

Supplementary File

Materials and Methods

Biological Assay

Cell Culture

Normal murine embryonic liver BNL CL.2 cell lines were maintained in Dulbecco's modified Eagle's medium (DMEM), supplemented with 10% fetal bovine serum (FBS) and 1% penicillin/streptomycin in CO₂ incubator with a humidified atmosphere of 95% air and 5% CO₂ at 37 °C.

Cell Cytotoxicity Assay Using MTT Assay

The effects of norcantharidin (**2**), compounds **9** and **18** on the cell viability were determined using 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide (MTT) assay. Briefly, murine embryonic liver cells BNL CL.2 (2.5×10^4 cells/well) were seeded in 96-well culture plates. After 24 h incubation to allow cell attachment, the cell were incubated with or without various concentrations of norcantharidin (**2**), compounds **9** and **18** for 24 h and 48 h. Ten microliter of MTT (5 mg/mL) was then added to each well, and the plates were incubated for an additional 2.5 h at 37 °C. The formazan crystals formed by MTT metabolism were solubilized by 150 μ L DMSO to each well. The absorbance at 540 nm was measured with a microplate ELISA reader (Molecular Devices spectramax 340 PC³⁸⁴). Values represent the mean $\pm$ SD form at least two independent experiments.

Figure S1. The effects of norcantharidin (**2**), compounds **9** and **18** on cell proliferative activity of normal cell line BNL CL.2 (murine embryonic liver cells) for (A) 24 h and (B) 48 h ($n = 3$).

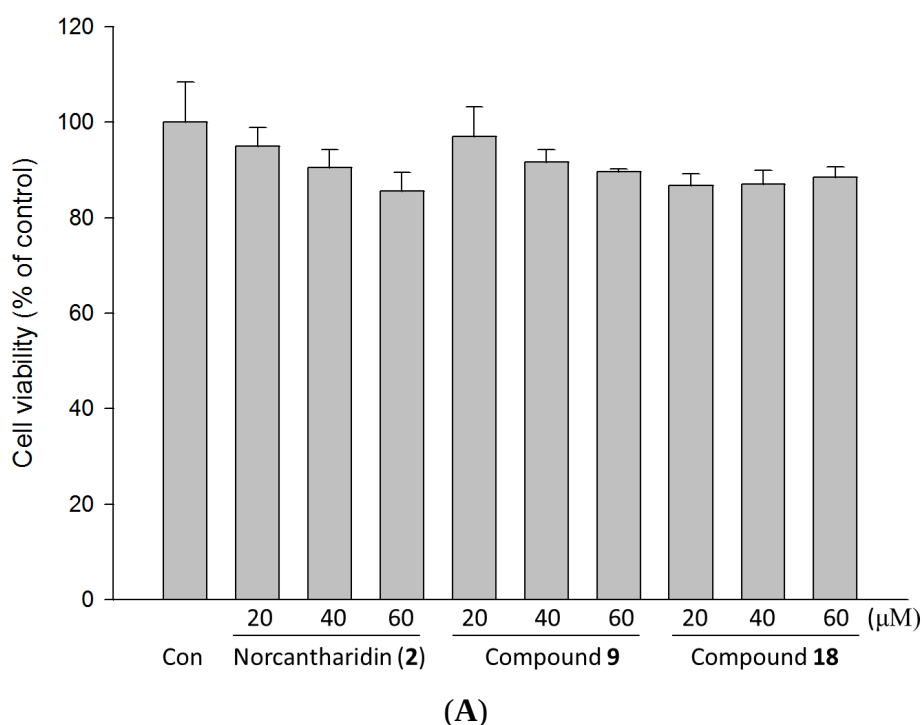


Figure S1. Cont.

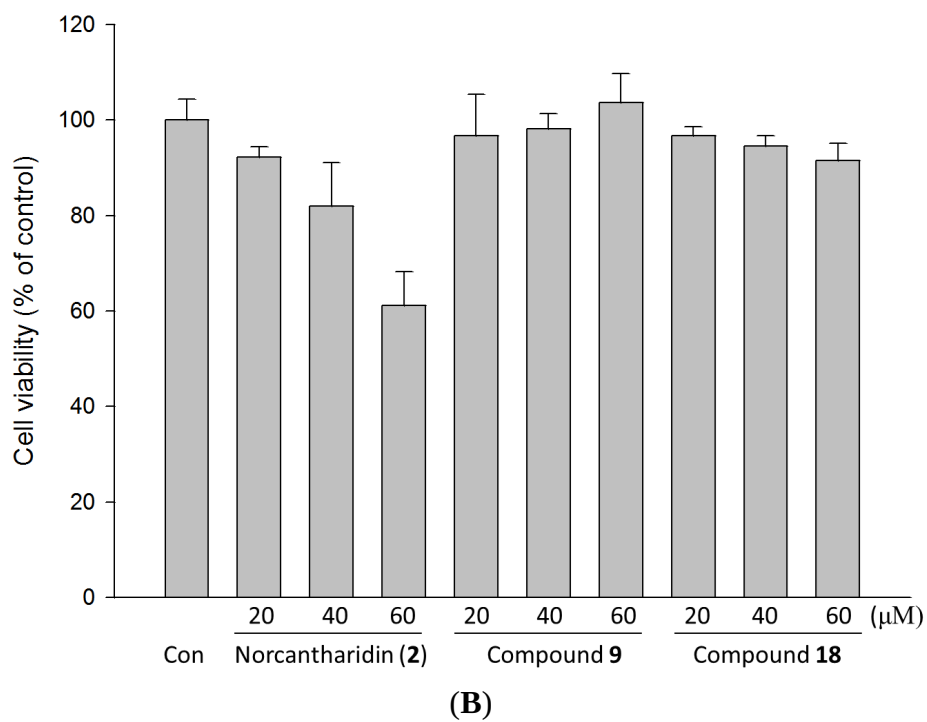


Figure S2. (A) ^1H -NMR spectra of norcantharidin (2); (B) ^{13}C -NMR spectra of norcantharidin (2).

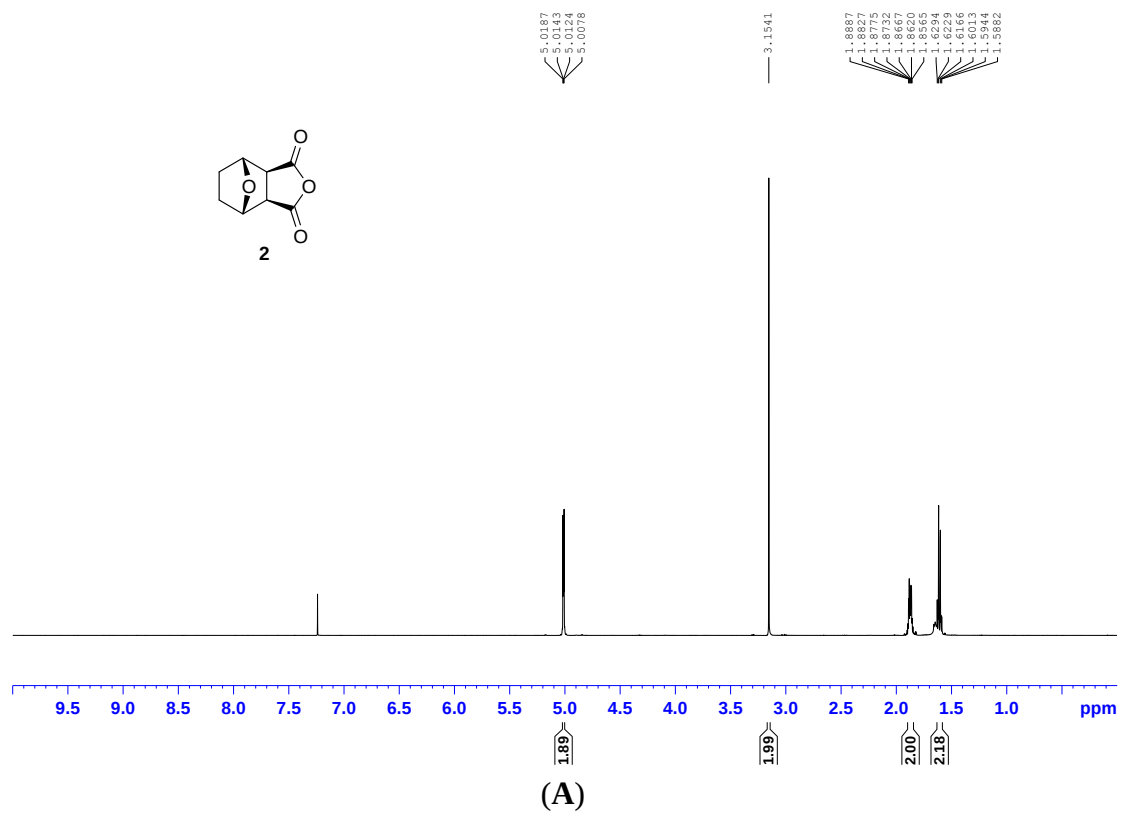


Figure S2. Cont.

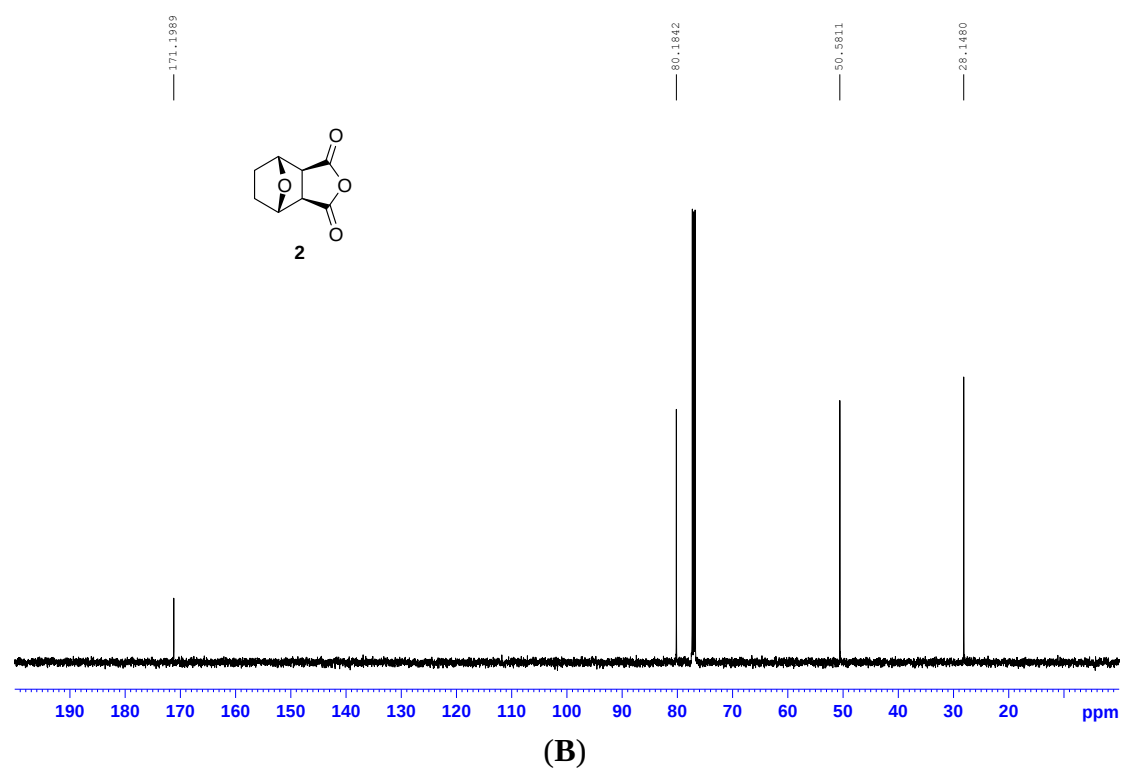
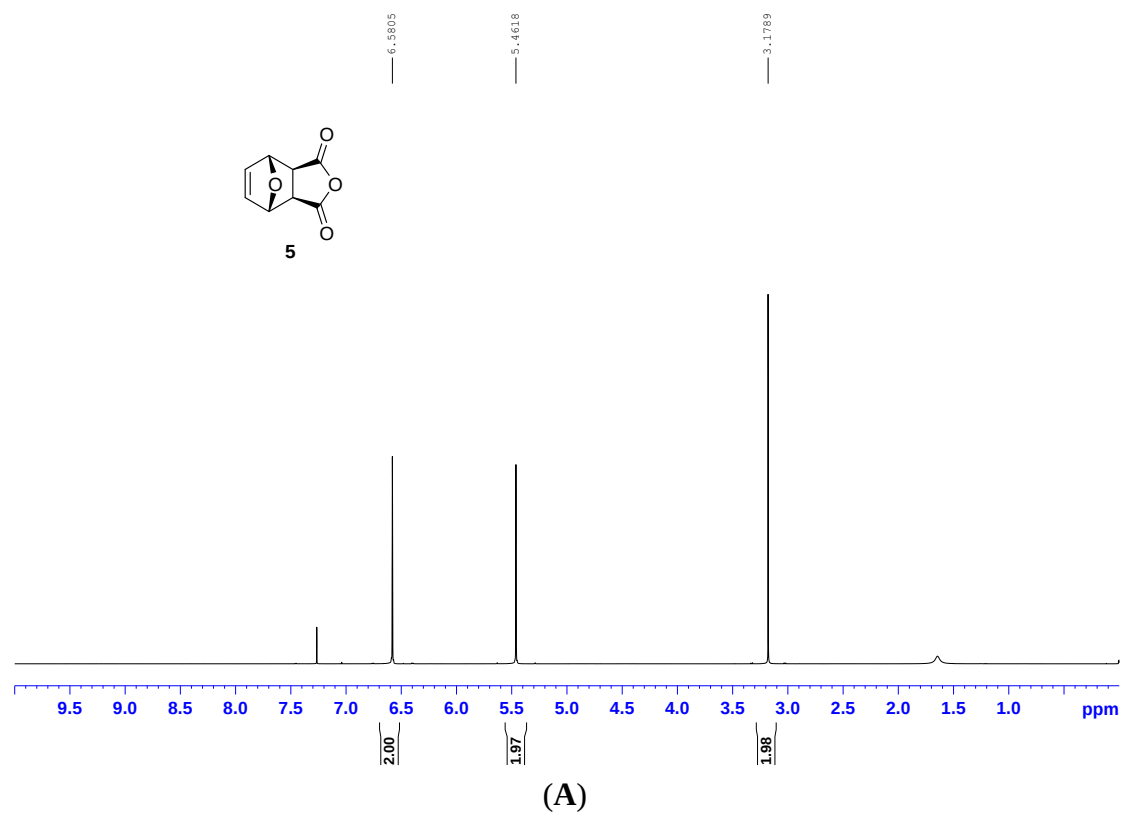
Figure S3. (A) ^1H -NMR spectra of compound 5; (B) ^{13}C -NMR spectra of compound 5.

Figure S3. Cont.

