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Inhibitors of phenylalanine ammonia-lyase: 1-aminobenzylphosphonic acids substituted in the benzene ring

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Abstract

Dextrorotatory 1-amino-3′,4′-dichlorobenzylphosphonic acid was found to be a potent inhibitor of the plant enzyme phenylalanine ammonia-lyase both in vitro and in vivo from among the ring-substituted 1-aminobenzylphosphonic acids and other analogues of phenylglycine. A structure activity relationship analysis of the results obtained permits predictions on the geometry of the pocket of the enzyme and is a basis in the strategy of better inhibitor synthesis. © 2002 Published by Elsevier Science Ltd.

Keywords: Phenylalanine ammonia-lyase; 1-Aminoalkylphosphonic acids; Enzyme inhibitors

1. Introduction

Phenylalanine ammonia-lyase (PAL; EC 4.3.1.5) catalyses the first committed step of phenylpropanoid metabolism in higher plants, yielding the precursor of a large number of compounds such as lignins, flavonoids, coumarins, stilbenes, benzoic acid derivatives, and even an alkaloid, with diverse biological functions (Hahlbrock and Scheel, 1989). We have previously identified 2-aminoindan-2-phosphonic acid (AIP) as a potent inhibitor of phenylalanine ammonia-lyase (Zoń and Amrhein, 1992) both in vitro and in vivo. Earlier, two other strong inhibitors of PAL had been identified: (±)-2-aminooxy-3-phenylpropanoic acid (Amrhein and Gödeke, 1977) and (*R*)-1-amino-2-phenylethylphosphonic acid (Janas et al., 1985; Laber et al., 1986). Of these three compounds, AIP appears to have the highest potential as a specific inhibitor in vivo and has been used as such in numerous studies, the most recent examples being those of Ruuhola and Julkunen-Tiitto (2000), Hrubcová et al. (2000), Schaller et al. (2000), Chen and McClure (2000), Chen and Chen (2000) and Kostenyuk et al. (2001). Thus, specific PAL inhibitors are valuable tools in the study of the phenylpropanoid pathway and its functions in vivo. A cinnamic acid derivative has recently been introduced as a potentially useful inhibitor as well (Hashimoto et al., 2000).

Additionally, derivatives of PAL inhibitors carrying, for example, a photoreactive or luminophoric group, may assist in the exploration of the enzyme's active site (Kotzyba-Hilbert et al., 1995).

In continuing our studies on phenylalanine ammonia-lyase (Zoń and Laber, 1988; Zoń and Amrhein, 1992; Zoń, 1996; Lewandowicz et al., 1999; Gloge et al., 2000), we report here the synthesis of a set of various analogues of phenylglycine (**1**–**41**, Fig. 1), mostly ring-substituted 1-aminobenzylphosphonic acids, and their evaluation as potential inhibitors of PAL both in vitro and in vivo.

2. Results and discussion

2.1. Synthesis

Our main effort was directed towards the synthesis of a wide spectrum of phosphonic and phosphinic analogues of α-phenylglycine and testing them as potential inhibitors of phenylalanine ammonia-lyase. To achieve this, we relied on various reports in the literature on the synthesis of ring-substituted 1-aminobenzylphosphonic and -phosphinic acids.

We have obtained a set of 1-aminobenzylphosphonic acids (**1**, **3**–**25**) using three synthetic routes: (i) direct

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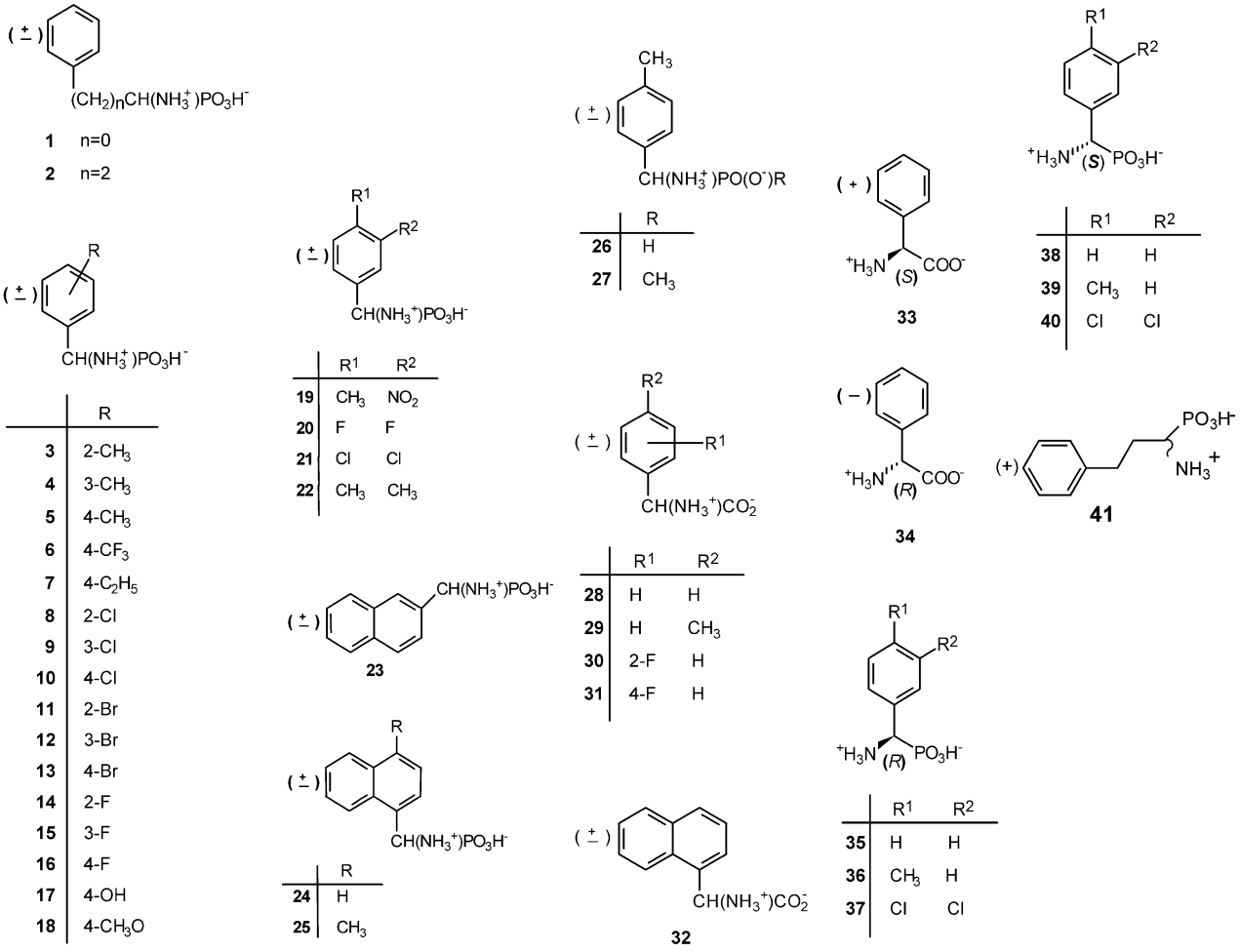

Fig. 1. Structures of the compounds tested **1**–**41**.

phosphonylo-amidation of aldehydes (Oleksyszyn, 1987; Soroka, 1990), (ii) phosphonylation of substituted benzylidenebisacetamides (Soroka et al., 1990), and (iii) hydrophosphonylation of substituted benzylidenebenzhydrylamines (Green et al., 1994), starting from ring-substituted benzaldehydes. The three methods (A–C as described in the Experimental) differed in their effectiveness, depending on the structure of the respective substituted benzaldehydes (Table 1 and Fig. 1). However, the most convenient route for the synthesis of substituted 1-aminobenzylphosphonic acids was found to be that proceeding via the benzylidenebenzhydrylamines (method C). So far, we have found only a single substituted benzaldehyde that did not provide the corresponding aminophosphonic acid via this method, i.e. anthracene-9-carboxaldehyde (not described in this paper).

