Electronic Supplementary Information

Design, synthesis, and evaluation of curcumin analogues as potential inhibitors of bacterial sialidase

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Affiliation

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[†]Both authors contributed equally to the work.

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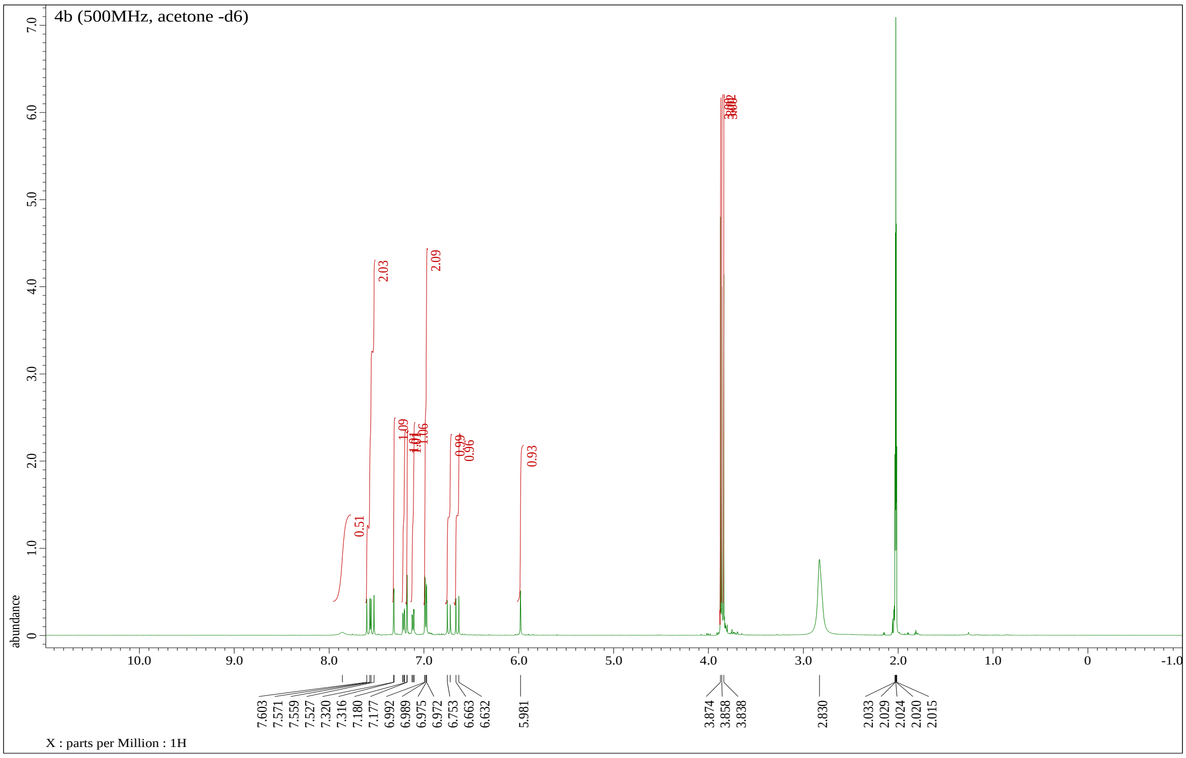
1. ^{1}H and ^{13}C NMR spectrum of curcumins



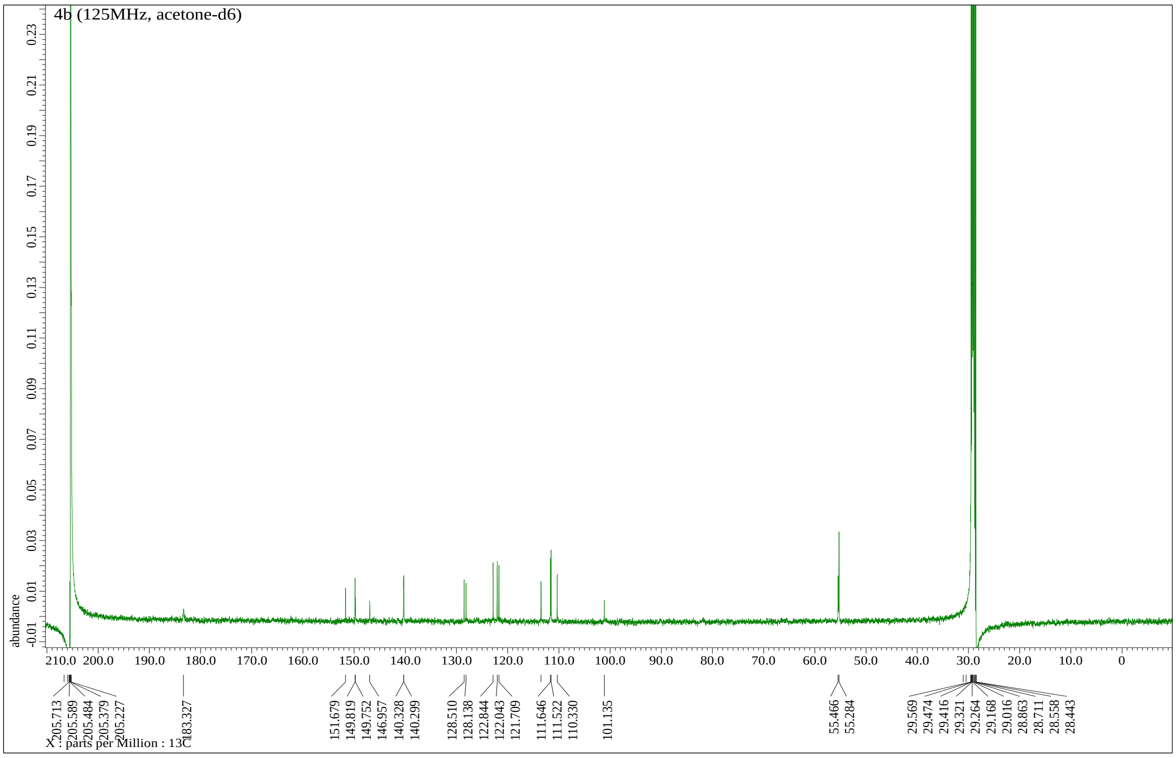
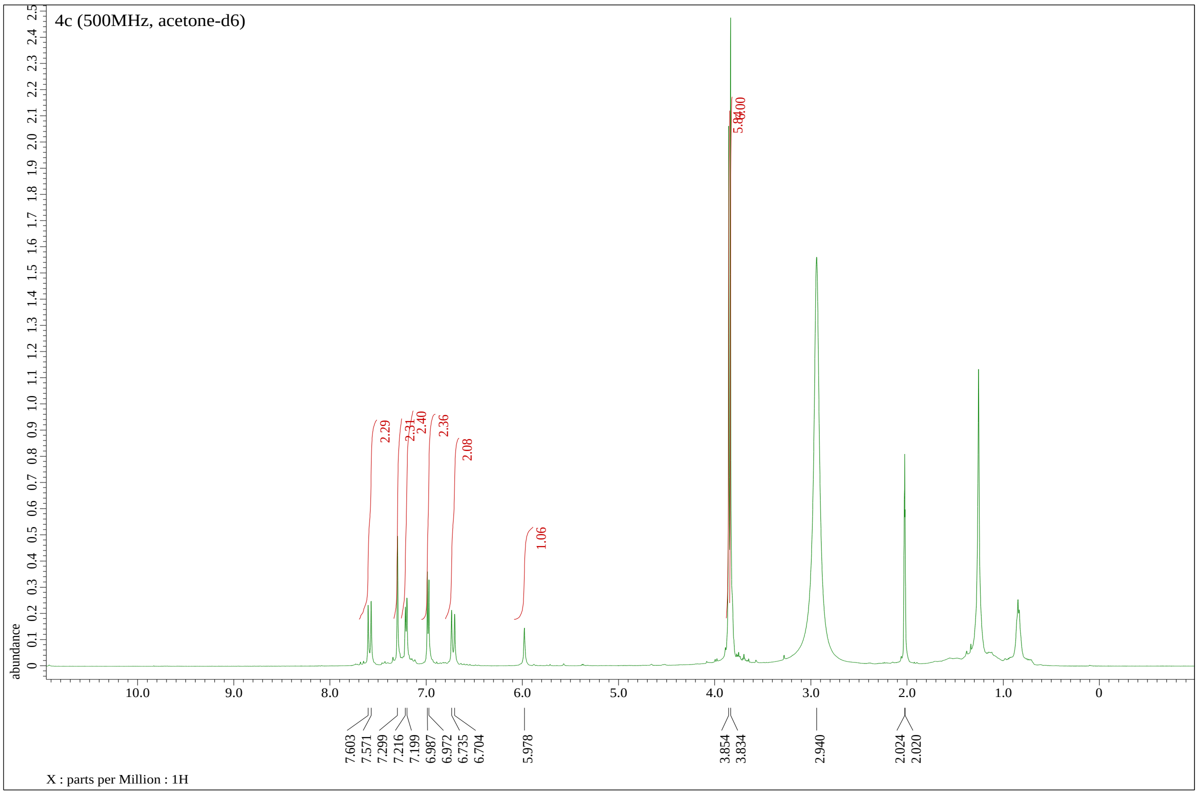


Figure S1. ^{1}H (top) and ^{13}C NMR (bottom) spectra in acetone-d_6 of **4b**



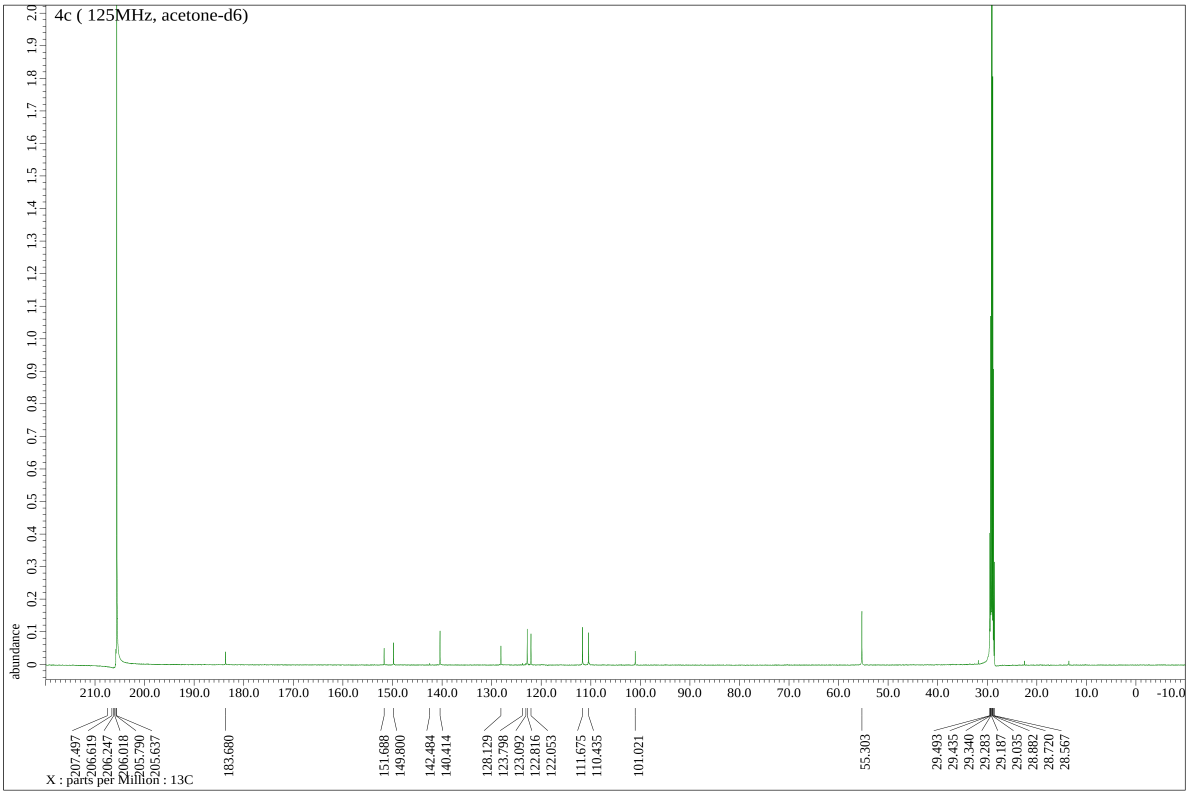
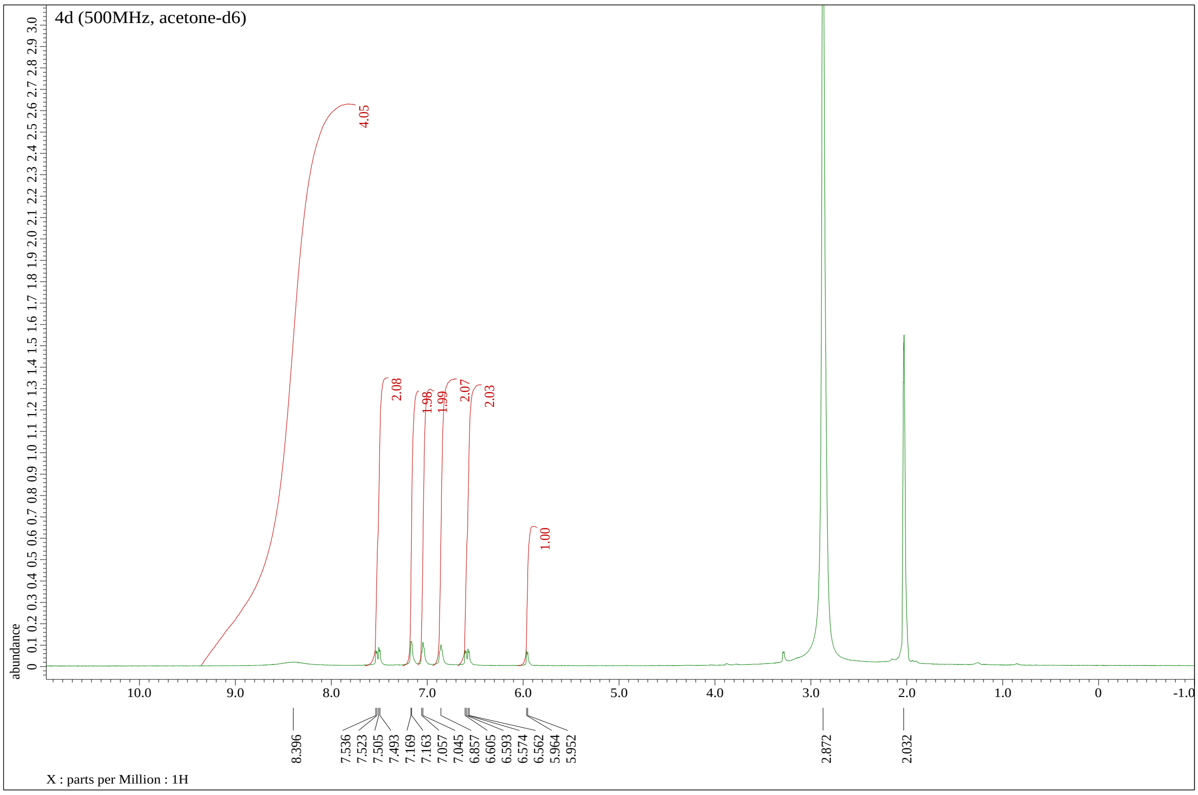


Figure S2. ^{1}H (top) and ^{13}C NMR (bottom) spectra in acetone-d_6 of **4c**



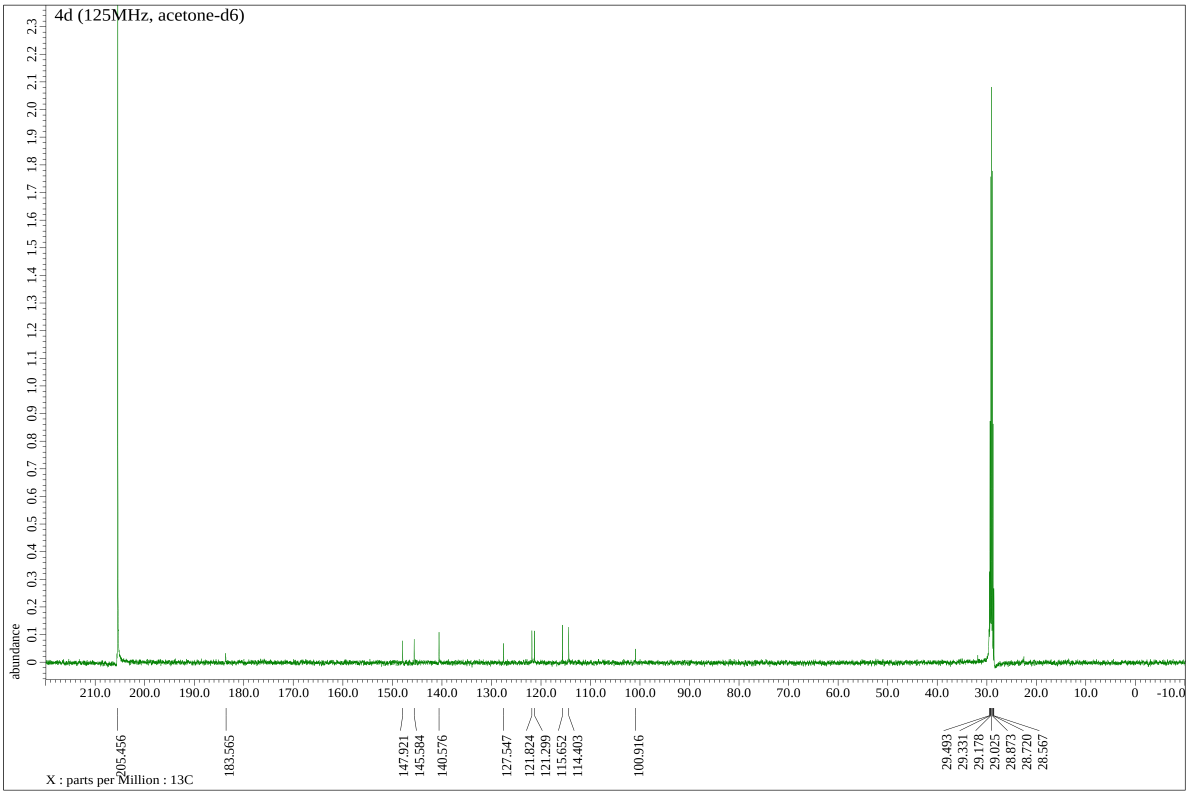
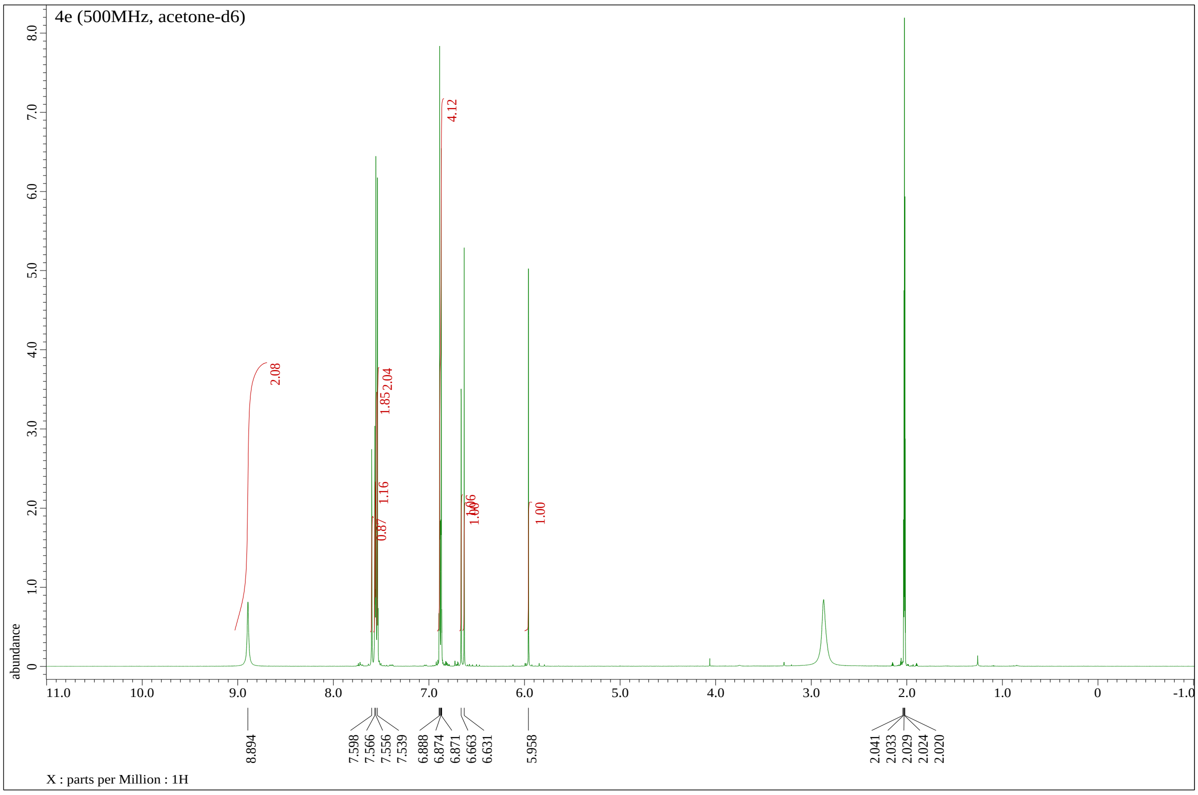


