Electronic Supplementary Information (ESI)

Synthesis of thiodisaccharides related to 4-thiolactose. Specific structural modifications increase the inhibitory activity against the *E. coli* β-galactosidase

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General Information

Column chromatography was carried out with silica gel 60 (230–400 mesh). Analytical thin-layer chromatography (TLC) was performed on silica gel 60 F_{254} aluminum-supported plates (layer thickness 0.2 mm). The spots were visualized by exposure to UV light and by charring with sulfuric acid (5% v/v in EtOH, containing 0.5% *p*-anisaldehyde). Optical rotations were measured at 25 °C in a 1 dm cell, in the solvent indicated. Nuclear magnetic resonance (NMR) spectra were recorded at 500 MHz (^{1}H) or 125.7 MHz (^{13}C). Chemical shifts were calibrated to tetramethylsilane or to a residual solvent peak. Assignments of ^{1}H and ^{13}C NMR spectra were assisted by 2D ^{1}H-COSY or NOESY, and 2D ^{1}H-^{13}C HSQC or HMBC experiments. High-resolution mass spectra (HRMS) were obtained using the electrospray ionization (ESI) technique and Q-TOF detection.

Determination of the configuration of thiodisaccharides based on NMR spectral data

The structure of the 3-deoxy-4-thio-hexopyranoside moiety of thiodisaccharides was deduced on the basis of their ^{1}H-NMR spectra, as follows.

Thiodisaccharide 6: The ^{1}H-NMR spectrum of **6** showed the H-1 signal as a doublet at 4.93 ppm with a coupling constant value ($J_{1,2}$ = 3.7 Hz), typical of an axial-equatorial arrangement. The coupling of H-2 with H-3ax ($J_{2,3ax}$ = 12.0 Hz) was characteristic of *trans* diaxial protons, while that with H-3eq ($J_{2,3eq}$ = 3.6 Hz) agreed with their axial-equatorial orientation. Hence, an *R* configuration was assigned to the new stereocenter at C-2. Similarly, the coupling constant values $J_{3eq,4}$ (2.8 Hz), $J_{3ax,4}$ (2.8 Hz) and $J_{4,5}$ (2.5 Hz) were consistent with the *R* configuration for C-4.

Thiodisaccharide 7: The ^{1}H NMR spectrum of **7** revealed large *J* values for the coupling of H-4 with H-3ax ($J_{3ax,4}$ = 12.0 Hz) and H-5 ($J_{4,5}$ = 11.5 Hz) in agreement with a *trans*-diaxial orientation for all these protons, while H-4 and H-3eq ($J_{3eq,4}$ = 5.6 Hz) maintained an axial-equatorial disposition. Comparison of the ^{1}H-NMR spectra of **4** and **7** revealed an upfield shifting of the H-4 signal from 3.70 ppm in **4** to 3.32 ppm in **7**. This could be attributed to the change in orientation of H-4 from an equatorial (in **4**) to an axial (in **7**) arrangement, which generally implies signal protection in such six-membered rings.[1]

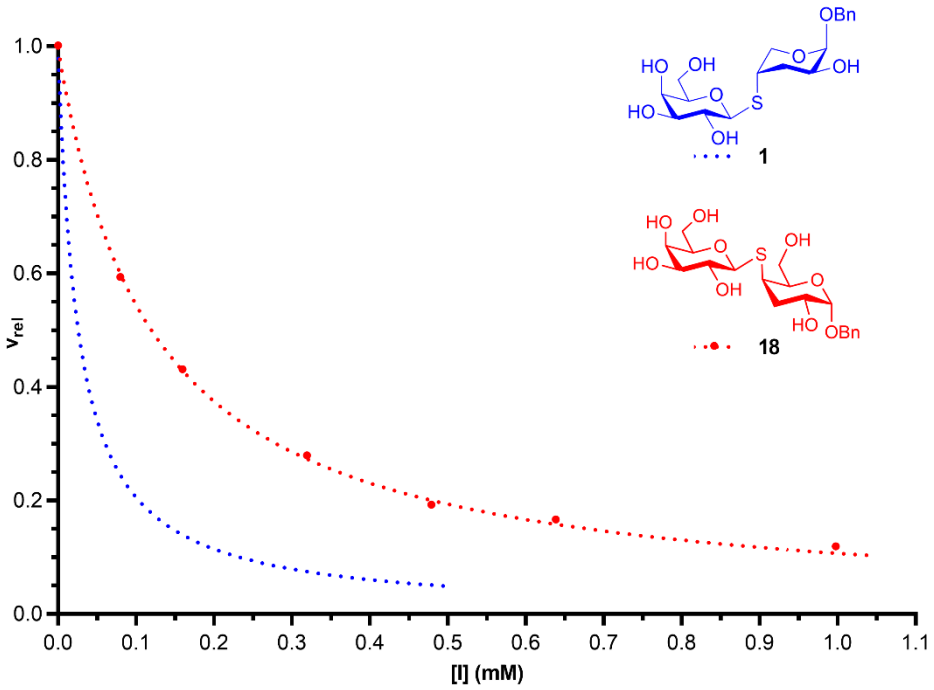
Thiodisaccharide 10: The ^{1}H-NMR spectrum of this compound showed a broad singlet (4.97 ppm) for H-2 and the downfield vinyl signals for H-4 and H-5 at 6.22 and 6.99 ppm, respectively. The coupling constant analysis suggested the enone adopted an ^{O}E conformation, since the small coupling constant value for the vinyl H-5 and allylic H-6 protons ($J_{5,6}$ = 1.6 Hz) suggested an almost perpendicular arrangement among them. Furthermore, H-4 exhibited long-range couplings with H-2 ($^4J_{2,4}$ = 0.3 Hz) and with H-6 ($^4J_{4,6}$ = 2.5 Hz). All these *J* values were in agreement with those predicted by the Garbisch equation for the ^{O}E conformation of **10**.[2]

Thiodisaccharides 11 and 12: The configuration at C-4 in **11** and **12** was determined according to relevant coupling constants values, which are summarized in Table S2. The *p*-nitrobenzyl derivative **11**, similar to analogue **4**, showed relatively small coupling constant values for H-4 with H-3 and H-5 and a long-range coupling between H-1 and H-3eq, confirming the *R* configuration for C-4. The isomer **12** had opposite configuration at C-4, according to the large *J* values of H-4 with H-3ax and H-5.

Compound	**Coupling constant (Hz)**				
	$J_{3eq,4}$	$J_{3ax,4}$	$J_{4,5}$	$^4J_{1,3eq}$	$J_{1',2'}$
11	2.4	4.9	2.4	0.9	10.0
12	5.4	12.0	10.7	-	9.8

Table S2: Coupling constant values for uloses **11** and 1**2**

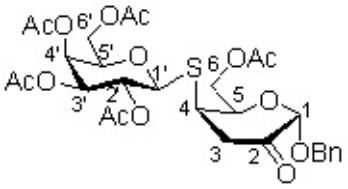
Effect of Concentration of Thioglycomimetics 1 and 18 on the enzymatic activity of the β-galactosidase from *E. coli*.

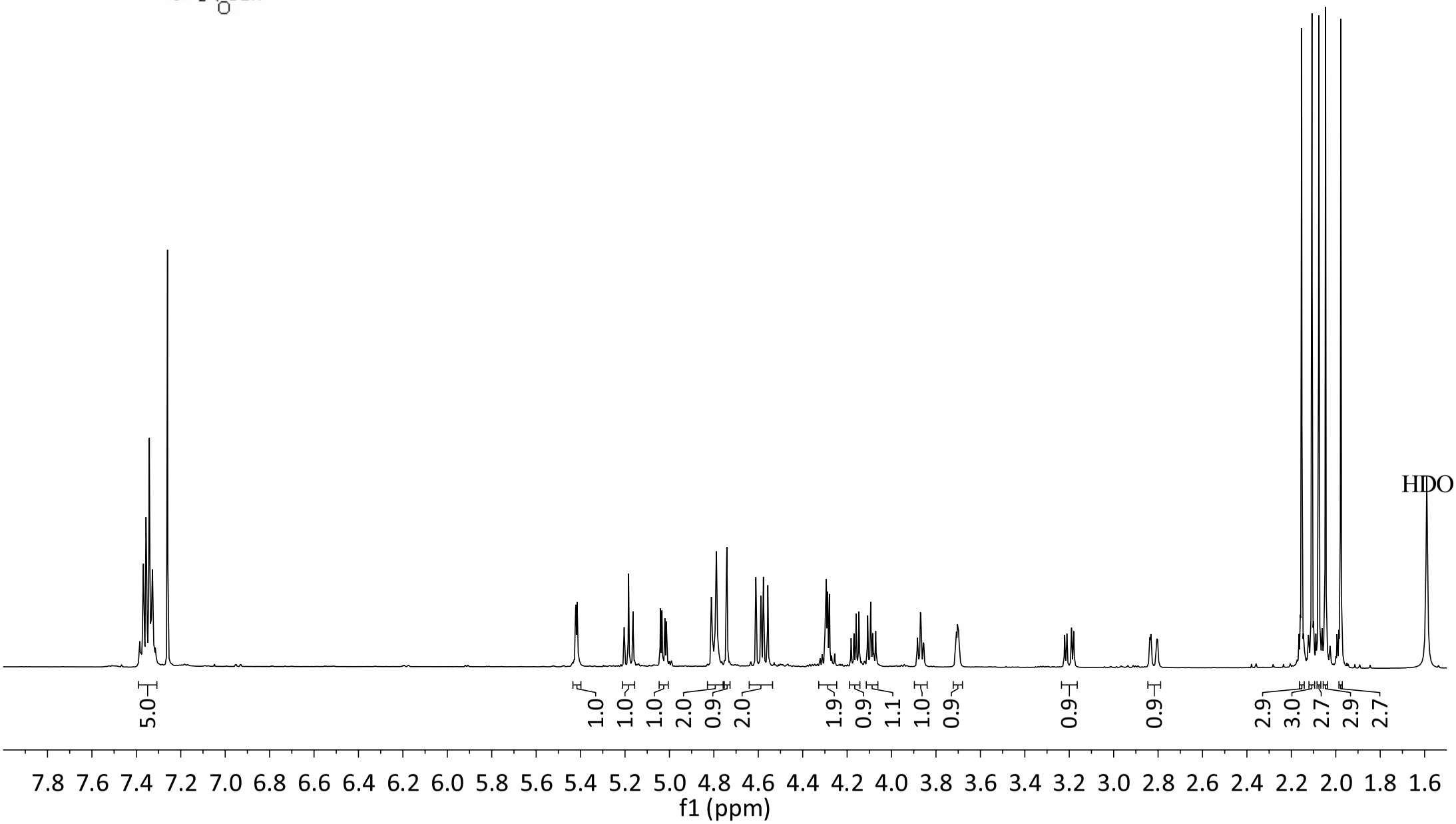


References

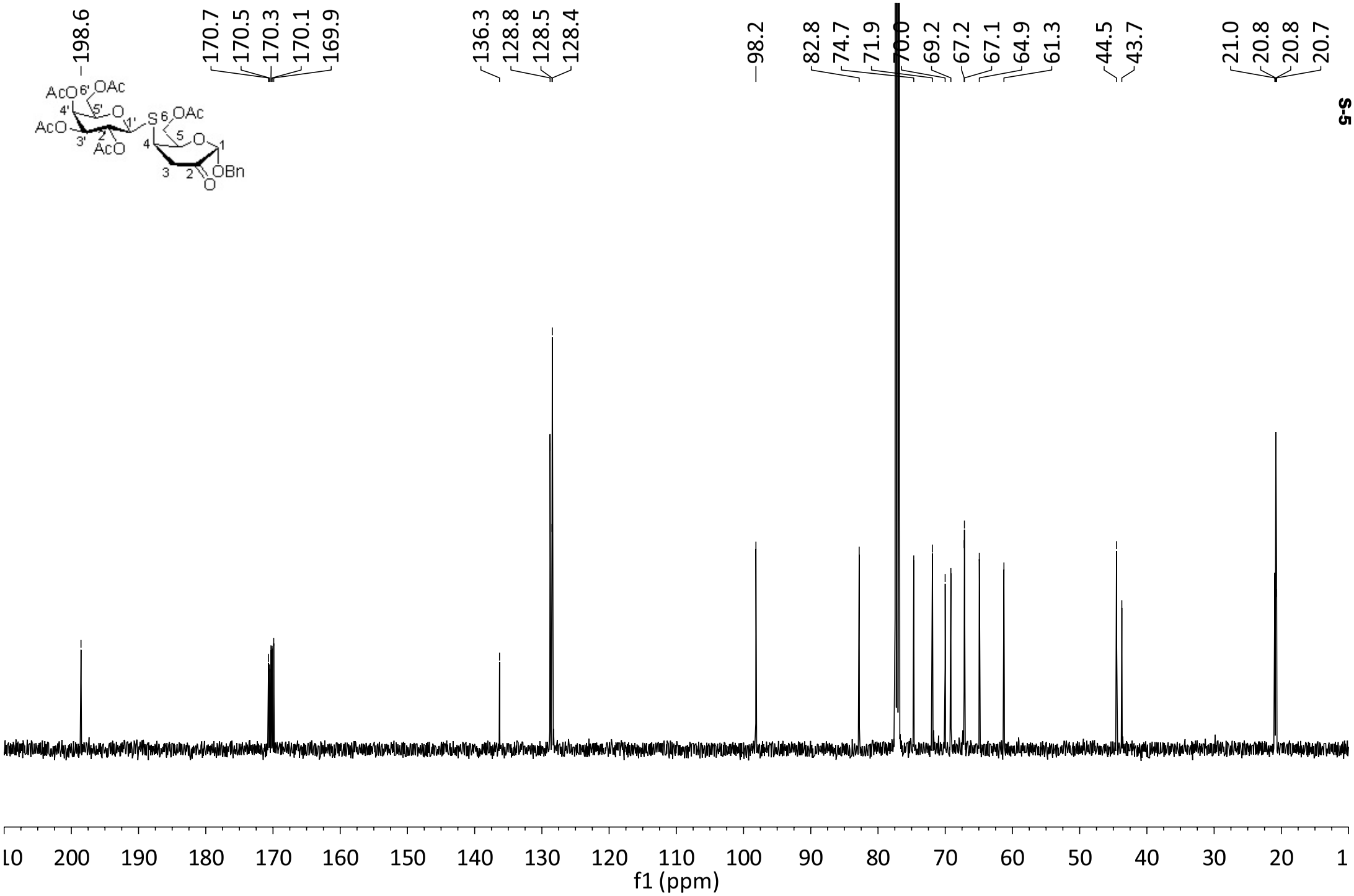
1 R. U. Lemieux, R. K. Kullnig, H. J. Bernstein and W. G. Schneider, *J. Am. Chem. Soc.*, 1958, **80**, 6098–6105.

