saddle field atom gun. Melting points were determined on a Büchi capillary melting point apparatus and are uncorrected.

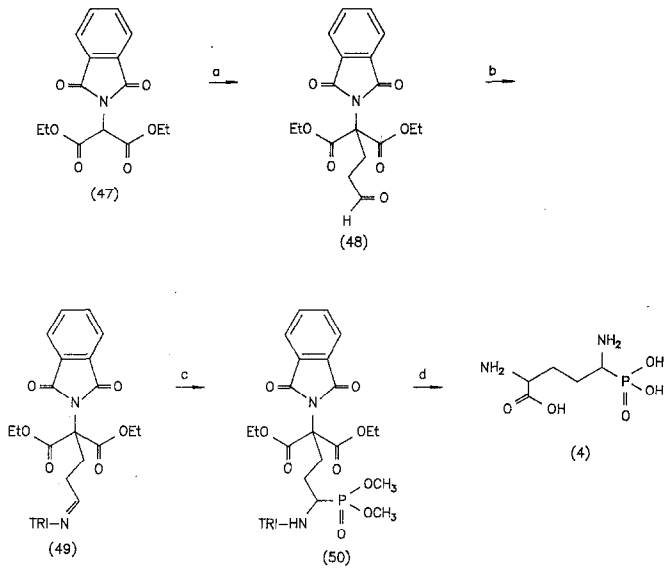
Syntheses

Pentanedial 18

A commercial solution of pentanedial (25%, 100 ml) was acidified with 12 N HCl (0.25 ml) and stirred for 2 h at room temperature. The solution was then neutralised with $NaHCO_3$ (2 g), saturated with NaCl (30 g) and extracted with CH_2Cl_2 (2 x 70 ml). The organic phases were collected, dried with Na_2SO_4, filtered and evaporated. The residue was distilled under reduced pressure. Pentanedial was obtained as a colourless, mobile liquid which solidifies quickly on standing and must be used immediately.

1,5-Diamino-1,5-pentanebis(phosphonic acid) ***5****, and 2,6-piperidinebis(phosphonic acid)* ***21****- amidoalkylation reaction*

Acetylchloride (7.8 g, 0.1 mol) was added dropwise in ≈ 10 min, with vigorous stirring and cooling (ice-water bath) to a mixture of freshly distilled pentanedial (5.0 g, 0.05 mol) **18**,



Scheme 4. (a) Acrolein, CH_3ONa, toluene; (b) tritylamine, Na_2SO_4, CH_2Cl_2; (c) dimethylphosphite; (d) HCl, methyloxirane.

acetamide (14.7 g, 0.25 mol) and AcOH (50 ml). The solution was stirred overnight at room temperature. PCl_3 (8.8 g, 0.1 mol) was then introduced dropwise to the cooled (0°C) mixture and stirring at 0°C was continued for 15 min with subsequent heating on a boiling water bath, under a reflux condenser, for 1 h. The volatile material was evaporated under reduced pressure on a boiling water bath and the oily residue refluxed overnight with 12 N HCl (100 ml). The hydrolysate was concentrated under reduced pressure on a boiling water bath and the residue extracted with CH_3OH (60 ml). The precipitated NH_4Cl was filtered off and washed twice with CH_3OH (20 ml). The collected filtrates were evaporated under reduced pressure, the combined residues dissolved in a minimum amount of water, placed on a strongly acid ion exchanger (Dowex 50 x 2-100, H^+ form) and eluted with H_2O. The ninhydrin positive fractions were collected and evaporated under reduced pressure. **21** and **5** were eluted consecutively (table I).

N,N'-Dimethyl-1,5-diamino-1,5-pentane bis(phosphonic acid) ***6***
This compound was prepared as described for **5** by an amido alkylation using *N*-methylacetamide (table I).

1,6-Diamino-1,6-hexane bis(phosphonic acid) ***13***
This compound was prepared as described for **5** using hexanedial as starting material (table I). Hexanedial was prepared by oxidation of 1,2-cyclohexanediol [38].

2,5-Pyrrolidine bis(phosphonic acid) ***22***
This compound was prepared as described for **21** by an amidoalkylation reaction using butanedial (table I). Butanedial was prepared by hydrolysis of dimethoxytetrahydrofurane [39].

