< Supplementary Information>

α-Aminophosphonates as novel antileishmanial chemotypes: synthesis, biological evaluation, and CoMFA studies

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Table of Contents

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1. Experimental Procedure about Biology

Screening Protocol

1.1. Chemicals

RPMI 1640 HEPES modified medium, antibiotics and DMSO was purchased from Sigma Chemicals Co. Foetal bovine serum (FBS) was purchased from Biological Industries. Absolute ethanol was supplied from Merck and had no effect on the morphology and proliferation of the renapromastigotes and macrophages.

1.2. Parasites and cell lines

Leishmania donovani wild type (WT, MHOM/80/IN/Dd8) promastigotes were cultured at 24°C in RPMI-1640, phenol red free medium supplemented with 0.2 % sodium bicarbonate, 100 µg/mL penicillin, 100 µg/mL streptomycin, 100 µg/mL gentamycin and 10 % heat inactivated foetal bovine serum and the pH of the medium was maintained at pH 7.2. The cells were washed in phosphate buffer saline (PBS) (pH 7.4) at 6000g for 10 mins at 24°C. The numbers of cells were counted on Neubauer hemocytometer. J774A.1 macrophages cell line was maintained in RPMI-1640 medium with 10% FBS (pH 7.2).

1.3. Antileishmanial assay / *In vitro* promastigote assay

Leishmanicidal activity of the compounds was investigated *in vitro* using MTT (Thiazolyl Blue Tetrazolium Bromide) assay[1] against *L. donovani* promastigotes. Exponentially growing cells were seeded to 96 well plate (*i.e.* $2x10^5$ cells /200 µL /well) and kept for growing at 24°C for 48 hours. Compounds were dissolved in absolute ethanol and the final concentration was maintained at 5% v/v. After 48 hours, different dilutions (10-100 µM) of each compound was prepared and added to wells in triplicates and further kept for incubation at 24°C for 48 hours. MTT was added to a final concentration of 400 µg/mL and further incubated at 37°C for 4 hours. The cells were centrifuged at 3000g for 10 minutes and the supernatant was removed. The resultant formazan formed was dissolved in 100 µL DMSO and absorbance was read at OD_{540} nm on a microplate reader. The IC_{50} values of the post treated viable cells were calculated relative to the untreated control cells and the results were expressed as the concentration of the compound inhibiting 50% of the parasite growth. Amphotericin B was used as the standard antileishmanial drug for data analysis

1.4. *In vitro* cytotoxicity assay

*J*774A.1 macrophage cell line was used for the *in vitro* cytotoxicity using MTT assay.[1] The cells were seeded on a 96 well tissue culture plate (*i.e.* $1x10^5$ cells / 100 µL / well) and incubated at 37°C in humidified air with 5 % CO_2 for 24 hours. The cells were then exposed to the different dilutions of the drugs and kept for incubation for 48 hours at 37°C. The cells were centrifuged at 3000g for 10 minutes and the supernatant was removed without disturbing the cell layer. The resultant formazan was dissolved in 100 µL DMSO in each well and microplate was kept for shaking for proper mixing at 100 rpm. The final absorbance was read at OD_{540} nm. The cytotoxicity was expressed as CC_{50} *i.e.* 50 % reduction in the viability of the cells after treatment with the drugs in comparison to the controls.

2. CoMFA Studies

2.1 Data Set:

For 3D-QSAR analyses, 23 compounds with defined IC_{50} values were employed. Total compounds were divided into a training set of 18 compounds, and a test set of 5 compounds. The most important step in the QSAR is the selection of a suitable training set with wide activity range, responsible for determining the quality of the generated QSAR model. The calculated pIC_{50} values (pIC_{50} = -logIC_{50}) of dataset spanned across a small range from 4.04 to 5.15. These activity values were rescaled to the range of four log units to develop statistically reliable model. To rescale the activity data following formula was employed.[2] The test compounds were selected manually considering the structural diversity and wide range of activity in the data set.

Rescaling data set

Existing series = a-b, Rescale series = x-y, Rescale value = x + (n-a)*[(y-x)/(b-a)]

=3.92+(n-4.04)*[3.6]

Where n is query. In our study, x= 3.92, y=7.92, a= 4.04, b= 5.15

2.2 Molecular modeling

All the molecular modeling calculations were performed using SYBYL 7.1 (Tripos Inc., USA) molecular modeling package installed on a Silicon Graphics Fuel Workstation running IRIX 6.5.[3] Structures of all monophosphonate derivatives were generated using sketch molecule module. There are mainly two lower energy conformations of compound **1** (R and S). Energy comparison showed that S conformation was more stable than R conformation. To better understand the activities of these compounds both the conformations were used to build the model. Compound **1** being the most potent was selected as a template molecule. (Fig S1) Two different sets each of S and R configuration were individually evaluated. Geometry-optimized was carried out by applying Tripos molecular mechanics force field with con*J*ugate gradient method. No constraints were applied on the internal geometries of the molecules. The minimization was terminated when the energy gradient convergence criterion of 0.001 kcal/mol was reached or when the 10,000 steps minimization cycle was exceeded. The fragment that was used as the common structure is shown in Fig. S2. Gasteiger- Hückel charges were applied to all the molecules of dataset, used for 3D-QSAR studies.

Figure S1. Confirmation of the template molecule compound **1**

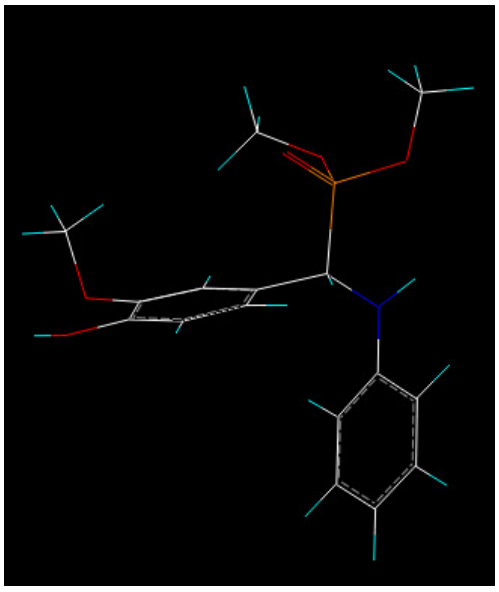
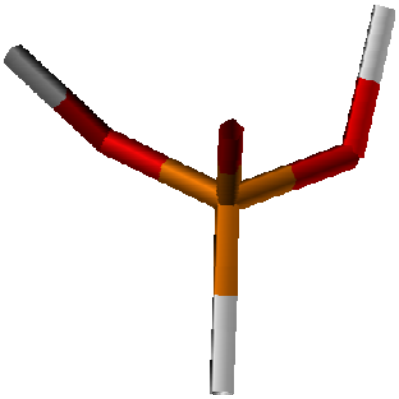


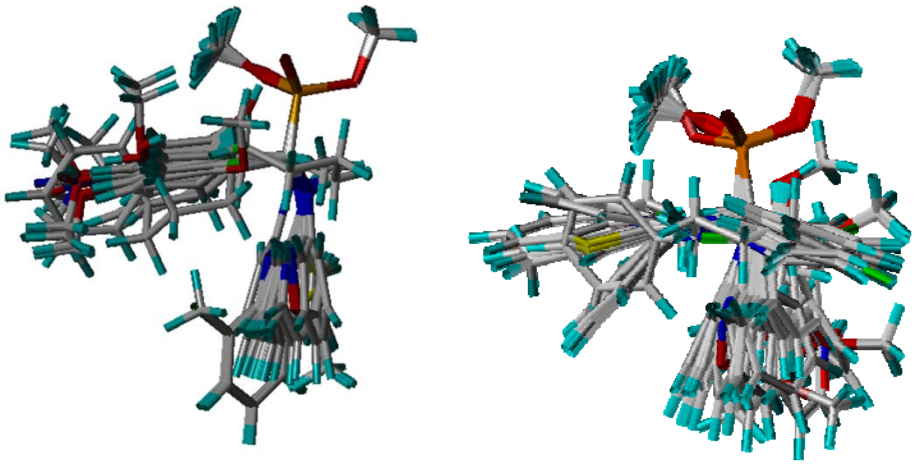
Figure S2. Common structure used during alignment of all monophosphonate derivatives



2.3 Molecular alignment for 3D-QSAR analysis

Molecular alignment is considered one of the most sensitive parameter in CoMFA analysis. Each molecule was aligned to the lowest energy conformation of the most active compound, **1**, by performing an rms fitting of the O-P-O backbone atoms of each conformer to those of the template using the alignment function of the Sybyl7.1. The aligned molecules obtained through pairwise superpositioning using the maximum common subgroup method, placed all molecules in the same reference frame as the reference compound shown in Fig. S3.

Figure S3. Alignment of all molecules used for CoMFA molecular field generation



S- Conformation

R- Conformation

1.5.Calculation of CoMFA descriptors

For generating CoMFA contour maps, a 3D cubic lattice with grid spacing of 2.0 Å was created around aligned molecule. For CoMFA, the steric (The Lennard-*J*ones potential) and electrostatic (Coulombic potential) field at each lattice point was calculated using the default probe, a sp^3 carbon atom with a charge of +1 and a van der Waals radius of 1.52 Å. The steric and electrostatic fields at these lattice points were calculated using Tripos force field. Energy values for these fields were truncated at 30 kcal/mol. The minimum column filtering was set to 2.5 kcal/mol to improve the signal-to noise ratio by retaining best fit model while omitting those lattice points whose energy variation was below this threshold. The optimal number of components in the final model was determined by using leave-one-out (LOO), a cross-validation method. The non-cross-validated conventional analysis was produced with the

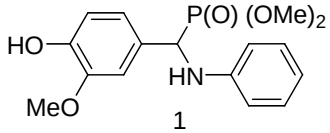
optimal number of components equal to that yielding the highest r^2_{cv}, Final model was evaluated based on the standard error of estimation values (SEE), non-cross validated r^2_{ncv} value and also by the F test value.

