

Synthesis, NMR, X-ray crystallography and bioactivity of some α -aminophosphonates

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Abstract

The synthesis of some new α -aminophosphonates (**4a-l**) was accomplished by one pot reaction of equimolar quantities of 2-amino-4-chlorophenol (**1**), various aromatic aldehydes (**2a-l**) and diethylphosphite (**3**) in dry toluene at reflux temperature. Products **4a-l** were characterized by IR, ¹H, ¹³C, ³¹P NMR and in the case of **4g** by X-ray crystal diffraction data.

Keywords: α -Aminophosphonates, antibacterial

Introduction

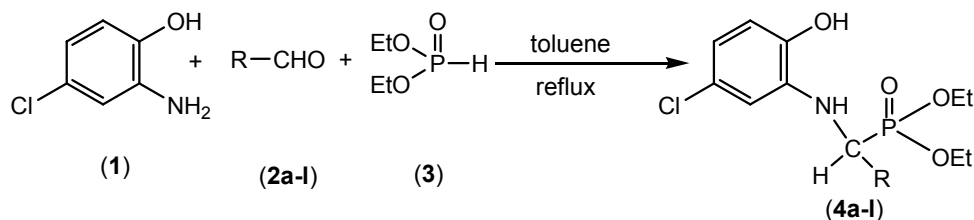
Organophosphorus compounds have found a wide range of application in the areas of industry, agriculture and medicinal chemistry owing to their unique physical and biological properties. Their utility in organic synthesis as reagents and potential synthons is gaining increased attention.¹

The synthesis of α -aminophosphonates has attracted much interest because of their biological activity and structural analogy to α -amino acids.² They also act as peptide mimics,³ enzyme inhibitors,⁴ haptens of catalytic antibodies,⁵ antibiotics and pharmacological agents⁶ and the analogues of amino acids, aminophosphonic, and aminophosphinic acids are of considerable interest due to their properties in agrochemistry as plant growth regulators and herbicides.⁷ As a result, a variety of synthetic approaches⁸ have been developed for the synthesis of α -aminophosphonates. Of these methods, the nucleophilic addition of phosphates with imines, catalyzed by an acid or a base is one of the most convenient methods. In our work we synthesized α -aminophosphonates under mild conditions without catalyst.

Results and Discussion

The synthetic route for the title compounds (**4a-l**) involves reaction of equimolar quantities of 2-amino-4-chlorophenol (**1**), various aromatic aldehydes (**2a-l**) and diethylphosphite (**3**) in toluene at reflux temperature (Scheme-1). The chemical structures of all the new compounds are confirmed by elemental analysis, IR, ^1H NMR, ^{13}C NMR and ^{31}P NMR spectral data. Compounds **4a-l** exhibited characteristic IR stretching frequencies in the region⁹ 1191-1264, 742-758 and 3274-3404 cm^{-1} for P=O, P-C_(aliphatic) and N-H respectively.

The aromatic protons of the two benzene rings of α -aminophosphonates (**4a-l**) showed a complex multiplet at δ 6.41-7.51. The P-C-H proton signal appeared as multiplet^{10a} at δ 4.09-4.19 due to its coupling with phosphorus and proton of N-H. The N-H proton signal appeared at δ 4.83-4.99 as doublet ($J = 23.6$ - 24.4 Hz) for the compounds **4a-c**, **4h-i** and **4l** and as a singlet in compounds **4d-g** and **4j-k**. The methylene protons of POCH_2CH_3 showed a multiplet and methyl protons of POCH_2CH_3 showed a triplet in the region 3.79-4.09 and 1.11-1.66 ppm respectively.¹⁰ The carbon chemical shifts for P-C-H, P-O- CH_2 - CH_3 and P-O CH_2 - CH_3 in the title compounds observed in the expected region.^{10b} ^{31}P NMR signals¹¹ appeared in the region 22.30-27.44 ppm for all the compounds **4a-l**.



