New class of diethyl substituted phosphoramidimidates and phosphonimidates: Synthesis, spectral characterization and antimicrobial activity

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**Supplemental Materials**

***Spectral data:***

***Diethyl N-2-hydroxyethyl-N'-(pyridin-2-yl)phosphoramidimidate (6a)***

Semi-solid, Yield:72%; IR (KBr, cm-1): 1354 (-P=N, str), 3267 (-P-NH, str), 3421 (-OH, str);1H NMR (CDCl3, 400 MHz): *δ* 1.27 (t, *J* = 6 Hz, 6H, H-16, H-18), 2.99-3.03 (q, 2H, H-3), 3.45 (s, 1H, H-4), 3.57 (t, *J* = 4.4 Hz, 2H, H-2), 3.88-3.92 (q, 4H, H-15, H-17), 5.22 (s, 1H, H-1), 7.07-7.51 (m, 3H, H-10, H-11, H-12), 8.41 (d, *J* = 6.8 Hz, 1H, H-13); 13C NMR (CDCl3, 100.6 MHz): *δ* 15.4 (C16, C18), 34.4 (C3), 44.2 (C15, C17), 61.3 (C2), 121.2 (C12), 122.7 (C10), 136.2 (C11), 148.3 (C13), 164.0 (C9).31P NMR (CDCl3,161.9 MHz,): *δ* 10.5.MS (m/z): 274 (M+H+). Anal.Calcd.forC11H20N3O3P: C 48.35, H 7.38, N 15.38; Found: C 48.23, H 7.40, N 15.42.



***Diethyl N'-5-bromopyridin-2-yl-N-(2-hydroxyethyl)phosphoramidimidate (6b)***

Solid, Yield: 80%; m.p**:** 187-189 oC. IR(KBr, cm-1): 1344 (-P=N, str), 3012 (-P-NH, str), 3412 (-OH, str);1H NMR(CDCl3, 400 MHz): *δ* 1.26 (t, *J* = 5.6 Hz, 6H, H-16, H-18**),** 3.07-3.14 (q, 2H, H3), 3.20-3.28 (q, 2H, H-2), 3.75 (s, 1H, H-4), 3.84 (s, 1H, H-1), 4.05-4.12 (q, 4H, H-15, H-17), 7.68 (d, *J* = 7.6 Hz, 1H, H-12), 7.85 (d, *J* = 7.2 Hz, 1H, H-13), 8.74 (d, *J* = 6.4 Hz, 1H, H-11);13C NMR(CDCl3,100.6 MHz): *δ* 18.0 (C16, C18), 40.3 (C3), 48.6 (C15, C17), 66.2 (C2), 120.3 (C14), 122.6 (C12), 135.5 (C13), 141.5 (C11), 153.4 (C9); 31P NMR (161.9 MHz, CDCl3): *δ* 10.6. MS(m/z): 353 (M+H+). Anal.Calcd.forC11H19BrN3O3P: C 37.52, H 5.44, N11.93; Found: C 37.56, H 5.38, N 11.86.



***Diethyl N'-cyclopropylmethyl-N-(2-hdroxyethyl)phosphoramidimidate (6d)***

Semi-solid, Yield:65%;IR (KBr, cm-1): 1354 (-P=N, str), 2987 (-P-NH, str), 3481 (-OH, str);1H NMR(CDCl3, 400 MHz): *δ* 0.42-0.48 (m, 5H, H-10, H-11, H-12), 1.20-1.26 (m, 2H, H-9), 1.29(t, *J* = 4.8 Hz, 6H, H-14, H-16), 3.02-3.09 (q, 2H, H-3), 3.15-3.17 (q, 2H, H-2),3.73 (s, 1H, H-4), 3.88 (s, 1H, H-1),4.07-4.13 (q, 4H, H-13, H-15); 13C NMR(CDCl3,100.6 MHz): *δ*13.2 (C10), 3.9 (C11, C12), 19.2 (C14, C16), 38.2 (C3), 51.4 (C13, C15), 66.4 (C2), 42.6 (C9); 31P NMR (CDCl3,161.9 MHz):*δ*12.6. MS(m/z): 251(M+H)+.Anal.Calcd.forC10H23N2O3P: C 47.99, H 9.26, N11.19; Found: C 47.89, H 9.30, N 11.25.



***Diethyl N-2-hydroxyethyl-N'-(thiazol-2-yl)phosphoramidimidate (6e)***

Semi-solid, Yield:72%; IR(KBr, cm-1): 1344 (-P=N, str), 2981 (-P-NH, str), 3535 (-OH, str);1H NMR (CDCl3,400 MHz,):*δ* 1.27 (t, *J* = 5.6 Hz,6H, H-15, H-17),3.06-3.15 (q, 2H, H-3),3.81-3.84 (t, *J* = 6.8 Hz, 2H, H-2), 3.92 (s, 1H, H-4),3.96-4.01 (q, 4H, H-14, H-16),5.29 (s, 1H, H-1), 7.55-7.56 (d, *J* = 2 Hz,1H, H-12),7.77 (d, *J* = 4.8 Hz, 1H, H-11);13C NMR(CDCl3,100.6 MHz): *δ* 16.2 (C15, C17), 36.5 (C3), 45.5 (C14, C16), 62.1 (C2), 121.4 (C12), 139.1 (C11), 156.3 (C9); 31P NMR(CDCl3, 161.9 MHz):*δ*12.8. MS (m/z): 280 (M+H+). Anal.Calcd.forC9H18N3O3PS: C 38.70, H 6.50, N15.04; Found: C 38.65, H 6.48, N 15.14.