The predictive abilities were determined from a test set of 5 compounds. pIC_{50} values for test molecules were predicted by using developed CoMFA. The predictive correlation coefficient (r^2_{pred}), based on the molecules of test set, was calculated according to the equation shown below;

$$r^2_{pred} = \frac{(SD - PRESS)}{(SD)}$$

Where SD is the sum of squared deviations between the inhibitory activities of the test set and mean activities of the training molecules and PRESS is the sum of squared deviations between predicted and actual activity values for each molecule in the test set.

3. Chemical structure of training and test set molecules (Test Set *)



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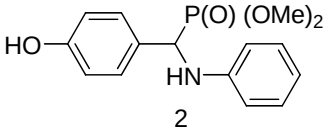

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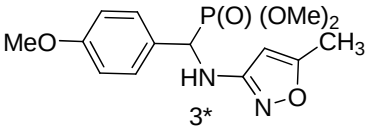

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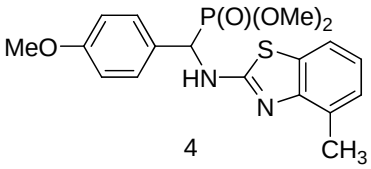

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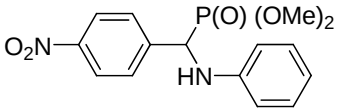

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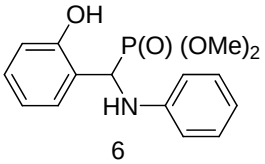

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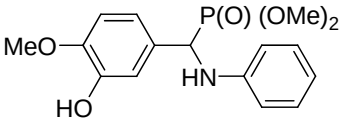

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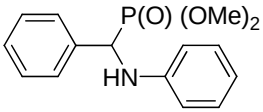

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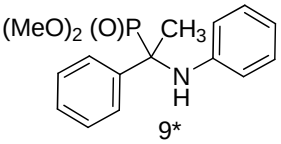

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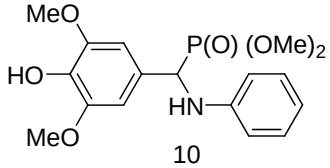

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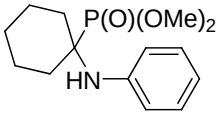

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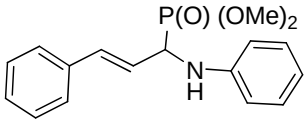

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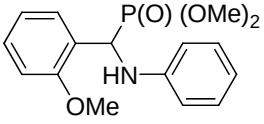

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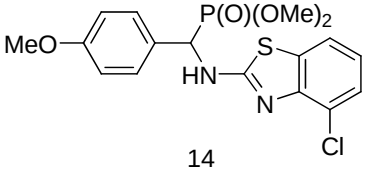

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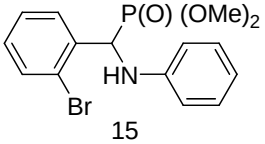

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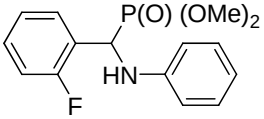

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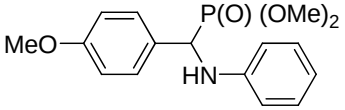

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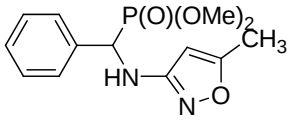

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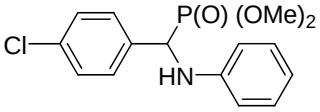

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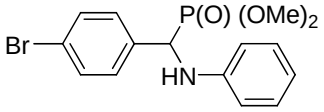

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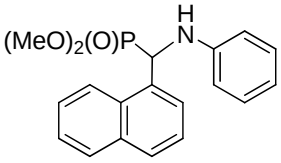

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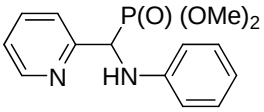

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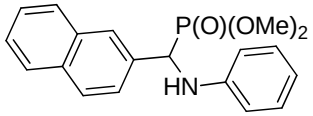

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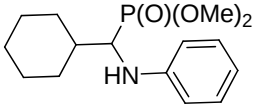

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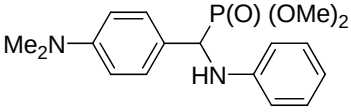

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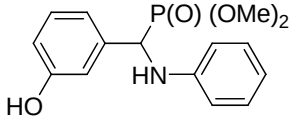


26

4. General Information about Synthesis

The glassware to be used in reactions was thoroughly washed and dried in an oven and the experiments were carried out with the required precautions. Chemicals and all solvents were commercially available (Aldrich Chemical, Merck AG, Fluka and S-D Fine Chemicals) and used without further purification/ drying unless otherwise mentioned. Ma*J*ority of the compounds are already reported in literature and the spectral characterization of these synthesized α-aminophosphonates are well comparable with them. ^{1}H and ^{13}C NMR spectra were recorded on a Bruker Avance 300/400 MHz NMR spectrometer in $CDCl_3$ with residual undeuterated solvent ($CDCl_3$: 7.26/77.0) using TMS as an internal standard. Chemical shifts (δ) are given in ppm and *J* values are given in Hz. Splitting patterns were designated as s, singlet; bs, broad singlet; d, doublet; dd, doublet of doublet; t, triplet; m, multiplet. Evaporation of solvents was performed at reduced pressure, using a Büchi rotary evaporator. The isolated crude product was either recrystallized with ethanol or column purified using flash chromatography (Model: SP-1; Make: Biotage, Swedane)

Synthetic Procedure

General procedure for the synthesis of α-aminophosphonates in the absence of any catalyst: The mixture of the aldehyde (2.5 mmol), amine (2.5 mmol) and dimethylphosphite (3 mmol) was stirred magnetically under neat condition at room temperature for the required time mentioned (table 1). After the completion of the reaction (TLC), the reaction mixture was extracted with EtOAc (3 × 10 mL) and the combined EtOAc extracts were washed with water (2 × 10 mL) to remove any unreacted dimethylphosphite. The EtOAc extracts were dried (anh Na_2SO_4), filtered, and concentrated under rotary vacuum evaporation to afford the crude product which was further purified either by recrystallization with ethanol or column chromatography (20:80 EtOAc-Hexane).

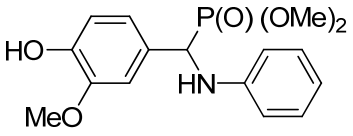
General procedure for $Mg(ClO_4)_2$-catalysed synthesis of α-aminophosphonates (3, 4, 9, 11, 14 and 18): The mixture of the carbonyl compound (2.5 mmol) and anh $Mg(ClO_4)_2$ (5 mol %) was magnetically stirred with for 10-15 min followed by the addition of the amine (2.5 mmol) and dimethyl phosphite (3 mmol). The resultant mixture was stirred magnetically at room temperature or 80°C for the specified time till completion of the reaction (TLC). The mixture was diluted with water (2 mL) and extracted with EtOAc (3 × 10 mL). The combined EtOAc extracts were washed with water (2 × 10 mL) to remove any unreacted dimethylphosphite, dried (anh Na_2SO_4), filtered, and concentrated under rotary vacuum evaporation to afford the crude product which was further purified either by recrystallization with ethanol or column chromatography (20:80 EtOAc-Hexane).

5. Compound Characterizations

Compound code **1, 2, 5, 6, 7, 8, 9, 10, 11, 12, 13, 14, 15, 17, 19, 20, 21, 22, 23, 25** are already known molecules.

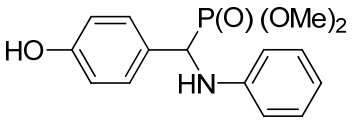
Spectral and physical property data:

[(4-Hydroxy-3-methoxy-phenyl)-phenyl amino-methyl]-phosphonic acid dimethyl ester, (table-1, entry 1)[9]:



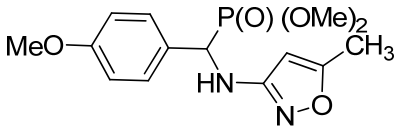
Whitish yellow solid, Yield: 0.296 g, 88%; ^{1}H NMR ($CDCl_3$, 300 MHz) δ: 3.47 (3H, d, *J* = 10.49Hz), 3.74 (3H, d, *J* = 10.6Hz), 3.83(3H, s) 4.68 - 4.76 (1H, d, *J* = 23.8 Hz,), 6.60-7.26 (8H, m). HRMS (ESI) m/z calcd for $C_{16}H_{20}NO_5PNa^+$ $[M+Na]^+$, 360.0971; Observed: 360.0977. Column purified: 70:30 EtOAc: Hexane.

Dimethyl (4-hydroxyphenyl) (phenylamino) methylphosphonate, (table 1, entry 2)[5]:



Fade white solid; Yield: 0.265 gm, 86%; ^{1}H NMR ($CDCl_3$, 300 MHz) δ: 3.48 (3H, d, *J* = 10.5 Hz), 3.7 (3H, d, *J* = 10.6 Hz), 4.71- 4.79(1H, d, $^1J_{PH}$ = 24 Hz), 6.5-7.25 (9H, m). MS (Maldi) m/z 308$(M+1)^+$, 198 [M^+-P(O)$(OMe)_2$]. Column purified with 80:20 EtOAc: Hexane.

