Synthesis of *N*-(phosphonomethyl)glycine derivatives and studies of their immunotropic activity

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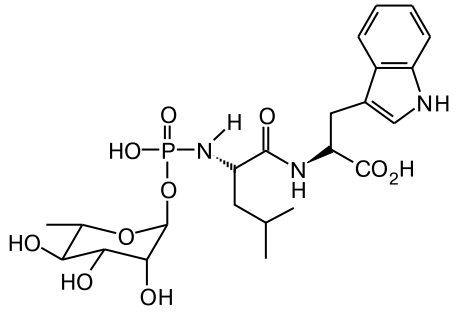
The Petasis reaction between glyoxylic acid, α-amino phosphonates, and organylboronic acid afforded *N*-phosphonomethyl-α-amino acids. This method has an advantage of preparative simplicity and high diastereoselectivity of the reactions. Immunotropic activity of the synthesized compounds was studied using the models *in vivo*.

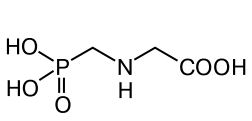
Key words: the Petasis reaction, α-amino phosphonates, *N*-(phosphonomethyl)glycines, immunotropic activity.

N-Phosphorylated peptides were found in the products of natural origin, they possess useful medicobiological properties.[1,2] For instance, phosphoramidon isolated from *Actynomycetes* is an efficient inhibitor of metalloproteases,[3] whereas some nucleoside phosphoramidates exhibit antitumor[4] and antiviral[5] activities. However, the P—N bond is very sensitive to hydrolysis, which limits the use of phosphorus amides *in vivo*. In this connection, *N*-phosphonomethylamino acids and peptides containing hydrolytically stable skeleton, whose phosphoryl group mimics the transition state of the reaction of hydrolytic cleavage of the peptide bond, are of significant interest. Among this type of compounds, a large number of practically useful substances were found, such as herbicide glyphosate,[6] antibacterial drug alaphosphaline,[7] and highly active antihypertensive drug CGS 24592.[8]

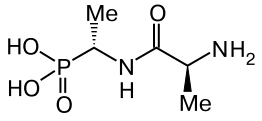
Until now, the three-component Kabachnick—Fields reaction based on the reaction of a carbonyl compound, amino acid ester, and dialkyl phosphite catalyzed by Lewis or Brønsted acids served as the most important method for the synthesis of *N*-phosphonomethylglycine derivatives.[2,9] The key step of this process is the addition of a hydrophosphoryl compound to the amino acid-derived azomethine generated in the course of the reaction. However, despite numerous advantages the Kabachnick—Fields reaction provides an access to *N*-phosphonomethylamino acids containing only secondary amino group. In addition, a classic version of this reaction involving amino acid



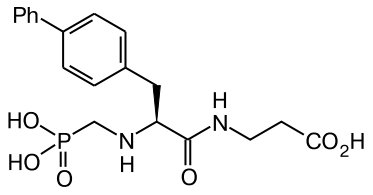
Phosphoramidon



Glyphosate



Alaphosphaline



CGS 24592

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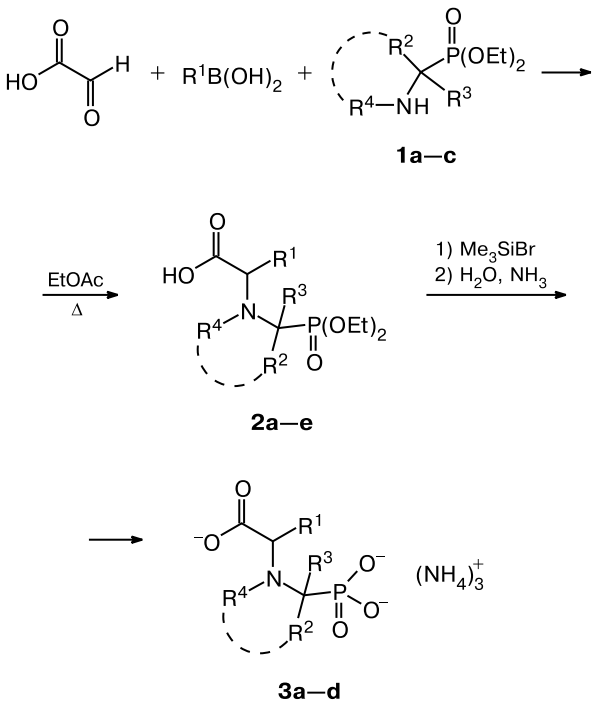
smoothly proceeds only with formaldehyde.[10,11] The use of aldehydes and ketones for the synthesis of *N*-phosphonomethylamino acids requires the use of amino acid *tert*-butyl esters as the amine component[12] or specific catalysts, such as aluminum tetra(*tert*-butyl)phthalocyanine chloride.[13–15] And finally, the use of the Kabachnick—Fields reaction for stereoselective synthesis of *N*-phosphonomethylamino acids with the chiral carbon atom at the phosphorus center, >P(O)—C*—, is a rather difficult problem.[16] Among other methods for the preparation of *N*-phosphonomethyl-α-amino acids, the following syntheses should be mentioned: through the functionalized azomethines,[17,18] 1,3-oxazolidin-2-ones,[19] L-pyroglutamine and 6-oxo-L-pipecolinic acids,[20] as well as desulfurization of thiocarbamoyl phosphonates[21] and reactions of 1,3,5-tris[*N*-(alkoxycarbonylalkyl)]hexahydrotriazines with PH-acids.[22] These methods can be used for the synthesis of specific types of *N*-phosphonomethylamino acids.