Racemic 1-amino-3-phenylpropylphosphonic acid (**2**) was synthesised by reduction of the corresponding oxime (method D). Racemic 1-amino-4′-methylbenzylphosphonous acid (**26**) was obtained as described previously from *p*-toluidenebisacetamide and hypophosphorous acid (method D). Racemic 1-amino-4′-methylbenzyl(methyl)-phosphinic acid (**27**) was obtained from *p*-toluidenebisacetamide and methyldichlorophosphine in acetic acid according to the published procedure (method D).

Racemic ring-substituted phenylglycines (**29** and **32**) were obtained by the Strecker synthesis (method E) and were used as reference compounds.

Optically active 1-aminobenzylphosphonic acids (**35**–**40**) and (+)-1-amino-3-phenylpropylphosphonic acid (**41**) were obtained by resolution of the diastereomeric amides of the corresponding diphenyl (±)-1-aminoalkylphosphonates and (−)-*O*,*O*′-dibenzoyl-*l*-tartaric acid according to known procedure (method F) and their enantiomeric purity (⩾95% ee) was estimated by $^{31}P\{^{1}H\}$ NMR of their corresponding diastereomeric amides (Kafarski et al., 1983).

The yields (Table 1) of the above mentioned amino acids (**1**–**27**, **29**, **32** and **35**–**41**) are given for analytically pure samples, and such samples were employed in the biological tests described below.

2.2. Inhibitory activity

The compounds **1**–**41** (Fig. 1) were evaluated as inhibitors of phenylalanine ammonia-lyase both in vitro

Table 1
Analytical data of the compounds **1**–**41**

Compound	Method of synthesis	Yield[a] (%)	M.p. (°C)	IR ν_{max}(KBr) (cm^{-1})	NMR (D_2O–DCl[b] or D_2O–D_2SO_4[c]) δ, (ppm)
1	C	26	274–277[d]	3132, 1621, 1539, 1270, 1213, 1080, 915	^1H (300 MHz)[b]: 4.77 (1 H, *d*, J_{HP} = 17.0 Hz, NC*H*P) and 7.52 (5 H, *bs*, C_6H_5); $^{31}P\{^1H\}$ (121 MHz)[b]: 13.86 (*s*)
2	D	24	285–287[d]	3027, 1603, 1533, 1249, 1179, 1033, 1017, 929	^1H (100 MHz)[b]: 2.40–3.00 (2 H, *m*, C_6CCH_2CN), 3.25–3.45 (2 H, *m*, $NCCCH_2C_6$), 4.00–4.35 (1 H, *m*, NC*H*P), 7.8 (5 H, *bs*, C_6H_5)
3	A	25	264–267[d]	3130, 1201, 1073, 909	^1H (100 MHz)[b]: 2.60 (3 H, *s*, C_6CH_3), 5.25 (1 H, *d*, J_{HP} = 17.0 Hz, NC*H*P) and 7.86 (4 H, *bs*, C_6H_4)
4	A	32	286–289[d]	3124, 1256, 1084, 912	^1H (100 MHz)[c]: 2.77 (3 H, *s*, C_6CH_3), 5.20 (1 H, *d*, J_{HP} = 18.0 Hz, NC*H*P), 7.66 (4 H, *bs*, C_6H_4)
5	A	25	254–256[d]	3139, 1205, 1081, 914	^1H (300 MHz)[b]: 2.52 (3 H, *s*, C_6CH_3), 5.03 (1 H, *d*, J_{HP} = 17.0 Hz, NC*H*P), 7.86 (4 H, *bs*, C_6H_4)
6	B	10	277–280[e]	3150, 1210, 1170, 1135, 1070, 910	^1H (300 MHz)[c]: 4.79 (1 H, *d*, J_{HP} = 17.2 Hz, NC*H*P), 7.68 (2 H, *d*, J_{HH} = 7.9 Hz, C_6H_2), 7.84 (2 H, *d*, J_{HH} = 8.1 Hz, C_6H_2); $^{31}P\{^1H\}$ (121 MHz)[c]: 12.31 (*s*)
7	B	11	273–277[e]	3140, 1620, 1533, 1250, 1085	^1H (300 MHz)[c]: 1.19 (3 H, *t*, J_{HH} = 6.5 Hz, CH_3C), 2.68 (2 H, *q*, J_{HH} = 7.5 Hz, CCH_2), 4.68 (1 H, *d*, J_{HP} = 18.0 Hz, NC*H*P), 7.35–7.45 (4 H, *m*., C_6H_4)
8	B	12	247–249[d]	3226, 1260, 1198, 1085, 905	^1H (80 MHz)[c]: 4.9 (1H, *d*, J_{HP} = 17.0 Hz, NC*H*P), 7.1–7.7 (4H, *m*, C_6H_4)
9	B	9	271–274[d]	3421, 1272, 1195, 1087, 914	^1H (300 MHz)[c]: 4.67 (1H, *d*, J_{HP} = 17.0 Hz, NC*H*P), 7.35–7.57 (4H, *m*, C_6H_4); $^{31}P\{^1H\}$ (121 MHz)[c]: 12.67 (s)
10	C	27	265–268[d]	3660, 3320, 1258, 1177, 1090, 920	^1H (300 MHz)[c]: 4.87 (1H, *d*, J_{HP} = 17.5 Hz, NC*H*P), 7.38–7.60 (4H, *m*, C_6H_4); $^{31}P\{^1H\}$ (121 MHz)[c]: 11.80 (*s*)
11	B	30	246–248[d]	3606, 3310, 1248, 1172, 1035, 933	^1H (100 MHz)[c]: 5.72 (1H, *d*, J_{HP} = 17 Hz, NC*H*P), 7.60–8.21 (4H, *m*., C_6H_4)
12	B	20	274–276[e]	3113, 1268, 1180, 1071, 900	^1H (100 MHz)[c]: 5.30 (1H, *d*, J_{HP} = 18.0 Hz, NC*H*P), 7.70–8.28 (4H, *m*., C_6H_4)
13	A	28	290–296[d]	3660, 3340, 1227, 1085, 917	^1H (60 MHz)[c]: 5.32 (1 H, *d*, J_{HP} = 17.0 Hz, NC*H*P), 7.67–8.33 (4 H, *m*., C_6H_4)
14	B	23	257–260[d]	3130, 1273, 1200, 1076, 925	^1H (300 MHz)[c]: 4.99 (1H, *d*, J_{HP} = 17.6 Hz, NC*H*P), 7.23–7.39 (2H, *m*, C_6H_4), 7.48–7.62 (2H, *m*, C_6H_4); $^{31}P\{^1H\}$ (121 MHz)[c]: 12.33 (*d*, J_{FP} = 3.8 Hz)
15	B	21	275–278[d]	3113, 1260, 1200, 1150, 1085, 990	^1H (300 MHz)[c]: 4.83 (1H, *d*, J_{HP} = 17.4 Hz, NC*H*P), 7.20–7.40 (3H, *m*, C_6H_4), 7.45–7.60 (1H, *m*, C_6H_4); $^{31}P\{^1H\}$ (121 MHz)[c]: 12.00 (*s*)
16	B	22	273–275[d]	3590, 3440, 1270, 1185, 1105, 1093, 1035, 980	^1H (300 MHz)[c]: 4.84 (1H, *d*, J_{HP} = 17.3 Hz, NC*H*P), 7.20–7.32 (3H, *m*, C_6H_4), 7.48–7.57 (1H, *m*, C_6H_4); $^{31}P\{^1H\}$ (121 MHz)[c]: 12.49 (*s*)

(*continued on next page*)

Table 1 (*continued*)

