

Supporting Information

Title: Synthesis of sulfated lactosyl glycosides for evaluation in vaccine adjuvant formulations

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Chemical synthesis:

The common precursor for the synthesis of the 3 SLGs is the thioglycoside **6**. Its synthesis has been previously reported. In brief, lactose **1** was subjected to a series of reactions involving protection, nucleophilic substitution and deprotection through intermediates **2-5** to yield compound **6**, as per the scheme below.¹

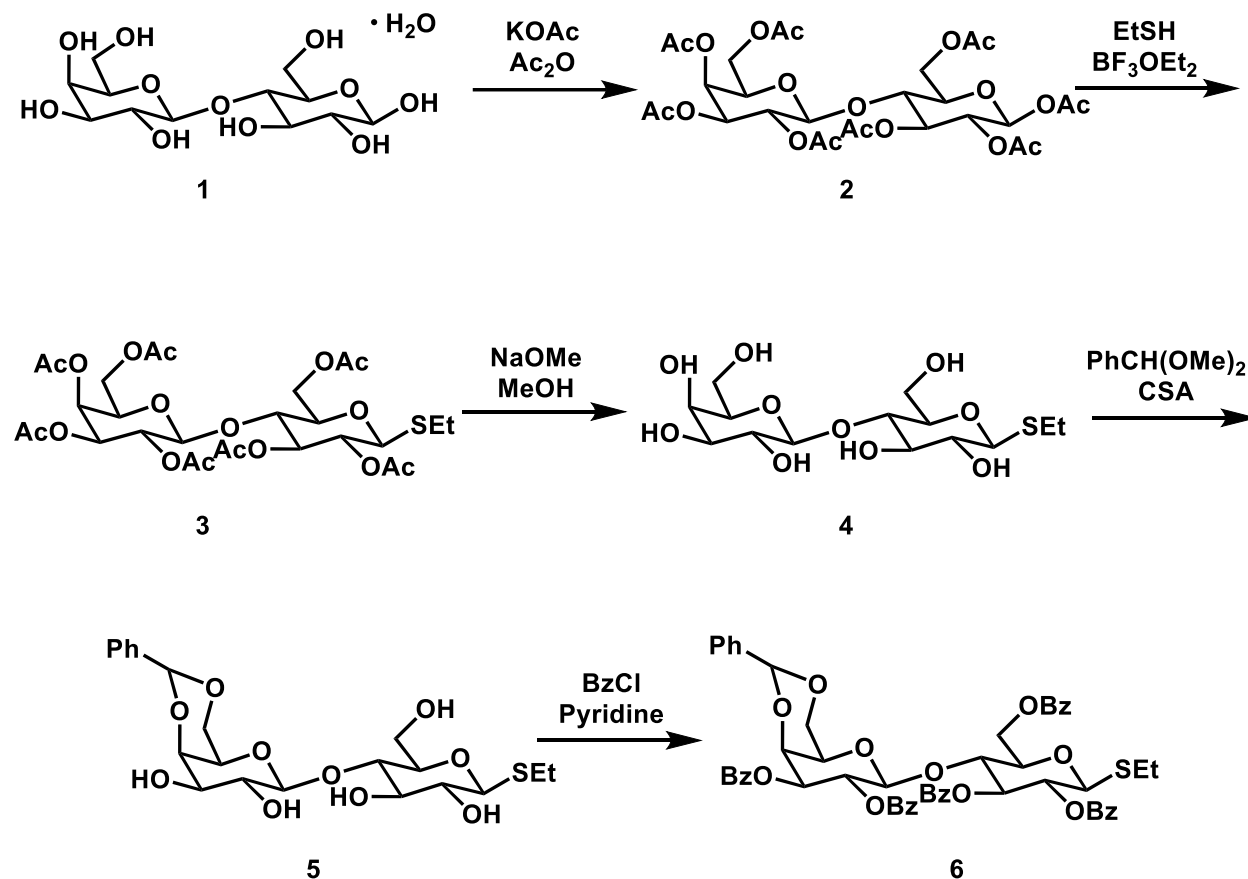


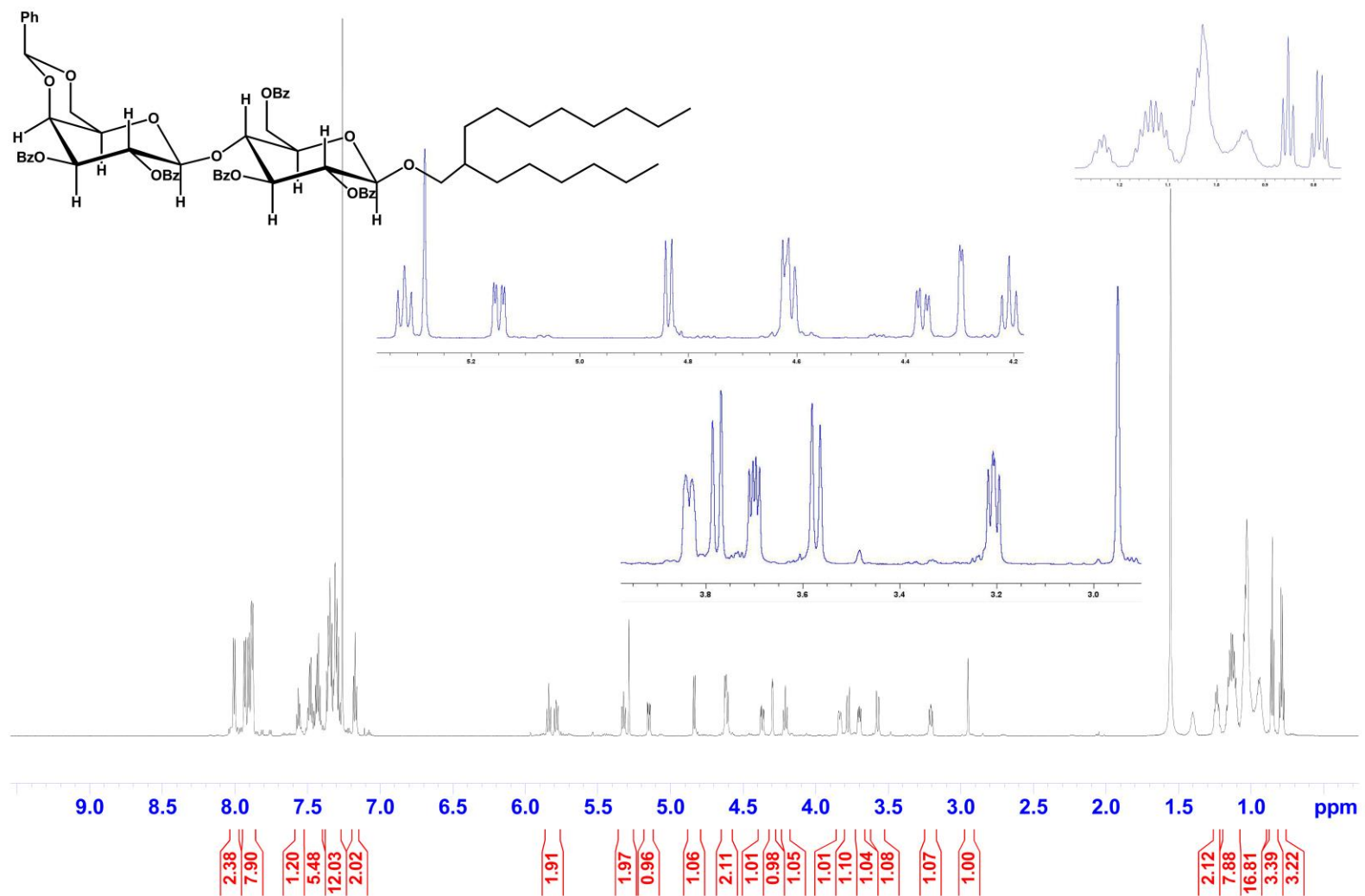
Table S1: Sample preparation for DLS and zeta potential measurements

Sample	Lipid concentration (mg/mL)	Volume (mL)	Total lipid available (mg)
DMPC: Chol	27.78 (DMPC: 20.83 mg/mL) (Chol: 6.94 mg/mL)	1.550	43,06 (DMPC: 32.29 mg) (Chol: 10.76 mg)
DMPC: Chol: 10A	52.92 (DMPC: 26.46 mg/mL) (Chol: 8.82 mg/mL) (10A: 17.64 mg/mL)	1.220	64,56 (DMPC: 32.28 mg) (Chol: 10.76 mg) (10A: 21.52 mg)
DMPC: Chol: 10B	41,67 (DMPC: 20.83 mg/mL) (Chol: 6.94mg/mL) (10B: 13.89 mg/mL)	0.737	30,72 (DMPC: 15.36 mg) (Chol: 5.12 mg) (10B: 10.24 mg)
DMPC: Chol: 10C	42,24 (DMPC: 21.12 mg/mL) (Chol: 7.04 mg/mL) (10C: 14.08 mg/mL)	1.750	73,92 (DMPC: 36.96 mg) (Chol: 12.32 mg) (10C: 24.64 mg)
SLA	17.96	1.400	25.1
DMPC: Chol: SLA	70,82 (DMPC: 35.41 mg/mL) (Chol: 11.80 mg/mL) (SLA: 23.61 mg/mL)	1.320	93,48 (DMPC: 46.74 mg) (Chol: 15.58 mg) (SLA: 31.16 mg)

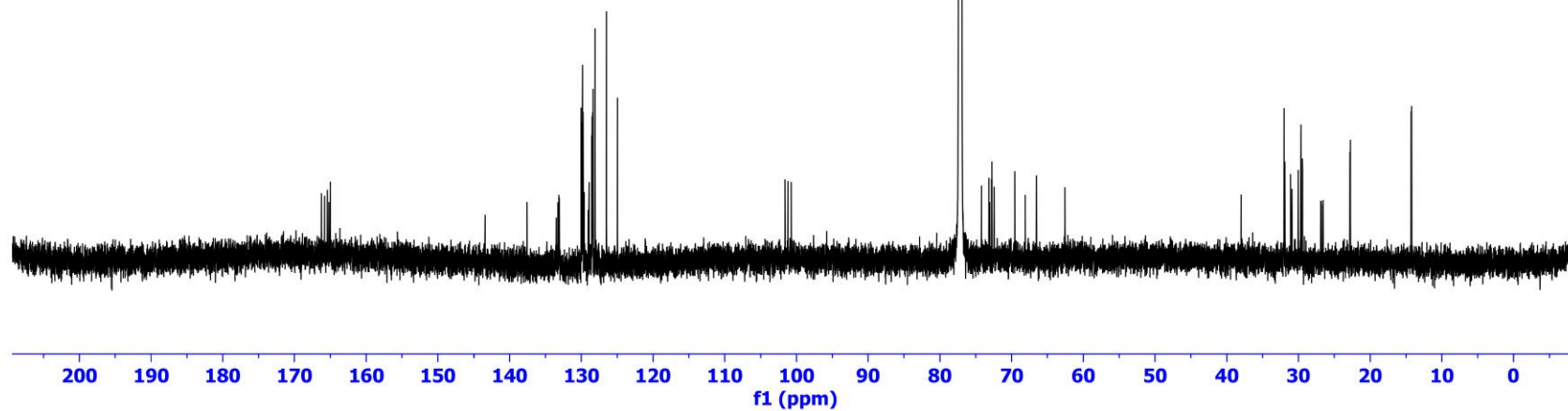
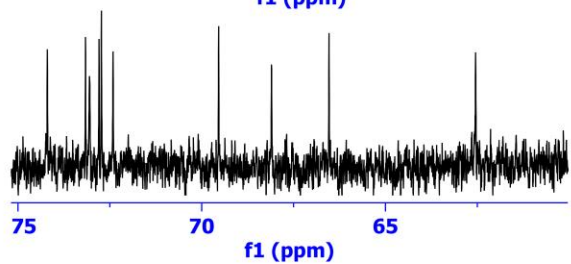
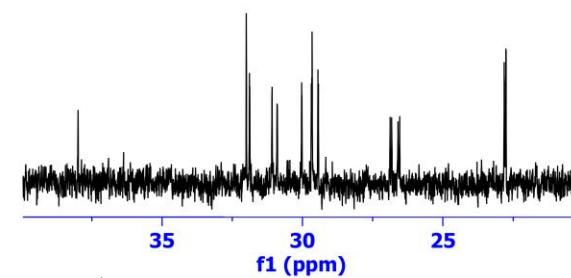
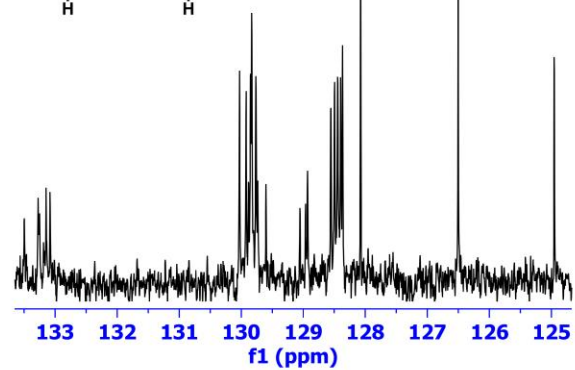
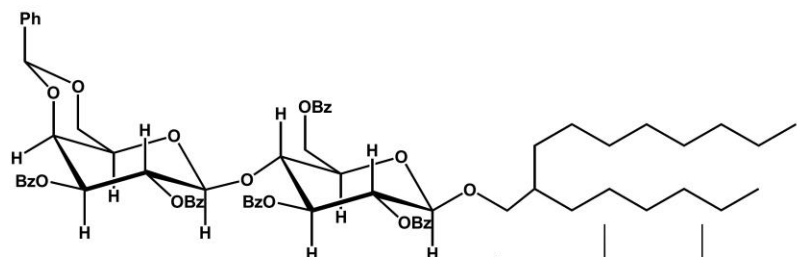
Table S2: Preparation of formulations on immunization day D0 and D21. The formulation preparation is for 5 mice. Measured volume of liposome (248 μ L of 1.25X liposomes) in corresponding vials and group# are provided and kept at 4°C until the day of injection.