A possibility of application of amino phosphonates as an amine component in the boronic version of the Mannich reaction (the Petasis reaction) was first reported in the patent literature.[23] In the preliminary communication,[24] we have shown that a three-component reaction of α-aminophosphonates **1** with glyoxylic acid and alkenyl- or arylboronic acids is an important method for the preparation of *N*-(diethoxyphosphorylmethyl)glycine derivatives **2** (Scheme 1). The present work is devoted to the synthesis of water-soluble salts of phosphonic acid **3** and studies of their immunotropic activity.

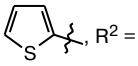
The general approach to the synthesis of compounds **3** is given in Scheme 1. The three-component one-pot reaction of α-aminophosphonates **1a—c**, glyoxylic acid, and boronic acids leads to *N*-phosphonomethylamino acid esters **2a—e**, whose yields depend on the nature of aminophosphonate, organylboronic acid, and the solvent used. On the whole, the regularities of the reaction are similar to those described earlier for the Petasis reaction involving organylamines.[25,26]: (a) for organylboronic acids, $RB(OH)_2$, the reactivity decreases in the following order of R: styryl > hetaryl > aryl; (b) diastereoselectivity of the reactions is higher in the case of aminophosphonates with secondary amino group (*dr* > 9 : 1) as compared to aminophosphonates with primary amino group (*dr* > 7 : 3); (c) aprotic solvents of moderate polarity are the optimum reaction media. When the reactants are refluxed in ethyl acetate, the reaction usually comes to completion within 2—5 h with the degree of conversion 90—95%. The use of alcohols (methanol, ethanol), dioxane, or acetonitrile leads to a decrease in the yields of the target products because of side processes. In dichloromethane, the reaction proceeds smoothly, however, at low rate (20 °C, 3—5 days). Phenylboronic acid under indicated conditions does not react. However, reactions involving arylboronic acids containing electron-enriched aryl groups (3,4-methylenedioxyphenyl, 4-methoxyphenyl) lead to compounds **2** in good

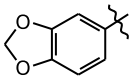
Scheme 1

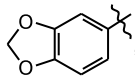


1: R^2 = 4-$MeOC_6H_4$, R^3 = H, R^4 = H (**a**);
R^2 = Ph, R^3 = H, R^4 = $PhCH_2$ (**b**);
R^2, R^4 = $-(CH_2)_3-$, R^3 = P(O)$(OEt)_2$ (**c**)

2, 3: R^1 = (*E*)-PhCH=CH, R^2 = 4-$MeOC_6H_4$, R^3 = H, R^4 = H (**a**)
R^1 = 4-$MeOC_6H_4$, R^2 = Ph, R^3 = H, R^4 = $PhCH_2$ (**b**);

R^1 =  , R^2 = Ph, R^3 = H, R^4 = $PhCH_2$ (**c**);

R^1 =  , R^2 = Ph, R^3 = H, R^4 = $PhCH_2$ (**d**);

R^1 =  , R^2, R^4 = $—(CH_2)_3—$, R^3 = P(O)$(OEt)_2$ (**e**)

yields. All the transformations are characterized by a high degree of diastereoselectivity (*dr* > 9 : 1), which was inferred from the ^{31}P NMR spectra of the reaction mixtures. In the case of *N*-benzylamino-α-amino phosphonate **1b**, the diastereoselectivity is higher than 95%. Purification of products **2** by column chromatography allowed us to isolate the major diastereomer in pure form.

The structures of compounds **2** were established based on the data from elemental analysis, mass spectrometry, and ^1H, ^{13}C, and ^{31}P NMR spectroscopy (see Experimental). The molecular structure of compound **2b** was confirmed by X-ray diffraction study. The general view[27] of molecule **2b** and its basic geometrical parameters are given in Fig. 1 and in Tables 1 and 2. The central fragment of the molecule C(12)—N(1)—C(1)—P(1) is nonplanar: the corresponding dihedral angle is 61.1(1)°. The configura-

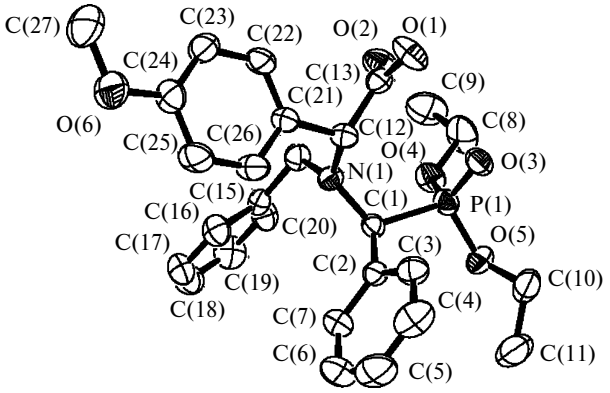


Fig. 1. The view of molecule **2b** (*ORTEP-3*) with representation of atoms by thermal ellipsoids ($p = 50\%$).

tion of the nitrogen atom is almost planar-trigonal (the sum of bond angles is 352.6°). The phosphorus atom P(1) has a distorted tetrahedral surrounding with the angle range from 100.3 to 116.15°. The bond distances at the nitrogen and phosphorus atoms are common for amines and phosphonic acid esters, respectively. The distance P(1)—O(3) 1.466(8) Å corresponds to the double P=O bond.