Figure S3. ^{1}H (top) and ^{13}C NMR (bottom) spectra in acetone-d_6 of **4d**



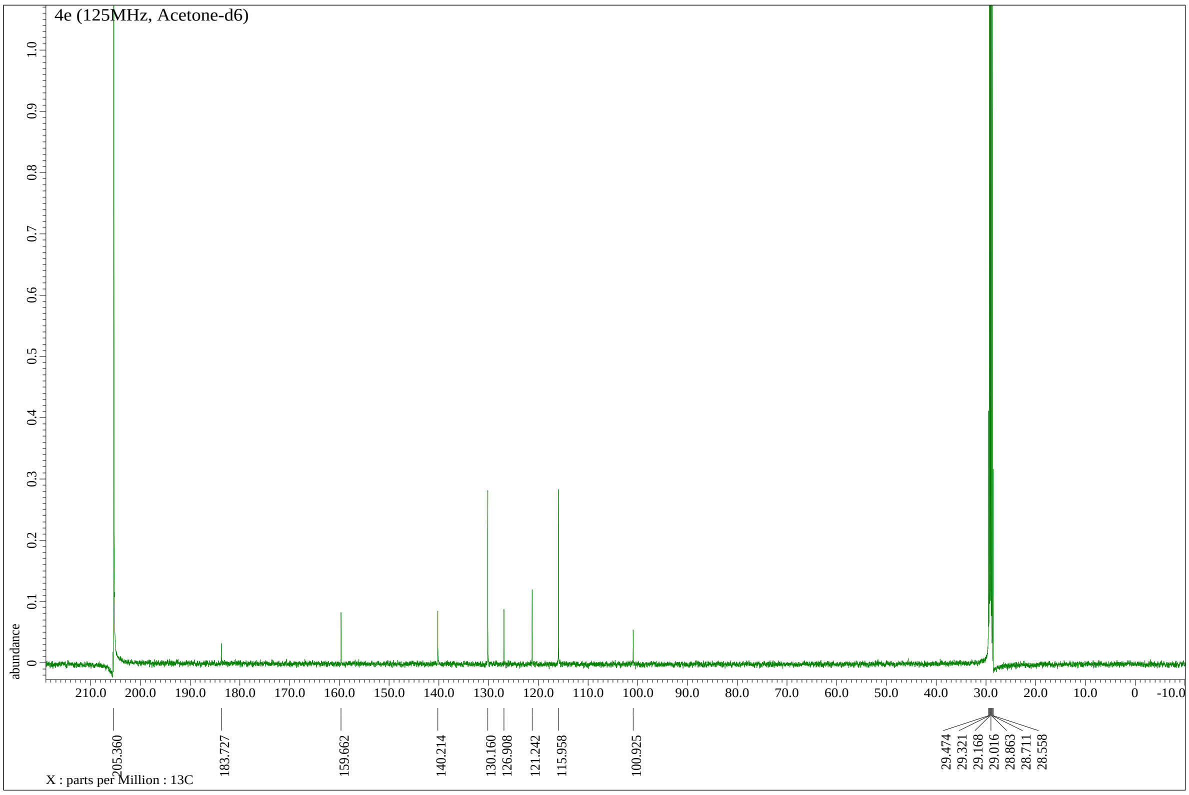
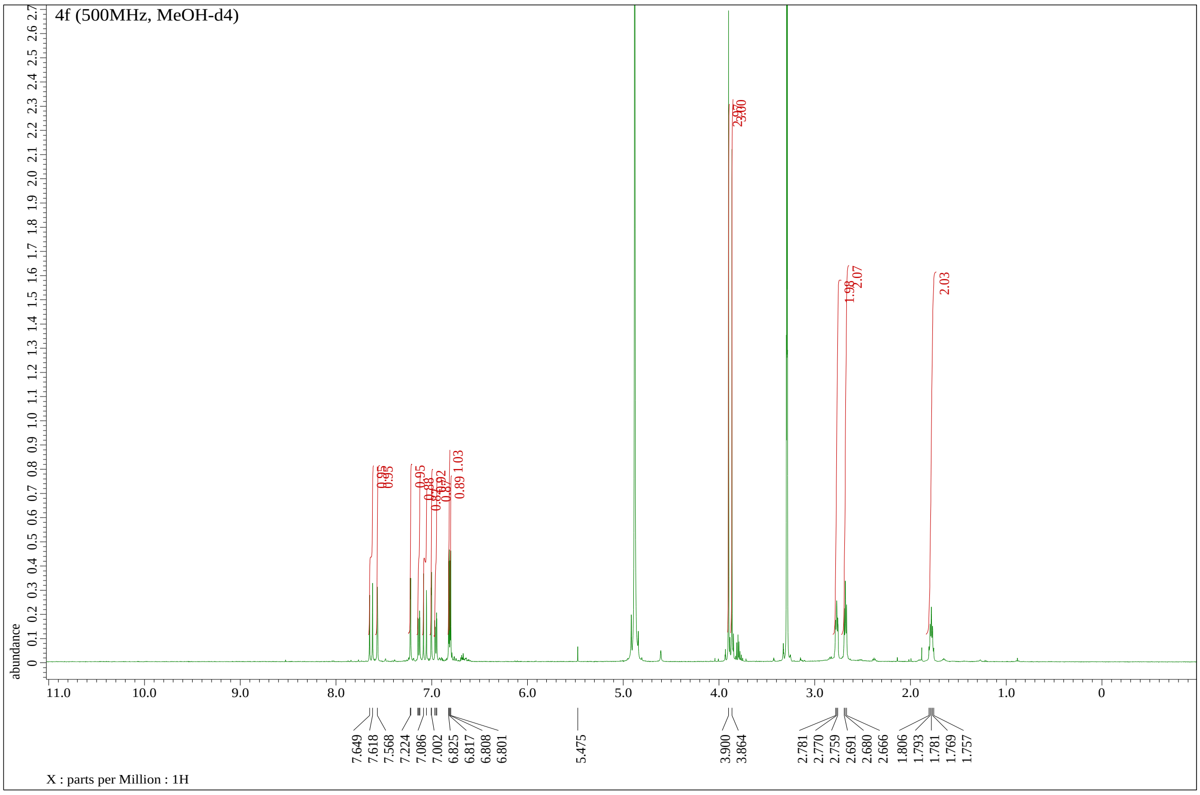


Figure S4. ^{1}H (top) and ^{13}C NMR (bottom) spectra in acetone-d_6 of **4e**



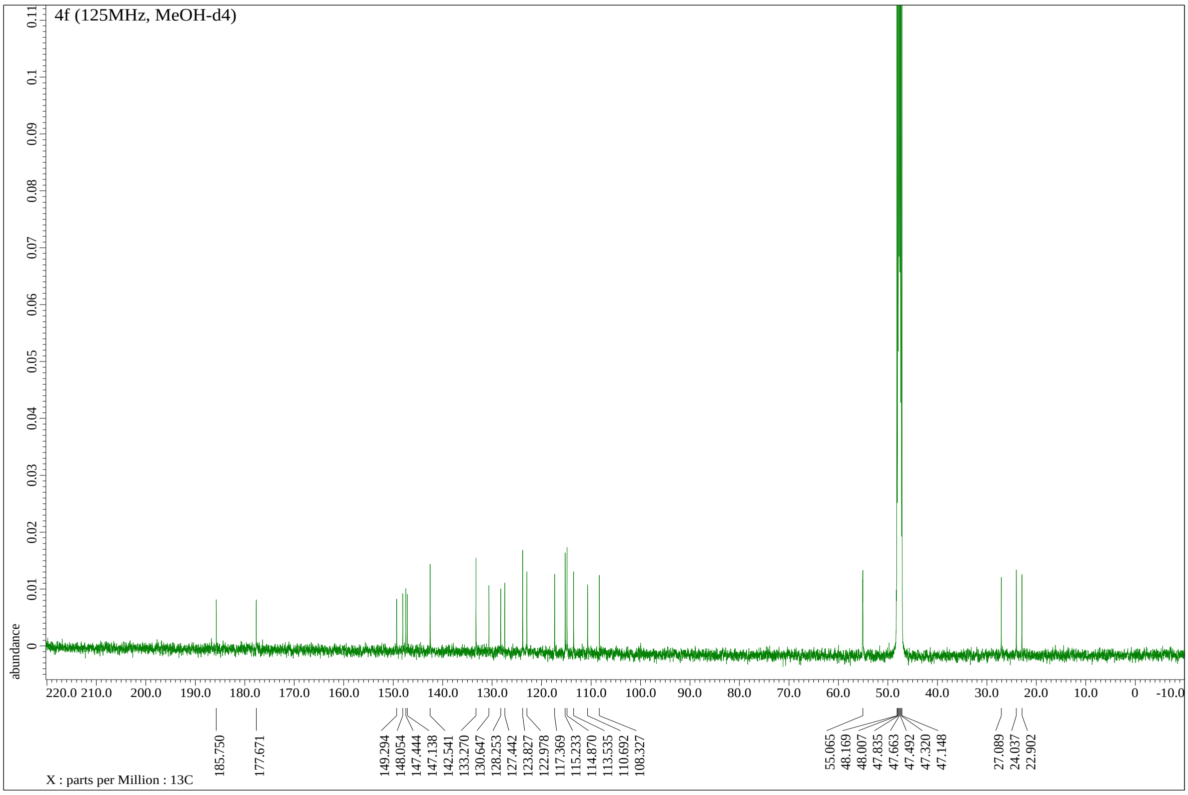


Figure S5. ^{1}H (top) and ^{13}C NMR (bottom) spectra in acetone-d_6 of **4f**

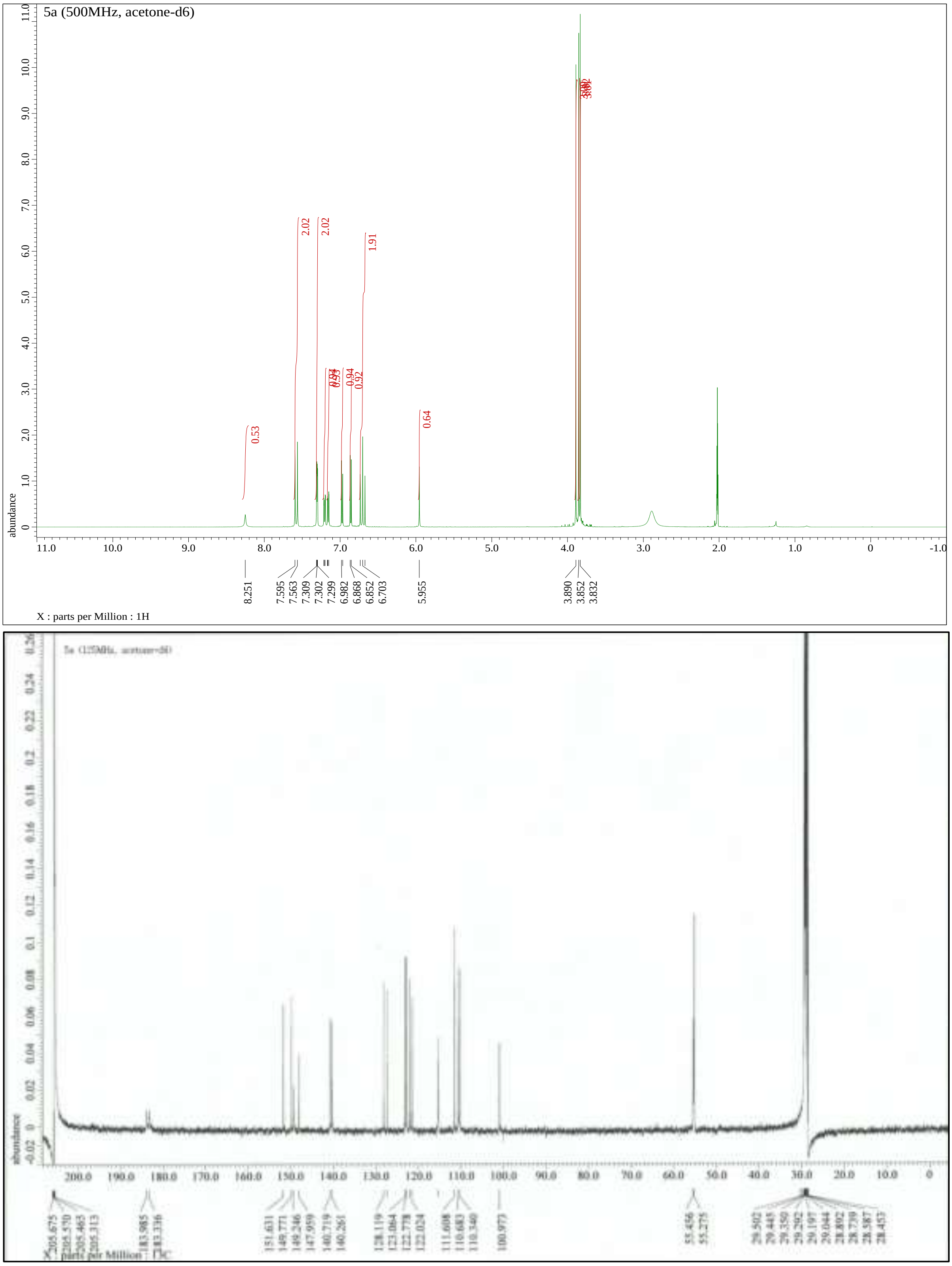
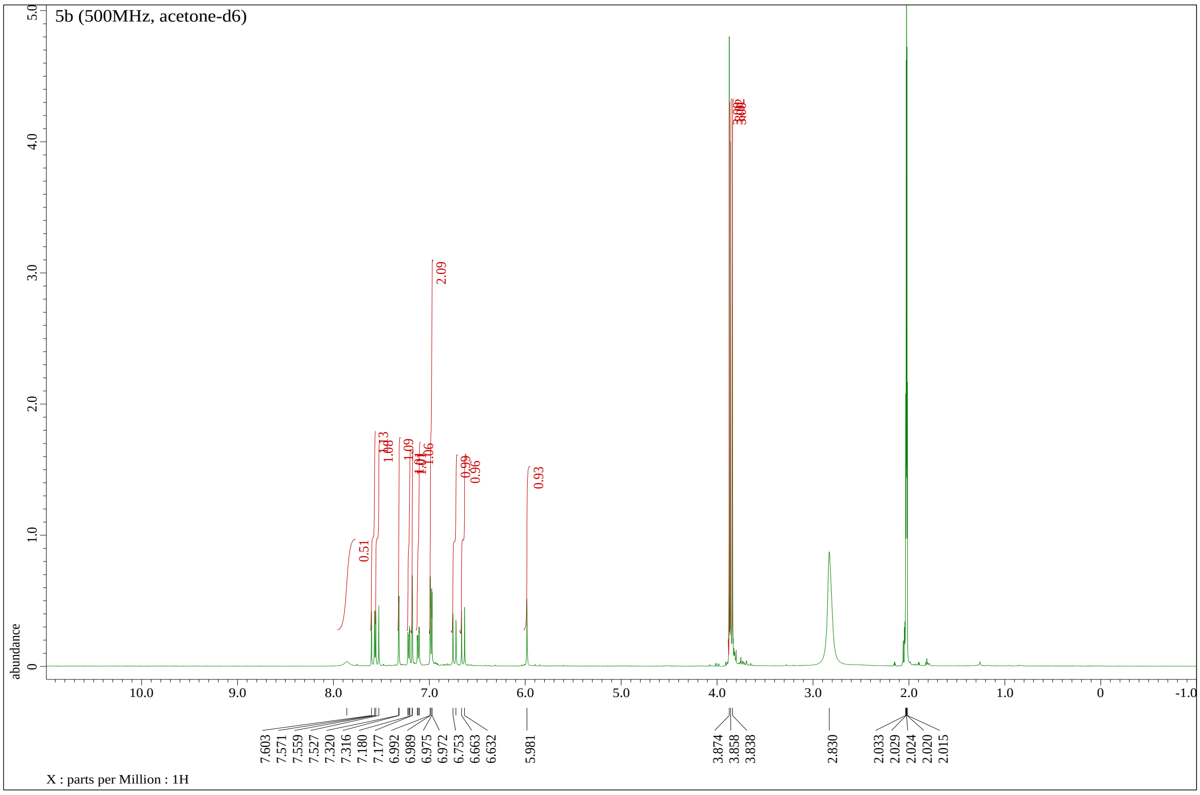


Figure S6. ^{1}H (top) and ^{13}C NMR (bottom) spectra in acetone-d_6 of **5a**



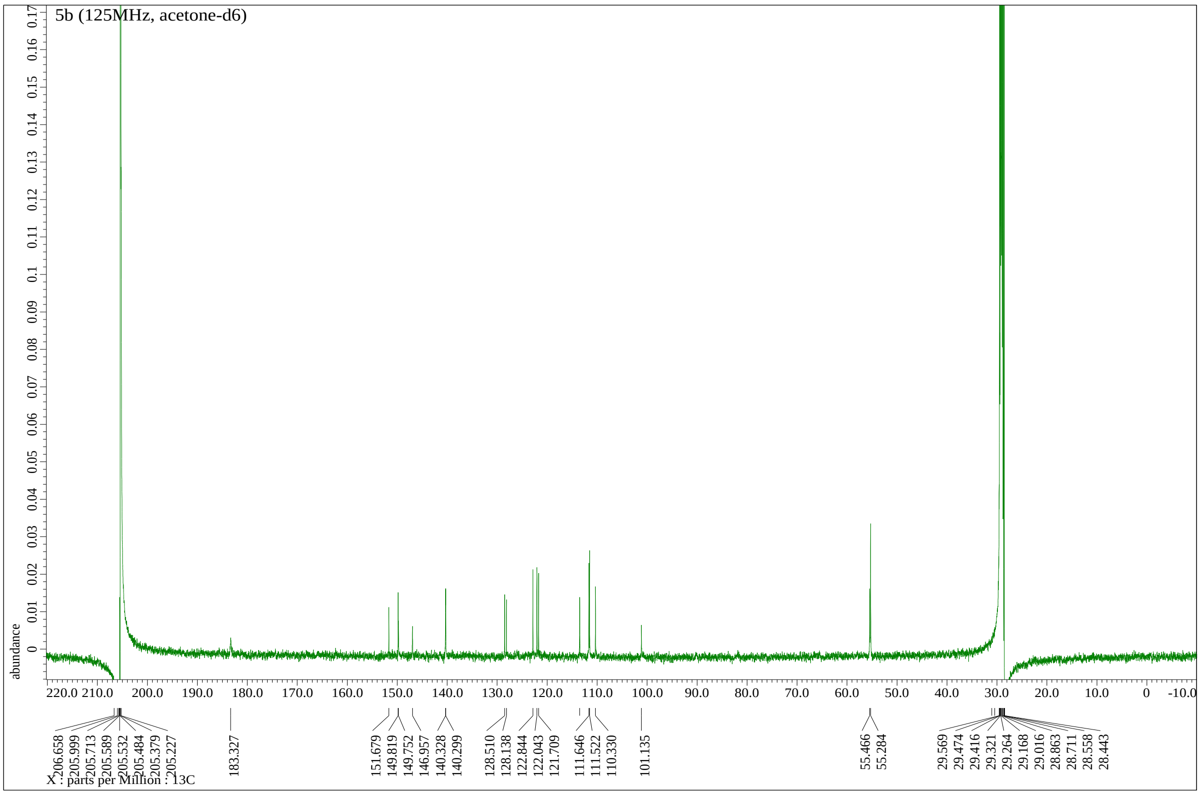
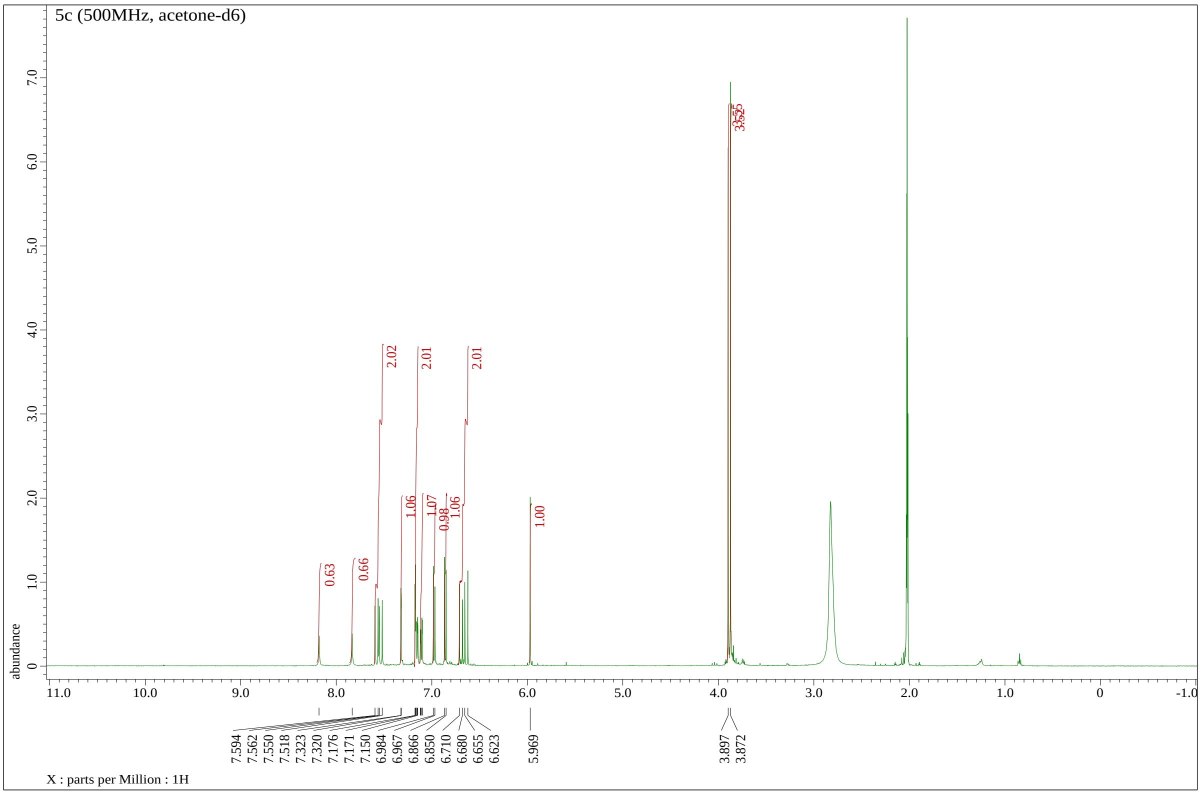


