

Studies Towards the Synthesis of Smenamide A, an Antiproliferative Metabolite from *Smenospongia aurea*. Total synthesis of *ent*-Smenamide A and 16-*epi*-Smenamide A

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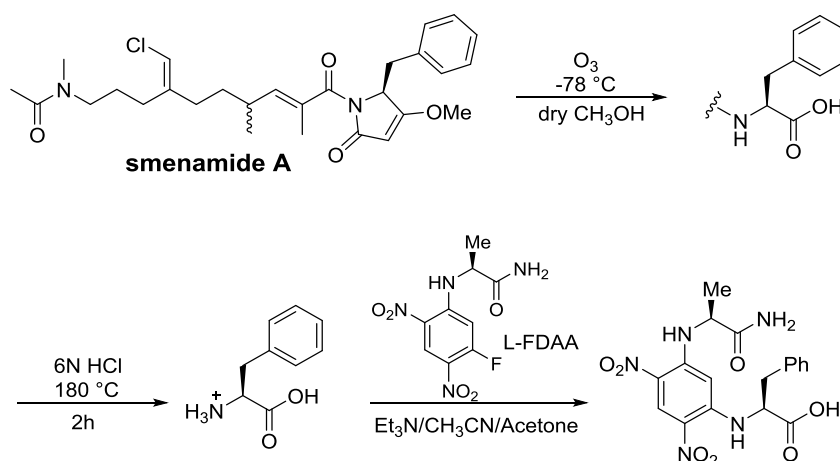


Figure S1. Ozonolysis, hydrolysis and derivatization of smenamide A with L-enantiomer of Marfey's reagent.

A small amount of smenamide A (5 μg) was suspended in ozone-saturated MeOH (300 μL) at -78 °C for 5 min (figure S1). The sample was dried under a N_2 stream to remove ozone, then treated with 6N HCl and heated in a sealed glass tube at 180 °C for 2 h. The residual HCl fumes were removed in vacuo. The hydrolysate was dissolved in Et_3N /acetone (2:3, 100 μL) and the solution was treated with 100 μL of 1% 1-fluoro-2,4-dinitrophenyl-5-L-alaninamide (L-FDAA) in CH_3CN /acetone (1:2). The vial was heated at 50 °C for 1 h. The mixture was dried, and the resulting L-FDAA-Phe derivative was redissolved in $\text{CH}_3\text{CN}/\text{H}_2\text{O}$ (5:95, 500 μL) for LC-MS analysis. An authentic L-Phe standard was treated with l-FDAA and d-FDAA as described above to give, respectively, the L-FDAA-L-Phe and D-FDAA-L-Phe standards used in the subsequent LC-MS analysis.

Marfey's derivatives were analyzed by LC-HRESIMS. A 5 μm Kinetex C18 column (50 \times 2.10 mm), maintained at 25 °C, was eluted at 200 $\mu\text{L min}^{-1}$ with H_2O and CH_3CN , using a gradient elution. The gradient program was as follows: 5% CH_3CN 3 min, 5-60% CH_3CN over 20 min, 90% CH_3CN 5 min. Mass spectra were acquired in positive ion detection mode and the data were analyzed using the suite of programs Xcalibur. The retention times of L-FDAA-L-Phe and D-FDAA-L-Phe standards were determined as 17.32

min and 18.60 min, respectively, on the basis of the extracted-ion chromatograms at m/z 418.1357. The retention time, measured in the same way for the L-FDAA-Phe sample obtained from smenamamide A, was 17.36 min, and was indicative of the L configuration of Phe residue in smenamamide A (figure S2).

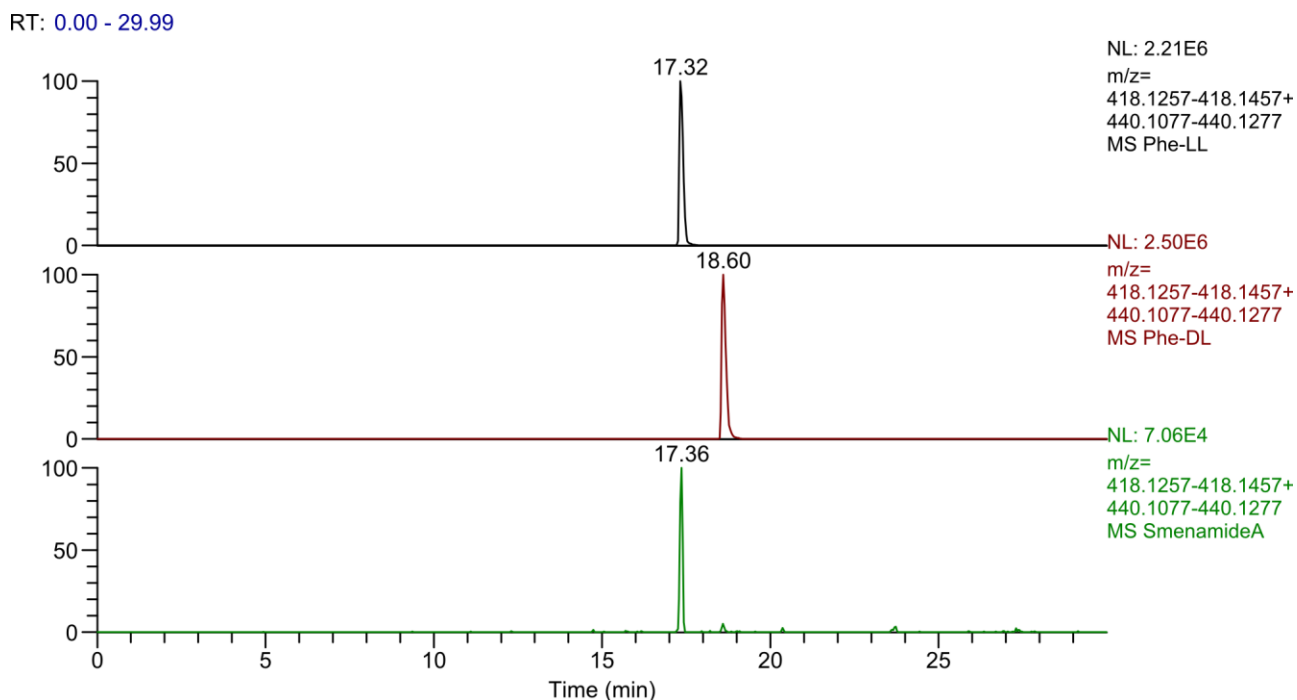
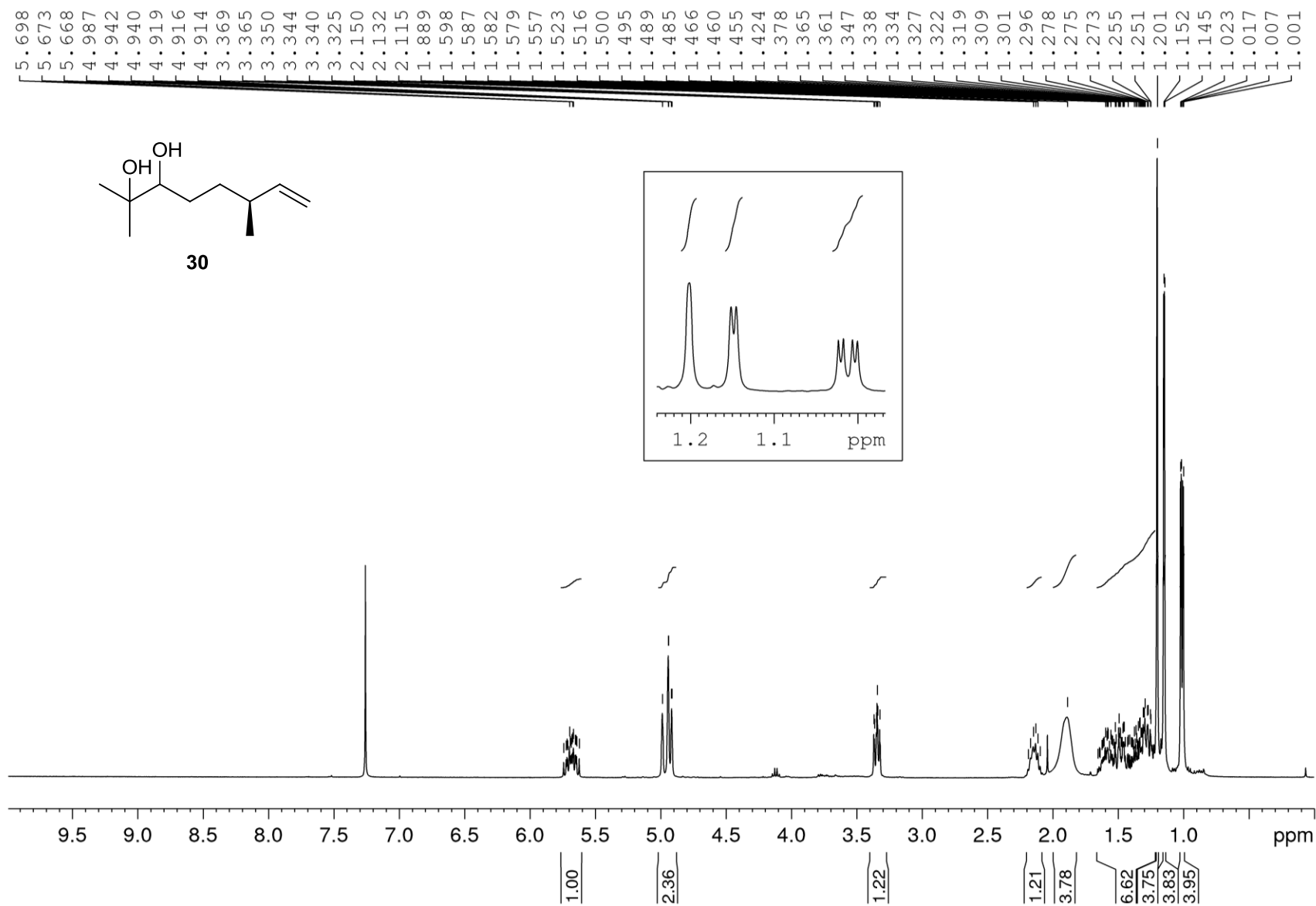
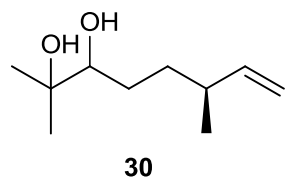


Figure S2. HR-ESI-MS-HPLC analysis of Marfey's derivative from smenamamide A. Extracted-ion chromatograms at m/z 418.1357 of authentic 1-fluoro-2,4-dinitrophenyl-5-alanine amide L-phenylalanine (L-FDAA-L-Phe), authentic D-FDAA-L-Phe and L-FDAA-Phe from smenamamide A.

Table S1. NMR data of natural smenamamide A (**1**) and *ent*-smenamamide A (**29**) (700 MHz, CD₃OD).

smenamamide A					<i>ent</i> -smenamamide A						
Position	Z-Conformer		E-Conformer		Position	Z-Conformer		E-Conformer			
	δ_{H} [Mult., <i>J</i> (Hz)]	δ_{C} [Mult.]	δ_{H} [Mult., <i>J</i> (Hz)]	δ_{C} [Mult.]		δ_{H} [Mult., <i>J</i> (Hz)]	δ_{C} [Mult.]	δ_{H} [Mult., <i>J</i> (Hz)]	δ_{C} [Mult.]		
1	–	135.6 (C)	–	135.6 (C)	1	–	135.7 (C)	–	135.7 (C)		
2/6	6.99 (m)	130.8 (CH)	6.99 (m)	130.8 (CH)	2/6	6.90 (m)	130.9 (CH)	6.90 (m)	130.9 (CH)		
3/5	7.23 (ovl)	129.4 (CH)	7.23 (ovl)	129.4 (CH)	3/5	7.23 (ovl)	129.3 (CH)	7.23 (ovl)	129.3 (CH)		
4	7.23 (ovl)	128.3 (CH)	7.23 (ovl)	128.3 (CH)	4	7.23 (ovl)	128.2 (CH)	7.23 (ovl)	128.2 (CH)		
7	a	3.37 (ovl)	34.8 (CH ₂)	3.37 (ovl)	34.8 (CH ₂)	7	a	3.36 (ovl)	34.7 (CH ₂)	3.36 (ovl)	34.7 (CH ₂)
	b	3.19 (m)		3.19 (m)			b	3.20 (m)		3.20 (m)	
8	5.02 (ovl)	60.5 (CH)	5.02 (ovl)	60.5 (CH)	8	5.02 (ovl)	60.5 (CH)	5.02 (ovl)	60.5 (CH)		
9	–	179.5 (C)	–	179.5 (C)	9	–	179.8 (C)	–	179.8 (C)		
10	5.04 (br. s)	95.5 (CH)	5.02 (br. s)	95.5 (CH)	10	5.04 (br. s)	95.5 (CH)	5.02 (br. s)	95.5 (CH)		
11	–	170.7 (C)	–	170.7 (C)	11	–	171.1 (C)	–	171.1 (C)		
12	–	172.3 (C)	–	172.2 (C)	12	–	172.7 (C)	–	172.7 (C)		
13	–	132.1 (C)	–	132.1 (C)	13	–	132.1 (C)	–	132.1 (C)		
14	1.77 (d, 1.5)	13.7 (CH ₃)	1.78 (d, 1.5)	13.7 (CH ₃)	14	1.77 (d, 1.4)	13.7 (CH ₃)	1.78 (d, 1.4)	13.7 (CH ₃)		
15	5.36 (br. d, 10.2)	144.1 (CH)	5.36 (br. d, 10.2)	144.1 (CH)	15	5.36 (br. d, 10.1)	144.1 (CH)	5.36 (br. d, 10.1)	144.1 (CH)		
16	2.45 (m)	33.4 (CH)	2.48 (m)	33.4 (CH)	16	2.45 (m)	33.4 (CH)	2.48 (m)	33.5 (CH)		
17	0.98 (d, 6.5)	20.4 (CH ₃)	1.00 (d, 6.5)	20.6 (CH ₃)	17	0.98 (d, 6.5)	20.6 (CH ₃)	1.00 (d, 6.5)	20.7 (CH ₃)		
18	a	1.51 (ovl)	36.1 (CH ₂)	1.52 (ovl)	35.9 (CH ₂)	18	a	1.51 (ovl)	36.4 (CH ₂)	1.51 (ovl)	36.2 (CH ₂)
	b	1.28 (ovl)		1.30 (ovl)			b	1.29 (ovl)		1.29 (ovl)	
19	a	2.19 (ovl)	33.2 (CH ₂)	2.23 (ovl)	33.2 (CH ₂)	19	a	2.19 (ovl)	33.2 (CH ₂)	2.23 (ovl)	33.3 (CH ₂)
	b	2.06 (ovl)		2.05 (ovl)			b	2.05 (ovl)		2.05 (ovl)	
20	–	143.1 (C)	–	142.8 (C)	20	–	143.4 (C)	–	143.2 (C)		
21	5.93 (br. s)	113.9 (CH)	5.97 (br. s)	114.1 (CH)	21	5.92 (br. s)	113.9 (CH)	5.96 (br. s)	114.1 (CH)		
22	a	2.22 (m)	28.1 (CH ₂)	2.26 (m)	28.0 (CH ₂)	22	a	2.22 (m)	28.1 (CH ₂)	2.26 (m)	27.9 (CH ₂)
	b	2.15 (m)		2.18 (m)			b	2.15 (m)		2.18 (m)	
23	1.64 (m)	25.9 (CH ₂)	1.70 (m)	26.6 (CH ₂)	23	1.65 (m)	25.7 (CH ₂)	1.70 (m)	26.6 (CH ₂)		
24	3.36 (ovl)	48.6 (CH ₂)	3.33 (ovl)	51.5 (CH ₂)	24	3.36 (ovl)	48.4 (CH ₂)	3.34 (ovl)	51.6 (CH ₂)		
25	–	172.9 (C)	–	172.7 (C)	25	–	172.7 (C)	–	172.7 (C)		
26	2.08 (s)	21.7 (CH ₃)	2.07 (s)	21.1 (CH ₃)	26	2.08 (s)	21.7 (CH ₃)	2.07 (s)	21.0 (CH ₃)		
27	3.03 (s)	36.6 (CH ₃)	2.88 (s)	33.7 (CH ₃)	27	3.03 (s)	36.6 (CH ₃)	2.88 (s)	33.7 (CH ₃)		
OMe	3.97 (s)	59.7 (CH ₃)	3.97 (s)	59.7 (CH ₃)	OMe	3.95 (s)	59.6 (CH ₃)	3.95 (s)	59.6 (CH ₃)		





144.525
144.297

112.976
112.758

78.893
78.433
77.308
76.994
76.675
73.448
73.290

37.943
37.722
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20.149

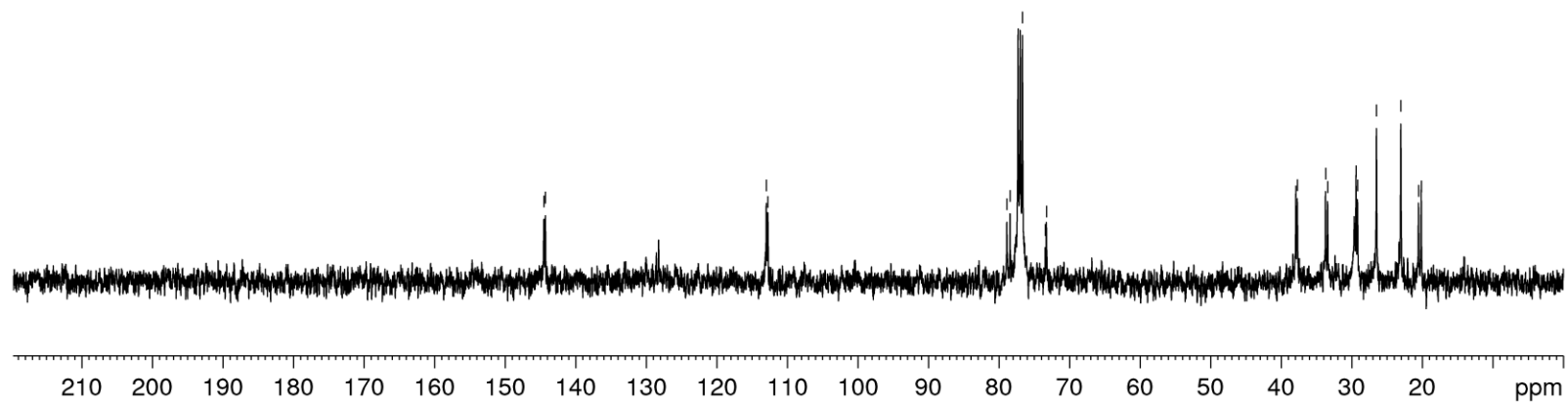


Figure S4. ^{13}C NMR spectrum of compound **30** (CDCl_3 , 100 MHz)

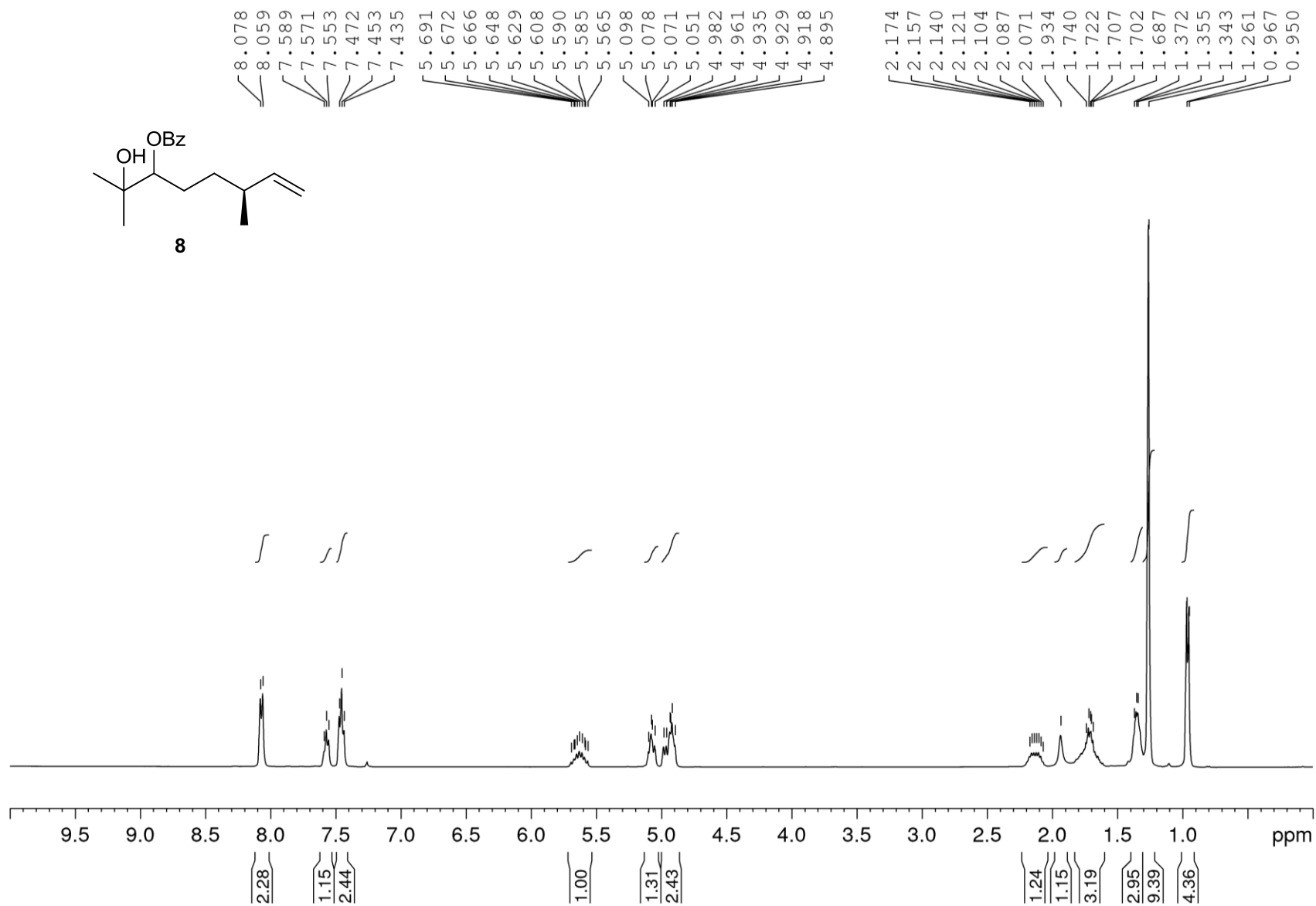


Figure S5. ^1H NMR spectrum of compound **8** (CDCl_3 , 400 MHz)