Transformation of phosphonates **2** to the corresponding phosphonic acids, isolated as ammonium salts **3**, was performed by transesterification of compounds **2** using bromotrimethylsilane and subsequent hydrolysis of the intermediate trimethylsilyl esters. The reactions gave quantitative yields in the case of phosphonates **2a—d**, however, treatment of compound **2e**, containing methylenebisphosphonate fragment incorporated into the pyrrolidine ring, with bromotrimethylsilane was accompanied by elimination of $P(OSiMe_3)_3$ and formation of (4,5-dihydropyrrol-2-yl)phosphonic acid derivatives. Preliminary studies of transesterification reactions of other *N*-substituted 2,2-bis-(diethoxyphosphoryl)pyrrolidines upon the action of Me_3SiBr showed that the ease of the C—P bond cleavage in these compounds is a specific feature of pyrrolidine-2,2-diylbisphosphonates.

Immunotropic activity of the synthesized compounds **3c**,**d** was studied using the models *in vivo*. The data obtained (Table 3) indicate that the studied compounds under conditions of the experiment do not affect proliferation processes in the animal spleen: no reliable changes in its weight and cellularity were revealed, rather they significantly intensify the processes of splenocyte differentiation, which are responsible for the functional state of the humoral part of immunity.[28] A significant (more than eightfold) increase in the antibody-synthesizing activity was registered as well. At the same time, when these compounds were introduced into the animal body, a significant (more than twofold) decrease in the thymus weight and cellularity was observed, whose lymphocytes functionally control the state of the cellular part of immunity.[29] The compounds had a negative effect on the nonspecific reactions of the immune response, significantly, virtually twofold, decreasing phagocytic reactivity of the animal polymorphonuclear blood lymphocytes. To sum up, the biological screening of the synthesized *N*-phosphonomethylglycine derivatives using the model *in vivo* showed that introduction of these compounds into the body leads to a disbalance of the animal immune system.

Table 1. Principal bond distances in molecule **2b**

Bond	*d*/Å	Bond	*d*/Å
C(1)—N(1)	1.466(2)	P(1)—O(3)	1.466(8)
C(1)—P(1)	1.830(5)	P(1)—O(4)	1.562(1)
N(1)—C(12)	1.450(2)	P(1)—O(5)	1.571(9)
N(1)—C(14)	1.462(6)	C(13)—O(1)	1.201(5)
		C(13)—O(2)	1.327(9)

Table 2. Principal bond angles in molecule **2b**

Angle	Value
Bond angle	ω/deg
C(1)—N(1)—C(12)	118.24(1)
C(1)—N(1)—C(14)	116.38(6)
C(12)—N(1)—C(14)	117.99(3)
Torsional angle	φ/deg
C(1)—N(1)—C(12)—C(13)	90.37(1)
C(12)—N(1)—C(1)—P	−61.11(7)

Experimental

All the experiments were carried out under argon. Acetonitrile was purified by distillation over P_2O_5. The course of the reactions was monitored by TLC on silica gel (Fluka). Preparative flash chromatography was performed on Merck 60 silica gel. Amino phosphonates were obtained using methods described in the literature.[30,31] Boronic acids and glyoxylic acid hydrate were purchased from Aldrich Co and used without additional purification.

^{1}H NMR spectra were recorded on Varian VXR-300 and Varian-400 spectrometers. ^{13}C and ^{31}P NMR spectra were recorded on a Varian-400 spectrometer (100 and 162 MHz, respectively). Chemical shifts are given in the δ-scale and measured with respect to internal standards: hexamethyldisiloxane ($\delta_H = 0.05$), residual signal of chloroform ($\delta_H = 7.26$, $\delta_C = 77.16$), or residual signal of methanol (CHD_2OD, $\delta_H = 3.31$); or external standard: 85% H_3PO_4 (δ = 0.0). Mass spectra were obtained on an Agilent 1100 LC MSD SL instrument (chemical ionization), signals of positive ions current are labeled as *pos.*, negative as *neg*. Melting points were measured on a Nagema Boetius PHMK05 apparatus from Rapido.

Single crystals of compound **2b** suitable for X-ray diffraction study were obtained by recrystallization from benzene. The X-ray diffraction experiment for crystals with linear sizes

Table 3. Effects of compounds **3c,d** on the weight and cellularity of lymphoid organs, reactions of antibody formations, and phagocytose using the models *in vivo* (M±*m*, *n* = 8)

Compound	Spleen		Amount of AT, $-\log_2 T$	Thymus		N' [b]
	M/mg	$N^a \cdot 10^8$		*M*/mg	$N \cdot 10^8$	
			1 Day after administration of compound			
Control	129.2±10.3	14.3±1.3	—	44.5±3.6	17.0±1.2	61.5±4.5
3c	136.3±12.4	11.9±1.0	—	24.6±2.0[c]	10.4±0.8[c]	60.7±4.0
3d	117.2±9.7	12.6±0.9	—	52.2±4.3	20.5±1.8	43.5±3.2[c]
			7 Days after administration of compound			
Control	114.3±9.8	13.4±1.2	3.0±0.2	45.0±3.9	16.2±1.4	63.3±3.8
3c	118.2±11.0	10.6±0.8	6.3±0.4[c]	49.0±4.0[c]	15.4±1.1	33.6±2.9[c]
3d	106.4±8.9	11.0±1.0	6.7±0.3[c]	9.1±0.7[c]	4.1±0.4[c]	19.7±1.2[c]

[a] Amount of cells per gram of an organ.
[b] Amount of phagocytes per 100 PMNL.
[c] The value reliable ($p < 0.05$) differs from the control one.