***Diethyl P-morpholino-N-(pyridin-2-yl)phosphonimidate (6f)***

Semi-solid, Yield:74%**;** IR(KBr, cm-1): 1313 (-P=N, str), 1023 (-P-O-C, str);1H NMR(CDCl3, 400 MHz):*δ*1.41 (t, *J* = 7.6 Hz, 6H, H12, H14), 3.14 (t, *J* = 7.2 Hz, 4H,H2, H4), 3.72 (t, *J* = 6 Hz, 4H, H-1, H-5),4.09-4.15 (q, 4H, H-11, H-13), 7.24-7.26 (m, 1H, H-20), 7.64-7.68 (m, 1H, H-18), 7.84-7.86 (m, 1H, H-19), 8.56 (m, 1H, H-17); 13C NMR(CDCl3, 100.6 MHz): *δ* 17.6 (C12, C14), 41.3 (C2, C4), 49.4 (C11, C13), 69.4 (C1, C5), 121.5 (C18), 123.3 (C20), 139.1 (C19), 146.8 (C17), 154.6 (C15); 31P NMR (CDCl3, 161.9 MHz): *δ*11.2. MS (m/z): 300 (M+H+).Anal.Calcd.forC13H22N3O3P: C 52.17, H 7.41, N 14.04; Found: C 52.12, H 7.39, N 14.14.



***Diethyl N-5-bromopyridin-2yl-P-morpholinophosphonimidate (6g)***

Solid, Yield:73%; m.p:132-134oC.IR (KBr, cm-1): 1315 (-P=N, str), 1044 (-P-O-C, str);1H NMR(CDCl3,400 MHz,): *δ* 1.26 (t, *J*= 7.2 Hz, 6H, H-12, H-14), 3.06(t, *J*= 6 Hz, 4H, H-2, H-4), 3.61(t, *J* = 4.8 Hz, 4H, H-1, H-5), 4.03-4.08 (q, 4H, H-11, H-13), 7.42 (d, *J* = 4.4 Hz, 1H, H-19), 7.90 (d, *J* = 6.8 Hz, 1H, H-18), 8.56 (s, 1H, H-17); 13C NMR(CDCl3, 100.6 MHz): *δ* 18.0 (C12, C14), 41.6 (C2, C4), 50.9 (C11, C13), 71.8 (C1, C5), 119.2 (C18), 125.2 (C20), 142.7 (C17), 152.7 (C19), 159.5 (C15); 31P NMR (CDCl3, 161.9 MHz): *δ* 14.2. MS (m/z): 378 (M+H+).Anal.Calcd.forC13H21BrN3O3P: C 41.28, H 5.60, N11.11;Found: C 41.32, H 5.55, N 11.17.



***Diethyl P-morpholino-N-(pyrazin-2-yl)phosphonimidate (6h)***

Semi-solid, Yield:74%; IR(KBr, cm-1): 1314 (-P=N, str), 1028 (-P-O-C, str); 1HNMR (CDCl3, 400 MHz):*δ* 1.28 (t, *J* = 5.6 Hz, 6H, H-12, H-14), 3.09-3.14 (q, 4H, H-2, H-4), 3.71 (t, *J* = 6 Hz, 4H, H-1,H-5), 3.88-3.94 (q, 4H, H-11, H-13), 8.18 (s, 1H, H-16), 8.40 (d, *J* = 6.8 Hz, 1H, H-19), 8.88 (d, *J* = 6.4 Hz, 1H, H-18); 13C NMR(CDCl3,100.6 MHz): *δ* 19.8 (C12, C14), 42.6 (C2, C4), 49.5 (C11, C13), 70.2 (C1, C5), 142.5 (C18), 146.2 (C19), 152.3 (C16), 157.5 (C15); 31P NMR (CDCl3, 161.9 MHz):*δ*11.8. MS (m/z): 301 (M+H+). Anal.Calcd.forC12H21N4O3P: C 48.00, H 7.05, N, 18.66; Found: C 48.11, H 7.01, N 18.58.



***Diethyl N-cyclopropylmethyl-P-morpholinophosphonimidate (6i)***

Semi-solid, Yield:64%;IR(KBr, cm-1): 1308 (-P=N, str), 1025 (-P-O-C, str);1H NMR(CDCl3, 400 MHz):*δ* 0.32-0.56 (m, 5H, H-16, H-17, H-18), 1.29 (t, *J* =8 Hz, 6H, H-12, H-14), 1.32-1.35 (m, 2H, H-15), 3.09 (t, *J* = 6.4 Hz, 4H, H-2, H-4), 3.52 (t, *J*= 5.6 Hz, 4H, H-1, H-5), 4.12-4.17 (q, 4H, H-11, H-13); 13C NMR(CDCl3,100.6 MHz): *δ* 3.9 (C17, C18), 14.2 (C16), 20.2 (C12, C14), 42.3 (C2, C4),43.5 (C15), 49.1 (C11, C13), 67.5 (C1, C5); 31P NMR (CDCl3,161.9 MHz): *δ* 12.6. MS (m/z): 277 (M+H+). Anal.Calcd.forC12H25N2O3P: C 52.16, H 9.12, N10.14; Found: C 52.15, H 9.08, N 10.18.



