Supporting Information

Regioselective Acylation of Levoglucosan Catalyzed by *Candida antarctica* (CaLB) lipase Immobilized on Epoxy Resin

Marcelo A. do Nascimento,[1] Larissa E. Gotardo,[1] Eduardo M. Bastos,[2] Fabio C. L. Almeida,[3] Raquel A. C. Leão,[1,4] Rodrigo O. M. A. de Souza,[1] Robert Wojcieszak[5] and Ivaldo Itabaiana Jr [6*]

1- BOSS Group - Biocatalysis and Organic Synthesis Group, Chemistry Institute, Federal University of Rio de Janeiro, CEP: 21941-909, Brazil;

2- Instituto de Macromoléculas Professora Eloisa Mano, Federal University of Rio de Janeiro, CEP 21941-598, Brazil.

3- Center of Structural Biology and Bioimaging I (CENABIO I), Federal University of Rio de Janeiro, Rio de Janeiro, Brazil.

4 – Pharmacy School, Federal Universityof Rio de Janeiro, CEP: 21941-170, Brazil;

5- Univ. Lille, CNRS, Centrale Lille, ENSCL, Univ. Artois, UMR 8181 - UCCS - Unité de Catalyse et Chimiedu Solide, F-59000, Lille, France;

6 - Department of Biochemical Engineering, School of Chemistry, Federal University of Rio de Janeiro, CEP: 21941-910, Brazil.

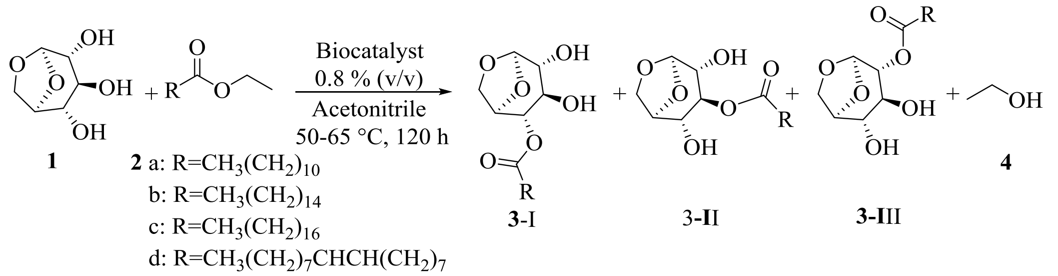
Corresponding author: ivaldo@eq.ufrj.br

Keywords: Levoglucosan, CaLB, lipase, transesterification, esters.

1. Reaction Optimization

The reaction of levoglucosan (1) and aliphatic esters (laurate, palmitate, stearate and oletate, respectively) was optimized for the formation of products with respect to different biocatalysts (N435, Calb_epoxy and PSIM) and incubation temperature.

Table 1: Regioselectivity in the enzymatic acetylation of levoglucosan in CH_3CN with ethyl aliphatic esters. Analyses were performed in a GC-MS.



Entry	Biocatalyst[1]	Acyl Donor[2]	Regioselectivity (%)								
			50°C			55°C			65°C		
			3-I	3-II	3-III	3-I	3-II	3-III	3-I	3-II	3-III
1	N435	a	99	-	-	48	-	52	51	-	49
2		b	46	-	54	50	-	50	50	-	50
3		c	23	23	54	51	-	49	60	-	40
4		d	72	-	28	68	-	32	66	-	34
5	CalLB_epoxy	a	73	-	27	64	-	36	99	-	-
6		b	51	-	49	73	-	27	94	-	6
7		c	-	-	-	78	-	22	81	-	19
8		d	99	-	-	83	-	17	99	-	-
9	PSIM	a	99	-	-	87	-	13	95	5	-
10		b	90	-	10	79	-	21	99	-	-
11		c	72	28	-	43	14	43	-	-	-
12		d	74	-	26	97	-	3	99	-	-

2. Analytical Methods

^{1}H-NMR, ^{13}C-NMR; COSY, NOEDIFF, HMBC, HSQC and Dept135 spectra were recorded on a Bruker Advance 400 and 500 MHz spectrometer. Reported chemical shifts (δ) are expressed in parts per million (ppm) down field from tetra methyl silane (TMS) and the coupling constant (J) in Hz.

[1] N435 = Novozym 435 (*Candida antarctica* lipase B), immobilized on macroporous acrylic type ion exchange resin; CaLB_epoxy = *Candida antarctica* lipase B immobilized on epoxy support by our research group and PSIM = lipase from *Pseudomonas cepacia* immobilized on ceramic particles.

[2] 2 equivalents, a = ethyl laurate, b = ethyl palmitate, c = ethyl stearate and d = ethyl oleate.

GC analysis was performed on a Shimadzu CG2010 – SLB-5MS capillary column 30 m, 0.25 mm ID, 0.25 um film thickness (MS) and with helium used as the carrier gas. The injector and detector temperatures were 250°C, and The oven was maintained at 80 °C for 4 min, increased to 200 °C at a rate of 6 °C/min for 10 min, increased to 310 °C at a rate of 8 °C/min, when it was held constant for 4 min, totaling 51.75 min. High Resolution Mass Spectrometric Analysis (HRMS).

Infrared spectra were obtained in KBr pellets (complexes), in NICOLET MAGNA FTIR-760 (4000-400 cm^{-1}) spectrophotometer.

2.1. GC-MS

In this section, we will show the illustrative chromatograms obtained in the steps of higher product conversions with their respective mass spectra, in the transesterification reactions with ethyl laurate, ethyl palmitate, ethyl stearate and ethyl oleate for the synthesis of levoglucosan esters.

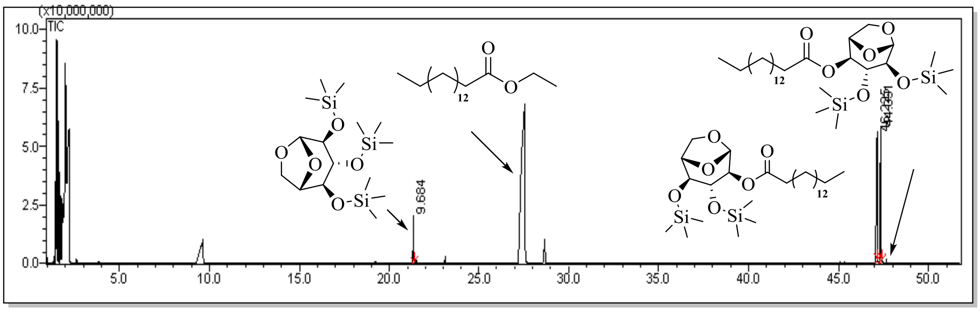


Figure 1: Chromatogram of levoglucosan reaction medium with ethyl palmitate using **N435** (derivatized) 55 °C. Peak with retention time at 9.2 for excess BSTFA.

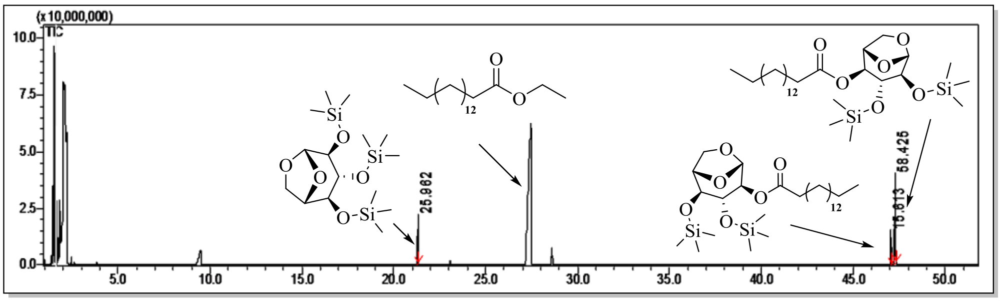


Figure 2: Chromatogram of levoglucosan reaction medium with ethyl palmitate using **PSIM** (derivatized) 55 °C. Peak with retention time at 9.2 for excess BSTFA