Compound	Method of synthesis	Yield[a] (%)	M.p. (°C)	IR ν_{max}(KBr) (cm^{-1})	NMR (D_2O–DCl[b] or D_2O–D_2SO_4[c]) δ, (ppm)
17	A	10	>350[d]	3130, 1245, 1170,1030, 953	^1H (60 MHz)[c]: 5.23 (1 H, *d*, J_{HP} = 17.0 Hz, NC*H*P), 7.40 (2 H, *d*, J_{HH} = 8.0 Hz, C_6H_2), 7.83 (2 H, *d*, J_{HH} = 8.0 Hz, C_6H_2)
18	A	45	242–245[d]	3570, 3380, 1255, 1180, 972	^1H (60 MHz)[c]: 4.18 (3 H, *s*, OCH_3), 5.20 (1 H, *d*, J_{HP} = 17.0 Hz, NC*H*P), 7.41 (2 H, *d*, J_{HH} = 8.0 Hz, C_6H_2), 7.85 (2 H, *d*, J_{HH} = 8.0 Hz, C_6H_2)
19	B	31	256–259[e]	3429, 3169, 1257, 1202, 1078, 933	^1H (100 MHz)[c]: 2.96 (3H, *s*, CH_3), 5.37 (1H, *d*, J_{HP} = 18.0 Hz, NC*H*P), 7.82–8.22 (2H, *m*, C_6H_3), 8.63 (1H, *s*, C_6H_3)
20	B	16	286–289[e]	3420, 3126, 1278, 1204, 1084, 921	^1H (300 MHz)[c] 4.68 (1H, *d*, J_{HP} = 16.9 Hz, NC*H*P), 7.25–7.50 (3H, *m*, C_6H_3)); $^{31}P\{^1H\}$ (121 MHz)[c]: 12.48 (*d*, J_{FP} = 3.9 Hz)
21	B	15	275–278[e]	3423, 1241, 1210, 1056, 942	^1H (300 MHz)[c]: 4.87 (1H, *d*, J_{HP} = 17.7 Hz, NC*H*P), 7.38–747 (1H, *m*, C_6H_3), 7.62–7.72 (2H, *m*, C_6H_3); $^{31}P\{^1H\}$ (121 MHz)[c]: 11.06 (*s*)
22	C	34	276–279[e]	3140, 1623, 1538, 1272, 1203, 1084, 910	^1H (300 MHz)[c]: 2.26 and 2.28 (6H, two overlapping singlets, 3′,4′-$(CH_3)_2C_6H_3$), 4.89 (1H, *d*, J_{HP} = 17.4 Hz, NC*H*P), 7.18–7.30 (3H, *m*, C_6H_3); $^{31}P\{^1H\}$ (121 MHz)[c]: 12.45 (*s*)
23	A	35	253–255[d]	3460, 3270, 1255, 1177, 1158, 1087, 913	^1H (300 MHz)[c]: 4.92 (1 H, *d*, J_{HP} = 17.0 Hz, NC*H*P), 7.50–7.75 (3 H, *m*, $C_{10}H_7$), 7.85–8.10 (4 H, *m*, $C_{10}H_7$)
24	A	13	235–238[d]	3425, 3240, 1264, 1197, 1183, 1065, 924	^1H (300 MHz)[c]: 5.80 (1 H, d, J_{HP} = 17.6 Hz, NC*H*P), 7.60–7.70 (4 H, m, $C_{10}H_7$), 8.00–8.15 (3 H, m, $C_{10}H_7$)
25	B	9	244–248[e]	3432, 1222, 1199, 1087, 943	^1H (300 MHz)[c]: 2.68 (3H, *s*, CH_3), 5.80 (1H, *d*, J_{HP} = 17.5 Hz, NC*H*P), 7.38–7.83 (4H, *m*, $C_{10}H_6$), 8.10–8.45 (2H, *m*, $C_{10}H_6$); $^{31}P\{^1H\}$ (121 MHz)[c]: 12.79 (*s*)
26	D	20	238–241[d]	2920, 2305, 1640, 1235, 1195, 1183, 1065, 952	^1H (300 MHz)[b]: 2.23 (3H, *s*, CH_3), 4.54 (1H, *d*, J_{HP} = 14.0 Hz, NC*H*P), 7.25 (4H, *bs*, C_6H_4); $^{31}P\{^1H\}$ (121 MHz)[b]: 22.43 (*t*, J_{PD} = 88.5 Hz) and small singlet for the nondeuteriated compounds 22.84 (*s*)
27	D	33	267–269[e]	2858, 2305, 1634, 1231, 1208, 1184, 1094, 1062, 885	^1H (300 MHz)[b]: 1.74 (3H,*d*, J_{HP} = 14.4 Hz, PCH_3), 2.48 (3H, *s*, C_6CH_3), 5.08 (1H, *d*, J_{HP} = 12.0 Hz, NC*H*P), 7.14–7.30 (4H, *m*, C_6H_4); $^{31}P\{^1H\}$ (121 MHz)[b]: 45.20 (*s*)
28	[f]				
29	E	9	247–250[d]	2980, 1585, 1520, 1357	^1H (300 MHz)[b]: 1.59 (3 H, *s*, C_6CH_3), 4.46 (1 H, *s*, NC*H*CO), 6.58 (2 H, *d*, J_{HH} = 8.3 Hz, C_6H_4), 6.63 (2 H, *d*, J_{HH} = 8.3 Hz, C_6H_4).
30	[f]				
31	[f]				
32	E	8	206–208[d]	2934, 1675, 1566, 1512, 1373	^1H (300 MHz)[b]: 5.89 (1H, *s*, NC*H*CO), 7.33–7.58 (4H, *m*, $C_{10}H_7$), 7.58–7.78 (2H, *m*, $C_{10}H_7$), 7.99 (1H, *d*, J_{HH} = 8.0 Hz, $C_{10}H_7$)
33	[f]				
34	[f]				

(*continued on next page*)

Table 1 (*continued*)

Compound	Method of synthesis	Yield[a] (%)	M.p. (°C)	IR ν_{max}(KBr) (cm^{-1})	NMR (D_2O–DCl[b] or D_2O–D_2SO_4[c]) δ, (ppm)
35[g]	F	5	280–285[c]	3132, 1621, 1539, 1270, 1213, 1080, 915	^1H (300 MHz)[b]: 4.70 (1 H, *d*, J_{HP} = 17.0 Hz, NC*H*P) and 7.50 (5 H, *bs*, C_6H_5); $^{31}P\{^1H\}$ (121 MHz)[b]: 13.76 (*s*)
36[g]	F	10	275–278	3139, 1205, 1081, 914	^1H (300 MHz)[b]: 2.52 (3 H, *s*, C_6CH_3), 5.03 (1 H, *d*, J_{HP} = 17.0 Hz, NC*H*P), 7.86 (4 H, *bs*, C_6H_4)
37[g]	F	5	265–267	3423, 1241, 1210, 1056, 942	^1H (300 MHz)[c]: 4.87 (1H, *d*, J_{HP} = 17.7 Hz, NC*H*P), 7.38–747 (1H, *m*, C_6H_3), 7.62–7.72 (2H, *m*, C_6H_3); $^{31}P\{^1H\}$ (121 MHz)[c]: 11.06 (*s*)
38[g]	F	10	280–283[d]	3132, 1621, 1539, 1270, 1213, 1080, 915	^1H (300 MHz)[b]: 4.70 (1 H, *d*, J_{HP} = 17.0 Hz, NC*H*P) and 7.50 (5 H, *bs*, C_6H_5); $^{31}P\{^1H\}$ (121 MHz)[b]: 13.76 (*s*)
39[g]	F	12	272–276	3139, 1205, 1081, 914	^1H (300 MHz)[b]: 2.52 (3 H, *s*, C_6CH_3), 5.03 (1 H, *d*, J_{HP} = 17.0 Hz, NC*H*P), 7.86 (4 H, *bs*, C_6H_4)
40[g]	F	4	255–257	3423, 1241, 1210, 1056, 942	^1H (300 MHz)[c]: 4.87 (1H, *d*, J_{HP} = 17.7 Hz, NC*H*P), 7.38–747 (1H, *m*, C_6H_3), 7.62–7.72 (2H, *m*, C_6H_3); $^{31}P\{^1H\}$ (121 MHz)[c]: 11.06 (*s*)
41[g]	F	9	273–277	3027, 1603, 1533, 1249, 1179, 1033, 1017, 929	^1H (100 MHz)[b]: 2.40–3.00 (2 H, *m*, C_6CCH_2CN), 3.25–3.45 (2 H, *m*, $NCCCH_2C_6$), 4.00–4.35 (1 H, *m*, NC*H*P), 7.8 (5 H, *bs*, C_6H_5)

[a] Yield was calculated for an analytically pure sample; for methods A, B, C, and E based on the aldehyde, for method D based on the carboxylic acid or the aldehyde, for method F based on the diphenyl (±)-1-aminoalkylphosphonate.

[b] NMR solvents.

[c] NMR solvents.

