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Convenient Syntheses of Phosphinic Analogues of γ-Aminobutyric- and Glutamic Acids[1]

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Abstract—Three-steps, one-pot synthesis of 2-amino-4-(hydroxyphosphinyl)butyric acid from dibutyl ester of vinylphosphinic acid was carried out with an overall yield of 66%. 3-Aminopropylphosphinic acid was prepared from allylamine in three steps with an overall yield of 56%. These improved protocols allowed to obtain these commercially unavailable phosphinic analogues of glutamic acid and GABA for testing on potential molecular targets.

Keywords: amino acids, phosphorus analogues, glutamic acid, γ-aminobutyric acid (GABA)
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INTRODUCTION

Phosphorus analogues of α-amino acids are compounds in which the carboxyl moiety of the amino acid is substituted with either a phosph*onic* (Fig. 1a, X = OH) or a phosph*inic* (Fig. 1a, X = H) group. Representatives of both types of phosphorus analogues of amino acids show broad spectra of biological activity, with the phosphonic ones being the most intensively investigated [1]. In addition to containing an unusual C–P bond (which is rarely occurring also in natural compounds), they present the phosphorus atom in two oxidation forms which affect their biochemical properties. Unlike the doubly-charged tetrahedral phosphonic group, the strongly flatted tetrahedral geometry of the singly-charged phosphinic group is structurally more similar to that of the planar, singly-charged carboxyl group (Fig. 1a). Hence, the aminophosphinic analogues of amino acids were found to be not only inhibitors but also substrates of enzymes of amino acid metabolism [2–5], especially of pyridoxal 5'-phosphate (PLP)-dependent enzymes [2, 6–8], whereas the aminophosphonic acids were only weak inhibitors of the corresponding enzymes.

This is also in line with the observation that cells can uptake aminophosphinic analogues, whereas the phosphonic analogues are unable to penetrate into the cells as such [1]. Indeed, 1-aminoethylphosphinic acid (the phosphinic analogue of Alanine) can be transaminated intracellularly to acetophosphinic acid (the phosphinic analogue of pyruvate), a nanomolar inhibitor of *E. coli* pyruvate dehydrogenase [9, 10]. On the other hand, 1-aminoethylphosphonic acid (the phosphonic analogue of alanine; Ala-*P*), which is a powerful inhibitor of alanine (Ala) racemase and D-Ala:D-Ala ligase [11–13], displays antibiotic activity as inhibitor of cell wall biosynthesis only when delivered intracellularly as Alafosfalin, i.e. an L-Ala-L-Ala-*P* dipeptide [14].

However the family of phosphorus analogues of amino acids should not include only α-amino phosphonates, but also the analogues of aspartic and glutamic acid with the phosphorus-containing group substituting the distal carboxylic moiety. Notably, these compounds were reported to be intermediates (i.e. only occurring in tiny amounts) in the biosynthetic pathway of 2-amino-4-(hydroxymethylphosphinyl)butyric acid (phosphinotricin, PT, Fig. 1b)

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Abbreviations: GABA, 4-aminobutyric acid; GABA-P_H, 3-aminopropylphosphinic acid; Glu-γ-P_H, 2-amino-4-(hydroxyphosphinyl)butyric acid; AIBN, α,α',-azoisobutyronitrile; PT, phosphinotricin, 2-amino-4-(hydroxymethylphosphinyl)butyric acid.