		# mice	Preparation		Final formulation ready for injection					
Gr#	Description			Volume (μL)	Stock solution concentration for injection			Per injection volume		
					Volume (μL)	Lipid concentration	Ova	Lipid (mg)	Ova (μg)	
								Per 50 μL inj (i.m.)		
1	DMPC: Chol	5	DMPC: Chol (1.25X)	248	310	20.0 mg/mL (DMPC: 15.0 mg/mL) (Chol: 5.00 mg/mL)	200 μg/mL	1.0 mg (DMPC: 0.75 mg) (Chol: 0.25 mg)	10	
			Ova 5x (1 mg/mL)	62						
2	DMPC: Chol: 10A	5	DMPC: Chol: 10A (1.25X)	248	310	30.0 mg/mL (DMPC: 15.0 mg/mL) (Chol: 5.0 mg/mL) (TS41: 10 mg/mL)	200 μg/mL	1.5 mg (DMPC: 0.75 mg) (Chol: 0.25 mg) (10A: 0.5 mg)	10	
			Ova 5x (1 mg/mL)	62						
3	DMPC: Chol: 10B	5	DMPC: Chol: 10B (1.25X)	248	310	30.0 mg/mL (DMPC: 15.0 mg/mL) (Chol: 5.0 mg/mL) (TS43: 10 mg/mL)	200 μg/mL	1.5 mg (DMPC: 0.75 mg) (Chol: 0.25 mg) (10B: 0.5 mg)	10	
			Ova 5x (1 mg/mL)	62						
			Ova 5x (1 mg/mL)	62						
4	SLA	5	SLA (1.25X)	248	310	10.0 mg/mL (SLA: 10 mg/mL)	200 μg/mL	0.5 mg (SLA: 0.5 mg)	10	
			Ova 5x (1 mg/mL)	62						
5	DMPC: Chol: SLA	5	DMPC: Chol: SLA	248	310	30.0 mg/mL (DMPC: 15.0 mg/mL) (Chol: 5.0 mg/mL) (SLA: 10 mg/mL)	200 μg/mL	1.5 mg (DMPC: 0.75 mg) (Chol: 0.25 mg) (SLA: 0.5 mg)	10	
			Ova 5x (1 mg/mL)	62						
6	Ova alone	5	PBS	248	310	n.a.	200 μg/mL	n.a.	10	
			Ova 5x (1 mg/mL)	62						
7	Naive	5	PBS ONLY	310	310					

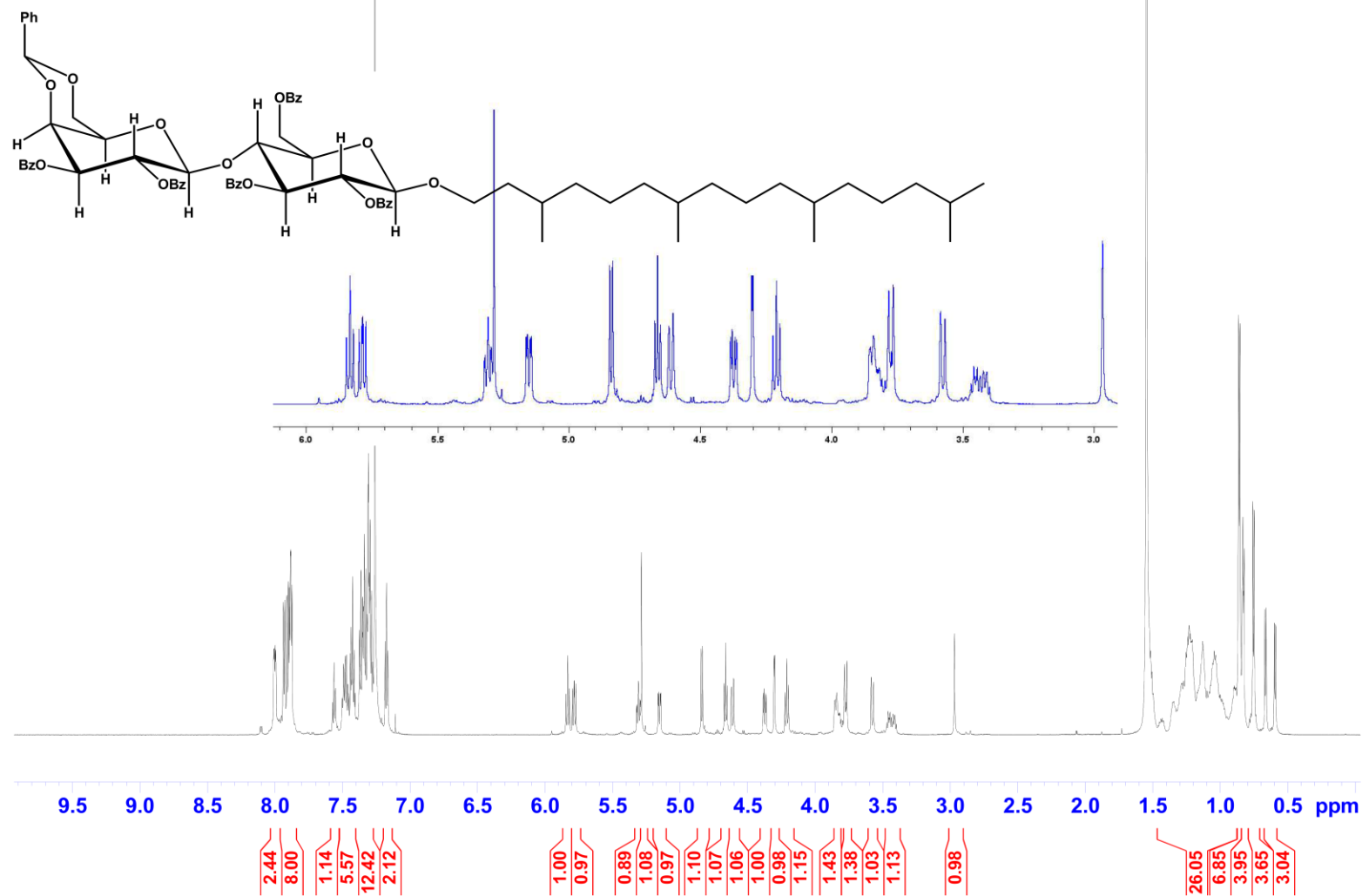
Compound 7a
 ^1H NMR (700 MHz, CDCl_3)



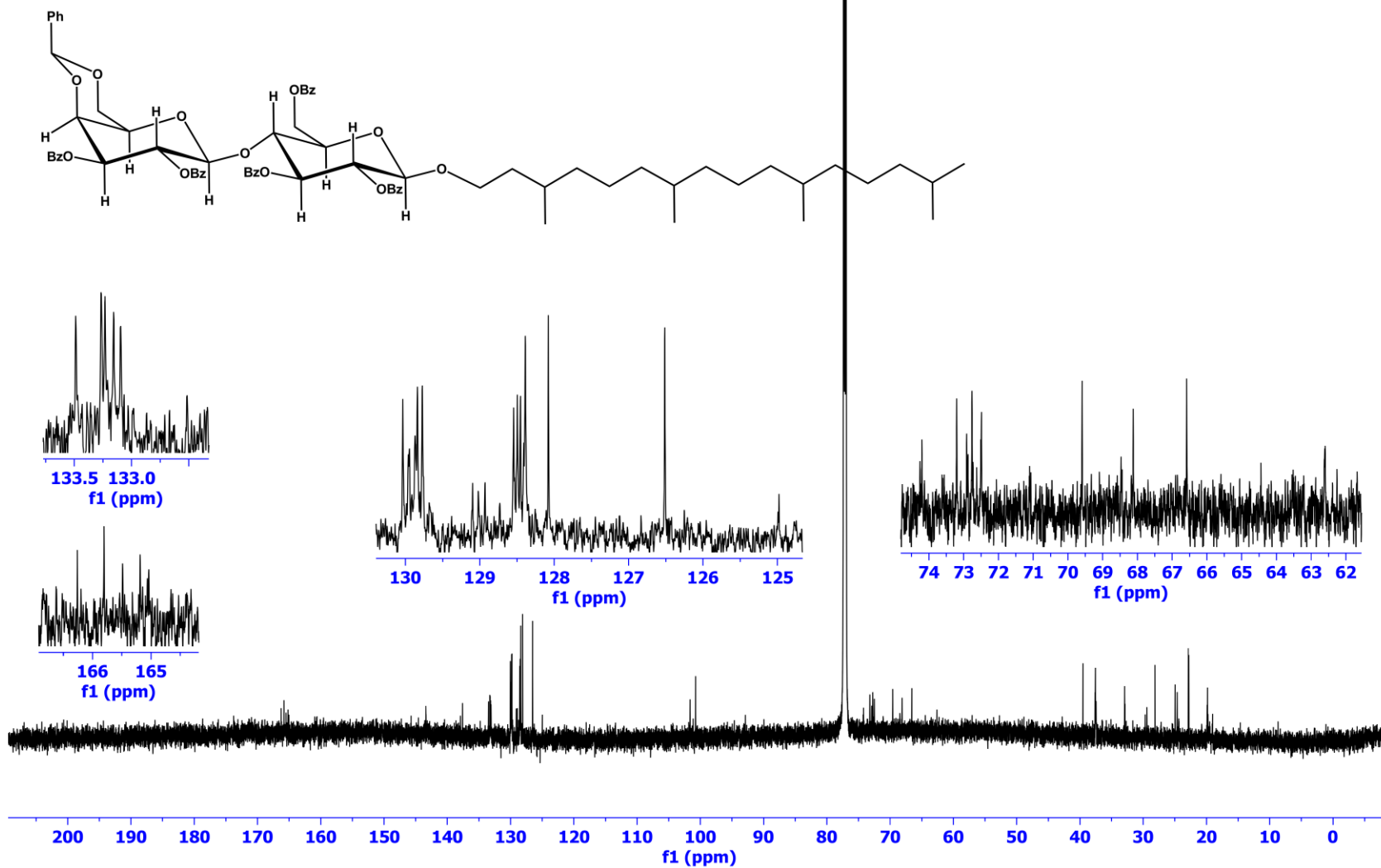
Compound 7a
 ^{13}C NMR (176 MHz, CDCl_3)