***Diethyl P-morpholino-N-(thiazol-2-yl)phosphonimidate (6j)***

Semi-solid, Yield:78%; IR(KBr, cm-1): 1272 (-P=N, str), 1018 (-P-O-C, str); 1H NMR(CDCl3,400 MHz): *δ* 1.37 (t, *J* =7.2 Hz, 6H, H-12, H-14), 3.15 (t, *J* = 7.2 Hz, 4H, H-2, H-4), 3.65 (t, *J* =7.2 Hz, 4H, H-1, H-5), 4.24-4.29 (q, 4H, H-11, H-13),7.25 (d, *J* = 8.8 Hz, 1H, H-18), 7.43 (t, *J* = 8.4 Hz, 1H, **H-17**);13C NMR(100.6 MHz, CDCl3): *δ* 20.7 (C12, C14), 41.6 (C2, C4), 49.5 (C11, C13), 69.5 (C1, C5), 123.2 (C18), 146.5 (C17), 166.8 (C15); 31P NMR (CDCl3,161.9 MHz): *δ* 6.76. MS (m/z): 304 (M-H+). Anal.Calcd.forC11H20N3O3PS: C 43.27, H 6.60, N13.76; Found: C 43.30, H 6.58, N 13.81.



***BIOLOGICAL ACTIVITY***

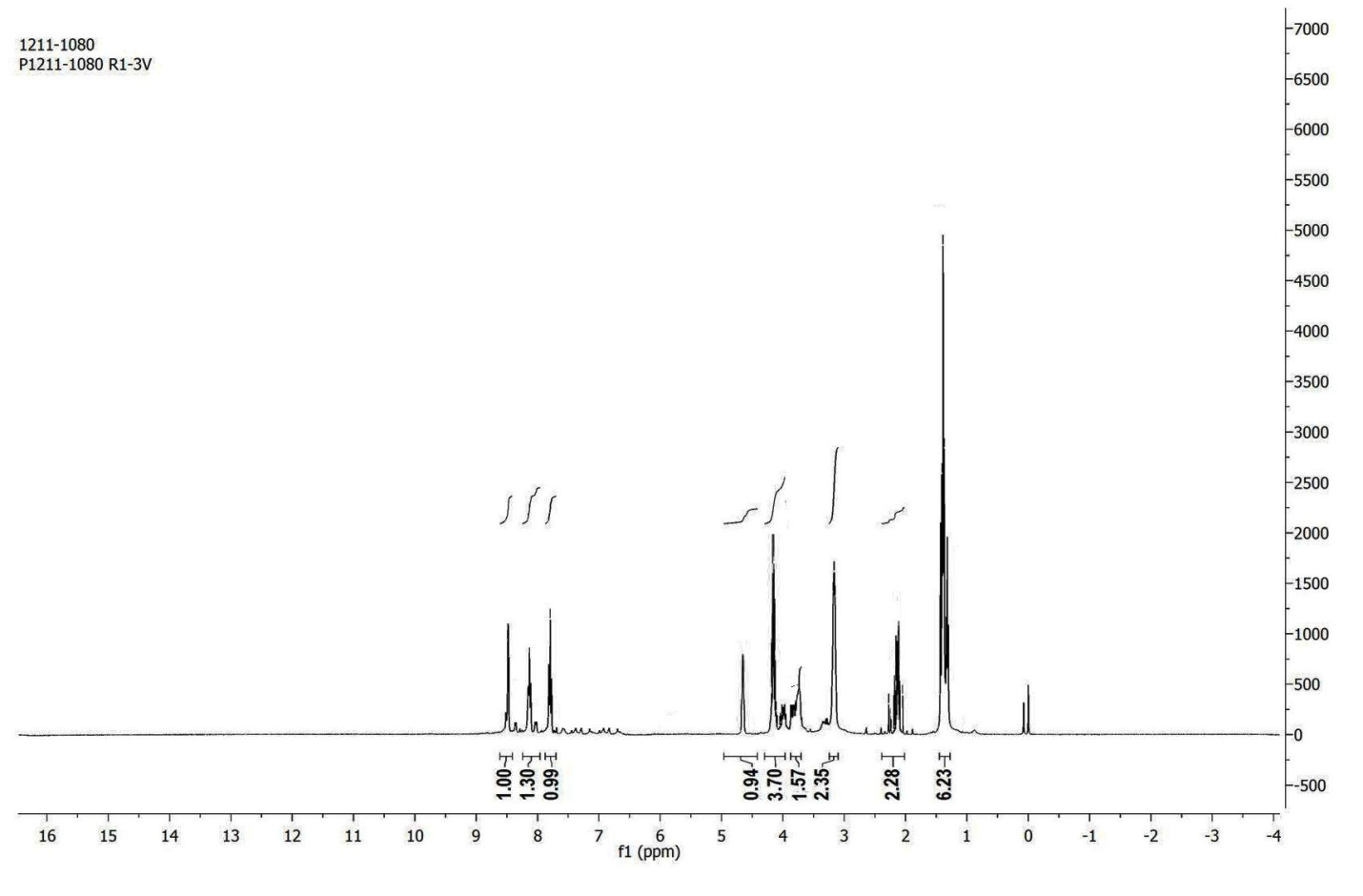
***Antimicrobial activity***

***Antibacterial activity***

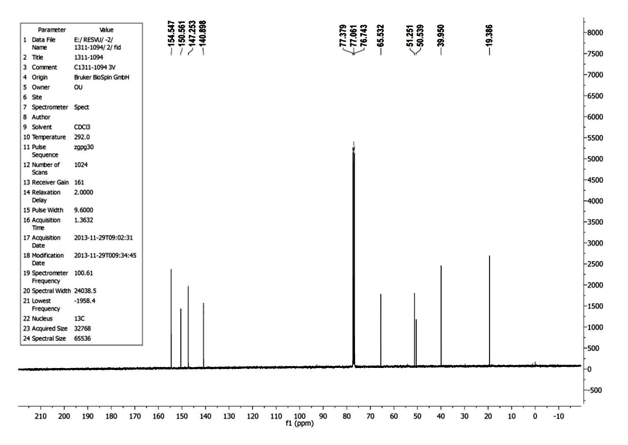
The antibacterial activity of the newly synthesized compounds **6(a-j)** was carried out using agar disc diffusion method20against the bacterial strains*Staphylococcus aureus*, *Lactobacillus acidophillus* (Gram positive) and *Escherichia coli*and *Vibrio cholerae* (Gram negative). Tetracycline was used as a standard drug for the comparison of the activity. The nutrient agar medium in each petri plate was homogenously spread with a bacterial strains and incubated at 25±2 oC for 24 h. All the test compounds and the standard (each 1.0 mg) were dissolved in DMSO and further diluted to required concentrations. The test solutions with concentration of 200 μg/mL) and negative control (DMSO) were introduced