Synthesis, and fungicidal activity of novel 1,2,4-triazol derivatives containing a pyrimidine moiety

Wen-Neng Wua, #, \*, Yang-ming Jiangb, #, Qiang-Fei a, Hai-tang Dua

aFood and Pharmaceutical Engineering Institute, Guiyang University, Guiyang 550003, PR China

bState Key Laboratory of Functions and Applications of Medicinal Plants, Guizhou Medical University, Guiyang 550014, PR China

# author: contribute equally to this work

Email: wuwenneng123@126.com.

**Supplemental Materials**

**Antibacterial Activity in Vitro**

**Antifungal biological assay**

The antifungal activities of all synthesized compounds were screened and evaluated against three pathogenic fungi, including *Botryosohaeria dothidea (B. dothidea)*, *Phompsis* sp. and *Botrytis cinerea* (*B. cinerea*) of kiwifruit soft rot disease, by the poison plate technique1-3. All the compounds were dissolved in 1 mL dimethyl sulfoxide before mixing with 90 mL potato dextrose agar. The compounds were tested at a concentration of 50 μg/mL. All the fungi were cultivated in PDA at 27 ± 1 °C for 4 days to make new mycelium for antifungal assay. Then mycelia dishes of approximately 4 mm diameter were cut from culture medium, and one of them was picked up with a germfree inoculation needle and inoculated in the middle of PDA plate aseptically. The inoculated plates were fostered at 27 ± 1 °C for 3-4 days. DMSO in sterile distilled water served as negative control, while Pyrimethanil acted as positive controls. For each treatment, three replicates were conducted. The radial growth of the fungal colonies was measured and the data were statistically analyzed. The inhibition rate of the test compounds against eight pathogenic fungi were calculated by the formula, where C represents the diameter of fungi growth on untreated PDA, T represents the diameter of fungi on treated PDA, and I means the inhibition rate.

I (%) = [(C − T)/(C − 0.4)] × 100

**Results and Discussion of antifungicidal activity**

The target compounds **8** and **9a−9o** were evaluated for their fungicidal activities *in vitro* against three kinds of plant pathogenic fungi of kiwifruit soft rot disease via the poison plate technique and the preliminary bioassay results were listed in **Table S 1**. As can be seen from **Table S 1**, the target compounds 8 and **9a−9o** exhibited moderate to good fungicidal activities against the test fungi compared with the commercial Pyrimethanil. Especially, compounds **9l**, **9n** and **9n** exhibited good fungicidal activities against *B. dothidea* (86.4%, 84.9%, 90.1%, respectively), *Phompsis* sp.(89.2%, 87.4%, 91.8%, respectively), and *B. cinerea* (88.5%, 82.6%, 89.5%, respectively) at 50 μg/mL, which were similar with Pyrimethanil (84.4%, 85.1% , 82.8% respectively). Moreover, compound **8** showed fungicidal activity against *B. dothidea*, *Phompsis* sp. and *B. cinerea* at 50 μg/mL, with inhibition rates of 80.6%, 83.6%, and 81.4%, respectively, which was less than that of Pyrimethanil.

**Table S 1** The fungicidal activities of the title compounds **(50 *µ*g/mL)**

|  |  |  |  |
| --- | --- | --- | --- |
| Compounds | Inhibition (%) | | |
| *B. dothidea* | *Phomopsis sp*. | *Botrytis cinerea* |
| **8** | 80.6±2.4 | 83.6±2.7 | 81.4±1.2 |
| **9a** | 41.2±1.0 | 45.0±1.3 | 43.8±1.9 |
| **9b** | 47.5±2.1 | 51.6±2.3 | 49.0±2.5 |
| **9c** | 58.5±2.6 | 60.1±3.0 | 58.9±1.5 |
| **9d** | 30.6±1.1 | 36.8±1.7 | 29.5±1.2 |
| **9e** | 42.3±1.9 | 46.0±3.0 | 49.6±2.2 |
| **9f** | 46.6±1.1 | 52.7±2.3 | 54.2±2.5 |
| **9g** | 55.9±1.8 | 62.4±1.2 | 63.2±1.9 |
| **9h** | 57.8±2.8 | 56.3±3.3 | 53.4±1.2 |
| **9i** | 67.5±2.1 | 66.2±2.1 | 62.1±1.6 |
| **9j** | 54.1±2.2 | 57.2±1.3 | 50.3±1.5 |
| **9k** | 64.9±1.1 | 68.0±2.2 | 62.1±3.1 |
| **9l** | 86.4±1.4 | 89.2±1.8 | 88.5±1.7 |
| **9m** | 71.1±1.9 | 67.5±1.0 | 70.8±2.2 |
| **9n** | 84.9±2.7 | 87.4±2.0 | 82.6±1.8 |
| **9o** | 90.1±2.6 | 91.8±1.4 | 89.5±2.5 |
| Pyrimethanil | 84.4±2.1 | 85.1±1.4 | 82.8±1.4 |

Based on the preliminary bioassays, the EC50 values of the target compounds **8** and **9a−9o** as well as the commercial Pyrimethanil against *B. dothidea and Phompsis* sp. were also tested and presented in **Table S 2, Table S 3** and **Table S 4**. **Table S 2, Table S 3** and **Table S 4** showed that compounds **9l**, 9**n** and **9o** showed good activities against *B. dothidea* with EC50 of 40.1, 48.2 and 33.6 μg/mL, respectively, which were better than that of Pyrimethanil (57.6 μg/mL). Meanwhile, compounds 9**n** and **9o** exhibited good fungicidal activity against *B. dothidea* and *B. cinerea* with the EC50 values of 25.4 and 55.1 μg/mL, respectively, which were similar with Pyrimethanil (32.1 and 62.8 μg/mL).