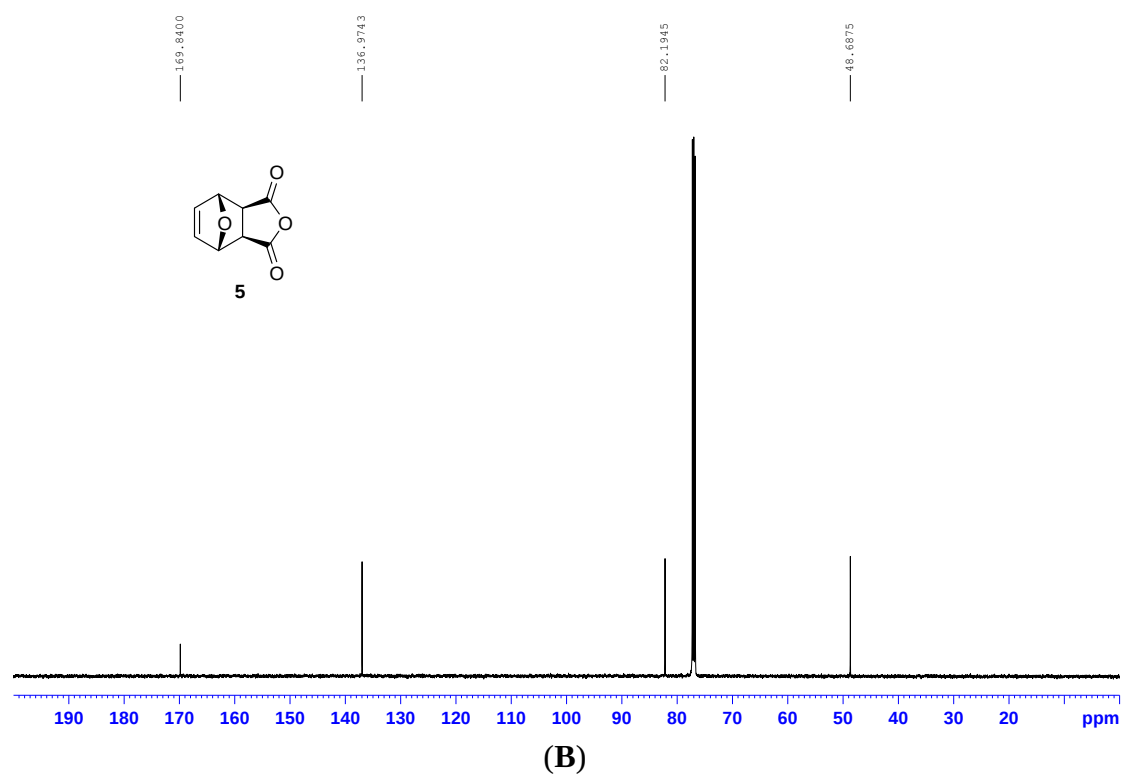
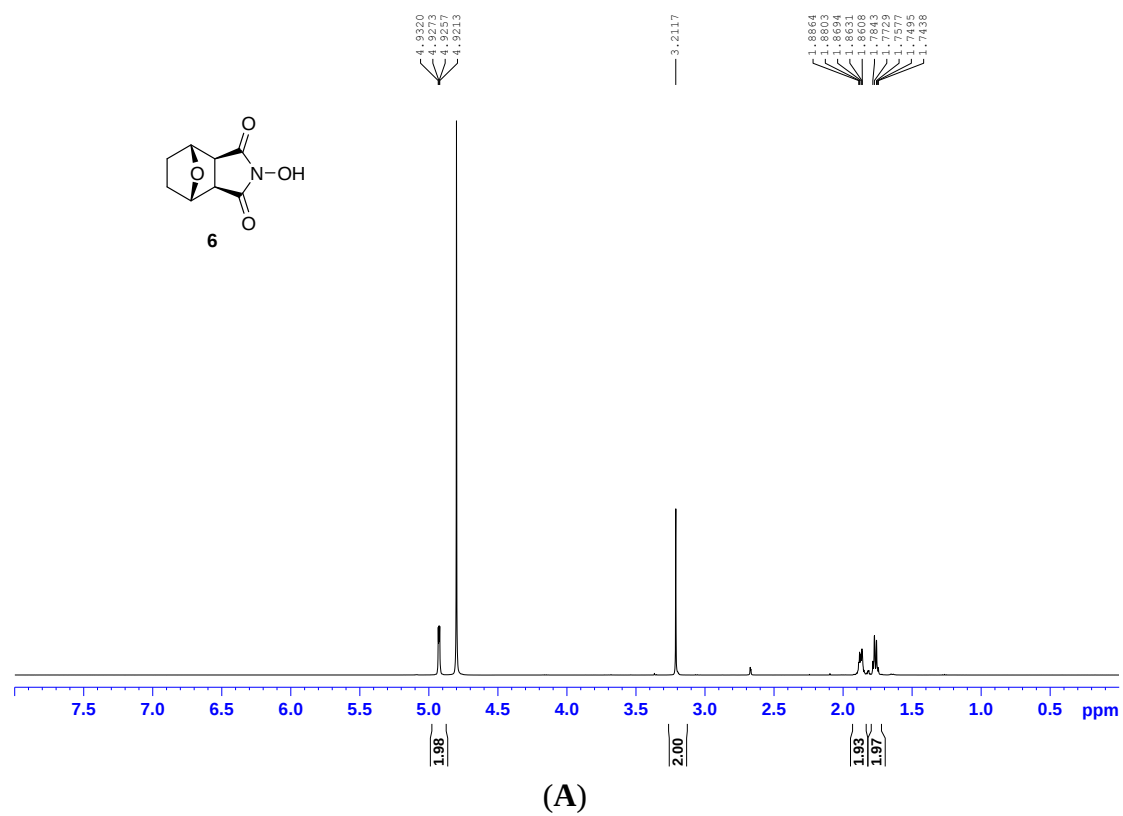
Figure S4. (A) ^1H -NMR spectra of compound 6; (B) ^{13}C -NMR spectra of compound 6.

Figure S4. Cont.

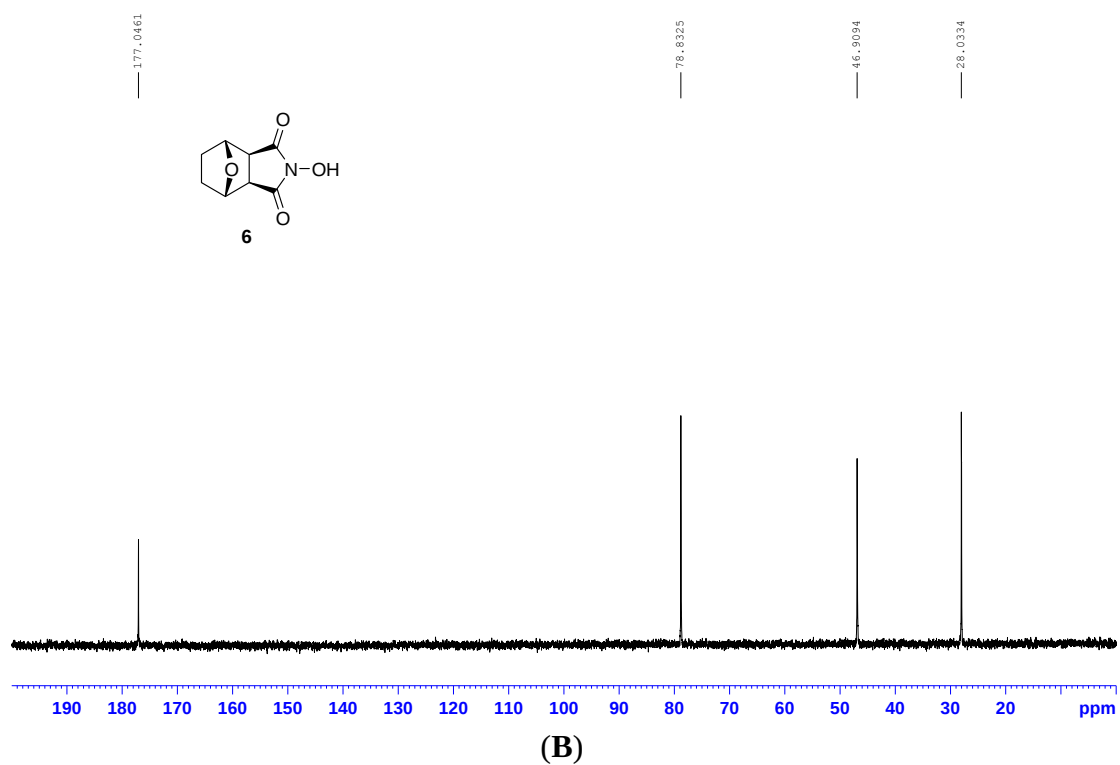
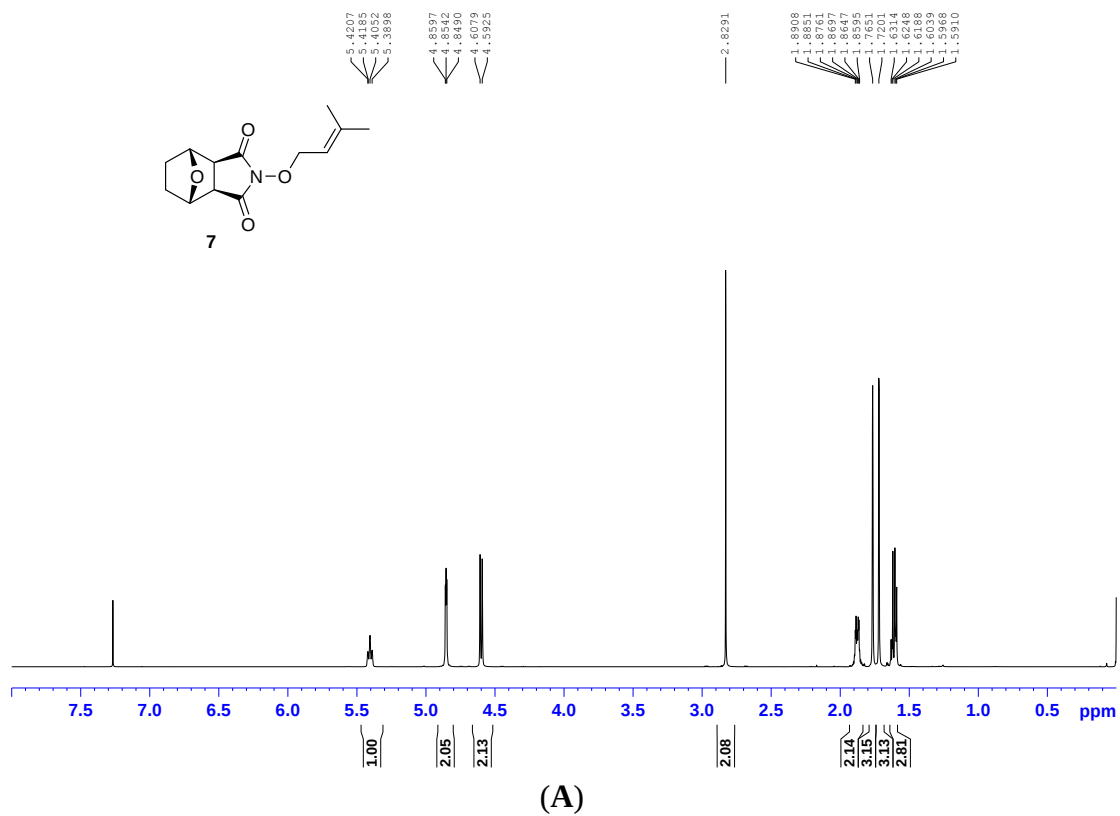
Figure S5. (A) ¹H-NMR spectra of compound 7; (B) ¹³C-NMR spectra of compound 7.

Figure S5. Cont.

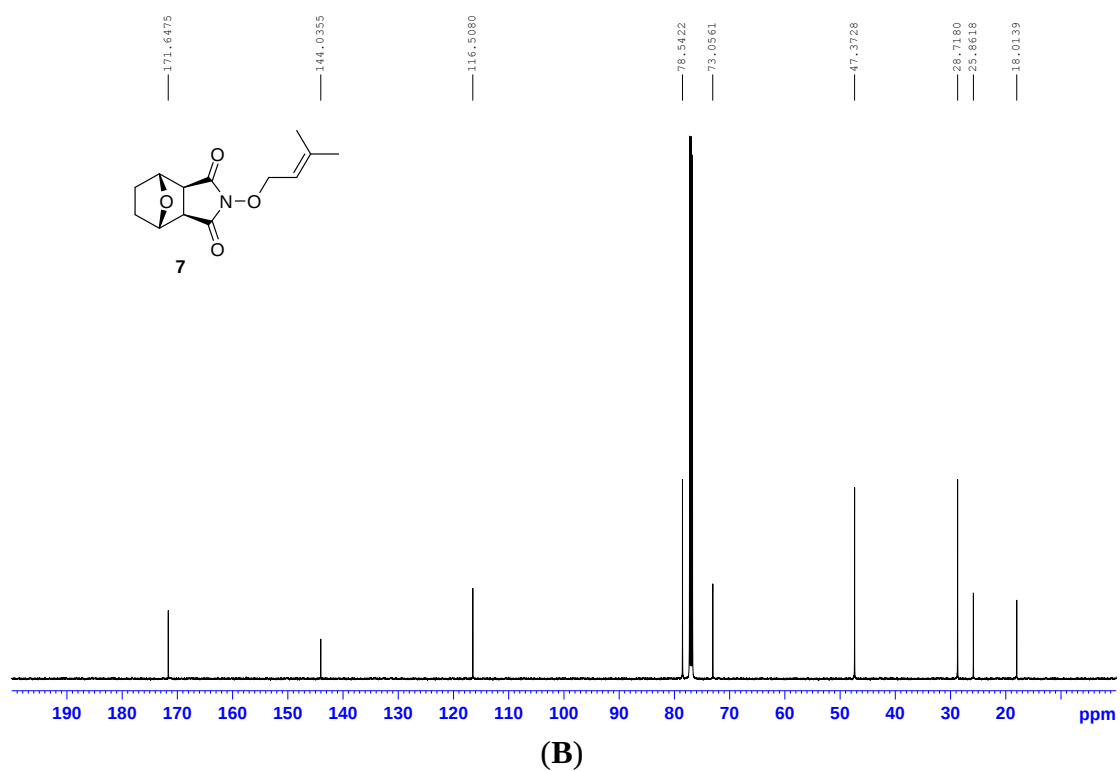
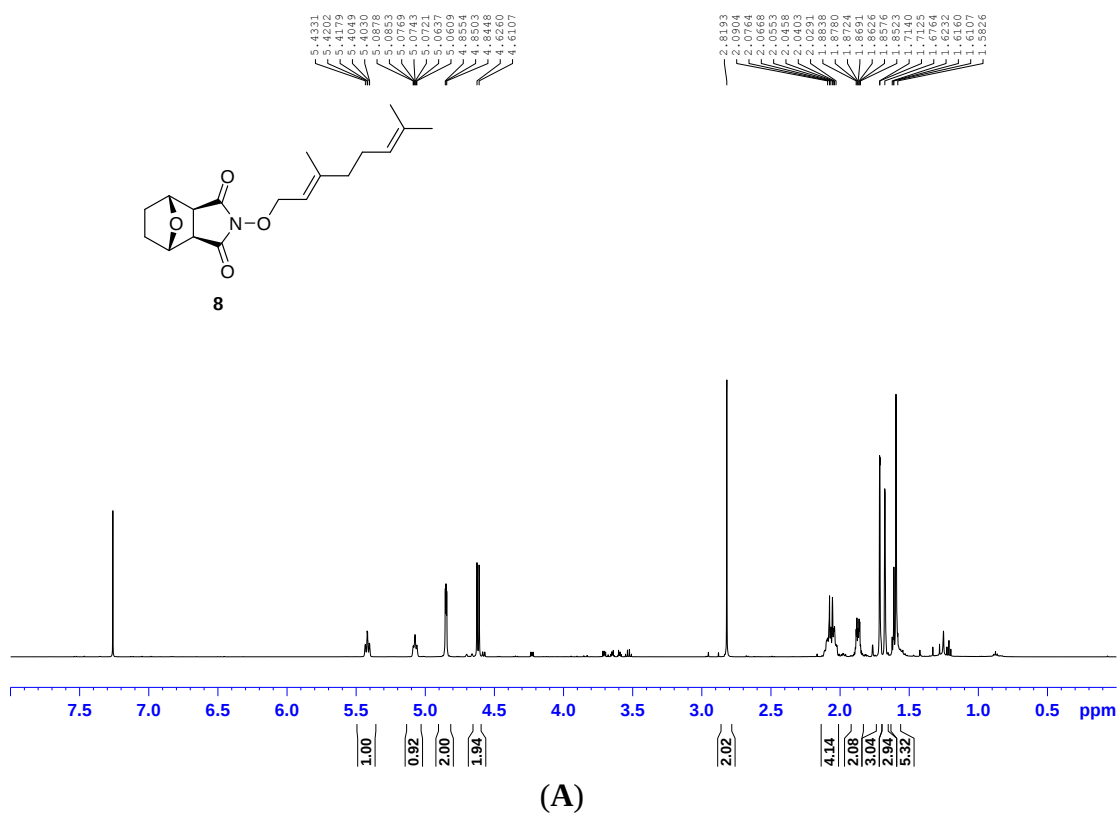
Figure S6. (A) ^1H -NMR spectra of compound 8; (B) ^{13}C -NMR spectra of compound 8.

Figure S6. Cont.