Compd.	R	Compd.	R
2a & 4a	4-FC ₆ H ₄	2g & 4g	3,4-(OMe) ₂ C ₆ H ₃
2b & 4b	4-ClC ₆ H ₄	2h & 4h	C ₆ H ₅
2c & 4c	4-BrC ₆ H ₄	2i & 4i	C ₄ H ₃ S
2d & 4d	4-MeC ₆ H ₄	2j & 4j	3-OH-4-OMeC ₆ H ₃
2e & 4e	4-AllyloxyC ₆ H ₄	2k & 4k	3-OHC ₆ H ₄
2f & 4f	4-OMeC ₆ H ₄	2l & 4l	2-OHC ₆ H ₄

Scheme 1

X-ray crystal structure determination of **4g**

Intensity data were collected on a Siemens SMART CCD diffractometer with monochromated Mo-K α radiation (λ 0.7107 Å). Structures were solved by direct methods and routinely developed and refined (on F^2) using the SHELX-97 programs.¹² Transparent colourless crystals were obtained from methanol. Crystal data: C₁₉H₂₅ClNO₆P, M_r 429.82 g mol^{-1} , monoclinic, space group P2(1)/n, a = 13.8079(19), b = 9.7872(14), c = 16.907(2) Å, β = 103.832(2)°,

$V = 2218.6(5) \text{ \AA}^3$, $D_{\text{calc}} = 1.287 \text{ g cm}^{-3}$, $Z = 4$, $\mu (\text{Mo K}\alpha) = 0.277 \text{ mm}^{-1}$, Size $0.16 \times 0.19 \times 0.53 \text{ mm}$, $F(000) = 904$, $T = 293 \text{ K}$. Total data 20878, unique data 3912 ($R_{\text{int}} = 0.0208$) in the range $2.18^\circ \leq \theta \leq 25^\circ$, 3912 with $I \geq 2 \sigma(I)$, empirically corrected for absorption and other effects. All non-H atoms anisotropic: H atoms in calculated positions. $R_1 (I > 2 \sigma(I)) = 0.0705$, wR_2 (all data) 0.2224, $\text{GoF} = 1.071$, largest residual feature $|1.154| \text{ e\AA}^{-3}$. The structure is illustrated in Figure 1. Full details of the structure determinations have been deposited with the Cambridge Crystallographic Data Centre as CCDC- 639459 (**4g**). Copies of this information may be obtained free of charge from The Director, CCDC, 12 Union Road, Cambridge CB2 1EZ, UK (fax: +44 1223 336 033; e-mail: deposit@ccdc.cam.ac.uk or www: <http://www.ccdc.cam.ac.uk>).