[d] Literature melting points: **1**, 285–286 °C (Green et al., 1994); **2**, 286–287 °C (Kafarski et al., 1995); **3**, 228–230 °C (Kafarski et al., 1995); **4**, 286–289 °C (Gancarz and Wieczorek, 1977); **5**, 254–256 °C (Łukszo and Tyka, 1977); **8**, 229–231 °C (Kafarski et al., 1995); **9**, 235–236 °C (Kafarski et al., 1995); **10**, 279–281 °C (Oleksyszyn and Gruszecka, 1981); **11**, 222–223 °C (Kafarski et al., 1995); **13**, 276–279 °C (Green et al., 1994); **14**, 256–258 °C (Kafarski et al., 1995); **15**, 279–280 °C (Kafarski et al., 1995); **16**, >300 °C (Maier and Diel, 1991); **17**, decomposition (Soroka et al., 1990); **18**, 286–288 °C (Green et al., 1994); **23**, 298–302 °C (Łukszo and Tyka, 1977); **24**, 249–251 °C (Soroka et al., 1990); **26**, 237–238 °C (Tyka and Hägele, 1989); **29**, 260–265 °C (Doyle et al., 1962); **32**, 199–201 °C (Bretscheider et al., 1988); **35**, 280–282 °C (Kafarski et al., 1983); **38**, 278–279 °C (Kafarski et al., 1983).

[e] Elemental analysis for new compounds: **6**, found N 5.40, P 12.00, $C_8H_9F_3NO_3P$ requires N 5.49, P 12.14; **7**, found N 6.43, P 14.25, $C_9H_{14}NO_3P$ requires N 6.51, P 14.39; **12**, found Br 29.95, N 5.22, P 11.78, $C_7H_9BrNO_3P$ requires Br 30.03, N 5.27, P 11.64; **19**, found N 11.23, P 12.97, $C_8H_{11}N_2O_5P$ requires N 11.38, P 12.58; **20**, found N 6.30, P 13.68, $C_7H_8F_2NO_3P$ requires N 6.28, P 13.88; **21**, found Cl 27.44, N 5.25, P 12.40, $C_7H_8Cl_2NO_3P$ requires Cl 27.70, N 5.47, P 12.10; **22**, found N 6.80, P 14.60, $C_9H_{14}NO_3P$ requires N 6.51, P 14.39; **25**, found N 5.40, P 12.20, $C_{12}H_{14}NO_3P$ requires N 5.58, P 12.33; **27**, found N 6.83, P 15.70, $C_9H_{14}NO_2P$ requires N 7.03, P 15.55; **36**, found N 6.90, P 15.31, $C_8H_{12}NO_3P$ requires N 6.96, P 15.40; **37**, found Cl 27.44, N 5.25, P 12.40, $C_7H_8Cl_2NO_3P$ requires Cl 27.70, N 5.47, P 12.10; **39**, found N 6.85, P 15.27, $C_8H_{12}NO_3P$ requires N 6.96, P 15.40; **40**, found Cl 27.51, N 5.28, P 12.31, $C_7H_8Cl_2NO_3P$ requires Cl 27.70, N 5.47, P 12.10; **41**, found N 6.43, P 14.30, $C_9H_{14}NO_3P$ requires N 6.51, P 14.39.

[f] Commercial products.

[g] Determined specific rotation: **35**, $[\alpha]_{578}^{25}$ + 18.0±1 ° (*c* 1, 1 M NaOH) (literature $[\alpha]_{578}^{20}$ + 19±1 ° (*c* 2, 1 M NaOH); Kafarski et al., 1983); **38**, $[\alpha]_{578}^{25}$ −21.5±1 ° (*c* 1, 1 M NaOH) (literature $[\alpha]_{578}^{20}$ −20±1 ° (*c* 2, 2 M NaOH); Kafarski et al., 1983); for new compounds **36**, **37**, **39**, **40**, and **41** see Experimental.

and in vivo. The inhibition constants (K_i) of buckwheat PAL, and the respective concentrations of the compounds which inhibited the anthocyanin production in illuminated buckwheat hypocotyls by 50% (I_{50} values), were determined as described previously (Zoń and Amrhein, 1992).

Compounds **1** and **2** are homologues with shorter and longer side chains, respectively, of 1-amino-2-phenylethylphosphonic acid, a known inhibitor of PAL (Janas et al., 1985; Laber et al., 1986). Even though compound **2** was a somewhat more potent inhibitor than **1**, we pursued the synthesis of the series of compounds (**3**–**18**) related to 1-aminobenzylphosphonic acid (**1**), because of their lower conformational flexibility. Racemic monosubstituted 1-aminobenzylphosphonic acids (**3**–**18**) containing the following groups: chloro, bromo, fluoro, methyl, trifluoromethyl, ethyl, hydroxy, and methoxy in *ortho*, *meta* and *para* position, respectively, were synthesised

(Fig. 1). Compounds **10** and **5** (chloro or methyl in *para* position) exhibited the highest inhibitory activities in this series of racemic monosubstituted 1-aminobenzylphosphonic acids (Table 2). For compound **10** it was established that, at 100 μM concentration, it causes a 21-fold increase in the endogenous concentration of soluble phenylalanine over a period of 24 h in illuminated buckwheat hypocoptyls (data not shown). This provides additional evidence that PAL is, indeed, an in vivo target of the inhibitor, as was previously shown for AIP (Zoń and Amrhein, 1992). Introducing the chloro or methyl group at the *meta* position (compounds **9** and **4**, respectively) affected the inhibitory activity only slightly (Table 2). In contrast, introduction of the same substituents at the *ortho* position significantly reduced the inhibitory activity (compounds **8** and **3**, Table 2). Racemic 1-amino-3′,4′-dichlorobenzylphosphonic acid (**21**) was identified as the most potent inhibitor both in vitro and in vivo in this subclass consisting of racemic mono- and disubstituted 1-aminobenzylphosphonic acids (Table 2). We obtained and evaluated separately dextrorotatory (**37**) and laevorotatory (**40**) 1-amino-3′,4′-dichlorobenzylphosphonic acid. The enantiomer **37** was a much more potent inhibitor of PAL in vitro and in vivo than its laevorotatory counterpart (Table 2). We propose the *R* configuration (corresponding to the natural *L*-configuration) for **37**, based on a stronger inhibitory activity of (*R*)-(+)-1-aminobenzylphosphonic acid (**35**) as compared to the enantiomer **38** (Głowiak et al., 1977). With the same argument, we propose *R*-configuration for dextrorotatory 1-amino-4′-methylbenzylphosphonic acid (**36**). Consequently, the *S*-configuration is proposed for the compounds **39** and **40**.

Table 2
Evaluation of compounds **1**–**41** as inhibitors of phenylalanine ammonia-lyase and anthocyanin synthesis

Compound	K_i PAL (μM)	I_{50} anthocyanin synthesis (μM)
1	6.5	250
2	5.0	110
3	8.5	4860
4	1.5	23
5	0.25	30
6	8.3	240
7	34	>1000
8	*a*	<1000
9	2.0	10
10	0.21	10
11	5.3	1710
12	1.0	15
13	0.29	22
14	*a*	*b*
15	3.3	67
16	1.8	25
17	*a*	*b*
18	40	>1000
19	4.0	82
20	1.0	16
21	0.21	5
22	0.89	24
23	3.7	220
24	0.30	140
25	7.5	*b*
26	*a*	*b*
27	25	550
28	9.0	<5000
29	0.24	640
30	*a*	*b*
31	1.3	1500
32	1.0	3000
33	2.3	3300
34	300	>5000
35	3.4	53
36	0.17	15
37	0.08	2.2
38	*a*	*b*
39	*a*	*b*
40	3.1	965
41	10	60

a = Less than 50% inhibition at 1 mM concentration. *b* = Less than 40% inhibition of light-induced anthocyanin synthesis in buckwheat hypocotyls at 1 mM concentration.

Incidentally, we did not observe a significant difference in the inhibitory activities of dextroratatory (**41**) and racemic (**2**) 1-amino-3-phenylpropylphosphonic acids, respectively (Table 2). The latter compound **2** was reported to inhibit potato phenylalanine ammonia-lyase and anthocyanin synthesis in buckwheat hypocotyls (Janas and Olechowicz, 1994).