2 E. W. Garbisch, *J. Am. Chem. Soc.*, 1964, **86**, 5561–5564.

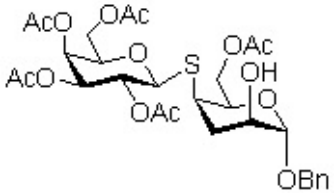




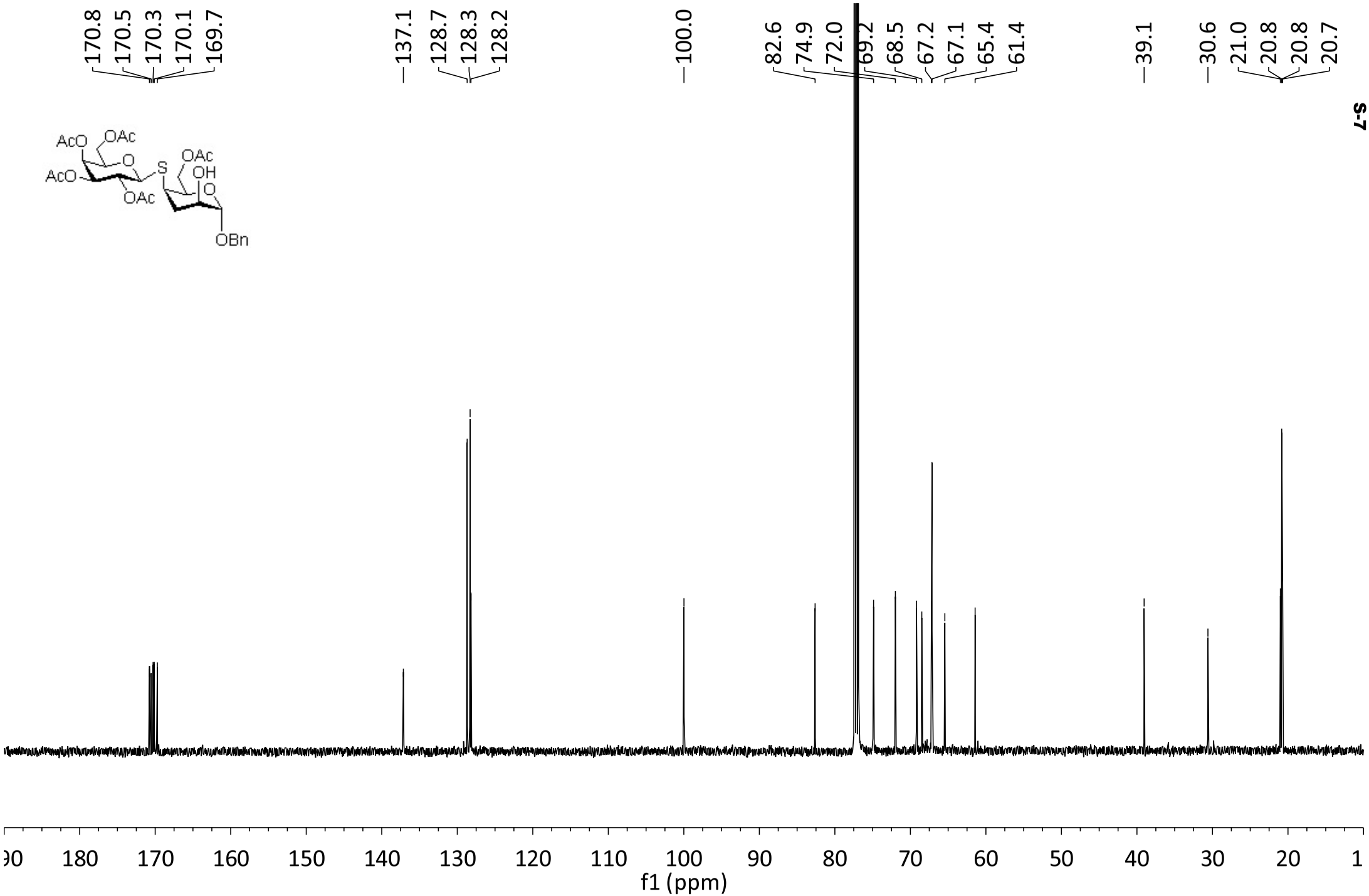
^{1}H-NMR Spectrum of compound **4** (500 MHz, $CDCl_3$).



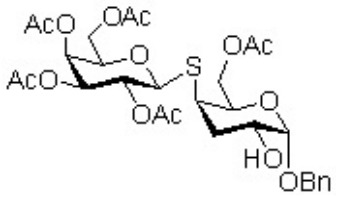
^{13}C-NMR Spectrum of compound **4** (125.7 MHz, $CDCl_3$).



^{1}H-NMR Spectrum of compound **5** (500 MHz, $CDCl_3$).



^{13}C-NMR Spectrum of compound **5** (125.7 MHz, $CDCl_3$).

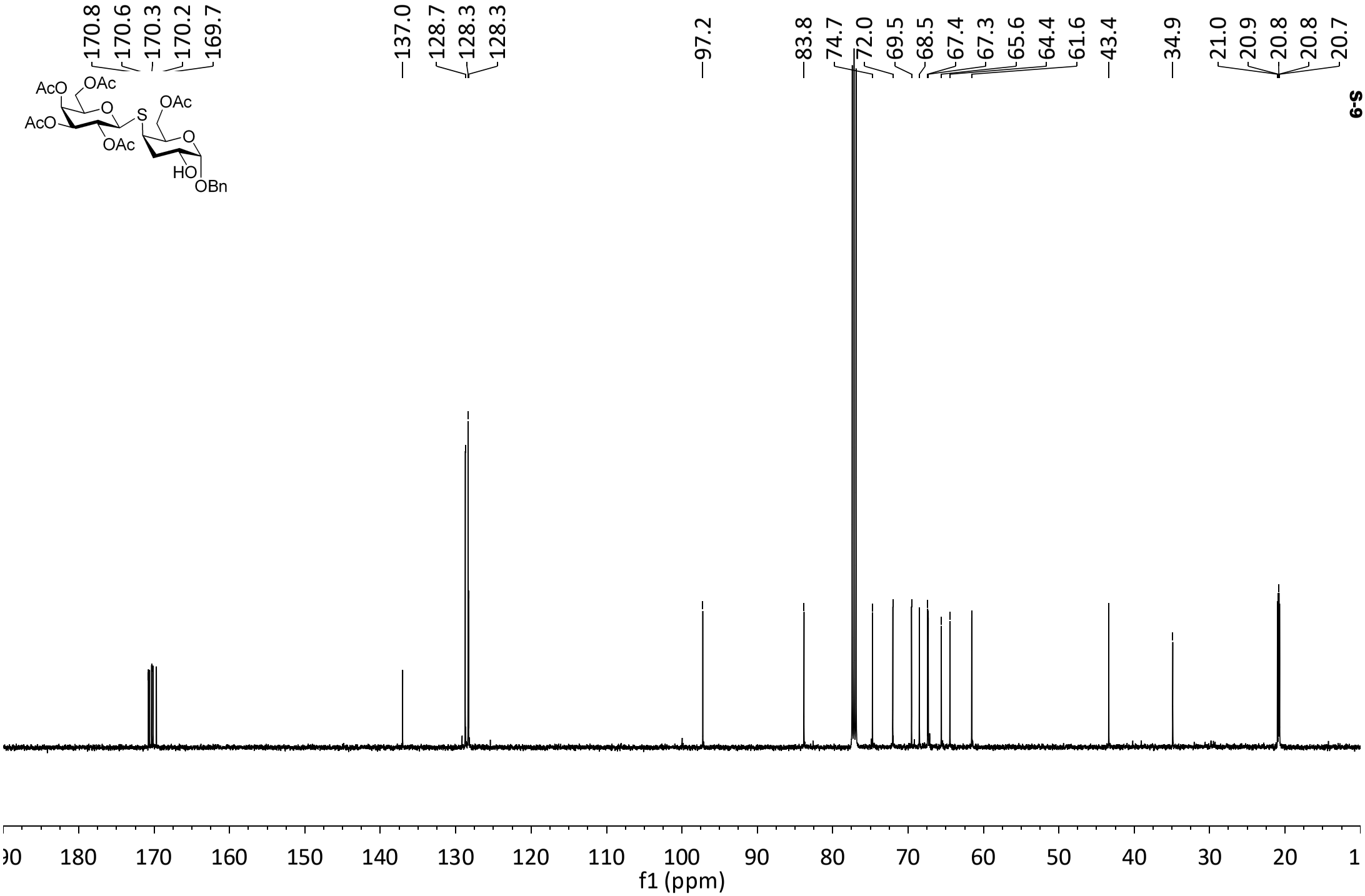


5.2 1.0 1.0 1.0 1.0 1.0 2.1 1.0 3.0 2.1 1.0 1.0 1.1 3.1 7.1 3.1 3.2

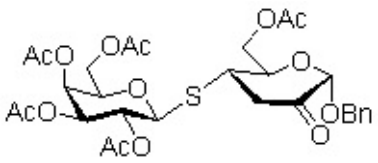
7.8 7.6 7.4 7.2 7.0 6.8 6.6 6.4 6.2 6.0 5.8 5.6 5.4 5.2 5.0 4.8 4.6 4.4 4.2 4.0 3.8 3.6 3.4 3.2 3.0 2.8 2.6 2.4 2.2 2.0 1.8 1.6

f1 (ppm)

^{1}H-NMR Spectrum of compound **6** (500 MHz, $CDCl_3$).



^{13}C-NMR Spectrum of compound **6** (125.7 MHz, $CDCl_3$).



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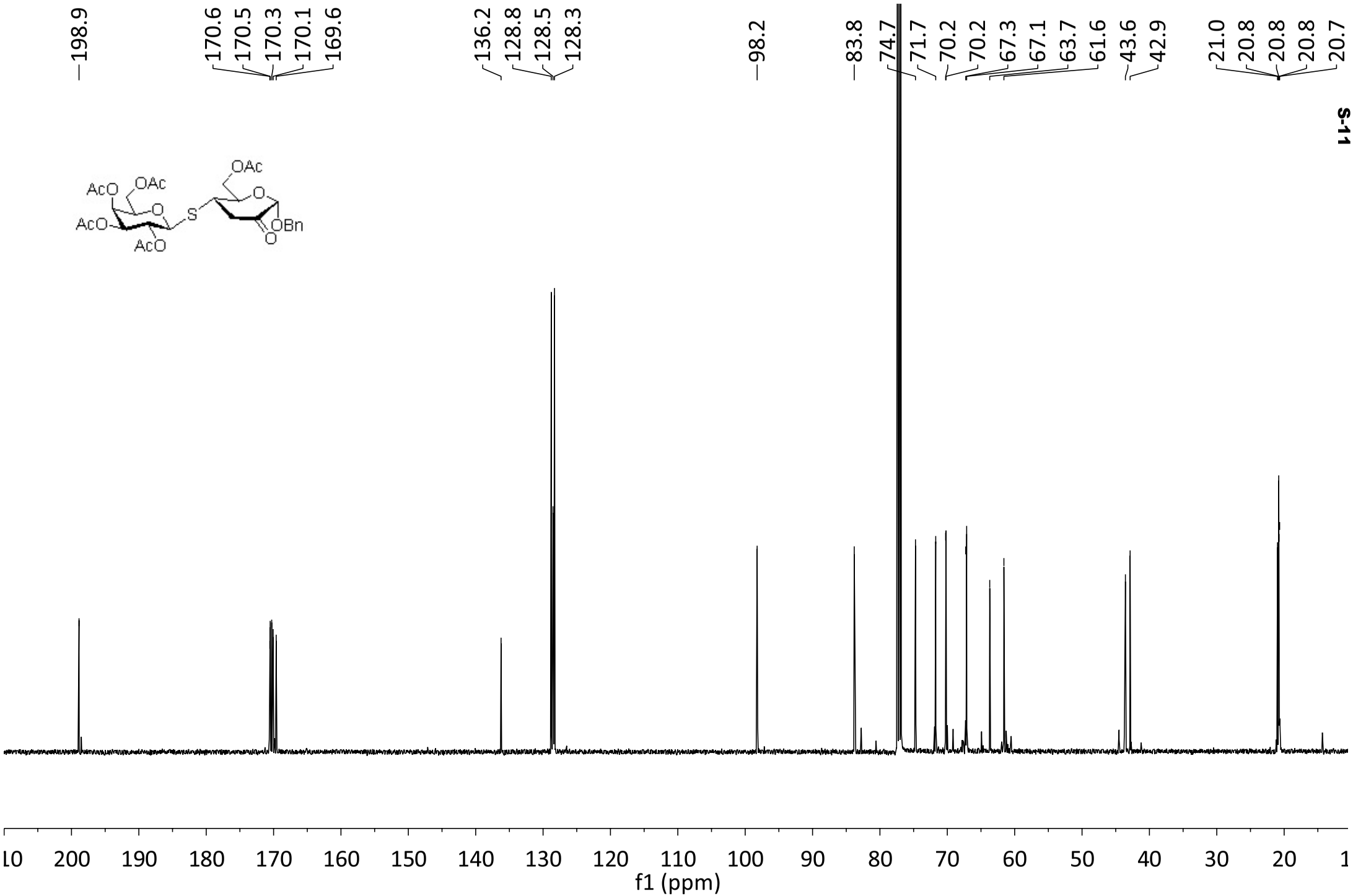
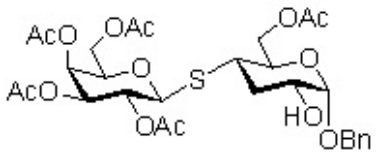
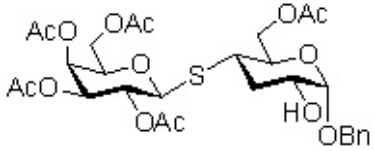


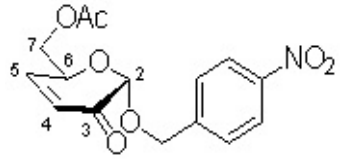
^{13}C-NMR Spectrum of compound **7** (125.7 MHz, $CDCl_3$).