onto the sterile disc (6 mm) and dried completely to saturate. These discs were placed onto the media and then incubated at 25±2 oC for 24 h. After incubation, zone of inhibition around the disc was calculated edge to edge zone of the disc and was measured in millimeters. All the tests were carried out in duplicate and average was taken as the final result. The results are tabulated in **Table S 1.**

***Antifungal activity:***

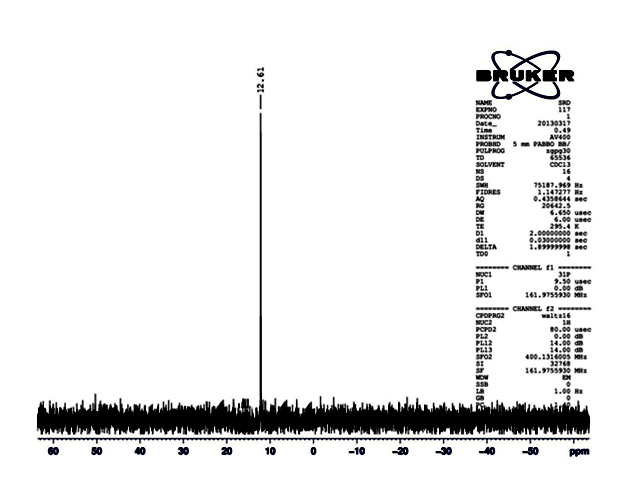
All the newly synthesized compounds were tested for the antifungal activity against the fungal strains *T. longibrachiatum, C. tetani, A. niger and A. fumigates* by the poison plate method21 using Fluconazole as a standard drug. The fungal strains were incubated in potato dextrose agar at 25 ±1 °C for 5 **d** to get new mycelium for antifungal assay, and then a mycelia disc of approximately 0.45 cm diameter cut from the culture medium was picked up with a sterilized inoculation needle and inoculated in the center of the PDA plate.The inoculated plates were incubated at 25 ±1 °C for 5 **d**. DMSO solvent was added as negative control to determine possible inhibitory activity of the solvent, while Fluconazole was used as a positive control.For each treatment, three replicates were carried out and the mean of the diameter of the inhibition zones was calculated. The results are shown in **Table S 2**.

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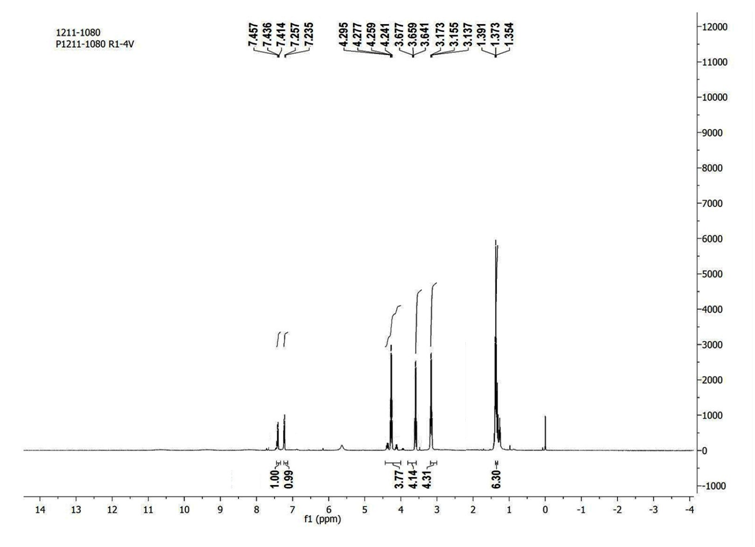
**Figure S 1:** 1H NMR spectrum of compound **(6c)**