1,5-Diamino-1,5-pentane bis(diphenylphosphinic acid) ***16***
This compound was prepared as described for **5** by an amidoalkylation reaction using dichlorophenylphosphine. **16** was eluted from the ion exchanger with 0.1 N HCl. The ninhydrin positive fractions were collected and evaporated under reduced pressure (table I).

N,N'-Ditrityl-1,5-pentanediimine ***23***
Freshly distilled pentanedial **18** (5.01 g, 0.05 mol) in benzene (25 ml) was added dropwise to a well stirred and cooled (ice-salt bath) mixture of tritylamine (25.9 g, 0.10 mol) and anhydrous Na_2SO_4 (16 g) in benzene (150 ml). The mixture was stirred for 5 h at 0°C and then overnight at room temperature. Na_2SO_4 was removed by filtration, washed with benzene and the combined filtrates concentrated under reduced pressure. The residue was treated with boiling cyclohexane (100 ml) and left for crystallization (table II).

N,N'-Ditritylbutane-1,4-diimine ***27***
This compound was prepared as **23** using butanedial as starting material (table II).

1,5-Diamino-1,5-pentane bis(phosphonic acid) 5- phosphite addition
A mixture of *N,N'*-ditrityl-1,5-pentanediimine **23** (23.6 g, 0.05 mol) and dimethyl phosphite (10 ml; 0.109 mol) was heated at 100°C for 1 h and the excess dimethyl phosphite removed from the clear yellow solution under reduced pressure. The oily residue **31*** was refluxed for 8 h in 12 N HCl (50 ml). The mixture was extracted with $CHCl_3$ and the aqueous phase evaporated under reduced pressure. The residue was dissolved in a minimum amount of H_2O and **5** isolated by ion-exchange chromatography as described in the amidoalkylation reaction. **5** can also be obtained by dissolving the above-mentioned residue in CH_3OH (20 ml) and precipitation with a solution of methyloxirane in CH_3OH (3 ml in 10 ml) (table I).

N,N'-Dialkyl-α,ω-alkanediimines ***24–26, 28–30***
These imines were prepared by adding a solution of the appropriate dialdehyde (0.02 mol) (pentanedial **18**, butanedial or hexanedial) in ether (20 ml) to a cooled mixture (ice-salt bath) of the amine (0.05 mol) and an excess anhydrous Na_2SO_4 in ether (50 ml). The mixture was stirred for 15 min and then for 1 h at room temperature. Na_2SO_4 was removed by filtration and washed with ether. The combined filtrates were concentrated under reduced pressure and the residue was distilled under reduced pressure. The alkanediimines are unstable liquids and their colour changes to yellow and then red on standing. They were used immediately (table II).

α,ω-Diamino-α,ω-alkane bis(phosphonic acids) and their N,N'-dialkyl analogues ***7–9, 12, 14–15***
These compounds were prepared by reaction with dimethyl phosphite as described for **5** (table I).

1,5-Diglycylamino-1,5-pentane bis(phosphonic acid) ***10***
The crude oily residue (0.025 mol tetramethyl 1,5-di (tritylamino)pentane-1,5-diphosphonate **31**) obtained after the addition of dimethyl phosphite to **23** (see preparation of **5**) was heated to boiling in 1 N HCl in CH_3OH (200 ml) for 15 min. The solution was evaporated at room temperature under reduced pressure and the residue treated with dry diethyl ether (100 ml). The ether was decanted and the oily product washed

*This oily residue **31** was also used for the preparation of tripeptides **10** and **11**.

