SUPPLEMENTARY DATA

Synthesis and biological evaluation of novel D-ring fused steroidal *N*(2)-substituted-1,2,3-triazoles

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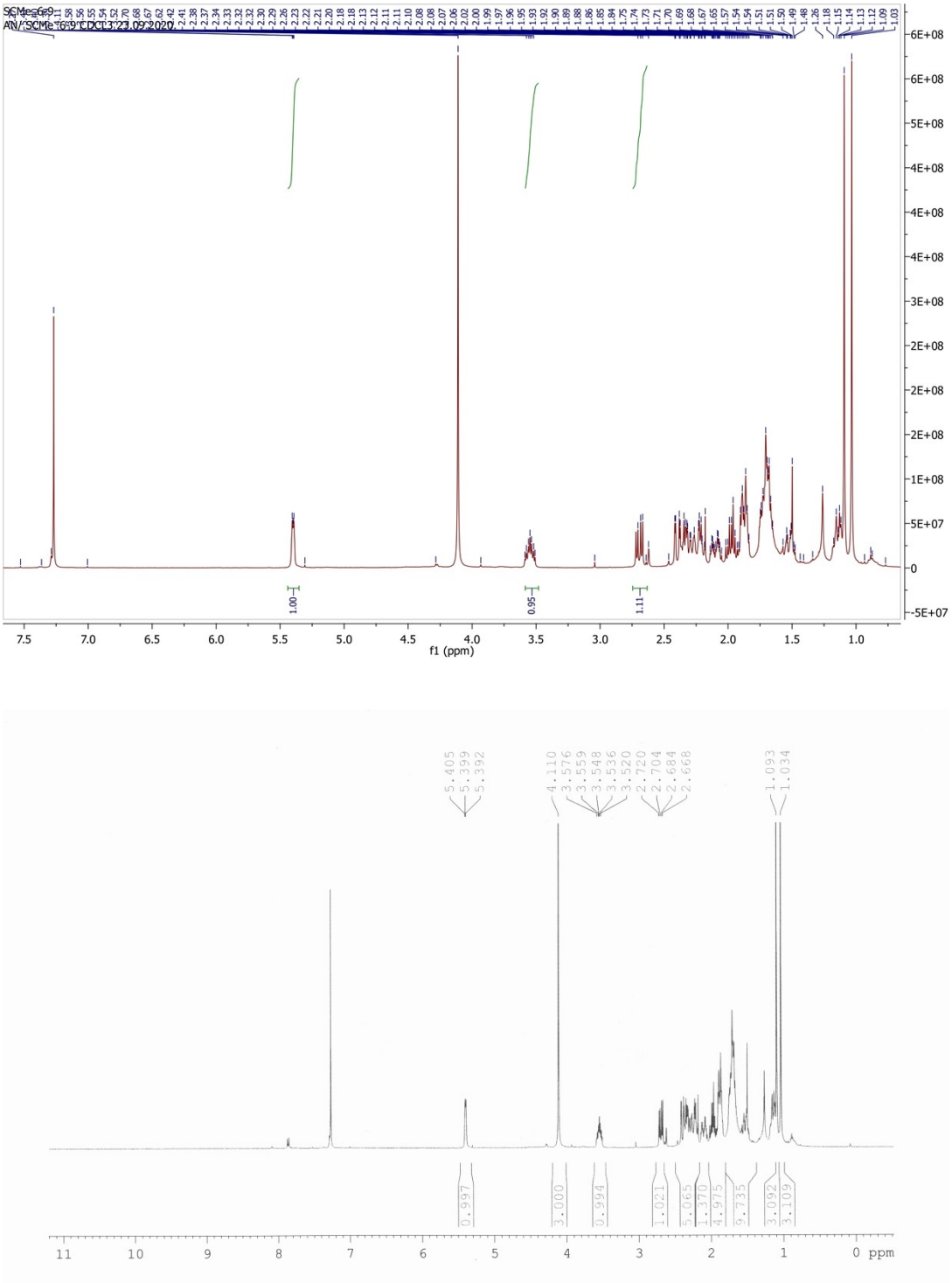


Fig S1. ^{1}H NMR of compound **3a**

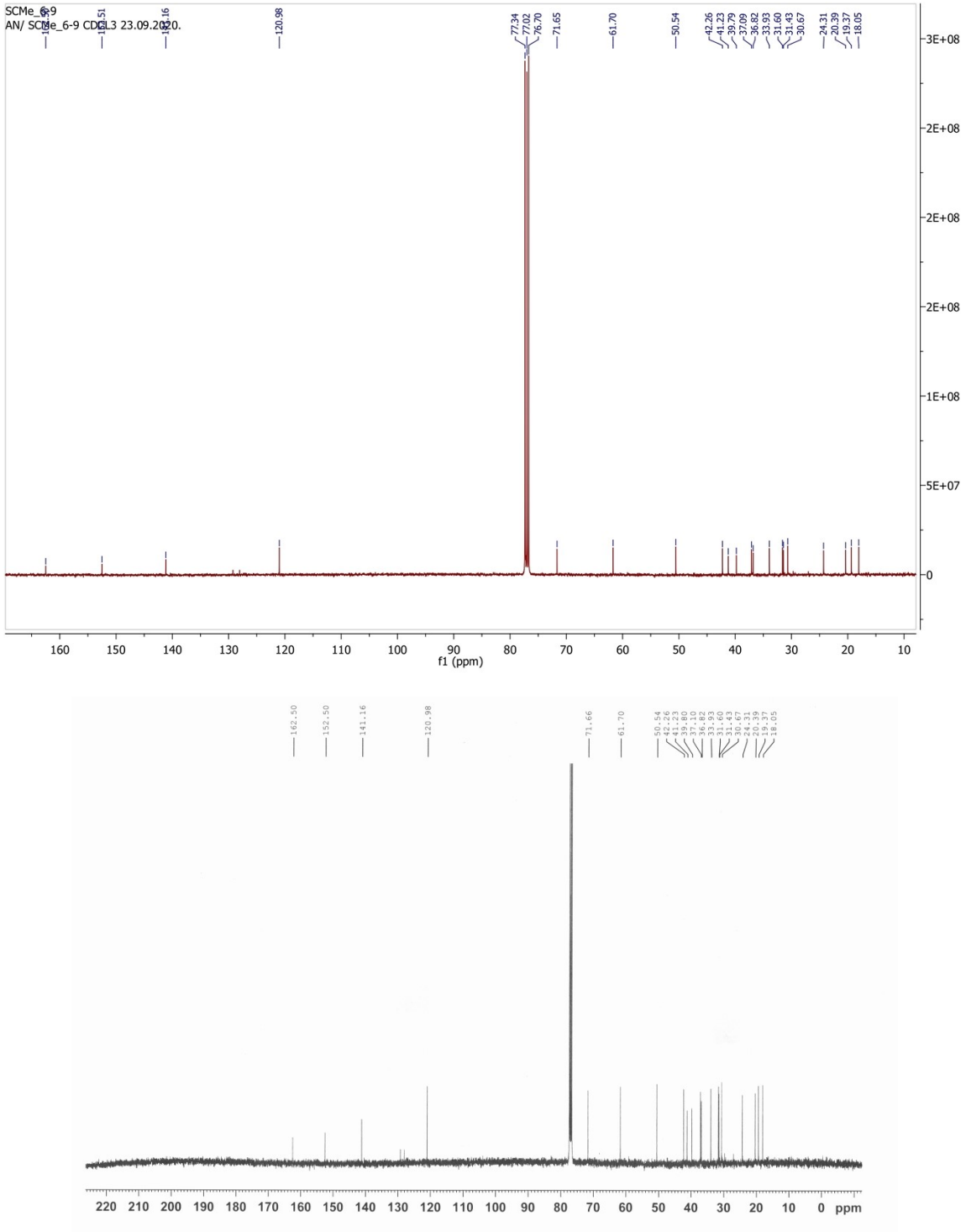


Fig S2. ^{13}C NMR of compound **3a**

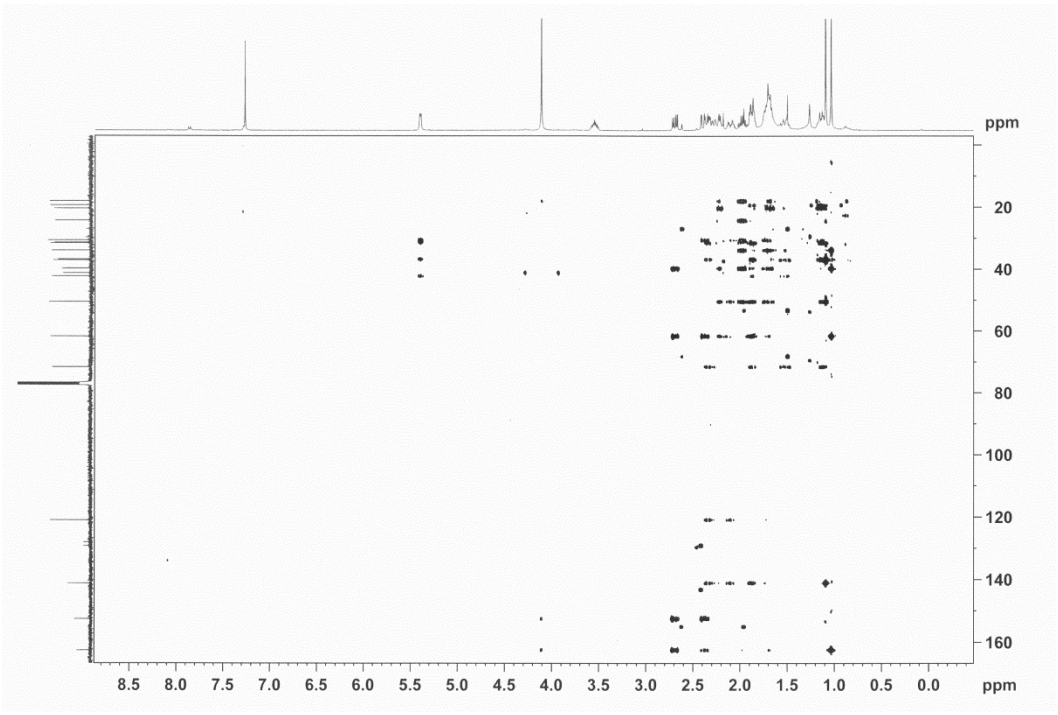


Fig S3. ^{1}H-^{13}C HMBC of compound **3a**

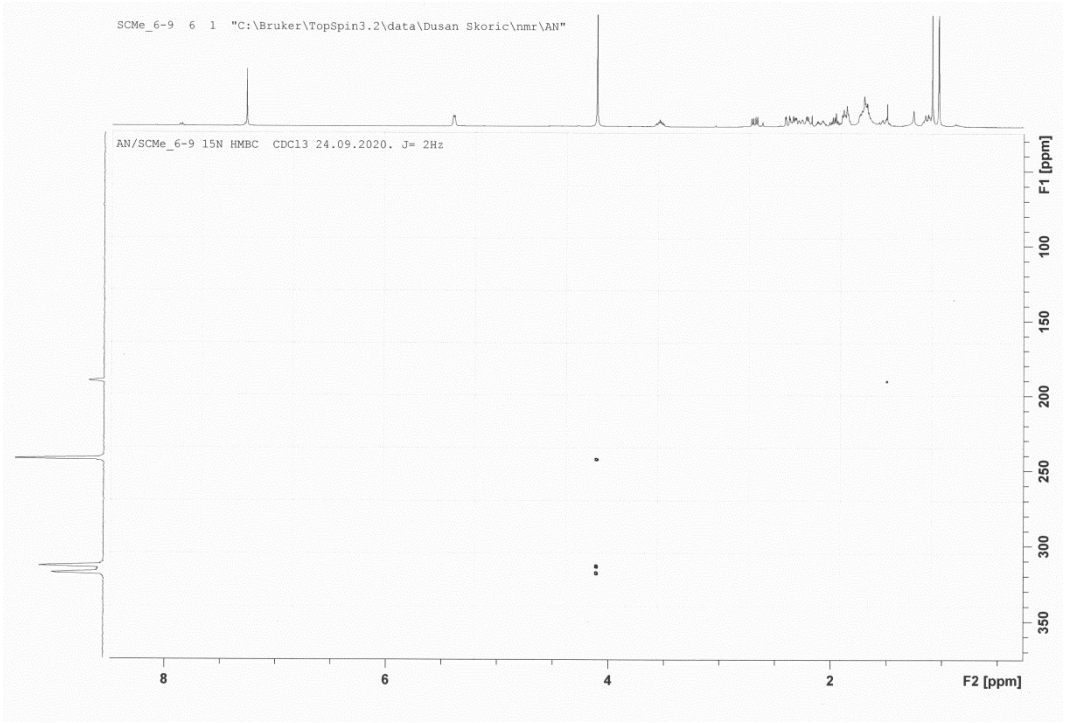


Fig S4. ^{1}H-^{15}N HMBC of compound **3a**

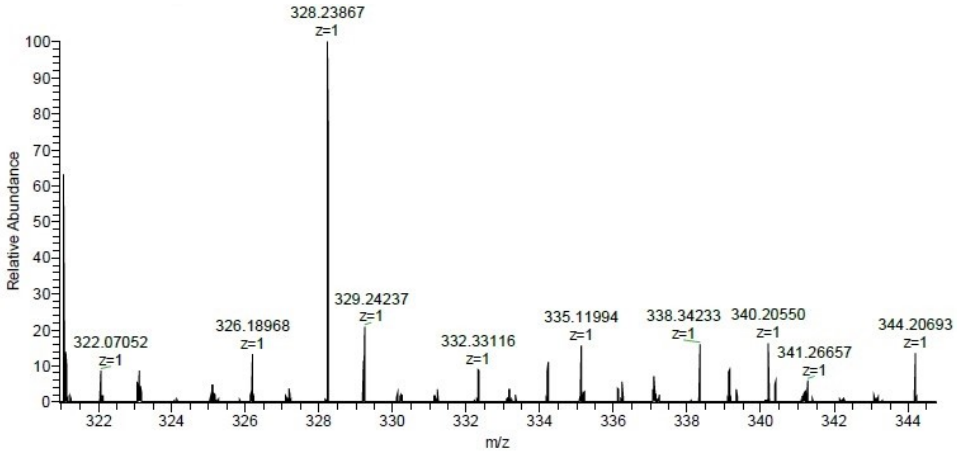
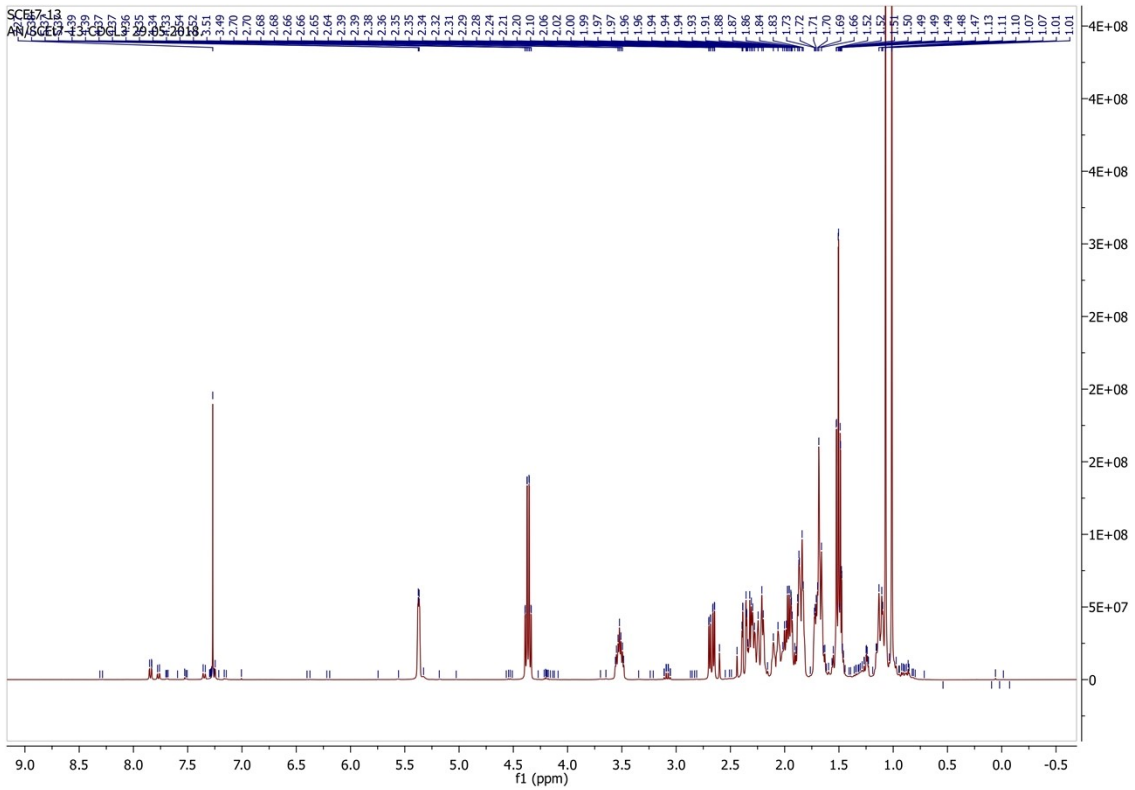


Fig S5. HRMS of compound **3a**



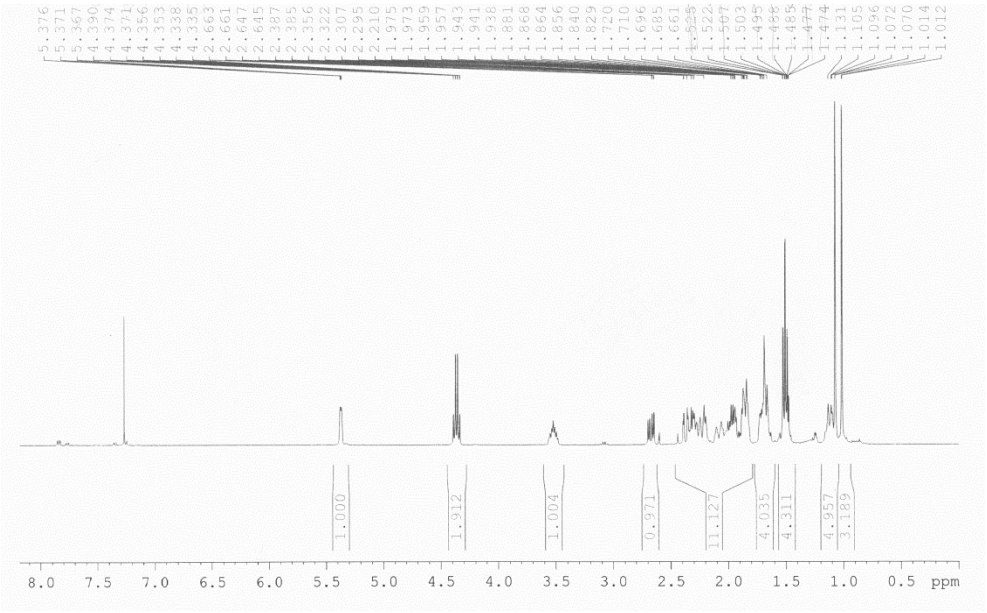
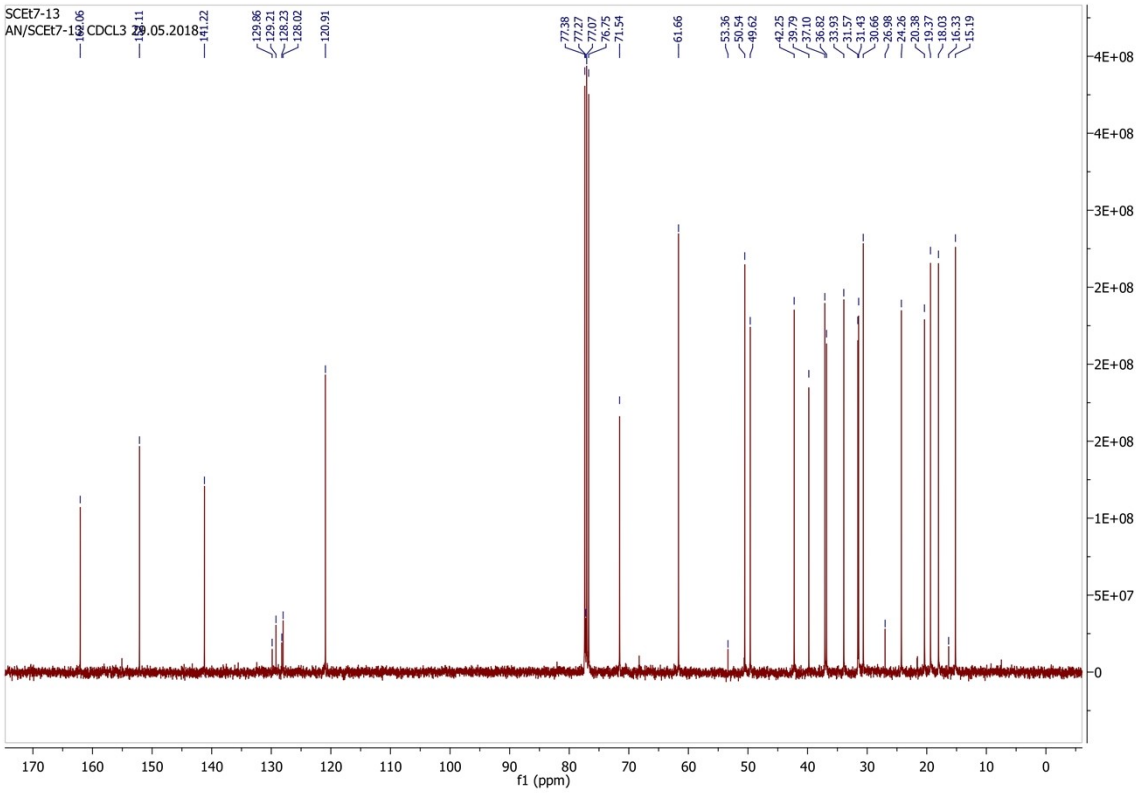


