SYNTHESIS OF A LIBRARY OF XYLOGLUCO-OLIGOSACCHARIDES FOR ACTIVE SITE MAPPING OF XYLOGLUCAN *ENDO*-TRANSGLYCOSYLASE

Régis Fauré,[†] Marc Saura-Valls,[‡] Harry Brumer III,[§] Antoni Planas,[‡] Sylvain Cottaz,[†] and Hugues Driguez[†]

General methods and materials. Roman numerals in ascending order are given to the residues from the reducing end. The same roman numeral is given to a glucosyl residue and its C-6 substituted xylosyl residue. NMR spectra were recorded on 300 or 400 MHz spectrometers. Proton chemical shifts (δ) are reported in ppm downfield from TMS. Coupling constants (*J*) are reported in Hertz (Hz) with singlet (s), doublet (d), doublet of doublet (dd), triplet (t), multiplet (m), broad (b). Carbon chemical shifts (δ) are reported in ppm with internal reference of solvent. Assignments were made on key protected compounds using COSY and relayed-COSY experiments. Structural characterization was based on comparison of different types of data obtained for the different compounds. The general procedure was to identify a characteristic signal and assign all of the protons of the same unit using homonuclear correlation COSY and relayed COSY. When the units exhibited the same characteristics, the assignments could be reversed. When overlaps occurred, the coupling constants were read on the 1D spectra or on the 2D COSY map. ^{13}C NMR data were assigned using model compounds. HMQC and HMBC experiments were executed to achieve the complete assignments of ^{1}H and ^{13}C data. Complete assignment of the oligomers **9**, **10**, **13**, **14** and **17** was performed using a combination of COSY, relayed-COSY, HMQC and HMBC experiments.

Low-resolution (MS) were recorded at CERMAV, and high-resolution mass spectra (HRMS) were recorded at the CRMPO (Rennes University, France). Microanalyses were performed by the "Laboratoire Central d'analyses du CNRS" (Vernaison, France).

Evolution of reactions was monitored by analytical thin-layer chromatography using silica gel 60 F254 precoated plates. All non protected compounds which were purified by column chromatography using silica gel Si 60 (63-200 μm) were subsequently filtrated with syringe driven filter unit 0.45 μm (Hydrophilic PVDF membrane) before lyophilisation.

All reactions in organic medium were carried out under argon using freshly distilled solvents. After work-up, organic phases were dried over anhydrous Na_2SO_4.

Preparation of ABEE-oligosaccharide derivatives. Oligosaccharides were coupled to 4-aminobenzoic acid ethyl ether (ABEE) by reductive amination.[49] A 0.5-1.0 mg amount of the lyophilized oligosaccharide and 165 mg of ABEE were placed in a Teflon-capped tube with 691 μL of coupling reagent (freshly prepared by mixing 35 mg of sodium cyanoborohydride ($NaBH_3CN$), 300 μL of water, 350 μL of methanol and 41 μL of acetic acid). The reaction mixture was heated at 80°C for 30 min. After the addition of 4 mL of water/dichloromethane (1:1 v/v), the excess ABEE was removed by washing (3 x 2 mL) with dichloromethane. The aqueous phase was subsequently loaded onto C18 cartridges. Salts were removed by washing with water (10 mL) and the derivatives were then eluted with a gradient of methanol in water.

LC/MS. HPLC separations were carried out with a system consisting of two pumps, a variable-wavelength detector and an injector with a 20 μL sample loop. A C18 columm (25 cm x 4.6 mm) was used for the chromo-labeled oligosaccharide derivatives. Absorbances were monitored at 304 nm and the on-column flow rate was 500 μL/min. The derivatives were eluted with a linear gradient of acetonitrile/water from 10 to 40% in 20 min (t_r is the retention time). A one-third splitting ratio was delivered at 200 μL/min into the electrospray ionization source. Positive ion ESI mass spectra were collected. The capillary was set to 3.5 kV and the cone voltage was 80 V. The ion source temperature was set to 90°C. The mass range was between m/z 1000 and 2048, and the scan time was set to 0.8 s.

HPCE. Reaction mixture consisting of 0.5 mM donor, 5 mM acceptor, 0.3–6 μM enzyme, in 50 mM phosphate/50 mM citrate buffer pH 5.5 at 30ºC, were analyzed by using a system equipped with a diode array UV-Vis detector (fused silica capillary, 50 μM phosphoric acid–triethylamine electrophoresis buffer, pH 2.5 [inverted EOF, anodic detection] at -30 kV, 30ºC) with UV detection at 270 nm. Reactions were performed in a final volume of 100 μL in

a thermostated bath at the desired temparature. 20 μL Aliquots were withdrawn at different time intervals, mixed with 20 μL of ManANTS 2 mM in water as internal reference, the mixture heated at 100°C for 10 min in a sealed tube, and finally samples were analyzed by HPCE (inverted EOF method).[35]

Table A. Summary of ^{1}H NMR data ($CDCl_3$, 400 MHz) of acetylated **9**.

	H-1	H-2	H-3	H-4	H-5a	H-5b	H-6a	H-6b
Glc^{I}	4.89 (7.8)	5.04	5.21 (9.3)	3.88	3.70 (2.1, 5.4)		4.52 (12.1)	4.10
Glc^{II}	4.56 (8.0)	4.85 (9.5)	5.14	4.98	3.65 (-, 2.9)		3.72	3.59 (10.7)
Xyl^{II}	5.00 (3.6)	4.79 (10.0)	5.42	5.01	3.85	3.71		

6.90-6.76 (m, 4H, $C_6H_4OCH_3$), 3.74 (s, 3H, $C_6H_4OCH_3$), 2.12-1.94 (m, 27H, $COCH_3$).

Table B. Summary of ^{1}H NMR data ($CDCl_3$, 400 MHz) of fluoride **10**.

	H-1	H-2	H-3	H-4	H-5a	H-5b	H-6a	H-6b
Glc^{I}	5.64 (2.7, 53.1)	4.81	5.45 (9.8)	3.88 (9.7)	4.08 (1.6,-)		4.54 (10.5)	4.12
Glc^{II}	4.55 (7.8)	4.85 (9.3)	5.14 (9.4)	4.97 (9.5)	3.64 (5.1, 3.4)		3.72 (10.6)	3.59
Xyl^{II}	5.01 (3.6)	4.78 (10.2)	5.41 (9.8)	4.96 (6.0,-)	3.80 (10.8)	3.64		

2.14-1.97 (m, 27H, $COCH_3$).

Table C. Summary of ^{1}H NMR data ($CDCl_3$, 400 MHz) of pentasaccharide **13**.

	H-1	H-2	H-3	H-4	H-5a	H-5b	H-6a	H-6b
Glc^{I}	4.89 (7.8)	5.04	5.21 (8.8)	3.84	3.66 (-, 5.6)		4.44	4.08 (12.0)
Glc^{II}	4.49 (8.0)	4.70 (9.8)	5.07	4.01 (9.6)	3.36		3.91	3.70
Glc^{III}	4.74 (8.1)	4.84	5.16 (8.9, 9.5)	3.82	3.88 (-, 2.9)		4.44	4.29 (12.5)
Gal	4.37 (7.9)	4.99	4.87	5.31 (1.1, 3.6)	3.82		4.06	
Xyl^{II}	5.08 (3.4)	4.83	5.35 (10.0)	4.97 (6.1, -)	3.94 (11.3)	3.70		

6.90-6.75 (m, 4H, $C_6H_4OCH_3$), 3.74 (s, 3H, $C_6H_4OCH_3$), 2.11-1.93 (m, 45H, $COCH_3$).

Table C. Summary of ^{1}H NMR data ($CDCl_3$, 400 MHz) of pentasaccharide **14**.

	H-1	H-2	H-3	H-4	H-5a	H-5b	H-6a	H-6b
Glc^{I}	5.61 (2.8; 53.1)	4.82	5.43 (9.5)	3.83	4.05 (-, 4.2)		4.48	4.13 (12.2)
Glc^{II}	4.49 (8.0)	4.71 (9.8)	5.07	4.02 (9.6)	3.39		3.93	3.69
Glc^{III}	4.75 (8.1)	4.84	5.16 (9.2)	3.82	3.89 (-, 2.8)		4.45	4.30 (12.6)
Gal	4.38 (7.8)	4.98 (10.4)	4.87	5.32 (1.1; 3.4)	3.82		4.06	
Xyl^{II}	5.07 (3.5)	4.85	5.34 (9.9)	4.97	3.94	3.71		

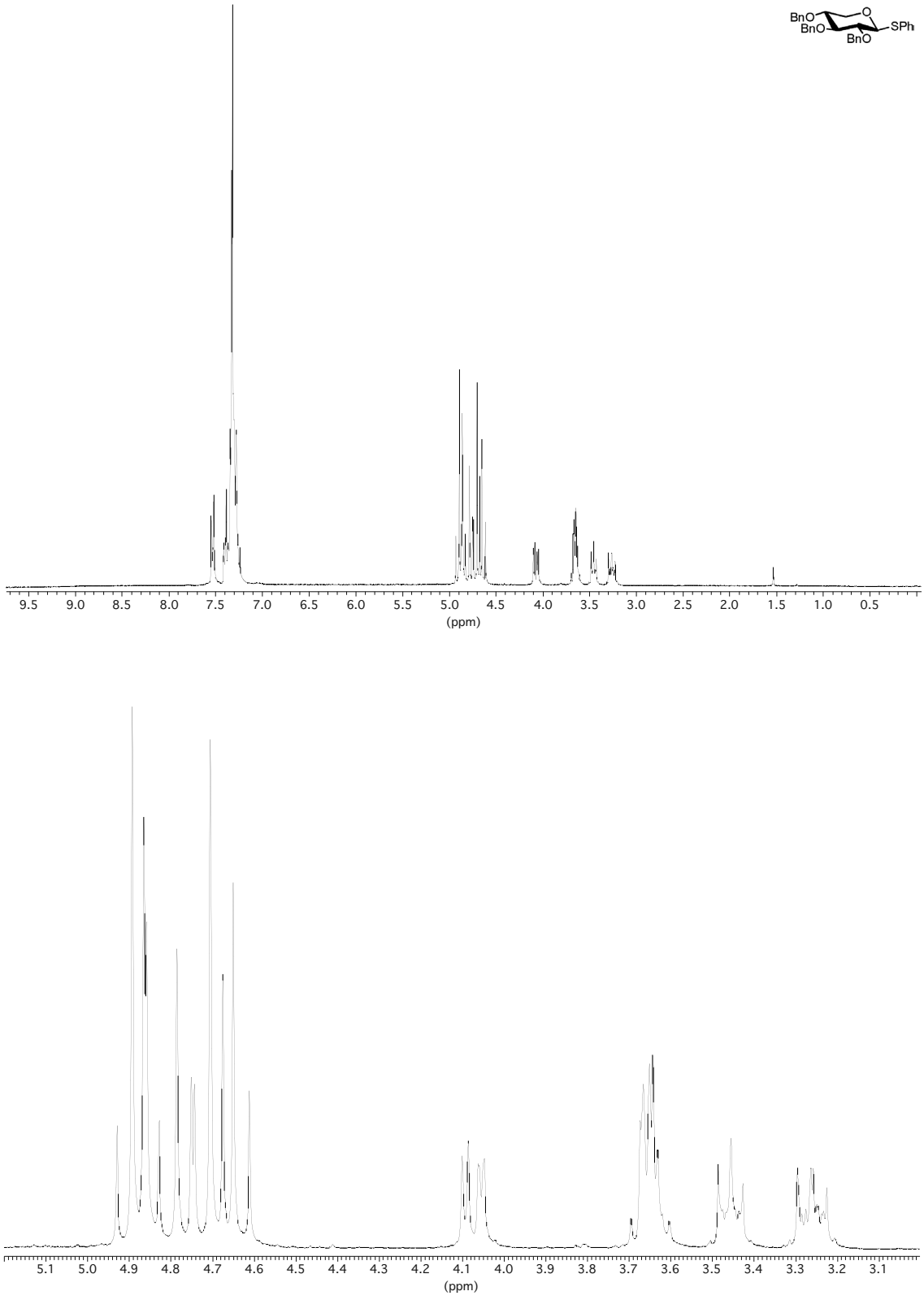
2.12-1.93 (m, 45H, COCH_3).

Table E. Summary of ^{1}H NMR data ($CDCl_3$, 400 MHz) of fluoride **17**.

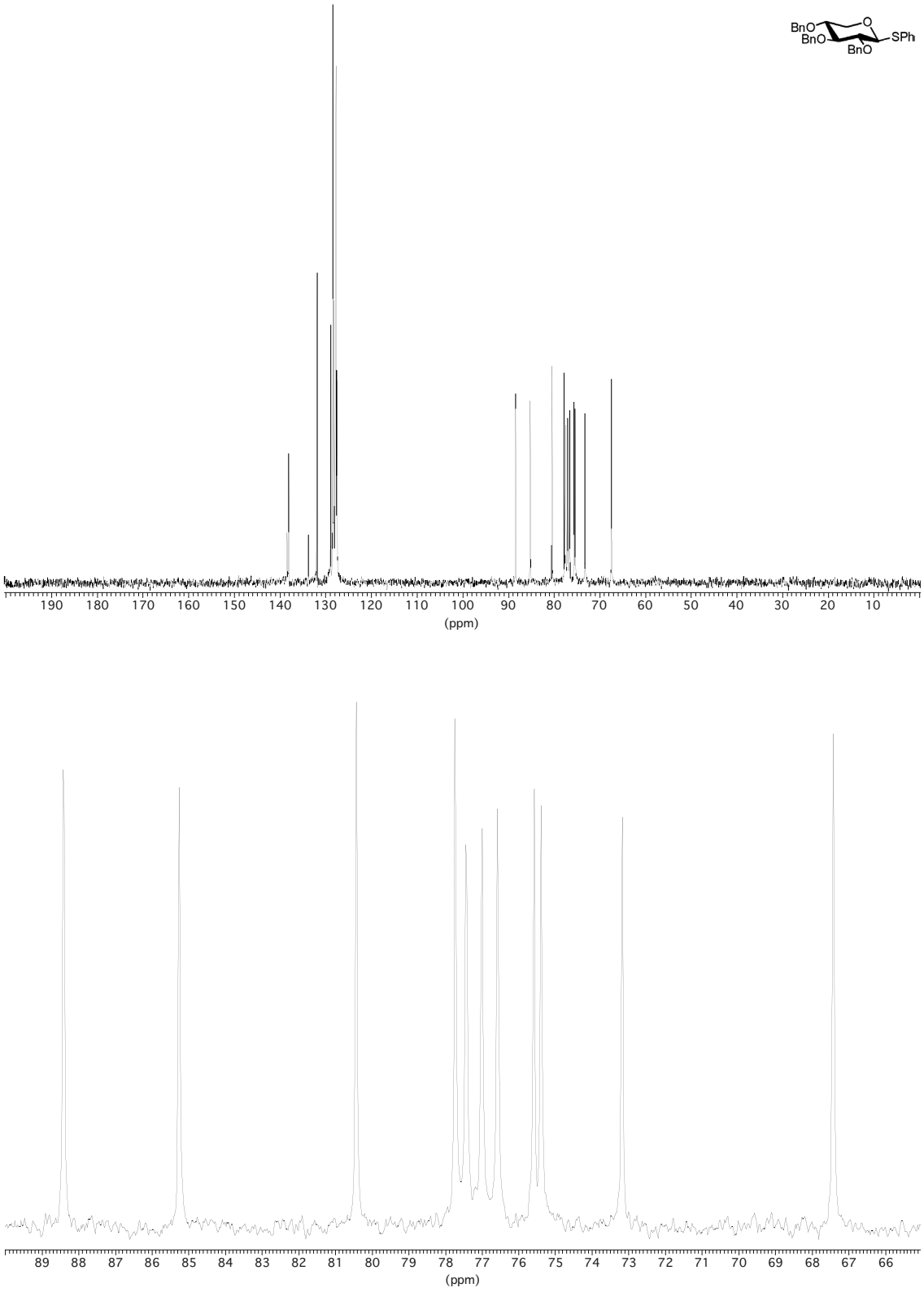
	H-1	H-2	H-3	H-4	H-5a	H-5b	H-6a	H-6b
Glc^{I}	5.60 (2.6; 53.1)	4.81	5.43 (9.5)	3.83	4.04 (-, 4.2)		4.47	4.12 (12.1)
Glc^{II}	4.51 (8.0)	4.66	5.06	3.98	3.35		3.98	3.66
Glc^{III}	4.70 (7.2)	4.68	5.11	4.06	3.74		4.08	3.77
Glc^{IV}	4.82 (7.7)	4.73	5.17 (9.3)	3.82	3.92 (-, 2.6)		4.44	4.30 (12.8)
Gal	4.37 (7.9)	4.97	4.86	5.32	3.80		4.07	
Xyl^{II*}	5.03 (3.3)	4.86	5.33	4.97	3.94	3.75		
Xyl^{III*}	5.06 (3.8)	4.84	5.33	4.93	3.93	3.68		

* Assignments may have to be reversed.