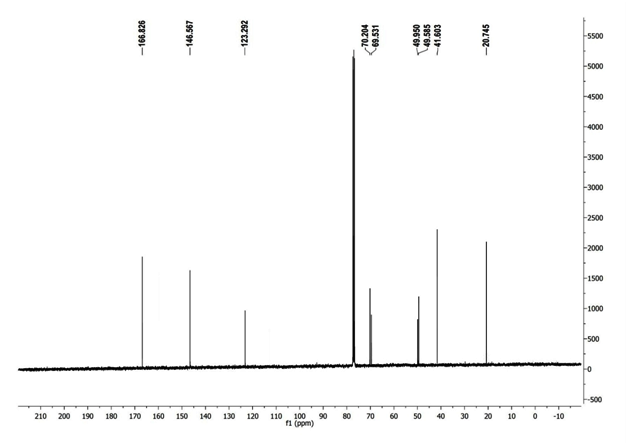
**Figure S 2** 13C NMR spectrum of compound **(6c)**



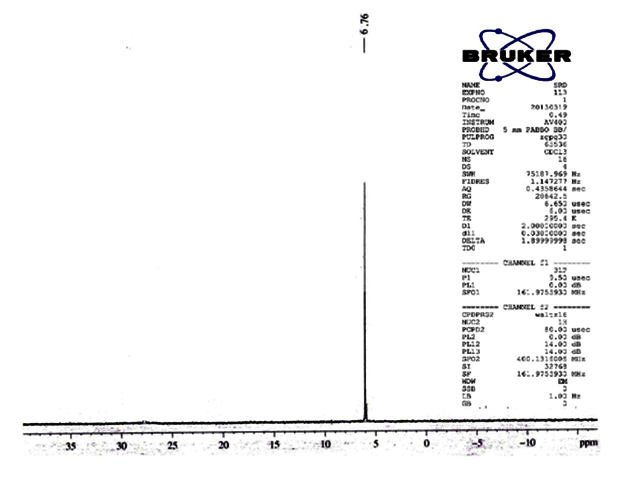
**Figure S 3** 31P NMR spectrum compound **(6c)**



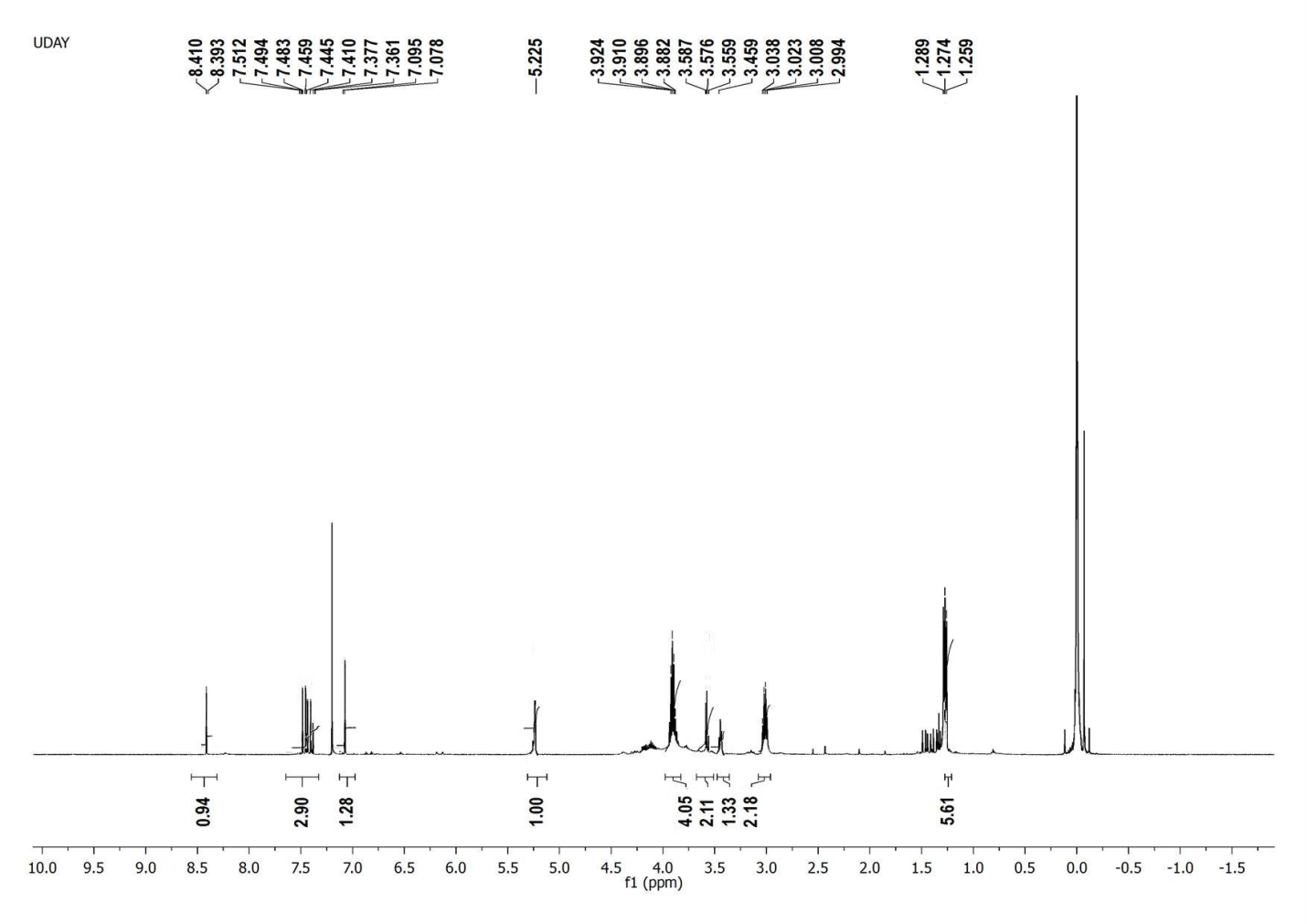
**Figure S 4** 1H NMR spectrum of compound **(6j)**