Fig S6. ^{1}H NMR of compound **3b**



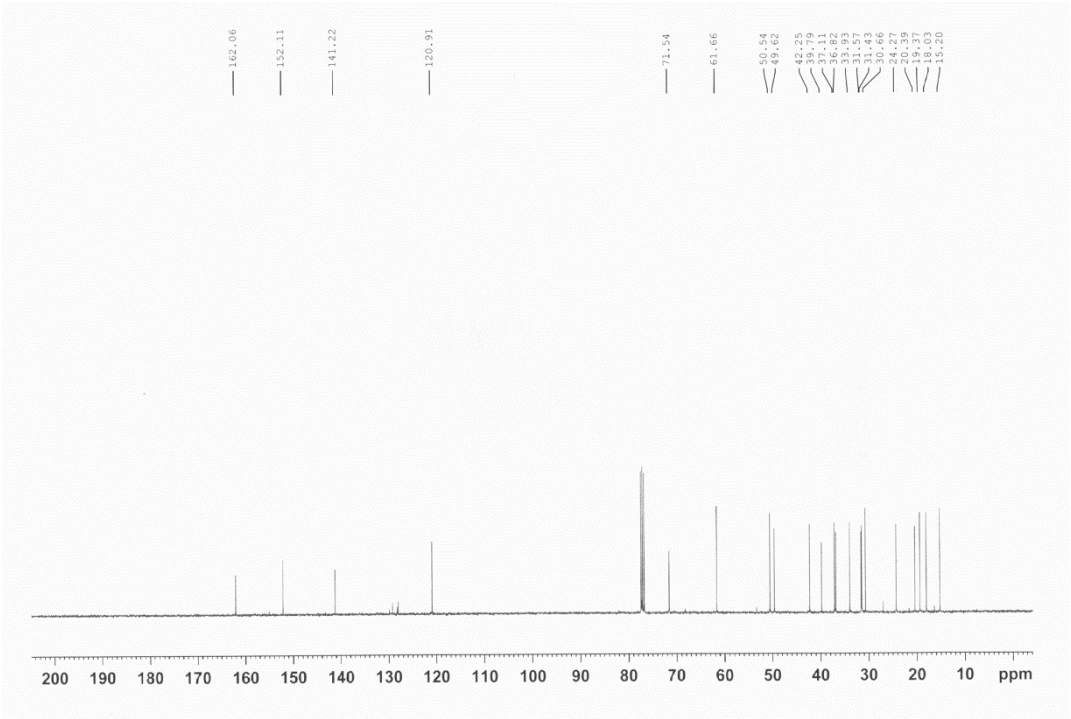


Fig S7. ^{13}C NMR of compound **3b**

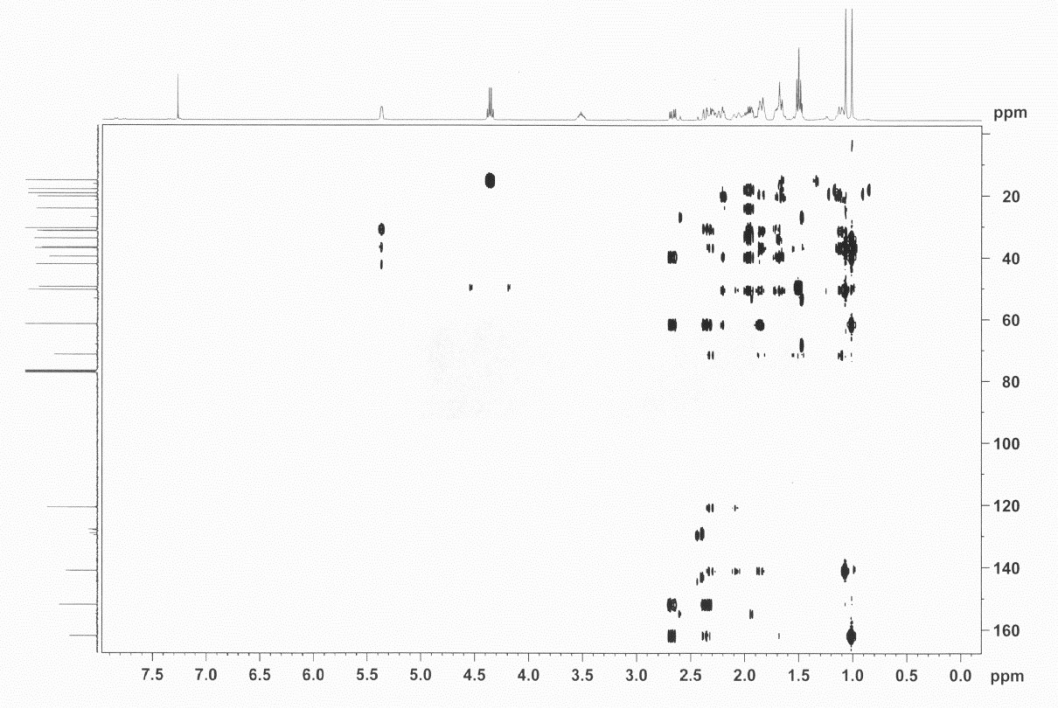


Fig S8. ^{1}H-^{13}C HMBC of compound **3b**

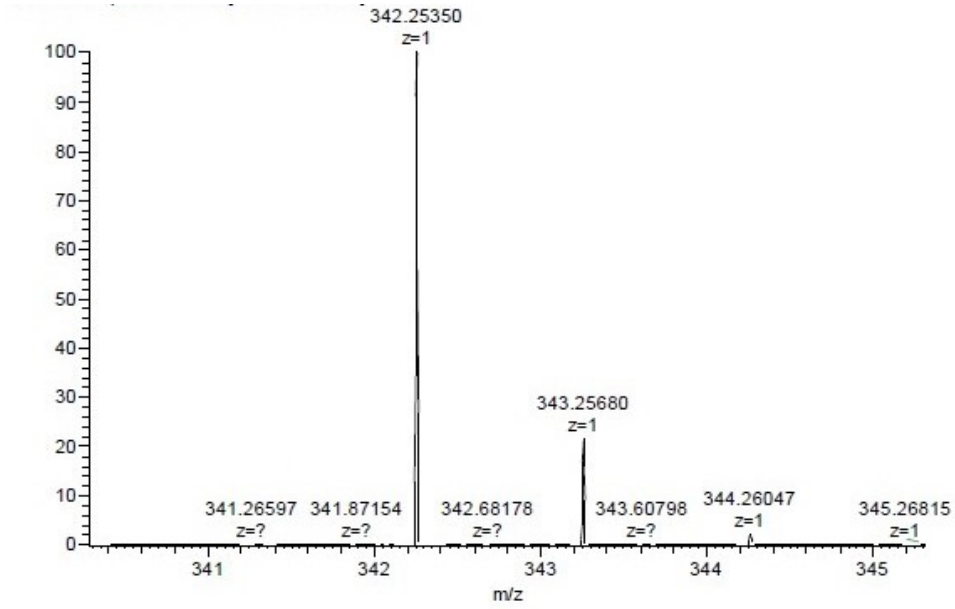
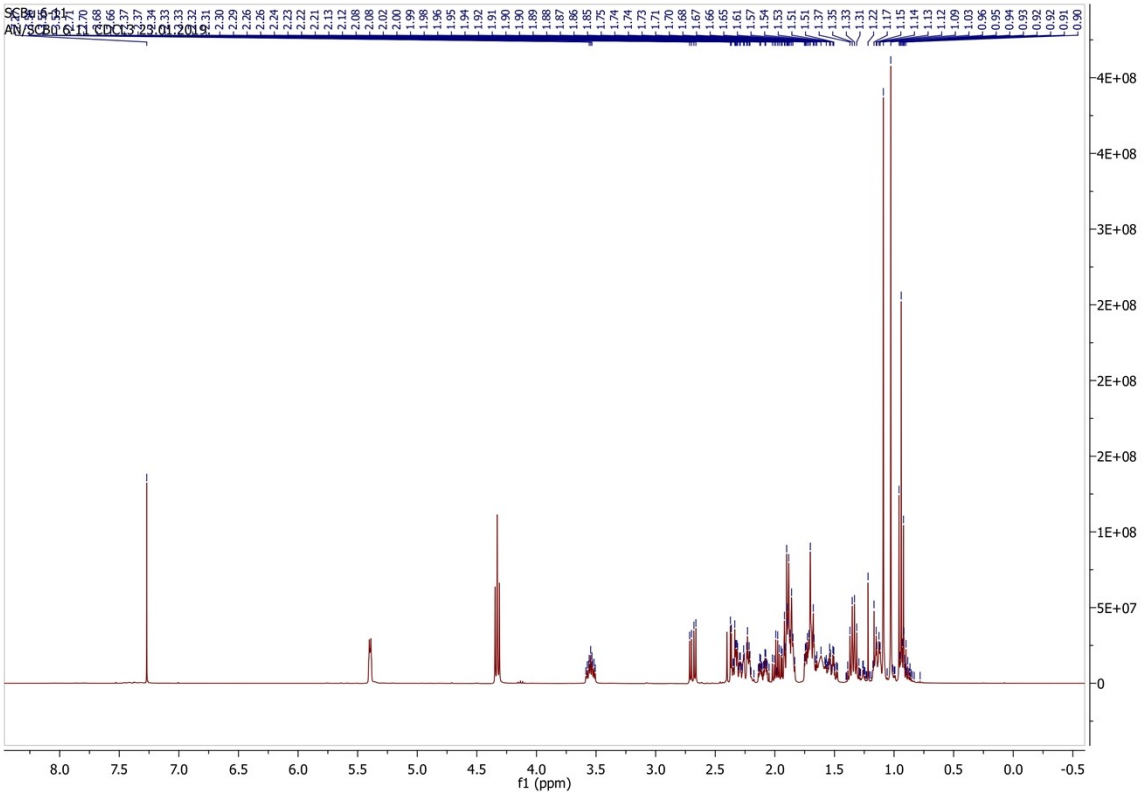


Fig S9. HRMS of compound **3b**



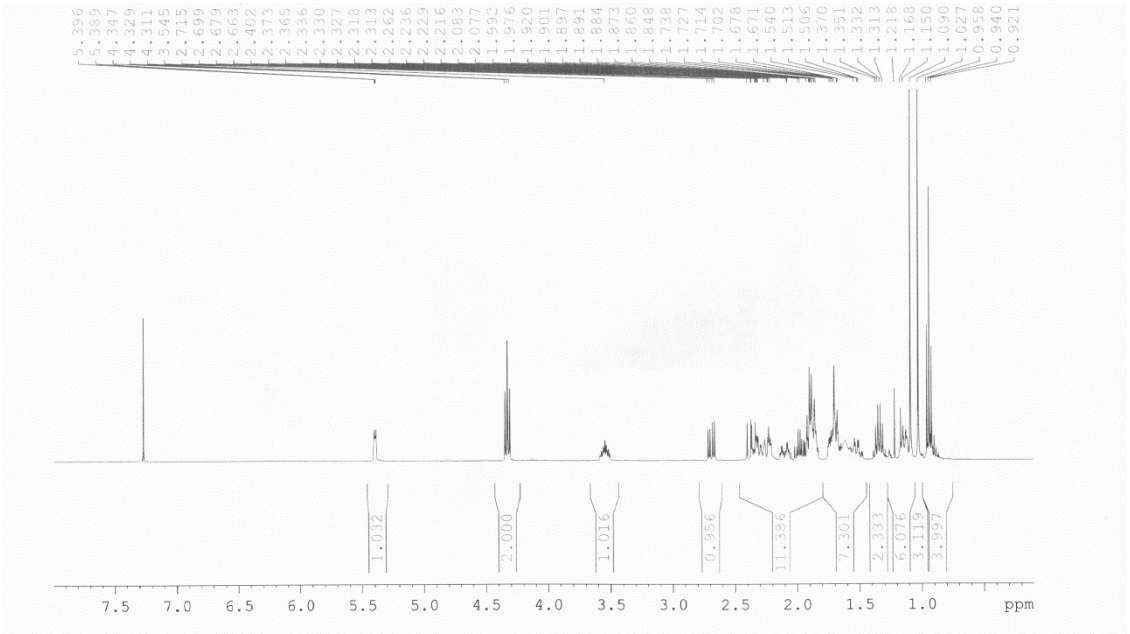
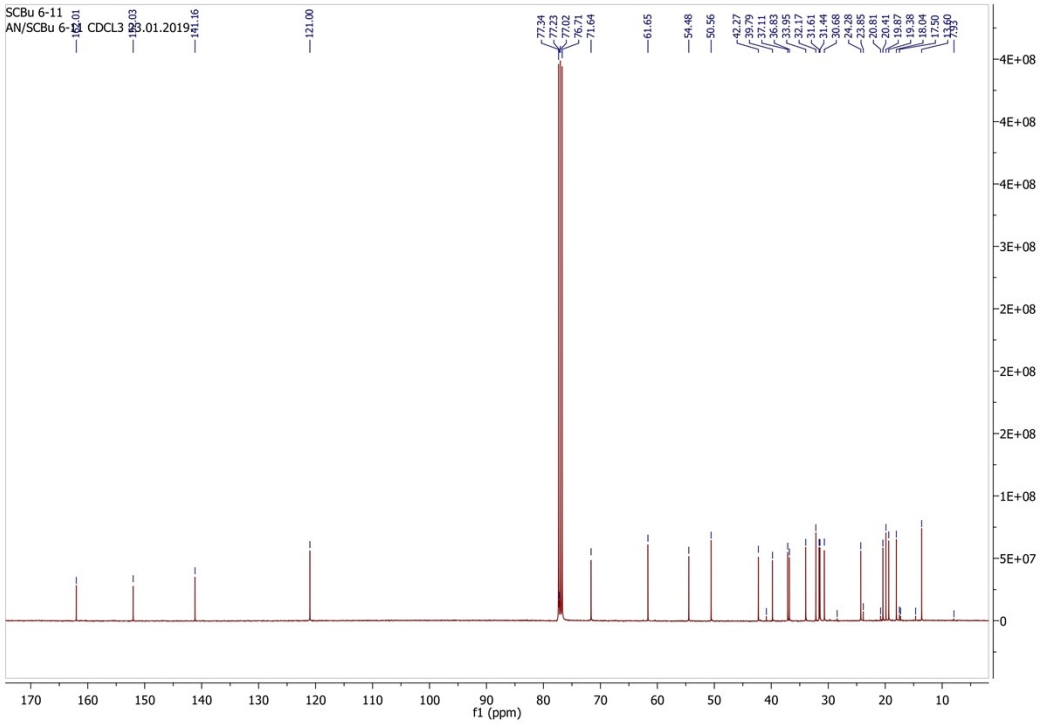


Fig S10. ^{1}H MNR of compound **3c**



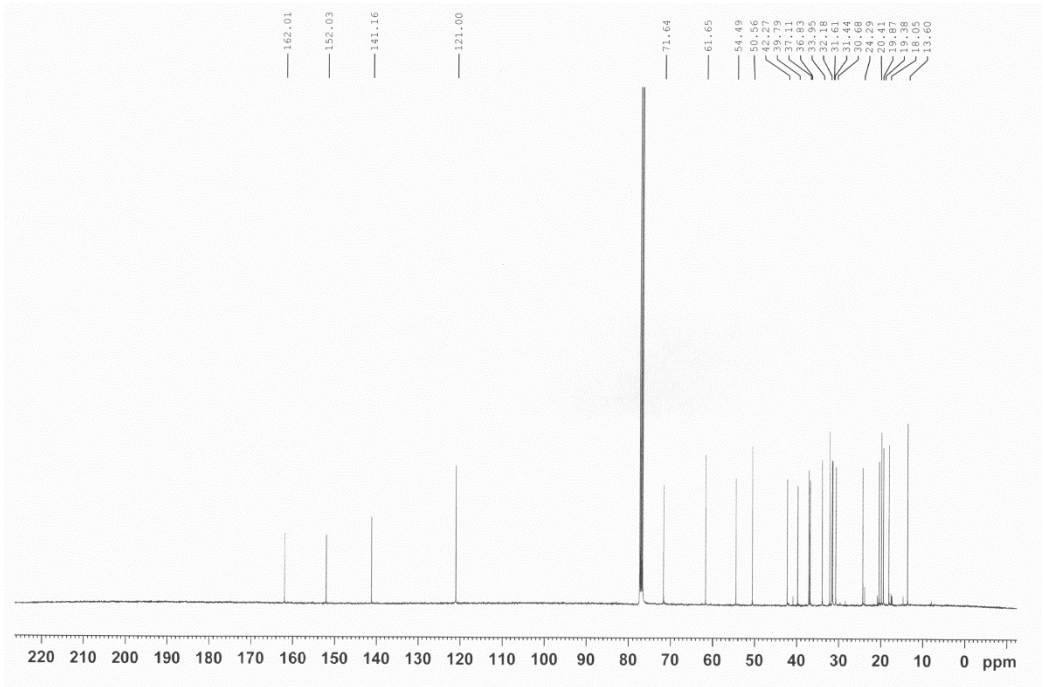


Fig S11. ^{13}C NMR of compound **3c**

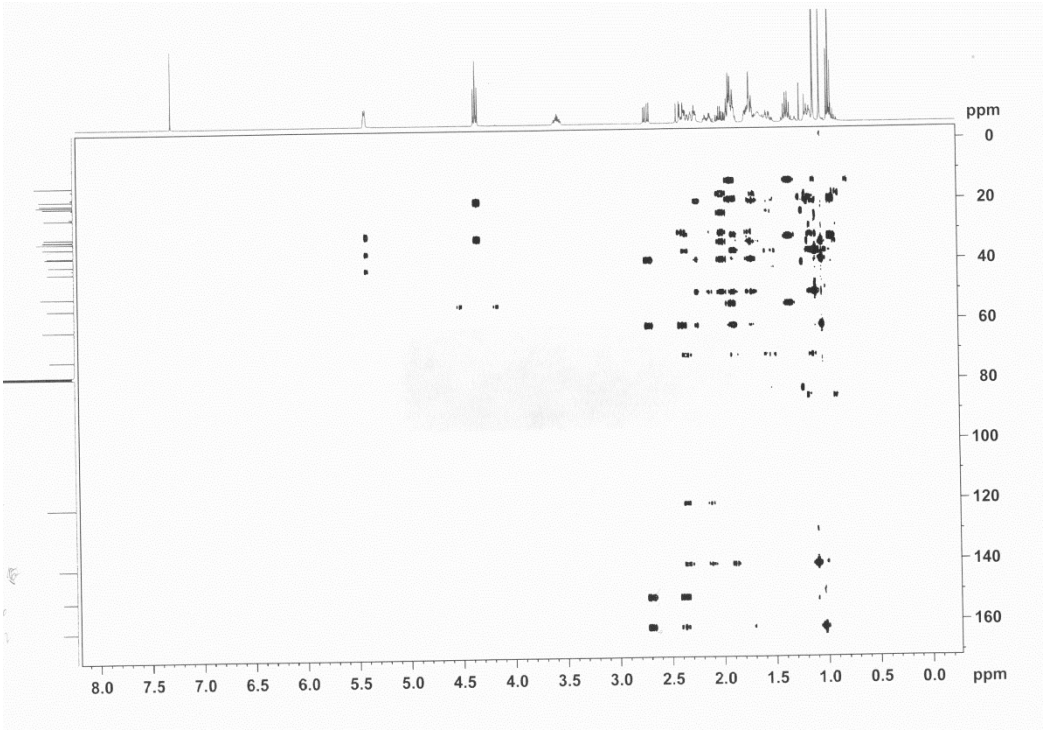


Fig S12. ^{1}H-^{13}C HMBC of compound **3c**

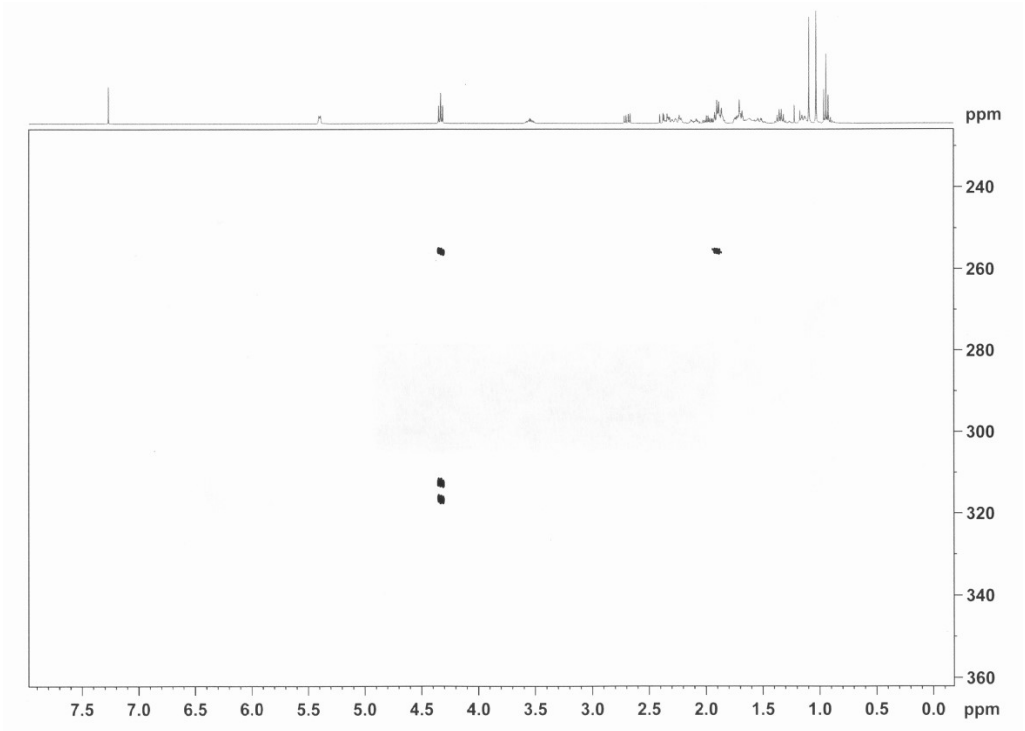


Fig S13. ^{1}H-^{15}N HMBC of compound **3c**

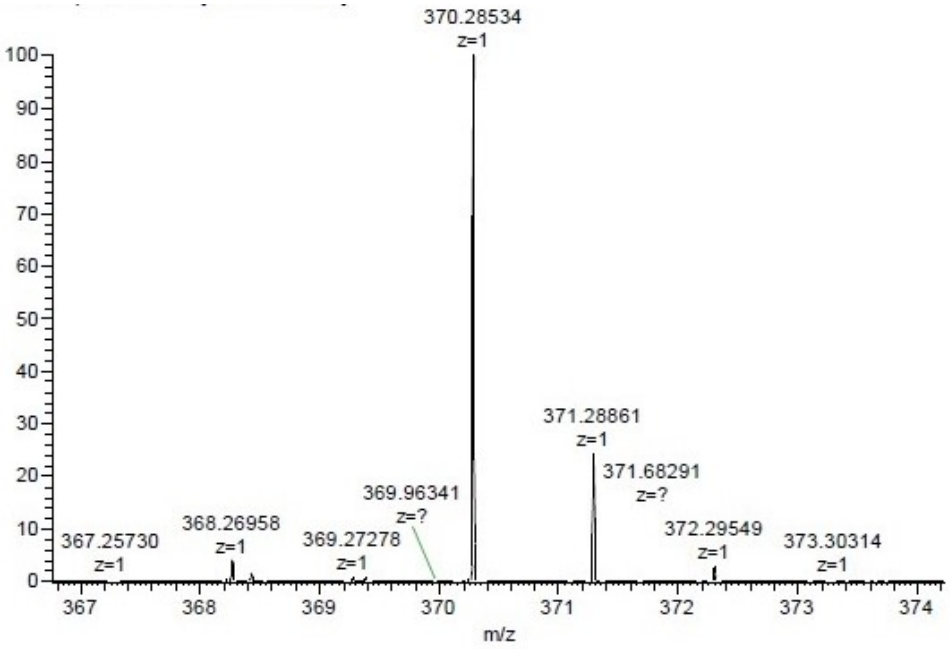
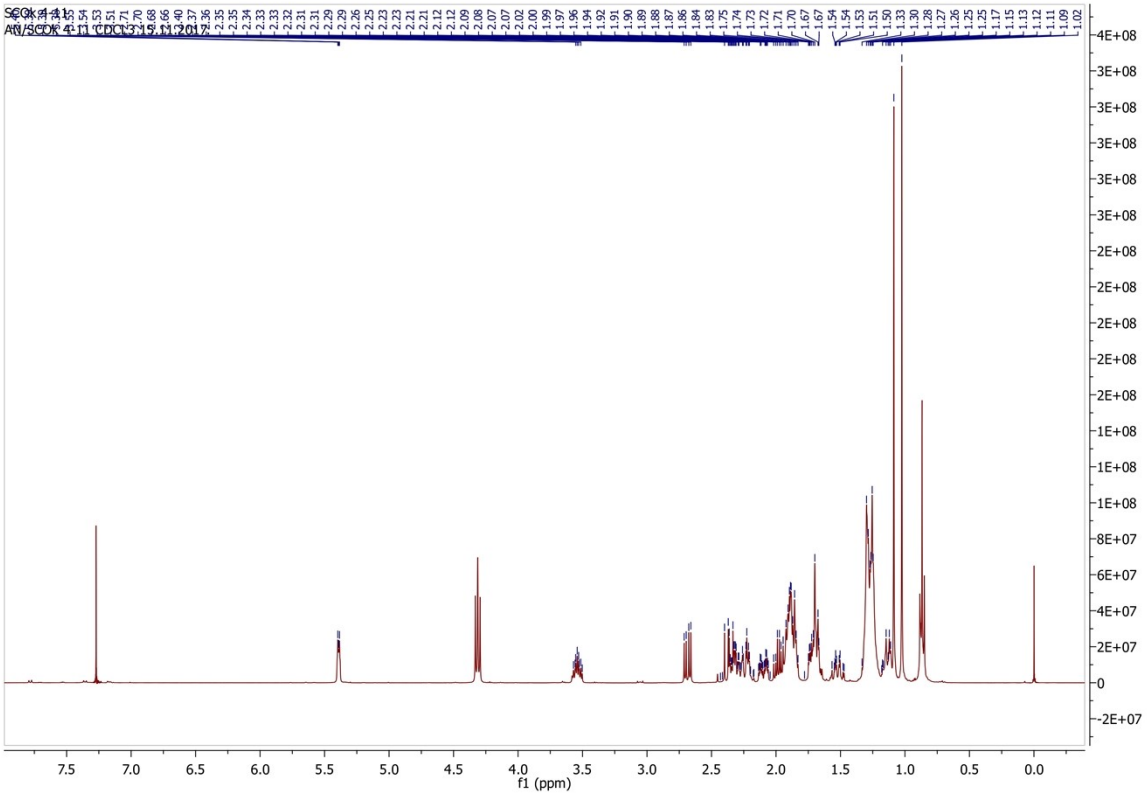


Fig S14. HRMS of compound **3c**



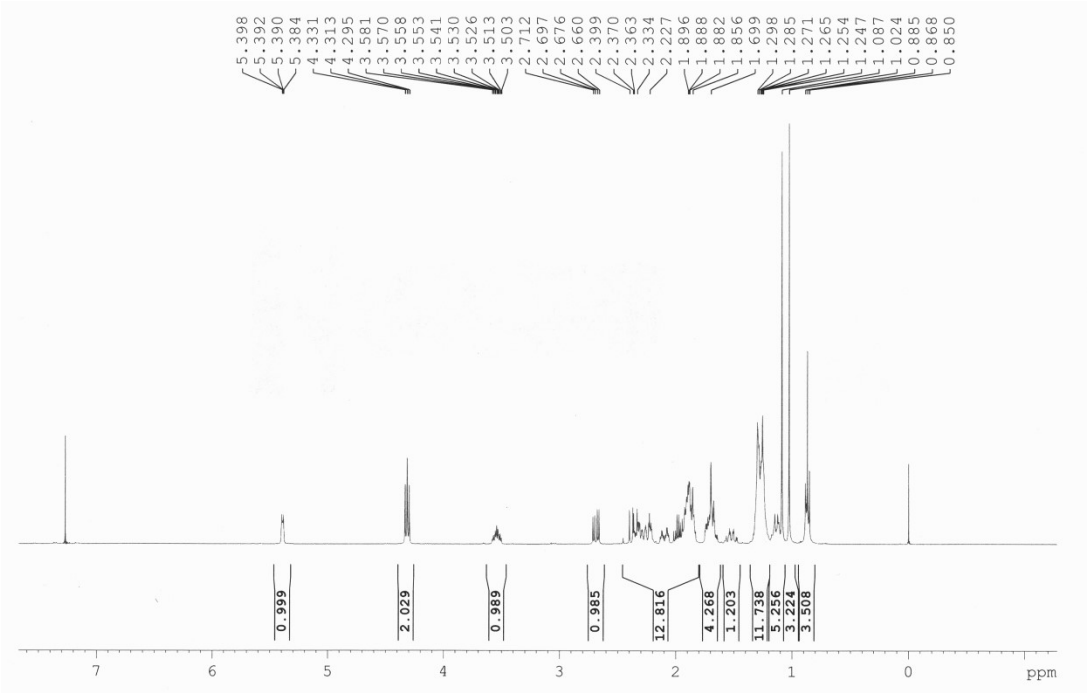
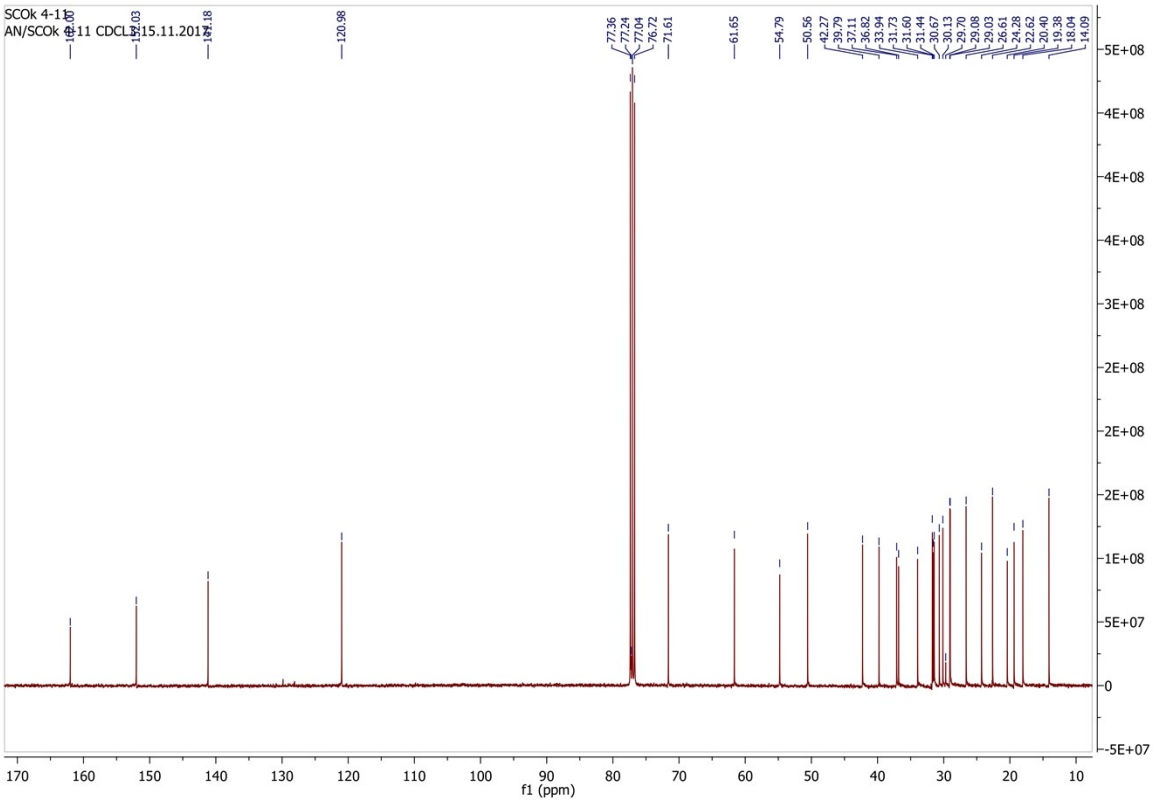