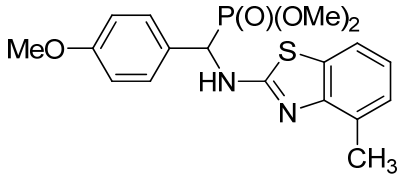
Dimethyl (4-methoxyphenyl)(5-methylisoxazol-3-ylamino)methylphosphonate, (table 1, entry 3):



White solid, Yield: 0.255 gm, 78%, **m.p: 153-154°C**; ^{1}H NMR ($CDCl_3$, 300 MHz) δ: 2.23(3H, S), 3.47(3H, d, *J* = 10.49 Hz), 3.78 and 3.80 (2S, 6H), 4.90-5.09 (1H, m, CH-P), 5.10 (1H, bs, -NH), 5.48 (1H, s), 6.86 (2H, d, *J* = 8.21 Hz), 7.40 (2H, d, *J* = 7.23 Hz); IR KBr) ν: 1632 cm-1, 3434.7 cm-1; MS

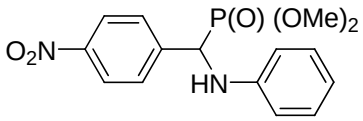
(GCMS, EI) m/z 327(M+1)$^+$, 217 [(M$^+$-P(O)(OMe)$_2$]. HRMS (ESI) m/z calcd for $C_{14}H_{19}N_2O_5PNa^+$ [M+Na]$^+$, 349.0924; Observed: 349.0922; Column purified using (70:30) EtOAc: Hexane.

Dimethyl (4-methoxyphenyl) (4-methylbenzo[d] thiazol-2-ylamino) methyl phosphonate, (table 1, entry 4):



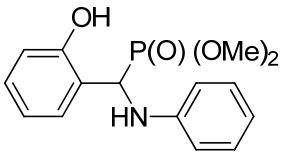
White solid, Yield: 0.291 gm, 76%, **m. p.: 85-86°C**; ^{1}H NMR ($CDCl_3$, 300 MHz) δ: 1.66 (1H, bs, NH), 2.54 (3H, s), 3.54(3H, d, *J* = 10.56 Hz), 3.77-3.81(6H, m), 5.30 (1H, d, *J* = 21.7 Hz), 6.88-7.49 (7H, m); IR (KBr) ν: 1537.4 cm^{-1}, 3233.7 cm^{-1}; MS (MALDI) m/z 393.6(M+1)$^+$, 283.5 [(M$^+$-P(O)(OMe)$_2$]. HRMS (ESI) m/z calcd for $C_{18}H_{21}N_2O_4PNa^+$ [M+Na]$^+$, 415.0852; Observed: 415.0857.

Dimethyl (4-nitrophenyl) (phenylamino) methyl phosphonate, (table 1, entry 5)[6]:



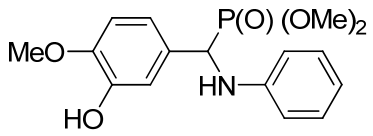
Yellow solid, Yield: 0.271gm, 83%; ^{1}H NMR ($CDCl_3$, 400 MHz) δ: 3.60 (3H, d, *J*= 10.76 Hz), 3.78 (3H, d, *J* = 10.76 Hz), 4.80 (H, d, $^1J_{PH}$ = 25.4 Hz), 6.53-8.21 (9H, m); ^{13}C NMR ($CDCl_3$, 100 MHz) δ: 53.87, 54.20, 54.81, 56.30 113.84, 126.59, 119.26, 123.91, 128.6, 129.4, 143.6, 145.3, 145.4, 147.6. MS (Maldi TOF TOF) m/z 337(M$^+$), 227 [(M$^+$-P(O)(OMe)$_2$].

Dimethyl (2-hydroxyphenyl) (phenylamino) methyl phosphonate, (table 1, entry 6)[7]:



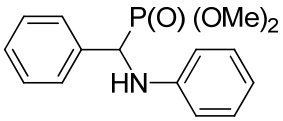
Light green solid, Yield: 0.271 gm, 88 %; ^{1}H NMR ($CDCl_3$, 400 MHz) δ: 3.67 (3H, d, *J* =10.6 Hz), 3.74(3H, d, *J* =10.5 Hz), 5.98(1H, d, *J* =22.9 Hz), 6.66 - 8.80 (9H, m); ^{13}C NMR ($CDCl_3$, 75 MHz) δ: 53.1, 53.8, 54.5, 114.5, 118.5, 119.6, 120.8, 121.2, 128.9, 129.3, 129.7 , 145.9, 155.7; MS (Maldi) m/z 308 (M+1)$^+$.

[(3-Hydroxy-4-methoxy-phenyl)-phenylamino-methyl]-phosphonic acid dimethyl ester, (table 1, entry 7)[10]:



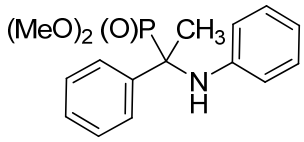
Greenish white solid, Yield: 0.29 gm, 86 %; ^{1}H NMR ($CDCl_3$, 400 MHz) δ: 3.45(3H, d, J = 10.64 Hz), 3.74 (3H, d, J = 10.5 Hz), 3.85(3H, s) 4.67 (1H, d, $^{1}J_{PH}$ = 24.3 Hz,), 6.58-7.11(8H, m); ^{13}C NMR ($CDCl_3$, 100 MHz) δ: 55.83, 55.9, 54.3, 55.9, , 110.9, 113.9, 114.2, 118.5, 119.4, 128.4, 129.2, 145.9, 146.0, 146.2, 146.6; MS (Maldi) m/z 338$(M+1)^+$. HRMS (ESI) m/z calcd for $C_{16}H_{20}NO_5PNa^+$ $[M+Na]^+$, 360.0971; Observed: 360.0978.

Dimethyl (phenyl-phenylaminomethyl) phosphonate, (table 1, entry 8)[5]:



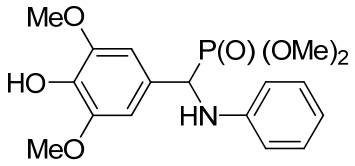
Fade white Solid, Yield: 0.273gm, 94%; ^{1}H NMR ($CDCl_3$, 300 MHz) δ: 3.43 (3H, d, J = 10.9 Hz), 3.73 (3H, d, J = 12.7 Hz), 4.76-4.8 (2H, m), 6.58-7.47 (10H, m); ^{13}C NMR ($CDCl_3$, 75 MHz) δ: 55.3, 55.4, 55.2, 57.2, 114.4, 119.1, 128.3, 128.6, 129.2, 129.7, 136.1, 146.5, 146.7; MS (APCI) m/z 291 $(M)^+$, 182 $[(M^+$-P(O)$(OMe)_2]$.

(1-Phenyl-1-phenylamino-ethyl)-phosphonic acid dimethyl ester, (table 1, entry 9)[4]:



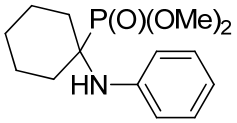
Fade yellow solid, Yield: 0.24 gm, 77 %; ^{1}H NMR ($CDCl_3$, 300 MHz) δ: 1.97 (3H, d, J = 16.5 Hz), 3.53-3.66(6H, m) , 6.38-7.81(10H, m); ^{13}C NMR ($CDCl_3$, 75 MHz) δ : 20.84, 54.69, 117.28, 119.06, 128.07, 128.65, 128.96, 129.22; MS (APCI) m/z 305(M^+), 196 $[(M^+$-P(O)$(OMe)_2]$.

[(4-Hydroxy-3, 5-dimethoxy-phenyl)- phenylamino-methyl]-phosphonic acid dimethyl ester, (table 1, entry 10)[4]:



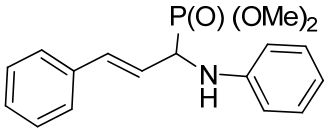
Brownish yellow solid, Yield: 0.29 gm, 79 %; IR (KBr) ν:1603 cm^{-1}, 2952 cm^{-1}, 3321 cm^{-1}, 3494.6 cm^{-1}; ^{1}H NMR ($CDCl_3$, 300 MHz) δ: 3.48 (3H, d, *J* = 10.56 Hz), 3.74 (3H, d, *J* = 10.64 Hz), 3.84(6H, s), 4.65-4.73(1H, d, $^1J_{PH}$ = 23.8 Hz), 6.60 - 7.14 (7H, m); ^{13}C NMR ($CDCl_3$, 75 MHz) δ: 54.30, 54.52, 55.38, 56.90, 57.40, 105.02, 114.43, 119.15, 127.02, 129.73, 135.09, 146.67, 146.86, 147.85; MS (Maldi) m/z 367(M^+), 257 [(M^+-P(O)$(OMe)_2$].

(1-Phenylamino-cyclohexyl)-phosphonic acid dimethyl ester, (table 1, entry 11)[4]:



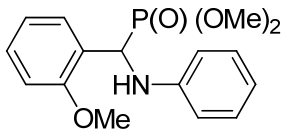
Fade yellow solid, yield: 0.26 gm, 91%; ^{1}H NMR ($CDCl_3$, 300 MHz)δ: 1.22-2.24 (10H,*m)*, 3.65 (6H, *d,J*= 10.15), 6.78-7.2 (5H,*m*); ^{13}C NMR ($CDCl_3$, 75 MHz) : 15.32, 15.47, 20.47, 25.78, 48.41, 48.52, 51.88, 54.01, 113.87, 114.96, 124.25, 141.13;MS (APCI) *m/z* 283 (M^+), 174[(M + -P(O)$(OMe)_2$].