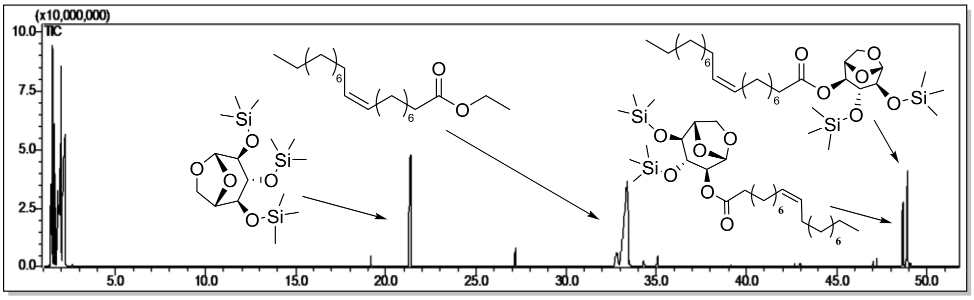
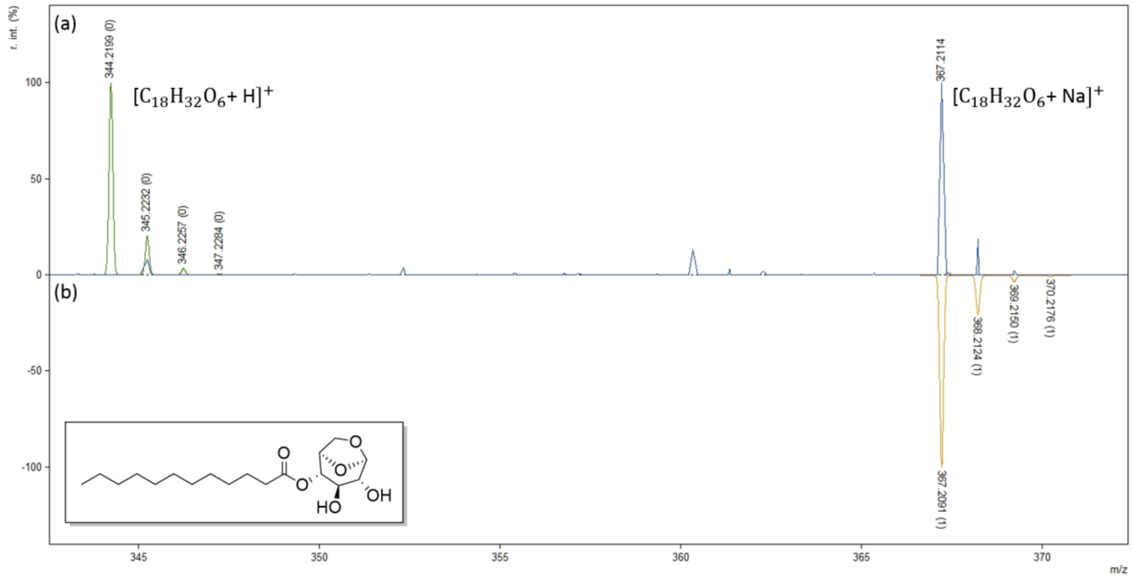


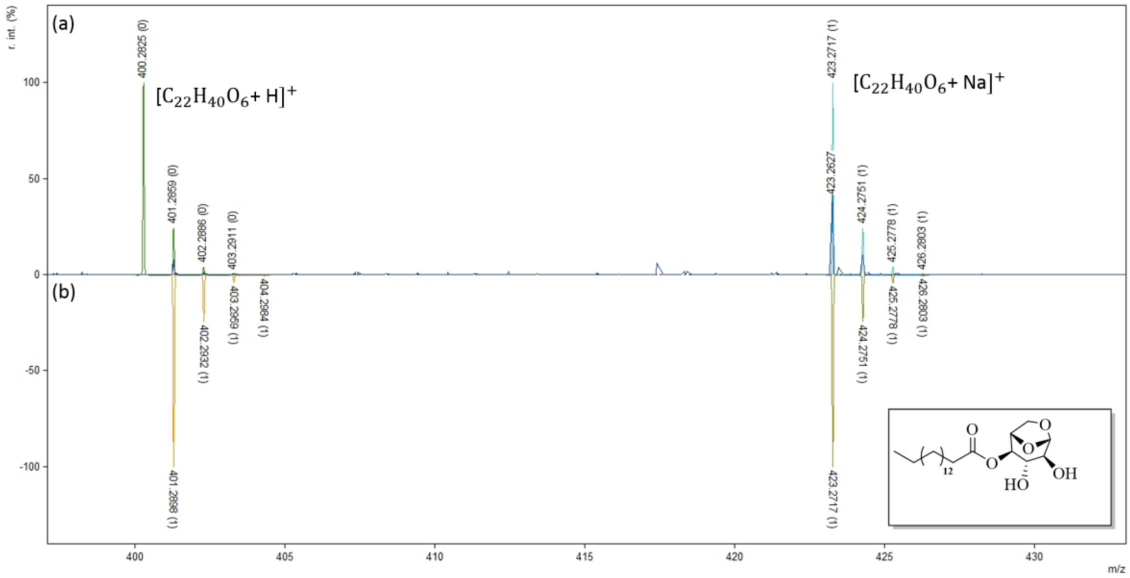
Figure 3: Chromatogram of levoglucosan reaction medium with ethyl oleate using **CaLB_epoxy** (derivatized) 55 °C.

2.2. HRMS

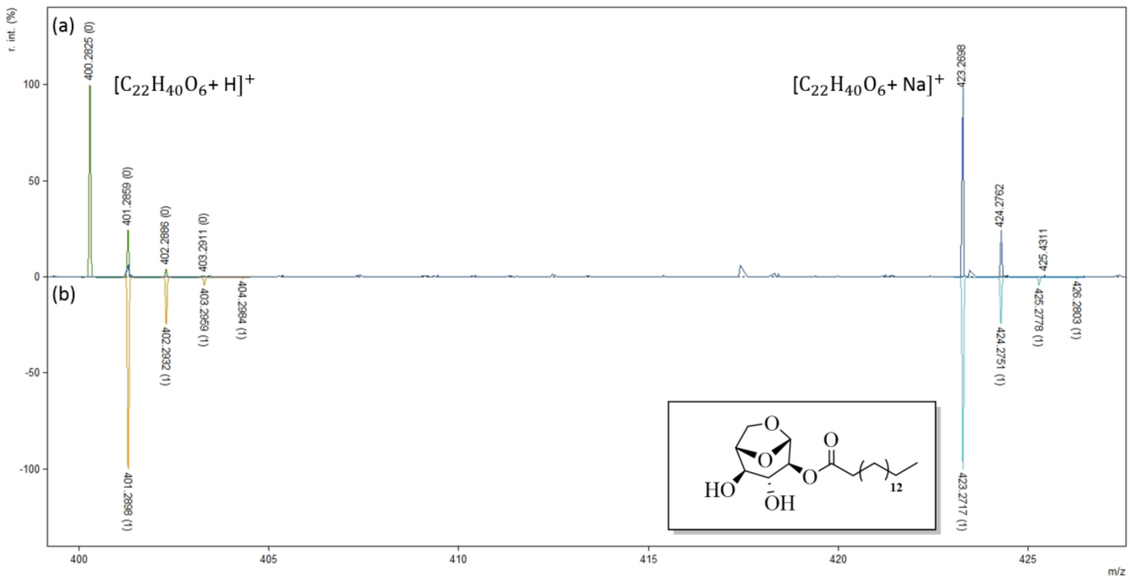
High Resolution Mass Spectrometric Analysis (HRMS) for 4-O-Lauryl-1,6-anhydroglucopyranose, where a) found and b) calculated.