Figure S7. ^{1}H (top) and ^{13}C NMR (bottom) spectra in acetone-d_6 of **5b**



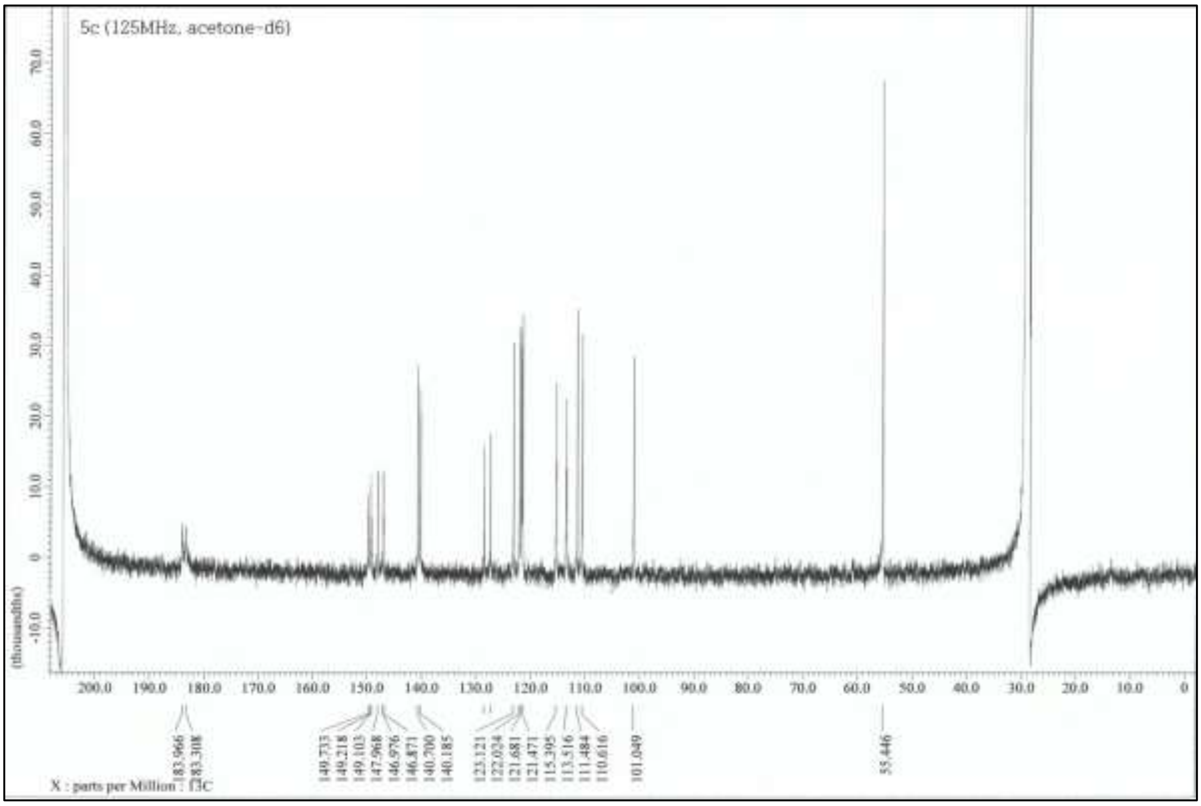
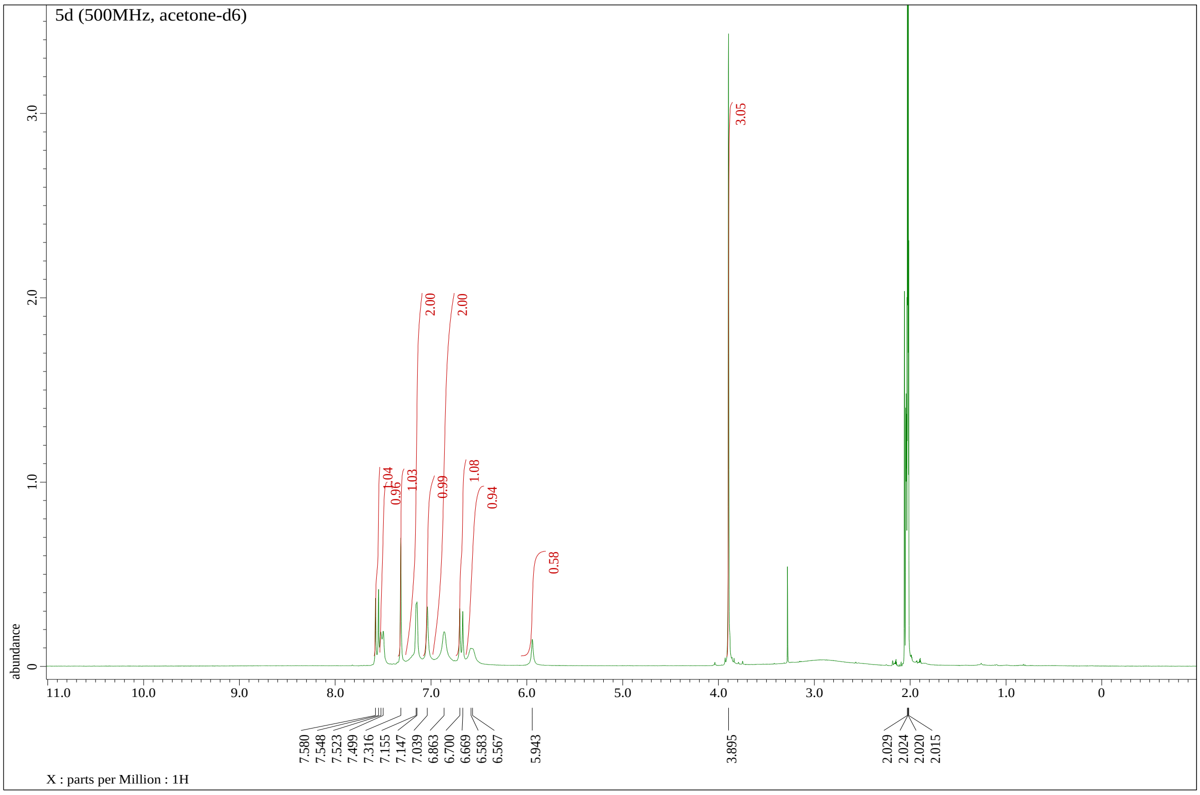


Figure S8. ^{1}H (top) and ^{13}C NMR (bottom) spectra in acetone-d_6 of **5c**



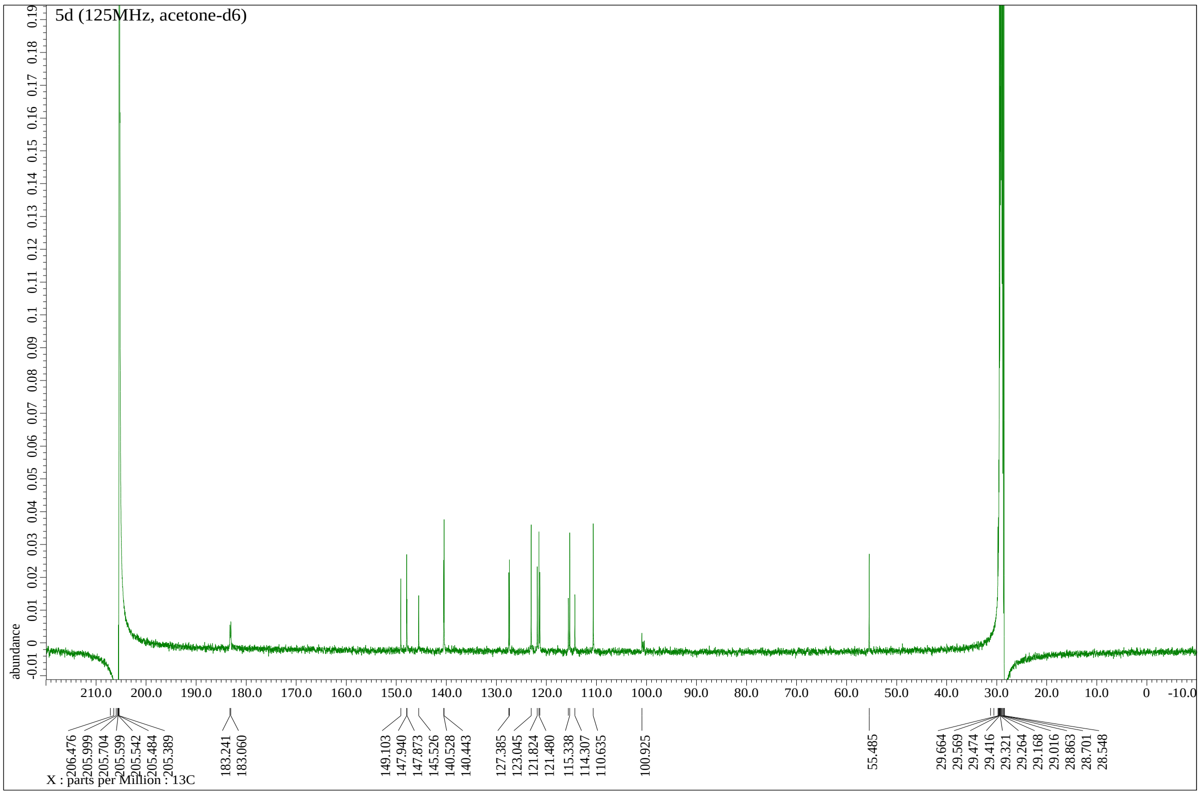


Figure S9. ^{1}H (top) and ^{13}C NMR (bottom) spectra in acetone-d_6 of **5d**

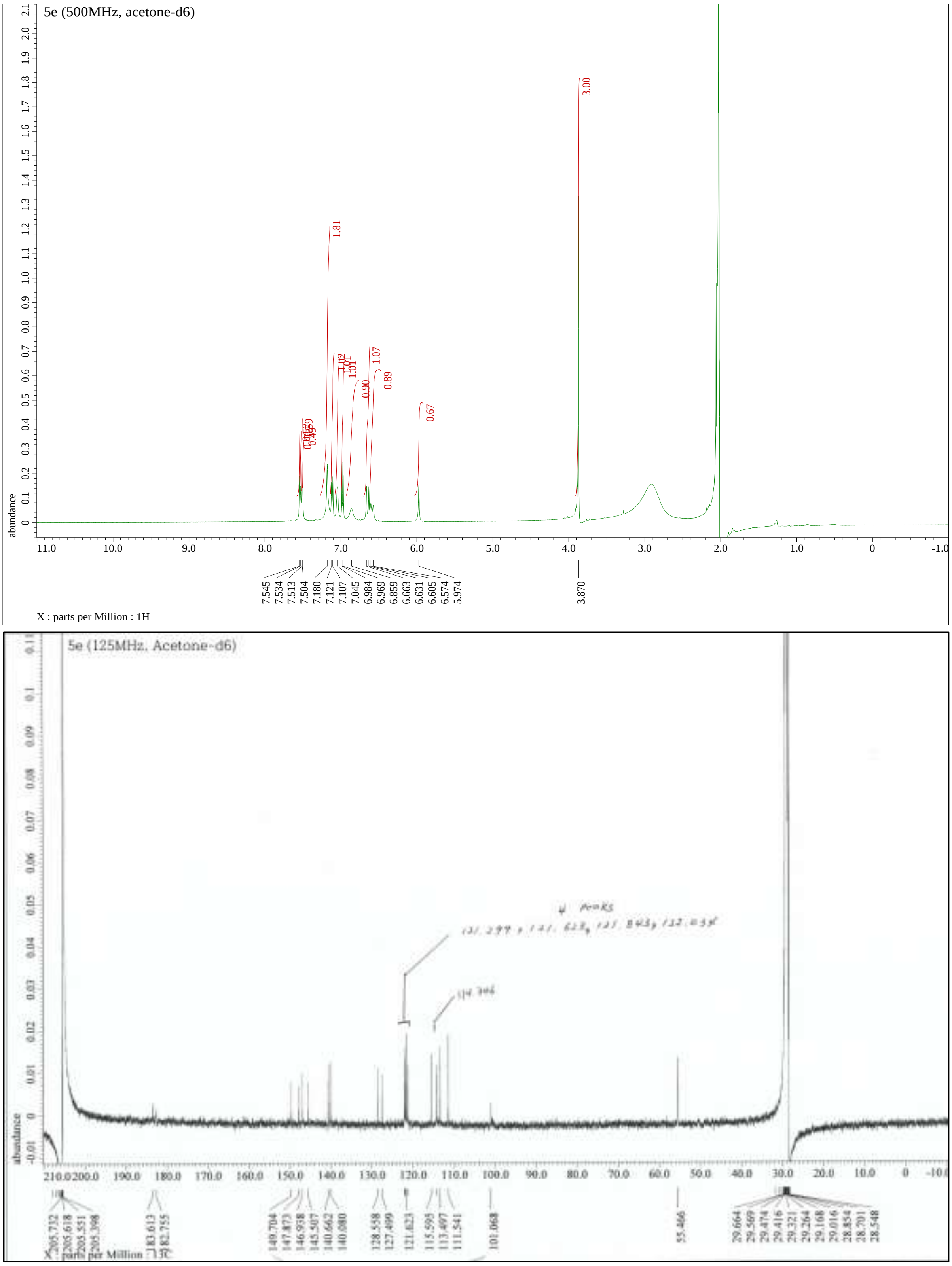
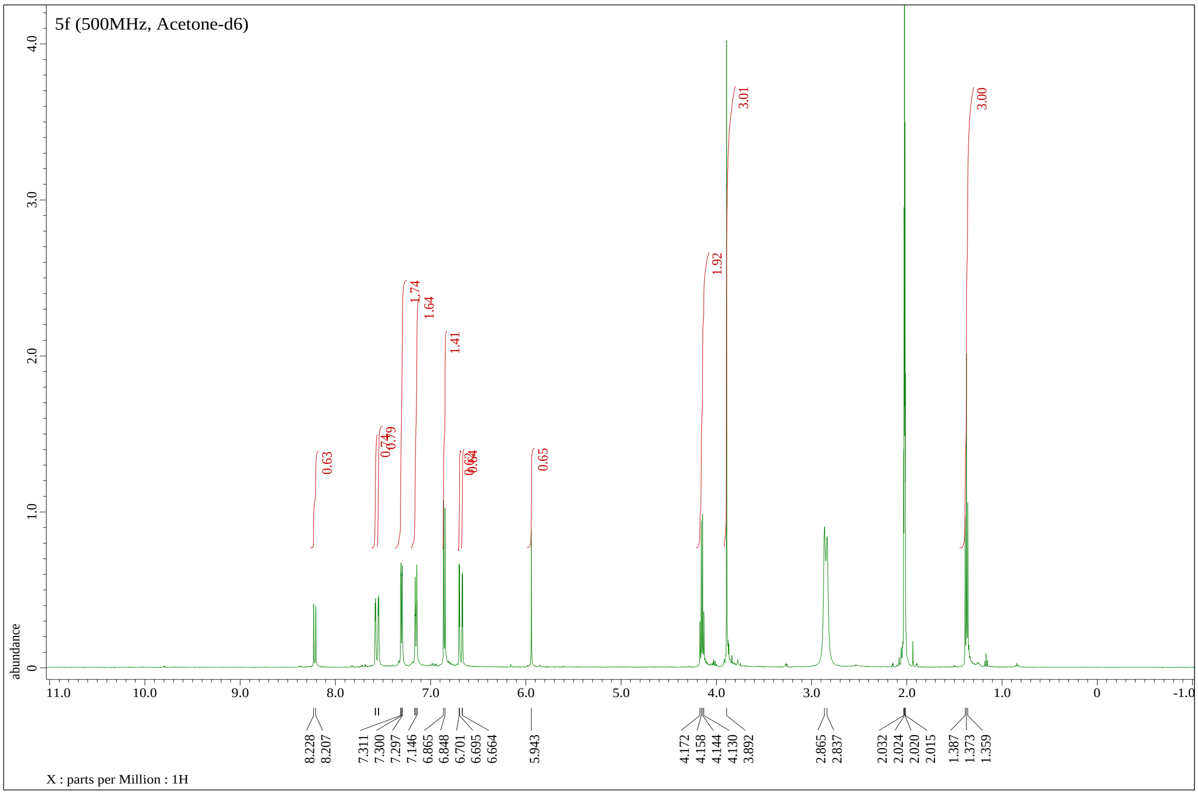


Figure S10. ^{1}H (top) and ^{13}C NMR (bottom) spectra in acetone-d_6 of **5e**



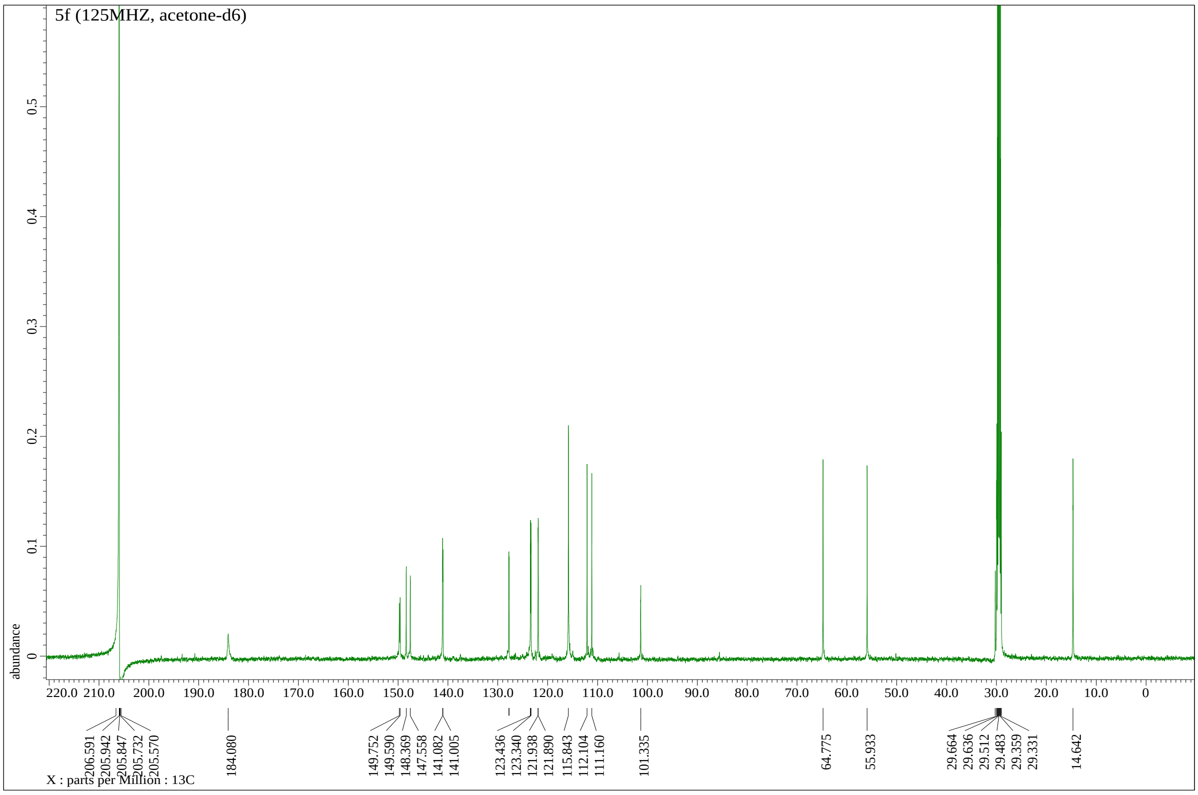
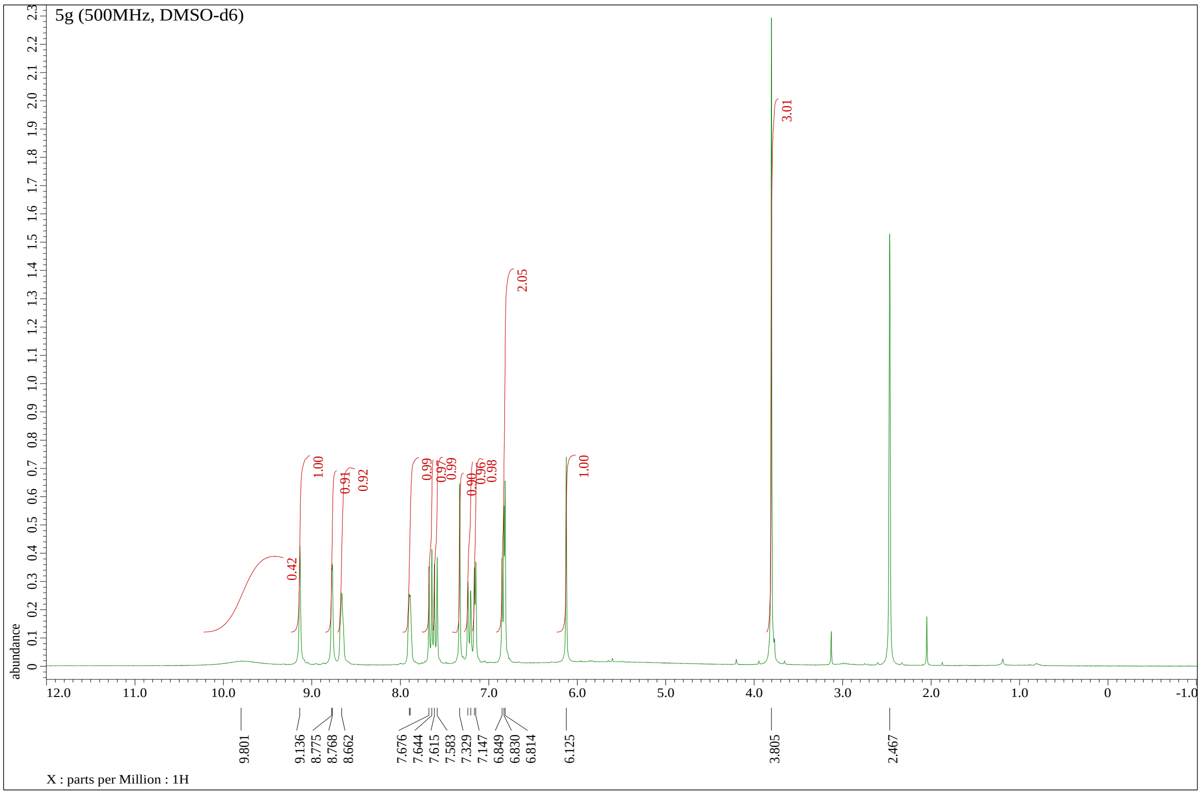