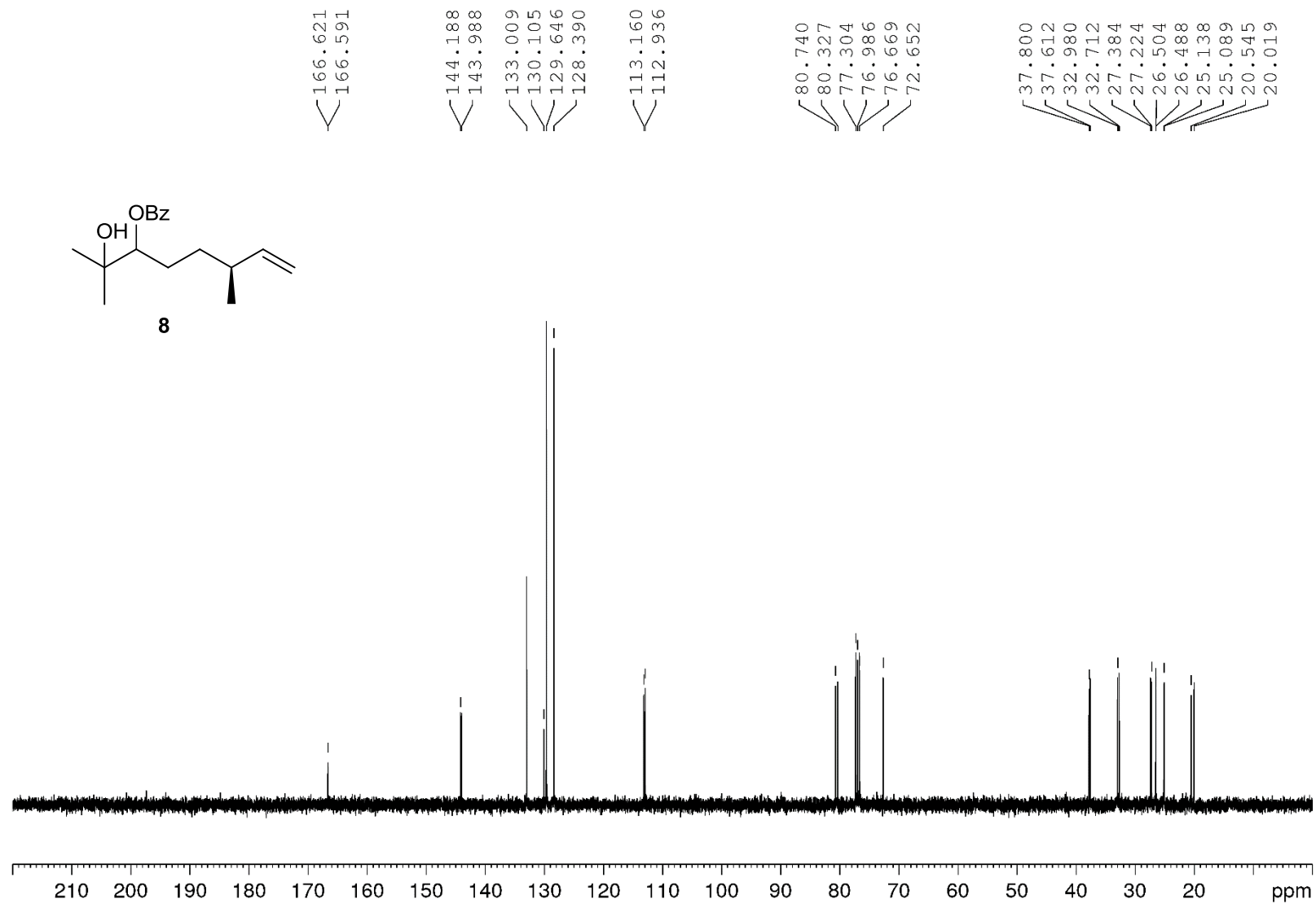
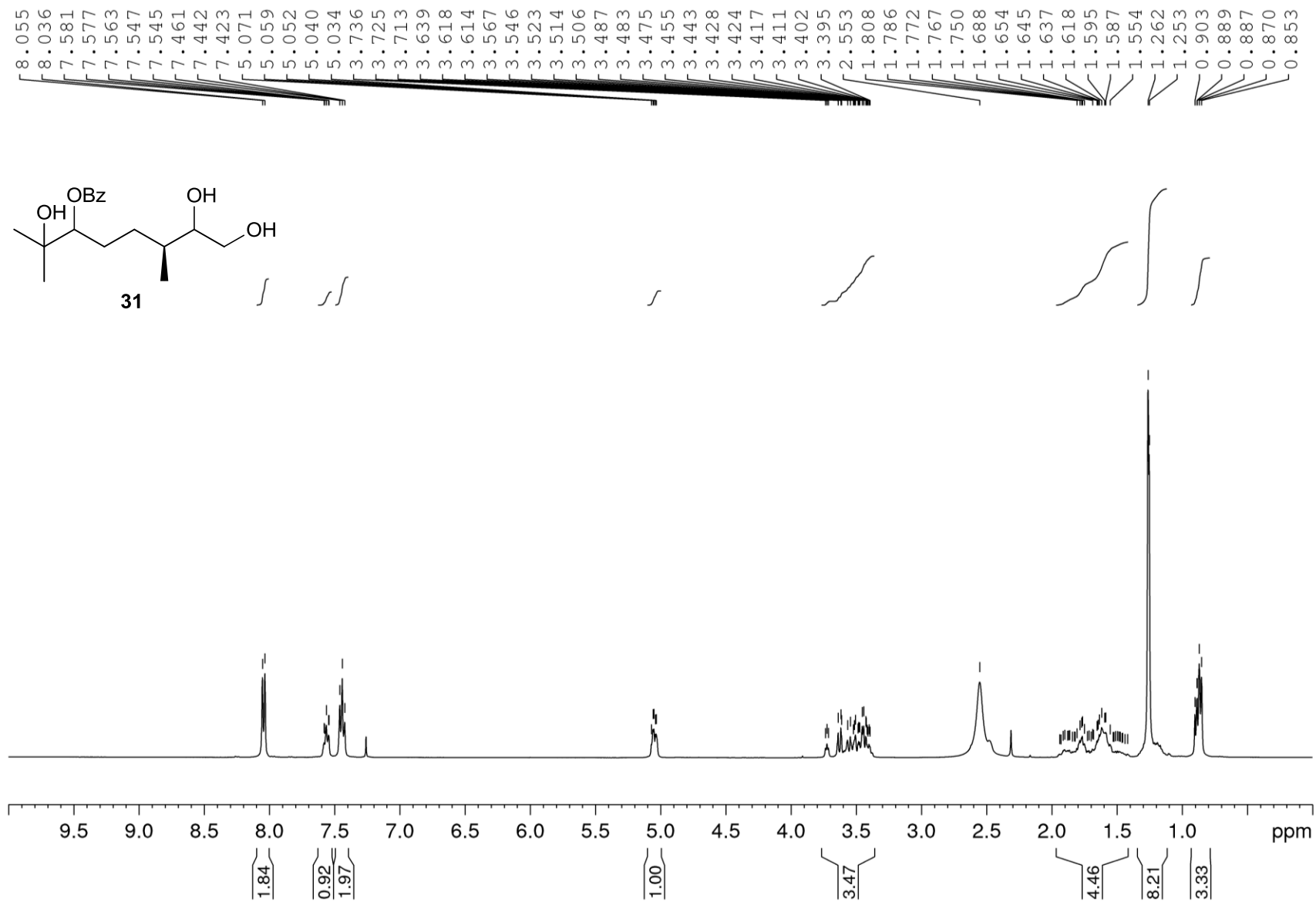


Figure S6. ^{13}C NMR spectrum of compound **8** (CDCl₃, 100 MHz)



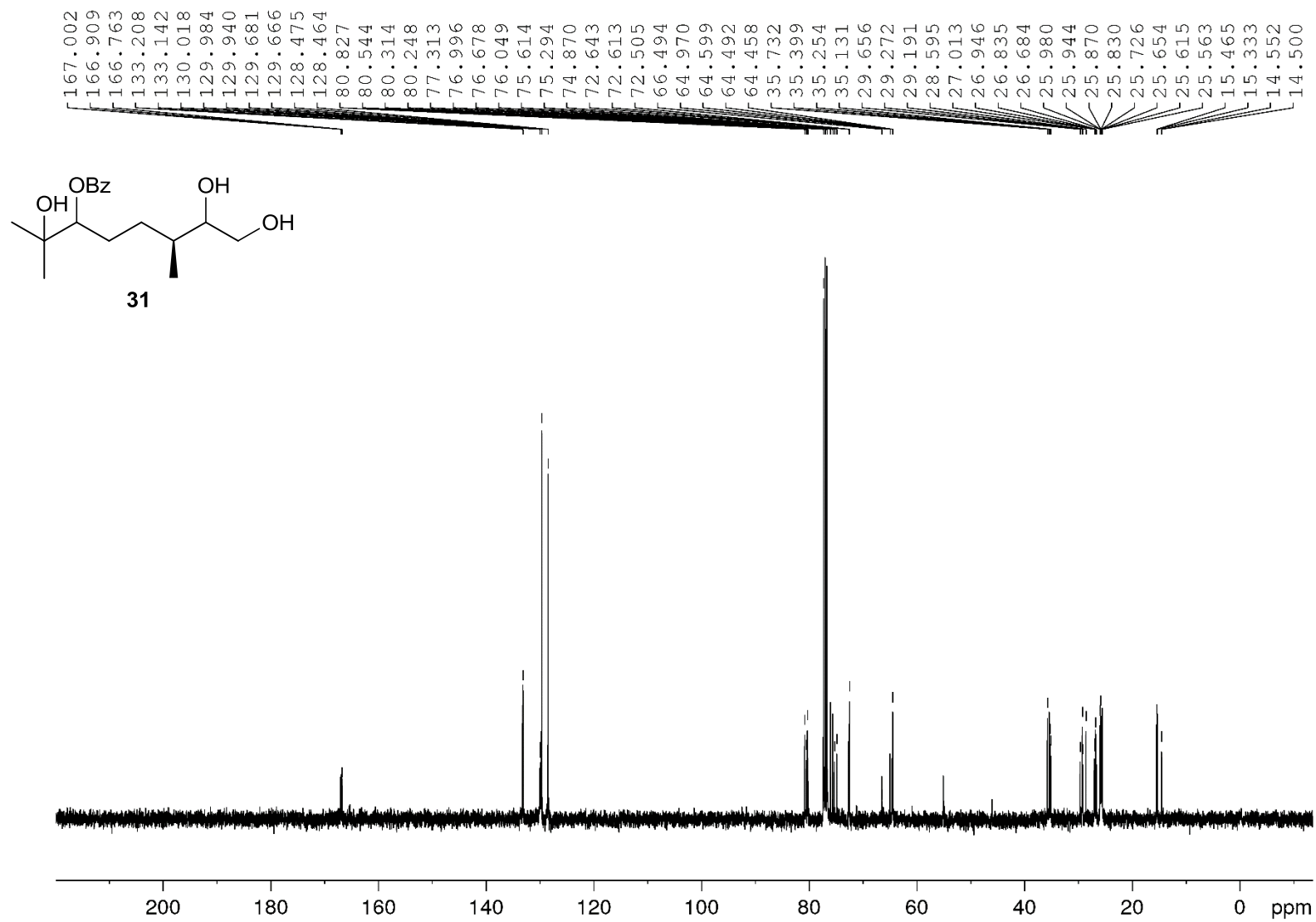
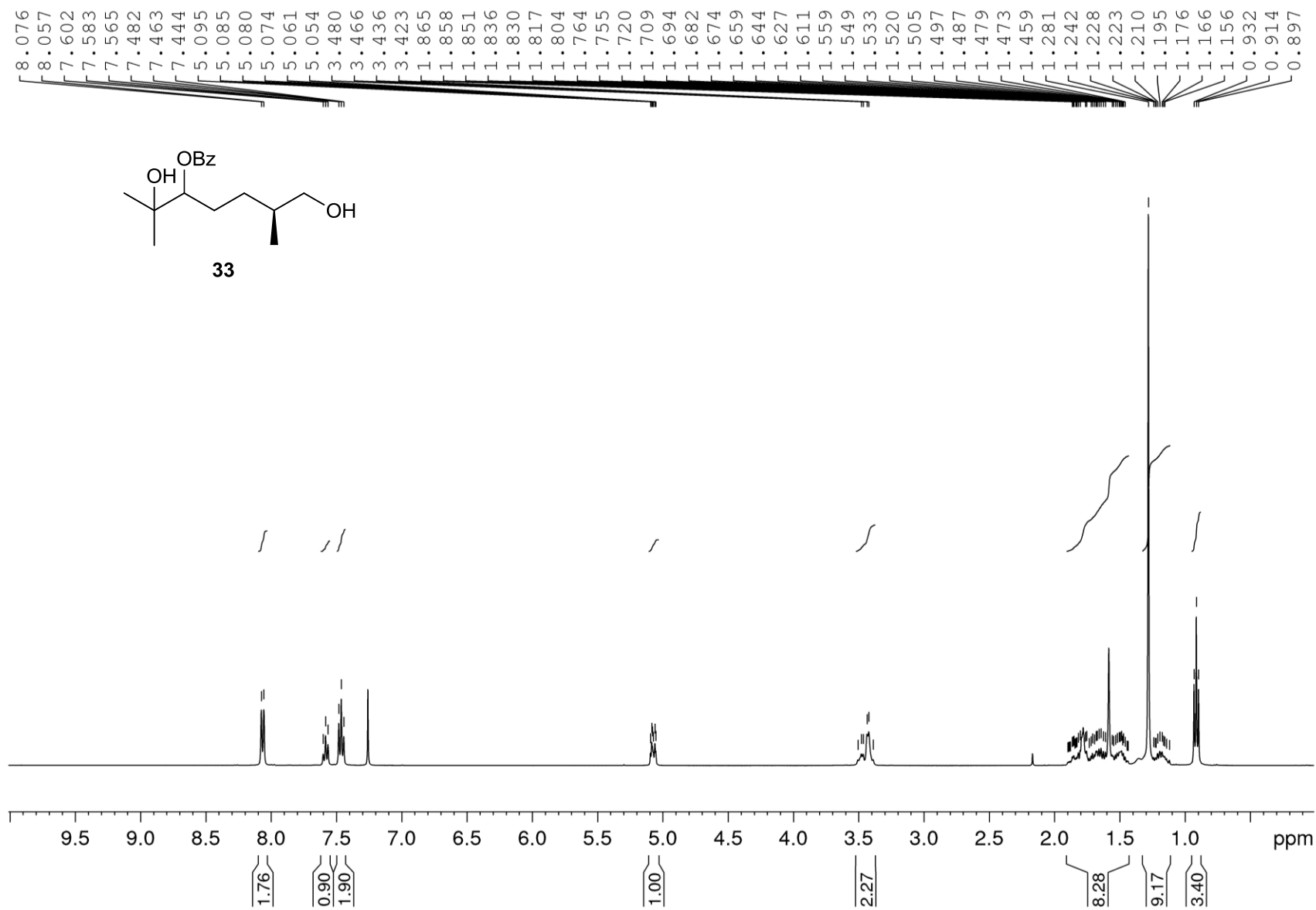


Figure S8. ¹³C NMR spectrum of compound **31** (CDCl₃, 100 MHz)



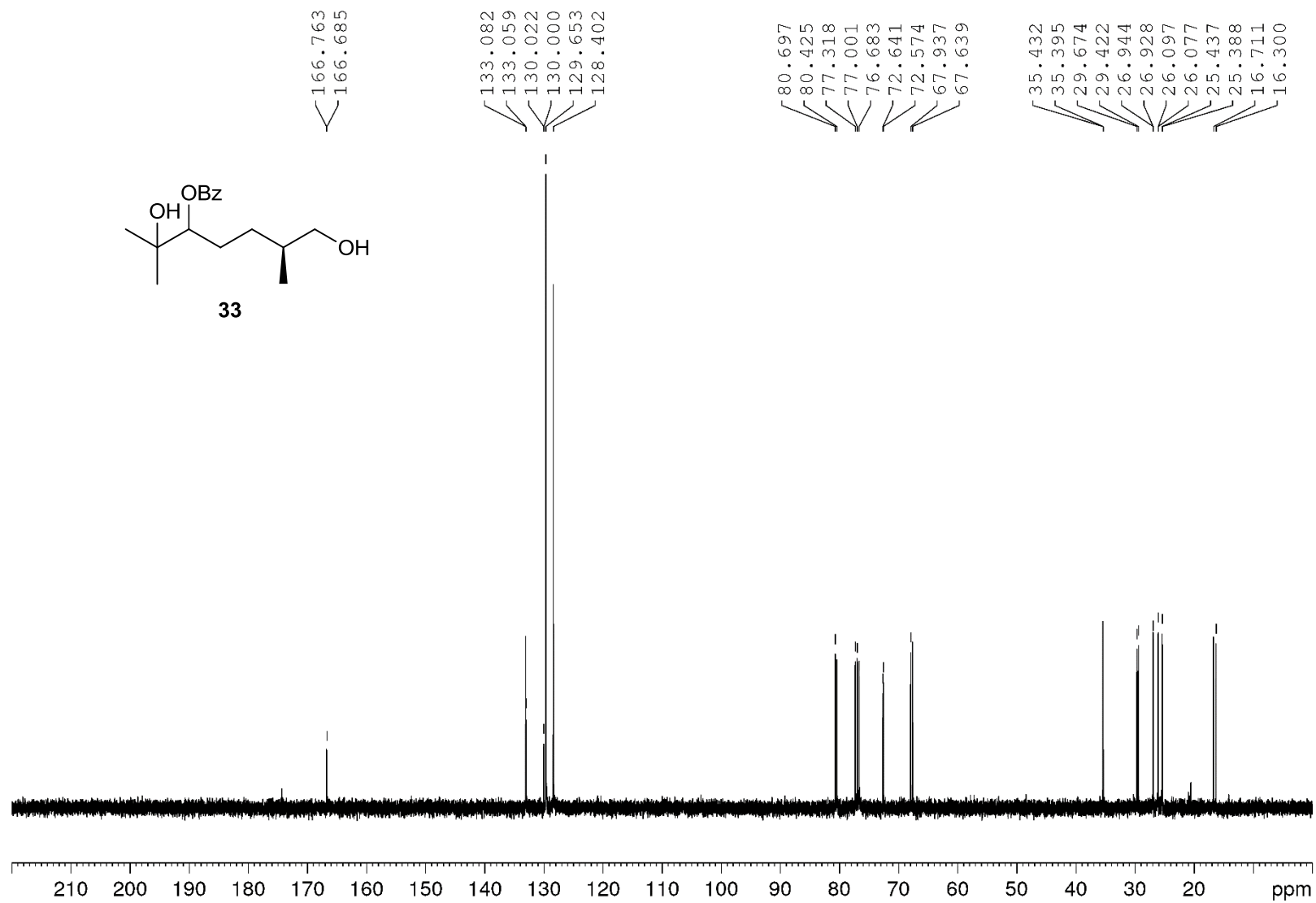


Figure S10. ^{13}C NMR spectrum of compound **33** (CDCl_3 , 100 MHz)

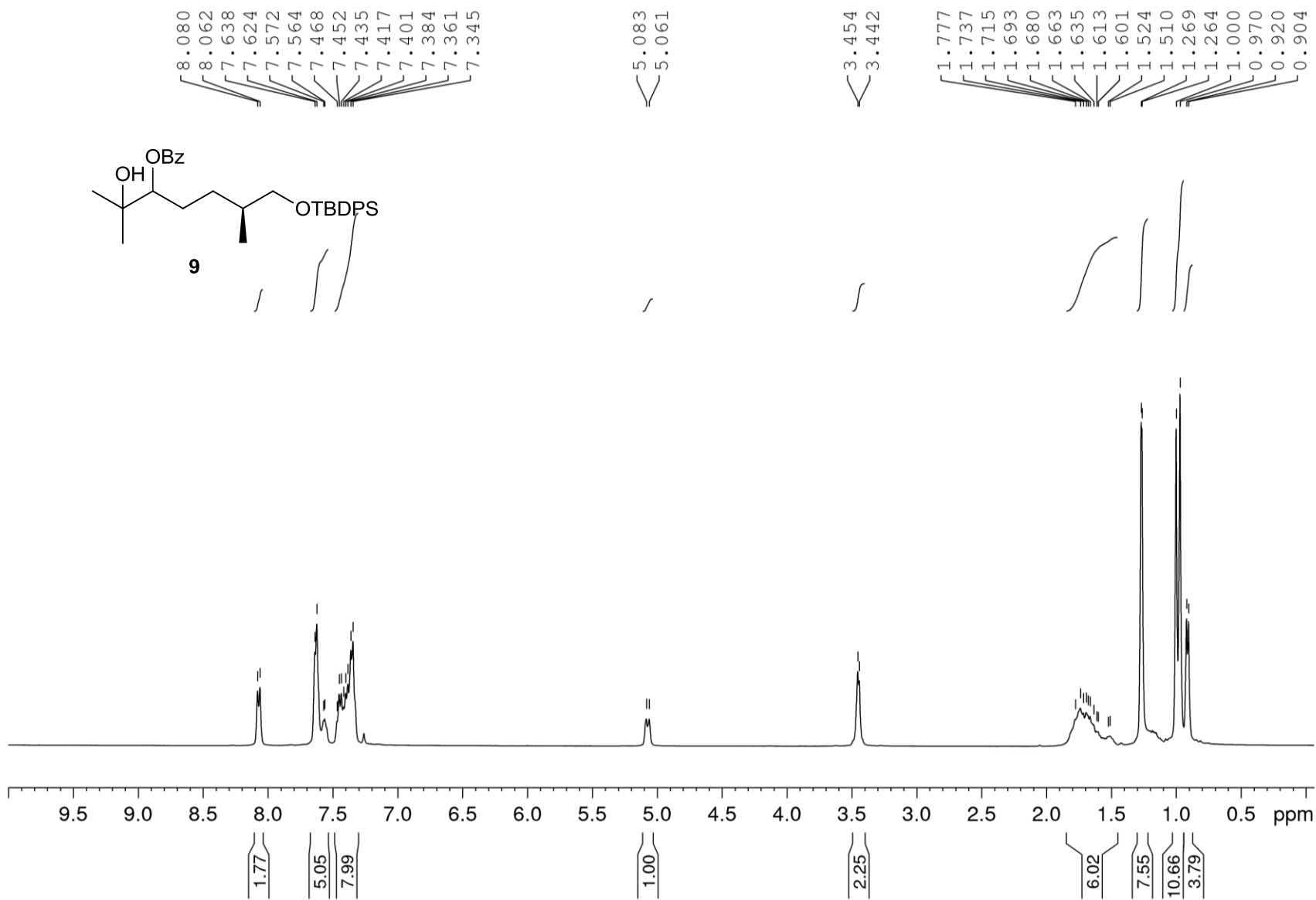


Figure S11. ^1H NMR spectrum of compound **9** (CDCl_3 , 400 MHz)

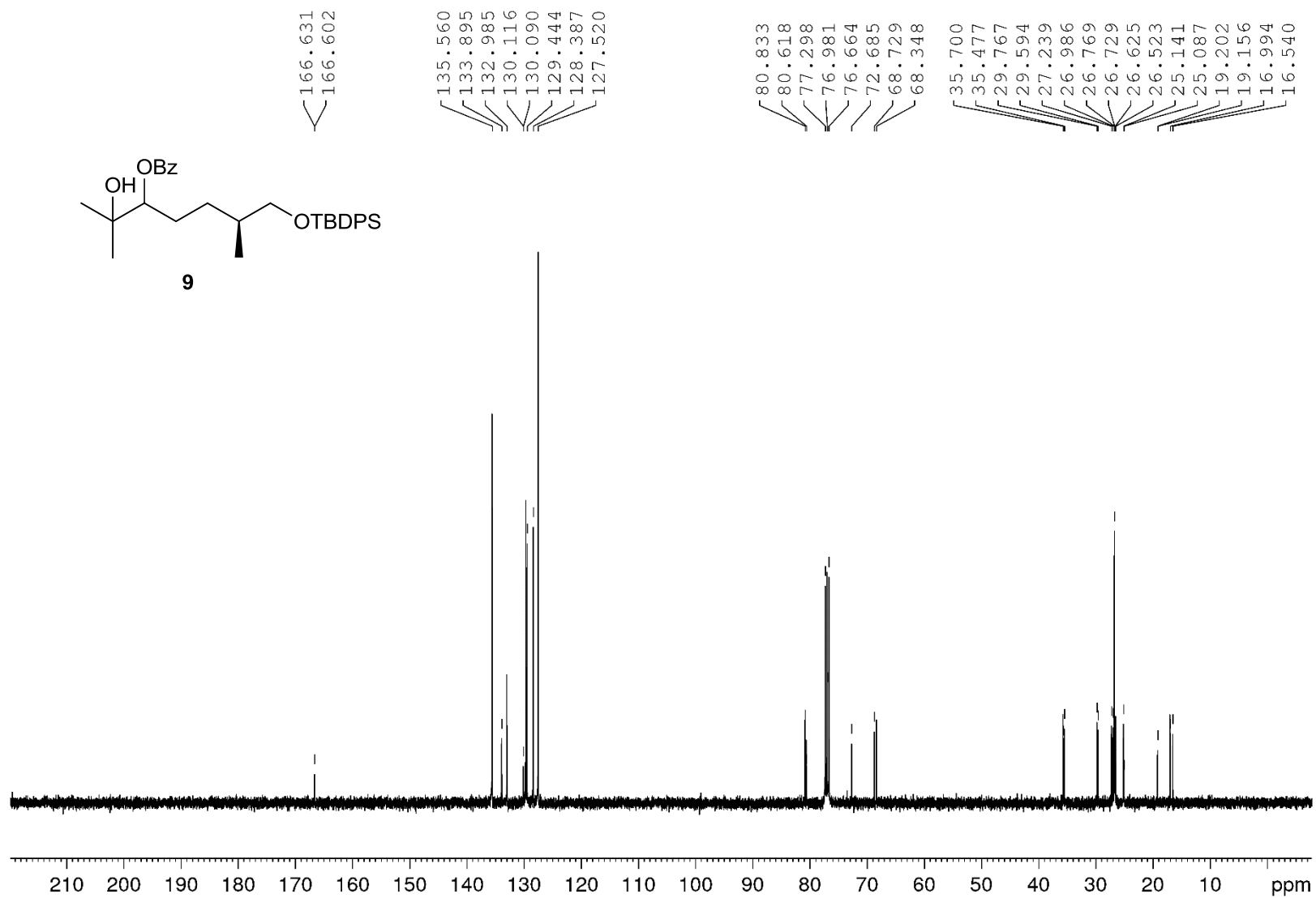


Figure S12. ^{13}C NMR spectrum of compound **9** (CDCl₃, 100 MHz)