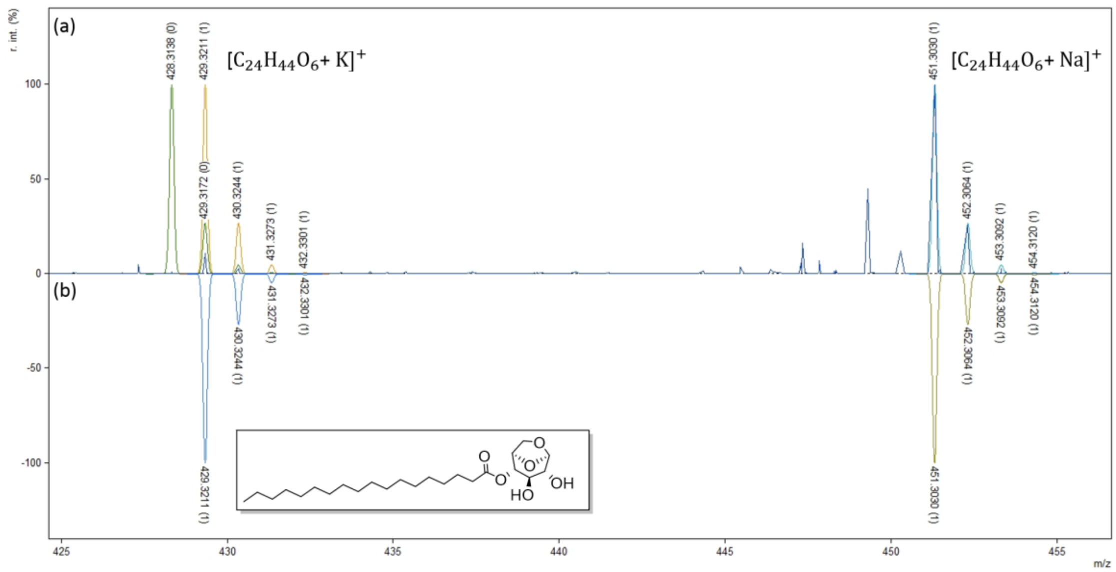
High Resolution Mass Spectrometric Analysis (HRMS) for 4-O-Palmitoyl-1,6-anhydroglucopyranose, where a) found and b) calculated.



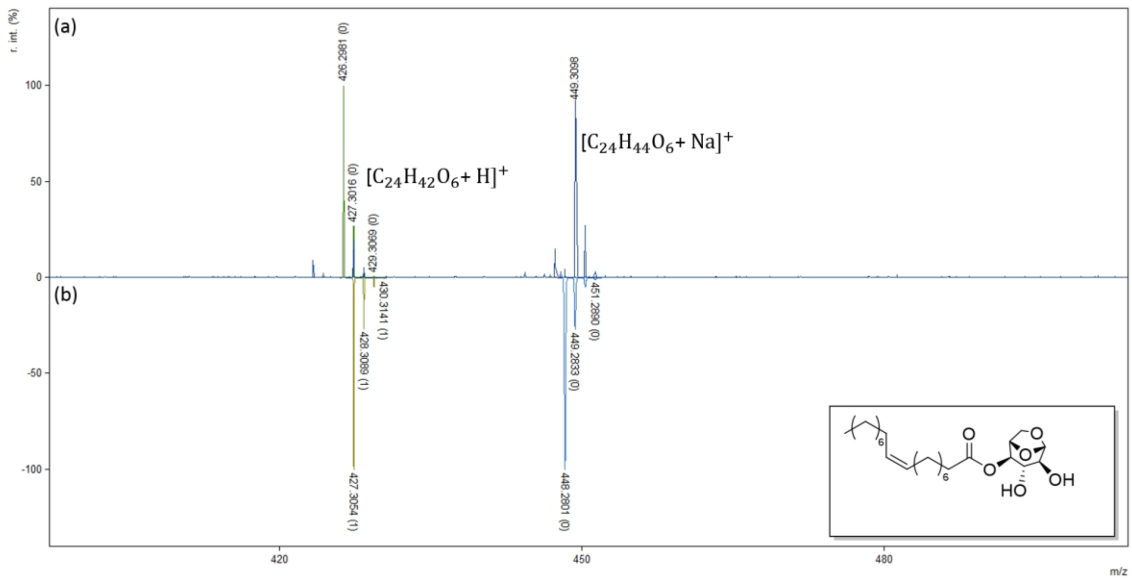
High Resolution Mass Spectrometric Analysis (HRMS) for 2-O-Palmitoyl-1,6-anhydroglucopyranose, where a) found and b) calculated.



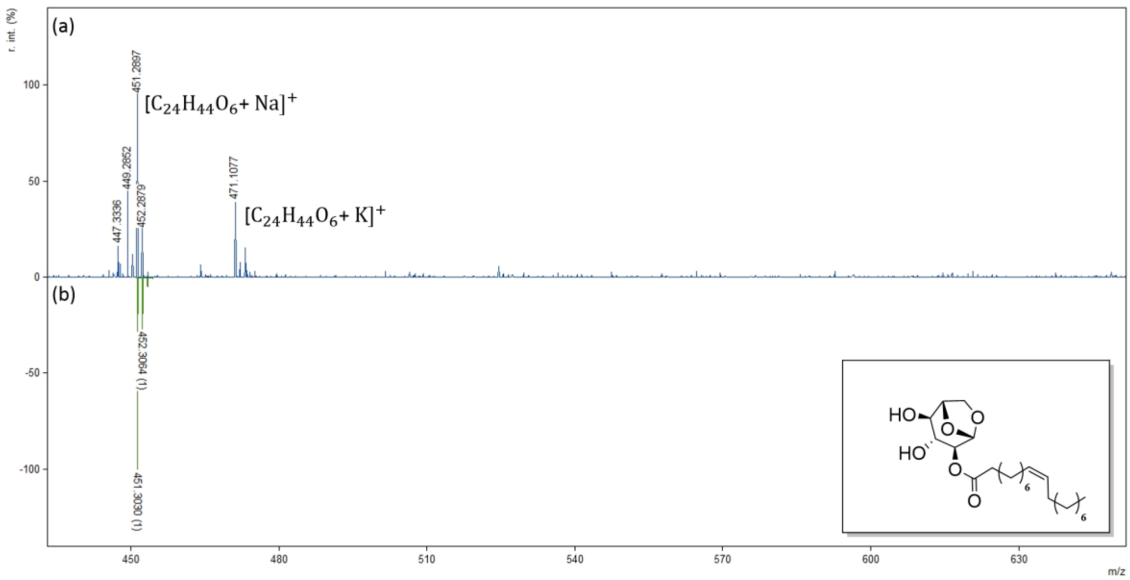
High Resolution Mass Spectrometric Analysis (HRMS) for 4-O-Stearyl-1,6-anhydroglucopyranose, where a) found and b) calculated.



High Resolution Mass Spectrometric Analysis (HRMS) for 4-O-Oleoyl-1,6-anhydroglucopyranose, where a) found and b) calculated.



High Resolution Mass Spectrometric Analysis (HRMS) for 2-O-Oleoyl-1,6-anhydroglucopyranose, where a) found and b) calculated.



2.3. Fourier Transform Infrared Spectroscopy (FTIR)

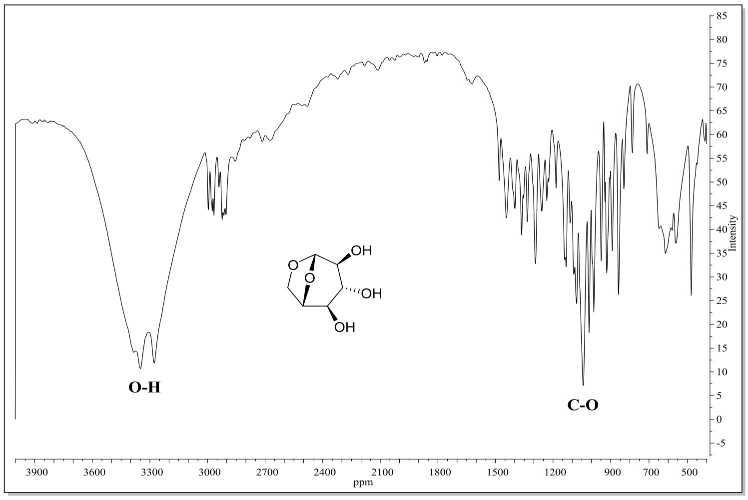


Figure 4: Infrared spectrum of levoglucosan.