(3-Phenyl-1-phenylamino-allyl)-phosphonic acid dimethyl ester, (table 1, entry 12)[5]:



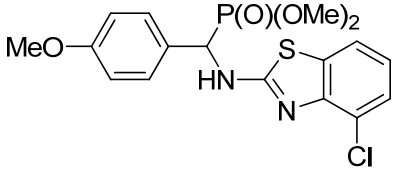
Yellowish brown solid, Yield: 0.28 gm, 87%; ^{1}H NMR ($CDCl_3$, 300 MHz) δ: 3.72-3.83 (6H, m,) 4.47-4.57 (1H, *dd*, *J*= 5.8 Hz, 25.46 Hz), 6.21-7.37(12H, m); ^{13}C NMR ($CDCl_3$, 75 MHz) δ:52.96, 53.96, 54.58, 55.01,114.22, 118.91, 123.35, 127.04, 128.23, 128.98, 129.60, 133.65, 133.82, 136.56, 146.66, 146.82; MS (MALDI TOF TOF) *m/z* 317 (M^+), 208 [(M^+-P(O)$(OMe)_2$].

Dimethyl (2-methoxyphenyl) (phenylamino) methylphosphonate, (table 1, entry 13)[8]:



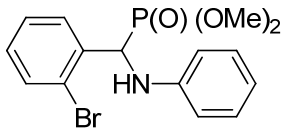
White solid, yield: 0.29 gm, 89%; ^{1}H NMR ($CDCl_3$, 400 MHz) δ: 3.41(3H, d, *J* = 10.5 Hz), 3.8 (3H, d, *J* = 10. 7), 3.94 (3H, s), 4.82 (1H, bs, NH), 5.4-5.5(1H, dd, *J* = 7.2 & 24.7 Hz), 6.6 -7.5 (9H, *m*); ^{13}C NMR ($CDCl_3$, 100 MHz) : 46.8(C-P, d, *J*= 154 Hz), 53.67, 53.8, 55.9, 110.6, 113.6, 118.3, 112.1, 124.1, 128.2, 129.1, 146.0, 157.2; MS (APCI) *m/z* 321 (M^+), 212 [(M^+-P(O)($OMe)_2$].

[(4-Chloro-benzothiazol-2-ylamino)-(4-methoxy-phenyl)-methyl]-phosphonic acid dimethyl ester, (table 1, entry 14)[4]:



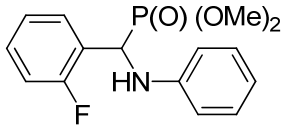
Fade white solid, Yield: 0.32 gm, 77%**;** ^{1}H NMR ($CDCl_3$, 300 MHz)δ: 3.56(3H, d, *J* = 10.5 Hz), 3.75 (3H, s), 3.82(3H, d, *J* = 10.8 Hz), 5.50-5.57, 1H, d, *J*= 21.5 Hz), 6.85-6.97(3H, m), 7.25(1H, d, *J* = 7.7 Hz), 7.36 (1H, d, *J* = 7.7 Hz), 7.5-7.56(2H, m), 7.9 (1H, bs); ^{13}C NMR ($CDCl_3$, 75 MHz) δ: 54.3, 54.7, 55.7, 56.4, 114.7, 119.7, 122.6, 124.3, 126.6, 127.1, 130.2, 132.9, 149.5, 160.2, 166.6; MS (MALDI) *m/z* 413(M^++1), 303 [(M^+-P(O)($OMe)_2$].

Dimethyl (2-bromophenyl) (phenylamino) methylphosphonate, (table 1, entry 15)[11]:



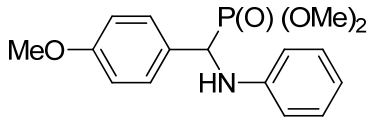
Greenish white solid, Yield: 0.30 gm, 82%; ^{1}H NMR ($CDCl_3$, 400 MHz) δ: 3.4 (3H, d, *J* = 10.6 Hz), 3.8 (3H, d, *J* = 10.8 Hz), 5.0 (1H, bs, NH), 5.36 (1H, d, *J* = 24.6 Hz), 6.5-7.6 (9H, m); ^{13}C NMR ($CDCl_3$, 100 MHz) : 53.2-54.7 (C-P, d, *J* = 152 Hz), 53.8, 53.9, 113.7, 118.7, 124.5, 124.6, 128.0, 129.1, 129.3, 129.6, 132.9, 135.3, 145.4, 145.6. HRMS (ESI) m/z calcd for $C_{15}H_{17}BrNO_3P$ $[M+Na]^+$, 392.0022; Observed: 392.0033.

Dimethyl (2-fluorophenyl) (phenylamino) methylphosphonate, (table 1, entry 16):



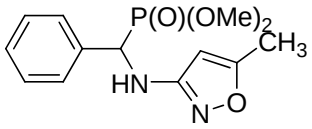
Light greenish blue solid, yield: 0.28 gm, 91%, **m. p.: 69-70°C;** ^{1}H NMR ($CDCl_3$, 400 MHz) δ: 3.5(3H, d, *J* = 10.6 Hz), 3.8 (3H, d, *J* = 10. 7), 5.2 (1H, d, *J* = 24.7 Hz), 6.6-7.5 (9H, *m*); ^{13}C NMR ($CDCl_3$, 100 MHz) : 46.9(C-P, d, J= 155 Hz), 53.7, 54.0, 113.6, 115.2, 115.4, 118.8, 123.2, 123.3, 124.7, 128.8, 129.3, 129.7,145.5, 145.7, 159.5, 161.9; MS (APCI) *m/z* 309 (M^+), 200[(M^+-P(O)($OMe)_2$]. HRMS (ESI) m/z calcd for $C_{15}H_{17}FNO_3PNa^+$ [M+Na^+], 332.0822; Observed: 392.0827.

[(4-Methoxy-phenyl) –phenylamino -methyl]-phosphonic acid dimethyl ester, (table 1, entry 17)[5]:



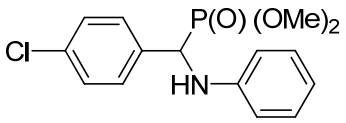
Green solid, Yield: 0.29 gm, 91%**;** ^{1}H NMR ($CDCl_3$, 300 MHz)δ: 3.47-3.50 (3H, *d*, *J*= 10.5Hz), 3.73-3.77 (6H, 2s), 4.70-4.78 (1H, d, $^{1}J_{PH}$ = 23.88 Hz). 6.58-7.39 (9H, m); ^{13}C NMR ($CDCl_3$, 75 MHz)δ: 54.31, 55.71, 56.52, 114.47, 114.74, 119.05, 127.87, 129.45, 129.73, 146.58, 146.78, 159.96; MS (EI) *m/z* 321 $(M)^+$, 212[(M^+-P(O)($OMe)_2$].

Dimethyl(5-methylisoxazol-3-ylamino) (phenyl) methylphosphonate, (table 1, entry 18):



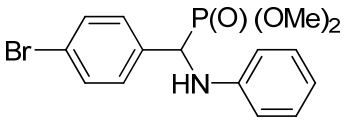
White solid: 0.23 gm, 76 %, **m. p.: 137-139°C;** ^{1}H NMR ($CDCl_3$, 300 MHz) δ: 2.24 (3H, S), 3.45 (3H, d, *J* = 10.54 Hz), 3.76 (3H, d, *J* = 10.7), 4.92-5.06 (2H, m), 5.5 (1H, s), 7.29-7.49 (5H, m); IR (KBr) ν: 1626.9 cm^{-1}, 3273.1 cm^{-1}; MS (GCMS, EI) m/z 297(M^++1), 187 [(M^+-P(O)($OMe)_2$]. HRMS (ESI) m/z calcd for $C_{13}H_{17}N_2O_4PNa^+$ [M + Na^+], 319.0818; Observed: 319.0816.

Dimethyl (4-chlorophenyl) (phenylamino) methylphosphonate, (table 1, entry 19)[5]:



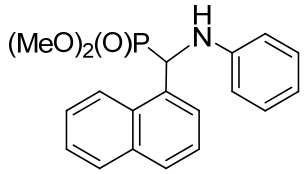
Yellowish green solid, Yield: 0.30 gm, 92%; ^{1}H NMR ($CDCl_3$, 300 MHz) δ: 3.52 (3H, d, *J* = 10.63 Hz),3.75 (3H, d, *J* = 10.79 Hz), 4.73-4.81 (1H, d, $^1J_{PH}$ = 24.51 Hz), 6.45-7.43(9H, m); MS (Maldi TOF TOF) *m/z* 370(M^+), 216 [(M^+-P(O)$(OMe)_2$].

Dimethyl (4-bromophenyl) (phenylamino) methylphosphonate, (table 1, entry 20)[8]:



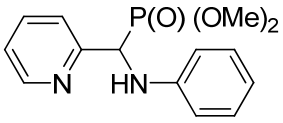
White solid, Yield: 0.31 gm, 81%, ^{1}H NMR ($CDCl_3$, 400 MHz) δ: 3.5(3H, d, *J* = 10.6 Hz), 3.7 (3H, d, *J* = 10.7 Hz), 4.7-4.8 (2H, m, merged proton with NH), 6.5-7.5 (9H, m); ^{13}C NMR ($CDCl_3$, 100 MHz) : 53.8, 53.9, 54.5 (C-P, d, J= 150 Hz), 113.8, 118.8, 122.1, 129.3, 129.4, 129.5, 131.9, 134.8, 145.6, 145.8.

(Naphthalen-1-yl-phenylamino-methyl)-phosphonic acid dimethyl ester, (table 1, entry 21)[4]:



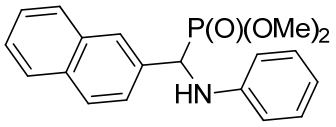
Light yellow solid, Yield: 0.28 gm, 83%; ^{1}H NMR ($CDCl_3$, 300 MHz)δ: 3.12(3H, d, *J*= 10.46Hz), 3.79(3H, *J*=10.75Hz), 5.62-5.70(1H, d, $^1J_{PH}$ = 24.091Hz), 5.62-8.23(12H, m); ^{13}C NMR ($CDCl_3$, 75 MHz) δ: 50.13, 52.16, 53.78, 113.62, , 118.45, 122.58, 125.75, 126.51, 126.90, 127.71, 128.71, 129.11, 129.22, 131.40, 133.87, 145.83, 146.02 ; MS (APCI) *m/z* 341(M^+), 232 [(M^+-P(O)$(OMe)_2$].