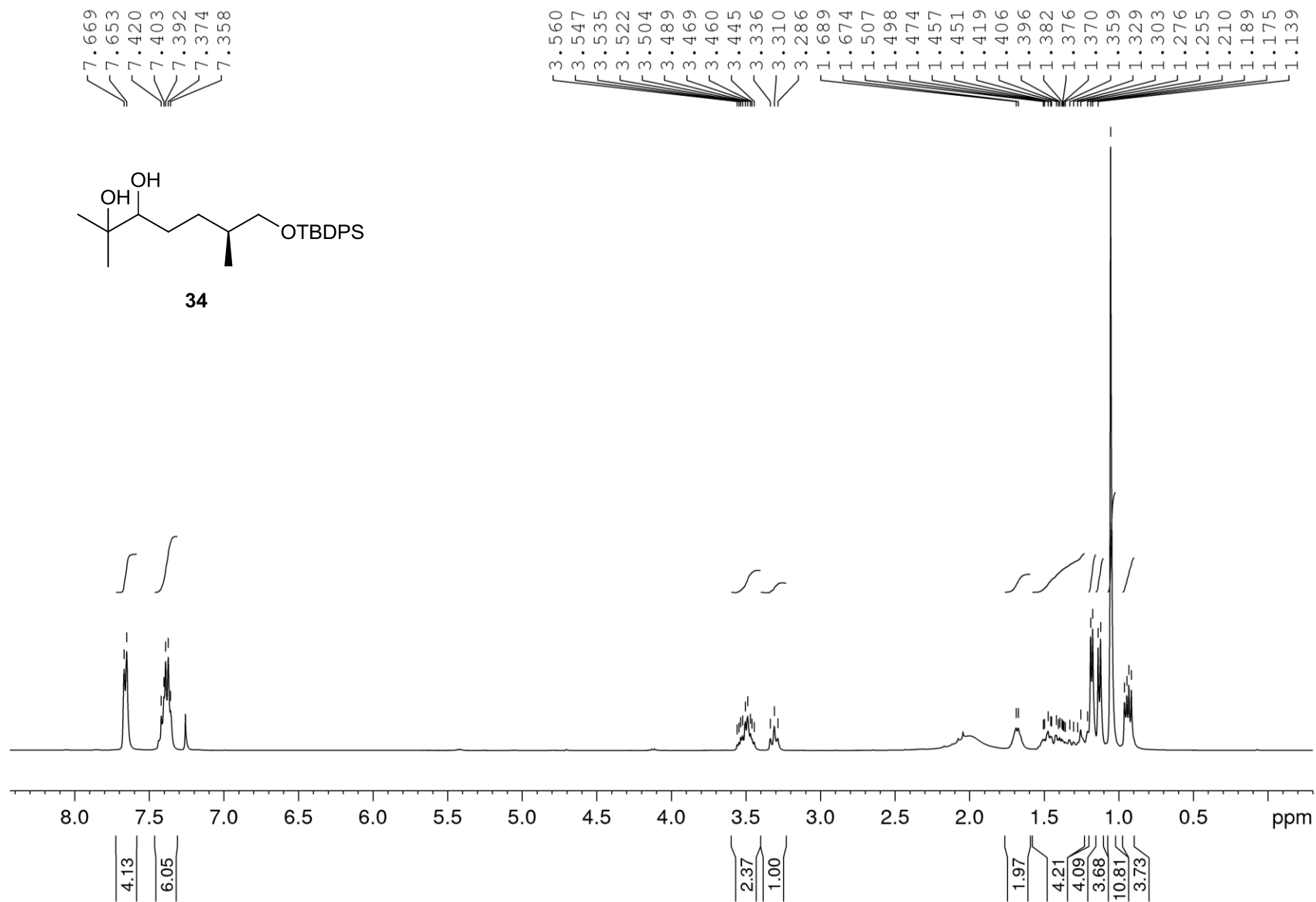


Figure S13. ^1H NMR spectrum of compound **34** (CDCl₃, 400 MHz)

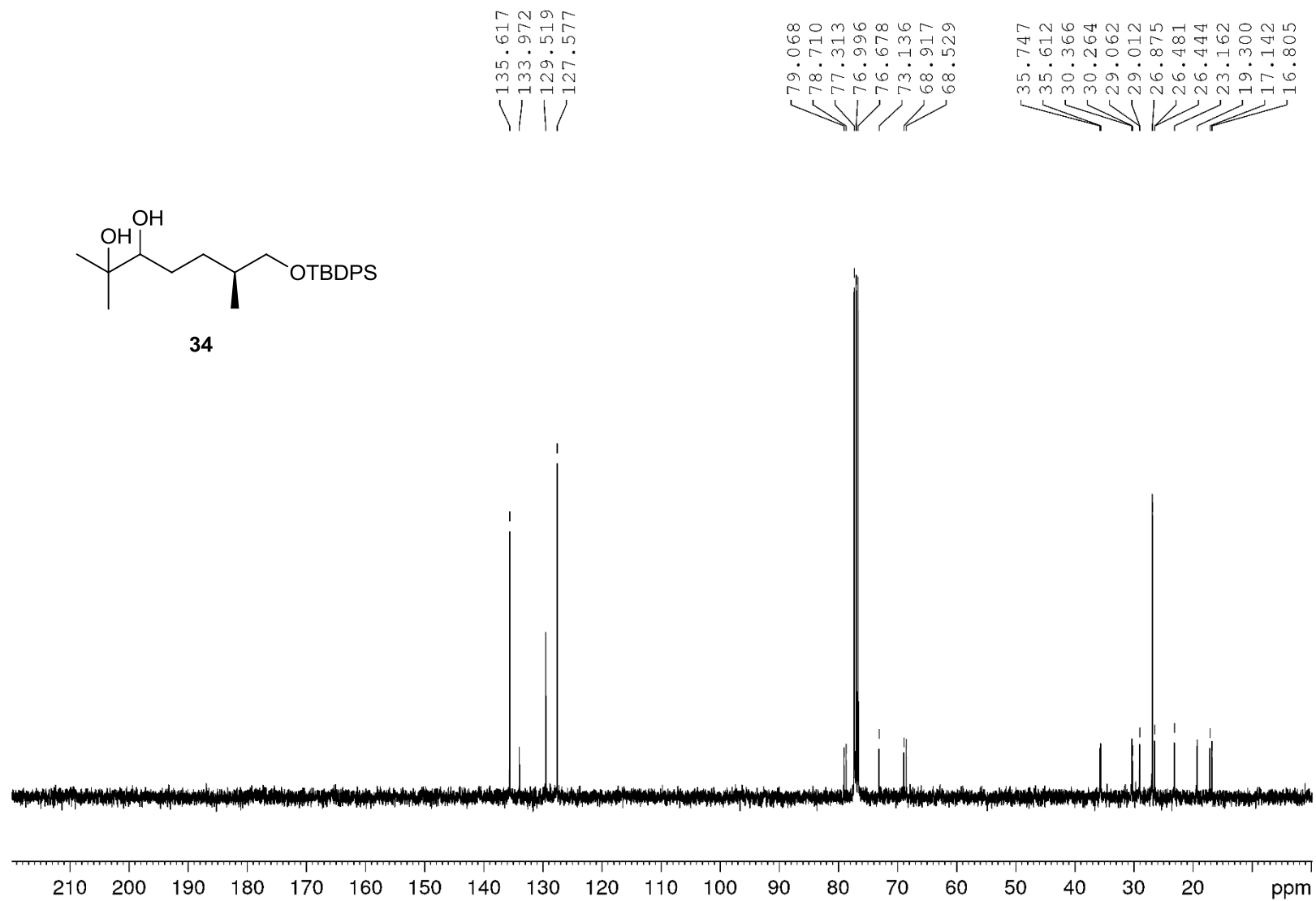


Figure S14. ^{13}C NMR spectrum of compound **34** (CDCl₃, 100 MHz)

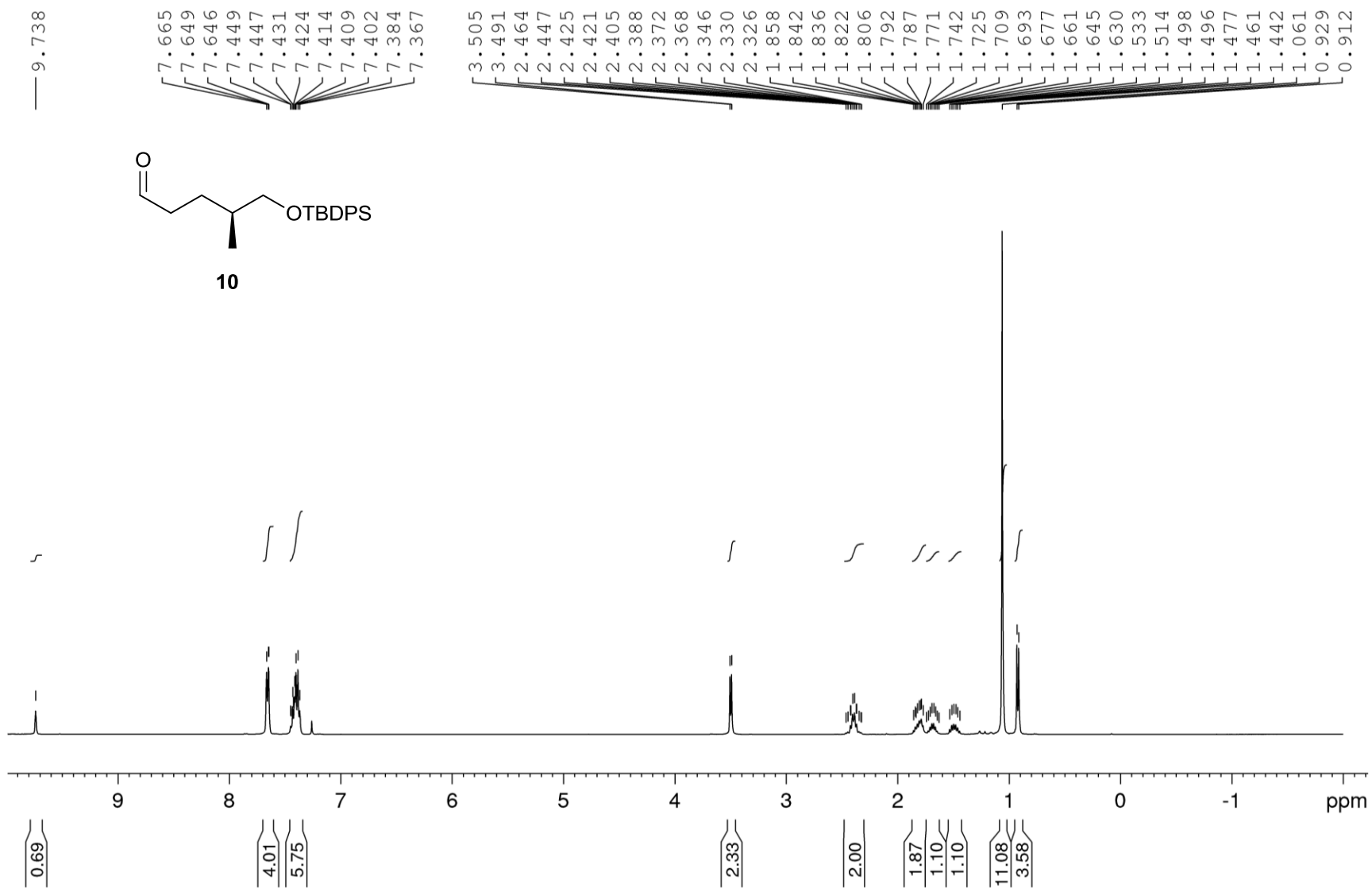


Figure S15. ¹H NMR spectrum of compound **10** (CDCl₃, 400 MHz)

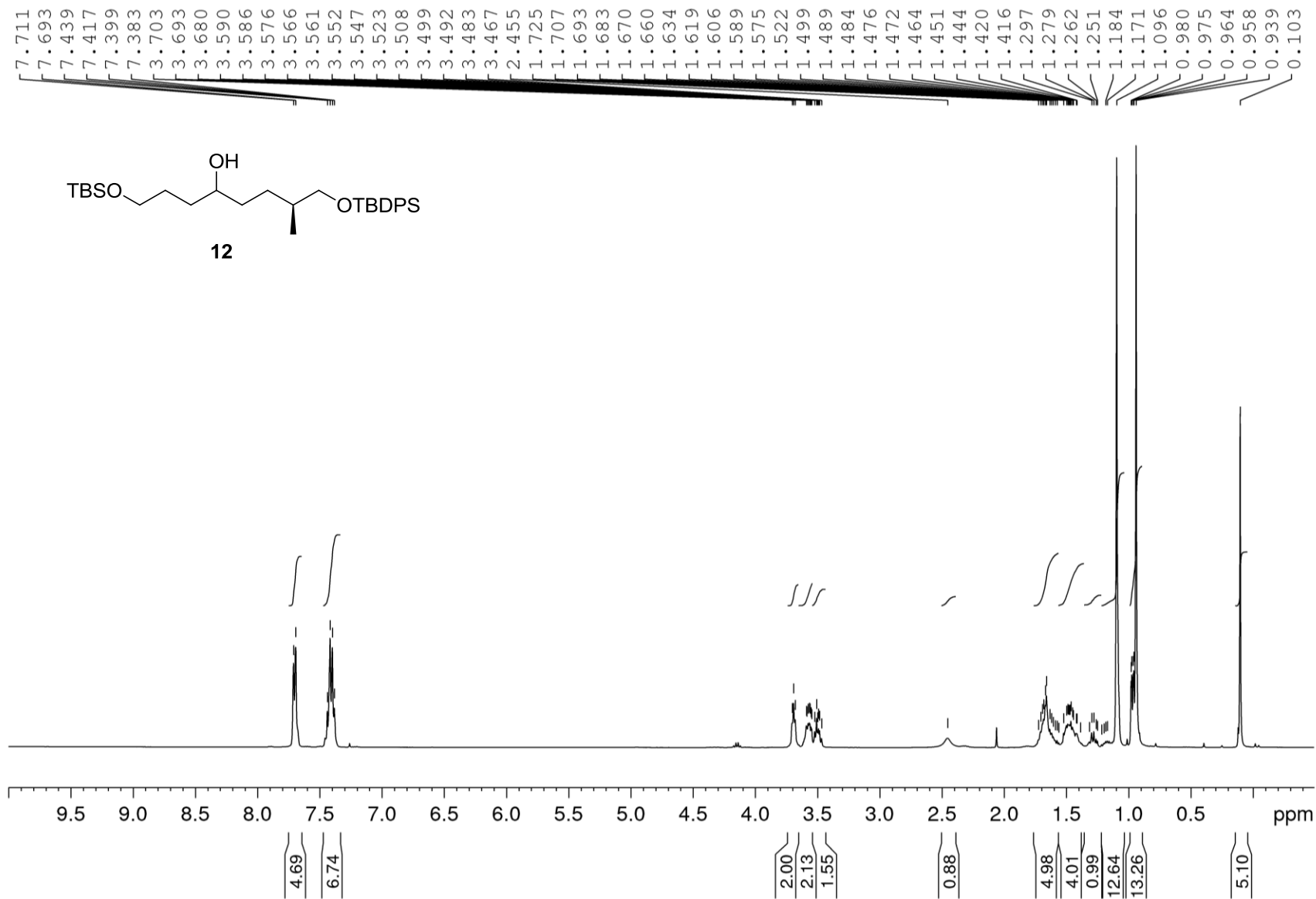


Figure S16. ^1H NMR spectrum of compound **12** (CDCl_3 , 400 MHz)

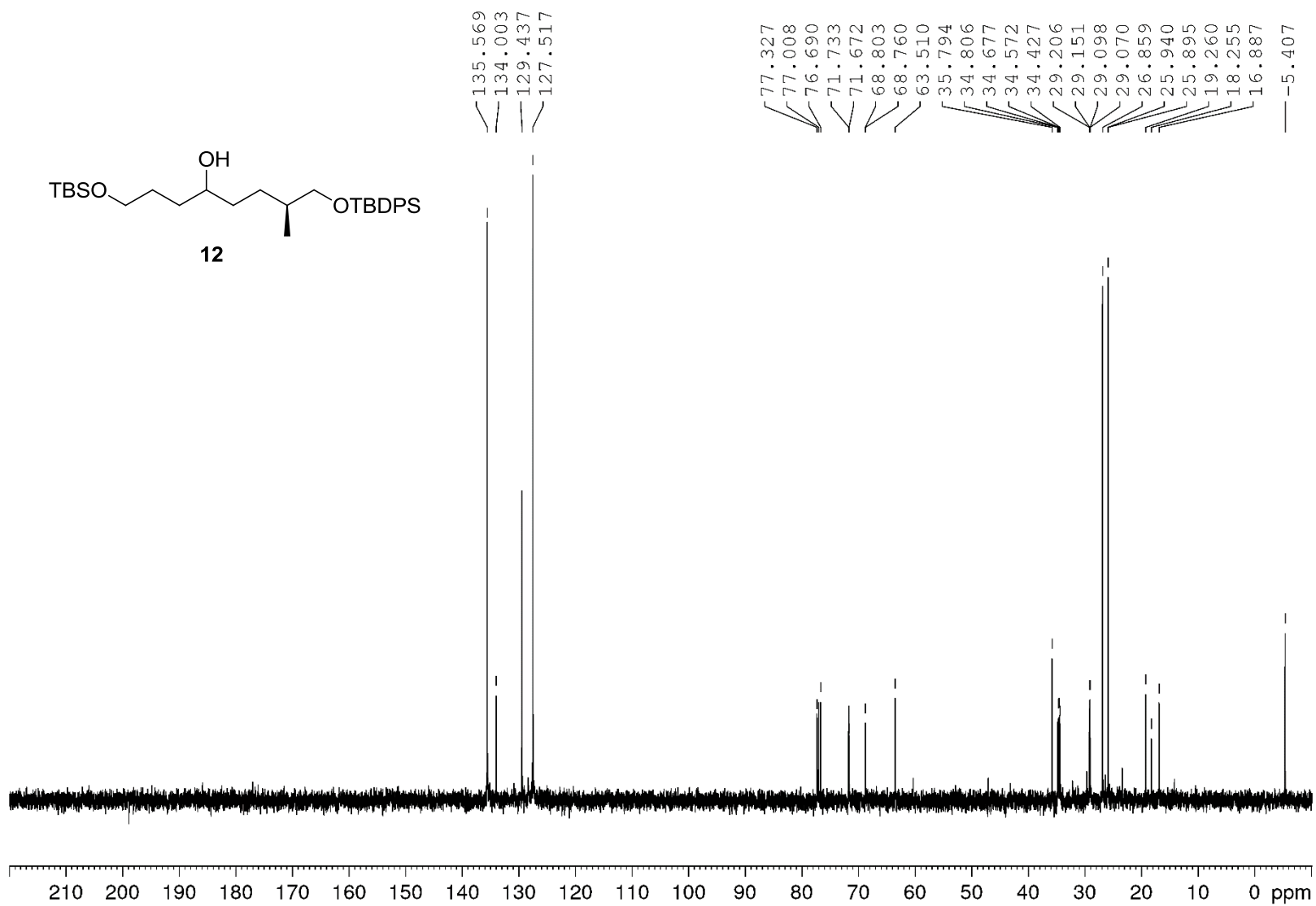


Figure S17. ¹³C NMR spectrum of compound **12** (CDCl₃, 100 MHz)

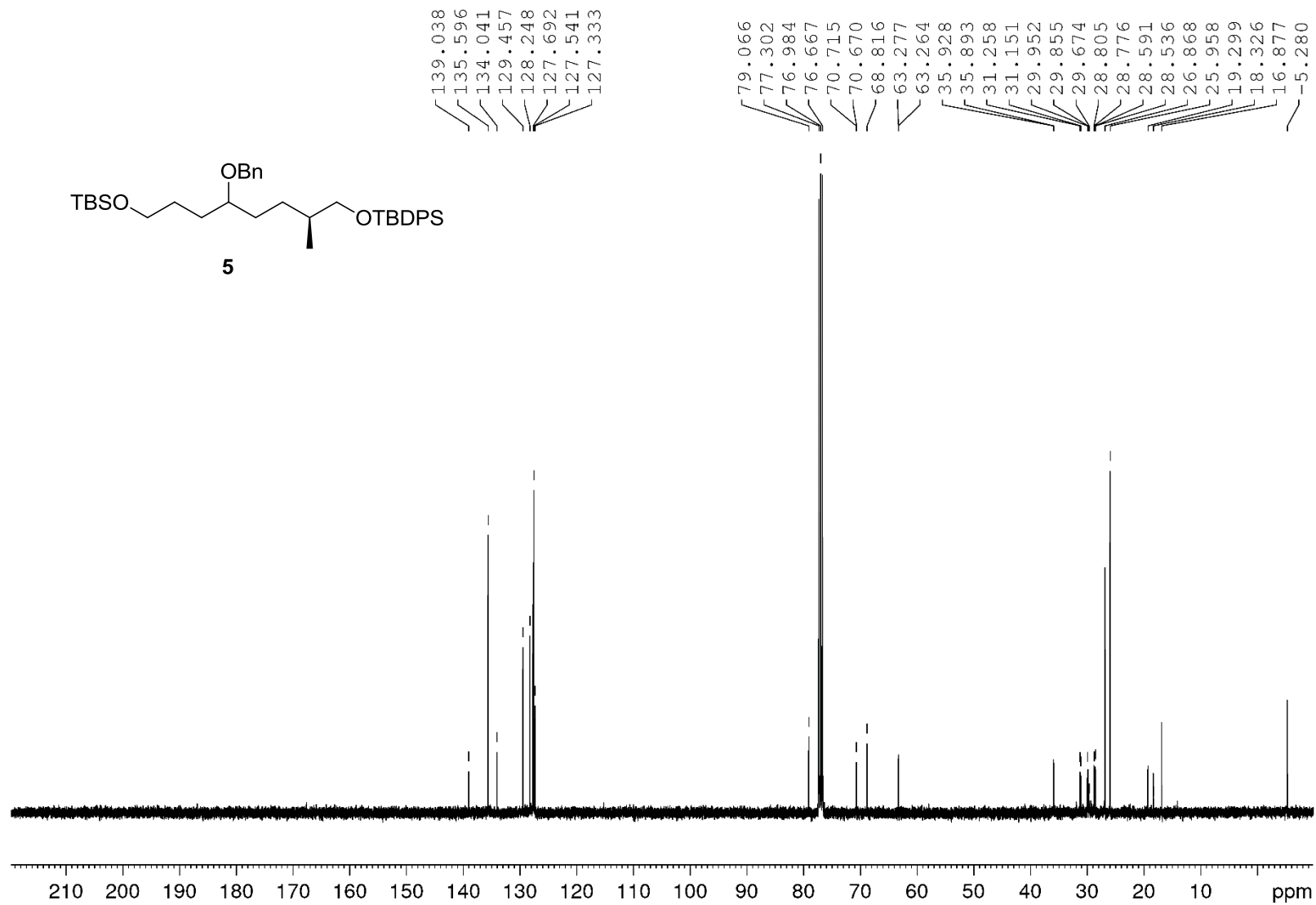
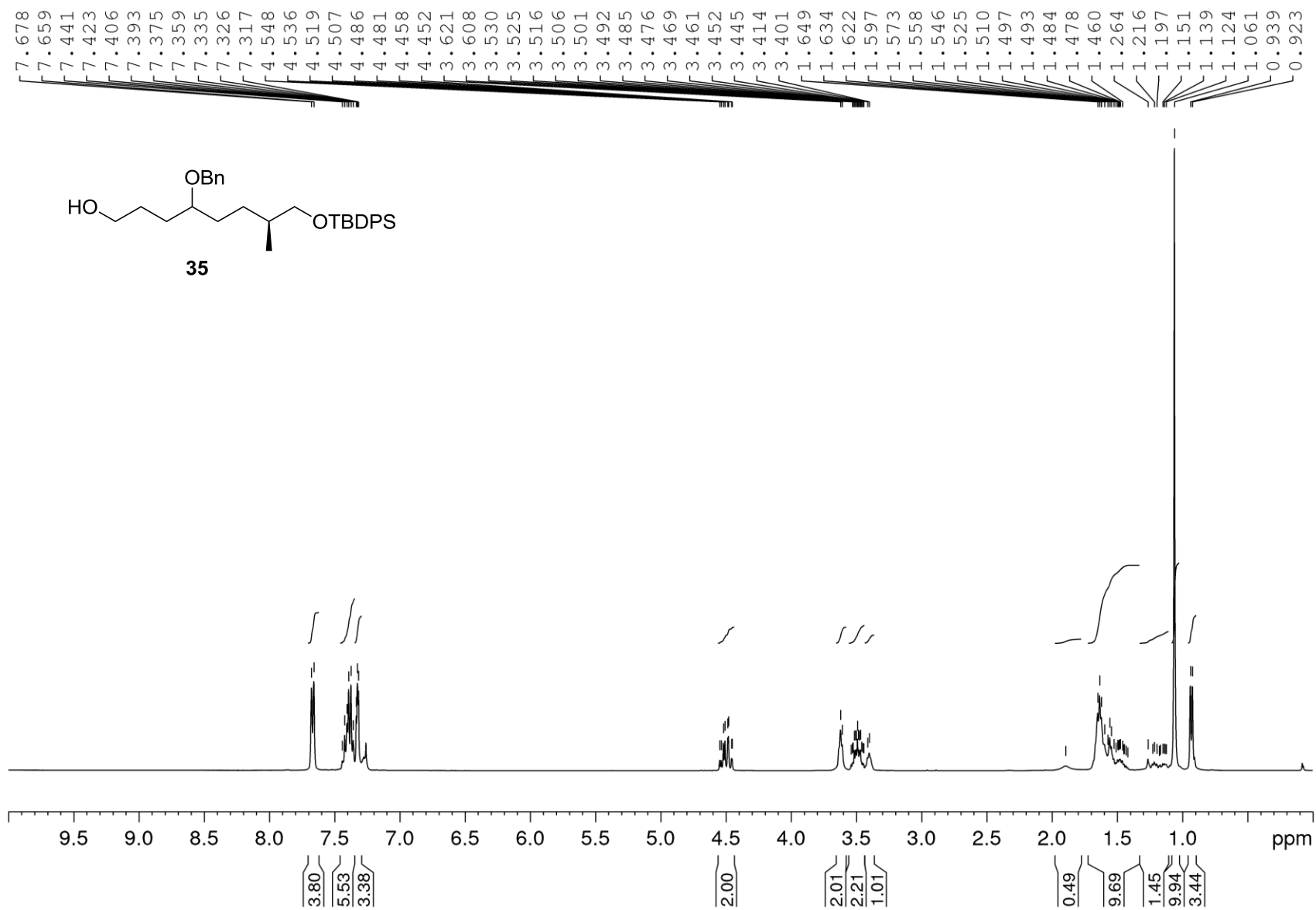


