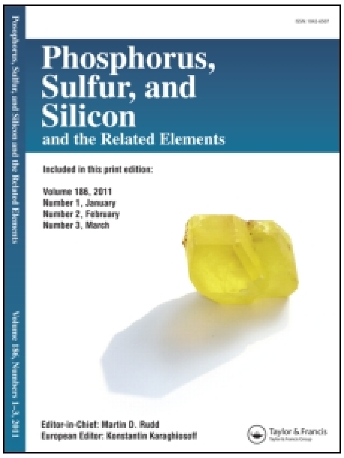

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ORGANIC PHOSPHORUS COMPOUNDS 76[1] SYNTHESIS AND PROPERTIES OF PHOSPHINOTHRICIN DERIVATIVES

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ORGANIC PHOSPHORUS COMPOUNDS 76[1] SYNTHESIS AND PROPERTIES OF PHOSPHINOTHRICIN DERIVATIVES

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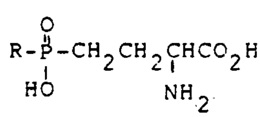
The synthesis, chemical and spectral properties of phosphinothricin analogues which either have other groups than methyl attached to phosphorus (**30** to **35**) or bear substituents on nitrogen (**43** to **50**) are described and the activity of some of these derivatives as glutamine synthetase inhibitors and contact herbicides is reported.

INTRODUCTION

It has been known for more than twenty years[2] that the phosphonic (A) and phosphinic acid (B) analogues of glutamic acid possess inhibitory properties towards glutamine synthetase, whereas the phenyl derivative (C) has only slight inhibitory activity.



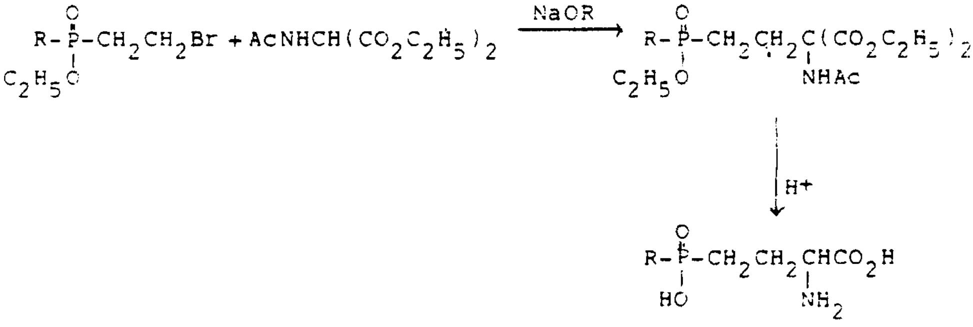
A, R = OH

B, R = C_2H_5

C, R = C_6H_5

D, R = CH_3

The synthesis of compounds B and C was accomplished by condensation of diethyl acetaminomalonate with the corresponding phosphinate followed by hydrolysis of the crude intermediate compound according to:[3]

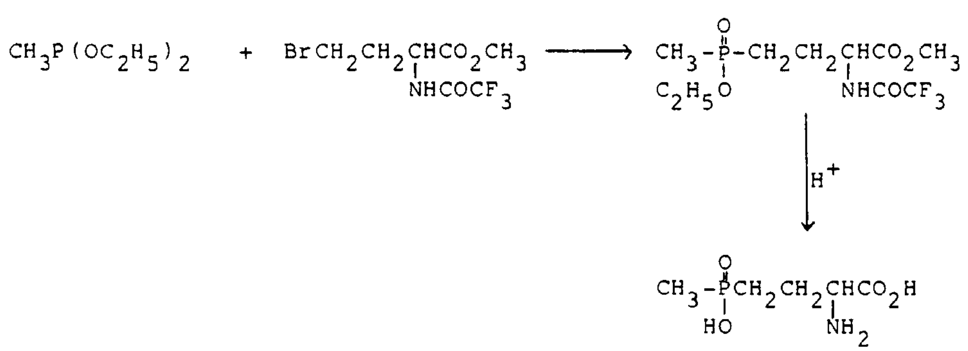


The phosphonic acid analogue (A) was synthesized in the same way starting from 2-bromoethylphosphonate.[4,5]

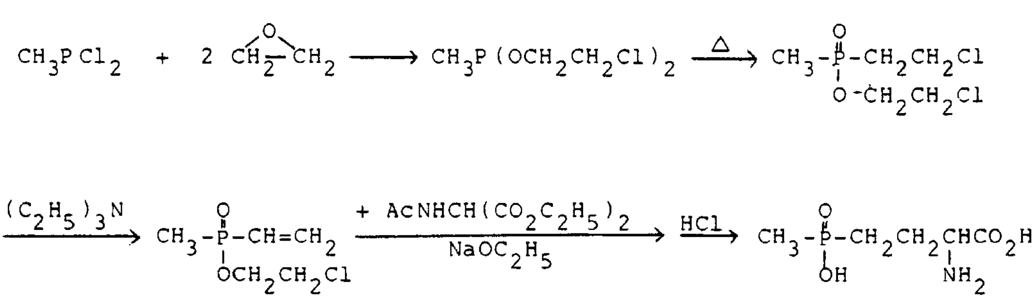
More recently phosphinothricin [2-amino-4-(methyl-hydroxyphosphinyl)-butanoic acid (D)] has been isolated from cultures of *Streptomyces viridochromogenes*[6] and *Streptomyces hygroscopicus*[7] as the tripeptide, phosphinothricyl-alanyl-alanine. This tripeptide is active against Gram-positive and Gram-negative bacteria and also against the fungi *Botrytis cinerea*,[6] sheath blight and rice blast.[8] The two alanine residues allow the penetration of this tripeptide through the cell wall. Inside the cell it is assumed that phosphinothricine is liberated ("lethal cleavage of an inactive material").[6]

D,L-Phosphinothricin is an active glutamine synthetase inhibitor[6,29] and shows herbicidal properties,[9] whereby the activity of the L-isomer has been said to be twice as high as that of the D,L form.[10] It has been claimed that the tripeptide phosphinothricyl-alanyl-alanine also exhibits herbicidal properties.[11]

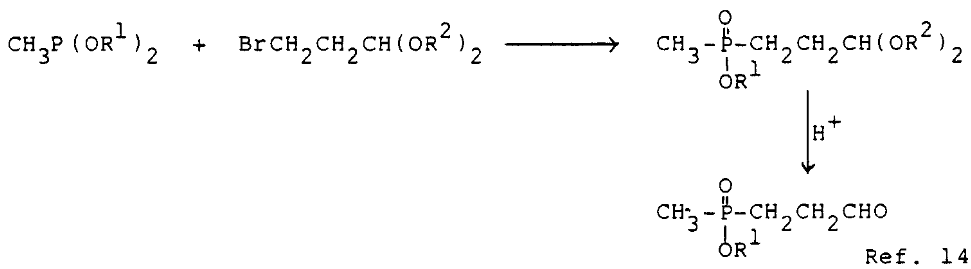
The first synthesis of phosphinothricin was reported by Zähner *et al.*[6] using in the final step an Arbuzov reaction of a homoserine derivative followed by hydrolysis. In another synthesis the acetaminomalonate procedure was used to prepare D.[12]