(Phenylamino-pyridin-2-yl-methyl)-phosphonic acid dimethyl ester, (table 1, entry 22)[5]:



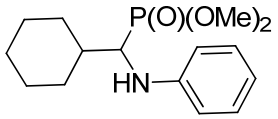
Fade brownish-white solid, Yield: 0.24 gm,81%**;** ^{1}H NMR ($CDCl_3$, 300 MHz) δ: 3.61(3H, *d, J* = 10.6 Hz), 3.76 (3H, *d*, *J* = 10.6 Hz), 4.98-5.07(1H, *dd, J* =6.7Hz , 22.0 Hz), 5.2 (1H, s, NH) 3.83(3H, s) 6.68-7.68 (8H, m), 8.6(1H, *d, J* = 4.7 Hz); MS (APCI) *m/z* 292$(M+1)^+$, 183 $[(M^+$-P(O) $(OMe)_2]$.

(Naphthalen-2-yl-phenylamino-methyl)-phosphonic acid dimethyl ester, (table 1, entry 23)[4]:



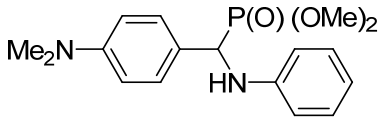
Yellowish green solid, Yield: 0.29 gm, 85 %; IR(KBr) ν:1521.9 cm^{-1} , 1603.5 cm^{-1}, 2950 cm^{-1}, 3293.5 cm^{-1}; ^{1}H NMR ($CDCl_3$, 300 MHz)δ: 3.43(3H, d, *J* = 10.57 Hz), 3.74(3H, d, *J* = 10.67 Hz), 4.92-5.00(1H, d, $^1J_{PH}$ = 24.45 Hz), 6.62-7.93 (12H, m) ; ^{13}C NMR ($CDCl_3$, 75 MHz) δ: 55.81, 54.90, 56.90, 125.42, 125.47, 126.16, 126.25, 126.86, 126.96, 127.68, 128.01, 128.56, 129.20, 133.13, 133.26, 145.98, 146.18; MS (Maldi TOF TOF) *m/z* 341(M^+), 232 $[(M^+$-P(O)$(OMe)_2]$.

Dimethyl cyclohexyl (phenylamino) methylphosphonate, (table 1, entry 24):



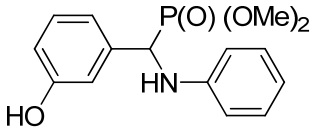
Fade white solid, Yield: 0.24 gm, 81 %, **m. p.: 74-75°C;** ^{1}H NMR ($CDCl_3$, 400 MHz)δ: 1.08-1.98 (10 H, m) 3.6(3H, d, *J* = 10.2 Hz), 3.7 (3H, d, *J* = 10.6 Hz), 3.84-3.88 (1H, m), 6.6-7.2 (5H, m); ^{13}C NMR ($CDCl_3$, 100 MHz) : 25.9, 26.1, 26.3, 28.3, 30.8, 30.9, 39.8, 39.9, 52.3, 53.4, 55.1-56.6 (C-P, d, J= 150 Hz), 113.1, 117.9, 129.4, 147.5. HRMS (ESI) m/z calcd for $C_{15}H_{24}NO_3P$ Na^+ $[M+Na]^+$, 320.1386; Observed: 320.1347.

[(4-Dimethylamino-phenyl)-phenylamino-methyl]-phosphonic acid dimethyl ester, (table 1, entry 25)[5]:



Yellowish orange solid, Yield: 0.31 gm, 93%; ^{1}H NMR ($CDCl_3$, 300 MHz) δ: 2.90 (6H, s) 3.45 (3H, d, *J* = 10.42 Hz), 3.72 (3H, d, *J* = 10.57 Hz), 4.66-4.74 (1H, d, $^1J_{PH}$ = 23.52 Hz), 6.59-7.32(9H, m); MS (Maldi TOF TOF) *m/z* 336$(M^+$+2), 225 $[(M^+$- P(O)$(OMe)_2]$.

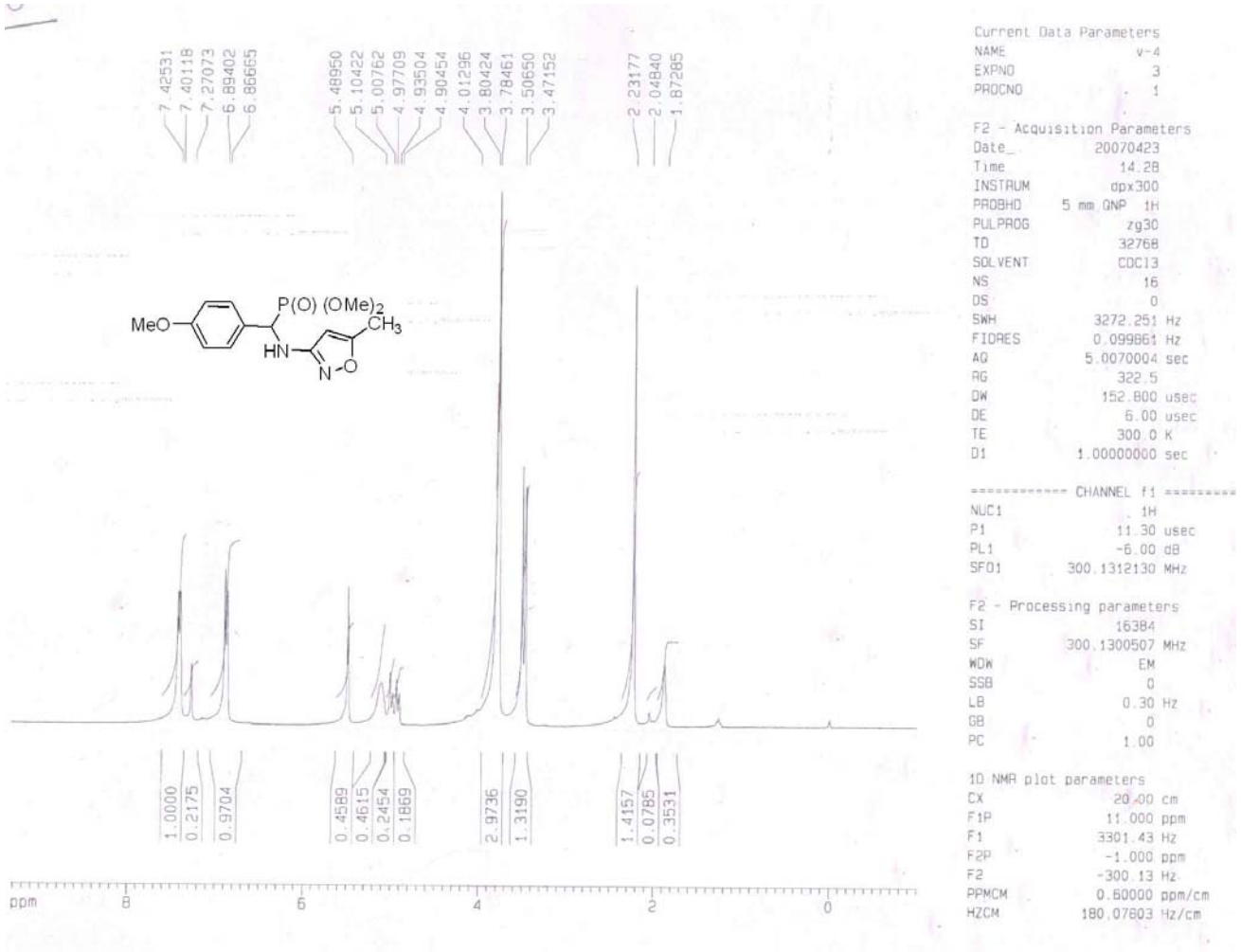
Dimethyl (3-hydroxyphenyl) (phenylamino) methylphosphonate, (table 1, entry 26):



Greenish-white solid, Yield: 0.25 gm, 82%, **m. p.: 120-121 °C**; ^{1}H NMR ($CDCl_3$, 400 MHz) δ: 3.4 (3H, d, *J* = 10.6 Hz), 3.7 (3H, d, *J* = 10.7 Hz), 4.73 (1H, d, *J* = 24.2 Hz), 6.5-7.21(9H, m); ^{13}C NMR ($CDCl_3$, 100 MHz): 54.0, 54.1, 54.7-56.3(C-P, d, *J* = 152 Hz), 113.9, 114.4, 115.8, 118.7, 119.7, 129.2, 129.9, 136.7, 145.9, 146.0, 157.3. HRMS (ESI) m/z calcd for $C_{15}H_{18}NO_4P$ Na^+ [M + Na^+], 330.0866; Observed: 330.0874.