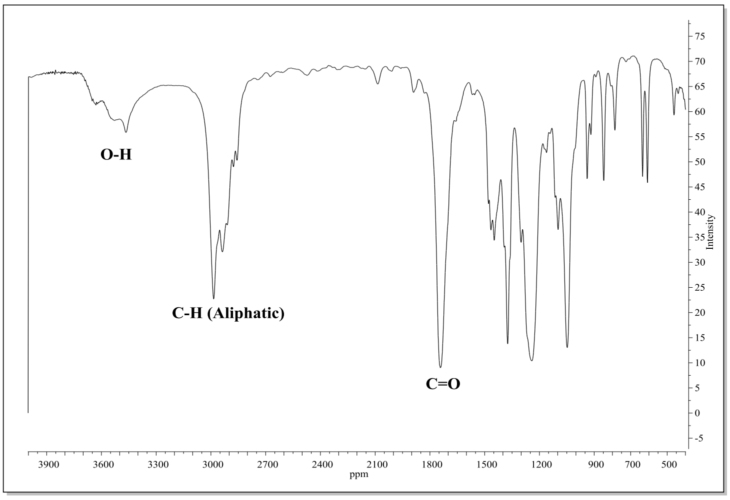


Figure 5: Infrared spectrum of the transesterification reaction of levoglucosan with ethyl oleate.

2.4. Nuclear Magnetic Resonance (NMR)

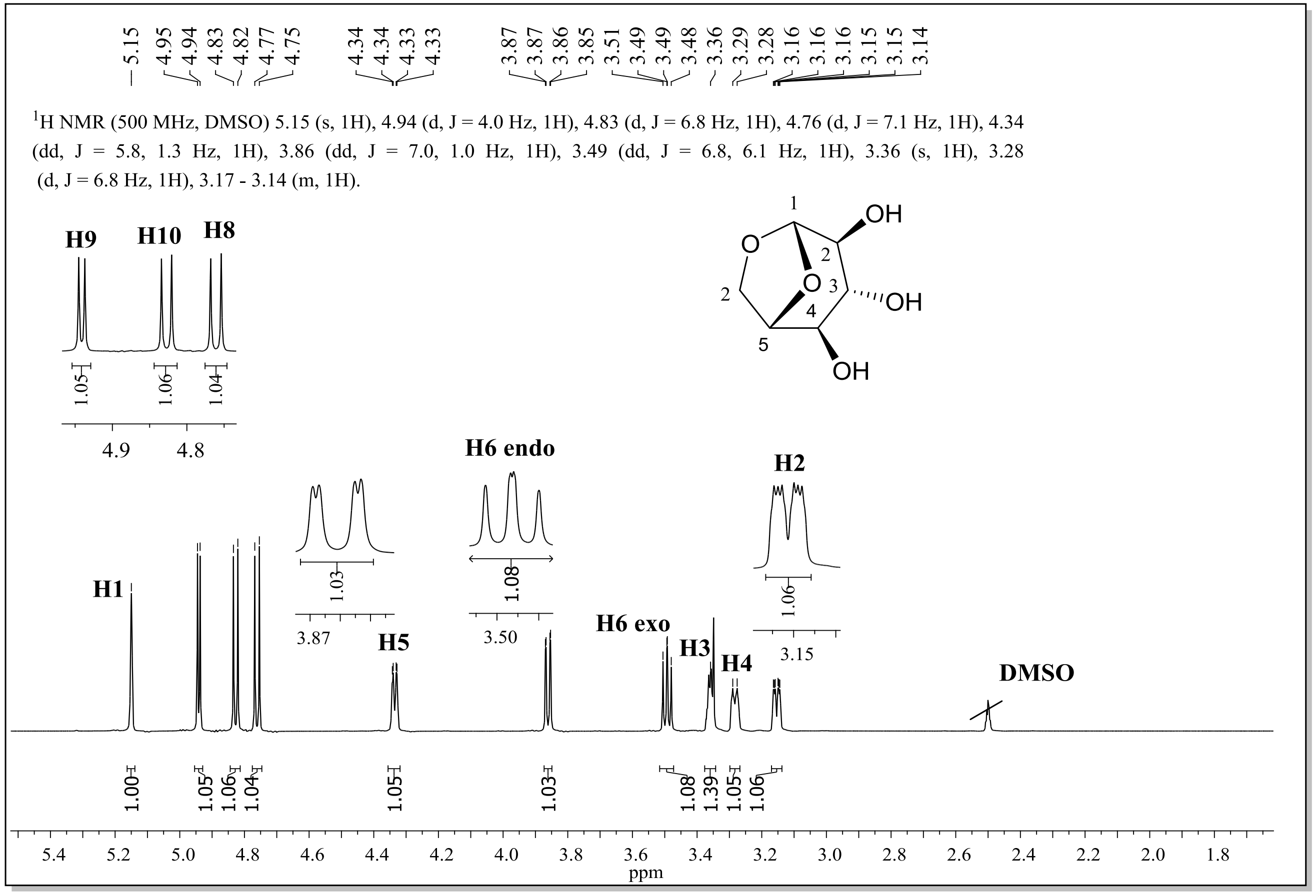


Figure 6: ^{1}H-NMR spectrum for levoglucosan (**DMSO**).

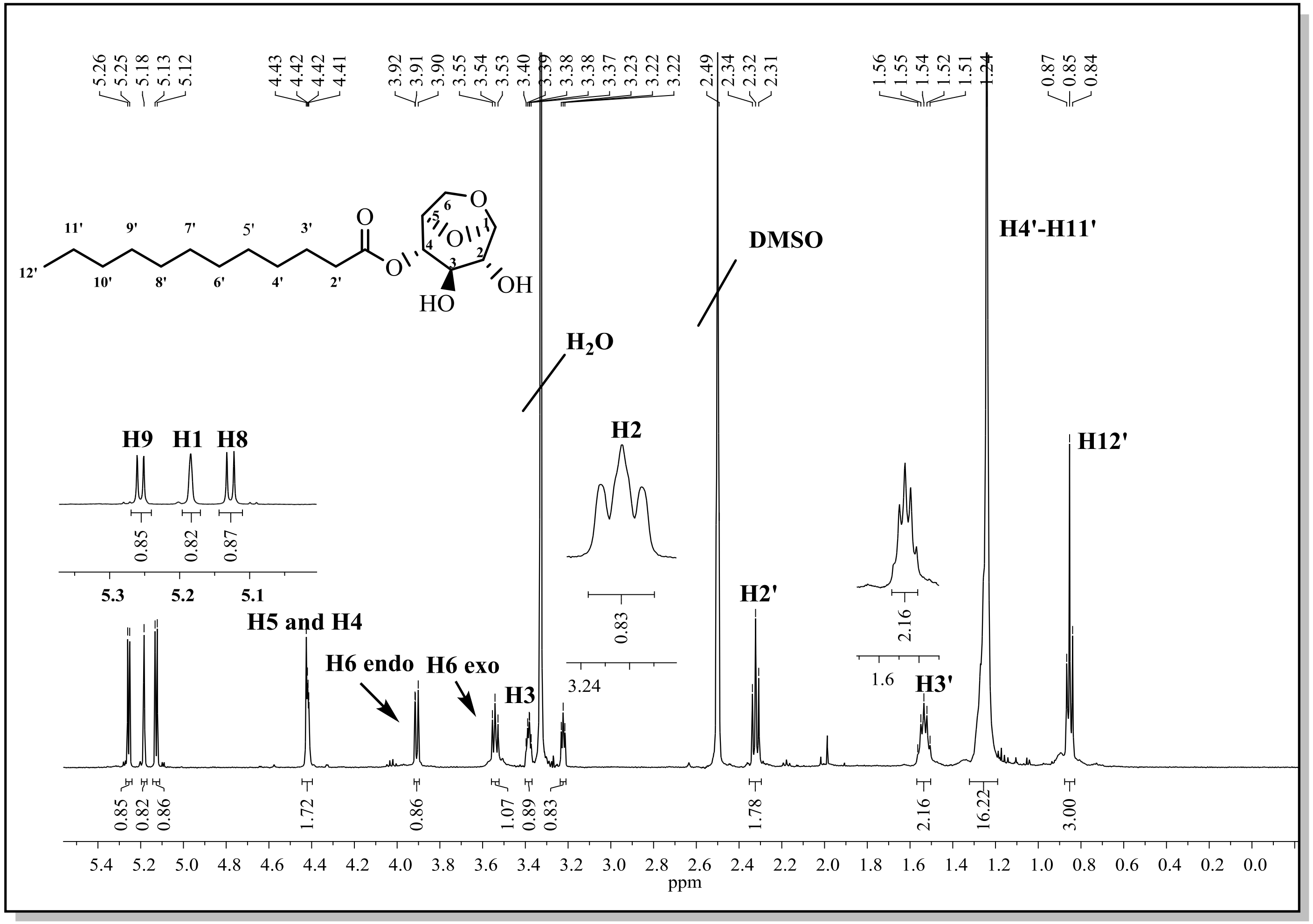


Figure 7: [1]H-NMR Spectrum for 4-O-Lauryl-1,6-anhydroglucopyranose (**CDCl**3).