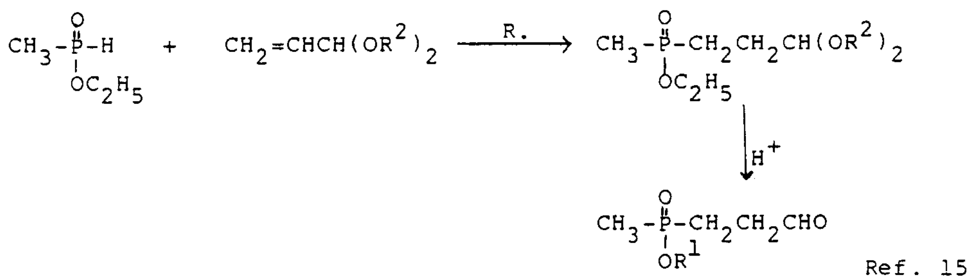
Because of problems encountered in the preparation of 2-bromoethyl-methylphosphinate needed as a starting material, this method gave only a few percent of D. A higher yield (35%) of phosphinothricin was obtained when *O*-2-chlorethyl-methylvinyl phosphinate was used as a starting material in the acetaminomalonate procedure.[13] Three other syntheses, all involving a Strecker reaction as the final step but



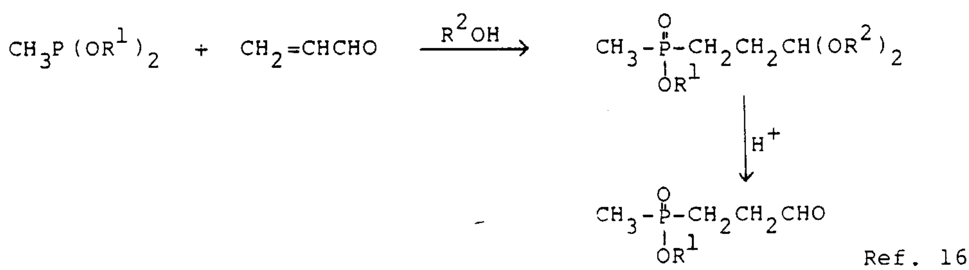
differing in the starting material (Arbuzov reaction[14] and addition reaction[15]), have also been reported:



Ref. 14

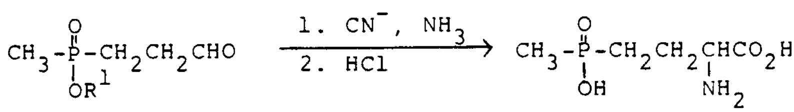


Ref. 15

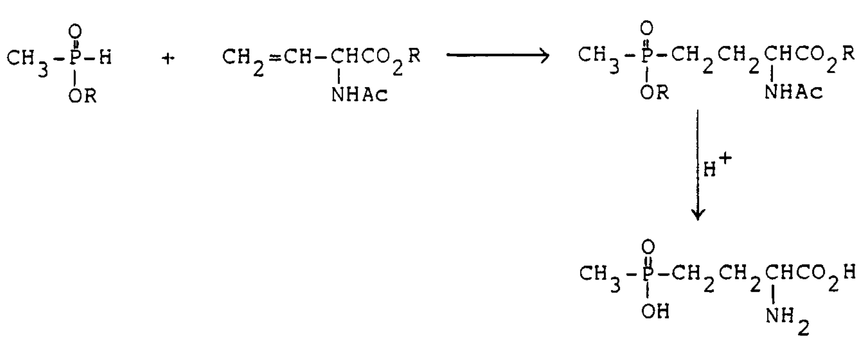


Ref. 16

then:



Another variation consisted in the addition of a phosphonite half-ester to acetamino-but-3-ene-oate.[17]



Finally phosphinothricin was also obtained by bromination of 4-methylphosphinylbutanoic acid followed by ammonolysis with aqueous ammonia solution:[18]

$$CH_3-\overset{O}{\underset{OH}{\overset{\|}{\underset{|}{P}}}}-CH_2CH_2CH_2CO_2H \xrightarrow[2.\ aq.\ NH_3]{1.\ Br_2/PCl_3} CH_3-\overset{O}{\underset{OH}{\overset{\|}{\underset{|}{P}}}}-CH_2CH_2\underset{NH_2}{\underset{|}{C}}HCO_2H$$

RESULTS AND DISCUSSION

Some of the procedures discussed above are also useful for the preparation of phosphinothricin derivatives which differ in the R group attached to phosphorus. Thus it was found that *O,O*-diethyl-2-chloroethylphosphonite[19] undergoes a Michaelis–Arbuzov reaction with alkyl halides and gives 2-chloroethyl-substituted phosphinates in good yields (**1** to **9**, Table I).

$$ClCH_2CH_2P(OC_2H_5)_2 \quad + \quad RX \longrightarrow R-\overset{O}{\underset{OC_2H_5}{\overset{\|}{\underset{|}{P}}}}-CH_2CH_2Cl \quad + \quad C_2H_5Cl$$

1 to **9**

Dehydrochlorination with triethylamine in refluxing toluene solution gave vinyl-substituted phosphinates in high yields (**10** to **15**, Table II).

$$ClCH_2CH_2-\overset{O}{\underset{OC_2H_5}{\overset{\|}{\underset{|}{P}}}}-R \quad + \quad (C_2H_5)_3N \longrightarrow R-\overset{O}{\underset{OC_2H_5}{\overset{\|}{\underset{|}{P}}}}-CH=CH_2 \quad + \quad (C_2H_5)_3N\ .HCl$$