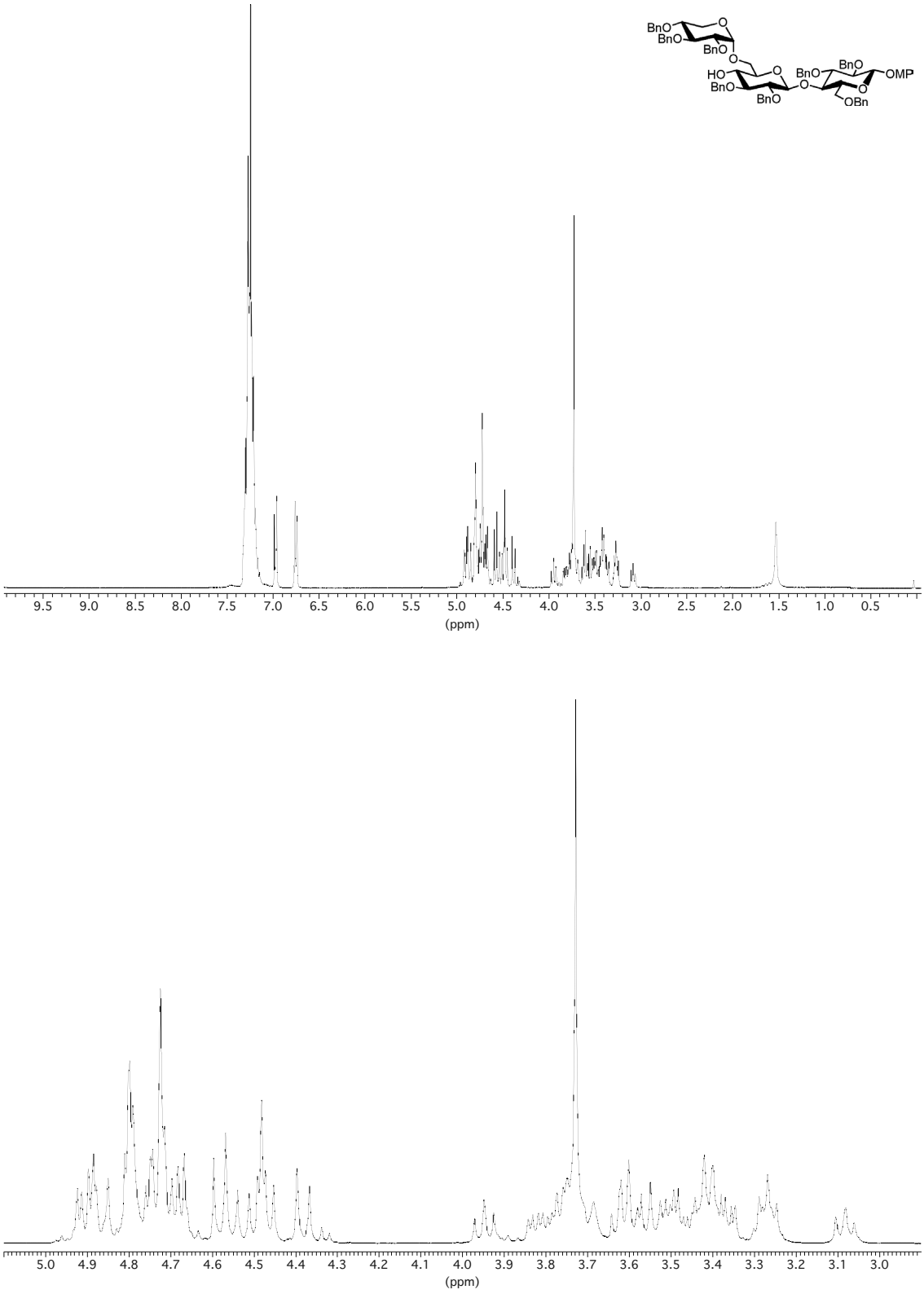
2.12-1.93 (m, 60H, COCH_3).



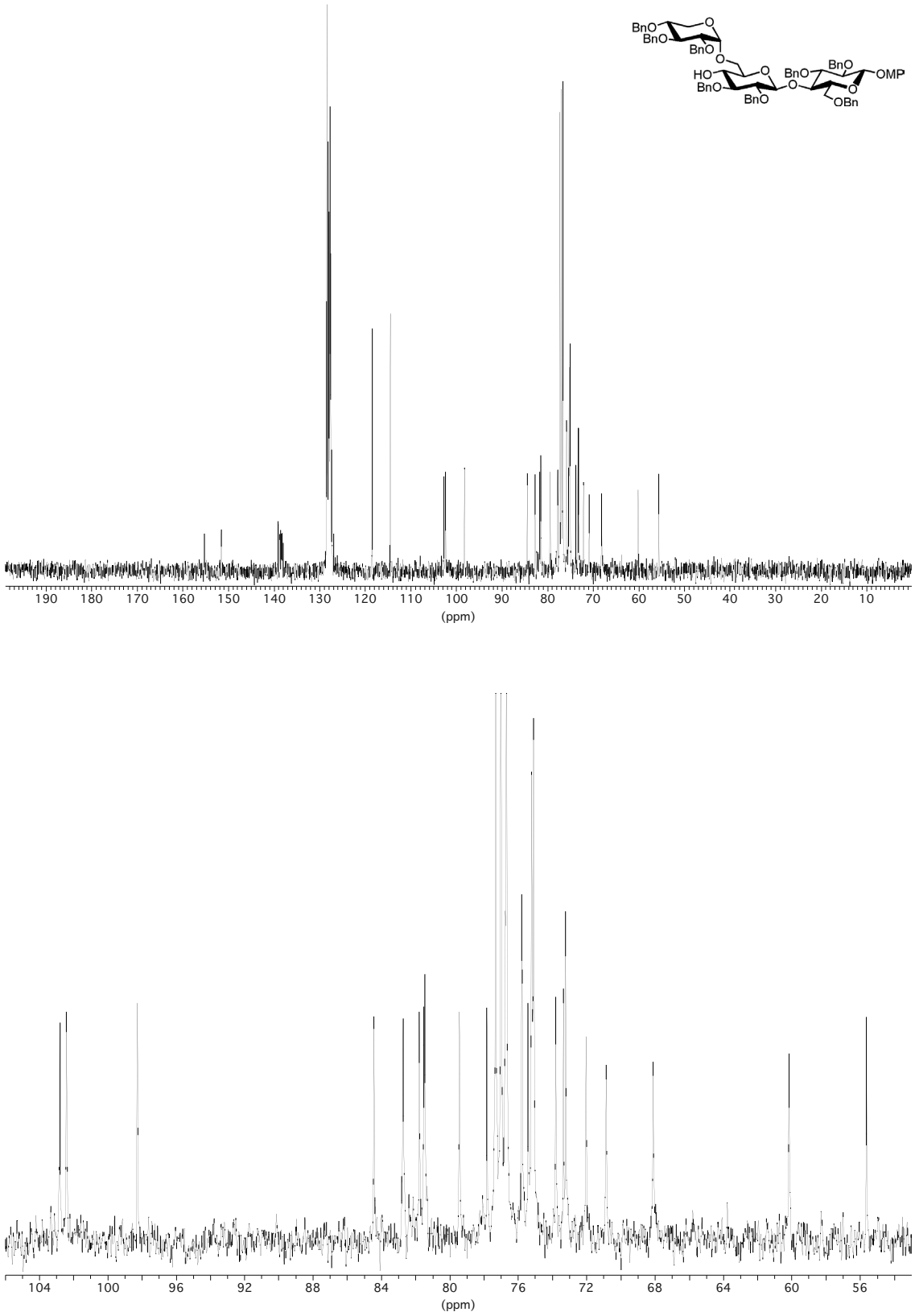
^{1}H NMR spectrum of compound **7** ($CDCl_3$, 300 MHz)



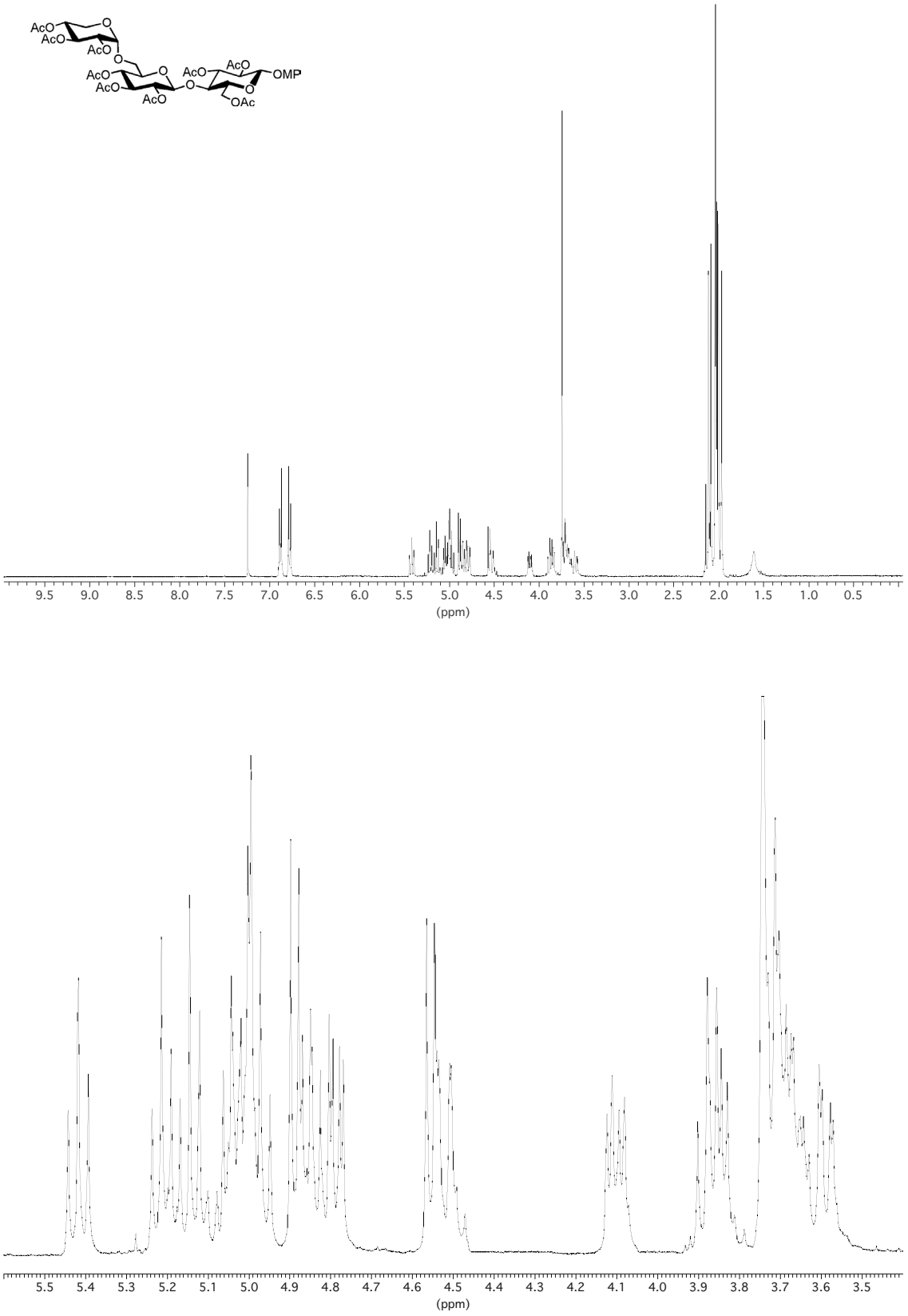
^{13}C NMR spectrum of compound **7** ($CDCl_3$, 75 MHz)



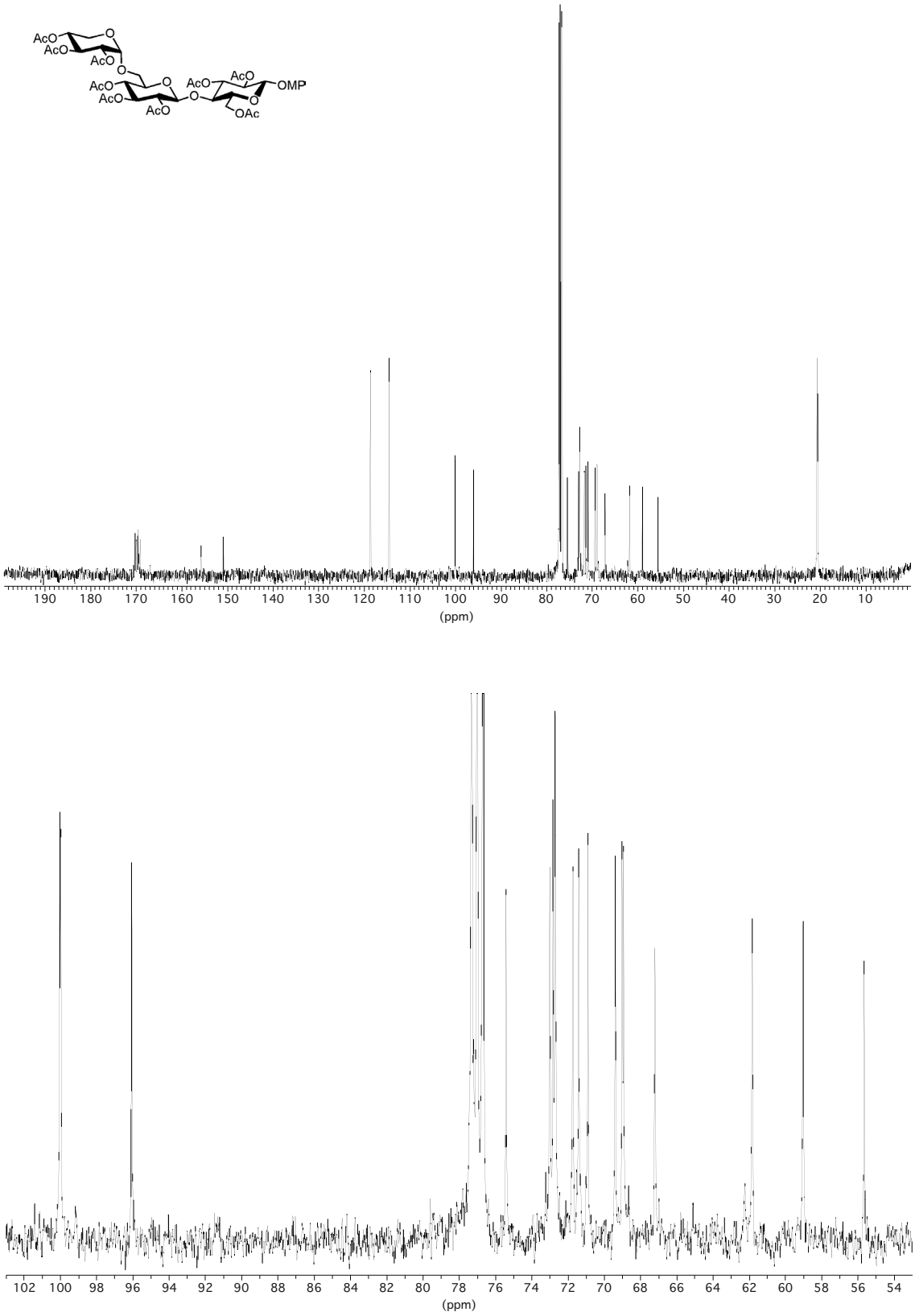
^{1}H NMR spectrum of compound **8** ($CDCl_3$, 400 MHz)