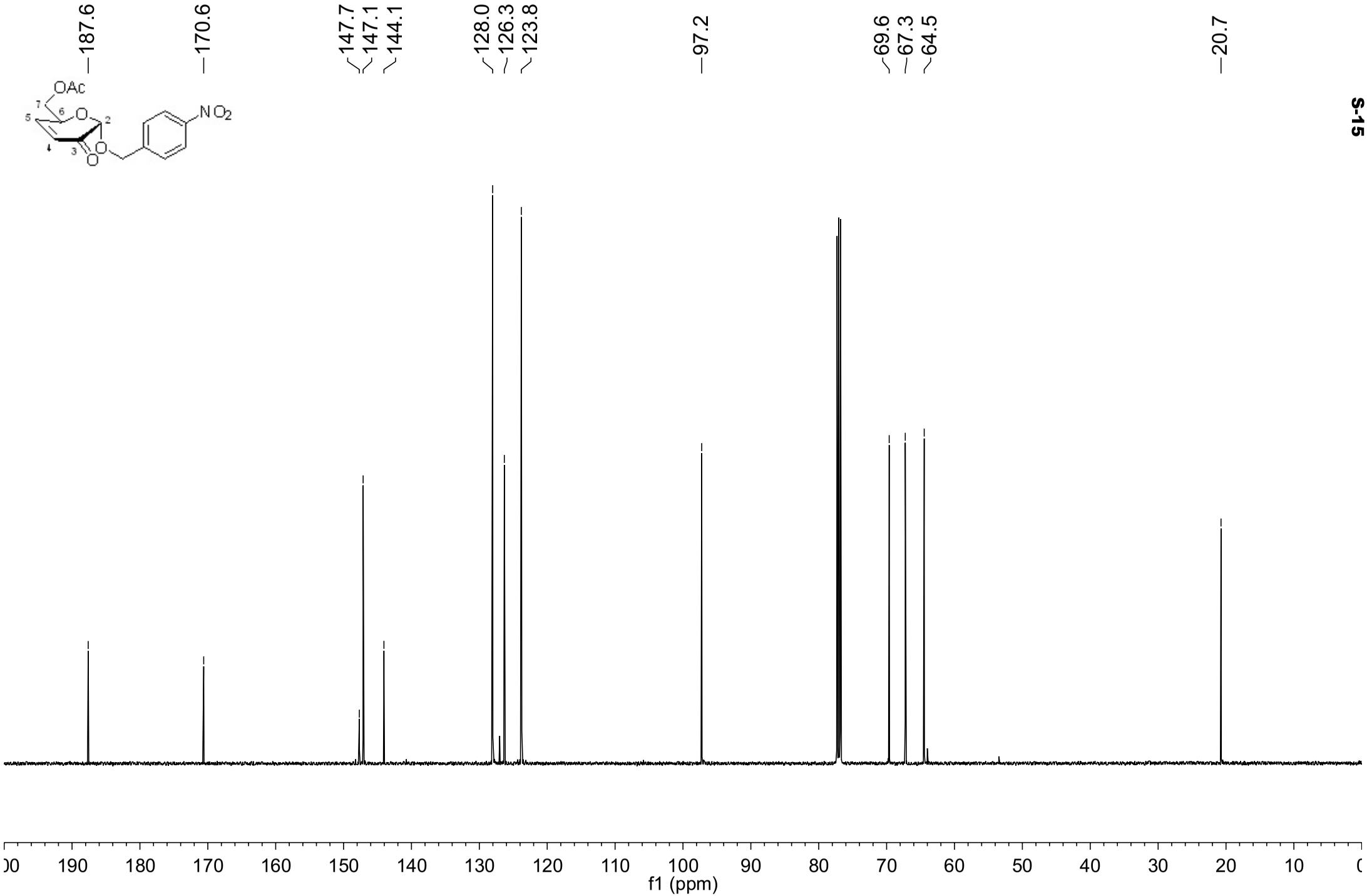
^{1}H-NMR Spectrum of compound **8** (500 MHz, $CDCl_3$).



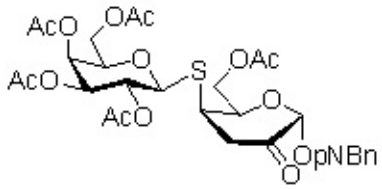
^{13}C-NMR Spectrum of compound **8** (125.7 MHz, $CDCl_3$).



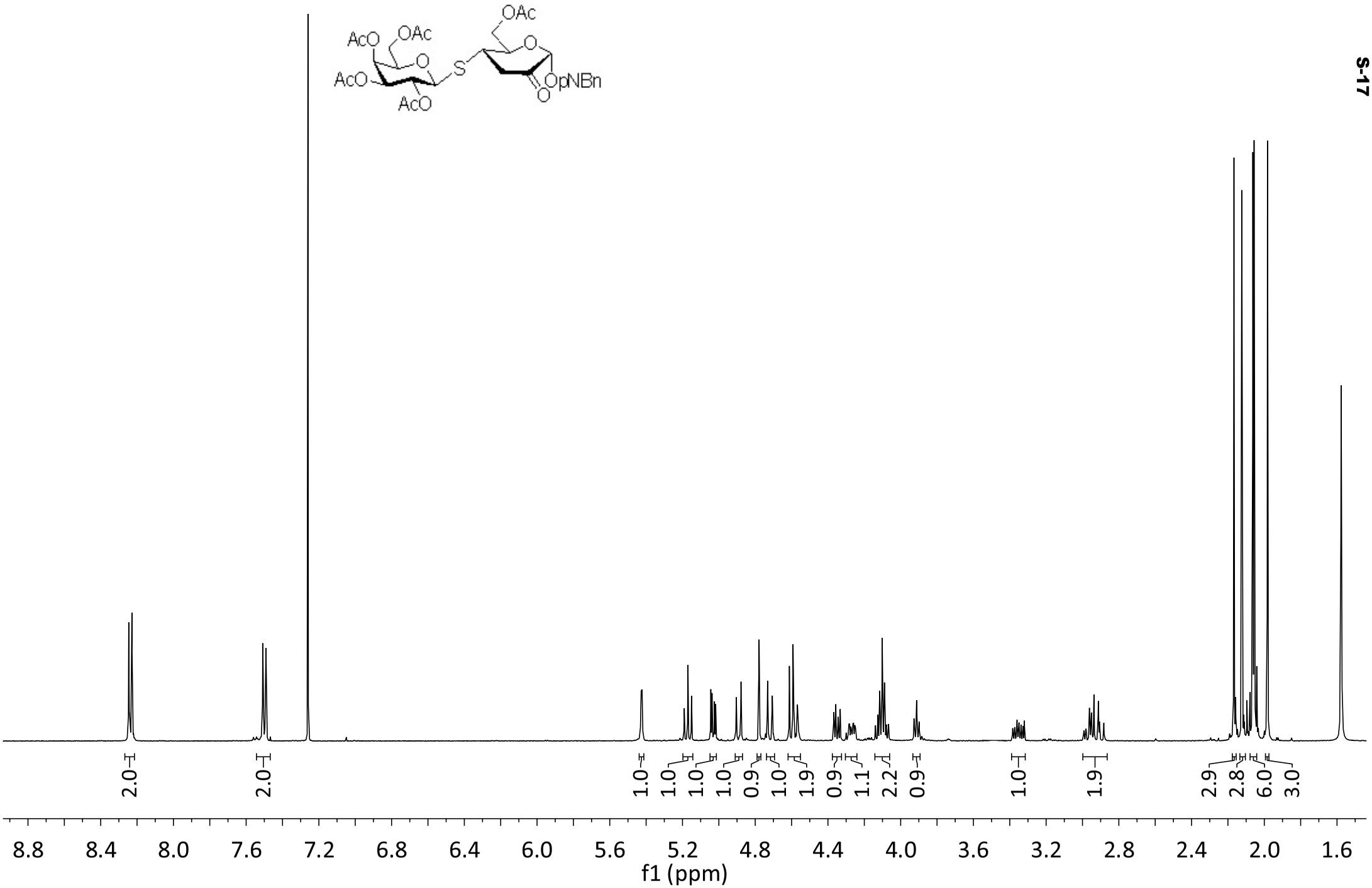
^{1}H-NMR Spectrum of compound **10** (500 MHz, $CDCl_3$).



^{13}C-NMR Spectrum of compound **10** (125.7 MHz, $CDCl_3$).



^{1}H-NMR Spectrum of compound **11** (500 MHz, $CDCl_3$).



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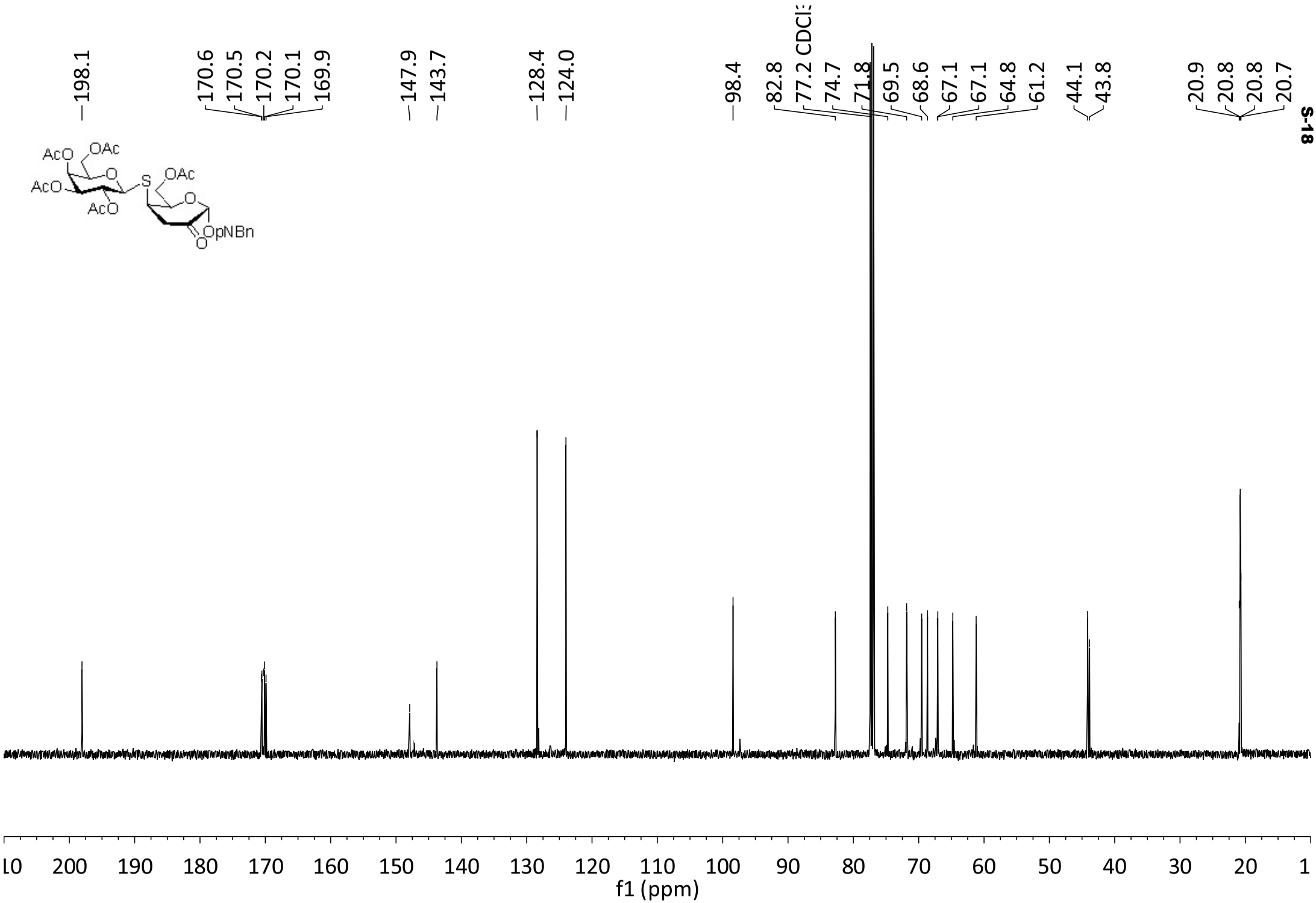
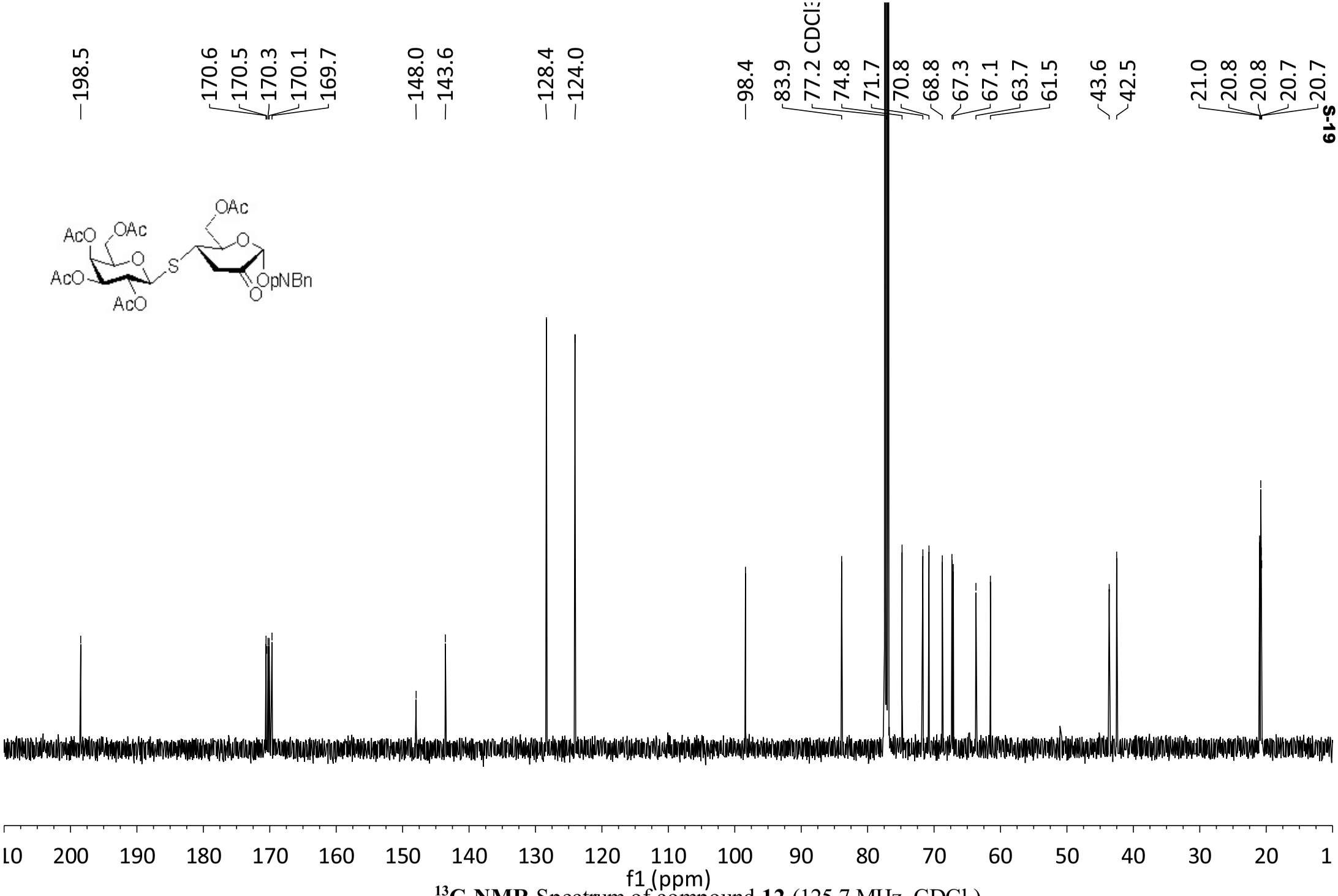
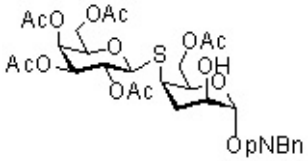


^{13}C-NMR Spectrum of compound **11** (125.7 MHz, $CDCl_3$).



^{13}C-NMR Spectrum of compound **12** (125.7 MHz, $CDCl_3$).