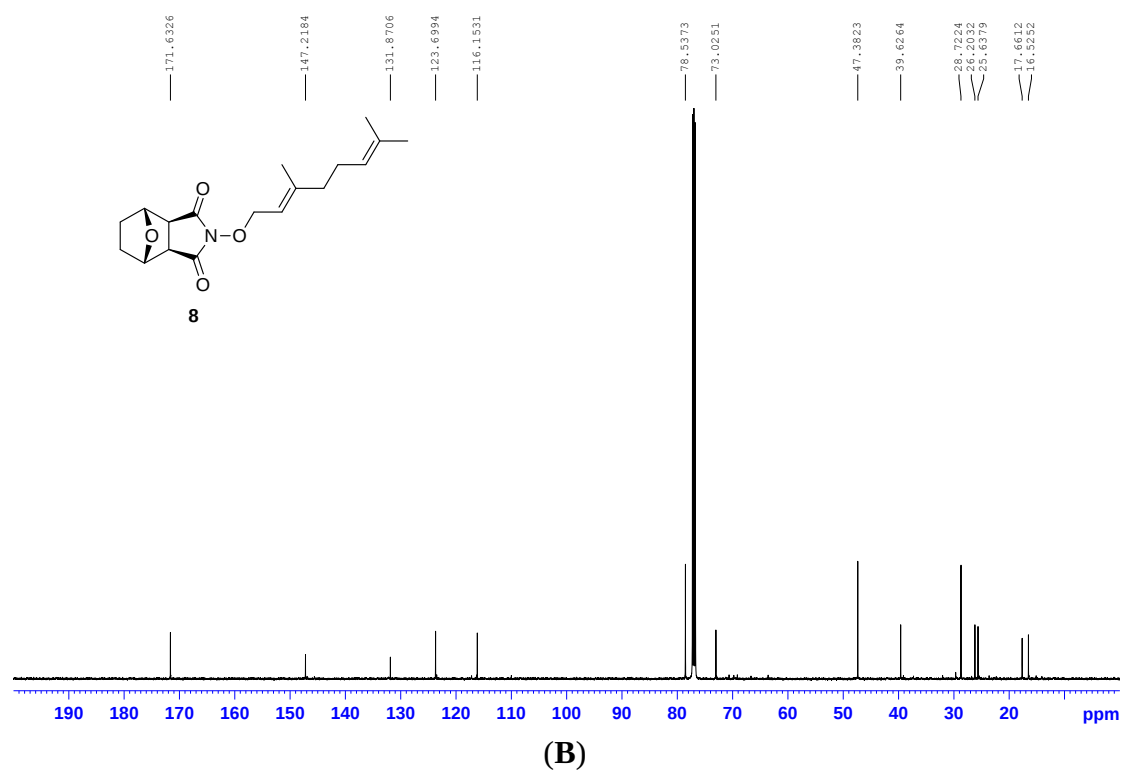
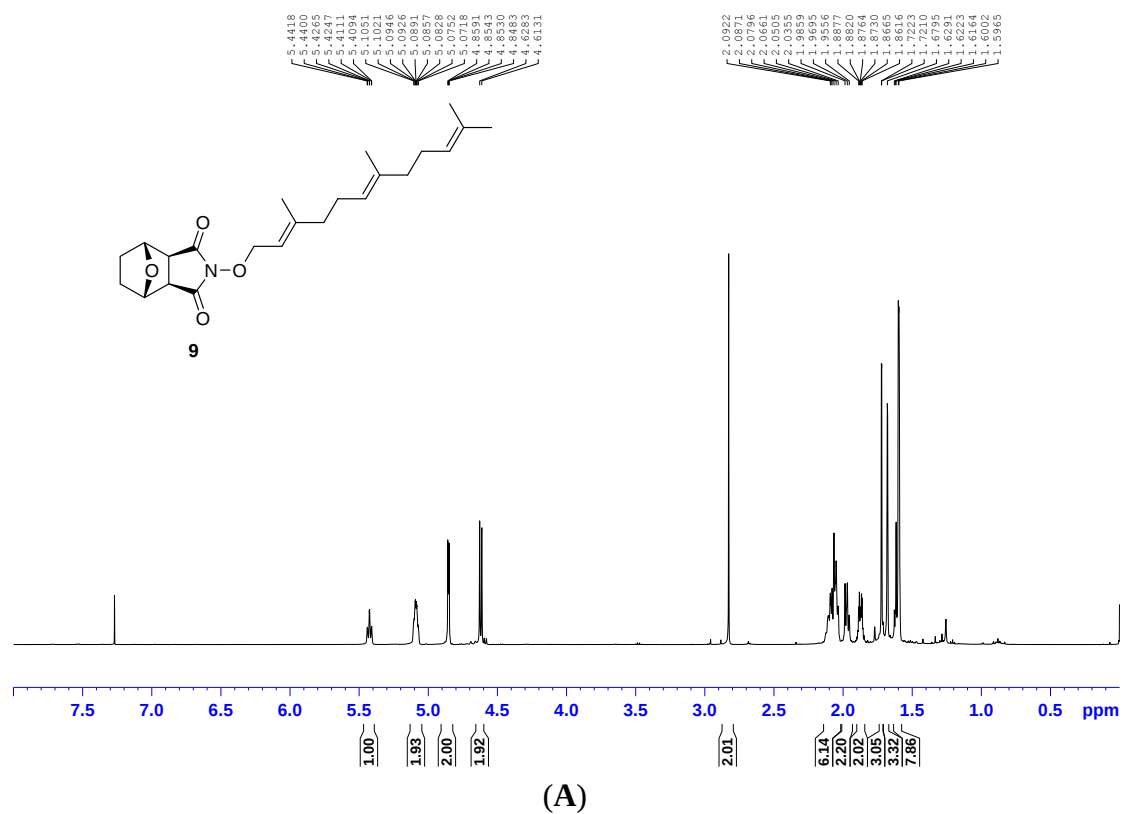
Figure S7. (A) ¹H-NMR spectra of compound **9**; (B) ¹³C-NMR spectra of compound **9**.

Figure S7. Cont.

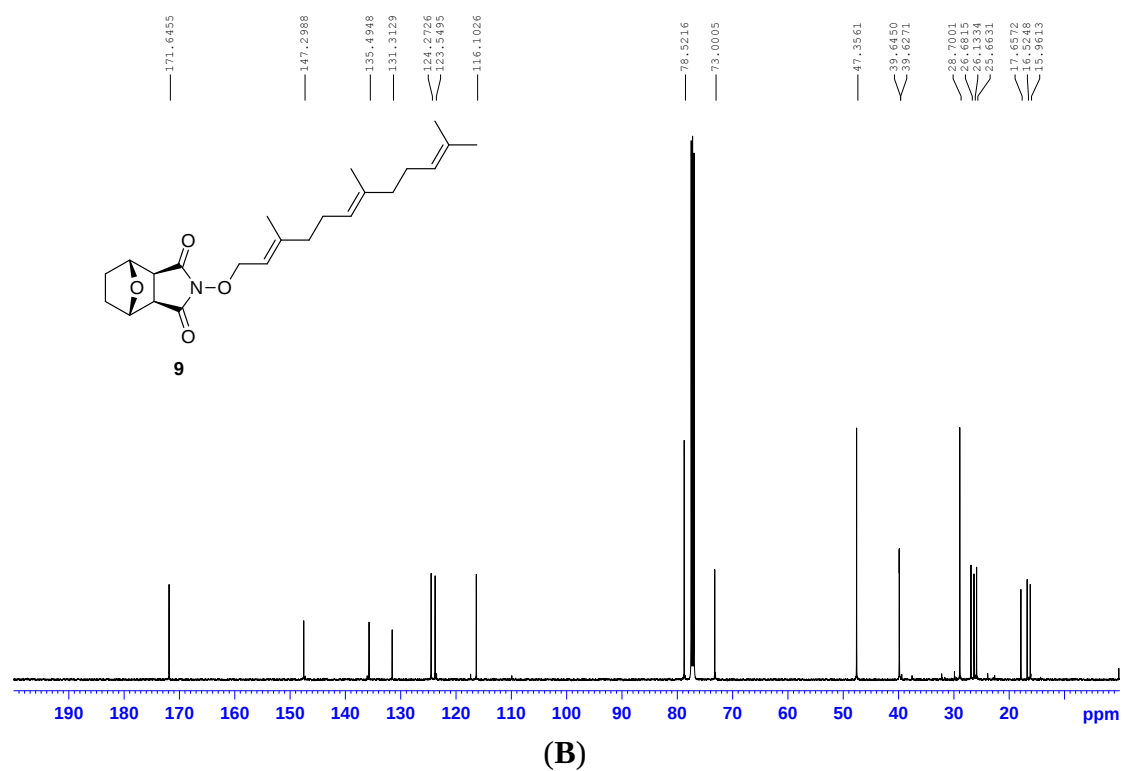
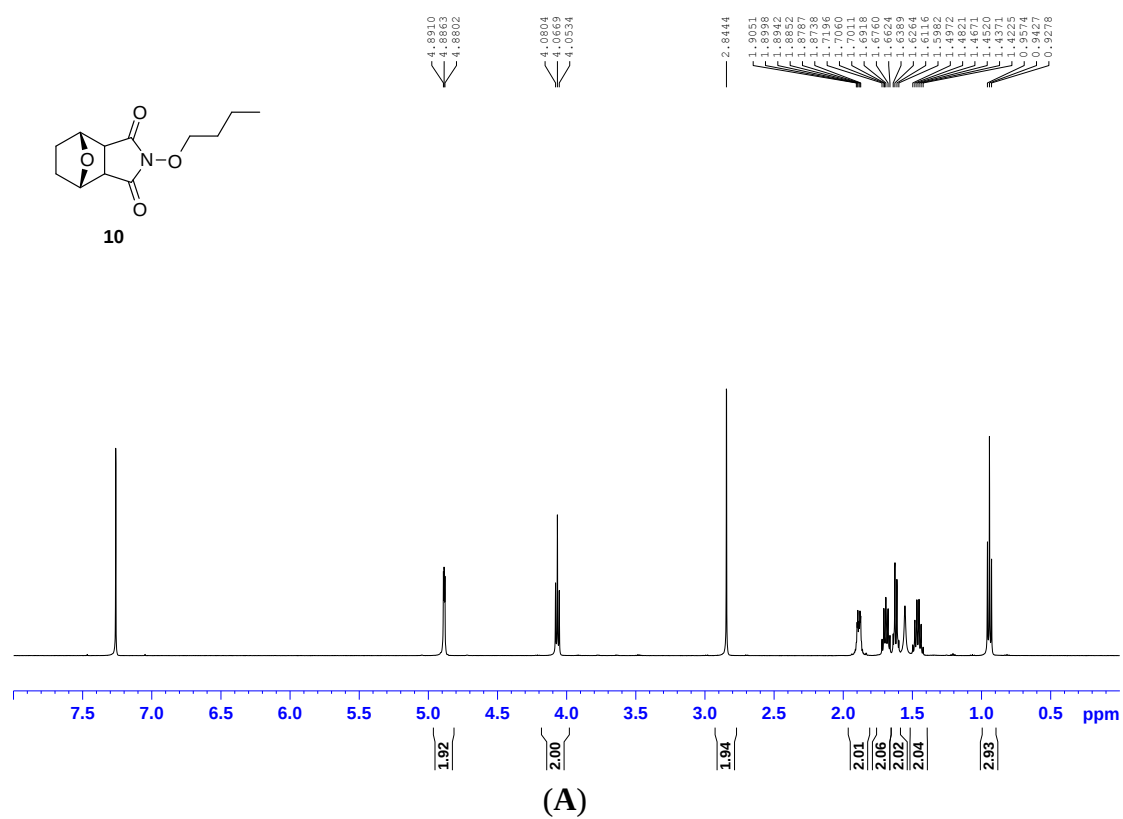
Figure S8. (A) ^1H -NMR spectra of compound **10**; (B) ^{13}C -NMR spectra of compound **10**.

Figure S8. Cont.

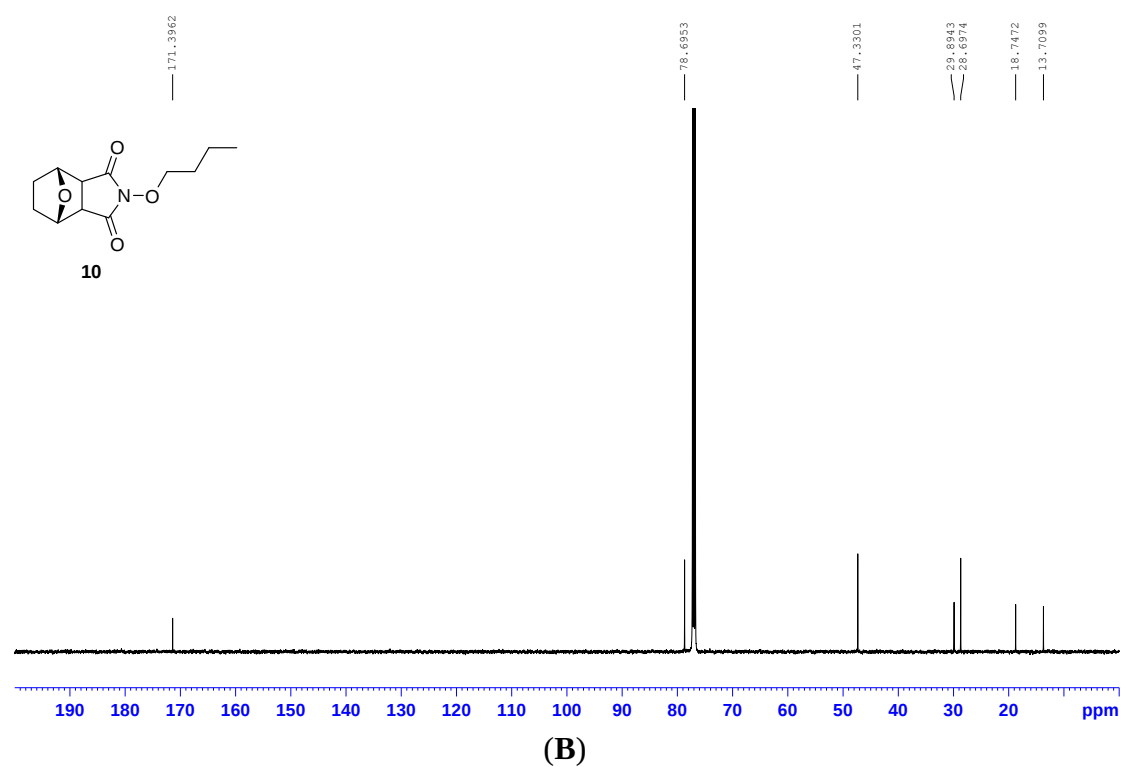
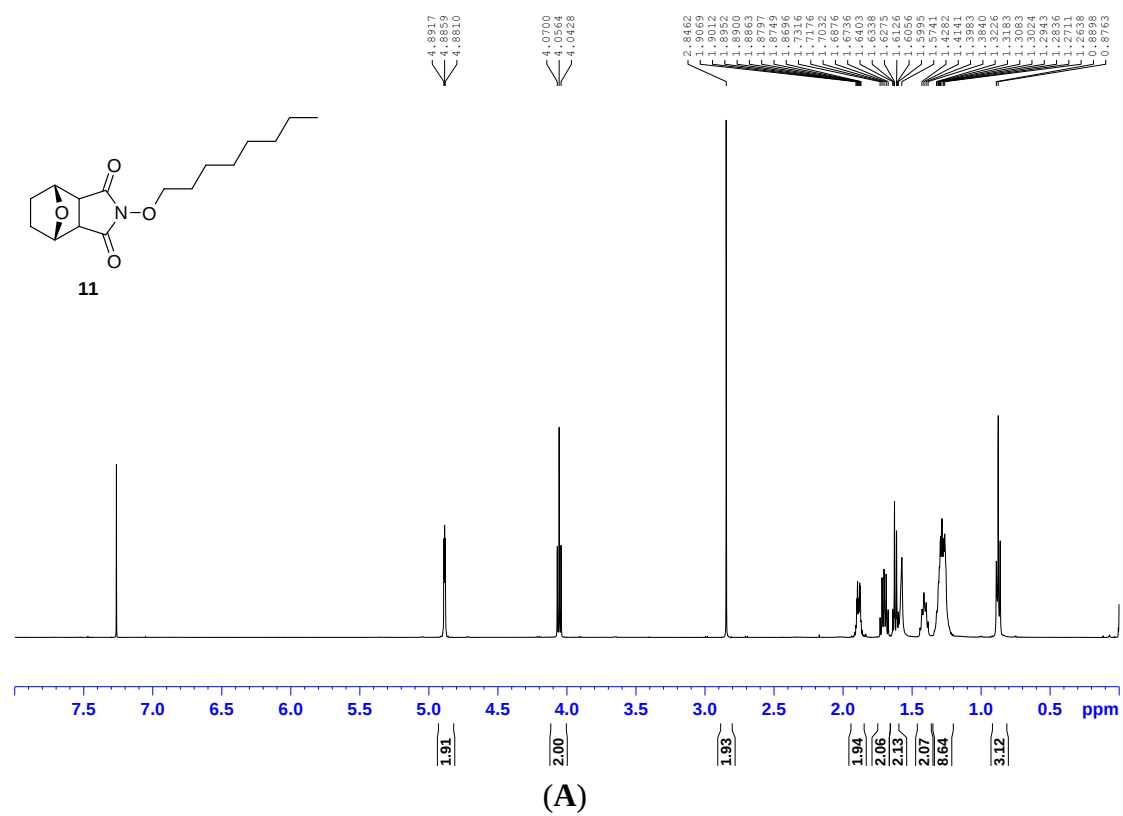
Figure S9. (A) ¹H-NMR spectra of compound 11; (B) ¹³C-NMR spectra of compound 11.

Figure S9. Cont.