Figure S19. ^{13}C NMR spectrum of compound **5** (CDCl_3 , 100 MHz)



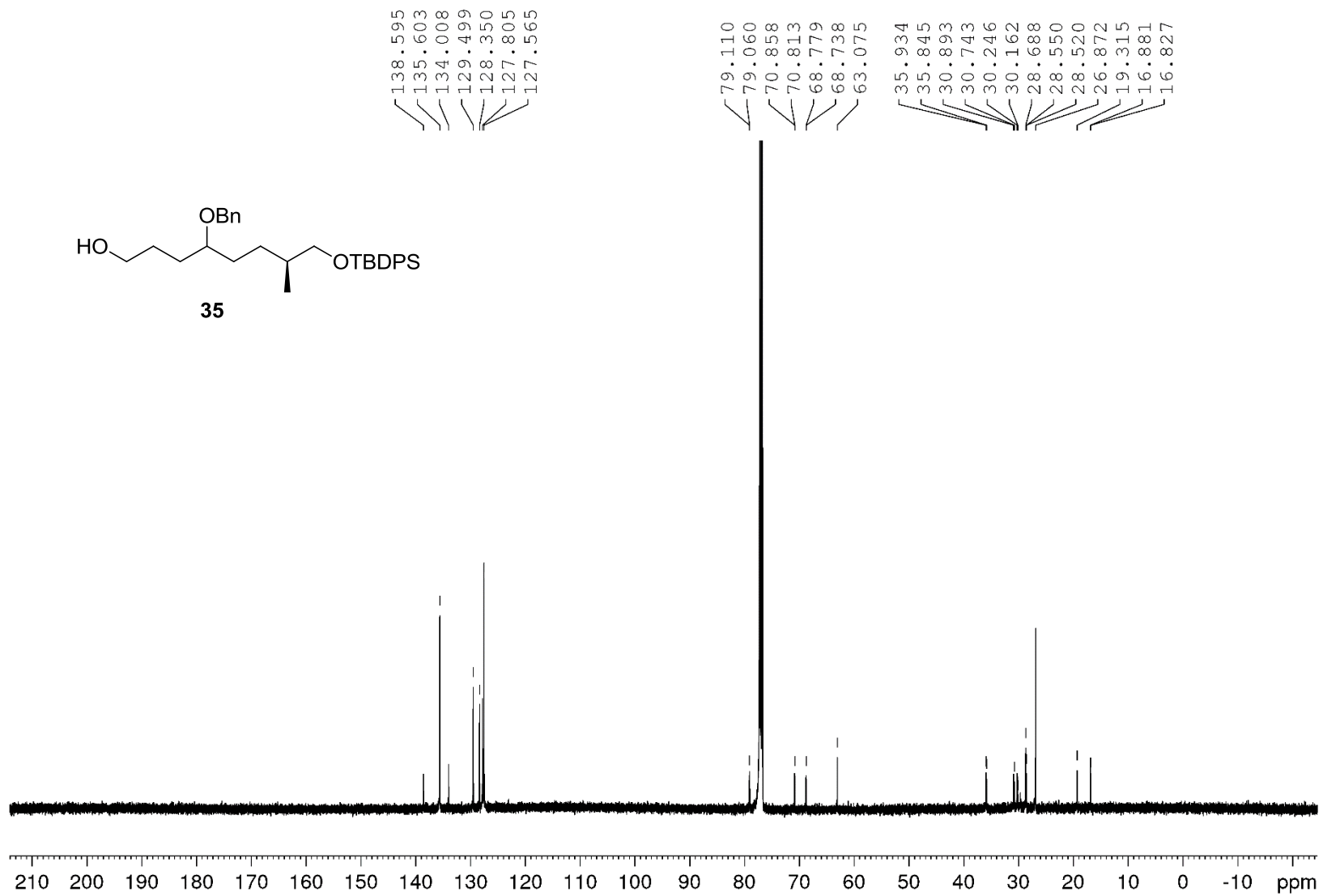


Figure S21. ^{13}C NMR spectrum of compound **35** (CDCl₃, 100 MHz)

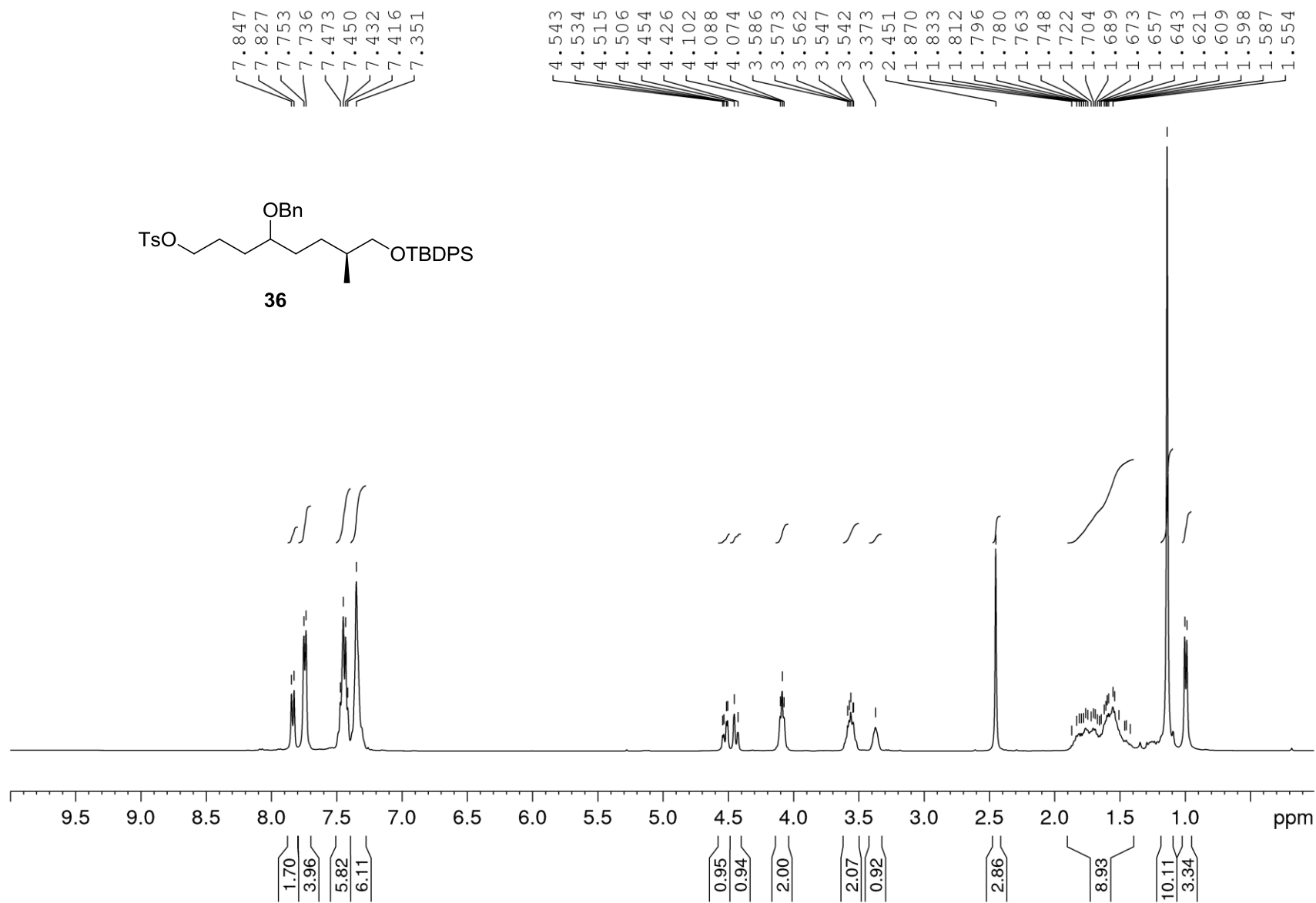


Figure S22. ¹H NMR spectrum of compound **36** (CDCl₃, 400 MHz)

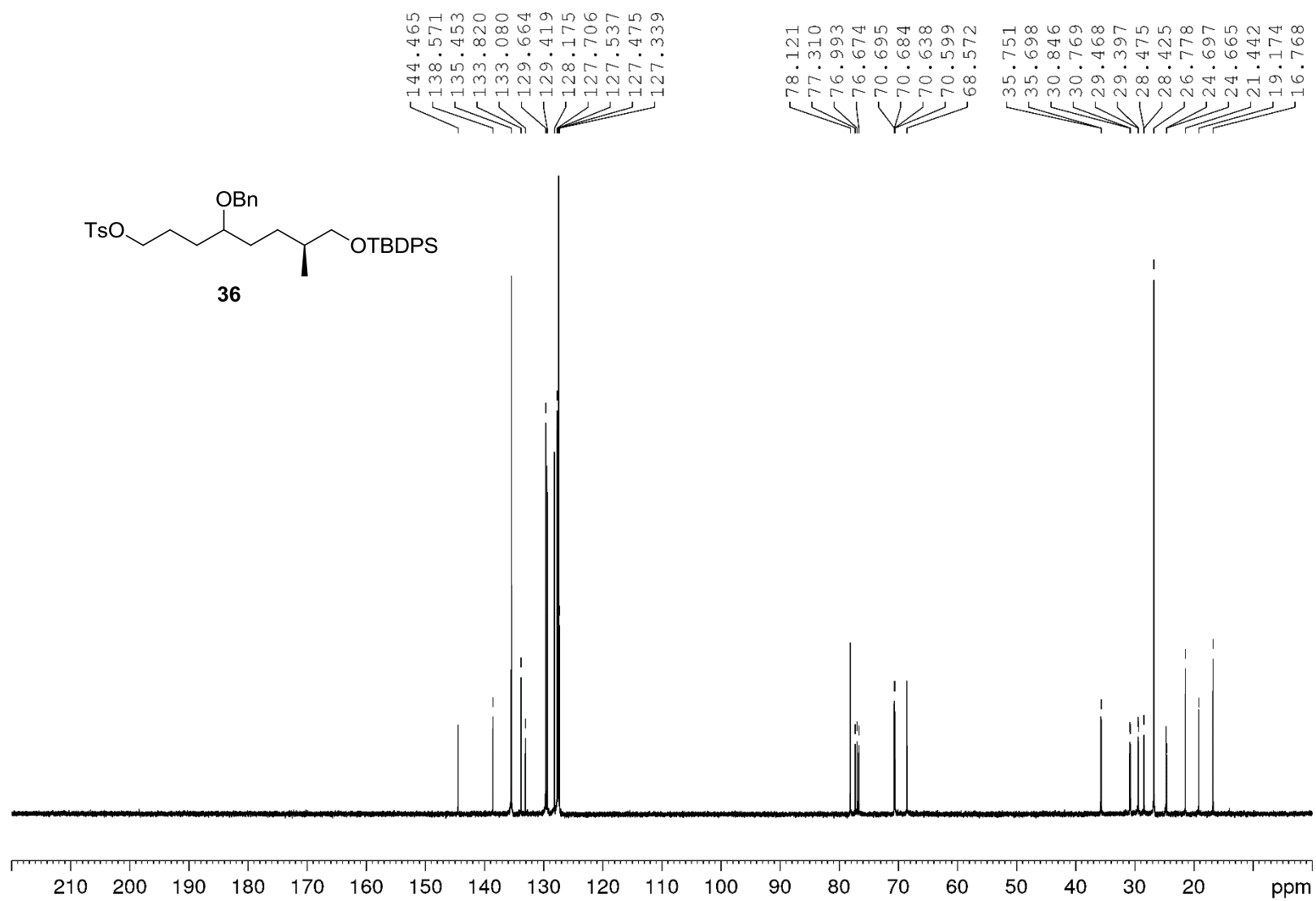


Figure S23. ¹³C NMR spectrum of compound **36** (CDCl₃, 100 MHz)

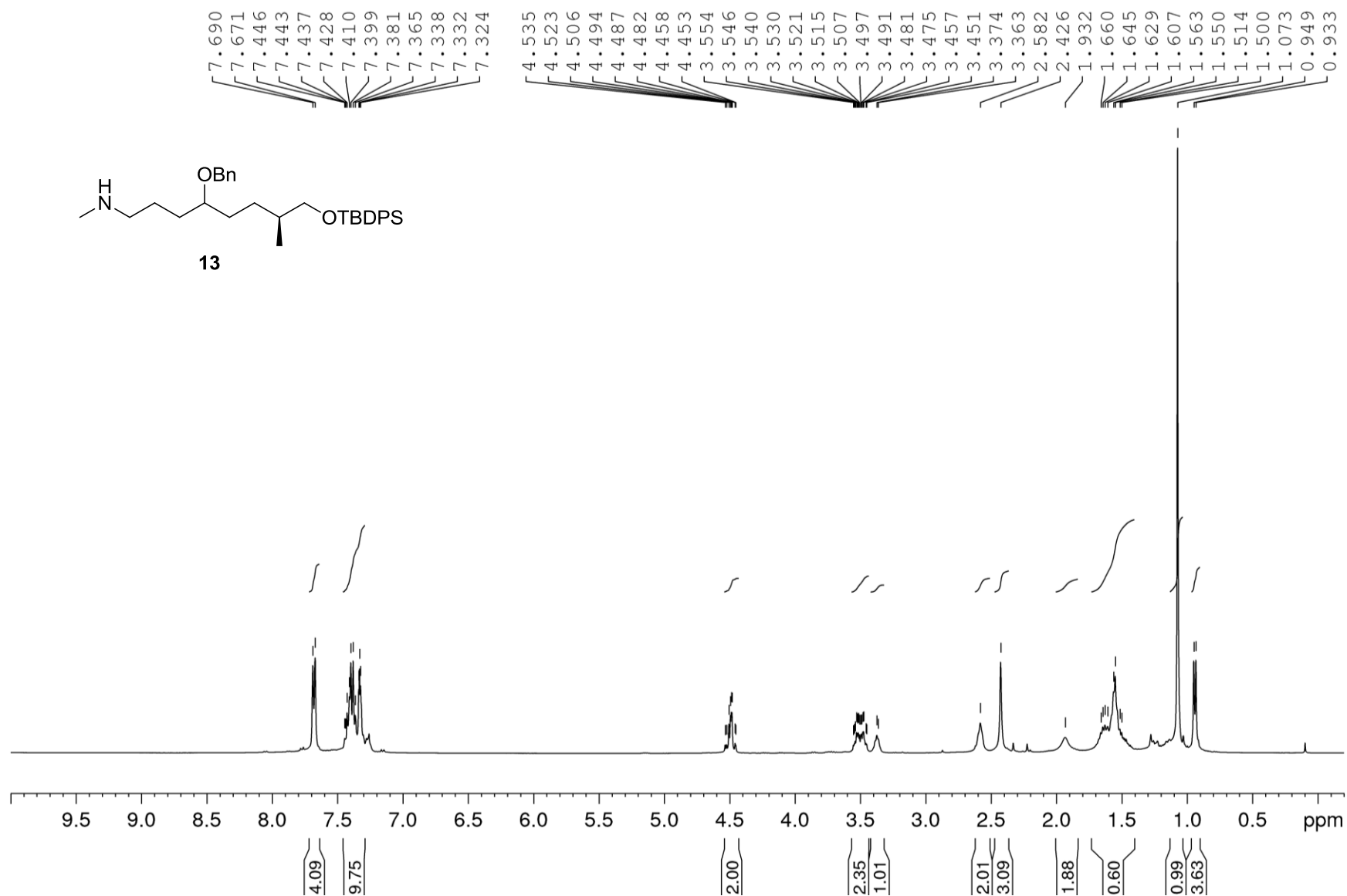


Figure S24. ¹H NMR spectrum of compound **13** (CDCl₃, 400 MHz)

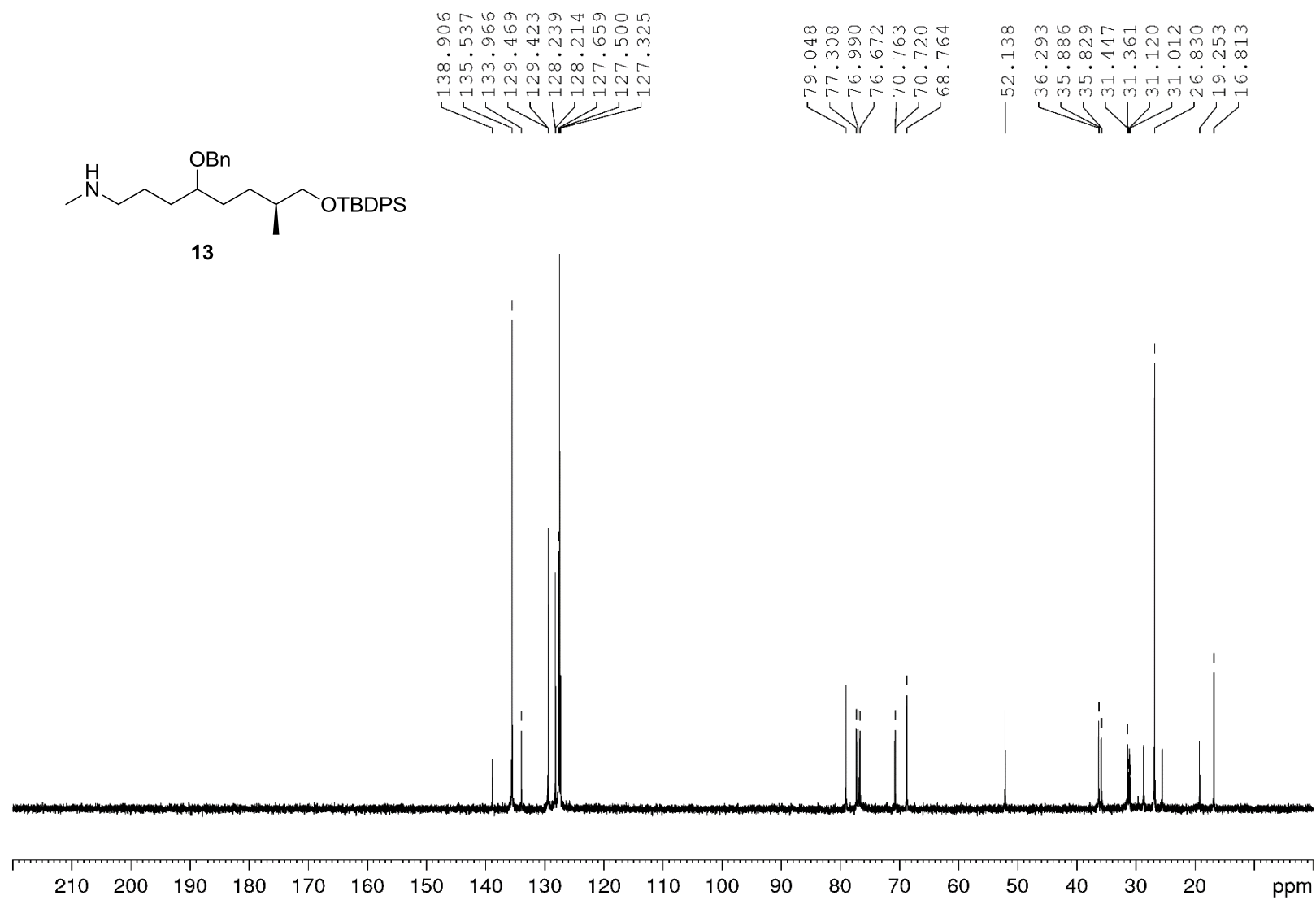


Figure S25. ^{13}C NMR spectrum of compound **13** (CDCl_3 , 100 MHz)

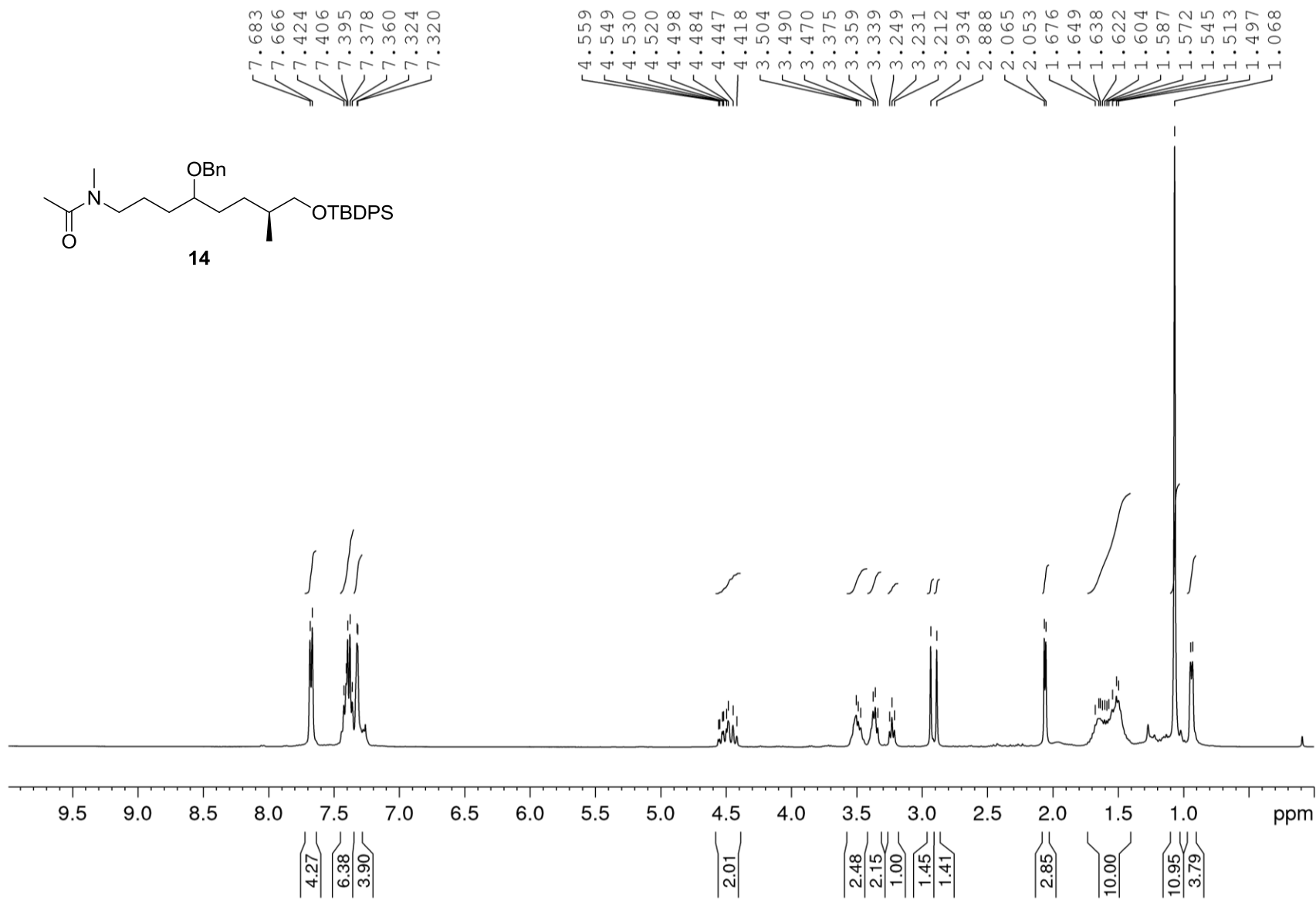


Figure S26. ¹H NMR spectrum of compound **14** (CDCl₃, 400 MHz)

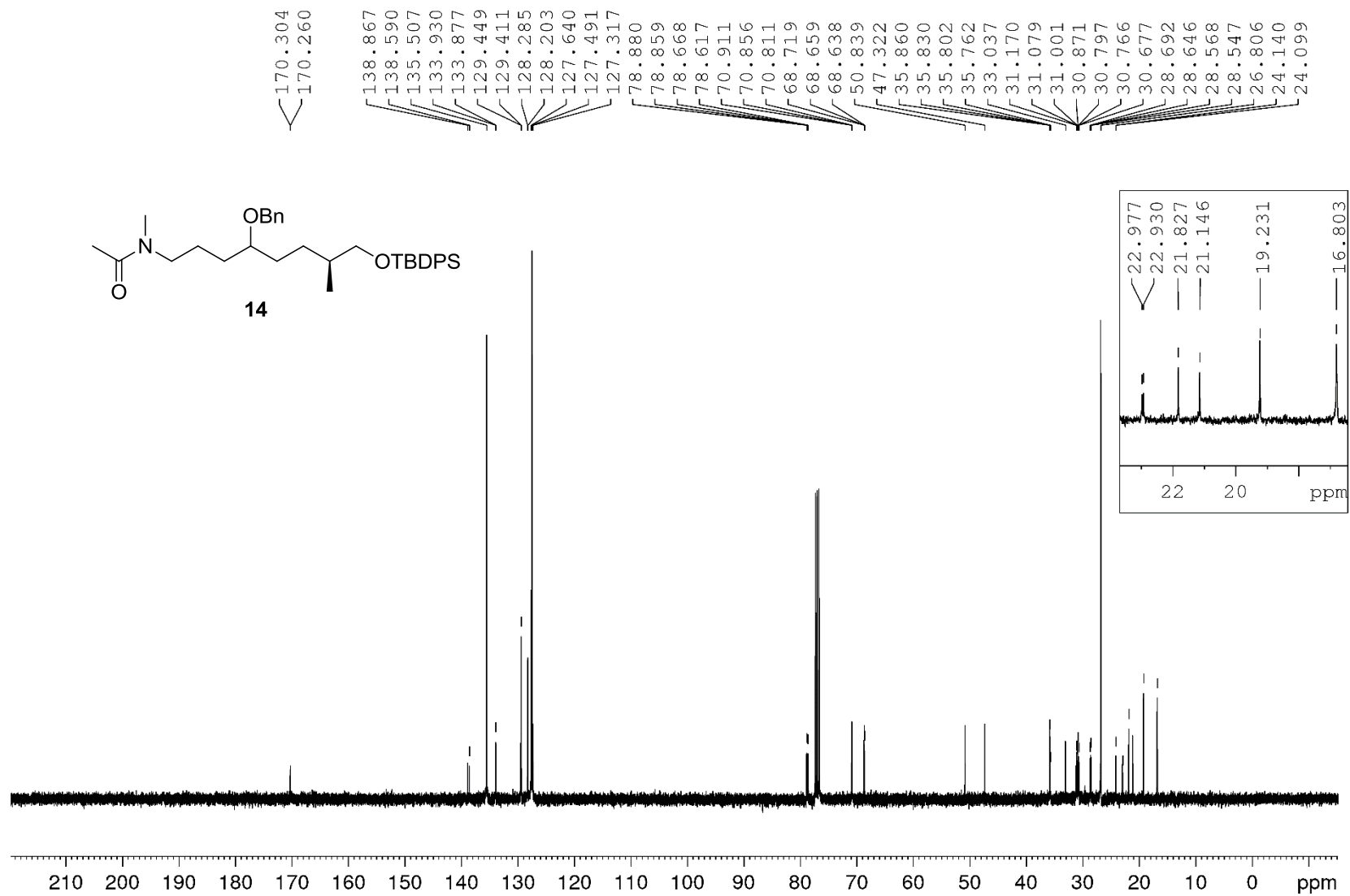


Figure S27. ^{13}C NMR spectrum of compound **14** (CDCl₃, 100 MHz)