**Table S 2**.The EC50 values of some of the target compounds against *B. dothidea*

|  |  |  |  |
| --- | --- | --- | --- |
| Compounds. | Toxic regression equation | *r* | EC50(μg/mL) |
| **8** | y =0.92 x +4.45 | 0.99 | 61.2±1.0 |
| **9l** | y = 0.67x + 3.68 | 0.99 | 40.1±1.4 |
| **9n** | y = 1.24x + 4.18 | 0.99 | 48.2±1.9 |
| **9o** | y = 0.88x + 5.62 | 0.99 | 33.6±2.1 |
| Pyrimethanil | y = 1.01x + 6.25 | 0.99 | 57.6±1.8 |

**Table S 3**.The EC50 values of the target compounds against *Phompsis* sp..

|  |  |  |  |
| --- | --- | --- | --- |
| Compounds. | Toxic regression equation | *r* | EC50(μg/mL) |
| **8** | y =1.48x + 3.62 | 0.99 | 35.1±1.1 |
| **9l** | y = 0.92x + 4.70 | 0.99 | 37.3±1.5 |
| **9n** | y = 0.74x + 4.31 | 0.98 | 31.6±1.6 |
| **9o** | y = 0.78x + 4.67 | 0.98 | 25.4±1.8 |
| Pyrimethanil | y = 2.18x + 8.25 | 0.99 | 32.1±2.0 |

**Table S 4** The EC50 values of some of the target compounds against *B. cinerea*

|  |  |  |  |
| --- | --- | --- | --- |
| Compounds. | Toxic regression equation | *r* | EC50 (μg/mL) |
| **8** | y =0.92 x +4.45 | 0.99 | 73.2±1.9 |
| **9l** | y = 1.15x + 3.91 | 0.99 | 61.6±2.5 |
| **9n** | y = 1.67x +3.82 | 0.99 | 67.5±1.4 |
| **9o** | y = 1.32x + 4.64 | 0.99 | 55.1±3.1 |
| Pyrimethanil | y = 1.46x +4.76 | 0.99 | 62.8±1.7 |

**References**

**References**

[1]Chattapadhyay, T. -K.; Dureja, P. Antifungal Activity of 4-Methyl-6-alkyl-2H-pyran-2-ones. J. Agric. Food Chem. **2006**, 54: 2129−2133. DOI: 10.1021/jf052792s.

[2]Xu, W. -M.; He, J.; He, M.; Han, F. -F.; Chen, X. -H.; Pan, Z. -X.; Wang, J.; Tong, M. -G. Synthesis and Antifungal Activity of Novel Sulfone Derivatives Containing 1,3,4-Oxadiazole Moieties. *Molecules*. **2011**, 16: 9129-9141. DOI: 10.3390/molecules16119129.

[3]Xu, W. -M.; Yang, S.; Bhadury, P.; He, J.; He, M.; Gao, L. -L.; Hu, D. -Y.; Song, B. -A. Synthesis and Bioactivity of Novel Sulfone Derivatives Containing 2,4-Dichlorophenyl Substituted 1,3,4-Oxadiazole/thiadiazole Moiety as Chitinase Inhibitors. *Pestic. Biochem. Phys*. **2011**,101: 6-15. DOI: 10.1016/j.pestbp.2011.05.006.