Fig S15. ^{1}H NMR of compound **3d**



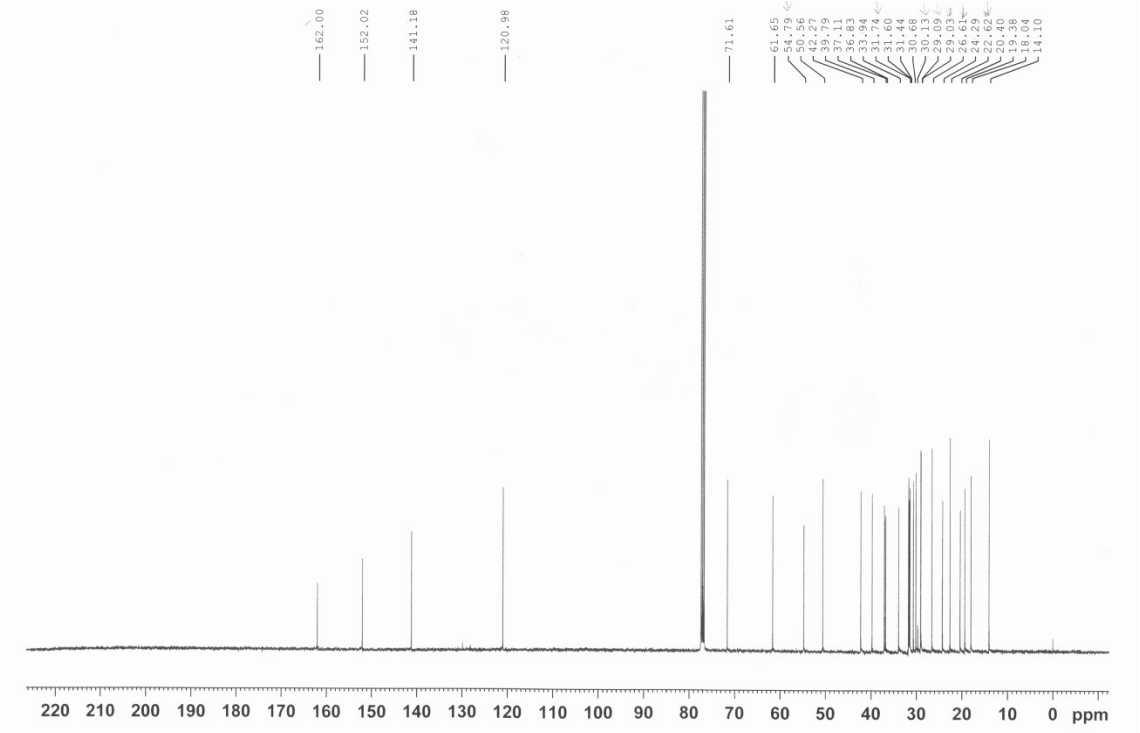


Fig S16. ^{13}C NMR of compound **3d**

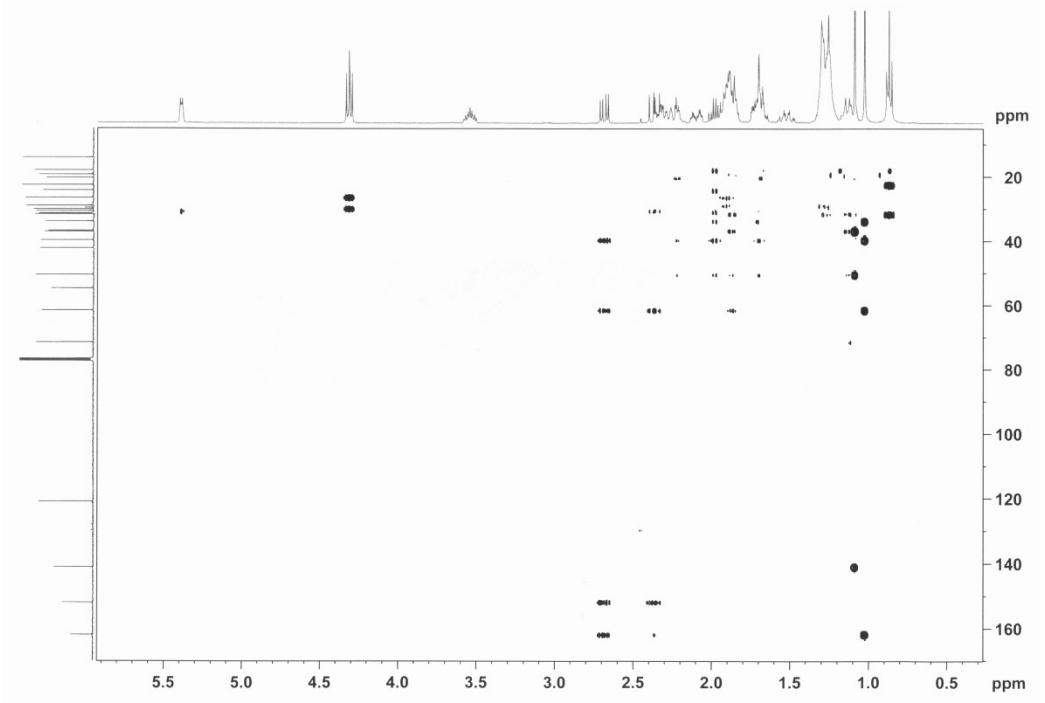
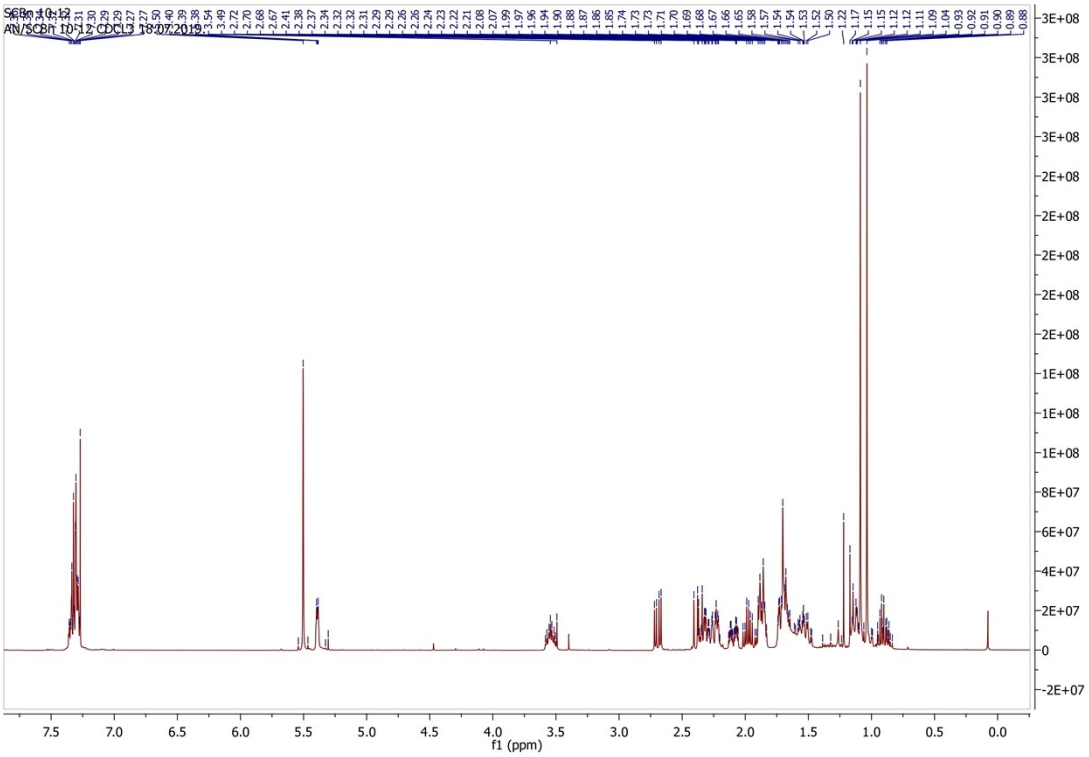


Fig S17. ^{1}H-^{13}C HMBC of compound **3d**



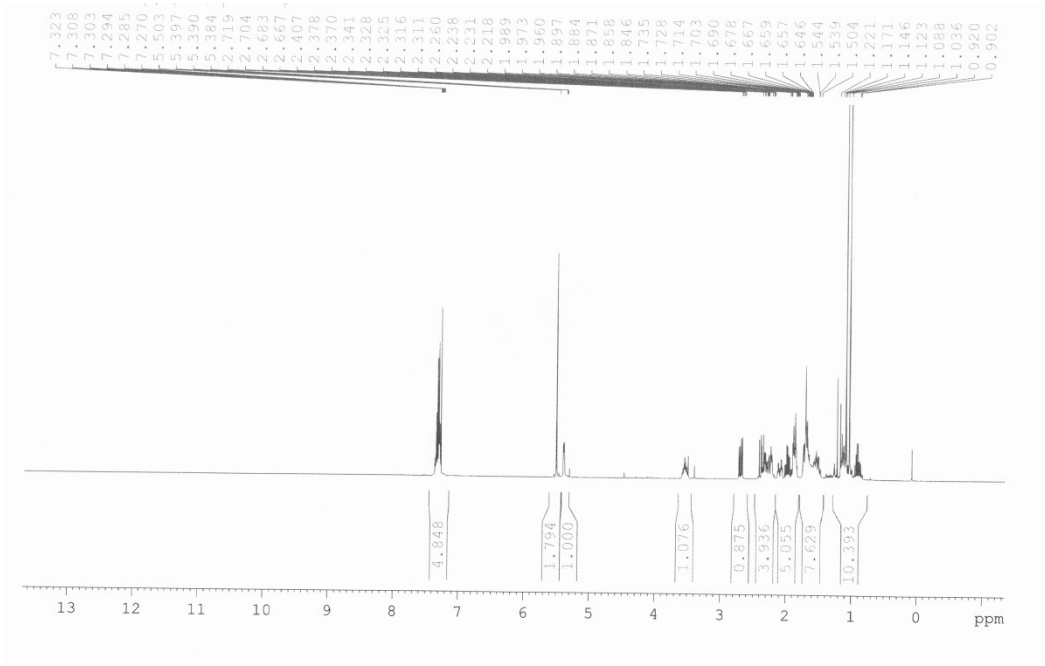
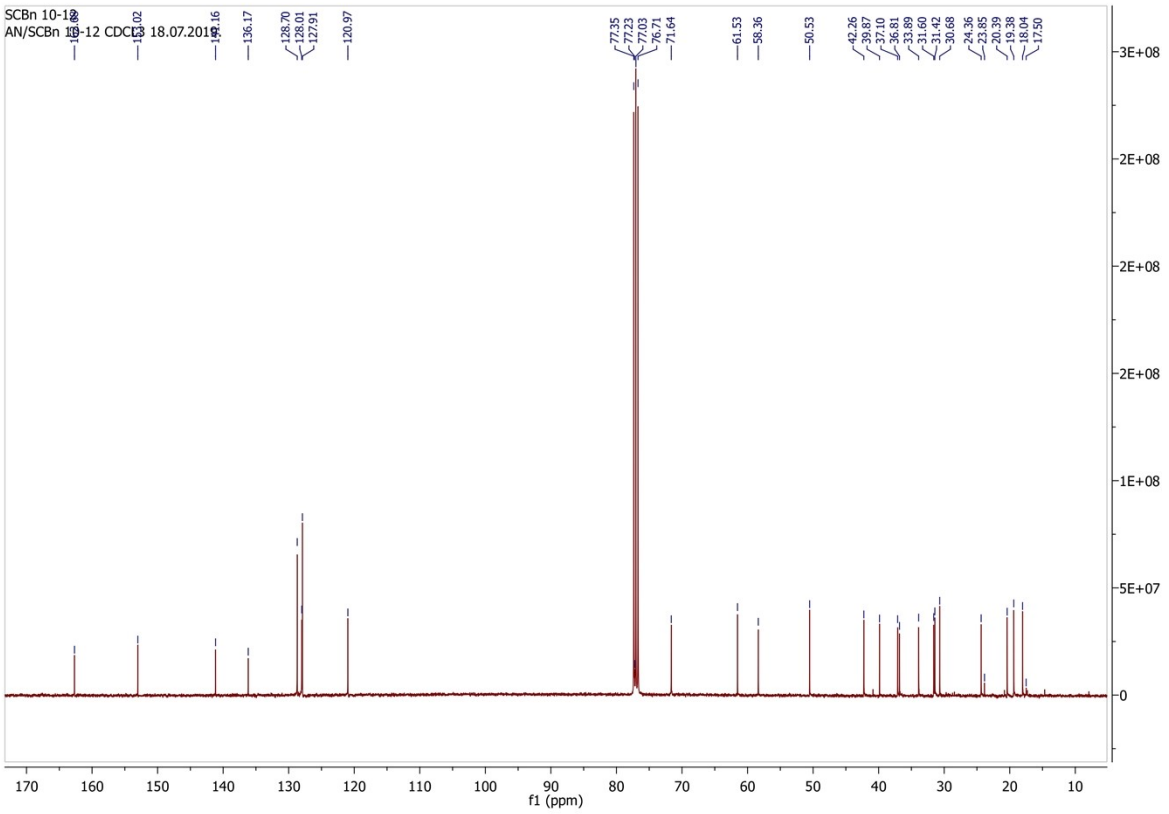


Fig S18. ^{1}H NMR of compound **3e**



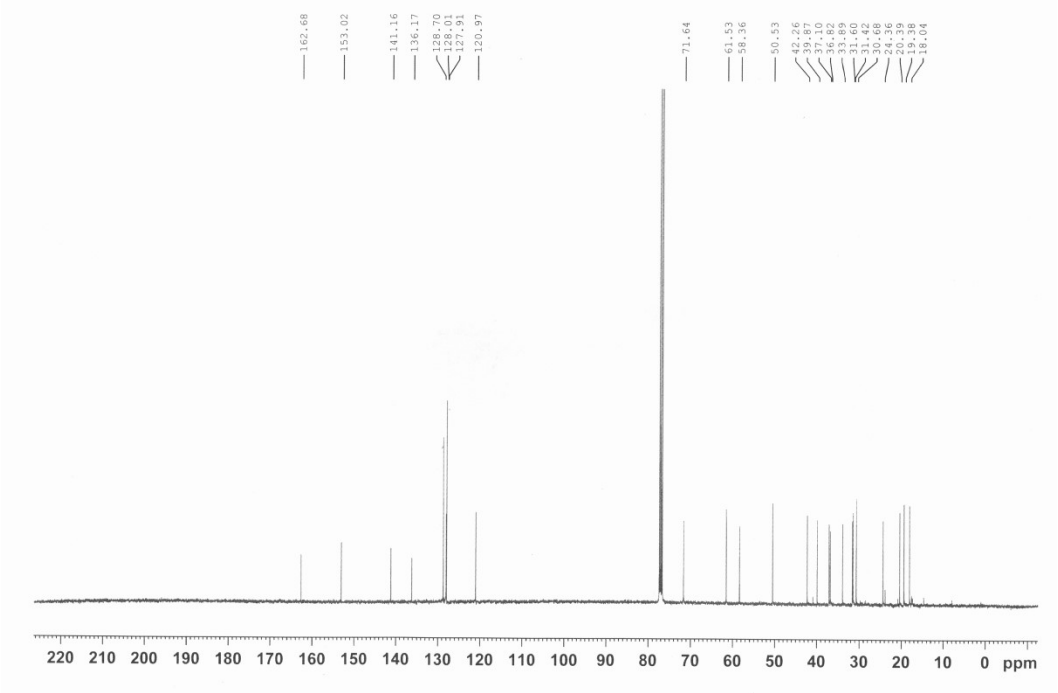


Fig S19. ^{13}C NMR of compound **3e**

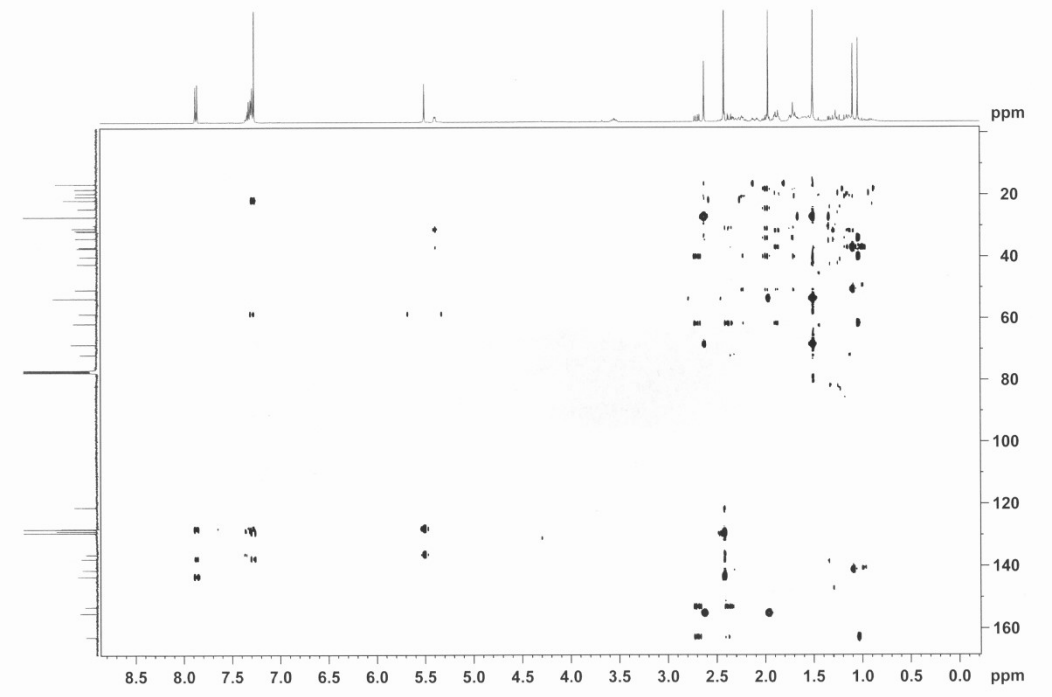


Fig S20. ^{1}H-^{13}C HMBC of compound **3e**

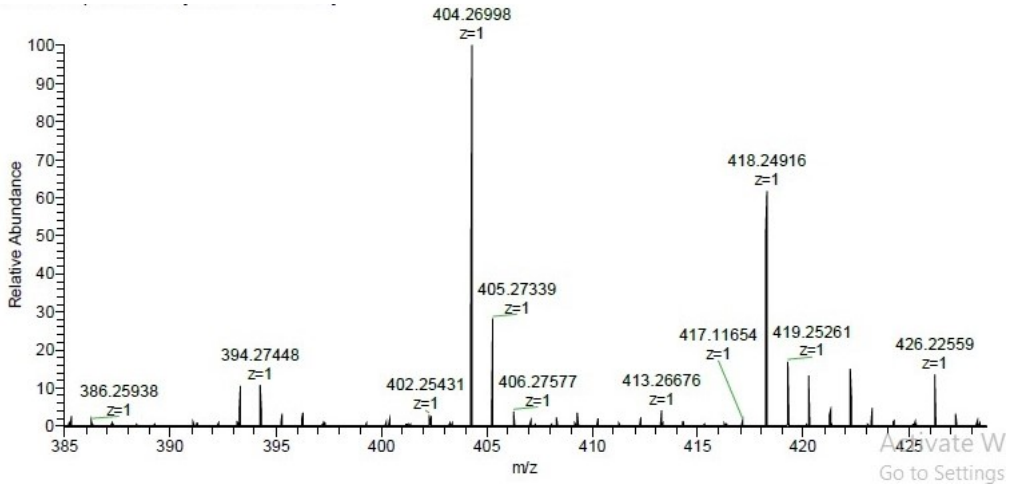


Fig S21. HRMS of compound **3e**

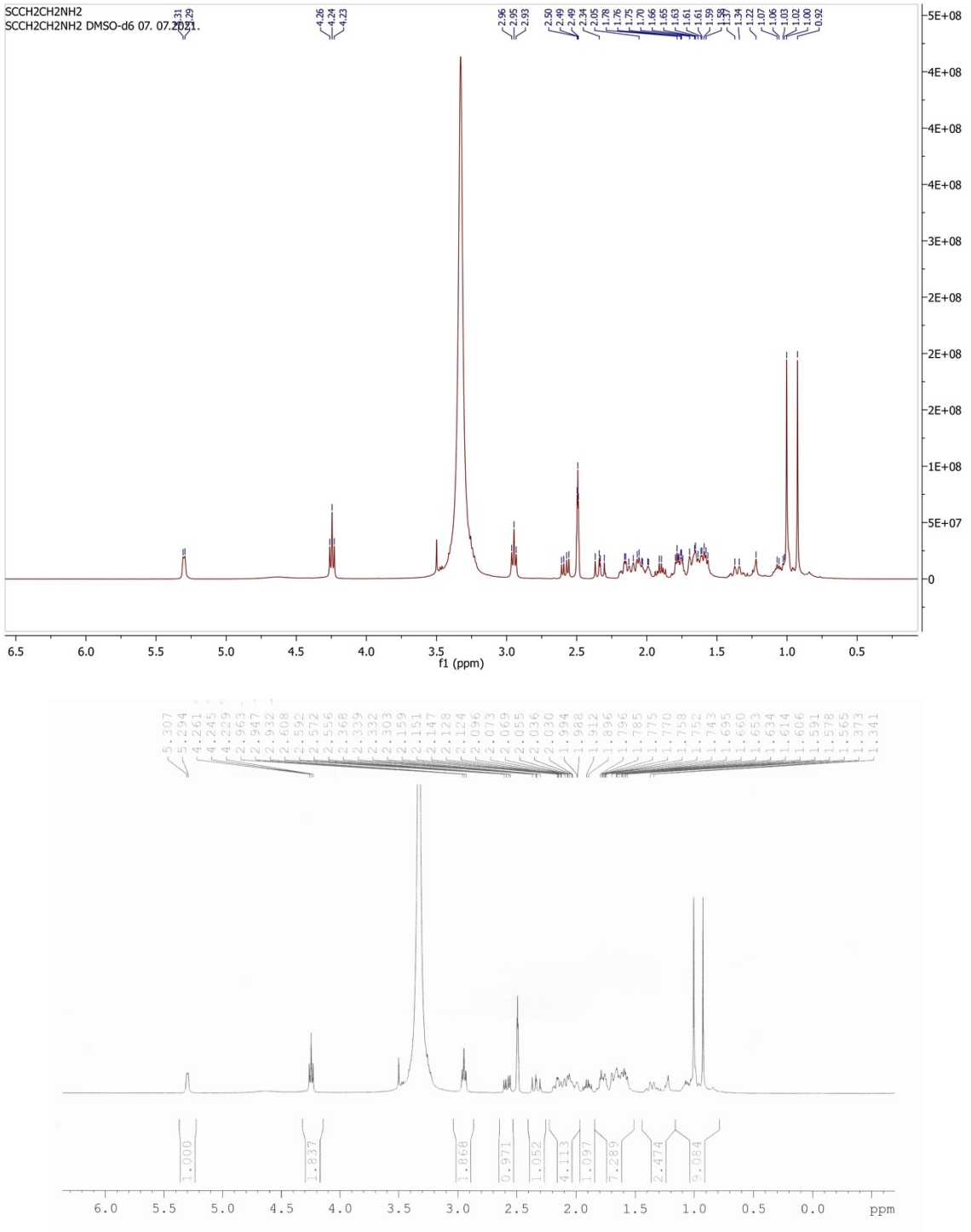
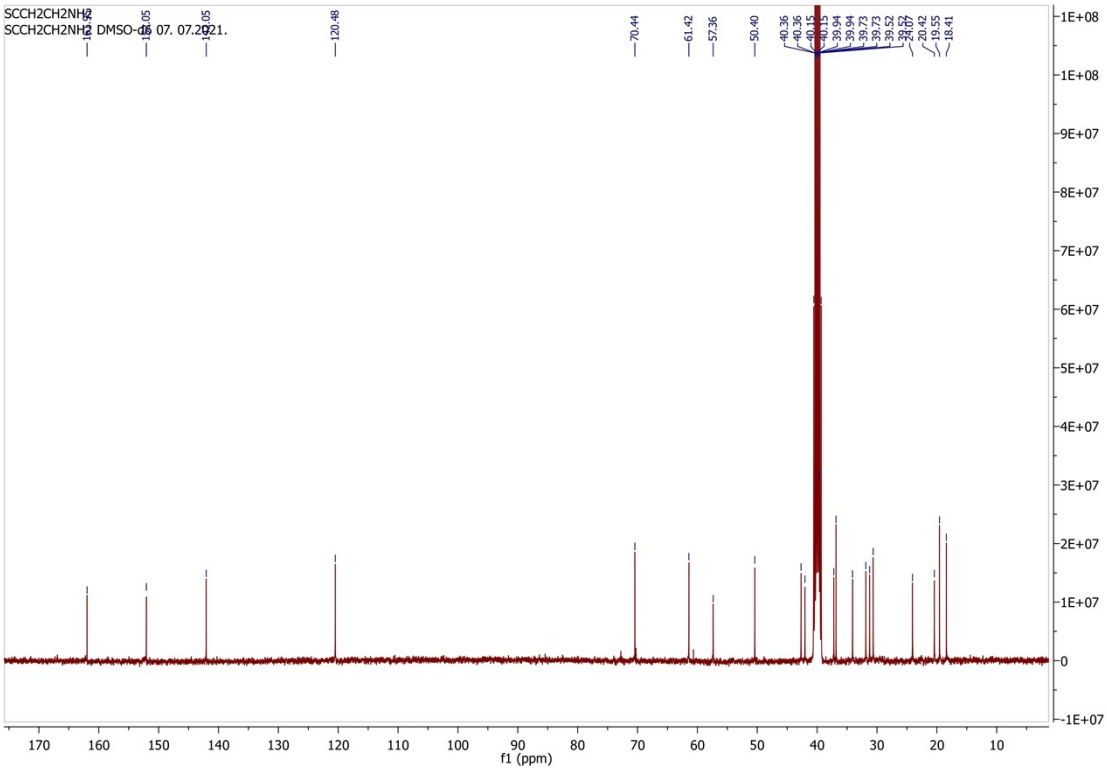