A comprehensive study of the inhibitory activities of substituted analogues of 1-amino-2-phenylethylphosphonic acid (the phosphonic acid analogue of phenylalanine), with a small substituent like fluorine in the benzene ring at any position, revealed that such a substituent lowers the inhibitory activity only insignificantly. This is not surprising considering the atomic radius of fluorine. Any other substituents at *para* position, however, substantially diminish the inhibitory activity (Maier and Diel, 1994).

It is worth mentioning that compounds **23**–**25** with the aminomethylphosphonic moiety adjacent to the naphthalene rather than the benzene scaffold had lower inhibitory activities (Table 2). This was particularly evident for the in vivo inhibition (I_{50} values), possibly reflecting poor uptake of these compounds. Neither phenylglycine nor phosphinic analogues of phenylglycine were suitable compounds in the search for PAL inhibitors (Table 2: **28**, **29** and **31**, as well as **26** and **27**).

2.3. Structure–activity relationship studies

In this study, two different biological activity factors have been analysed, i.e. the inhibition constant (K_i) of buckwheat PAL ("in vitro" activity), and the I_{50} value for the inhibition of anthocyanin synthesis ("in vivo" activity). In the latter assay, anthocyanins in illuminated buckwheat hypocotyls represent one of the ultimate products of phenylpropanoid metabolism, and in vivo inhibition of PAL will, therefore, lead to a reduction of

anthocyanin accumulation. In this test, other factors, such as differential uptake and metabolism of an inhibitor, will also contribute to its biological activity. Furthermore, targets other than PAL may be affected, and one cannot a priori expect a correlation between the in vitro and in vivo activities, respectively, of a set of inhibitors. In fact, the plot shown in Fig. 2 reveals, that K_i and I_{50} values are not strongly correlated for all data points. There are, however, clusters of points, which can be interpreted as follows. The aminocarboxylic acids (filled squares), the aminophosphonic acids with substituents longer than one carbon atom at *para* position (open squares), the aminophosphonic acids with substituents in *ortho* and *meta* position other than a fused benzene ring (asterisks), are very distinct from each other and also from the rest of the aminophosphonic acids (filled circles). Analysis of the outliners from the best correlation line allows to formulate the following conclusions: (i) the main factor influencing the activity is just the presence of a aminomethylphosphonic group attached to benzene ring, (ii) the biological activity drops when a substituent longer than one carbon atom is attached at position *para* in benzene ring, which can suggest that the PAL active site is not very deep, (iii) the activity drops when substituent at position *ortho* is other than fused benzene ring, which might indicate that in this region the pocket is relatively flat.

We have also analysed the correlation between each of the biological responses and electronic, steric and lipophilic parameters of the substituents (Table 3). The correlation is very small, and correlation factors did not exceed a value of 0.27, meaning that there was no correlation at all.

3. Conclusions

We have found that (+)-1-amino-3′,4′-dichlorobenzylphosphonic acid (**37**) is quite a strong inhibitor of phenylalanine ammonia-lyase both in vitro and in vivo. Its absolute configuration is proposed to be *R*. This conformationally more flexible compound **37** and the conformationally locked 2-aminoindan-2-phosphonic acid ($K_i = 0.08$ μM; $I_{50} = 1.5$ μM) (Zoń and Amrhein, 1992) are of comparable potency.

Presently, little information is available on the structure of the active site of PAL, but both site-directed mutagenesis (Schuster and Rétey, 1994; Langer et al., 1997) and comparison with the recently determined three-dimensional structure of histidine ammonia-lyase (Schwede et al., 1999) will advance our understanding of this important enzyme of higher plants and provide the basis for an understanding of the action of the PAL inhibitor.

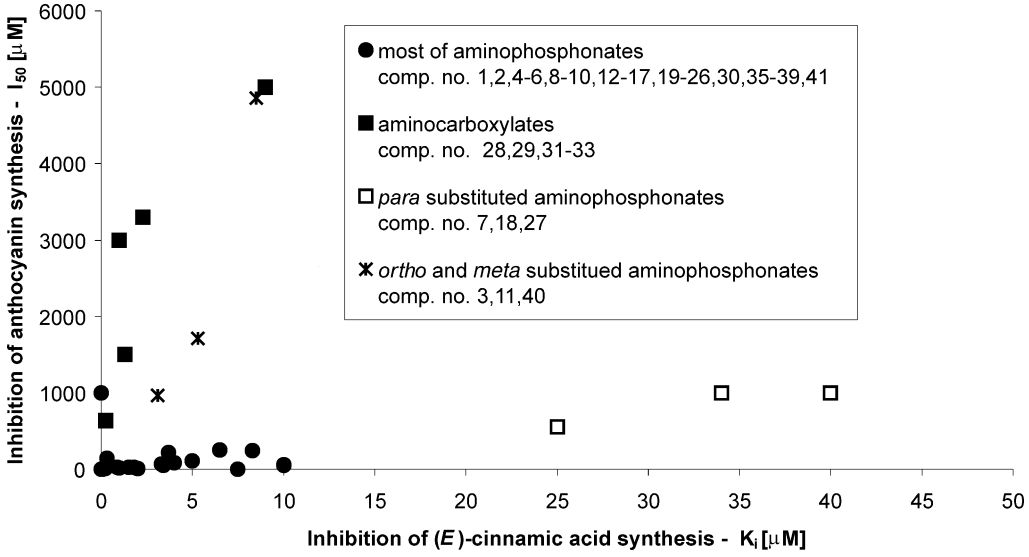

Fig. 2. Correlation analysis of potency of compounds to inhibit PAL in vitro (K_i) and anthocyanin synthesis in vivo (I_{50}).

Table 3
Correlation coefficients[a] between structural parameters and biological activity of evaluated PAL inhibitors

Biological test	Electronic parameters (σ)	Steric parameters (E_s)	Lipophilic parameters (π)
Antocyanin synthesis reduce (I_{50})	−0.0215	0.2684	0.1494
Inhibition constant (K_i)	0.0624	0.2007	0.0981

[a] Correlation coefficients given in the Table 3 were calculated using statistical package STATGRAPH 6.0 and are significant at the confidence level $P = 0.05$.

On the other hand, our inhibitor studies may allow the design of inhibitors containing a photoreactive group, and such compounds may then serve as probes into the active site of PAL.

4. Experimental

^{1}H NMR spectra were recorded at 100 MHz with a Tesla BS 497, at 80 MHz with a Tesla BS 587A, and at 300 MHz with a Bruker DRX 300 instrument. ^{31}P{^{1}H} NMR spectra with broad band proton decoupling were recorded at 121 MHz with a Bruker DRX 300 instrument. IR spectra were recorded with a Perkin Elmer 2000 FT–IR spectrometer. Optical rotations were determined on an Optical Activity LTD Automatic polarimeter using 5 cm cell. GC analyses were performed on a Perkin Elmer F-11 chromatograph. GC–MS analyses were performed on a Hewlett Packard 5890 series II chromatograph with mass selective detector 5971A. Melting points were determinated using Boëtius apparatus and are uncorrected. Elemental analyses were performed by Laboratory of Elemental Analysis of Institute Organic Chemistry, Biochemistry and Biotechnology, Wrocław. TLC was carried out on commercially available pre-coated plates (Merck Kieselgel 60). THF was freshly distilled from lithium aluminium hydride. The following commercially available amino acids were tested: (±)-phenylglycine (**28**) (Sigma), (±)-2-amino-2′-fluorophenylacetic acid (**30**) (Fluka), (±)-2-amino-4′-fluorophenylacetic acid (**31**) (Fluka), (*S*)-(+)-phenylglycine (**33**) (Fluka) and (*R*)-(−)-phenylglycine (**34**) (Sigma). All other amino acids were synthesised by one of the method described below (method A, B, C, D, E, and F). After synthesis, the respective aminophosphonic acid was recrystallised from water in the following manner. It was dissolved in a minimum volume of boiling water, the solution treated with charcoal, filtered and filtrate was concentrated under atmospheric pressure to about one thirds of starting volume. The crystals of aminophosphonic acid was filtered and dried on air. The yield, melting point, elemental analysis, IR selected band, ^{1}H NMR spectra and inhibitory activity were determined (Table 1). The inhibition constants (K_i) of buckwheat PAL, and the respective concentrations of the compounds which inhibited the anthocyanin production in illuminated buckwheat hypocotyls by 50% (I_{50} values), were determined as described previously (Zoń and Amrhein, 1992). Standard deviations for K_i values were in the range of 5–8%, for I_{50} values in the range of 10–15%. Assays for compounds with $I_{50} > 1000$ μM were performed only once.