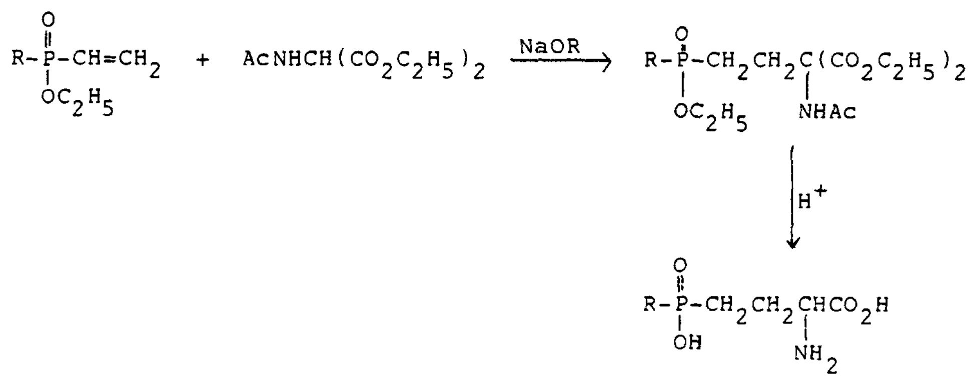
10 to **15**

Substituted aminomalonates were obtained by reaction of amines with bromomalonates as described in the literature.[20] The physical properties of the compounds prepared are summarized in Table III, (**16** to **29**).

$$(C_2H_5O_2C)_2CHBr \quad + \quad 2\ HNRR^1 \longrightarrow (C_2H_5O_2C)_2CHNRR^1 \quad + \quad RR^1NH\ .HBr$$

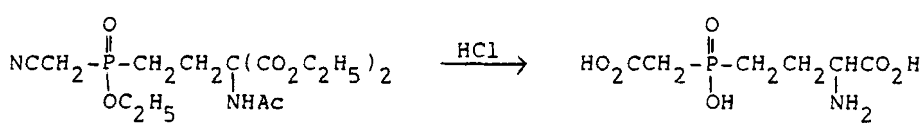
16 to **29**

When the vinylphosphinates were treated with diethyl acetaminomalonates in alcoholic solution in the presence of sodium ethoxide similar to a procedure described in the literature,[13] the substituted malonates were produced. The crude esters were hydrolyzed by heating with concentrated hydrochloric acid, and after removal of excess acid, the crude acids **30** to **35** were dissolved in alcohol–water and treated with an excess of propylene oxide to remove hydrochloric acid.[4]



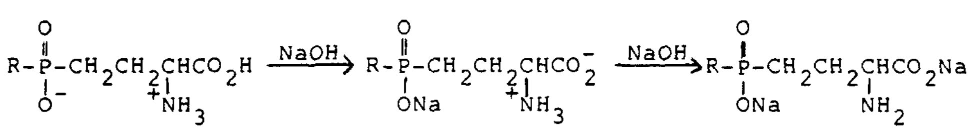
30 to **35**

All acids were obtained in a crystalline form (**30** to **35**, Table IV). The cyanomethyl-substituted derivative could not be obtained. Instead, during the hydrolysis step, this group was hydrolyzed as well and the acid **31** was isolated.



31

Like in other aminosubstituted phosphinic acid compounds,[21] the ^{31}P-chemical shift of these phosphinothricin derivatives is strongly dependent on the pH of the solution (Table V). Very likely all acids **30** to **35** possess the betaine structure, and on neutralization with sodium hydroxide, produce the disodium salt.



During a related study we observed that phosphinothricin is also formed by the base-catalyzed addition of aminomalonate to methylvinylphosphinate followed by hydrolysis.

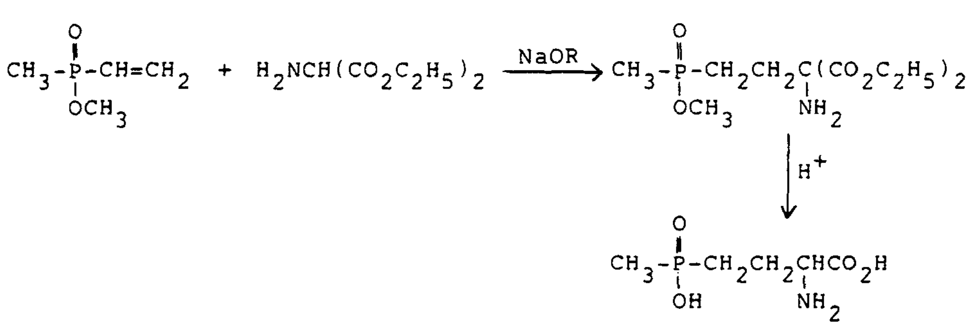
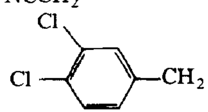
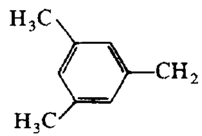
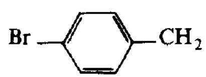
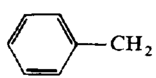


TABLE I

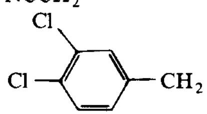
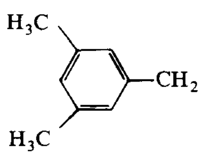
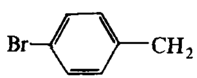
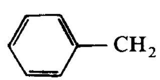
Properties of some 2-chloroethylphosphinates, $R—P(=O)(OC_2H_5)—CH_2CH_2Cl$

No.	R	b.p. °C/torr	Yield %	^{1}H-NMR of R group in $CDCl_3$ [ppm][a]	^{31}P in $CDCl_3$ [ppm] (85% H_3PO_4 as ref.)
1	CH_3OCH_2	87–90/0.04	63	OCH_2P 3.75 (d, *J* 7 H); CH_3O 3.54 (s)	45.21
2	$NCCH_2$	125/0.1[b]	67	CH_2P 3.13 (d, *J* 16 Hz)	38.79
3		163–172/0.06	30	CH_2P 3.13 (d, *J* 16 Hz); C_6H_3 7.5 (m)	45.2
4		125/0.2[bc]	69	CH_2P 3.15 (d, *J* 17 Hz); CH_3 2.3 (s), C_6H_3 6.9 (m)	48.0[d]
5		130/0.08[b]	79	CH_2P 3.15 (d, *J* 17 Hz); C_6H_4 7.4 (m)	46.61
6		110/0.04	100	CH_2P 3.25 (d, *J* 17 Hz); C_6H_5 7.3 (m)	47.73
7	$C_2H_5O_2CCH_2$	100/0.04[b]	92.7	CH_2P 3.05 (d, *J* 17.5 Hz); CH_3 1.35 (t), OCH_2 4.2 (qu)	42.42
8	$C_2H_5O_2C—C(=CH_2)—CH_2$[e]	100/0.08[b]	79.3	CH_2P 3.05 (d, *J* 18 Hz); $C{=}CH_2$ 5.9, 6.45; CH_3 1.37 (t); OCH_2 4.2 (qu)	47.08
9	*n*-C_6H_{13}	135–140/0.15	21.2	0.8–2.6 (m)	52.29

[a] Position of the other groups about the same as in **1** (see Experimental, example **1**).
[b] Molecularly distilled.
[c] m.p. 50–55°C.
[d] The corresponding phosphinic acid exhibits a ^{31}P-chem. shift of 51.08 ppm.
[e] The Michaelis–Arbuzov reaction proceeds exothermically at 30–40°C.