0.50×0.20×0.12 mm was carried out on a Smart APEX automatic diffractometer with graphite monochromator (Mo-Kα-irradiation, λ = 0.71073 Å) at room temperature. Allowance for the absorption in crystal was made using the multiscanning method. The structure was solved by the direct method and refined by the least squares method in the full-matrix anisotropic approximation for all the nonhydrogen atoms using the SHELXS97[32] and SHELXL97[33] program packages. All the hydrogen atoms were localized geometrically. The residual electron density after the final cycle of refinement was 0.46 and −0.37 e Å^{-3}. The crystallographic data, parameters of the X-ray diffraction experiment and refinement for compound **2b** are given in Table 4.

In biological experiments, we used female outbred mice with the weight 18—21 g. Equimolar concentrations of compounds under study were administered intraabdominally to the animals in one portion in a doze $2 \cdot 10^{-4}$ mol per 1 kg of body weight in saline.[34] An aliquot of saline was introduced to the control animals. Testing were performed 1 and 7 days after administration of compounds. The murine thymus and spleen were isolated and weighed, a suspension of lymphoid cells were washed off in the nutrient medium 199 for culturing, their amount was calculated in the Goryaev chamber.[35] The results obtained refer to 1 g of the organ weight. The content of antibodies (AB) to the thymus-dependent antigen, the ram erythrocytes (RE), were determined in the blood serum of immunized mice by the direact hemagglutination reaction[36] in the seventh day after simultaneous administration of compounds under study and RE into mice. The results of the reaction were given as $-\log_2 T$ (T is the final twofold dilution of the blood serum that manifested agglutination). Phagocytic reactivity of the animal blood polymorphonuclear lymphocytes (PMNL) was determined by the known method using the *Staphylococcus aureus* culture.[34] All the results obtained were proceeded statistically.

(*E*)-2-[(Diethoxyphosphoryl)(4-methoxyphenyl)methylamino]-4-phenylbut-3-enoic acid (2a) and ammonium (*E*)-2-[(phosphonato)(4-methoxyphenyl)methylamino]-4-phenylbut-3-enoate (3a). Amino phosphonate **1a** (273 mg, 1.00 mmol) and after 5 min (*E*)-styrylboronic acid (155 mg, 1.05 mmol) were added to a suspension of glyoxylic acid monohydrate (97 mg, 1.05 mmol) in ethyl acetate (5 mL) with stirring. The mixture was refluxed for 2—2.5 h, monitoring the progress of the reaction by TLC (5% methanol in chloroform). After the reaction has come to completion, the suspension obtained was diluted with ethyl acetate (10—15 mL), the reaction mixture was washed with brine (2×3 mL) and dried with Na_2SO_4. The solvent was evaporated to obtain a yellow oil crystallized on standing. The solid substance formed was recrystallized from ethyl acetate—hexane (4 : 1). The yield of compound **2a** was 325 mg (75%), colorless crystals,

Table 4. Crystallographic data and principal geometric parameters of the X-ray diffraction experiment for **2b**

Parameters	Value
Molecular formula	$C_{27}H_{32}NO_6P$
Molecular weight	497.51
Crystal system	Monoclinic
Space group	$P2(1)/n$
T/K	296(2)
a/Å	10.6098(13)
b/Å	11.5274(14)
c/Å	21.894(3)
β/deg	103.859(4)
V/Å^3	2599.7(6)
Z	4
d_{calc}/g cm^{-3}	1.271
$F(000)$	1056
$2\theta_{max}$/deg	26.63
Number of collected reflections	17842
Number of independent reflections	5425
R_{int}	0.0941
Number of observed reflections with $I > 2\sigma(I)$	2673
Number of refined parameters	322
Absorption coefficient/mm^{-1}	0.147
R_1 ($I > 2\sigma(I)$)	0.0643
wR_2	0.1015
GOOF	0.962