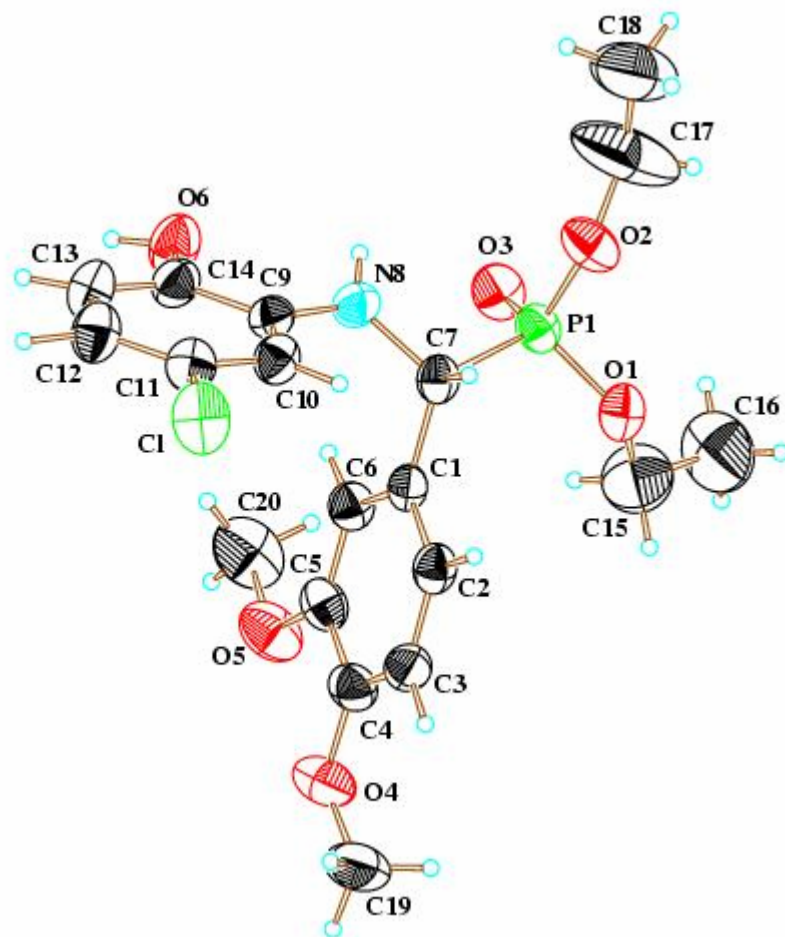


Figure 1. Molecular structures of **4g** showing the atom-labelling scheme

The O3-P1-O1 [$115.4(2)^\circ$], O3-P1-O2 [$115.1(2)^\circ$] and O3-P1-C7 [$112.5(1)^\circ$] bond angles are larger than O1-P1-O2 [$102.6(2)^\circ$], O1-P1-C7 [$106.4(1)^\circ$] and O2-P1-C7 [$103.6(4)^\circ$] bond angles indicating a distorted tetrahedral configuration for the phosphorus atom. The deformation of the PO_3C tetrahedron in this molecule may be due to the steric effects of ethoxy groups in which their atoms exist in distorted conformation. The $\text{C}(\text{sp}^3)\text{-N}$ bond length $1.445(4) \text{ \AA}$ is

intermediate between the normal C-N single¹³ [1.47 (1) Å] and double bonds¹⁴ [1.28 (1) Å] which is indicative of its partial double bond character. The interplanar angle between the aniline moiety and the phenyl ring bonded to the stereogenic center C7 is 83.7(1)°. The methoxy groups are almost coplanar with the benzene ring to which they are substituted, with a C6–C5–O5–C20 & C3–C4–O4–C19 torsion angles of 4.8(6)° & -1.6(5)°. Chloro and hydroxy groups are also coplanar with aniline moiety. In the crystal structure, molecules are linked by intramolecular N–H···O and intermolecular C–H···O and O–H···O hydrogen bonds.

Antibacterial activity

The compounds were diluted in DMSO for bioassay. Solvent control was included although no antibacterial activity has been noted for the solvent employed. Ciprofloxacin (Hi-media) controls were included to compare its activity with that of **4a-l**. All samples were tested in triplicate and average results were recorded.

The compounds were assayed for antibacterial activity against six registered bacterial isolates which were obtained from the NCIM (National Collection of Industrial Microorganisms, National Chemical Laboratories, Pune-411 003, India). The bacteria included Gram positive bacterial isolates-*Staphylococcus aureus* (NCIM No: 5021, ATCC No. 25923), *Bacillus subtilis* (NCIM No: 2063, ATCC No. 6633) and four Gram negative bacteria-*Escherichia coli* (NCIM No: 2931, ATCC No. 25922), *Pseudomonas aeruginosa* (NCIM No: 5029, ATCC No: 27853), *Salmonella typhimurium* (NCIM No: 2501, ATCC No: 23564) and *Klebsiella pneumoniae* (NCIM No: 2957). The bacteria were grown on Hi-media nutrient agar and sub cultured as needed.

Experimental Section

General Procedures. Chemicals procured were of commercial quality and were distilled / recrystallised before use. Elemental analyses were performed by the Central Drug Research Institute, Lucknow, India. IR spectra were recorded as KBr pellets or Nujol mulls on a Perkin Elmer 283 unit. The ¹H, ¹³C and ³¹P NMR spectra were taken on AMX 400 MHz spectrometer operating at 400 MHz for ¹H, 100 MHz for ¹³C and 161.9 MHz for ³¹P. All these compounds were dissolved in methanol-*d*₄. The chemical shifts in δ were referenced to TMS (¹H and ¹³C) and 85% H₃PO₄ (³¹P).