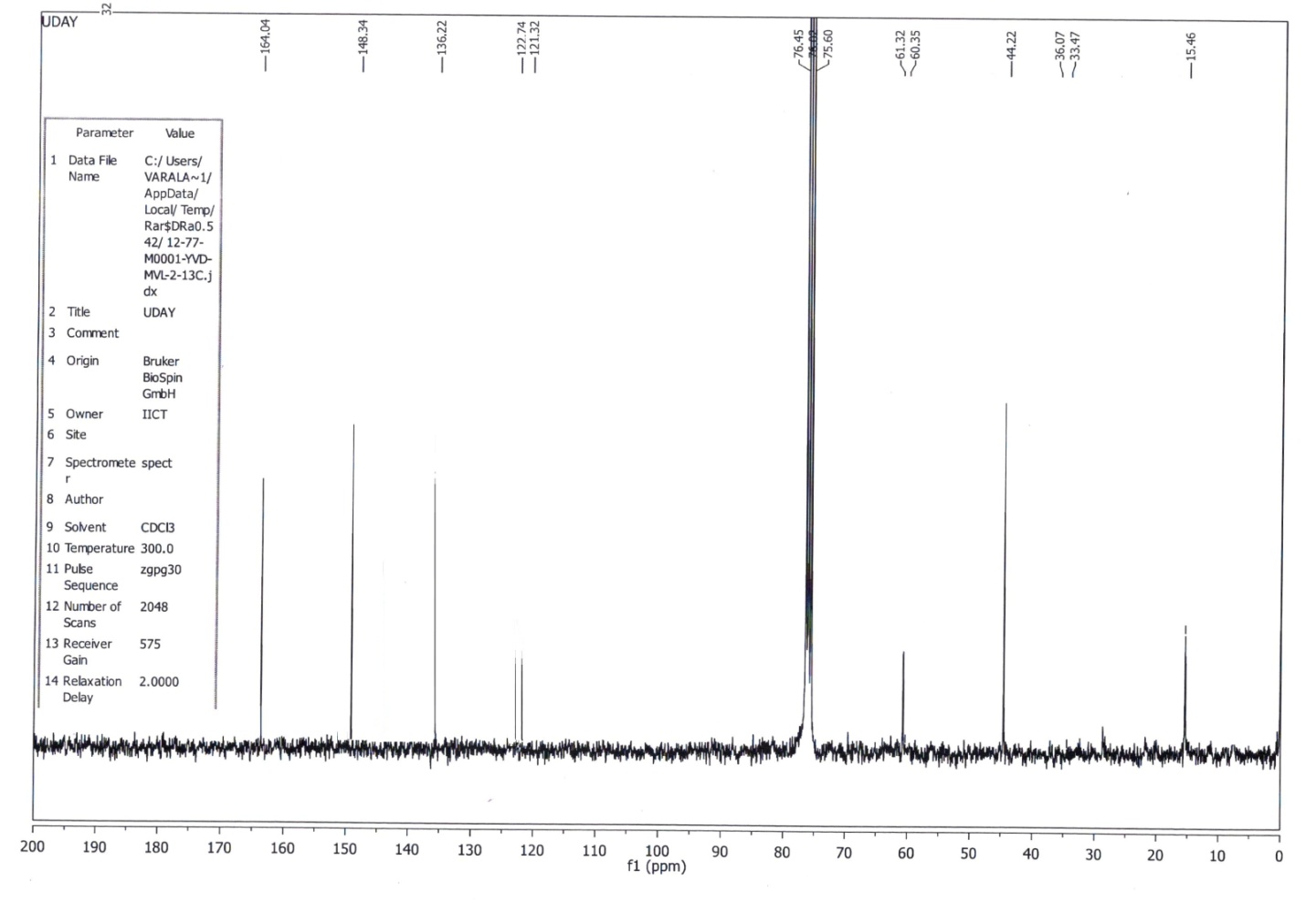
**Figure S 5** 13C NMR spectrum of compound **(6j)**