TABLE II

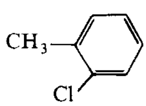
Properties of some vinylphosphinates $R-P(=O)(OC_2H_5)-CH=CH_2$

No.	R	b.p. °C/torr	Yield %	^{1}H-NMR of R group in $CDCl_3$ [ppm][a]	^{31}P in $CDCl_3$ [ppm] (85% H_3PO_4 as ref.)
10	CH_3OCH_2	49–53/0.04	72	CH_2P 3.75 (d, J 7.5 Hz), CH_3O 3.5 (s)	34.79
11	$NCCH_2$	81–87/0.02	93	CH_2P 2.97 (d, J 17 Hz)	27.82
12		140/0.1	83	CH_2P 3.25 (d, J 18 Hz); C_6H_3 7.5 (m)	36.19
13		114–119/0.04	41	CH_2P 3.1 (d, J 18 Hz); CH_3 2.3 (s); C_6H_3 6.9 (m)	37.96
14		110/0.08[b]	78.5	CH_2P 3.1 (d, J 19 Hz); C_6H_4 7.4 (m)	36.84
15		120/0.15[b]	62	CH_2P 3.2 (d, J 18 Hz); C_6H_5 7.3 (br. s)	37.68

[a]Position of the other groups about the same as in **10** (see Experimental, example **10**).
[b]Molecularly distilled.

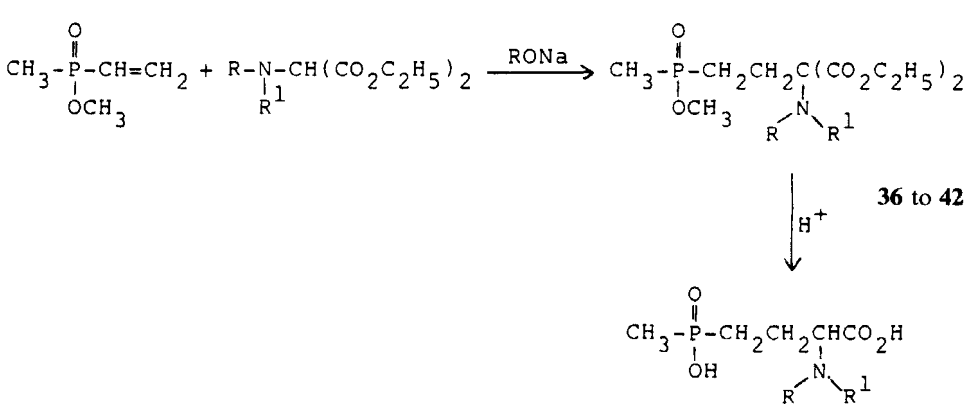
TABLE III

Properties of some substituted aminomalonates, $R^1RNCH(CO_2C_2H_5)_2$

No.	R	R^1	b.p. °C/torr	Yield %	^{1}H-NMR (in $CDCl_3$) (ppm)[a] R	R^1	CH
16	CH_3	CH_3	57–60/0.05	61	2.5 (s)	2.5 (s)	4.06 (s)
17	CH_3	$CH_2C_6H_5$	126/0.08[b]	72	2.43	CH_2 3.8; C_6H_5 7.3	4.2
18	CH_3	H[c]	53–59/0.02	86	2.43	2.3	3.95
19	CH_3	CH_3CO[d]	108–112/0.07	92	3.1	2.17	6.0
20	C_2H_5	$CH_2C_6H_5$	132–135/0.1	55	CH_3 1.1; CH_2 2.77	CH_2 3.9; C_6H_5 7.3	4.3
21	C_2H_5	C_2H_5	93–95/0.09	60	CH_3 1.05; CH_2 2.7	CH_3 1.05; CH_2 2.7	4.23
22	$C_2H_5O_2CCH_2$	$CH_2C_4H_5$	153–157/0.02	58	CH_3 1.27; CH_2O 4.23 CH_2N 3.65	CH_2 4.03; C_6H_5 7.33	4.27
23	$C_2H_5O_2CCH_2$	H[e]	114–118/0.02	79	CH_3 1.27; CH_2O 4.2 CH_2N 3.5	2.63	4.05
24		H	72–75 (m.p.)	52	CH_3 1.3; C_6H_3 6.3–7.1	4.73	4.73
25	$-(CH_2)_4-$		93–95/0.09	60	$(CH_2)_2$ 1.88	CH_2NCH_2 2.9	4.2
26	$-(CH_2)_5-$		92/0.04	72	$(CH_2)_3$ 1.53	CH_2NCH_2 2.63	4.0
27	$-(CH_2)_6-$		117–119/0.1	66	$(CH_2)_4$ 1.6	CH_2NCH_2 2.83	4.1
28	$-CH_2CH_2OCH_2CH_2-$		107–109/0.1	74	CH_2OCH_2 3.7	CH_2NCH_2 2.73	4.0
29	$-CH_2CH_2CH(CH_3)CH_2CH_2-$		103–104/0.15	76.5	$CH_2CH(CH_3)CH_2$ 0.9–1.7	CH_2NCH_2 2.83	4.05

[a] The ester groups have the same chemical shift as given for sample **16** (see Experimental).
[b] Lit. [20] b.p. 132–135°C/0.3 torr; obtained from **17** by debenzylation with $H_2/Pd/C$.
[d] Obtained from **18** by acylation; obtained from **22** by debenzylation.

The latter procedure was also successfully applied for the synthesis of amino substituted phosphinothricin derivatives (Tables VI and VII).

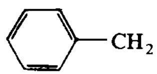
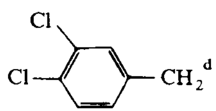
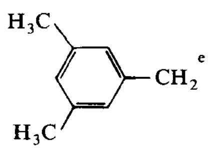
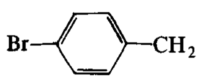


36 to **42**

43 to **50**

TABLE IV

Properties of some 2-amino-4-(alkyl- and aralkyl-hydroxyphosphinyl) butanoic acids $R-P(O)(OH)CH_2CH_2CH(NH_2)CO_2H$

No.	R	m.p. °C (dec.)	Yield %	Solvent	^1H-NMR of R group[a]	^{31}P in ppm (D_2O/DCl, ref. 85% H_3PO_4)
30		206–212	71	D_2O/DCl	CH_2P 3.7 (d, J 18 Hz); Ph 7.7 (m)	49.96 (pH 1)
31	HO_2CCH_2[b]	103–105	65	D_2O	CH_2P 2.87 (d, J 17 Hz)	35.35 (in D_2O)
32	CH_3OCH_2[c]	211–215	30	D_2O/DCl	CH_2P 4.1 (d, J 7 Hz); CH_3O 3.7 (s)	37.86 (pH 1)
33	 [d]	136–141	61	$D_2O/NaOD$	CH_2P 3.1 (d, J 17 Hz); C_6H_3 7.5	43.91 (pH 1)
34	 [e]	219–220	29	$D_2O/NaOD$	CH_2P 3.27 (d, J 16 Hz); C_6H_3 7.26 (m)	49.77 (pH 1)
35		234–235	34.5	D_2O/DCl	CH_2P 3.45 (d, J 17 Hz); C_6H_4 7.5 (m)	47.91 (pH 1)

[a] The other groups have about the same chemical shift as given for sample **30** (see Experimental).