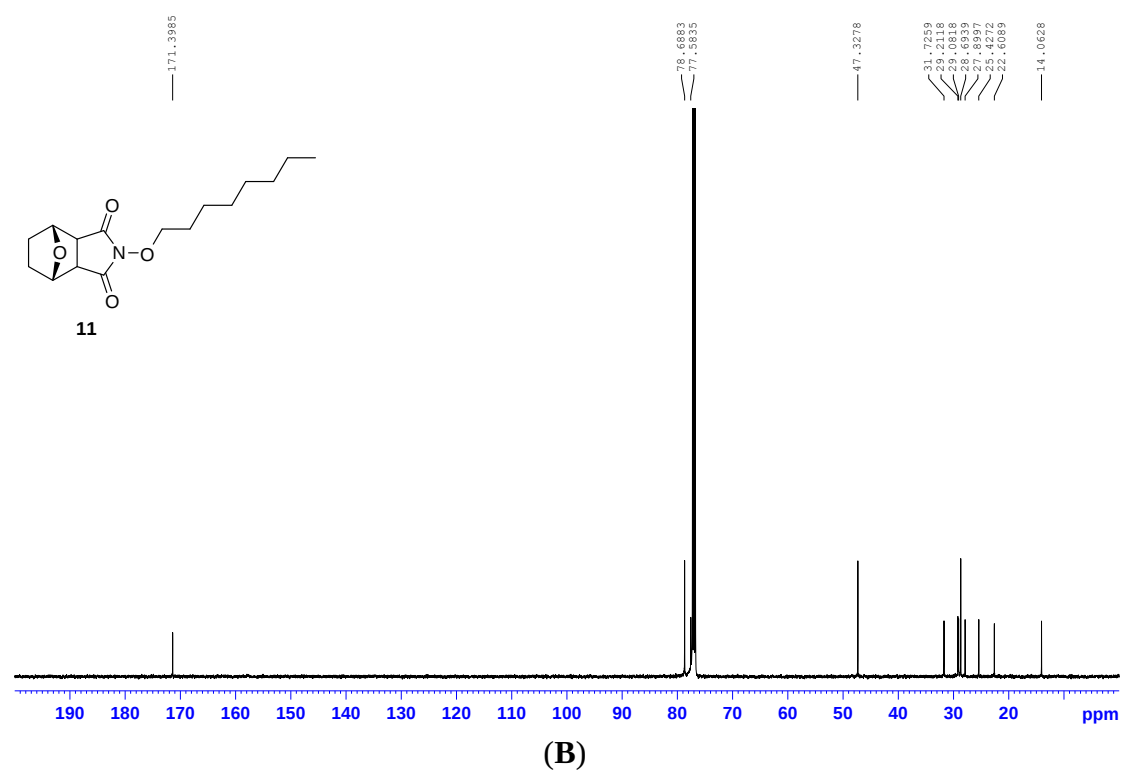
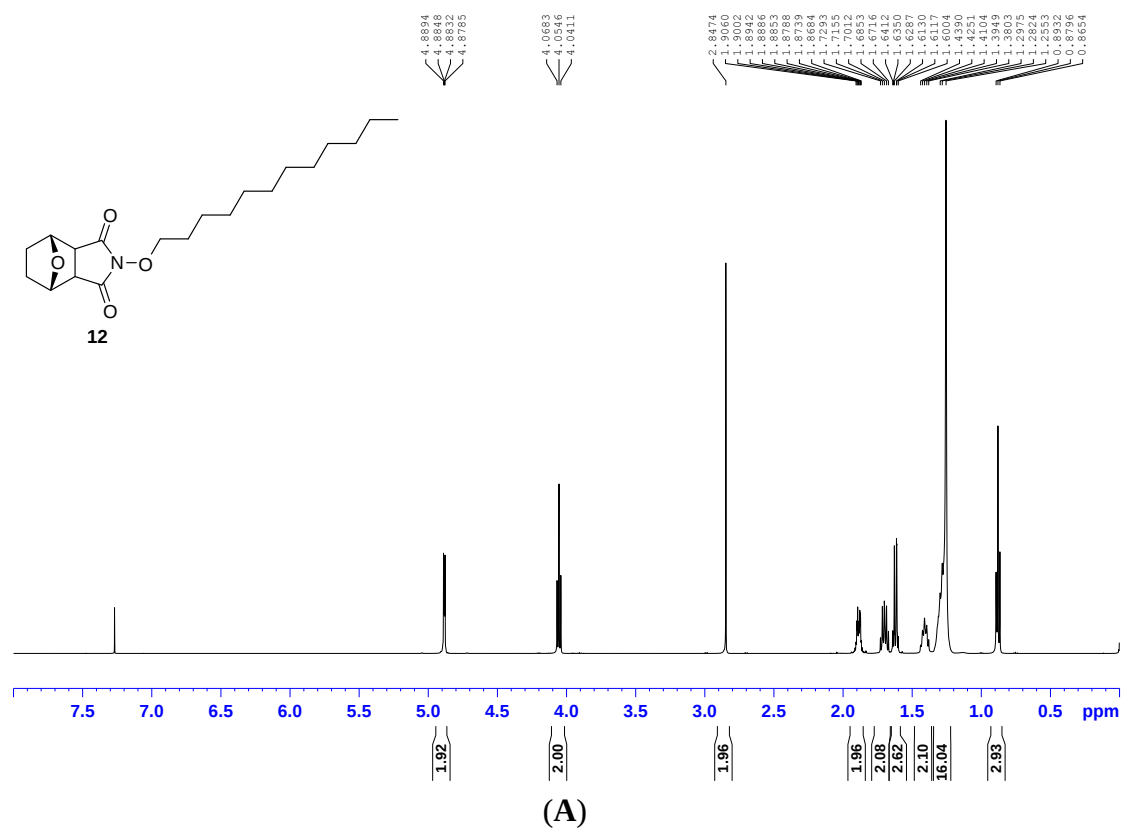
Figure S10. (A) ¹H-NMR spectra of compound 12; (B) ¹³C-NMR spectra of compound 12.

Figure S10. Cont.

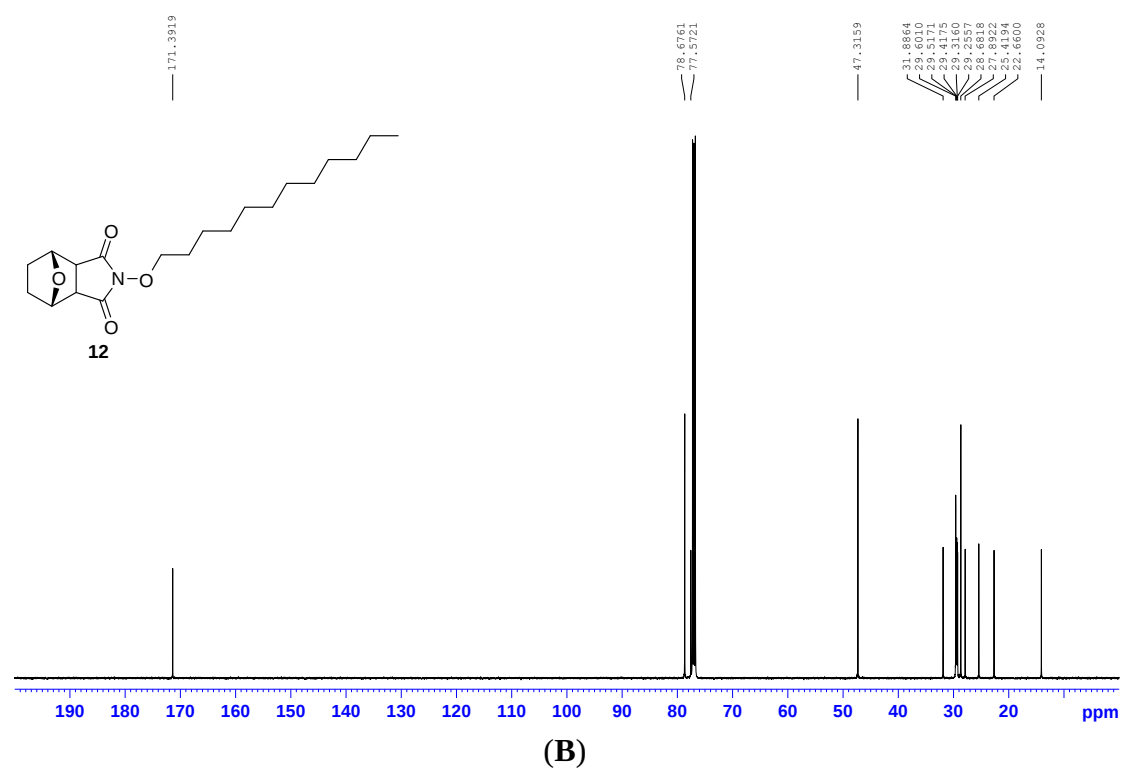
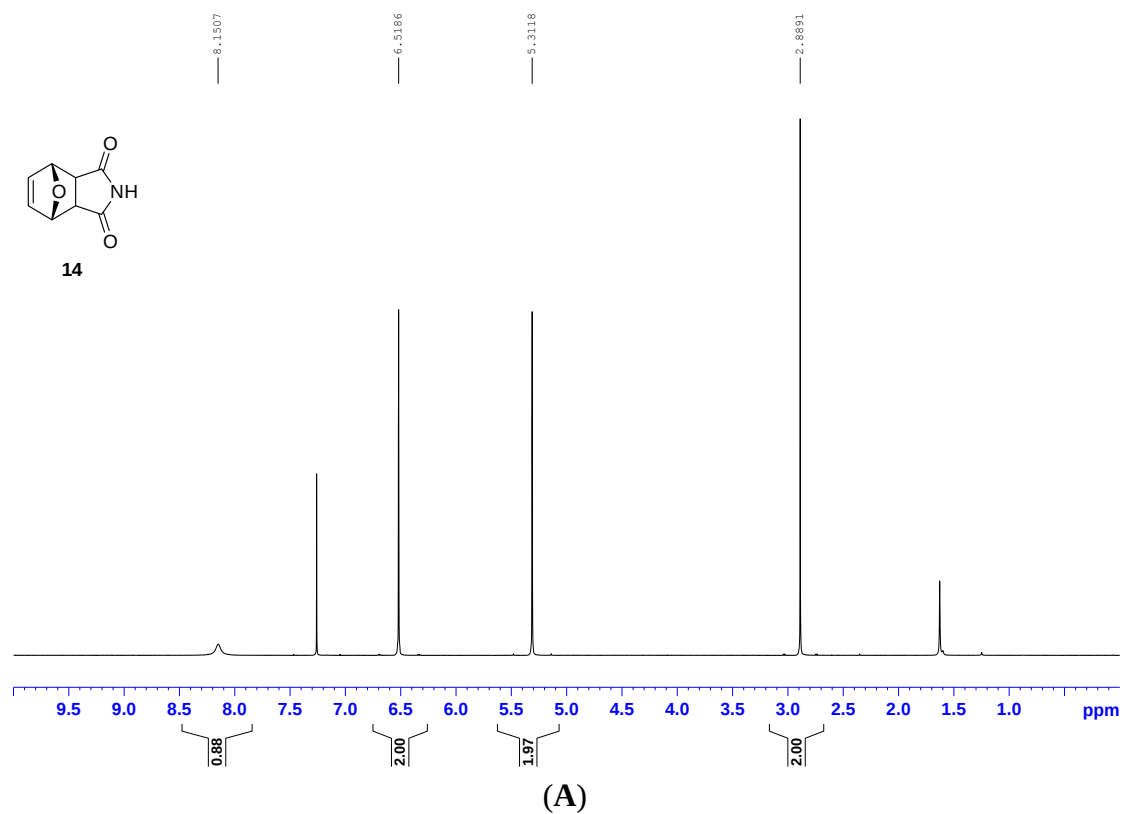
Figure S11. (A) ¹H-NMR spectra of compound **14**; (B) ¹³C-NMR spectra of compound **14**.

Figure S11. Cont.

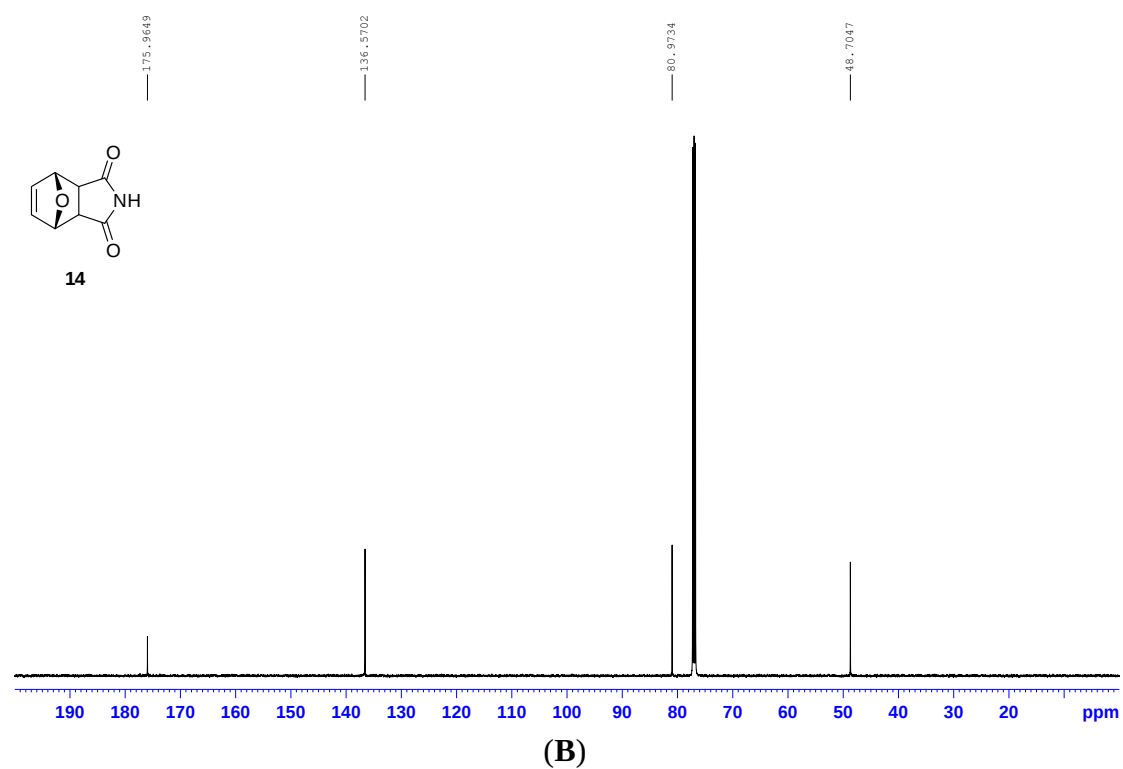
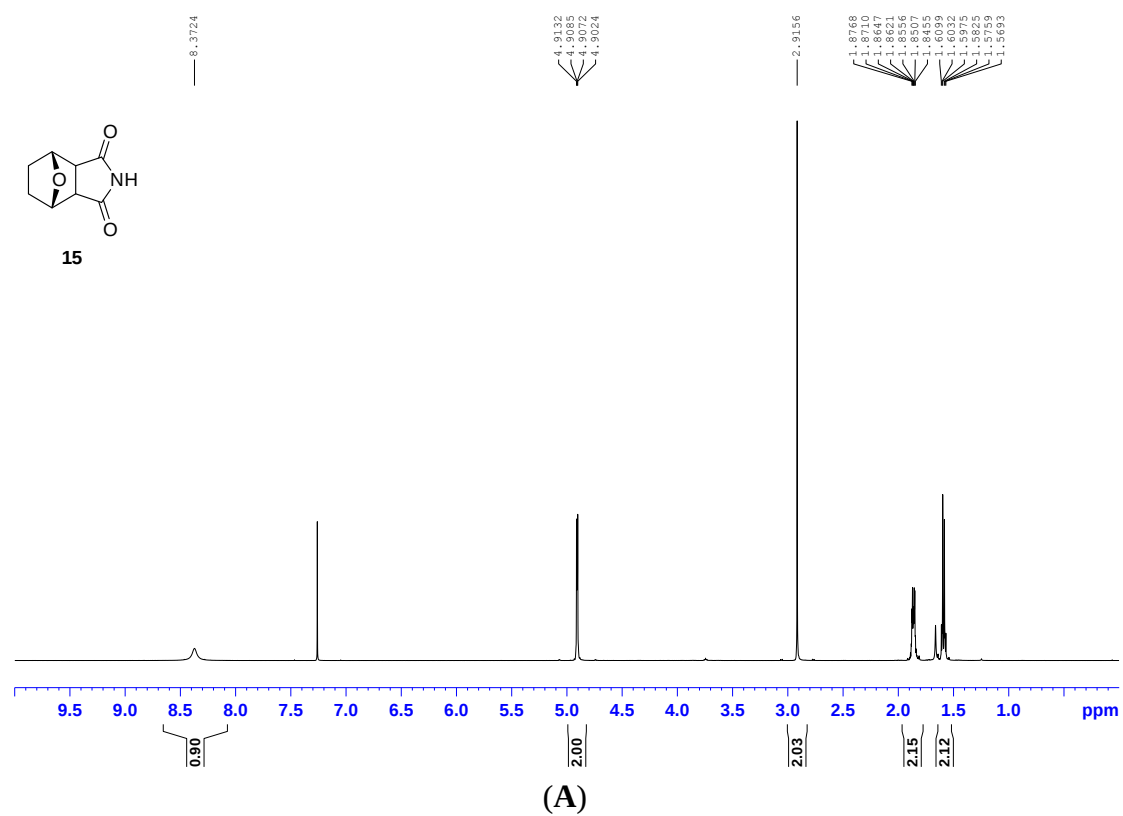
Figure S12. (A) ¹H-NMR spectra of compound 15; (B) ¹³C-NMR spectra of compound 15.

Figure S12. Cont.