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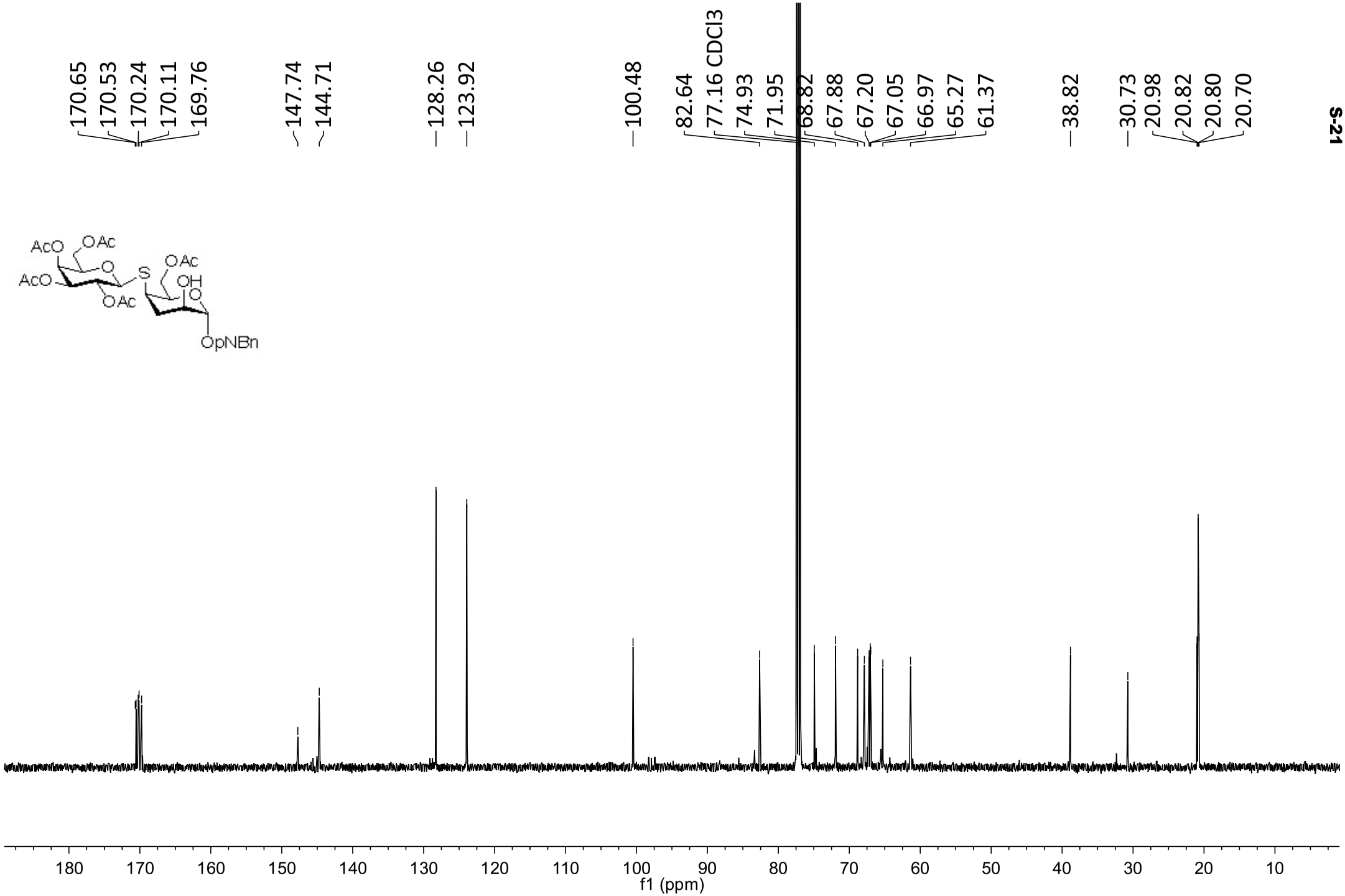
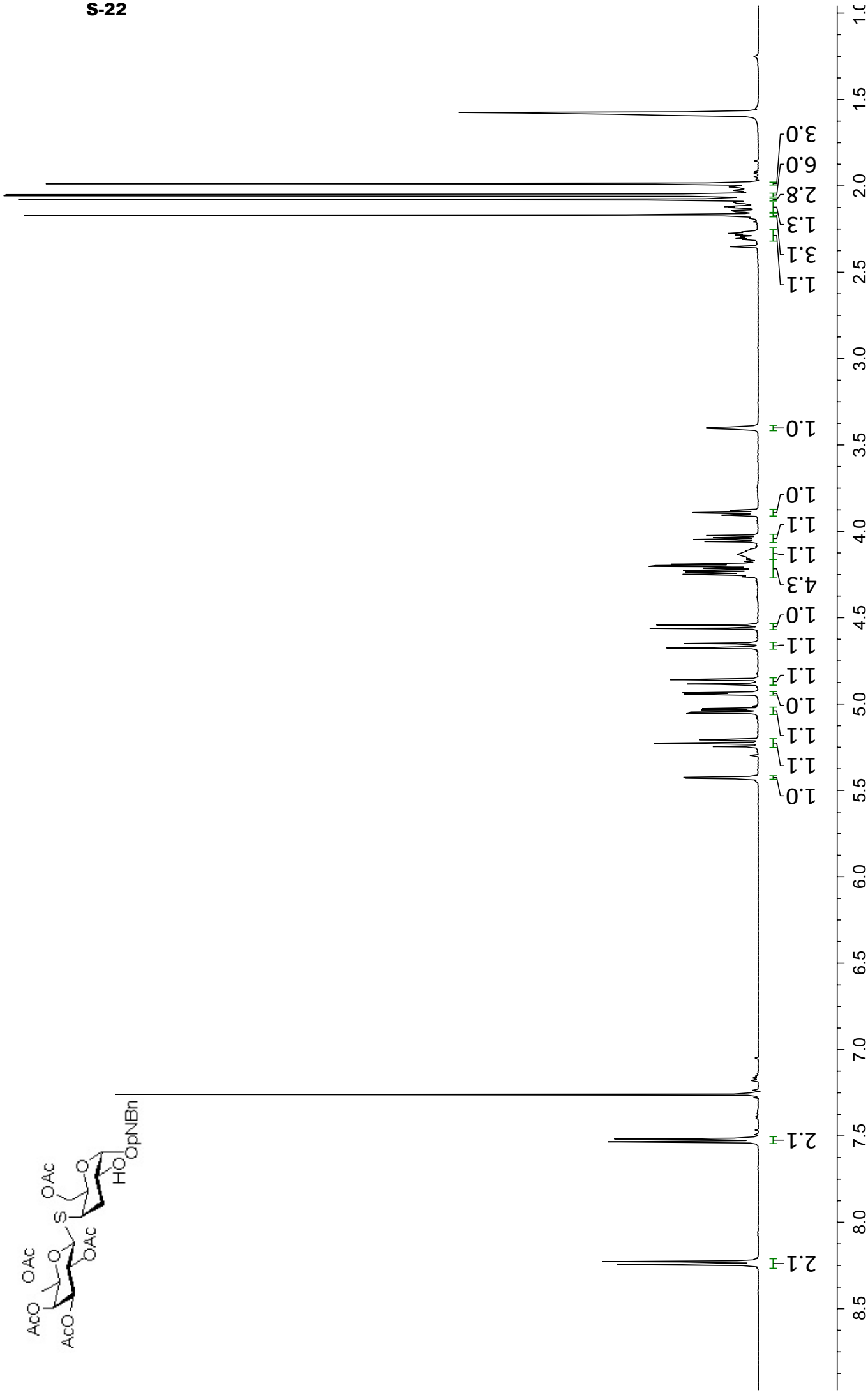
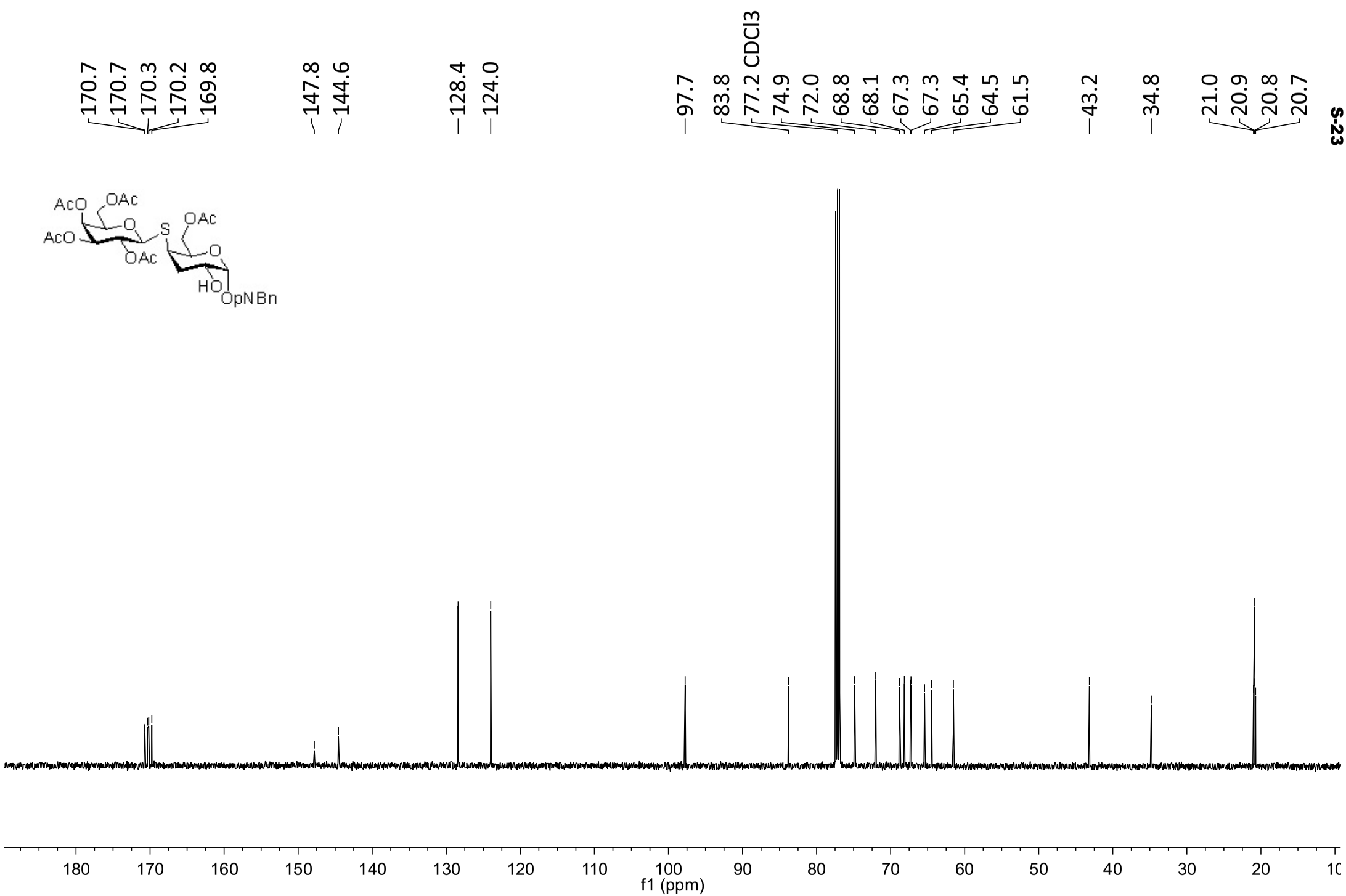


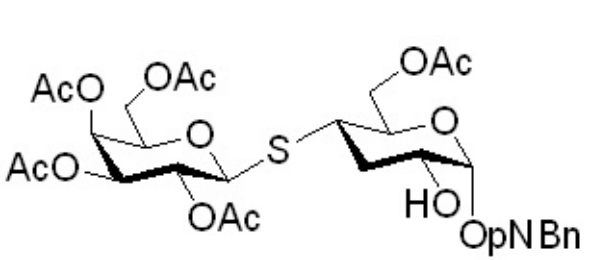
^{13}C-NMR Spectrum of compound **13** (125.7 MHz, $CDCl_3$).



^{1}H-NMR Spectrum of compound **14** (500 MHz, $CDCl_3$).



^{1}H-NMR Spectrum of compound **14** (500 MHz, $CDCl_3$).



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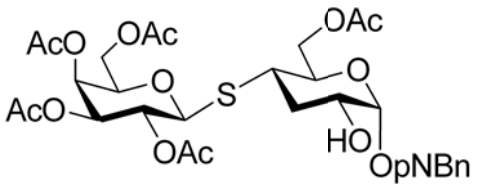
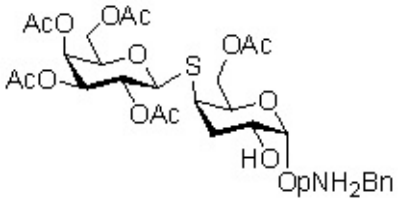


^{13}C-NMR Spectrum of compound **15** (125.7 MHz, $CDCl_3$).



