# Phosphinic Acid Analogues of Methylaspartic and Methylglutamic Acids as Antibacterials

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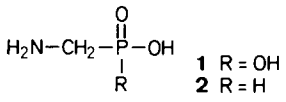
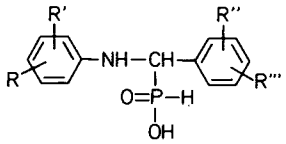
**Abstract:** DL-1-Amino-1-methyl-3-carboxypropanephosphinic acid, a bioisostere of α-methylglutamic acid, was synthesized. This compound, the corresponding α-methylaspartic acid analogue and their precursors were tested for antibacterial activity. The methylaspartic acid analogue gave a MIC of 400 and 800 μg/ml against *B. subtilis* and *P. aeroginosa*, respectively.

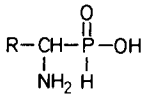
Phosphorus analogues of amino acids have been previously investigated; however, most studies have involved aminophosphonic acids, which bear the $P(O)(OH)_2$ grouping (1–3), such as aminomethanephosphonic acid (**1**). Only in recent years have aminophosphinic acids, which possess a P(O)H(OH) moiety, been prepared. The glycine analogue (**2**) was synthesized in 1964 by a procedure that yields limited types of products (4). In 1961 Linfield et al. prepared phosphinic acids bearing substituted α-anilino and phenyl groups (**3**) by the addition of hypophosphorous acid to the appropriate Schiff bases and reported antibacterial properties associated with some of these derivatives (5). Except for the glycine compound the only *N*-unsubstituted α-aminophosphinic acids reported are those by Khomutov and Osipova (6) and Bayliss et al. (7) that were synthesized by the addition of hypophosphorous acid to oximes and diphenylmethylimines, respectively. The amino acid analogues resulting from the latter process were investigated for antibacterial properties with alanine (**4**), valine (**5**) and methionine (**6**) derivatives displaying significant activity. In addition, the effect of the valine analogue was reversed by valine, an indication that a false substrate mechanism was operative.



**1** R = OH
**2** R = H



**3**



**4** R = $CH_3$
**5** R = $CH(CH_3)_2$
**6** R = $(CH_2)_2SCH_3$

A minor modification of the structure of biologically important substances often results in an inhibitory effect. This is true for α-methylaspartic and glutamic acids which have shown antibacterial properties (8, 9) and ability to inhibit many enzyme systems such as transaminases (10, 11).

## Materials and Methods

Melting points were taken on a Thomas Hoover apparatus and are corrected to reference standards. IR spectra were determined on a Perkin-Elmer 283 spectrophotometer, and absorptions are reported in $cm^{-1}$. $^1H$-NMR spectra were recorded on a Varian FT-80A spectrometer using trimethylsilane as an internal standard. Chemical shifts are reported in δ units and coupling constants in Hz. Elemental analyses were performed by Atlantic Microlab Inc., Atlanta, GA. Sensi-Discs from BBI, Cockeysville, MD were used as standard in the antibacterial screening. AG50W-X8 resin was used for chromatography.

### *Syntheses*

*DL-1-Benzhydrylamino-1-methyl-3-carbethoxypropanephosphinic Acid* (**12**). According to a previously reported procedure (7), a suspension of benzhydrylammonium hypophosphite (12.4 g, 50 mmoles) in *p*-dioxane (60 ml) was heated to 100° C, and ethyl levulinate (7.2 g, 50 mmoles) in *p*-dioxane (10 ml) was added dropwise under nitrogen. The mixture was heated to 120 °C and distilled with azeoptropic removal of water using a Dean-Stark receiver. The remaining solution was cooled to 25°C and diluted with an equal volume of ethanol. The resulting solid was collected on a filter, washed with ethanol and dried to yield 6.5 g (35 %) of **12** as a white powder: mp 161–163° C; IR (KBr): 2600 (P-OH), 2300 (P-H), 1730 (C=O), 1590 (C=C), 1200 (P=O); NMR (DMSO-$d_6$): 1.11–1.34 (d,3H,$CH_3$,$J_{P\text{-}CH}$ =18), 1.43 (t,3H,$CH_3$), 1.91–2.62 (bm,4H,$2CH_2$), 3.88 (q,2H,$CH_2$), 5.43 (s,1H,CH), 7.16–7.64(m,10H,2Ph), 3.84, 10.16 (1:1d,lH, $J_{P\text{-}H}$=502).

Anal. Calc. for $C_{20}H_{26}NO_4P$: C, 63.96; H, 6.98; N, 3.73. Found: C, 64.06; H, 7.01; N, 3.71.

*DL-1-Benzhydrylamino-l-methyl-3-carboxypropanephosphinic Acid* (**11**). Compound **12** (8.0 g, 21.3 mmoles) was dissolved in 0.5 N sodium hydroxide (150 ml) and the solution was heated at 85–95° C for 4 h. The solution was cooled, filtered and the filtrate adjusted to pH <1 with conc. hydrochloride acid. The resulting precipitate was collected on a filter, washed repeatedly with water and then with ethanol and ether. After drying for 12 h in a 55–60°C vacuum oven, 7.1 g (96 %) of the product was obtained as a white powder: mp 154–156° C; IR (KBr): 3450 (OH), 2500–2700 (P-OH, COOH), 2320 (P-H), 1690 (C=O), 1595 (C=C), 1180 (P=O); NMR (DMSO-$d_6$): 1.17–1.38 (d,3H,$CH_3$,$J_{2P\text{-}CH}$=17), 2.09–2.66 (m,4H,$2CH_2$), 5.47 (s,1H,CH), 7.21–7.68 (m,10H, 2Ph), 3.93, 10.25 (1:1d, 1H,$J_{P\text{-}H}$= 502).