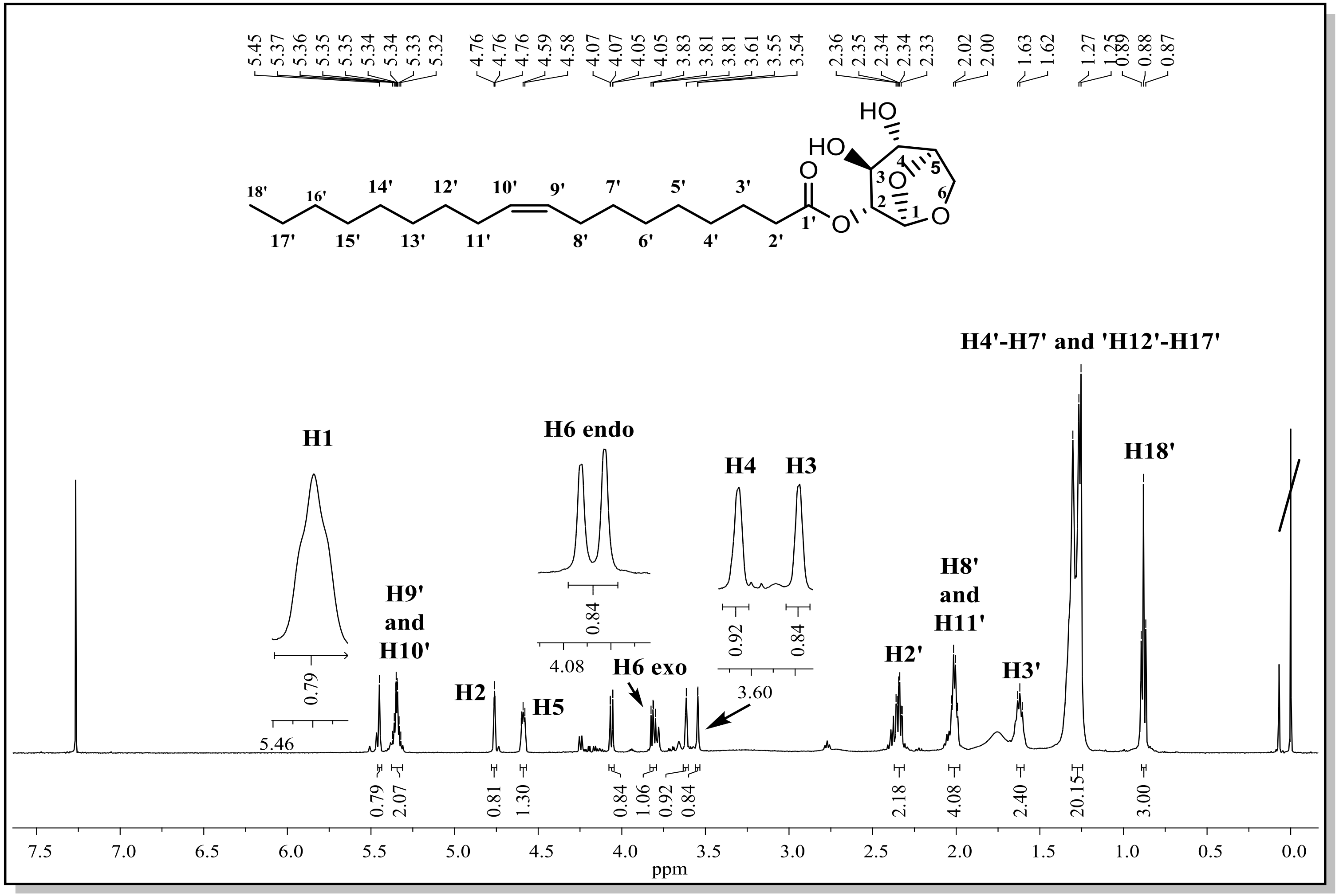


Figure 8: [1]H-NMR Spectrum for 2-O-Oleoyl-1,6-anhydroglucopyranose (**CDCl3**).

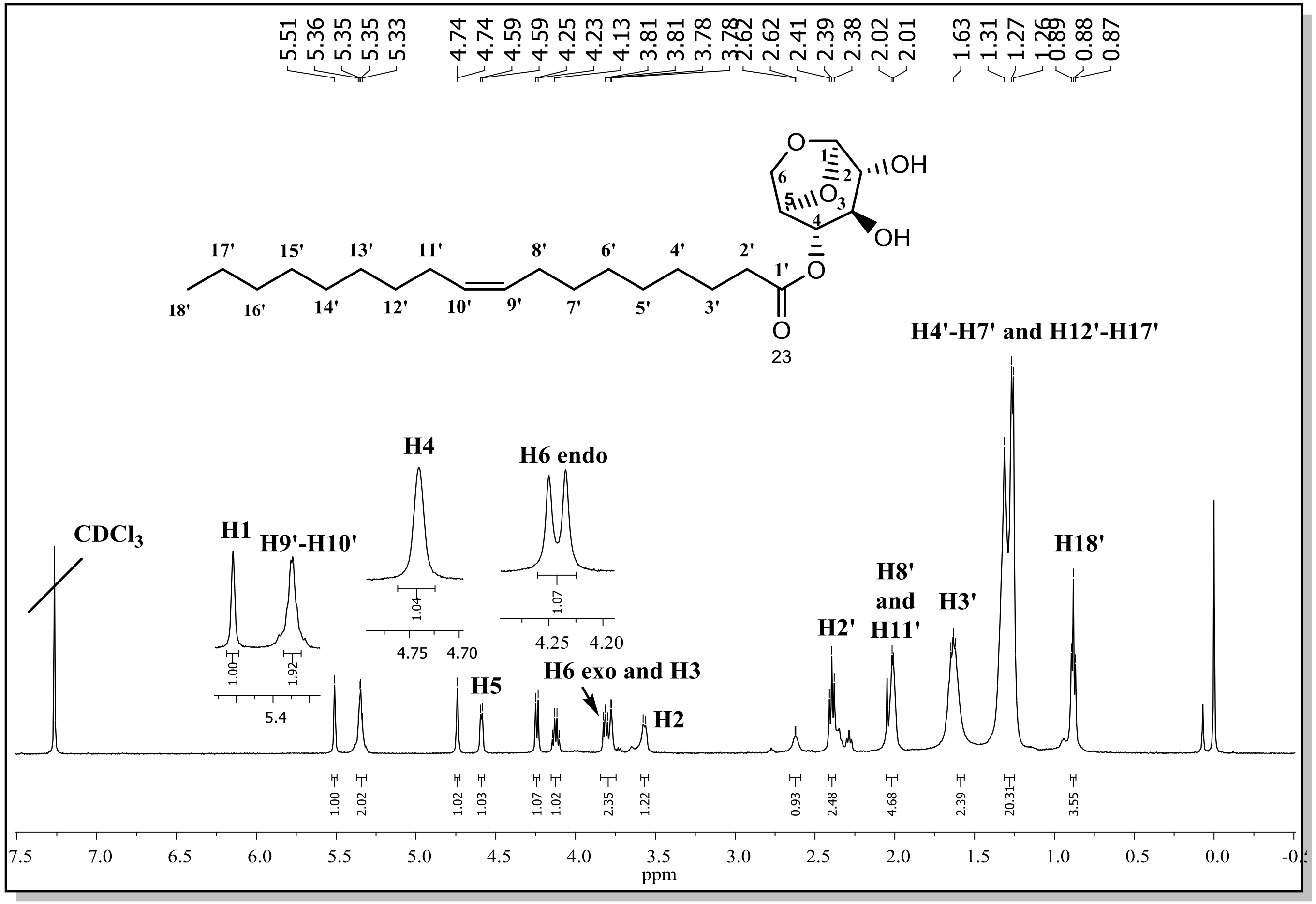


Figure 9: ^{1}H-NMR Spectrum for 4-O-Oleoyl-1,6-anhydroglucopyranose (**$CDCl_3$**).