General experimental procedure for products 4a-l

To a stirred solution of 2-amino-4-chlorophenol (**1**) (0.72 g, 0.005 mol) various aromatic aldehydes (**2a-l**) (0.005 mol) in anhydrous toluene (15 mL) was added dropwise. Stirring was continued at room temperature for 1 h. Then diethylphosphite (**3**) (0.7 g, 0.005 mol) in anhydrous toluene (15 mL) was added dropwise. Stirring was continued at room temperature for another 0.5 h, later the mixture was heated under reflux for 4-6 h. After completion of reaction (monitored by TLC) and the solvent was removed under reduced pressure. The resulting residue

was purified by column chromatography on silica gel using petroleum ether-ethylacetate (8:2) as eluent. The spectral and analytical data for these compounds are presented below.

[(5-Chloro-2-hydroxyphenylamino)-(4-fluorophenyl)methyl]diethylphosphonate (4a). Yield (%): 65. mp: 145-147°C. IR: 743 (P-C_{aliphatic}), 1217 (P=O), 3379 (N-H) cm⁻¹. ¹H NMR: δ 1.28 (t, *J* = 7.0 Hz, 3H, P-OCH₂CH₃), 1.18 (t, *J* = 7.0 Hz, 3H, P-OCH₂CH₃), 3.86-4.04 (m, 4H, P-OCH₂CH₃), 4.13-4.17 (m, 1H, P-CH), 4.98 (d, *J* = 24.1 Hz, 1H, NH), 6.47 (d, *J* = 2.1 Hz, 2H, Ar-H), 6.60 (d, *J* = 8.9 Hz, 1H, Ar-H), 7.08 (t, *J* = 8.6 Hz, 2H, Ar-H), 7.47 (t, *J* = 2.8 Hz, 2H, Ar-H). ³¹P NMR: δ 22.88. Anal. Calcd for C₁₇H₂₀ClFNO₄P: C, 52.34; H, 5.20; N, 3.61. Found: C, 52.16; H, 5.15; N, 3.54.

[(5-Chloro-2-hydroxyphenylamino)-(4-chlorophenyl)methyl]diethylphosphonate (4b). Yield (%): 71. mp: 161-163°C. IR: 744 (P-C_{aliphatic}), 1199 (P=O), 3399 (N-H) cm⁻¹. ¹H NMR: δ 1.28 (t, *J* = 7.0 Hz, 3H, P-OCH₂CH₃), 1.18 (t, *J* = 7.0 Hz, 3H, P-OCH₂CH₃), 3.88-4.05 (m, 4H, P-OCH₂CH₃), 4.12-4.17 (m, 1H, P-CH), 4.99 (d, *J* = 24.4 Hz, 1H, NH), 6.45 (t, *J* = 2.0 Hz, 1H, Ar-H), 6.47 (d, *J* = 2.1 Hz, 1H, Ar-H), 6.61 (d, *J* = 8.1 Hz, 1H, Ar-H), 7.34 (d, *J* = 8.3 Hz, 2H, Ar-H), 7.43 (dd, *J* = 6.5, 2.0 Hz, 2H, Ar-H). ³¹P NMR: δ 22.59. Anal. Calcd for C₁₇H₂₀Cl₂NO₄P: C, 50.51; H, 4.99; N, 3.47. Found: C, 50.32; H, 4.93; N, 3.40.

