Supporting Information

for

Influence of the 4'-substituent on the efficiency of flavonol-based fluorescent indicators of β-glycosidase activity

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Fluorescence spectra at pH 5.2; results of the ESP analysis; NMR spectra of compounds considered.

Table of Contents

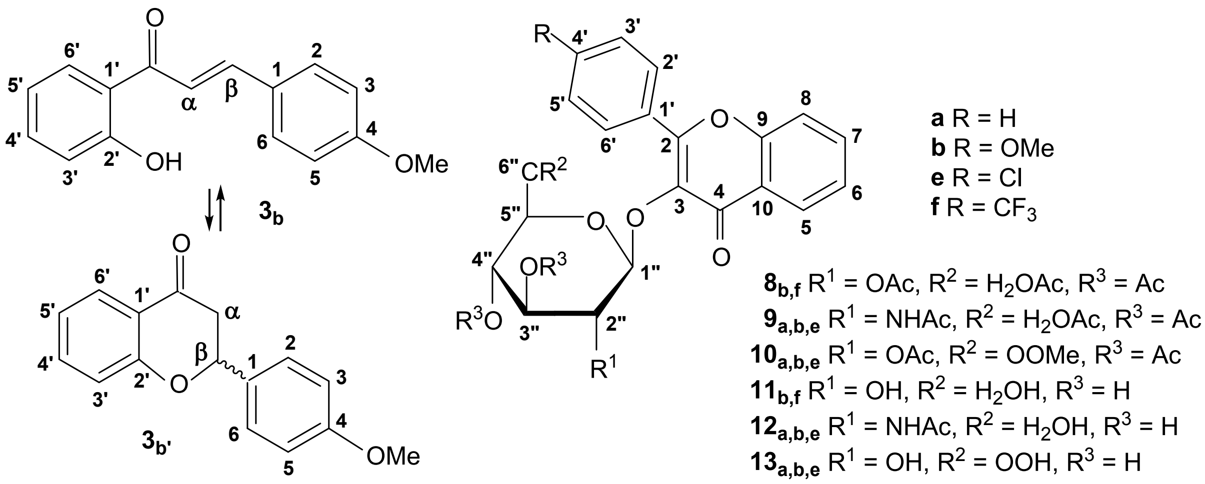
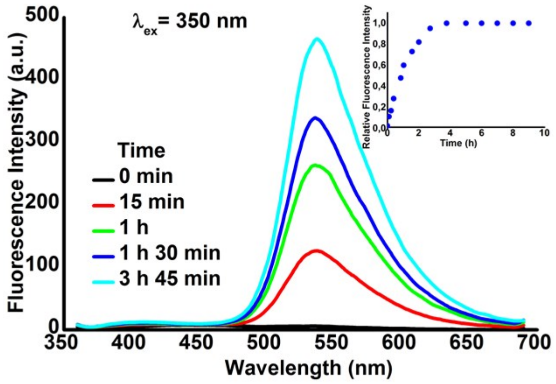
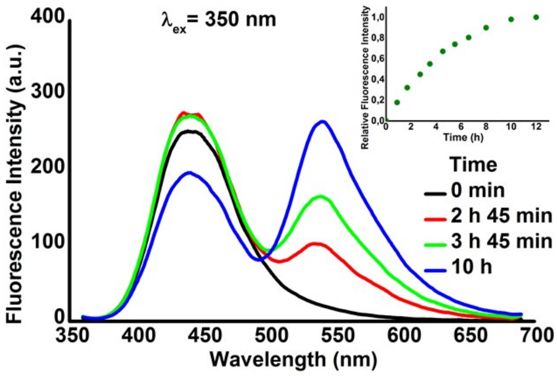


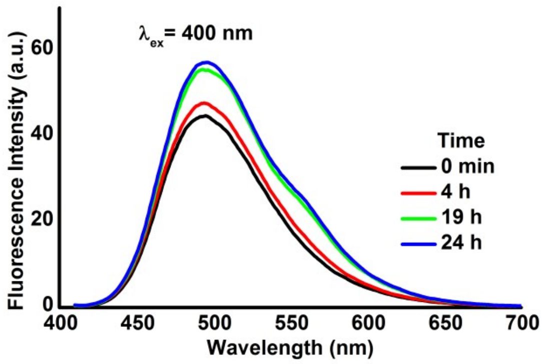
Figure S1. The atom numbering system for compounds considered.



$\mathbf{11_a}$



$\mathbf{11_b}$



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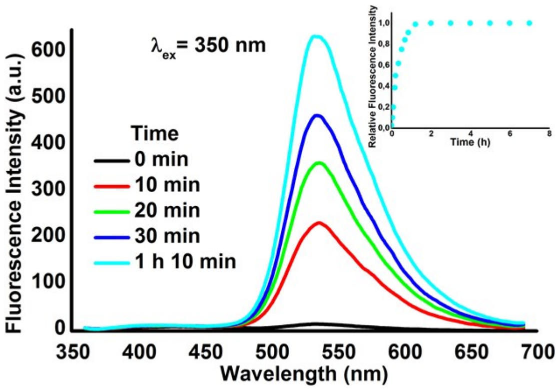

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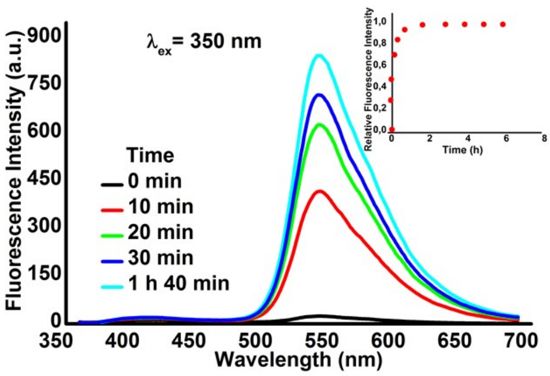
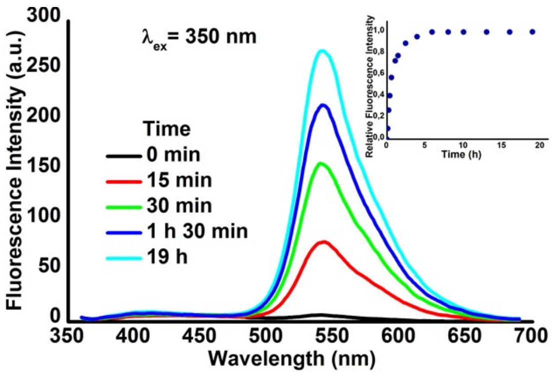
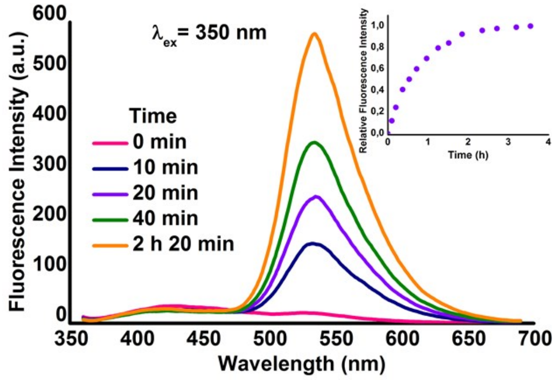


$\mathbf{11_e}$

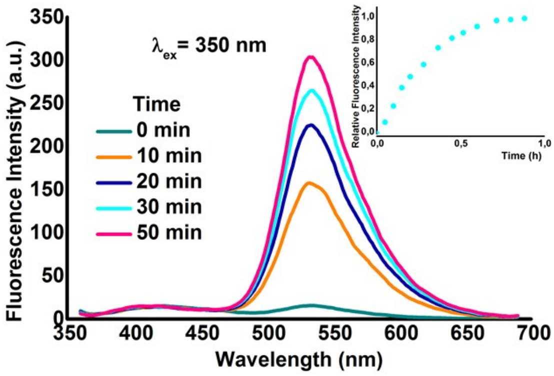


$\mathbf{11_f}$

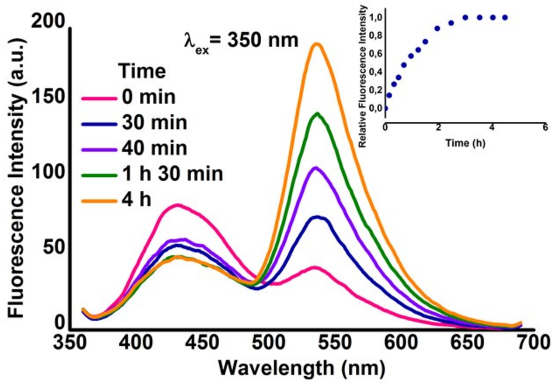
Figure S2. Changes in the fluorescence spectra during enzymatic hydrolysis of probes $\mathbf{11_{a\text{-}f}}$ at pH 5.2. Insets: plots of relative intensity of the T* fluorescence (I_i^{T*}/ I_{max}^{T*}) versus time.



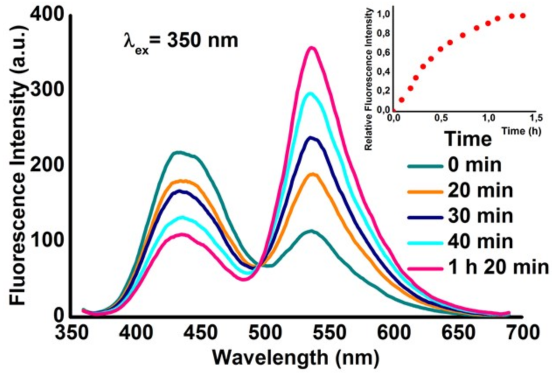
12_a



13_a



12_b



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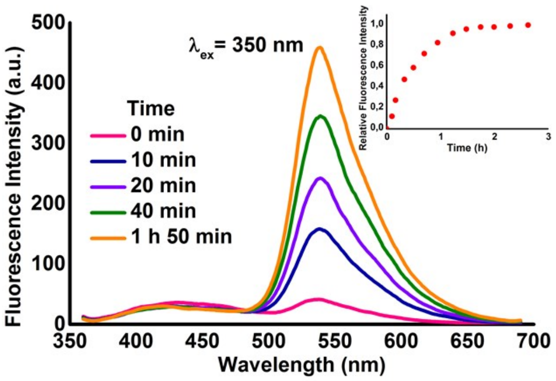
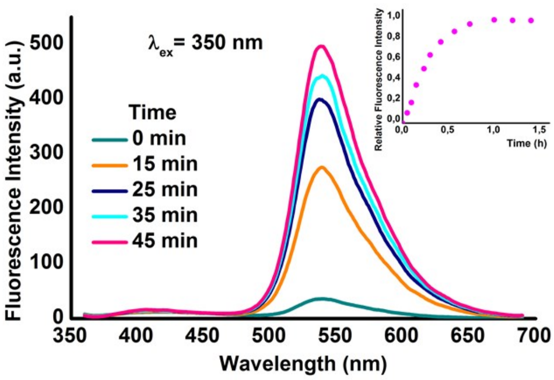
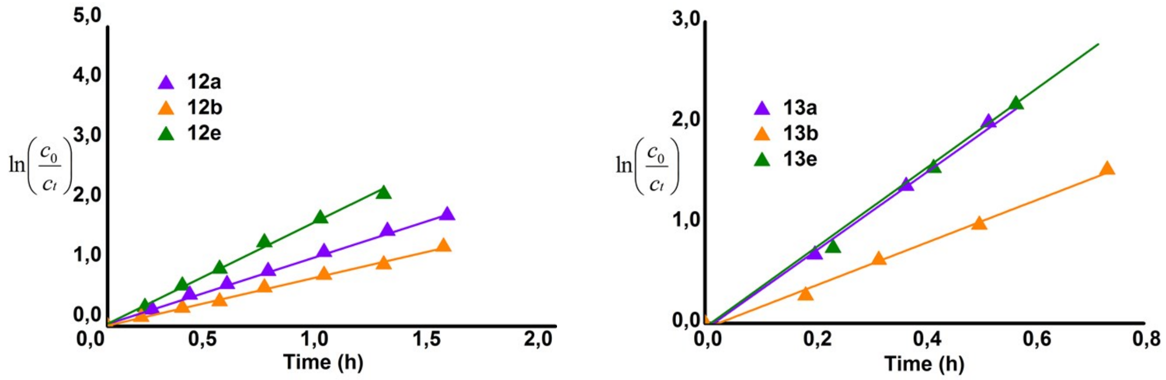


12_e



13_e

Figure S3. Changes in the fluorescence spectra during enzymatic hydrolysis of probes **$12_{a,b,e}$** and **$13_{a,b,e}$** at pH 5.2. Insets: plots of relative intensity of the T* fluorescence (I_i^{T*}/ I_{max}^{T*}) versus time.

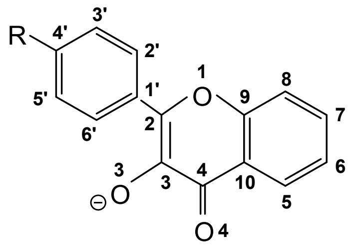


$\mathbf{12}_{a,b,e}$ $\mathbf{13}_{a,b,e}$

Figure S4. The plots of ln c_o/c_t vs time for enzymatic hydrolysis of $\mathbf{12}_{a,b,e}$ and $\mathbf{13}_{a,b,e}$ at pH 5.2.

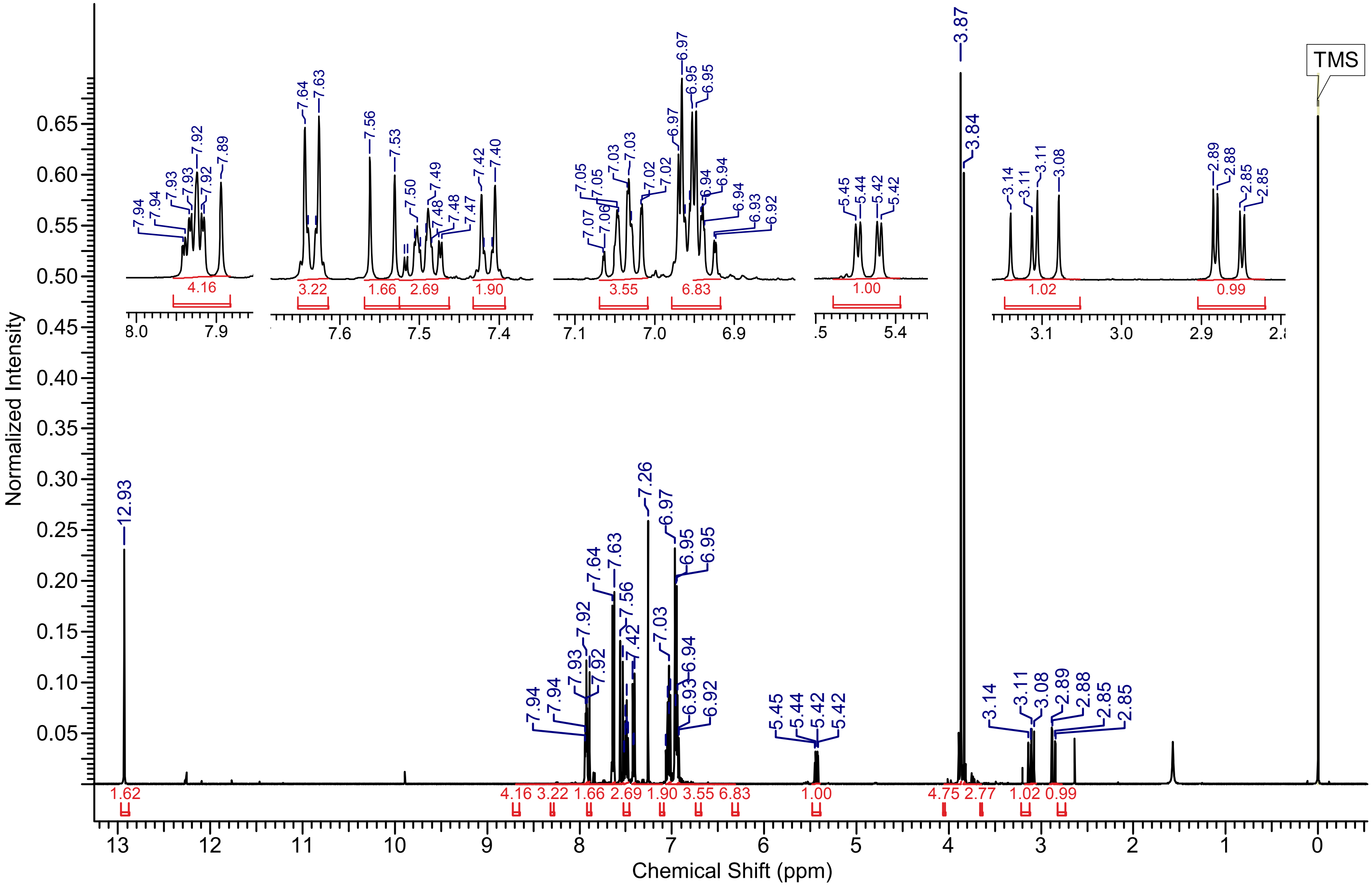
Table S1. The charge distribution in the anionic forms of $\mathbf{4}_a$-$\mathbf{4}_f$ from the ESP analysis.