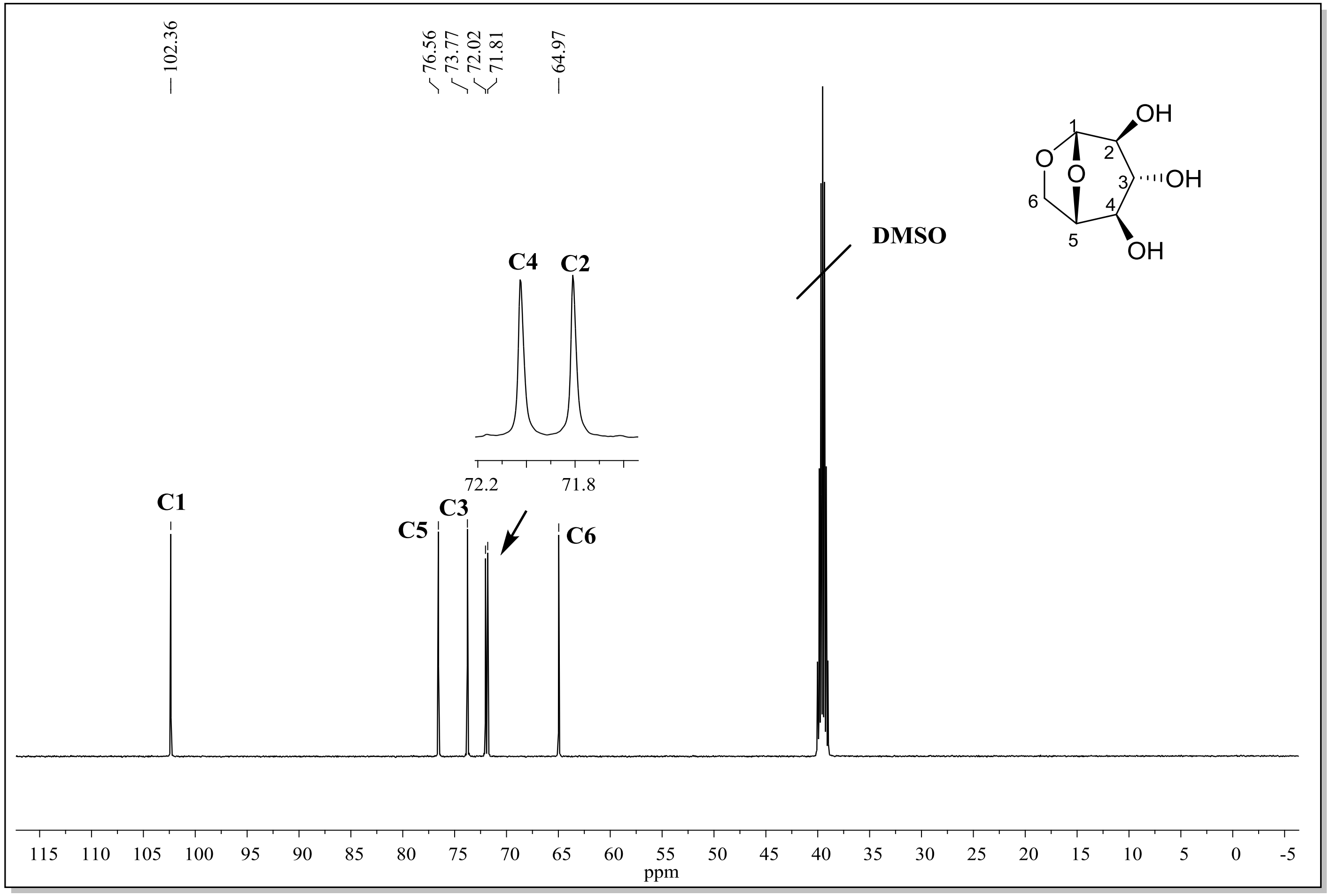


Figure 10: ^{13}C-NMR Spectrum for levoglucosan (**DMSO**).

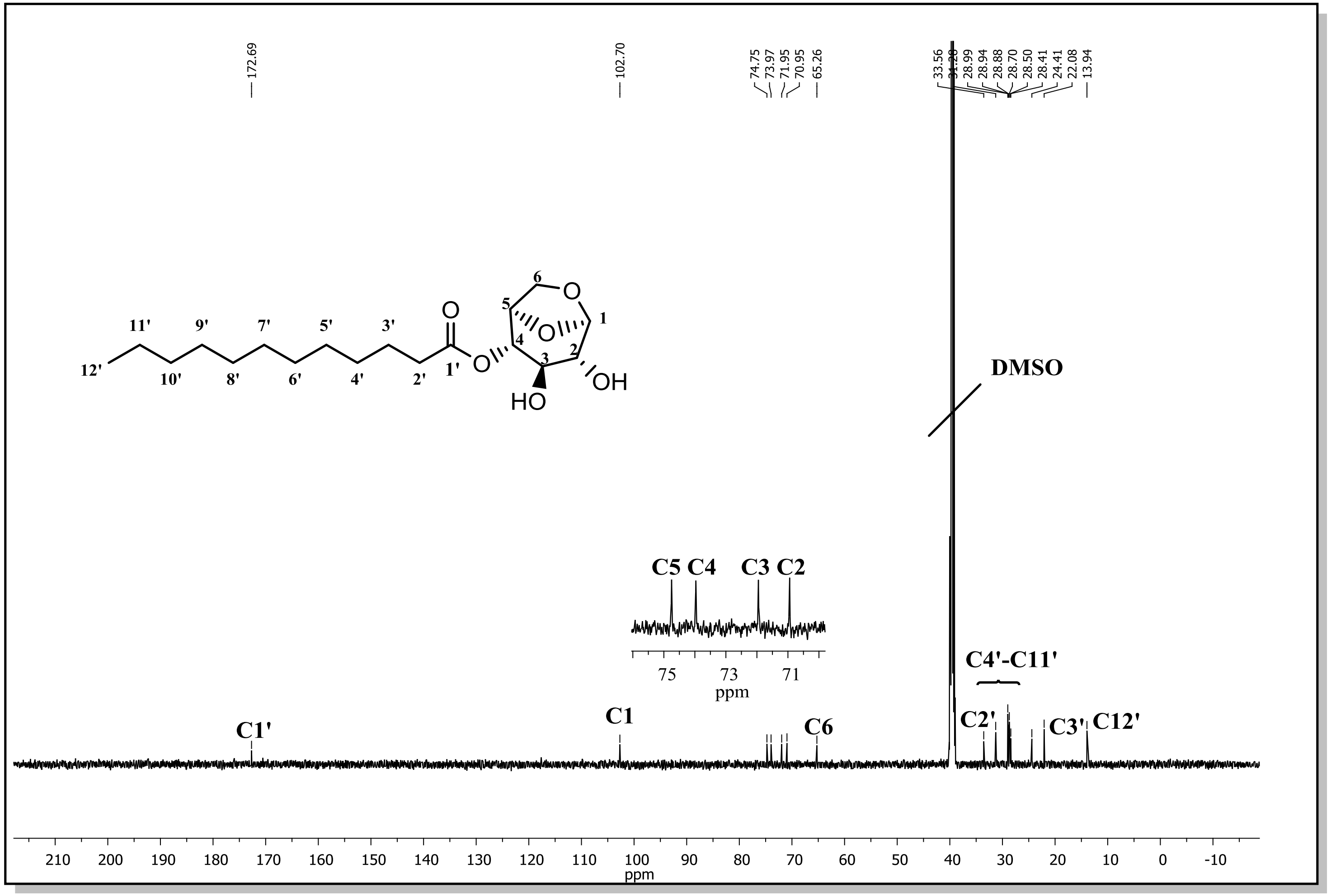


Figure 11: ^{13}C-NMR Spectrum for 4-O-Lauryl-1,6-anhydroglucopyranose (**CDCl$_3$**).