[(5-Chloro-2-hydroxyphenylamino)-(4-bromophenyl)methyl]diethylphosphonate (4c). Yield (%): 78. mp: 188-190°C. IR: 743 (P-C_{aliphatic}), 1213 (P=O), 3399 (N-H) cm⁻¹. ¹H NMR: δ 1.28 (t, *J* = 7.0 Hz, 3H, P-OCH₂CH₃), 1.19 (t, *J* = 7.0 Hz, 3H, P-OCH₂CH₃), 3.88-4.05 (m, 4H, P-OCH₂CH₃), 4.11-4.17 (m, 1H, P-CH), 4.98 (d, *J* = 24.4 Hz, 1H, NH), 6.45 (t, *J* = 2.2 Hz, 1H, Ar-H), 6.48 (d, *J* = 2.3 Hz, 1H, Ar-H), 6.61 (d, *J* = 8.1 Hz, 1H, Ar-H), 7.37 (dd, *J* = 6.5, 2.1 Hz, 2H, Ar-H), 7.50 (d, *J* = 8.4 Hz, 2H, Ar-H). ¹³C NMR: δ 16.5 (d, *J* = 8.2 Hz), 55.9 (d, *J* = 151.3 Hz), 65.0 (d, *J* = 6.5 Hz), 113.1, 115.5, 118.6, 122.9, 125.8, 130.9, 132.6, 136.4, 144.9. ³¹P NMR: δ 22.32. Anal. Calcd for C₁₇H₂₀BrClNO₄P: C, 45.51; H, 4.49; N, 3.12. Found: C, 45.68; H, 4.43; N, 3.09.

[(5-Chloro-2-hydroxyphenylamino)-*p*-tolylmethyl]diethylphosphonate (4d). Yield (%): 69. mp: 148-150°C. IR: 742 (P-C_{aliphatic}), 1198 (P=O), 3399 (N-H) cm⁻¹. ¹H NMR: δ 1.28 (t, *J* = 7.0 Hz, 3H, P-OCH₂CH₃), 1.15 (t, *J* = 7.0 Hz, 3H, P-OCH₂CH₃), 2.30 (s, 3H, CH₃), 3.79-4.00 (m, 4H, P-OCH₂CH₃), 4.09-4.14 (m, 1H, P-CH), 4.92 (s, 1H, NH), 6.43 (d, *J* = 2.2 Hz, 2H, Ar-H), 6.50 (d, *J* = 8.9 Hz, 1H, Ar-H), 7.16 (d, *J* = 7.8 Hz, 2H, Ar-H), 7.32 (dd, *J* = 6.0, 2.0 Hz, 2H, Ar-H). ¹³C NMR: δ 16.5 (d, *J* = 8.5 Hz), 21.1, 56.2 (d, *J* = 152.1 Hz), 64.7 (d, *J* = 7.6 Hz), 113.1, 115.4, 118.3, 125.8, 129.0, 130.2, 133.7, 139.1, 144.9. ³¹P NMR: δ 23.32. Anal. Calcd for C₁₈H₂₃ClNO₄P: C, 56.33; H, 6.04; N, 3.65. Found: C, 56.44; H, 6.12; N, 3.58.

[(5-Chloro-2-hydroxyphenylamino)-(4-allyloxyphenyl)methyl]diethylphosphonate (4e). Yield (%): 67. mp: 120-122°C. IR: 747 (P-C_{aliphatic}), 1161 (P=O), 3388 (N-H) cm⁻¹. ¹H NMR: δ 1.28 (t, *J* = 7.0 Hz, 3H, P-OCH₂CH₃), 1.56 (t, *J* = 7.0 Hz, 3H, P-OCH₂CH₃), 3.79-3.86 (m, 4H, P-OCH₂CH₃), 4.09-4.14 (m, 1H, P-CH), 4.90 (s, 1H, NH), [4.51 (d, *J* = 5.1 Hz, 2H), 5.22 (d, *J* = 10.6 Hz, 1H), 5.37 (d, *J* = 17.2 Hz, 1H), 5.99-6.07 (m, 1H)] allyloxy-H, 6.44 (d, *J* = 2.3 Hz, 1H, Ar-H), 6.45 (d, *J* = 9.1 Hz, 1H, Ar-H), 6.59 (d, *J* = 8.0 Hz, 1H, Ar-H), 6.91 (d, *J* = 8.4 Hz, 2H, Ar-H), 7.35 (dd, *J* = 6.5, 2.0 Hz, 2H, Ar-H). ¹³C NMR: δ 16.6 (d, *J* = 9.0 Hz), 55.8 (d, *J* =

153.0 Hz), 64.7 (d, $J = 6.9$ Hz), 69.9, 113.1, 115.4, 115.9, 117.5, 118.3, 125.8, 128.8, 130.2, 134.8, 144.9, 160.0. ^{31}P NMR : δ 23.45. Anal. Calcd for $\text{C}_{20}\text{H}_{25}\text{ClNO}_5\text{P}$: C, 56.41; H, 5.92; N, 3.29. Found : C, 56.38; H, 5.88; N, 3.24.