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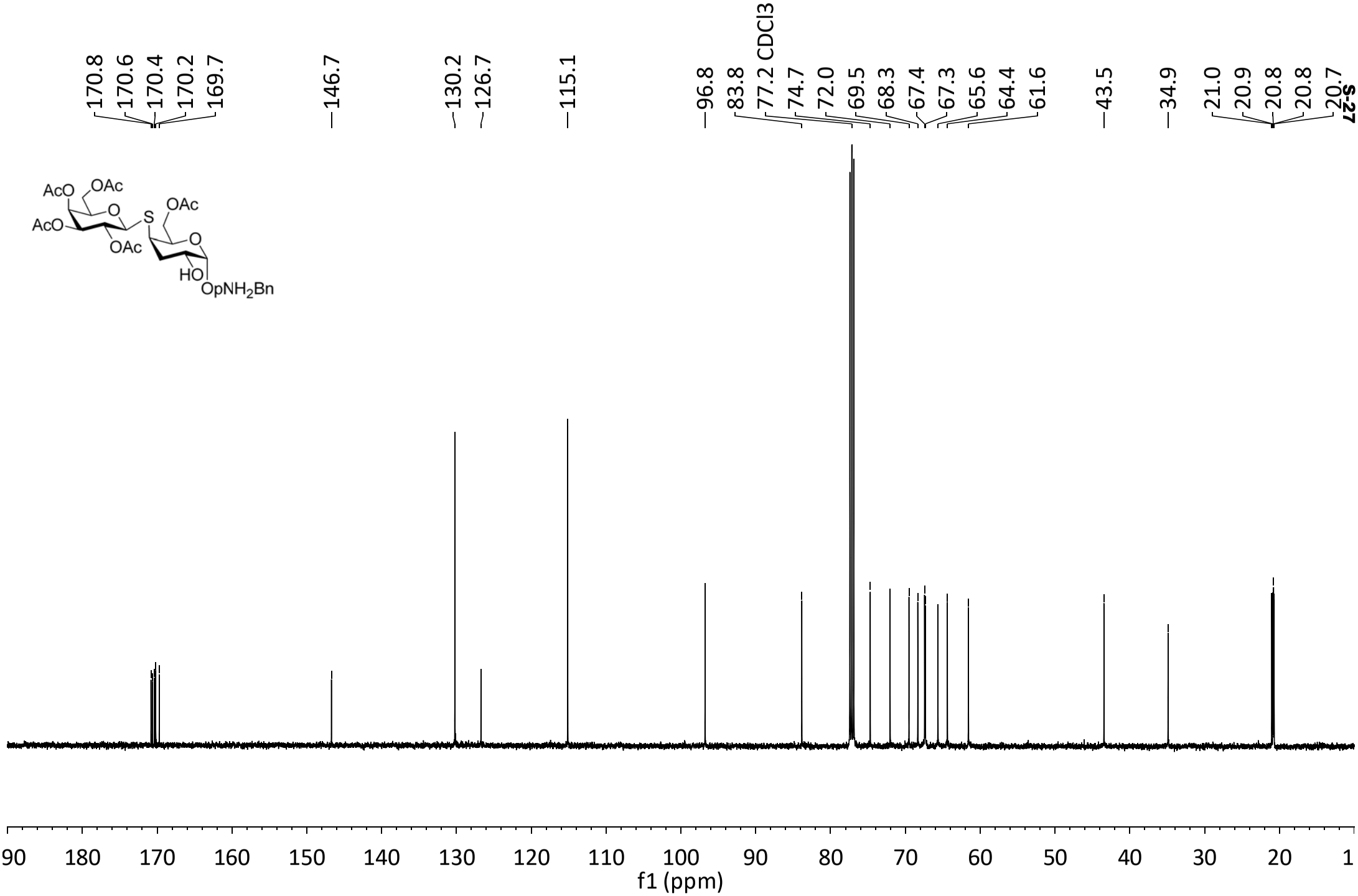
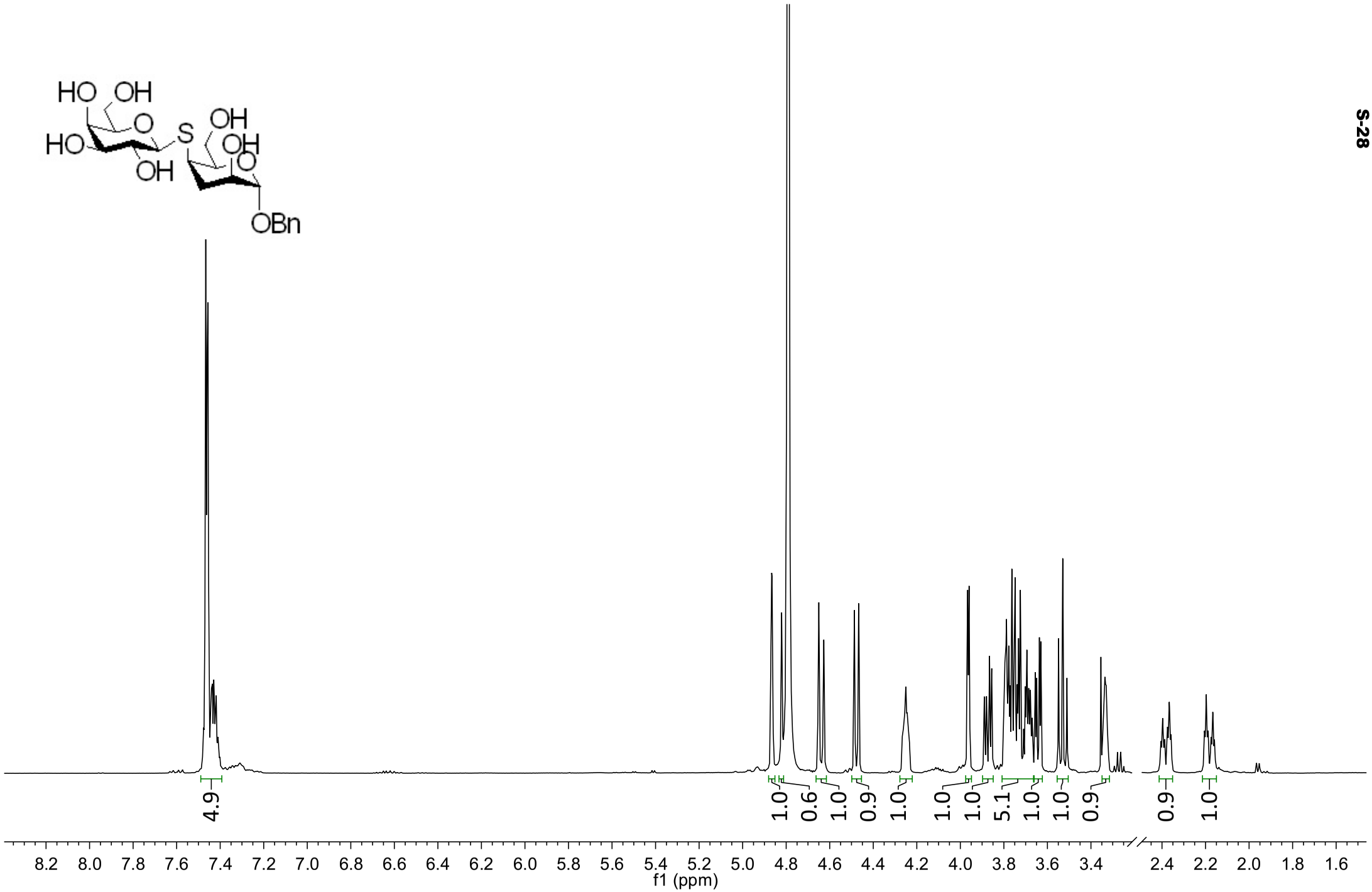
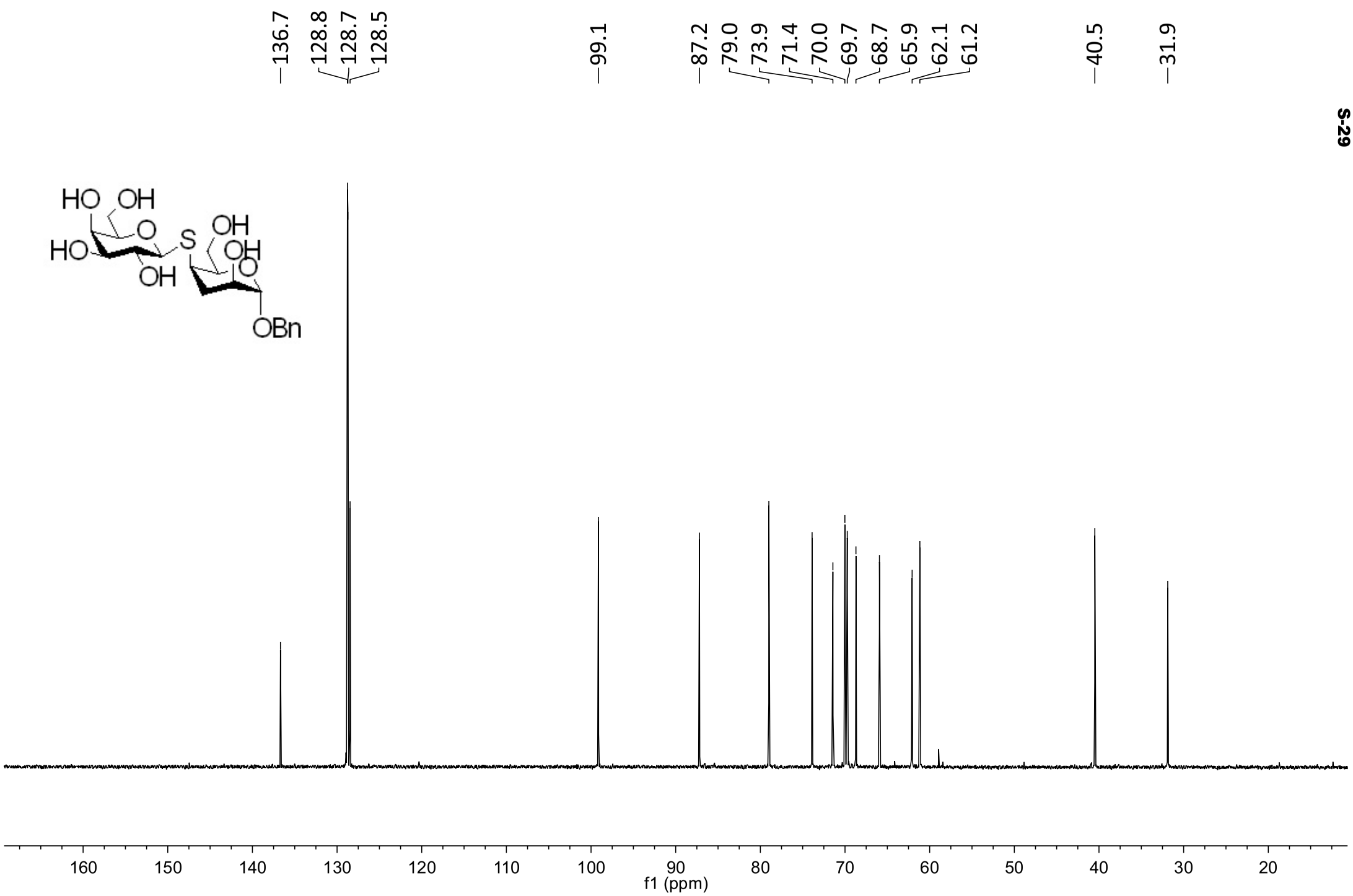


^{13}C-NMR Spectrum of compound **16** (125.7 MHz, $CDCl_3$).



^{1}H-NMR Spectrum of compound **17** (500 MHz, $CDCl_3$).



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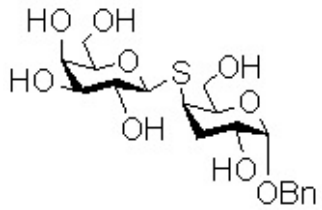

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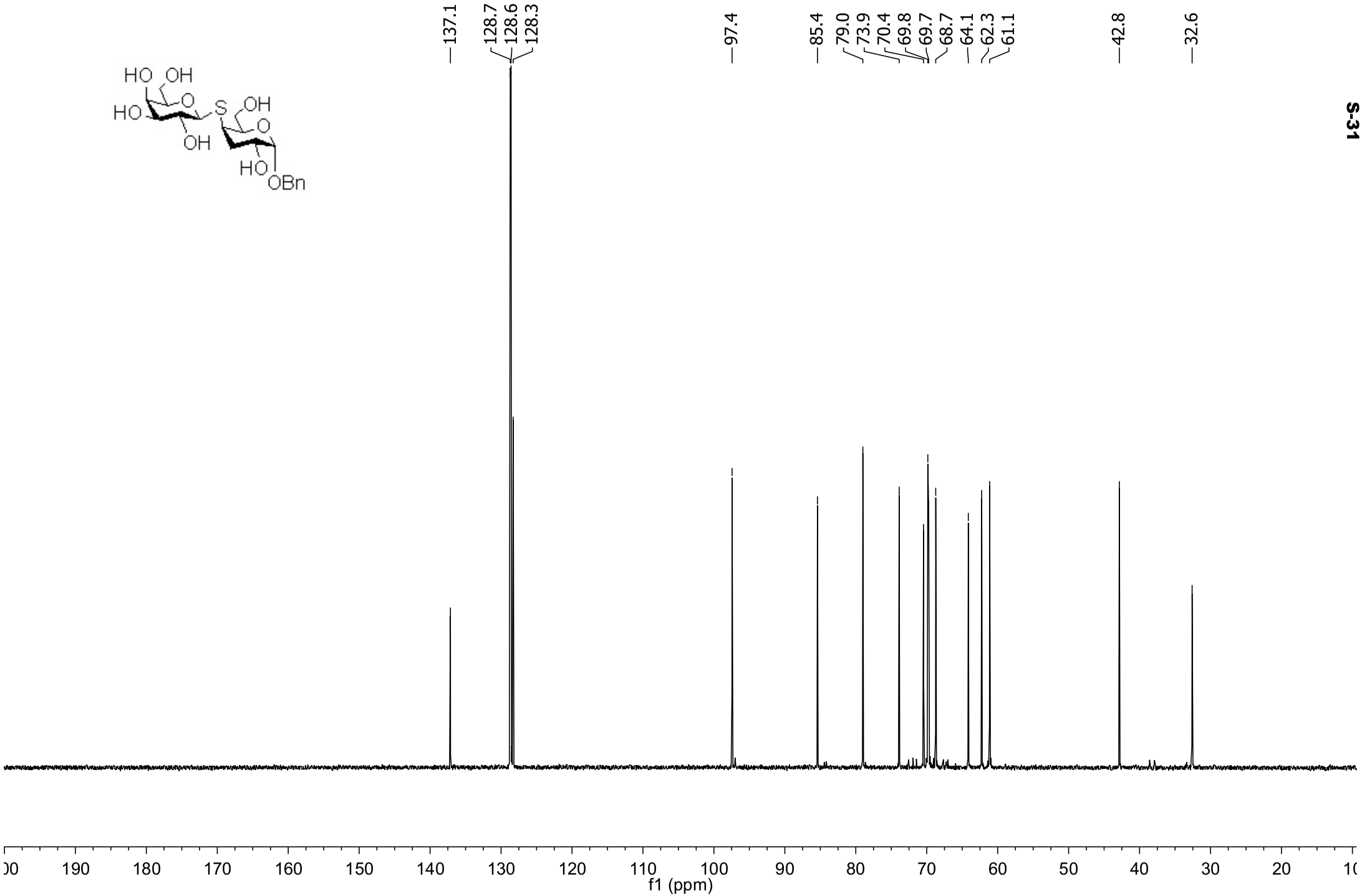
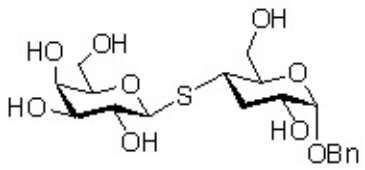
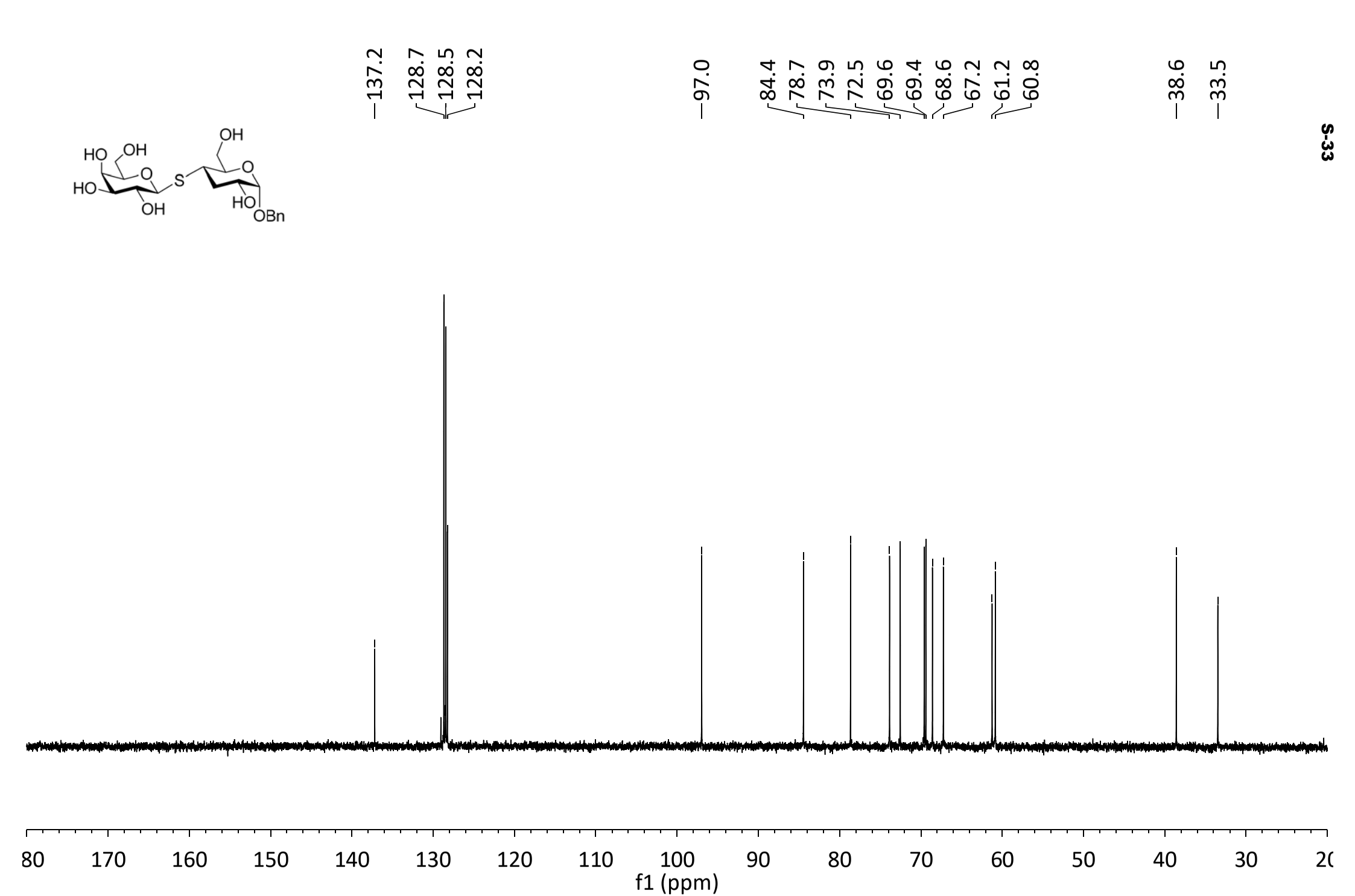


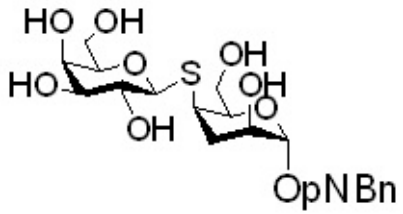
^{13}C-NMR Spectrum of compound **18** (125.7 MHz, $CDCl_3$).