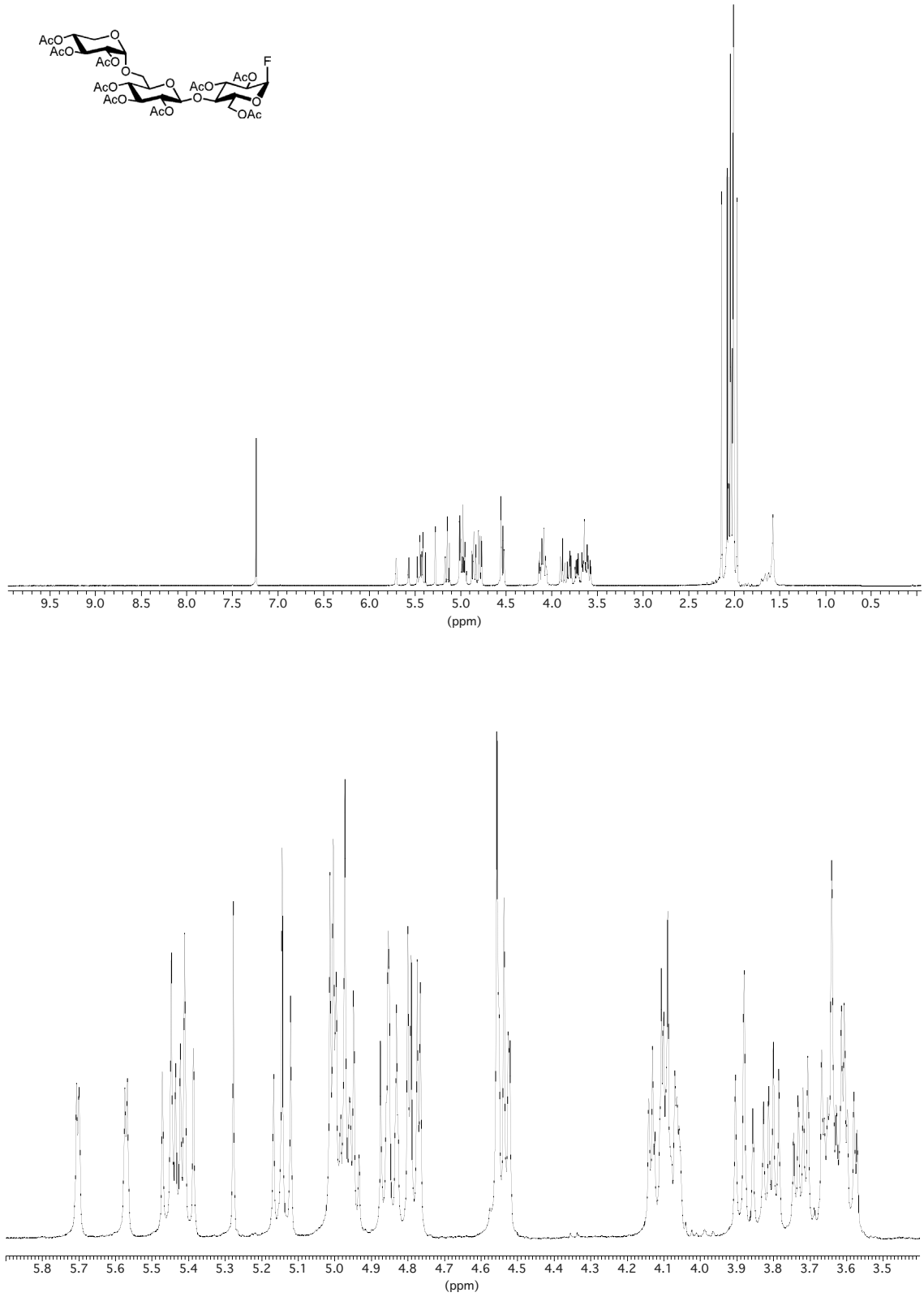
^{13}C NMR spectrum of compound **8** ($CDCl_3$, 100 MHz)



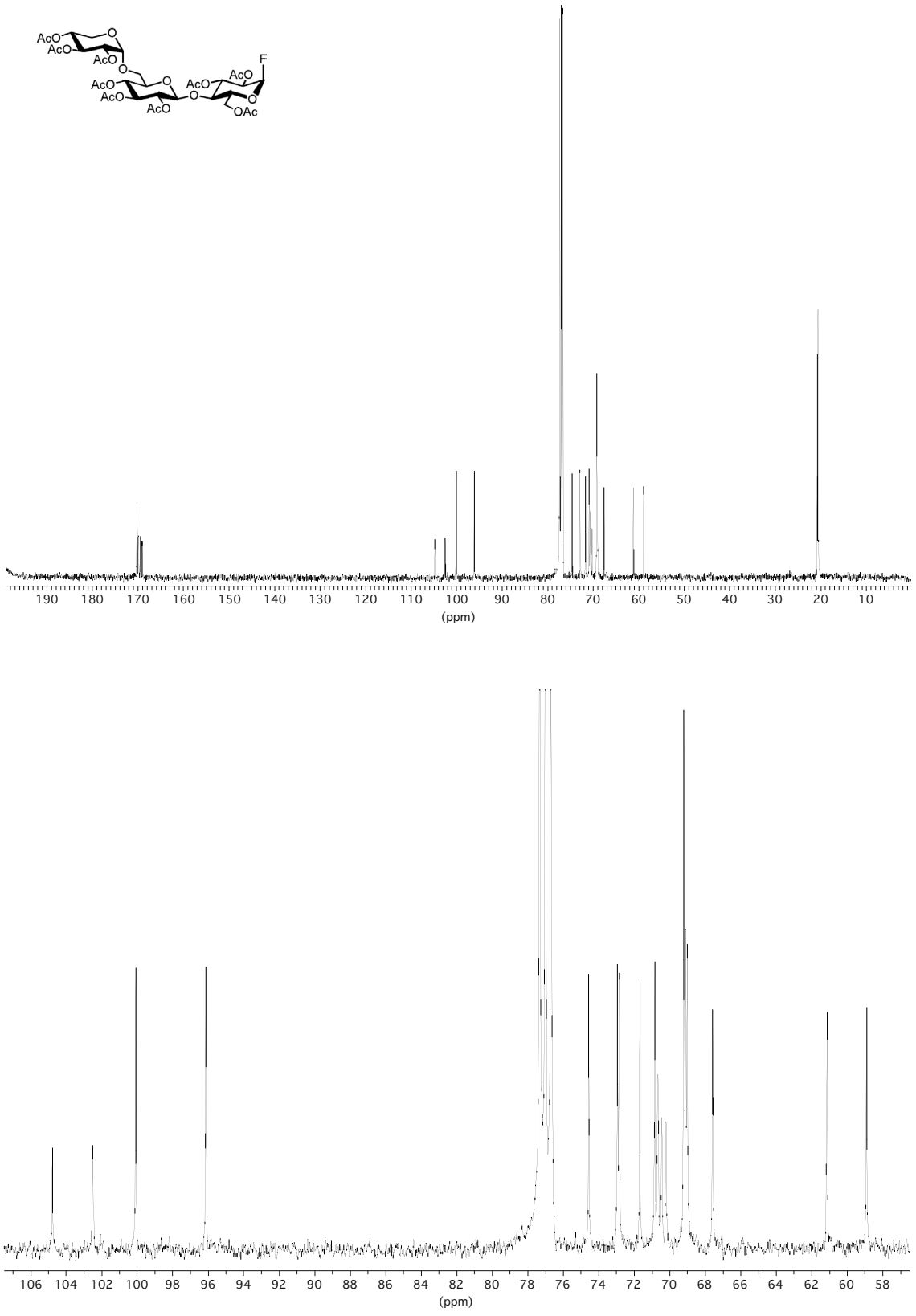
^{1}H NMR spectrum of compound **9** ($CDCl_3$, 400 MHz)



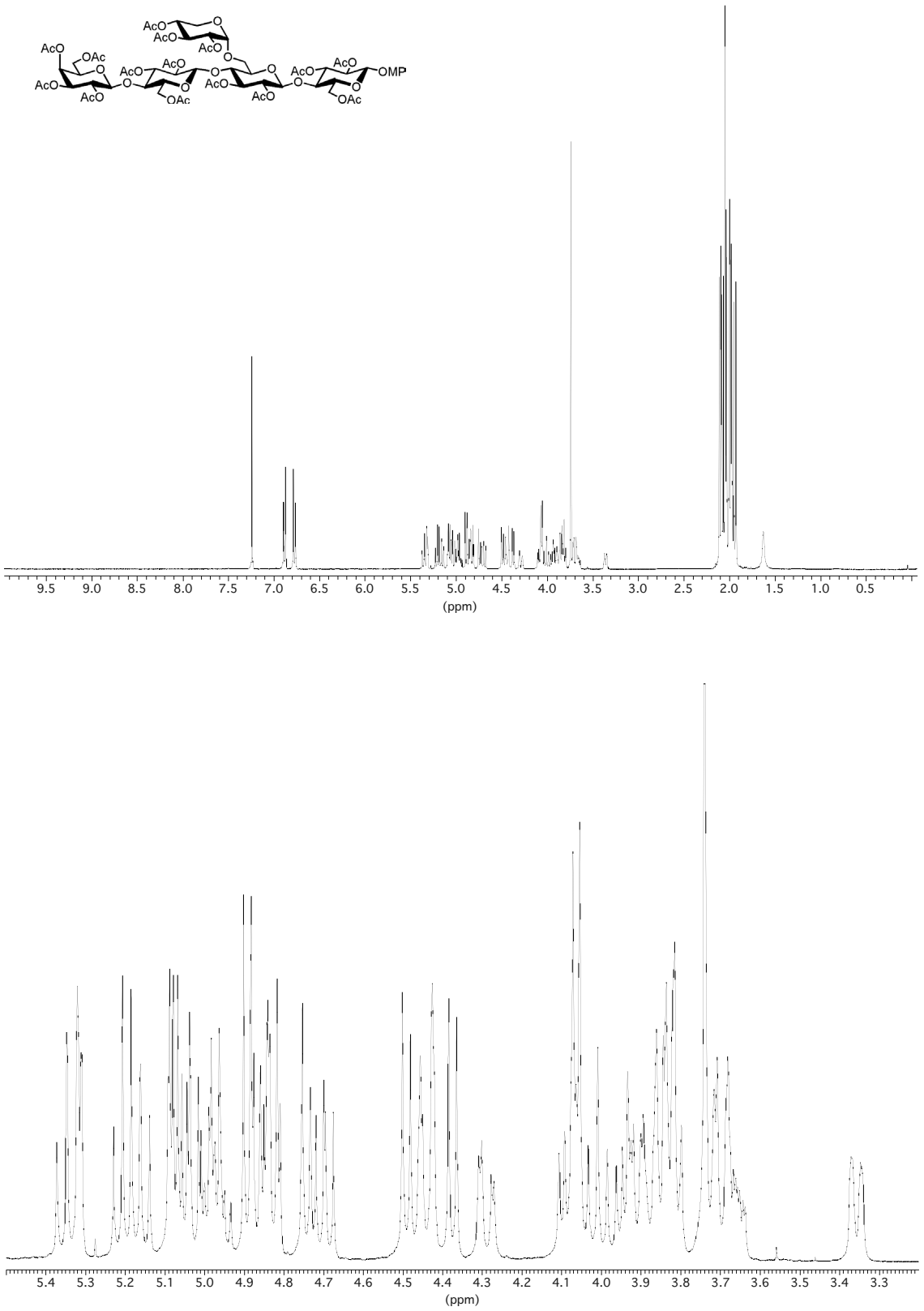
^{13}C NMR spectrum of compound **9** ($CDCl_3$, 100 MHz)



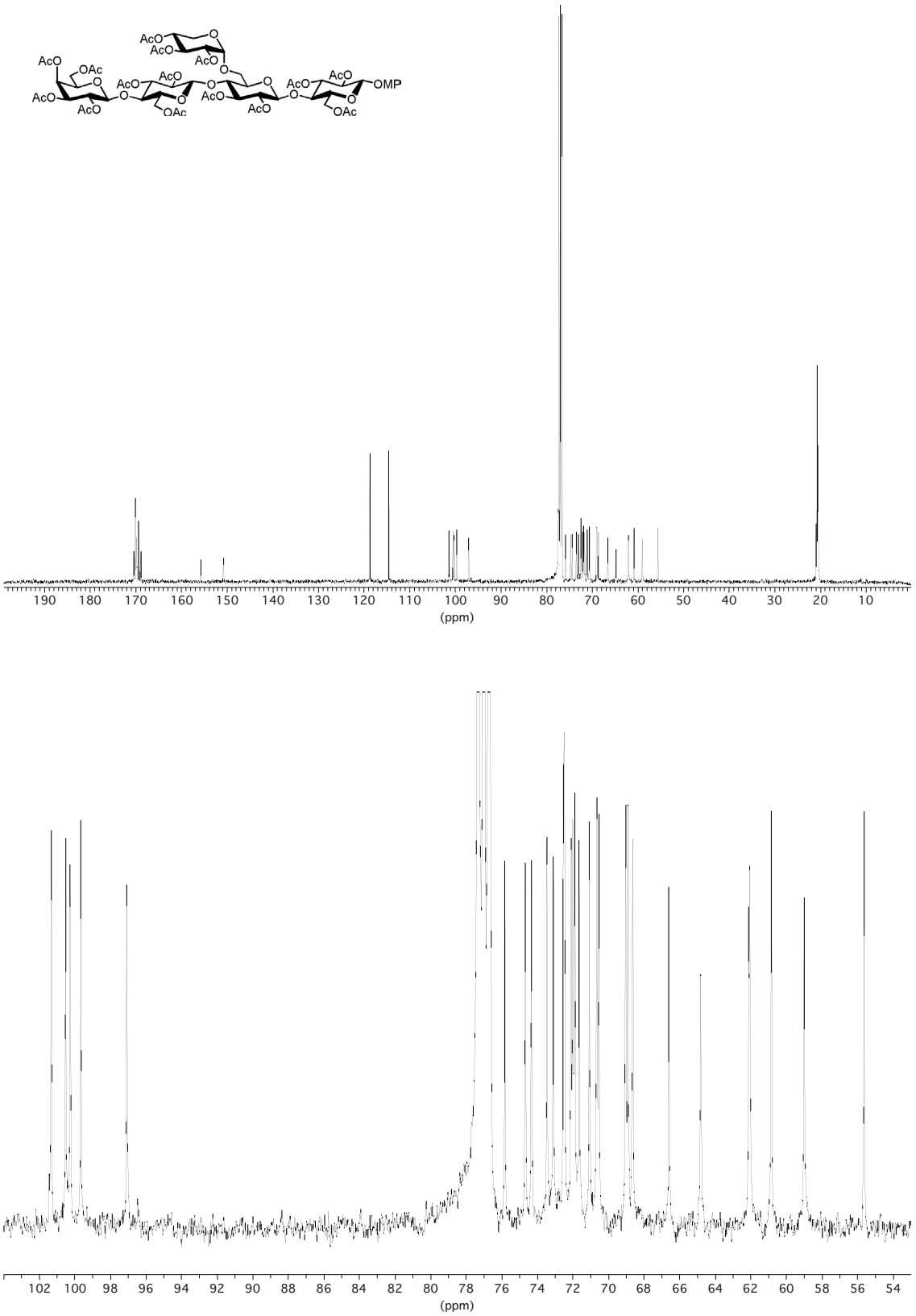
^{1}H NMR spectrum of compound **10** ($CDCl_3$, 400 MHz)