Fig S22. ^{1}H NMR of compound **3f**



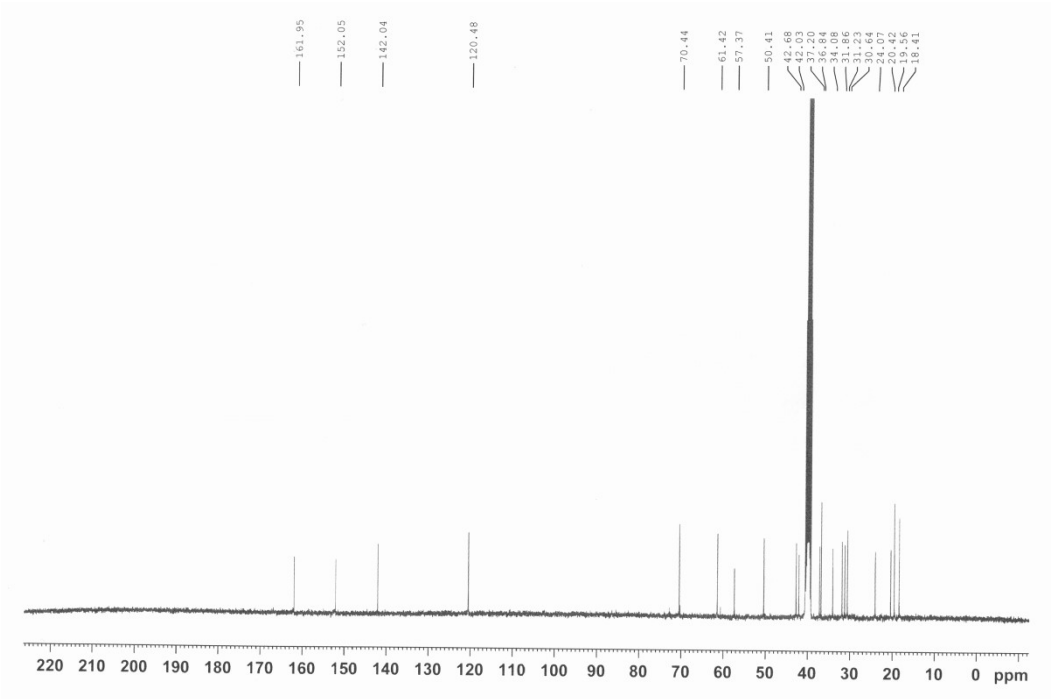


Fig S23. ^{13}C NMR of compound **3f**

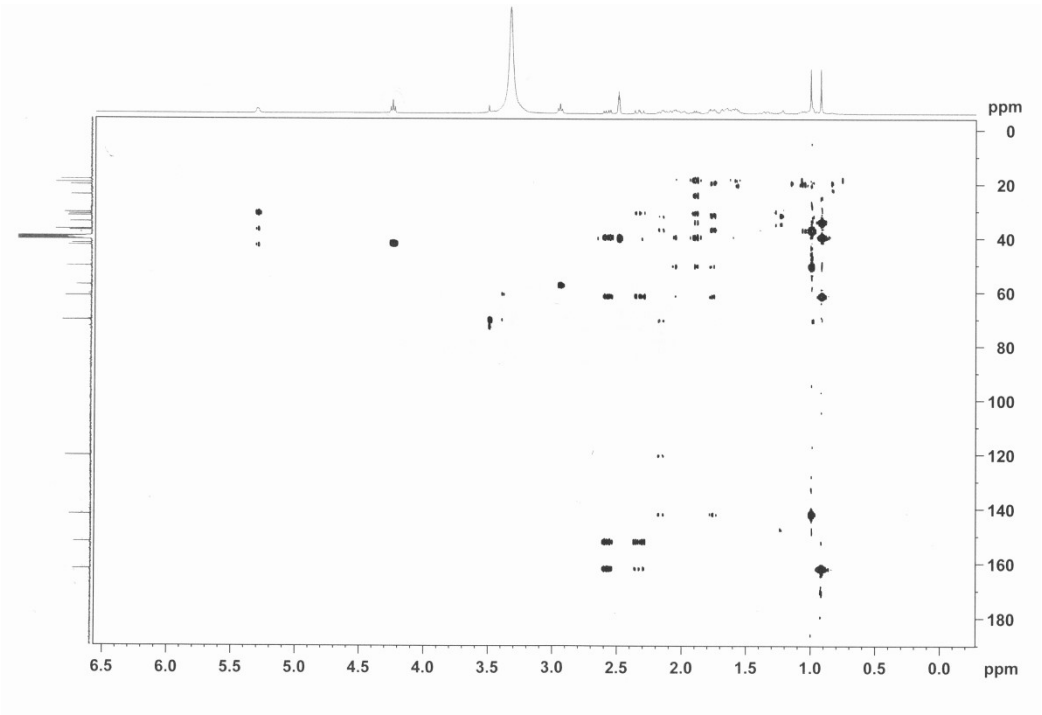


Fig S24. ^{1}H-^{13}C HMBC of compound **3f**

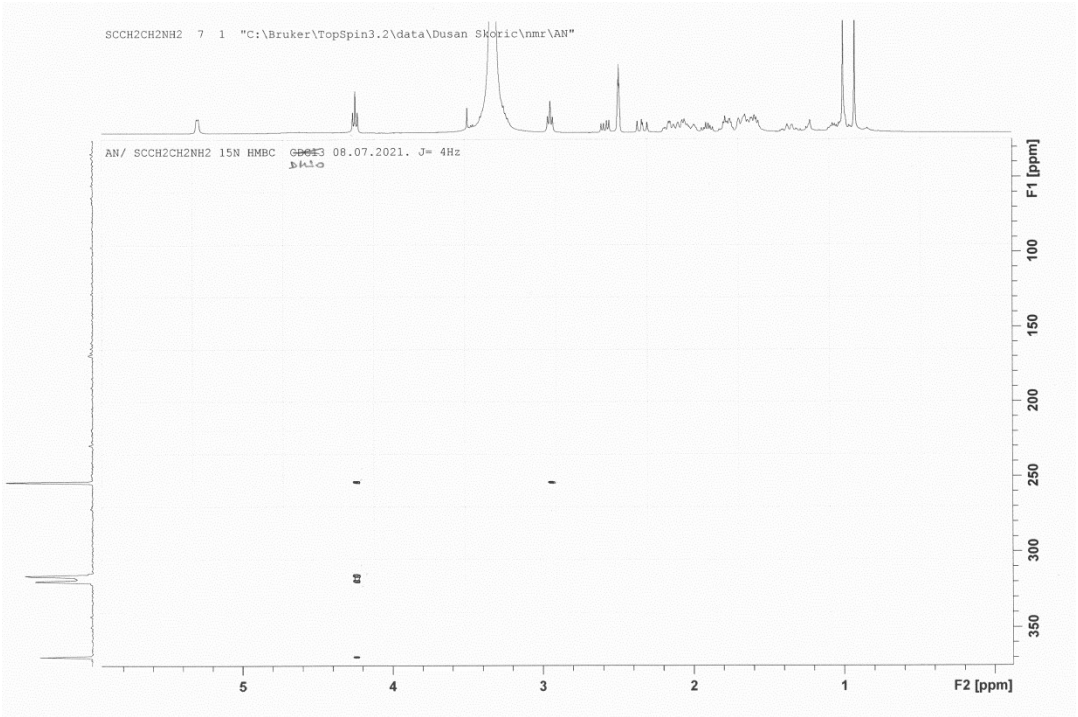


Fig S25. ^{1}H-^{15}N HMBC of compound **3f**

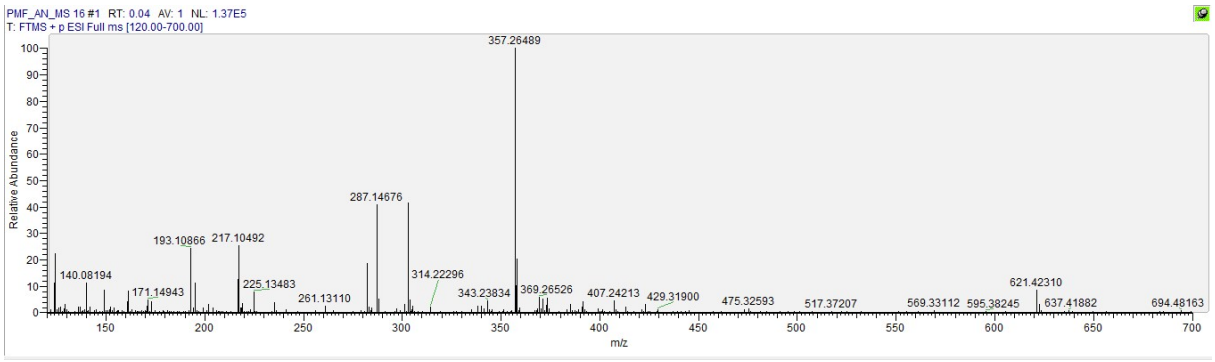
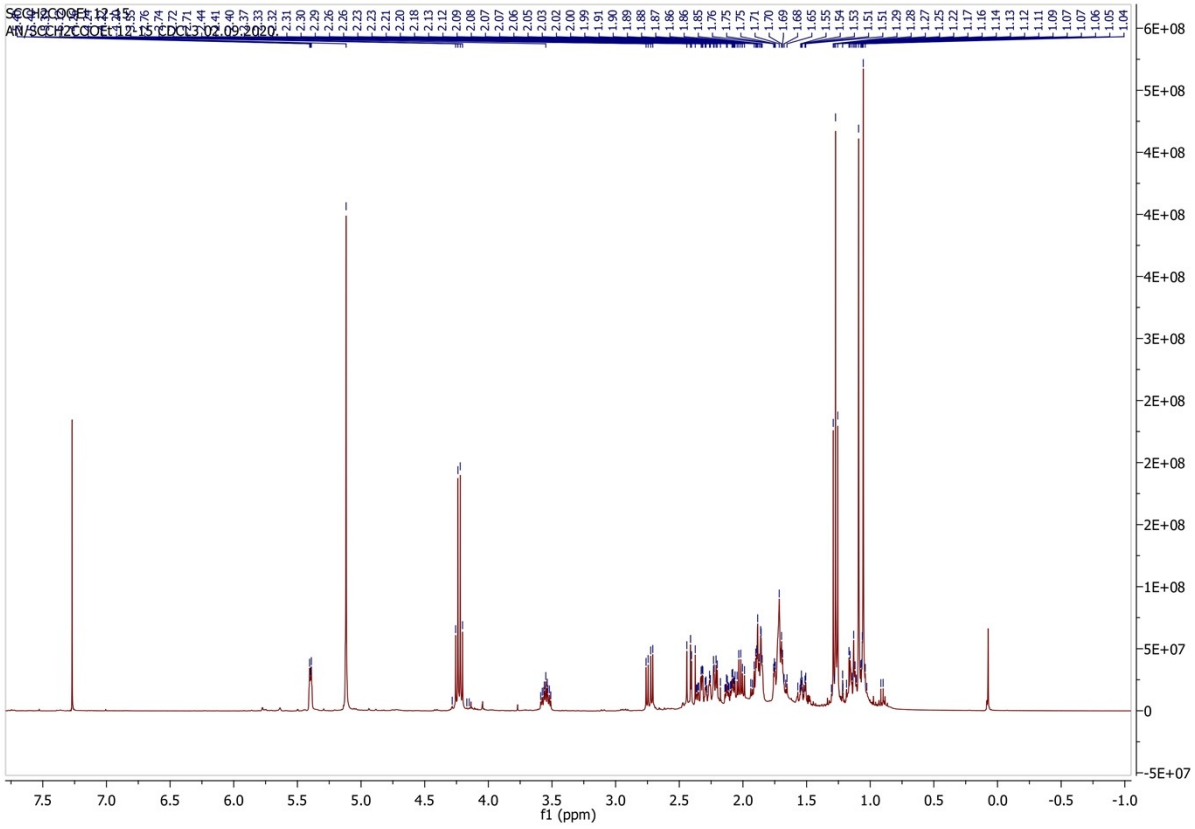


Fig S26. HRMS of compound **3f**



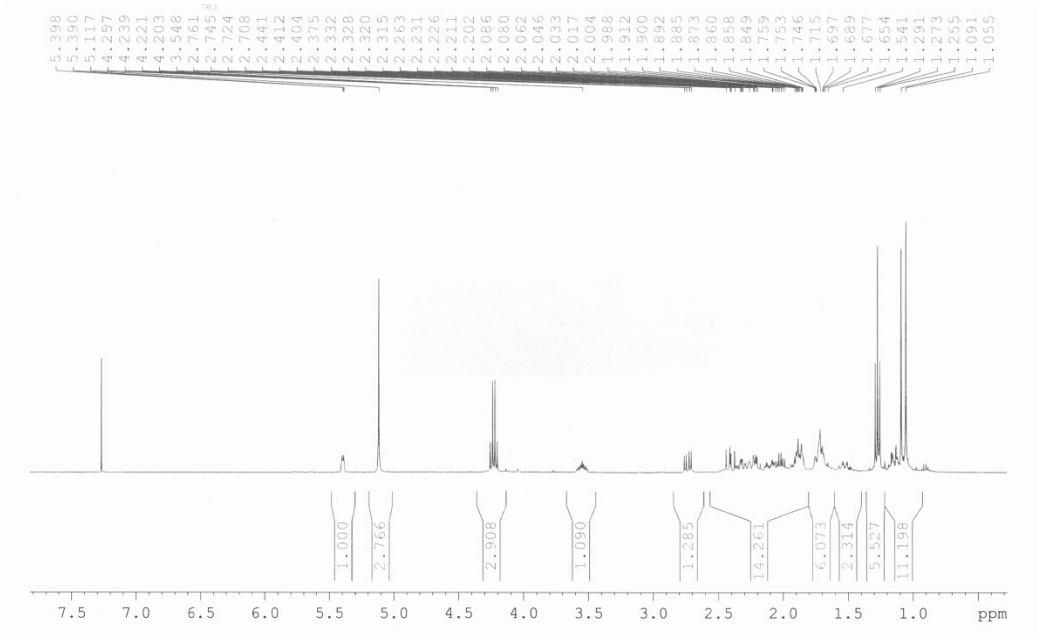
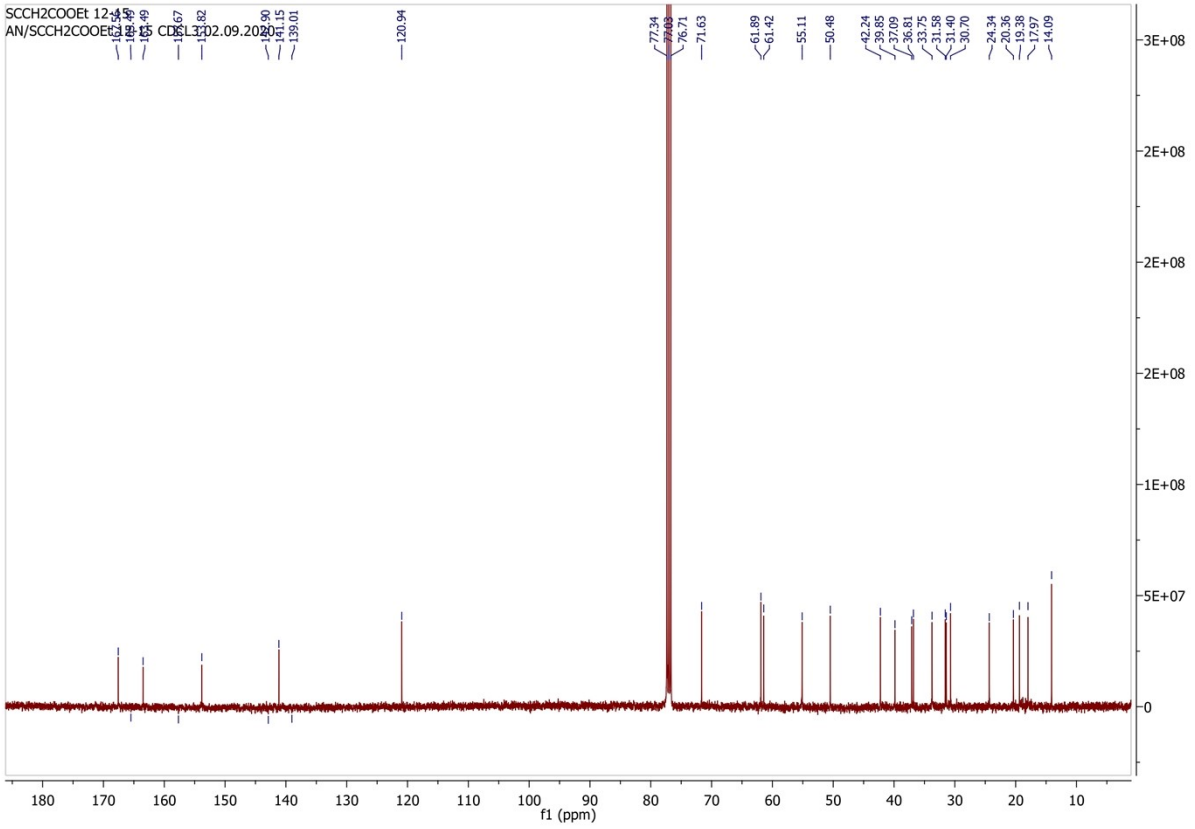


Fig S27. ^{1}H NMR of compound **3g**



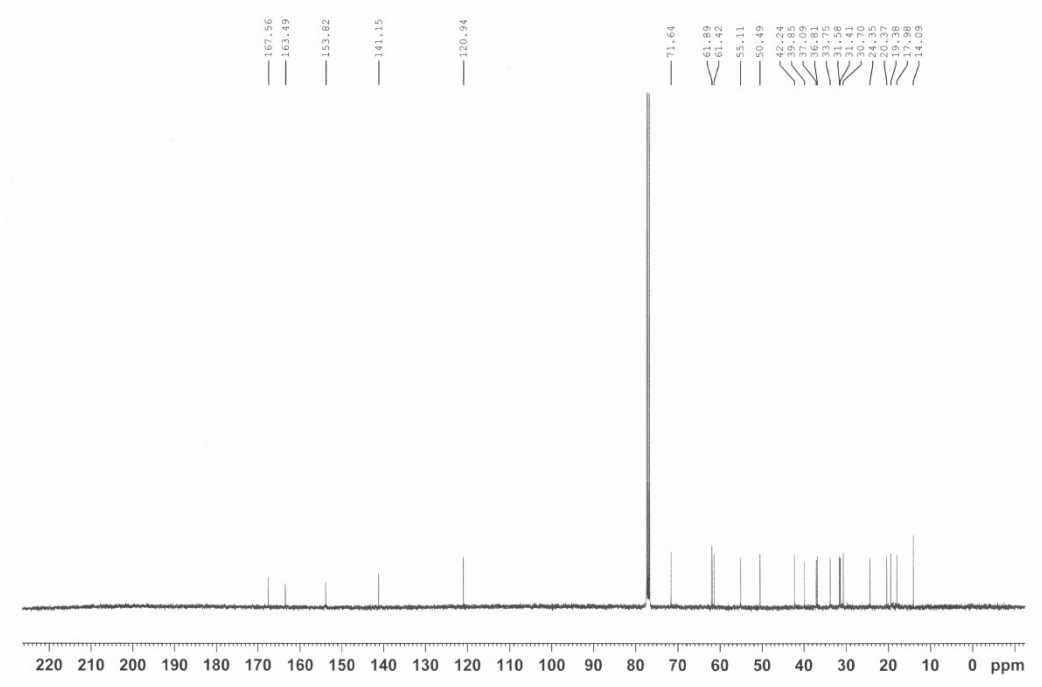


Fig S28. ^{13}C NMR of compound **3g**

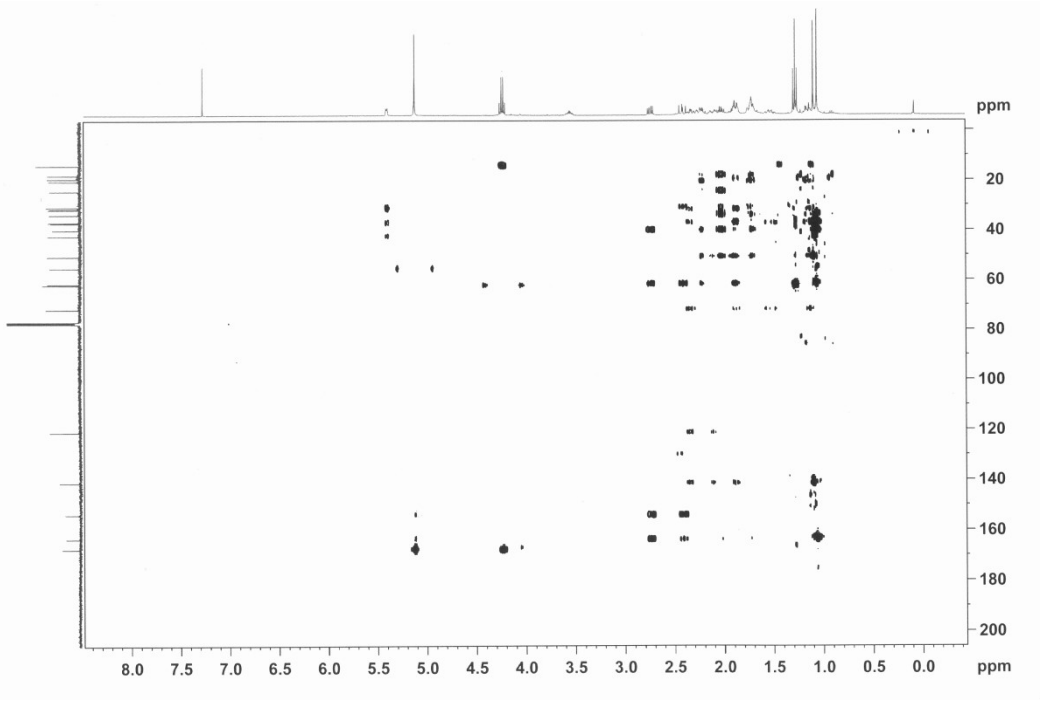


Fig S29. ^{1}H-^{13}C HMBC of compound **3g**

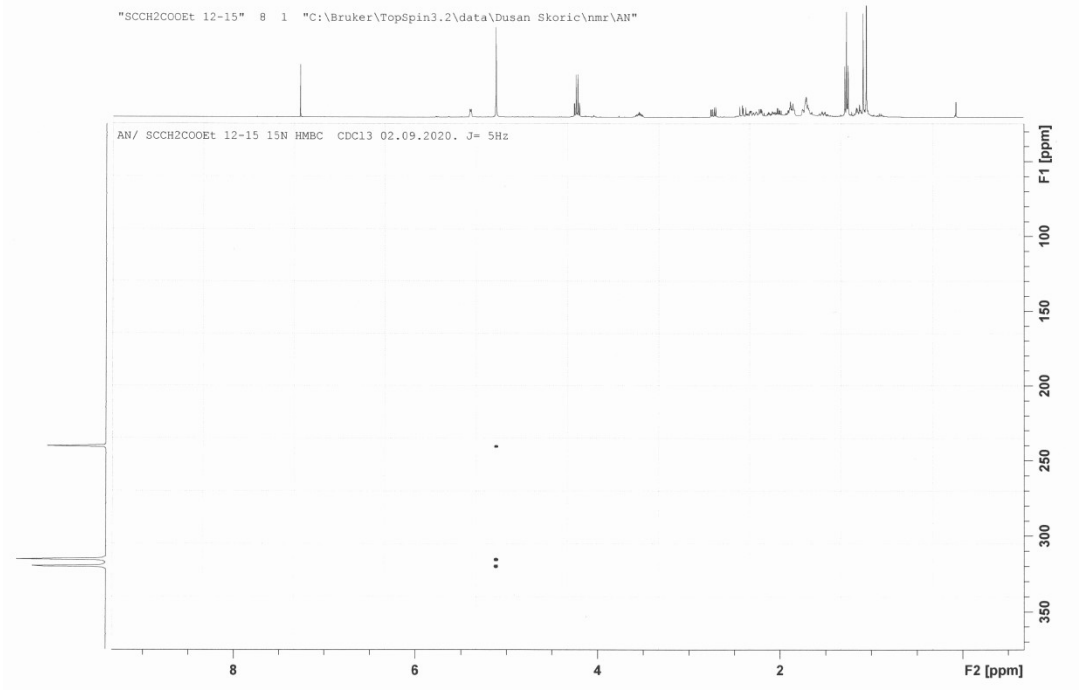


Fig S30. ^{1}H-^{15}N HMBC of compound **3g**

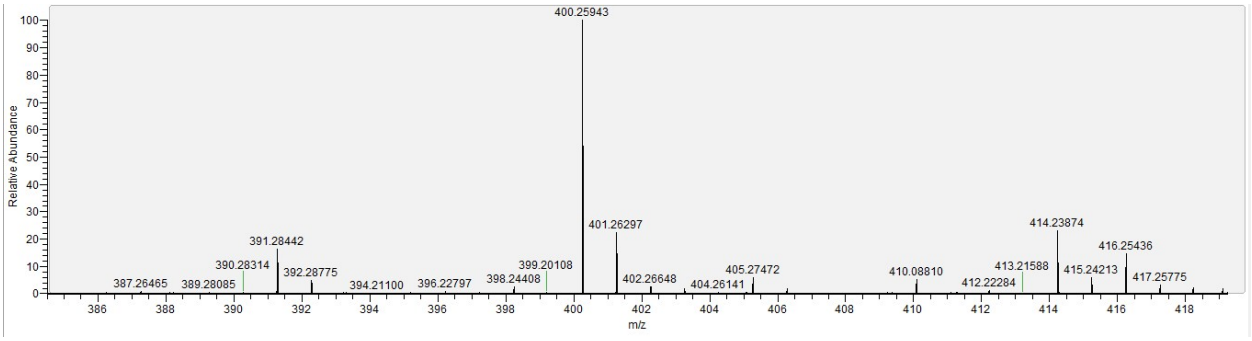
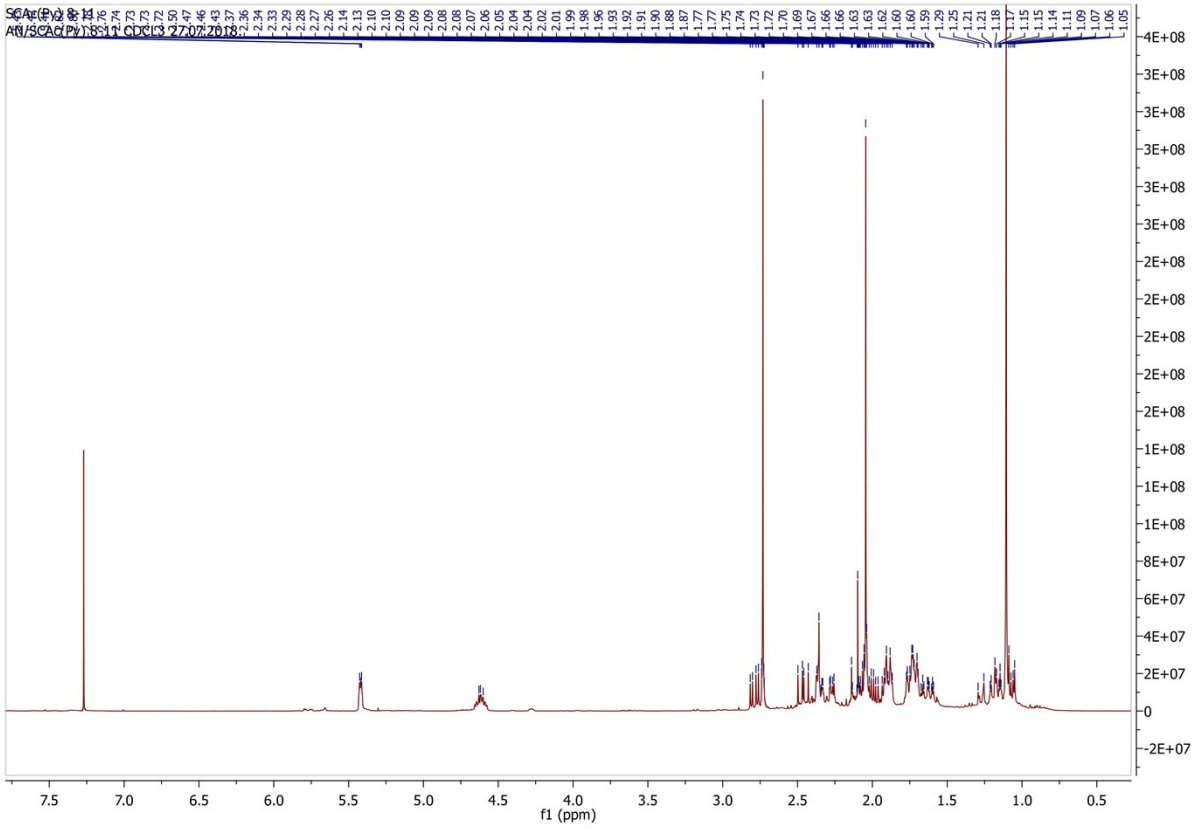