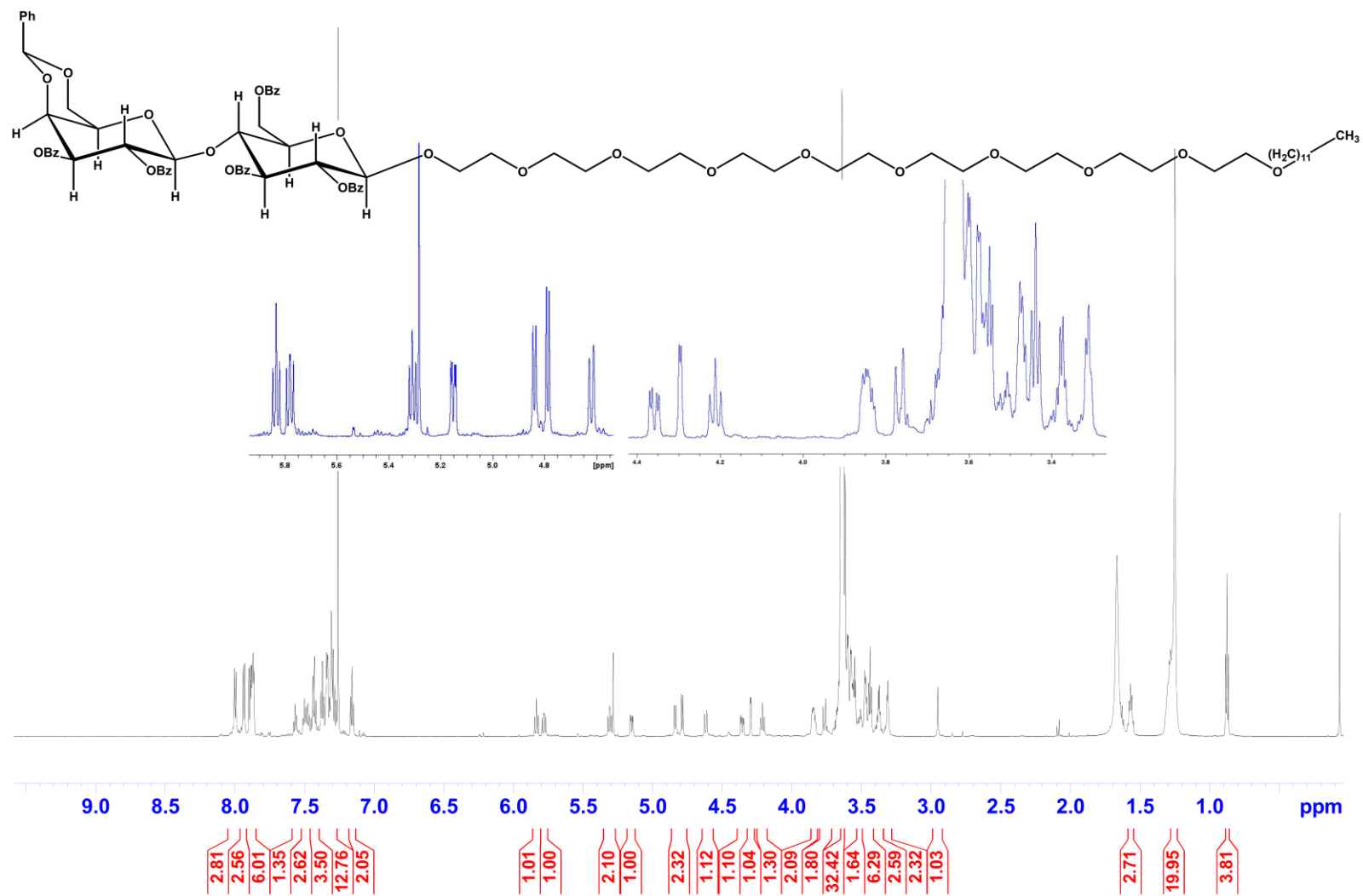
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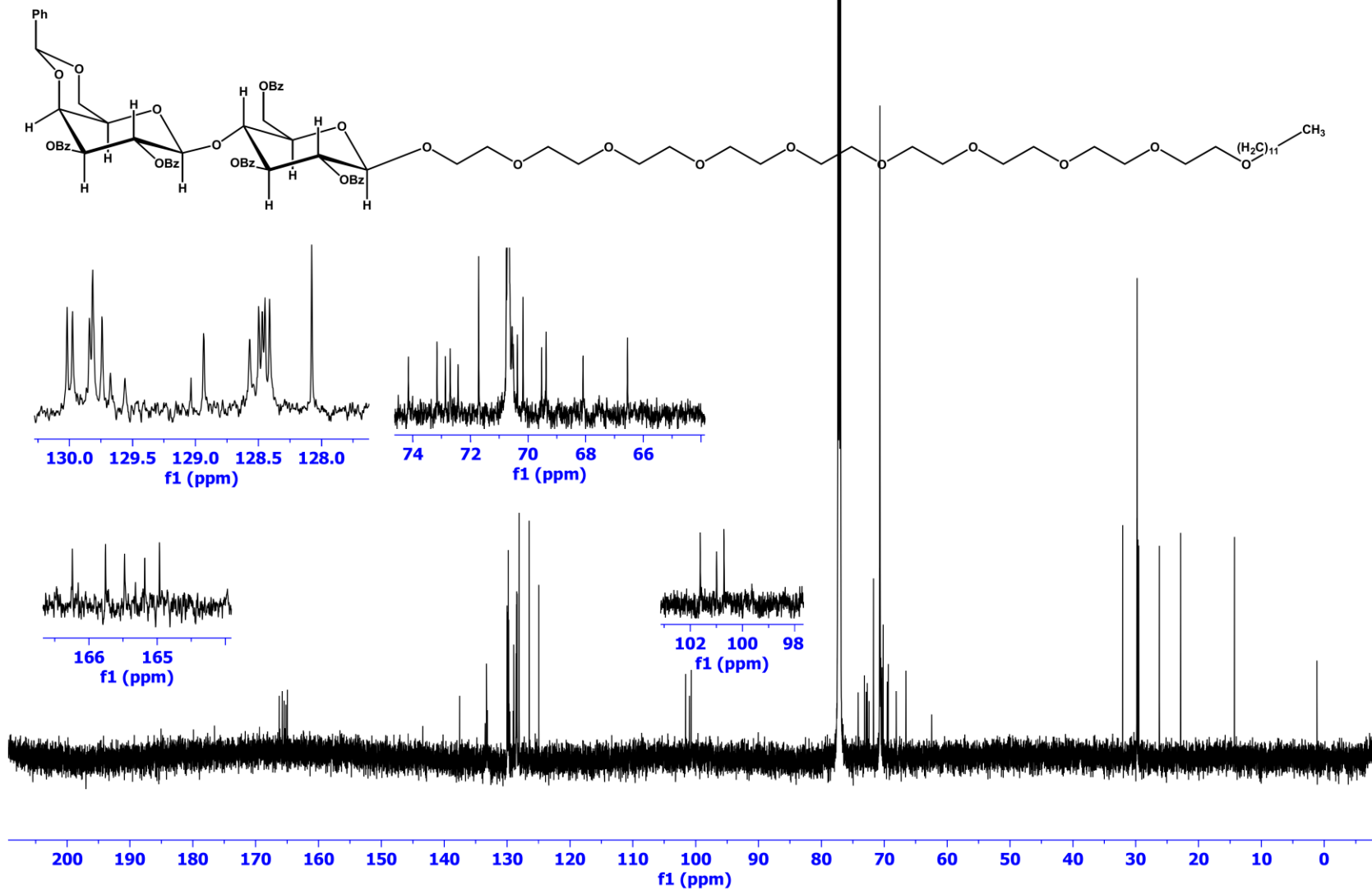
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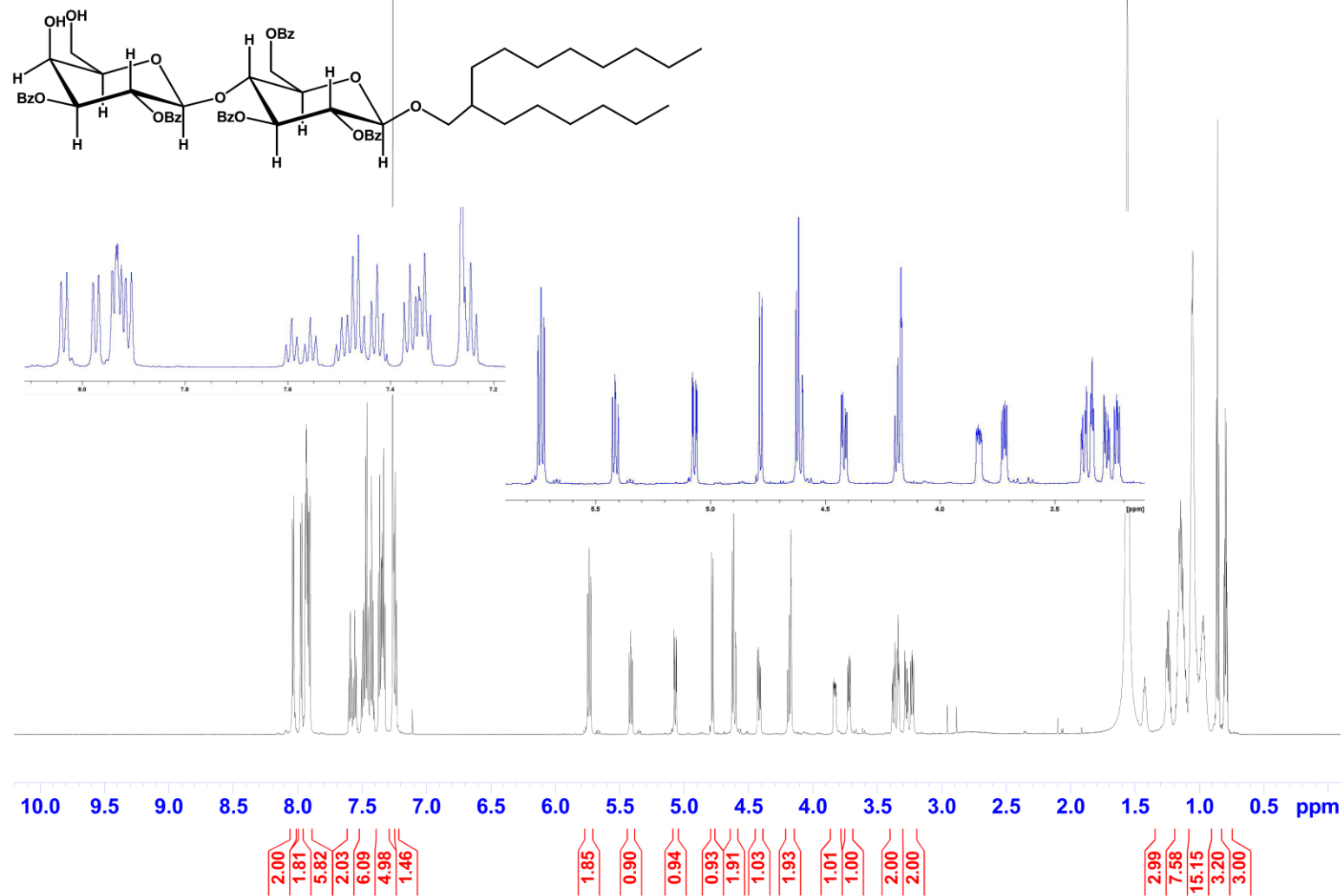
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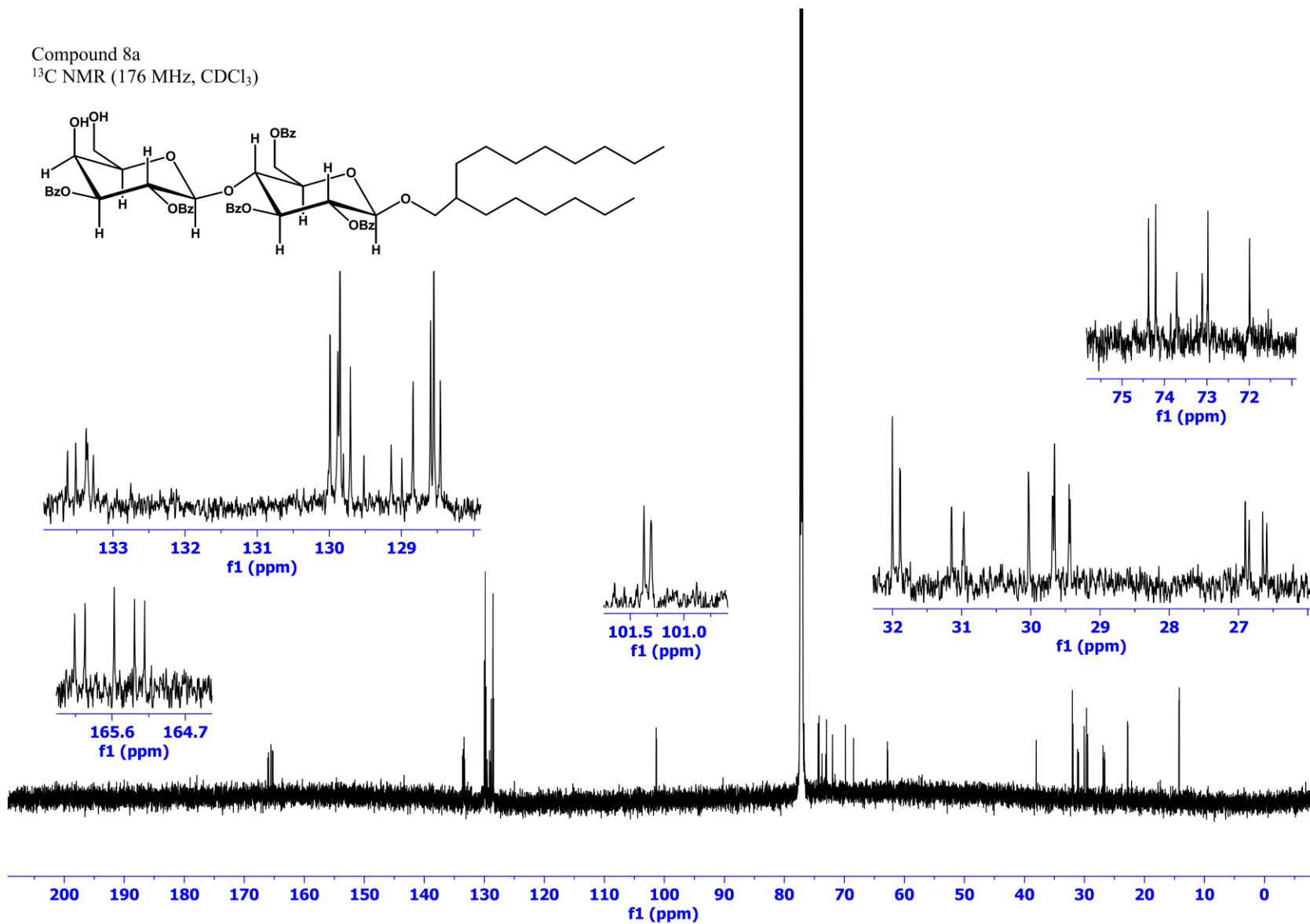