**1H NMR and 13C NMR spectra**

**C:\Users\ADMINI~1\AppData\Local\Temp\360zip$Temp\360$0\甲基-H.tiff**

**Figure S 1:** 1H NMR of 9a

**C:\Users\ADMINI~1\AppData\Local\Temp\360zip$Temp\360$1\甲基-C.tiff**

**Figure S 2:** 13C NMR of 9a

C:\Users\ADMINI~1\AppData\Local\Temp\360zip$Temp\360$2\乙基-H.tiff

**Figure S 3:** 1H NMR of 9b

C:\Users\ADMINI~1\AppData\Local\Temp\360zip$Temp\360$3\乙基-C.tiff

**Figure S 4:** 13C NMR of 9b

**C:\Users\ADMINI~1\AppData\Local\Temp\360zip$Temp\360$4\W-2-H.tiff**

**Figure S 5:** 1H NMR of 9c

**C:\Users\ADMINI~1\AppData\Local\Temp\360zip$Temp\360$5\W-2-C.tiff**

**Figure S 6:** 13C NMR of 9c

**H:\修改数据\W-5-H.tiff**

**Figure S 7:** 1H NMR of 9d

**H:\修改数据\W-5-C.tiff**

**Figure S 8:** 13C NMR of 9d

C:\Users\Administrator\Documents\Tencent Files\564368711\FileRecv\W-11-H.tiff

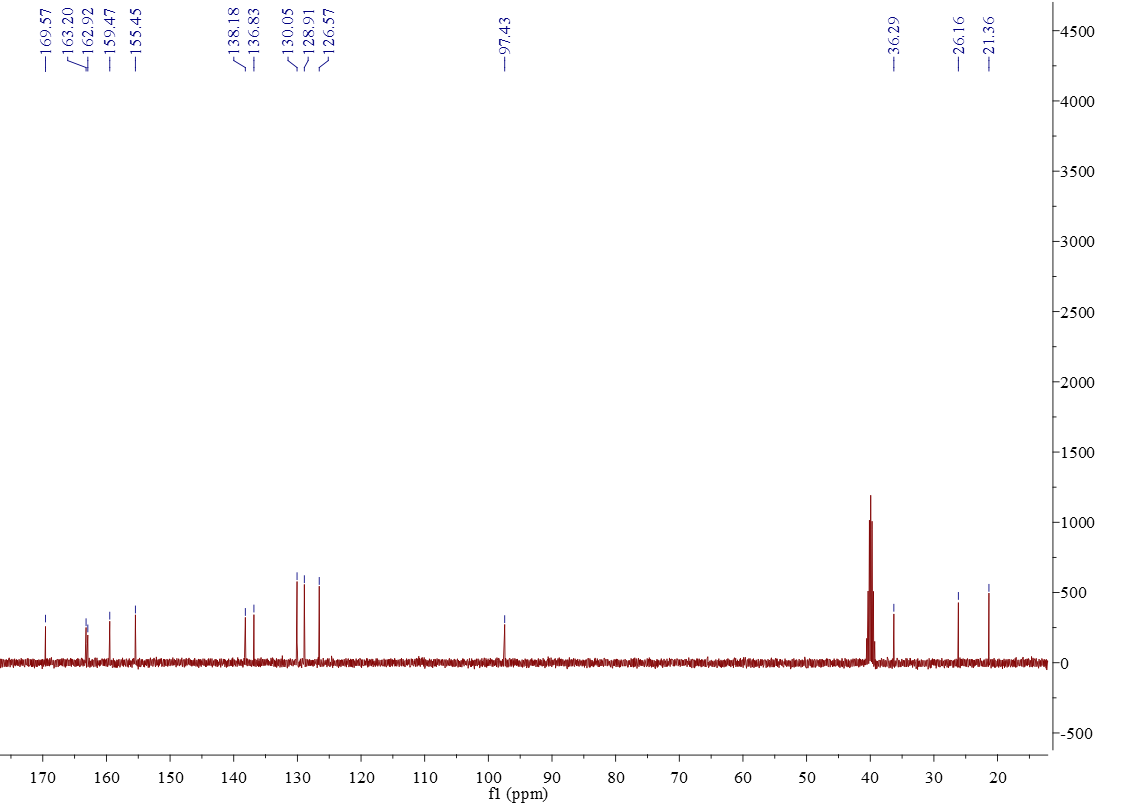