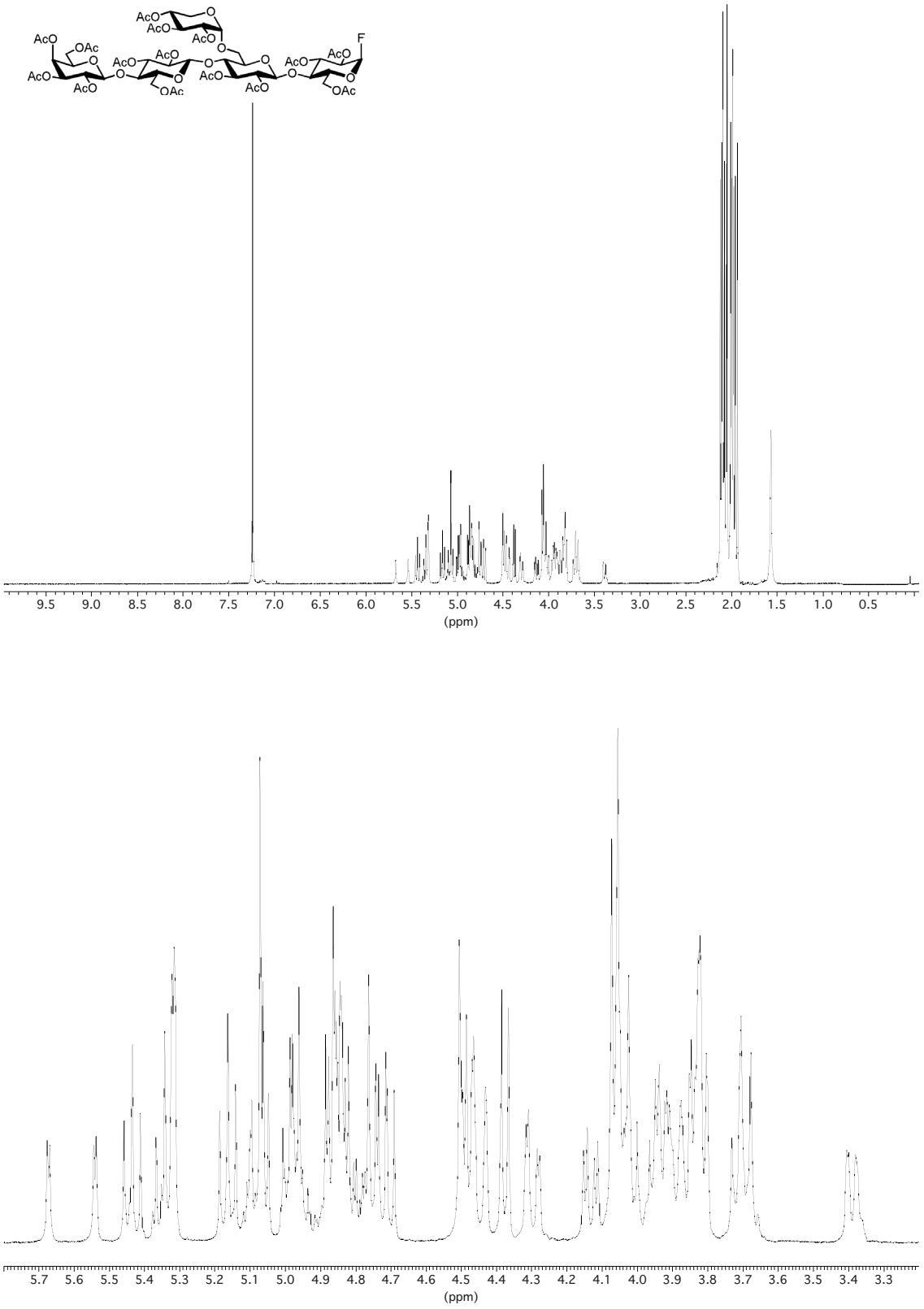
^{13}C NMR spectrum of compound **10** ($CDCl_3$, 100 MHz)



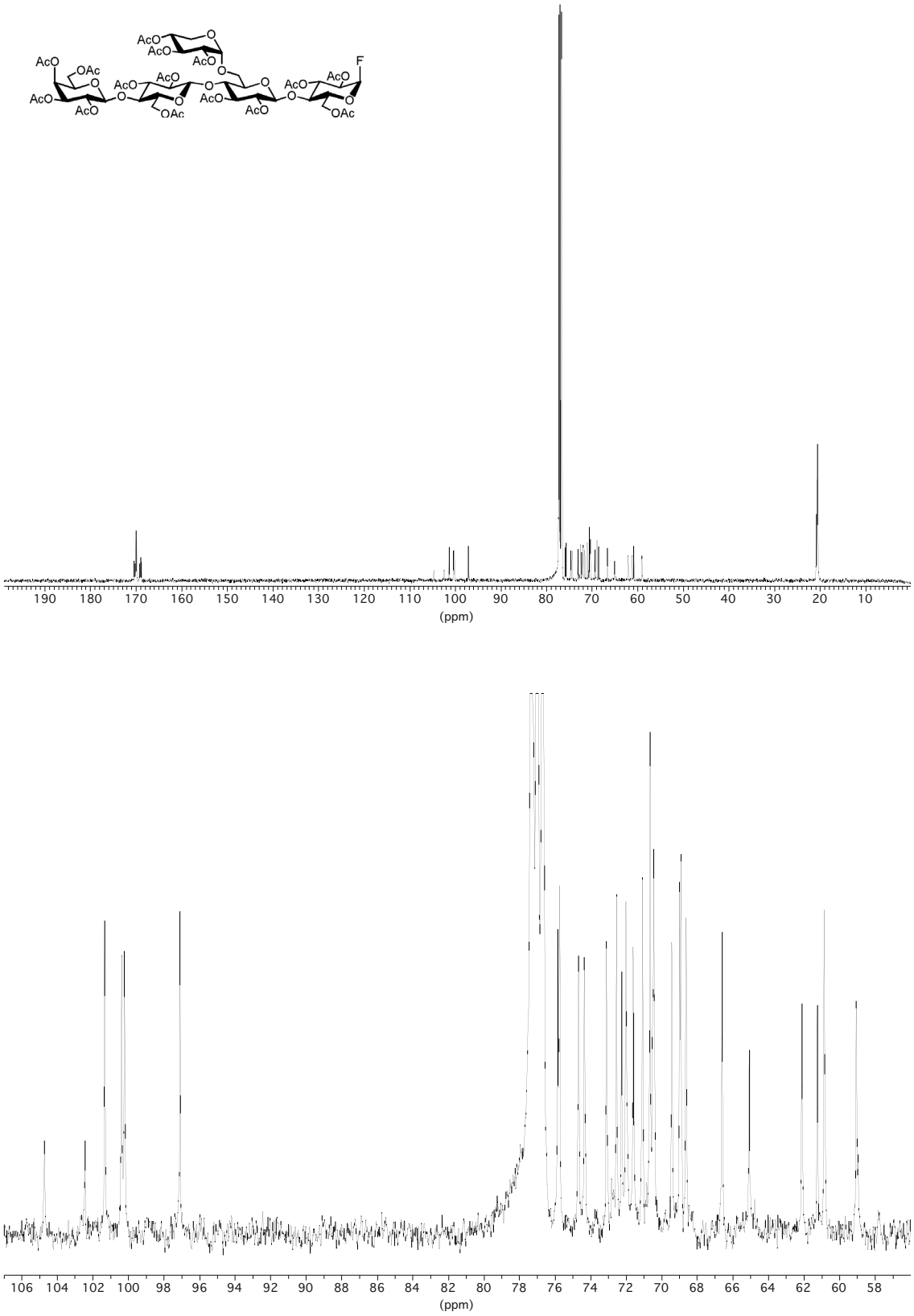
^{1}H NMR spectrum of compound **13** ($CDCl_3$, 400 MHz)



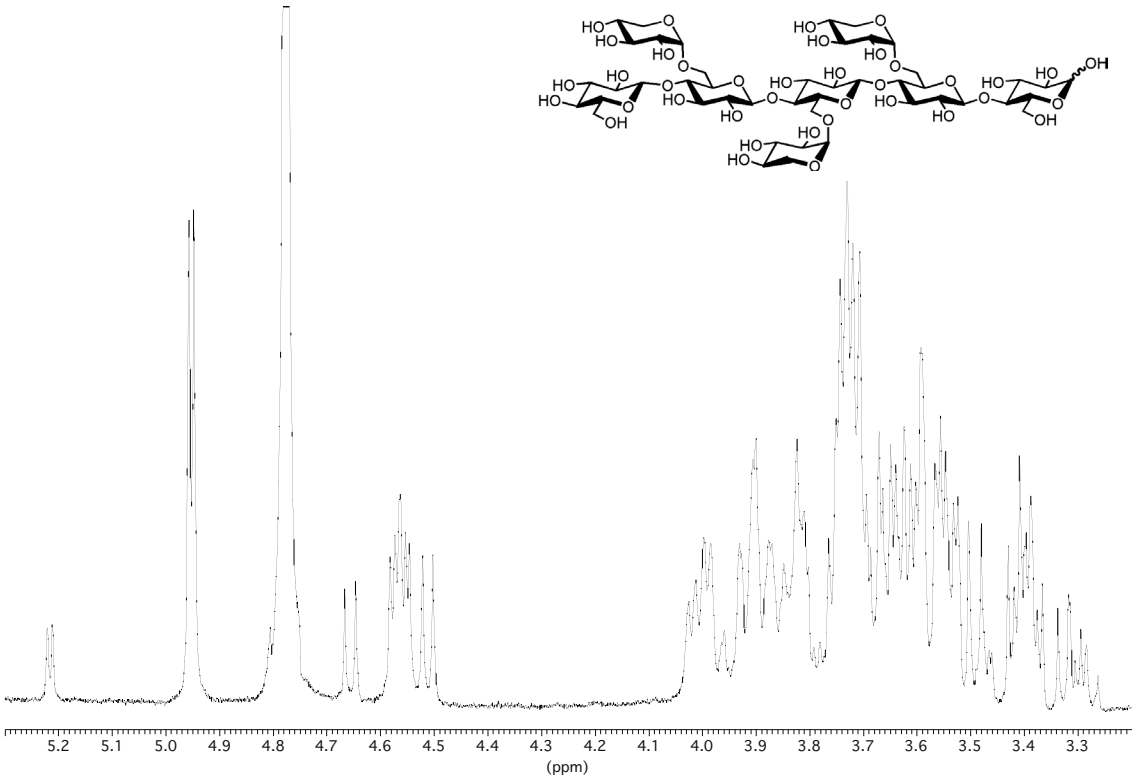
^{13}C NMR spectrum of compound **13** ($CDCl_3$, 100 MHz)



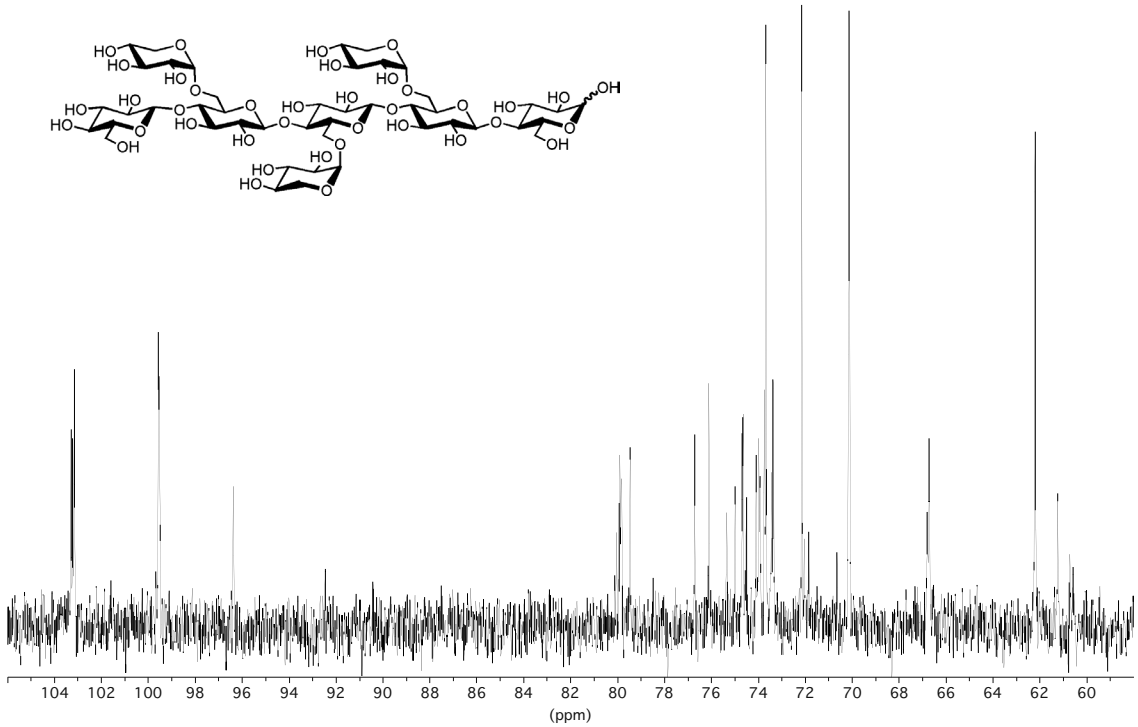
^{1}H NMR spectrum of compound **14** ($CDCl_3$, 400 MHz)