^{1}H-NMR Spectrum of compound **19** (500 MHz, $CDCl_3$).



^{13}C-NMR Spectrum of compound **19** (125.7 MHz, $CDCl_3$).



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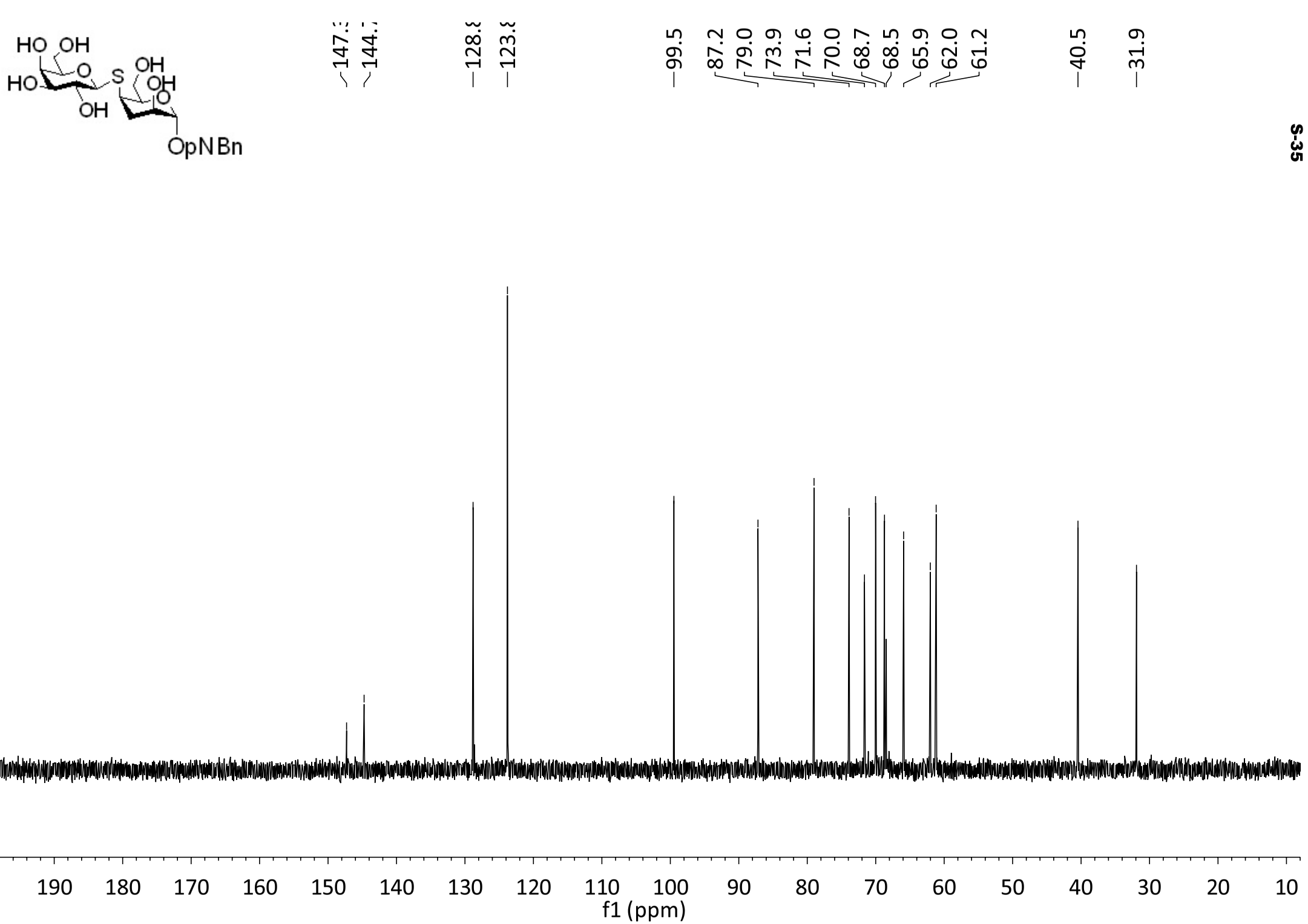
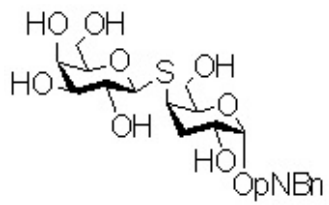
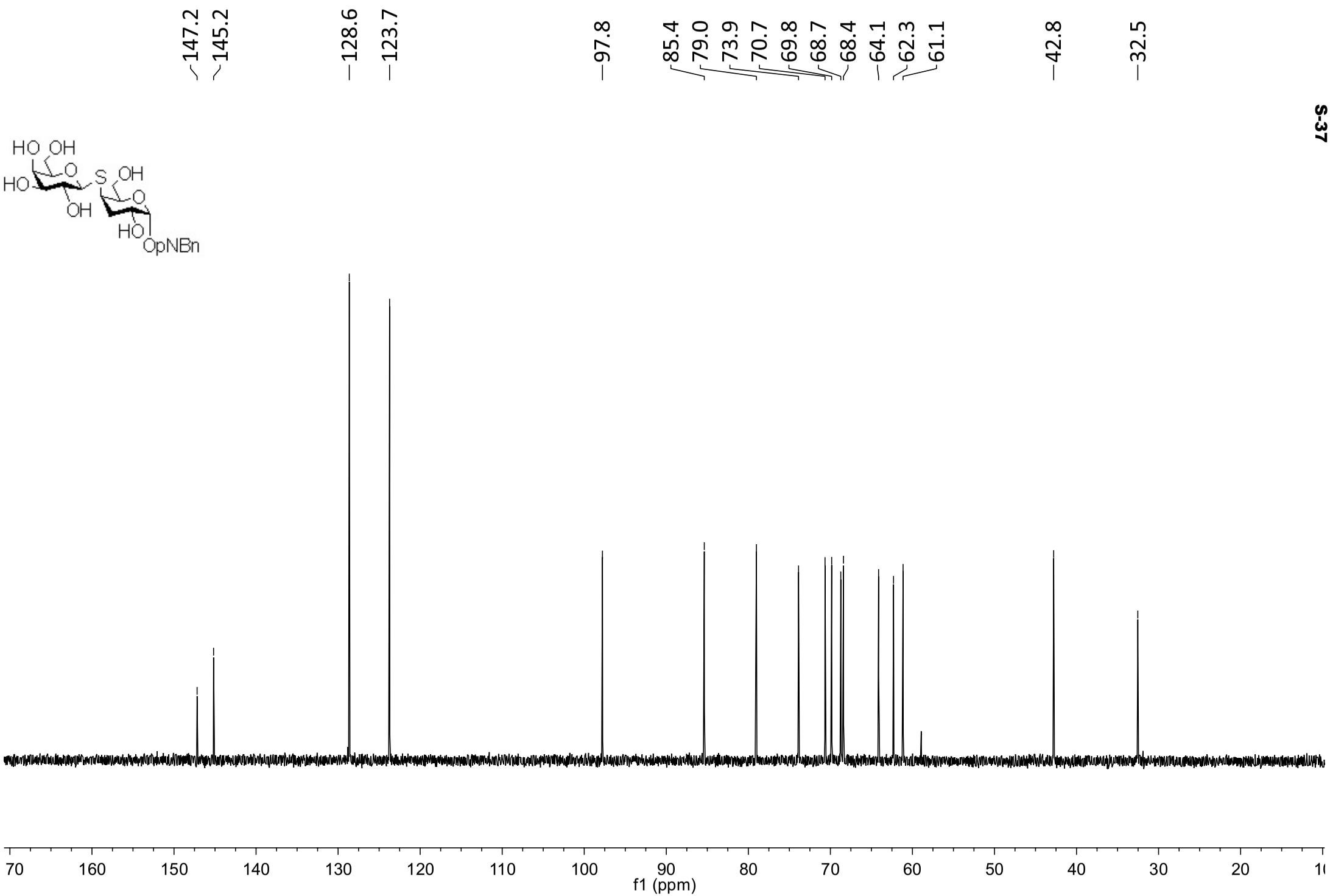


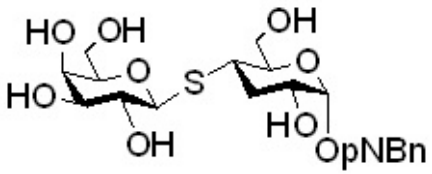
^{13}C-NMR Spectrum of compound **20** (125.7 MHz, $CDCl_3$).



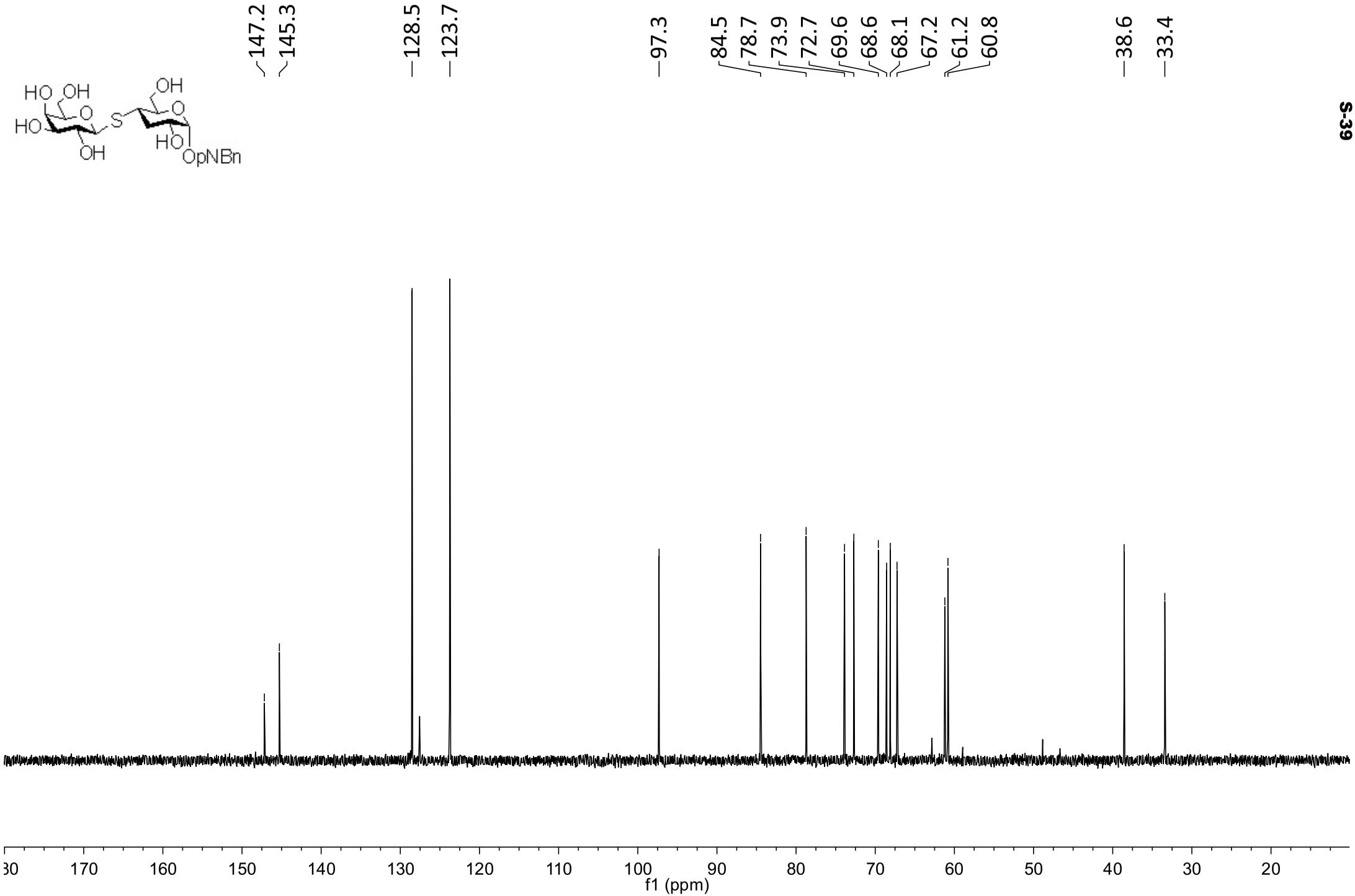
^{1}H-NMR Spectrum of compound **21** (500 MHz, $CDCl_3$).



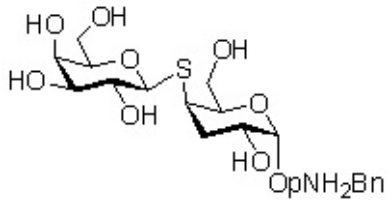
^{13}C-NMR Spectrum of compound **21** (125.7 MHz, $CDCl_3$).



^{1}H-NMR Spectrum of compound **22** (500 MHz, $CDCl_3$).



^{13}C-NMR Spectrum of compound **22** (125.7 MHz, $CDCl_3$).