m.p. 62 °C. Found (%): C, 60.96; H, 6.51; N, 3.23. $C_{22}H_{28}NO_6P$. Calculated (%): C, 60.80; H, 6.45; N, 3.19. ^{1}H NMR ($CDCl_3$), δ: 1.14 (t, 3 H, $OCH_2C\underline{H}_3$, J = 7.0 Hz); 1.20 (t, 3 H, $OCH_2C\underline{H}_3$, J = 7.0 Hz); 3.71 (s, 3 H, $OC\underline{H}_3$); 3.81—4.09 (m, 5 H, $OC\underline{H}_2CH_3$, C<u>H</u>COOH); 4.17 (d, 1 H, ArC<u>H</u>P, J = 18.4 Hz); 6.12 (dd, 1 H, PhCH=C<u>H</u>, J = 16.0 Hz, J = 6.4 Hz); 6.58 (d, 1 H, PhC<u>H</u>=CH, J = 16.0 Hz); 6.70 (br.s, 2 H, N<u>H</u>); 6.80 (d, 2 H, Ar, J = 8.0 Hz); 7.14—7.34 (m, 7 H, Ar). ^{13}C NMR ($CDCl_3$), δ: 16.3 ($OCH_2\underline{C}H_3$); 55.3 ($O\underline{C}H_3$); 58.2 (d, Ar<u>C</u>HP, J = 154.8 Hz); 61.2 (d, <u>C</u>HCOOH, J = 16.8 Hz); 63.3 (d, $O\underline{C}H_2CH_3$, J = 7.4 Hz); 63.7 (d, $O\underline{C}H_2CH_3$, J = 7.4 Hz); 114.2, 125.6, 126.6 (d, J = 5.5 Hz); 126.8, 128.1, 128.7, 130.1, 133.2, 136.5, 159.9 (Ar), 174.5 (COOH). ^{31}P NMR ($CDCl_3$), δ: 23.2. MS (CI), m/z (I_{rel} (%)): *pos.* 434 $[M + H]^+$ (45), 296 $[M + H - HP(O)(OEt)_2]^+$ (100).

2,4,6-Trimethylpyridine (0.33 mL, 2.5 mmol) and freshly distilled bromotrimethylsilane (0.33 mL, 2.5 mmol) were added to a solution of compound **2a** (108 mg, 0.25 mmol) in anhydrous CH_2Cl_2 (1 mL). The suspension that formed was stirred for 16 h, then the volatile products were evaporated *in vacuo* (0.05 Torr). The residue was diluted with aqueous 1 *M* NaOH (3 mL), the mixture was stirred for 30 min and lyophilized. A solid substance obtained was washed with acetone (5 mL) and diethyl ether (10 mL), dissolved in water (5 mL), and acidified with dilute hydrochloric acid (~1%) to pH 1. A white precipitate formed was filtered off, dissolved in concentrated aq. ammonia (1 mL), and concentrated in air to obtain compound **3a** (87 mg, 44%), colorless crystals, m.p. 182—184 °C (decomp.). ^{1}H NMR (CD_3OD), δ: 3.80 (s, 3 H, $OC\underline{H}_3$); 3.98 (d, 1 H, C<u>H</u>COOH, J = 9.8 Hz); 4.20 (d, 1 H, ArC<u>H</u>P, J = 16.1 Hz); 6.29 (dd, 1 H, PhCH=C<u>H</u>, J = 15.9 Hz, J = 9.8 Hz); 6.47 (d, 1 H, PhC<u>H</u>=CH, J = 15.9 Hz); 6.94 (d, 2 H, Ar, J = 8.3 Hz); 7.22—7.35 (m, 4 H, Ar); 7.44—7.52 (m, 3 H, Ar). ^{31}P NMR (CD_3OD), δ: 10.3 (d, J = 16.1 Hz).

2-{*N*-[(Diethoxyphosphoryl)(phenyl)methyl]benzylamino}-2-(4-methoxyphenyl)acetic acid (2b) and ammonium 2-{*N*-[(phenyl)(phosphonato)methyl]benzylamino}-2-(4-methoxyphenyl)acetate (3b). Compound **2b** was obtained similarly to compound **2a** starting from aminophosphonate **1b** (333 mg, 1.00 mmol), 4-methoxyphenylboronic acid (152 mg, 1.00 mmol), and glyoxylic acid (92 mg, 1.00 mmol). The yield was 472 mg (95%), colorless crystals, m.p. 138—139 °C (ethyl acetate). Found (%): C, 64.95; H, 6.43; N, 2.78. $C_{27}H_{32}NO_6P$. Calculated (%): C, 65.18; H, 6.48; N, 2.82. ^{1}H NMR ($CDCl_3$), δ: 1.01 (t, 3 H, $OCH_2C\underline{H}_3$, J = 7.0 Hz); 1.41 (t, 3 H, $OCH_2C\underline{H}_3$, J = 7.0 Hz); 3.64—3.75 (m, 1 H, $OC\underline{H}_2CH_3$); 3.72 (s, 3 H, $OC\underline{H}_3$); 3.84—3.92 (m, 1 H, $OC\underline{H}_2CH_3$); 3.94 (d, 1 H, PhC<u>H</u>$_2$N, J =14.2 Hz); 4.17—4.23 (m, 1 H, $OC\underline{H}_2CH_3$); 4.29 (d, 1 H, PhC<u>H</u>P, J = 20.5 Hz); 4.31—4.39 (m, 1 H, $OC\underline{H}_2CH_3$); 4.44 (dd, 1 H, PhC<u>H</u>$_2$N, J = 14.2 Hz, J = 4.9 Hz); 5.09 (s, 1 H, C<u>H</u>COOH); 6.70 (d, 2 H, Ar, J = 8.5 Hz); 6.84 (d, 2 H, Ar, J = 8.5 Hz); 7.12 (d, 2 H, Ar, J = 6.4 Hz); 7.16—7.33 (m, 8 H, Ar); 10.45 (br.s, CO<u>O</u>H). ^{13}C NMR ($CDCl_3$), δ: 16.4 (d, $OCH_2\underline{C}H_3$, J = 5.7 Hz); 16.8 (d, $OCH_2\underline{C}H_3$, J = 6.5 Hz); 53.1 (Ph<u>C</u>H$_2$N); 55.4 ($O\underline{C}H_3$); 58.9 (d, Ph<u>C</u>HP, J = 150.0 Hz); 62.5 (d, $O\underline{C}H_2CH_3$, J = 7.6 Hz); 63.1 (d, $O\underline{C}H_2CH_3$, J =7.6 Hz); 64.1 (<u>C</u>HCOOH); 113.7, 127.5, 128.3, 128.4, 128.7, 129.6, 130.7, 130.7 (d, J = 9.0 Hz); 135.1 (d, J = 9.0 Hz); 159.2, 174.73 (d, <u>C</u>OOH, J = 3.8 Hz). ^{31}P NMR ($CDCl_3$), δ: 24.1. MS (CI), m/z (I_{rel} (%)): *pos.* 498 $[M + H]^+$ (70), 360 $[M + H - HP(O)(OEt)_2]^+$ (100), 196 $[M + H - HP(O)(OEt)_2 - MeOC_6H_4CHCOOH]^+$ (45), 165 $[M + H - HP(O)(OEt)_2 - PhCH_2NCHPh]^+$ (10).