$\mathbf{4}_a$ R = H
$\mathbf{4}_b$ R = OCH_3
$\mathbf{4}_c$ R = $N(CH_3)_2$
$\mathbf{4}_d$ R = F
$\mathbf{4}_e$ R = Cl
$\mathbf{4}_f$ R = CF_3

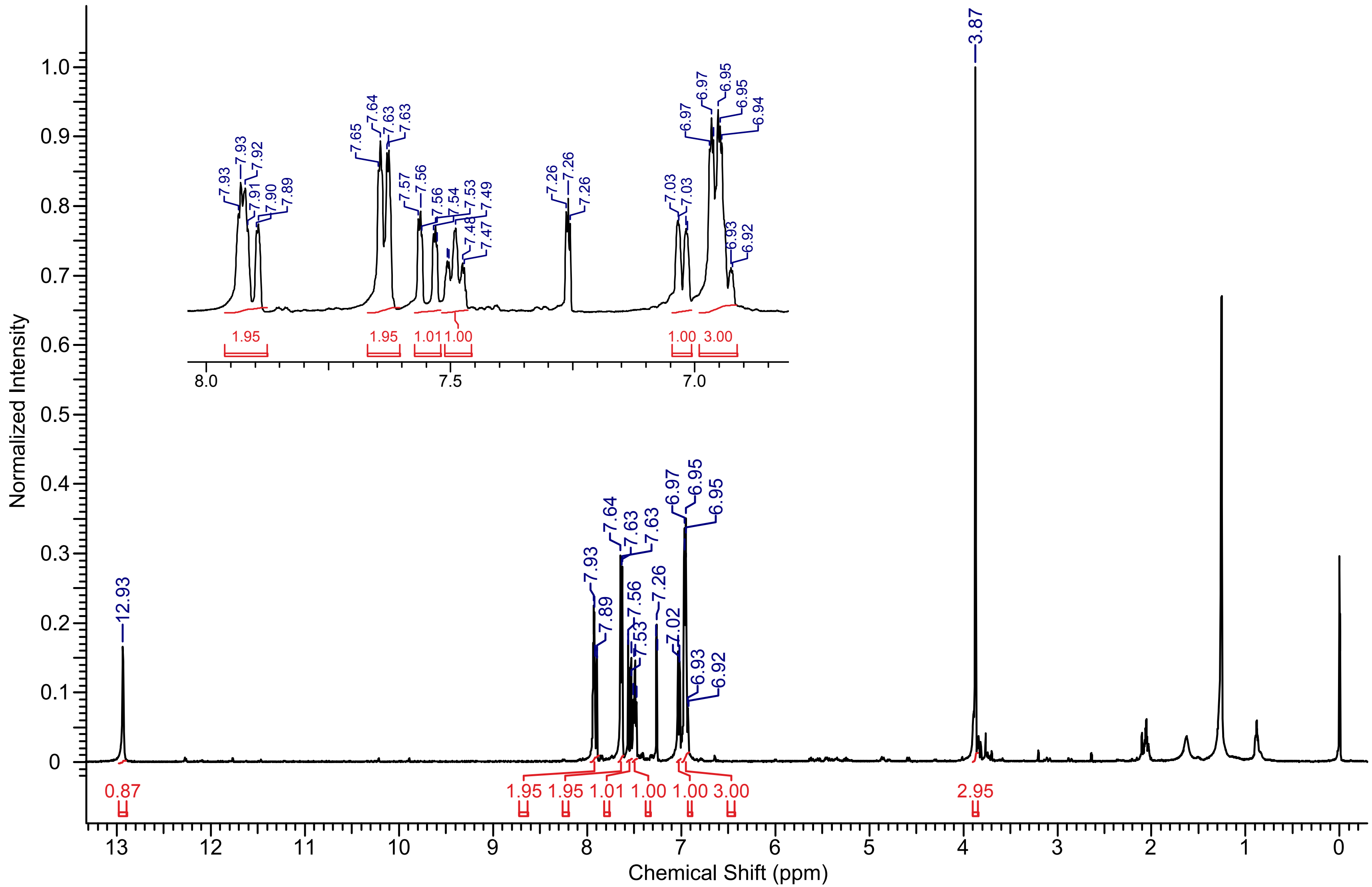


Atom	$\mathbf{4}_a$	$\mathbf{4}_b$	$\mathbf{4}_c$	$\mathbf{4}_d$	$\mathbf{4}_e$	$\mathbf{4}_f$
O1	-0.2289	-0.2365	-0.2336	-0.2522	-0.2469	-0.2396
C2	-0.4015	-0.0413	-0.3763	-0.0564	-0.1514	-0.2827
O3	-0.6036	-0.5948	-0.6116	-0.5878	-0.5838	-0.5634
O4	-0.5186	-0.5436	-0.5268	-0.5389	-0.5321	-0.5437
C4	0.4074	0.5275	0.4132	0.5311	0.5102	0.5778
C9	0.4638	0.3905	0.4613	0.3958	0.4260	0.5035
C10	-0.2307	-0.3404	-0.2353	-0.3166	-0.3138	-0.3738
C1’	0.4683	-0.0607	0.4254	0.0181	0.1949	0.3562
C2’	-0.3303	-0.0321	-0.2964	-0.0950	-0.2178	-0.2348
C3’	-0.1184	-0.3781	-0.3169	-0.4018	-0.1120	-0.1744
C4’	-0.2284	-0.4821	-0.1803	-0.4130	-0.0062	-0.0812
C5’	-0.0807	-0.4857	-0.2656	-0.3682	-0.1297	-0.1678
C6’	-0.2818	-0.1160	-0.2462	-0.0937	-0.2390	-0.2351
R	0.1010 (H)	-0.3527 (O) -0.0476 (C)	-0.0764 (N) -0.1184 (C) -0.1349 (C)	-0.2622 (F)	-0.2013 (Cl)	0.6092 (C) -0.2253 (F) -0.2404 (F) -0.2404 (F)

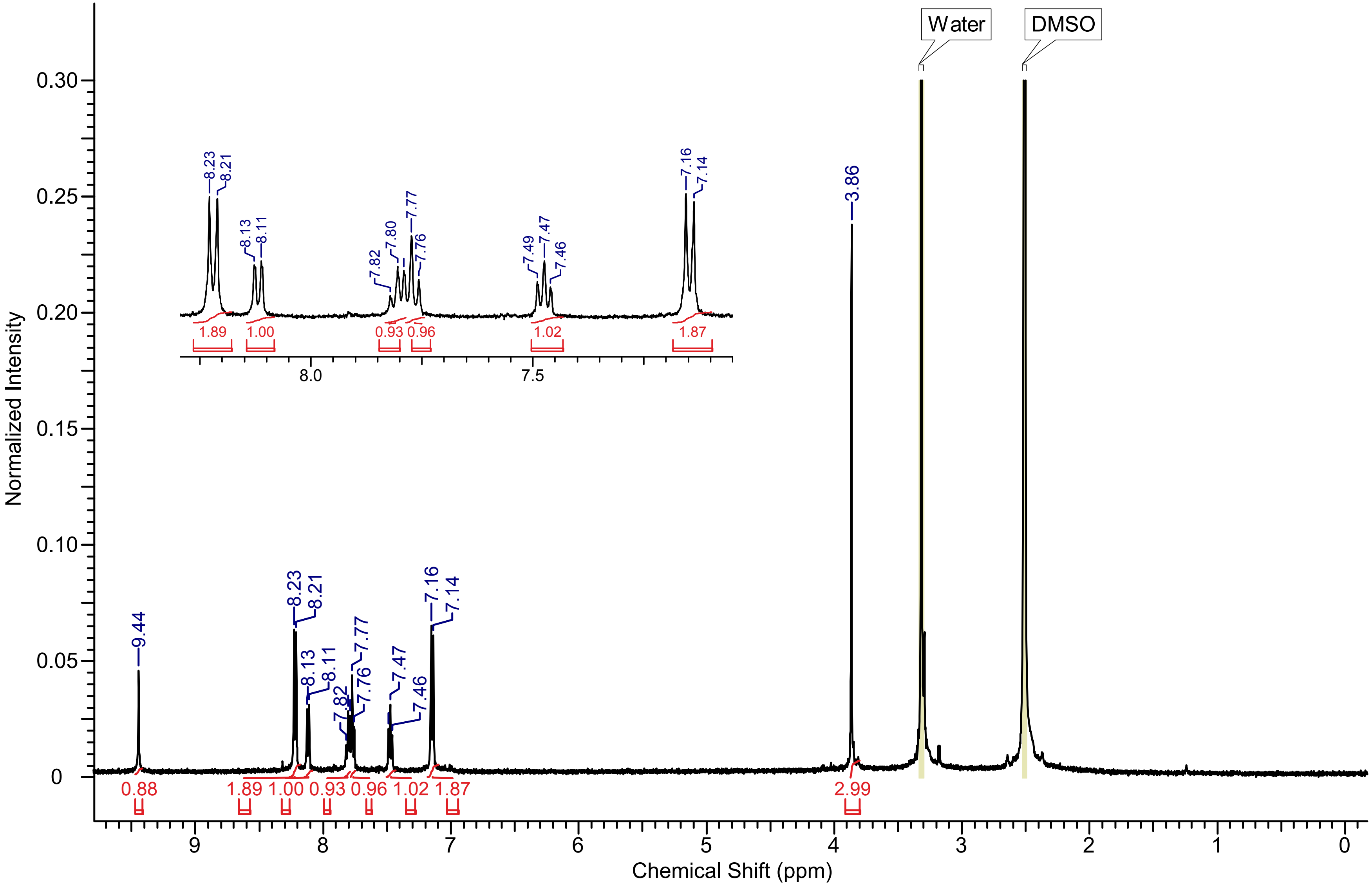
1. ^{1}H NMR spectrum of 3_b+$3_{b'}$ (500 MHz, $CDCl_3$)



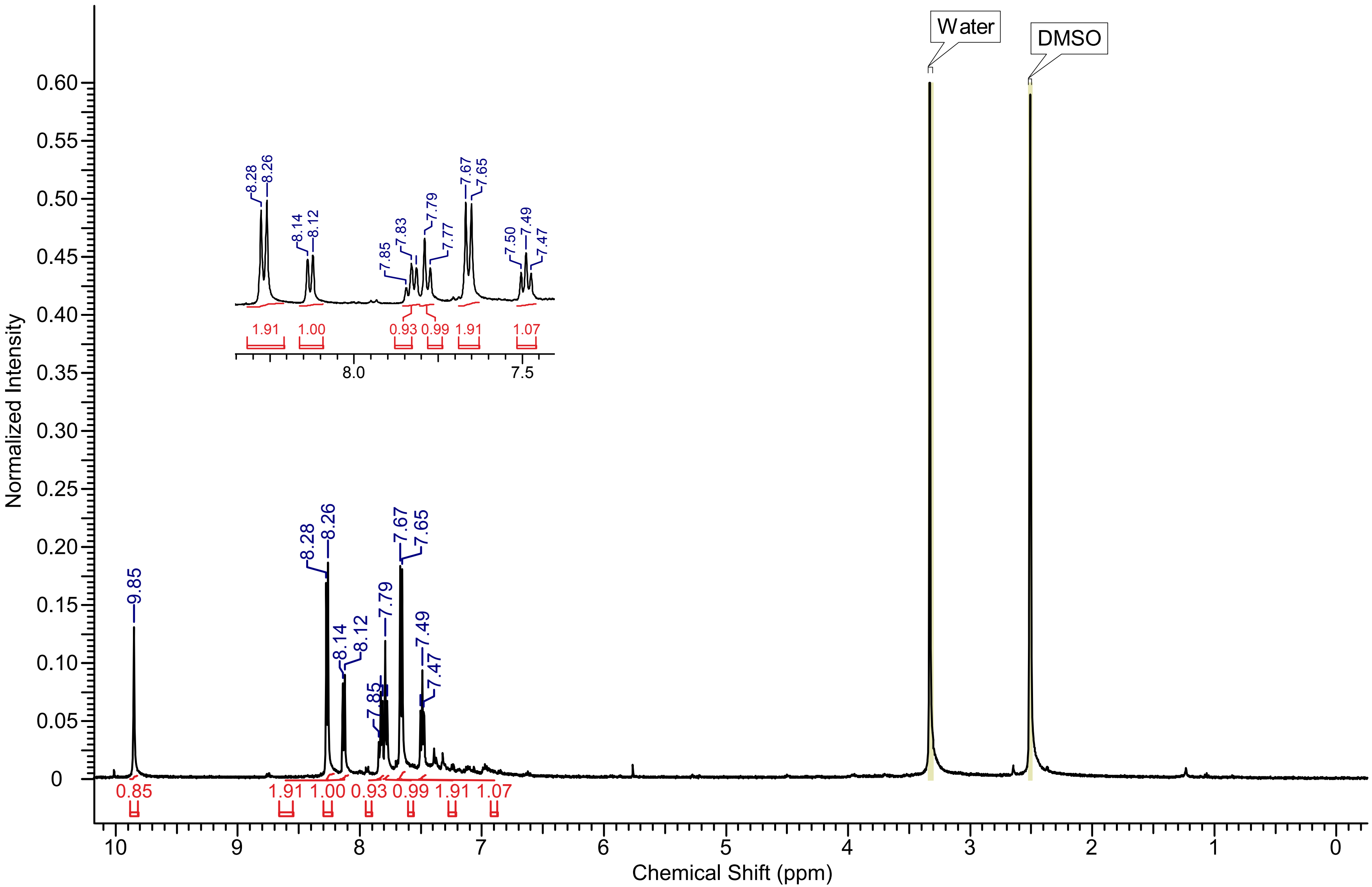
2. ¹H NMR spectrum of 3_b (500 MHz, $CDCl_3$)



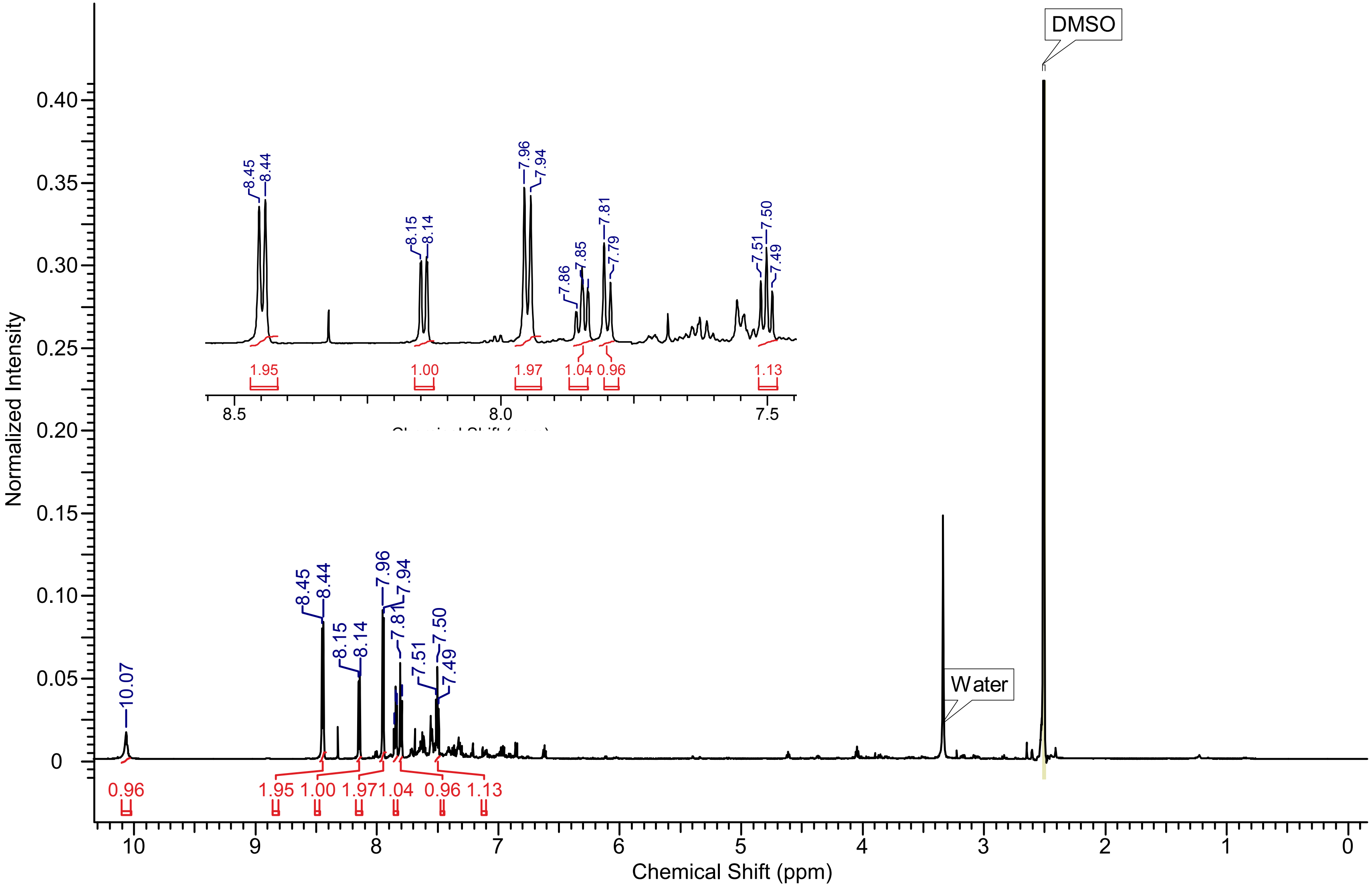
3. ^{1}H NMR spectrum of 4_b (500 MHz, DMSO-d_6)



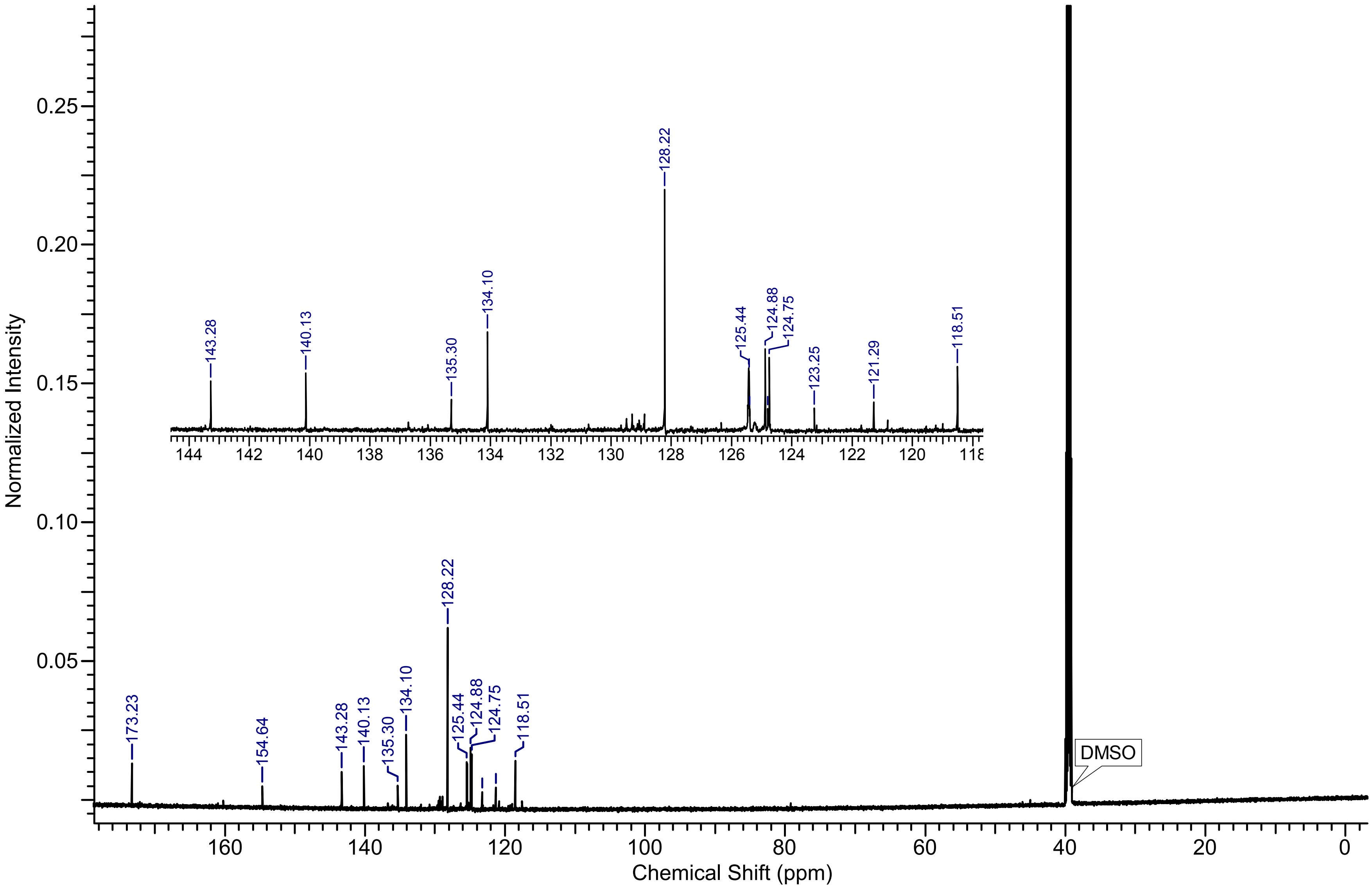
4. ^{1}H NMR spectrum of 4_e (500 MHz, DMSO-d_6)