**Figure S 9:** 1H NMR of 9e

C:\Users\ADMINI~1\AppData\Local\Temp\360zip$Temp\360$5\W-11-C.tiff

**Figure S 10:** 13C NMR of 9e

C:\Users\ADMINI~1\AppData\Local\Temp\360zip$Temp\360$2\W-10-H.tiff

**Figure S 11:** 1H NMR of 9f



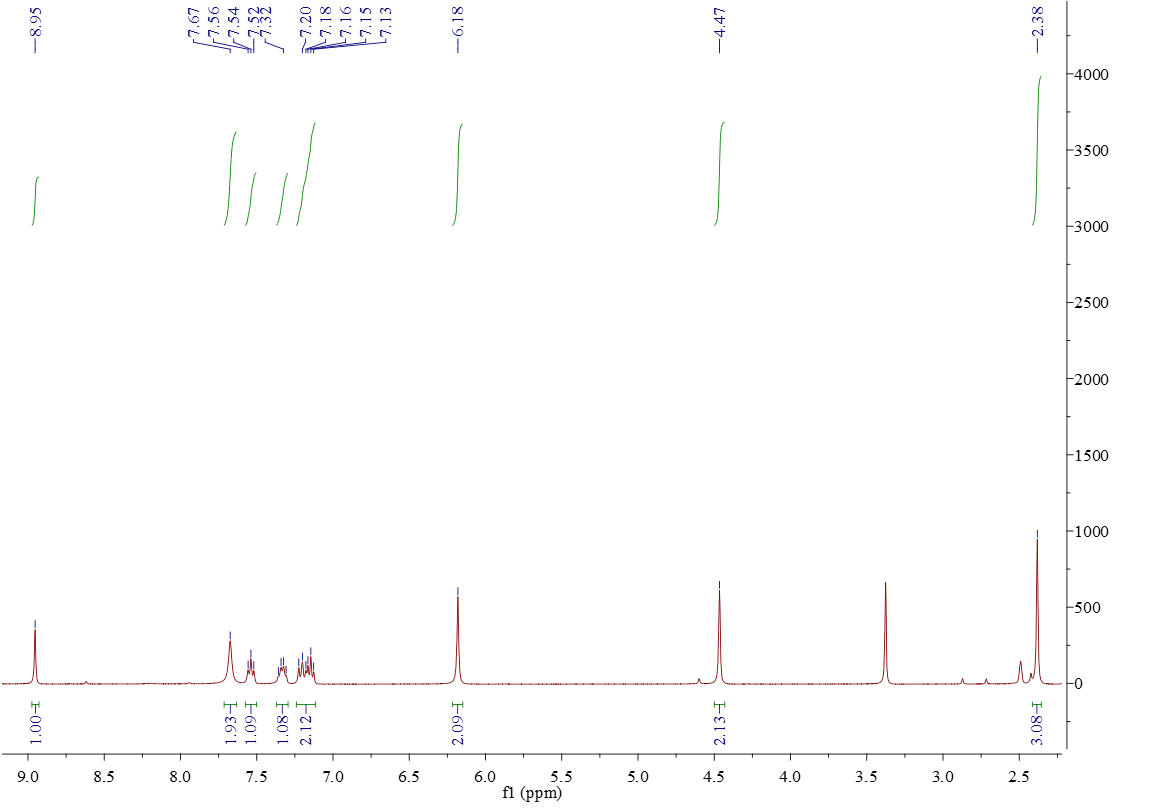
**Figure S 12:** 13C NMR of 9f

C:\Users\Administrator\Documents\Tencent Files\564368711\FileRecv\W-8-H.tiff

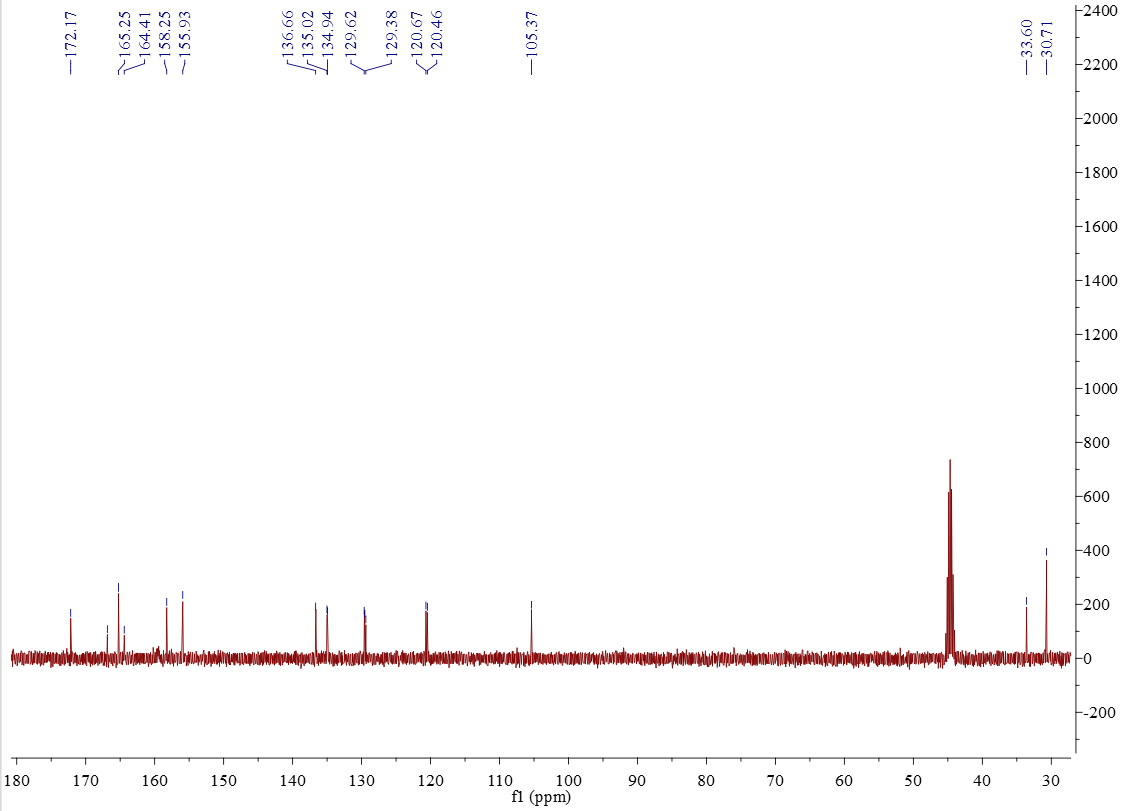