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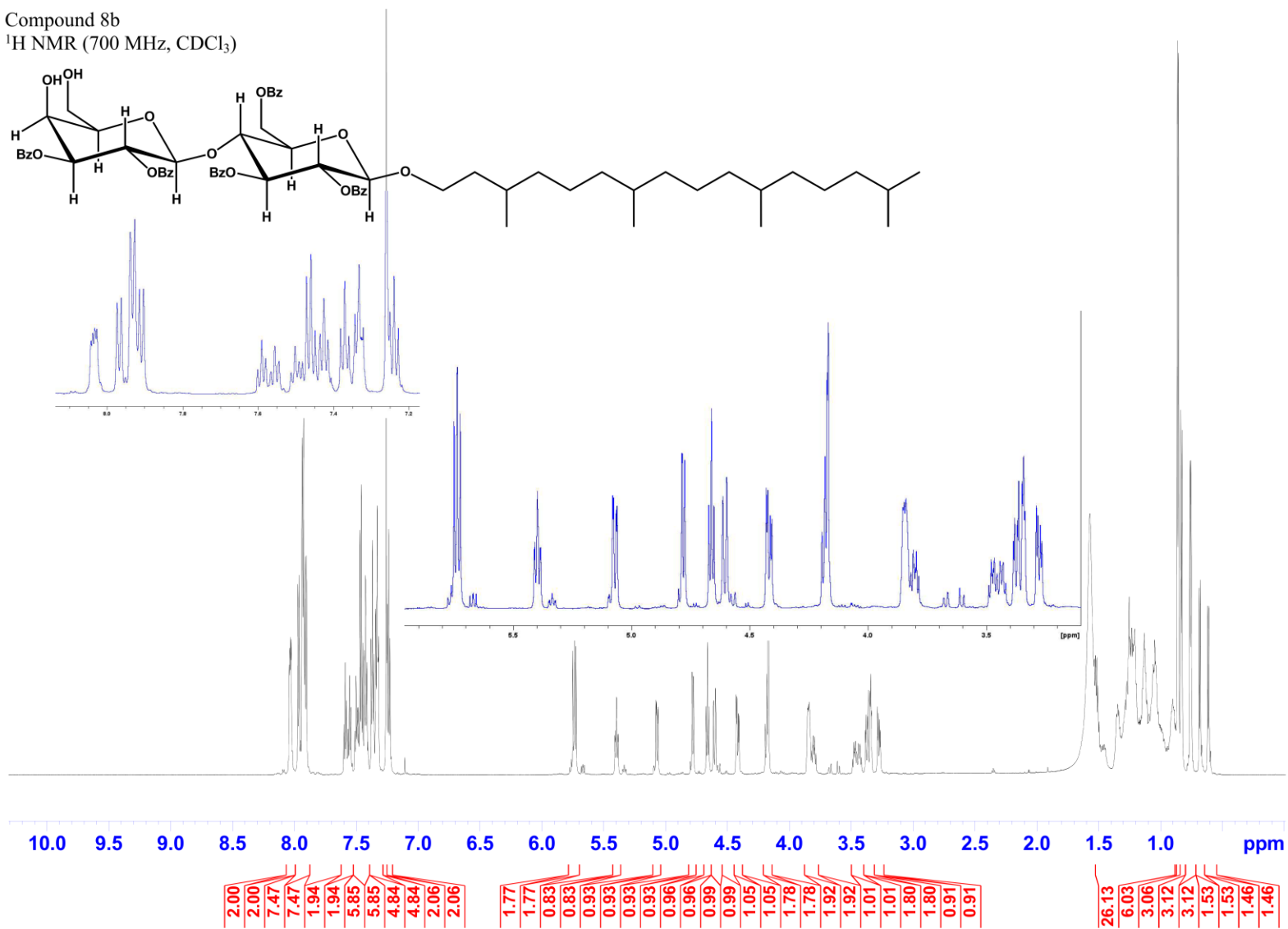
Compound 8a
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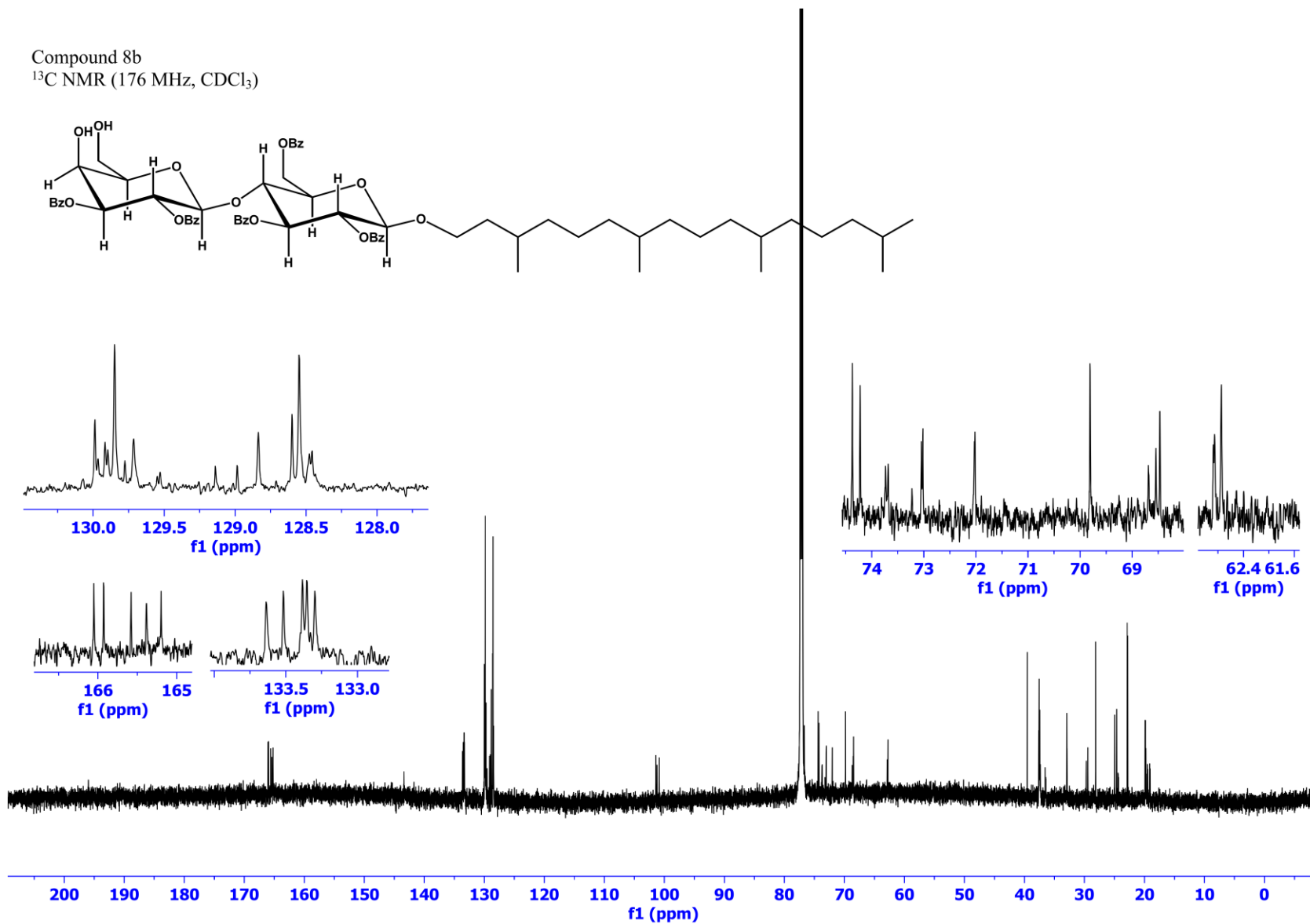
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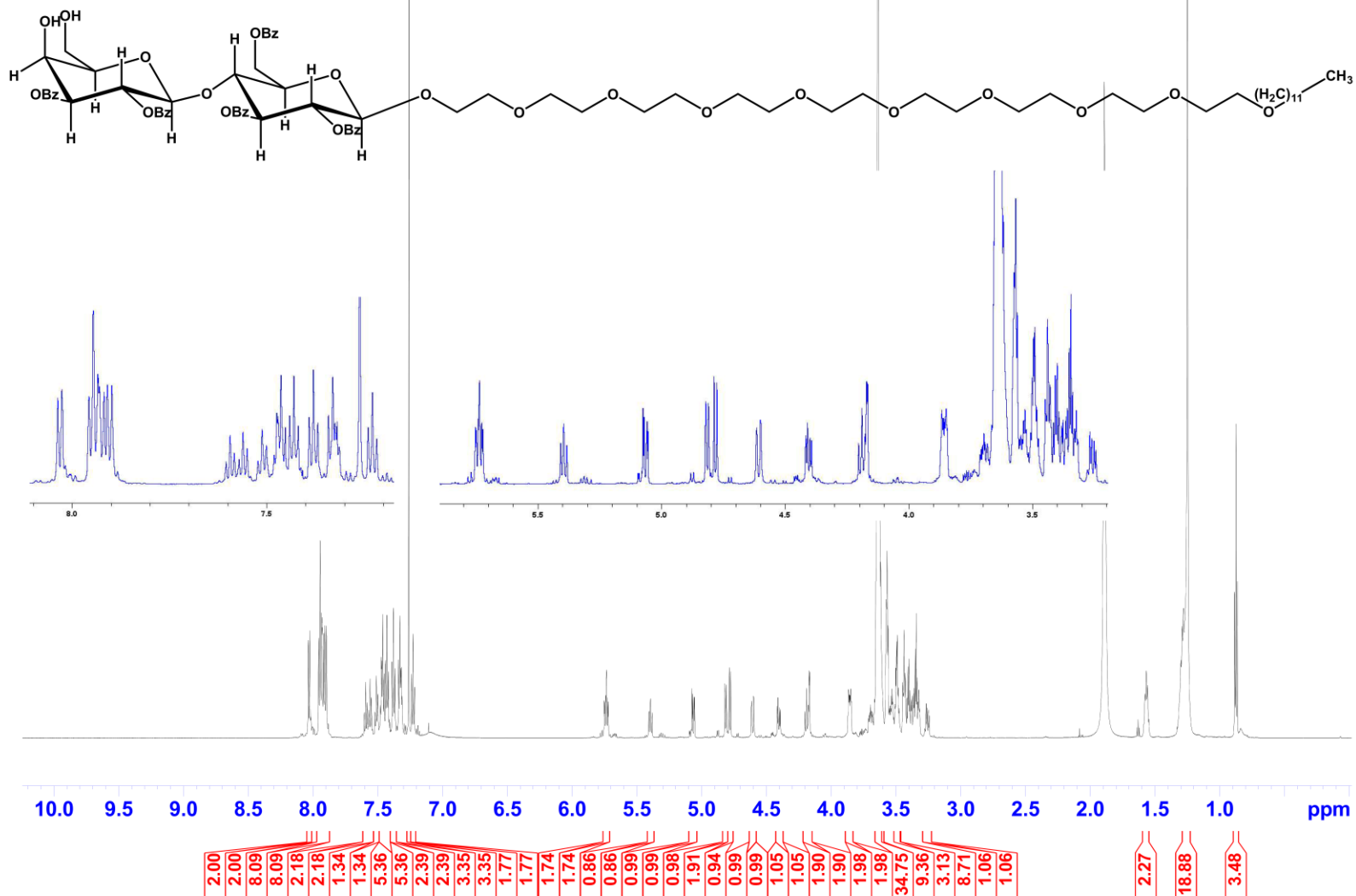
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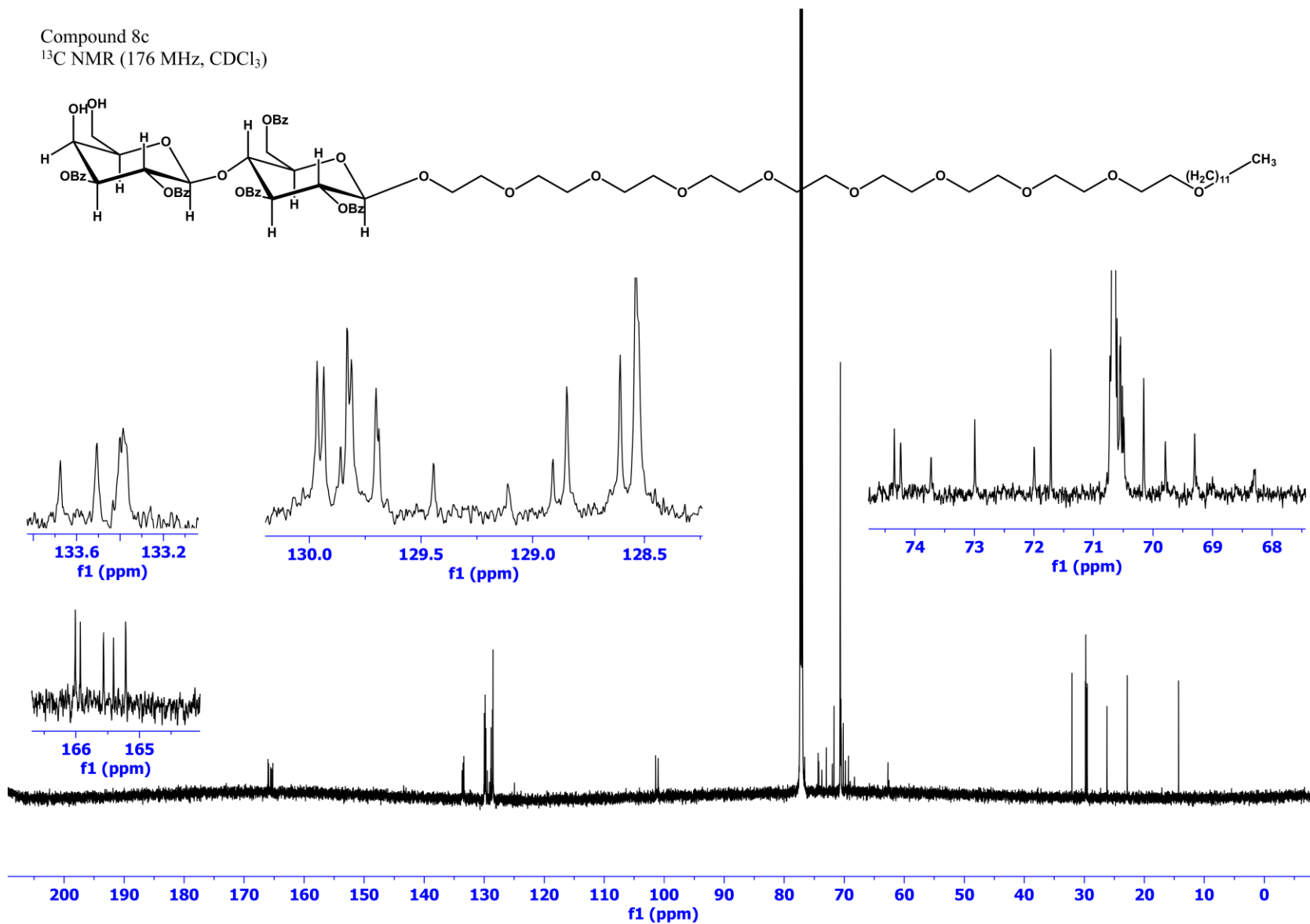