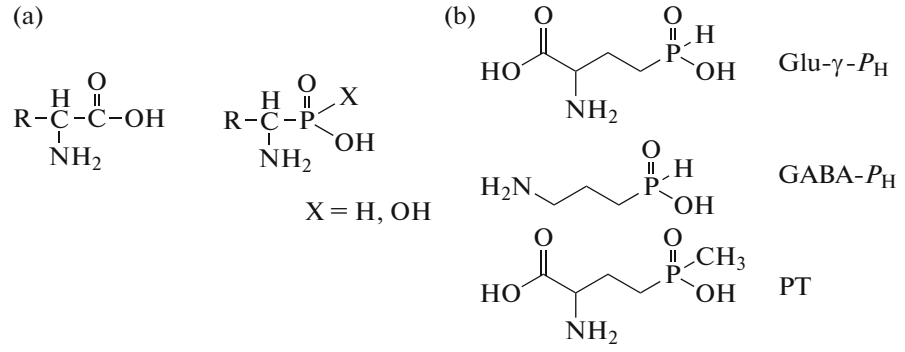


Fig. 1. (a) α-Amino acids and their phosphorus-containing analogues: aminophosphonic (X = OH) and aminophosphinic (X = H) acids. (b) Phosphinotricin (PT) and phosphinic analogues of γ-aminobutyric and glutamic acids, GABA-P_H and Glu-γ-P_H, respectively.

and its tripeptide derivative, L-PT-L-Ala-L-Ala (Bialaphos), two well-known herbicides produced by *Streptomyces viridochromogenes* and *S. hygroscopicus* [15, 16].

L-2-Amino-4-(hydroxyphosphinyl)butyric acid (hereafter Glu-γ-P_H) is a key intermediate of PT biosynthesis. However this molecule has hardly been investigated for its substrate/inhibitor properties towards other enzymes than aspartate- and GABA-aminotranferases, on which it acts as a substrate [2, 8]. When tested in vitro, the potency of L-Glu-γ-P_H on group III metabotropic L-Glu receptors (i.e. mostly presynaptic and controlling glutamate release at the synaptic level) was found to be in the same range of L-Glu [17].

With the aim of investigating the effect of Glu-γ-P_H on potential molecular targets, herein we present optimized synthetic protocols for the preparation of the racemate of this phosphinic analogue of Glutamic acid and of GABA-P_H (Fig. 1b), both commercially unavailable.

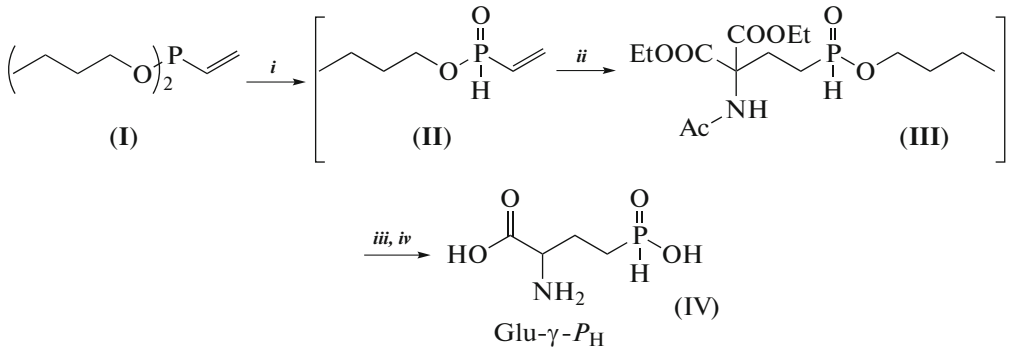
RESULTS AND DISCUSSION

L-Glu-γ-P_H (initially named MP101) was originally isolated in the fermentation broth of cultures from the soil microorganisms *S. viridochromogenes* and *S. hygroscopicus* where it occurs in tiny amounts when PT and Bialaphos production was blocked either by specific inhibitors or by knocking-out genes of the corresponding biosynthetic pathways [15, 16].

First chemical synthesis of racemic Glu-γ-P_H was performed starting from ethyl ester of 2-chloroethylphosphinic acid, which was converted into ethyl ester of vinylphosphinic acid in 78% yield. Subsequent addition of diethyl acetamidomalonate and acidic hydrolysis to remove protection groups afforded target Glu-γ-P_H (**IV**) in 30% overall yield [18].