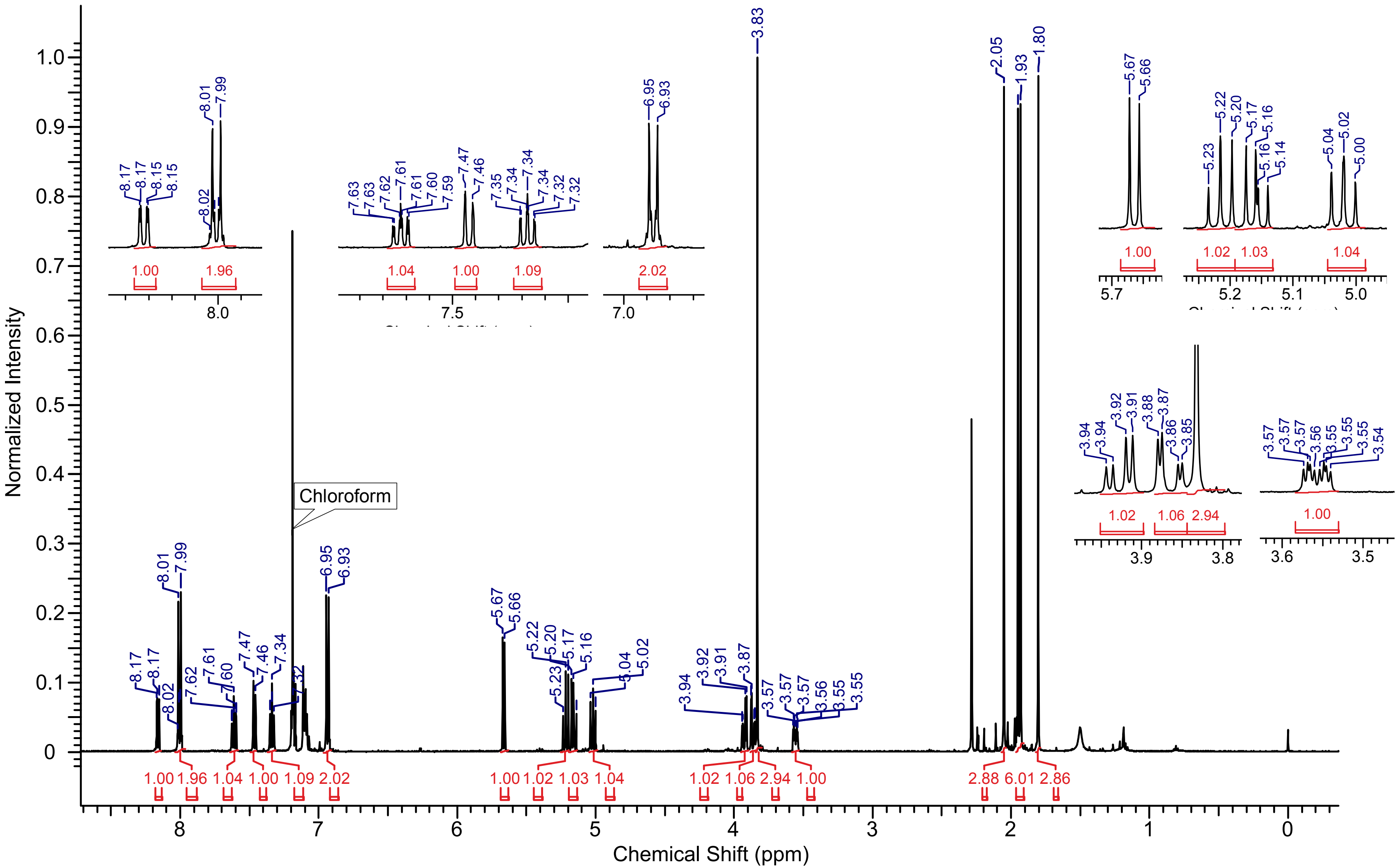
5. ^{1}H NMR spectrum of 4_f (500 MHz, DMSO-d_6)



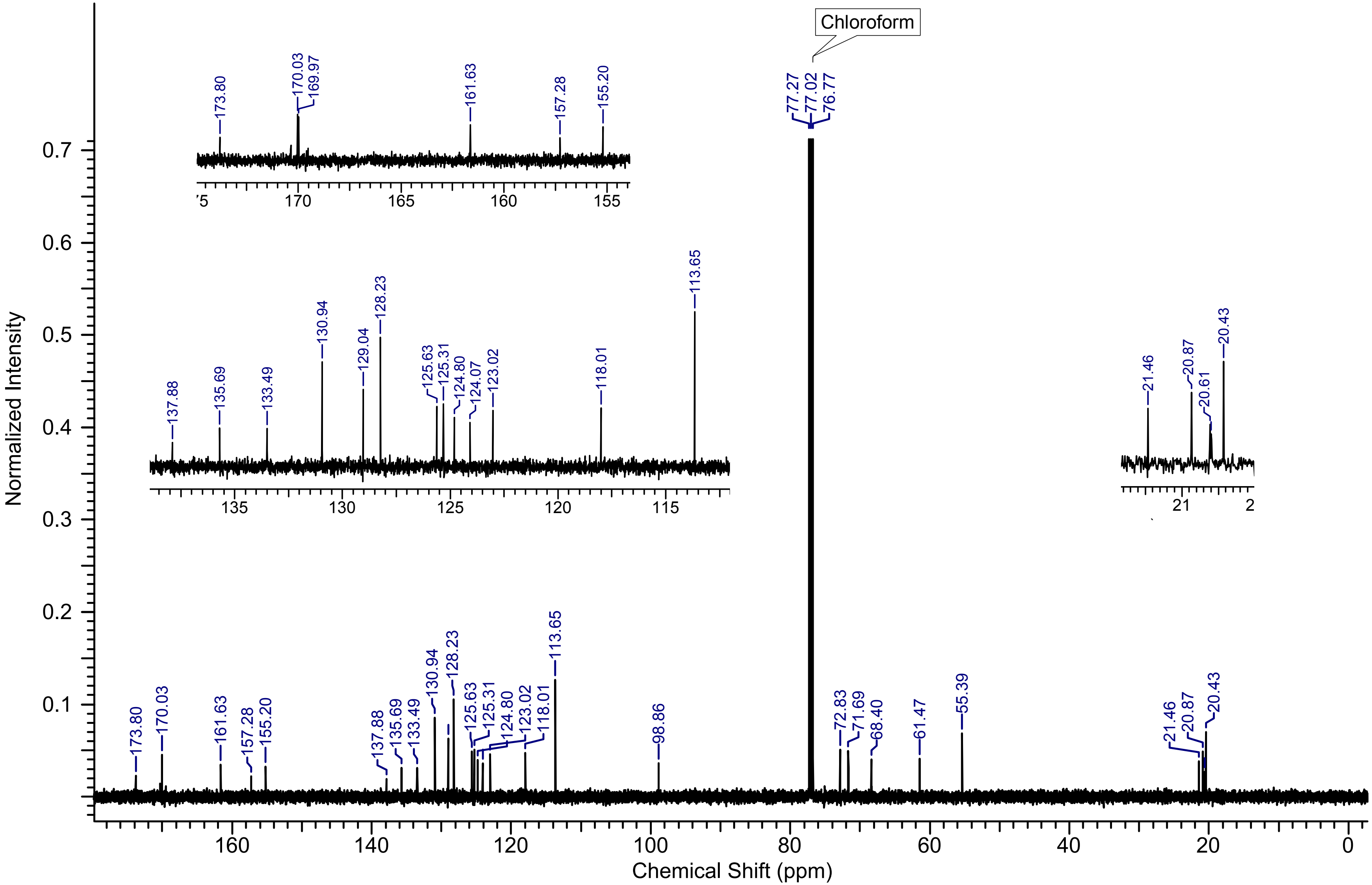
6. ^{13}C NMR spectrum of 4_f (125 MHz, DMSO-d$_6$)



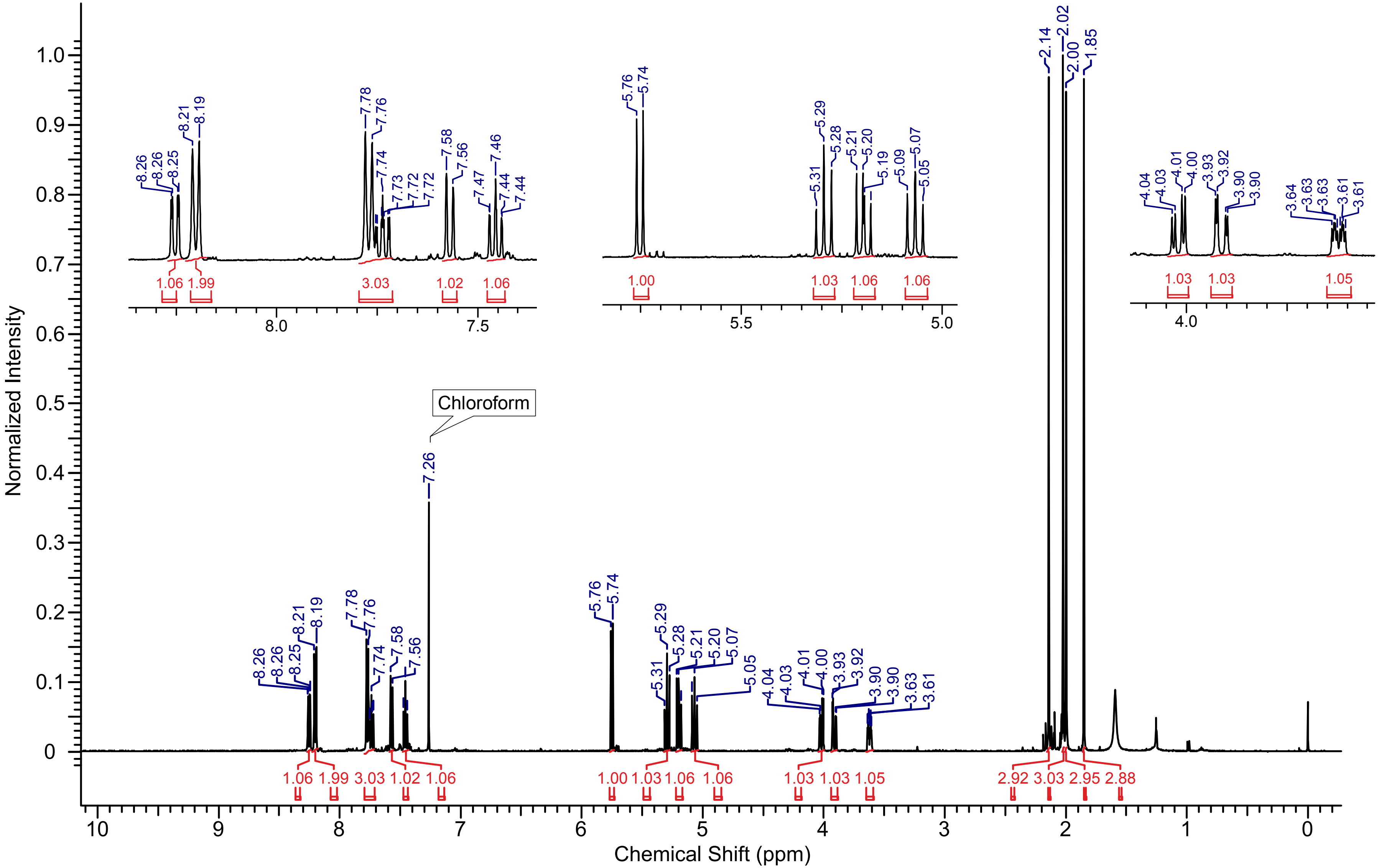
7. ^{1}H NMR spectrum of 8_b (500 MHz, $CDCl_3$)



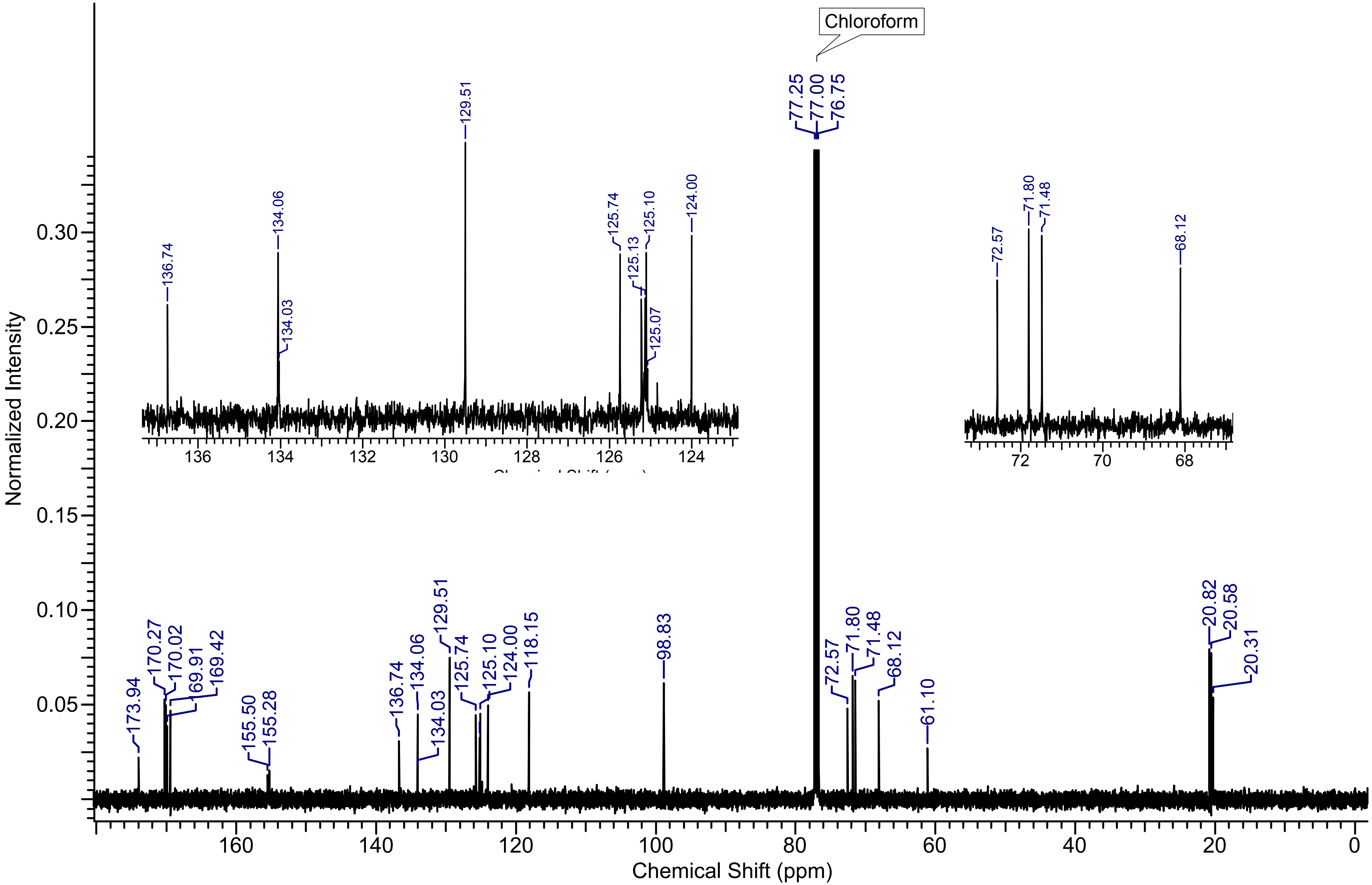
8. ^{13}C NMR spectrum of 8_b (125 MHz, $CDCl_3$)



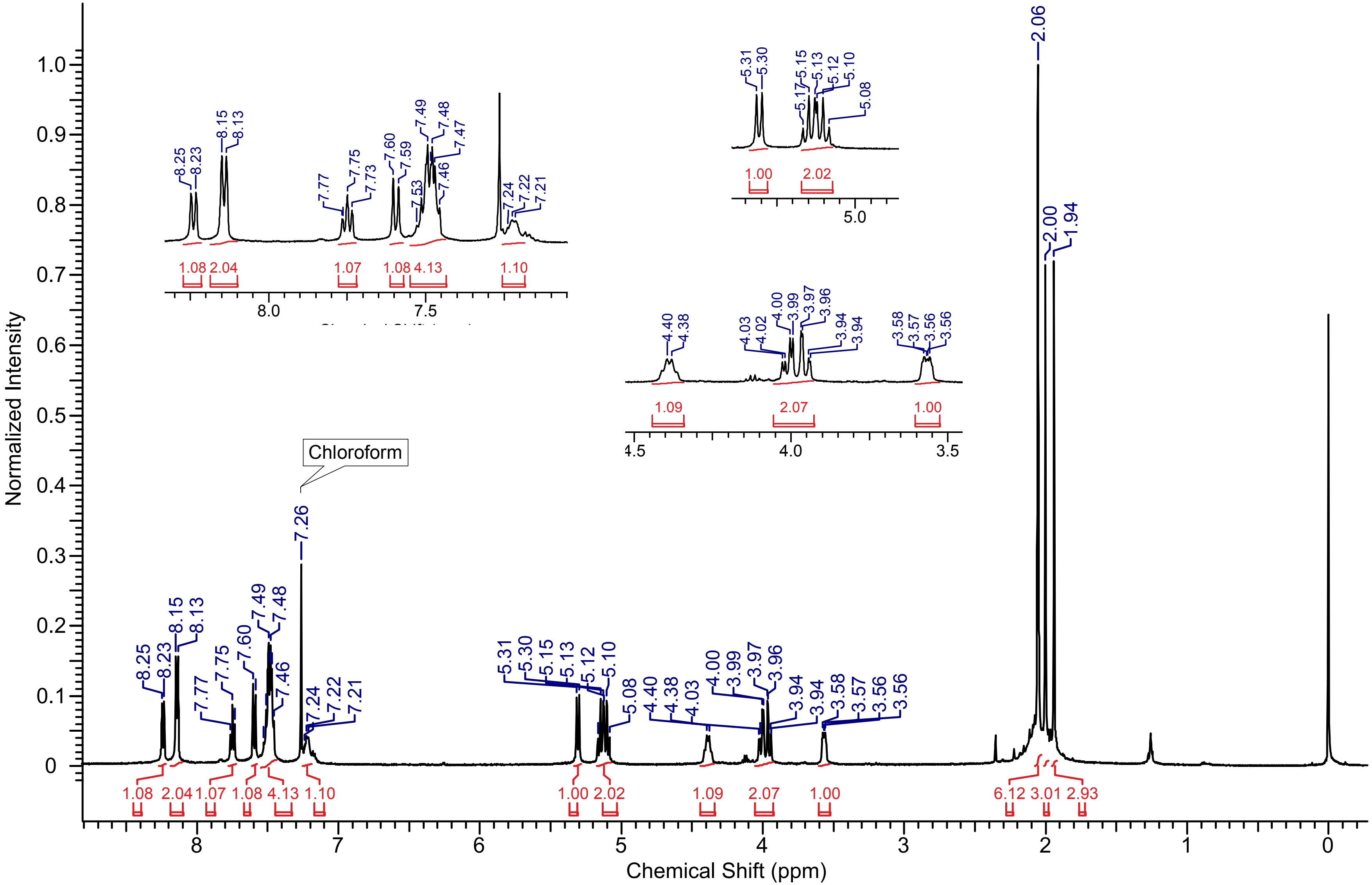
9. ^{1}H NMR spectrum of 8_f (500 MHz, $CDCl_3$)