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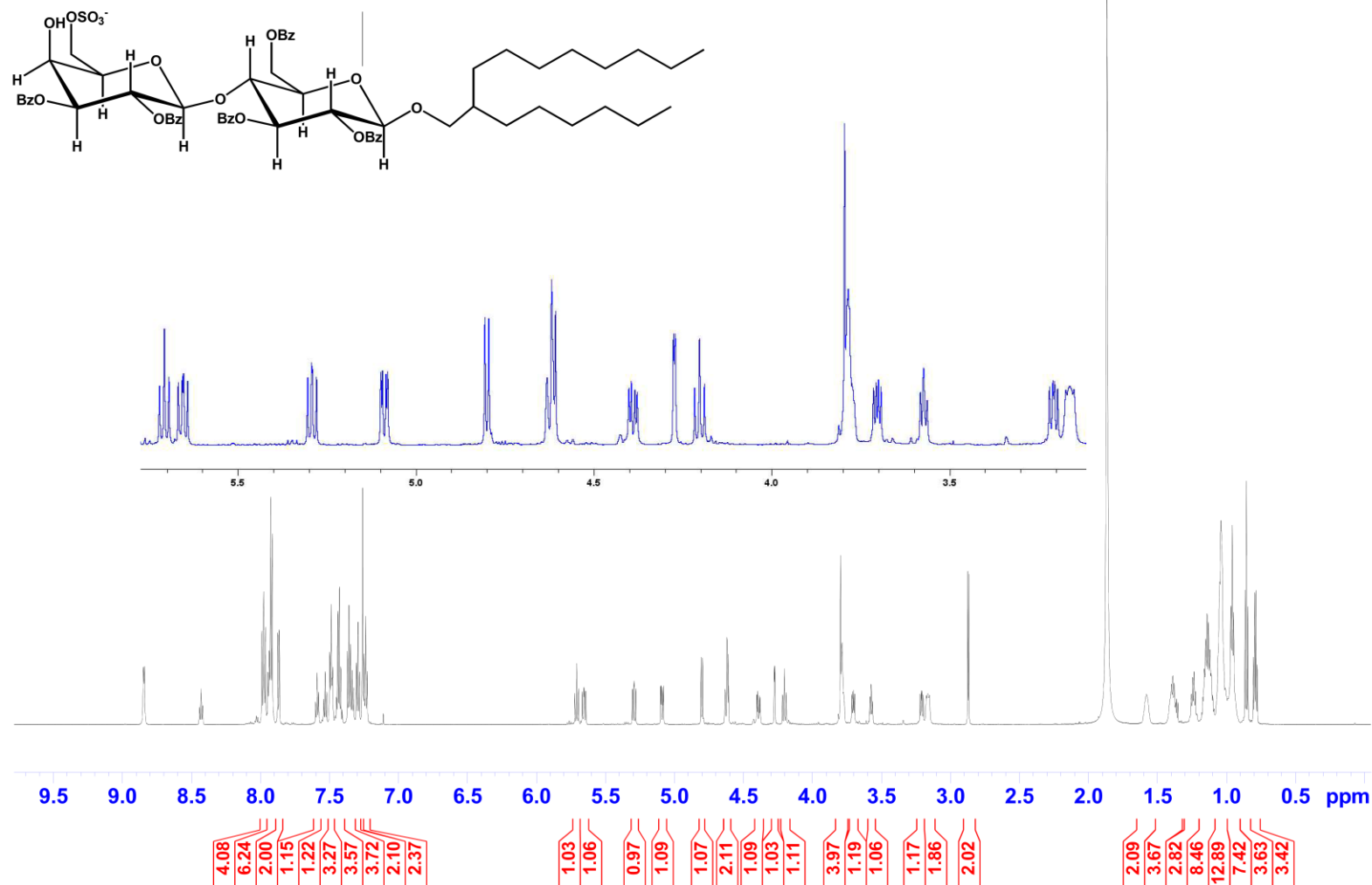
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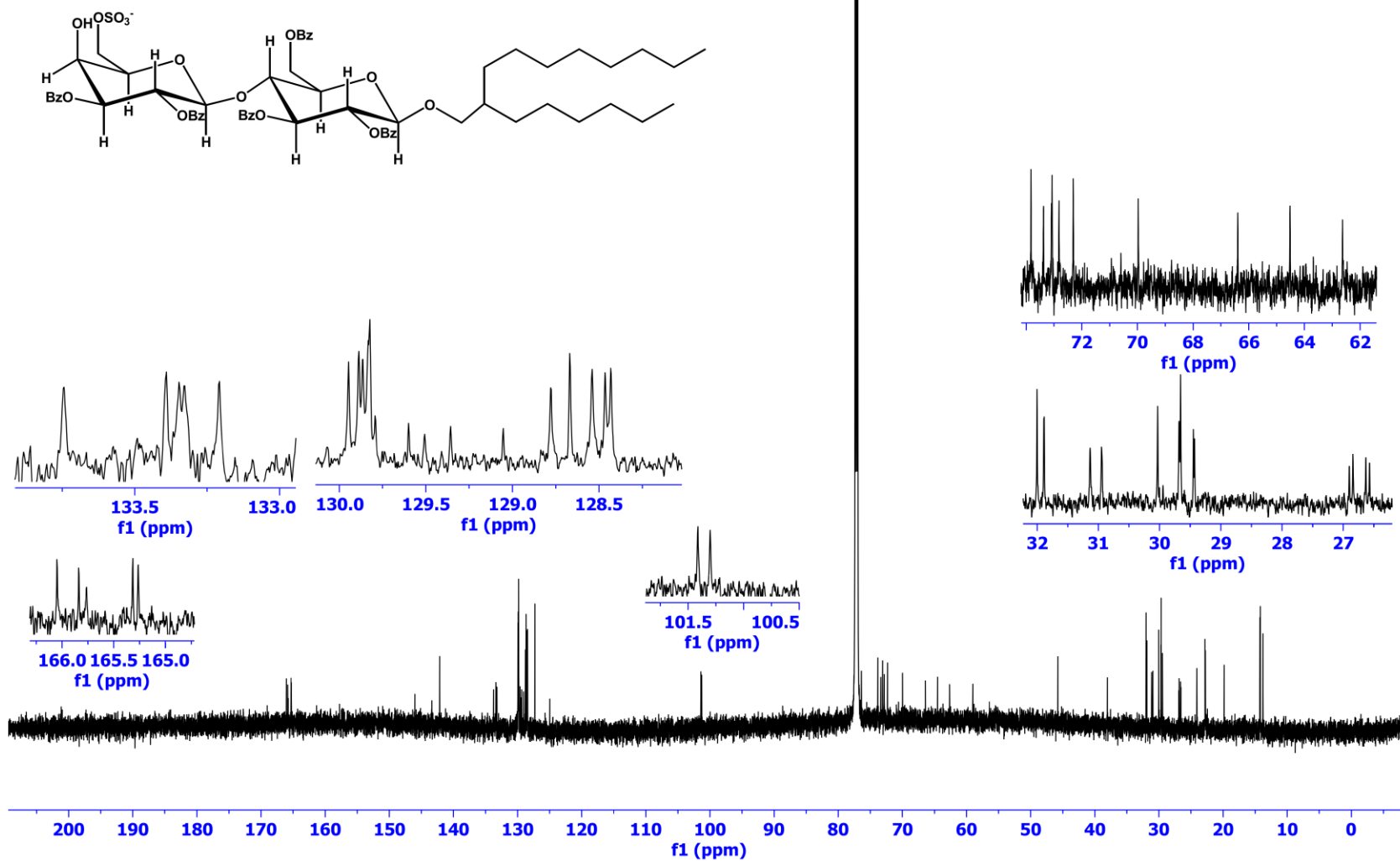
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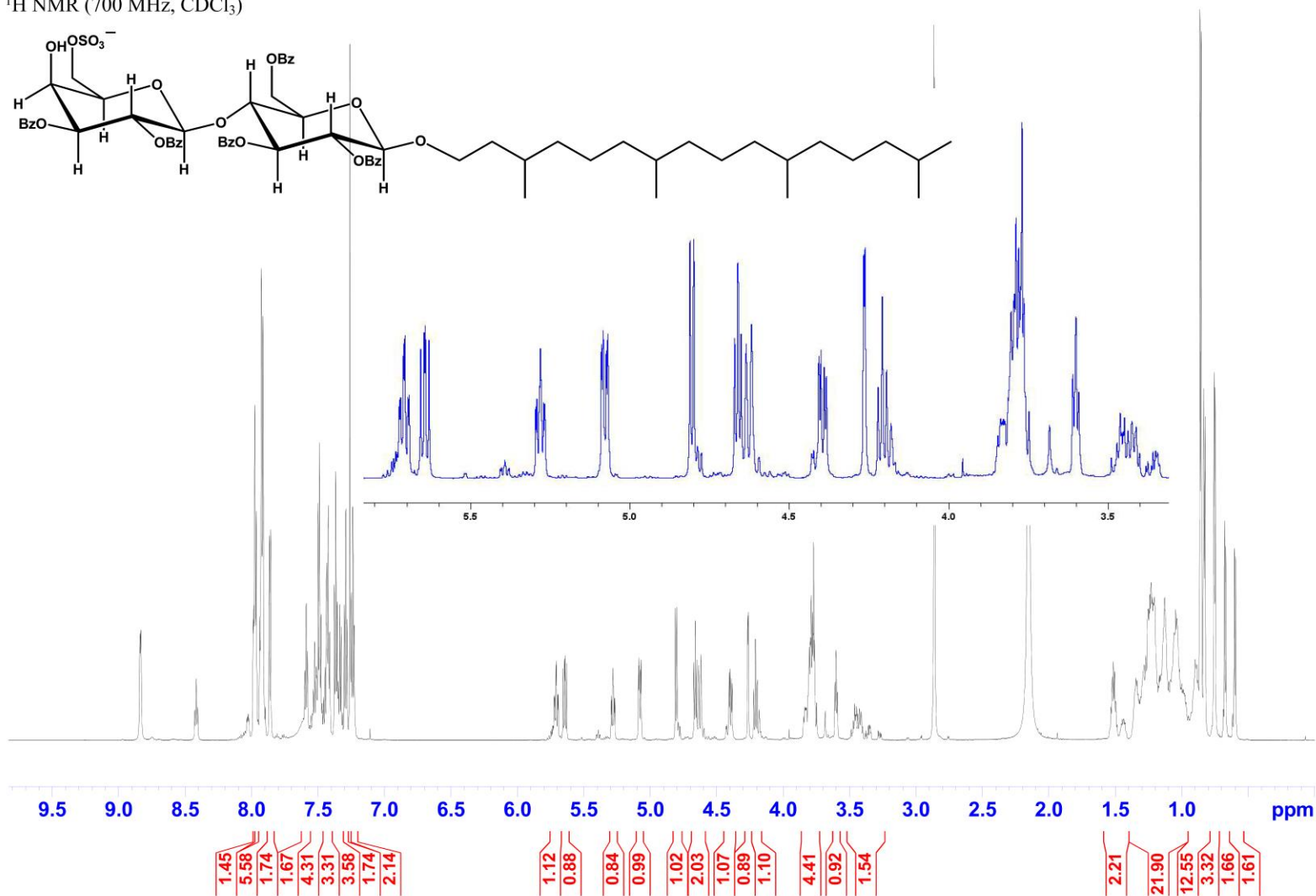