According to alternative synthetic protocol [20] a completely protected Glu-γ-P_H was obtained by addition of diethyl acetamidomalonate to butyl ester of vinylphosphinic acid [19] and isolated from reaction mixture by column chromatography on silica gel in 77% yield. The refluxing of this compound in 20% HCl afforded "crude" Glu-γ-P_H hydrochloride, which was converted into its *N*,*O*-tetra-trimethylsilyl derivative. The latter was purified by high vacuum distillation that greatly decreased the overall yield. Subsequent methanolysis gave Glu-γ-P_H (**IV**) with a 33% overall yield, as calculated from butyl ester of vinylphosphinic acid [20].

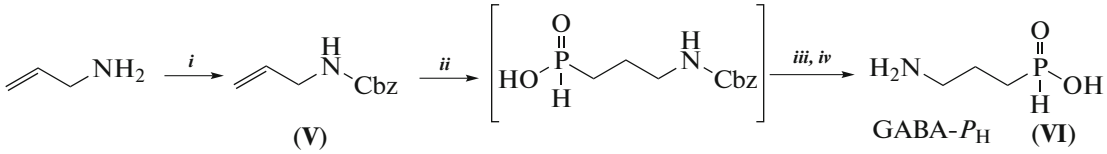
In the present report we describe a simple three-step "one-pot" synthesis of Glu-γ-P_H (Scheme 1) starting from dibutyl ester of vinylphosphinic acid (**I**), which was initially converted into the corresponding monobutyl ester (**II**) upon treatment with equimolar amount of H_2O and catalytic amounts of H_2SO_4 in dioxane. Subsequent addition of diethyl acetamidomalonate followed (after 36 hours) by refluxing in 20% HCl to remove protecting groups gave the target compound, Glu-γ-P_H (**IV**), which was isolated by chromatography on Dowex 50Wx8 resin (H^+-form) using 15% isopropanol as an eluent. The suggested procedure avoided laborious isolation and purification of intermediate compounds, as reported by others [20], and gave target Glu-γ-P_H, contaminated with less than 4% of 4-phosphono-2-aminobutyric acid, with an overall yield of 66%, as calculated from starting dibutyl ester (**I**). To remove from Glu-γ-P_H the trace amounts of the corresponding phosphonate, one additional chromatographic step on Dowex 50Wx8 resin was added, with 15% isopropanol as an eluent.



Scheme 1. Synthesis of Glu-γ-P_H. (***i***) H_2O/cat.H_2SO_4/dioxane; (***ii***) $AcNHCH(COOEt)_2$/cat.EtONa/dioxane; (***iii***) $HCl/H_2O/\Delta$; (***iv***) Dowex50x8 (H^+)/H_2O.

Given that L-Glu-γ-P_H was reported to be a substrate also of GABA-aminotransferase [8], the phosphinic derivative of GABA, hereafter GABA-P_H, was also synthesized using a convenient protocol. Published three-step synthesis of GABA-P_H [21] comprised the addition of ethyl diethoxymethylene phosphinic acid [22] to acrylonitrile, further Ni-Raney reduction of nitrile and finally, deprotection of aminophosphinite by refluxing in 20% HCl. An overall yield of GABA-P_H was 32% as calculated from starting ethyl diethoxymethylene phosphinic acid [21].

In the present report, GABA-P_H was prepared by an essentially different approach (Scheme 2), consisting in the anti-Markovnikov addition of H_3PO_2 to *N*-(benzyloxycarbonyl)allylamine (**V**) by refluxing in 80% aq methanol, in the presence of α,α'-azoisobutyronitrile (AIBN) as a catalyst, to yield *N*-Cbz-GABA-P_H. GABA-P_H was obtained after the removal of the *N*-Cbz group by refluxing in 20% HCl and was purified by column chromatography on Dowex 50Wx8 resin (H^+-form) using 0.5 N HCl as eluent. Subsequent neutralization of GABA-P_H hydrochloride with propylene oxide in EtOH and recrystallization from H_2O/EtOH gave target compound GABA-P_H (**VI**) with an overall yield of 56% as calculated from allylamine.