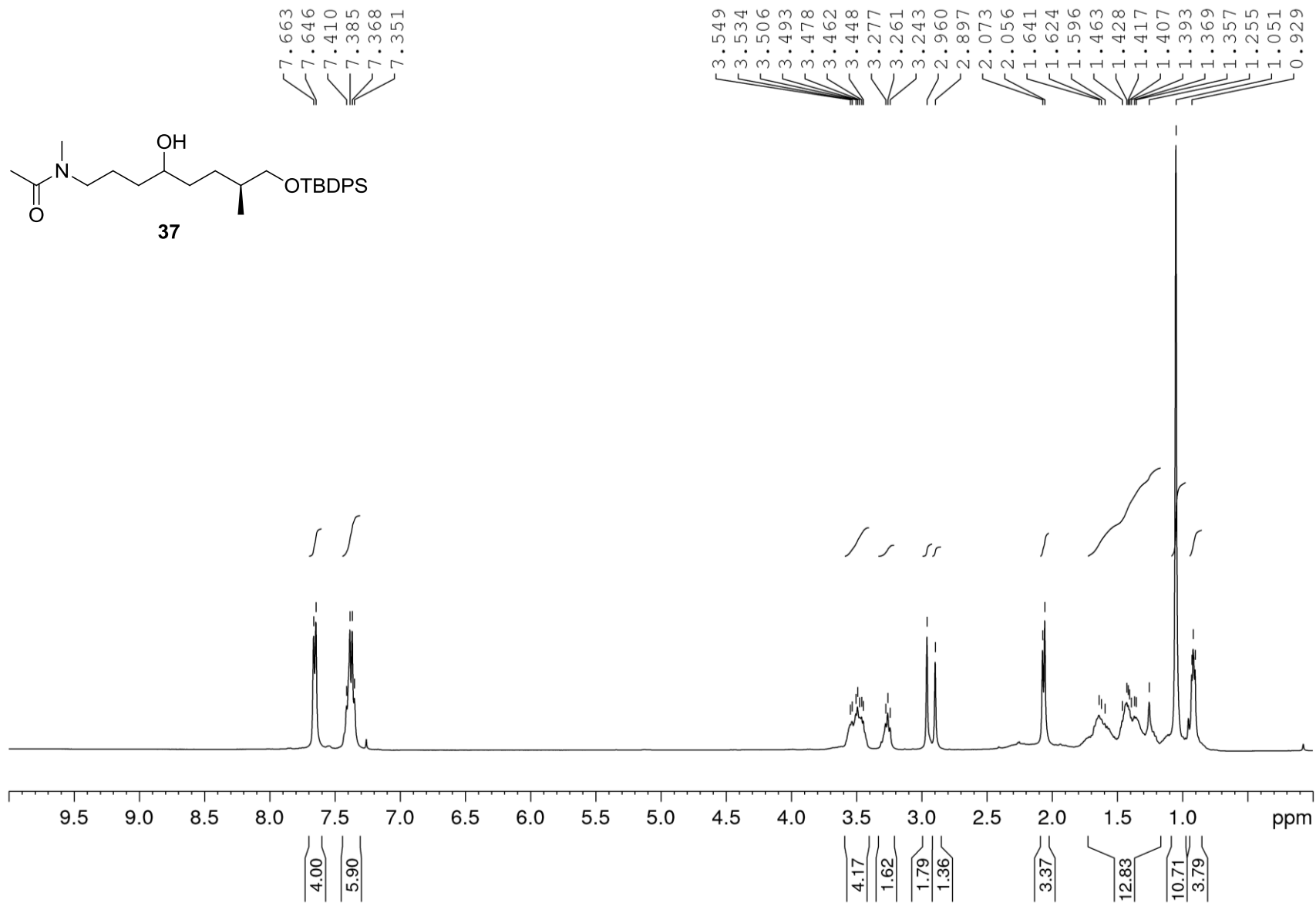


Figure S28. ^1H NMR spectrum of compound **37** (CDCl_3 , 400 MHz)

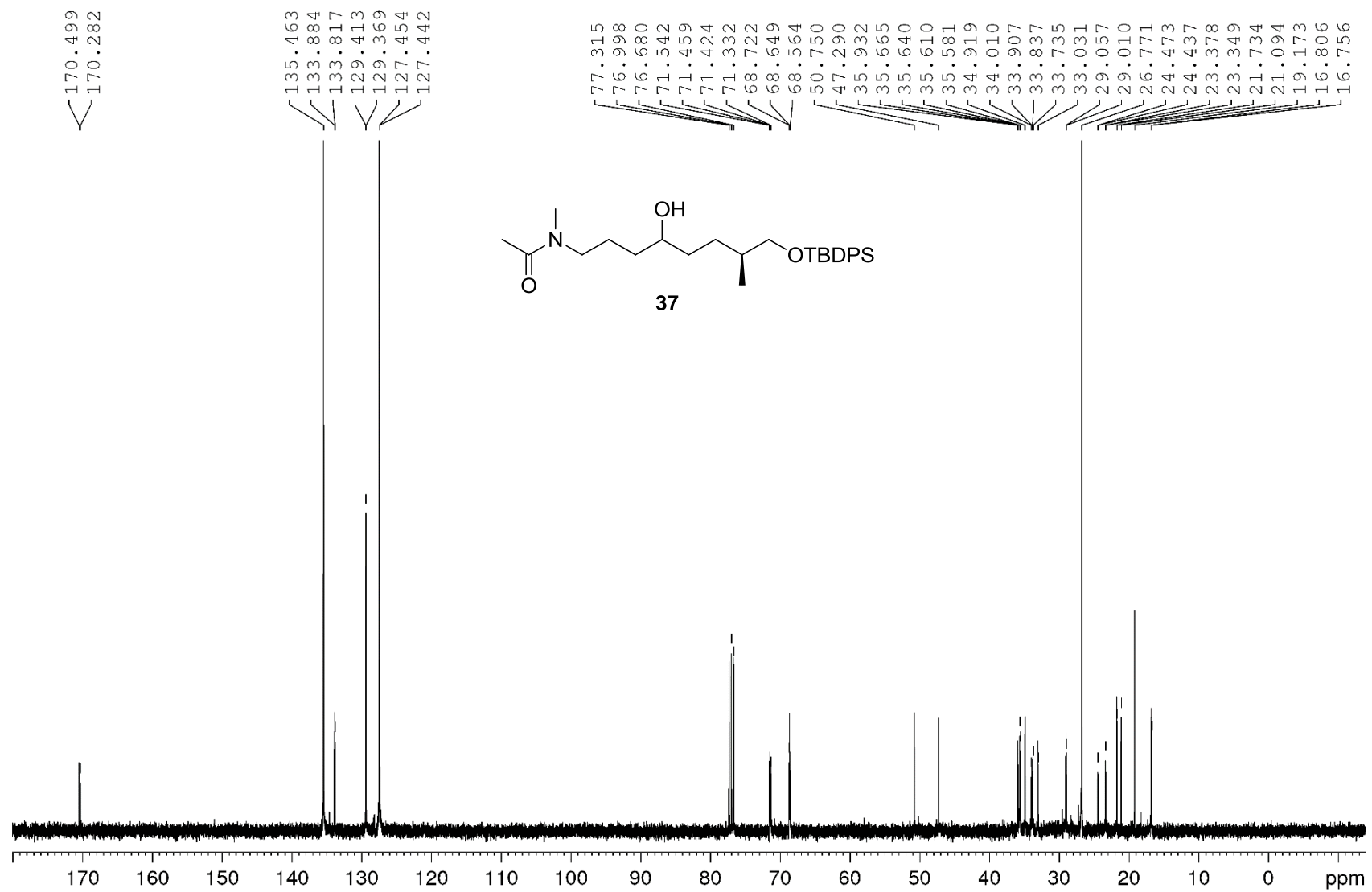


Figure S29. ¹³C NMR spectrum of compound **37** (CDCl₃, 100 MHz)

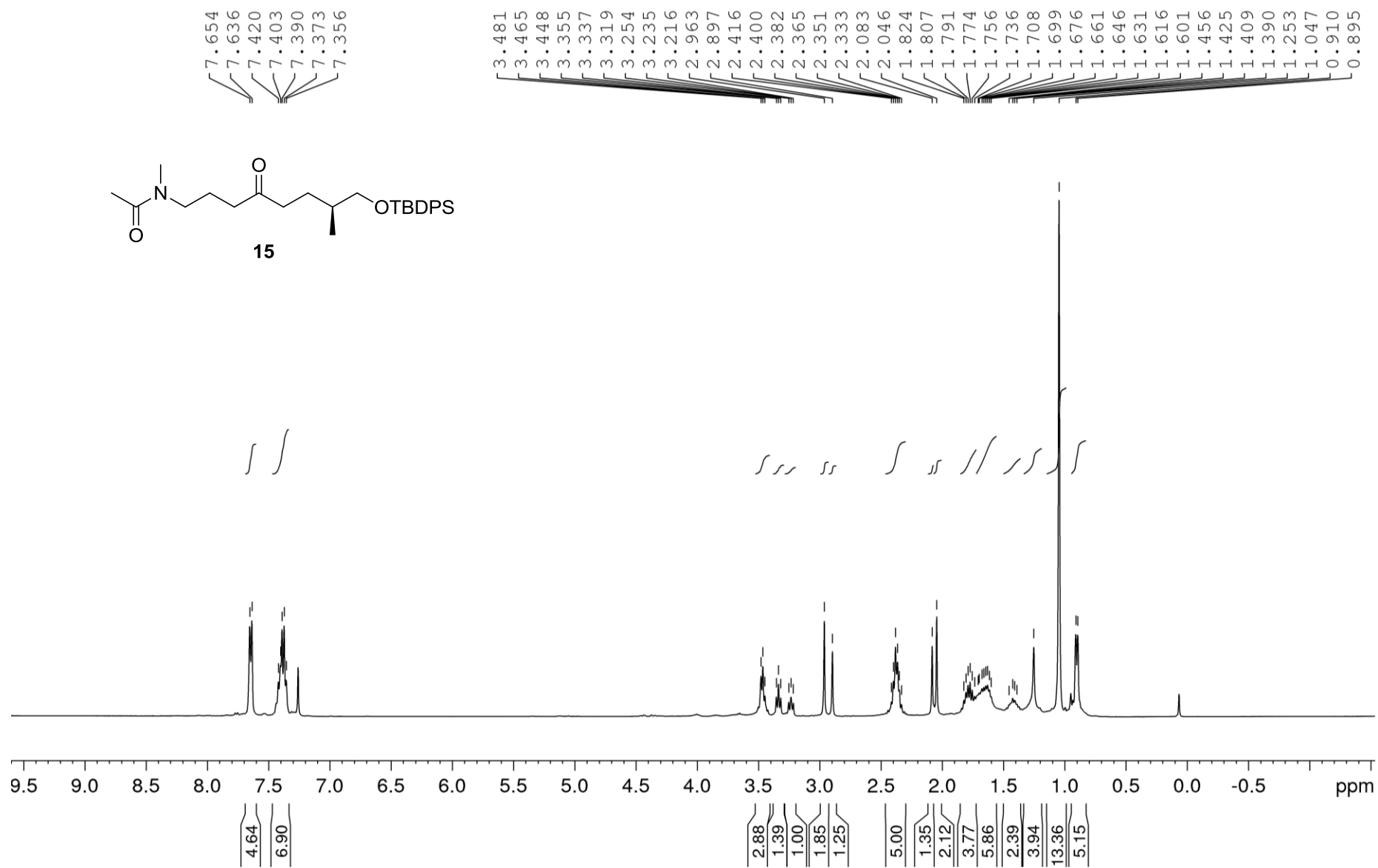


Figure S30. ^1H NMR spectrum of compound **15** (CDCl_3 , 400 MHz)

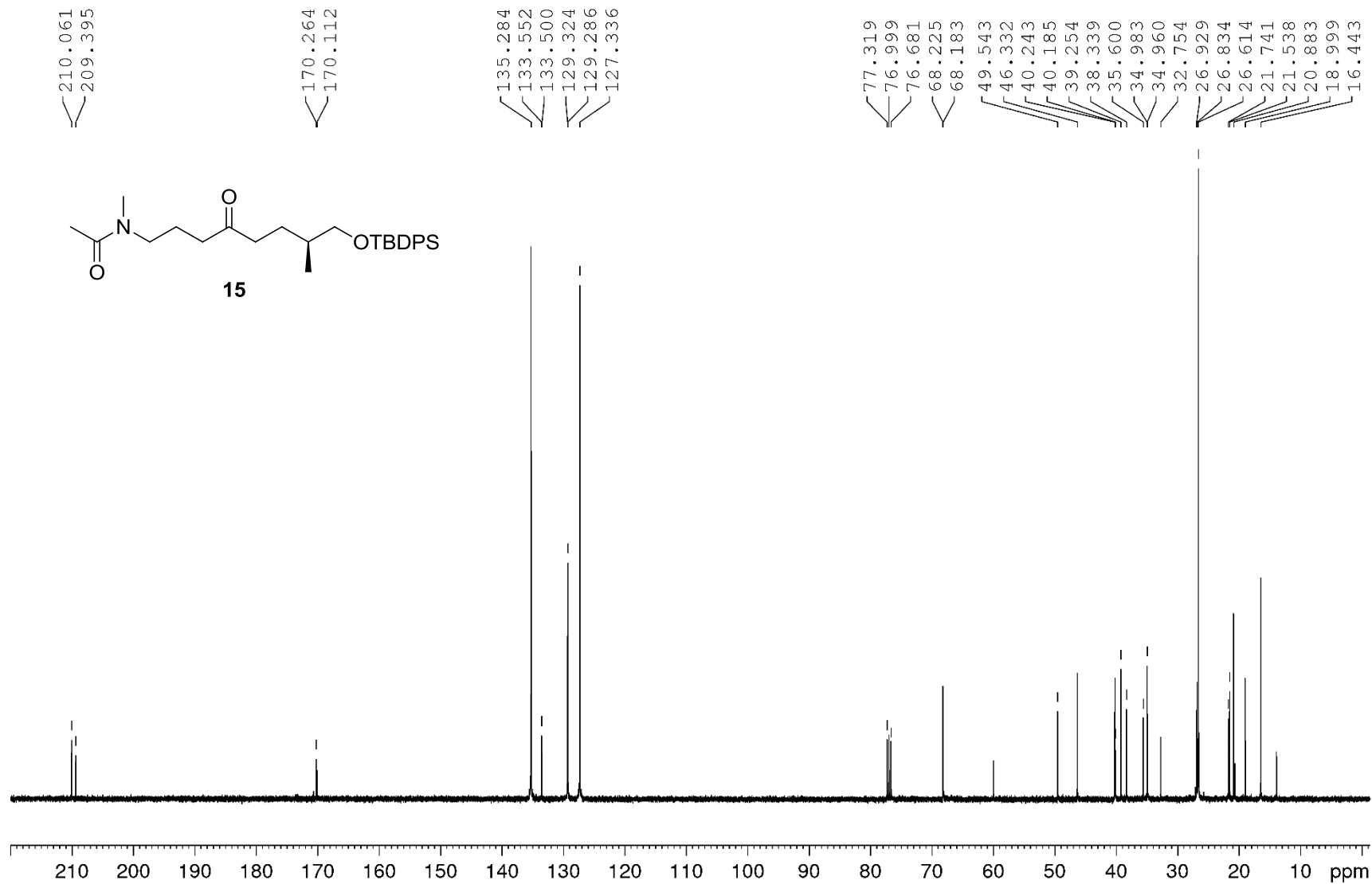


Figure S31. ^{13}C NMR spectrum of compound **15** (CDCl₃, 100 MHz)

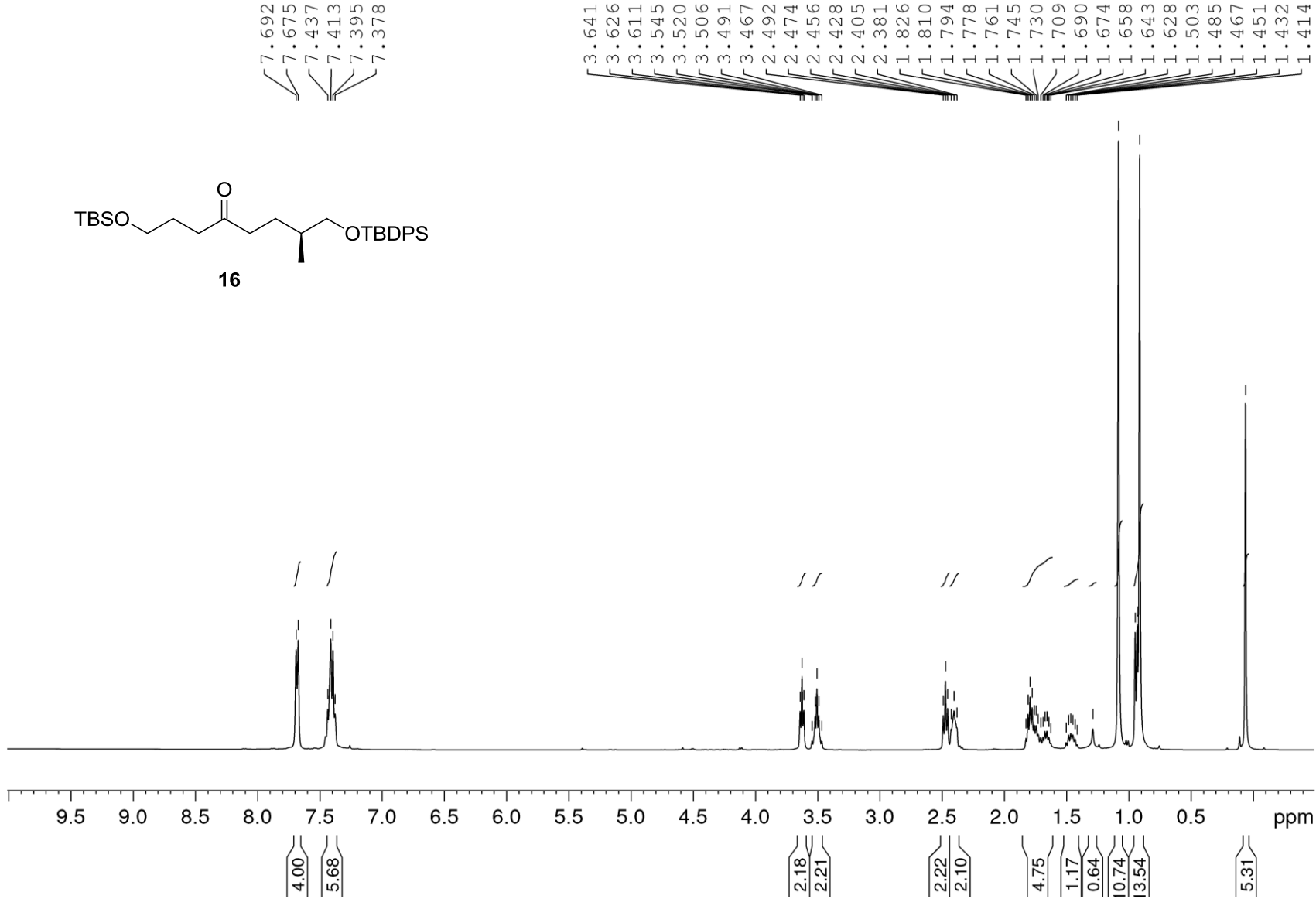


Figure S32. ¹H NMR spectrum of compound **16** (CDCl₃, 400 MHz)

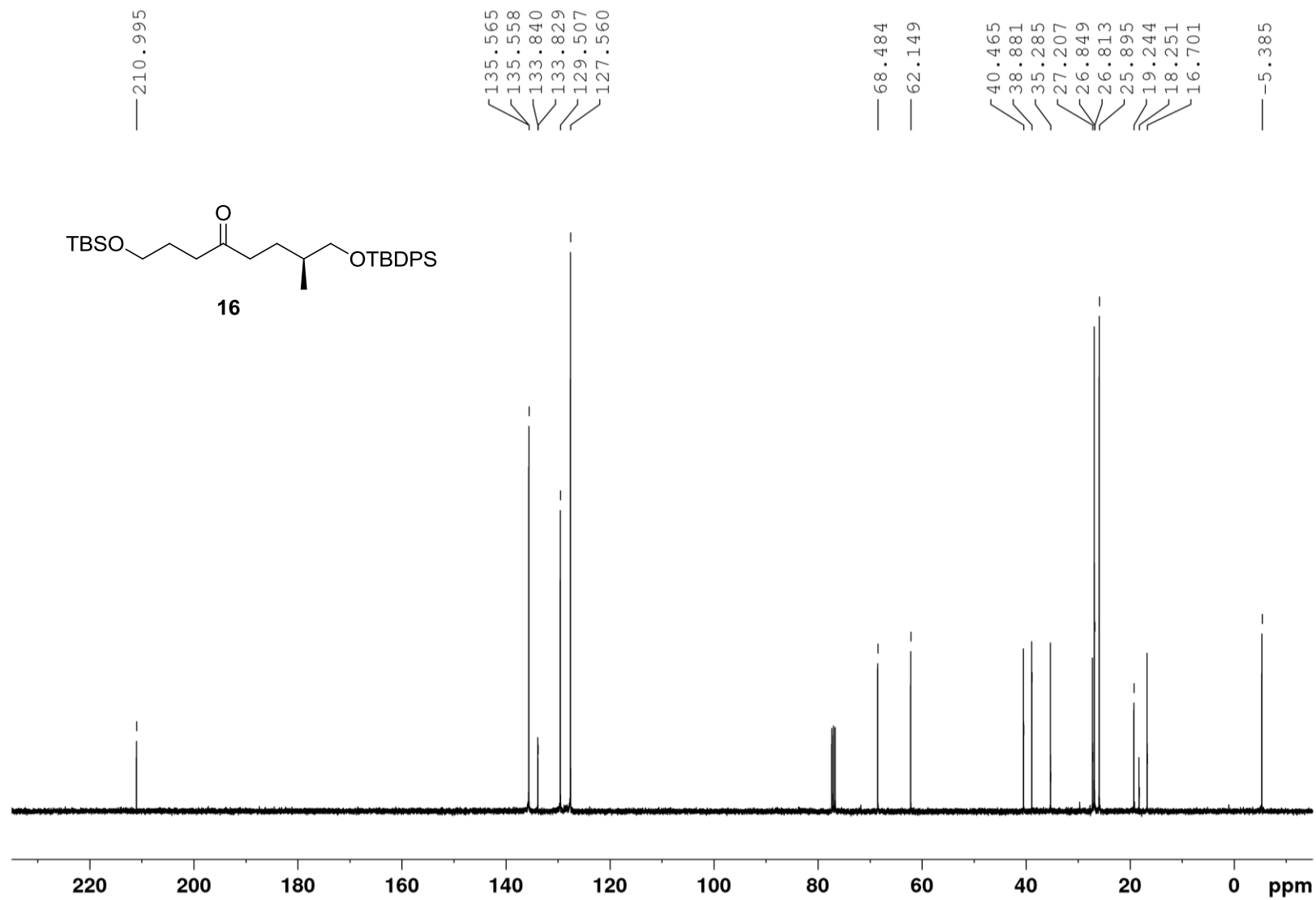


Figure S33. ^{13}C NMR spectrum of compound **16** (CDCl₃, 100 MHz)