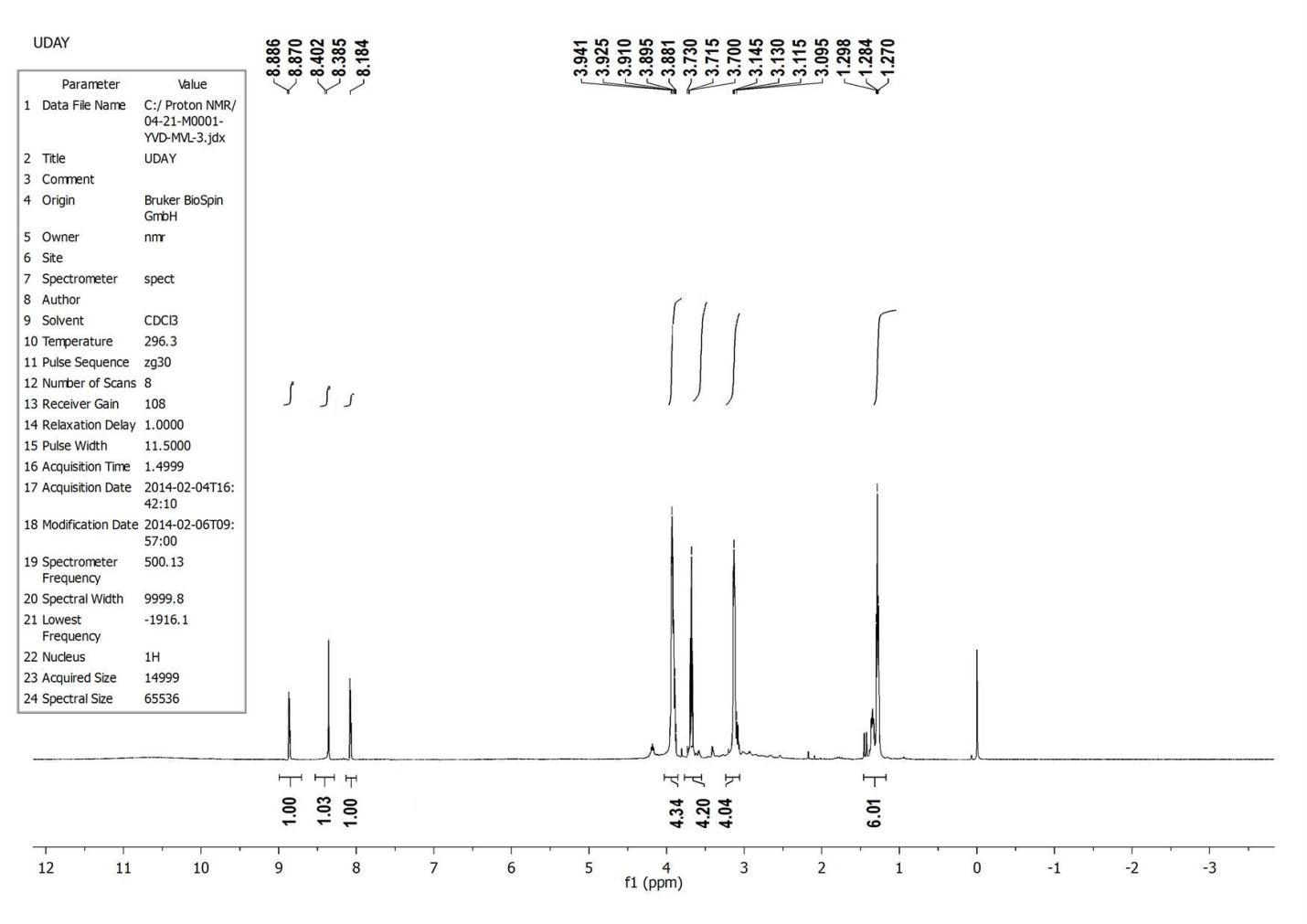
**Figure S 6** 31P NMR spectrum of compound **(6j)**