Compound **3b** was obtained similarly to compound **3a**. The yield was 63%, colorless crystals, m.p. 149—151 °C (decomp.). ^{1}H NMR (CD_3OD), δ: 3.63 (d, 1 H, PhC<u>H</u>$_2$N, J = 13.9 Hz); 3.70 (s, 3 H, $OC\underline{H}_3$); 4.53 (d, 1 H, PhC<u>H</u>P, J = 18.8 Hz); 4.74 (s, 1 H, C<u>H</u>COOH); 5.53 (d, 1 H, PhC<u>H</u>$_2$N, J = 13.9 Hz); 6.50 (d, 2 H, Ar, J = 8.3 Hz); 6.72 (d, 2 H, Ar, J = 8.3 Hz); 7.01—7.11 (m, 5 H, Ar); 7.52 (m, 3 H, Ar); 7.82 (m, 2 H, Ar). ^{31}P NMR (CD_3OD), δ: 8.9 (d, J = 18.8 Hz).

2-{*N*-[(Diethoxyphosphoryl)(phenyl)methyl]benzylamino}-2-(2-thienyl)acetic acid (2c) and ammonium 2-{*N*-[(phenyl)(phosphonato)methyl]benzylamino}-2-(thiophen-2-yl)acetate (3c). Compound **2c** was obtained similarly to compound **2a** starting from aminophosphonate **1b** (397 mg, 1.19 mmol), 2-thienylboronic acid (153 mg, 1.20 mmol), and glyoxylic acid (110 mg, 1.20 mmol). The chloroform—ethyl acetate—methanol (35 : 55 : 5) solvent system was used for flash-chromatography. The yield was 390 mg (69%), colorless crystals, m.p. 219—220 °C (decomp.). Found (%): C, 61.68; H, 5.95; S, 7.09. $C_{24}H_{28}NO_5PS$. Calculated (%): C, 60.88; H, 5.96; S, 6.77. ^{1}H NMR ($CDCl_3$), δ: 1.04 (t, 3 H, $OCH_2C\underline{H}_3$, J = 7.0 Hz); 1.42 (t, 3 H, $OCH_2C\underline{H}_3$, J = 7.0 Hz); 3.74—3.85 (m, 1 H, $OC\underline{H}_2CH_3$); 3.93 (m, 1 H, $OC\underline{H}_2CH_3$); 4.00 (d, 1 H, PhC<u>H</u>$_2$N, J =14.2 Hz); 4.21 (m, 1 H, $OC\underline{H}_2CH_3$); 4.31 (d, 1 H, PhC<u>H</u>P, J = 22.0 Hz); 4.35 (m, 1 H, $OC\underline{H}_2CH_3$); 4.47 (dd, 1 H, PhC<u>H</u>$_2$N, J = 14.2 Hz, J = 3.5 Hz); 5.43 (s, 1 H, C<u>H</u>COOH); 6.74 (s, 1 H, $\underline{H}_{thiophene}$); 6.82 (s, 1 H, $\underline{H}_{thiophene}$); 7.13 (s, 1 H, $\underline{H}_{thiophene}$); 7.28—7.50 (m, 10 H, Ar); 11.00 (br.s, 1 H, CO<u>O</u>H). ^{13}C NMR ($CDCl_3$), δ: 16.4 (d, $OCH_2\underline{C}H_3$, J = 6.0 Hz); 16.74 (d, $OCH_2\underline{C}H_3$, J = 6.0 Hz); 53.3 (d, Ph<u>C</u>H$_2$N, J = 4.0 Hz); 58.3 (d, Ph<u>C</u>HP, J = 152.0 Hz); 59.8 (<u>C</u>HCOOH); 62.8 (d, $O\underline{C}H_2CH_3$, J = 8.0 Hz); 63.3 (d, $O\underline{C}H_2CH_3$, J = 8.0 Hz); 126.1, 126.3, 127.6, 127.8, 128.6, 128.6, 128.9, 130.0, 130.6 (d, J = 9.0 Hz); 134.4 (d, $J_{C,P}$ = = 8.0 Hz); 138.9, 140.1, 172.8 (<u>C</u>OOH). ^{31}P NMR ($CDCl_3$), δ: 24.1. MS (CI), m/z (I_{rel} (%)): *pos.* 474 $[M + H]^+$, 336 $[M + H - HP(O)(OEt)_2]^+$ (100), 196 $[M + H - HP(O)(OEt)_2 - (C_4H_3S)CHCOOH]^+$ (35); *neg.* 472 $[M - H]^-$ (100).