[b] Obtained from the cyanomethylphosphinyl-derivative, $NCCH_2P(O)(OC_2H_5)CH_2CH_2C(NHAc)(CO_2C_2H_5)_2$ (Calcd.: C, 47.87; H, 6.70; N, 7.44; P, 8.23. Found: C, 47.36; H, 6.77; N, 7.39; P, 8.67%) by hydrolysis with conc. HCl.

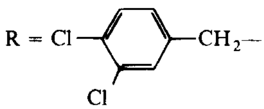
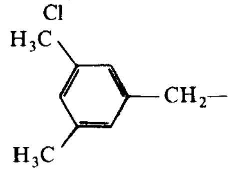
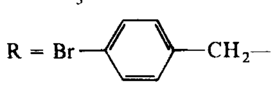
[c] Analysis $C_6H_{14}NO_5P \times 0.65\ H_2O$ (222.85). Calcd.: C, 32.34; H, 6.92; N, 6.29; P, 13.9; H_2O, 5.25%. Found: C, 32.1; H, 6.5; N, 6.20; P, 13.6; H_2O, 5.23%.

[d] Analysis $C_{11}H_{14}Cl_2NO_4P \times 1.09\ H_2O$ (345.75). Calcd.: C, 38.21; H, 4.73; N, 4.05; Cl, 20.51; P, 8.96; H_2O, 5.68%. Found: C, 38.19; H, 4.60; N, 4.17; Cl, 20.35; P, 8.94; H_2O, 5.68%.

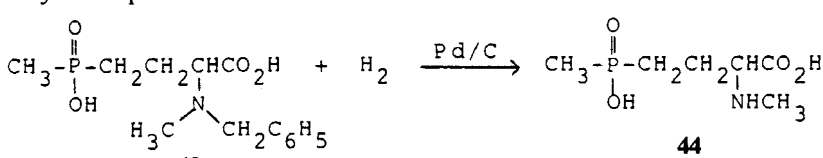
[e] Analysis $C_{13}H_{20}NO_4P$ (285.28). Calcd.: C, 54.73; H, 7.07; N, 4.91; P, 10.86%. Found: C, 54.41; H, 6.72; N, 5.04; P, 10.56%; $pK_1 < 2.5$; $pK_2 = 2.84$; $pK_3 = 9.5$.

TABLE V

Dependence of the [31]P-chemical shift of some phosphinothricin derivatives, $R{-}P(=O)(OH){-}CH_2CH_2CH(NH_2)CO_2H$, on the pH in D_2O-solution (in ppm)

	pH	1	4	9	11
33	R = 	43.91	38.89	38.79	39.72
34	R = 	49.77	41.21	40.84	41.21
35	R = 	47.91	38.79	39.82	40.28

The acid **44** was obtained by debenzylation of **43** with H_2 in the presence of Pd/C as a catalyst in aqueous solution.



43 **44**

Sometimes recrystallization of an acid from alcohol water and other purification procedures failed to give the pure acid. It was however, found that silylation of the crude hydrochloride by refluxing with excess hexamethyldisilazane, then distillation, followed by hydrolysis with ethanol produced the acid in a crystalline state and excellent purity. The successful preparation of the disilylesters of **43**, **46**, **48** and **50** (Table VIII) seems to indicate that this silylation reaction can generally be used for the purification of phosphinic acids. The silylation procedure has also been used successfully by us for the purification of phosphonic and phosphonous acids.[22] Silylation of aminoalkylphosphonic acids has been used previously for the gas chromatographic characterization of these compounds.[23]

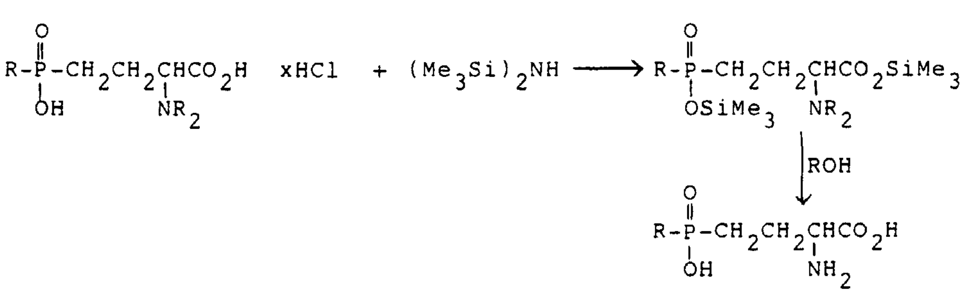
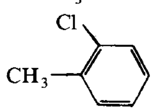


TABLE VI

Properties of some 2-subst. amino-2-ethoxycarbonyl-4-(*O*-ethyl-methylphosphinyl)-ethyl-butanoates:

$$\underset{\displaystyle \mid \atop \displaystyle OC_2H_5}{CH_3-P(O)}CH_2CH_2\underset{\displaystyle \mid \atop \displaystyle N(R)R^1}{C}(CO_2C_2H_5)_2{}^{a}$$

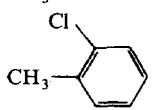
No.	R	R^1	b.p. °C/torr	Yield %	^{1}H-NMR of R and R^1 groups[b] in $CDCl_3$	^{31}P in $CDCl_3$ (ppm) P—OCH_3 ester	^{31}P in $CDCl_3$ (ppm) POC_2H_5 ester
36	CH_3	$CH_2C_6H_5$	190–197/0.05	62	CH_3N 2.2; CH_2N 3.75; C_6H_5 7.3		54.33
37		H	wax	52	CH_3—C 2.25; C_6H_3 6.4–7.4; NH 4.3		c
38	—$(CH_2)_4$—		157.8/0.02	65	$(CH_2)_2$ 1.3; CH_2NCH_2 2.93	56.1	54.33
39	—$(CH_2)_5$—		161–3/0.01	51	$(CH_2)_3$ 1.4; CH_2NCH_2 2.5	56.1	54.43
40	—$(CH_2)_6$—		164–174/0.02	48	$(CH_2)_4$ 1.5; CH_2NCH_2 2.6	56.47	54.71
41	—$CH_2CH_2\underset{\mid \atop CH_3}{C\underline{H}}CH_2CH_2$—		156–158/0.03	61	$CH_2\underset{\mid \atop CH_3}{C}HCH_2$ 0.8–1.5; CH_2NCH_2 2.7	56.1	54.43
42	—$CH_2CH_2OCH_2CH_2$—		172–178/0.015	57.5	CH_2OCH_2 3.8; CH_2NCH_2 2.75	55.64	53.87

[a]All the products contained also small amount of the P—OCH_3 ester.
[b]The other groups have about the same chemical shift as given for sample **36** (see Experimental).
[c]Not determined.