Figure S11. ^{1}H (top) and ^{13}C NMR (bottom) spectra in acetone-d_6 of **5f**



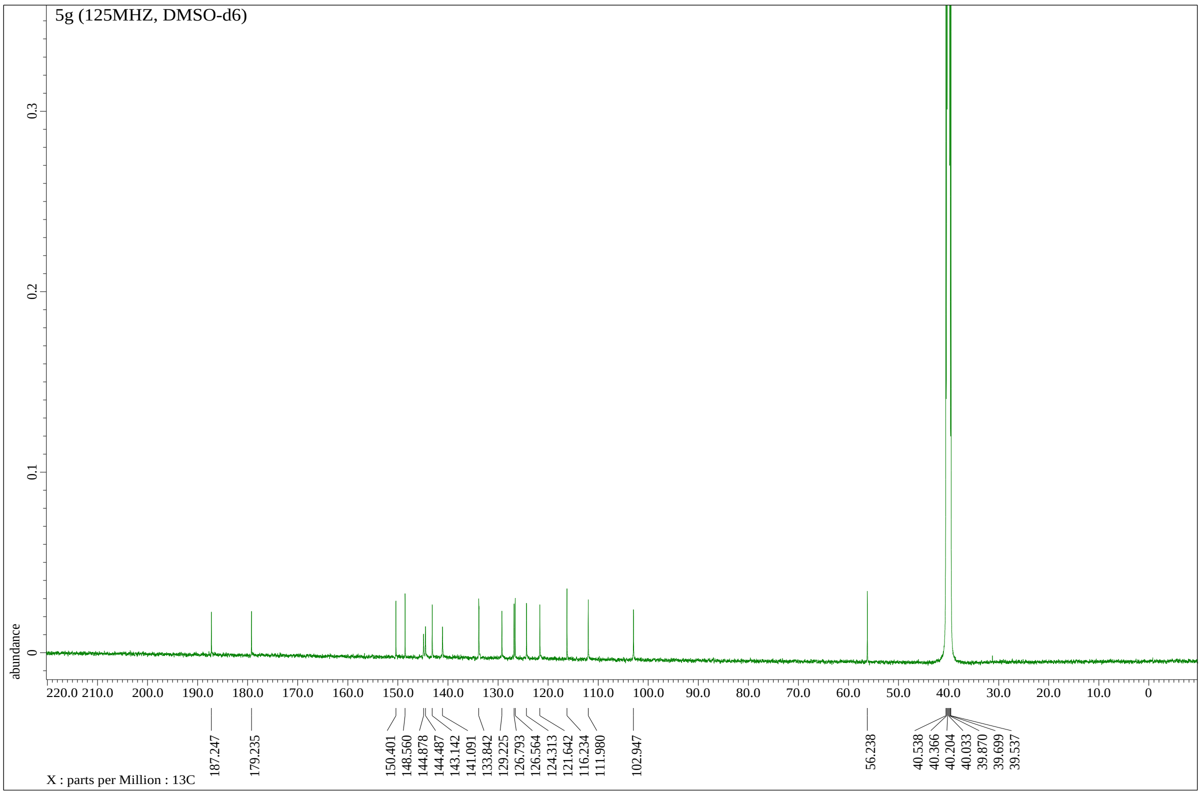
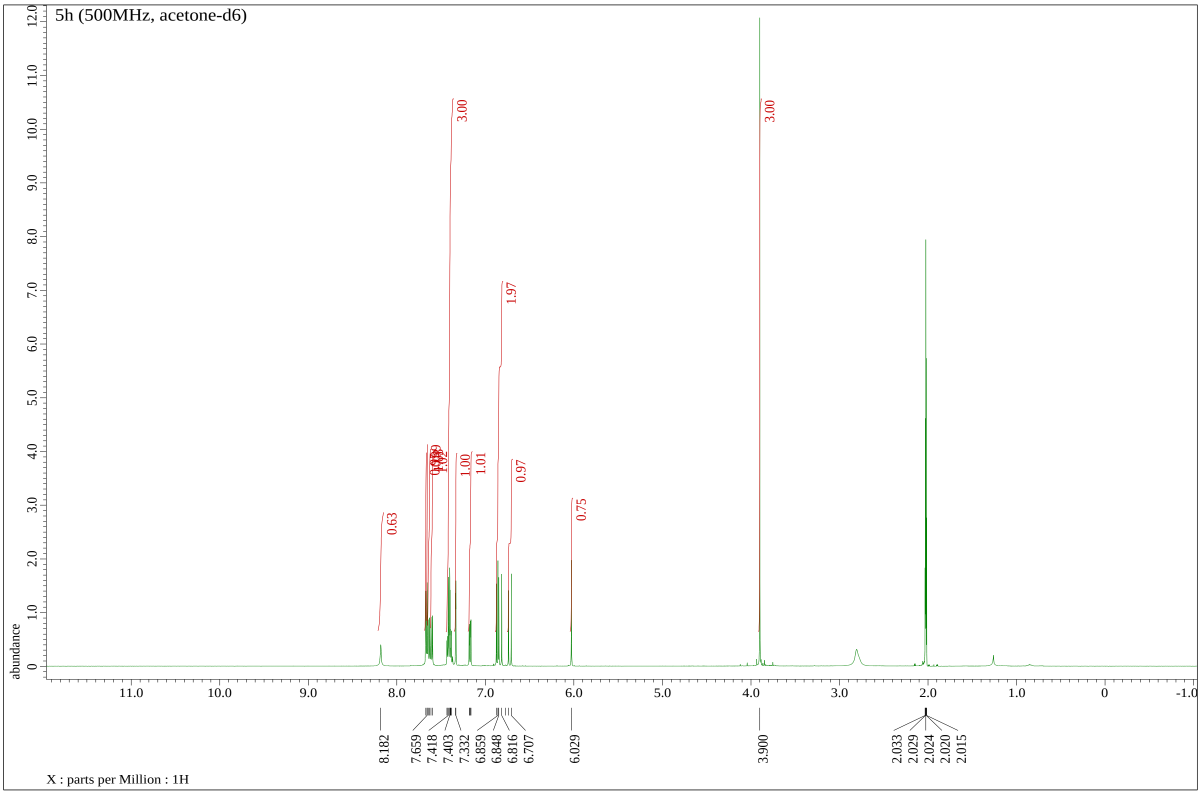


Figure S12. ^{1}H (top) and ^{13}C NMR (bottom) spectra in dimethylsulfoxide-d_6 of **5g**



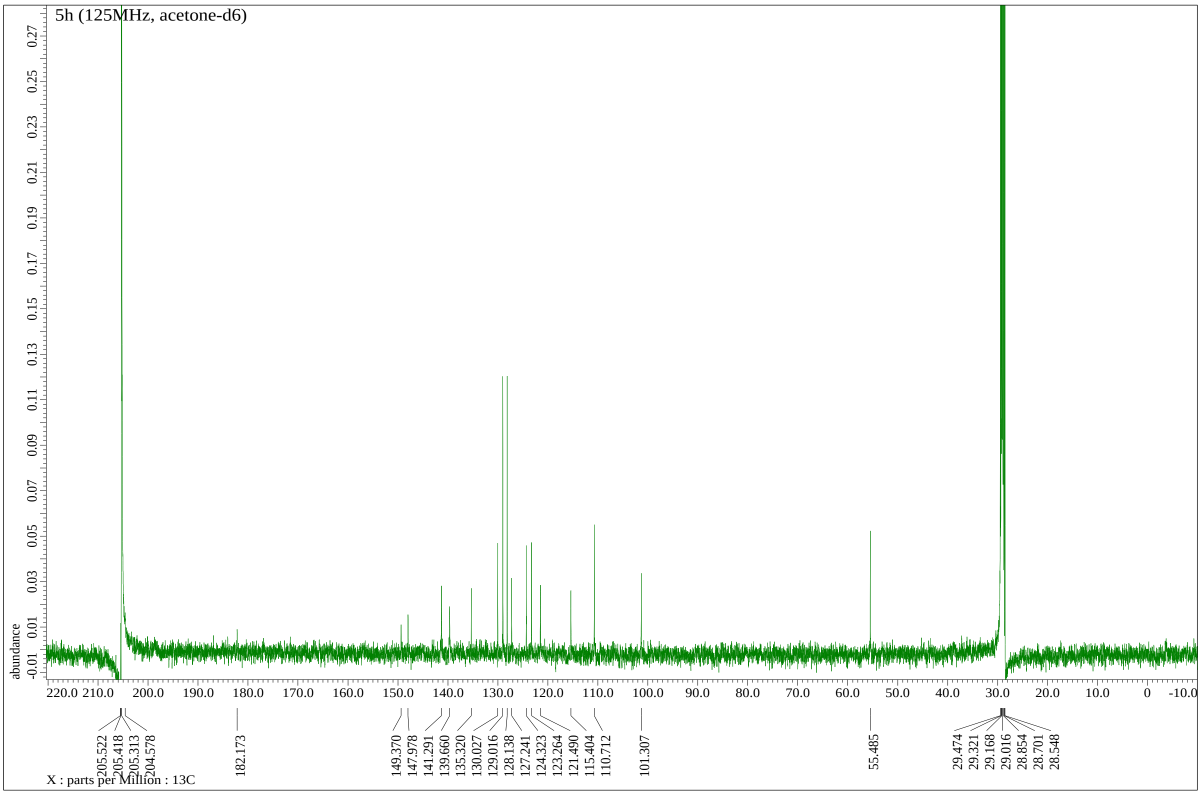
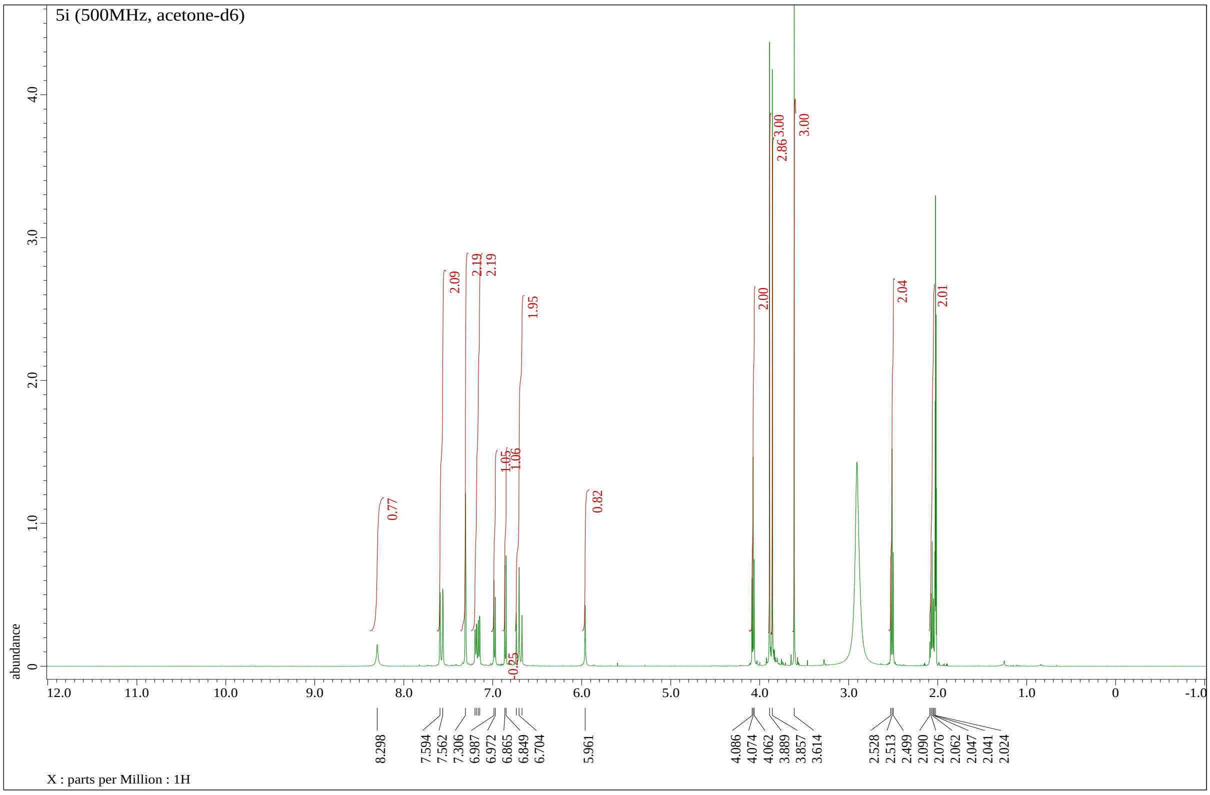


Figure S13. ^{1}H (top) and ^{13}C NMR (bottom) spectra in acetone-d_6 of **5h**



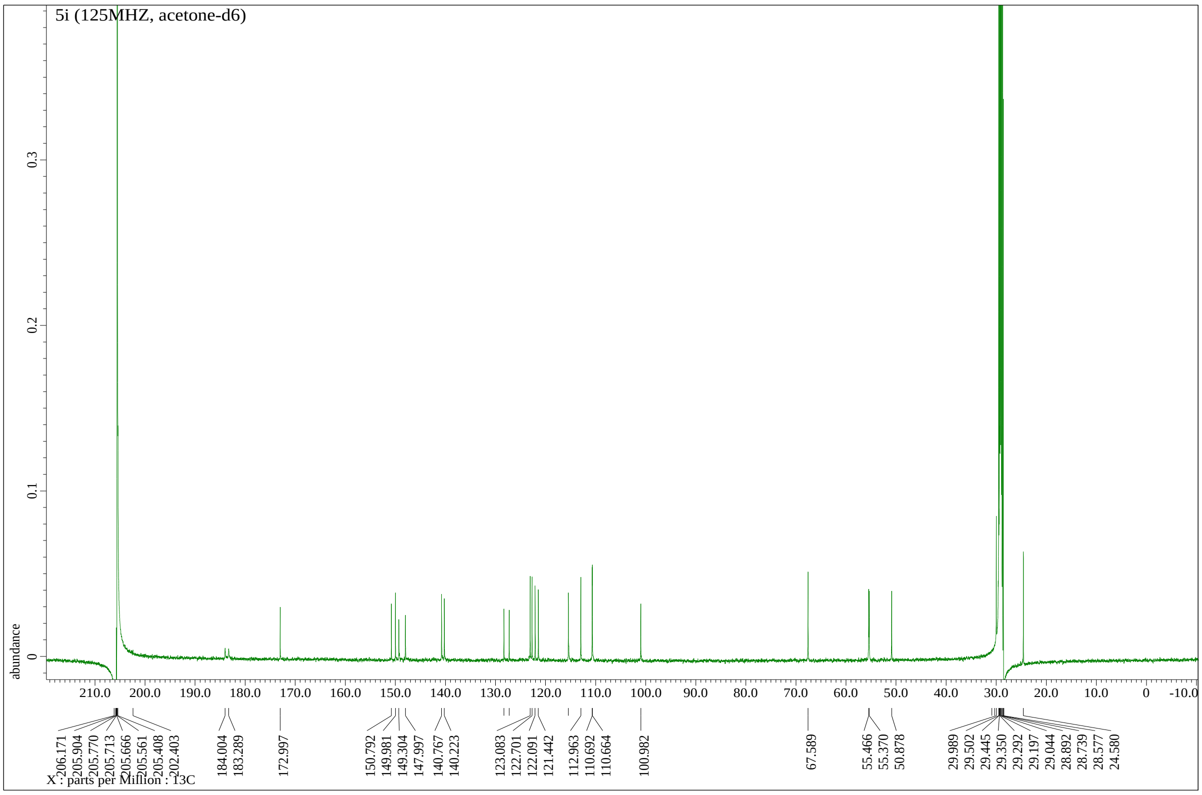
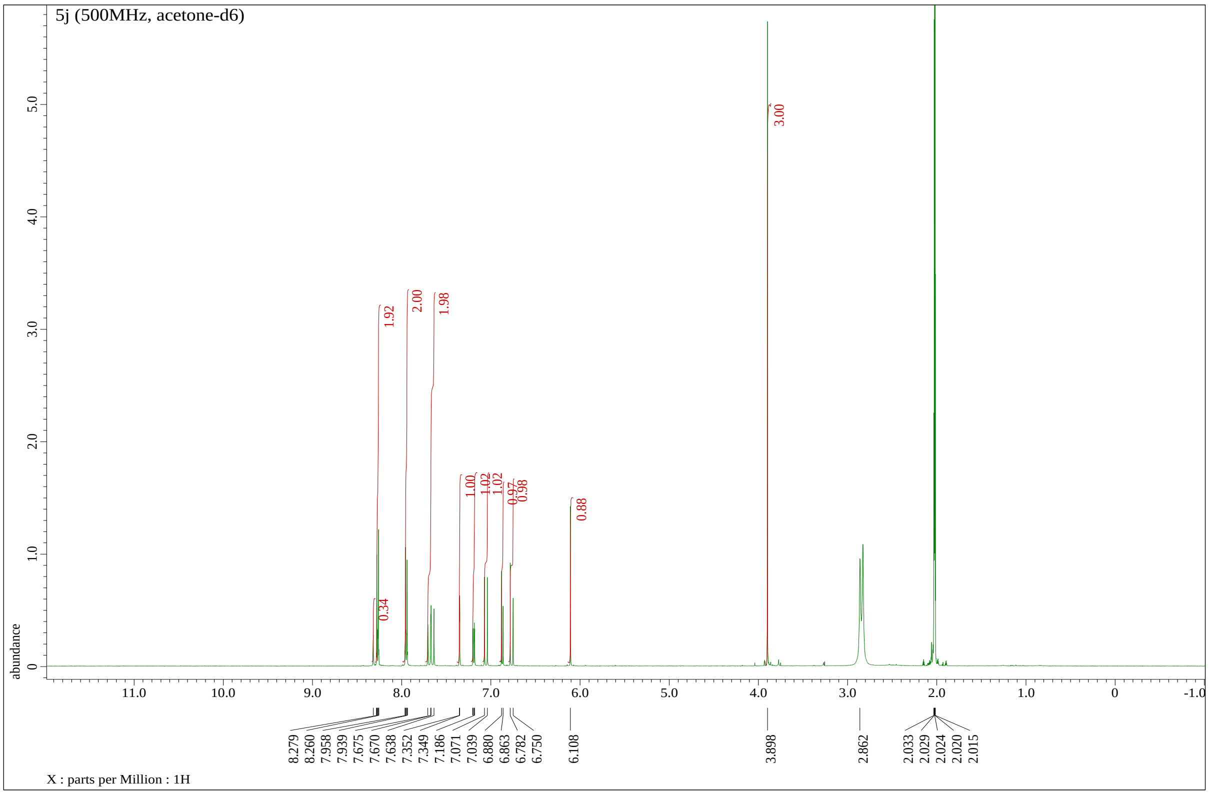