1-Methyl-4-naphthaldehyde was obtained from commercially available 1-methylnaphthalene and α,α-dichloromethyl methyl ether in presence of titanium (IV) chloride according to Rieche et al. (1960) procedure. Yield: 21.92 g; 56%; bp 132–134 °C (2 mm Hg). GC analysis shows 94% of 1-methyl-4-naphthaldehyde (retention time 7.9 min) and 6% of 1-methylnaphthalene (retention time 1.1 min on column filled with 2% DEGS on Chromosorb G, 1 m, 180 °C). ^{1}H NMR (60 MHz, CCl_4, $Me_3SiOSiMe_3$ external): δ 2.67 (3 H, *s*, CH_3), 7.20–8.08 (5 H, *m*, C$_{10}H_6$), 9.23 (1 H, *d*, $J = 9$ Hz, C$_{10}H_6$), 10.13 (1H, *s*, C*H*O). The aldehyde was used to obtain compound **25** (method B).

3,4-Dimethylbenzaldehyde was obtained from commercially available 3,4-dimethylbenzoic acid in a three-step synthesis. 3,4-Dimethylbenzoic acid was esterified by methanol-dry hydrochloride yielding corresponding methyl 3,4-dimethylbenzoate. To the suspension of lithium aluminium hydride (2.00 g, 0.0527 mol) in dry THF (45 ml) a solution of methyl 3,4-dimethylbenzoate (6.86 g, 0.0418 mol) in dry THF (15 ml) was added dropwise under stirring and under nitrogen. The mixture was refluxed for 3 h. Then, second portion of lithium aluminium hydride (0.543 g, 0.01431 mol) was added and the mixture was refluxed for an additional 6 h. After that time water (1.8 ml) was carefully added under stirring to the cold reaction mixture (0–5 °C), and then 10% sulfuric acid (19.5 ml) was added dropwise. After filtration, the solid residue was extracted with methylene chloride (50 ml). The organic extract and the filtrate were combined, dried with anhydrous potassium carbonate and the solvents were evaporated under reduced pressure. The residue (5.319 g) was dissolved in boiling hexane (20 ml) and let to stand to crystallise, yielding 3,4-dimethylbenzyl alcohol: 4.515 g; 79%; mp 58–61 °C. Elemental analysis: found C 79.40, H 8.90, $C_9H_{12}O$ requires C 79.37, H 8.88. In the subsequent step 3,4-dimethylbenzyl alcohol (1.000 g, 0.00734 mol) was stirred at room temperature for 90 h in dry methylene chloride (30 ml) with manganese (IV) oxide (5.00 g). The reaction was monitored by TLC: hexane-diethyl ether (4:1 v/v), iodine, R_f for the aldehyde 0.35 and for the alcohol 0.09. After filtration out manganese (IV) oxide the methylene chloride was evaporated providing crude 3,4-dimethylbenzaldehyde (0.8774 g, 89%) sufficiently pure for the next reaction. ^{1}H NMR (300 MHz, $CDCl_3$): δ 2.28 and 2.25 (6H, two overlapping singlets, CH_3 and CH_3), 7.18–7.25 (1H, *m*, C$_6H_3$), 7.51–7.58 (2H, *m*, C$_6H_3$), 9.87 (1H, *s*, C*H*O). IR ν_{max}^{film} cm^{-1}: 1694 (C=O). 3,4-Dimethylbenzaldehyde spontaneously oxidised to the corresponding acid even in a freezer. So fresh 3,4-dimethylbenzaldehyde (0.7603 g, 0.00566 mol) and benzhydrylamine (1.0386 g, 0.00566 mol) were dissolved in methylene chloride (20 ml) and left for 2 h. Then the solution was dried with anhydrous potassium carbonate (0.6 g), and was evaporated to get solid 3,4-(dimethylbenzylidene)bezhydrylamine: 1.4039 g; 83%; mp 72–73 °C. The last compound was used to obtain (±)-1-amino-3′,4′-dimethylbenzylphosphonic acid (**22**) (method C).

4.1. Synthesis of racemic 1-aminobenzylphosphonic acids from the corresponding substituted benzaldehyde, acetamide, acetyl chloride and phosphorous(III) chloride according to Oleksyszyn (1987) and Soroka (1990) procedures

A solution of substituted benzaldehyde (0.02 mol) and acetamide (2.36 g, 0.04 mol) in glacial acetic acid (10 ml) was cooled on a water-ice bath. At a temperature below 15 °C acetyl chloride (1.42 ml, 0.02 mol) was added dropwise while stirring. The reaction mixture was stirred for 3 h on the bath, then phosphorus (III) chloride (1.74 ml, 0.02 mol) was added dropwise while keeping the temperature below 15 °C. Then mixture was gradually heated to 60 °C during 1 h and then to 90 °C during the next 2 h. Volatile by-products were evaporated under reduced pressure. The residue was refluxed by 10 h with concentrated hydrochloric acid (20 ml) and water (20 ml). Tarry semisolid or oily impurities were separated by decantation or extraction with chloroform. An acidic solution was decolourised by a charcoal. Then evaporated under reduced pressure, and the residue was treated with methanol (10 ml). The insoluble ammonia chloride was filtered off and the filtrate was treated with propylene oxide (1.40 ml, 0.02 mol) which yielded a crude crystalline product. The last one was recrystallised one or more time from water until analytically pure aminophosphonic acids was obtained (Table 1).

4.2. Synthesis of racemic 1-aminobenzylphosphonic acids from N,N′-benzylidenebisacetamides and phosphorous(III) chloride in acetic acid followed by acidic hydrolysis as described by Soroka et al. (1990)

A substituted benzaldehyde (0.06 mol), acetamide (7.01 g, 0.12 mol), glacial acetic acid (7 ml) and acetic anhydride (7 ml) were refluxed for 3 h. Then, after cooling the mixture to room temperature, a crude bisacetamide was crystallised. The crystals were filtered off, washed twice with methanol and dried in open air to yield sufficiently pure derivatives of *N,N′*-(benzylidene)bisacetamides for the next reaction. To a solution of the crude derivatives of *N,N′*-(benzylidene)bisacetamide (0.02 mol) in glacial acetic acid (10 ml), phosphorus (III) chloride (1.74 ml, 0.02 mol) was added dropwise keeping temperature below 15 °C while stirring. Then the mixture was gradually heated up to 60 °C during 1 h and finally up to 90 °C within additional 2 h. Then the residue was worked up analogously as in the method A.

4.3. Synthesis of racemic 1-aminobenzylphosphonic acid by addition of diethyl phosphite to N-benzylidenebenzhydrylamine and subsequent acidic hydrolysis according to Green et al. (1994) procedure

A solution of benzaldehyde (0.05 mol) and diphenylmethylamine (8.61 ml, 0.05 mol) in methylene chloride (25 ml) was left for 3 h at room temperature, then was dried with anhydrous potassium carbonate (5 g) and a residue was treated with hexane. A resulted crude (benzylidene)diphenylmethylamine (0.02 mol) and diethyl phosphite (2.58 ml, 0.02 mol) were heated for 4 h at 100±3 °C to obtain a crude diethyl 1-diphenylmethylaminobenzylphosphonate. The crude product was treated with hexane. A solid diethyl 1-diphenylmethylaminobenzylphosphonate was filtered, and dried in open air. The solid diethyl 1-diphenylmethylaminobenzylphosphonate, were refluxed for 4 h with concentrated hydrochloric acid (20 ml) and water (20 ml). The hydrolysate was extracted with methylene chloride (2×15 ml), and the aqueous fraction was concentrated under reduced pressure. The solution was evaporated under reduced pressure, and residue was treated with methanol (20 ml), evaporated under reduced pressure and again dissolved in methanol (20 ml). The solution was treated with propylene oxide (1.40 ml, 0.02 mol) yielding a crude crystalline product. The last one was recrystallised one or more time from water until analytically pure aminophosphonic acids was obtained (Table 1).