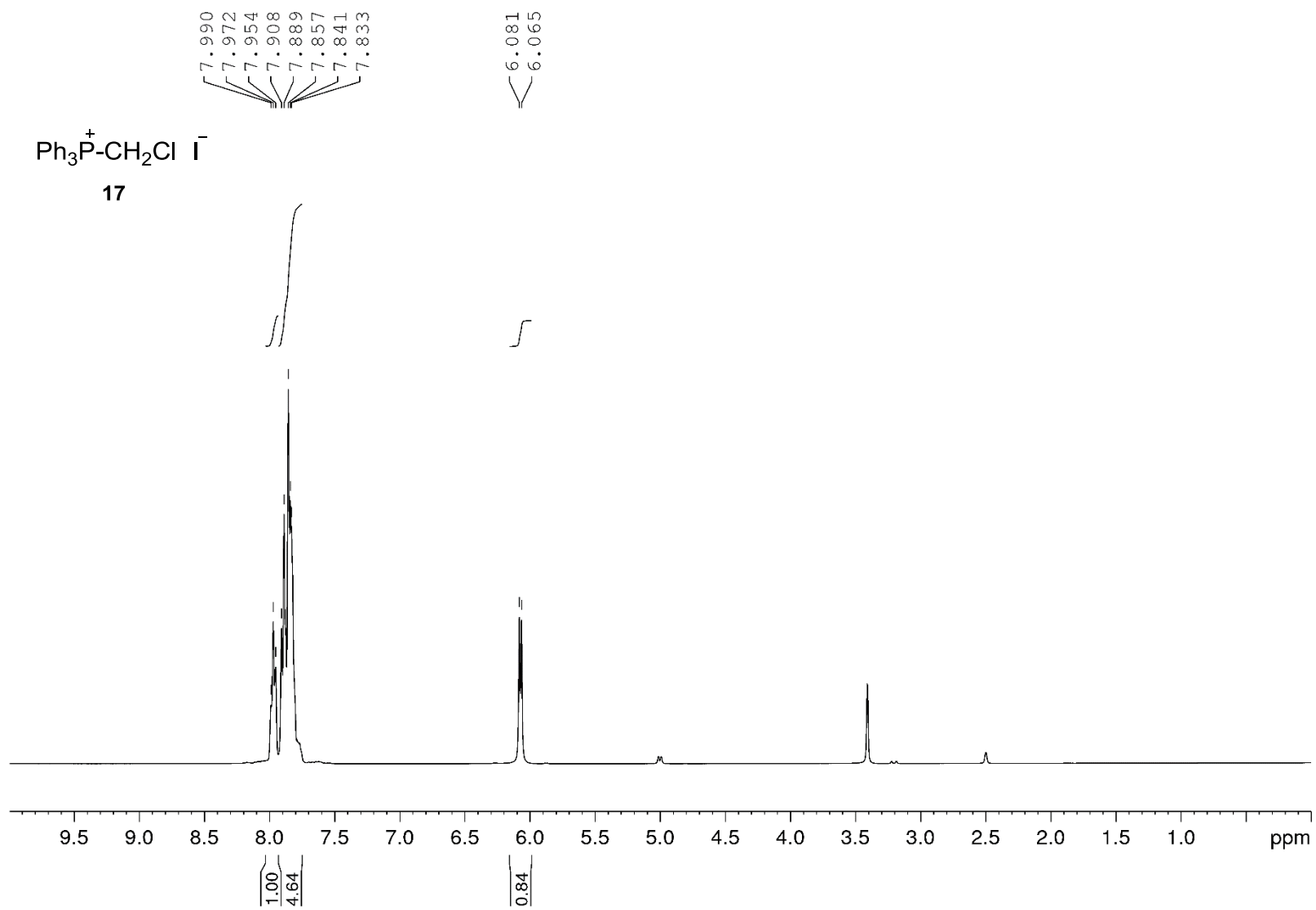


Figure S34. ^1H NMR spectrum of compound **17** (DMSO, 400 MHz)

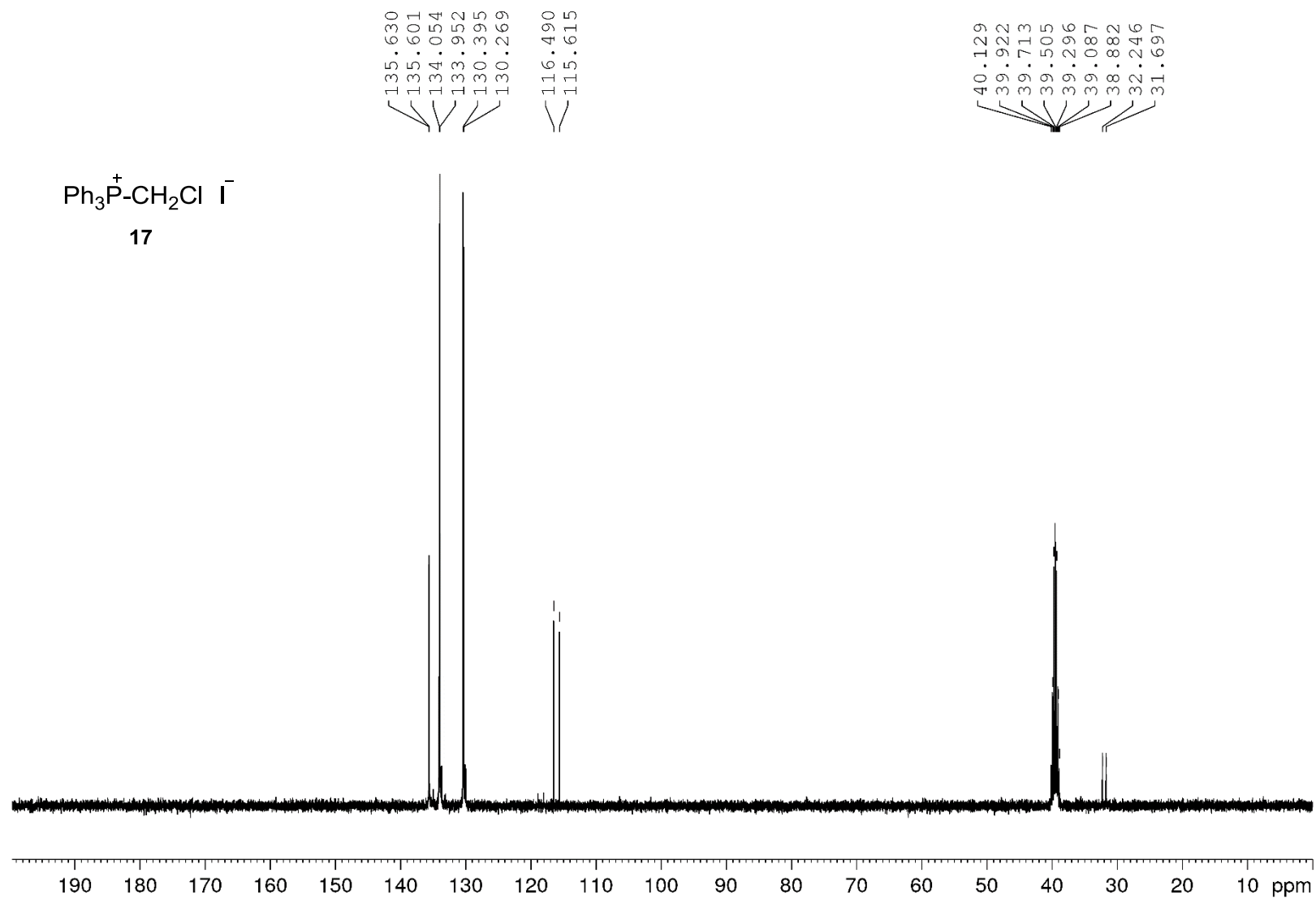


Figure S35. ^{13}C NMR spectrum of compound **17** (DMSO, 100 MHz)

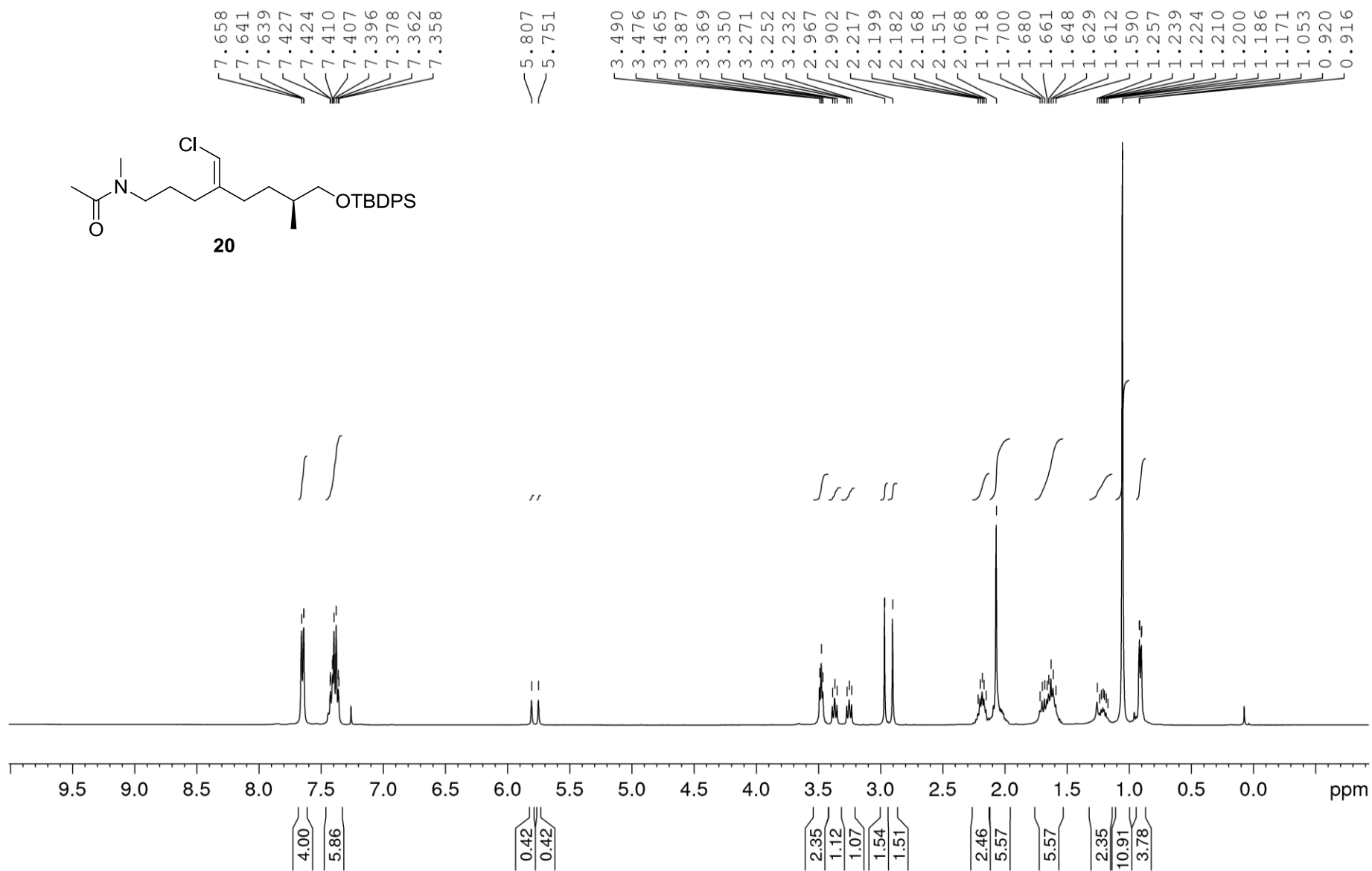


Figure S37. ^1H NMR spectrum of compound **20** (CDCl₃, 400 MHz)

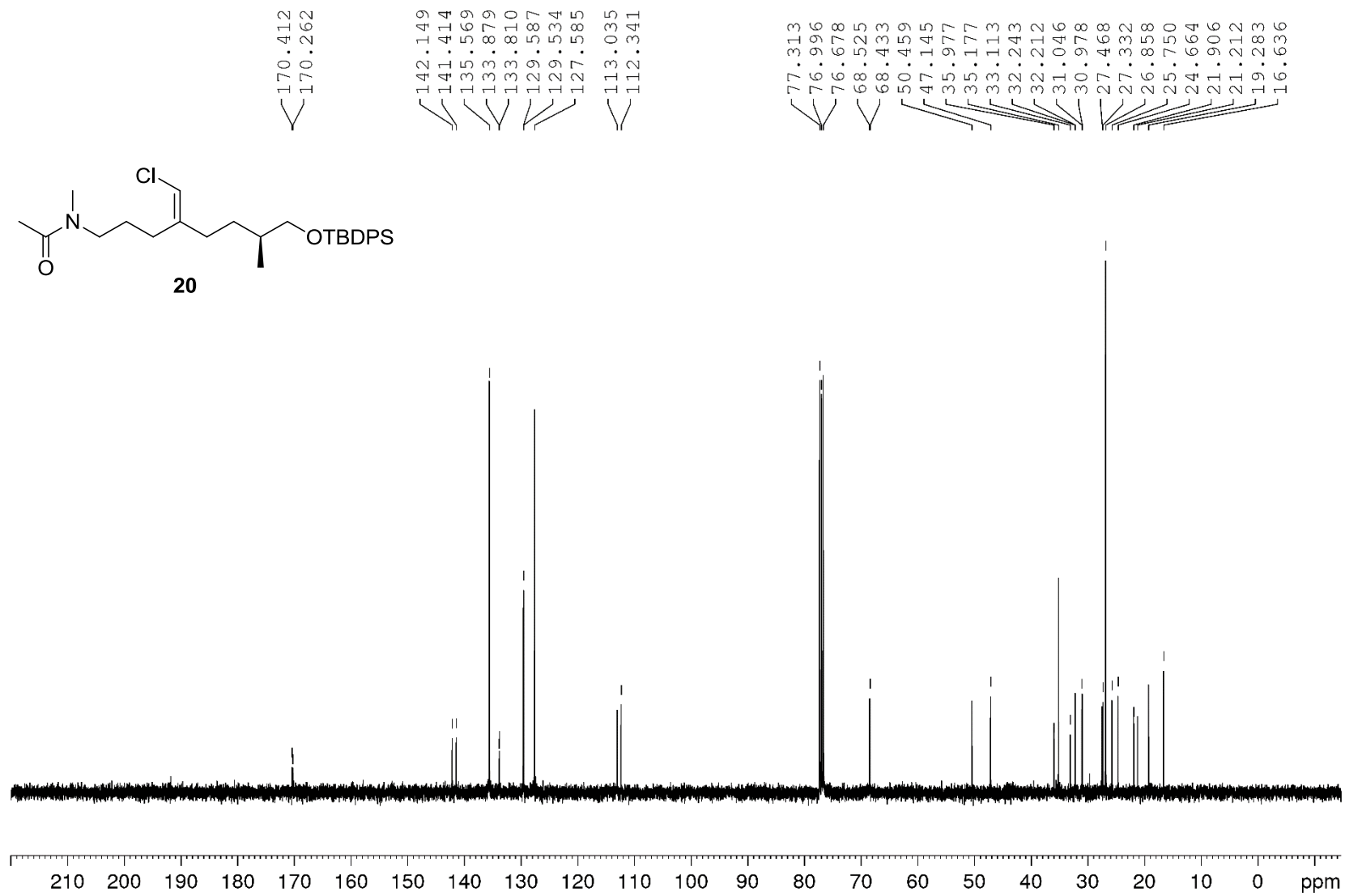


Figure S38. ^{13}C NMR spectrum of compound **20** (CDCl₃, 100 MHz)

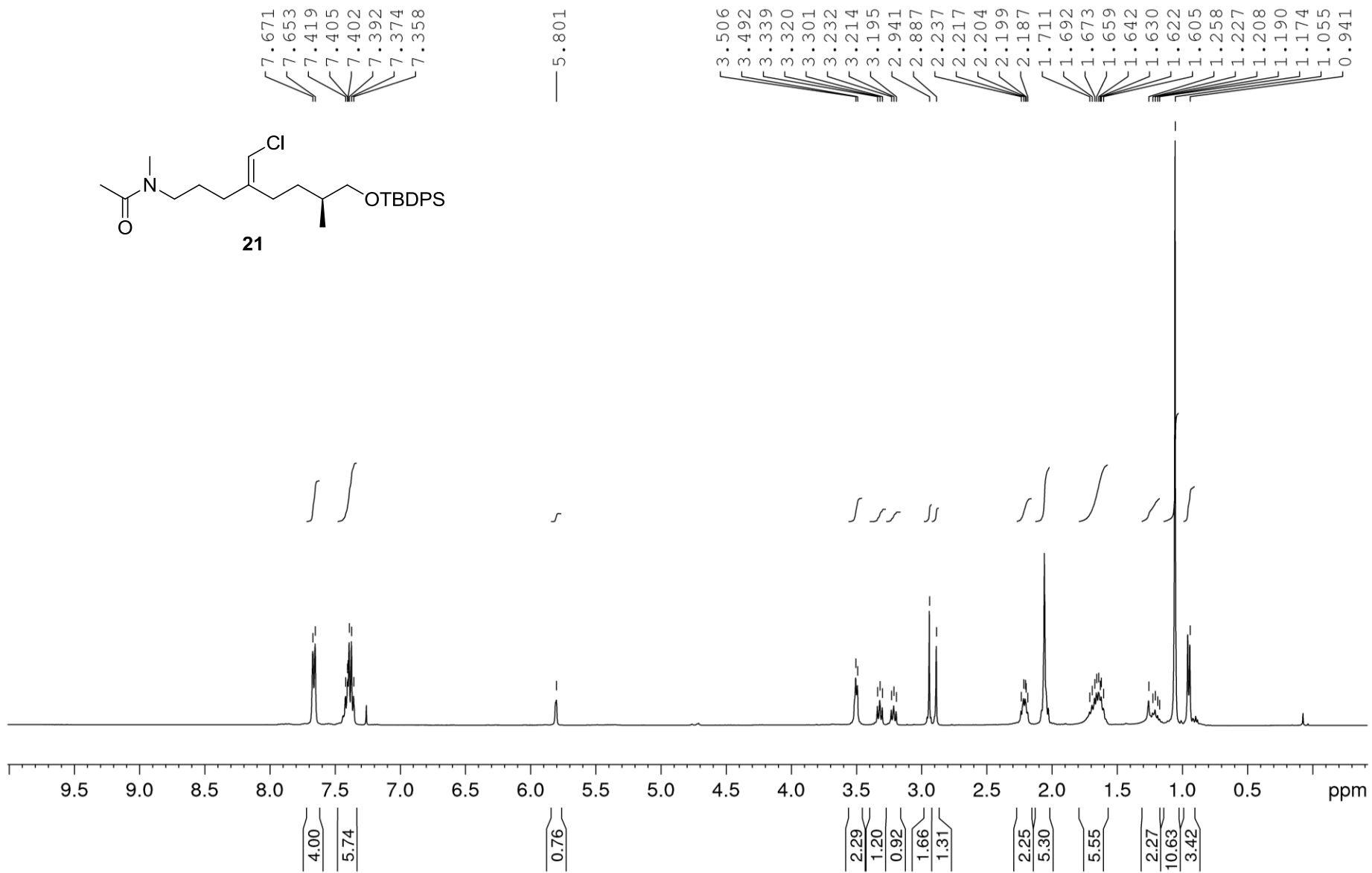


Figure S39. ^1H NMR spectrum of compound **21** (CDCl₃, 400 MHz)

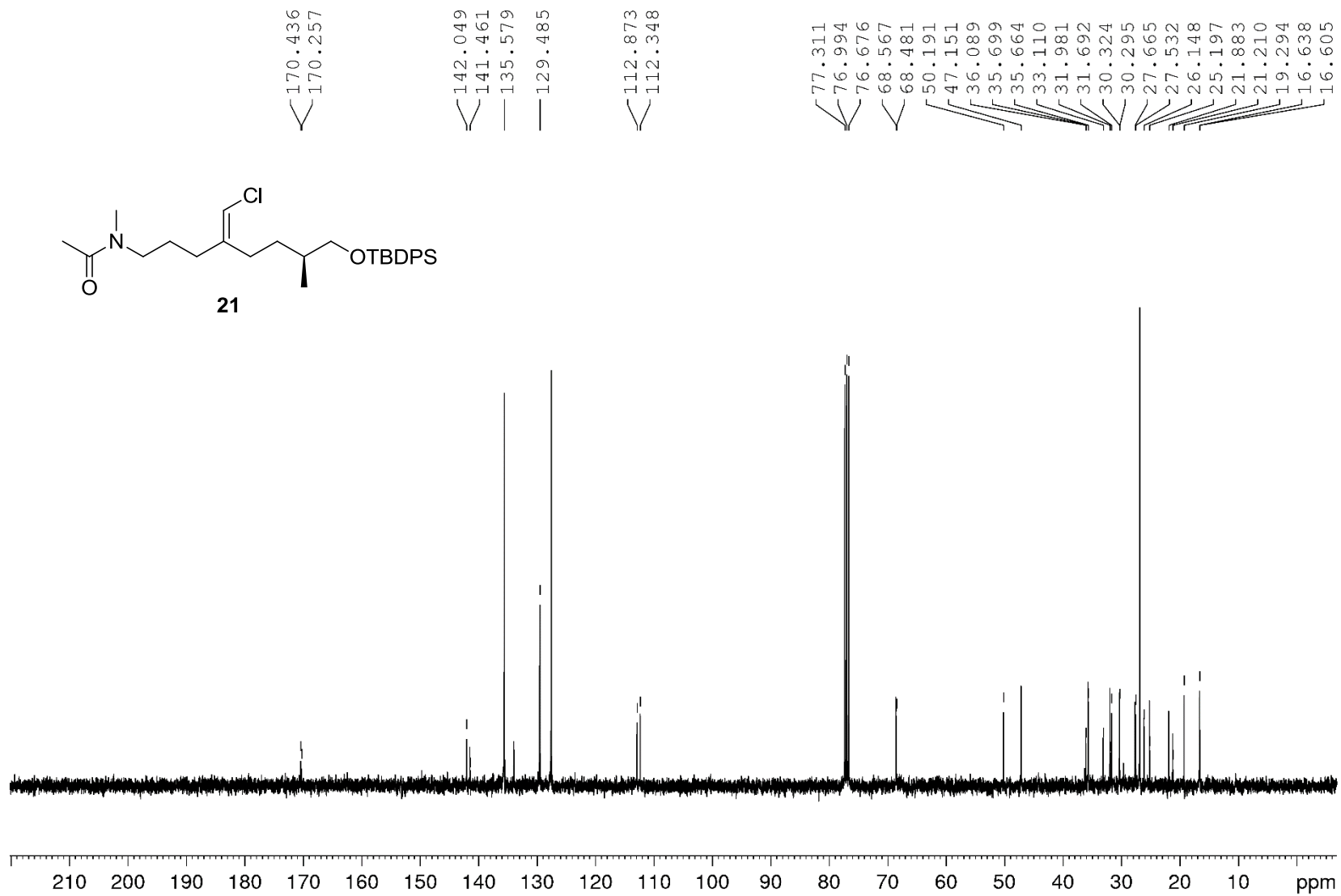


Figure S40. ^{13}C NMR spectrum of compound **21** (CDCl₃, 100 MHz)

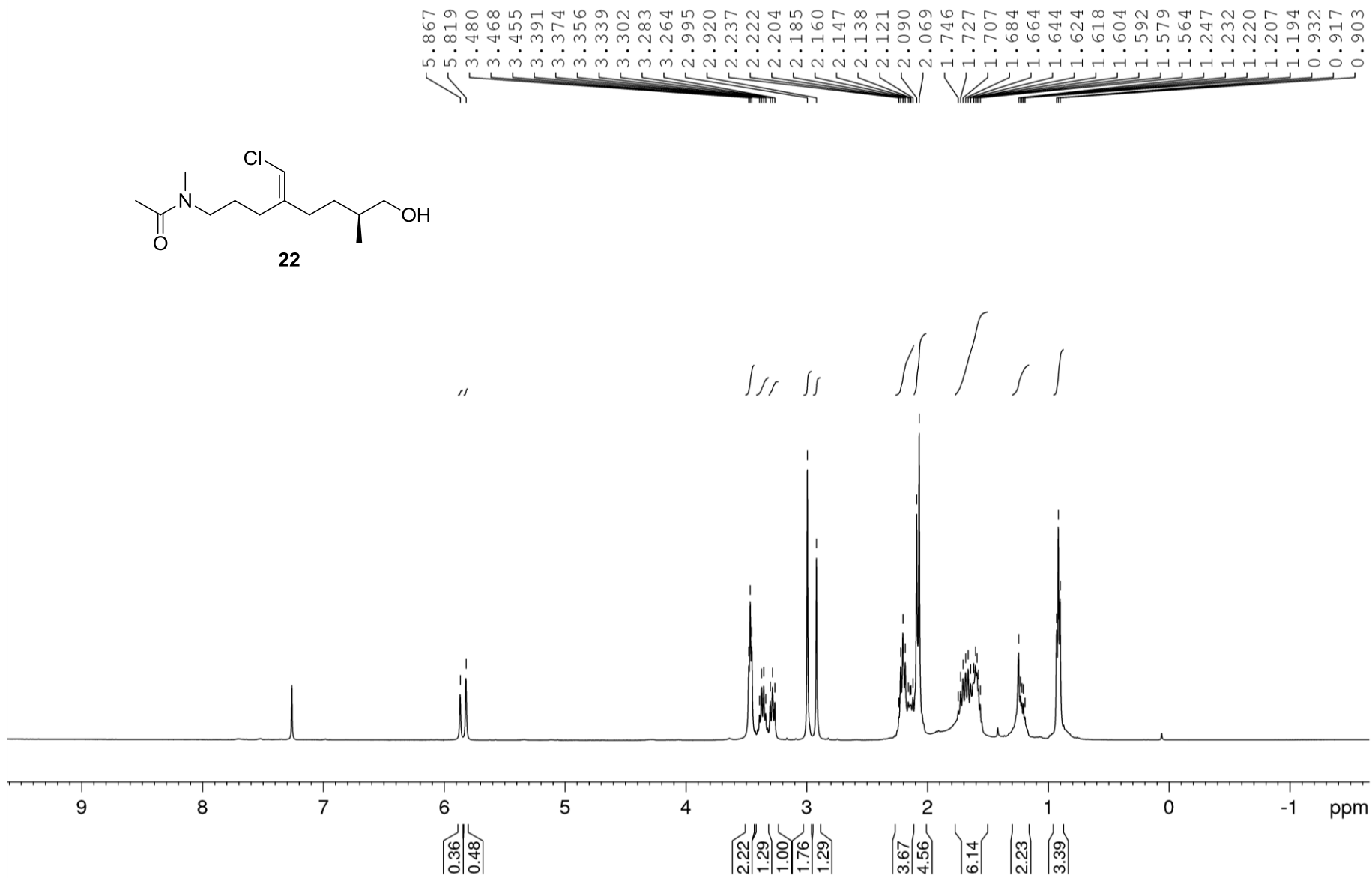


Figure S41. ¹H NMR spectrum of compound **22** (CDCl₃, 400 MHz)