Fig S31. HRMS of compound **3g**



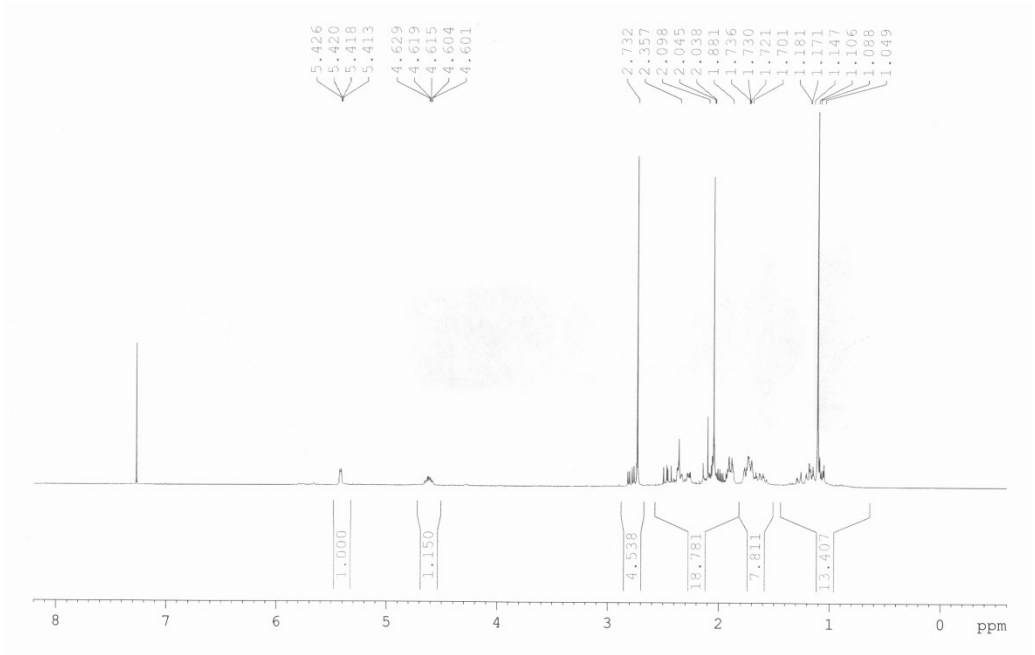
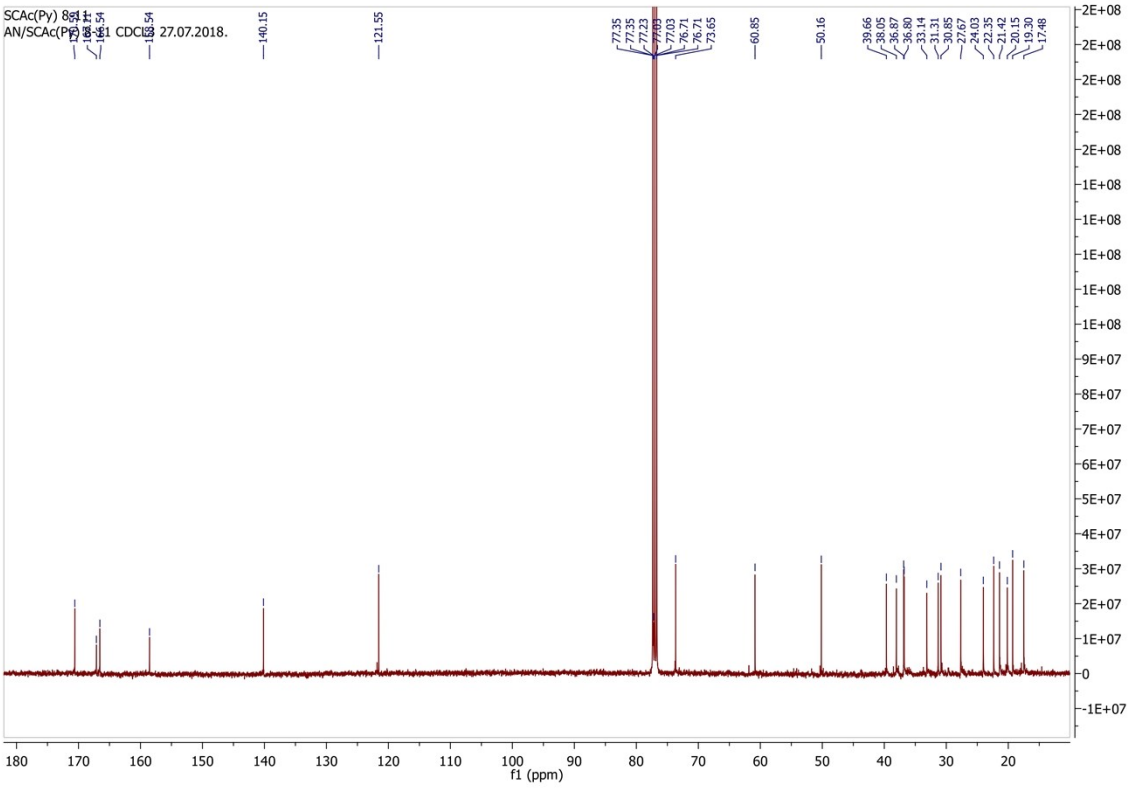


Fig S32. ^{1}H NMR of compound **3h**



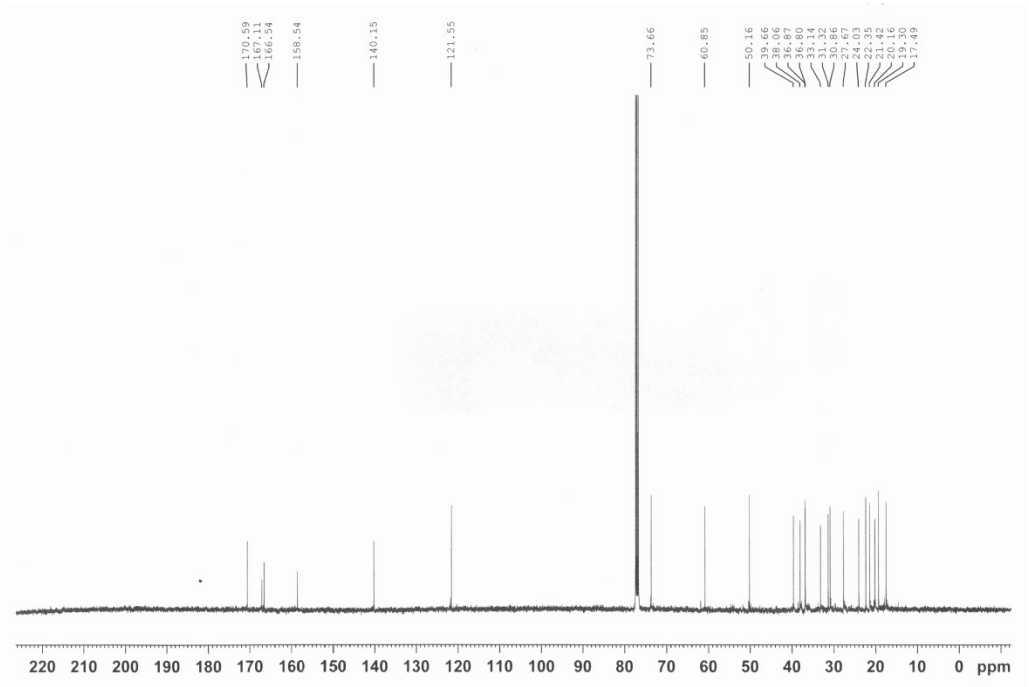


Fig S33. ^{13}C NMR of compound **3h**

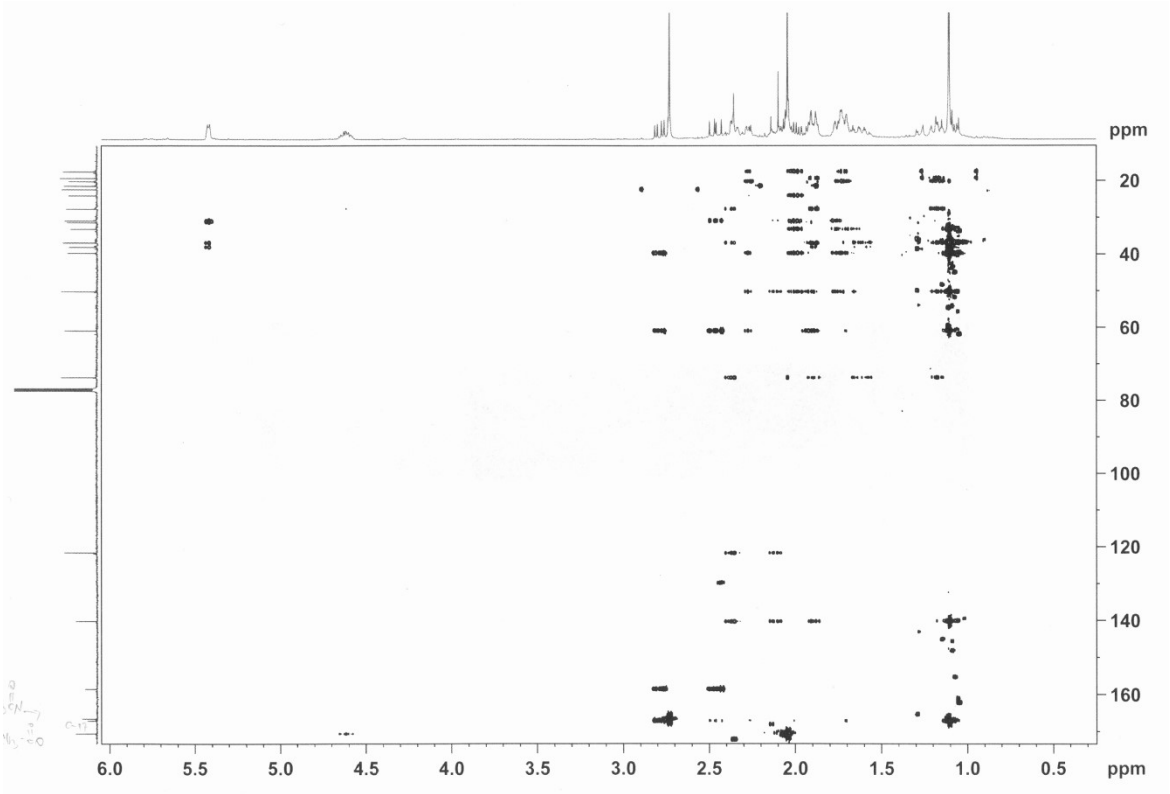


Fig S34. ^{1}H-^{13}C HMBC of compound **3h**

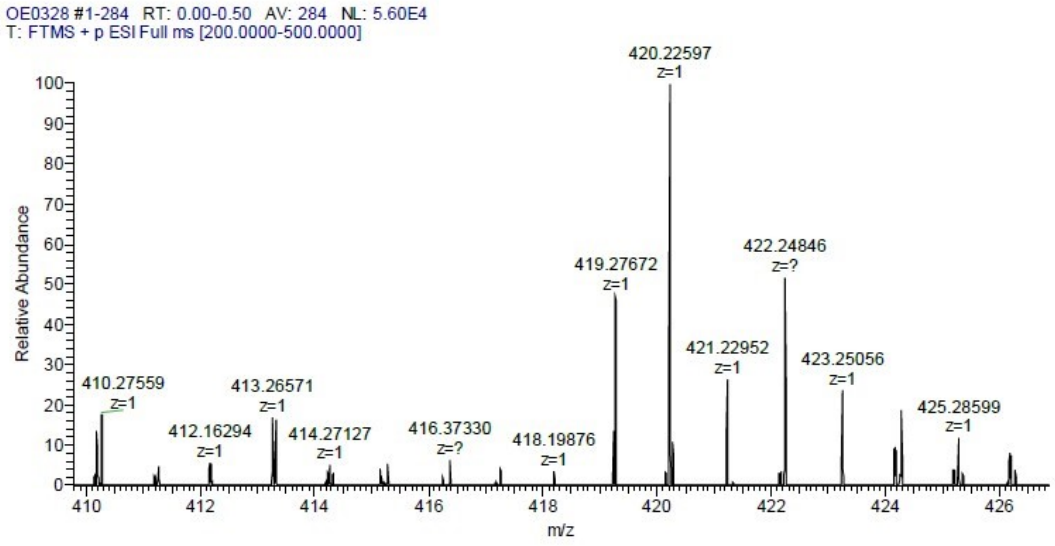
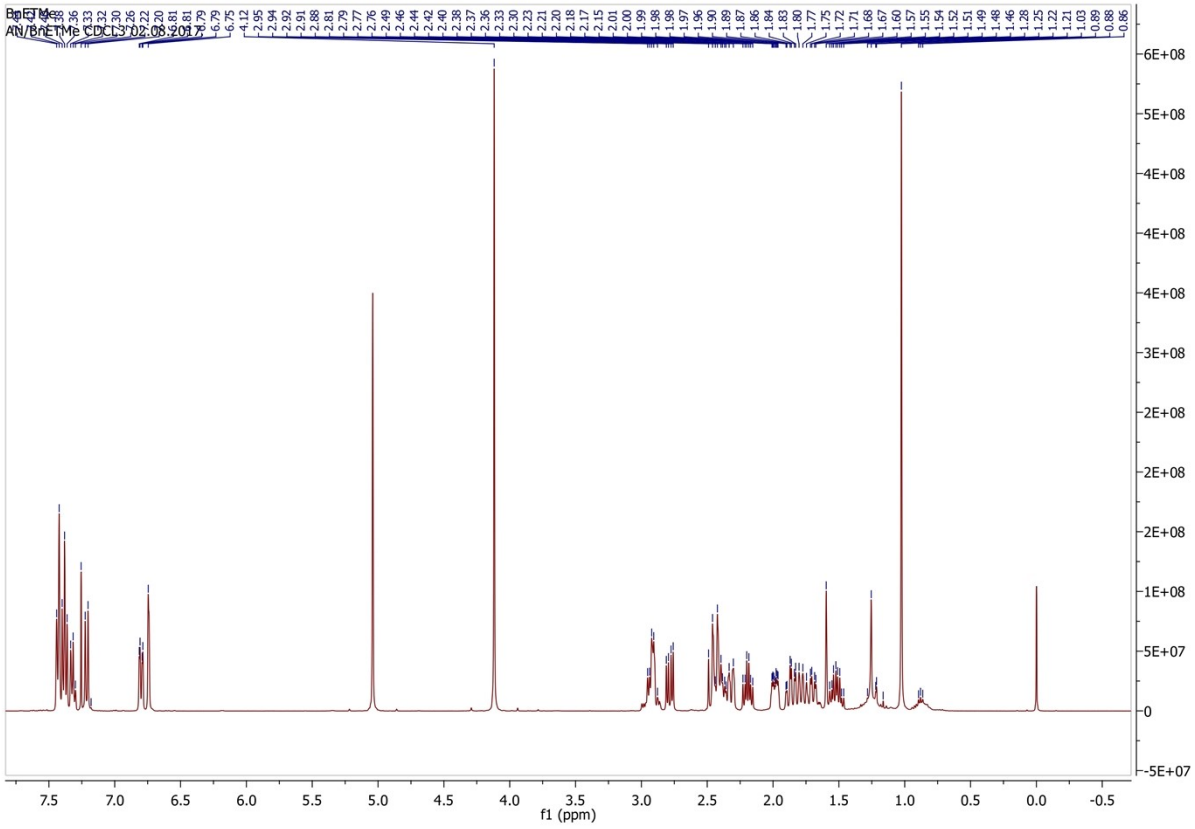


Fig S35. HRMS of compound **3h**



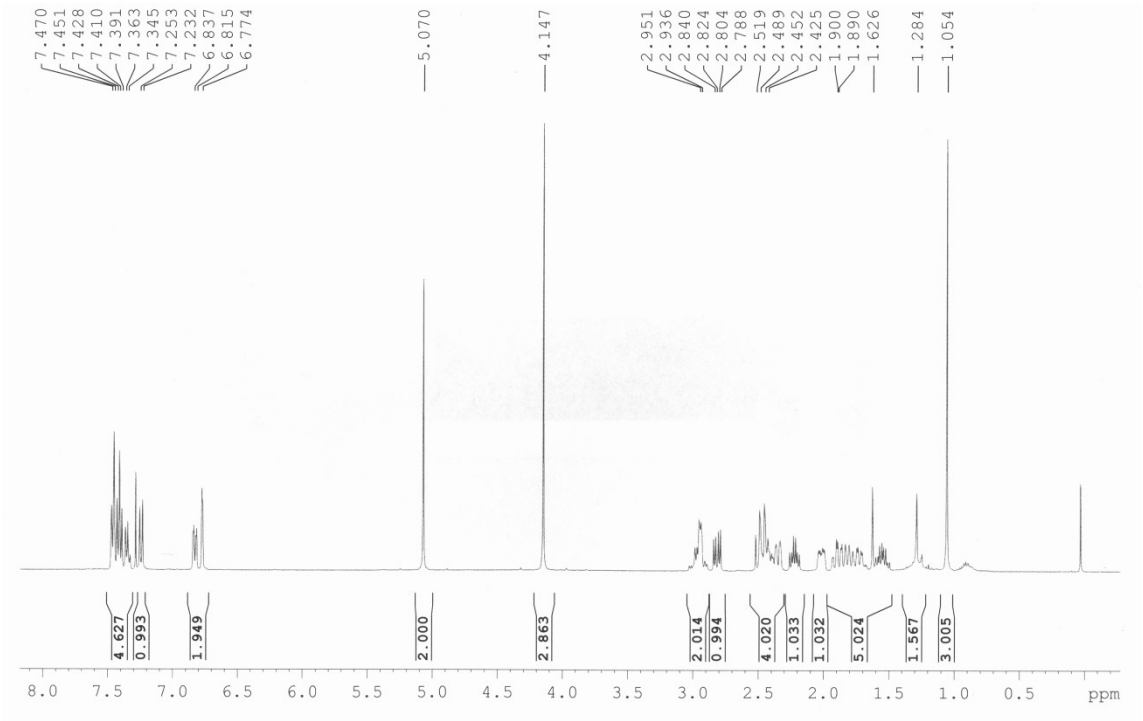
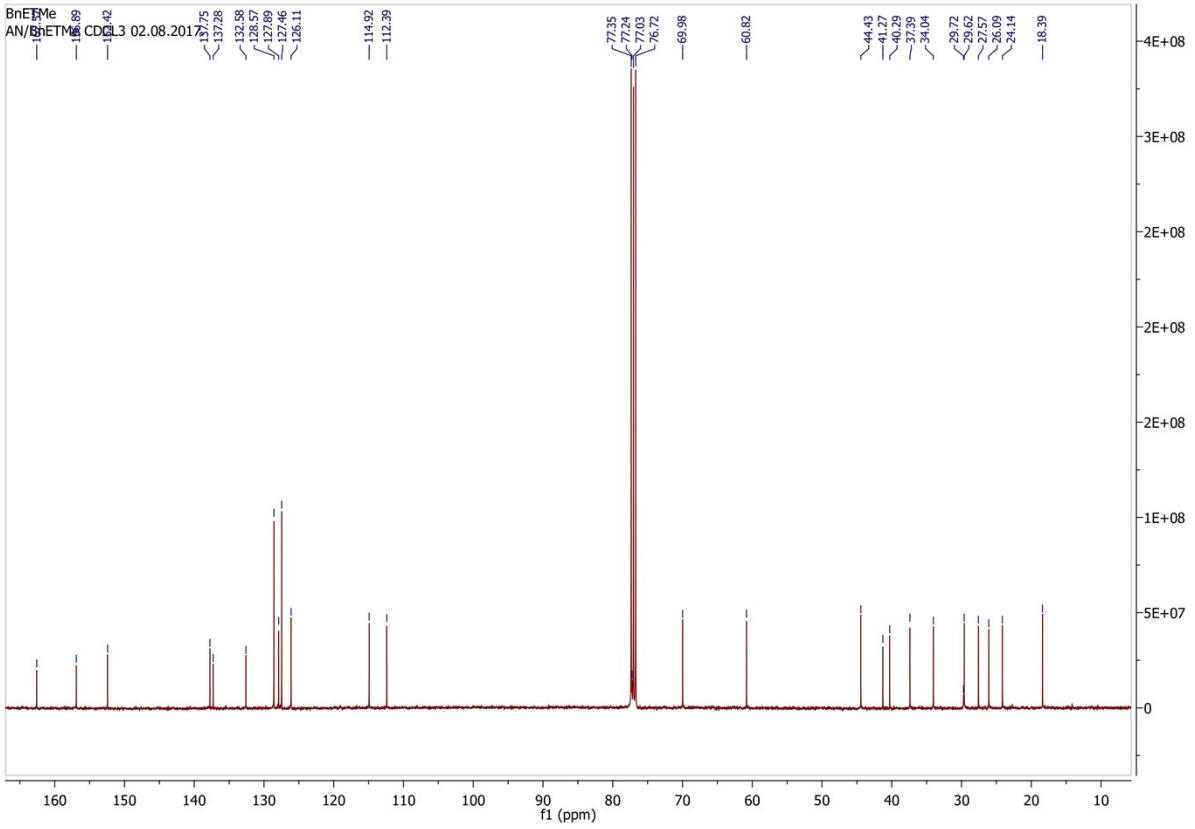