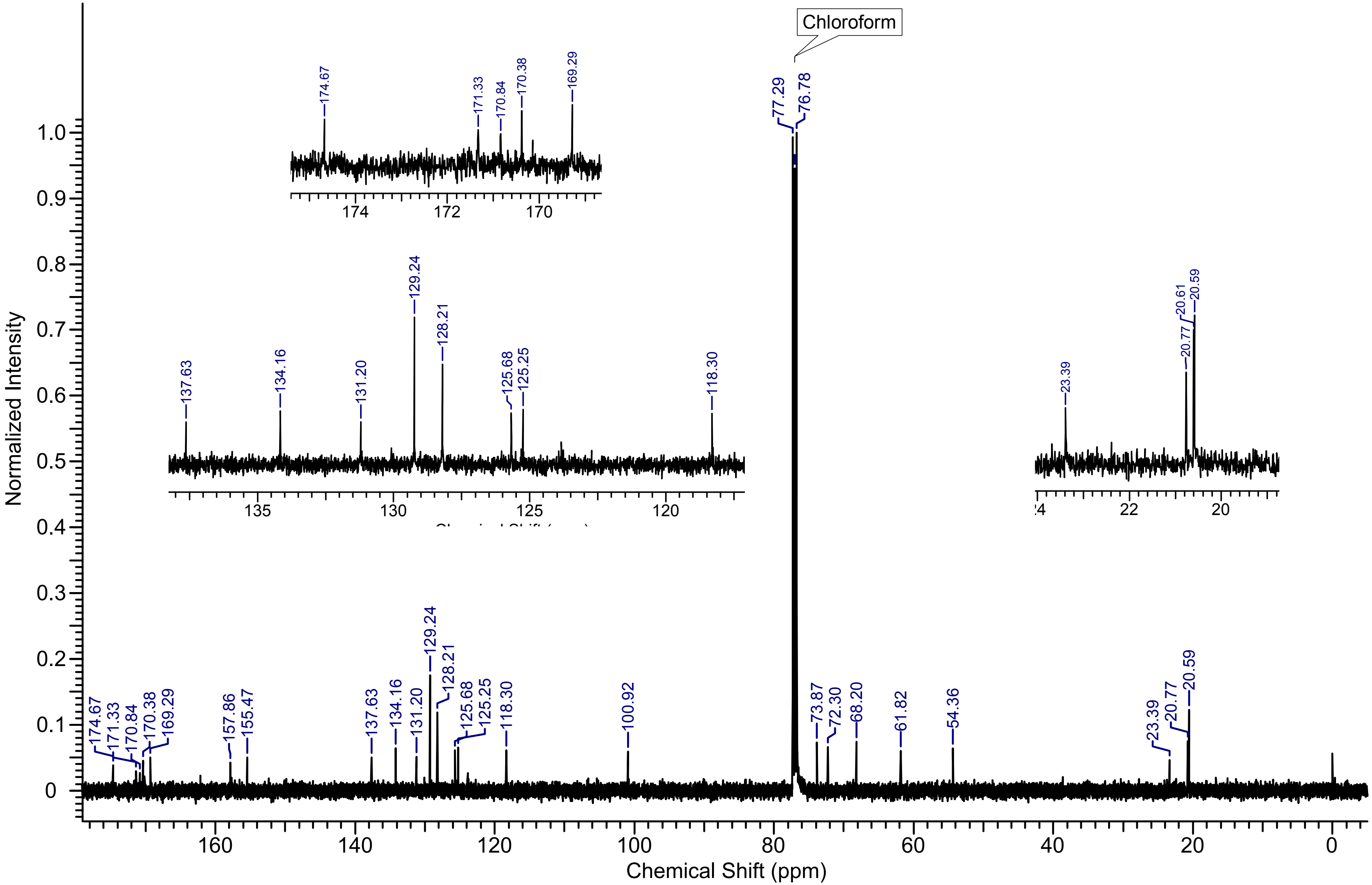
10. ^{13}C NMR spectrum of 8$_f$ (125 MHz, CDCl$_3$)



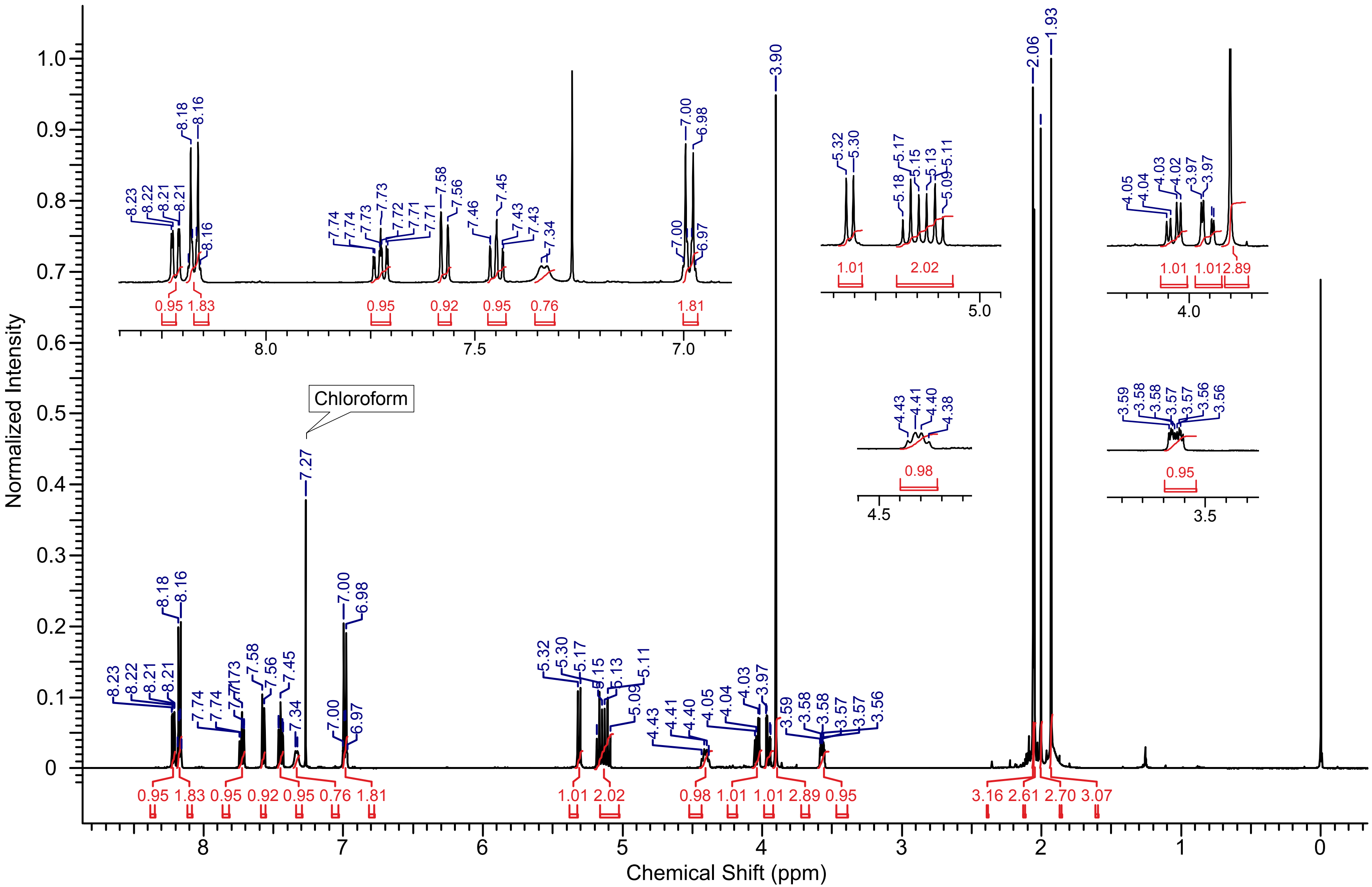
11. [1]H NMR spectrum of 9_a (500 MHz, $CDCl_3$)



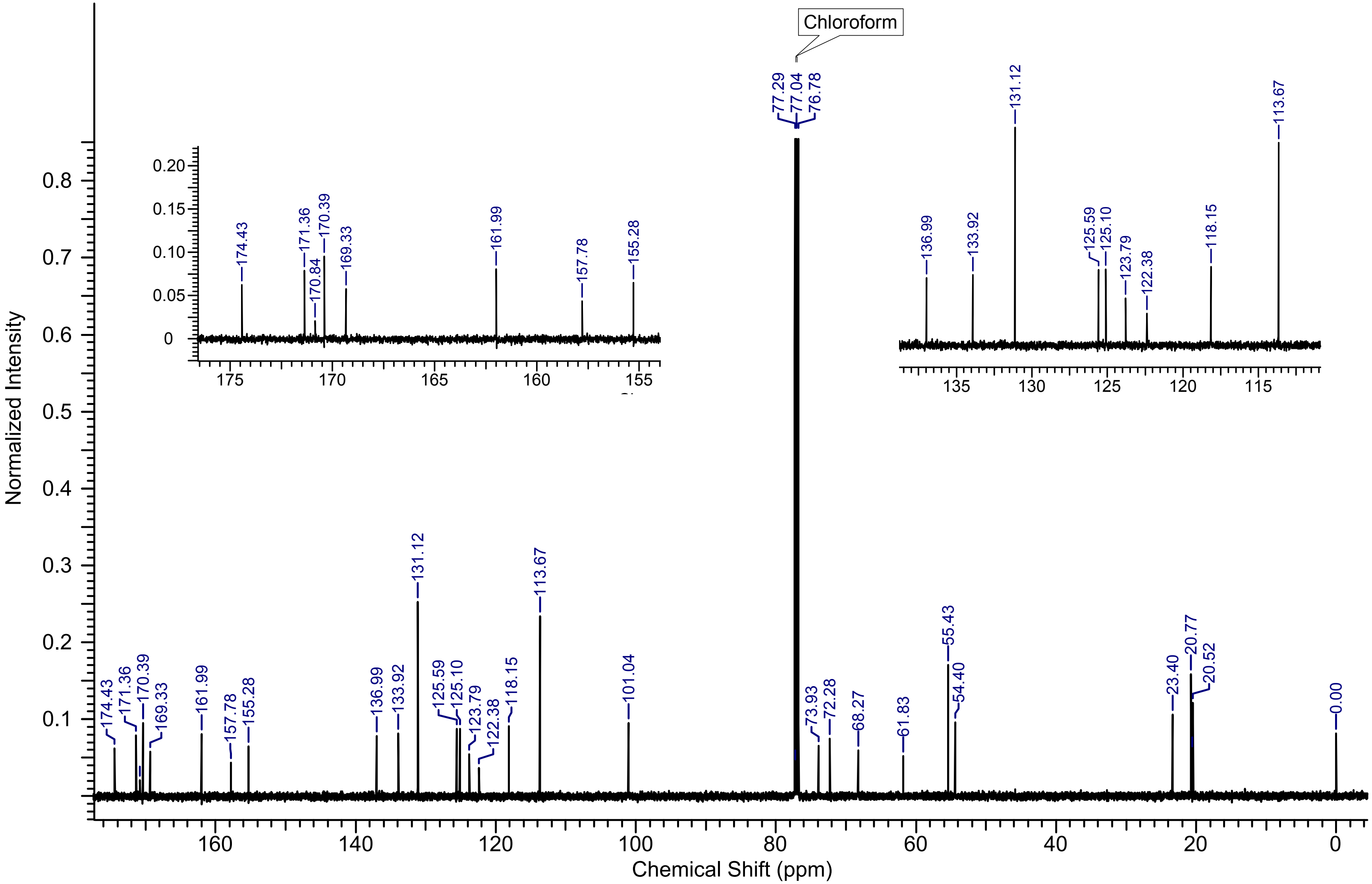
12. ^{13}C NMR spectrum of 9_a (125 MHz, $CDCl_3$)



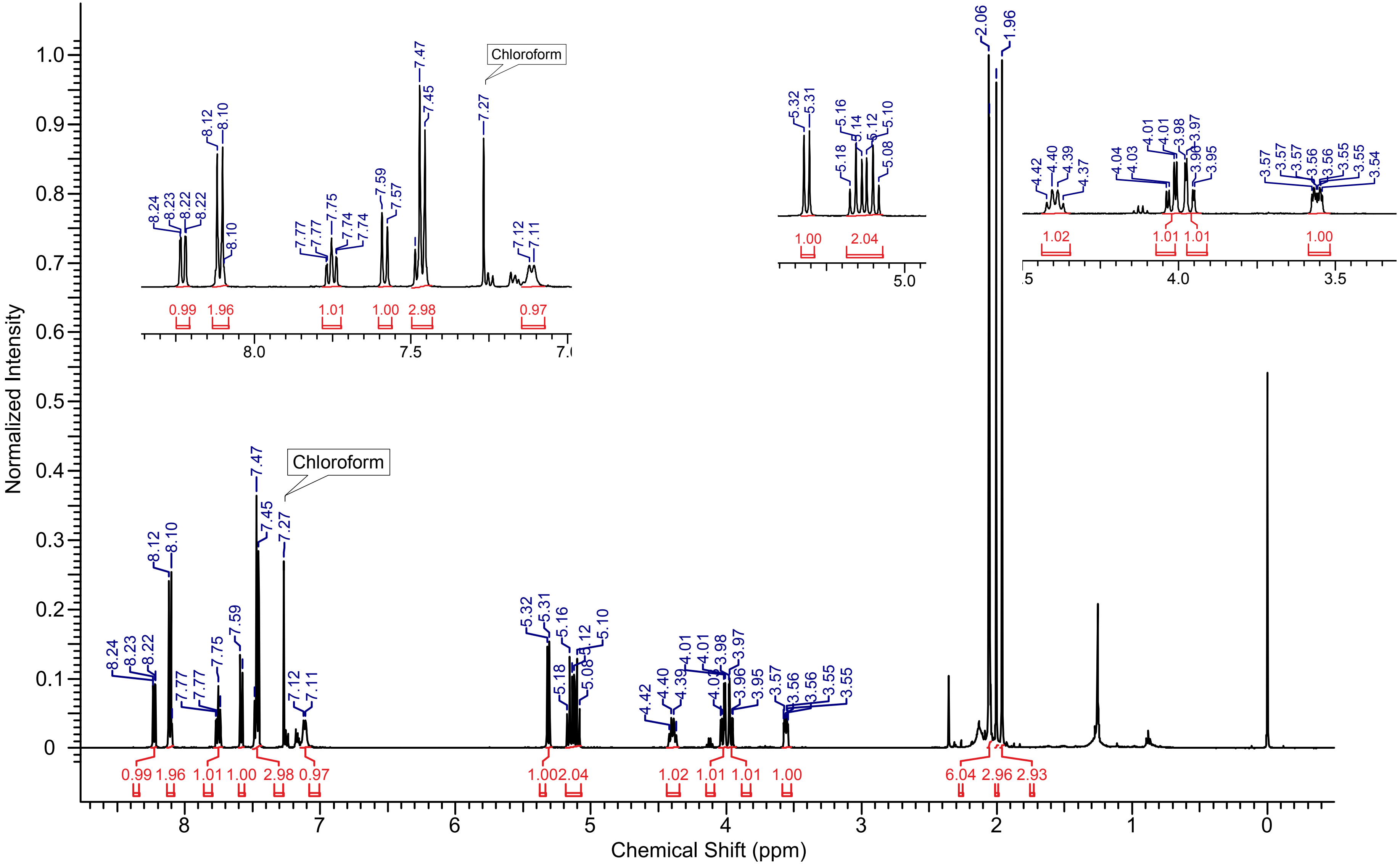
13. ¹H NMR spectrum of 9_b (500 MHz, $CDCl_3$)



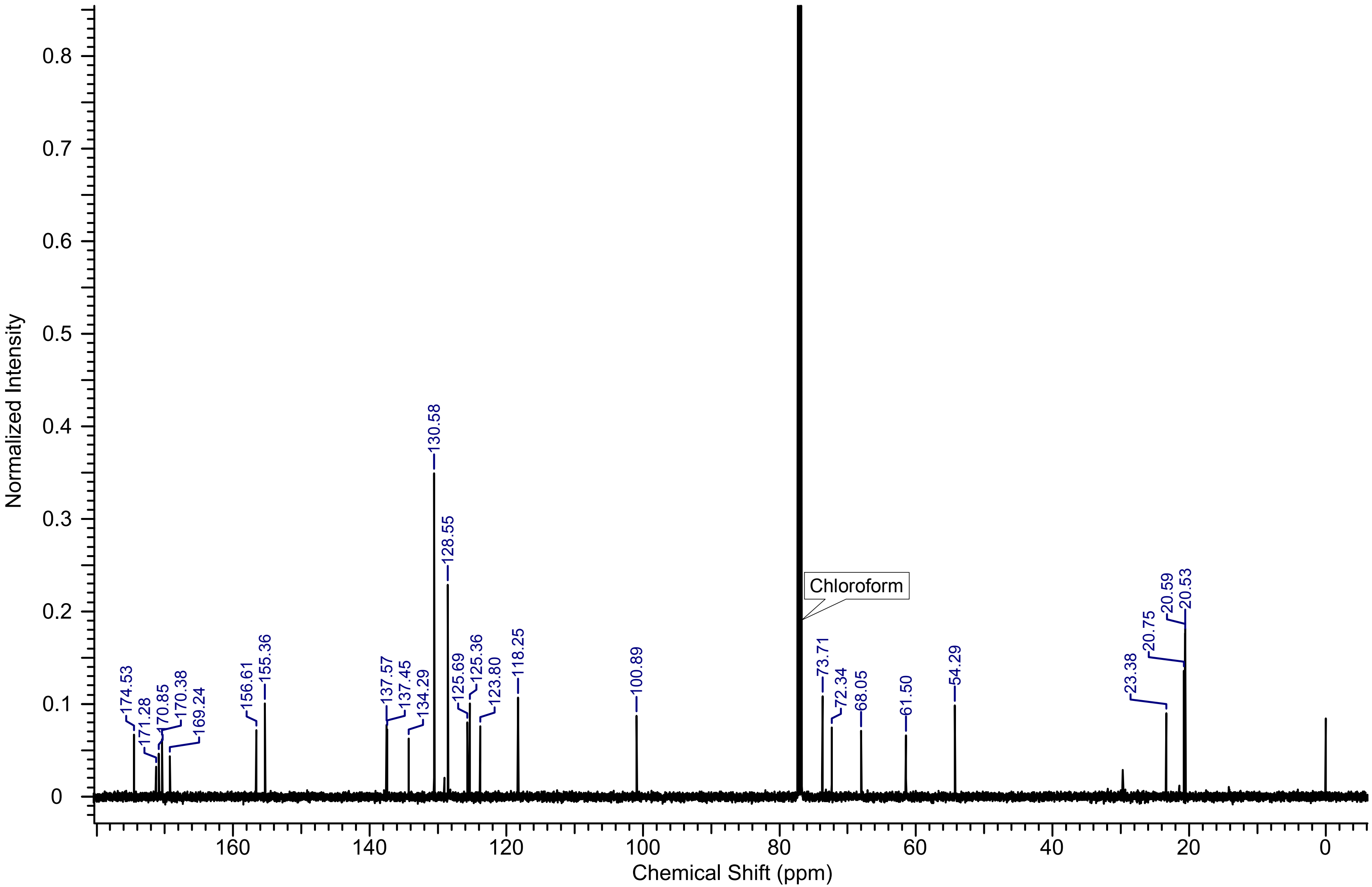
14. ^{13}C NMR spectrum of 9_b (125 MHz, $CDCl_3$)



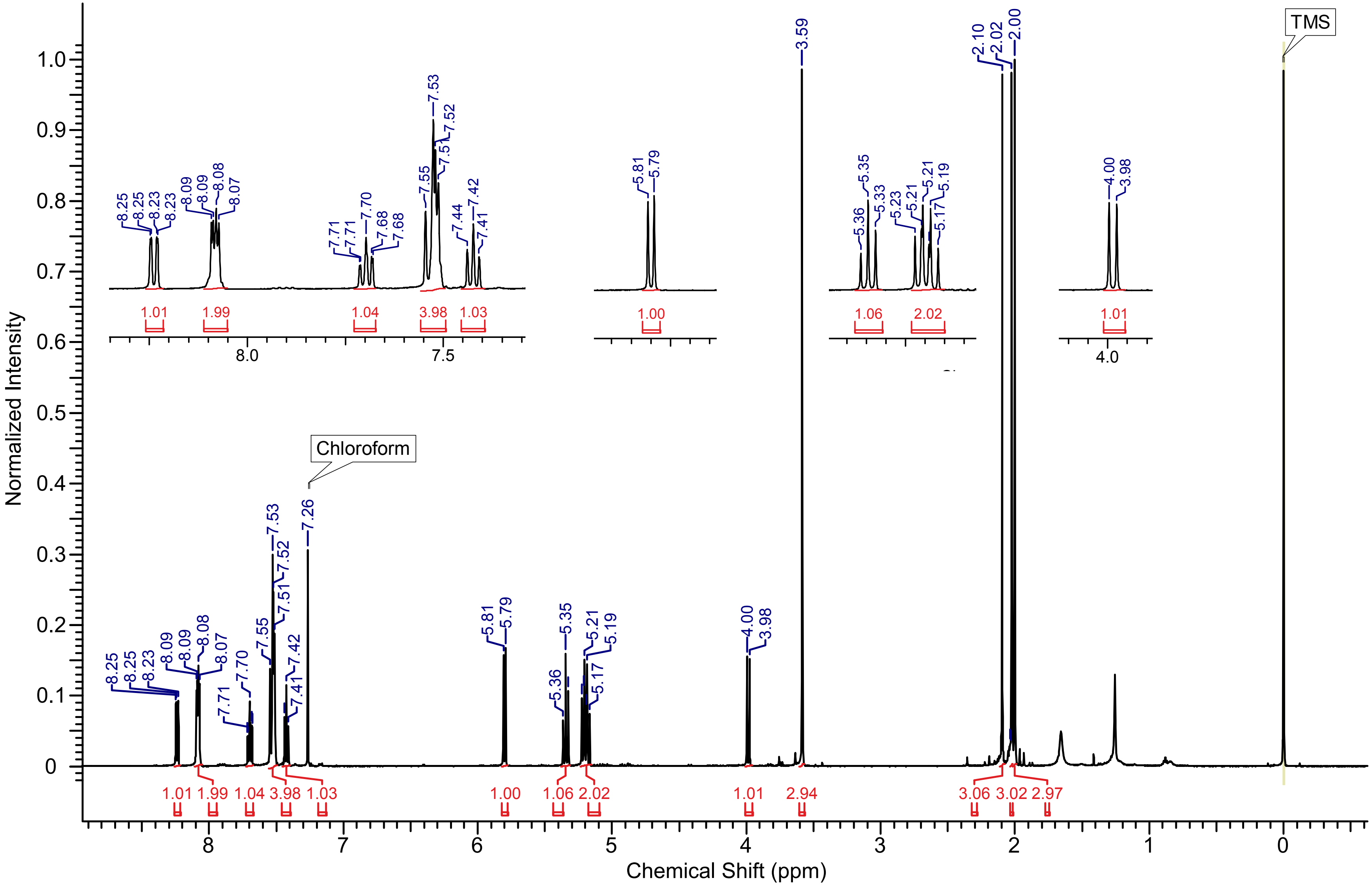
15. ^{1}H NMR spectrum of 9_e (500 MHz, $CDCl_3$)