Scheme 2. Synthesis of GABA-P_H. (***i***) $CbzCl/CH_2Cl_2$; (***ii***) H_3PO_2/MeOH/H_2O/AIBN/Δ; (***iii***) $HCl/H_2O/\Delta$; (***iv***) propylene oxide/EtOH.

EXPERIMENTAL

Allylamine, benzyl chloroformate (CbzCl), diethyl acetamidomalonate, 50 wt % hypophosphorous acid solution in H_2O, α,α',-azoisobutyronitrile (AIBN) and Dowex 50Wx8 hydrogen form, 100–200 mesh, were supplied by Aldrich. Dibutyl ester of vinylphosphinic acid was synthesized as described [19]. Diethyl acetamidomalonate was recrystallized from benzene before use.

Ion-exchange chromatography was carried out on Dowex 50Wx8, 100–200 mesh (H^+-form). Systems for elution are specified in the text. TLC was carried out on Cellulose F_{254} plates (Merck) in the system: *i*-PrOH–25% NH_4OH–H_2O, 7 : 1 : 2 (A) and Kieselgel 60 F_{254} plates (Merck) in: $CHCl_3$ (B). Aminophosphinates were detected on TLC plates with either ammonium molibdate reagent or by ninhydrin (0.4% in acetone) staining, while Cbz-derivative by UV absorbance.

NMR spectra were registered on a Bruker Avance 400 DRX (Germany) instrument with 400.1 MHz for ^1H, 100.6 MHz for ^{13}C and 162 MHz for ^{31}P, either in D_2O with sodium 3-trimethyl-1-propanesulfonate as internal standard and 85% H_3PO_4 as external standard, or in $CDCl_3$ with Me_4Si as internal standard. Chemical shifts are given in ppm, and spin–spin coupling constants in Hz. Melting points were determined in open capillary tubes on Electrothermals Mel-Temp 1202D instrument and are uncorrected.

2-Amino-4-(hydroxyphosphinyl)butyric acid (Glu-γ-P_H) (IV). A solution of 10.8 g (53 mmol) vinylphosphinic acid dibutyl ester and 0.5% aq H_2SO_4 (1.04 g) in dry dioxane (30 mL) was incubated at 20°C for

30 min, evaporated to one-half in vacuo and then diluted with dry dioxane to the initial volume, giving solution (**S**).

To 11.5 g (53 mmol) of diethyl acetamidomalonate in dry dioxane (80 mL) 2.0 M EtONa/EtOH (3 mL) was added that gave white amorphous gel-like solution, which was evaporated to one-third in vacuo and then diluted with dry dioxane to the initial volume. To this, solution (**S**) was added and the reaction mixture became slightly cloudy after stirring at 20°C for 35 h under Argon atmosphere. Reaction mixture was evaporated to dryness in vacuo, 20% HCl (200 mL) was added to the residue, refluxed under argon atmosphere for 3 h, evaporated to dryness in vacuo and coevaporated in vacuo with H_2O (3 × 30 mL). This latter residue was dissolved in 15% *i*-PrOH (50 mL) and purified on a Dowex 50Wx8 (H^+-form) resin (V = 300 mL) by eluting with 15% *i*-PrOH. Fractions containing Glu-γ-P_H (**IV**) were combined, evaporated to dryness in vacuo, crystallized from H_2O/EtOH and dried in vacuo over P_2O_5 to give (**II**) (5.86 g, 66%), contaminated with less than 4% (according to ^{31}P-NMR) of 2-amino-4-phosphonobutyric acid. Analytical sample (after additional purification on Dowex 50Wx8 resin, elution with 15% *i*-PrOH and subsequent H_2O/EtOH crystallization): mp 216–218°C, dec. (lit.: 193–197°C, dec. [18]; 208–210°C, dec. [20]; 221–222°C, dec. (*L*-isomer) [15]), R_f 0.24 (A). ^{1}H NMR (D_2O) δ: 6.98 (dt, 1 H, J_{HP} 523, J_{HH} 1.5, P-H), 4.09 (t, 1 H, J_{HH} 6.1, CH), 2.22–2.07 (m, 2 H, C$\underline{H}_2$P), 1.83–1.61 (m, 2 H, C$\underline{H}_2$CH). ^{13}C NMR (D_2O) δ: 174.91 s, 56.39 d (J_{CP} 16.1), 29.48 d (J_{CP} 88.9), 25.35 s. ^{31}P NMR (D_2O) δ: 29.67.