with dry diethyl ether (2 x 50 ml) and finally dried under reduced pressure. This crude hydrochloride of tetramethyl 1,5-diamino-1,5-pentane bis(phosphonate) was dissolved in dry $CHCl_3$ (40 ml) and triethylamine (7.5 ml). This solution was added at – 5°C to a solution of the mixed anhydride of *Z*-glycine (prepared by dissolving *Z*-glycine (10.45 g, 0.05 mol) in dry $CHCl_3$ (100 ml) containing triethylamine (7.5 ml), adding ethyl chloroformate at – 5°C (5.0 ml, 0.055 mol) and stirring for 20 min). The mixture was slowly heated to boiling, cooled after 30 min and washed successively with H_2O (100 ml), 5% HCl (100 ml), H_2O (100 ml), saturated $NaHCO_3$ solution (100 ml), and saturated NaCl solution (100 ml). The organic solution was dried with Na_2SO_4, concentrated under reduced pressure and the oily residue dissolved in a solution of HBr (30%) in AcOH (100 ml) and left at room temperature overnight. The volatile compounds were then removed under reduced pressure and the residue dissolved in H_2O (60 ml). Benzylbromide was removed by extraction with ether (2 x 40 ml) and the water removed under reduced pressure. The residue was dissolved in CH_3OH (100 ml) and methyloxirane was added (10 ml). **10** precipitated after a few hours at 0°C. Recrystallization from H_2O–EtOH (table I).

1,5-Di-L-alanylamino-1,5-pentane bis(phosphonic acid) ***11***
This compound was prepared in the same way as **10** using *Z*-L-alanine (table I).

5-Methoxycarbonyl-5-phthalimidopentanoic acid ***43***
To a solution of triethylamine (2.43 g, 0.0241 mol) in absolute $CHCl_3$ (50 ml) was added 2-phthalimidoadipic acid **42** (7 g, 0.0241 mol) and dimethyl sulfate (2.5 ml, 0.0265 mol) and the mixture stirred overnight at room temperature. The solvent was evaporated under reduced pressure, and the residue dissolved in ethyl acetate (120 ml), washed with H_2O (3 x 40 ml) and dried over Na_2SO_4. After concentration under reduced pressure, the residue was dissolved in a small amount of ether, and the same volume of petroleum ether (bp = 35–70°C) was added; and crystallization was induced by cooling and scratching (6.6 g, 78%): mp = 131°C; IR 2950, 2910, 1735, 1710, 1470, 1425, 1250, 1170, 720, 910 cm^{-1}; ^1H NMR (DMSO-d_6), 1.55 (m, 2 H, CH_2), 2.00–2.50 (m, 4 H, CH_2COOH, $CHCH_2$), 3.65 (s, 3 H, $COOCH_3$), 4.90 (t, 1 H, CH), 7.89 (m, 4H, ArH). Anal $C_{15}H_{15}NO_6$ (C, H, N).

Methyl 5-formyl-2-phthalimidopentanoate ***44***
In a flask provided with a septum inlet and a reflux condenser attached to a connecting tube leading to a mercury bubbler was placed 5-methoxy-carbonyl-5-phthalimidopentanoic acid **43** (5 g, 0.164 mol) and dry THF (30 ml). The mixture was vigorously stirred and boranedimethyl sulfide (1.67 ml) was added dropwise from a syringe. After completion of the addition, the mixture was heated under reflux for 1 h, the solvent was removed under reduced pressure (room temperature), and the residue dissolved in CH_2Cl_2 (20 ml). This solution was added dropwise to a well-stirred suspension of pyridinium chlorochromate (3.91 g, 0.018 mol) in CH_2Cl_2 (30 ml) and heated under reflux for 1 h. The mixture was diluted with CH_2Cl_2, passed through a Florisil column and the eluates were collected and concentrated yielding an oil (2.8 g, 60%). Crystallization from different solvent systems failed, but the ^1H NMR spectrum indicated the presence of the aldehyde function (peak at 9.68 ppm). This crude aldehyde was used further without purification.