Anal. Calc. for $C_{18}H_{22}NO_4P{\cdot}H_2O$: C, 59.17; H, 6.62; N, 3.83. Found C, 59.02; H, 6.66; N, 3.77.

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*DL-1-Amino-1-methyl-3-carboxypropanephosphinic Acid* (**10**).
According to a previously described method (12) compound **11** (4.5 g, 13.0 mmoles) and 48% aqueous hydrobromic acid (50 ml) were heated at 80° C for 4 h. The benzhydryl bromide was extracted with ether and then benzene and the aqueous phase evaporated to dryness at 30° C and 1 mm pressure. The residue was dissolved in ethanol (20 ml), cooled to 5–10° C and treated with propylene oxide until the precipitation was complete. The precipitate was collected on a filter and washed with ethanol to yield a white powder, which was chromatographed using aqueous hydrochloric acid (pH 3.0) as the eluent to yield 1.06 g (46%) of **10** as a white powder: mp 164–165°C (dec.); IR (KBr): 3150 ($NH^+_3$), 2500–2700 (P-OH, COOH), 2340 (P-H), 1700 (C=O), 1170, 1180, 1225 (P=O); NMR (DMSO-$d_6$): 1.08 (d,3H,$CH_3$,$J_{P\text{-}CH}$ = 15.6); 1.67–2.40 (m,4H,$2CH_2$), 3.43, 9.71 (1; 1d,1H,$J_{P\text{-}H}$ = 500.2).

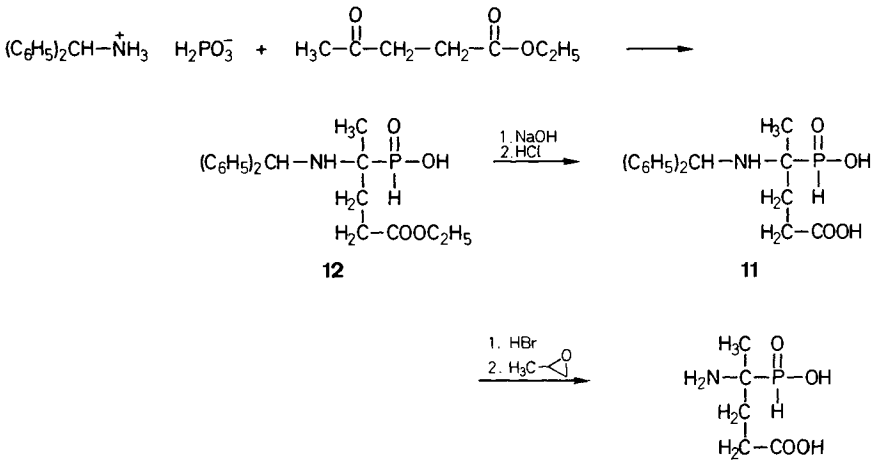
Anal. Calc. for $C_5H_{12}NO_4P$: C, 33.17; H, 6.63; N, 7.74. Found: C, 33.21; H, 6.69; N, 7.63.

*Antibacterial Testing*

Screening was conducted using a previously reported method (13). The discs were treated with a solution of 2.5 mg/ml of **8, 9, 11** or **12** in 1:1 Tris buffer (pH 8) – ethanol or of **7** or **10** in Tris buffer (pH 8), and the solvents were used as standards. Cephalothin, gentamicin and ampicillin discs were used as standards. The serial broth dilution tubes were examined daily during three days of incubation.

## Results and Discussion

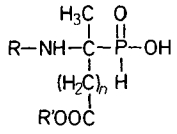
In a previous paper (12) the synthesis of DL-1-methyl-2-carboxyethanephosphinic acid (**7**) and its precursors (**8** and **9**) were reported. In this present study the preparation of **10**, the next higher homolog of **7**, and its intermediates **11** and **12** are shown. In the present case ethyl levulinate is employed in lieu of ethyl acetoacetate for condensation with the hypophosphorous salt of 1,1-diphenylmethylamine (Scheme 1). Compound **10**, despite its higher carbon content, was more hygroscopic than **7** and required cation exchange column chromatography for purification.




**Scheme 1**

Both **7** and **10** represent the bioisosteric replacement of the carboxy group of an amino acid with a phosphinic acid moiety. Phosphinic acids can be considered the closest analogues of carboxylic acids and this relationship can be demonstrated, for example, by a comparison of $pK_a$ values. Propylphosphinic acid has a $pK_a$ value of 3.46 compared to butyric and propylphosphonic acid with values of 4.82 and 2.49, respectively (14).

In view of the antibacterial activity previously reported for α-aminophosphinic acid (**3–6**), compounds **7–12** were screened against four microorganisms, *S. aureus, E. coli, B. subtilis* and *P. aeroginosa* using the disc method. Only **7** produced zones of inhibition and was subsequently shown by the serial broth dilution method to have weak antibacterial activity with minimum inhibition concentrations of 400 and 800 μg/ml against the latter two organisms.



**7** R=H, R'=H, n=1
**8** R=$(C_6H_5)_2CH$, R'=H, n=1
**9** R=$(C_6H_5)_2CH$, R'=$C_2H_5$, n=1
**10** R=H, R'=H, n=2
**11** R=$(C_6H_5)_2CH$, R'=H, n=2
**12** R=$(C_6H_5)CH$, R'=$C_2H_5$, n=2

**Acknowledgment**

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