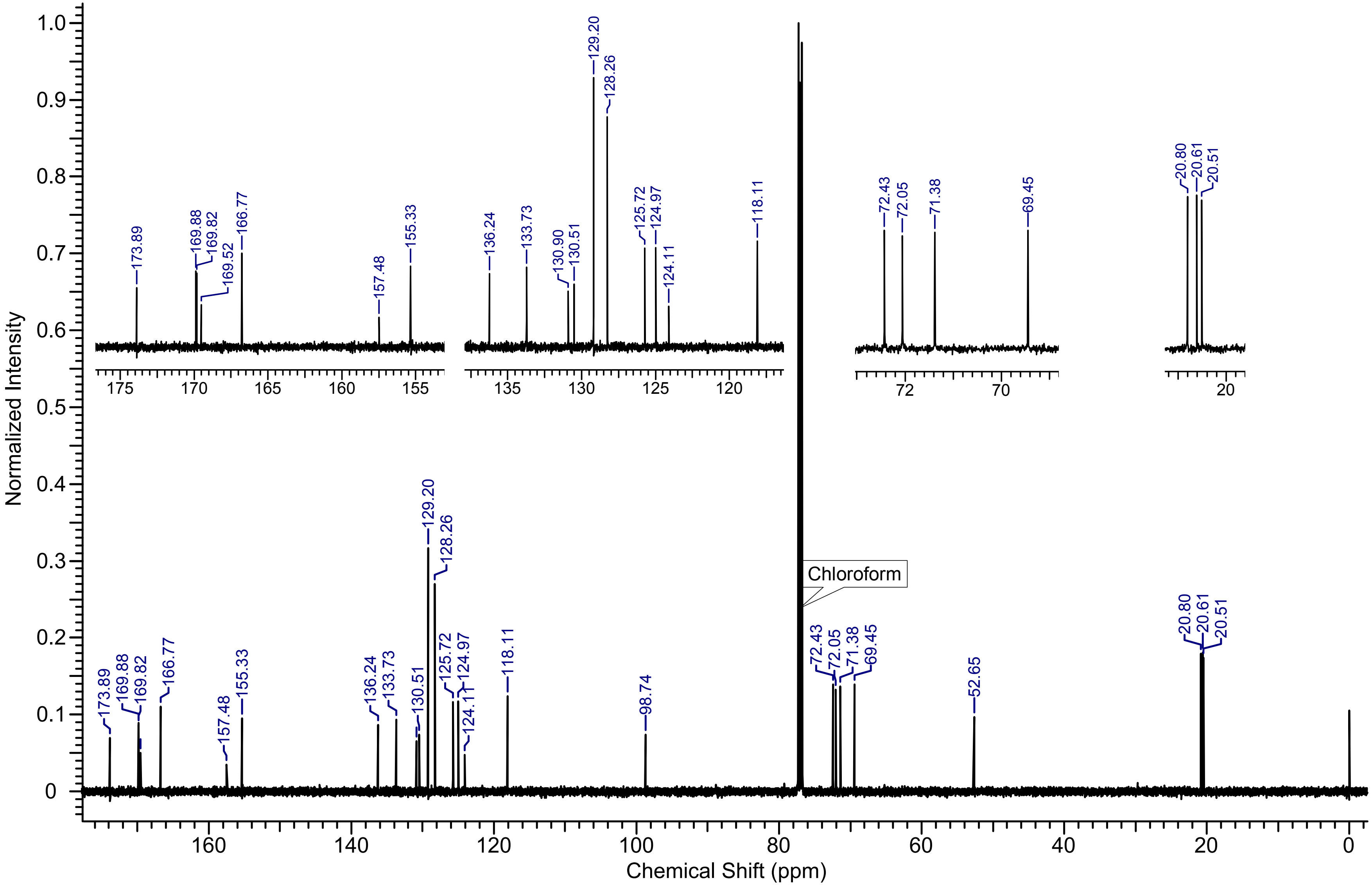
16. ^{13}C NMR spectrum of 9_e (125 MHz, $CDCl_3$)



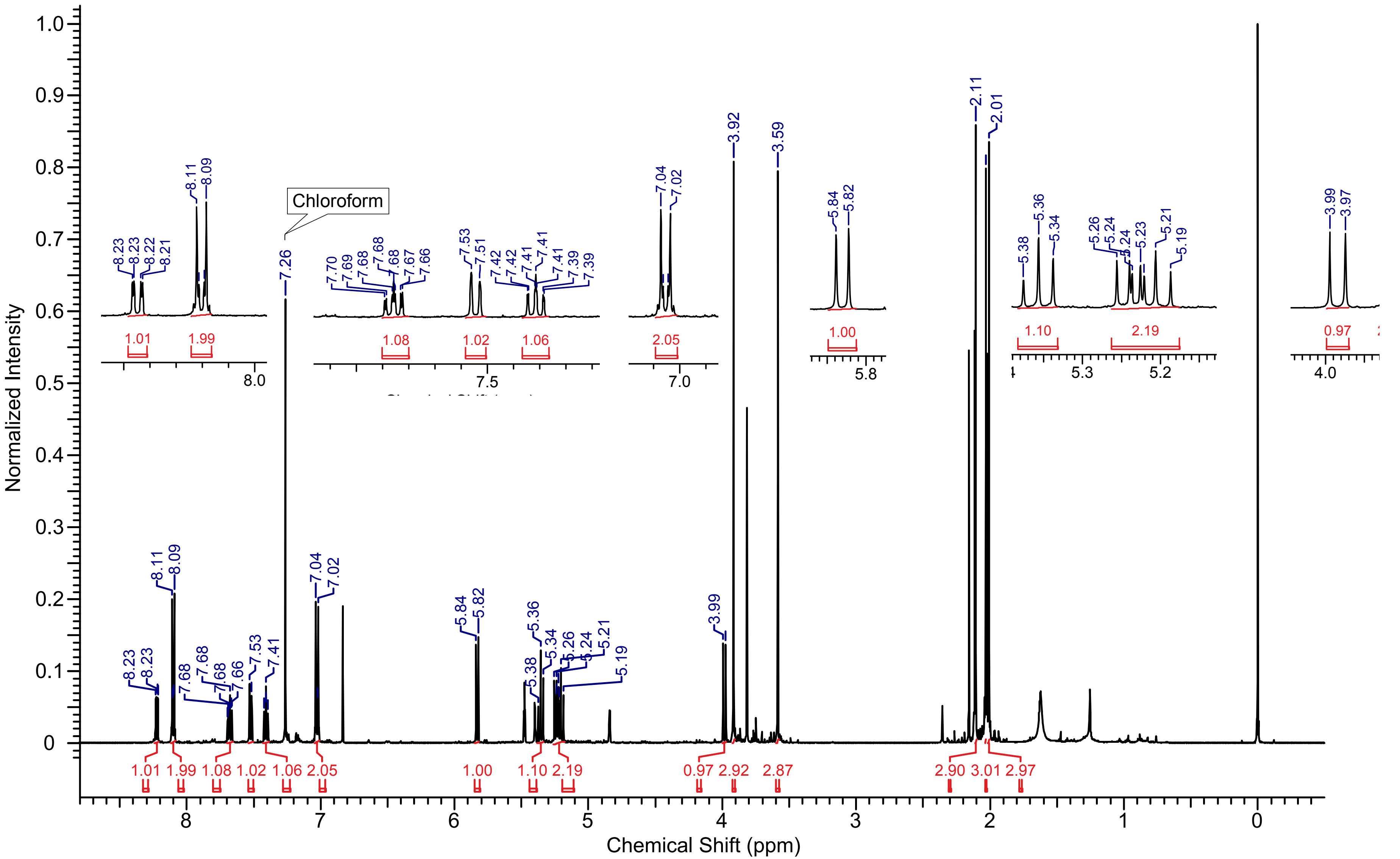
17. ^{1}H NMR spectrum of 10_a (500 MHz, $CDCl_3$)



18. ^{13}C NMR spectrum of 10_a (125 MHz, $CDCl_3$)



19. ^{1}H NMR spectrum of 10_b (500 MHz, $CDCl_3$)



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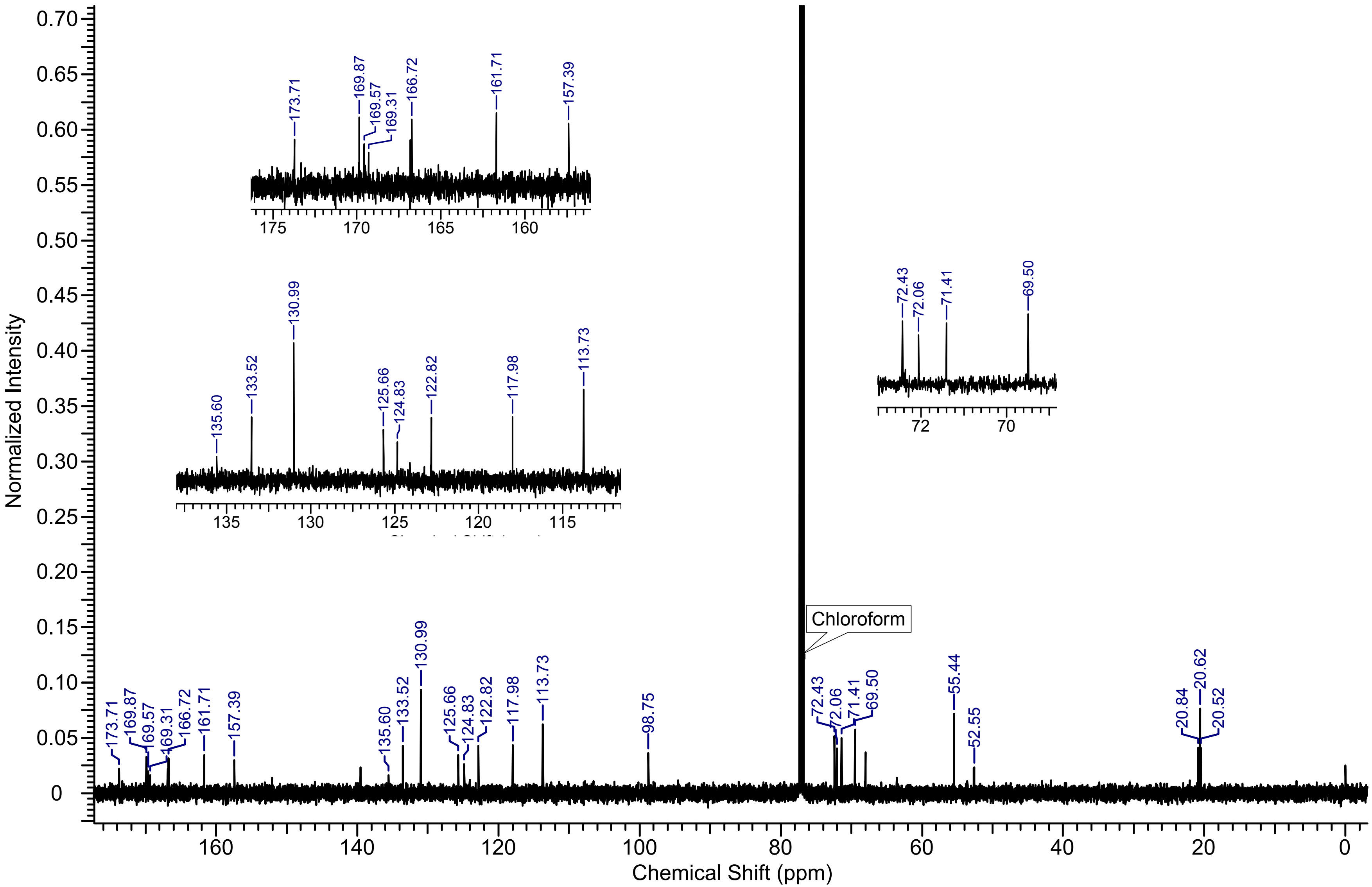
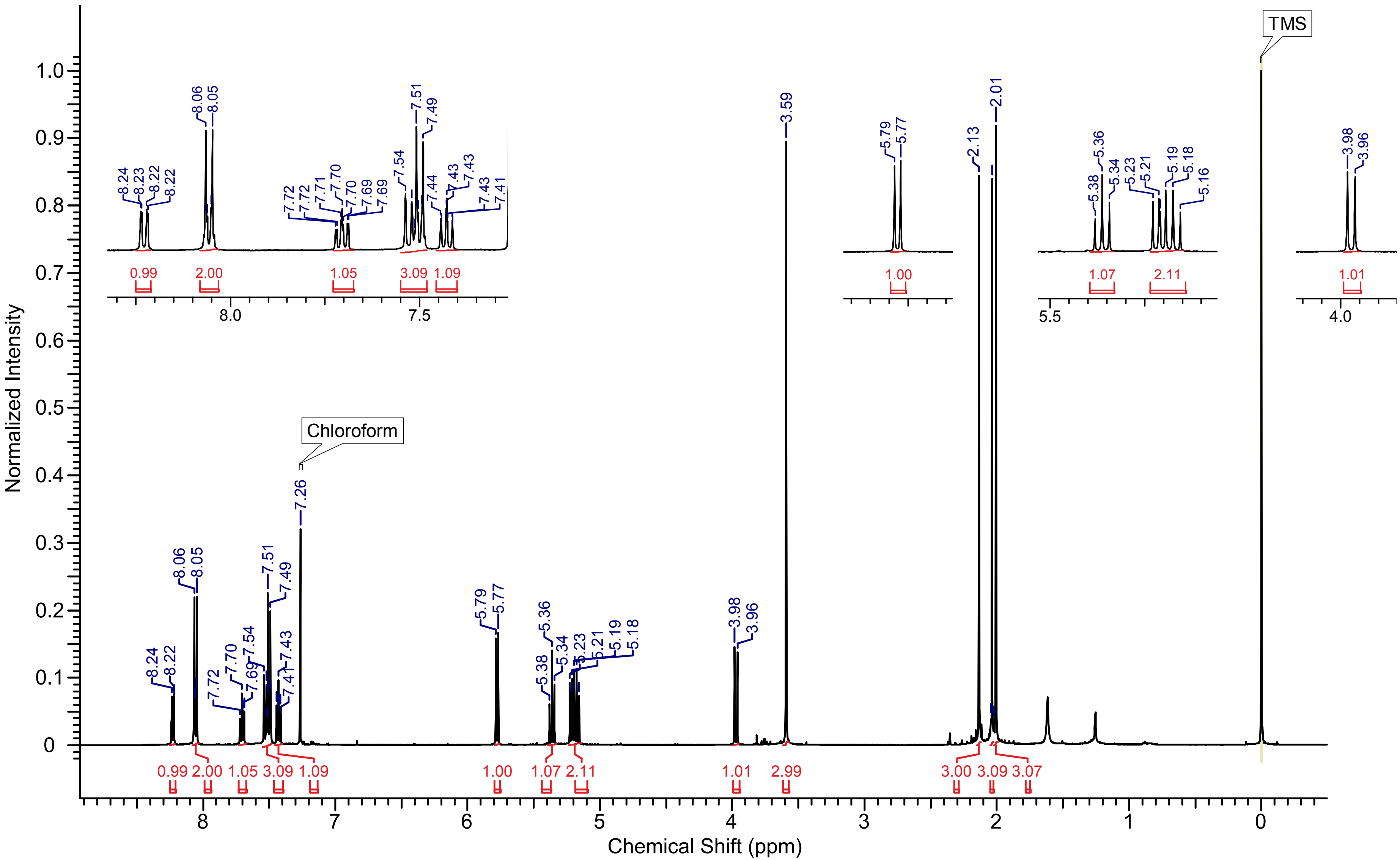
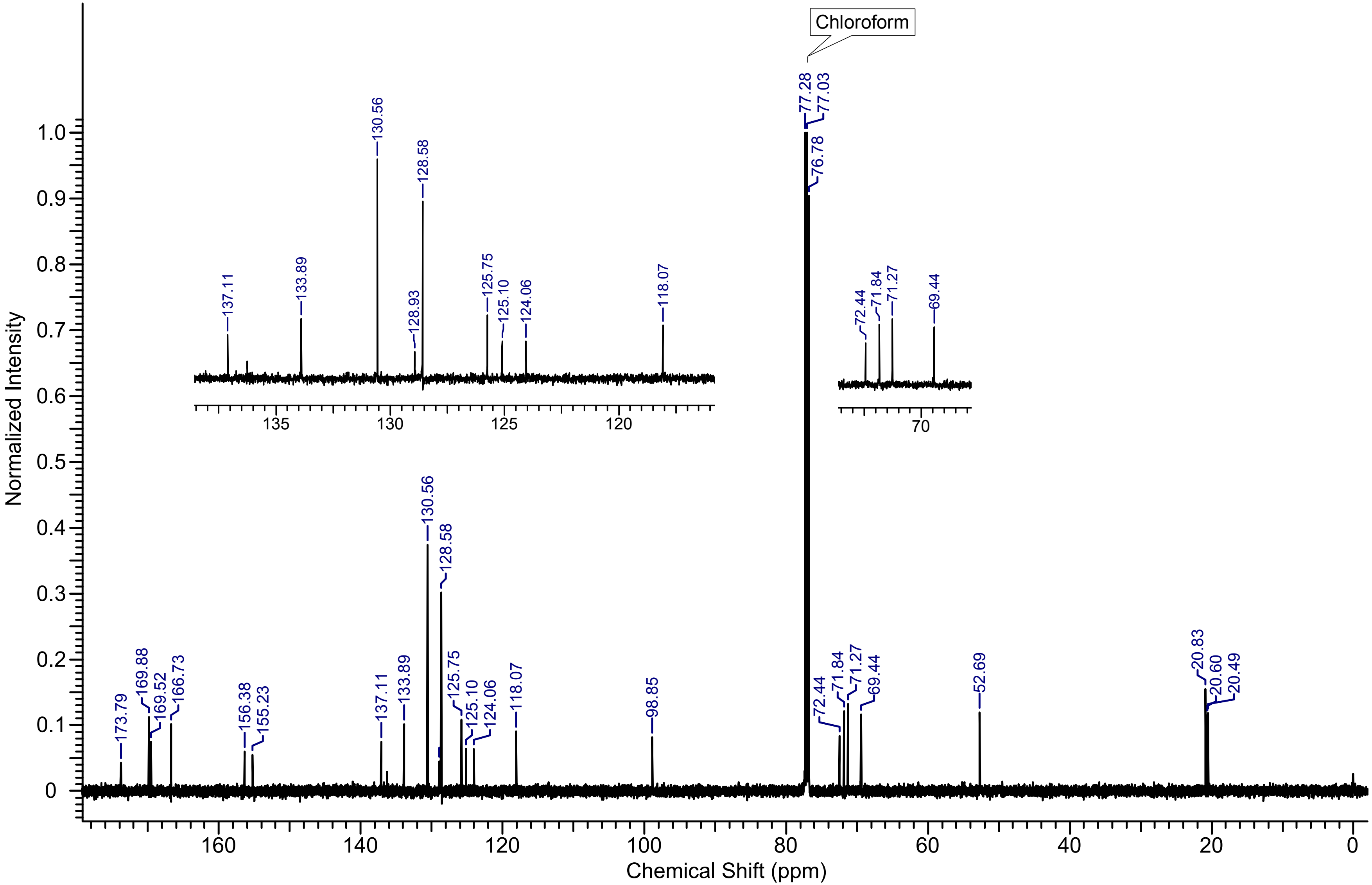