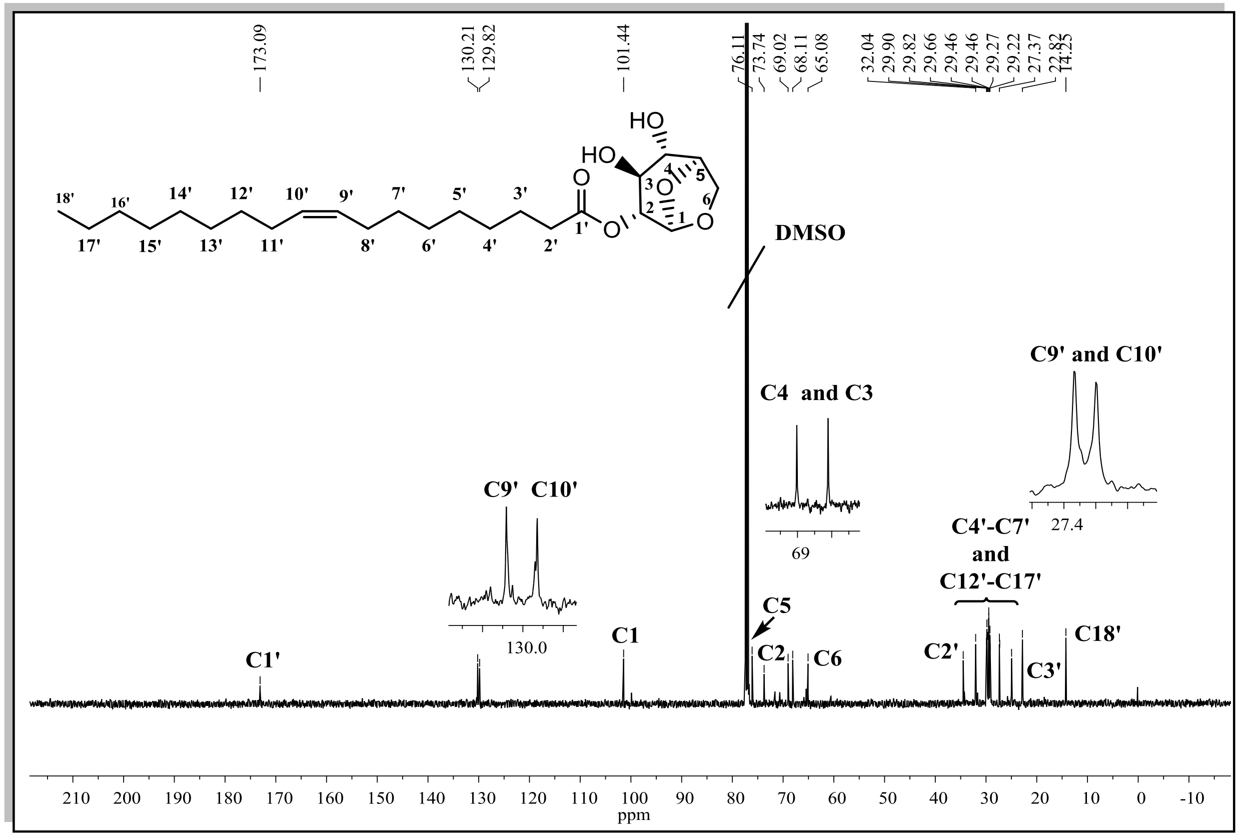


Figure 12: ^{13}C-NMR Spectrum for 2-O-Oleoyl-1,6-anhydroglucopyranose **(CDCl$_3$).**

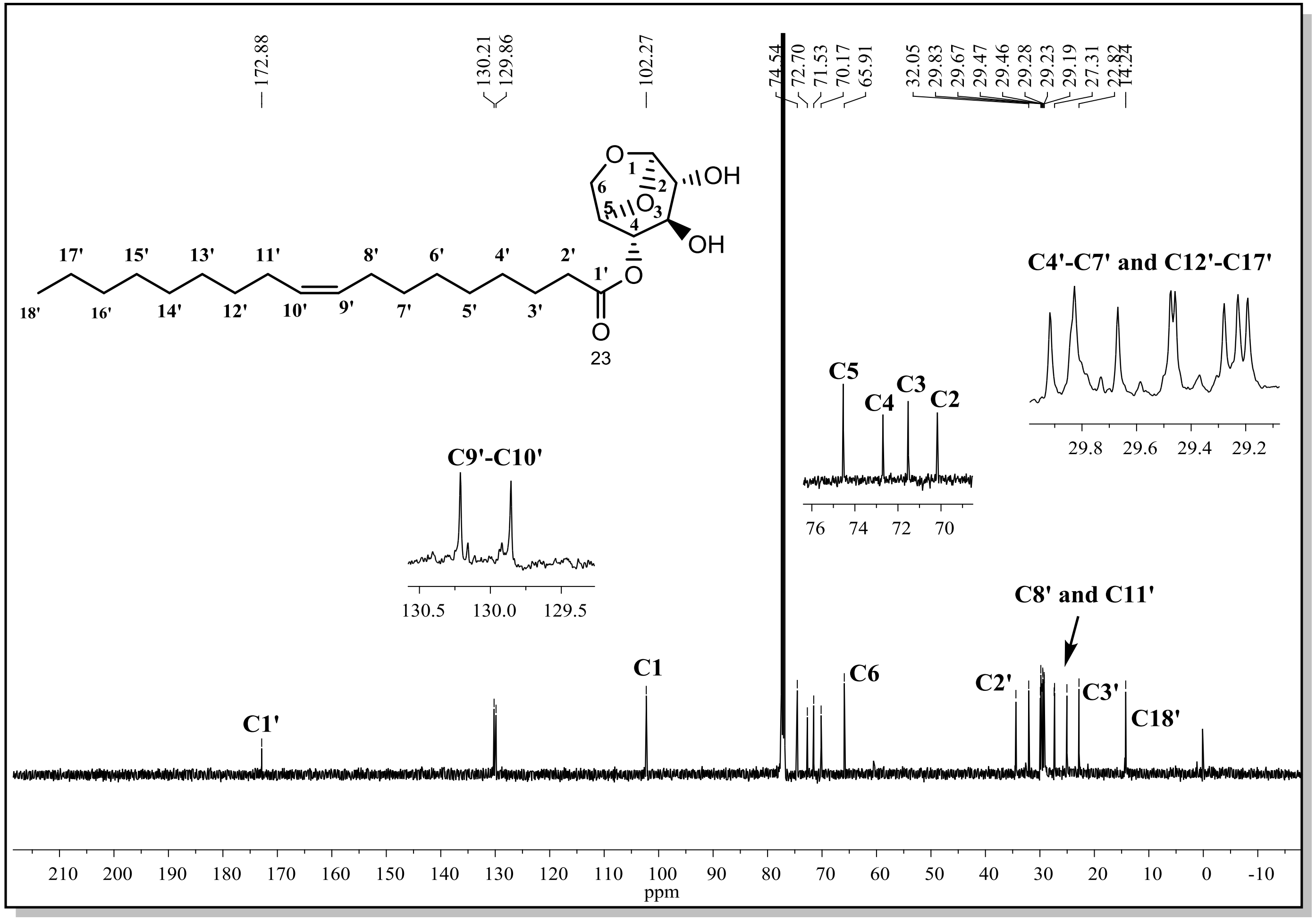


Figure 13: [13]C-NMR Spectrum for 4-O-Oleoyl-1,6-anhydroglucopyranose **($CDCl_3$).**