**Figure S 13:** 1H NMR of 9g

C:\Users\ADMINI~1\AppData\Local\Temp\360zip$Temp\360$1\W-8-C.tiff

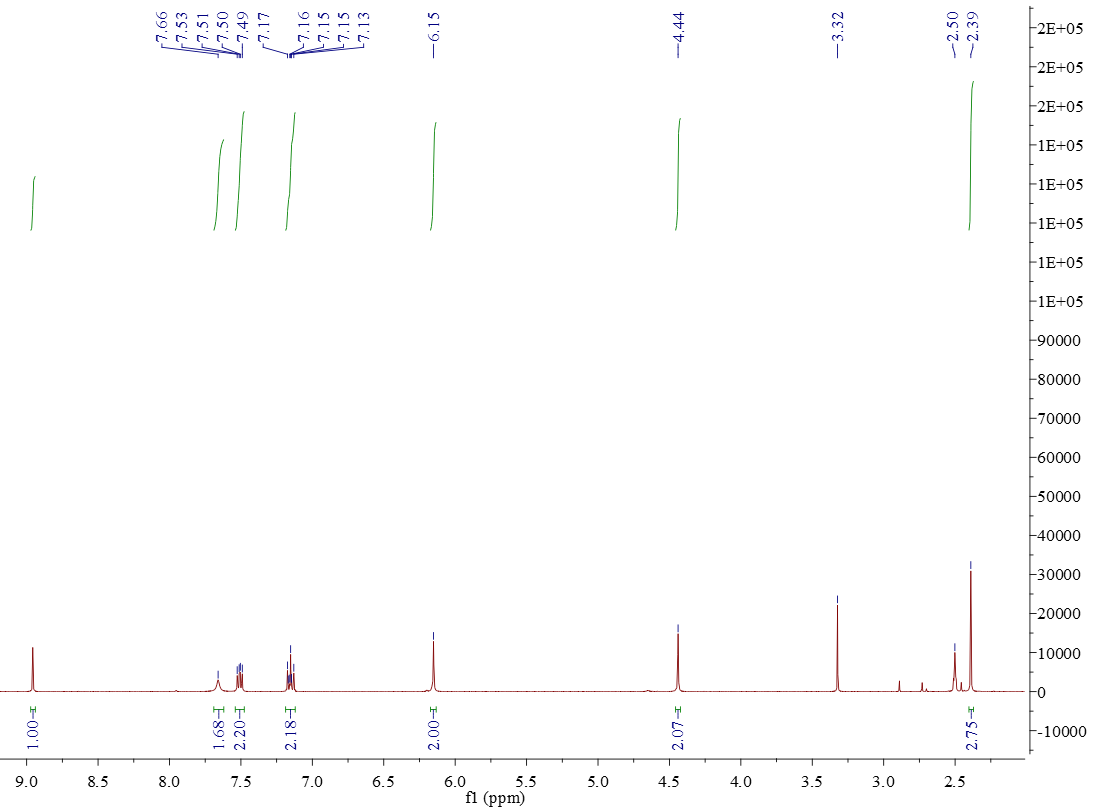
**Figure S 14:** 13C NMR of 9g



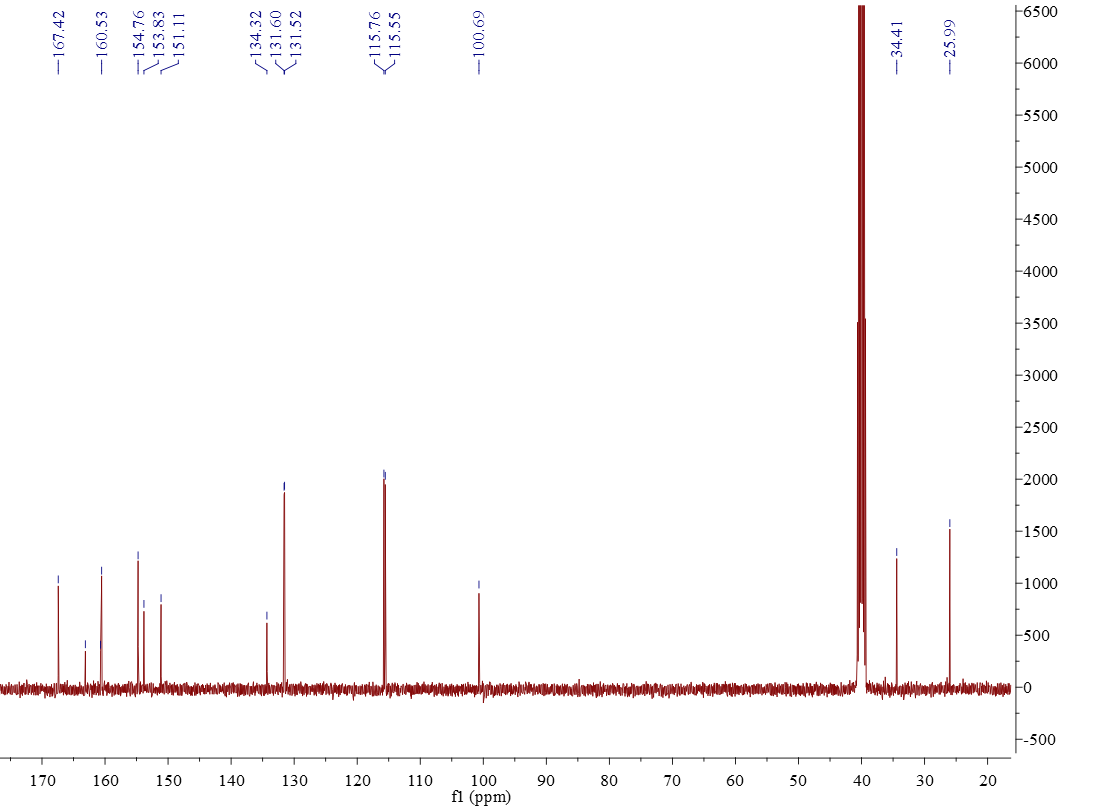
**Figure S 15:** 1H NMR of 9h



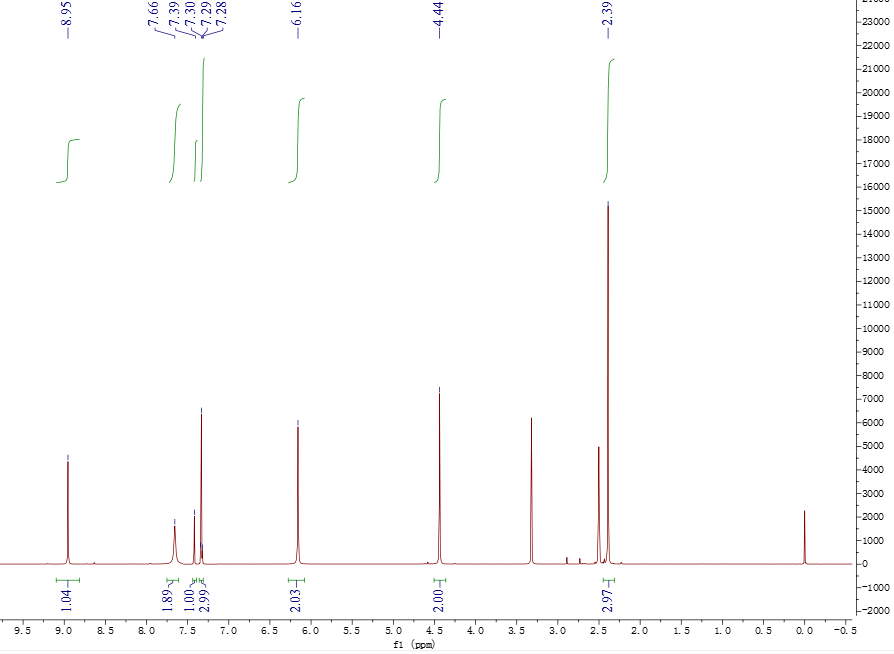
**Figure S 16:** 13C NMR of 9h



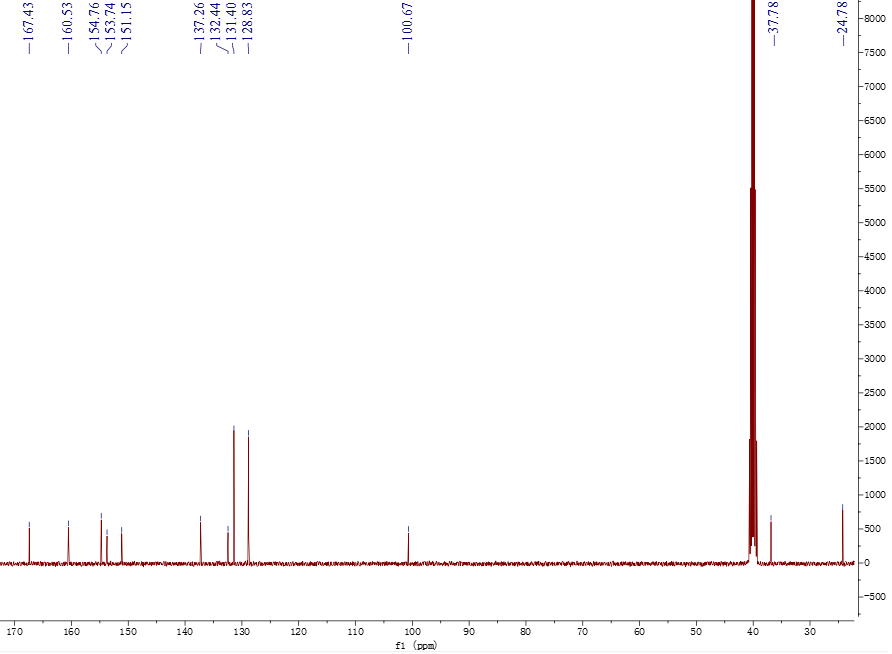
**Figure S 17:** 1H NMR of 9i



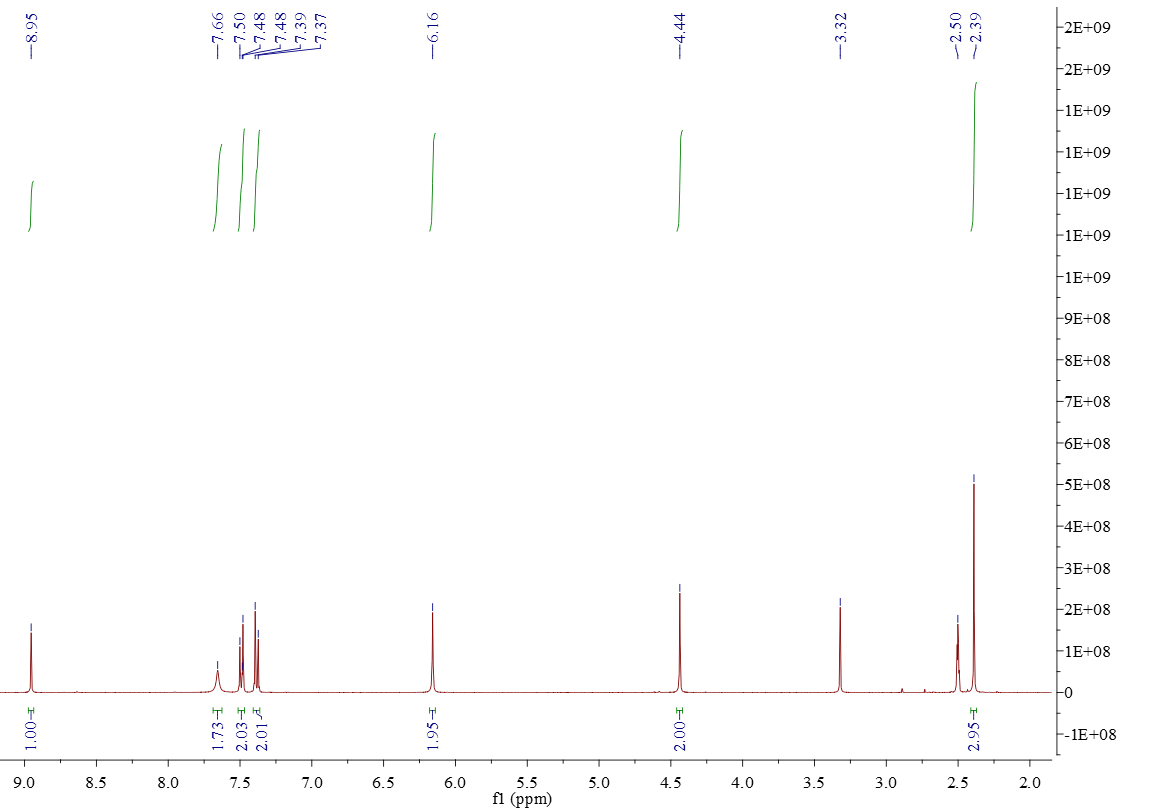