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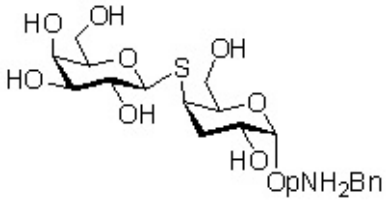
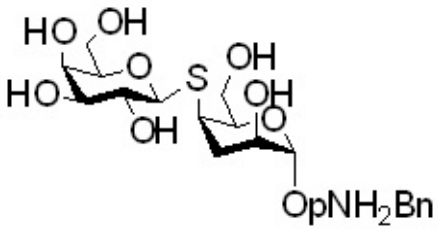
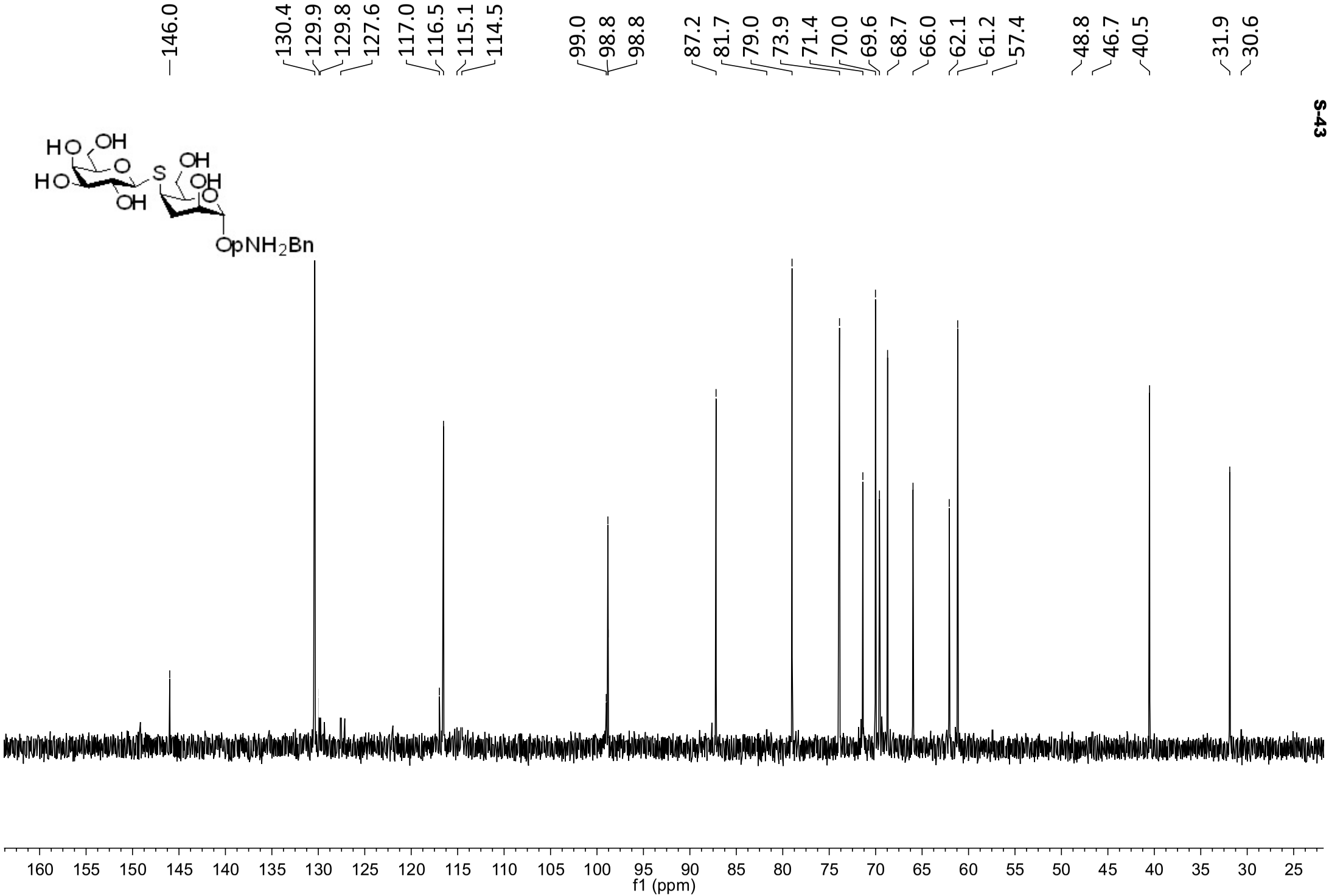


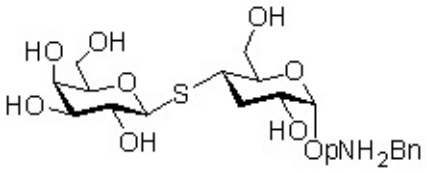
^{13}C-NMR Spectrum of compound **23** (125.7 MHz, $CDCl_3$).



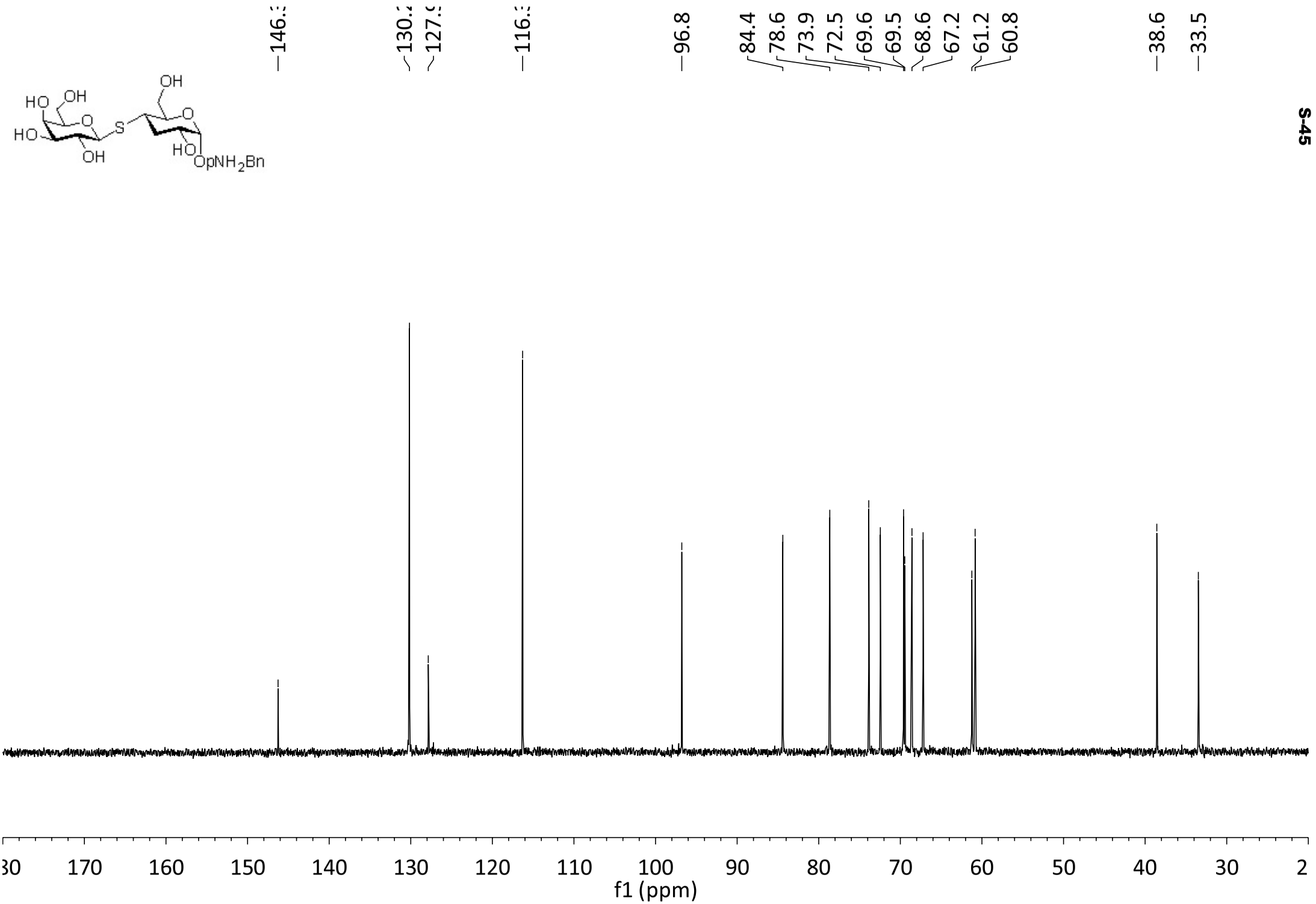
^{1}H-NMR Spectrum of compound **24** (500 MHz, $CDCl_3$).



^{13}C-NMR Spectrum of compound **24** (125.7 MHz, $CDCl_3$).

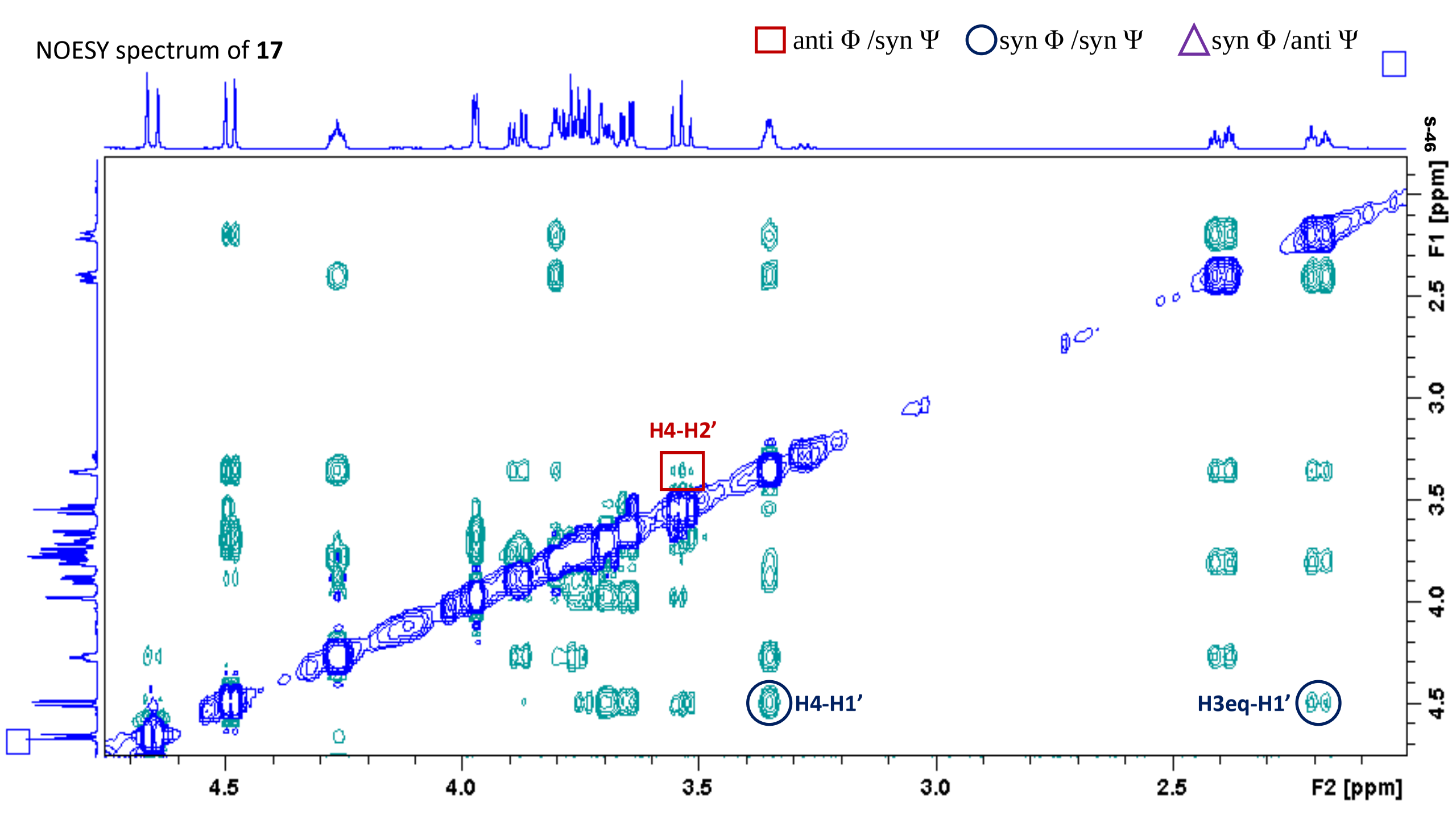


^{1}H-NMR Spectrum of compound **25** (500 MHz, $CDCl_3$).

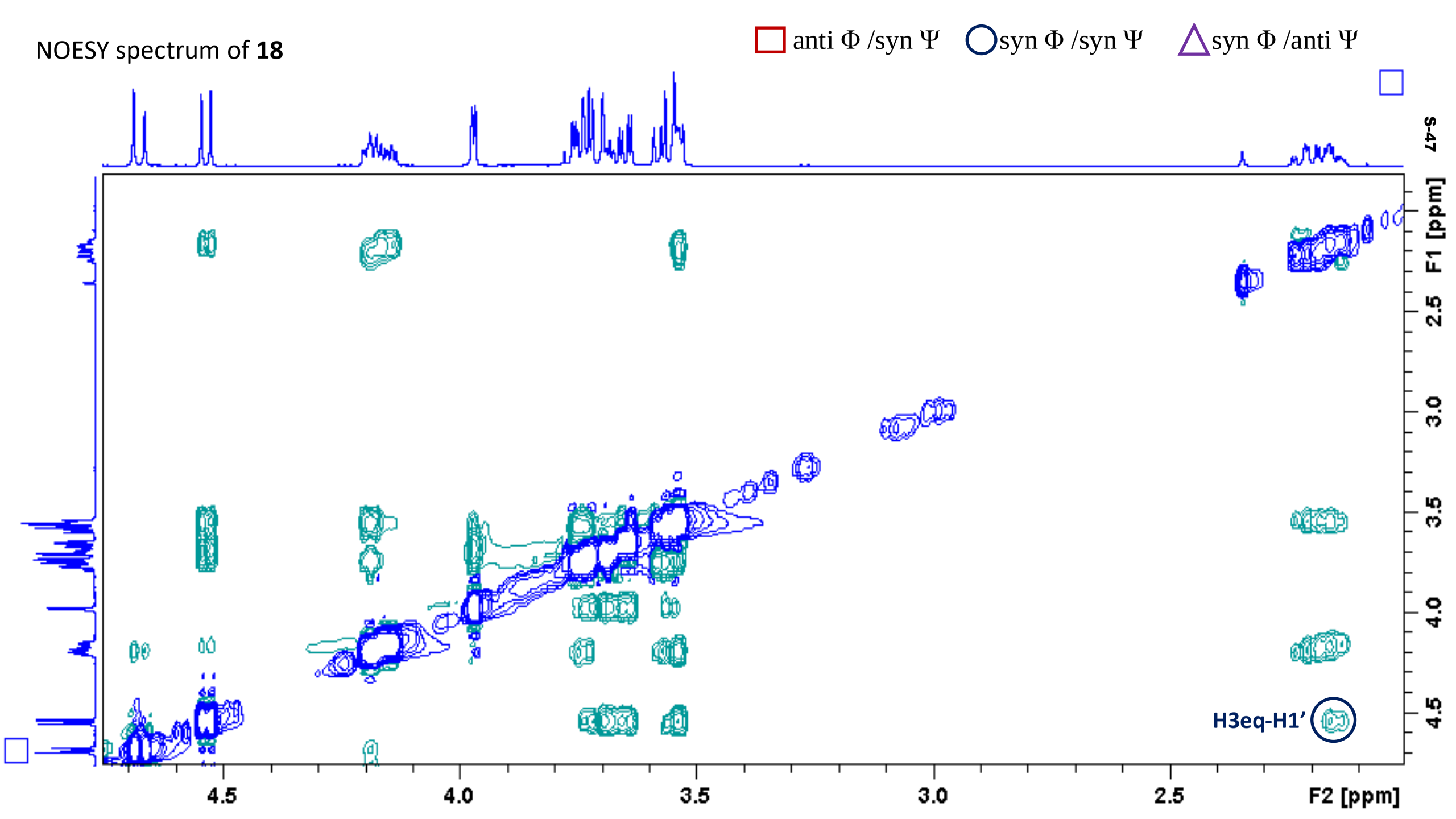


^{13}C-NMR Spectrum of compound **25** (125.7 MHz, $CDCl_3$).