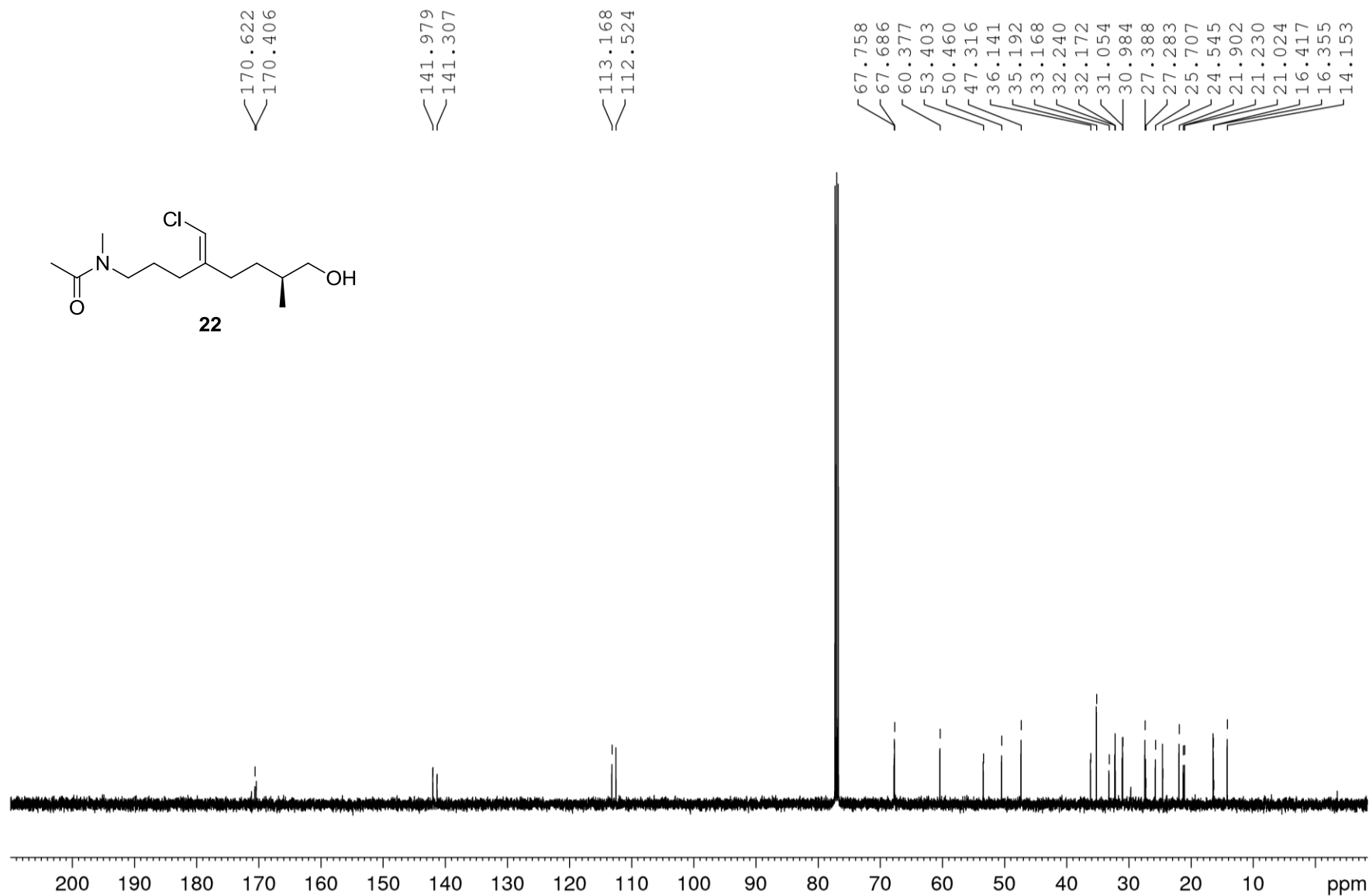


Figure S42. ^{13}C NMR spectrum of compound **22** (CDCl₃, 100 MHz)

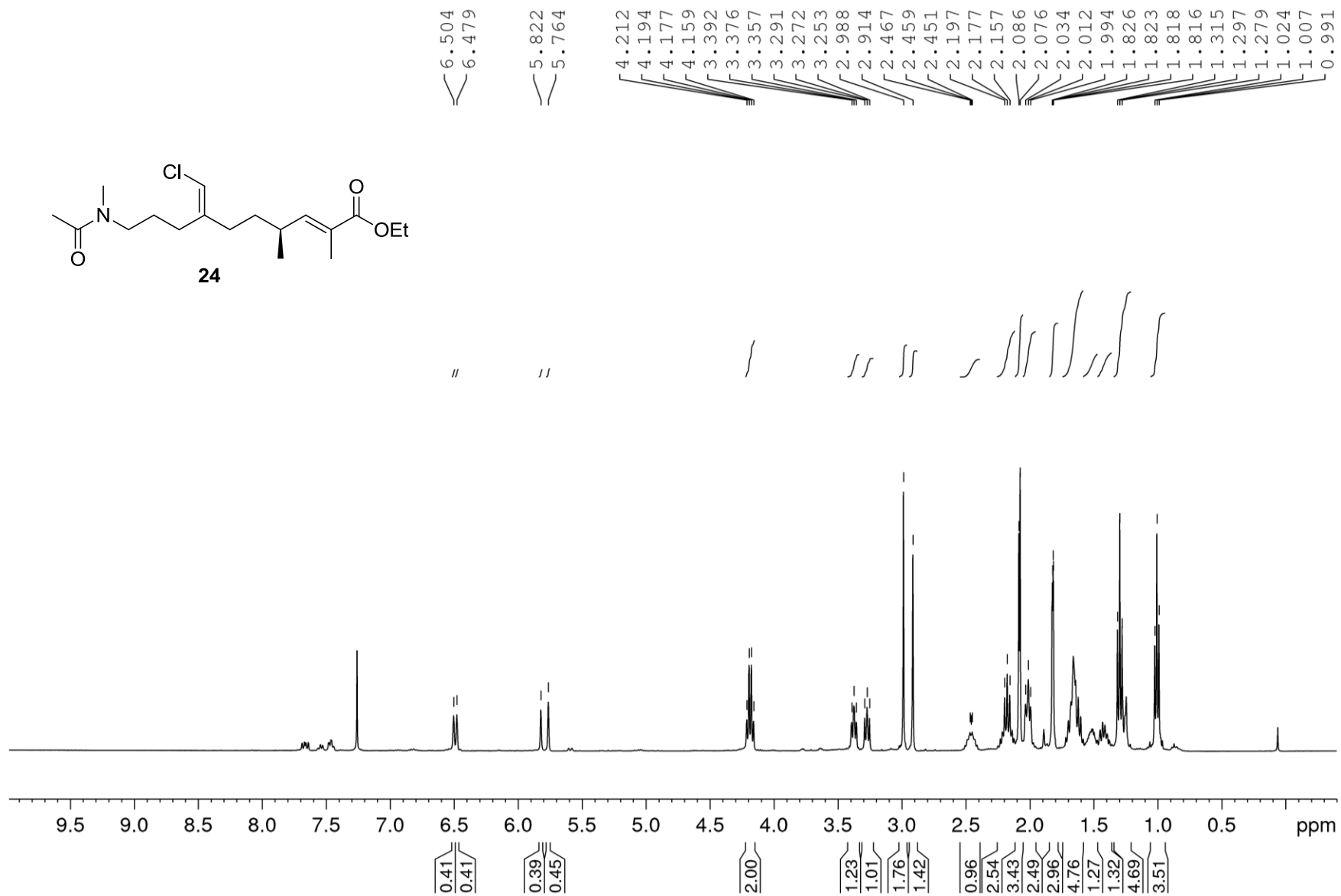


Figure S43. ¹H NMR spectrum of compound **24** (CDCl₃, 400 MHz)

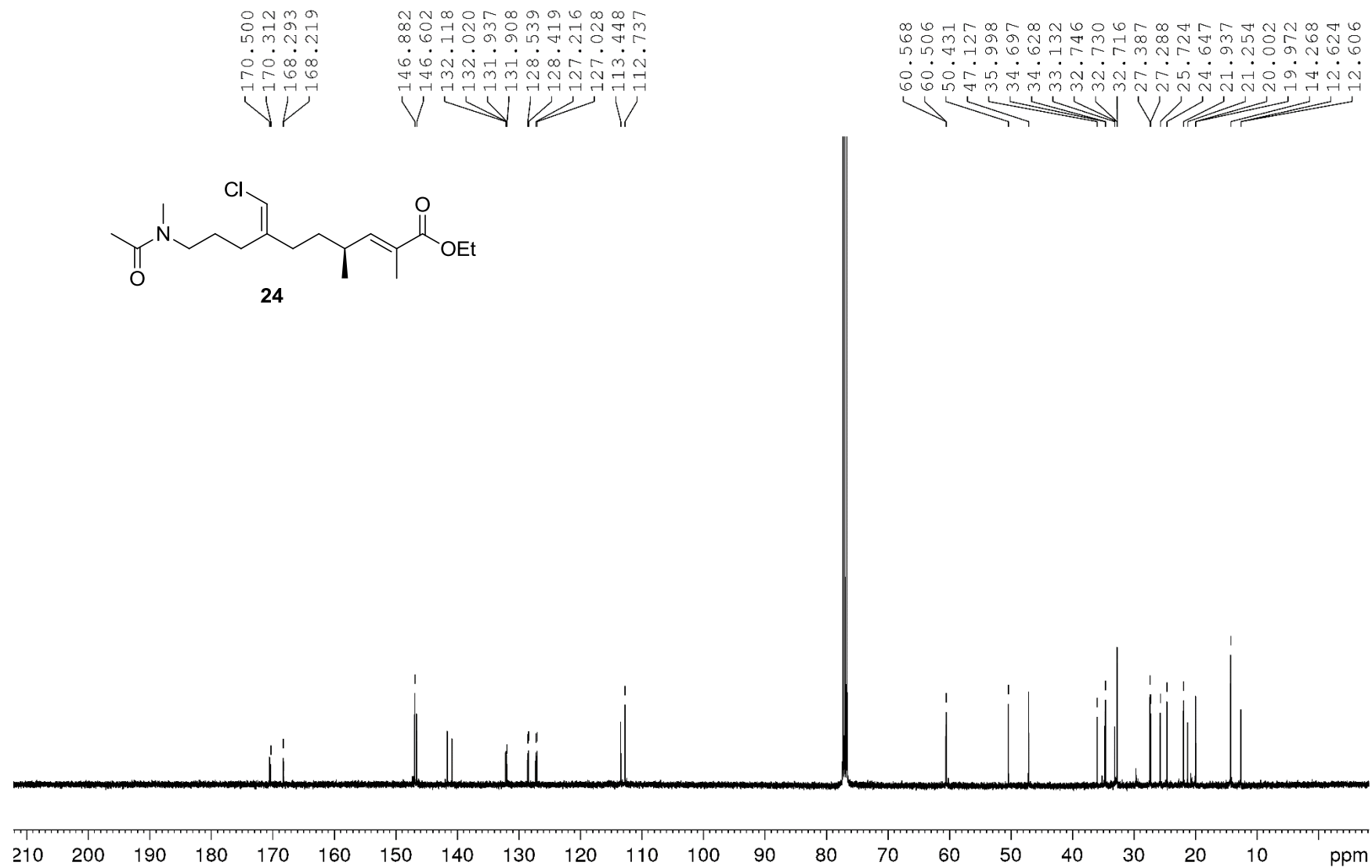


Figure S44. ^{13}C NMR spectrum of compound **24** (CDCl_3 , 100 MHz)

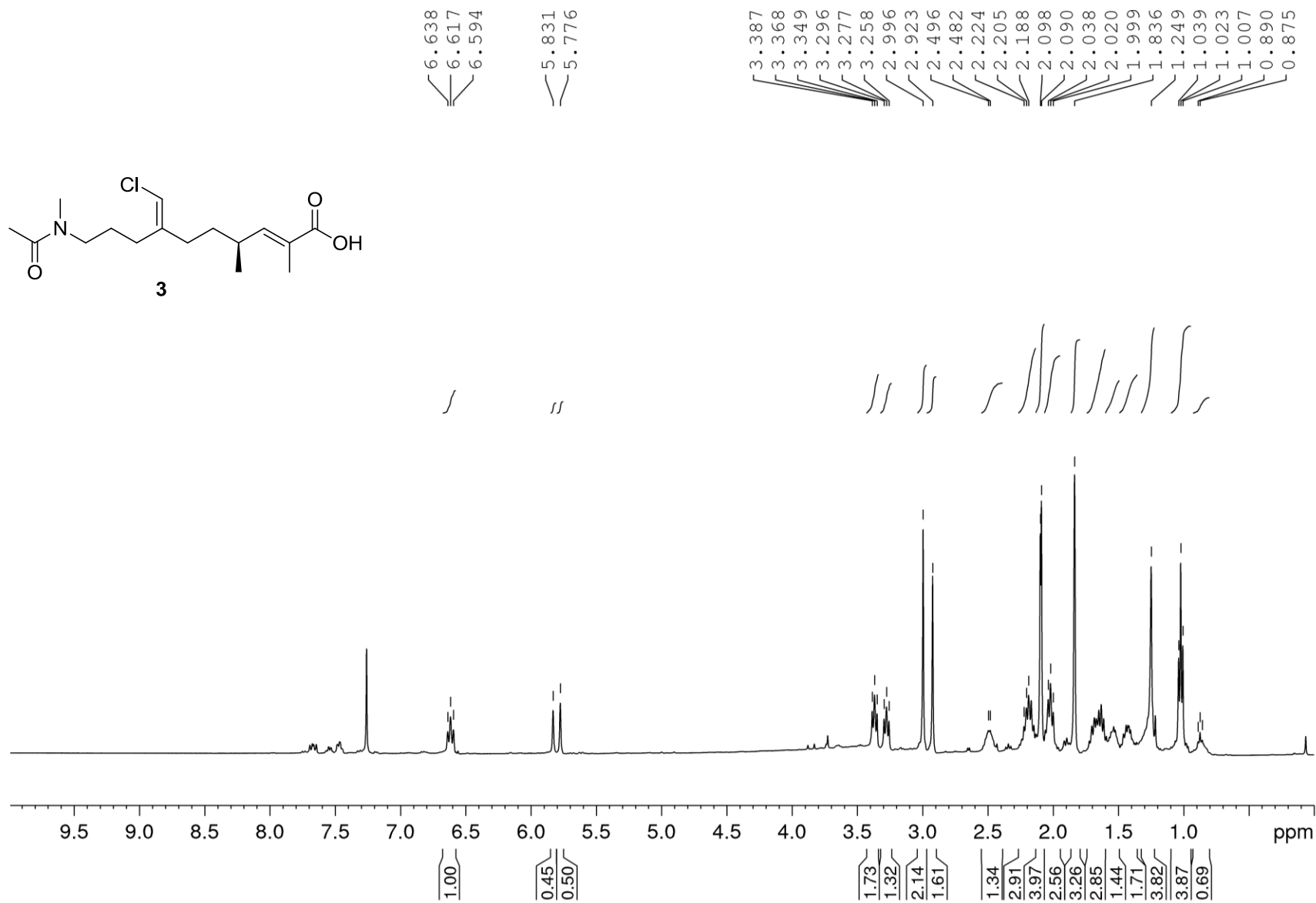


Figure S45. ¹H NMR spectrum of compound **3** (CDCl₃, 400 MHz)

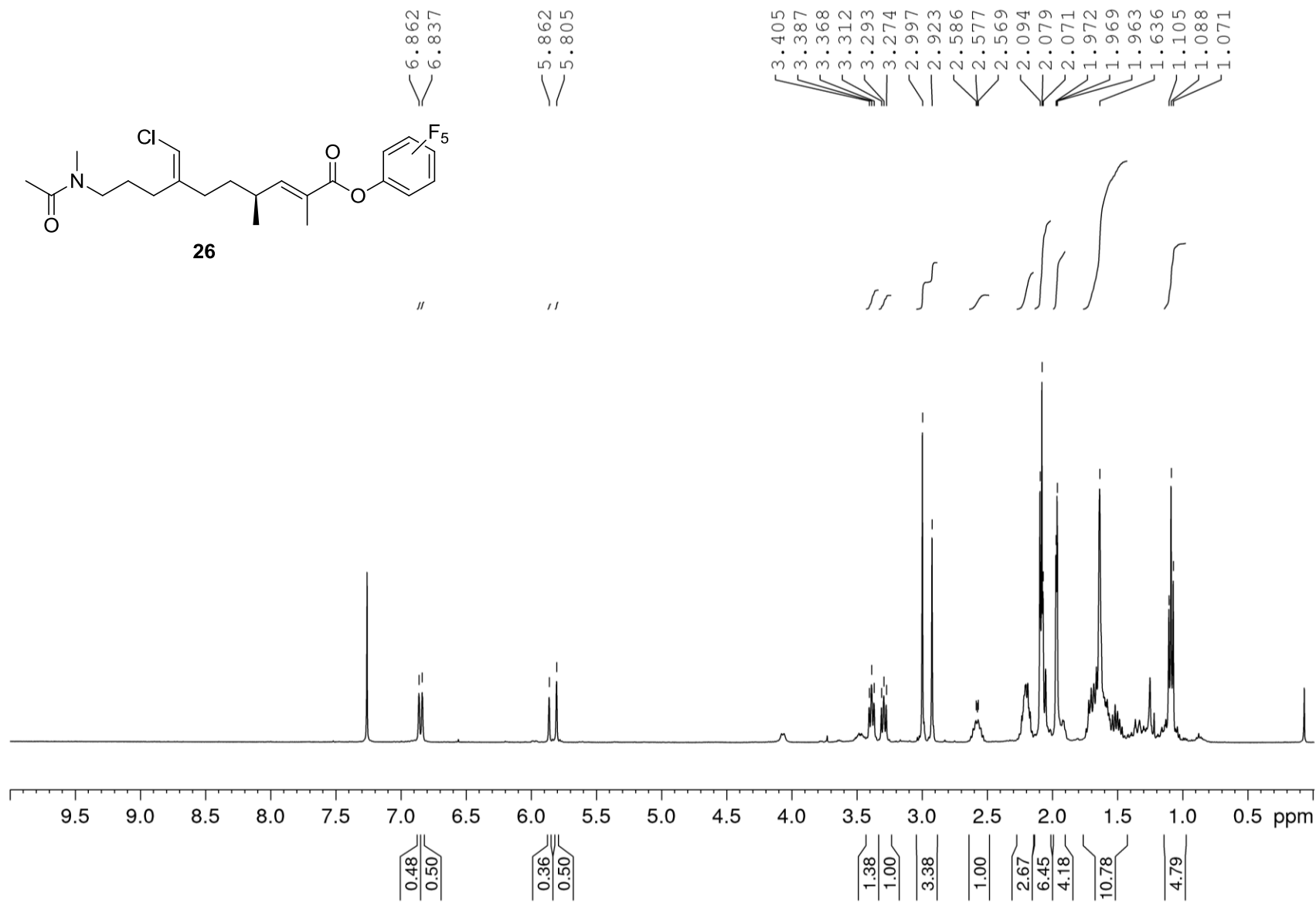


Figure S46. ^1H NMR spectrum of compound **26** (CDCl_3 , 400 MHz)

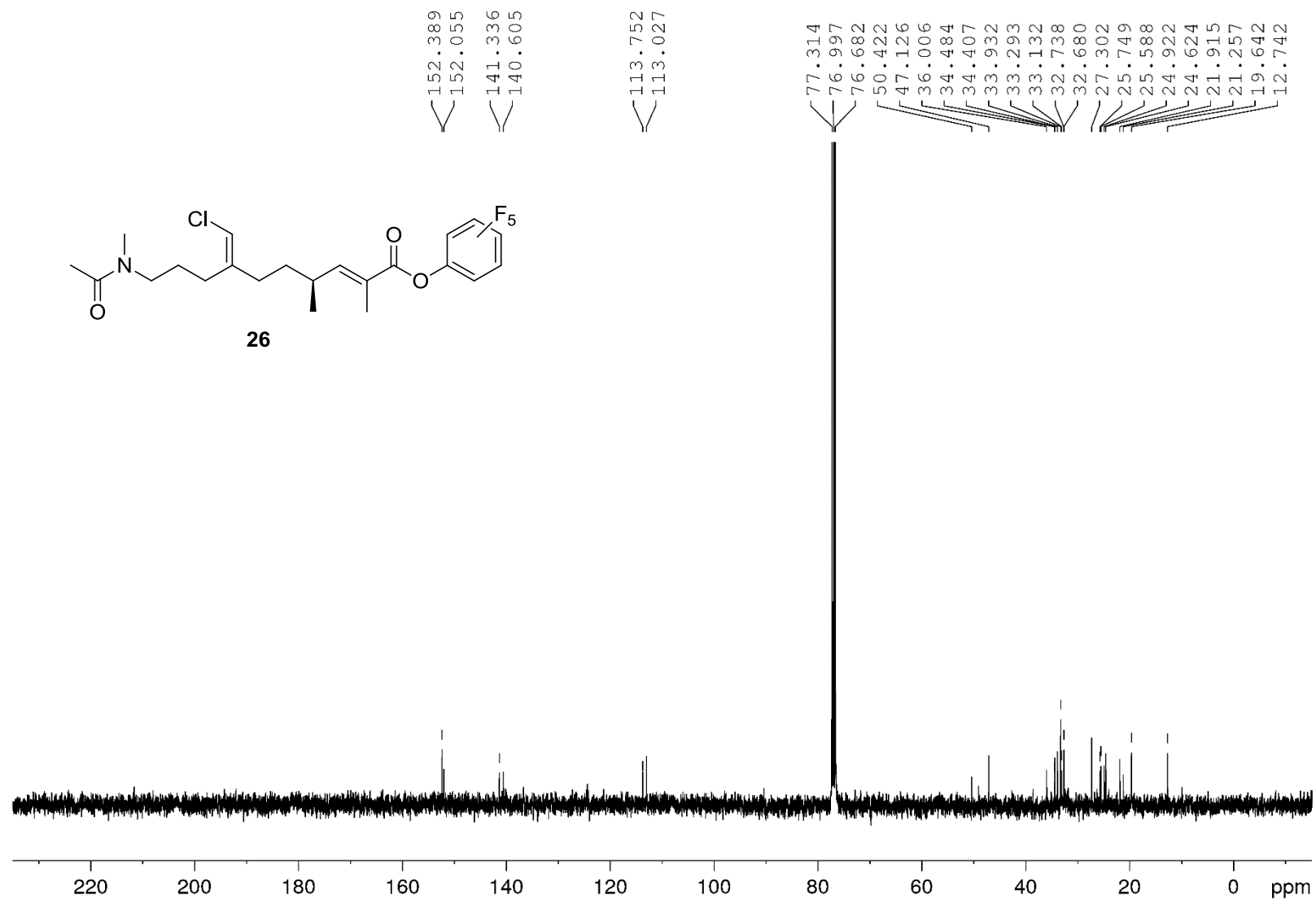
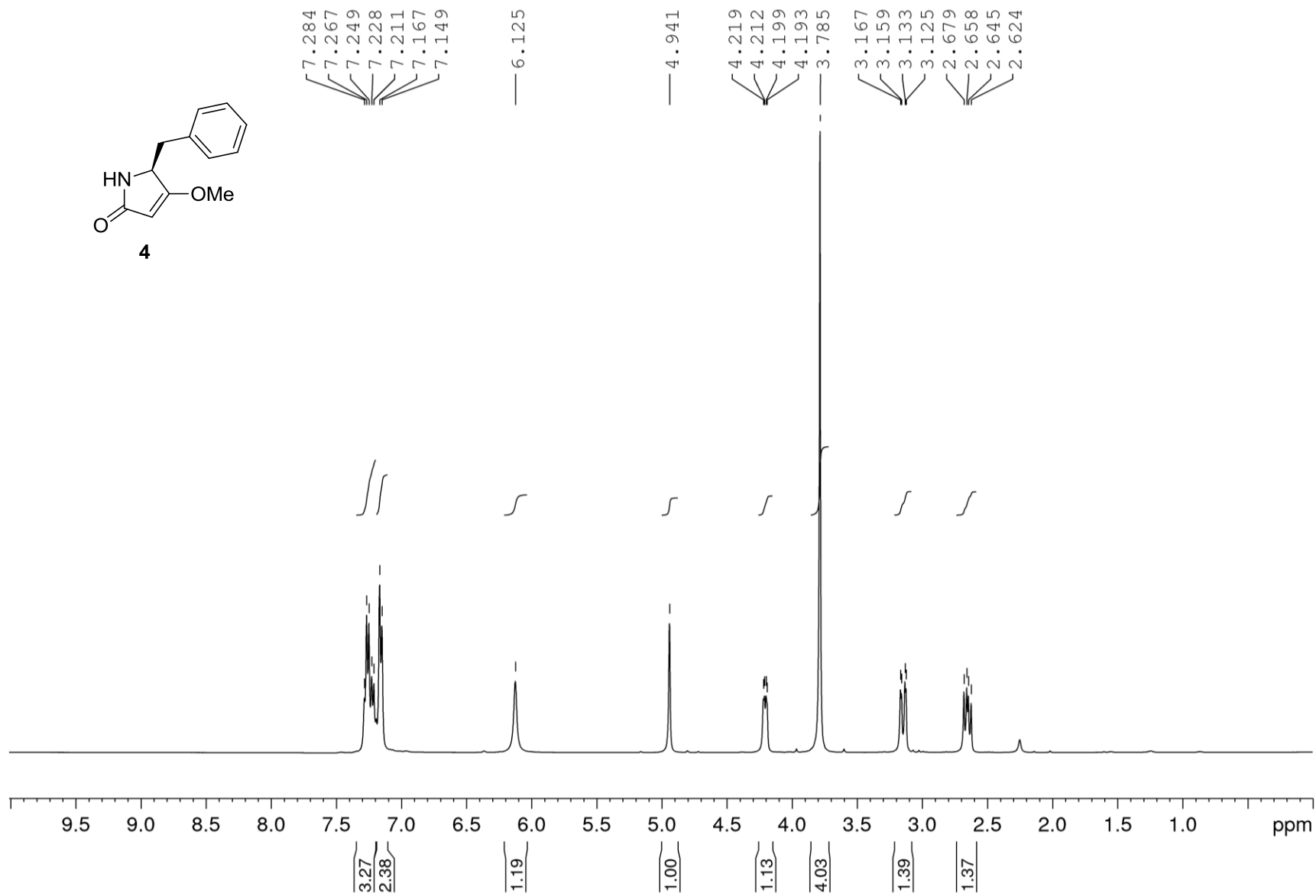