Figure S14. ^{1}H (top) and ^{13}C NMR (bottom) spectra in acetone-d_6 of **5i**



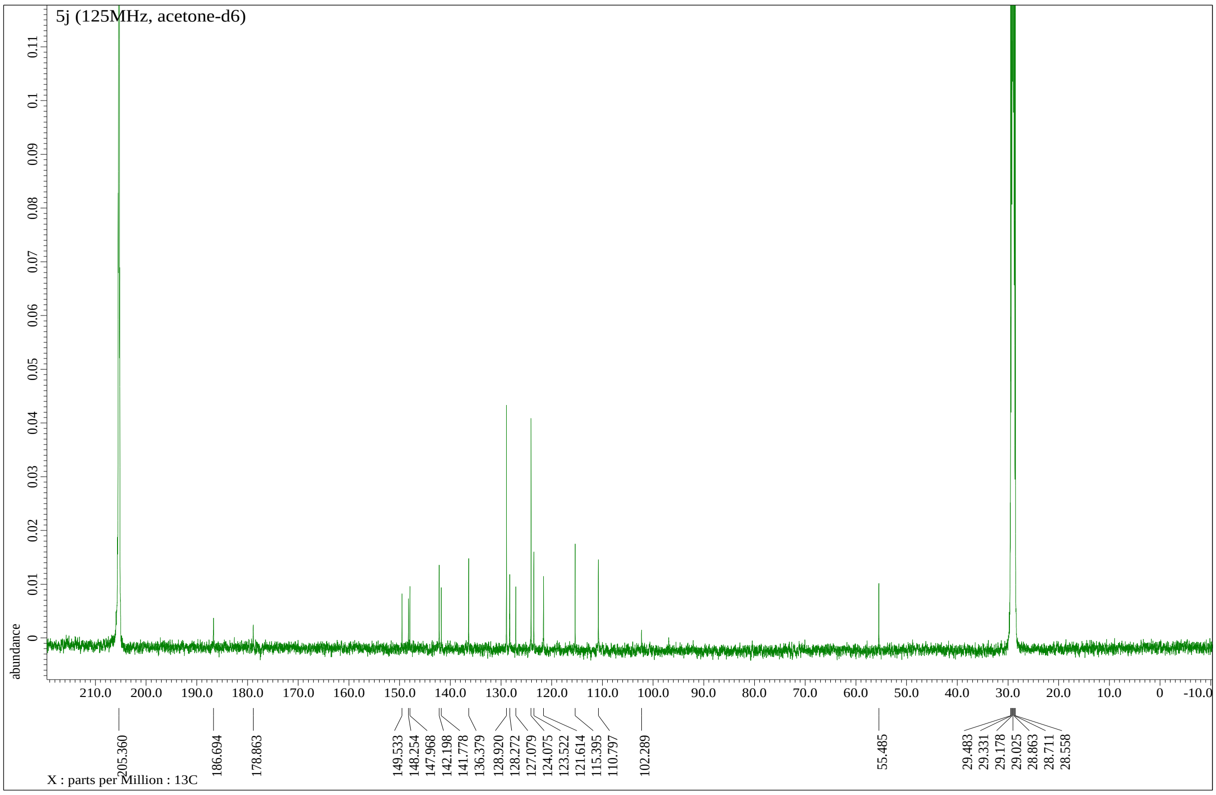
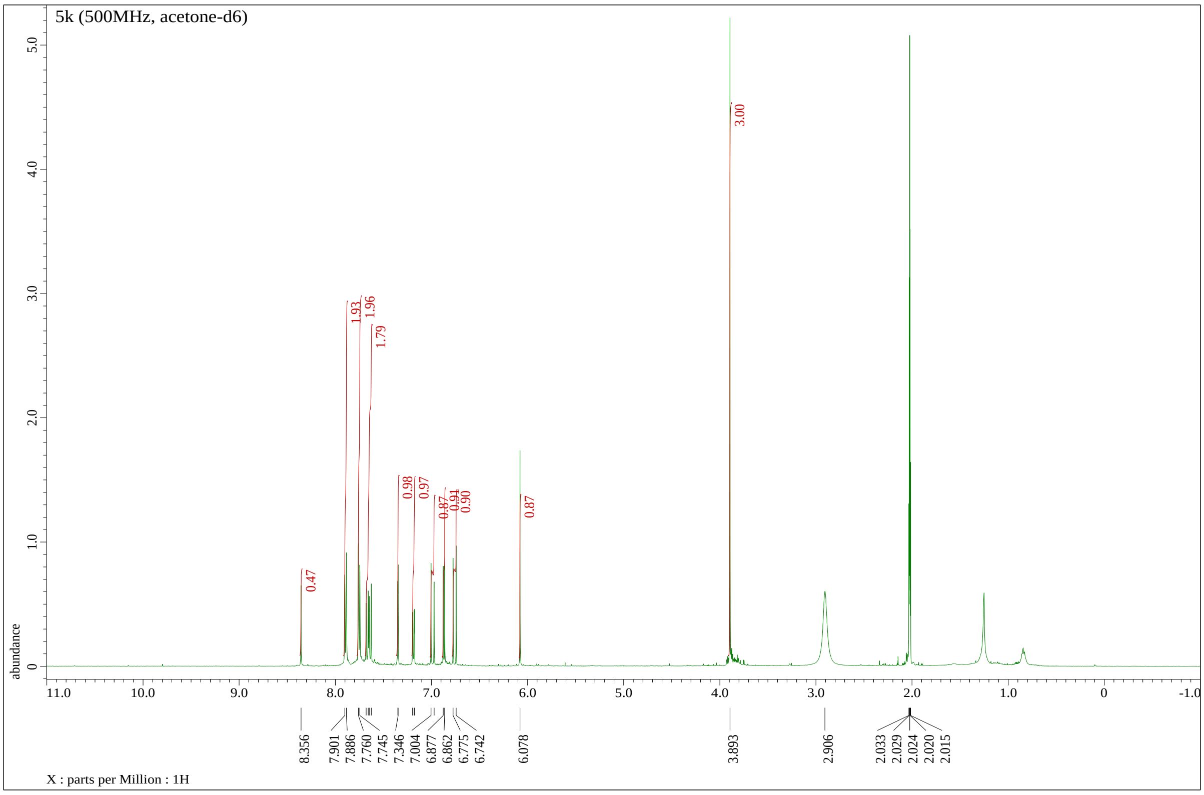


Figure S15. ^{1}H (top) and ^{13}C NMR (bottom) spectra in acetone-d_6 of **5j**



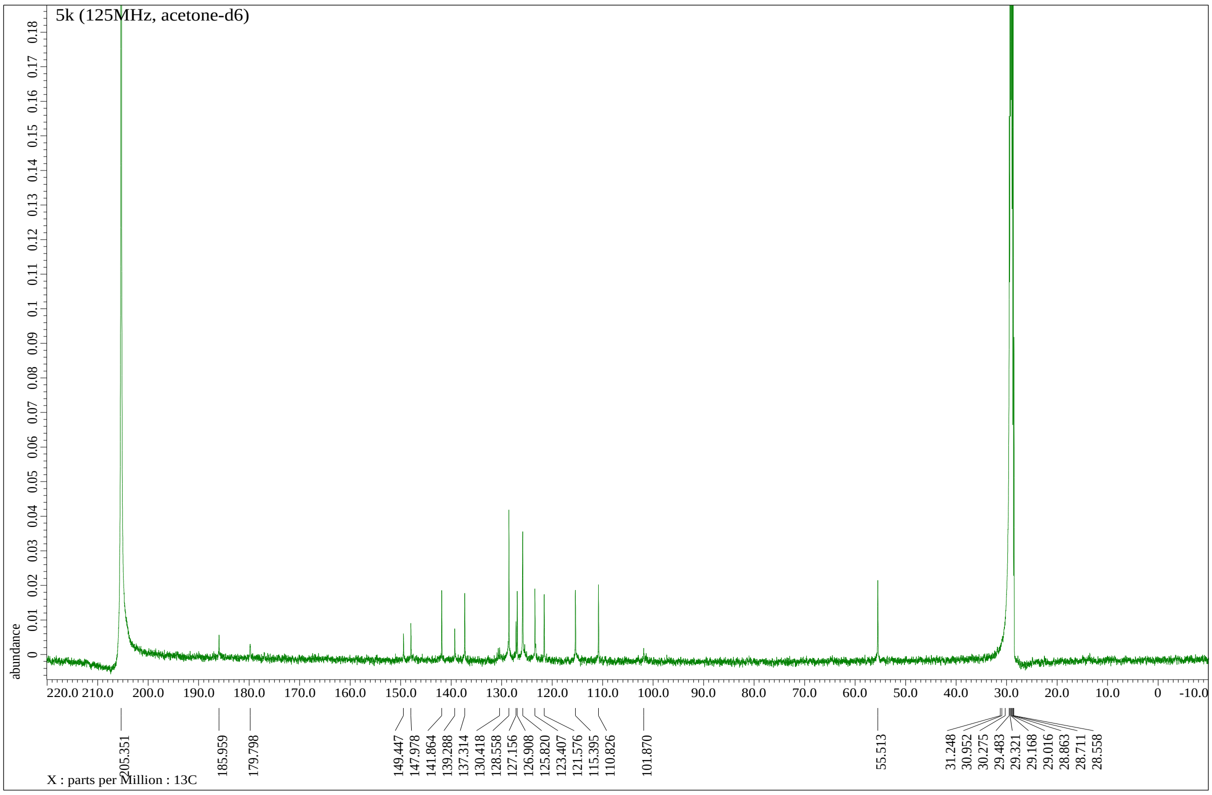
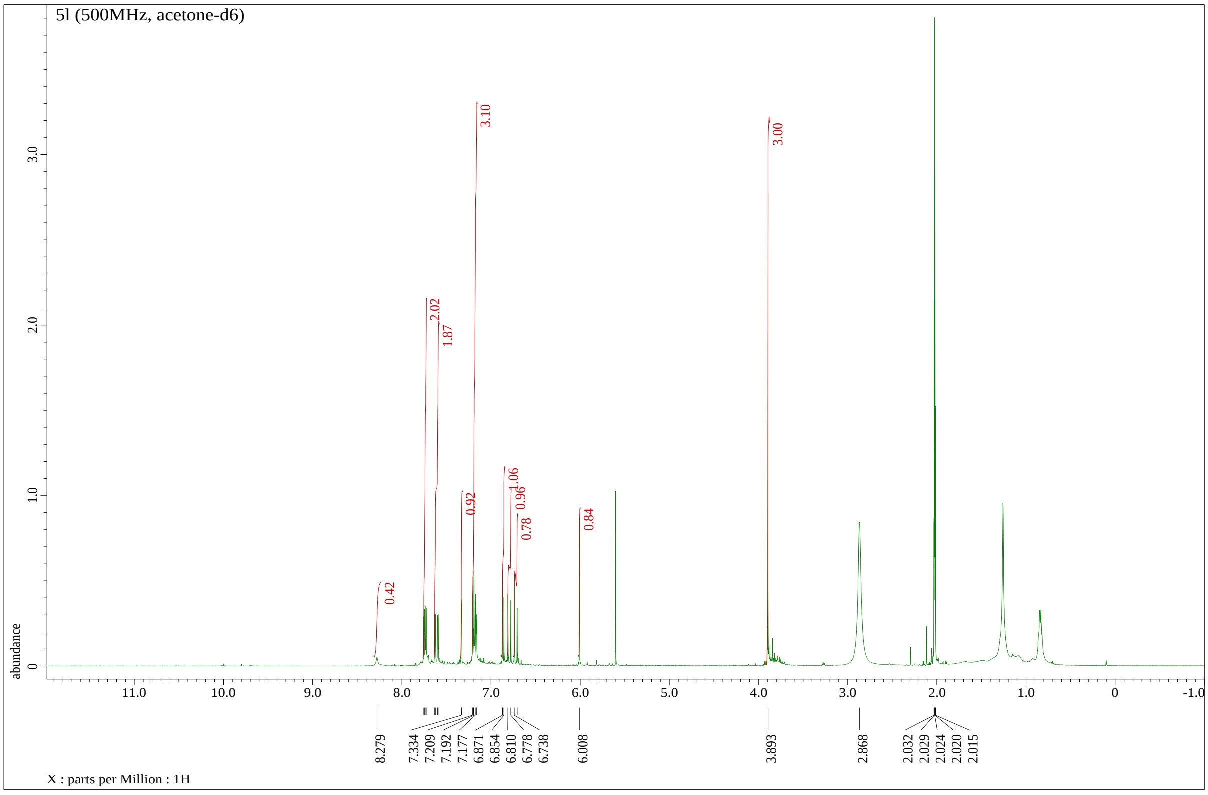


Figure S16. ^{1}H (top) and ^{13}C NMR (bottom) spectra in acetone-d_6 of **5k**



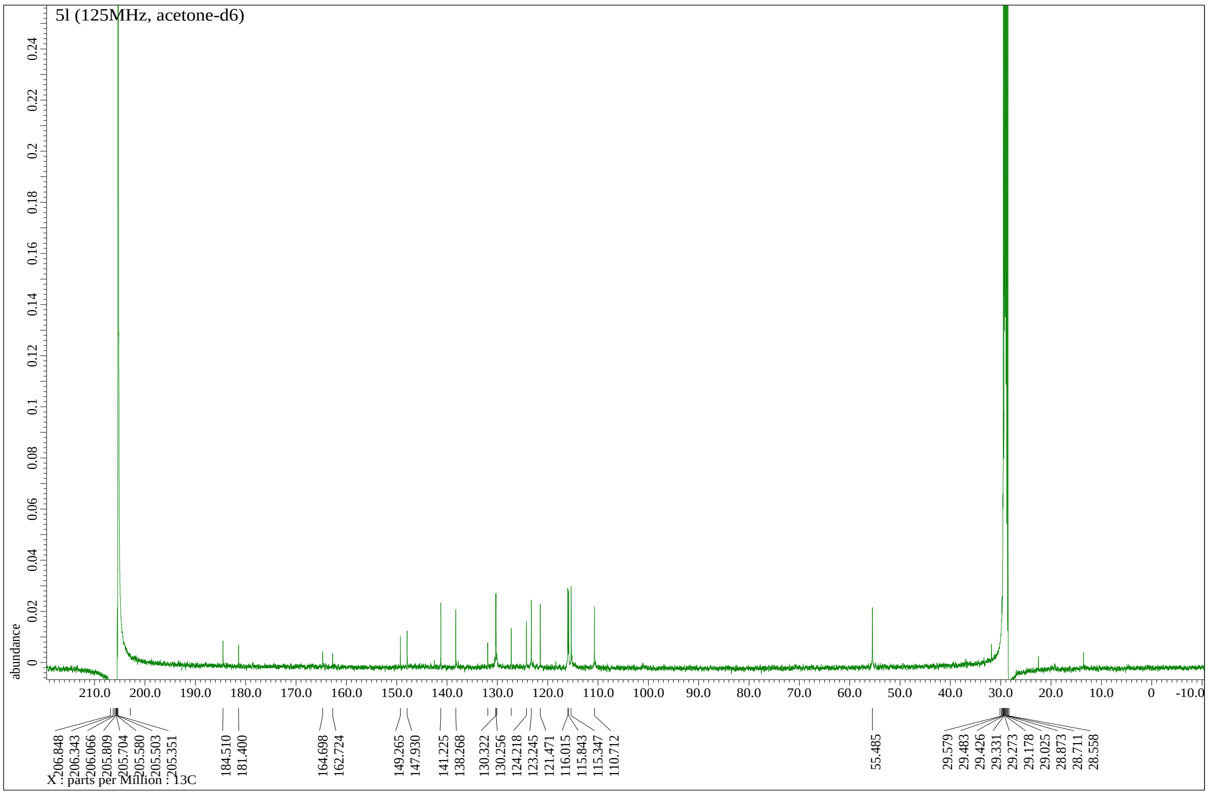
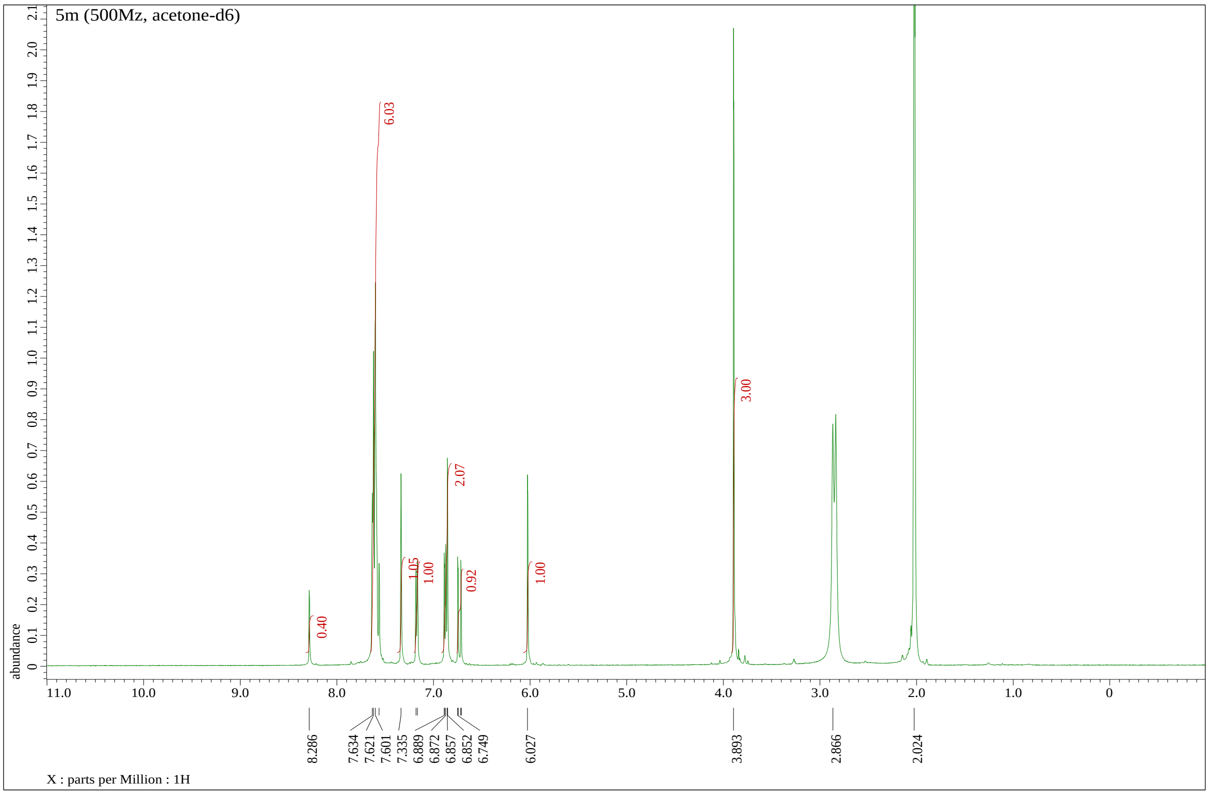


Figure S17. ^{1}H (top) and ^{13}C NMR (bottom) spectra in acetone-d_6 of **5l**



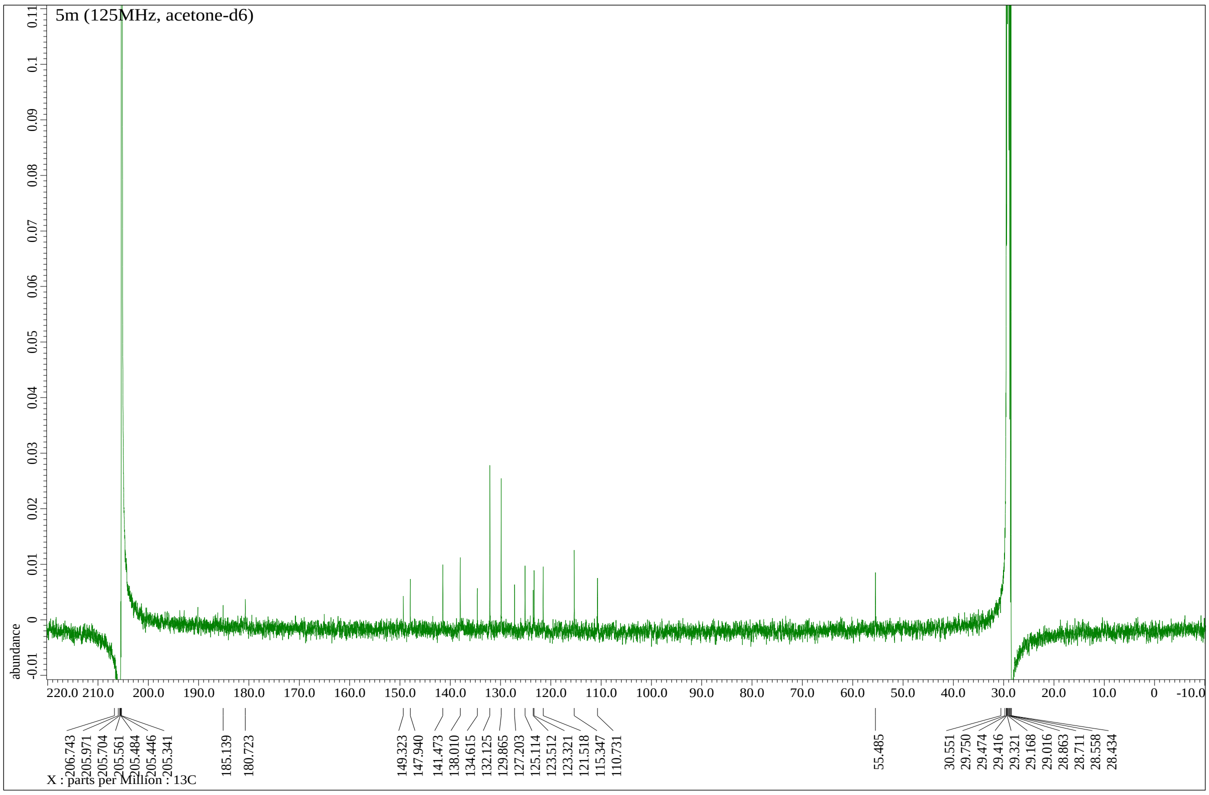
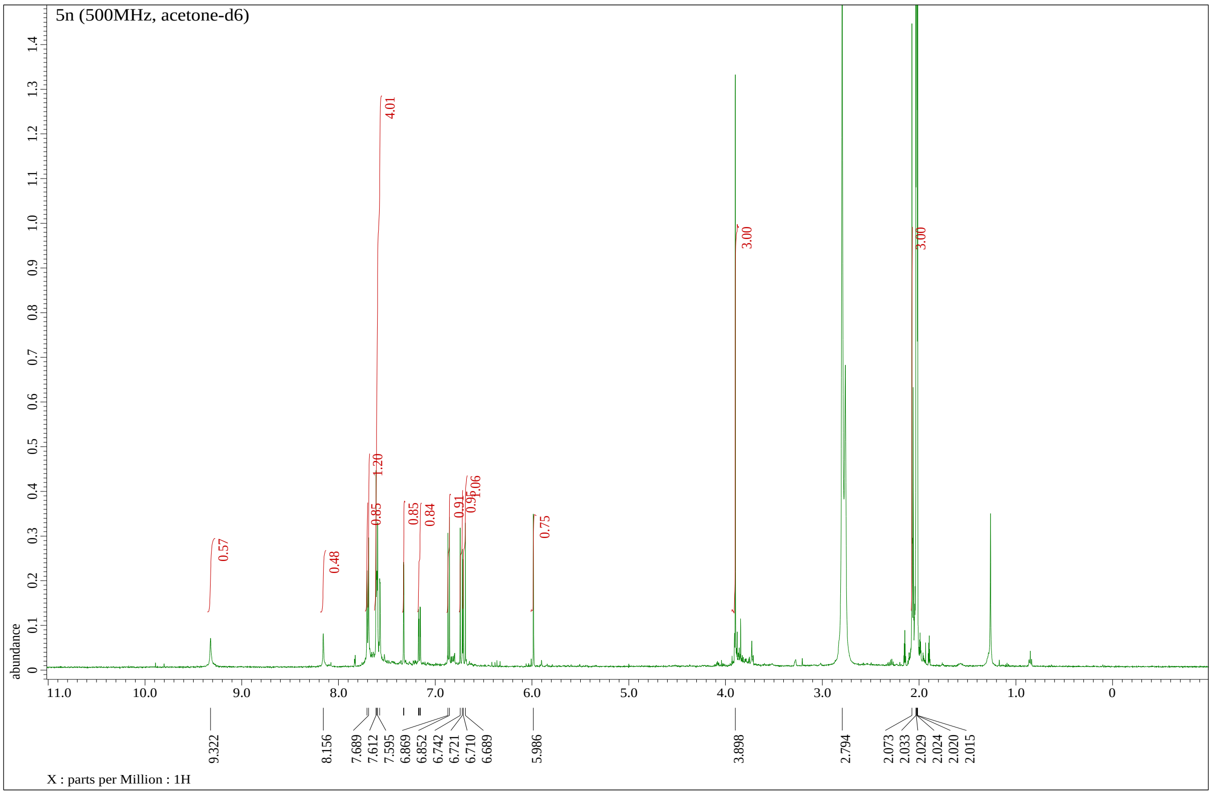