7. Scanned NMR spectral data of unknown compounds

7.1. ^{1}H NMR; Dimethyl (4-methoxyphenyl)(5-methylisoxazol-3-ylamino)methylphosphonate, (table 1, entry 3):

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Current Data Parameters
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EXPNO                   3
PROCNO                  1

F2 - Acquisition Parameters
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Time                 14.28
INSTRUM              dpx300
PROBHD     5 mm QNP  1H
PULPROG               zg30
TD                   32768
SOLVENT              CDCl3
NS                      16
DS                       0
SWH               3272.251 Hz
FIDRES            0.099861 Hz
AQ                5.0070004 sec
RG                   322.5
DW                 152.800 usec
DE                   6.00 usec
TE                  300.0 K
D1                1.00000000 sec

========== CHANNEL f1 =========
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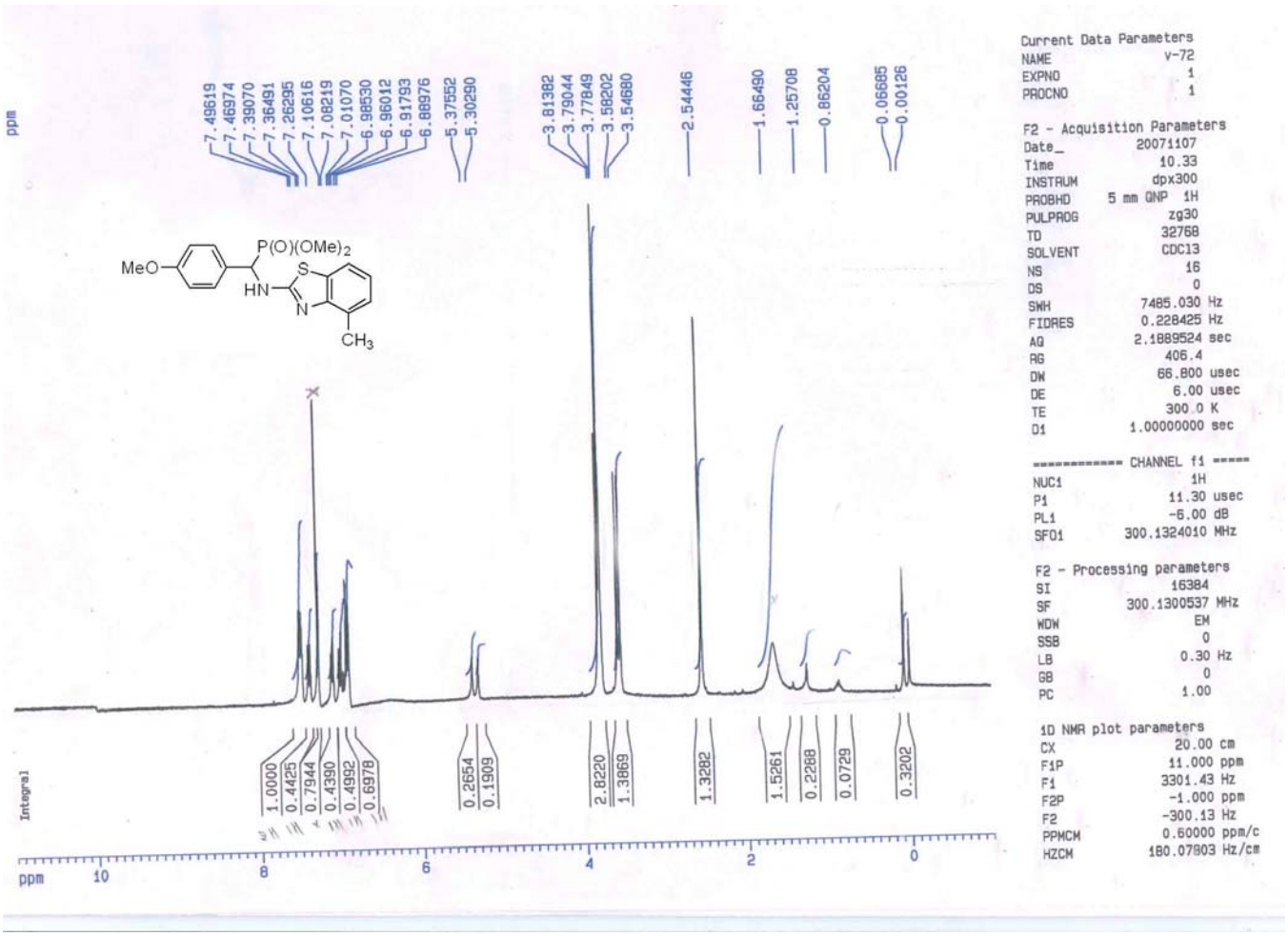
P1                  11.30 usec
PL1                 -6.00 dB
SFO1          300.1312130 MHz

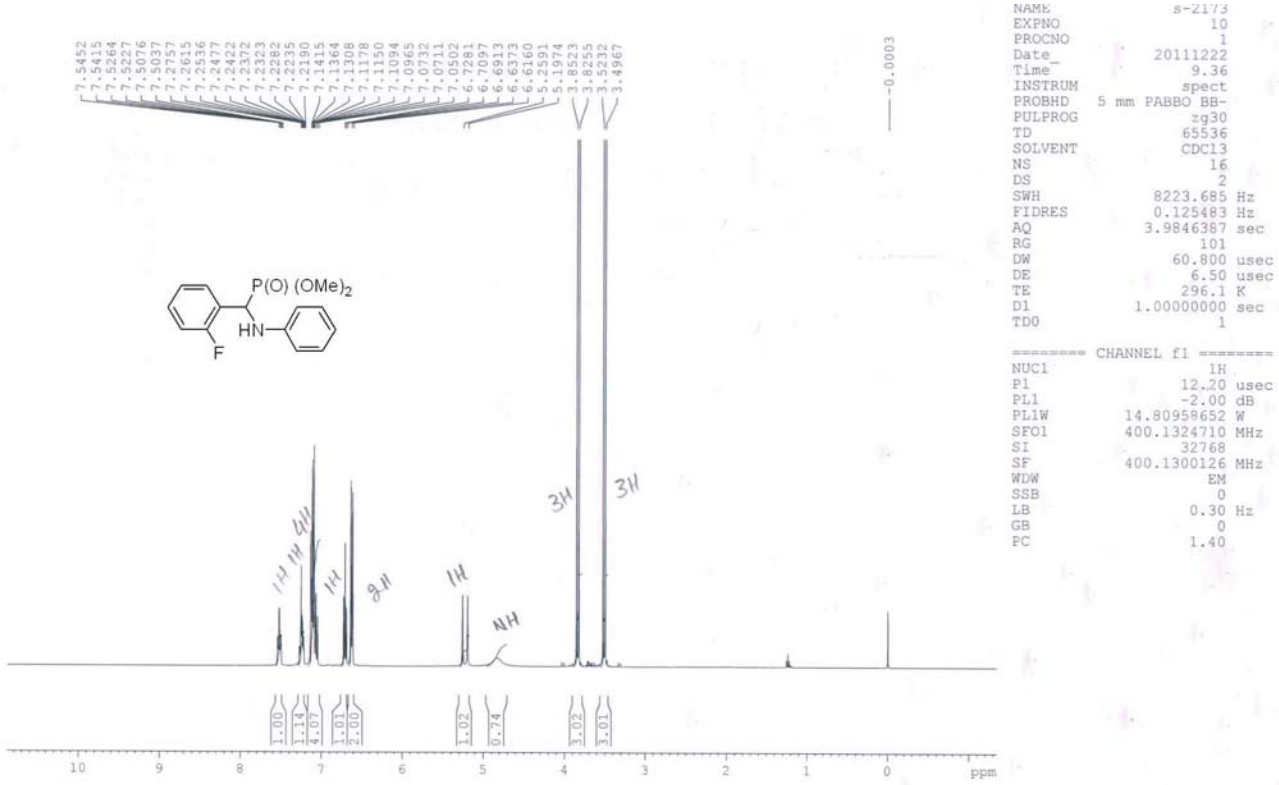
F2 - Processing parameters
SI                   16384
SF            300.1300507 MHz
WDW                    EM
SSB                     0
LB                   0.30 Hz
GB                      0
PC                   1.00