Figure S47. ¹³C NMR spectrum of compound **26** (CDCl₃, 100 MHz)



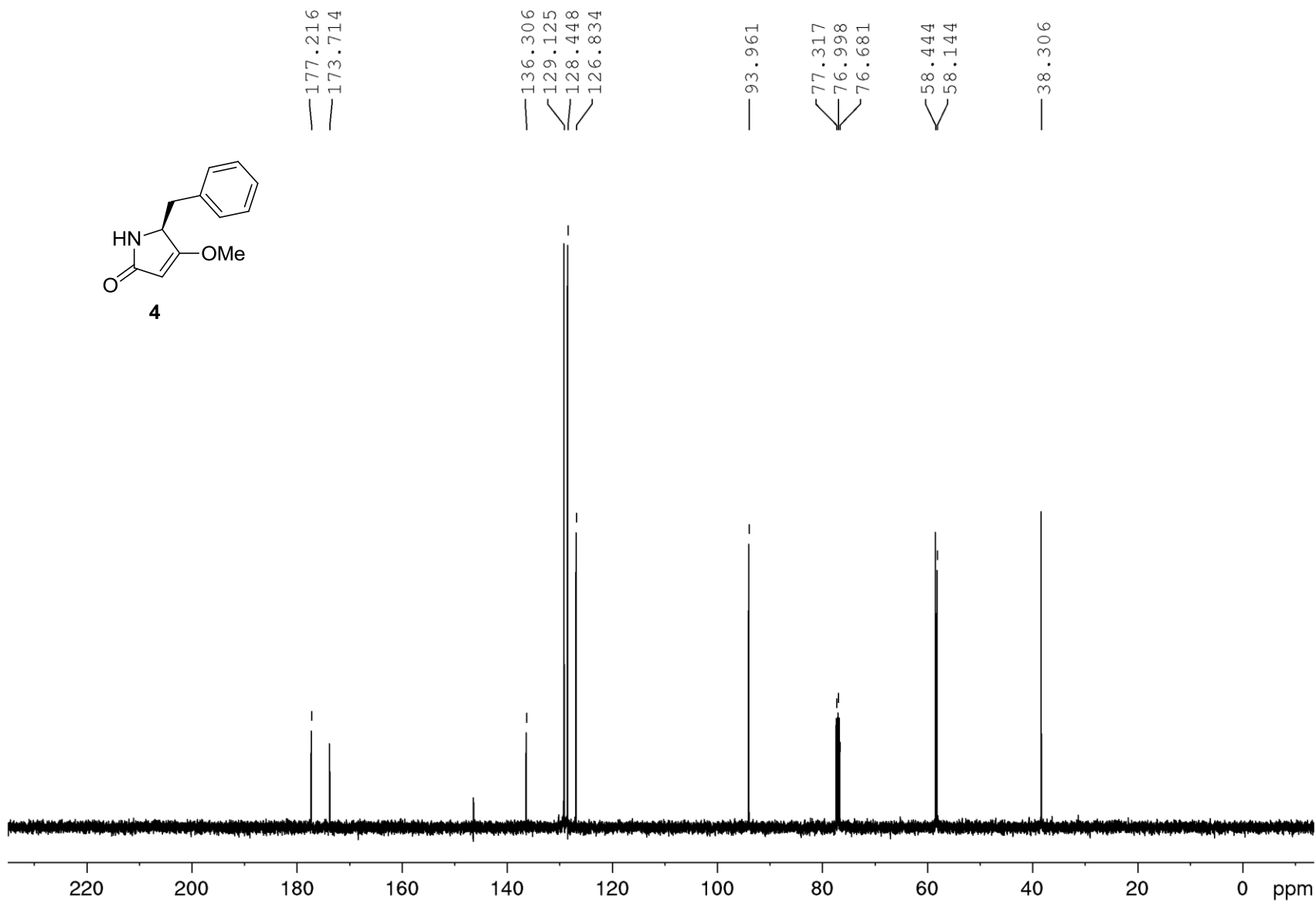


Figure S49. ¹³C NMR spectrum of compound 4 (CDCl₃, 100 MHz)

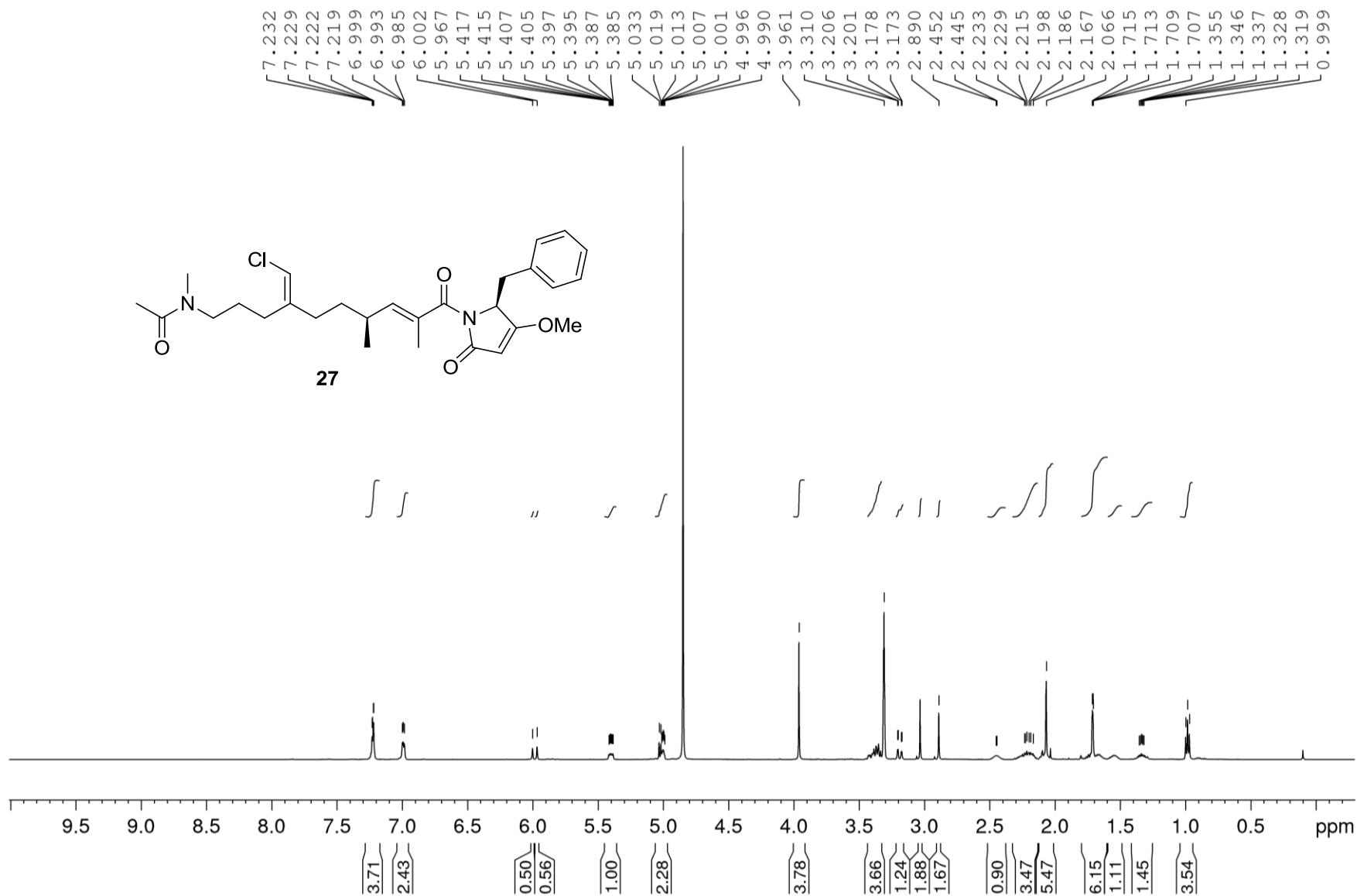


Figure S50. ¹H NMR spectrum of compound **27** (CD₃OD, 500 MHz)

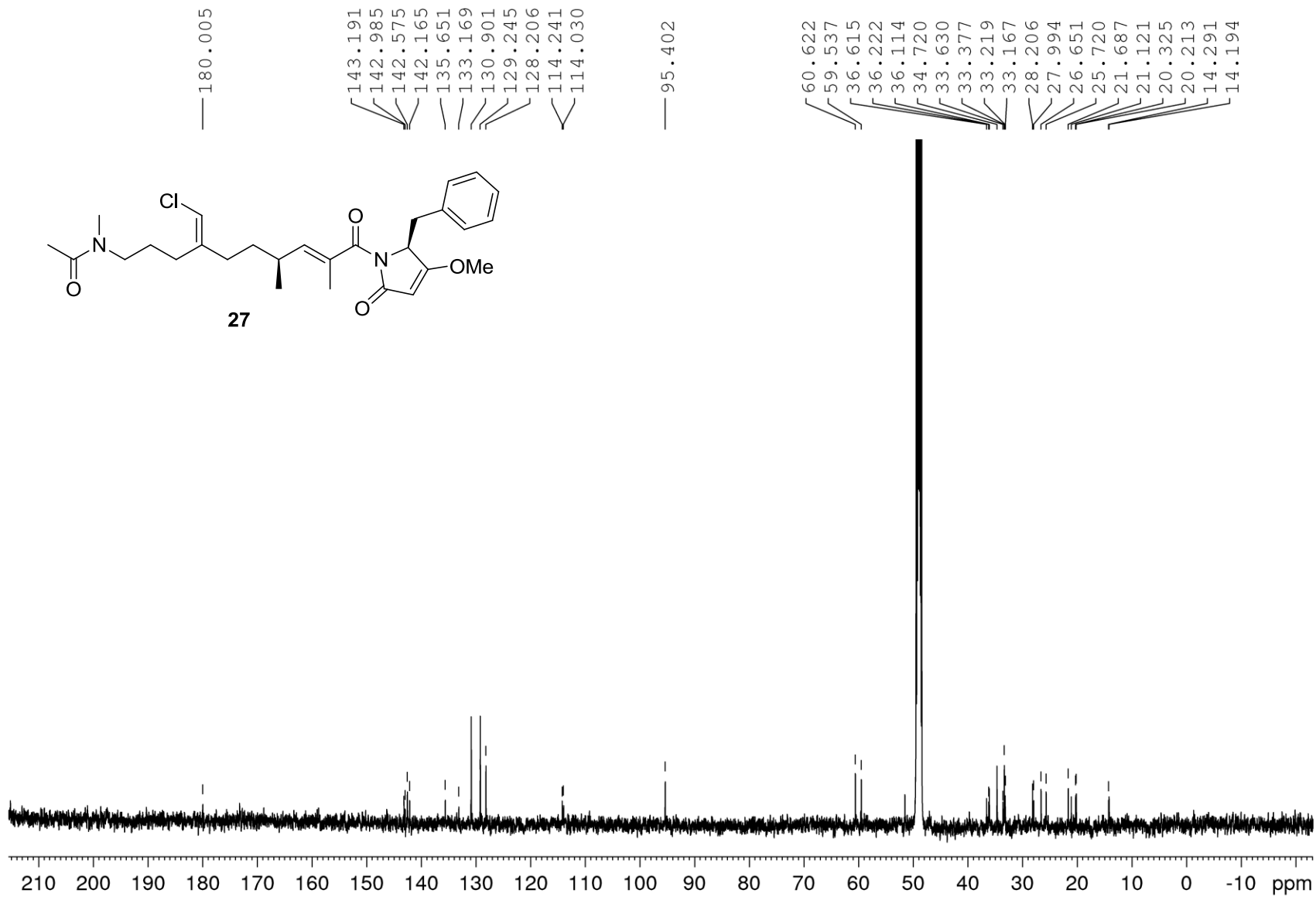


Figure S51. ^{13}C NMR spectrum of compound **27** (CD_3OD , 125 MHz)

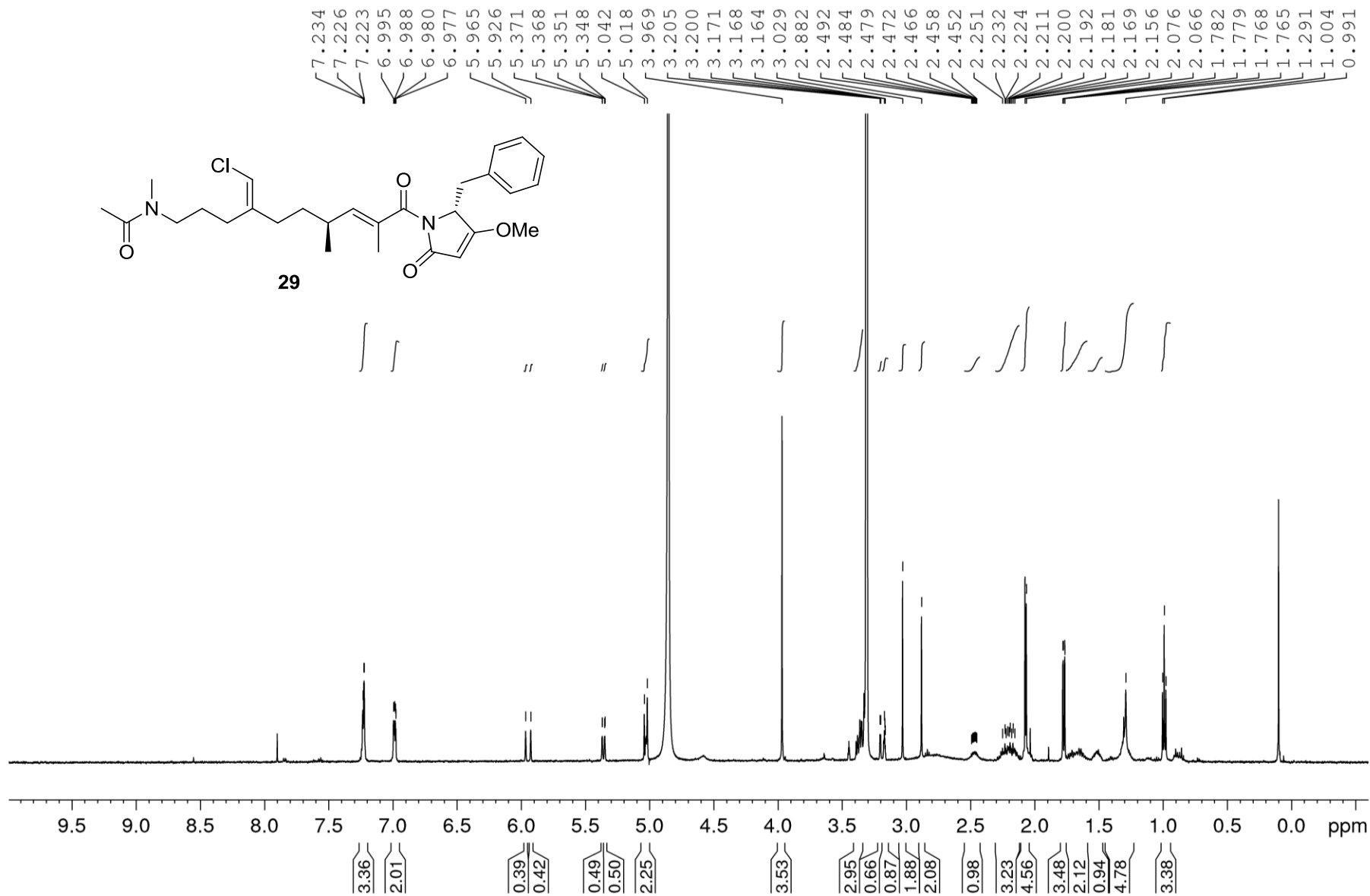


Figure S52. ¹H NMR spectrum of compound **29** (CD₃OD, 700 MHz)

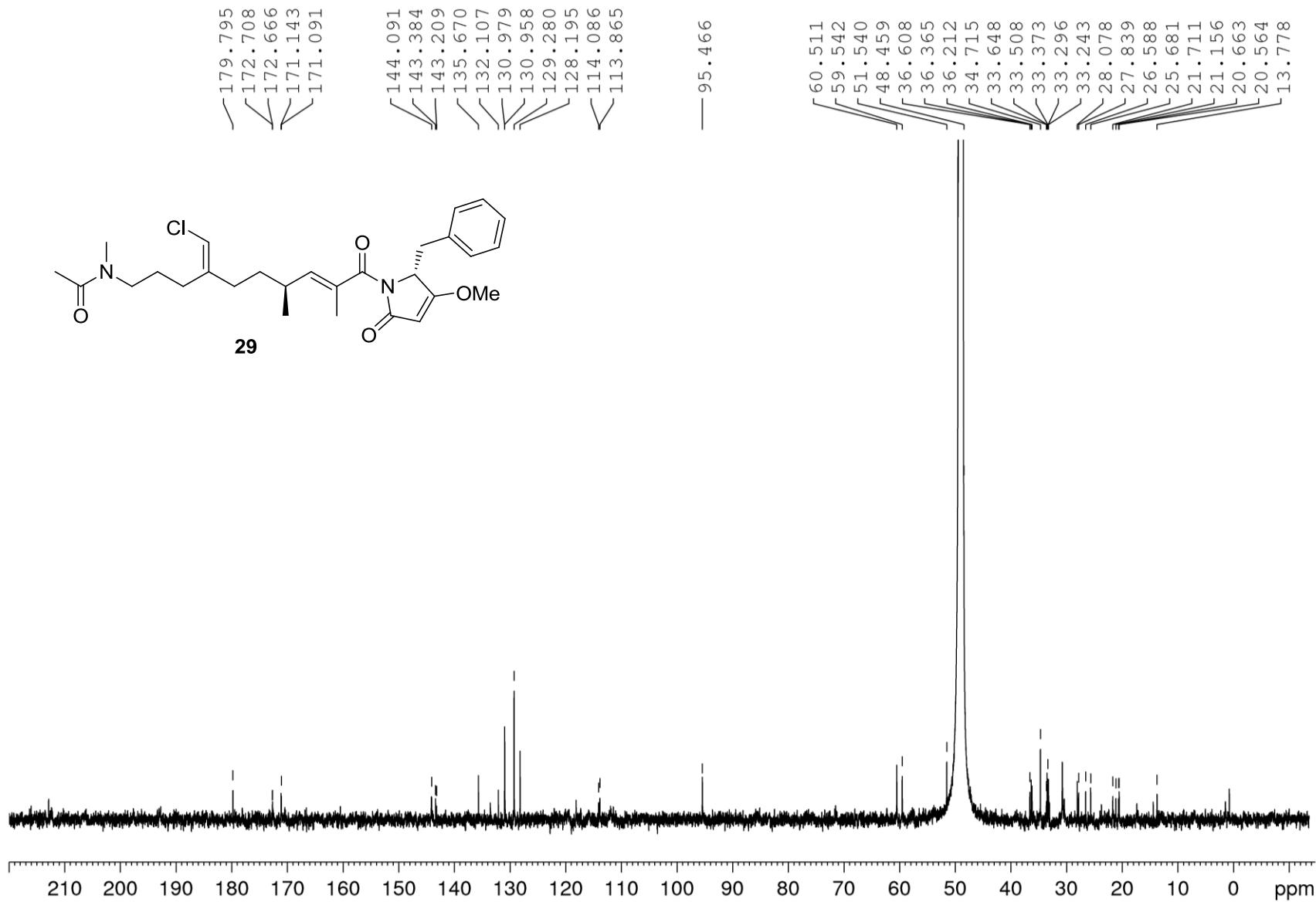


Figure S53. ¹³C NMR spectrum of compound **29** (CD₃OD, 175 MHz)

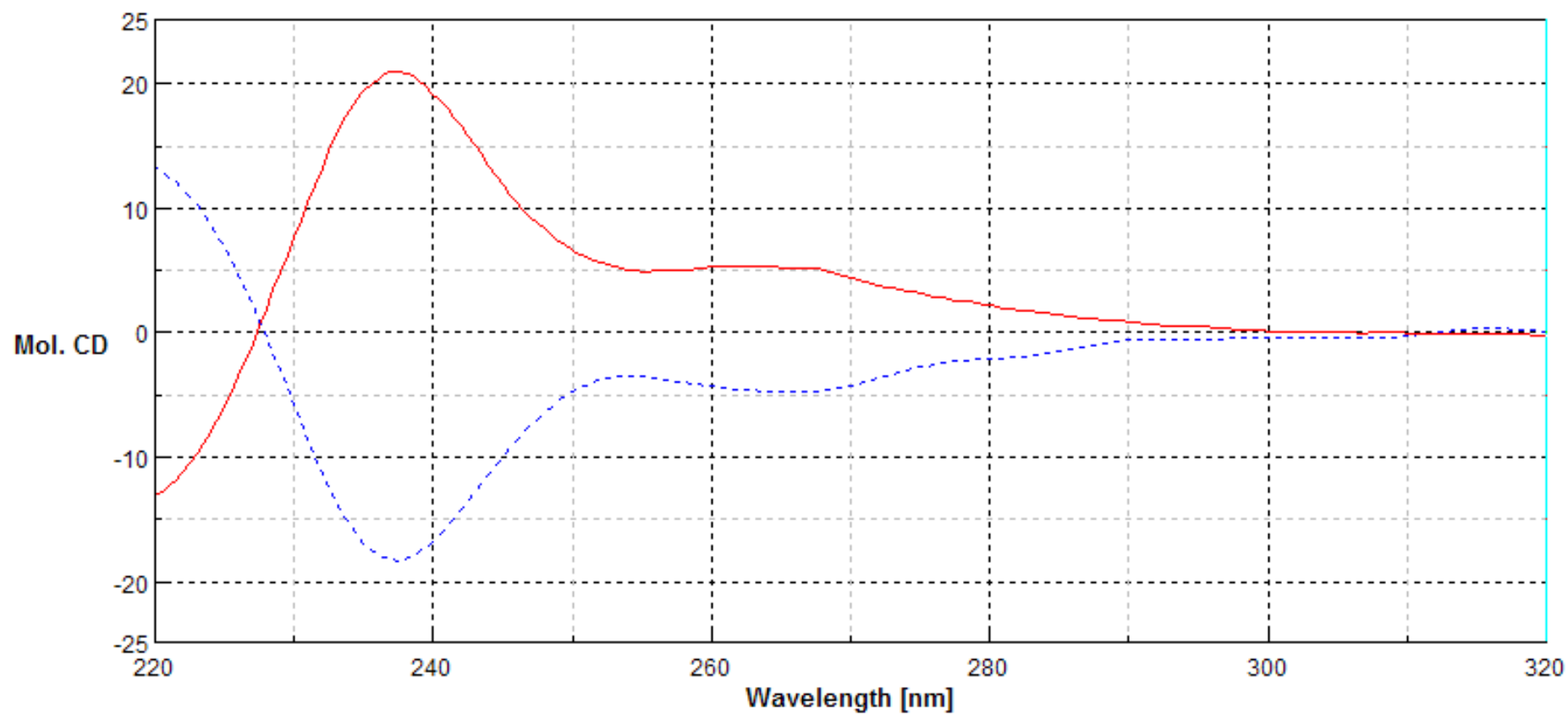


Figure S54. ECD spectra of natural smenamides A (solid red line) and *ent*-smenamides A (**29**) (dashed blue line).