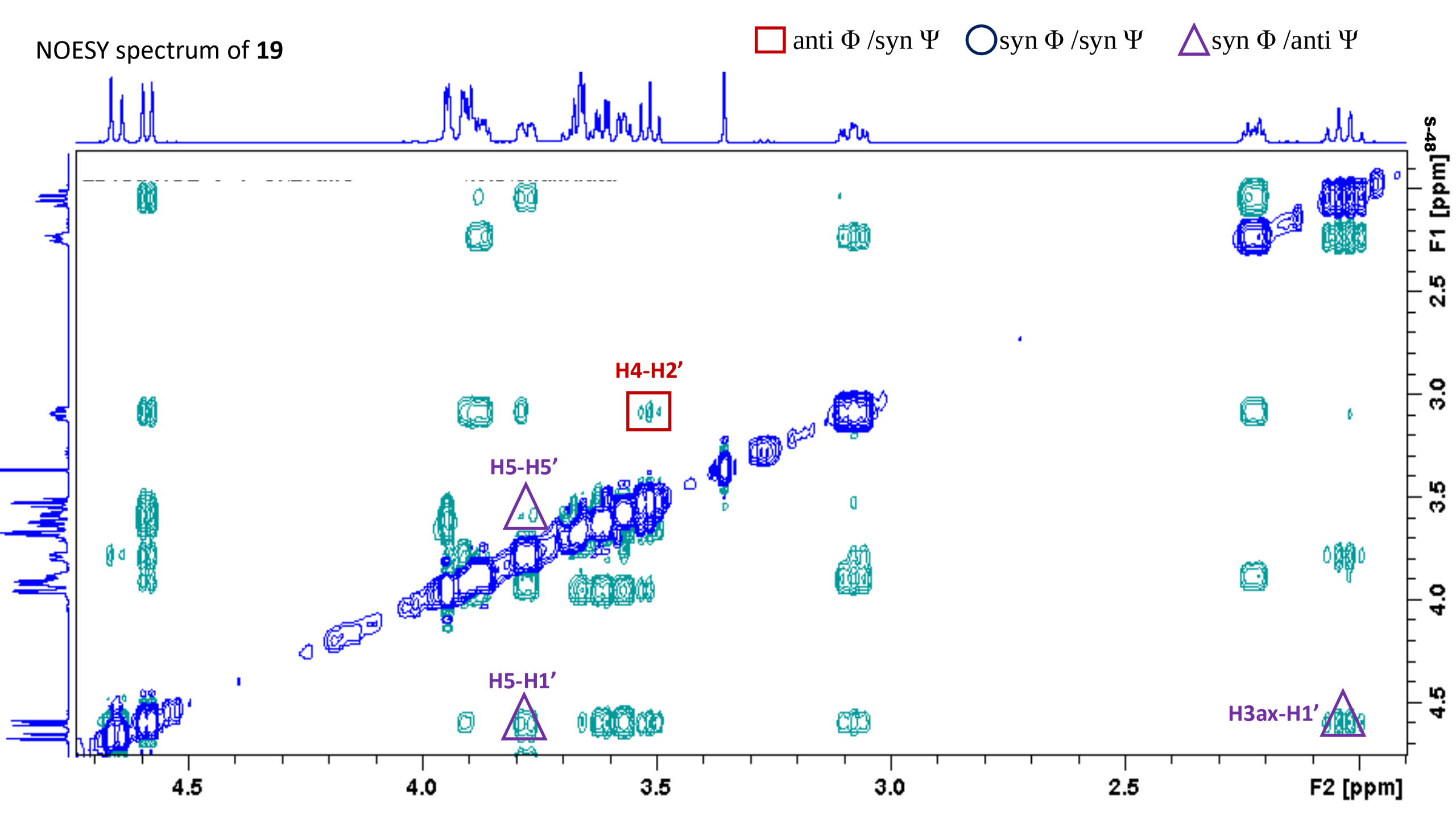
NOESY spectrum of **17**



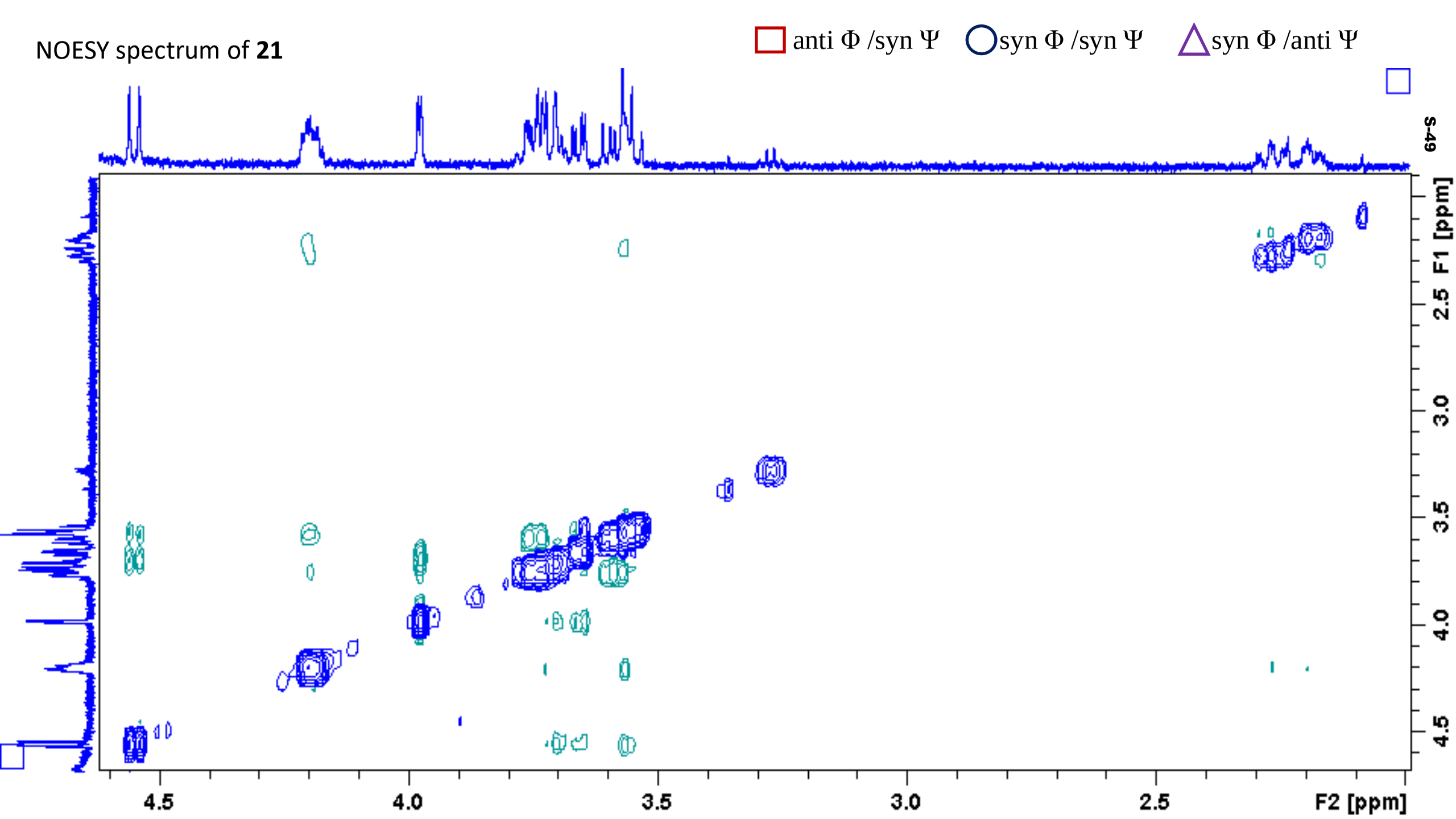
NOESY spectrum of **18**



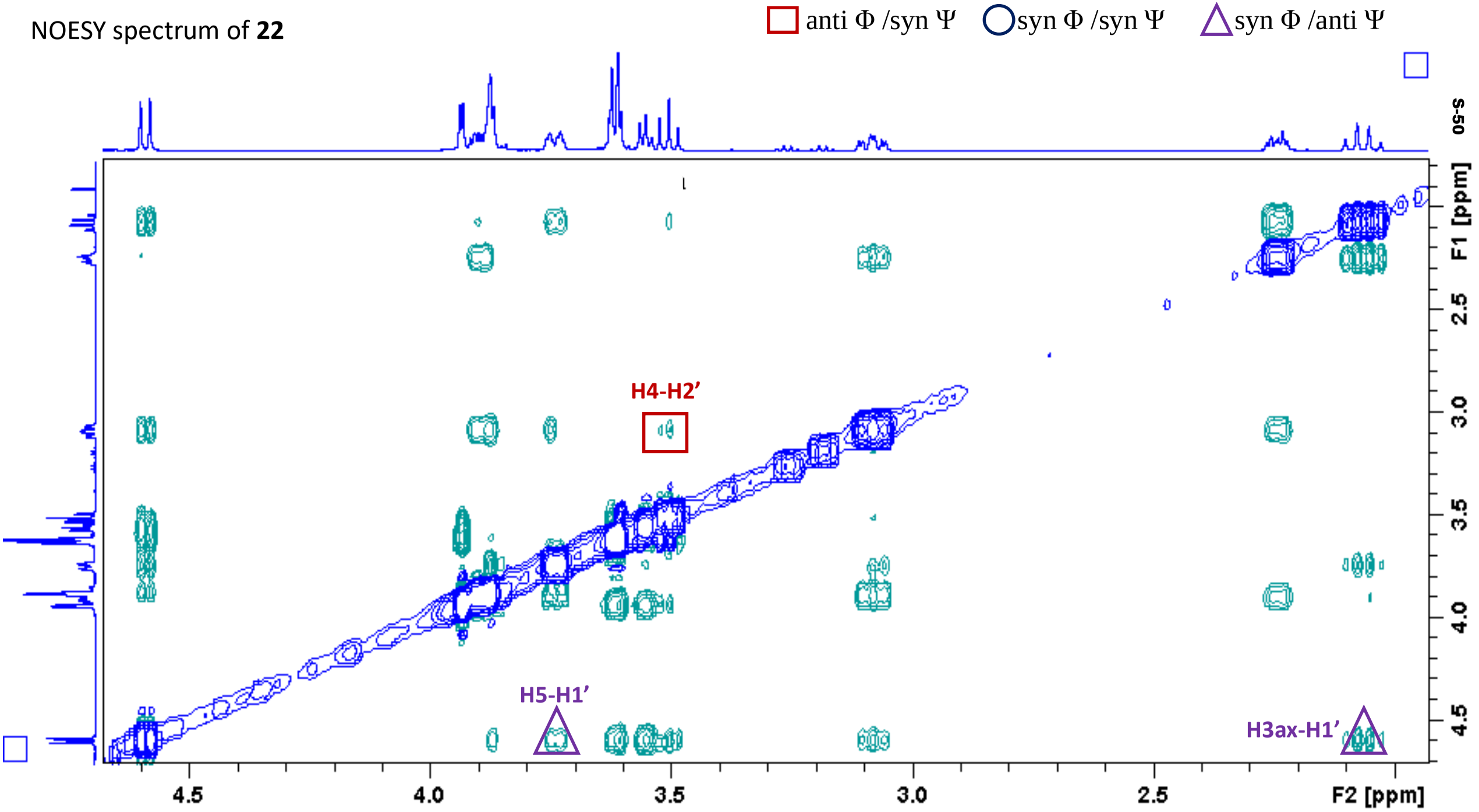
NOESY spectrum of **19**



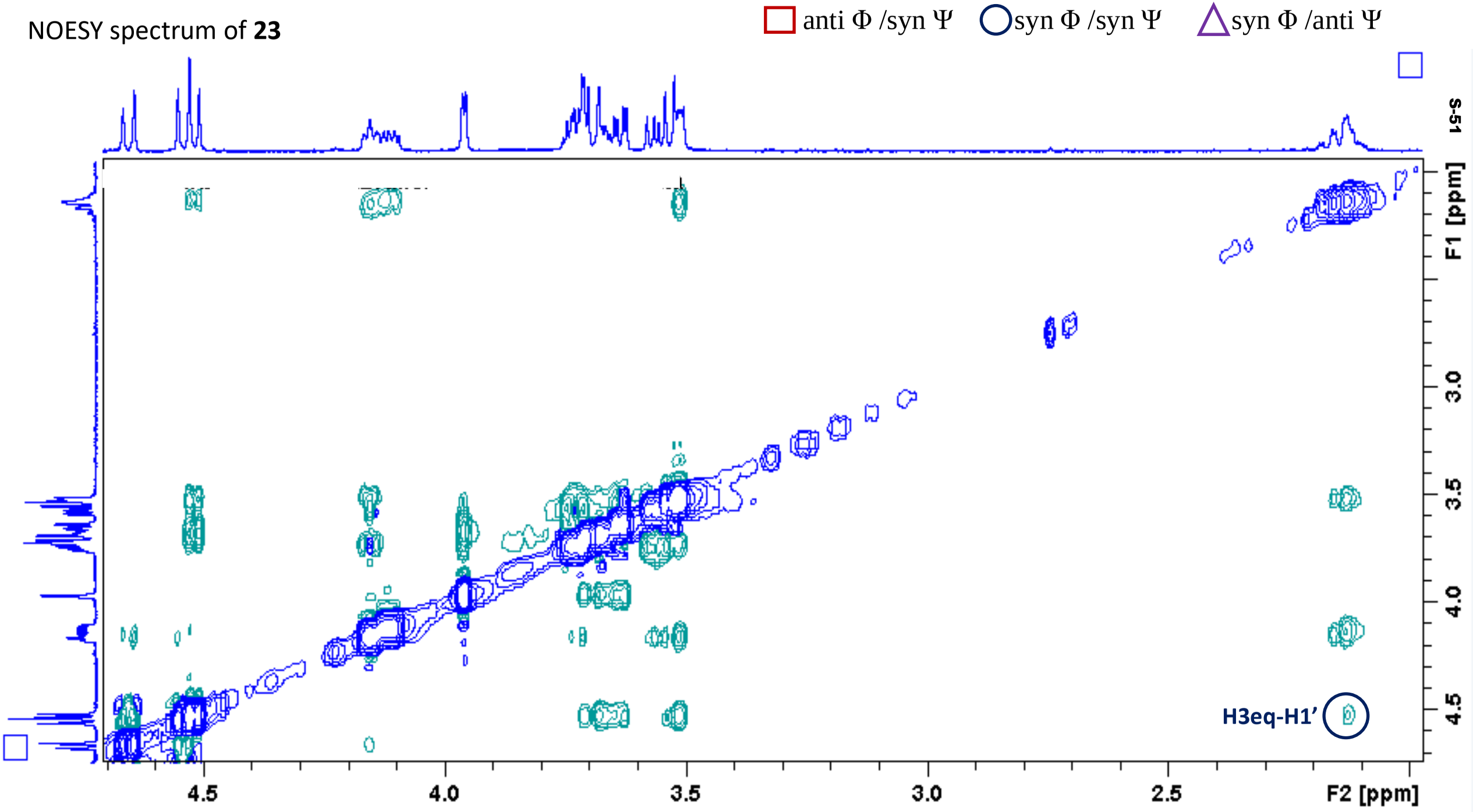
NOESY spectrum of **21**



NOESY spectrum of **22**



NOESY spectrum of **23**



NOESY spectrum of **25**