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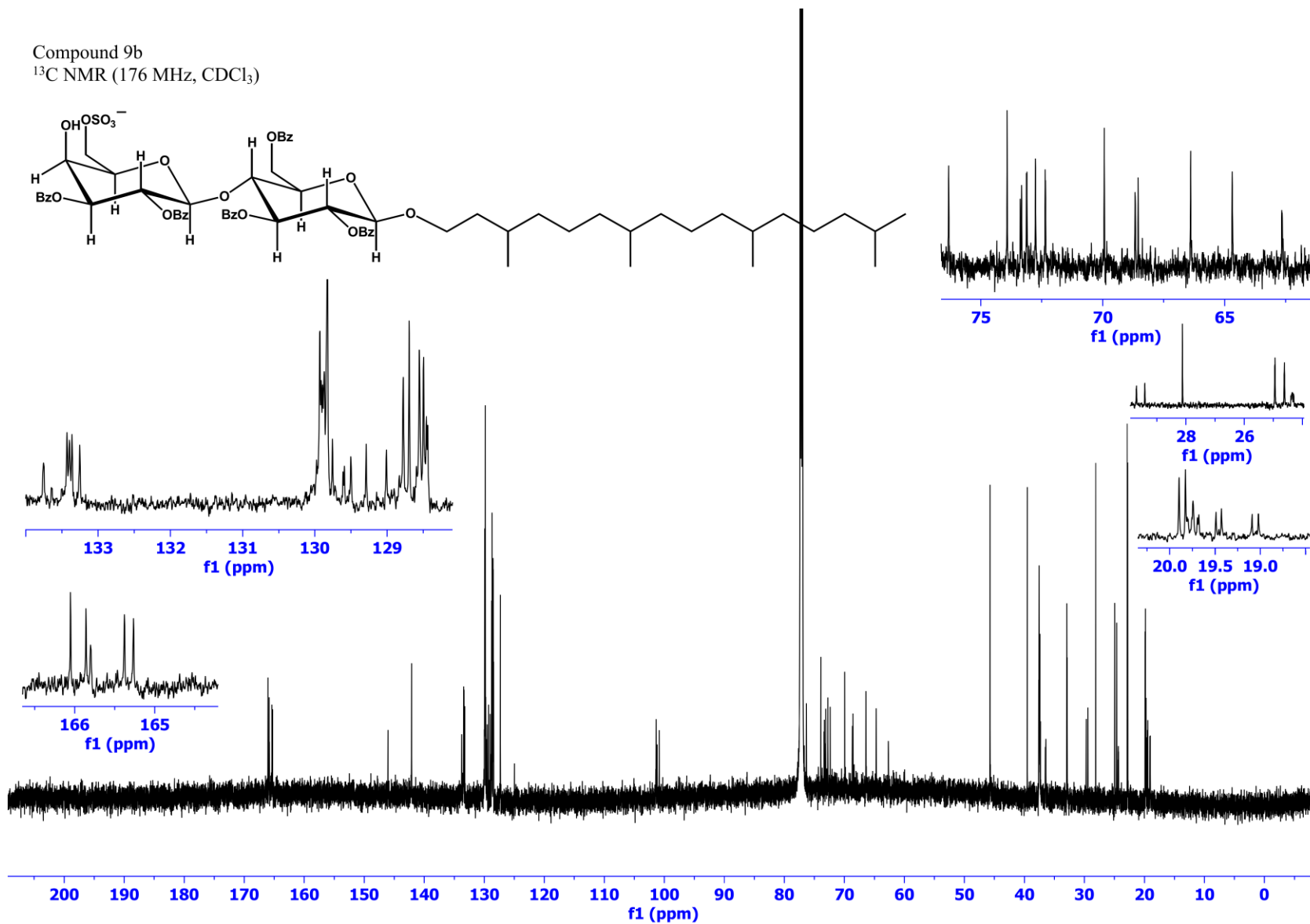
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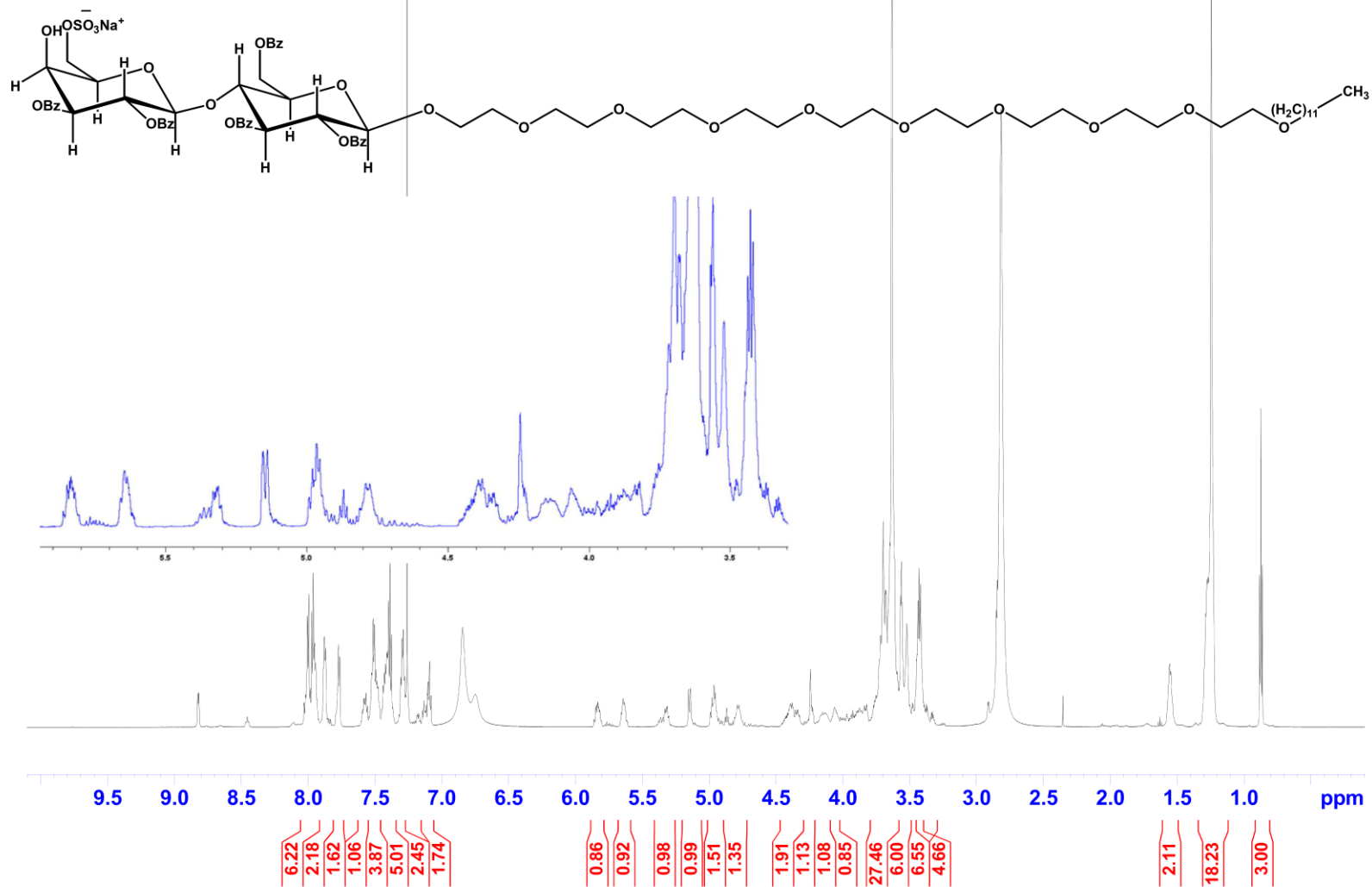
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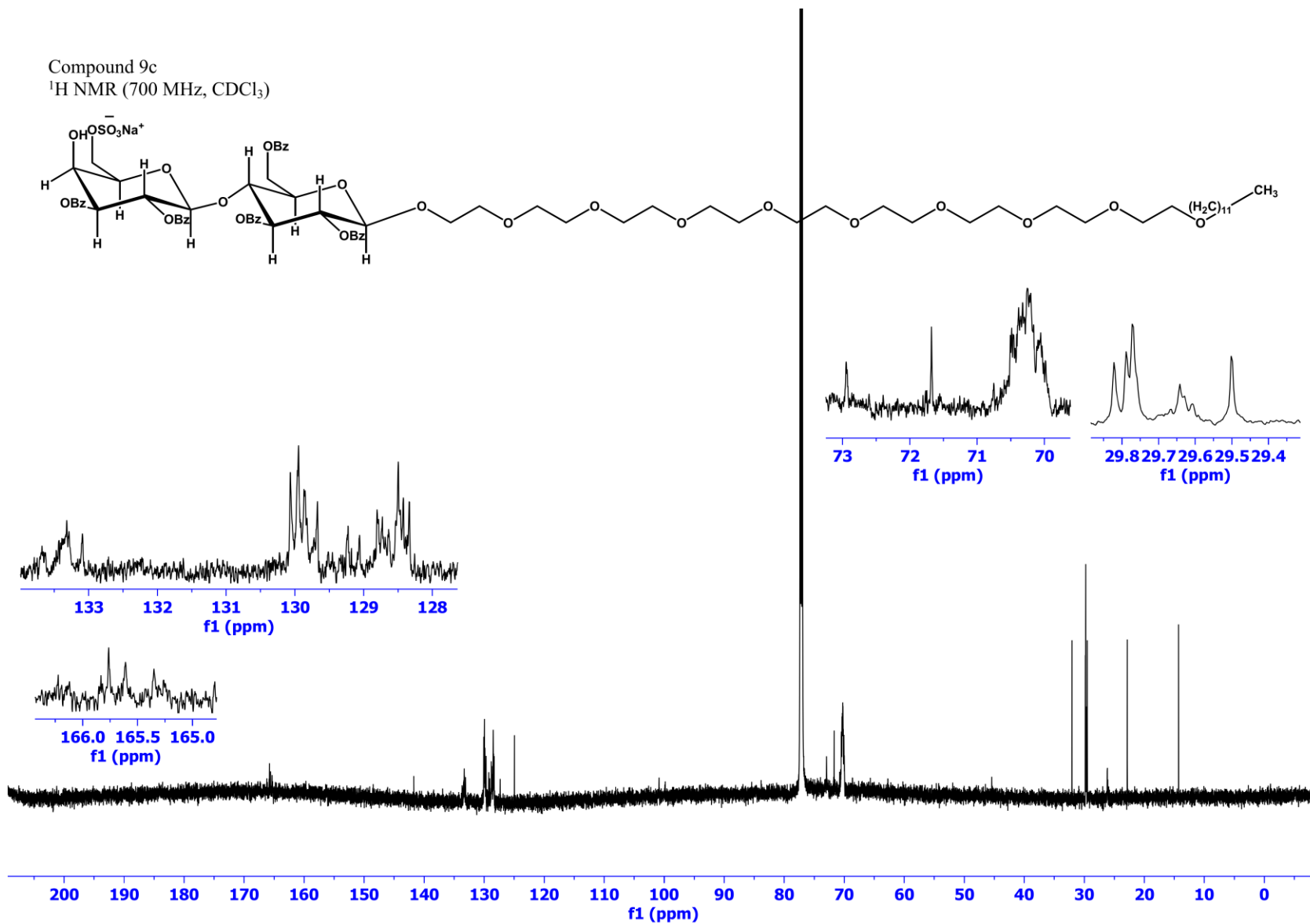
Compound 9b
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Compound 9c
¹H NMR (700 MHz, CDCl₃)