Figure S18. ^{1}H (top) and ^{13}C NMR (bottom) spectra in acetone-d_6 of **5m**



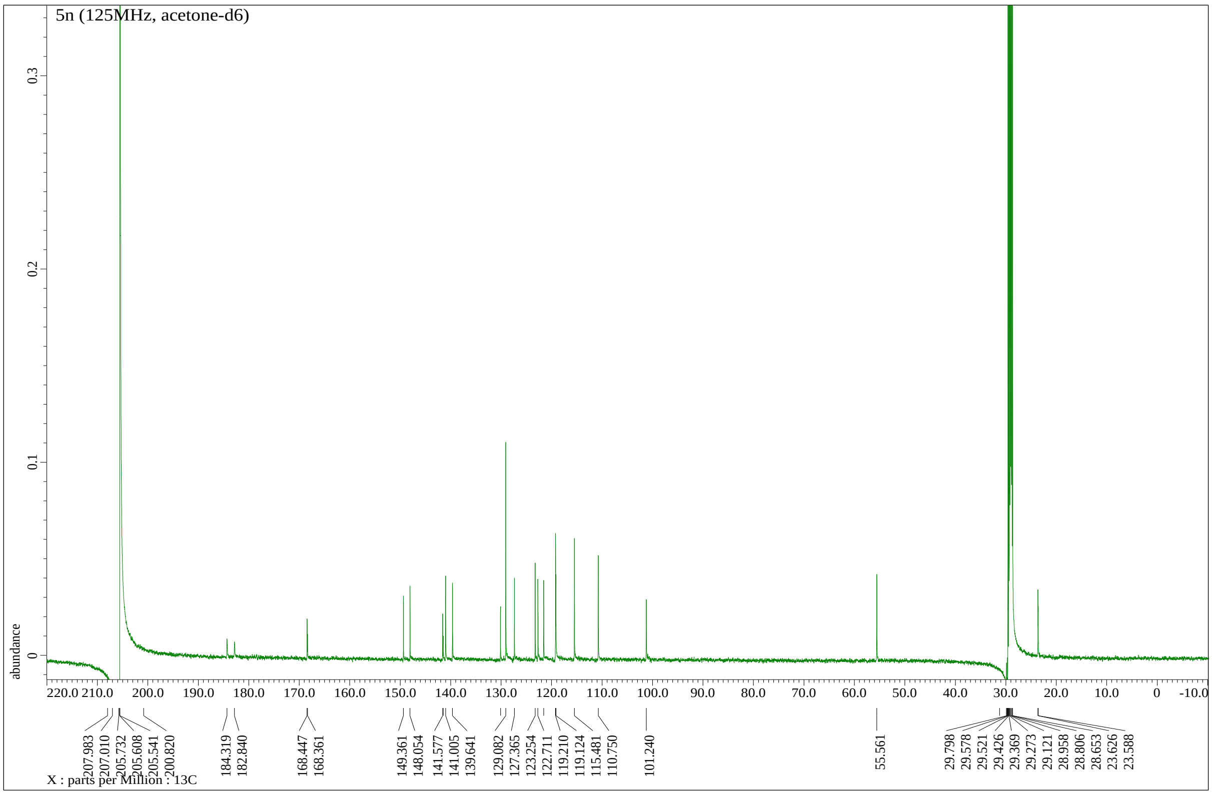
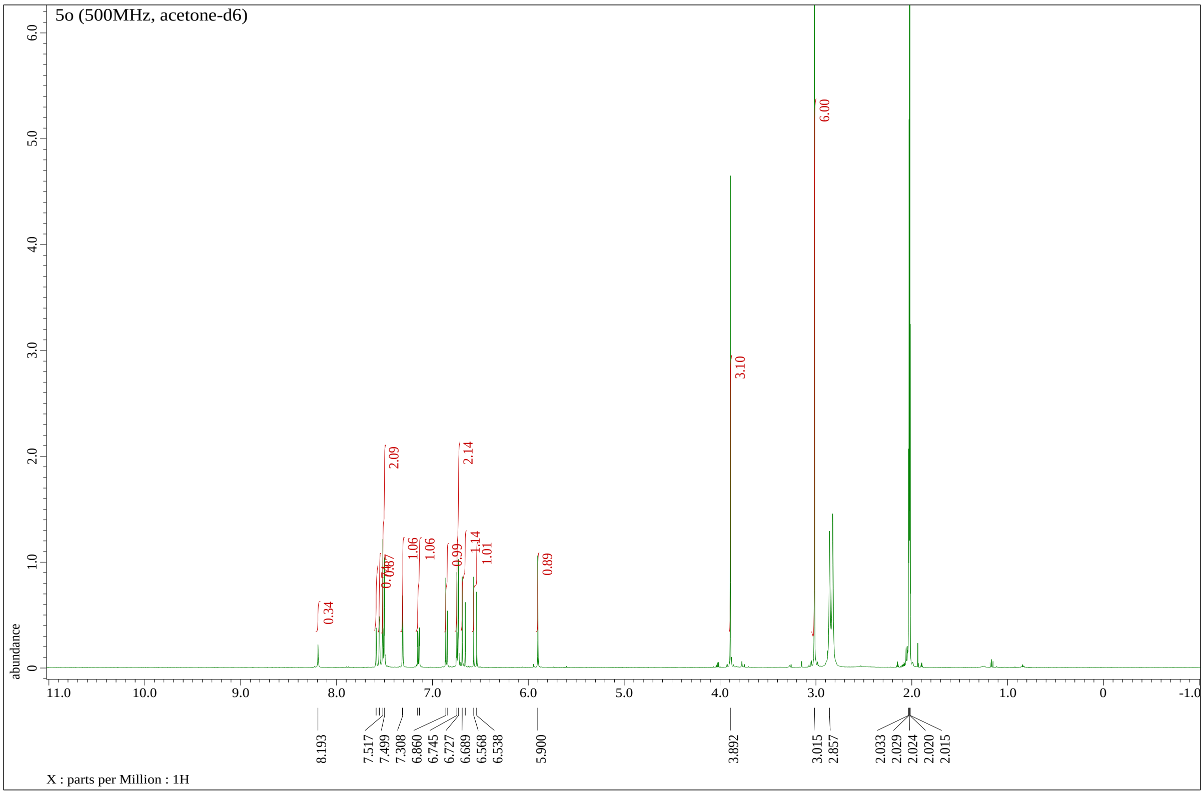


Figure S19. [1]H (top) and [13]C NMR (bottom) spectra in acetone-d_6 of **5n**



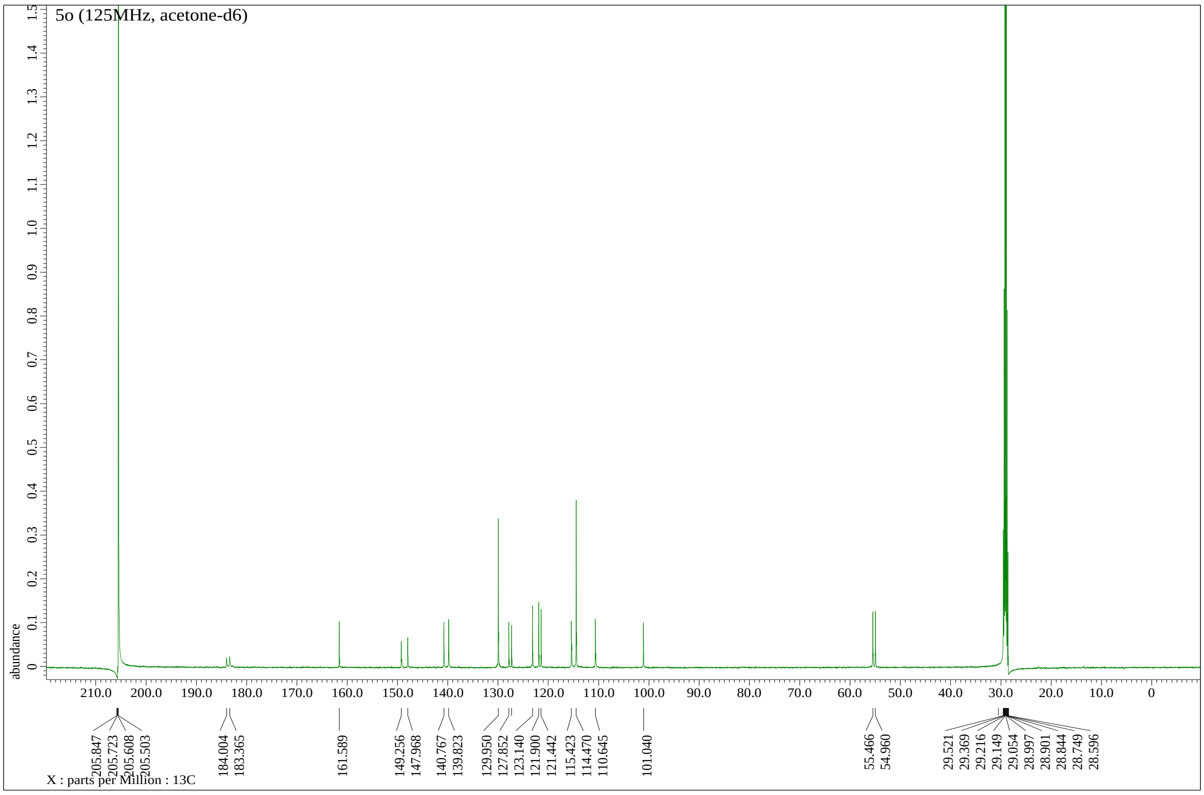
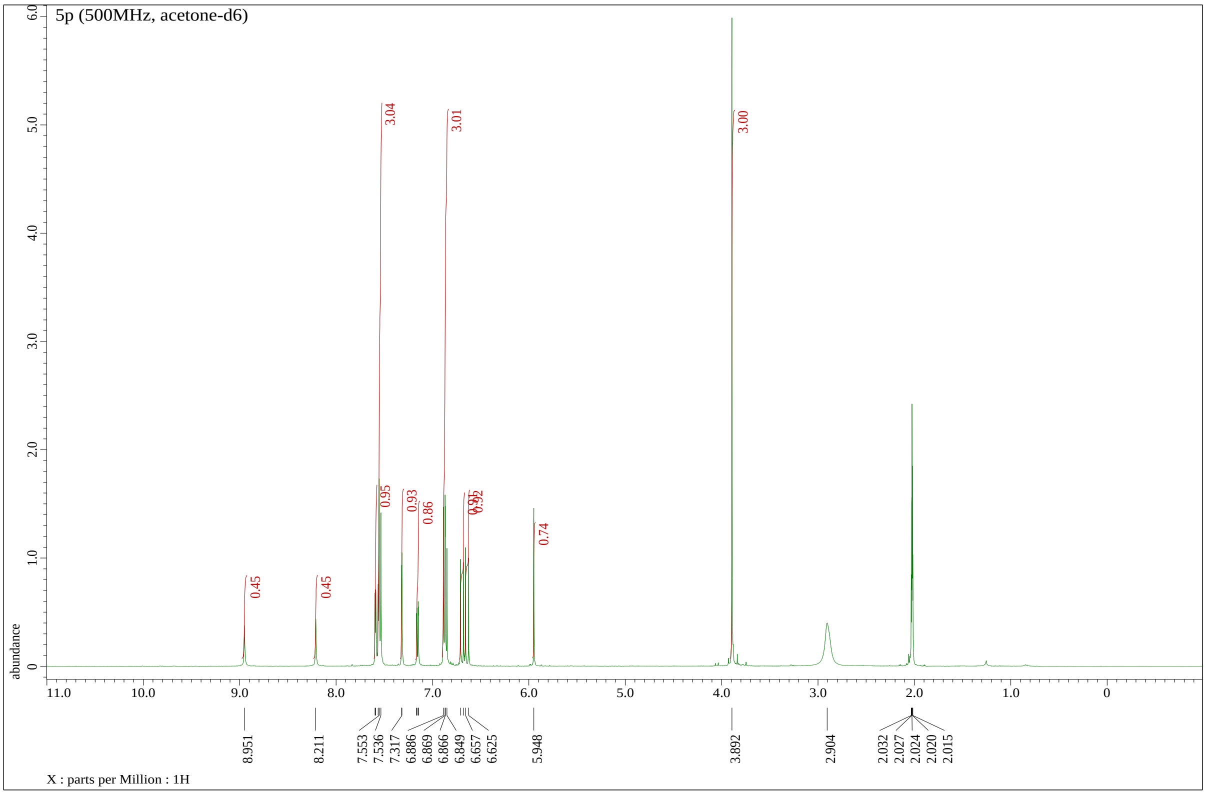


Figure S20. ^{1}H (top) and ^{13}C NMR (bottom) spectra in acetone-d_6 of **5o**



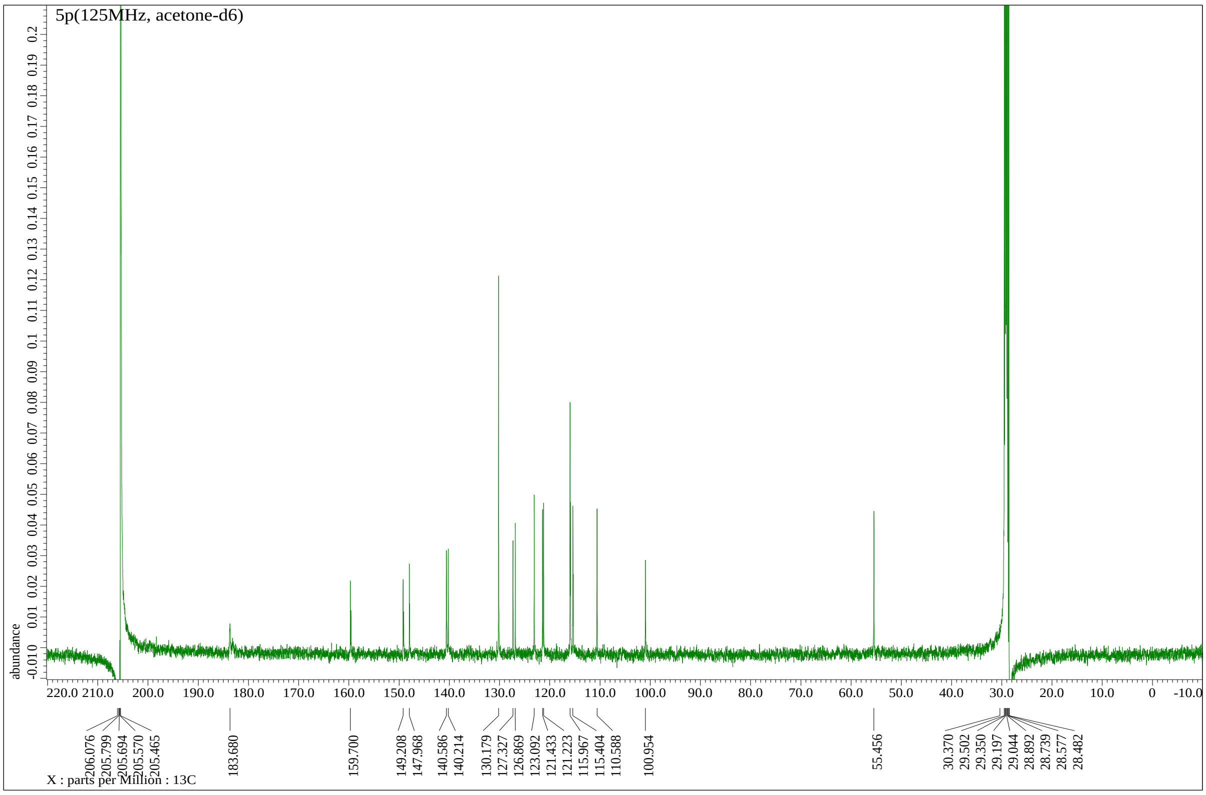
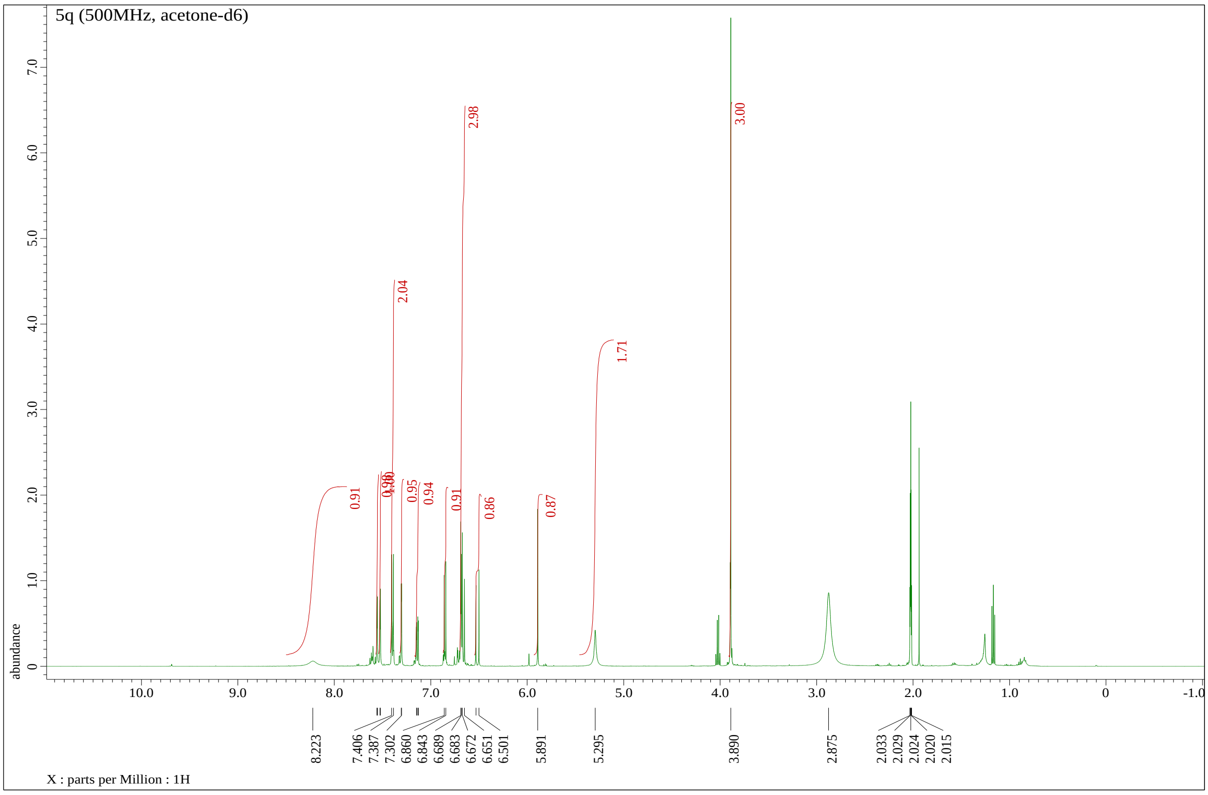