Fig S36. ^{1}H NMR of compound **4a**



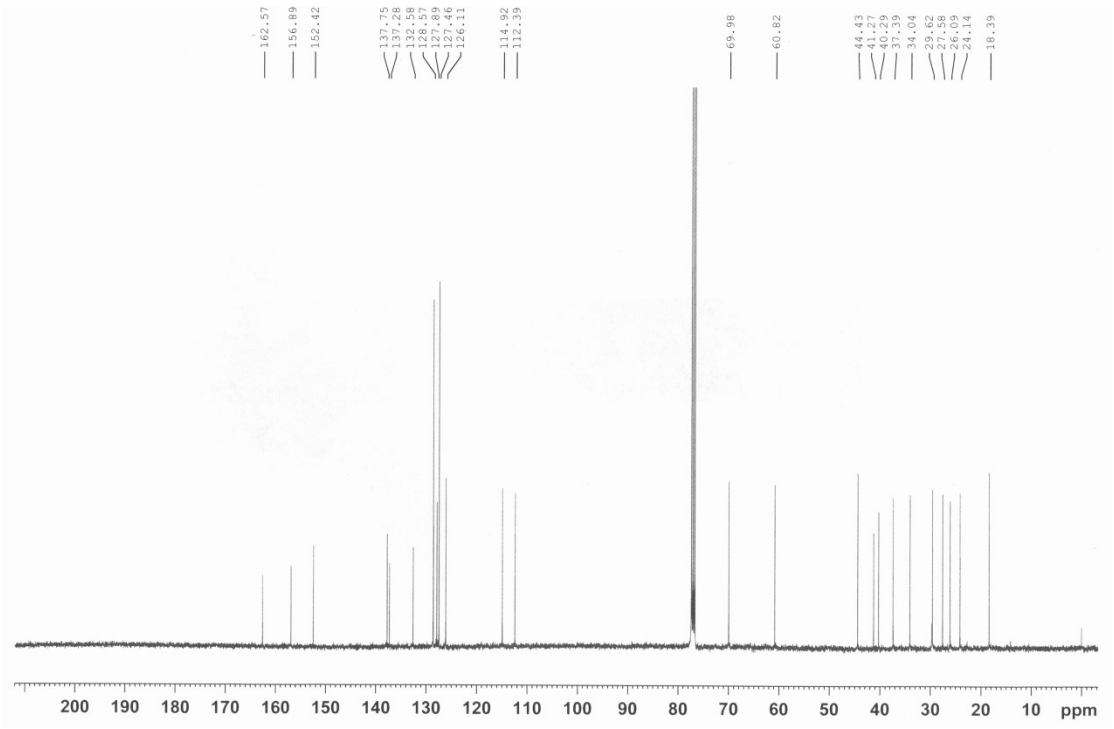


Fig S37. ^{13}C NMR of compound **4a**

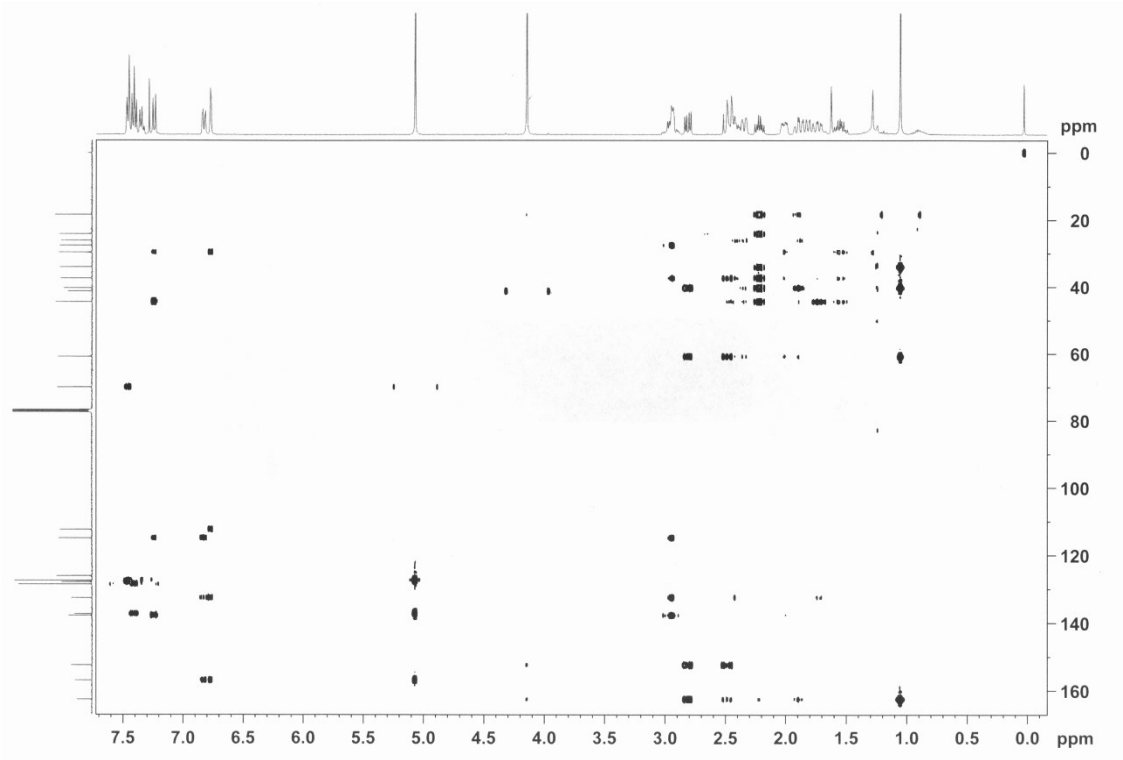


Fig S38. ^{1}H-^{13}C HMBC of compound **4a**

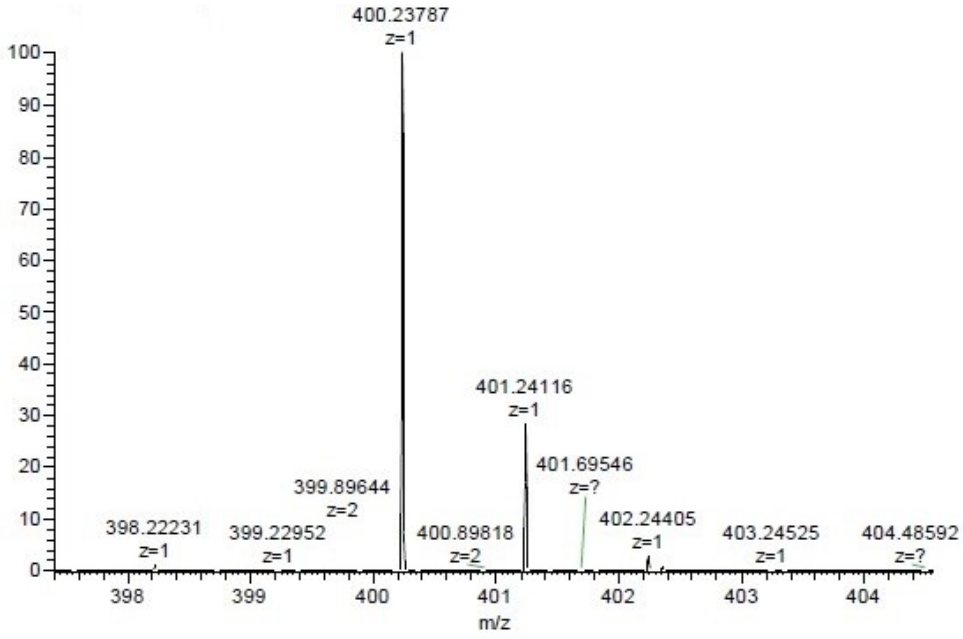
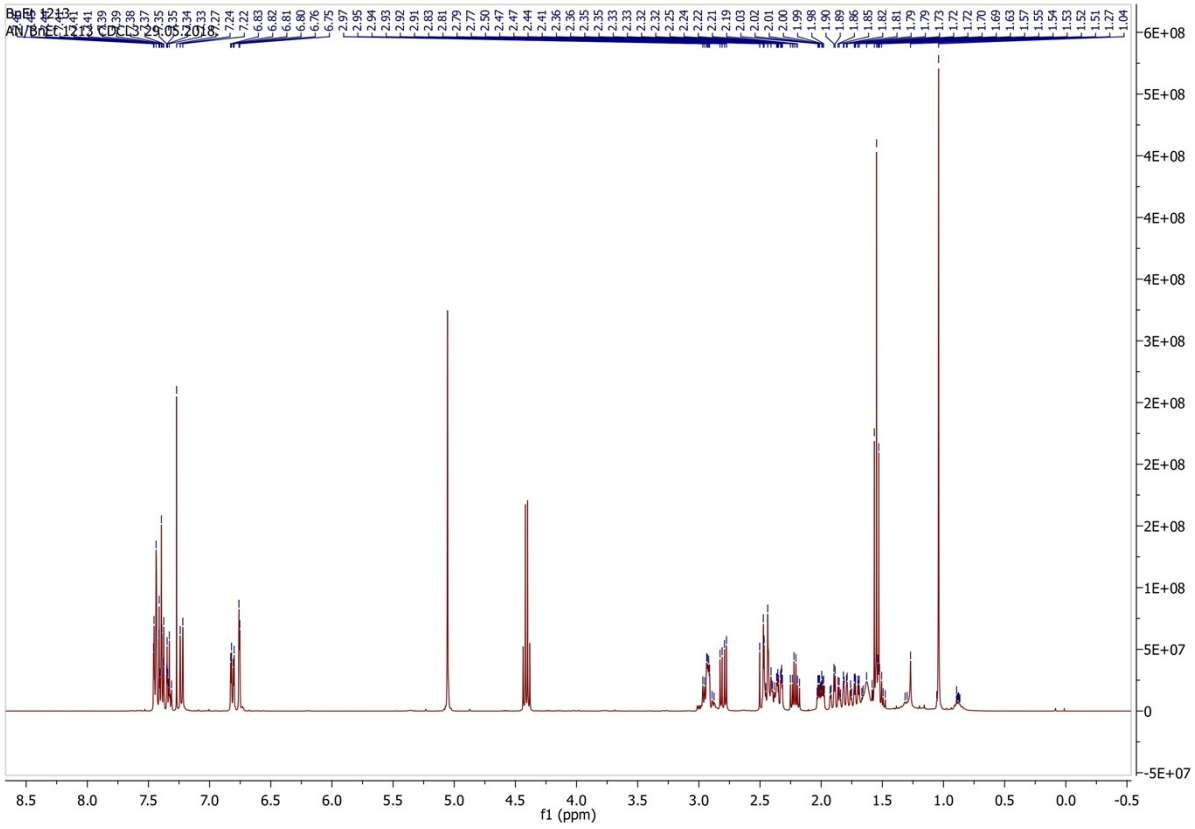


Fig S39. HRMS of compound **4a**



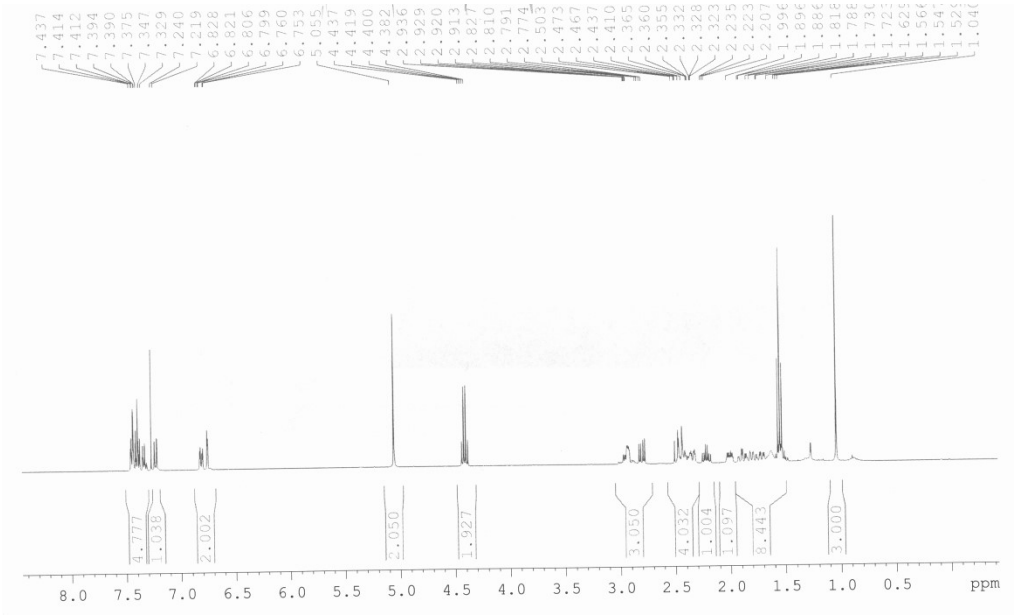
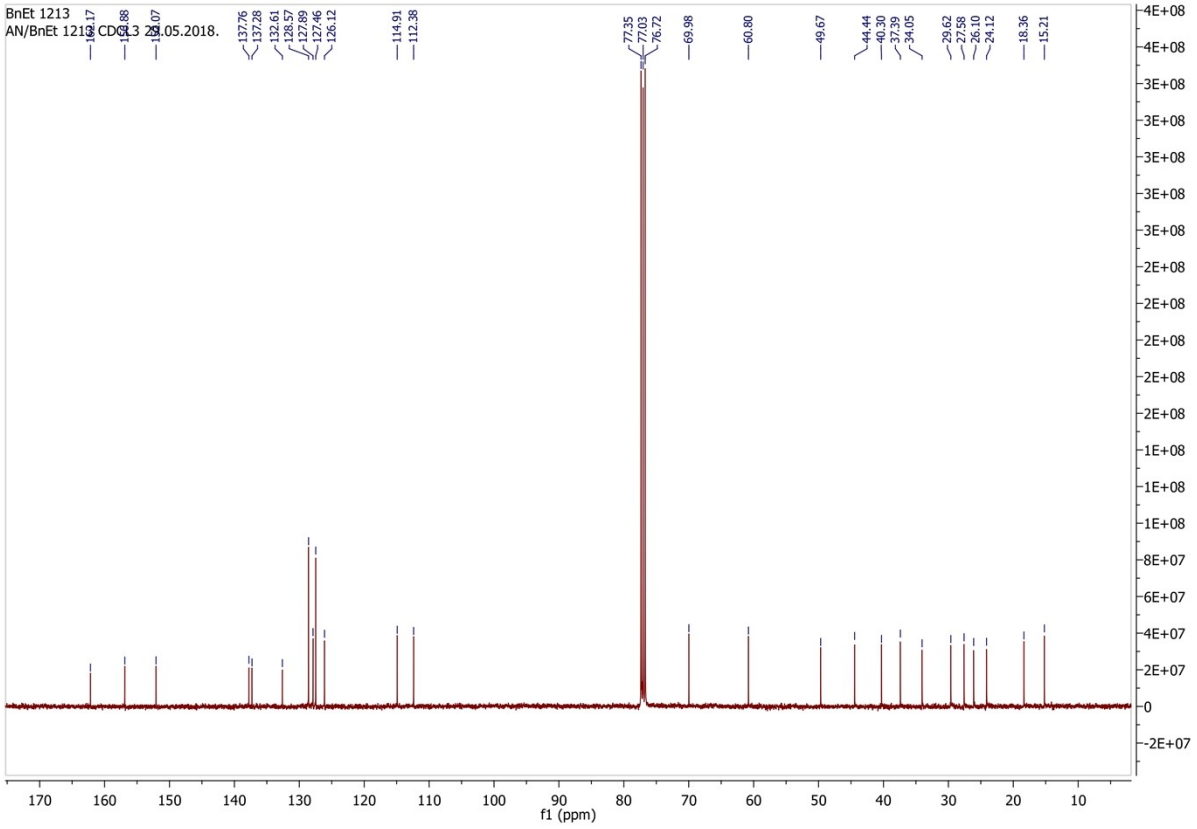


Fig S40. ^{1}H NMR of compound **4b**



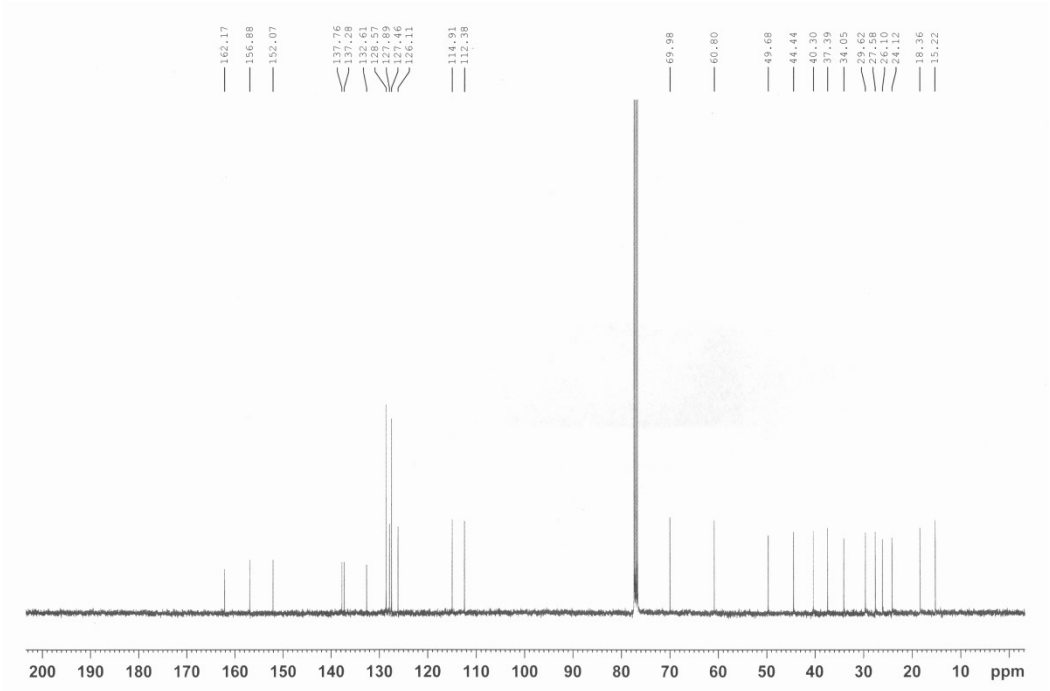


Fig S41. ^{13}C NMR of compound **4b**

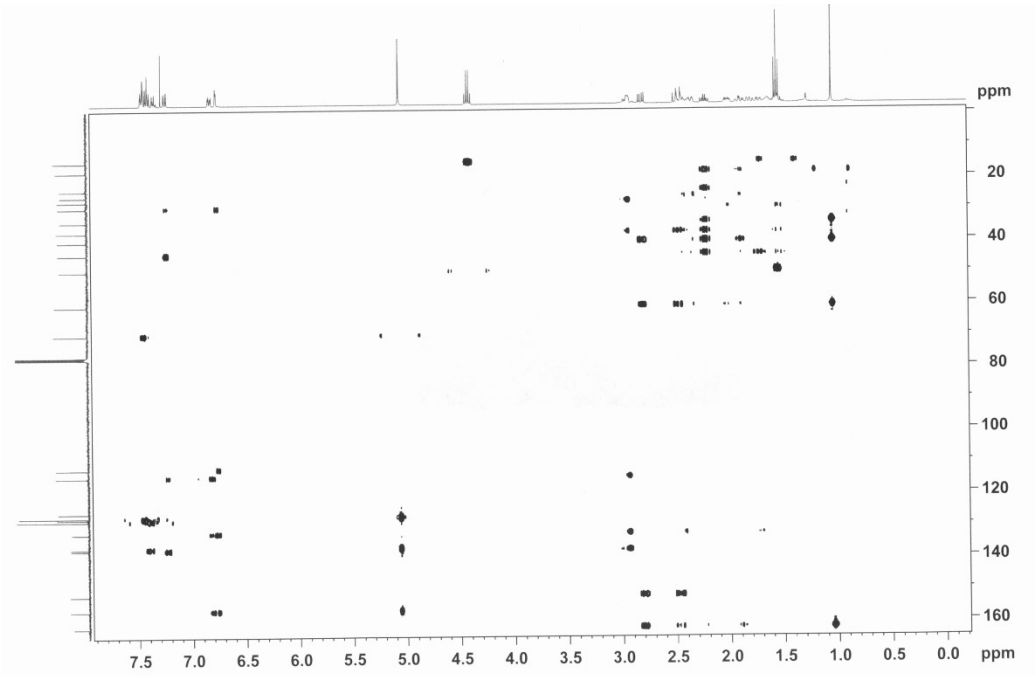


Fig S42. ^{1}H-^{13}C HMBC of compound **4b**

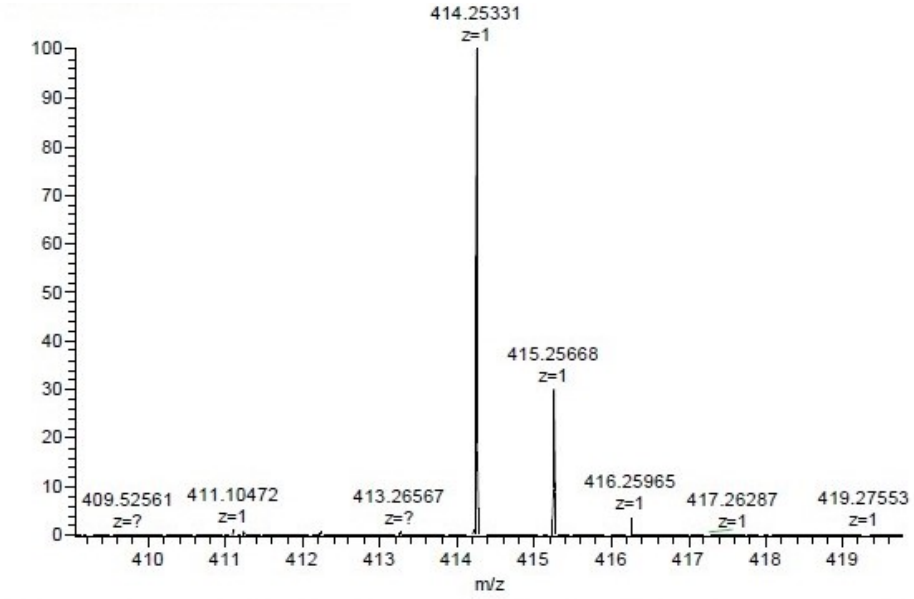
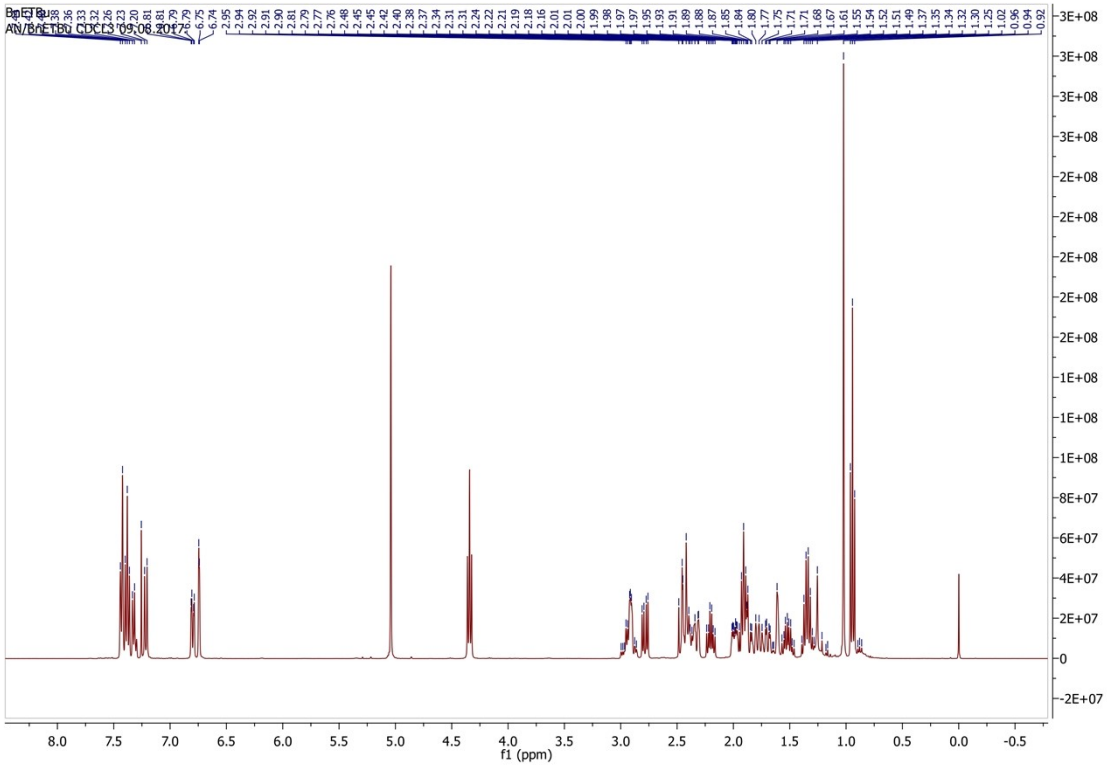


Fig S43. HRMS of compound **4b**



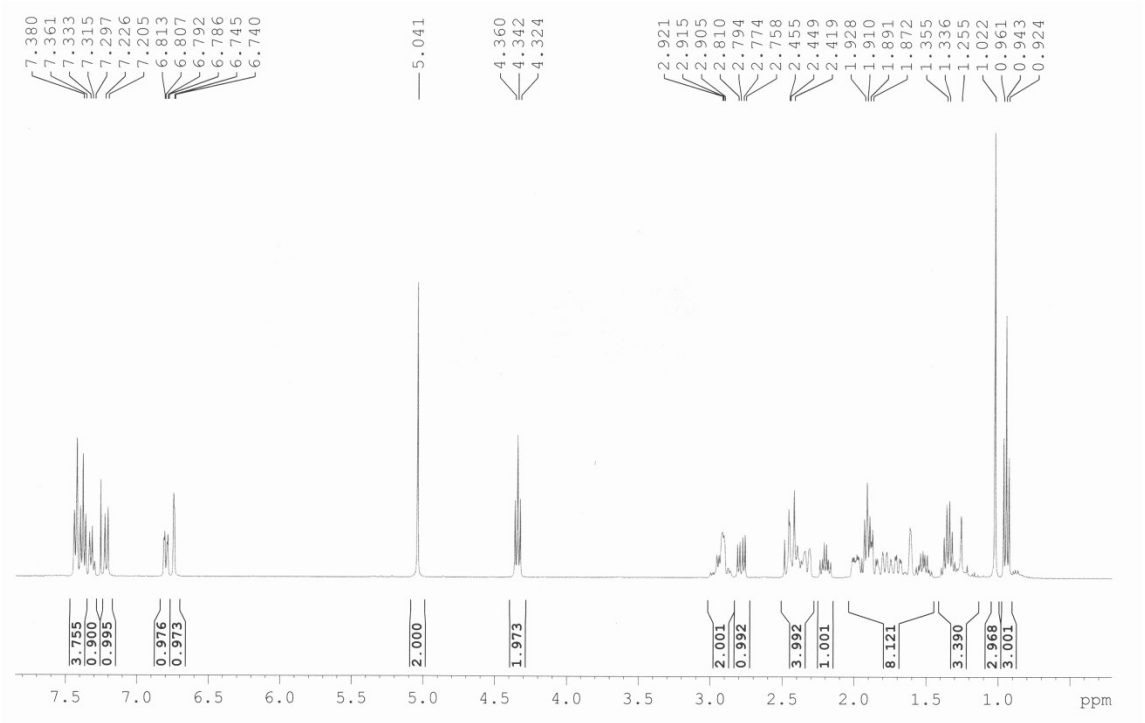
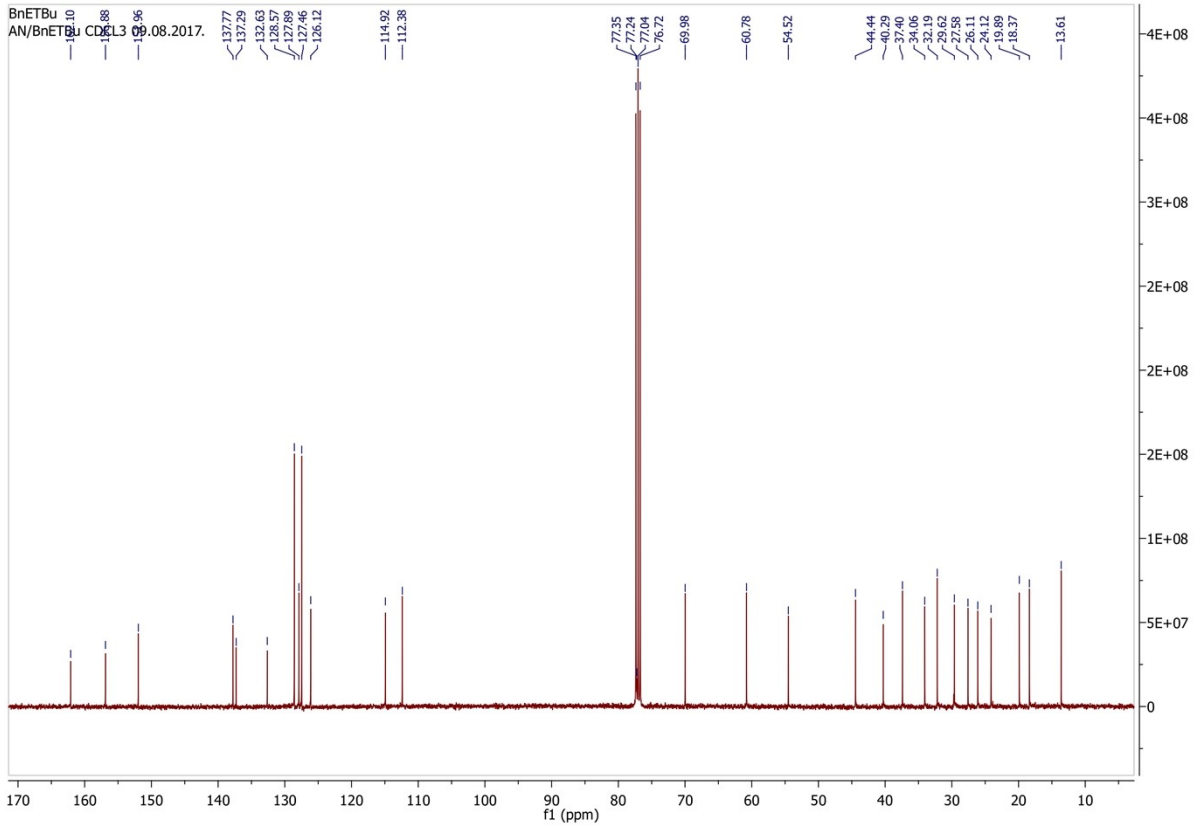