TABLE VII

Properties of some 2-substituted amino-4-(methyl-hydroxyphosphinyl)-butanoic acids,

CH_3—P(O)(OH)CH_2CH_2CH(N(R)R[1])CO_2H

No.	R	R[1]	m.p. °C (dec.)	Yield %	[1]H-NMR of R and R[1] groups in D_2O[a]	[31]P in D_2O, pH = 1 ref. 85% H_3PO_4
43	CH_3	$CH_2C_6H_5$	145–147[b]	69	CH_3N 2.65; CH_2N 4.32; C_6H_5 7.33	50.8
44	CH_3	H[c]	glassy	96	CH_3N 2.83; NH, OH 4.9	49.31
45		H	glassy	52	p-CH_3 2.6; C_4H_3 7.4; NH, OH 5.3	54.24
46	—$(CH_2)_4$—(×HCl)		glassy	65	$(CH_2)_2$ 2.9; CH_2NCH_2 4.3; OH 5.8	54.15
47	—$(CH_2)_5$—(×HCl)		glassy	43	$(CH_2)_3$ 2.1; CH_2NCH_2 3.6; OH 5.1	54.33
48	—$(CH_2)_6$—[d]		179–183[b]	48	$(CH_2)_4$ 2.6; CH_2NCH_2 4.3; OH 5.8	54.24
49	—$CH_2CH_2CH(CH_3)CH_2CH_2$— (×HCl)		glassy	61	$CH_2CH(CH_3)CH_2$ 0.8–1.5; CH_2NCH_2 3.7; OH 5.2	54.33
50	—$CH_2CH_2OCH_2CH_2$—[e]		198–201[b]	55	CH_2OCH_2 4.05; CH_2NCH_2 3.55; OH 4.9	50.8

[a] The other groups have about the same chemical shift as given for sample **43** (see Experimental).
[b] Purified through the disilylester followed by hydrolysis.
[c] Obtained from **43** by debenzylation with $H_2/Pd/C$ in water.
[d] Analysis $C_{11}H_{22}NO_4P$ (263.2). Calcd.: C, 50.19; H, 8.42; N, 5.32; P, 11.77%. Found: C, 50.05; H, 8.64; N, 5.09; P, 11.97%. $pK_1 = 2.5$; $pK_2 = 2.79$; $pK_3 = 10.2$.
[e] Analysis $C_9H_{18}NO_5P$ (251.2). Calcd.: C, 43.03; H, 7.22; N, 5.58; P, 12.33%. Found: C, 43.39; H, 7.14; N, 5.66; P. 12.09%. $pK_1 = 2.64$; $pK_2 = 7.31$.

TABLE VIII

Physical properties of some phosphinothricin-disilylester derivatives

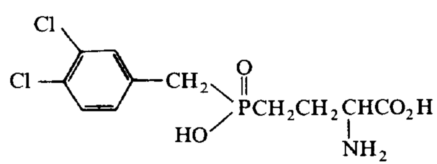
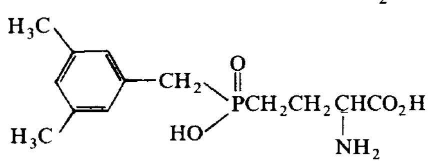
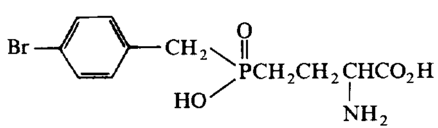
$$[(CH_3)_3SiO]_2\text{—}\underset{\displaystyle CH_3}{\underset{|}{P}}(O)CH_2CH_2\underset{\displaystyle R\diagup N \diagdown R^1}{\underset{|}{C}}HCO_2Si(CH_3)_3$$

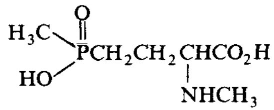
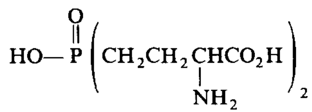
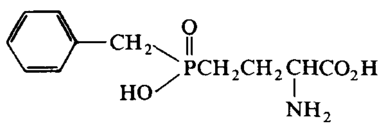
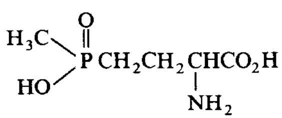
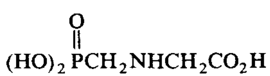
No.	R	R^1	b.p. °C/torr	Yield %	^{1}H-NMR of R and R^1 groups in $CDCl_3$, (ppm)[a]	^{31}P in ppm ($CDCl_3$) (85% H_3PO_4 as ref.)
51	CH_3	$CH_2C_6H_5$	184/0.2	36		
52	$—(CH_2)_4—$		139/0.1	61.4	$(CH_2)_2$ 1.25 (m); CH_2NCH_2 2.6 (m)	44.28
53	$—(CH_2)_6—$		143–149/0.015	62	$(CH_2)_4$ 1.38 (m); CH_2NCH_2 2.48 (m)	45.21
54	$—CH_2CH_2OCH_2CH_2—$		151–152/0.05	68	CH_2OCH_2 3.5 (m); CH_2NCH_2 2.47 (m)	

[a] The other groups have about the same chemical shift as given for sample **53** (see Experimental).

TABLE IX

Glutamine-synthetase inhibition and herbicidal activity

Compound	Glutamine synthetase inhibition at 12.5 mM[a]	Contact herbicidal activity (post)[b]							Pre-emergent activity
		Avena	Setaria	Lolium	Solanum	Sinapis	Stellaria	Phaseolus	
$(HO)_2P(=O)CH_2CH_2CH(NH_2)CO_2H$	0	9	7	9	5	6	6	8	9
$H(HO)P(=O)CH_2CH_2CH(NH_2)CO_2H$	65	5	6	6	5	2	6	8	7
$HO_2CCH_2(HO)P(=O)CH_2CH_2CH(NH_2)CO_2H$	35	7	7	8	8	4	4	7	7
	20	9	6	9	4	6	8	8	9
	20	9	9	9	9	9	9	9	9
	20	9	9	9	9	9	9	9	9

	0	7	9	6	8	7	9	7	9
	10	9	9	9	9	8	9	9	9
	30	9	9	9	9	9	9	6	9
	100	1	1	2	1	1	2	1	2
	0	2	1	2	1	2	2	1	8

[a]No compound shows glutamate-dehydrogenase inhibition.
[b]Percent control: 1 = 100% control; 9 = 0% control.