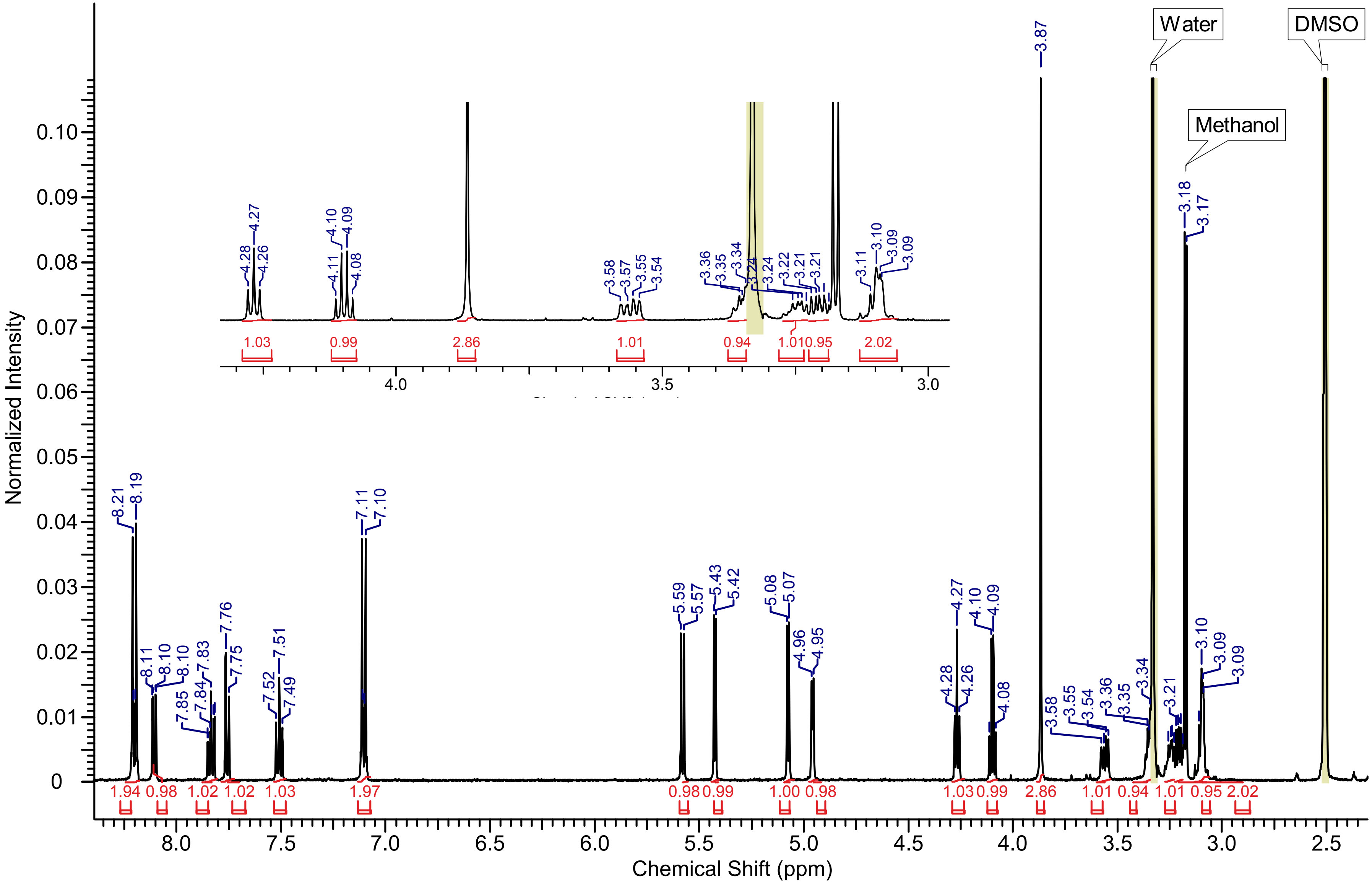
21. ^{1}H NMR spectrum of 10_e (500 MHz, $CDCl_3$)



22. ^{13}C NMR spectrum of 10$_e$ (125 MHz, CDCl$_3$)



23. [1]H NMR spectrum of 11_b (500 MHz, DMSO-d_6)



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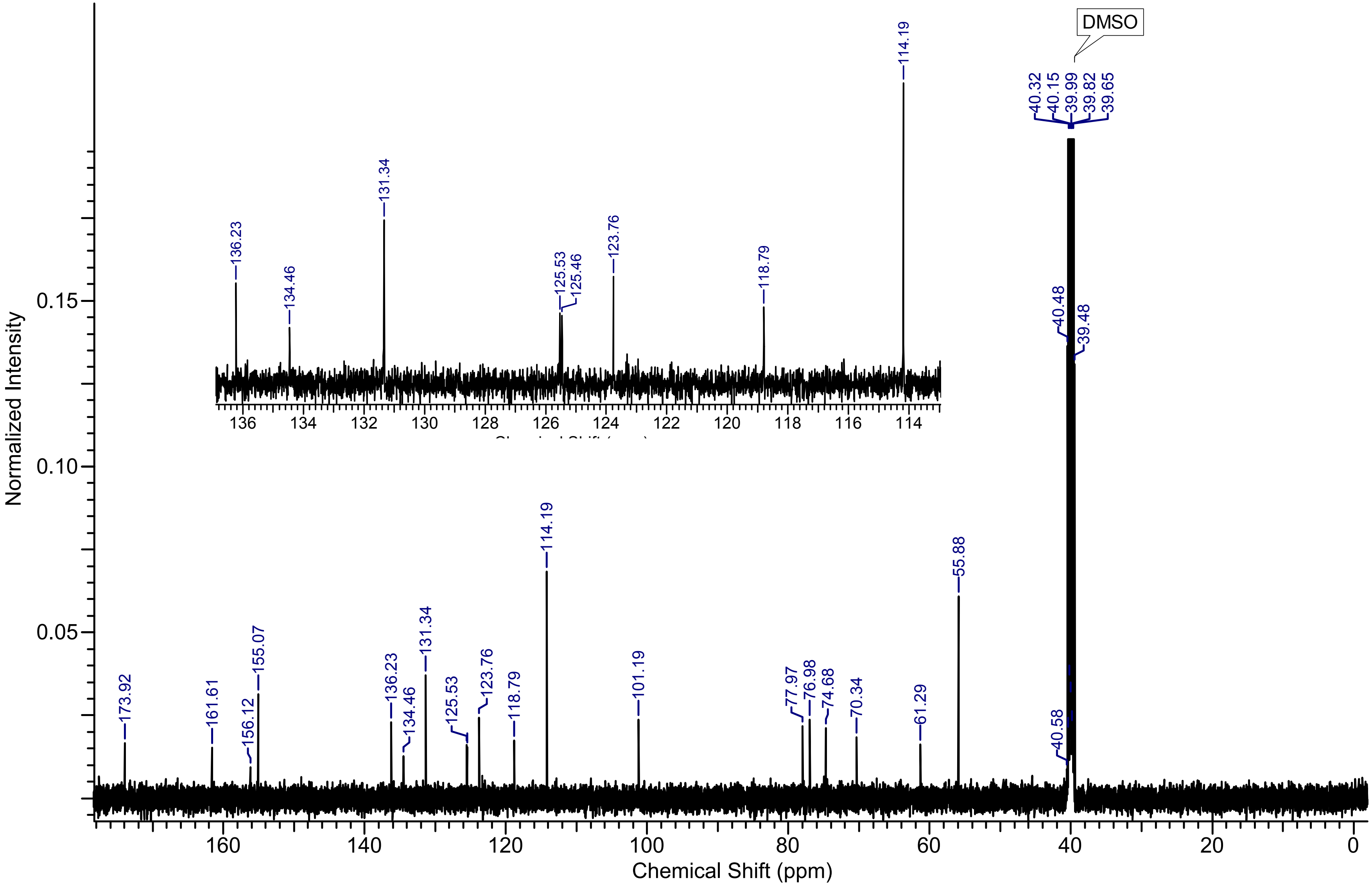
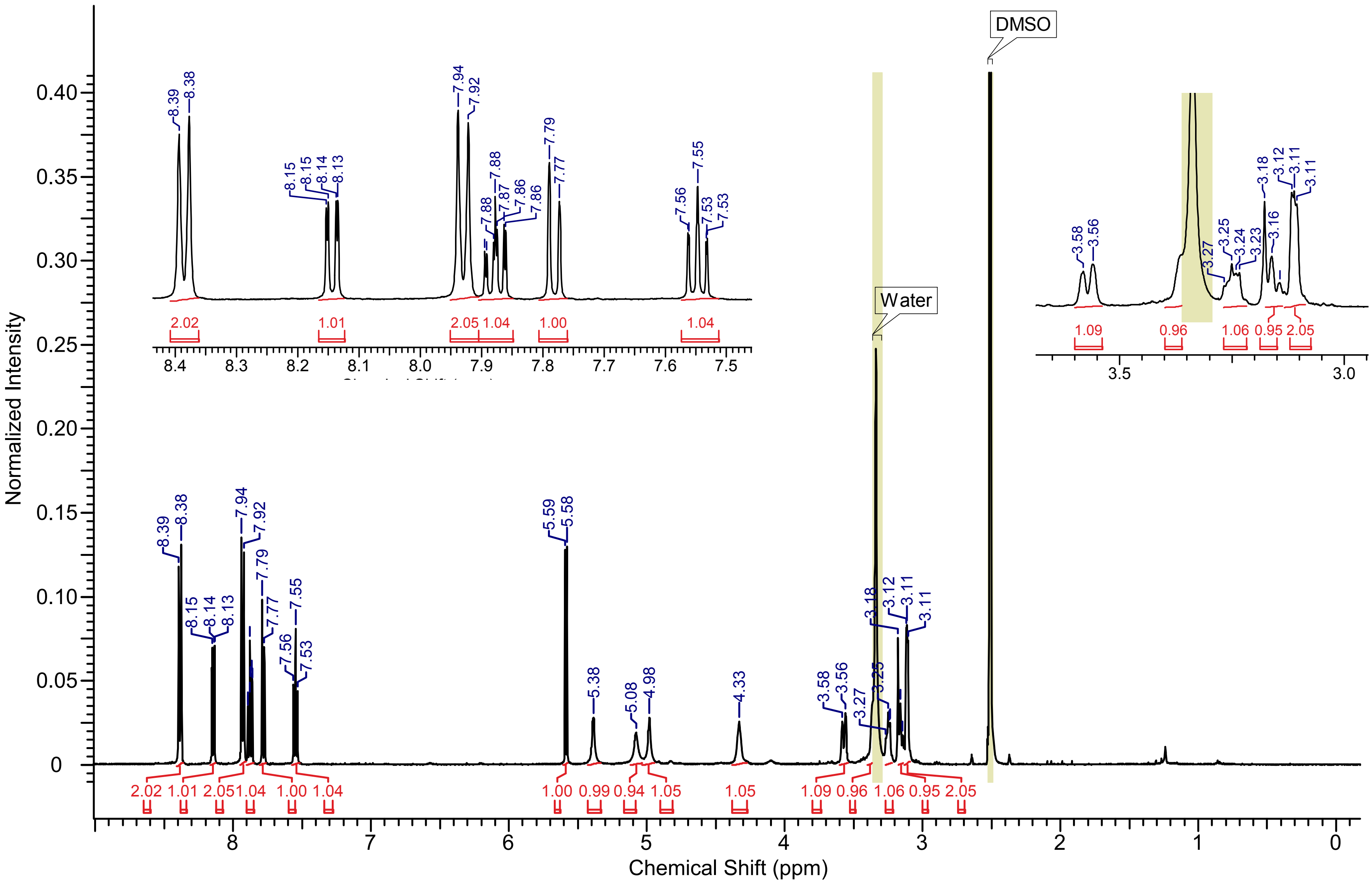
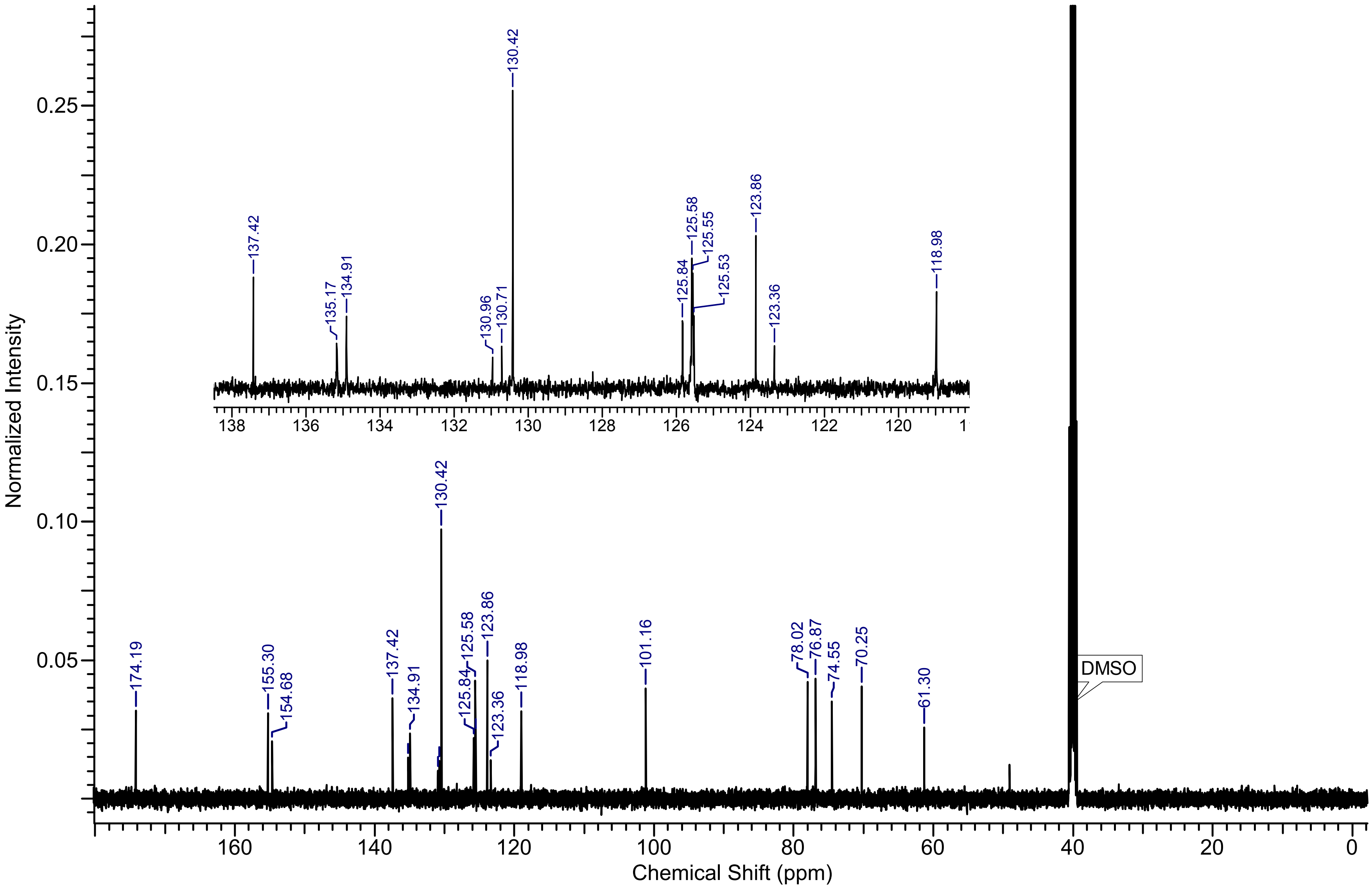


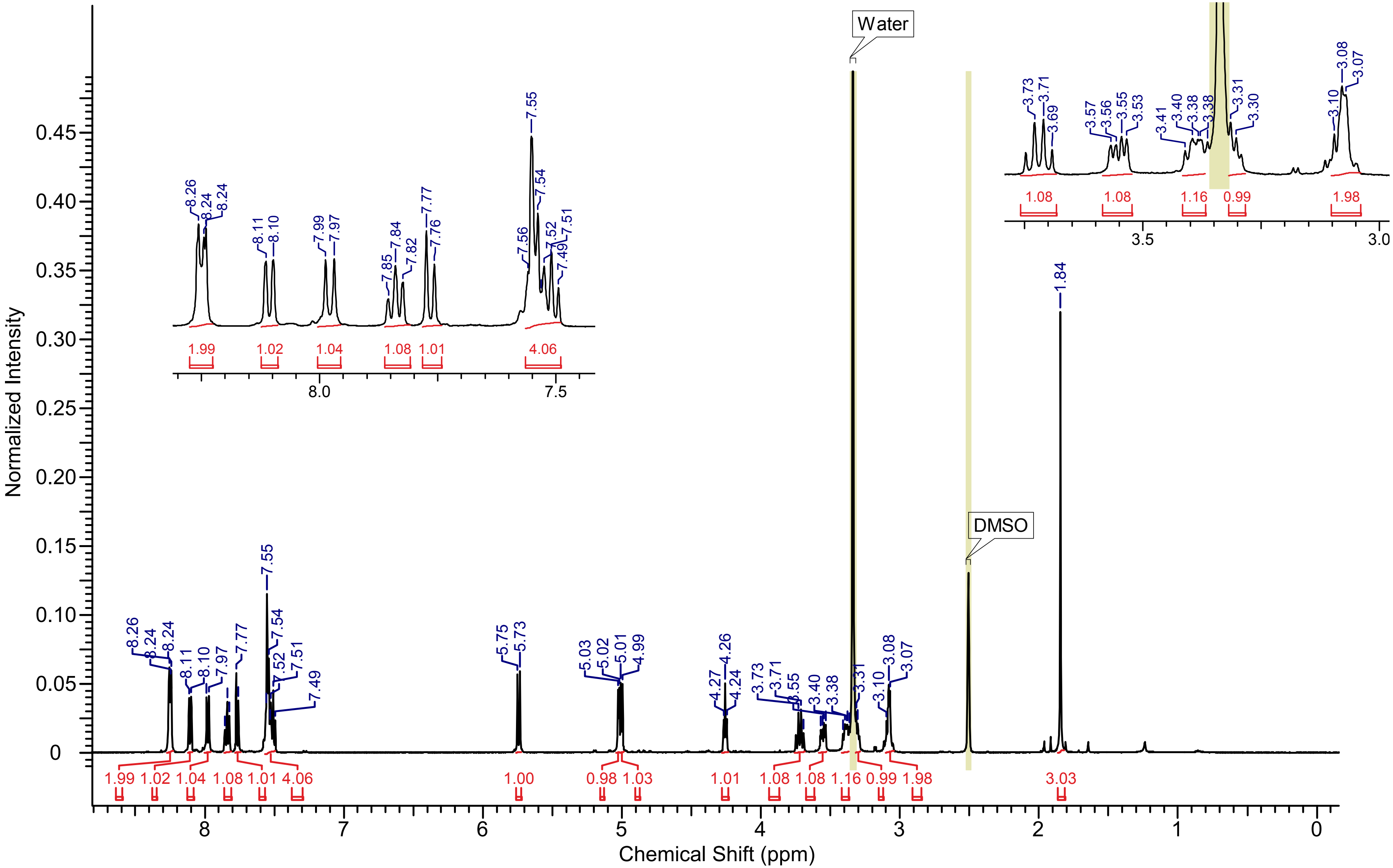
25. [1]H NMR spectrum of 11_f (500 MHz, DMSO-d_6)