1D NMR plot parameters
CX                  20.00 cm
F1P                 11.000 ppm
F1                3301.43 Hz
F2P                -1.000 ppm
F2                -300.13 Hz
PPMCM             0.60000 ppm/cm
HZCM            180.07803 Hz/cm
```

7.2. ^{1}H NMR; Dimethyl (4-methoxyphenyl) (4-methylbenzo[d] thiazol-2-ylamino) methyl phosphonate, (table 1, entry 4):



7.3. ^{1}H NMR ; Dimethyl (2-fluorophenyl) (phenylamino) methylphosphonate, (table 1, entry 16):



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PROCNO	1
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Time_	9.36
INSTRUM	spect
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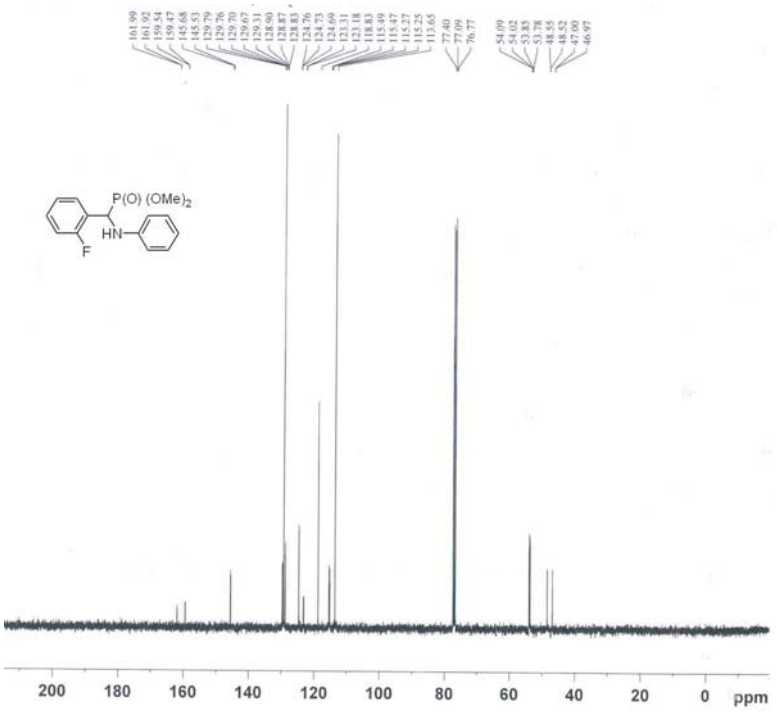
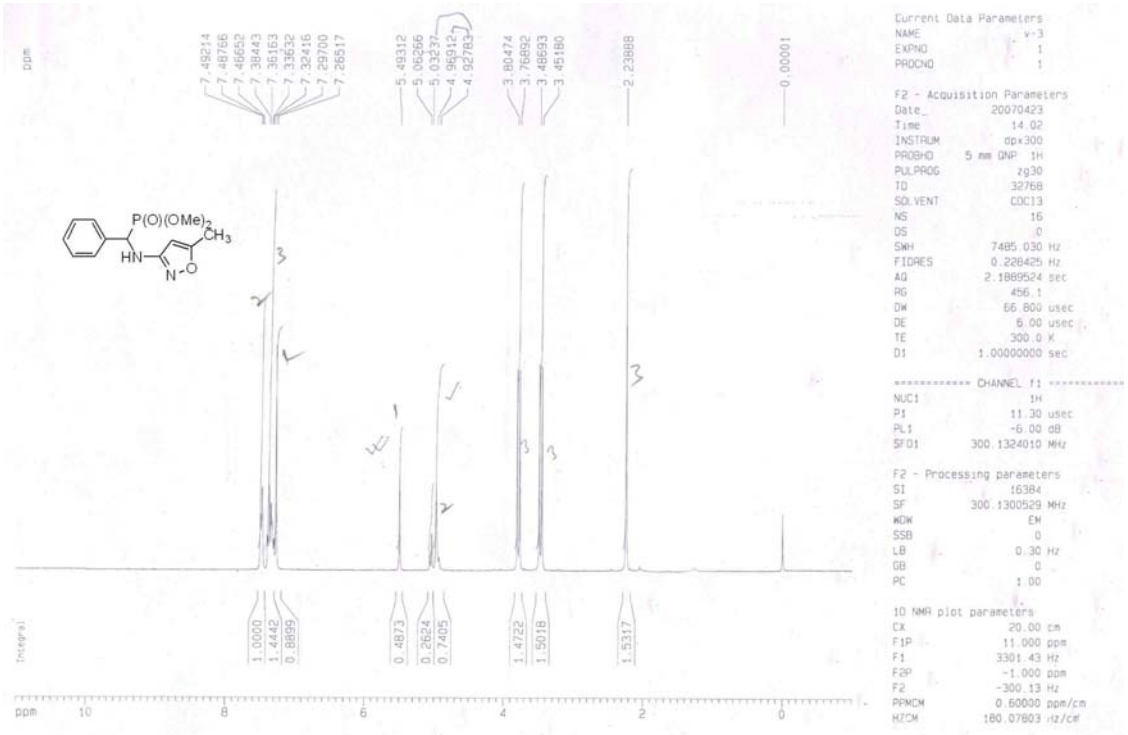
PULPROG	zg30
TD	65536
SOLVENT	CDCl3
NS	16
DS	2
SWH	8223.685 Hz
FIDRES	0.125483 Hz
AQ	3.9846387 sec
RG	101
DW	60.800 usec
DE	6.50 usec
TE	296.1 K
D1	1.00000000 sec
TD0	1
======== CHANNEL f1 ========	
NUC1	1H
P1	12.20 usec
PL1	-2.00 dB
PL1W	14.80958652 W
SFO1	400.1324710 MHz
SI	32768
SF	400.1300126 MHz
WDW	EM
SSB	0
LB	0.30 Hz
GB	0
PC	1.40

^{13}C NMR; Dimethyl(5-methylisoxazol-3-ylamino) (phenyl) methylphosphonate, (table 1, entry 16):

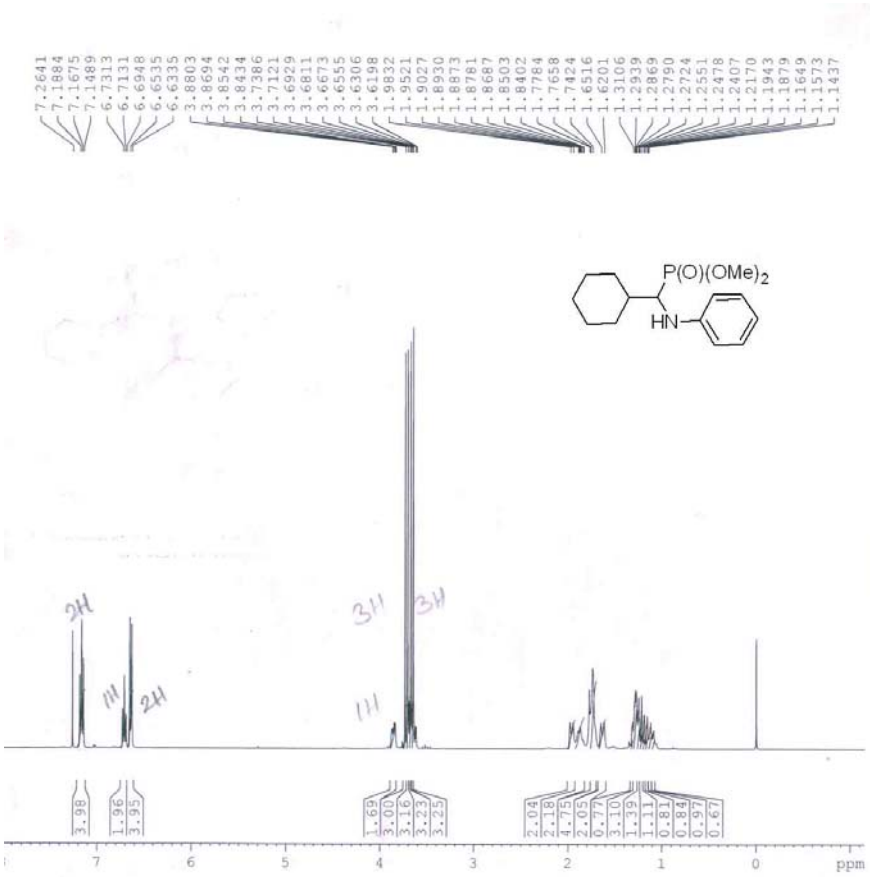


NAME	s-2173
EXPNO	11
PROCNO	1
Date_	20111222
Time_	17.10
INSTRUM	spect
PROBHD	5 mm PABBO BB-
PULPROG	zgpg30
TD	65536
SOLVENT	CDCl3
NS	128
DS	4
SWH	24038.461 Hz
FIDRES	0.366798 Hz
AQ	1.3631988 sec
RG	203
DW	20.800 usec
DE	6.50 usec
TE	295.0 K
D1	2.00000000 sec
D11	0.03000000 sec
TD0	1
======== CHANNEL f1 ========	
NUC1	13C
P1	9.50 usec
PL1	-1.00 dB
PL1W	44.90434265 W
SFO1	100.6228298 MHz
======== CHANNEL f2 ========	
CPDPRG2	waltz16
NUC2	1H
PCPD2	80.00 usec
PL2	-2.00 dB
PL12	14.33 dB
PL13	18.33 dB
PL2W	14.80958652 W
PL12W	0.34478071 W
PL13W	0.13725966 W
SFO2	400.1316005 MHz
SI	32768
SF	100.6127690 MHz
WDW	EM
SSB	0
LB	1.00 Hz
GB	0
PC	1.40

7.4 ^{1}H NMR; Dimethyl(5-methylisoxazol-3-ylamino) (phenyl) methylphosphonate, (table 1, entry 18):



7.5. ^{1}H NMR; Dimethyl cyclohexyl (phenylamino) methylphosphonate, (table 1, entry 24):

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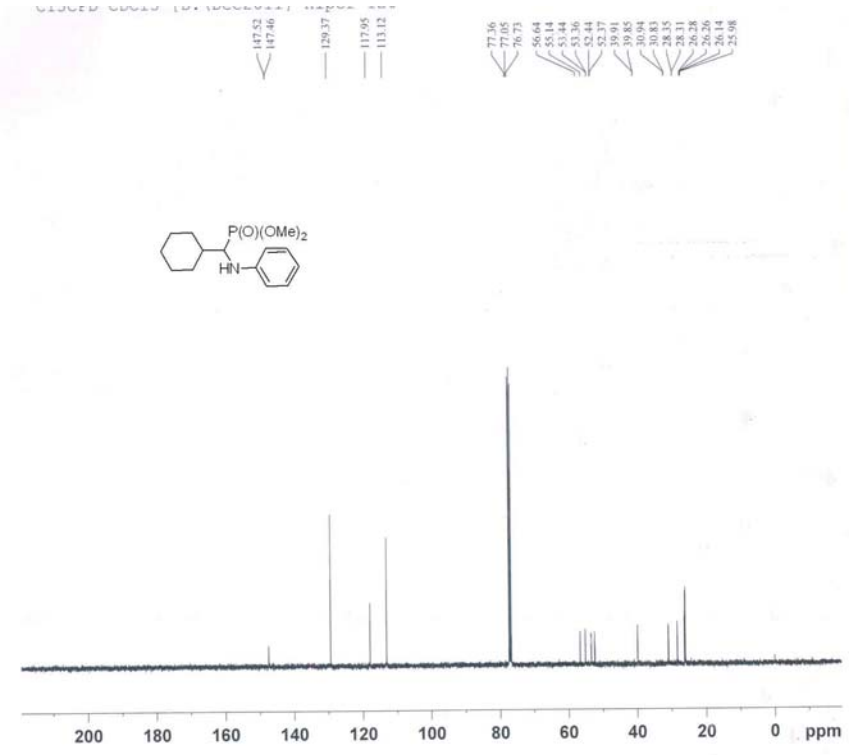
EXPNO                    10
PROCNO                    1
Date_             20111222
Time                  10.25
INSTRUM               spect
PROBHD   5 mm PABBO BB-
PULPROG               zg30
TD                   65536
SOLVENT              CDCl3
NS                       16
DS                        2
SWH               8223.685 Hz
FIDRES            0.125483 Hz
AQ                3.9846387 sec
RG                      128
DW                 60.800 usec
DE                  6.50 usec
TE                  295.0 K
D1                1.00000000 sec
TD0                       1

======== CHANNEL f1 ========
NUC1                    1H
P1                  12.20 usec
PL1                 -2.00 dB
PL1W         14.80958652 W
SFO1         400.1324710 MHz
SI                   32768
SF           400.1300085 MHz
WDW                    EM
SSB                     0
LB                  0.30 Hz
GB                      0
PC                   1.40
```