[(5-Chloro-2-hydroxyphenylamino)-(4-methoxyphenyl)methyl]diethylphosphonate (4f). Yield (%) : 76. mp : 177-179°C. IR : 754 (P-C_{aliphatic}), 1247 (P=O), 3382 (N-H) cm^{-1} . ^1H NMR : δ 1.27 (t, $J = 7.0$ Hz, 3H, P-OCH₂CH₃), 1.14 (t, $J = 7.0$ Hz, 3H, P-OCH₂CH₃), 3.81-4.00 (m, 4H, P-OCH₂CH₃), 3.76 (s, 3H, OCH₃), 4.09-4.15 (m, 1H, P-CH), 4.90 (s, 1H, NH), 6.43 (d, $J = 2.2$ Hz, 1H, Ar-H), 6.44 (d, $J = 8.0$ Hz, 1H, Ar-H), 6.59 (d, $J = 7.9$ Hz, 1H, Ar-H), 6.89 (d, $J = 8.5$ Hz, 2H, Ar-H), 7.35 (dd, $J = 6.5, 2.1$ Hz, 2H, Ar-H). ^{13}C NMR : δ 16.7, 55.8 (d, $J = 153.4$ Hz), 56.1, 65.0, 113.2, 115.2, 115.7, 118.6, 128.5, 130.2. ^{31}P NMR : δ 26.77. Anal. Calcd for $\text{C}_{18}\text{H}_{23}\text{ClNO}_5\text{P}$: C, 54.07; H, 5.80; N, 3.50. Found : C, 54.17; H, 5.75; N, 3.48.

[(5-Chloro-2-hydroxyphenylamino)-(3,4-dimethoxyphenyl)methyl]diethylphosphonate (4g). Yield (%) : 82. mp : 109-111°C. IR : 752 (P-C_{aliphatic}), 1264 (P=O), 3372 (N-H) cm^{-1} . ^1H NMR : δ 1.29 (t, $J = 7.0$ Hz, 3H, P-OCH₂CH₃), 1.17 (t, $J = 7.0$ Hz, 3H, P-OCH₂CH₃), 3.80 (s, 3H, OCH₃), 3.82 (s, 3H, OCH₃), 3.84-4.03 (m, 4H, P-OCH₂CH₃), 4.11-4.16 (m, 1H, P-CH), 4.92 (s, 1H, NH), 6.45 (dd, $J = 6.5, 2.3$ Hz, 1H, Ar-H), 6.48 (d, $J = 9.0$ Hz, 1H, Ar-H), 6.60 (d, $J = 8.2$ Hz, 1H, Ar-H), 6.91-7.06 (m, 3H, Ar-H). ^{31}P NMR : δ 26.67. Anal. Calcd for $\text{C}_{19}\text{H}_{25}\text{ClNO}_6\text{P}$: C, 53.09; H, 5.86; N, 3.26. Found : C, 53.12; H, 5.78; N, 3.17.

[(5-Chloro-2-hydroxyphenylamino)-phenylmethyl]diethylphosphonate (4h). Yield (%) : 81. mp : 125-127°C. IR : 758 (P-C_{aliphatic}), 1216 (P=O), 3404 (N-H) cm^{-1} . ^1H NMR : δ 1.28 (t, $J = 7.0$ Hz, 3H, P-OCH₂CH₃), 1.15 (t, $J = 7.0$ Hz, 3H, P-OCH₂CH₃), 3.79-4.01 (m, 4H, P-OCH₂CH₃), 4.11-4.15 (m, 1H, P-CH), 4.95 (d, $J = 24.2$ Hz, 1H, NH), 6.43 (d, $J = 2.3$ Hz, 1H, Ar-H), 6.45 (d, $J = 6.5$ Hz, 1H, Ar-H), 6.60 (d, $J = 8.9$ Hz, 1H, Ar-H), 7.26-7.46 (m, 5H, Ar-H). ^{31}P NMR : δ 26.44. Anal. Calcd for $\text{C}_{17}\text{H}_{21}\text{ClNO}_4\text{P}$: C, 55.22; H, 5.72; N, 3.79. Found : C, 55.13; H, 5.63; N, 3.68.