4.4. Synthesis of racemic 1-aminoalkylphosphonic acids by miscellaneous procedures

(±)-1-Amino-3-phenylpropylphosphonic acid (**2**)

This was obtained from 3-phenylpropanoic acid in four steps. Thus, 3-phenylpropanoic acid was converted to 3-phenylpropanoyl chloride by phosphorous (III) chloride using standard method. Then, diisopropyl 1-hydroxyimino-3-phenylpropylphosphonate was obtained according to Zoń (1984) procedure starting from 3-phenylpropanoyl chloride (0.02 mol) yielding corresponding oxime: 3.0 g; 48%; mp 97–99 °C (hexane); ^{1}H NMR (60 MHz, $CDCl_3$, Me_4Si): δ 1.4 (12 H, *dd*, $J=2.5$ Hz, $J=7$ Hz, OCCH_3), 2.6–3.1 (4 H, *m*, CCH_2 CH_2C(=NOH)P), 4.83 (2 H, *dq*, $J=14$ Hz, $J=14$ Hz, OC*H*C), 7.2 (5 H, *bs*, C_6H_5) and 11.5 ppm (1 H, *s*, C=NO*H*). The oxime (3.0 g) was subjected to reduction with zinc in formic acid according to Kowalik et al. (1981) procedure to give the oxalate of diisopropyl (±)-1-amino-3-phenylpropylphosphonate: 2.2 g; 60%; mp 160–165 °C. The oxalate was hydrolysed by a refluxing mixture of conc HCl (20 ml) and water (20 ml) for 10 h. After standard work-up crude **2** (1.3 g) was isolated. The crude aminophosphonic acid was dissolved in water (800 ml), treated with charcoal and the solution was concentrated by evaporation under atmospheric pressure to a volume of about 150 ml. After crystallization (±)-1-amino-3-phenylpropylphosphonic acid (**2**) was collected by filtration and dried (Table 1).

(±)-1-Amino-4′-methylbenzylphosphonous acid (**26**) was obtained from *p*-tolualdehyde by Tyka and Hägele procedure (Tyka and Hägele, 1989).

(±)-1-Amino-4′-methylbenzyl(methyl)phosphinic acid (**27**) was obtained as follow. Methyldichlorophosphine (2.7 ml, 0.03 mol) was added dropwise to glacial acetic acid (20 ml) at room temperature while stirring. Stirring was continued for 30 min, then *N,N′*-(4-methylbenzylidene)bisacetamide (6.60 g, 0.03 mol) was added in a one portion. Within a few minutes the bisamide had dissolved. The reaction mixture was heated at 60–70 °C for 2 h. Volatile components including acetic acid were pumped off with a water respirator keeping the reaction mixture in hot water bath under to give a light-yellow residue. The water (30 ml) and concentrated hydrochloric acid (30 ml) were added to the residue and the mixture was refluxed for 10 h. Tarry semisolid or oily impurities were separated by decantation or extraction with chloroform. An acidic solution was decolourised by a charcoal. The solution was evaporated under reduced pressure, and residue was treated with methanol (20 ml) and concentrated hydrochloric acid (0.5 ml). The insoluble ammonia chloride was filtered off. The filtrate was treated with propylene oxide (2.1 ml, 0.03 mol) which yielded a crude crystalline aminophosphinic acid **27** which was isolated by filtration, 4.50 g (75%). The recrystallisation from water yielded the analytically pure product (Table 1).

4.5. Synthesis of racemic phenylglycines according to Greenstein and Winitz (1961) procedure

Potassium cyanide (3.0 g, 0.046 mol) and ammonium chloride (2.5 g, 0.047 mol) were dissolved in a pressure flask, in a minimum volume of water (15 ml). Then, *p*-tolualdehyde or 1-naphthaldehyde (0.045 mol) and methanol (20 ml) was added until reaction mixture became homogenous. The flask was corked up and shacked from time to time, and left for 8 h at room temperature. Then the flask was opened (caution: hydrogen cyanide), and the mixture was extracted with ether (20 ml). Ethereal fraction was washed with water (5 ml). The ether layer was evaporated and the residue treated with 10% hydrochloric acid (10 ml) and finally evaporated. The rest was hydrolysed with 6 M hydrochloric acid (45 ml) for 8 h. The aqueous solution was decolourised with a charcoal, and evaporated to dryness. The residue was dissolved in methanol (15 ml), filtered, and treated with propylene oxide (2.8 ml, 0.04 mol). A crude amino acid (**29** or **32**) was separated by filtration. Then, the amino acid was recrystallised from acetic acid–water mixture to get the analytically pure sample.

4.6. Synthesis of optically active 1-aminobenzylphosphonic acids. Synthesis of racemic diphenyl 1-aminoalkylphosphonates according to Oleksyszyn et al. (1979) procedure

Racemic diphenyl 1-aminoalkylphosphonates were obtained by heating the corresponding aldehydes (0.15 mol), benzyl carbamate (0.10 mol) and triphenyl phosphite (0.10 mol) in glacial acetic acid (15 ml), followed by bromohydrogenolysis and treatment of the resulting hydrobromide esters with gaseous ammonia in ethereal suspension or neutralisation with aqueous 2 M sodium hydroxide and extracting the free ester with chloroform.

Diphenyl (±)-1-amino-4′-methylbenzylphosphonate was obtained from *p*-tolualdehyde: 8.10 g; 21%; mp 74–79 °C (diethyl ether); ^{1}H NMR (80 MHz, CCl_4, Me_4Si): 2.09 (2 H, *bs*, NH_2), 2.35 (3 H, *s*, C_6CH_3), 4.60 (1 H, *d*, $J=16$ Hz, NC*H*P), 6.6–7.6 (14 H, *m*, NC(C_6H_5)$_2$ and C_6H_4).

Diphenyl (±)-1-amino-3′,4′-dichlorobenzylphosphonate was obtained from 3,4-dichloro-benzaldehyde: 20.4 g; 50%; mp 104–107 °C (diethyl ether); ^{1}H NMR (300 MHz, CD_3Cl): 2.1 (2H, *bs*, NH_2), 4.63 (1H, *d*, $J=16.7$ Hz, NC*H*P), 7.00–7.70 (13 H, *m*, NC(C_6H_5)$_2$ and $C_6H_3Cl_2$).

Diphenyl (±)-1-amino-3-phenylpropylphosphonate was obtained from hydrocinnamaldehyde: 5.91 g; 23%; mp 83–85 °C (diethyl ether); ^{1}H NMR (300 MHz, CD_3Cl): 1.59 (2H, *bs*, NH_2), 1.83–2.02 (1H, *m*, CC*H*C), 2.18–2.37 (1H, *m*, CC*H*C), 2.69–2.83 (1H, *m*, CCH_2C_6), 2.89–3.02 (1H, *m*, CCH_2C_6), 3.18–3.32 (1H, *m*, NC*H*P), 6.92–7.25 (15H, *m*, P(OC_6H_5)$_2$ and CC_6H_5); ^{31}P{^{1}H} NMR(121 MHz, CD_3Cl): 23.45 (*s*).

4.7. Resolution of the amides from diphenyl 1-aminobenzylphosphonates and O,O′-dibenzoyl-l-tartaric anhydride according to Kafarski et al. (1983) procedure

The racemic mixture of diphenyl 1-aminoalkylphosphonates were treated by (−)-*O,O′*-dibenzoyl-*l*-tartaric acid anhydride yielding corresponding diastereoizomers according to Kafarski procedure (Kafarski et al., 1983). The diastereoizomers were additionally recrystalised from a solvent (for details see below) until no further improvement of separation were achieved as seen by their ^{31}P{^{1}H} NMR.