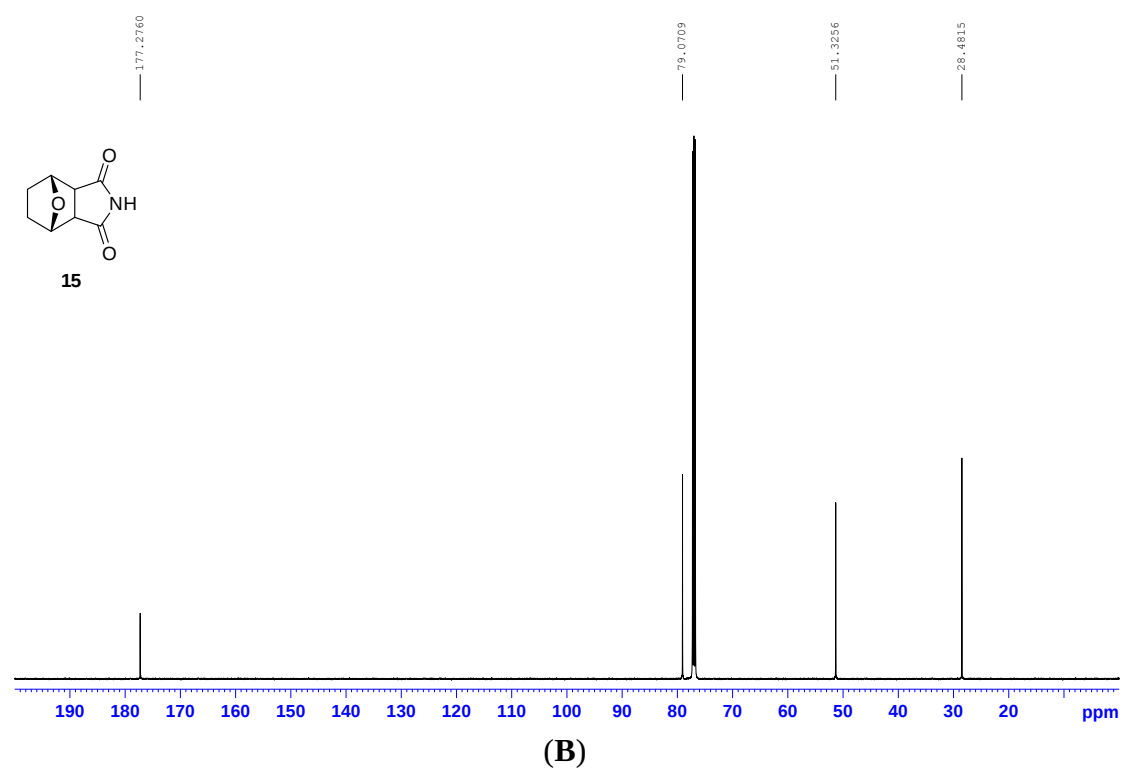
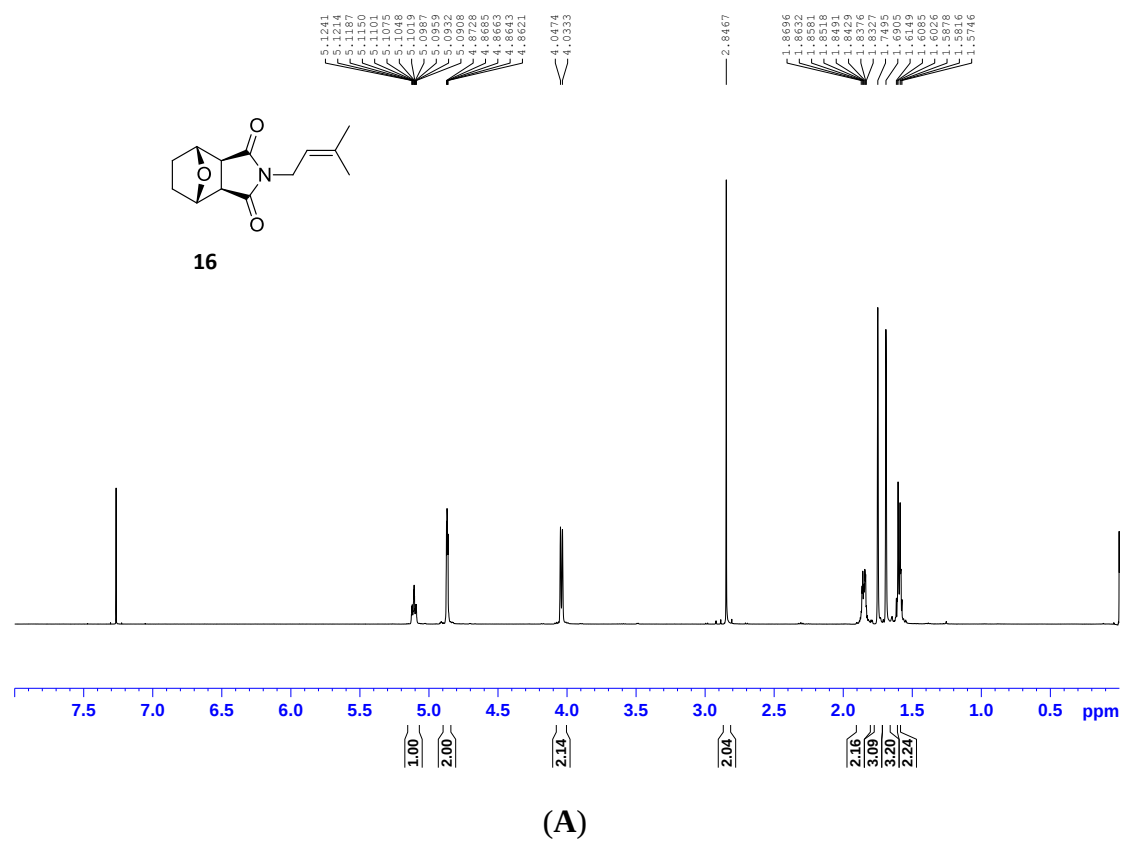
Figure S13. (A) ¹H-NMR spectra of compound 16; (B) ¹³C-NMR spectra of compound 16.

Figure S13. Cont.

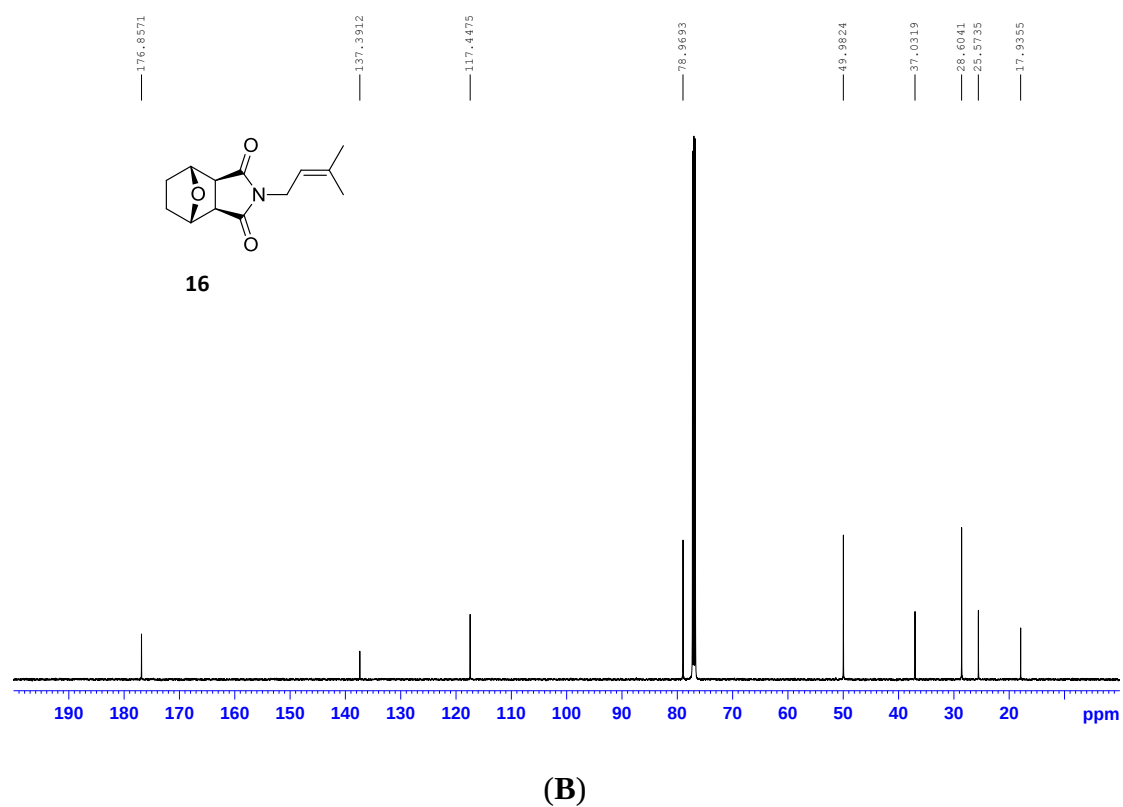
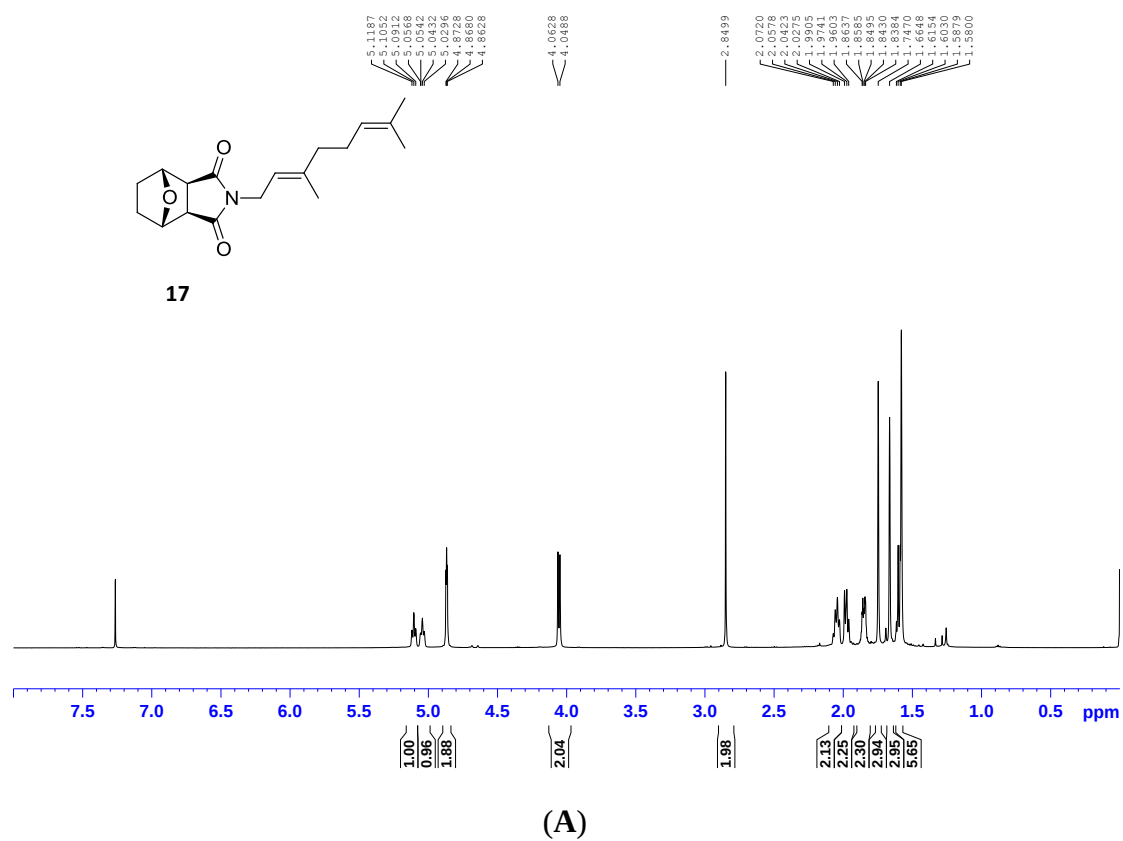
Figure S14. (A) ^1H -NMR spectra of compound 17; (B) ^{13}C -NMR spectra of compound 17.

Figure S14. Cont.

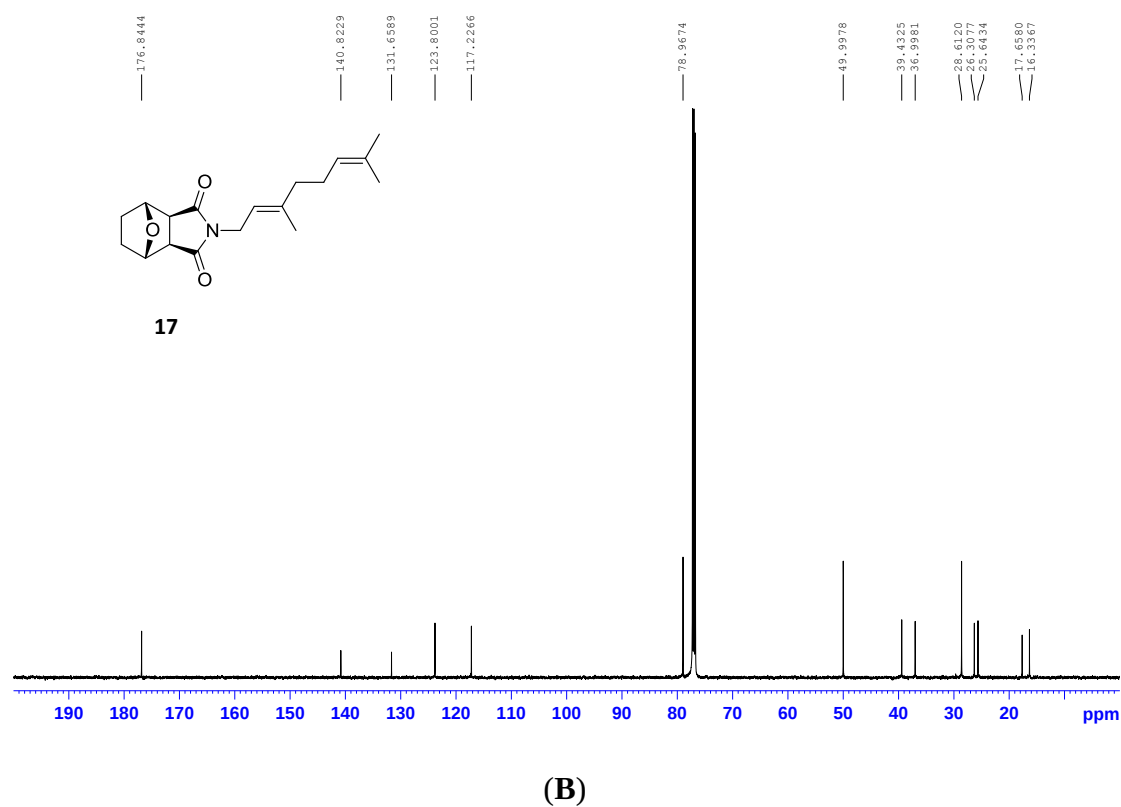
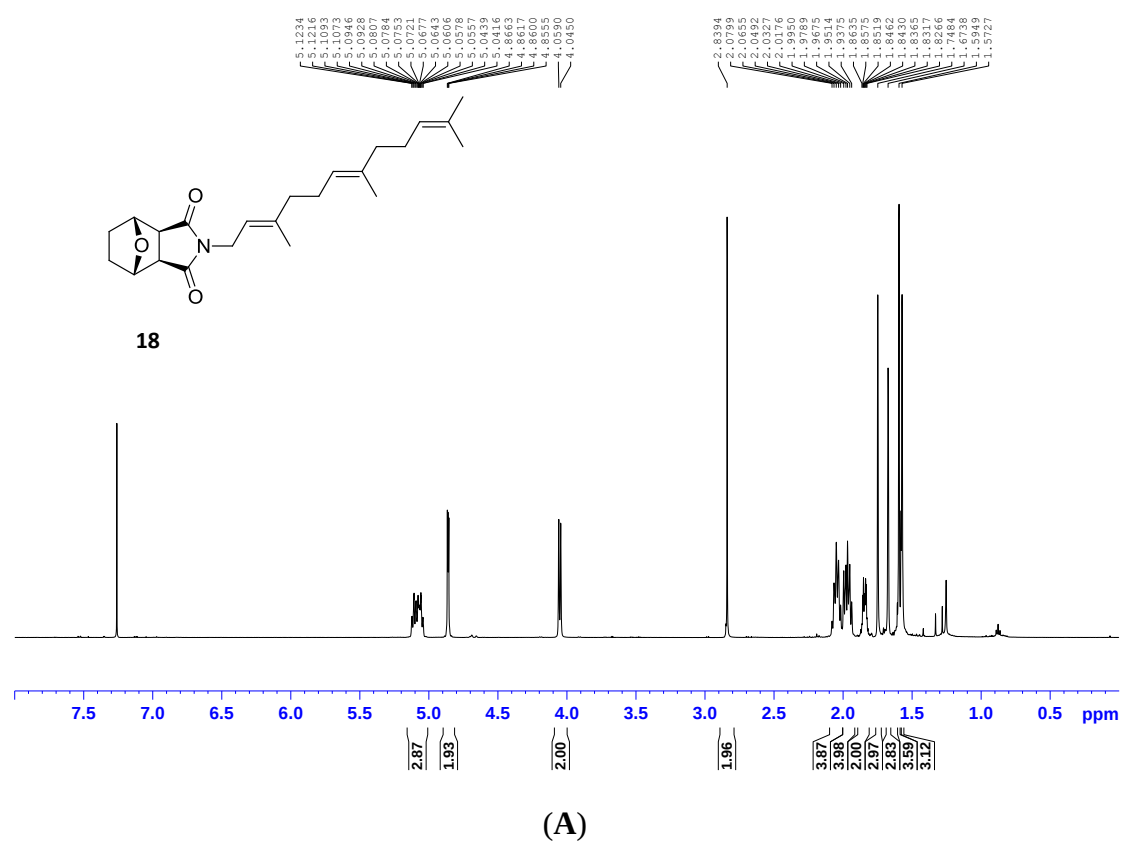
Figure S15. (A) ^1H -NMR spectra of compound 18; (B) ^{13}C -NMR spectra of compound 18.

Figure S15. Cont.