[(5-Chloro-2-hydroxyphenylamino)-thiophen-2-yl-methyl]diethylphosphonate (4i). Yield (%) : 66. mp : 105-107°C. IR : 751 (P-C_{aliphatic}), 1214 (P=O), 3348 (N-H) cm^{-1} . ^1H NMR : δ 1.28 (t, $J = 7.0$ Hz, 3H, P-OCH₂CH₃), 1.20 (t, $J = 7.0$ Hz, 3H, P-OCH₂CH₃), 3.91-4.09 (m, 4H, P-OCH₂CH₃), 4.11-4.19 (m, 1H, P-CH), 5.28 (d, $J = 23.6$ Hz, 1H, NH), 6.49 (dd, $J = 6.2, 2.3$ Hz, 1H, Ar-H), 6.1 (d, $J = 8.3$ Hz, 1H, Ar-H), 6.63 (d, $J = 2.2$ Hz, 1H, Ar-H), 6.98-7.34 (m, 3H, Ar-H). ^{31}P NMR : δ 22.30. Anal. Calcd for $\text{C}_{15}\text{H}_{19}\text{ClNO}_4\text{PS}$: C, 47.94; H, 5.10; N, 3.73. Found : C, 48.06; H, 5.17; N, 3.80.

[(5-Chloro-2-hydroxyphenylamino)-(3-hydroxy-4-methoxyphenyl)methyl]diethylphosphonate (4j). Yield (%) : 70. mp : 143-145°C. IR : 745 (P-C_{aliphatic}), 1196 (P=O), 3359 (N-H) cm^{-1} . ^1H NMR : δ 1.28 (t, $J = 7.0$ Hz, 3H, P-OCH₂CH₃), 1.17 (t, $J = 7.0$ Hz, 3H, P-OCH₂CH₃), 3.83 (s, 3H, OCH₃), 3.95-4.01 (m, 4H, P-OCH₂CH₃), 4.11-4.18 (m, 1H, P-CH), 4.84 (s, 1H, NH), 6.45 (dd, $J = 6.1, 2.0$ Hz, 1H, Ar-H), 6.50 (s, 1H, Ar-H), 6.60 (d, $J = 8.2$ Hz, 1H, Ar-H), 6.77 (d, $J = 7.9$ Hz, 1H, Ar-H), 6.88 (d, $J = 6.0$ Hz, 1H, Ar-H), 6.89 (d, $J = 2.0$ Hz, 1H, Ar-H), 7.03 (s, 1H, Ar-H). ^{31}P NMR : δ 26.94. Anal. Calcd for $\text{C}_{18}\text{H}_{23}\text{ClNO}_6\text{P}$: C, 51.99; H, 5.58; N, 3.37. Found : C, 52.09; H, 5.56; N, 3.43.

[(5-Chloro-2-hydroxyphenylamino)-(3-hydroxyphenyl)methyl]diethylphosphonate (4k).
Yield (%): 74. mp : 128-130°C. IR : 756 (P-C_{aliphatic}), 1201 (P=O), 3314 (N-H) cm⁻¹. ¹H NMR : δ 1.28 (t, *J* = 7.0 Hz, 3H, P-OCH₂CH₃), 1.66 (t, *J* = 7.0 Hz, 3H, P-OCH₂CH₃), 3.80-4.01 (m, 4H, P-OCH₂CH₃), 4.11-4.16 (m, 1H, P-CH), 4.84 (s, 1H, NH), 6.45 (d, *J* = 2.0 Hz, 2H, Ar-H), 6.59 (d, *J* = 8.7 Hz, 1H, Ar-H), 6.70-7.17 (m, 4H, Ar-H). ³¹P NMR: δ 22.60. Anal. Calcd for C₁₇H₂₁ClNO₅P : C, 52.93; H, 5.49; N, 3.63. Found : C, 53.06; H, 5.55; N, 3.70.