***N*-(Benzyloxycarbonyl)allylamine (V).** A solution of 6.88 g (50 mmol) CbzCl in dry CH_2Cl_2 (20 mL) was added within 20 min at +4°C to a stirred solution of 6.38 g (110 mmol) allylamine in dry CH_2Cl_2 (80 mL) and the reaction mixture was stirred at +4°C for 30 min and then for 1 h at 20°C. Reaction mixture was washed with H_2O (3 × 20 mL), 1 M HCl (2 × 20 mL), H_2O (10 mL), 1 M $NaHCO_3$ (10 mL), H_2O (10 mL), 5 M NaCl (10 mL), dried (Na_2SO_4) and concentrated in vacuo. The residue was dried in vacuo over P_2O_5 to give (**V**) (9.0 g, 94%) as a viscous oil, R_f 0.38 (B). ^{1}H NMR ($CDCl_3$) δ: 7.40–7.28 (m, 5 H, C_6H_5), 5.87–5.80 (m, 1 H, $CH_2C\underline{H}$), 5.19 (d, 1 H, J_{HH} 17, C$\underline{H}_2$CH), 5.15–5.08 (m, 3 H, C$\underline{H}_2$CH and C$\underline{H}_2C_6H_5$), 4.85 (bs, 1 H, NH), 3.80 (m, 2 H, C$\underline{H}_2$NH).

3-Aminopropylphosphinic acid (GABA-P_H), (VI). A solution of 1.32 g (7.1 mmol) (**V**), 50% aq H_3PO_2 (6.06 g) and 0.043 g (0.26 mmol) AIBN in MeOH (20 mL) was refluxed under argon atmosphere for 3 h, diluted with H_2O (30 mL), evaporated to 15 mL in vacuo and extracted with EtOAc (4 × 7 mL). Combined EtOAc extracts were washed with H_2O (3 mL), evaporated to dryness in vacuo and the residue was coevaporated with H_2O (15 mL) in vacuo. To the final residue 20% HCl (60 mL) was added, refluxed under argon atmosphere for 3 h and extracted with Et_2O (2 × 15 mL). The aqueous layer was evaporated to dryness in vacuo, the residue was dissolved in H_2O (20 mL) and purified on Dowex 50Wx8 (H^+-form) resin (*V* = 35 mL) by eluting first with H_2O and then with 0.5 M HCl to isolate pure GABA-P_H. Fractions containing GABA-P_H hydrochloride were combined, evaporated to dryness in vacuo, the residue was dissolved in minimal volume of EtOH and propylene oxide added dropwise until precipitation started. The mixture was allowed to stand at +4°C until complete precipitation, the solid was filtered off, recrystallized from H_2O/EtOH and dried in vacuo over P_2O_5 to give (**VI**) (0.53 g, 60%), mp 208–211°C (lit.: 209–213°C [22]), R_f 0.42 (A). ^{1}H NMR (D_2O) δ: 6.89 (dt, 1 H, J_{HP} 510.7, J_{HH} 1.5, P-H), 3.00 (t, 1 H, J_{HH} 7.4, C$\underline{H}_2$NH$_2$), 1.85–1.74 (m, 2 H, C$\underline{H}_2$CH$_2$NH$_2$), 1.59–1.49 (m, 2 H, C$\underline{H}_2$P). ^{13}C NMR (D_2O) δ: 42.83 d (J_{CP} 17.5), 30.99 d (J_{CP} 89.8), 22.15 s. ^{31}P NMR (D_2O) δ: 28.57.

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