**Figure S 18:** 13C NMR of 9i



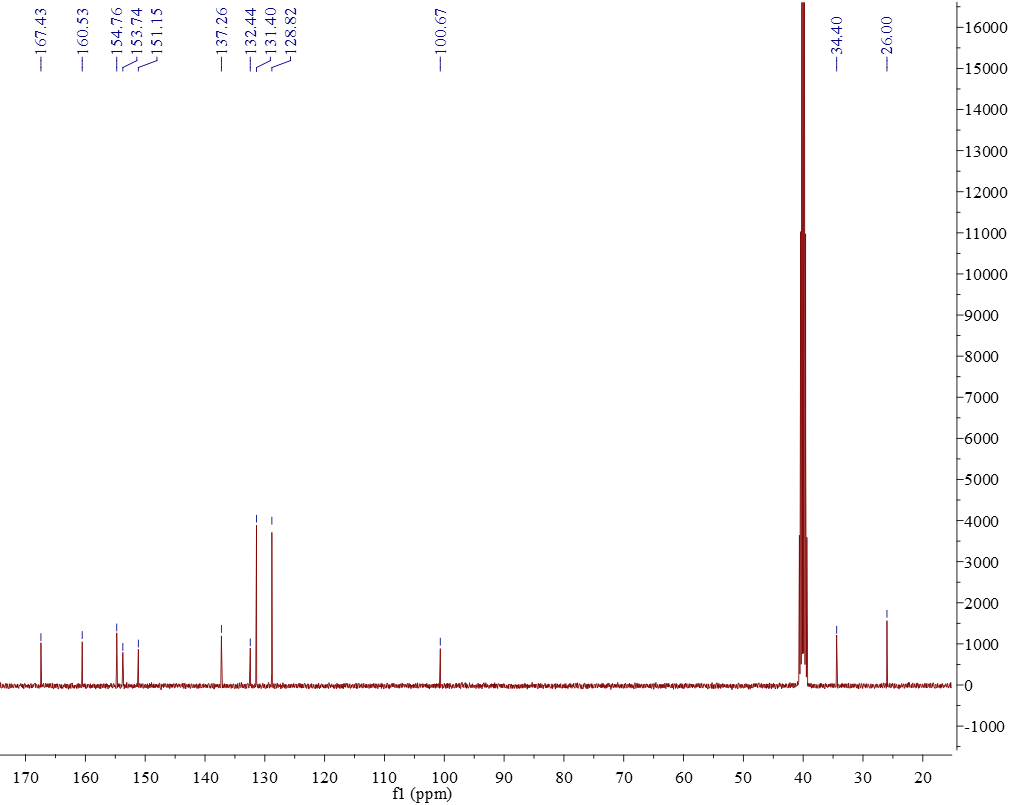
**Figure S 19:** 1H NMR of 9j



**Figure S 20:** 13C NMR of 9j



**Figure S 21:** 1H NMR of 9k



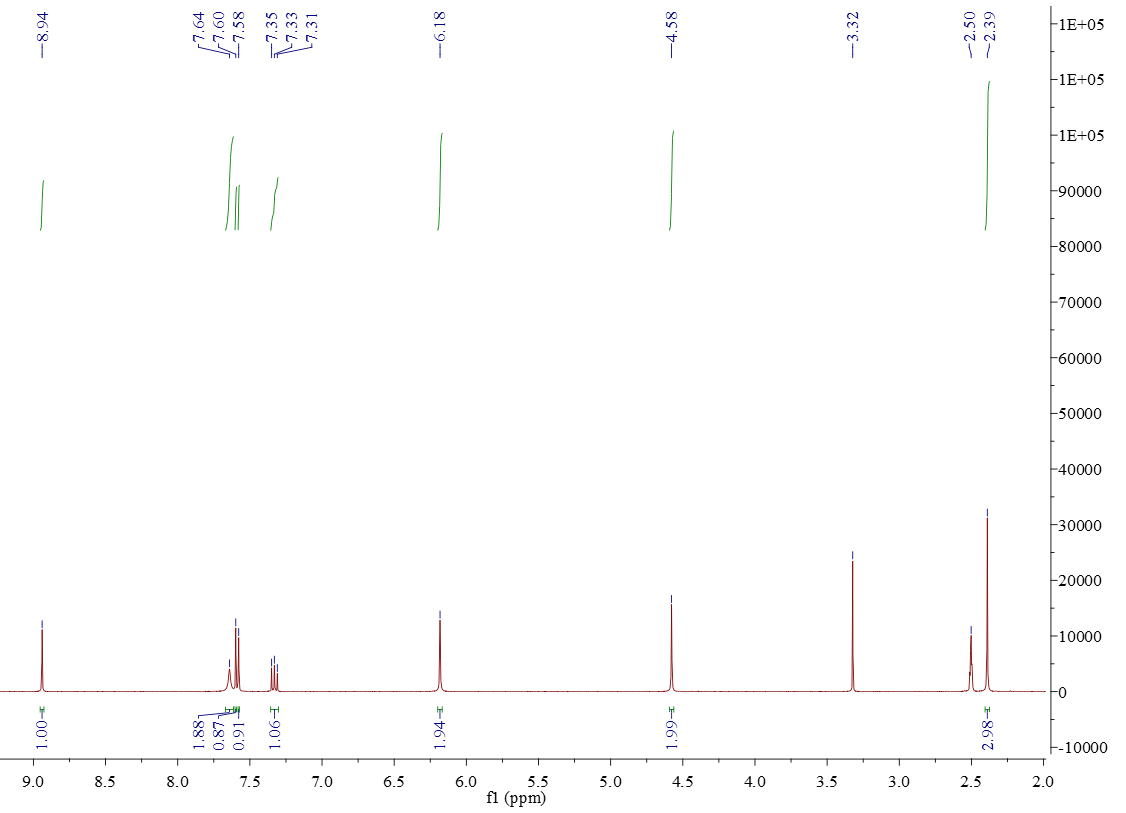
**Figure S 22:** 13C NMR of 9k

**H:\修改数据\W-6-H.tif**

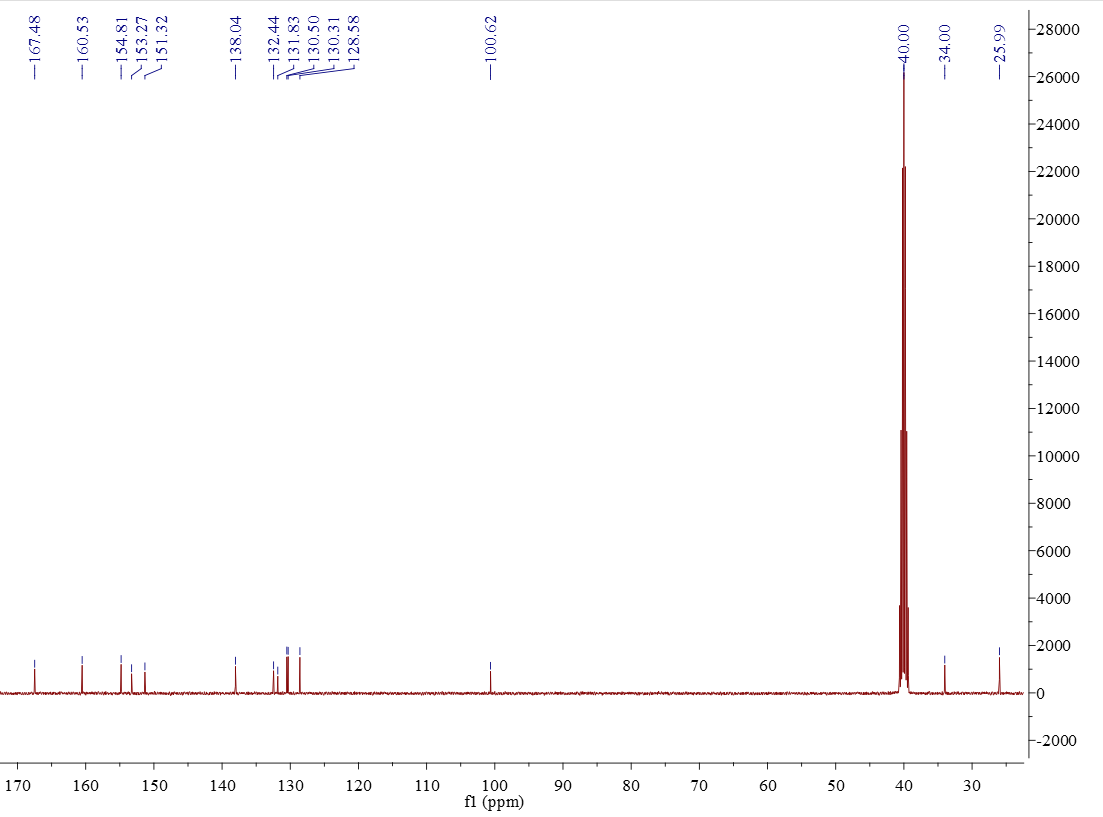
**Figure S 23:** 1H NMR of 9l

**C:\Users\Administrator\Documents\Tencent