Methyl 2-phthalimido-6-N-tritylliminohexanoate ***45***
Tritylamine (3.37 g, 0.013 mol) was added to a solution of aldehyde **44** (3.76 g, 0.013 mol) in CH_2Cl_2 (20 ml) at 0°C. Anhydrous Na_2SO_4 (2.8 g) was added and the mixture was stirred overnight. The Na_2SO_4 was removed by filtration and the filtrate concentrated under reduced pressure. The imine was crystallized from ether (4.48 g, 65%): mp = 96°C; IR 2945, 2920, 1740, 1725, 1665, 1495, 1450, 1250, 1215, 760, 720, 705 cm^{-1}; ^1H NMR ($CDCl_3$), 1.66 (m, 2 H, CH_2), 2.30 (m, 2 H, $CH_2CH{=}N$), 2.44 (m, 2 H, $CHCH_2$), 3.71 (s, 3 H, CH_3), 4.88 (t, 1 H, CH), 7.21 (m, 15 H, CH=N – ArH), 7.80 (m, 4H, ArH); ^{13}C NMR ($CDCl_3$), 22.42 (CH_2), 28.38 ($CH_2CH{=}N$), 35.45 ($CHCH_2$), 51.86 (OCH_3), 52.62 (CH), 123.45 - 134.02 - 145.59 - 167.49 (C_6H_4), 126.49 - 127.48 - 129.58 - 131.69 (C_6H_5), 159.84 ($COOCH_3$). Anal $C_{34}H_{30}N_2O_4$ (C, H, N).

2,6-Diamino-6-phosphonohexanoic acid ***3***
A mixture of imine **45** (5.3 g, 0.01 mol) and dimethyl phosphite (5.5 g, 0.05 mol) was heated at 100°C for 2 h. The excess dimethyl phosphite was then removed under reduced pressure (100°C) and the residue **46** refluxed overnight in 12 N HCl (20 ml). The acidic solution was filtered and the filtrate was extracted with $CHCl_3$ (3 x 10 ml). The aqueous phase was concentrated under reduced pressure, the residue dissolved in a minimum amount of H_2O and placed on an ion exchange resin (Dowex 50X-200, H^+ form). The column was washed with H_2O and the product eluted with 2 N HCl. The eluate was evaporated under reduced pressure, treated with CH_3OH (20 ml) and excess methyloxirane. The mixture was kept at 0°C for a few hours and the precipitated **3** isolated and dried (table I).

Ethyl 2-ethoxycarbonyl-4-formyl-2-phthalimidobutanoate ***48***
Acrolein (2 ml, 0.03 mol), dissolved in toluene (2 ml) was added dropwise to diethyl phthalimidomalonate **47** (9.16 g, 0.03 mol) in toluene (100 ml), containing a catalytic amount of sodium methoxide and the mixture cooled in a waterbath. The mixture was stirred for 2 h and then concentrated under reduced pressure. The residue was warmed with 2 N H_2SO_4 (25 ml) for 5 min and extracted with CH_2Cl_2 (3 x 30 ml). The organic layer was concentrated under reduced pressure and the residue placed on a silicagel 60 (230–340 mesh) column which was eluted with CH_2Cl_2. Evaporation afforded **48** as an oil (8.71 g, 83%): ^1H NMR ($CDCl_3$), 1.30 (t, 6 H, CH_3), 2.78 (m, 4 H, CH_2CH_2), 4.30 (q, 2H, CH_2), 7.79 (m, 4H, ArH), 9.65 (t, 1H, CHO).

Ethyl 2-ethoxycarbonyl-2-phthalimido-5-N-tritylimino-pentanoate ***49***
This compound was prepared from **48** in the same way as described for **45**. Crystallization from ether–hexane (4.66 g, 86%): mp = 124°C; IR 1760, 1725, 1665 cm^{-1}; ^1H NMR ($CDCl_3$), 1.28 (t, 6 H, CH_3), 2.60 (m, 2 H, $CH_2CH{=}N$), 2.88 (t, 2H, CCH_2), 4.32 (q, 4 H, OCH_2), 7.22 (m, 16 H, ArH - CH=N), 7.80 (m, 4 H, ArH); ^{13}C NMR ($CDCl_3$), 13.78 (CH_3), 28.85 ($CH_2CH{=}N$), 31.65 (CCH_2), 62.20 (OCH_2), 123.45 - 131.40 - 134.20 (C_6H_4), 126.49 - 127.48 - 129.58 - 145.53 (C_6H_5), 1611.82 (CH=N). Anal $C_{35}H_{30}N_2O_4$ (C, H, N).

2,5-Diamino-5-phosphonopentanoic acid ***4***
This compound was prepared *via* **50** in the same way as **3** (table I).