Figure S21. ^{1}H (top) and ^{13}C NMR (bottom) spectra in acetone-d_6 of **5p**



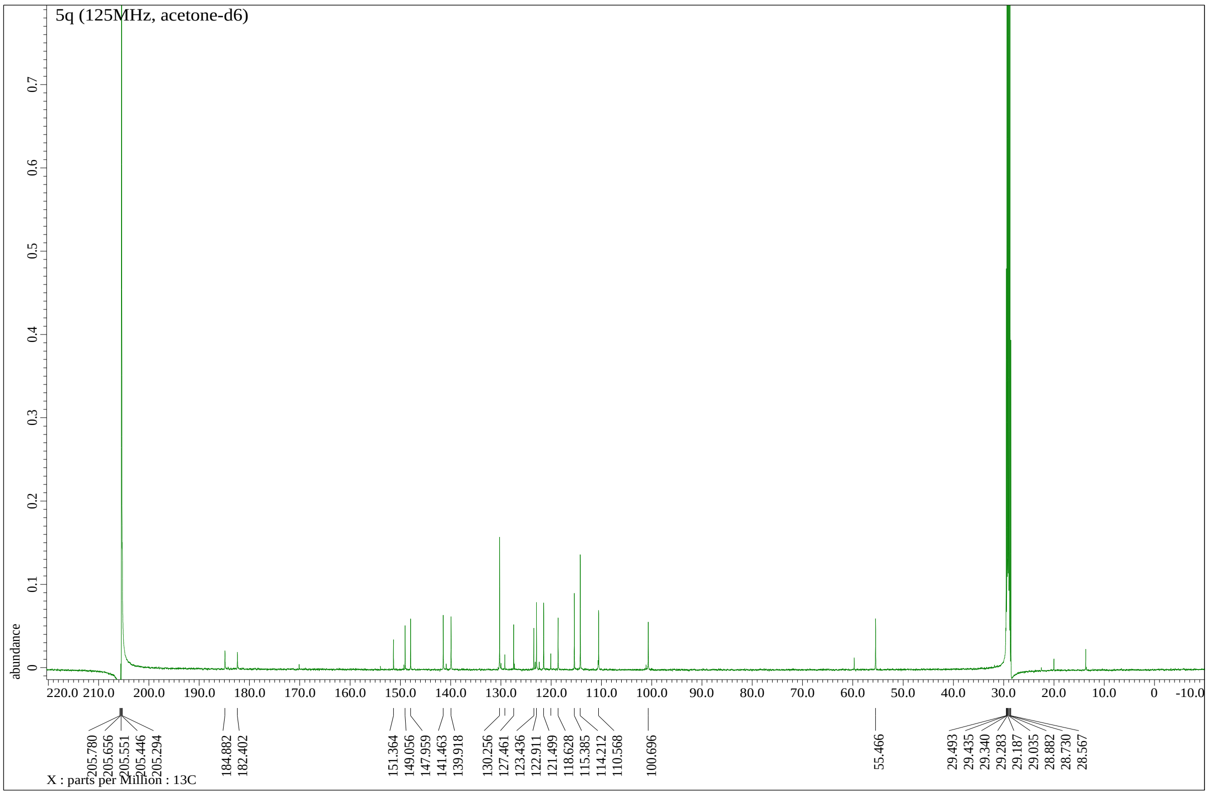
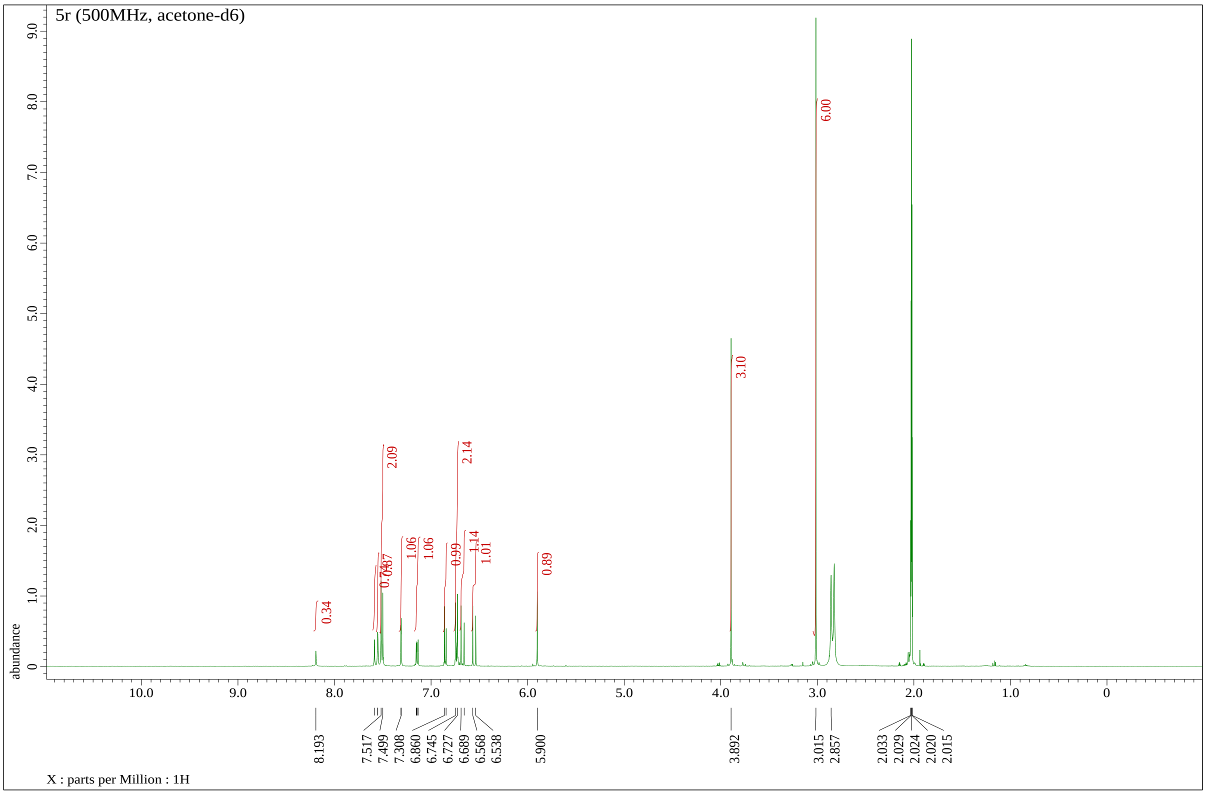


Figure S22. ^{1}H (top) and ^{13}C NMR (bottom) spectra in acetone-d_6 of **5q**



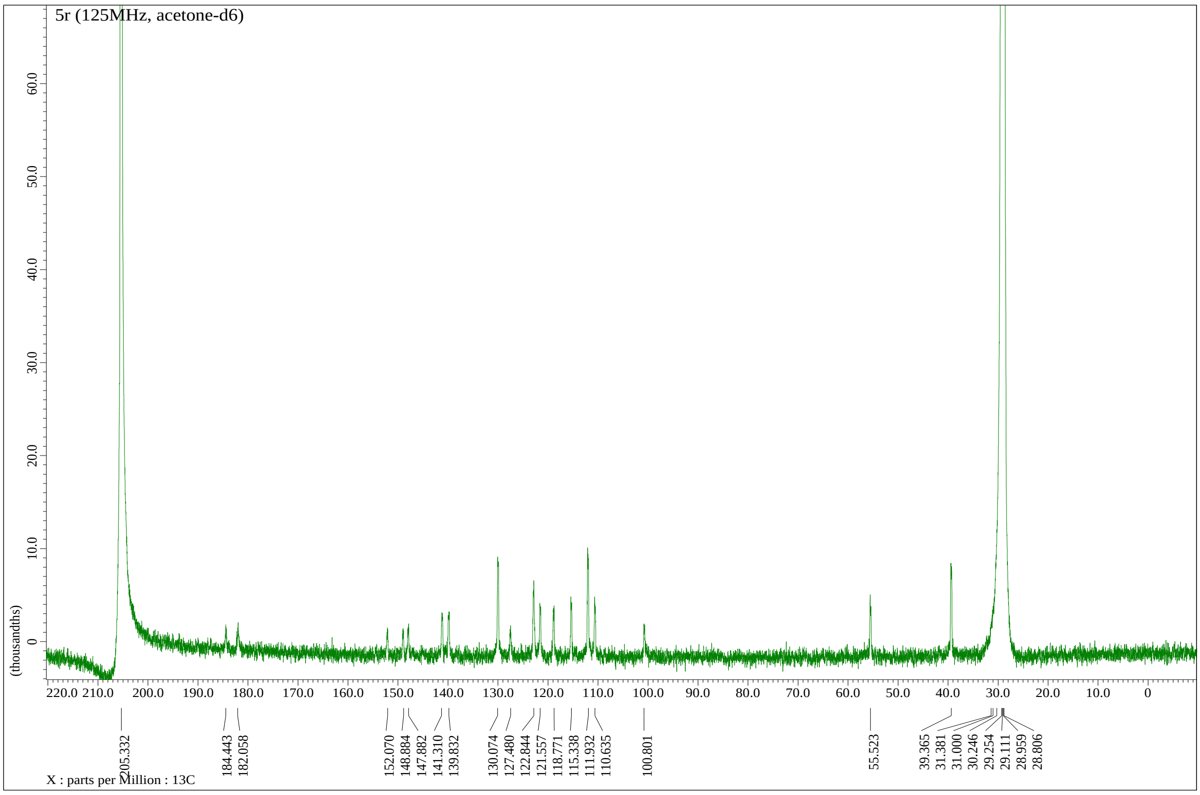
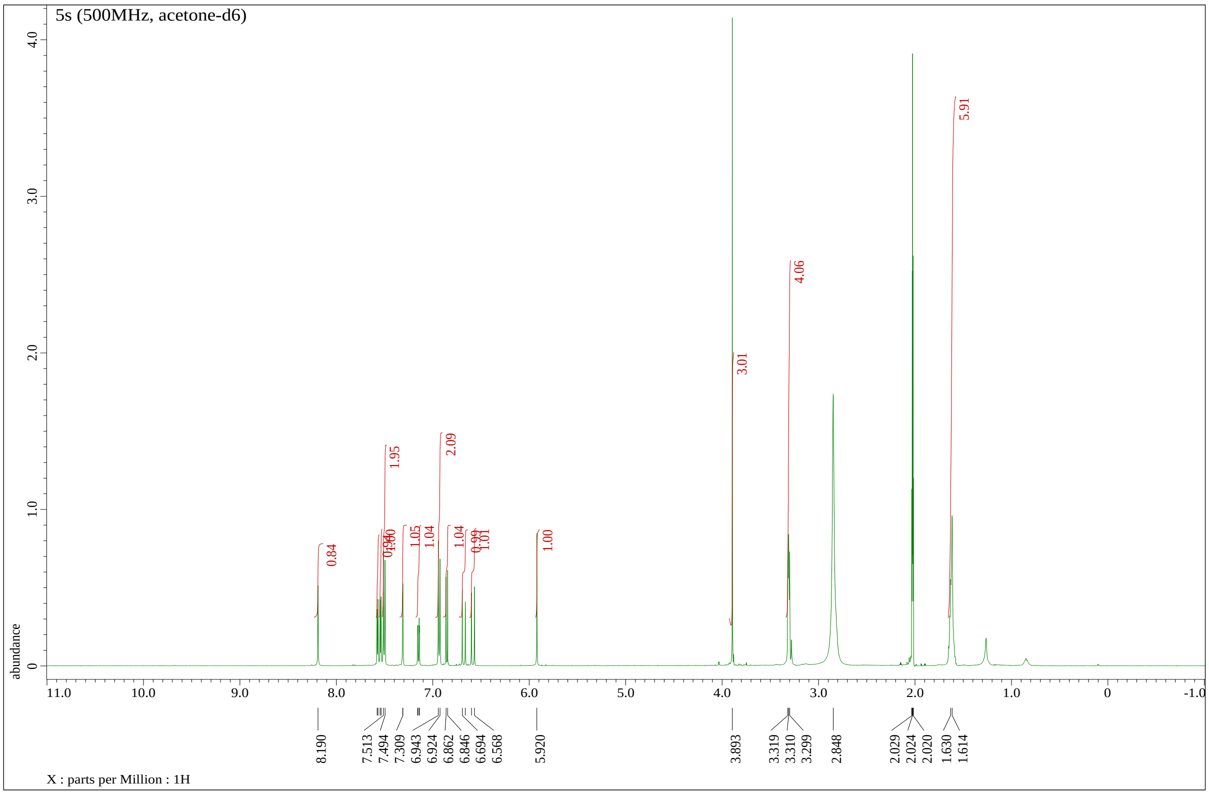


Figure S23. ^{1}H (top) and ^{13}C NMR (bottom) spectra in acetone-d_6 of **5r**



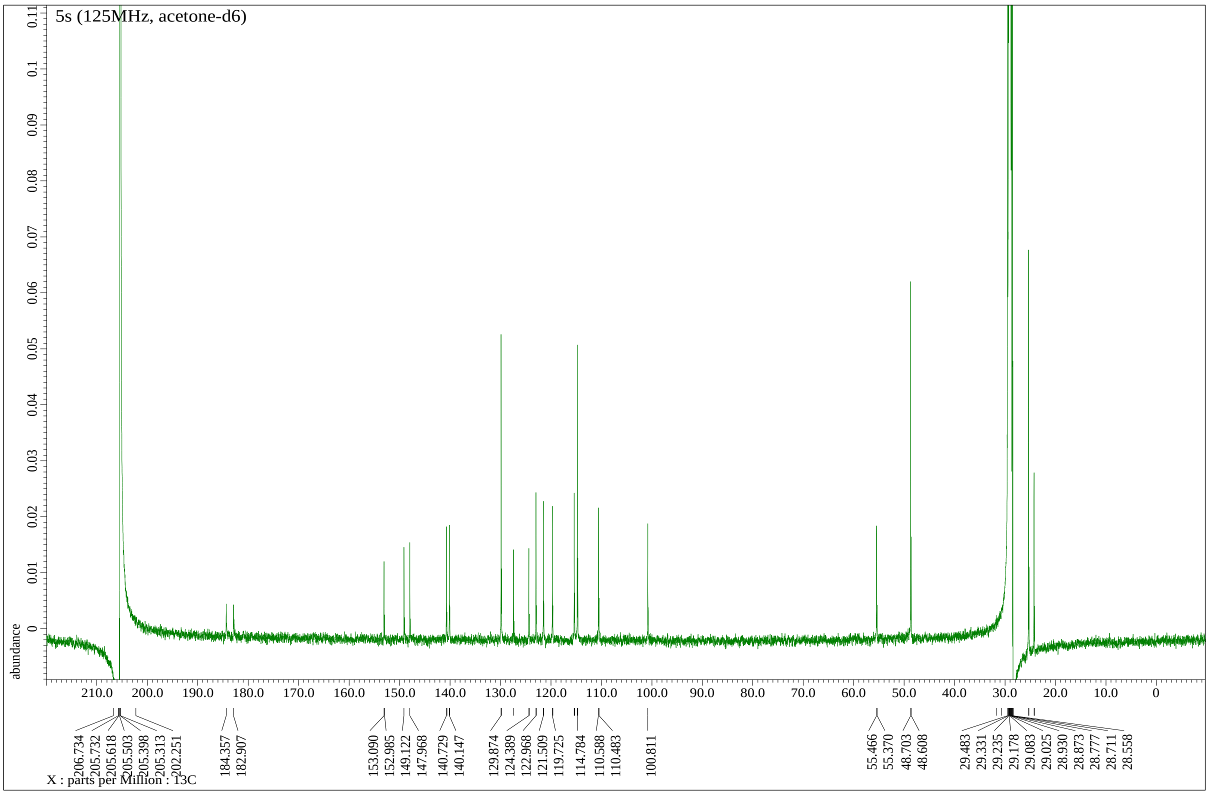
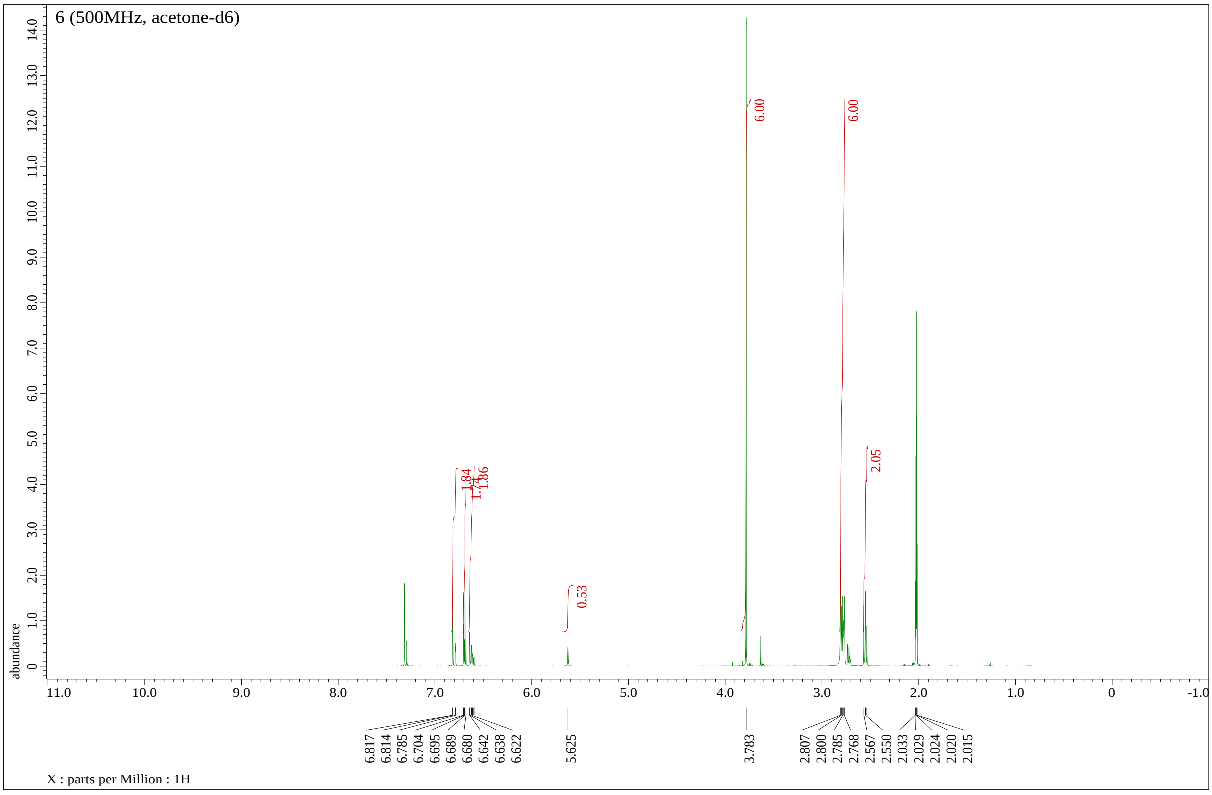


Figure S24. ^{1}H (top) and ^{13}C NMR (bottom) spectra in acetone-d_6 of **5s**



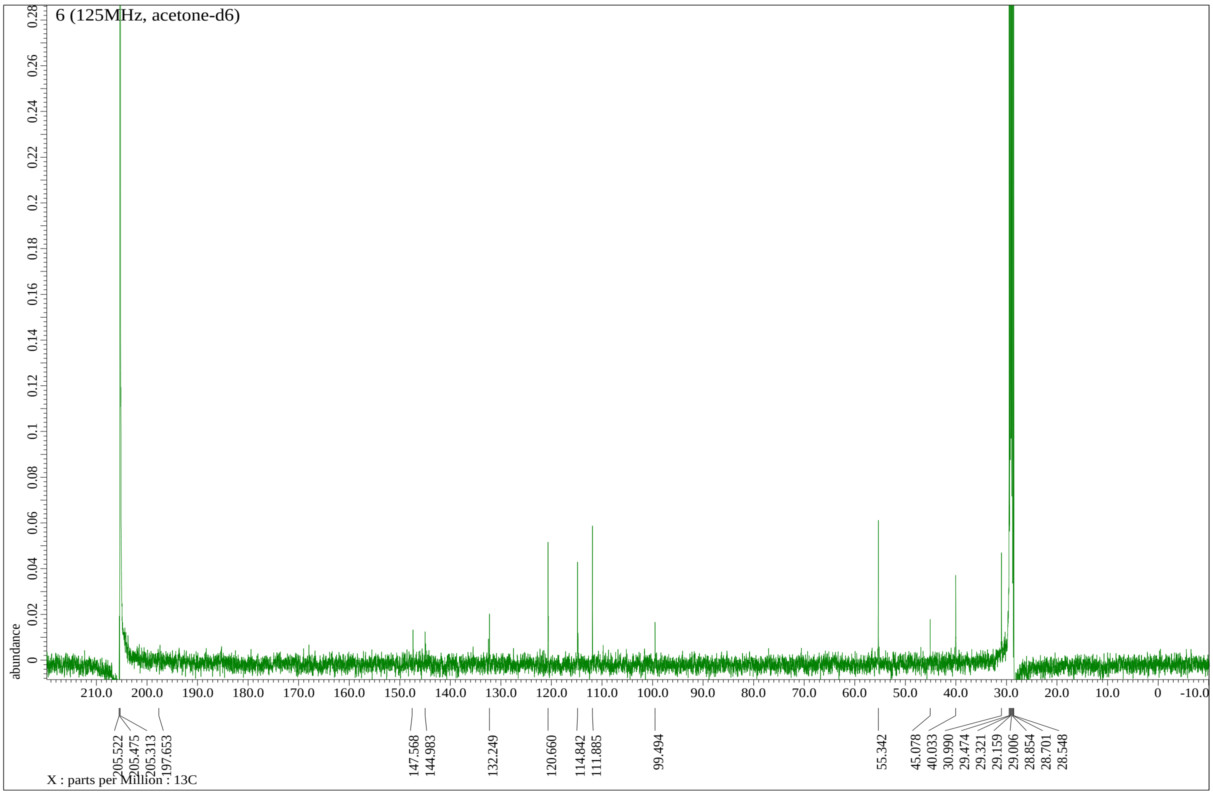
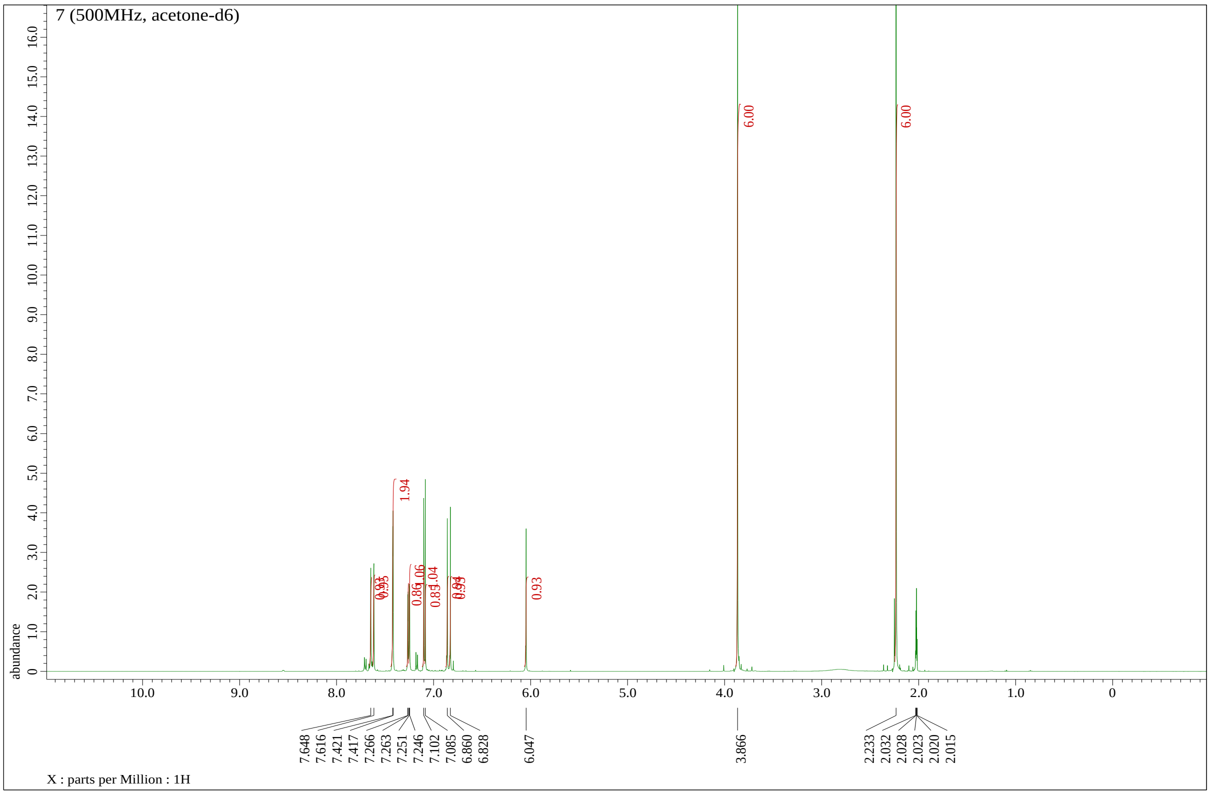