Files\564368711\FileRecv\W-6-C.tiff**

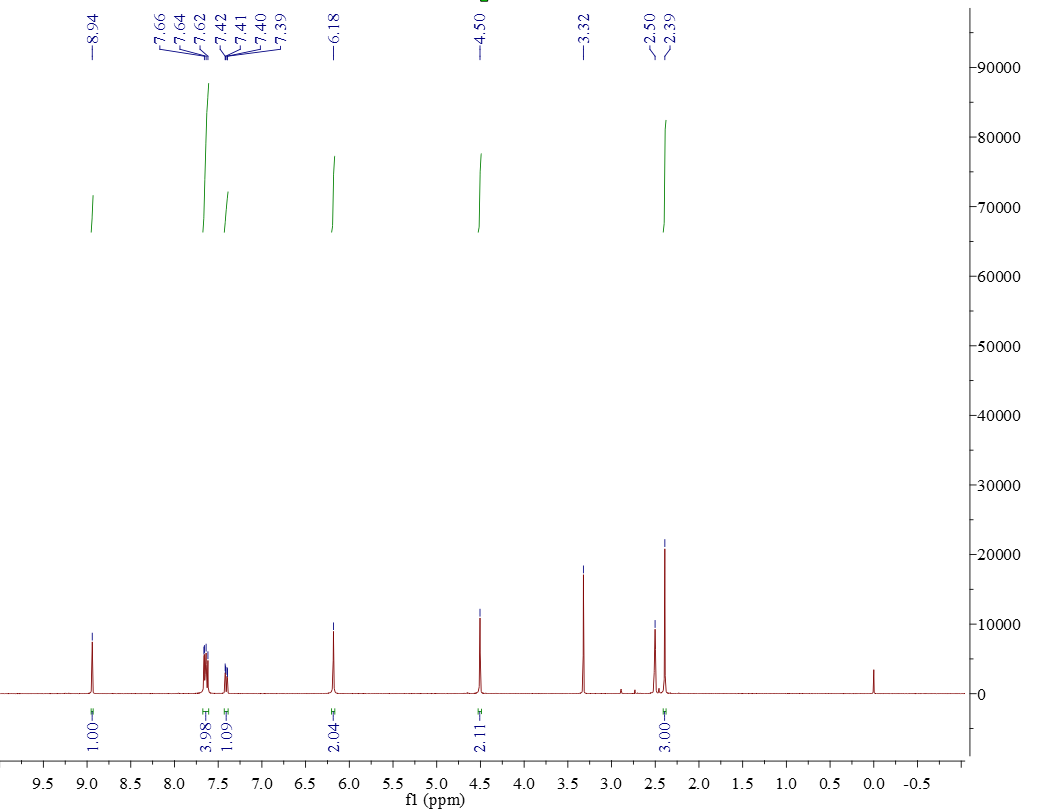
**Figure S 24:** 13C NMR of 9l



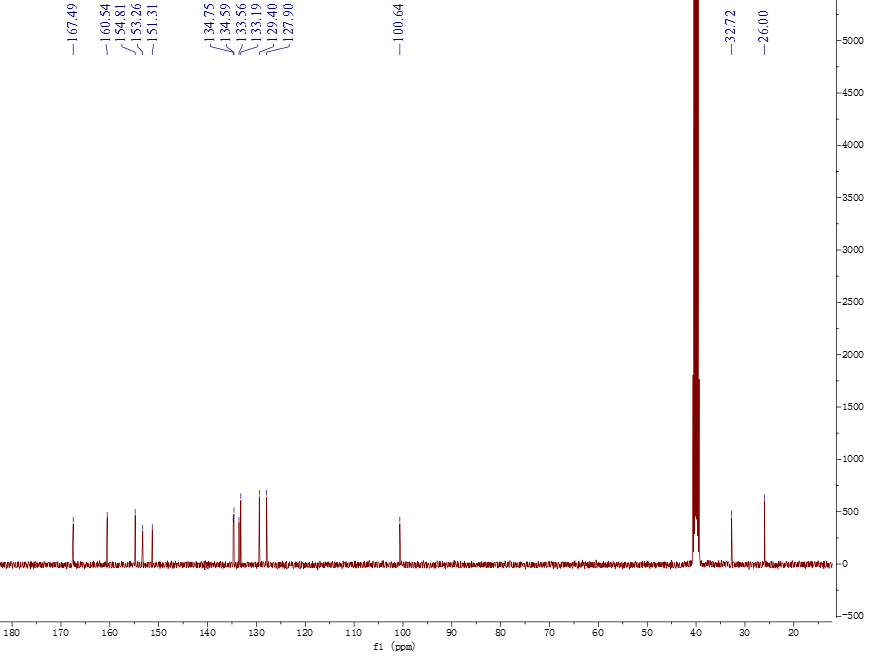
**Figure S 25:** 1H NMR of 9m



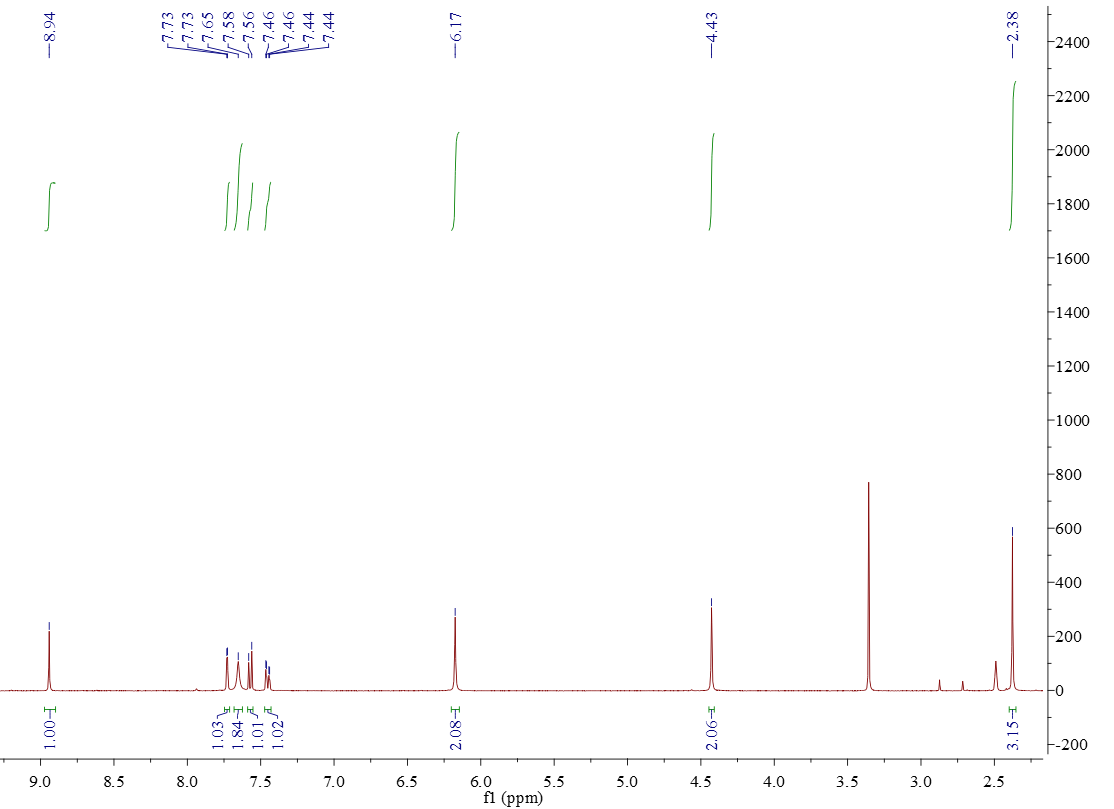
**Figure S 26:** 13C NMR of 9m



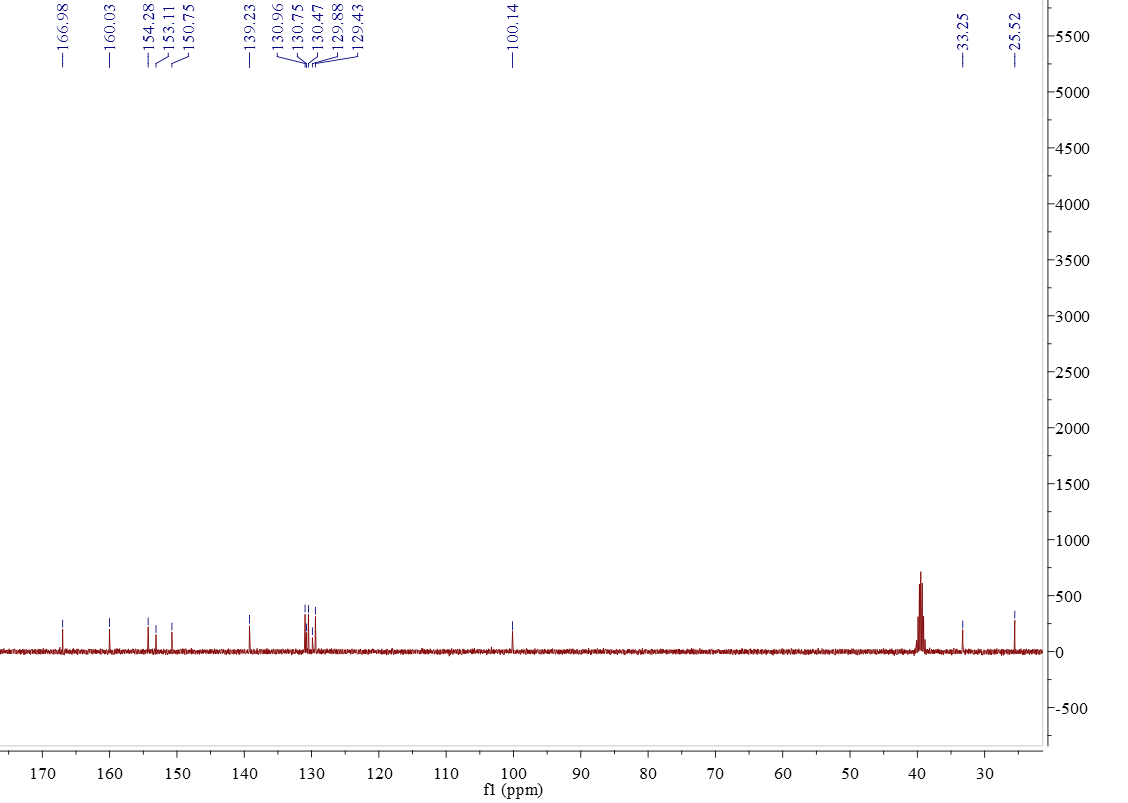
**Figure S 27:** 1H NMR of 9n



**Figure S 28:** 13C NMR of 9n



**Figure S 29:** 1H NMR of 9o



**Figure S 30:** 13C NMR of 9o