Biological activity of some of the derivatives

The major mechanism by which plants are able to assimilate ammonia is via the consecutive action of two enzymes glutamine synthetase and glutamate synthase.[24] As phosphinothricin is a potent inhibitor of glutamine synthetase it has been proposed that the herbicidal properties of the compound are due to the liberation of toxic levels of ammonia within the plant cell.[25] This proposal has recently been confirmed by Lea *et al.*[26] who showed that phosphinothricin caused the ammonia concentration inside the leaves of a number of plants to increase rapidly to a level that chloroplast metabolism was severely inhibited.

From Table IX it can be seen that there is a general correlation between the herbicidal properties of the ten compounds tested and their ability to inhibit pea leaf glutamine synthetase. The final compound in Table IX, glyphosate—which is known to have a different mode of action[27] does not inhibit glutamine synthetase.

From Table IX it can be seen that the phosphonous[28] and phosphinic acid derivatives are inhibitors of glutamine synthetase whilst the phosphonic acid derivative has no action. There is a certain amount of latitude on the group that is able to substitute on the phosphorus atom, but the methyl group is clearly the most potent. The importance of a methyl group in this position has been noted for another inhibitor of glutamine synthetase namely methionine sulphoximine.[29]

A second enzyme that has the potential to assimilate ammonia in higher plants is glutamate dehydrogenase although there is little evidence to suggest that it operates under normal physiological conditions.[24] There was no indication that any of the compounds tested could inhibit this enzyme, thus its involvement in the herbicidal action of phosphinothricin is unlikely.

EXPERIMENTAL

D,L-phosphinothricin[2-amino-4-(methyl-hydroxyphosphinyl)-butanoic acid] and its hydrochloride were prepared as described in the literature.[7,9,13] 2-Chloroethyldichlorophosphine[30] and *O,O*-diethyl-2-chloroethylphosphonite[19] were prepared as described previously by us. Phosphorus NMR spectra were recorded using a Bruker WP 90 spectrometer at 32.28 MHz, and the chemical shifts are reported in units relative to external 85% phosphoric acid, with negative values being upfield of the standard and positive downfield.

A. Preparation of alkyl- and aralkyl-2-chloroethylphosphinates by the Michaelis–Arbuzov reaction

1. *O-Ethyl-methoxymethyl-2-chloroethylphosphinate*, $CH_3OCH_2P(O)(OC_2H_5)(CH_2CH_2Cl)$, **1**. A mixture of 38.8 g (0.2 mol) of $ClCH_2CH_2P(OC_2H_5)_2$, 14.9 ml of $ClCH_2OCH_3$ and 0.5 g of $NiCl_2$ is heated with stirring. At 75–80°C reaction ensues and ethyl chloride is evolved. In the course of 1.5 h the reaction temperature is increased to 135°C. Then the volatile products are distilled off on a rotavapor and the residue fractionated in a vacuum. There is obtained 25.2 g (= 63%) of **1**, a colorless liquid, b.p. 87–90°C/0.04 torr. ^{1}H-NMR (in $CDCl_3$): CH_3 1.35 (t, 3 H); C—CH_2P 2.35 (m, 2 H); CH_3O 3.45 (s, 3 H); OCH_2P 3.75 (d, *J* 7 Hz); $ClCH_2$ 3.7 (m) and $POCH_2$ 4.2 (m) (6 H) [ppm]. ^{31}P 45.21 ppm (in $CDCl_3$). The compounds listed in Table I have been prepared in the same way.

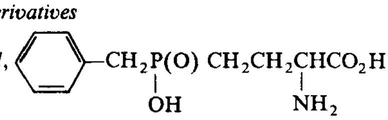
B. Preparation of alkyl- and substituted benzyl-vinylphosphinates

10. *O-Ethyl-methoxymethyl-vinylphosphinate*, $CH_3OCH_2{-}\underset{\substack{| \\ CH=CH_2}}{P}(O)(OC_2H_5)$, **10**. A mixture of 20 g (0.1 mol) of **1**, 15.3 ml of triethylamine and 100 ml of toluene is stirred and refluxed for 8 h. Then the precipitated amine hydrochloride is filtered and the filtrate fractionally distilled. There is obtained 11.8 g (= 72%) of **10**, a colorless liquid b.p. 49–53°C/0.04 torr. ^{1}H-NMR (in $CDCl_3$): CH_3 1.4 (t, 3 H); OCH_3 3.5 (s, 3 H) OCH_2P 3.75 (d, J 7 Hz); $C{-}CH_2O$ 4.2 (qu., 2 H); $CH_2{=}CH$ 5.9–6.8 (m, 3 H) [ppm] ^{31}P 34.79 ppm (in $CDCl_3$). The vinylphosphinates, listed in Table II have been prepared in the same way. To stabilize the vinylphosphinates, a trace of hydroquinone was added at the preparation and after the distillation.

C. Preparation of substituted aminomalonates

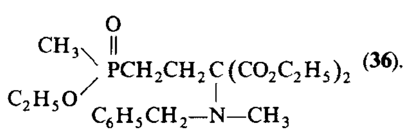
16. *Dimethylamino-diethylmalonate*, $(CH_3)_2NCH(CO_2C_2H_5)_2$, **16**. To 180 ml of a 33% solution of dimethylamine (0.5 mol) in ethanol is added with stirring and cooling 83 ml of diethylbromomalonate, the mixture stirred for 15 h at 20°C, then the amine salt filtered and the filtrate evaporated on a rotavapor. To the residue is added diisopropyl ether, again filtered and the filtrate fractionally distilled. There is obtained 61.8 g (= 61%) of **16**, a colorless liquid b.p. 57–60°C/0.05 torr. ^{1}H-NMR (in $CDCl_3$): CH_3 1.3 (t, 6 H); $(CH_3)_2N$ 2.5 (s, 6 H); CH 4.06 (s, 1 H) OCH_2 4.3 (qu., 4 H) [ppm]. The compounds listed in Table III have been prepared in the same way.