Compound **3c** was obtained similarly to compound **3a**. The yield was 32%. ^{31}P NMR (CD_3OD), δ: 9.1 (d, J = 19.1 Hz).

2-{*N*-[(Diethoxyphosphoryl)(phenyl)methyl]benzylamino}-2-(benzo[*d*][1,3]dioxol-5-yl)acetic acid (2d) and ammonium 2-{*N*-[(phenyl)(phosphonato)methyl]benzylamino}-2-(benzo[*d*][1,3]-dioxol-5-yl)acetate (3d). Compound **2d** was obtained similarly to compound **2a** starting from aminophosphonate **1b** (333 mg, 1.00 mmol), 3,4-methylenedioxyphenylboronic acid (166 mg, 1.00 mmol), and glyoxylic acid (92 mg, 1.00 mmol). The product was recrystallized from ethyl acetate. The yield was 450 mg (88%), colorless crystals, m.p. 176—179 °C. Found (%): C, 63.71; H, 5.99; N, 2.79. $C_{27}H_{30}NO_7P$. Calculated (%): C, 63.40; H, 5.91; N, 2.74. ^{1}H NMR ($CDCl_3$), δ: 1.00 (t, 3 H, $OCH_2C\underline{H}_3$, J = 7.0 Hz); 1.43 (t, 3 H, $OCH_2C\underline{H}_3$, J = 7.0 Hz); 3.66—3.75 (m, 1 H, $OC\underline{H}_2CH_3$); 3.85—3.94 (m, 1 H, $OC\underline{H}_2CH_3$); 3.99 (d, 1 H, PhC<u>H</u>$_2$N, J = 14.2 Hz); 4.18—4.27 (m, 1 H, $OC\underline{H}_2CH_3$); 4.28 (d, 1 H, PhC<u>H</u>P, J = 20.0 Hz); 4.35—4.45 (m, 1 H, $OC\underline{H}_2CH_3$); 4.49 (dd, 1 H, PhC<u>H</u>$_2$N, J = 14.2 Hz, J = 4.9 Hz); 5.04 (s, 1 H, C<u>H</u>COOH); 5.85 (d, 2 H, $OC\underline{H}_2O$, J = 7.0 Hz); 6.32 (s, 1 H, Ar); 6.46 (d, 1 H, Ar, J = 7.9 Hz); 6.62 (d, 1 H, Ar, J = 7.9 Hz); 7.14 (d, 2 H, Ar, J = 7.2 Hz); 7.16—7.33 (m, 8 H, Ar); 11.00 (br.s, CO<u>O</u>H). ^{13}C NMR ($CDCl_3$), δ: 16.3 (d, $OCH_2\underline{C}H_3$, J = 5.0 Hz); 16.7 (d, $OCH_2\underline{C}H_3$, J = 8.0 Hz); 52.9 (Ph<u>C</u>H$_2$N); 58.8 (d, Ph<u>C</u>HP, J = 150.0 Hz); 62.5 (d, $O\underline{C}H_2CH_3$, J = 7.0 Hz); 63.1 (d, $O\underline{C}H_2CH_3$, J =7.0 Hz); 64.0 (<u>C</u>HCOOH);

101.1 (O<u>C</u>H$_2$O); 108.0, 110.0, 122.8, 127.5, 128.4, 128.5, 128.7, 129.6, 130.7 (d, J = 9.0 Hz); 131.5, 135.2 (d, J = 9.0 Hz); 139.6, 147.2, 147.6, 174.4 (d, <u>C</u>OOH, J = 3.0 Hz). ^{31}P NMR ($CDCl_3$), δ: 23.8. MS (CI), m/z (I_{rel} (%)): *pos.* 512 $[M + H]^+$ (55), 374 $[M + H - HP(O)(OEt)_2]^+$ (100), 334 $[M + H - (CH_2O_2C_6H_3)CHCOOH]^+$ (10), 196 $[M + H - HP(O)(OEt)_2 - (CH_2O_2C_6H_3)CHCOOH]^+$ (35); *neg.* 510 $[M - H]^-$ (100).