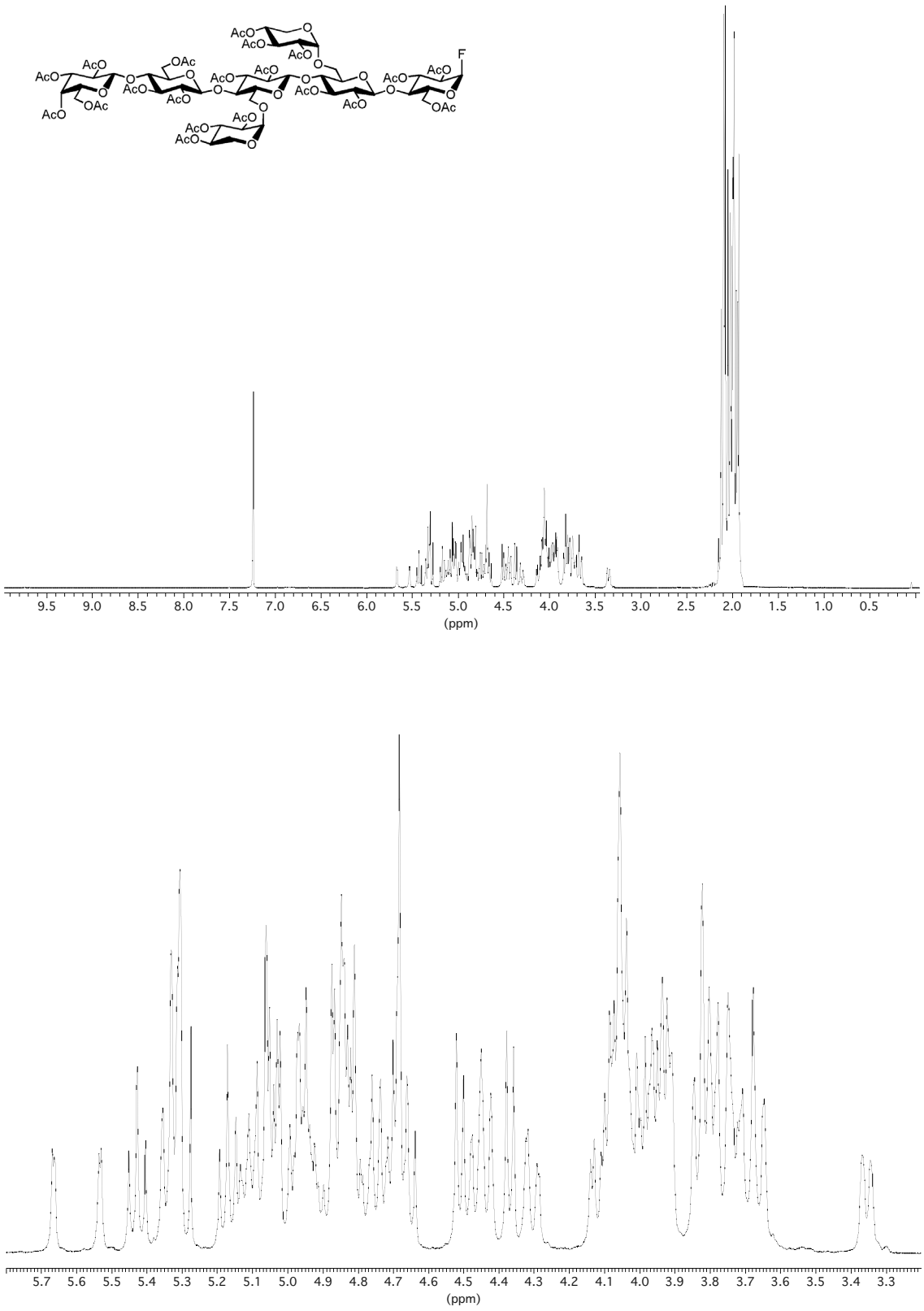
^{13}C NMR spectrum of compound **14** ($CDCl_3$, 100 MHz)



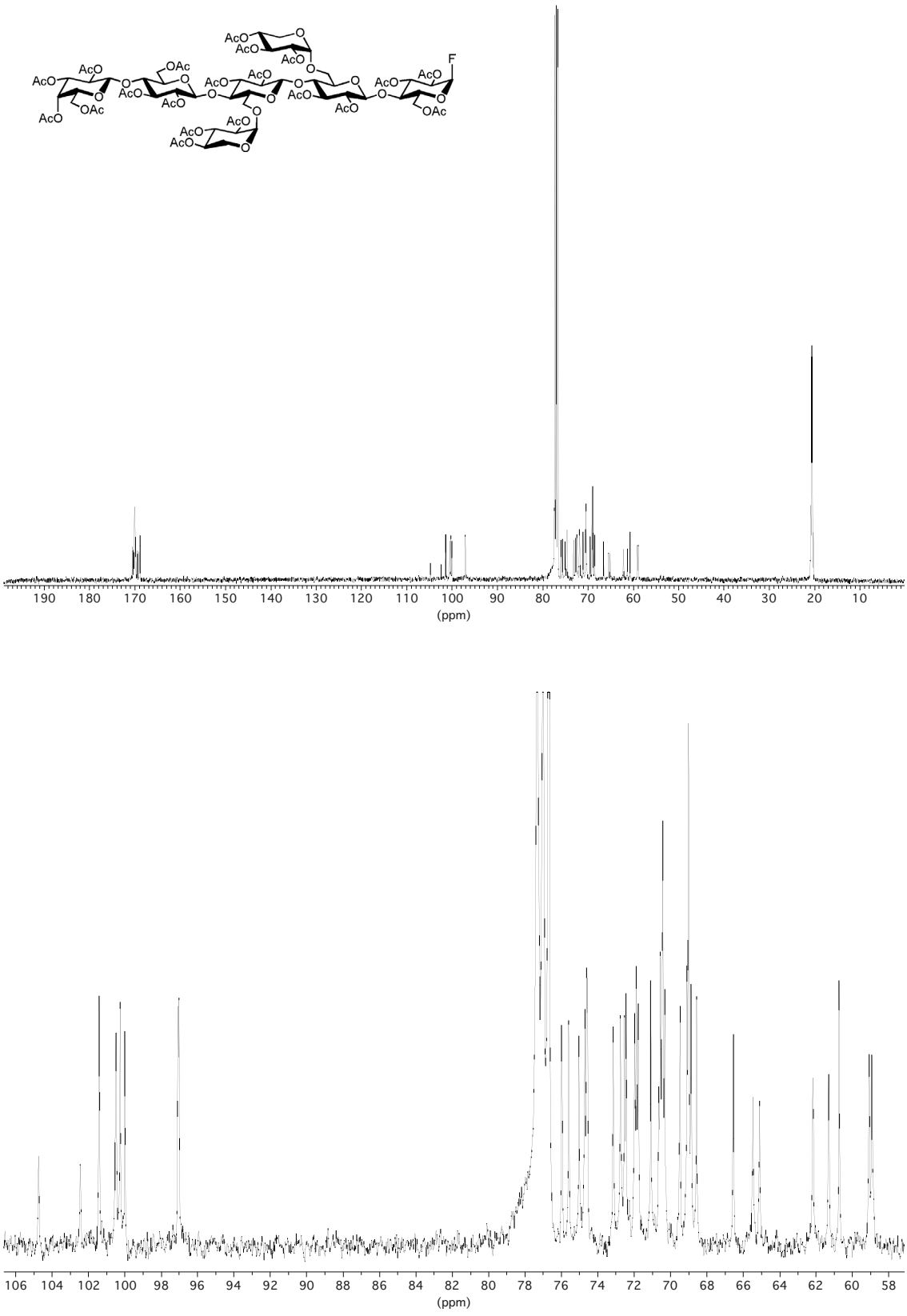
^{1}H NMR spectrum of compound **16** (D_2O, 400 MHz)



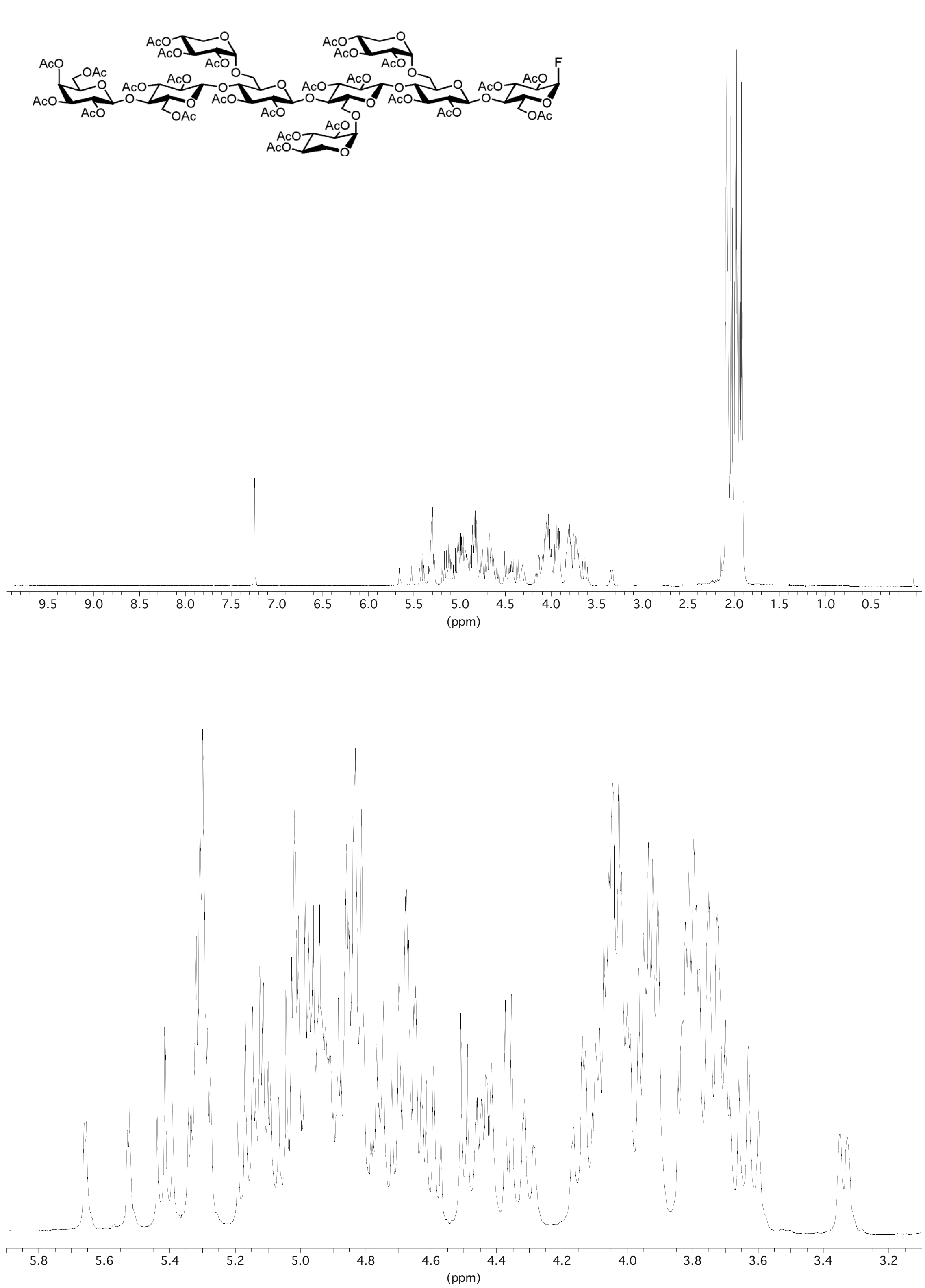
^{13}C NMR spectrum of compound **16** (D_2O, 100 MHz)



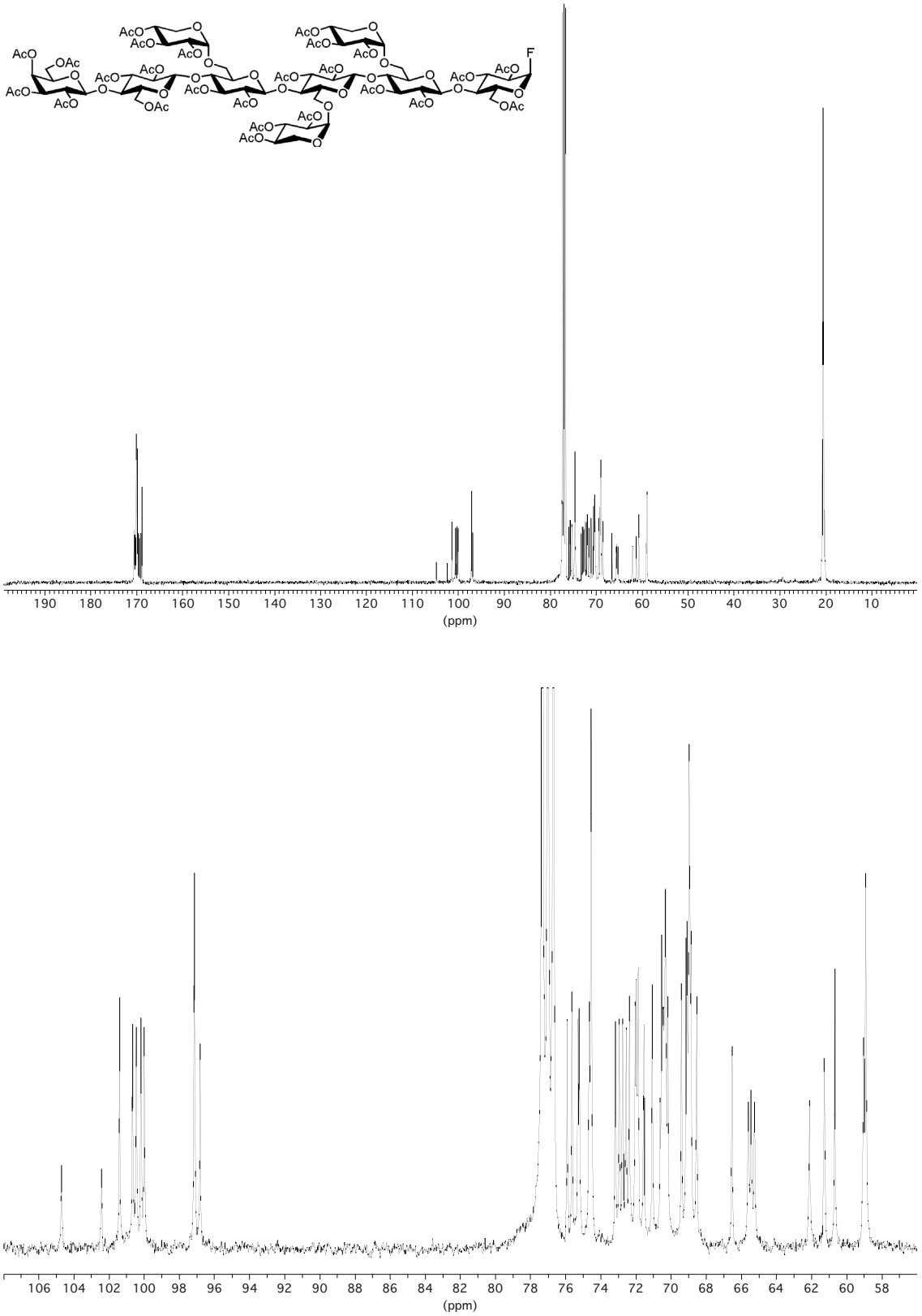
^{1}H NMR spectrum of compound **17** ($CDCl_3$, 400 MHz)