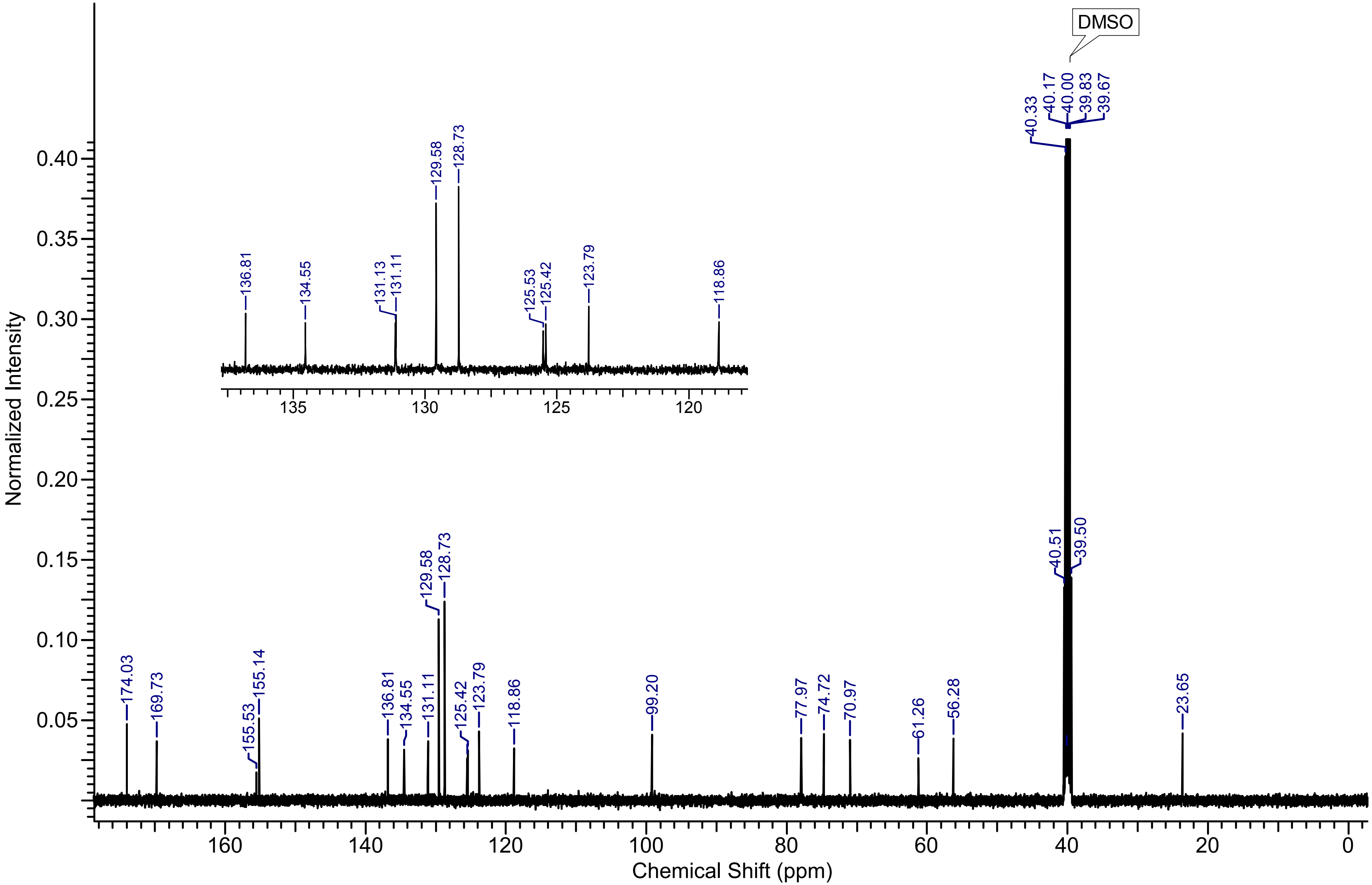
26. ^{13}C NMR spectrum of 11_f (125 MHz, DMSO-d_6)



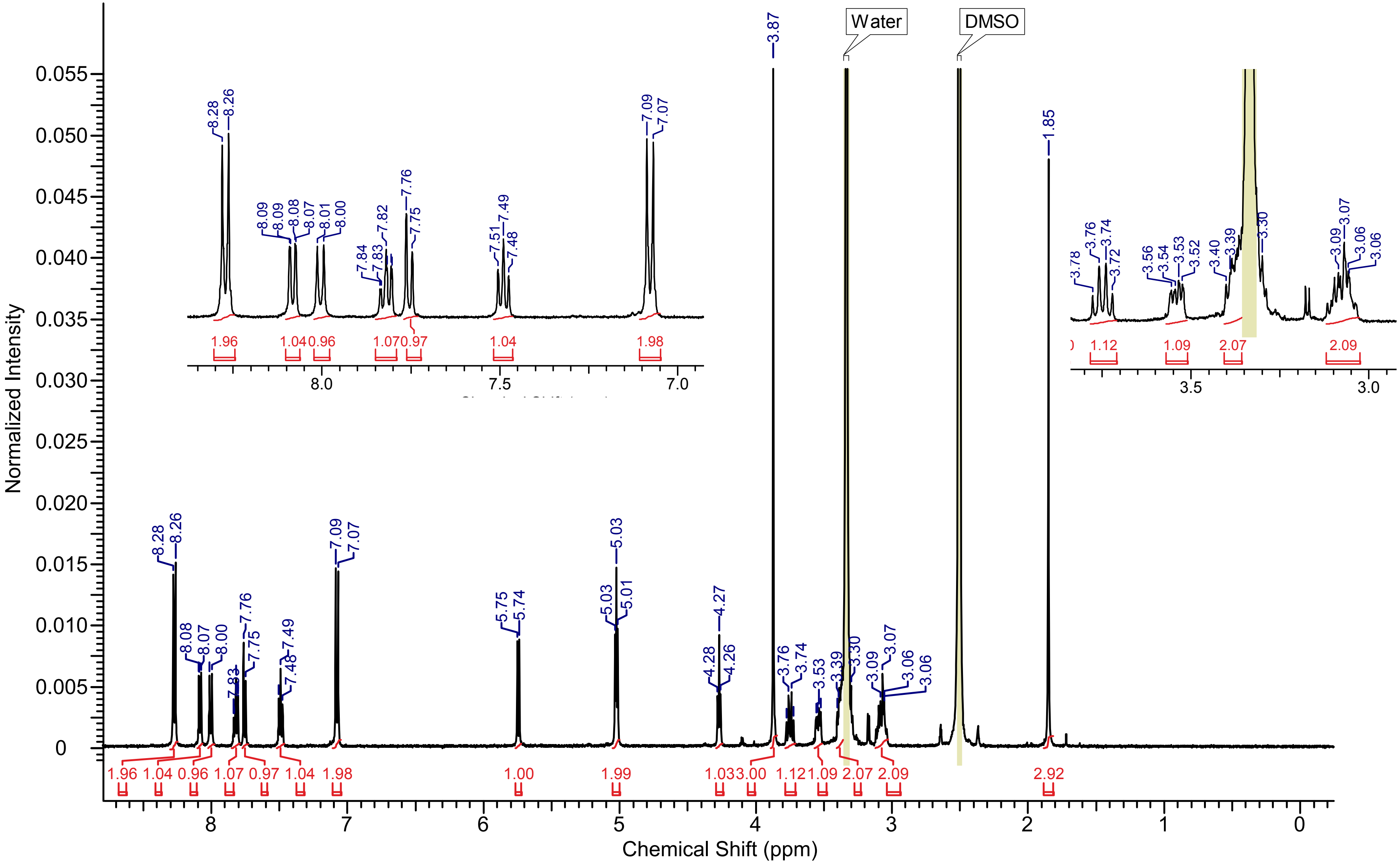
27. ^{1}H NMR spectrum of 12_a (500 MHz, DMSO-d_6)



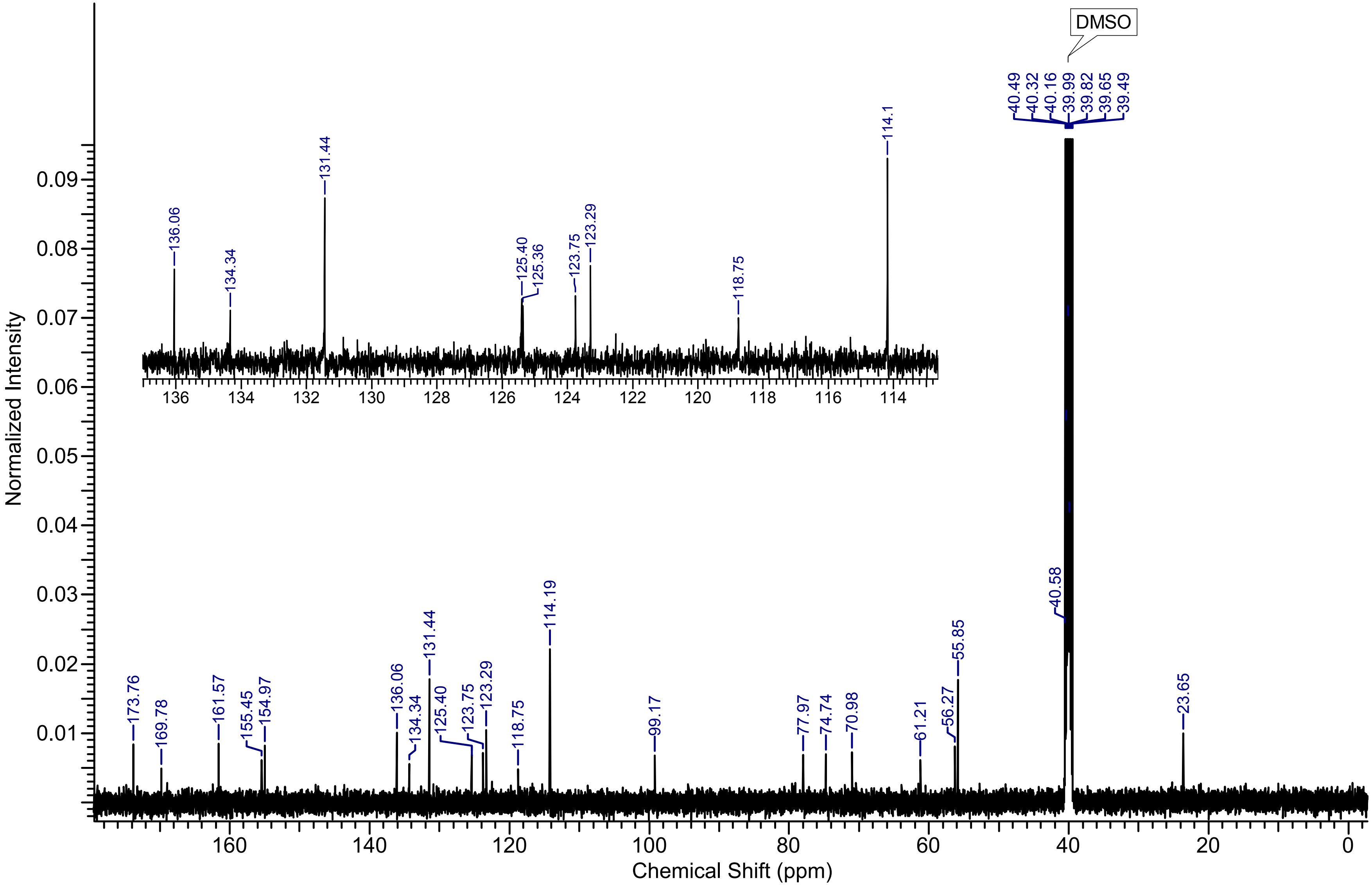
28. ^{13}C NMR spectrum of 12_a (125 MHz, DMSO-d_6)



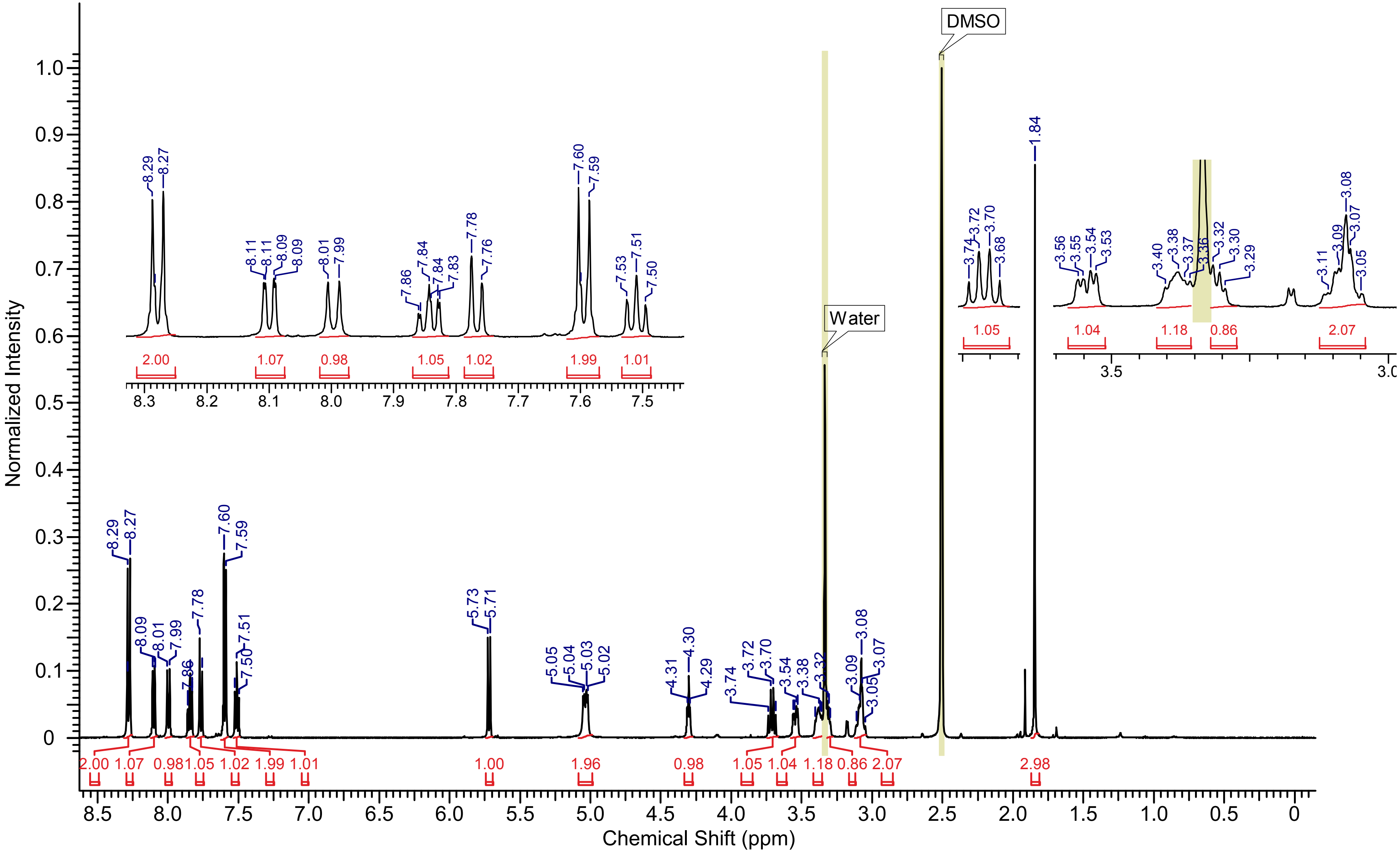
29. [1]H NMR spectrum of 12_b (500 MHz, DMSO-d_6)



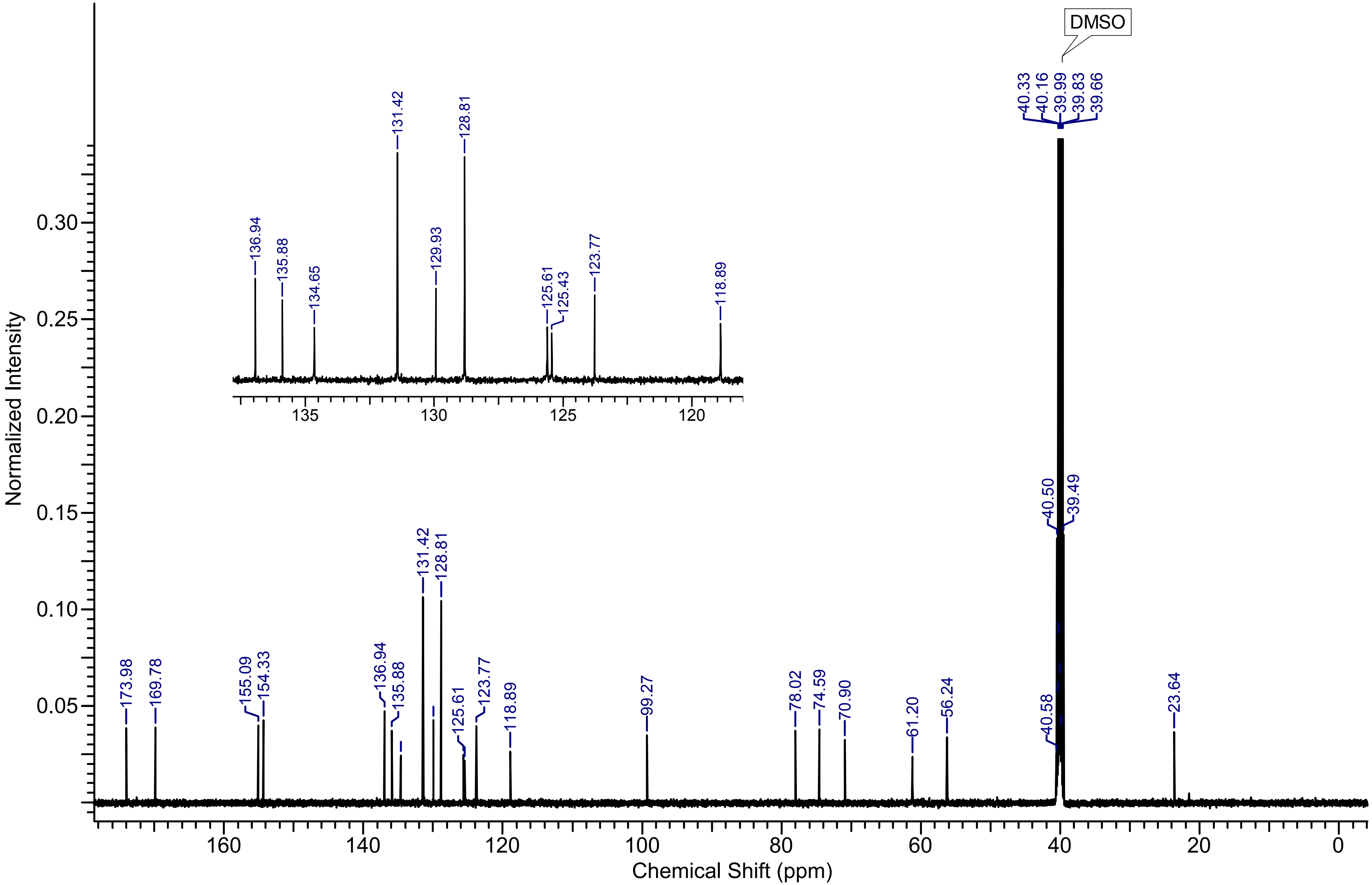
30. ^{13}C NMR spectrum of 12_b (125 MHz, DMSO-d_6)