[(5-Chloro-2-hydroxyphenylamino)-(2-hydroxyphenyl)methyl]diethylphosphonate (4l).
Yield (%): 65. mp : 134-135°C. IR : 754 (P-C_{aliphatic}), 1191 (P=O), 3274 (N-H) cm⁻¹. ¹H NMR : δ 1.30 (t, *J* = 7.0 Hz, 3H, P-OCH₂CH₃), 1.11 (t, *J* = 7.0 Hz, 3H, P-OCH₂CH₃), 3.79-4.00 (m, 4H, P-OCH₂CH₃), 4.13-4.18 (m, 1H, P-CH), 5.32 (d, *J* = 24.2 Hz, 1H, NH), 6.42 (dd, *J* = 6.0, 2.3 Hz, 1H, Ar-H), 6.51 (d, *J* = 2.3 Hz, 1H, Ar-H), 6.57 (d, *J* = 8.2 Hz, 1H, Ar-H), 6.77-7.31 (m, 4H, Ar-H). ³¹P NMR: δ 27.44. Anal. Calcd for C₁₇H₂₁ClNO₅P : C, 52.93; H, 5.49; N, 3.63. Found : C, 53.04; H, 5.54; N, 3.71.

Disc diffusion bioassay

Suspension of approximately 1.5 x 10⁸ bacterial cells per mL were used. In sterile normal saline was prepared as described by Forbes *et al*¹⁵ and 1.5 mL of it was uniformly spreaded on Nutrient Agar (Hi-media) in 12 x 1.2 cm glass Petri dishes, left aside for 15 min and excess of suspension was then drained and discarded. For the agar disc diffusion method, the test compound was introduced onto the disc and then allowed to dry to completely saturate the disc with the test compound. Then the disc was introduced onto the upper layer of the medium with the bacteria. The petridishes were incubated overnight at 37°C for 24 hrs. Bioactivity was determined by measuring Diameter of Inhibition Zones (DIZ) in mm. The compounds **4a-l** were taken as 20 and 40 µg / mL concentrations and were evaluated by disc method. Each test was done in triplicate and the mean of the diameter of the inhibition zones was calculated. Controls included the use of solvent without test compounds although no antibacterial activity was noted for the solvent employed in the test.¹⁶

Determination of minimum inhibitory concentration (MIC)

The lowest concentration (highest dilution) of the compounds **4a-l** that produced no visible bacterial growth (no turbidity) when compared with the control tubes¹⁶ MIC was determined for the compounds **4a-l**. Compounds **4a-l** in concentrations of 0.1-4.1 mg / mL in steps of 100 µg / mL were evaluated. Specifically 0.1 mL of standardized inoculum (1-2 x 10⁷ CFU / mL) was added to each tube. The tubes were incubated aerobically at 37°C for 18-24 hr. Two control tubes were maintained for each test batch. These included antibiotic control (tube containing compounds **4a-l** and the growth medium without inoculum) and organism control (the tube containing the growth medium, physiological saline and the inoculum).

Conclusions

We synthesized new α -aminophosphonates **4a-l** in high yields by Kabachnik-Fields reaction without using any catalyst. All of them showed low antibacterial activity against selected bacteria, while compounds **4a** exhibited significant antibacterial activity even at lower concentration against both Gram negative and Gram positive bacteria. X-ray crystallographic studies for **4g** proved distorted tetrahedral configuration for P in these compounds.

Acknowledgements

The authors express thanks to Prof. C. Devendranath Reddy and Dr. C. Naga Raju, Dept. of Chemistry, S. V. University, Tirupati, for helpful discussions and SIF, IISc, Bangalore, for providing the NMR spectra.

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