D. Preparation of 2-amino-4-phosphinyl-butanoic acid derivatives

30. *2-Amino-4-(benzyl-hydroxyphosphinyl)-butanoic acid*,  (**30**). To 5.95 g (0.03 mol) of **15** and 6.5 g of acetaminomalonate is added at 85°C 3 ml of a 6% $NaOC_2H_5$-solution in ethanol. A slight exothermic reaction ensues. The mixture is stirred and heated to 95–100°C for four hours. The dark brown reaction mixture is hydrolyzed with 30 ml conc. HCl by heating to reflux for 4 h. The clear brown solution is evaporated on a rotavapor and the residue recrystallized from H_2O/C_2H_5OH and addition of propylene oxide. There is obtained 5.5 g (= 71%) of **30**, a white solid, m.p. 206–212°C (dec.). ^{1}H-NMR (in D_2O/DCl)PCH_2CH_2 2.5 (m, 4 H); $PhCH_2P$ 3.7 (d, J_{PCH} 18 Hz, 2 H); CH 4.57 (m, 1 H); OH, NH_2 5.05 (s); C_6H_5 7.7 (m, 5 H) [ppm]. ^{31}P 49.96 ppm (in D_2O + DCl, pH = 1). $C_{11}H_{16}NO_4P \times 0.4\ H_2O$ (264.79). Calcd.: C, 49.91; H, 6.43; N, 5.29; P, 11.7; H_2O 2.83%. Found: C, 48.76; H, 6.13; N, 5.69; P, 11.36; H_2O 2.83%. The compounds listed in Table IV have been prepared in a similar way.

E. Preparation of 2-alkyl- and -arylamino-4-(methyl-hydroxyphosphinyl)-butanoic acid derivatives

43. *2-(N-methyl-N-benzylamino)-4-(methyl-hydroxyphosphinyl)-butanoic acid*,
$CH_3\underset{\substack{| \\ HO}}{P}(O)CH_2CH_2\underset{\substack{| \\ C_6H_5CH_2-N-CH_3}}{CH}CO_2H$ (**43**)

(a) *Malonate-derivative*  (**36**). A mixture of 24 g (0.2 mol) of $CH_3P(O)(OCH_3)(CH{=}CH_2)$, 55.8 g (0.2 mol) of **17** and 10 ml ethanol containing 6% $NaOC_2H_5$ is stirred and heated to 130°C for 14 h. The volatile products are evaporated on a rotavapor and the residue distilled on a wiped wall molecular still. During the reaction trans-esterification occurred. There is obtained 51.3 g (= 62%) of **36**, a colorless oil, b.p. 190–197°C/0.05 torr. ^{1}H-NMR (in $CDCl_3$): CH_3 1.3 (t, 9 H); CH_3P 1.5 (d, J 14 Hz, 3 H) CH_2CH_2P 1.9 (m, 4 H); CH_3N 2.2 (s, 3 H); $PhCH_2$ 3.75 (s, 2 H); OCH_2 4.2 (m, 6 H); C_6H_5 7.3 (m, 5 H) [ppm]. ^{31}P 54.33 ppm (in $CDCl_3$). The compounds listed in Table VI have been prepared in a similar way.

(b) *Acid* **43**. A mixture of 51.3 g (0.124 mol) of **36** and 200 ml of conc. HCl is refluxed with stirring for 12 h and then evaporated on a rotavapor. There is obtained 40 g (= 100%) of crude **43** which is purified by conversion into the disilyester (b.p. 184°C/0.2 torr, 36% yield, procedure see under (c)). Hydrolysis of the disilyester, evaporation, and recrystallization of the residue from H_2O/C_2H_5OH/acetone yields 8.9 g (= 69%) of **43**, white crystals, m.p. 145–147°C. ^{1}H-NMR (in D_2O): CH_3P 1.33 (d, J 14 Hz, 3 H); CH_2CH_2P 1.9 (m, 4 H); CH_3N 2.65 (s, 3 H); CH 3.65 (m, 1 H); $PhCH_2$ 4.32 (s, 2 H); OH 4.6 (s); C_6H_5 7.33 (br. s, 5 H) (ppm). ^{31}P 50.8 ppm (in D_2O, pH = 1); ^{31}P-of hydrochloride 54.5 ppm (in D_2O, pH = 1). $C_{13}H_{20}NO_4P \times H_2O$ (303.3). Calcd.: C, 51.48; H, 7.31; N, 4.62; P, 10.21; H_2O 5.9%. Found: C, 51.41; H, 6.82; N, 4.73; P, 10.39; H_2O 5.6%. The waterfree acid gave on titration with 0.1 N NaOH two inflection points, equivalent weight found 287, calcd 285.2; half-neutralization potentials $pK_1 = 2.5$; $pK_2 = 2.73$; $pK_3 = 8.78$. The compounds listed in Table VII have been prepared in a similar way.

(c) *Purification of 2-(N-hexamethylene-amino)-4-(methyl-hydroxyphosphinyl)butanoic acid*, $CH_3{-}P(O)(OH)CH_2CH_2CH(N(CH_2)_6)CO_2H$, *by silylation.* A mixture of 15 g (0.05 mol) of crude **48** (×HCl) and 50 ml of $[(CH_3)_3Si]_2NH$ is refluxed with stirring for 4 h. Thereby the NH_4Cl formed sublimes into the condenser. The residual brown liquid is evaporated on a rotavapor. There is obtained 12.6 g (= 62%) of the di-silylester, $CH_3P(O)(OSi(CH_3)_3)CH_2CH_2CH(N(CH_2)_6)CO_2Si(CH_3)_3$, **53**, b.p. 143–149°C/0.015 torr, as a clear, slightly yellow oil. ^{1}H-NMR (in $CDCl_3$): $(CH_3)_3Si$ 0.03 (s, 18 H); CH_3P 1.1 (d, J 14 H, 3 H); CH_2CH_2P 1.38 (m) $(CH_2)_4$ 1.38 (m) (12 H); CH_2NCH_2 2.48 (m, 4 H); CH 2.95 (t, 1 H) (ppm). ^{31}P 45.21 ppm (in $CDCl_3$). The compounds listed in Table VIII have been prepared in a similar way.

The disilylester is hydrolyzed by refluxing with 30 ml ethanol for 15 min. Then 100 ml acetone is added to the clear, slightly yellow solution. Thereby the acid **48** crystallizes, 6.2 g (= 78.5%), m.p. 179–183°C (dec.).

Glutamine synthetase activity was isolated from the leaves of *Pisum sativum* cv Feltham First as described by Leason *et al.*[29] Inhibition studies were carried out at equal concentrations of glutamate (12.5 mM) and the test compounds.

Glutamate dehydrogenase activity was isolated from the leaves of *Pusum sativum* cv Feltham First as described by Cunliffe *et al.*[31] Inhibition studies were carried out in both the glutamate formation and glutamate deamination directions. Inhibition studies were carried out at either equal concentrations of glutamate (12.5 mM) or 2-oxoglutarate (6 mM) and the test compound.

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