Fig S44. ^{1}H NMR of compound **4c**



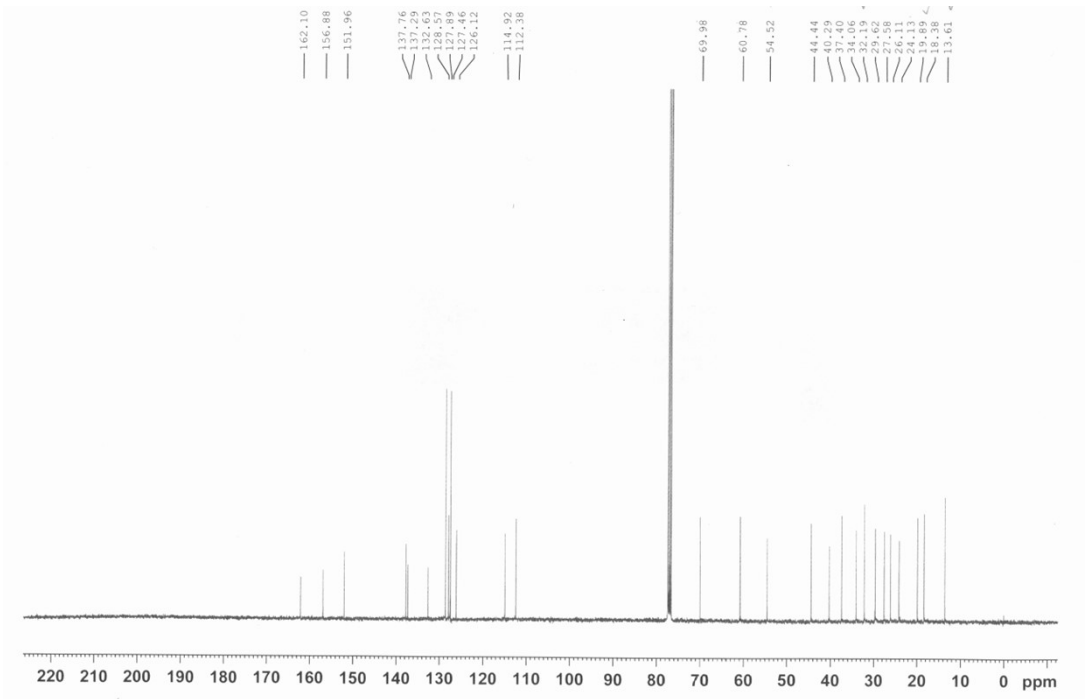


Fig S45. ^{13}C NMR of compound **4c**

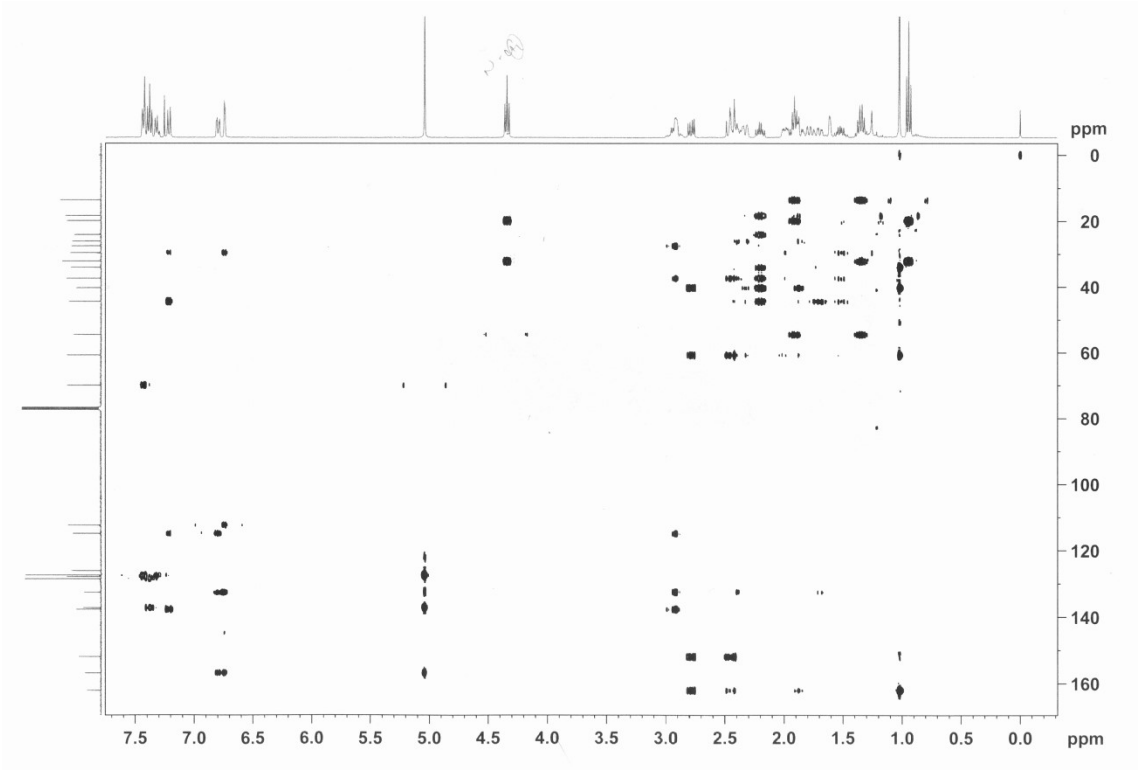


Fig S46. ^{1}H-^{13}C HMBC of compound **4c**

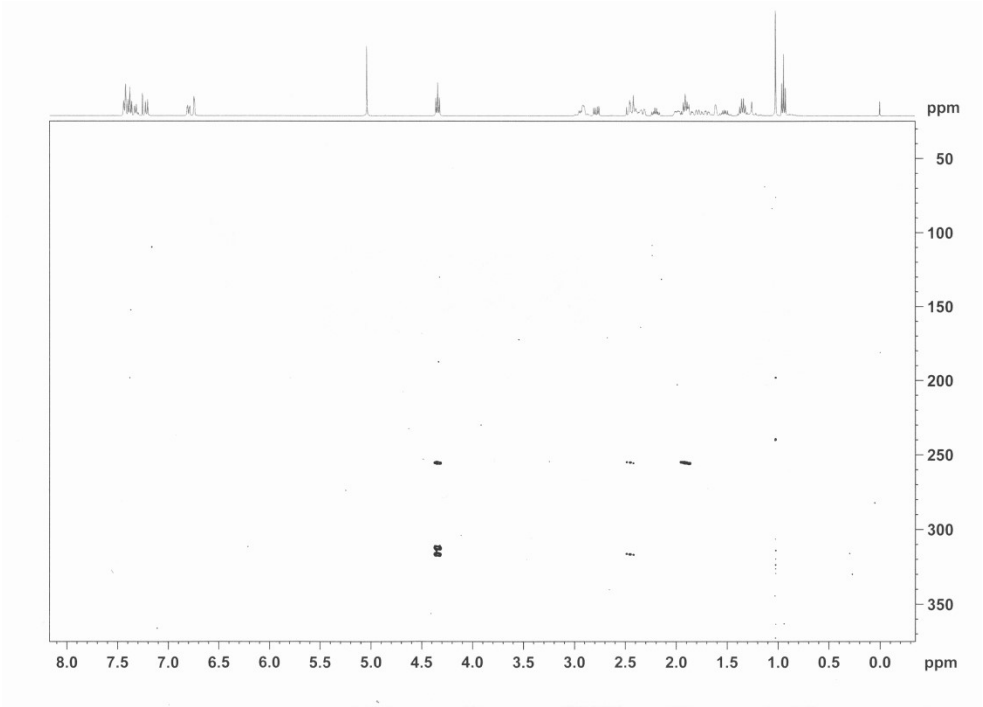
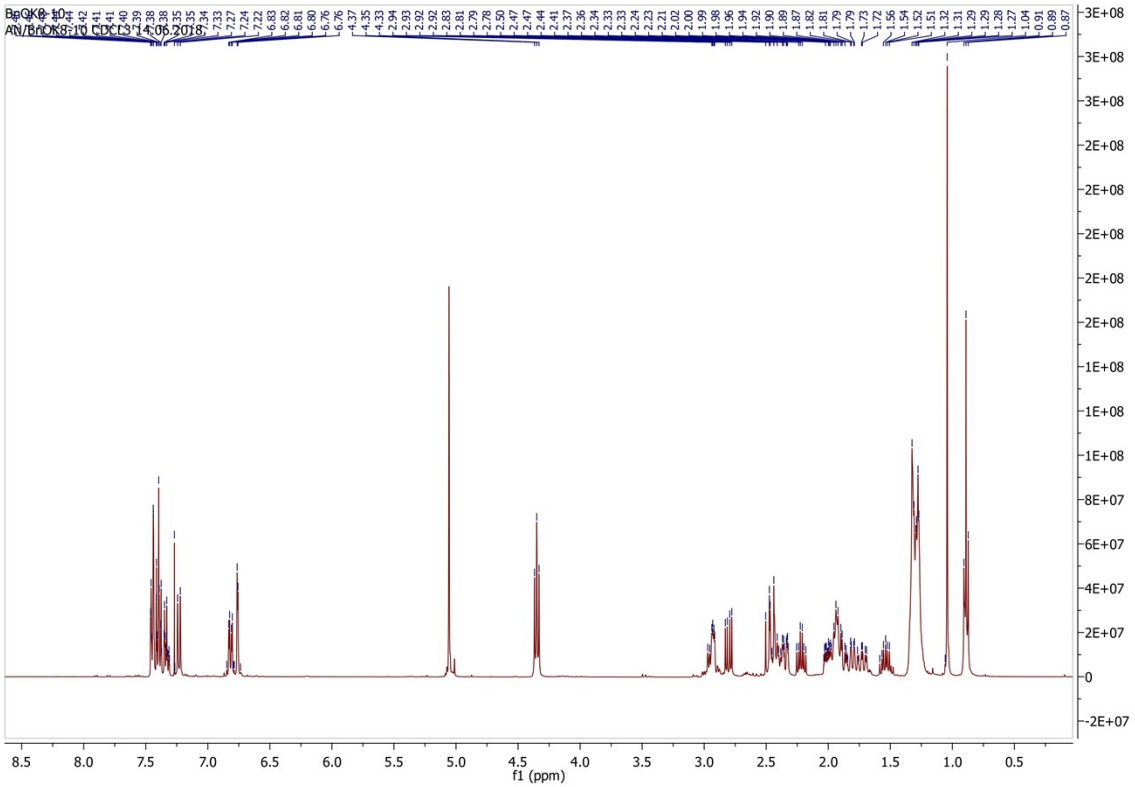


Fig S47. ^{1}H-^{15}N HMBC of compound **4c**



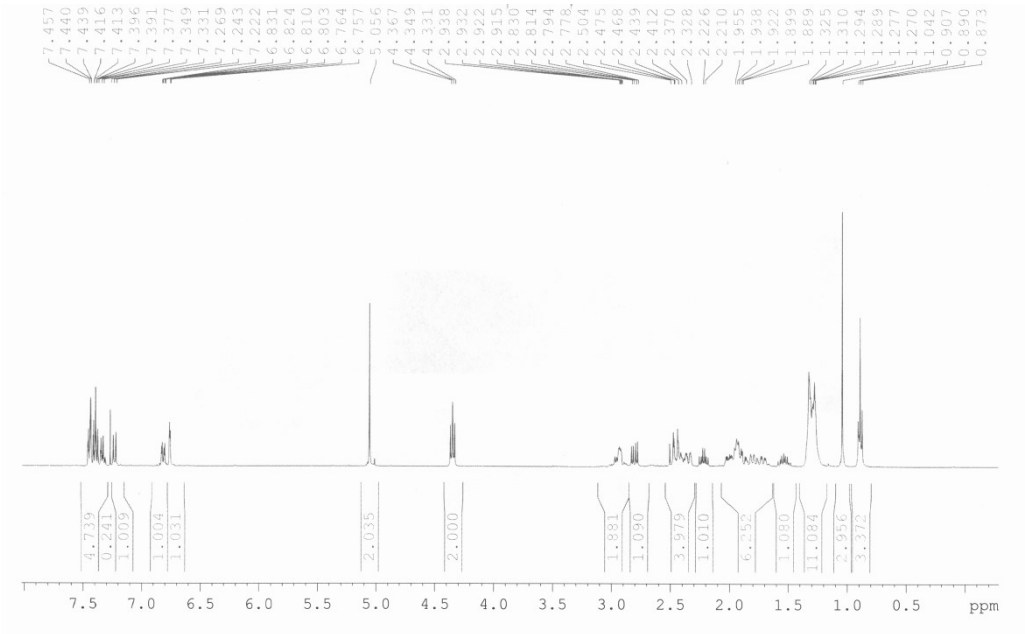
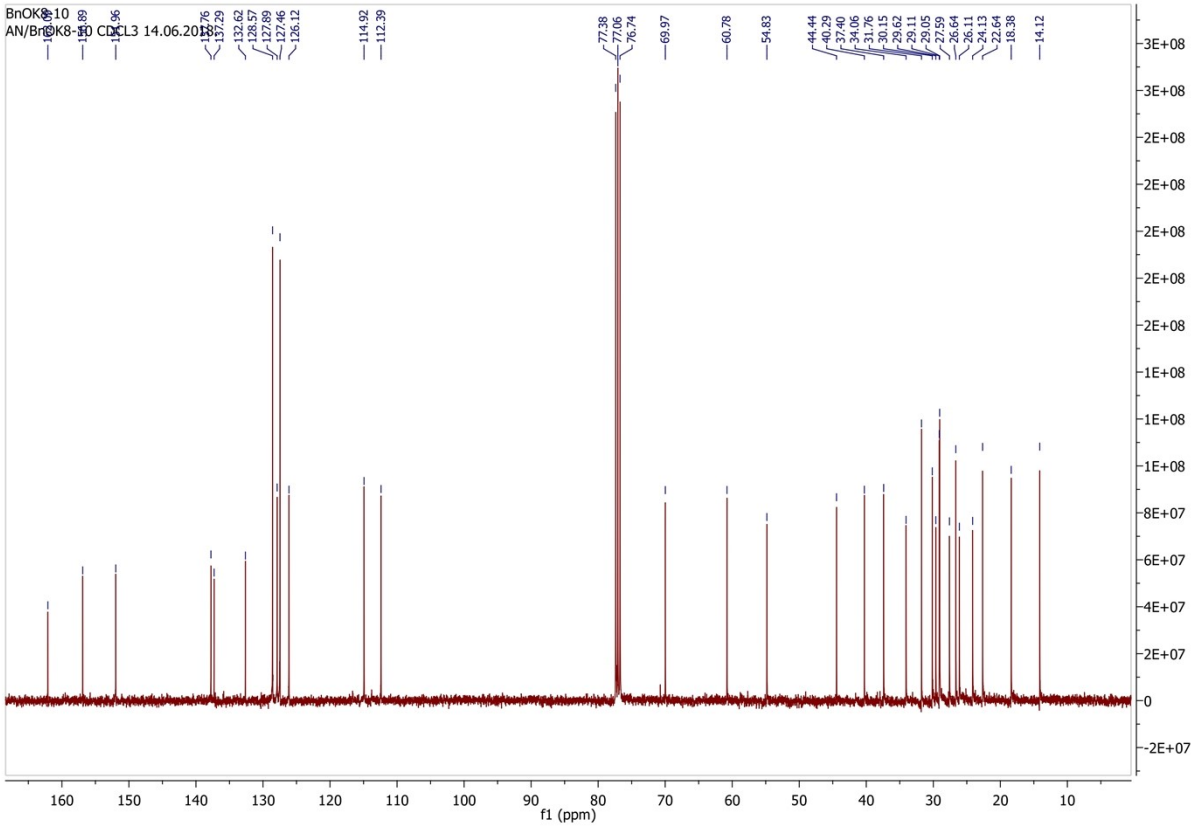