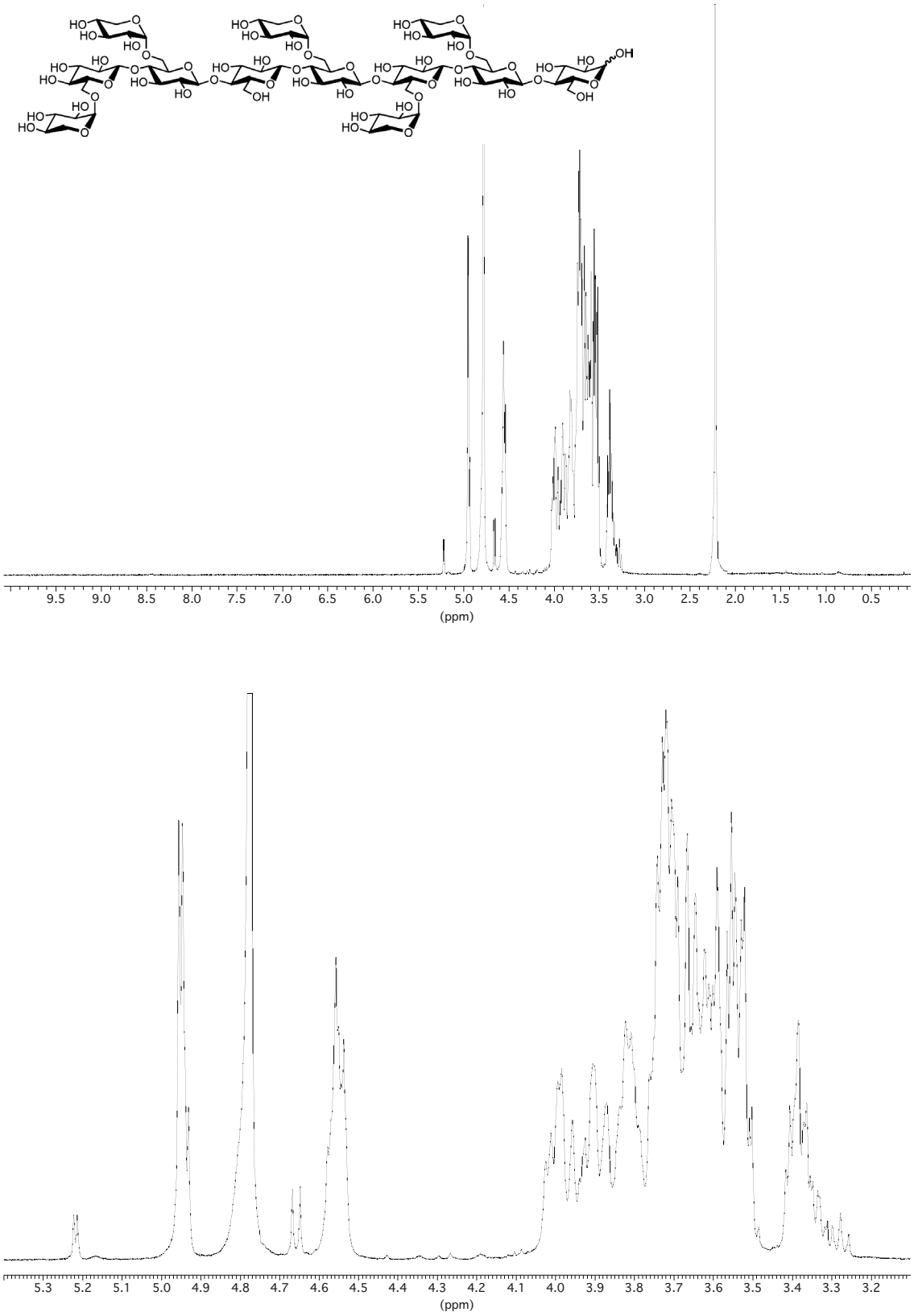
^{13}C NMR spectrum of compound **17** ($CDCl_3$, 100 MHz)



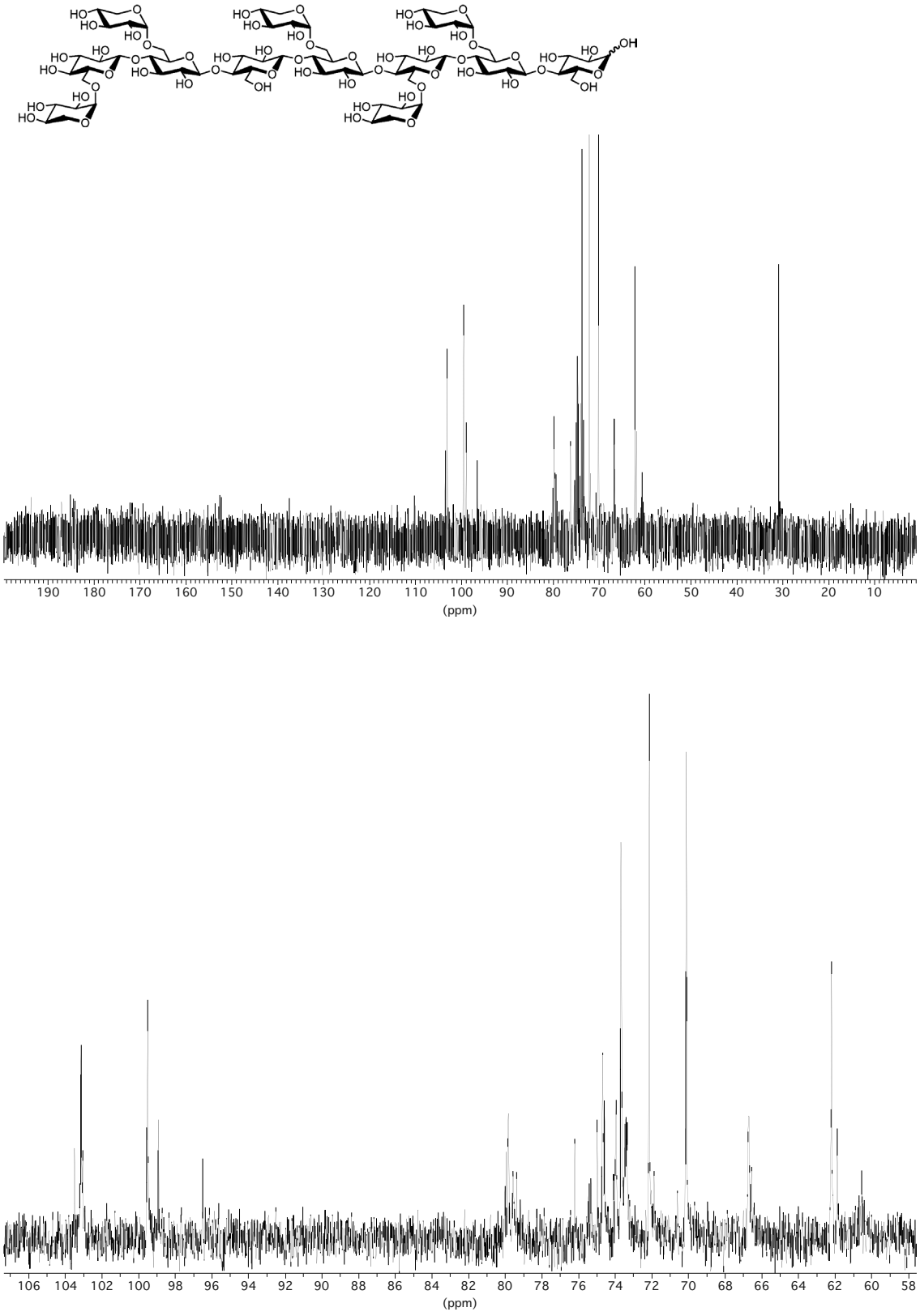
^{1}H NMR spectrum of compound **18** ($CDCl_3$, 400 MHz)



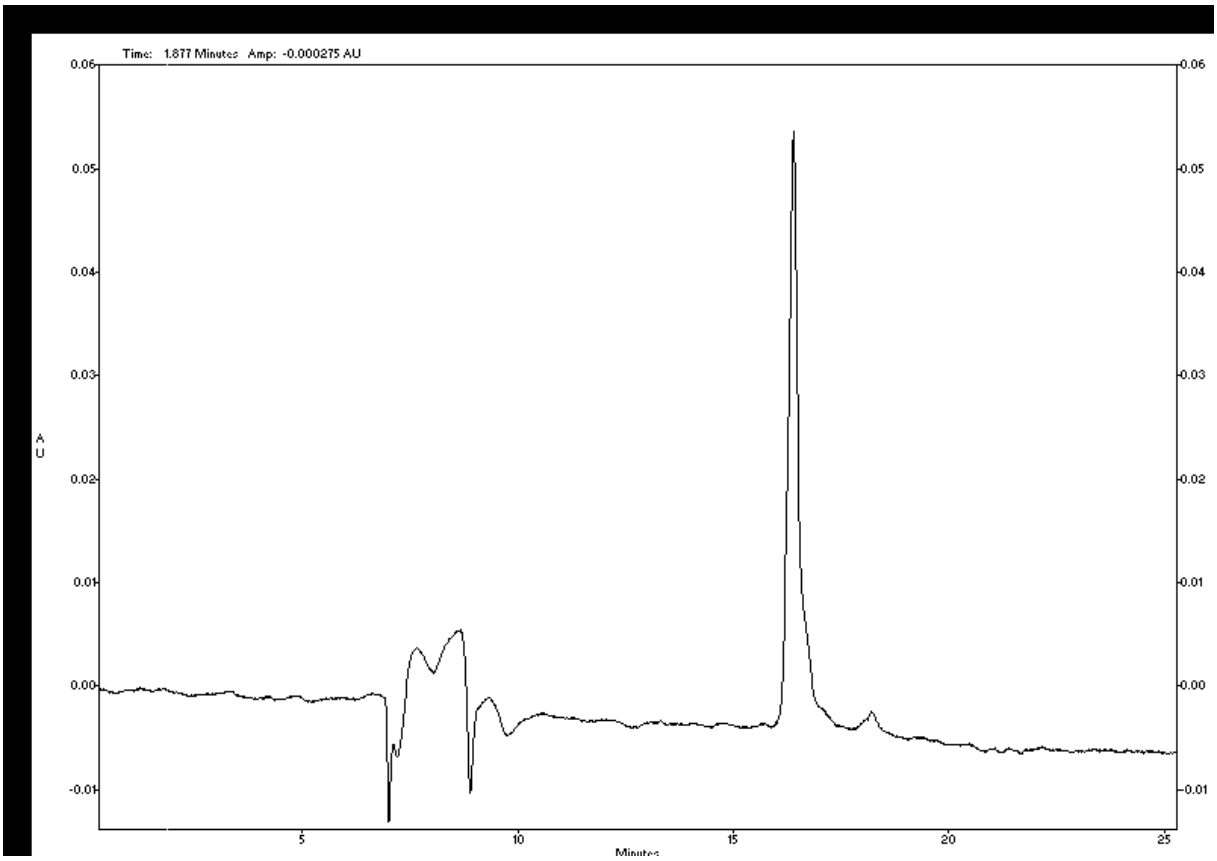
^{13}C NMR spectrum of compound **18** ($CDCl_3$, 100 MHz)



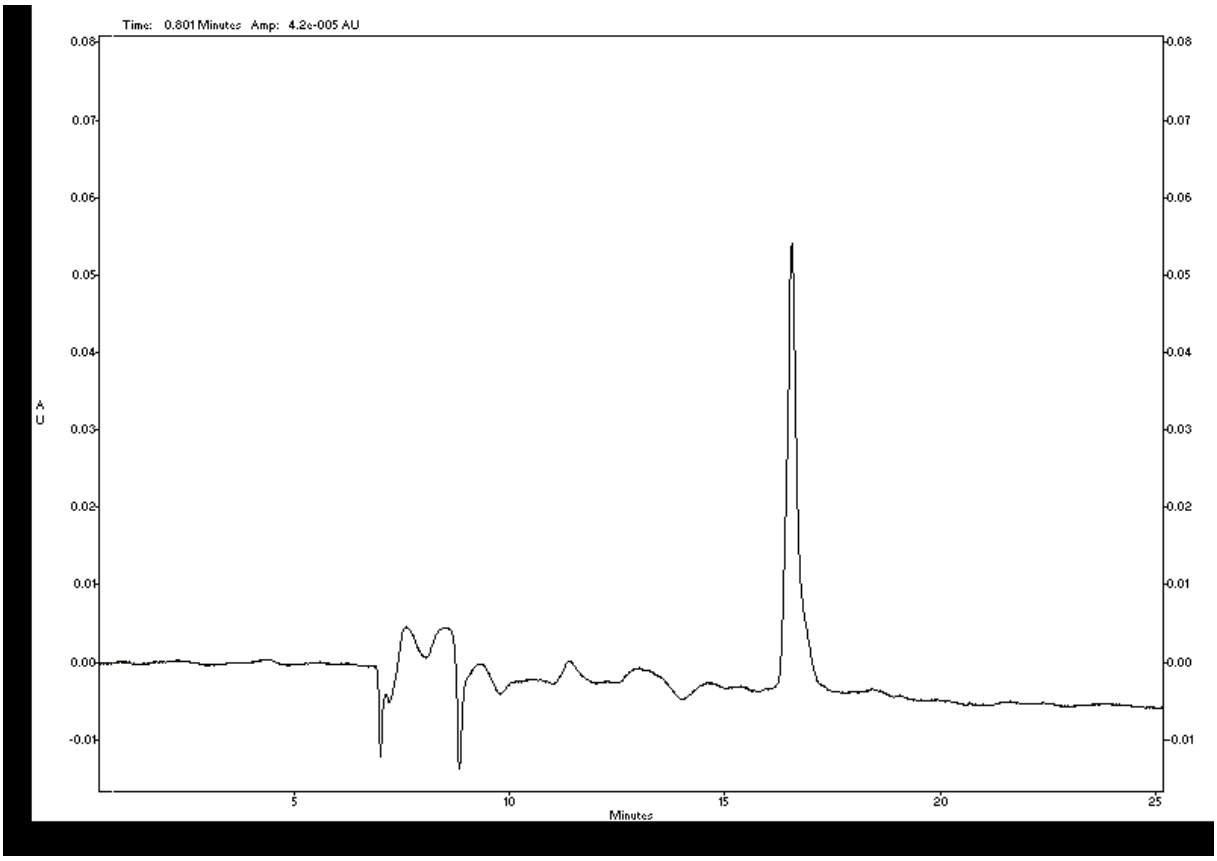
^{1}H NMR spectrum of compound **26** (D_2O, 400 MHz)



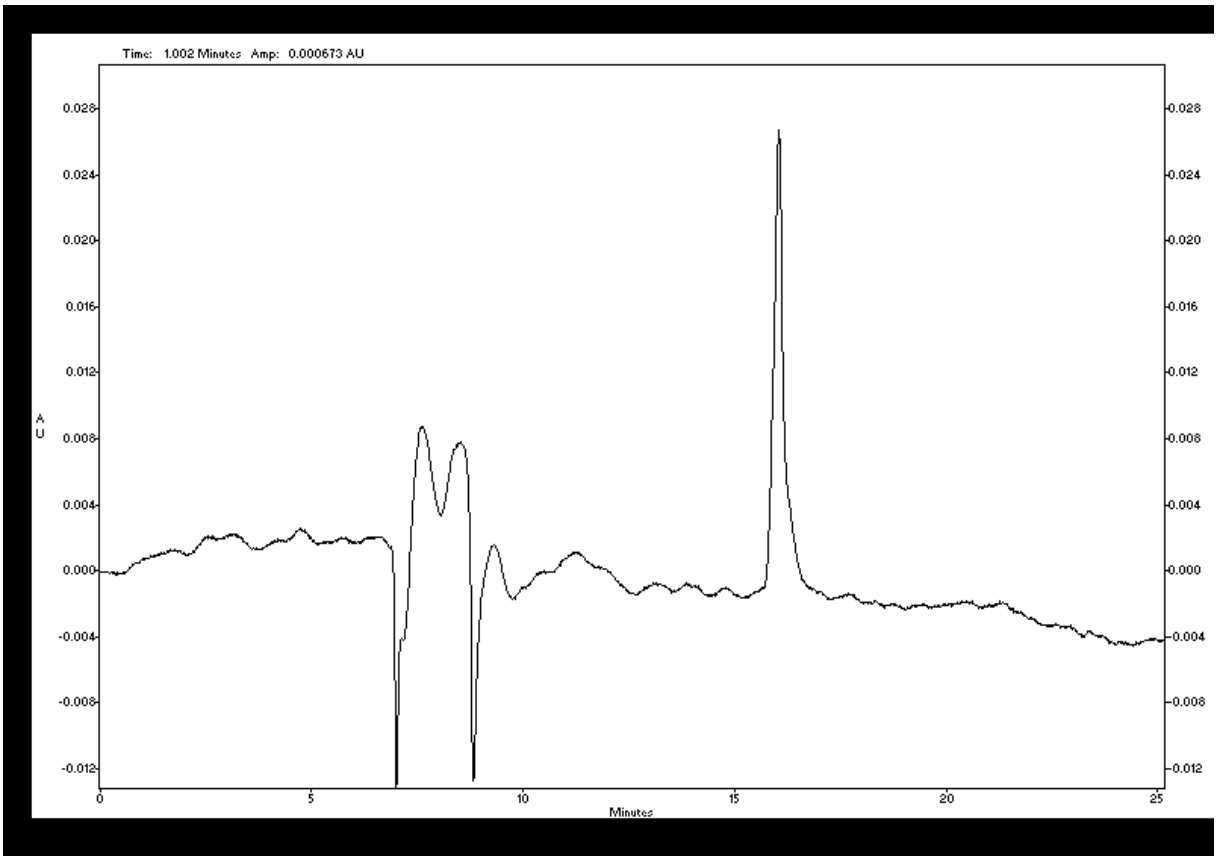
^{13}C NMR spectrum of compound **26** (D_2O, 100 MHz)