Compound **3d** was obtained similarly to compound **3a**. The yield was 30%, colorless crystals, m.p. 154—158 °C (decomp.). ^{1}H NMR (CD_3OD), δ: 3.65 (d, 1 H, PhC<u>H</u>$_2$N, J = 13.9 Hz); 4.53 (d, 1 H, PhC<u>H</u>P, J = 19.3 Hz); 4.65 (s, 1 H, C<u>H</u>COOH); 5.51 (d, 1 H, PhC<u>H</u>$_2$N, J = 13.9 Hz); 5.75 (s, 1 H, OCH$_2$O); 5.84 (s, 1 H, OCH$_2$O); 6.27 (s, 1 H, Ar); 6.36 (d, 1 H, Ar, J = 8.1 Hz); 6.50 (d, 1 H, Ar, J = 8.1 Hz); 7.13 (s, 5 H, Ar); 7.51 (s, 3 H, Ar); 7.81 (s, 1 H, Ar). ^{31}P NMR (CD_3OD), δ: 9.2 (d, J = 19.7 Hz). MS, m/z (I_{rel} (%)): *pos.* 456 $[M + H - NH_3]^+$ (65), 374 $[M + H - 3NH_3 - HP(O)(OH)_2]^+$ (30), 330 $[M + H - 3NH_3 - HP(O)(OH)_2 - CO_2]^+$ (15), 278 $[M + H - 3\ NH_3 - (CH_2O_2C_6H_3)CHCOOH]^+$ (20), 196 $[M + H - 3\ NH_3 - HP(O)(OH)_2 - (CH_2O_2C_6H_3)CHCOOH]^+$ (45), 91 $[M + H - 3\ NH_3 - HP(O)(OH)_2 - (CH_2O_2C_6H_3)CHCOOH - PhNH_2]^+$ (40); *neg.* 454 $[M - H]^-$, 276 $[M - H - 3\ NH_3 - (CH_2O_2C_6H_3)CHCOOH]^-$ (20).

2-[2,2-Bis(diethoxyphosphoryl)pyrrolidino]-2-(benzo[*d*]-[1,3]dioxol-5-yl)acetic acid (2e). Compound **2e** was obtained similarly to compound **2a** starting from tetraethyl pyrrolidine-2,2-diyldiphosphonate **1c** (412 mg, 1.20 mmol), 3,4-methylenedioxyphenylboronic acid (210 mg, 1.25 mmol), and glyoxylic acid (112 mg, 1.22 mmol). The dichloromethane—methanol (96 : 4) solvent systemt was used for flash-chromatography. The yield was (460 mg, 73%), colorless crystals, m.p. 134—135 °C. Found (%): C, 48.71; H, 6.23. $C_{21}H_{33}NO_{10}P_2$. Calculated (%): C, 48.37; H, 6.38. ^{1}H NMR ($CDCl_3$), δ: 1.06 (t, 3 H, OCH$_2$C<u>H</u>$_3$, J = 7.1 Hz); 1.24 (t, 3 H, OCH$_2$C<u>H</u>$_3$, J = 7.1 Hz); 1.35 (t, 3 H, OCH$_2$C<u>H</u>$_3$, J = 7.1 Hz); 1.36 (t, 3 H, OCH$_2$C<u>H</u>$_3$, J = 7.1 Hz); 1.79—1.92 (m, 2 H, C<u>H</u>$_2$); 2.29—2.53 (m, 2 H, C<u>H</u>$_2$); 2.78—2.87 (m, 1 H, C<u>H</u>$_2$); 3.11—3.22 (m, 1 H, C<u>H</u>$_2$); 3.87—4.02 (m, 2 H, OC<u>H</u>$_2$CH$_3$); 4.03—4.33 (m, 6 H, OC<u>H</u>$_2$CH$_3$); 5.54 (s, 1 H, C<u>H</u>COOH); 5.90 (d, 2 H, OC<u>H</u>$_2$O, J = 2.0 Hz); 6.73 (d, 1 H, 5-Ar, J = 8.0 Hz); 6.93 (dd, 1 H, 6-Ar, J = 8.0 Hz, J = 1.6 Hz); 6.95 (s, 1 H, 2-Ar). ^{13}C NMR ($CDCl_3$), δ: 16.1 (d, OCH$_2$<u>C</u>H$_3$, J = 6.0 Hz); 16.5 (d, OCH$_2$<u>C</u>H$_3$, J = 6.0 Hz); 16. 6 (d, OCH$_2$<u>C</u>H$_3$, J = 6.0 Hz); 16.6 (d, OCH$_2$<u>C</u>H$_3$, J = 6.0 Hz); 23.9 (t, <u>C</u>H$_2$, J = 3.5 Hz); 32.1 (t, <u>C</u>H$_2$, J = 3.5 Hz); 48.7 (d, <u>C</u>H$_2$, J = 5.0 Hz); 62.0 (d, O<u>C</u>H$_2$CH$_3$, J = 8.0 Hz); 63.2 (d, O<u>C</u>H$_2$CH$_3$, J = 8.0 Hz); 63.6 (d, O<u>C</u>H$_2$CH$_3$, J = 8.0 Hz); 64.1 (s, <u>C</u>HCOOH); 64.1 (dd, P<u>C</u>P, J = 148.0 Hz, J = 154.0 Hz); 64.72 (d, O<u>C</u>H$_2$CH$_3$, J = 7.0 Hz); 101.1 (s, O<u>C</u>H$_2$O); 107.7, 110.6, 123.8, 130.0, 147.1, 147.2, 174.0 (s, <u>C</u>OOH). ^{31}P NMR ($CDCl_3$), δ: 21.5 (d, 1 P, J = 92.0 Hz); 23.6 (d, 1 P, J = 92.0 Hz). MS, m/z (I_{rel} (%)): *pos.* 384 $[M + H - HP(O)(OEt)_2]^+$ (100), 206 $[M + H - HP(O)(OEt)_2 - CH_2O_2C_6H_3CHCOOH]^+$ (10); *neg.* 520 $[M - H]^-$, (100), 382 $[M - H - HP(O)(OEt)_2]^-$ (20), 338 $[M - H - HP(O)(OEt)_2 - CO_2]^-$ (10).

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