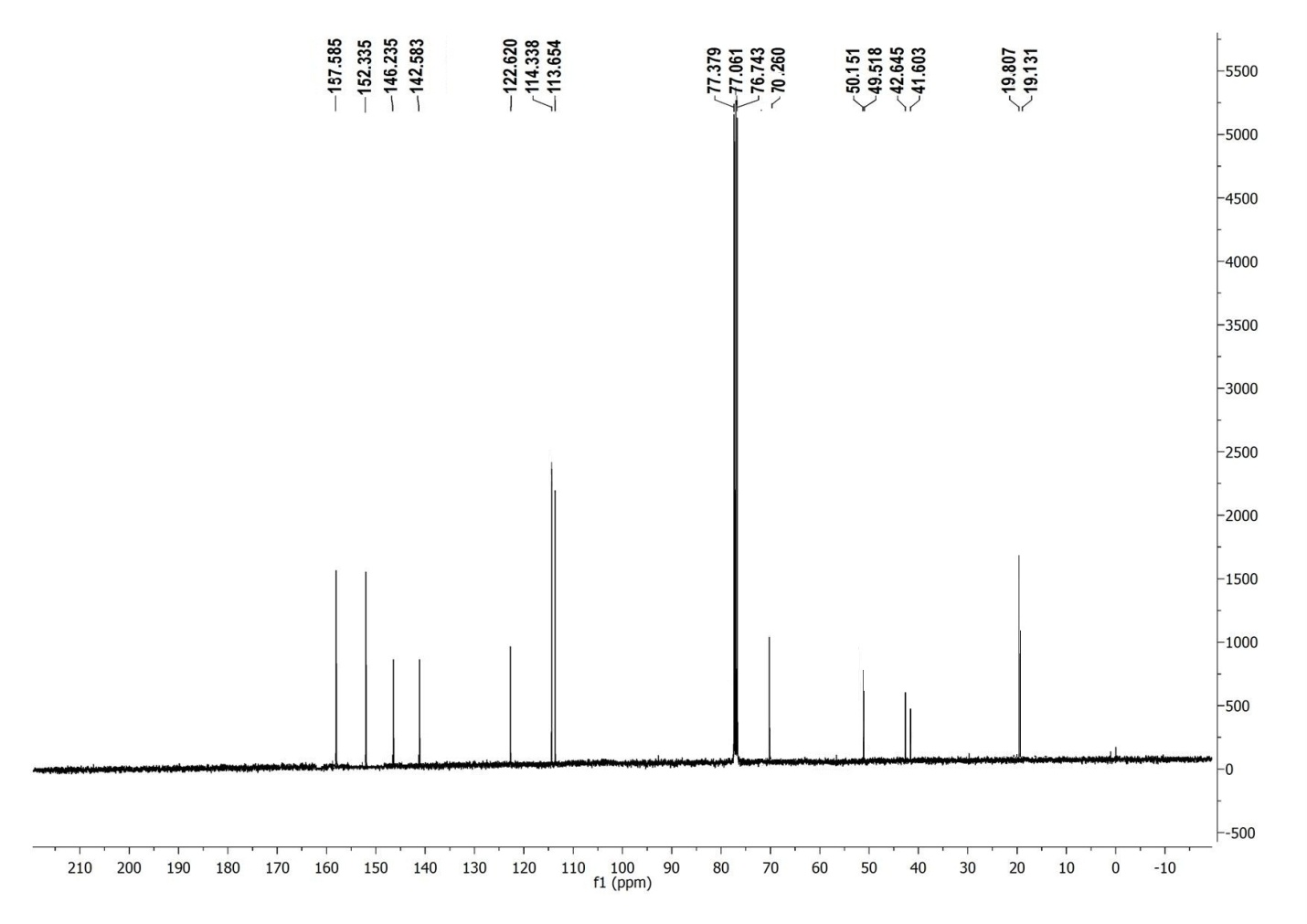
**Figure S 7** 1H NMR spectrum of compound **(6a)**

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**Figure S 8** 13C NMR spectrum of compound **(6a)**



**Figure S 9** 1H NMR spectrum of compound **(6h)**



**Figure S 10** 13C NMR spectrum of compound **(6h)**

**Table S 1:** Antibacterial activity of the diethyl substituted phosphoramidimidates and phosphonimidates **6(a-j)**

|  |  |  |  |  |
| --- | --- | --- | --- | --- |
| **Compd.** | **Zone of inhibition (mm)** | | | |
| ***G +Ve bacteria*** | | ***G –Ve bacteria*** | |
| ***S. aureus*** | ***L. acidophilus*** | ***E. coli*** | ***V. cholerae*** |
| **6a** | 18 | 22 | 12 | 17 |
| **6b** | 23 | 31 | 17 | 25 |
| **6c** | 22 | 28 | 16 | 24 |
| **6d** | 19 | 23 | 12 | ND |
| **6e** | 23 | 31 | 17 | 25 |
| **6f** | 18 | 23 | 11 | 16 |
| **6g** | 23 | 27 | 16 | 23 |
| **6h** | 22 | 28 | 15 | 20 |
| **6i** | 16 | 16 | ND | ND |
| **6j** | 21 | 26 | 15 | 24 |
| **Tetracycline** | 25 | 32 | 18 | 27 |

**Table S 2.** Antifungal activity of the diethyl substituted phosphoramidimidates and phosphonimidates **6(a-j)**

|  |  |  |  |  |
| --- | --- | --- | --- | --- |
| **Zone of inhibition (mm)** | | | | |
| **Compd.** | ***T. longibrachiatum*** | ***C. tetani*** | ***A. niger*** | ***A. fumigates*** |
| **6a** | 9 | 8 | 7 | 5 |
| **6b** | 15 | 11 | 14 | 9 |
| **6c** | 15 | 12 | 13 | 9 |
| **6d** | 8 | ND | 8 | 5 |
| **6e** | 15 | 12 | 14 | 8 |
| **6f** | ND | ND | ND | ND |
| **6g** | 12 | 10 | 10 | 7 |
| **6h** | 11 | 11 | 9 | 7 |
| **6i** | ND | ND | 5 | ND |
| **6j** | 12 | 11 | 13 | 9 |
| **Fluconazole** | 16 | 13 | 15 | 10 |