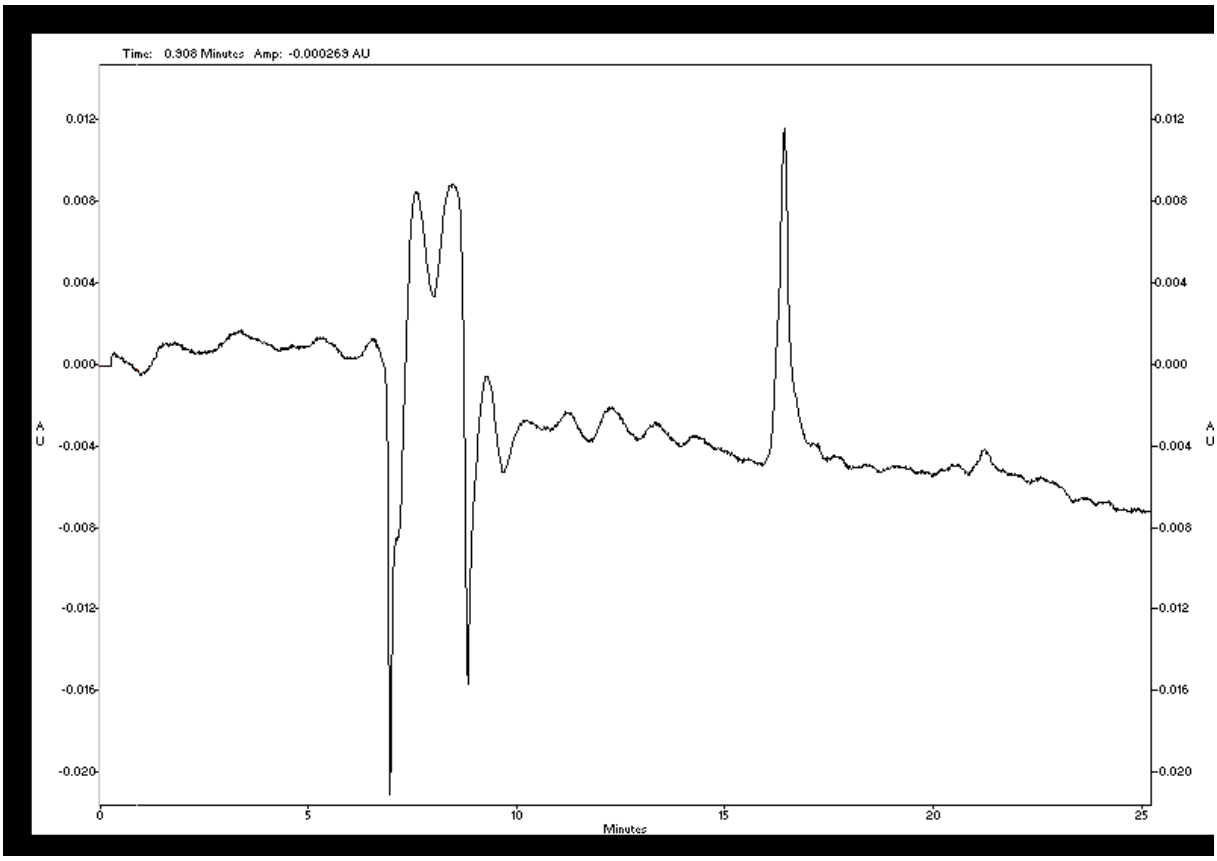
Quantitative HPLC-ESMS of ABEE derivative of compound **15**



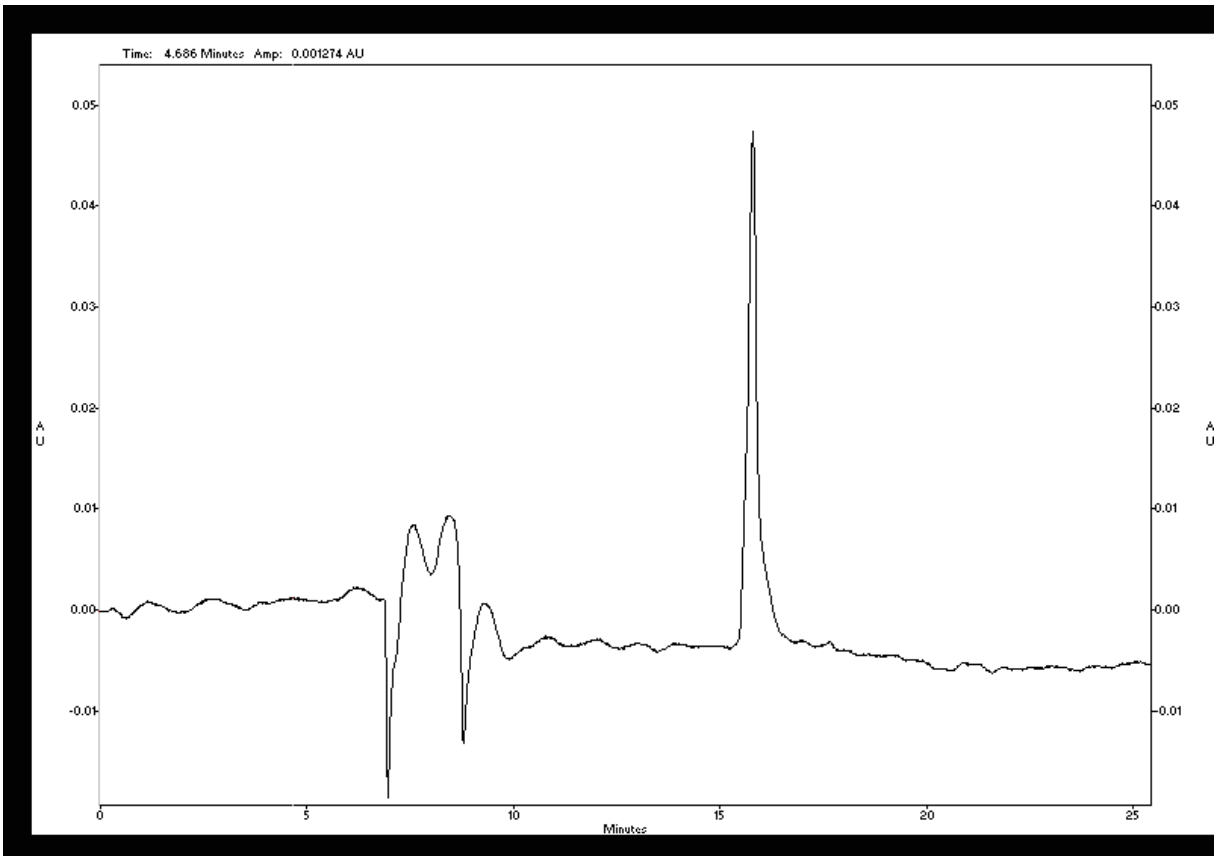
Quantitative HPLC-ESMS of ABEE derivative of compound **20**



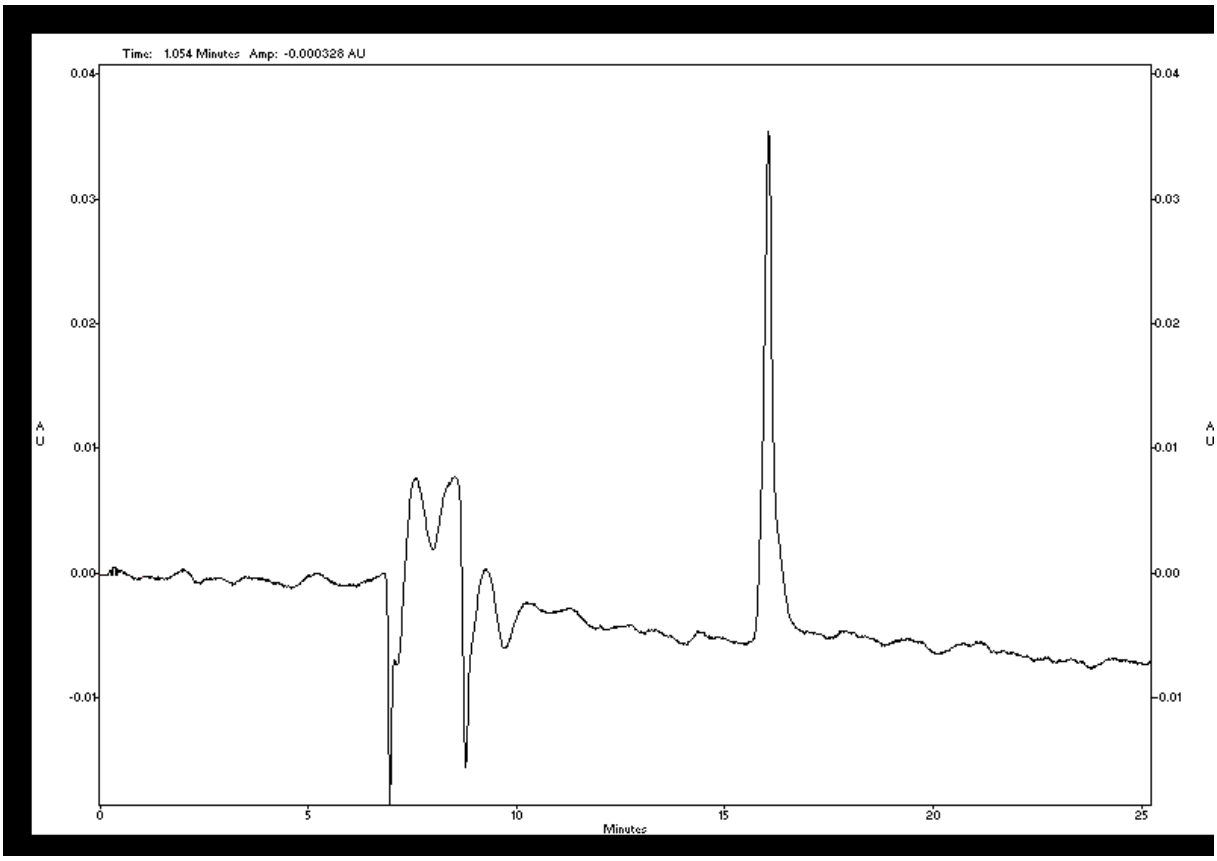
Quantitative HPLC-ESMS of ABEE derivative of compound **21**



Quantitative HPLC-ESMS of ABEE derivative of compound **23**



Quantitative HPLC-ESMS of ABEE derivative of compound **24**



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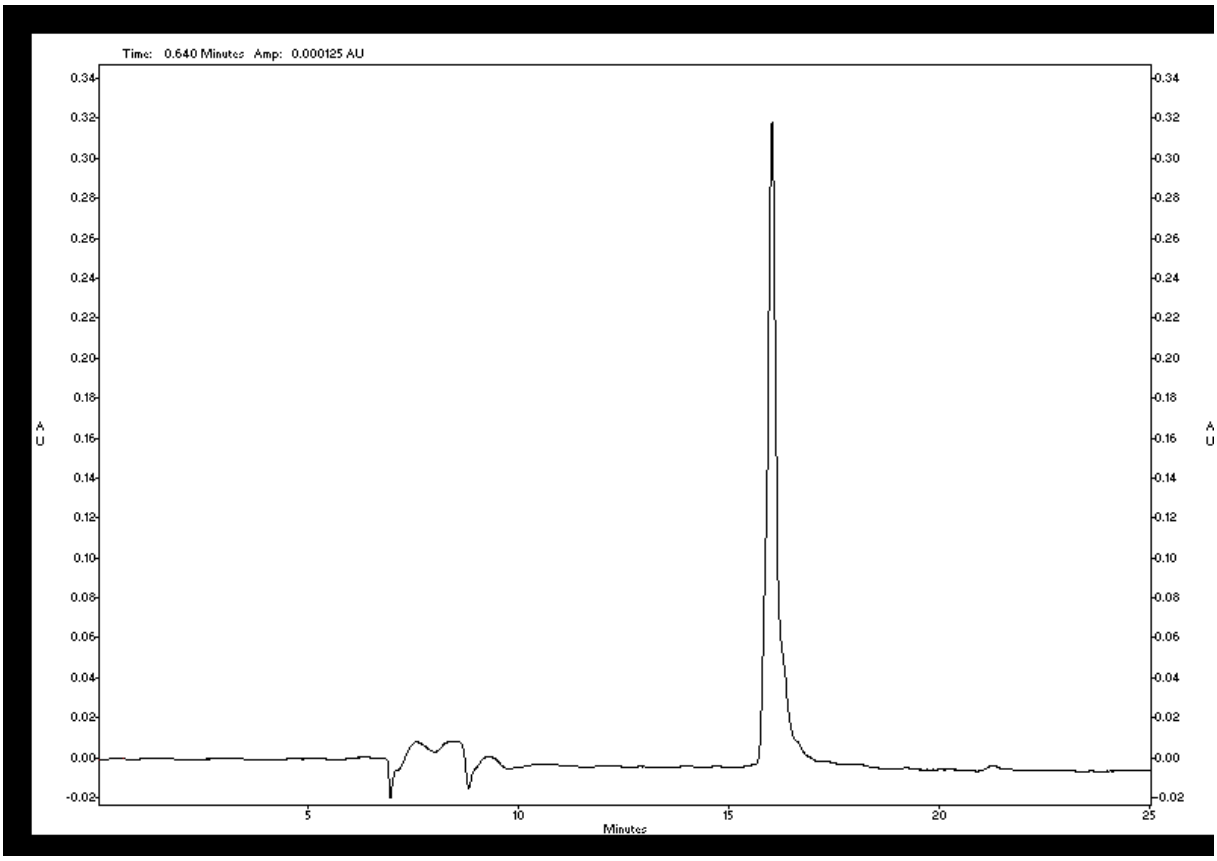