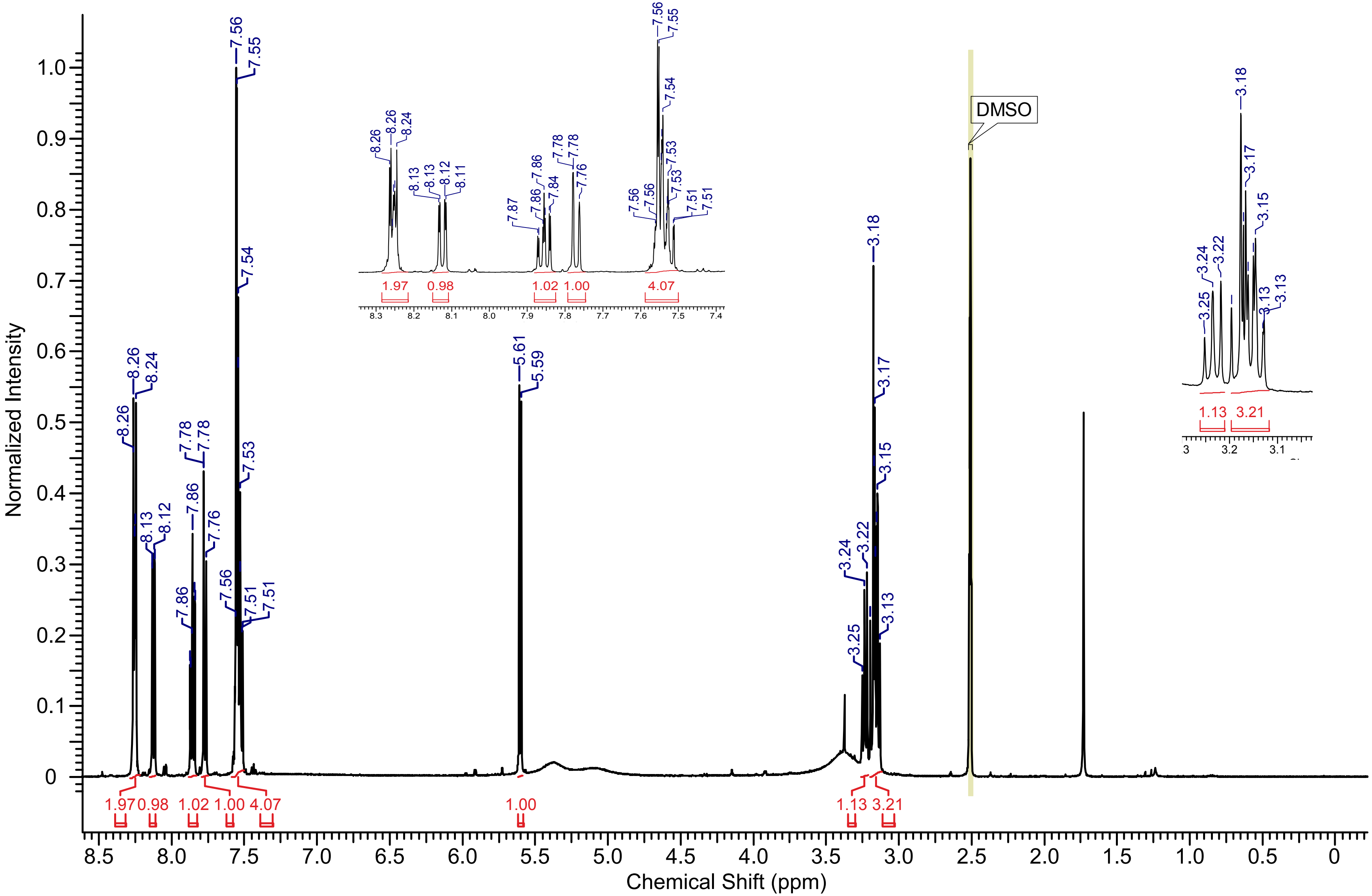
31. ^{1}H NMR spectrum of 12$_e$ (500 MHz, DMSO-d$_6$)



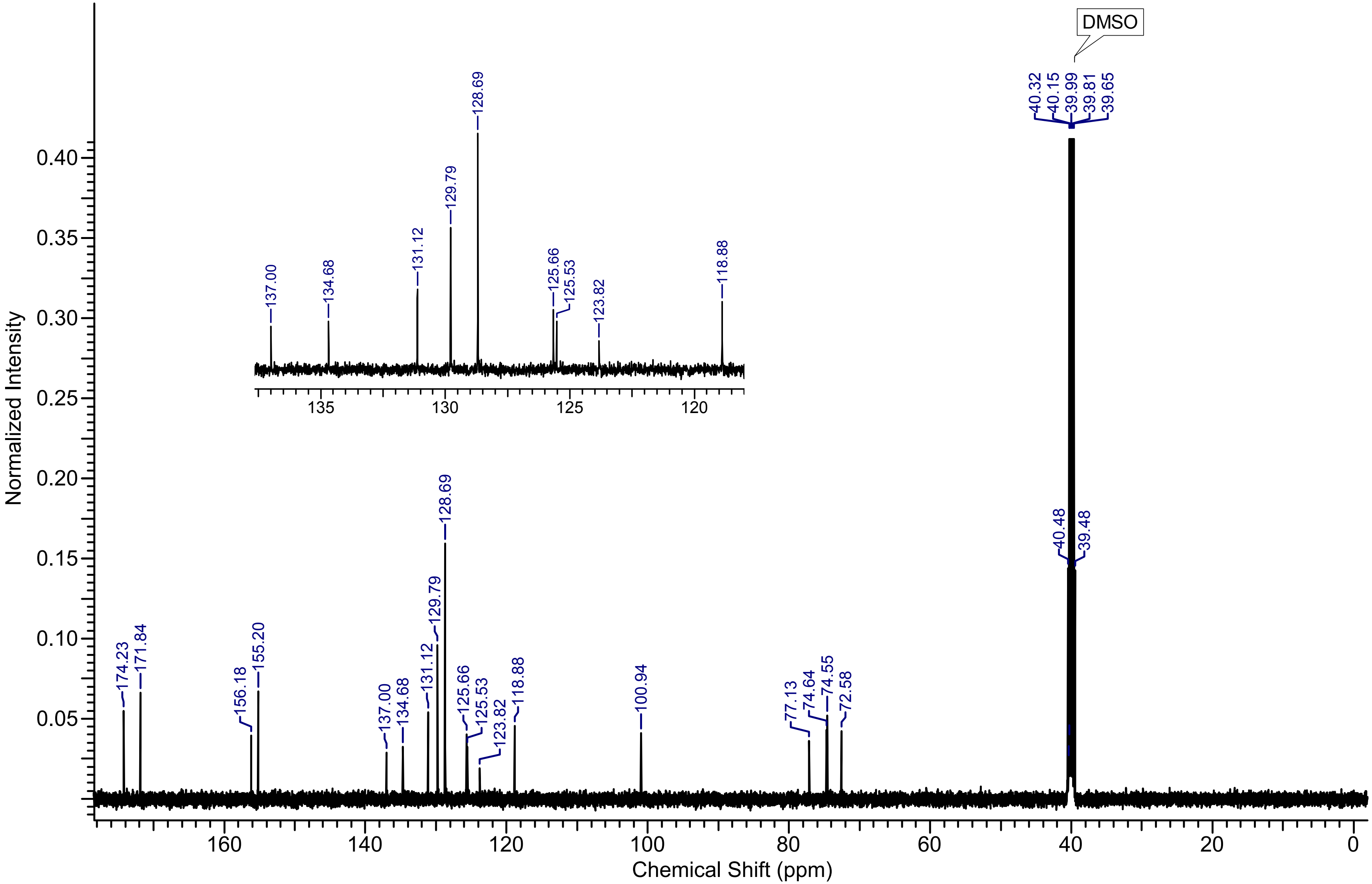
32. ^{13}C NMR spectrum of 12_e (125 MHz, DMSO-d_6)



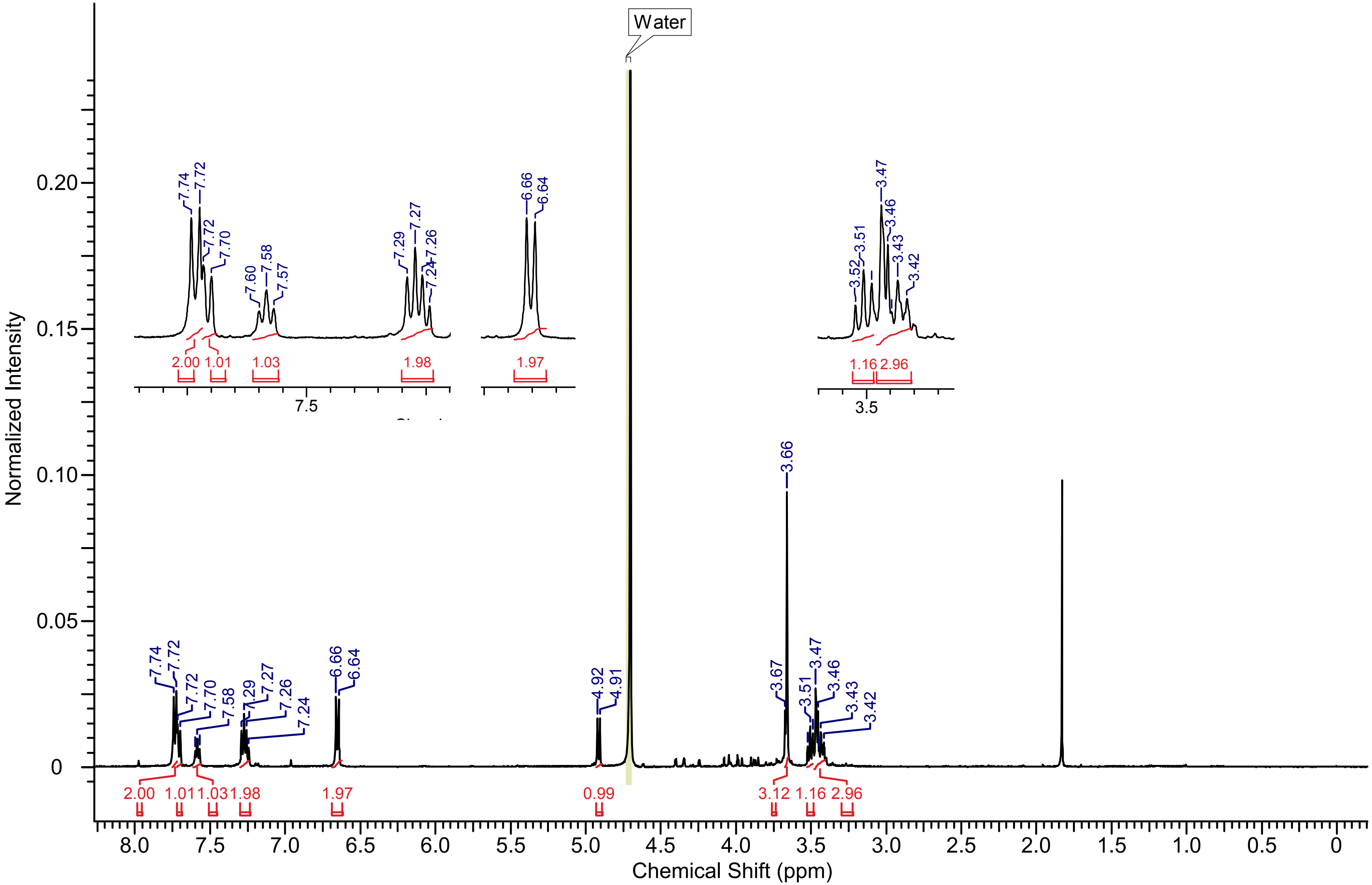
33. ^{1}H NMR spectrum of 13_a (500 MHz, DMSO-d_6)



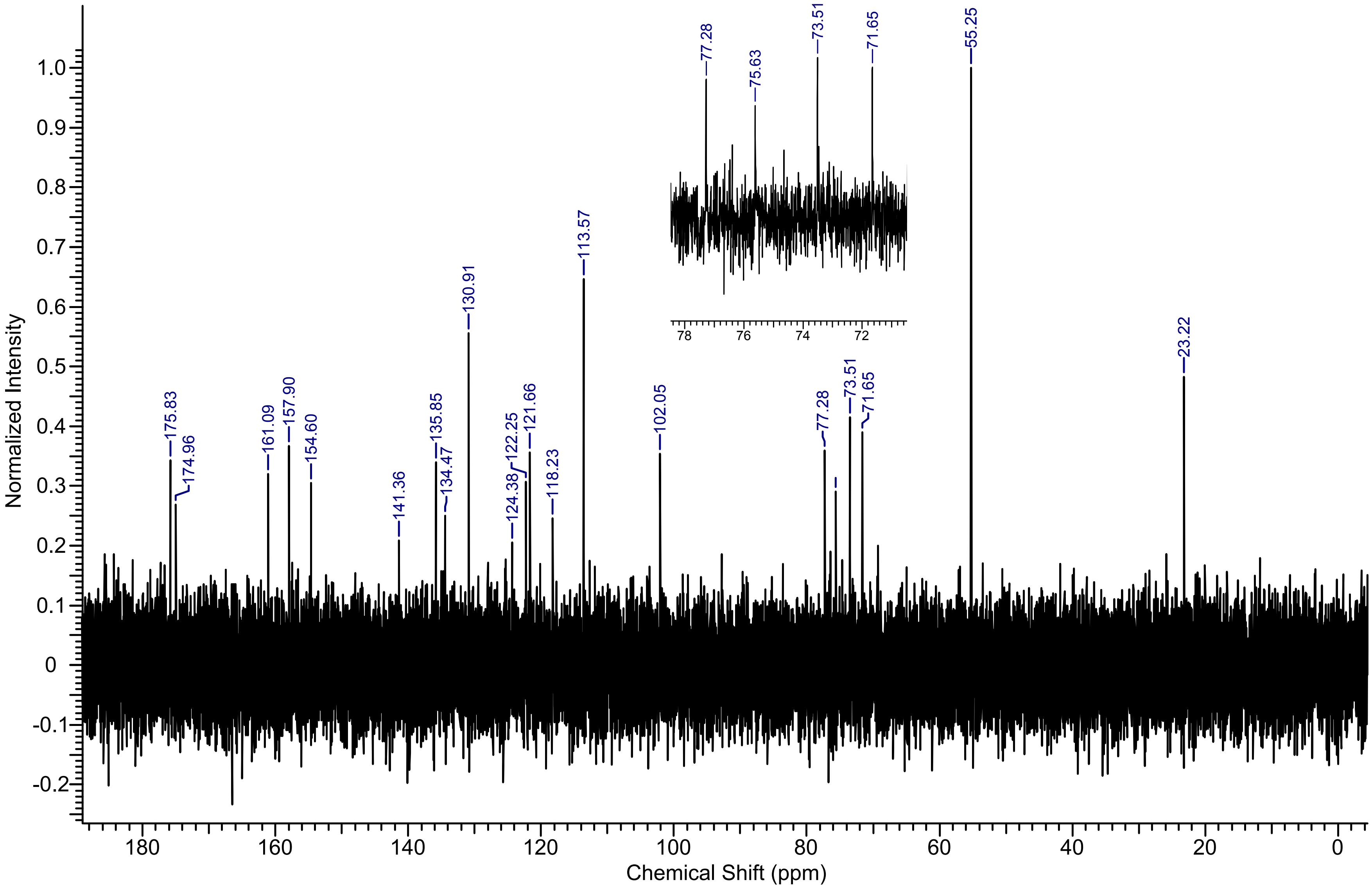
34. ^{13}C NMR spectrum of 13$_a$ (125 MHz, DMSO-d$_6$)



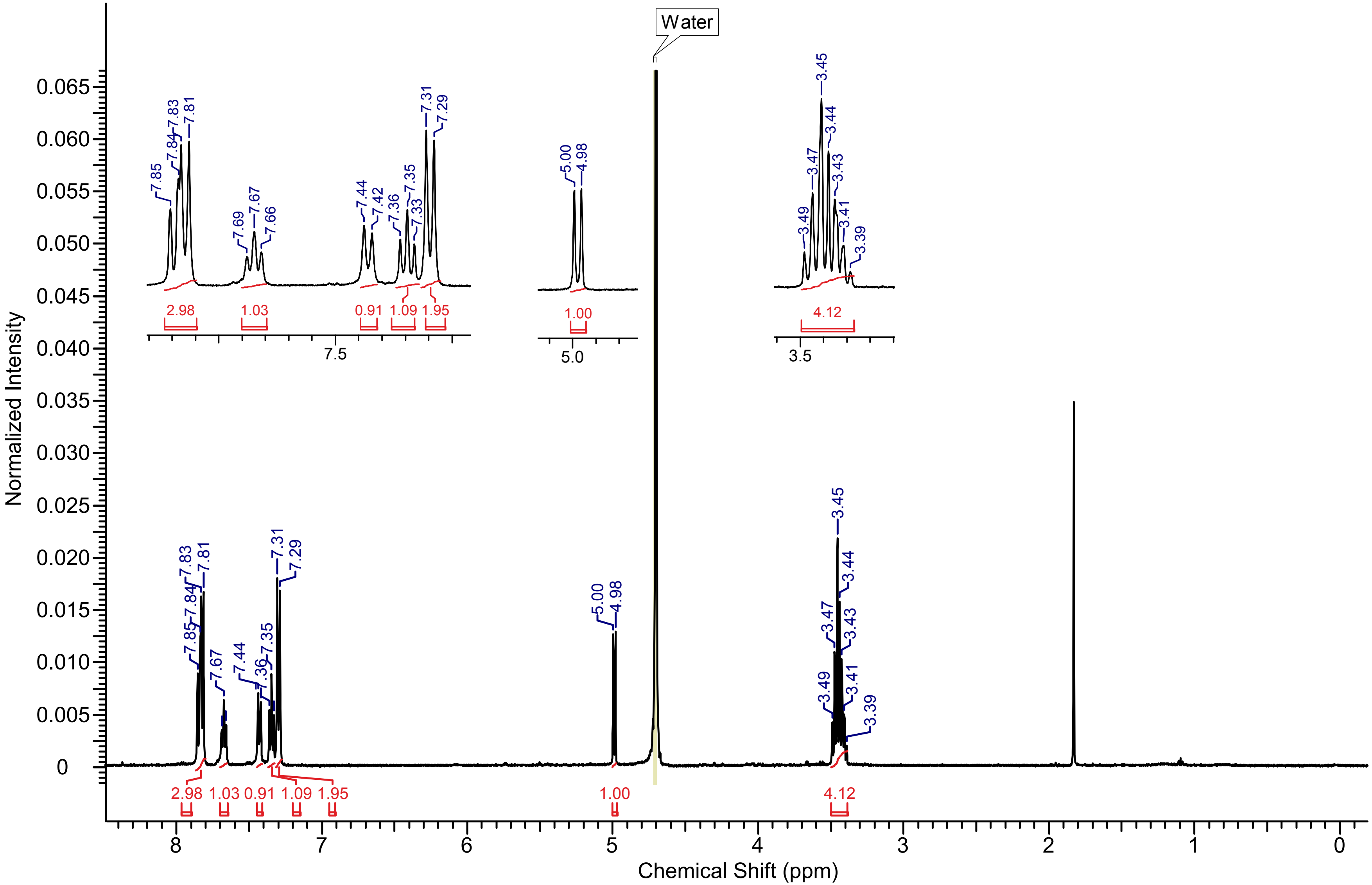
35. [1]H NMR spectrum of 13$_b$ (500 MHz, D_2O)



36. ^{13}C NMR spectrum of 13_b (125 MHz, D_2O)



37. [1]H NMR spectrum of 13$_e$ (500 MHz, D_2O)



38. ^{13}C NMR spectrum of 13_e (125 MHz, D_2O)