^{13}C NMR; Dimethyl cyclohexyl (phenylamino) methylphosphonate, (table 1, entry 24):

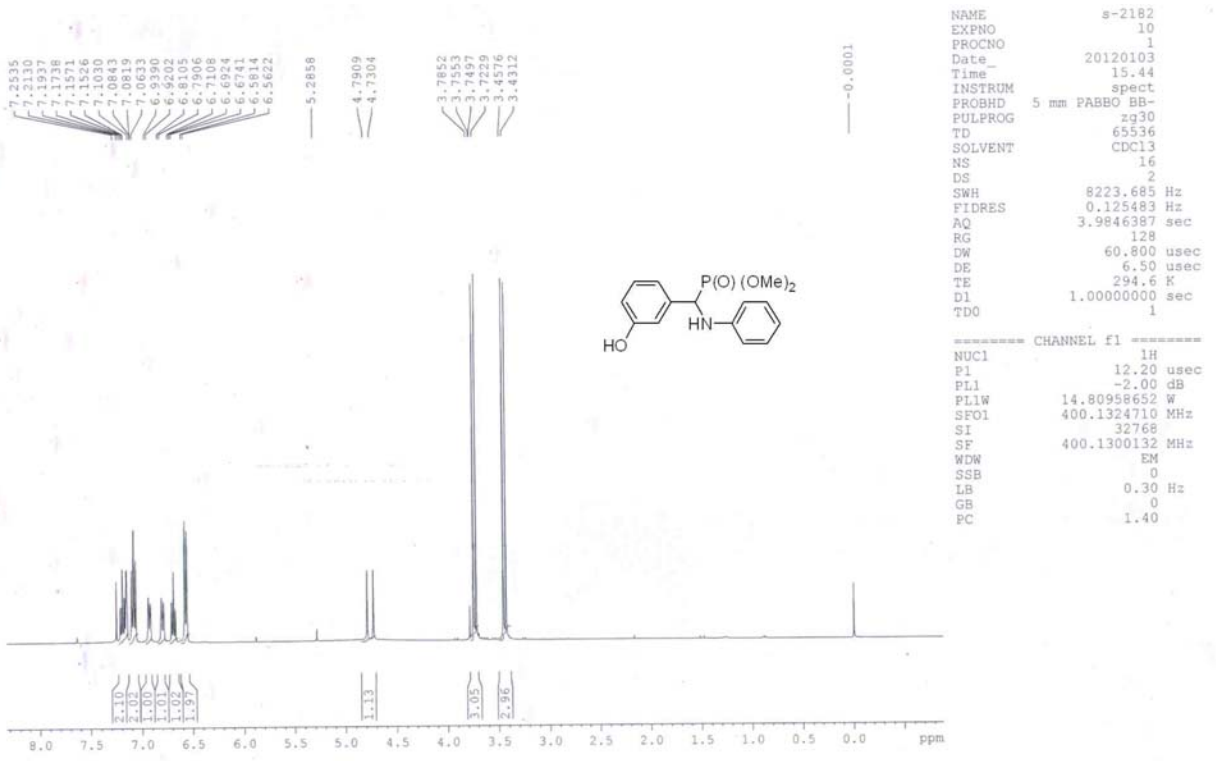



```
NAME                 s-2181
EXPNO                    11
PROCNO                    1
Date_             20111222
Time                  14.23
INSTRUM               spect
PROBHD   5 mm PABBO BB-
PULPROG             zgpg30
TD                   65536
SOLVENT              CDCl3
NS                      184
DS                        4
SWH              24038.461 Hz
FIDRES            0.366798 Hz
AQ                1.3631988 sec
RG                      203
DW                 20.800 usec
DE                  6.50 usec
TE                  295.8 K
D1                2.00000000 sec
D11               0.03000000 sec
TD0                       1

======== CHANNEL f1 ========
NUC1                   13C
P1                  9.50 usec
PL1                 -1.00 dB
PL1W         44.90434265 W
SFO1         100.6228298 MHz

======== CHANNEL f2 ========
CPDPRG2             waltz16
NUC2                    1H
PCPD2              80.00 usec
PL2                 -2.00 dB
PL12                14.33 dB
PL13                18.33 dB
PL2W         14.80958652 W
PL12W         0.34478071 W
PL13W         0.13725966 W
SFO2         400.1316005 MHz
SI                   32768
SF           100.6127690 MHz
WDW                    EM
SSB                     0
LB                  1.00 Hz
GB                      0
PC                   1.40
```

7.6. ^{1}H NMR; Dimethyl (3-hydroxyphenyl) (phenylamino) methylphosphonate, (table 1, entry 26):

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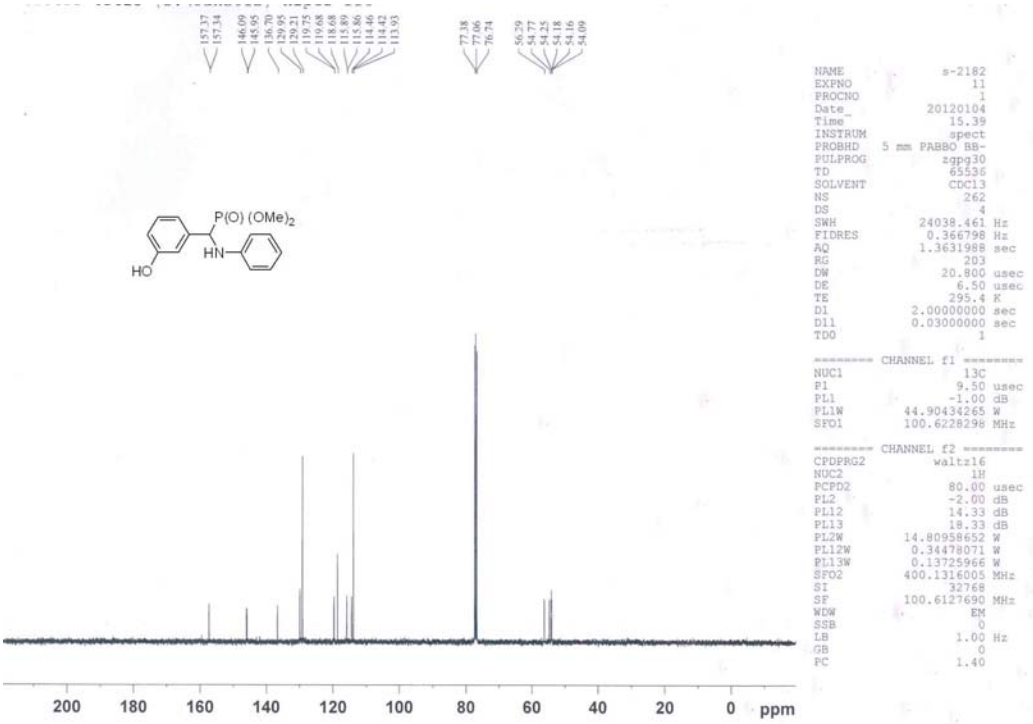
NAME                 s-2182
EXPNO                    10
PROCNO                    1
Date_             20120103
Time                  15.44
INSTRUM               spect
PROBHD   5 mm PABBO BB-
PULPROG                zg30
TD                    65536
SOLVENT               CDCl3
NS                       16
DS                        2
SWH                8223.685 Hz
FIDRES             0.125483 Hz
AQ                 3.9846387 sec
RG                      128
DW                   60.800 usec
DE                    6.50 usec
TE                   294.6 K
D1                1.00000000 sec
TD0                       1

======== CHANNEL f1 ========
NUC1                      1H
P1                    12.20 usec
PL1                   -2.00 dB
PL1W             14.80958652 W
SFO1             400.1324710 MHz
SI                     32768
SF               400.1300132 MHz
WDW                       EM
SSB                        0
LB                    0.30 Hz
GB                         0
PC                    1.40
```

^{13}C NMR; Dimethyl (3-hydroxyphenyl) (phenylamino) methylphosphonate, (table 1, entry 26):




```
NAME                 s-2182
EXPNO                    11
PROCNO                    1
Date_             20120104
Time                  15.39
INSTRUM               spect
PROBHD   5 mm PABBO BB-
PULPROG             zgpg30
TD                    65536
SOLVENT               CDCl3
NS                      262
DS                        4
SWH               24038.461 Hz
FIDRES             0.366798 Hz
AQ                 1.3631988 sec
RG                      203
DW                   20.800 usec
DE                    6.50 usec
TE                   295.4 K
D1                2.00000000 sec
D11               0.03000000 sec
TD0                       1

======== CHANNEL f1 ========
NUC1                     13C
P1                    9.50 usec
PL1                  -1.00 dB
PL1W             44.90434265 W
SFO1             100.6228296 MHz

======== CHANNEL f2 ========
CPDPRG2              waltz16
NUC2                      1H
PCPD2                 80.00 usec
PL2                   -2.00 dB
PL12                  14.33 dB
PL13                  18.33 dB
PL2W             14.80958652 W
PL12W             0.34478071 W
PL13W             0.13725966 W
SFO2             400.1316005 MHz
SI                     32768
SF               100.6127690 MHz
WDW                       EM
SSB                        0
LB                    1.00 Hz
GB                         0
PC                    1.40
```

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