(+)-1-Amino-4′-methylbenzylphosphonic acid (**36**) was obtained as follows. The one from two diphenyl 1-[(1′,2′-dibenzoyloxy-2′-carboxyethanocarbonyl)amino]-4″-methyl-benzylphosphonate diastereoisomers having lower melting point was obtained from the benzene solution, after separation of the crystalline product and evaporation of the solvent. The residue (13.3 g) was crystallized from diethyl ether (30 ml) providing the amide with lower melting point: 7.65 g; 37%; mp 96–102 °C. The amide was extracted with boiling *tert*-butylmethyl ether (4×100 ml), filtered and the combined extracts were concentrated to a final volume of about 130 ml. After crystallization, the amide was filtered giving crystals of the amide: 6.18 g; 30%; mp 102.5–107.5 °C; $[\alpha]_{578}^{20}-41.6\pm1$ ° (*c* 1, acetone); ^{31}P{^{1}H} NMR (121 MHz, $CDCl_3$): δ 16.04 (*s*) and 16.17 (*s*) with a ratio of >20:1.

(+)-1-Amino-4′-methylbenzylphosphonic acid (**36**) was obtained from the amide (4.47 g, 0.00644 mol) by hydrolysis in a mixture of acetic acid and 40% hydrobromic acid, followed by ion exchange chromatography purification and recrystallisation from water yielding: 0.43 g; 33%; mp 275–278 °C; $[\alpha]_{578}^{25}+20.0\pm1$ ° (*c* 1, 1 M NaOH).

(−)-1-Amino-4′-methylbenzylphosphonic acid (**39**) was obtained as follows. The corresponding diphenyl 1-[(1′,2′-dibenzoyloxy-2′-carboxyethanocarbonyl)amino]-4″-methylbenzylphosphonate diastereoisomer with higher melting point was isolated as crystalline product from the benzene (60 ml) solution of the reaction mixture of diphenyl (±)-1-amino-4′-methylbenzylphosphonates (10.41 g, 0.0295 mol) and *O,O′*-dibenzoyl-*l*-tartaric anhydride (Butler and Cretcher, 1933) (10.03 g, 0.02995 mol) yielding: 9.62 g, 47%, mp 171–178 °C. The amide was recrystallised from benzene (150 ml) to get the amide: 9.20 g, 45%, mp 173.5–177.7 °C. The product was dissolved in boiling diisopropyl ether (320 ml), and the solution was concentrated to a final volume of about 180 ml. After crystallisation, crystals were filtered yielding the amide: 6.50 g; 32%; mp 173.5–175.5 °C; $[\alpha]_{578}^{20}$ -47.5 ± 1 ° (*c* 1, acetone); $^{31}P\{^{1}H\}$ NMR (121 MHz, $CDCl_3$): δ 16.16 (*s*) and 16.04 (*s*) with a ratio of >25:1.

(−)-1-Amino-4′-methylbenzylphosphonic acid (**39**) was obtained from the amide (3.81 g, 0.00549 mol) by hydrolysis in a mixture of acetic acid and 40% hydrobromic acid, followed by ion exchange chromatography purification and crystallisation from water yielding: 0.42 g, 38%, mp 272–276 °C, $[\alpha]_{578}^{25}-22.0\pm1$ ° (*c* 1, 1 M NaOH).

(+)-1-Amino-3′,4′-dichlorobenzylphosphonic acid (**37**) was obtained as follows. The corresponding diphenyl 1-[(1′,2′-dibenzoyloxy-2′-carboxyethanocarbonyl)amino]-3″,4″-dichloro-benzylphosphonate diastereoisomer with higher melting point was isolated as crystalline product from the benzene (46 ml) solution of the reaction mixture of diphenyl (±)-1-amino-3′,4′-dichlorobenzylphosphonate (9.50 g, 0.023 mol) and *O,O′*-dibenzoyl-*l*-tartaric anhydride (Butler and Cretcher, 1933) (8.0 g, 0.023 mol) yielding: 4.60 g, 26%, mp 102–198 °C. The product was dissolved in boiling diisopropyl ether (1000 ml), and the solution was concentrated to a final volume of about 400 ml. After crystallization, crystals were filtered providing the amide: 1.91 g, 11%, mp 199–207 °C, $[\alpha]_{578}^{20}$ -25.5 ± 1 ° (*c* 1, acetone).

(+)-1-Amino-3′,4′-dichlorobenzylphosphonic acid (**37**) was obtained from the amide (1.87 g, 0.00251 mol) by hydrolysis in a mixture of acetic acid and 40% hydrobromic acid, followed by ion exchange chromatography (0.2 M hydrochloric acid was used as eluent). The eluate was evaporated to dryness, the residue was dissolved in methanol (5 ml) and treated with propylene oxide (0.17 ml, 0.0017 mol). The crystalline product was finally purified by crystallization from water yielding: 0.25 g, 39%, mp 265–267 °C, $[\alpha]_{578}^{25}+15.5\pm1$ ° (*c* 1, 1 M NaOH).

(−)-1-Amino-3′,4′-dichlorobenzylphosphonic acid (**40**) was obtained as follows. The corresponding diphenyl 1-[(1′,2′-dibenzoyloxy-2′-carboxyethanocarbonyl)amino]-3″,4″-dichloro-benzylphosphonate diastereoisomer with lower melting point was obtained from the benzene solution left after separation of the crystalline product and evaporation of the solvent. The residue (12.8 g) was crystallised from diethyl ether (23 ml) providing the amide with lower melting point: 3.20 g; mp 167–175 °C. The amide was extracted with boiling *tert*-butylmethyl ether (150 ml), filtered and the solution was concentrated to a final volume of about 10 ml. After recrystallisation from *tert*-butylmethyl ether, the amide was filtered, dried in open air providing the amide: 1.20 g, 7%, mp 172–175.5 °C; $[\alpha]_{578}^{25}-57.0\pm1$ ° (*c* 1, acetone).

(−)-1-Amino-3′,4′-dichlorobenzylphosphonic acid (**40**) was obtained from the amide (1.15 g, 0.00154 mol) by hydrolysis in a mixture of acetic acid and 40% hydrobromic acid, followed by ion exchange chromatography purification and recrystallisation from water yielding: 0.10 g, 25%, mp 255.5–257 °C, $[\alpha]_{578}^{25}$ -23.0 ± 1 ° (*c* 1, 1 M NaOH).

(+)-1-Amino-3-phenylpropylphosphonic acid (**41**) was obtained as follows. The reaction of diphenyl (±)-1-amino-3-phenylpropylphosphonate (5.12 g, 0.0139 mol) with *O,O′*-dibenzoyl-*l*-tartaric anhydride (Butler and Cretcher, 1933) (4.74 g, 0.0139 mol) in benzene solution.(28 ml) resulted in a mixture of both amide diastereoisomers of diphenyl 1-[(1′,2′-dibenzoyloxy-2′-carboxyethanocarbonyl)amino] - 3 - phenylpropylphosphonate as crystalline product: 9.70 g, 98%, mp 130–181 °C, $[\alpha]_{578}^{25}-79.0\pm1$ ° (*c* 1, acetone). The amide (8.49 g) was treated with boiling benzene (50 and 25 ml) to obtain the insoluble crystals: 4.53 g, 46%, mp 89–177 °C, $[\alpha]_{578}^{25}-57.0\pm1$ ° (*c* 1, acetone) and the solution. The crystalline product was treated again with boiling diisopropyl ether (500 ml). The insoluble crystals were mainly one diastereoisomer of diphenyl 1-[(1′,2′-dibenzoyloxy - 2′ - carboxyethanocarbonyl)amino] - 3 - phenylpropylphosphonate which was used in preparation of (+)-1-amino-3-phenylpropylphosphonic acid: 2.74 g, 28%, mp 98–165 °C, $[\alpha]_{578}^{25}$ -78.6 ± 1 ° (*c* 1, acetone).

(+)-1-Amino-3-phenylpropylphosphonic acid (**41**) was obtained from the amide (2.68 g, 0.00379 mol) by hydrolysis in a mixture of acetic acid and 40% hydrobromic acid, followed by ion exchange chromatography purification and recrystallisation from water yielding: 0.10 g, 31%, mp 273–277 °C, $[\alpha]_{578}^{25}$ -26.2 ± 1 ° (*c* 1, 1 M NaOH). Enantiomeric purity of the compound **41** was determined by capillary electrophoresis with α-cyclodextrin (Dżygiel et al., 2000) and was estimated to be at least 99% (only one pick on chromatogram, whereas two of them were observed for the racemate in the same conditions).

The diisopropyl ether (500 ml) solution contained a mixture of diastereoisomers as determined by capillary

electrophoresis with α-cyclodextrin of the product after hydrolysis.

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