Figure S25. ^{1}H (top) and ^{13}C NMR (bottom) spectra in acetone-d_6 of **6**



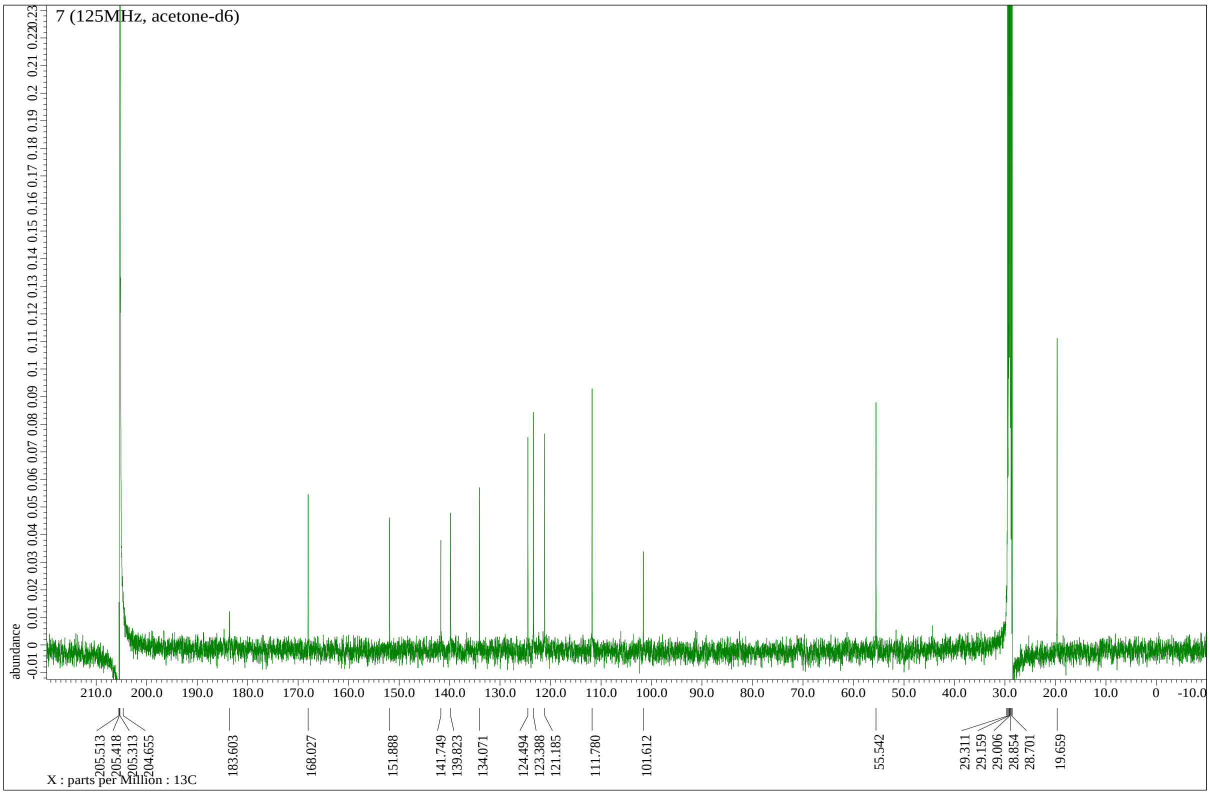
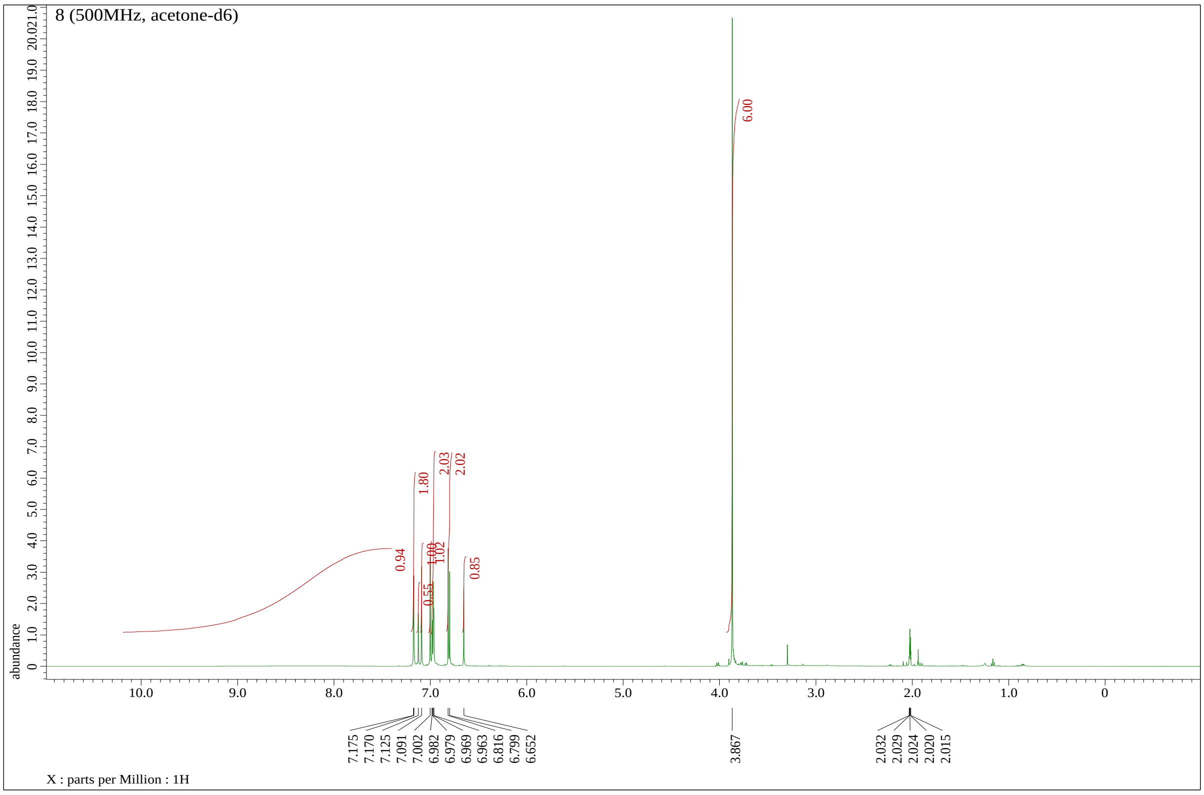


Figure S26. ^{1}H (top) and ^{13}C NMR (bottom) spectra in acetone-*d*$_6$ of **7**



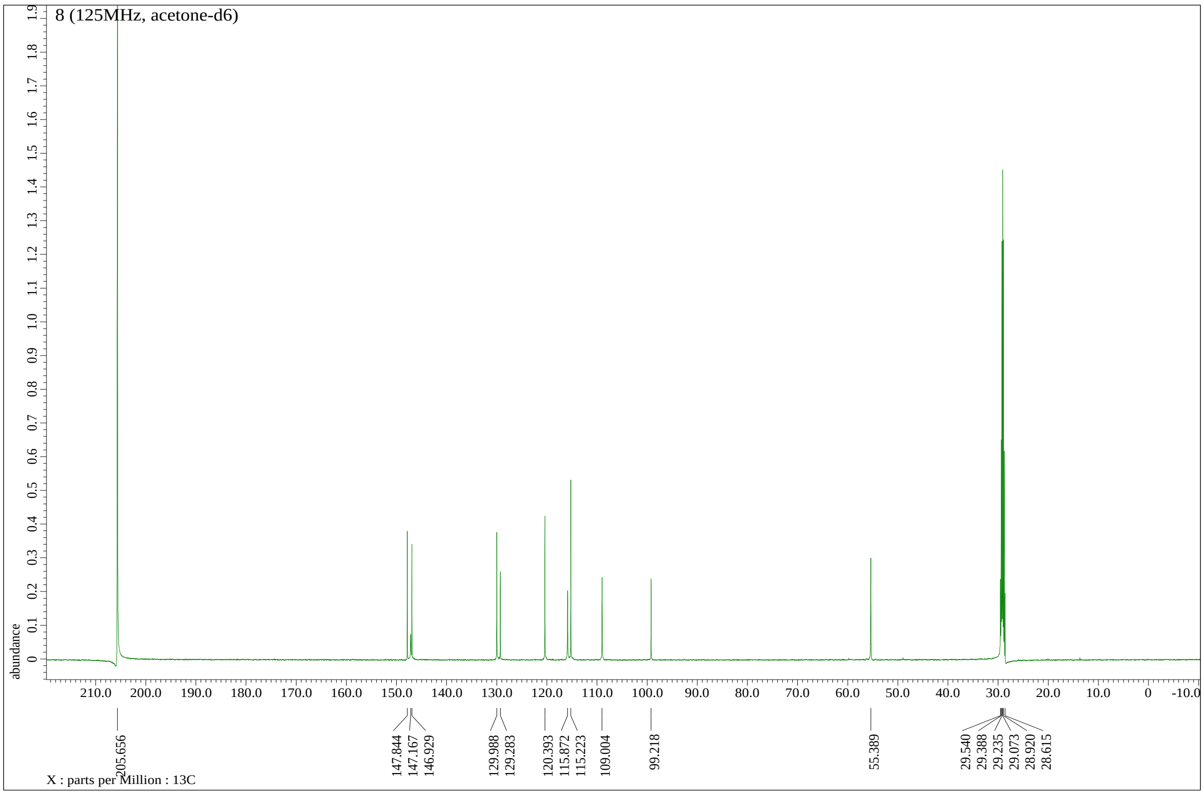


Figure S27. ^{1}H (top) and ^{13}C NMR (bottom) spectra in acetone-d_6 of **8**

2. Preparation of enzyme

```
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1021 aaactctttatgcgtggtttgactggagatcttcaggttgctacaagtaaagacggagga
     K  L  F  M  R  G  L  T  G  D  L  Q  V  A  T  S  K  D  G  G
1081 gtgacttgggagaaggatatcaaacgttatccacaggttaaagatgtctatgttcaaatg
     V  T  W  E  K  D  I  K  R  Y  P  Q  V  K  D  V  Y  V  Q  M
```
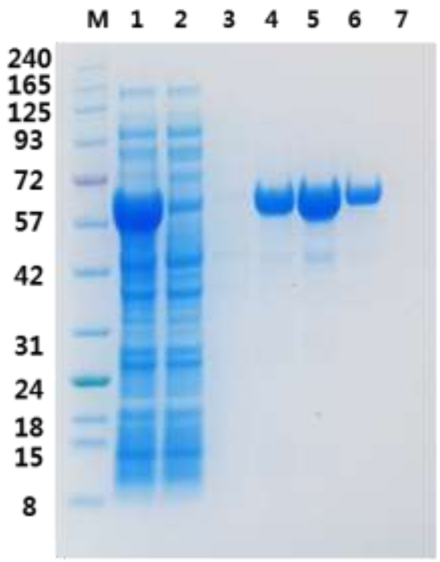

```
1141 tctgctatccatacgatgcacgaaggaaaagaatacatcatcctcagtaatgcaggtgga
     S  A  I  H  T  M  H  E  G  K  E  Y  I  I  L  S  N  A  G  G
1201 ccgaaacgtgaaaatgggatggtccacttggcacgtgtcgaagaaaatggtgagttgact
     P  K  R  E  N  G  M  V  H  L  A  R  V  E  E  N  G  E  L  T
1261 tggctcaaacacaatccaattcaaaaaggagagtttgcctataattcgctccaagaatta
     W  L  K  H  N  P  I  Q  K  G  E  F  A  Y  N  S  L  Q  E  L
1321 ggaaatggggagtatggcatcttgtatgaacatactgaaaaaggacaaaatgcctatacc
     G  N  G  E  Y  G  I  L  Y  E  H  T  E  K  G  Q  N  A  Y  T
1381 ctatcatttagaaaatttaattgggactttttgagcaaagatctgatttctcctaccgaa
     L  S  F  R  K  F  N  W  D  F  L  S  K  D  L  I  S  P  T  E
1441 gcgaaagtgaagcgaactagagagatgggcaaaggagttattggcttggagttcgactca
     A  K  V  K  R  T  R  E  M  G  K  G  V  I  G  L  E  F  D  S
1501 gaagtattggtc
     E  V  L  V
```

Figure S28. Nucleotide sequence and protein sequence of synthesized *S. pneumoniae* neuraminidase A (NanA)



lane 1 : Crude NanA
lane 2 : Flow through
lane 3 : washing (Binding buffer)
lane 4 : Elution (50 mM imidazole)
lane 5 : Elution (100 mM imidazole)
lane 6 : Elution (200 mM imidazole)
lane 7 : Elution (300 mM imidazole)

Figure S29. Expression and purification of *S. pneumoniae* NanA.

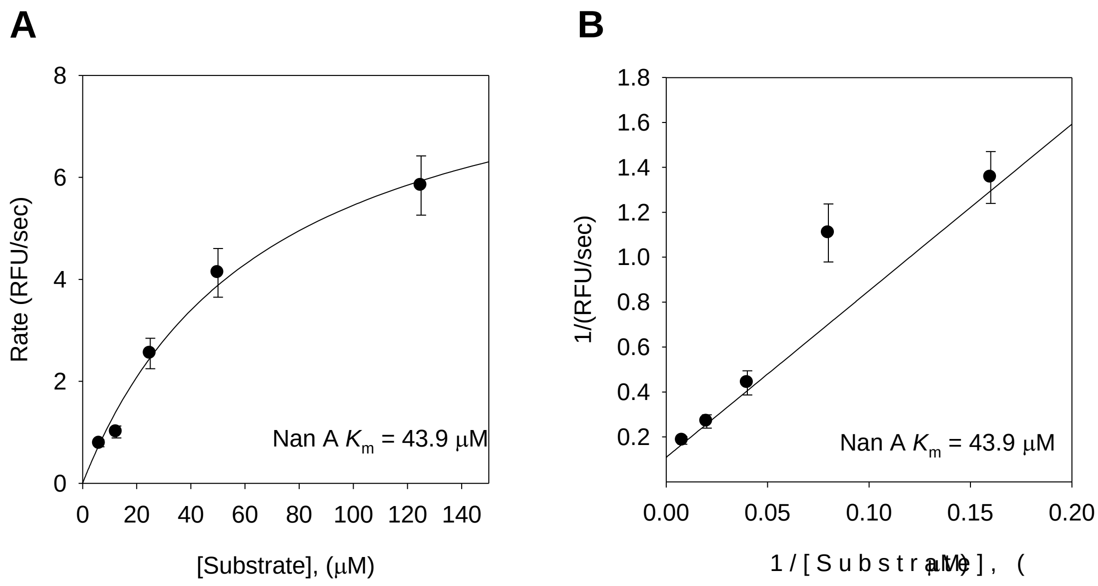
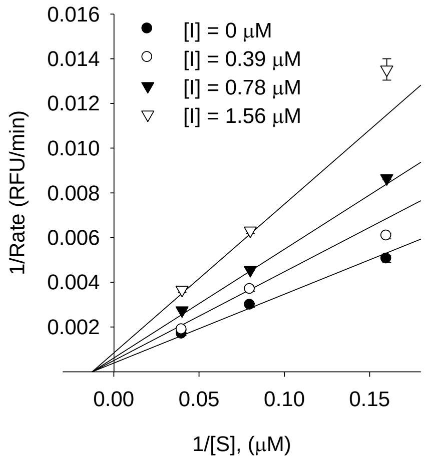
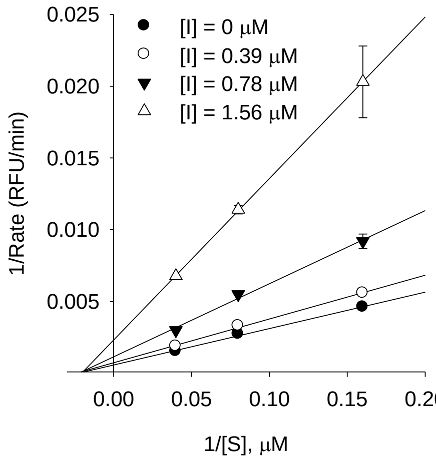


Figure S30. Michaelis-Menten plots (A) and Linewever-Burk plot (B) of *S. pneumoniae* NanA K_m values. The reaction was performed at various substrate concentrations to obtain enzyme K_m values. SigmaPlot was used to fit the kinetic data using Michaelis-Menten and Lineweaver-Burk double reciprocal plots.

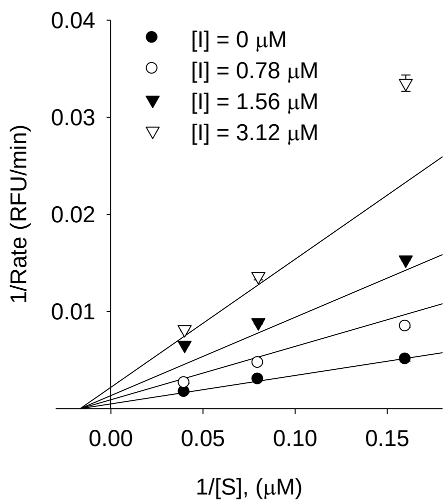
[A] **4a**



[B] **4e**



[C] **5q**



[D] **5e**

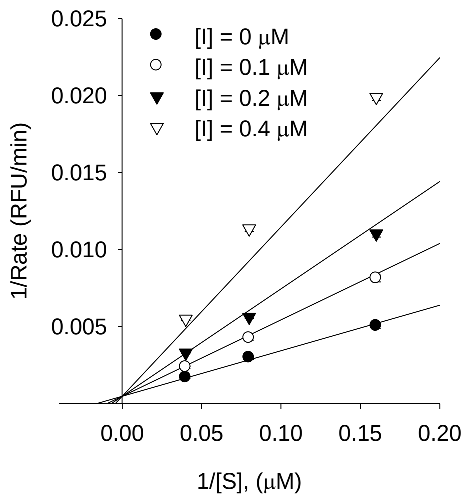
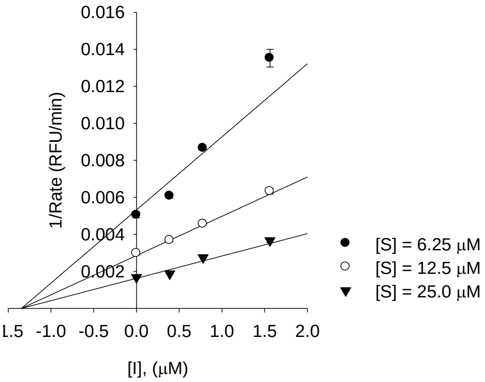
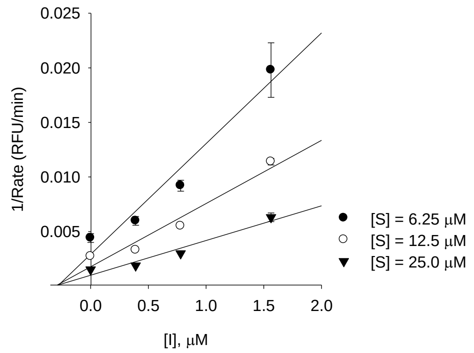


Figure S31. Graphical determination of the inhibition type for compounds **4a**, **4e**, **5q** and **5e**. Lineweaver-Burk (A-D) and Dixon (E-H) plots for the inhibitory activity of compounds **4a**, **4e**, **5q** and **5e**, respectively, against *S. pneumoniae* NanA hydrolysis activity in the presence of different substrate concentrations.

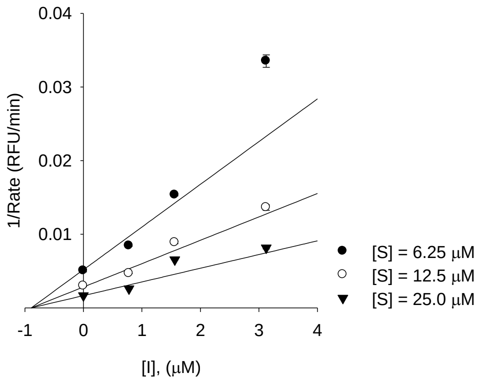
[E] **4a**



[F] **4e**



[G] **5q**



[H] **5e**

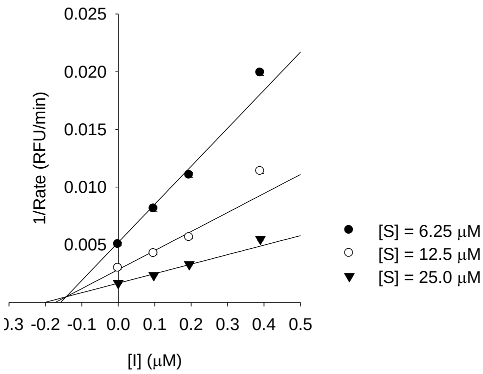


Figure S31. Graphical determination of the inhibition type for compounds **4a**, **4e**, **5q** and **5e**. Lineweaver-Burk (A-D) and Dixon (E-H) plots for the inhibitory activity of compounds **4a**, **4e**, **5q** and **5e**, respectively, against *S. pneumoniae* NanA hydrolysis activity in the presence of different substrate concentrations (continued).