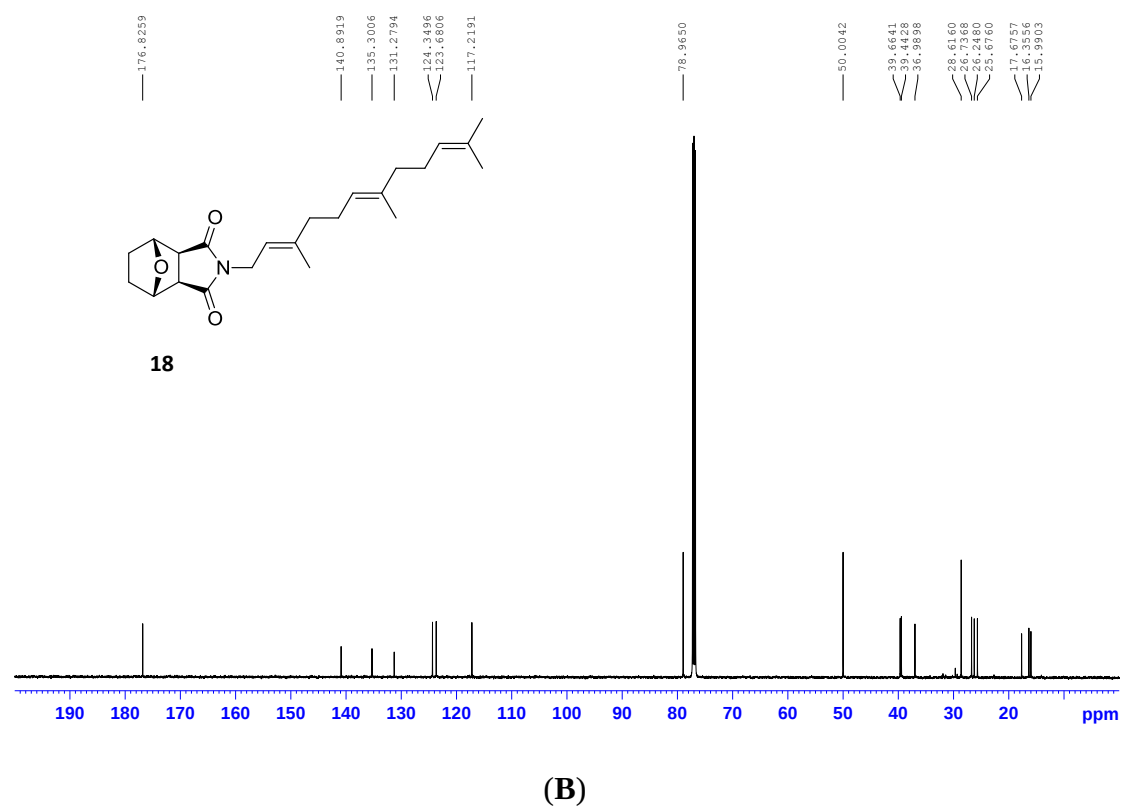
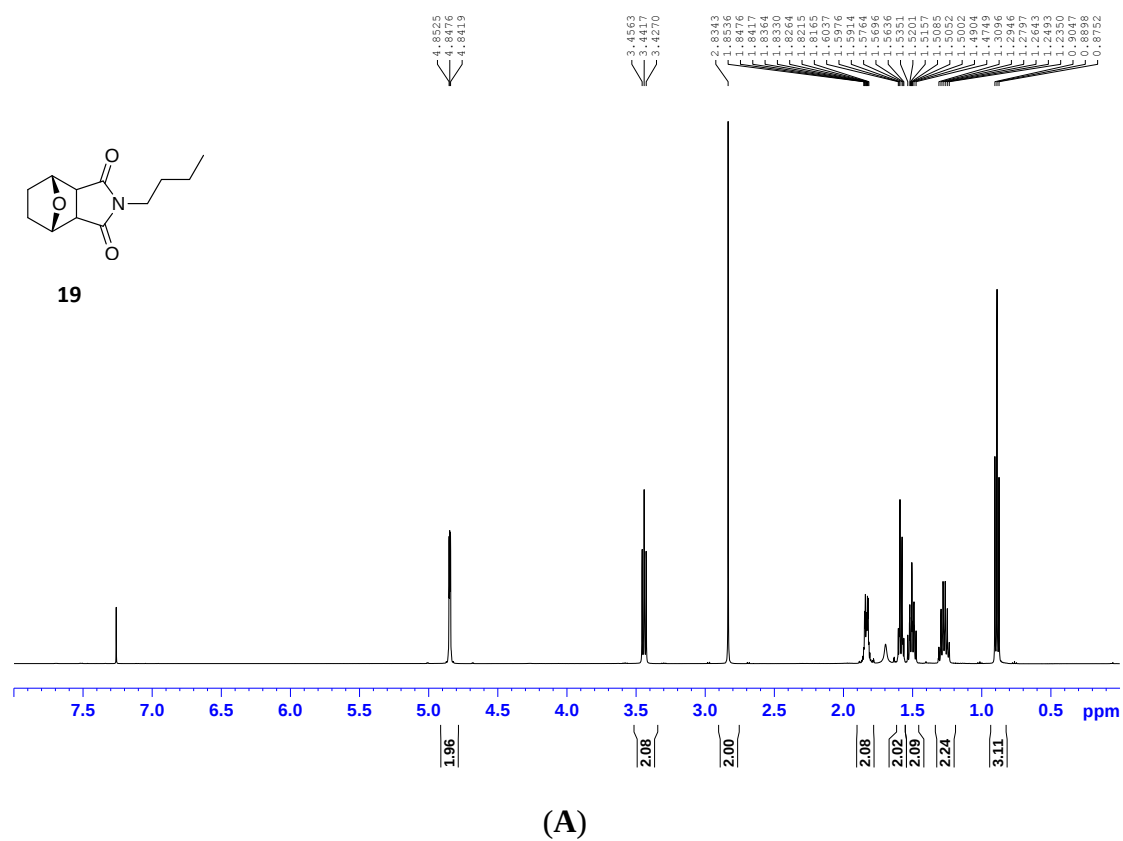
Figure S16. (A) ^1H -NMR spectra of compound 19; (B) ^{13}C -NMR spectra of compound 19.

Figure S16. Cont.

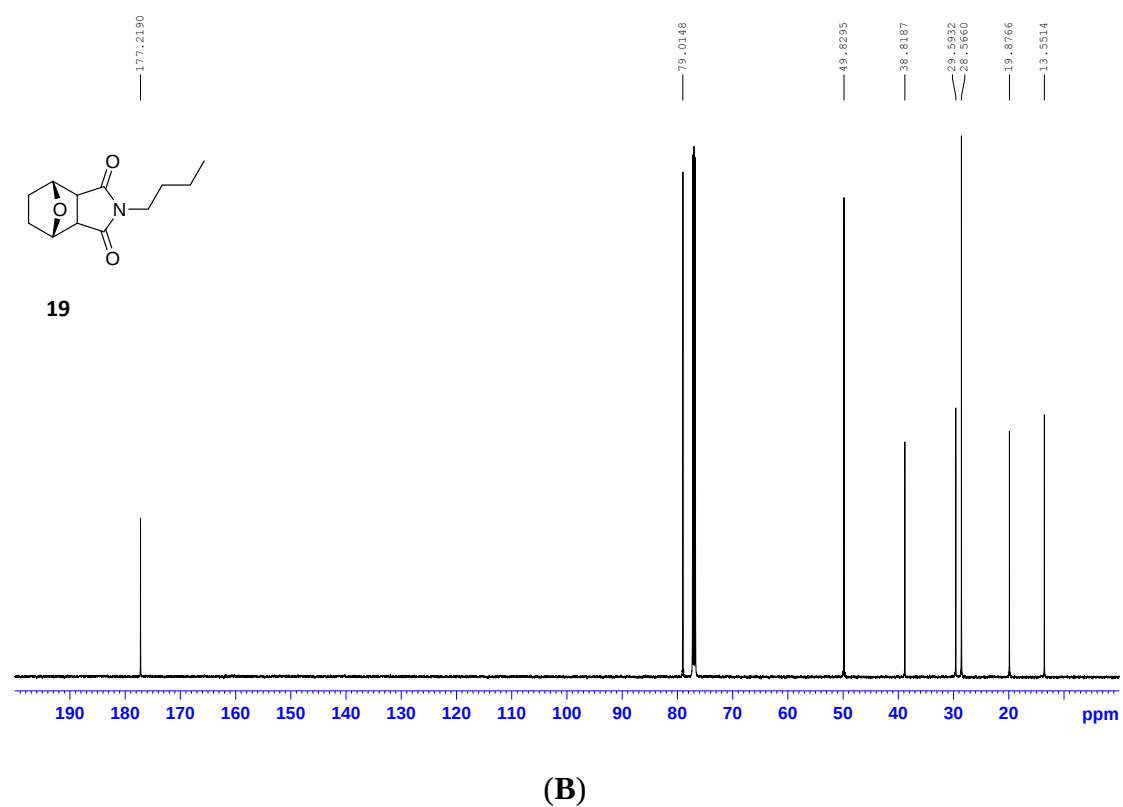
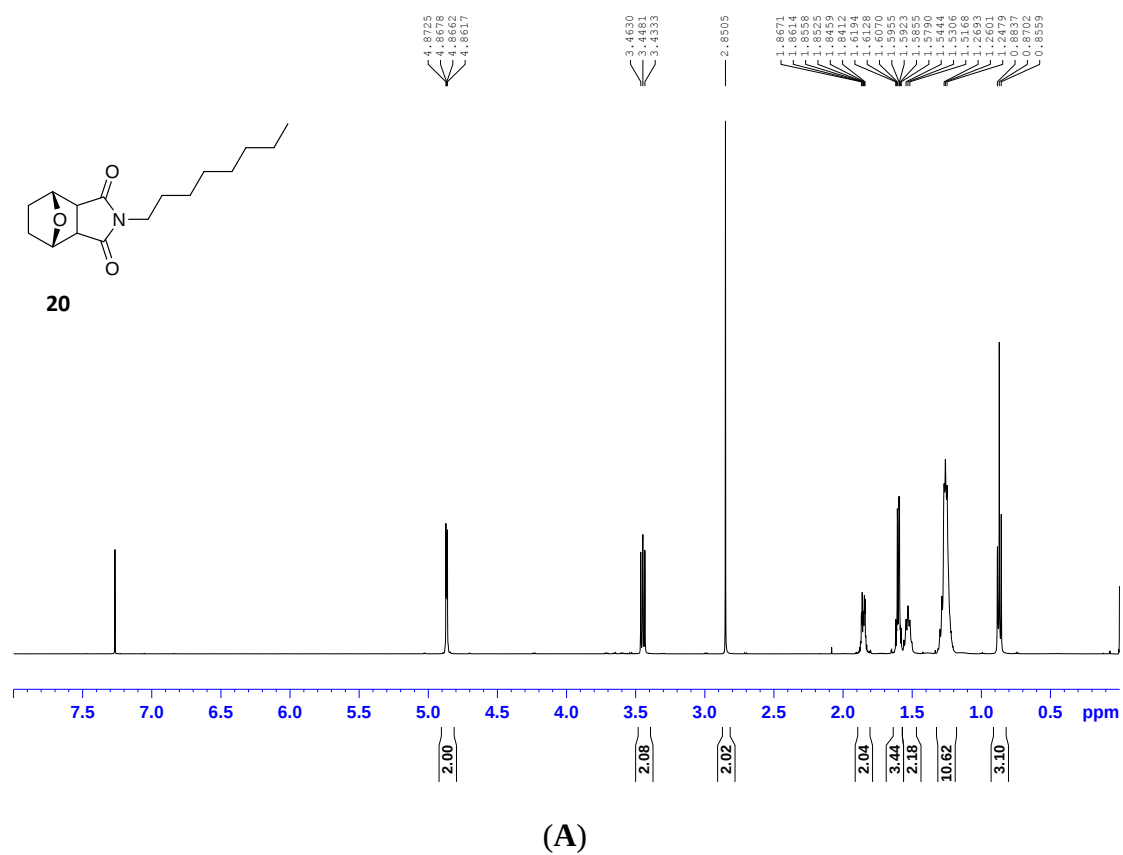
Figure S17. (A) ^1H -NMR spectra of compound **20**; (B) ^{13}C -NMR spectra of compound **20**.

Figure S17. Cont.

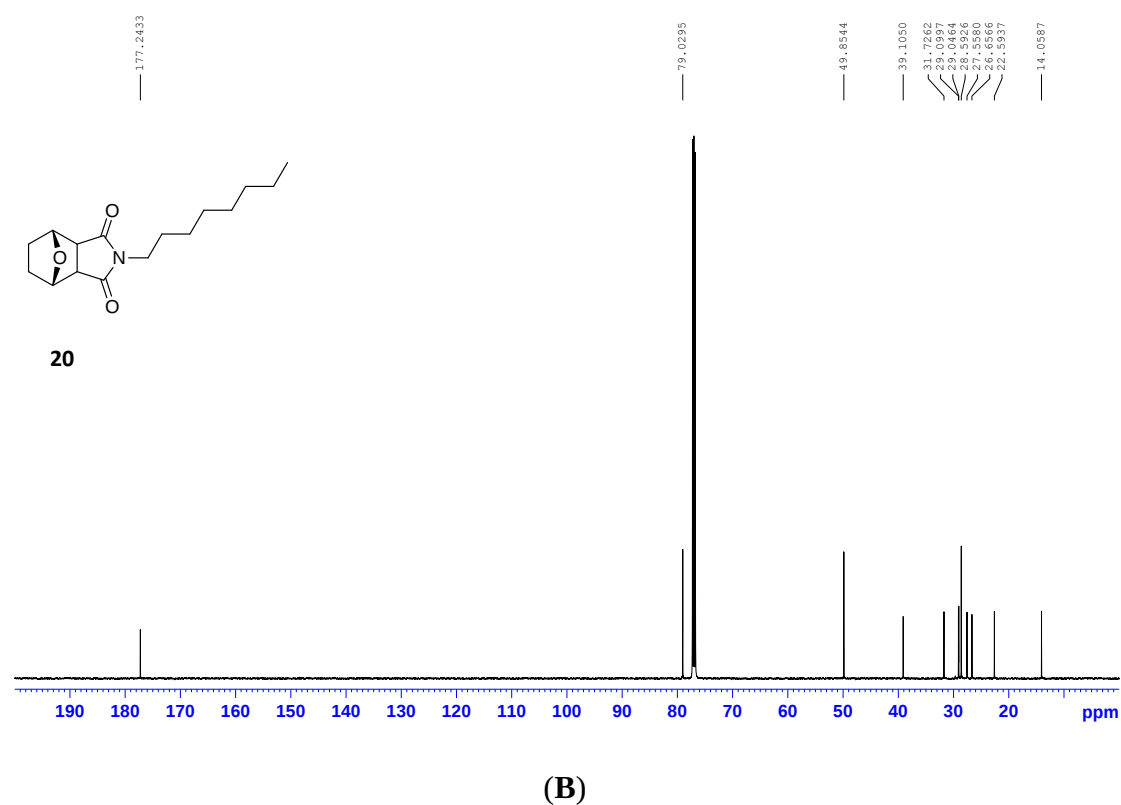
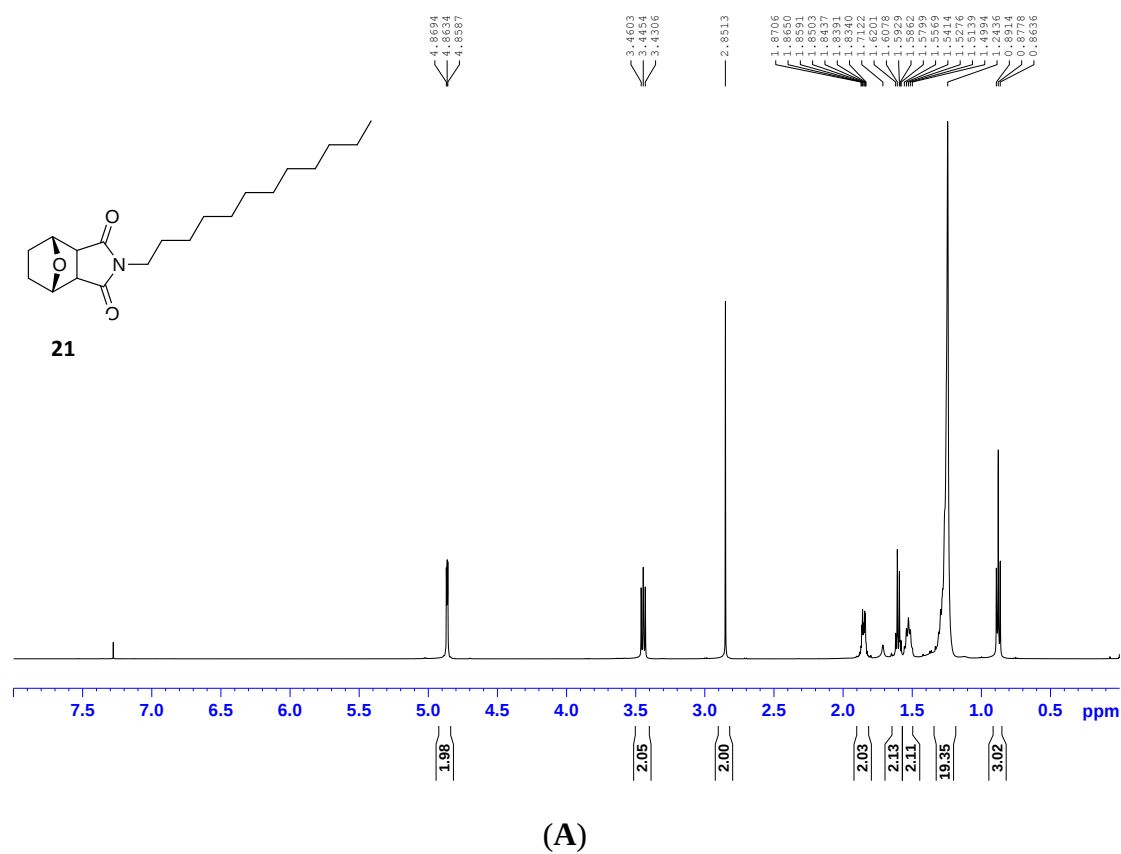
Figure S18. (A) ^1H -NMR spectra of compound 21; (B) ^{13}C -NMR spectra of compound 21.

Figure S18. Cont.