Mass spectra
FAB mass spectra of the diphosphonic acids from the C5 and C4 series were all characterized by a $[MH]^+$ and a $[(MH)-H_3PO_3-NH_2R]^+$ peak where *R* is H **5**, alkyl **6–9**, **12** or acyl **10**, **11**. When *R* is ethyl **7**, isopropyl **8** or *t*-butyl **9**, $[RNH_2{=}CH{-}CH{=}CH_2]^+$ is also an important peak in the C5 group. In the C6

Table II. α,ω-alkanediimines.

n°	*R*	*n*	*Molecular Formula*	*mp°C* bp$_1$°C*	*Yield %*	*IR*	*^{1}H NMR (CDCl$_3$)*	*^{13}C NMR (CDCl$_3$)*
23	TRI	1	$C_{43}H_{38}N_2$	134°C*	85	3030,2000,1700, 1665,1600,1500, 1450,760,710	1.99(q,2H,CH_2),2.48 (m,4H,$\underline{CH_2}CH_2\underline{CH_2}$),7.15-7.25(m,3H,ArH,CH=N)	21.98(CH_2),36.41 ($\underline{CH_2}CH_2\underline{CH_2}$),126.73-129.81-145.92(ArC), 163.33(CH=N)
24	CH_2CH_3	1	$C_9H_{18}N_2$	51-53°C	29	-	1.20(t,6H,CH_3);1.88 (q,2H,CH_2);2.24(m,4H, $\underline{CH_2}CH$);3.40(q,4H, $\underline{CH_2}CH_3$);7.76(t,2H, N=CH)	-
25	$CH(CH_3)_2$	1	$C_{11}H_{22}N_2$	52-57°C	65	-	1.18(d,12H,CH_3);1.90 (q,2H,CH_2);2.24(m,4H, $CH\underline{CH_2}$);3.34(m,2H,CH); 7.74(t,2H,N=$\underline{CH}$)	-
26	$C(CH_3)_3$	1	$C_{13}H_{26}N_2$	68-72°C	60	-	1.22(s,18H,CH_3);1.84 (q,2H,CH_2);2.29(m,4H, $CH\underline{CH_2}$);7.80(t,2H,N= $\underline{CH}$)	-
27	TRI	0	$C_{42}H_{36}N_2$[b]	205°C*	81	3030,2000,1700, 1165,1600,1500, 1450,760,710	2.80(m,4H,CH_2CH_2); 7.20-7.22(m,30H,ArH), 7.38(m,2H,CH=N)	32.24(CH_2),126.49-127.4-129.58-145.64 (ArC),162.75(CH=N)
28	$CH(CH_3)_2$	0	$C_{10}H_{20}N_2$	48-50°C	32	-	1.15(d,12H,CH_3);1.50 (d,4H,$\underline{CH_2}$,J_{HH}=7.6Hz); 3.33(m,2H,CH);7.83 (t,2H,N=CH)	-
29	$CH(CH_3)_2$	2	$C_{12}H_{24}N_2$	58-66°C	85	-	1.18(d,12H,CH_3);1.68 (m,4H,CH_2CH_2);2.26(m,4H, $CH\underline{CH_2}$);3.10(m,2H,CH); 7.82(t,2H,N=CH)	-
30	$C(CH_3)_3$	2	$C_{14}H_{28}N_2$	75-81°C	70	-	1.20(s,18H,CH_3);1.66 (m,4H,CH_2);2.30(m,4H, $CH\underline{CH_2}$);8.20(t,2H,N=CH)	-

[a]TRI = Trityl; [b]analysis C, H.

series **13–15** $[MH]^+$, $[(MH)\text{-}H_3PO_3]^+$, $[(MH)\text{-}H_3PO_3\text{-}NH_2R]^+$ were found. The cyclic compounds **21**, **22** were characterized by a $[MH]^+$ and a $[(MH)\text{-}H_3PO_3]^+$ peak. The monophosphono-monocarboxylic acids **3**, **4** both showed a $[MH]^+$ peak. The C5 compound **3** also showed a $[(MH)\text{-}H_3PO_3]^+$ and a $[(MH)\text{-}H_3PO_3\text{-}NH_3]^+$ peak.

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