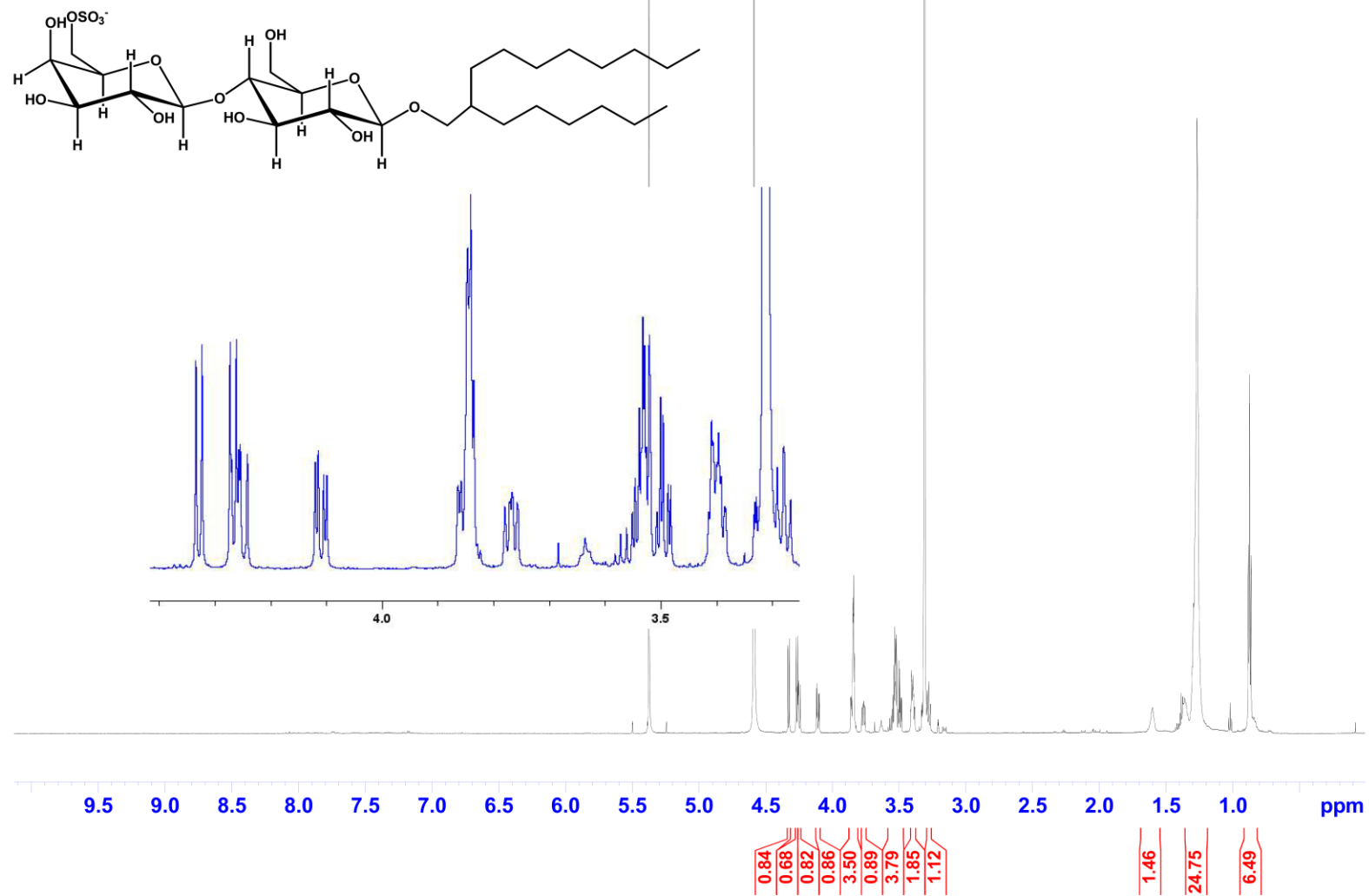


Compound 9c
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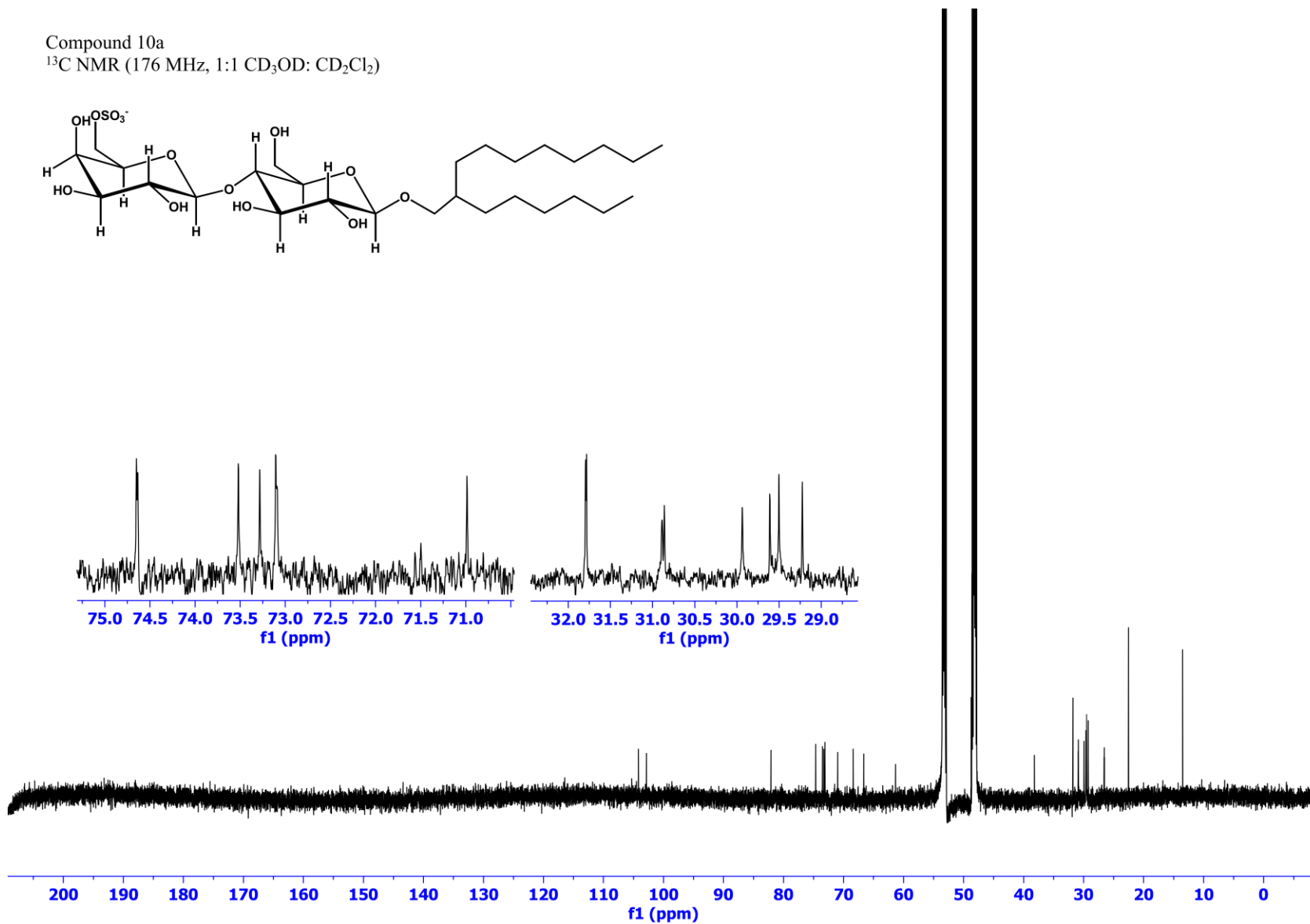
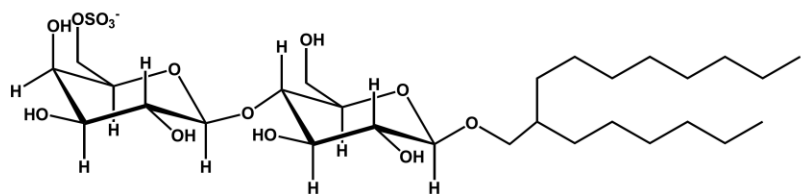
Compound 10a

^1H NMR (700 MHz, 1:1 CD_3OD : CD_2Cl_2)



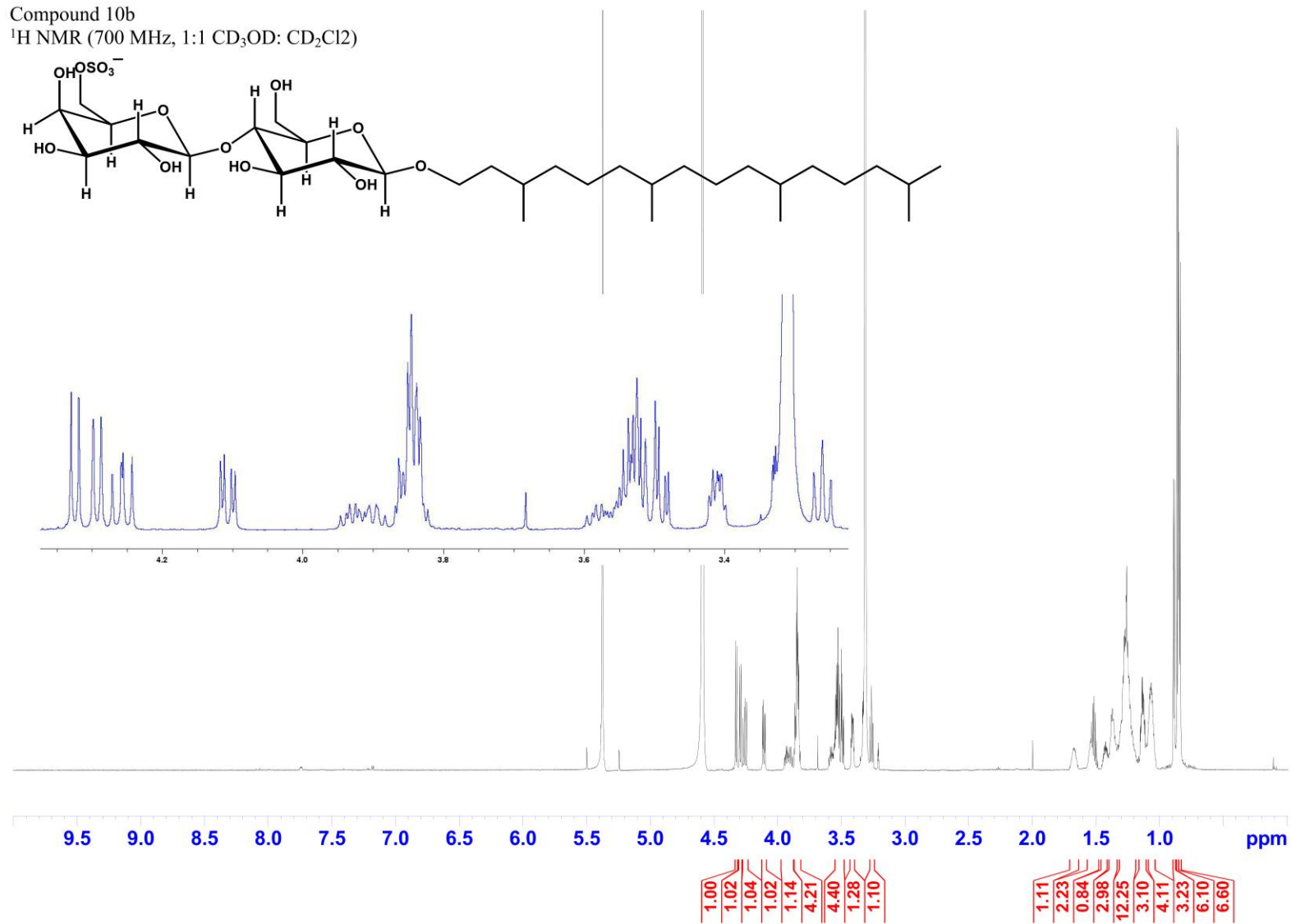
Compound 10a

^{13}C NMR (176 MHz, 1:1 CD_3OD : CD_2Cl_2)