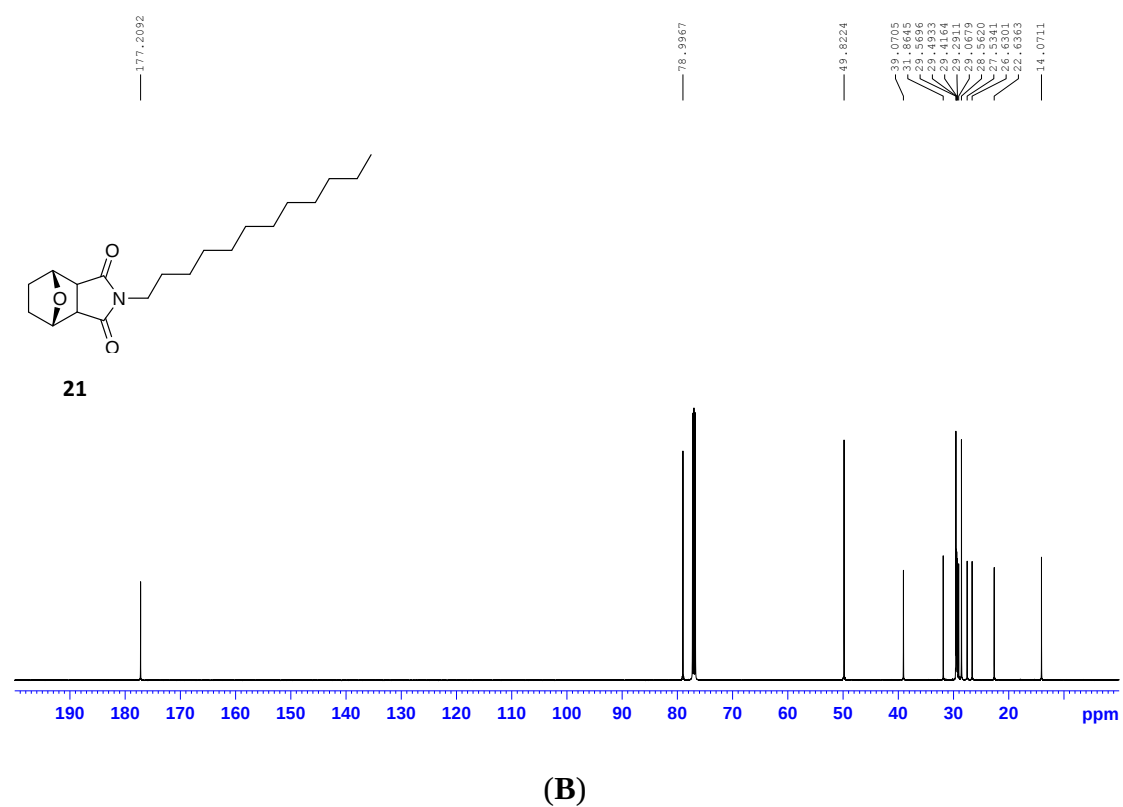
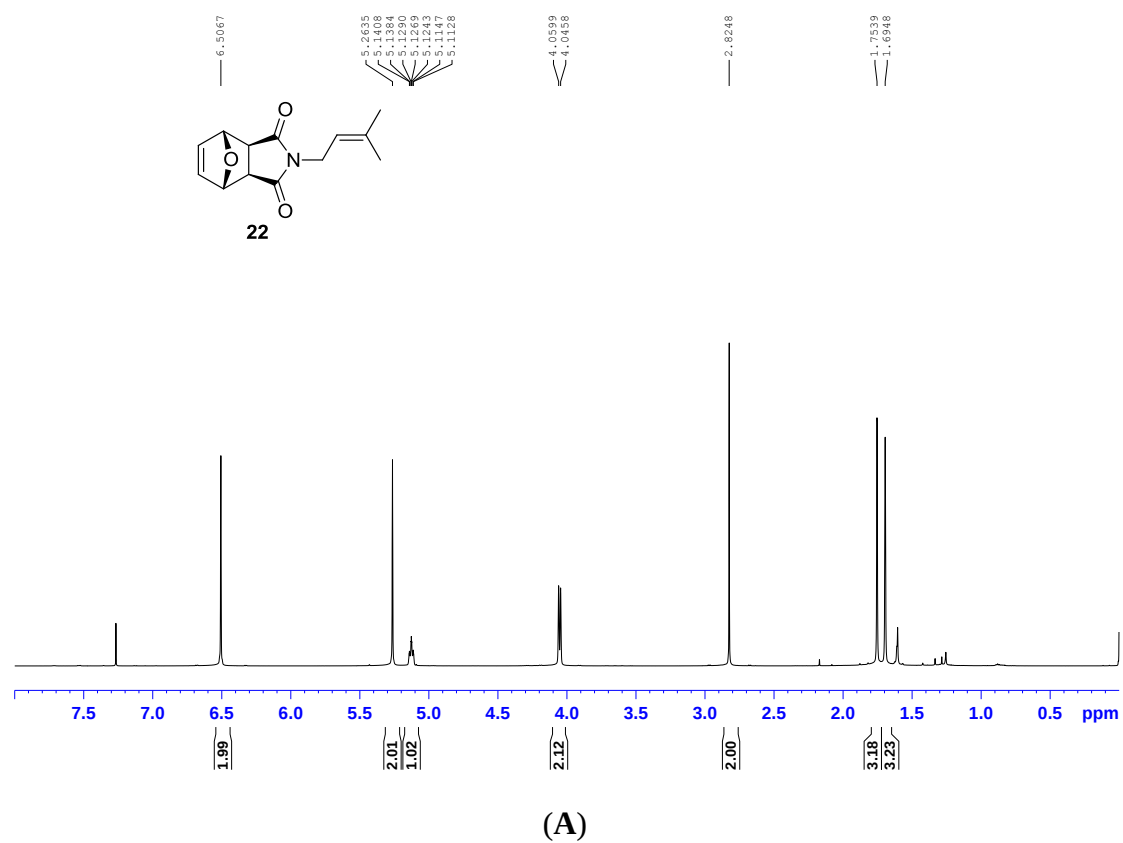
Figure S19. (A) ^1H -NMR spectra of compound **22**; (B) ^{13}C -NMR spectra of compound **22**.

Figure S19. Cont.

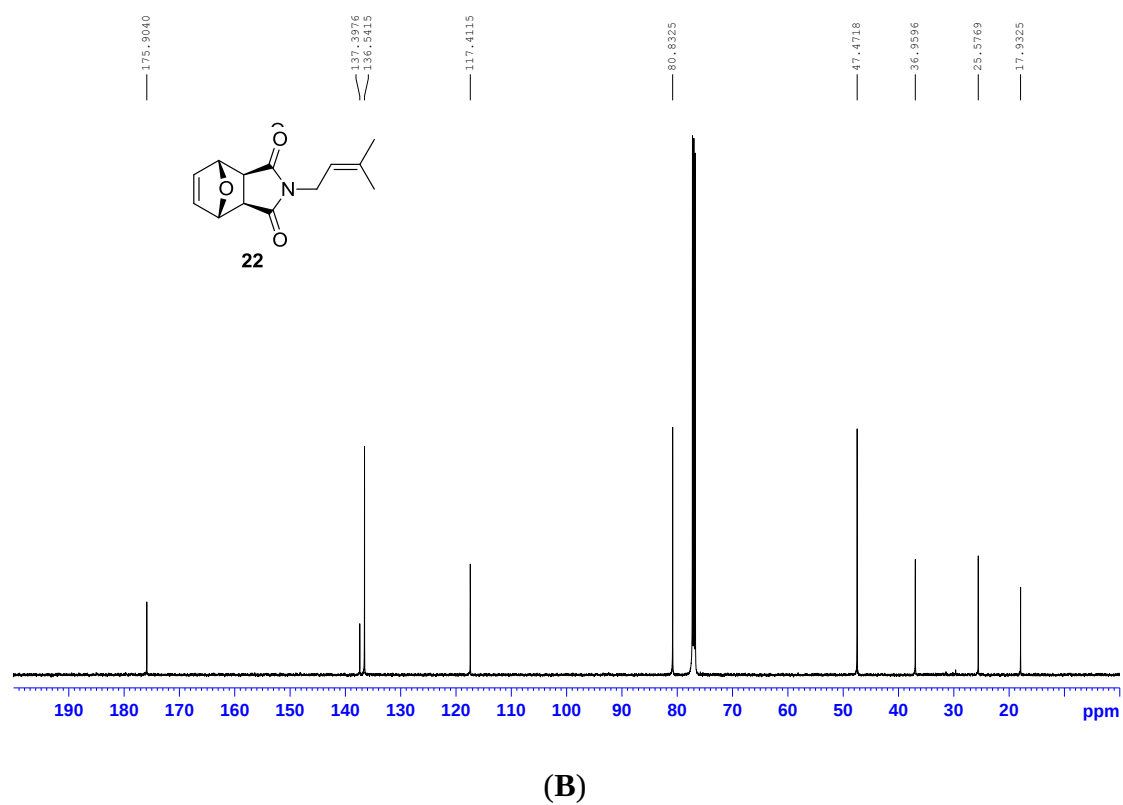
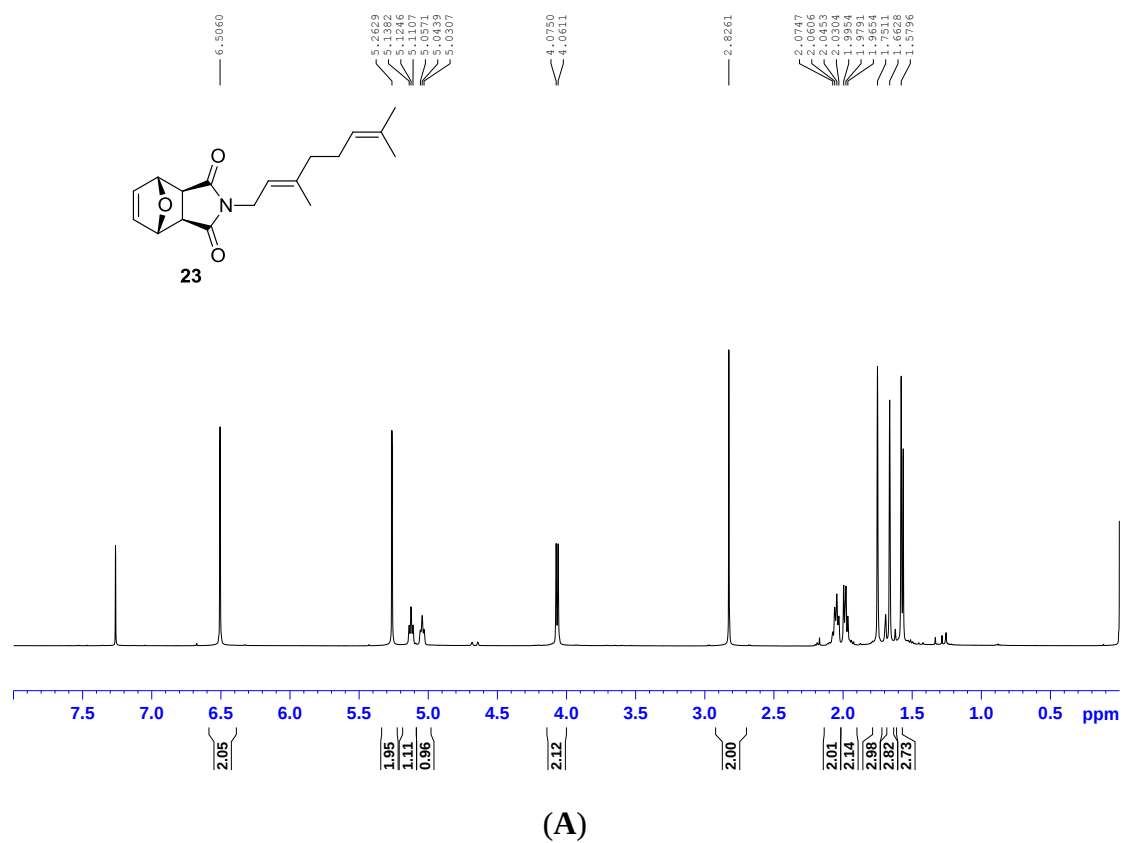
Figure S20. (A) ^1H -NMR spectra of compound 23; (B) ^{13}C -NMR spectra of compound 23.

Figure S20. Cont.

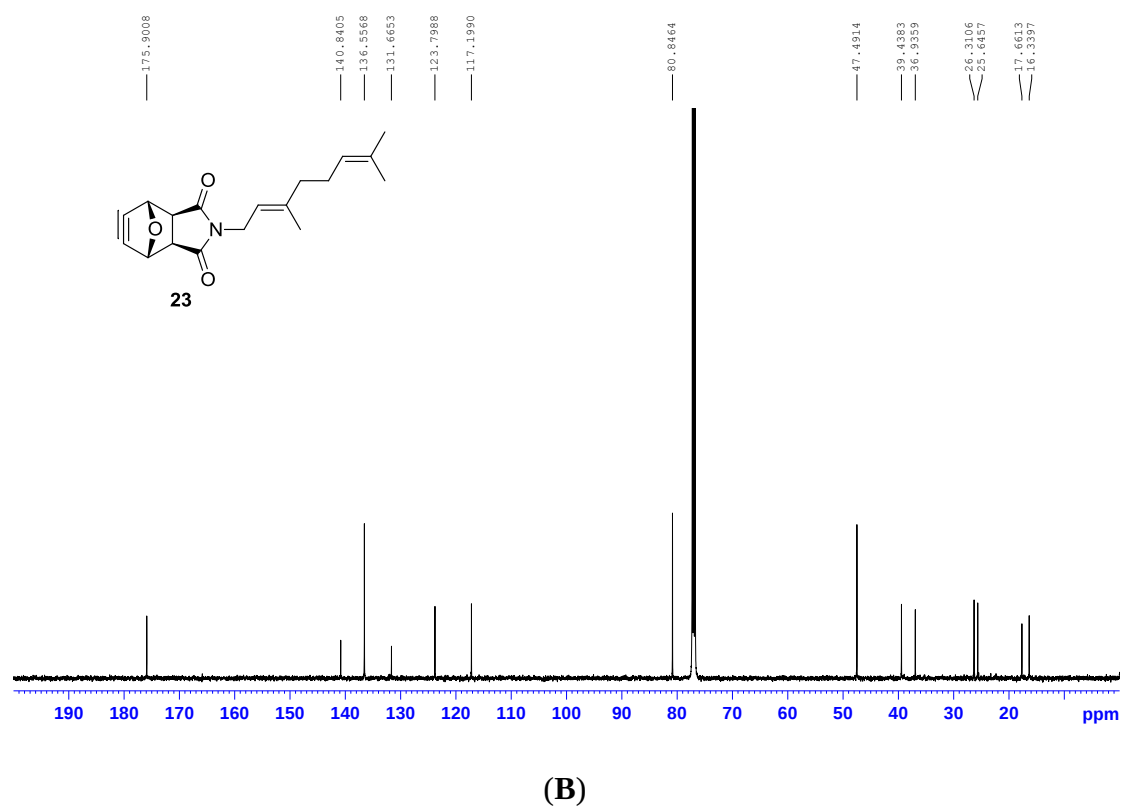
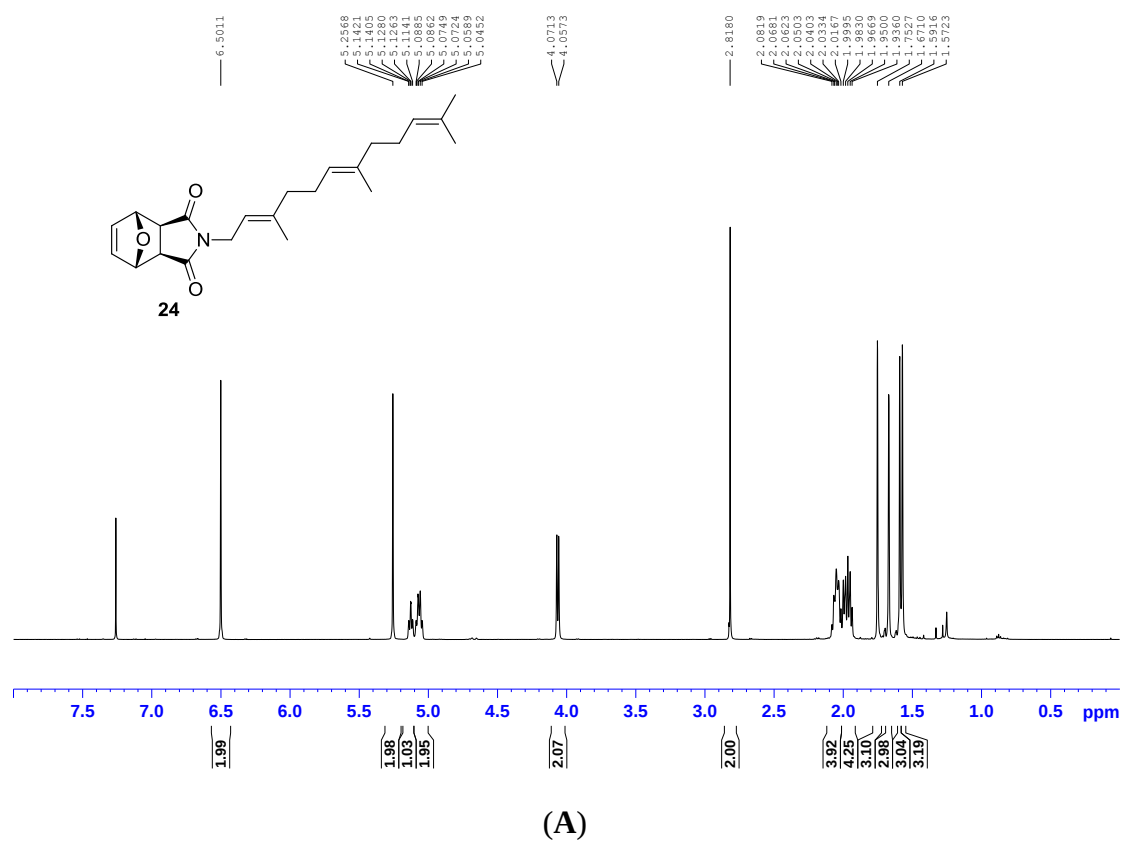
Figure S21. (A) ^1H -NMR spectra of compound **24**; (B) ^{13}C -NMR spectra of compound **24**.

Figure S21. Cont.