Fig S48. ^{1}H NMR of compound **4d**



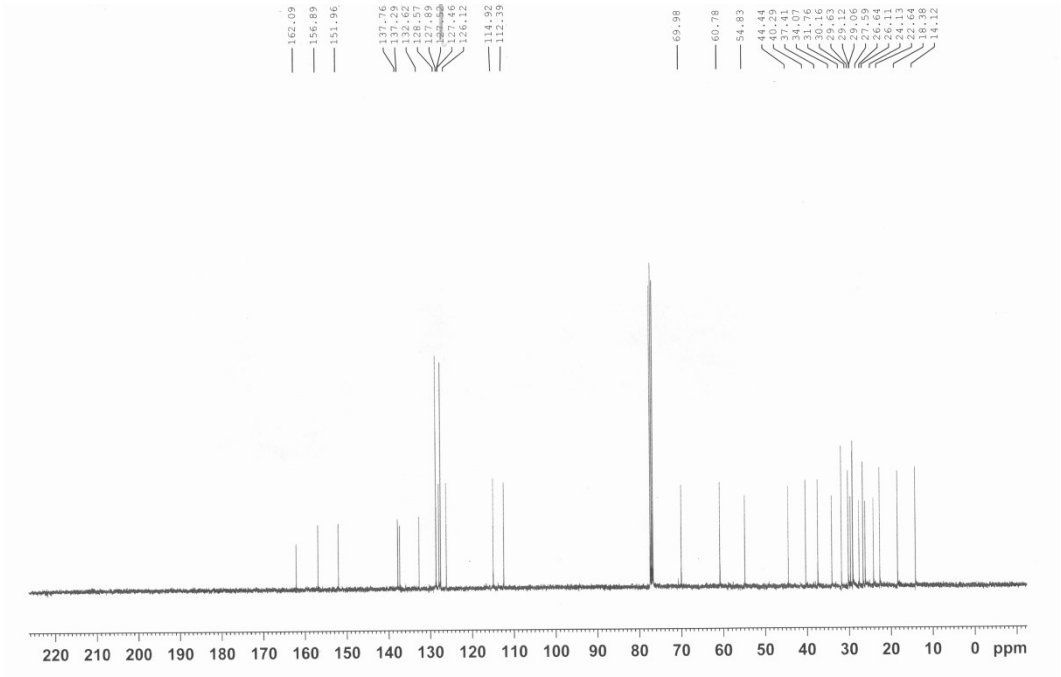


Fig S49. ^{13}C NMR of compound **4d**

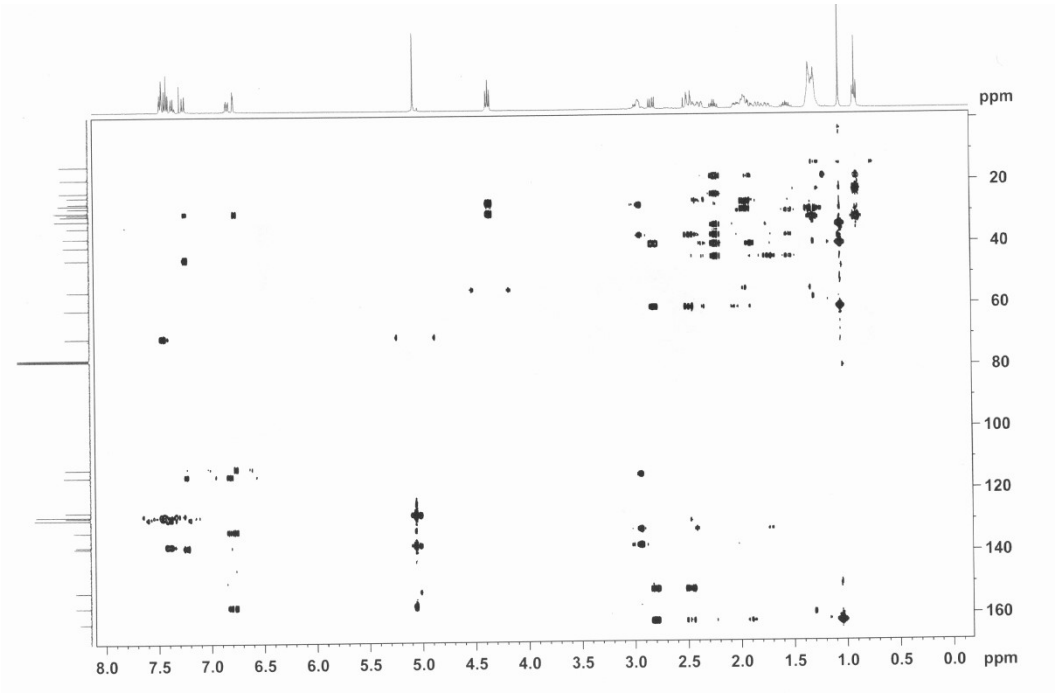


Fig S50. ^{1}H-^{13}C HMBC of compound **4d**

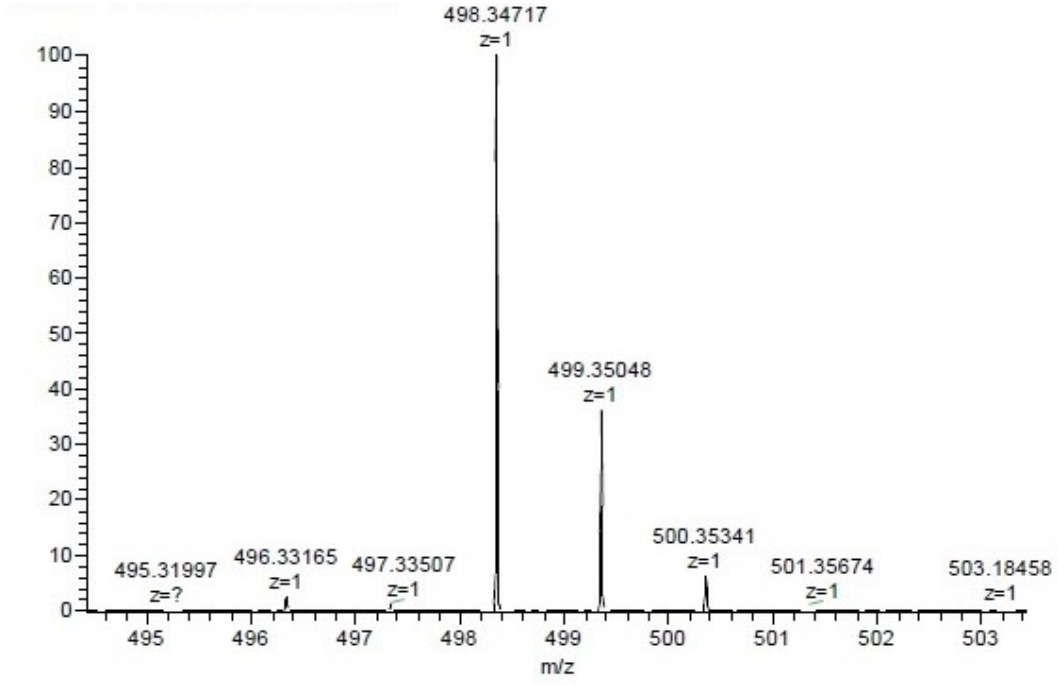


Fig S51. HRMS of compound **4d**

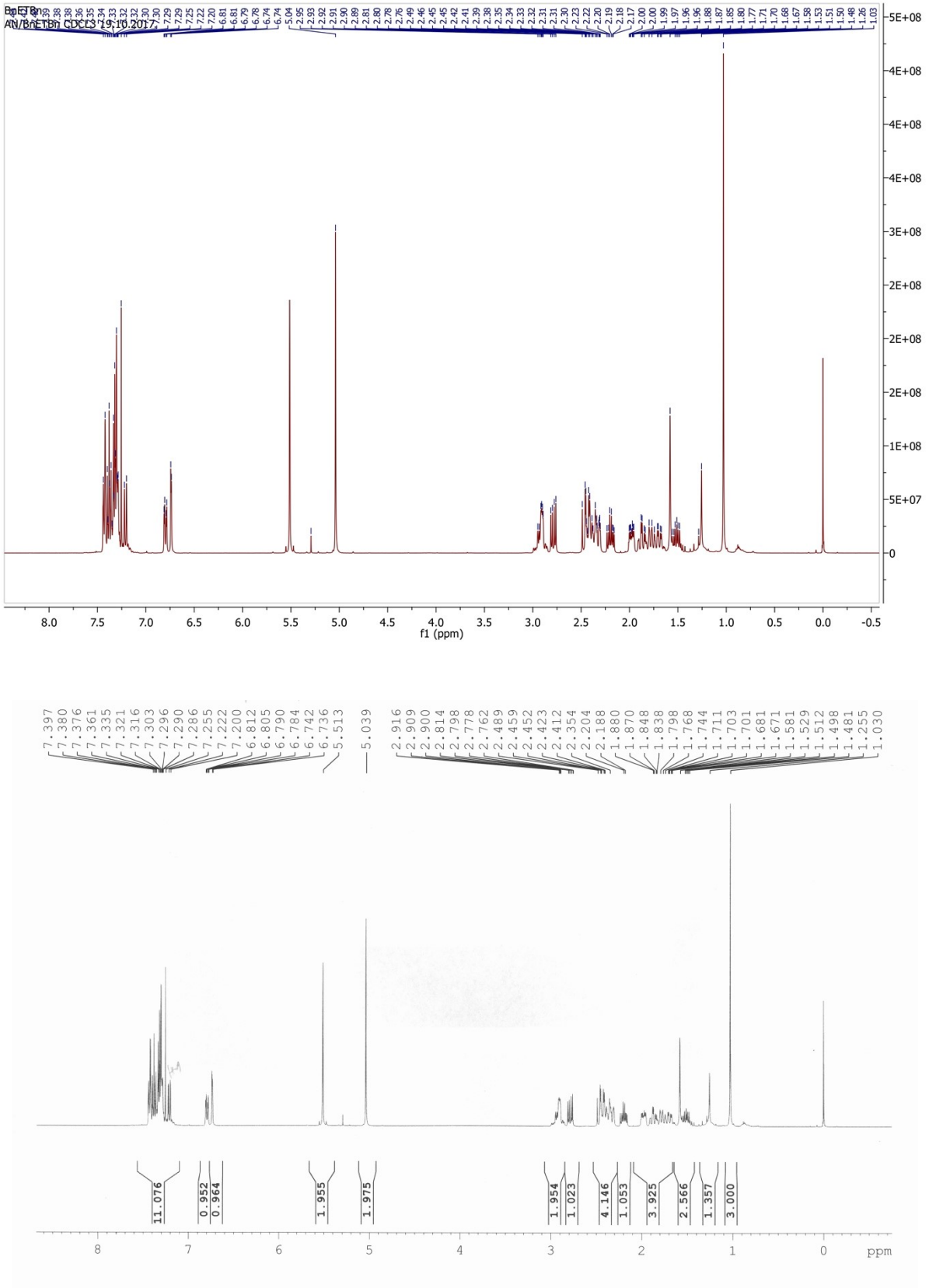
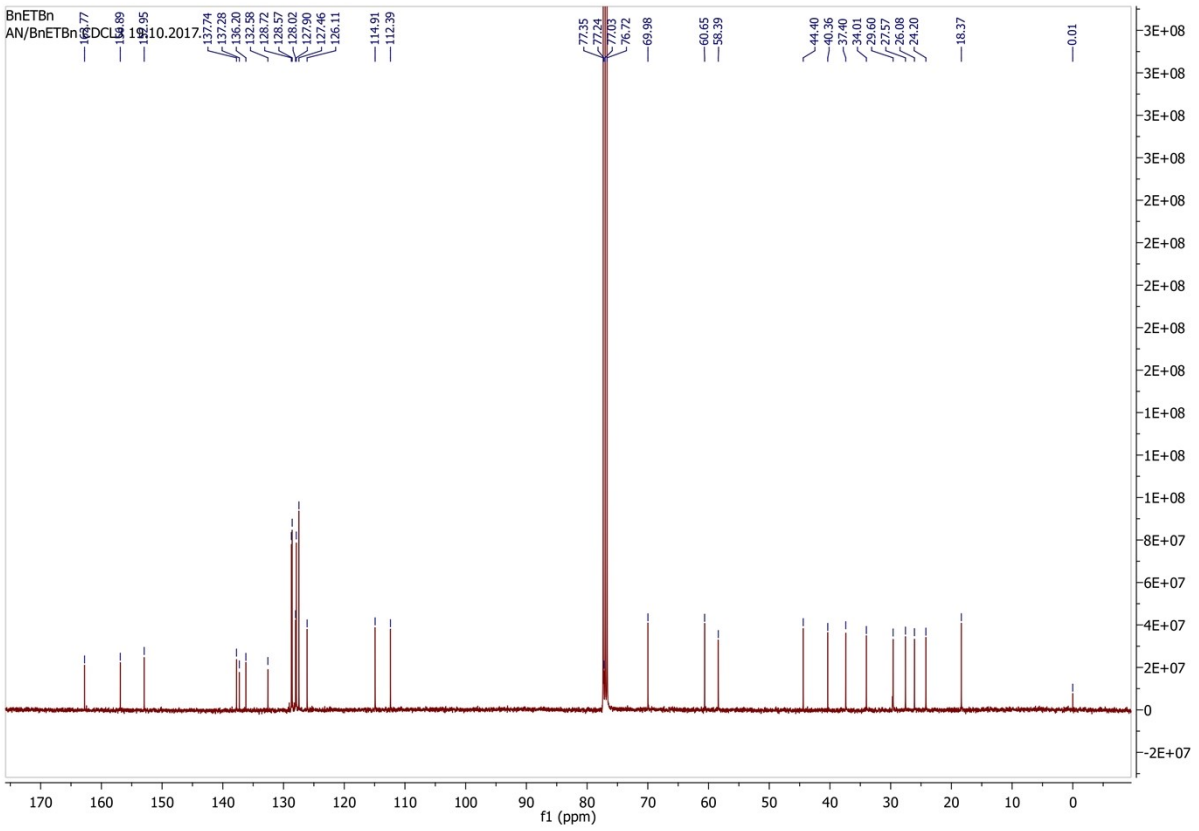


Fig S52. ^{1}H NMR of compound **4e**



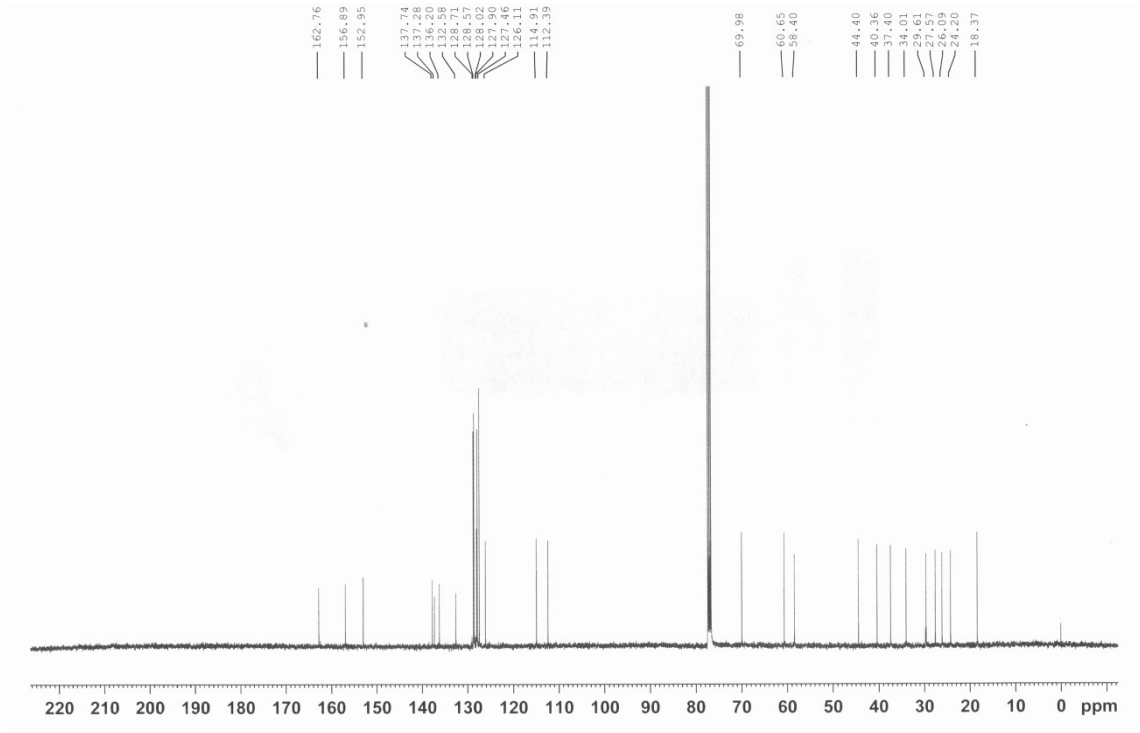


Fig S53. ^{13}C NMR of compound **4e**

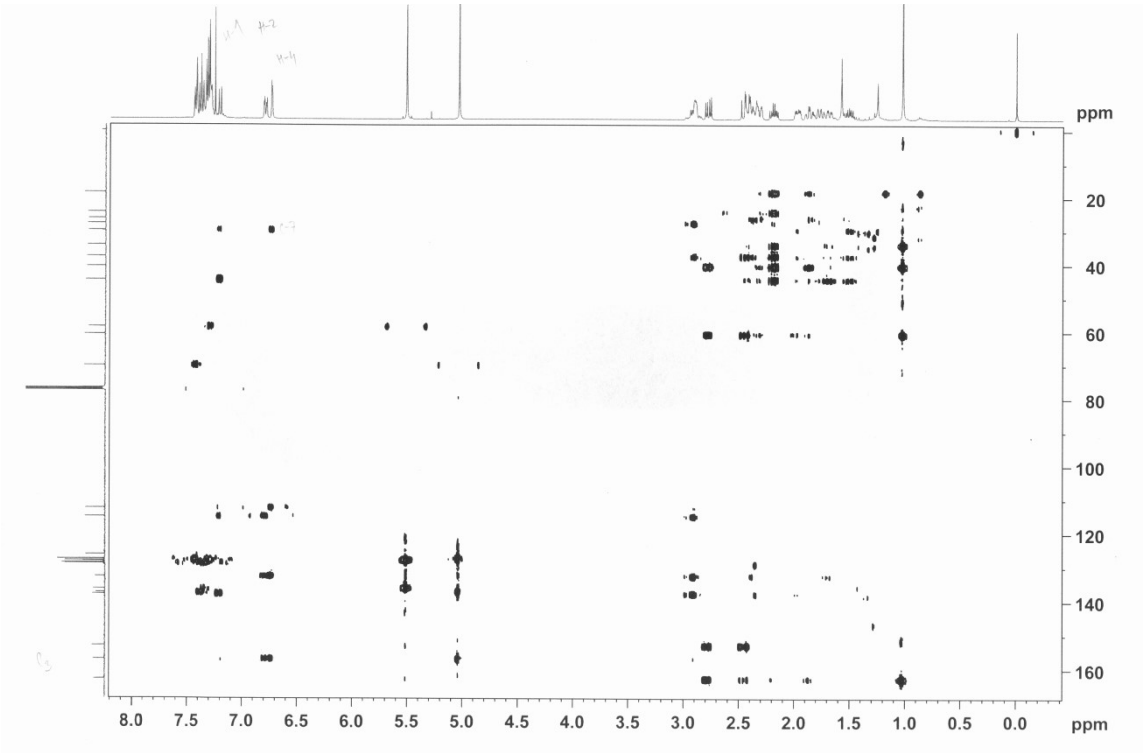


Fig S54. ^{1}H-^{13}C HMBC of compound **4e**

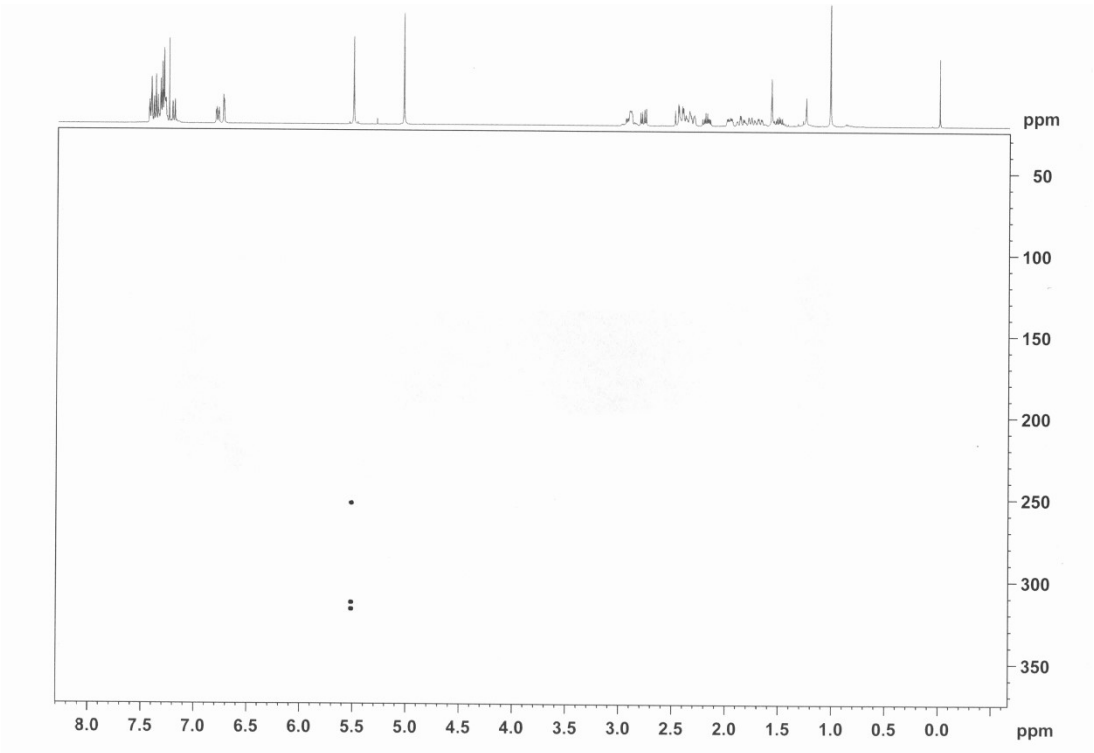


Fig S55. ^{1}H-^{15}N HMBC of compound **4e**

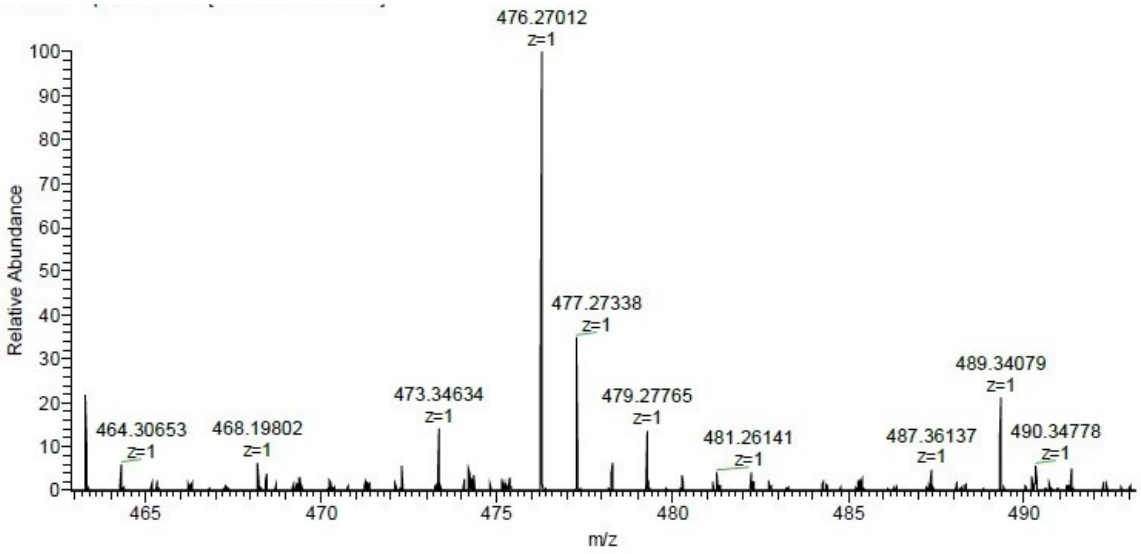


Fig S56. HRMS of compound **4e**

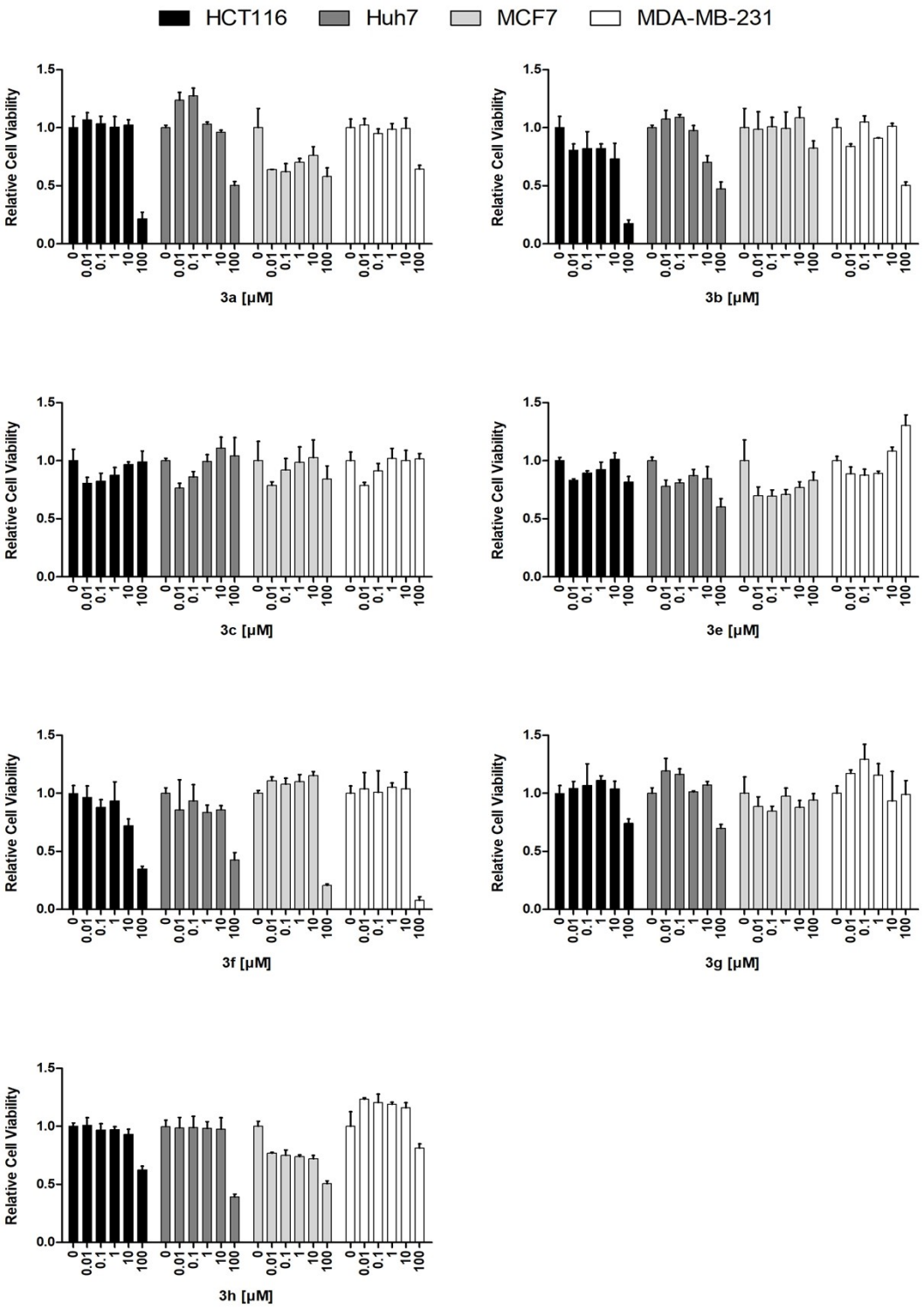


Fig S57. Cell viability assays with compounds **3a-c, 3e-h** in indicated human cancer cell lines

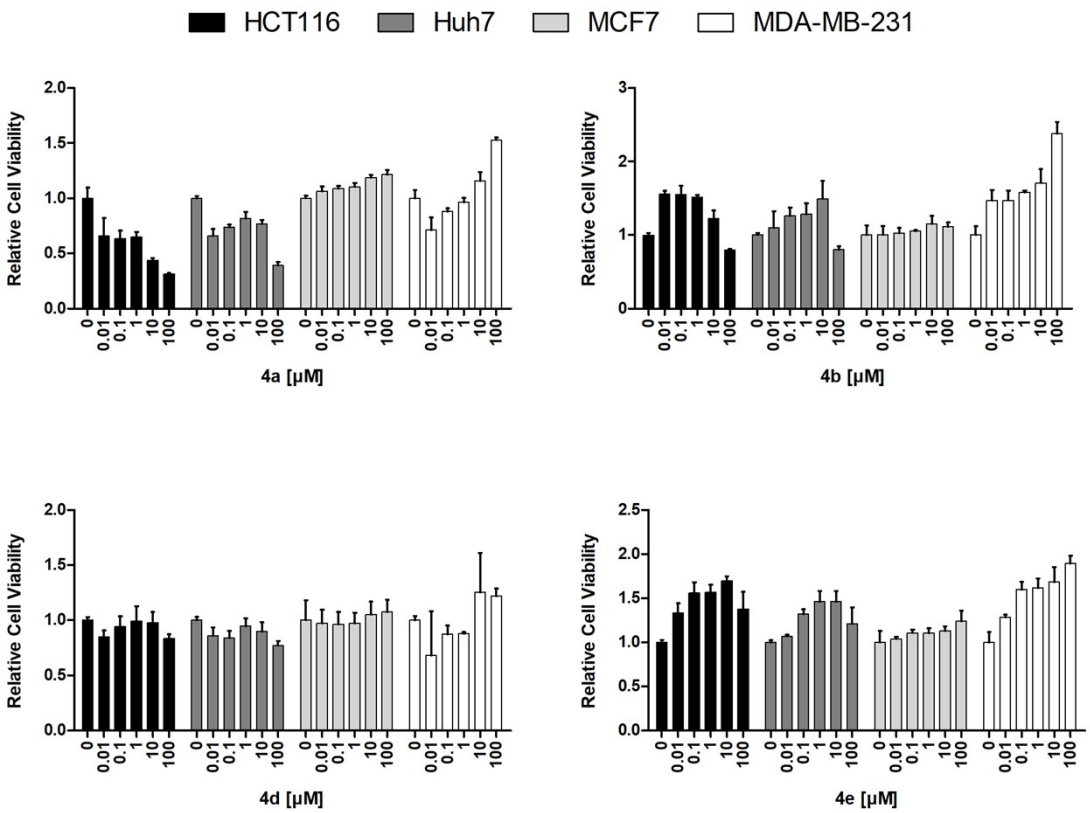


Fig S58. Cell viability assays with compounds **4a-b, 4d-e** in indicated human cancer cell lines.

Table S1. IC_{50} values [µM] of compound **3f** in indicated cancer cells lines.

Cell line	IC_{50} of compound 3f [µM]
HCT116	22.3
Huh7	93.6
MCF7	33.9
MDA-MB-231	28.7

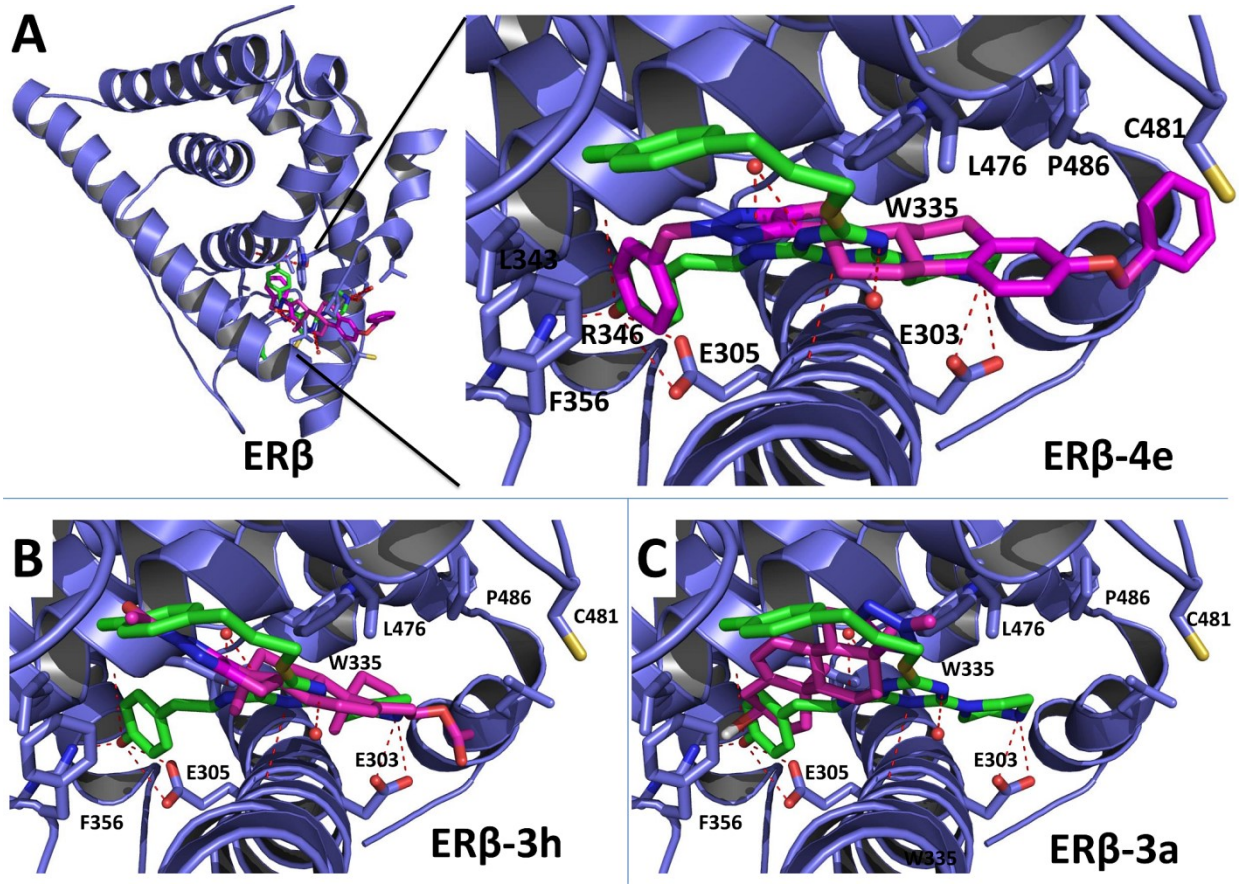


Figure S59. Molecular docking of compounds **3a**, **3h** and **4e** against ERβ-LBD. The top ranking pose predicted for **4e** (magenta sticks) is shown in **panel A**, in the context of the entire receptor (left) and zoomed into the ligand binding site (right). The top ranking docking poses for **3h** and **3a** are shown in **panels B** and **C**, respectively. ERβ residues involved in ligand binding are labeled. Autodock Vina was used for docking as described in the methods for AKR1C3, but with the following modifications: coordinates from a structure of ERβ in complex with a 1,3,5-triazine compound (PDB 1NDE) were used as receptor [1]. Docking simulations were centered on the ERβ active site (x=110.144, y=8.060, z=-108.215) with an exhaustiveness setting of 16. Based on molecular docking, compounds **4e**, **3h** and **3a** could bind in a similar position as the 1,3,5-triazine compound present in the X-ray structure (green sticks), with the exception that 3a is rotated in comparison with 4e and 3h. Binding energies predicted

by docking for 4e (-10.5 kcal/mol), 3h(-8.5 kcal/mol) and 3a (-10.0 kcal.mol) are energetically favorable, suggesting that they have affinity for ERβ LBD, in agreement with experimental results from the yeast cell fluorescence assay shown in Figure 1.

1. Henke BR, Consler TG, Go N, Hale RL, Hohman DR, Jones SA, Lu AT, Moore LB, Moore JT, Orband-Miller LA, Robinett RG, Shearin J, Spearing PK, Stewart EL, Turnbull PS, Weaver SL, Williams SP, Wisely GB, Lambert MH. A new series of estrogen receptor modulators that display selectivity for estrogen receptor beta. J Med Chem. 2002;45(25):5492-505.