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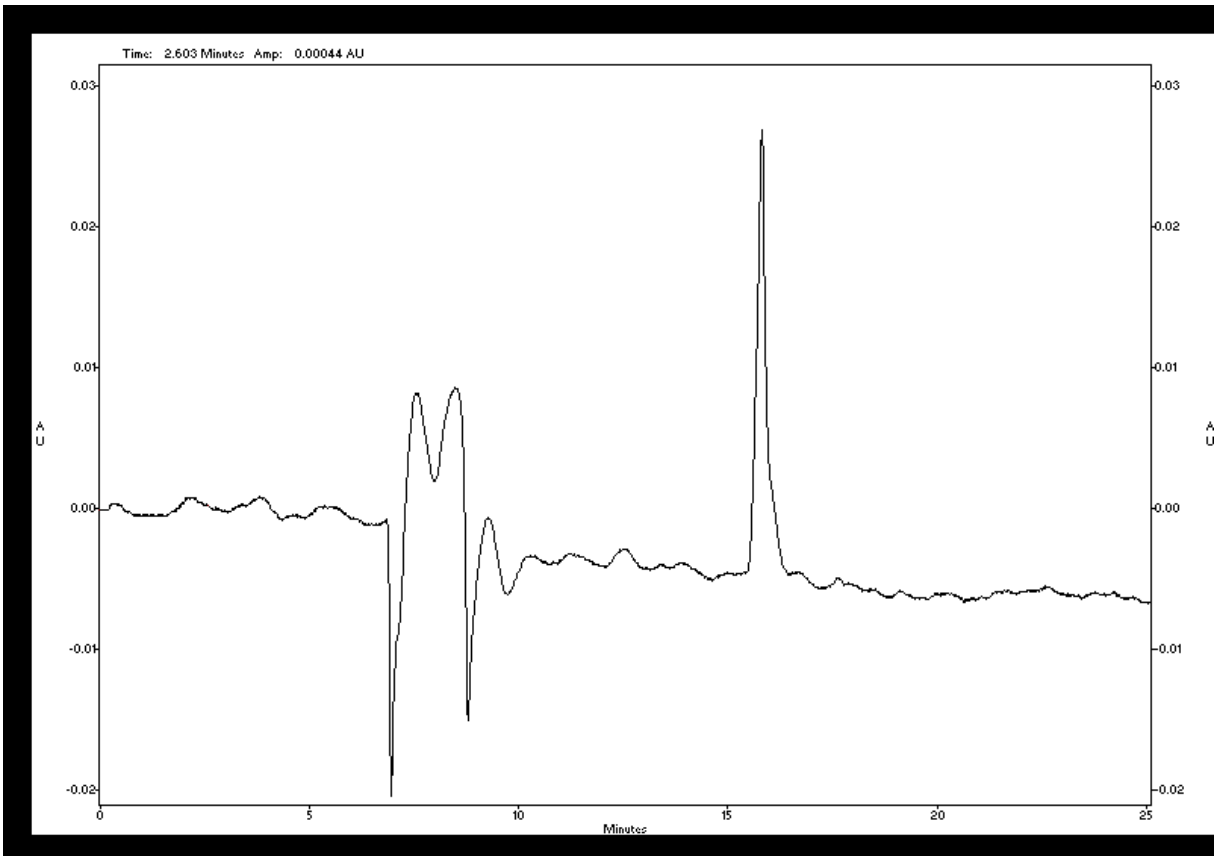

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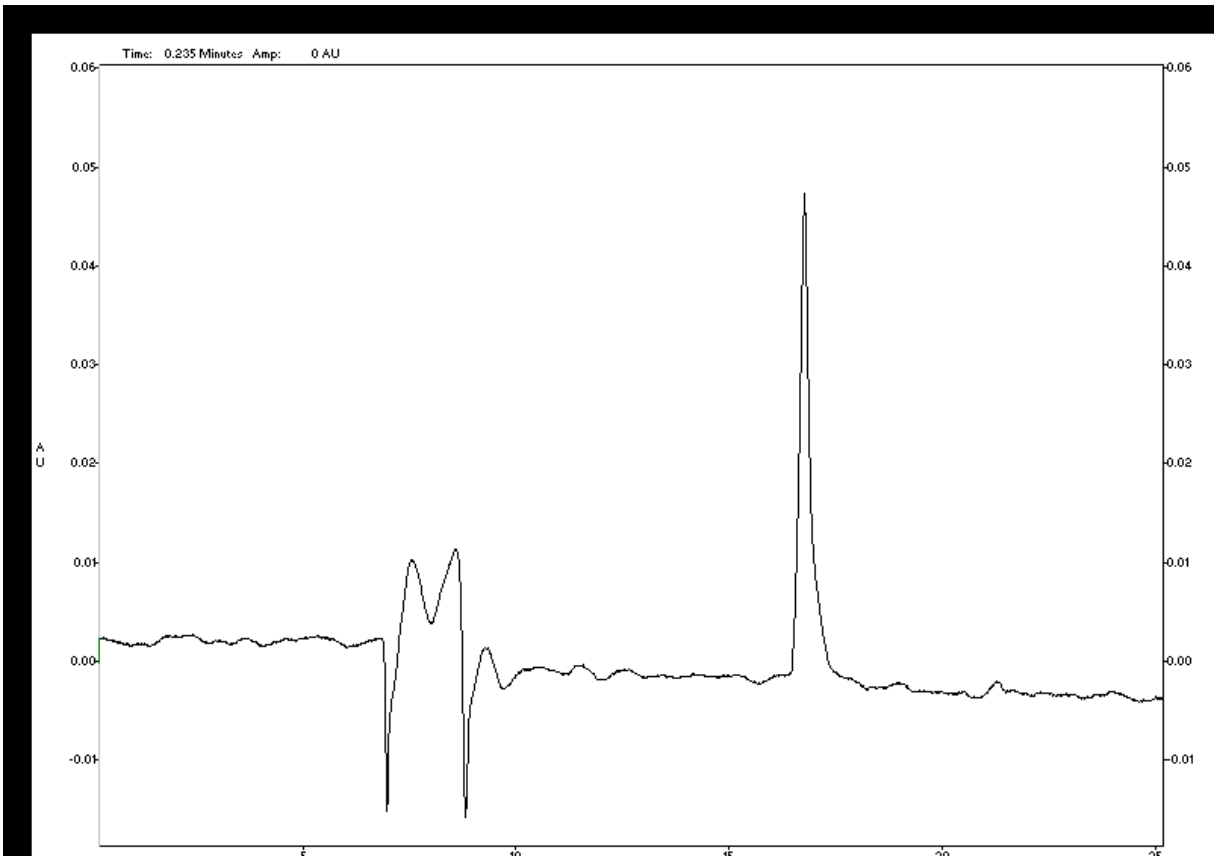
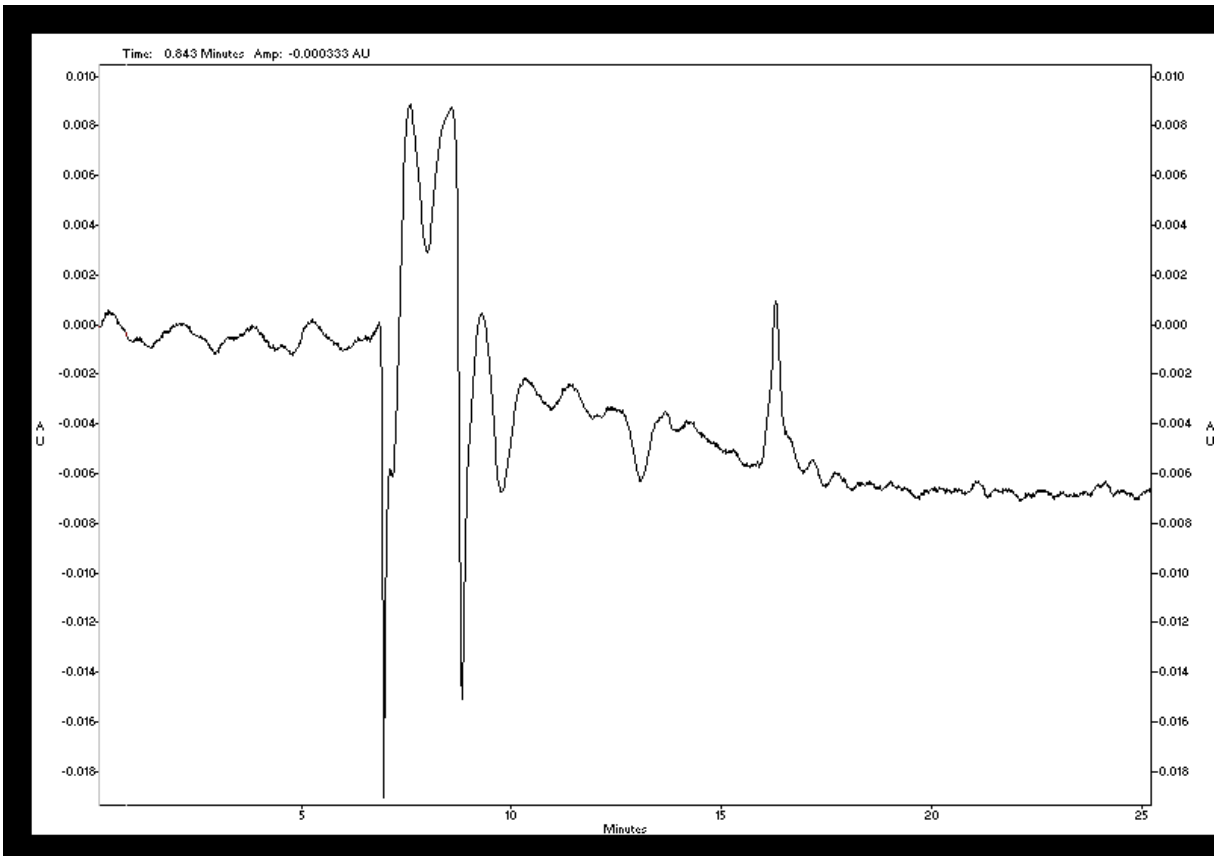


Quantitative HPLC-ESMS of ABEE derivative of compound **31**



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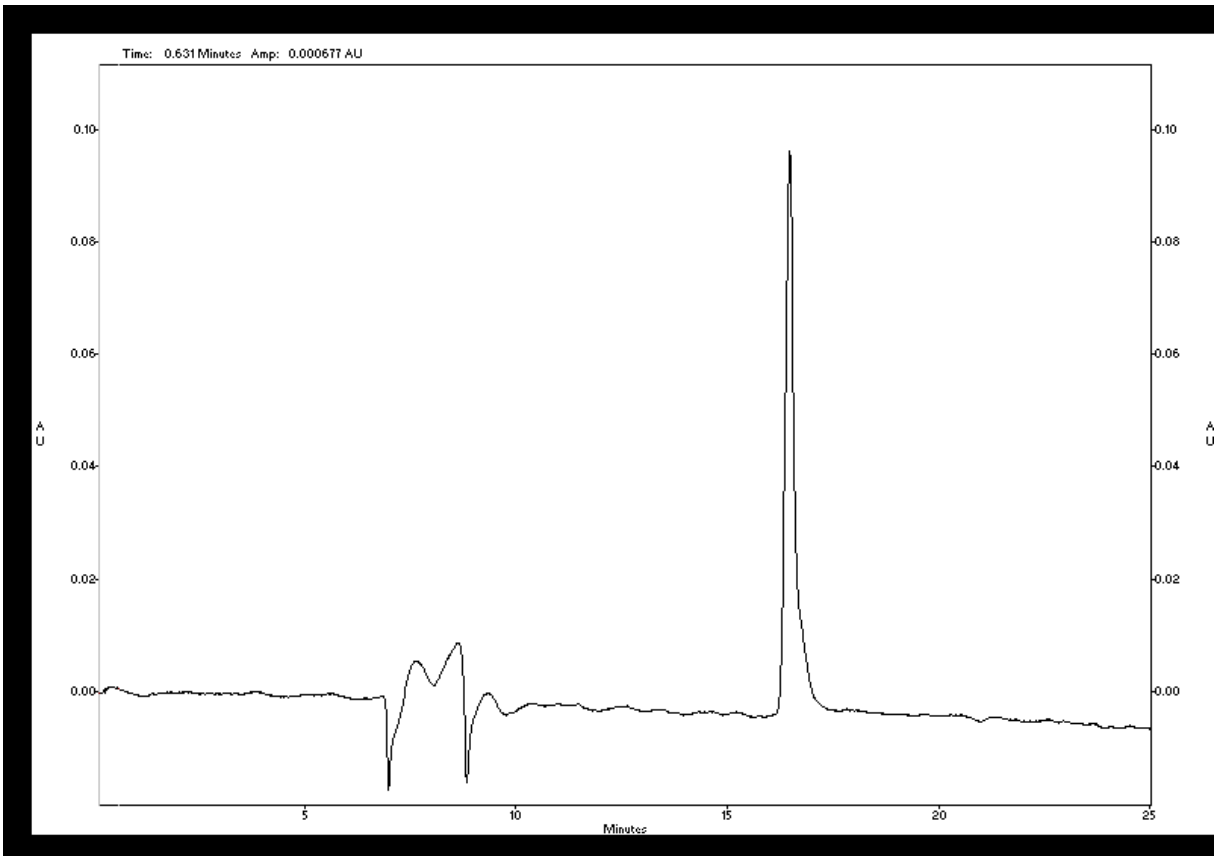

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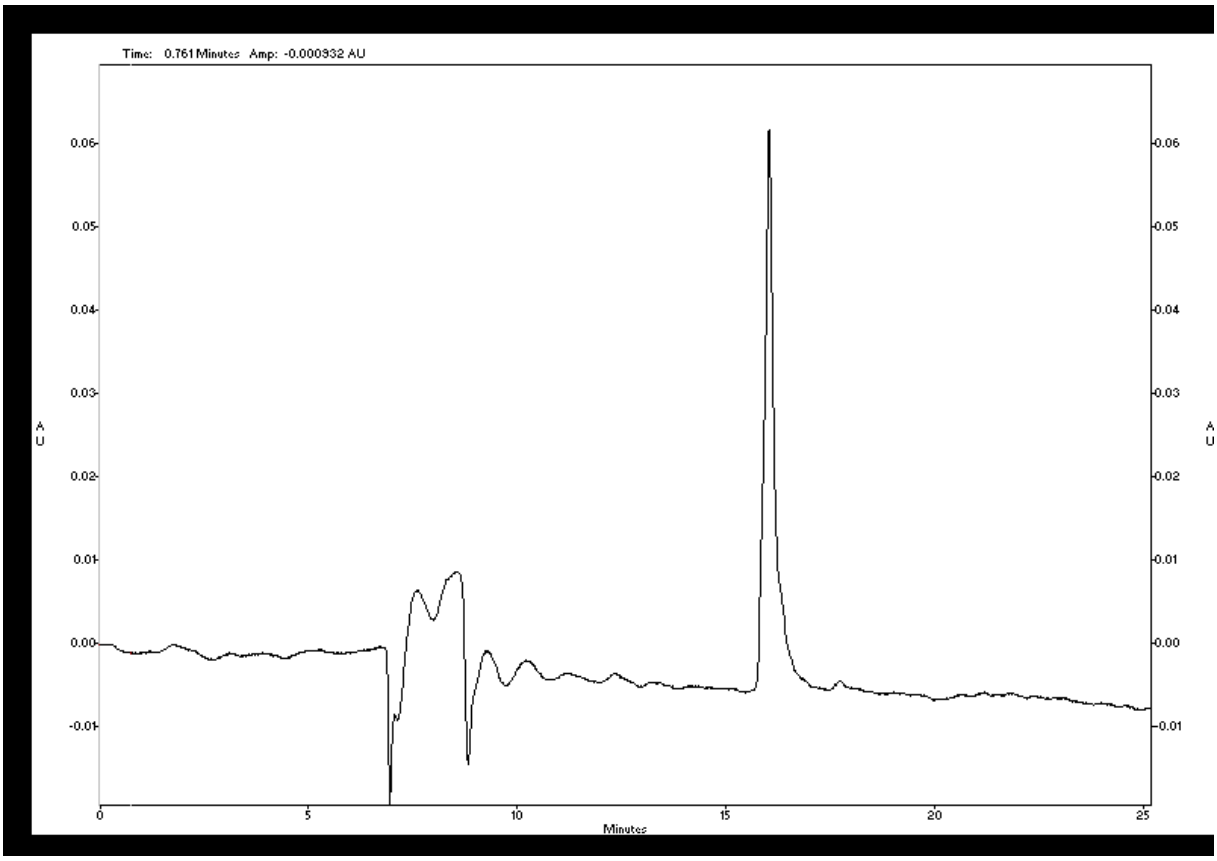

Quantitative HPLC-ESMS of ABEE derivative of compound **34**