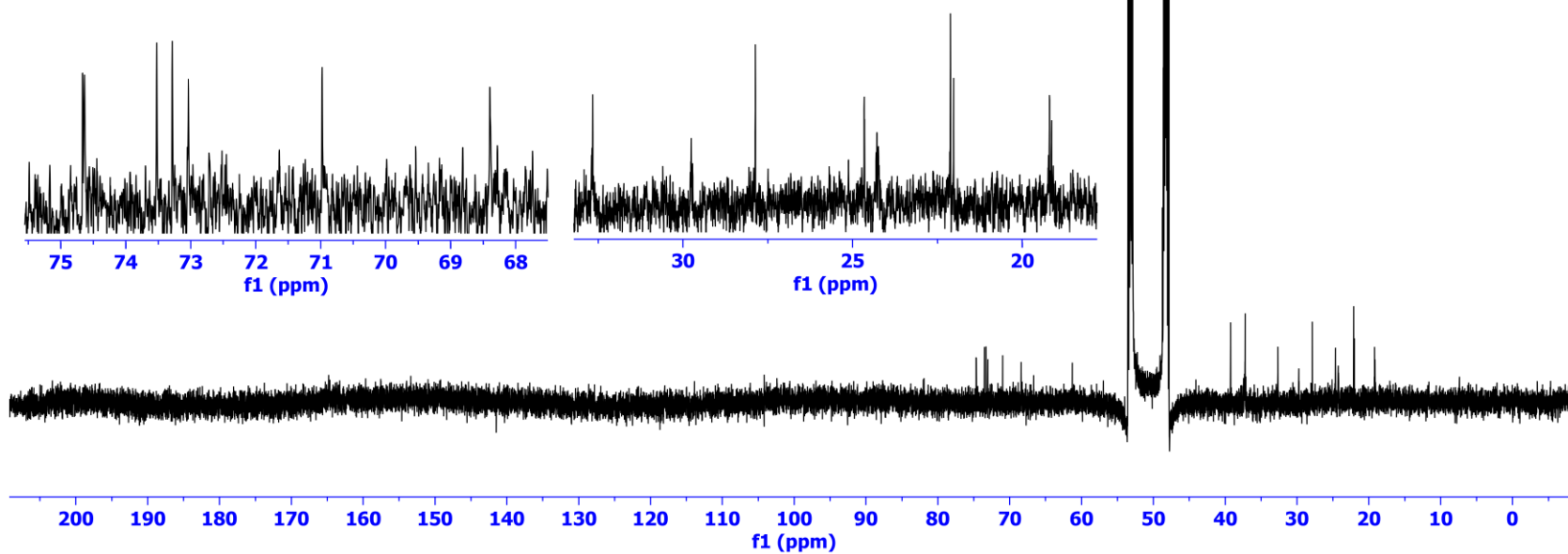
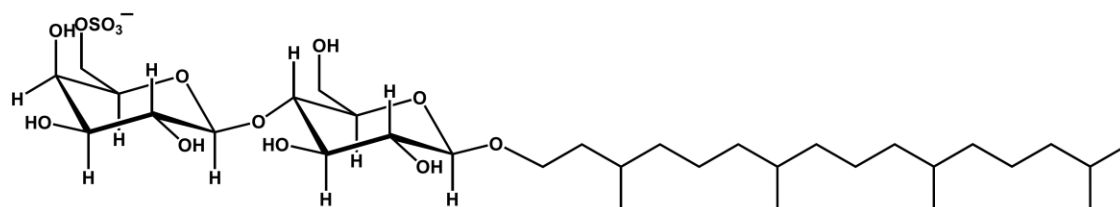
Compound 10b

^1H NMR (700 MHz, 1:1 CD_3OD : CD_2Cl_2)

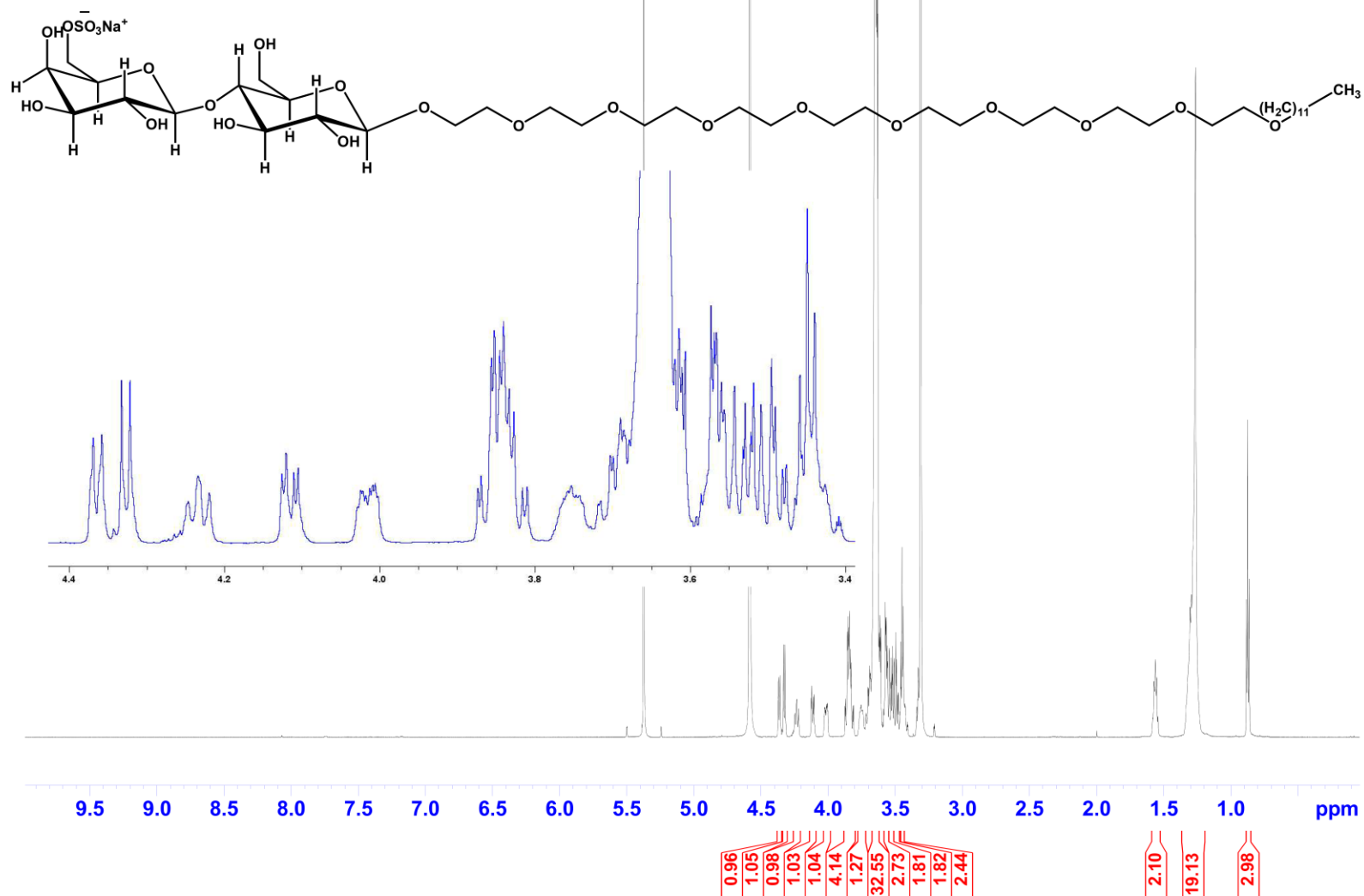


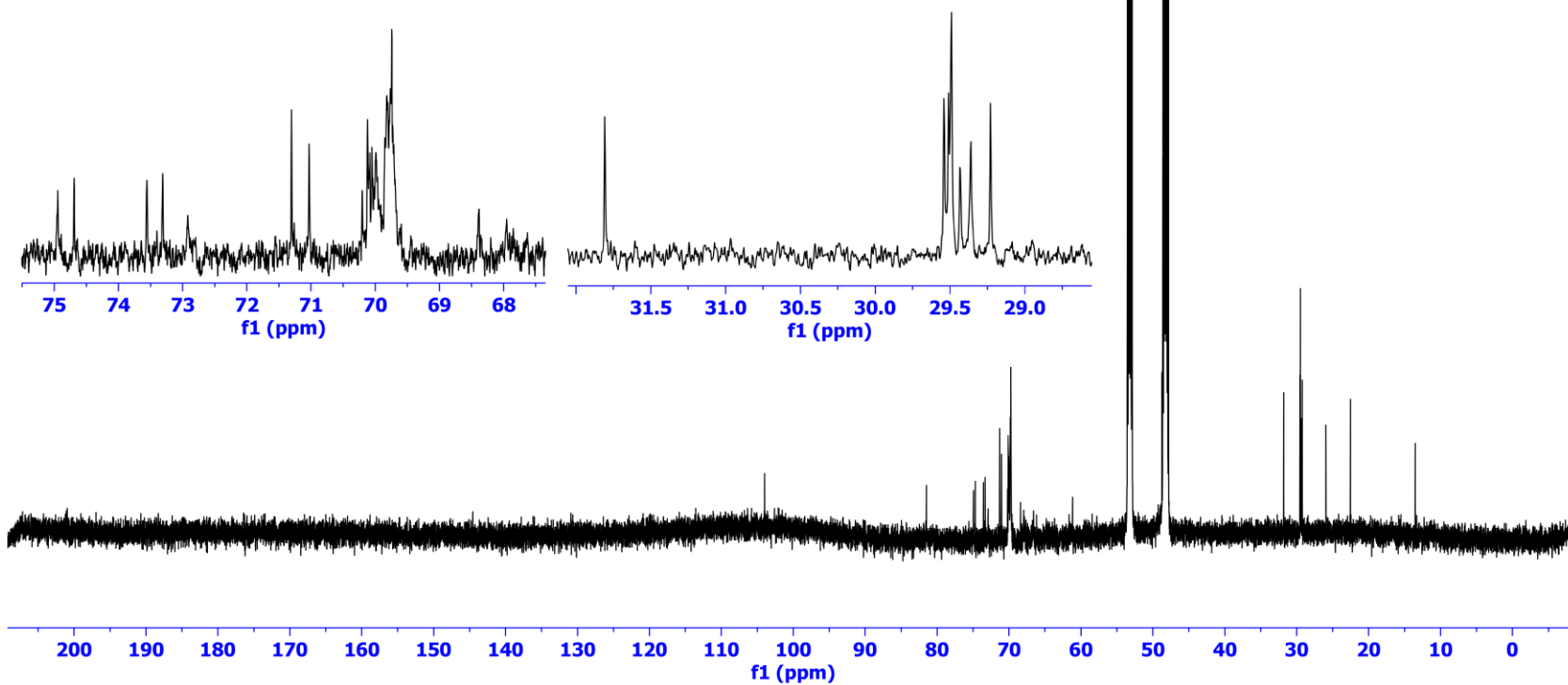
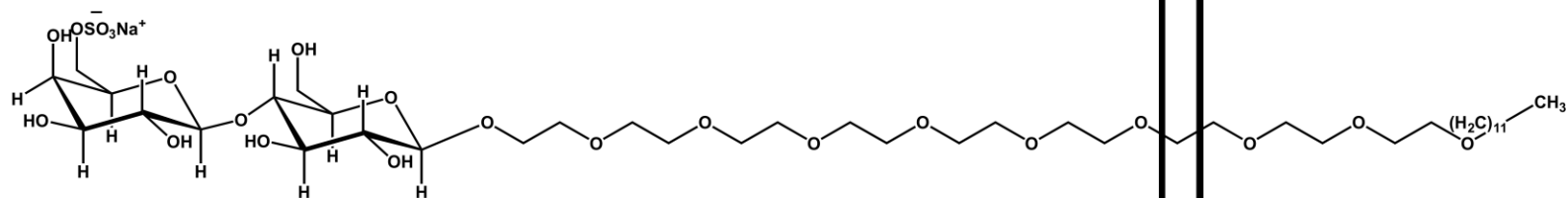
Compound 10b

^{13}C NMR (176 MHz, 1:1 CD_3OD : CD_2Cl_2)



Compound 10c
 ^1H NMR (700 MHz, 1:1 CD_3OD : CD_2Cl_2)



¹³C NMR (176 MHz, 1:1 CD₃OD: CD₂Cl₂)

References:

1. S. Regnier, E. Lam, V. Vasquez, C. F. Martinez-Farina, F. C. Stark, G. Agbayani, L. Deschatelets, R. Dudani, B. A. Harrison, B. Akache, M. J. McCluskie and U. D. Hemraz, *J Med Chem*, 2022, **65**, 8332-8344.