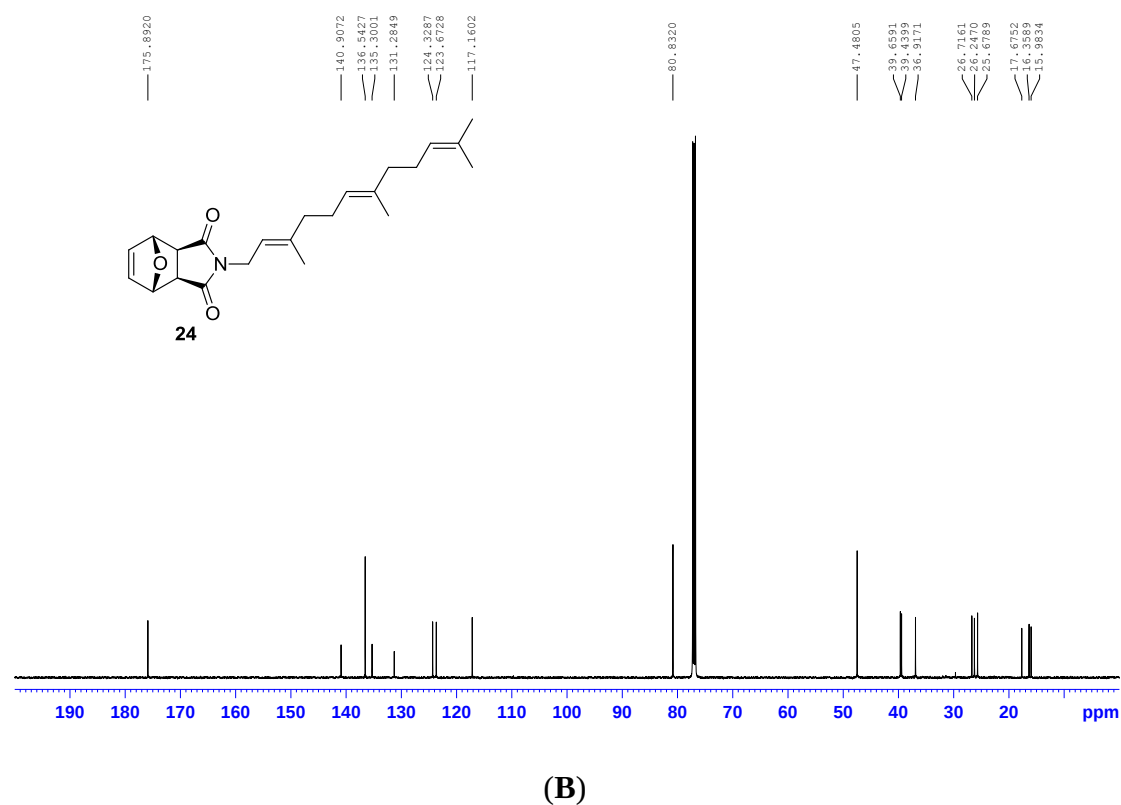
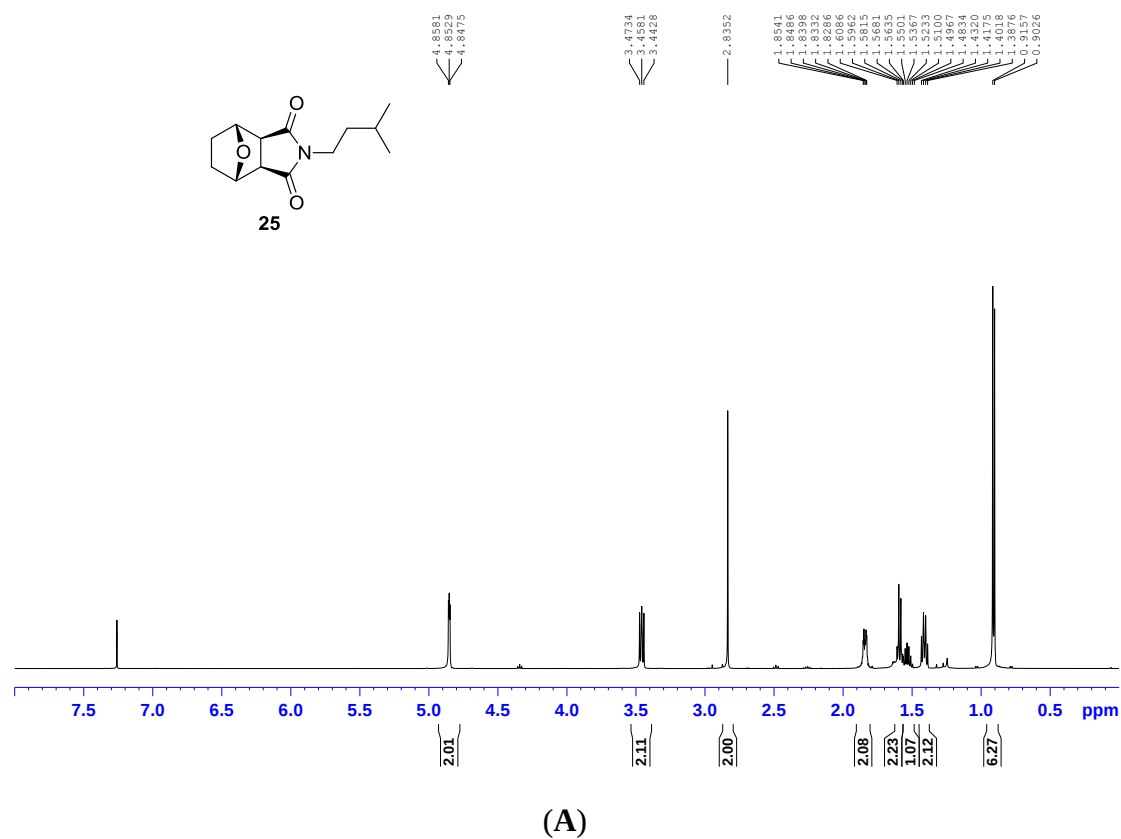
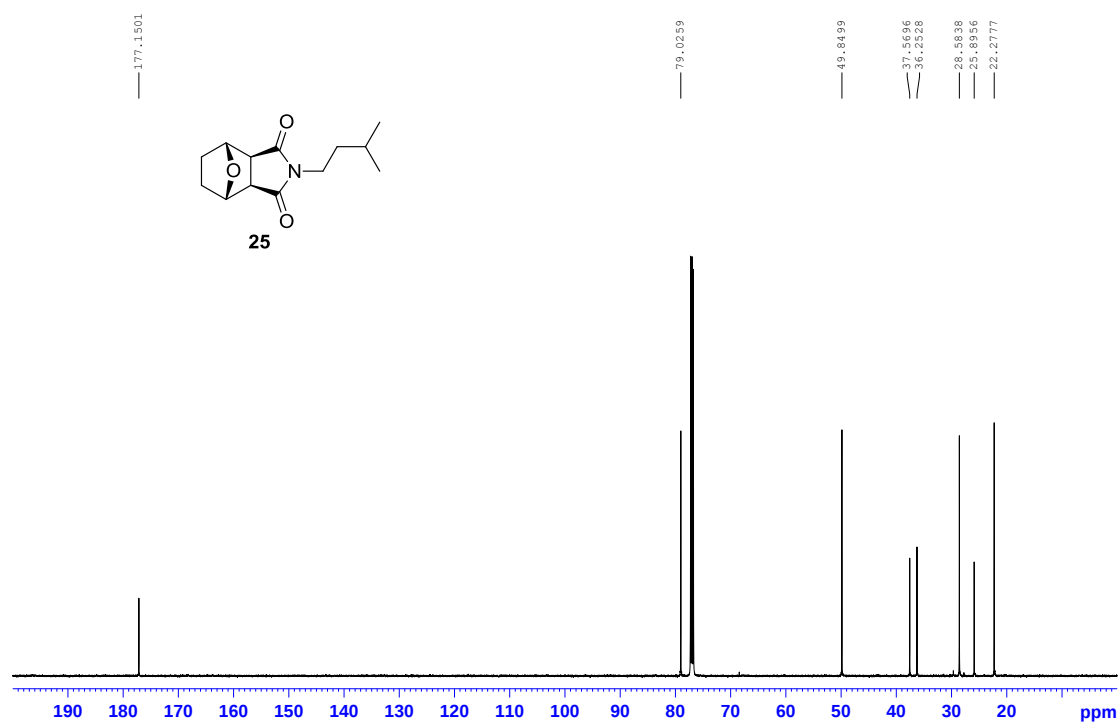
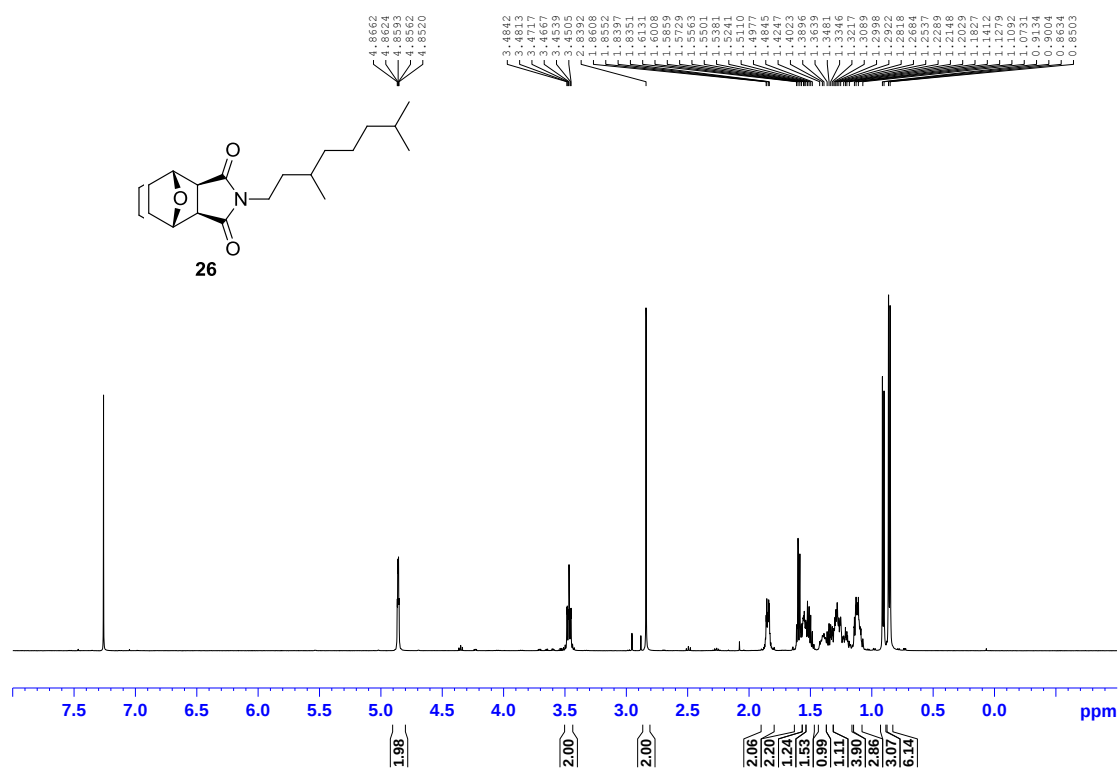
Figure S22. (A) ^1H -NMR spectra of compound 25; (B) ^{13}C -NMR spectra of compound 25.

Figure S22. Cont.



(B)

Figure S23. (A) ¹H-NMR spectra of compound 26; (B) ¹³C-NMR spectra of compound 26.

(A)

Figure S23. Cont.

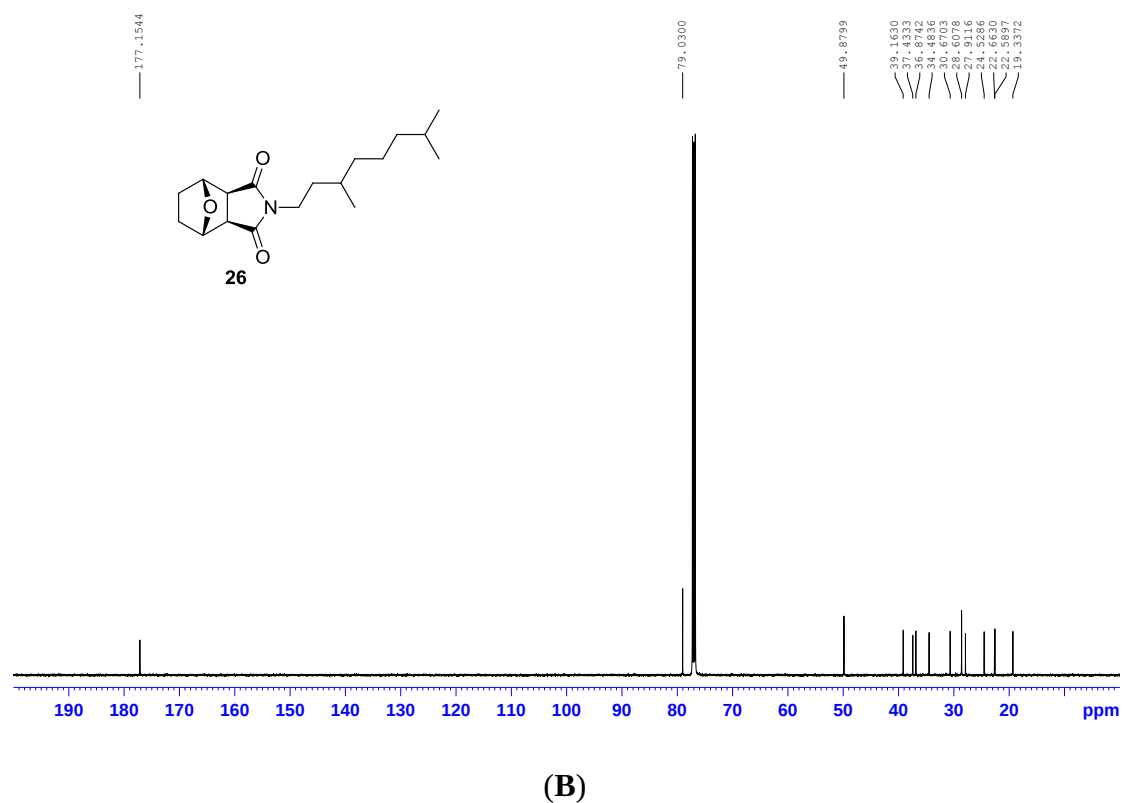
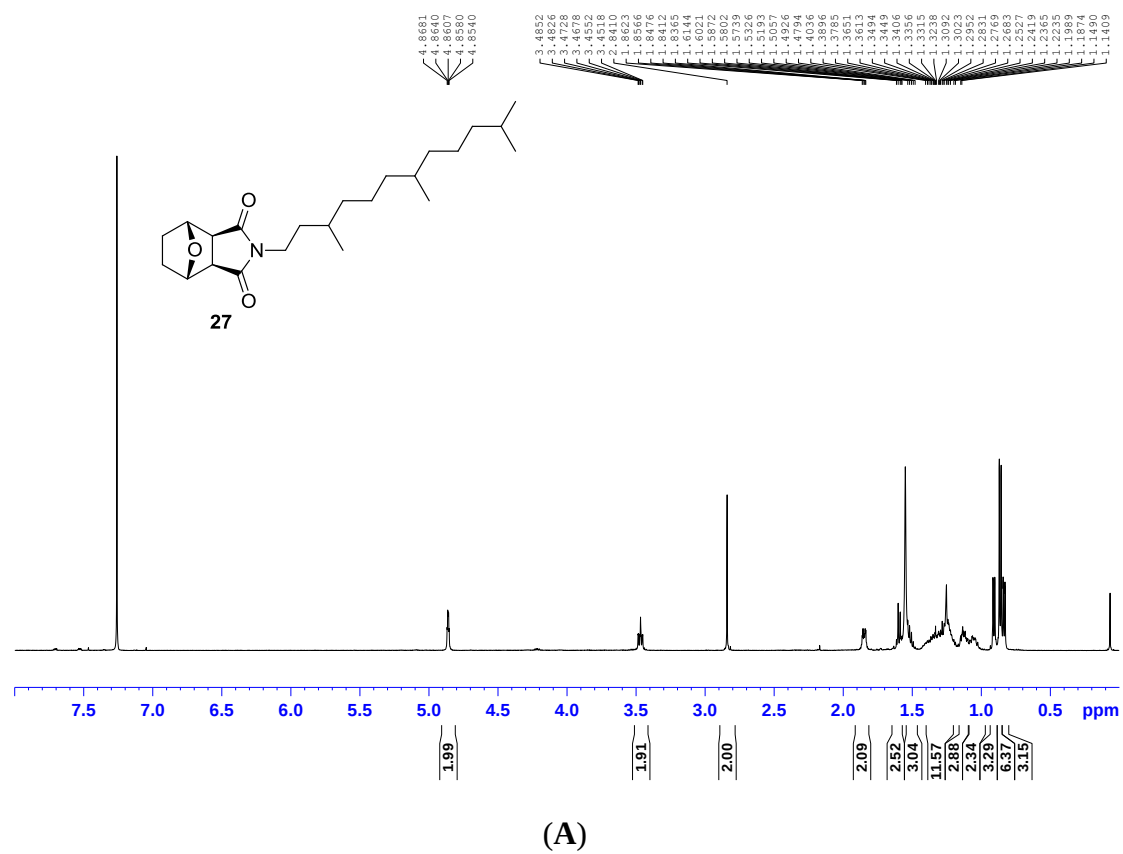
Figure S24. (A) ¹H-NMR spectra of compound 27; (B) ¹³C-NMR spectra of compound 27.

Figure S24. Cont.