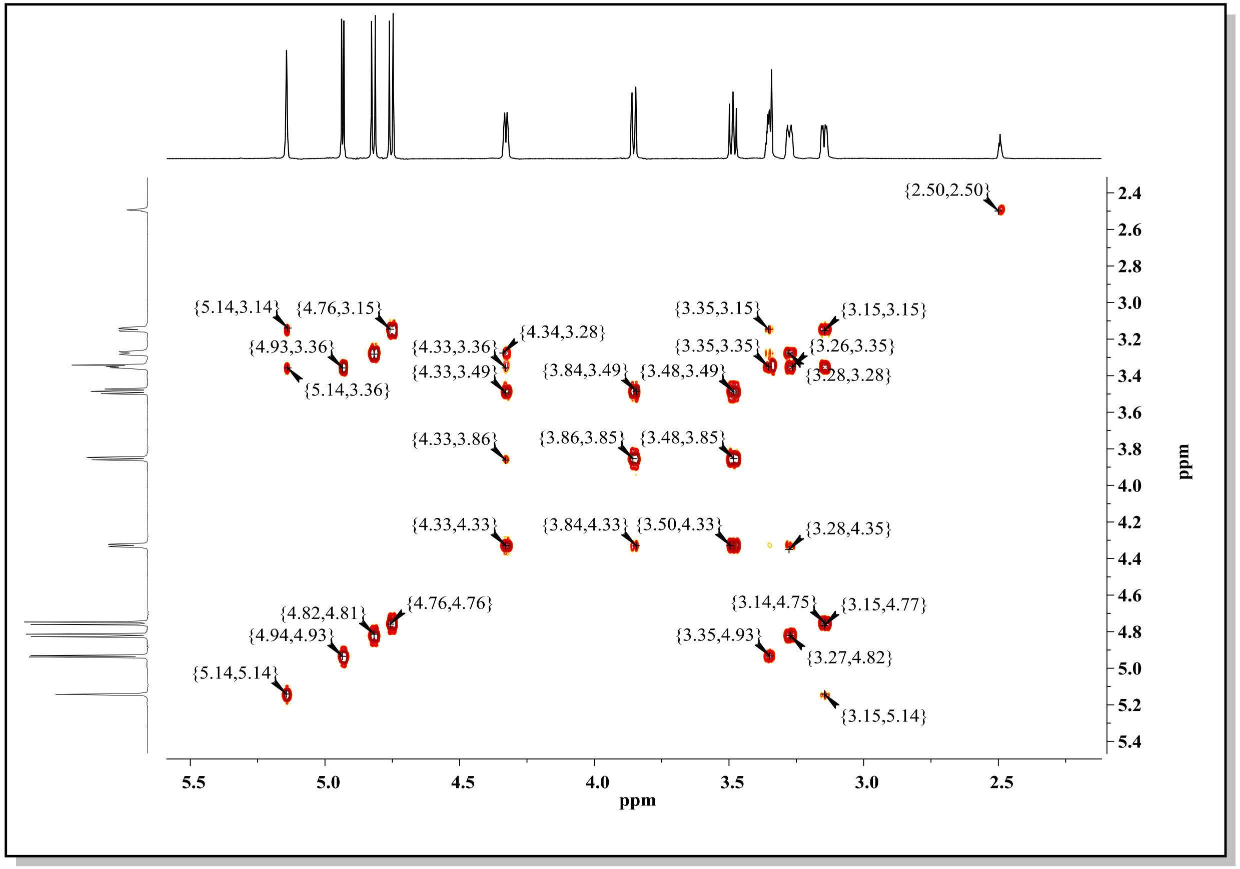


Figure 14: ^{1}H, ^{1}H COSY for levoglucosan (**DMSO**).

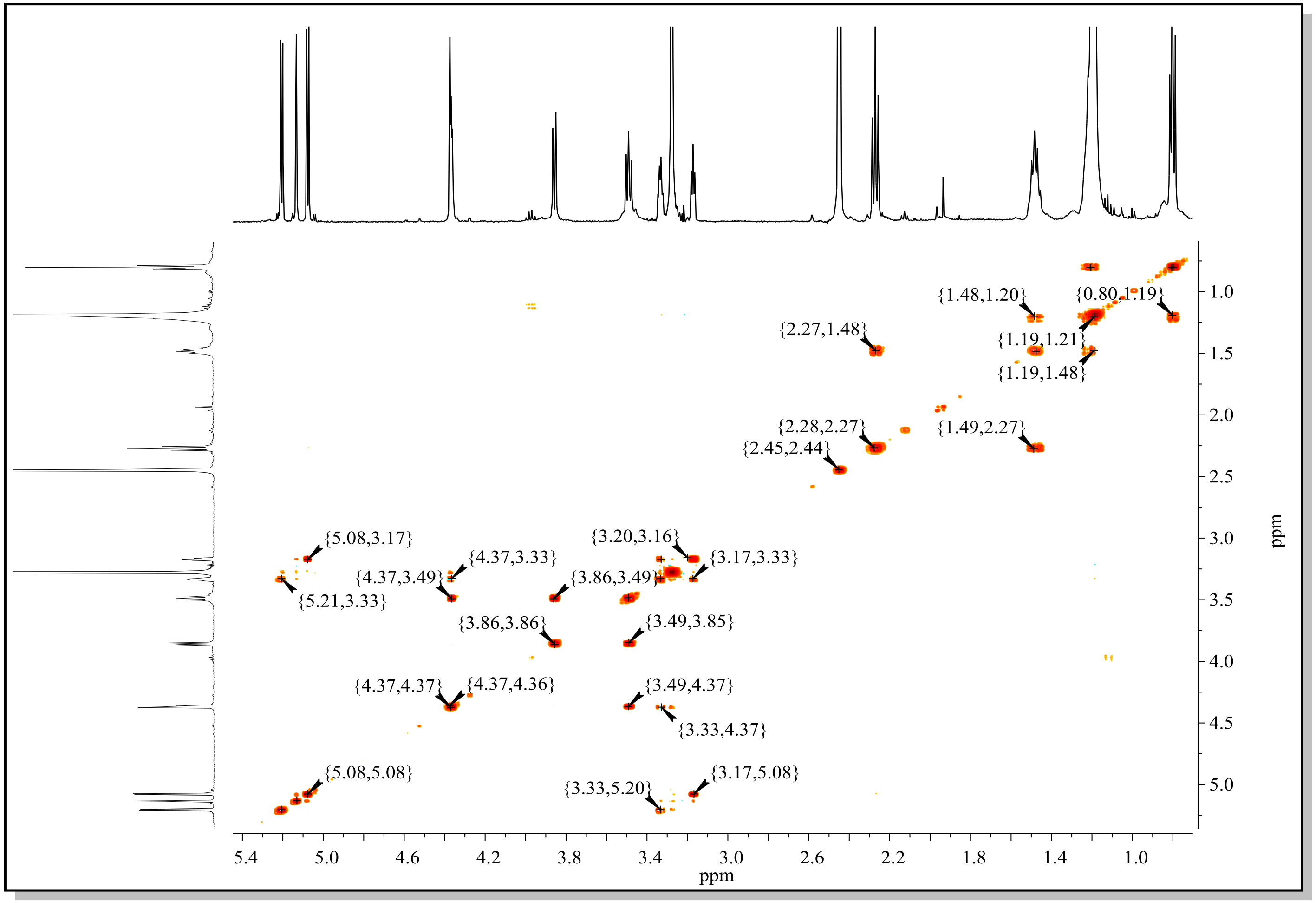


Figure 15: ^{1}H, ^{1}H COSY for 4-O-Lauryl-1,6-anhydroglucopyranose **($CDCl_3$).**

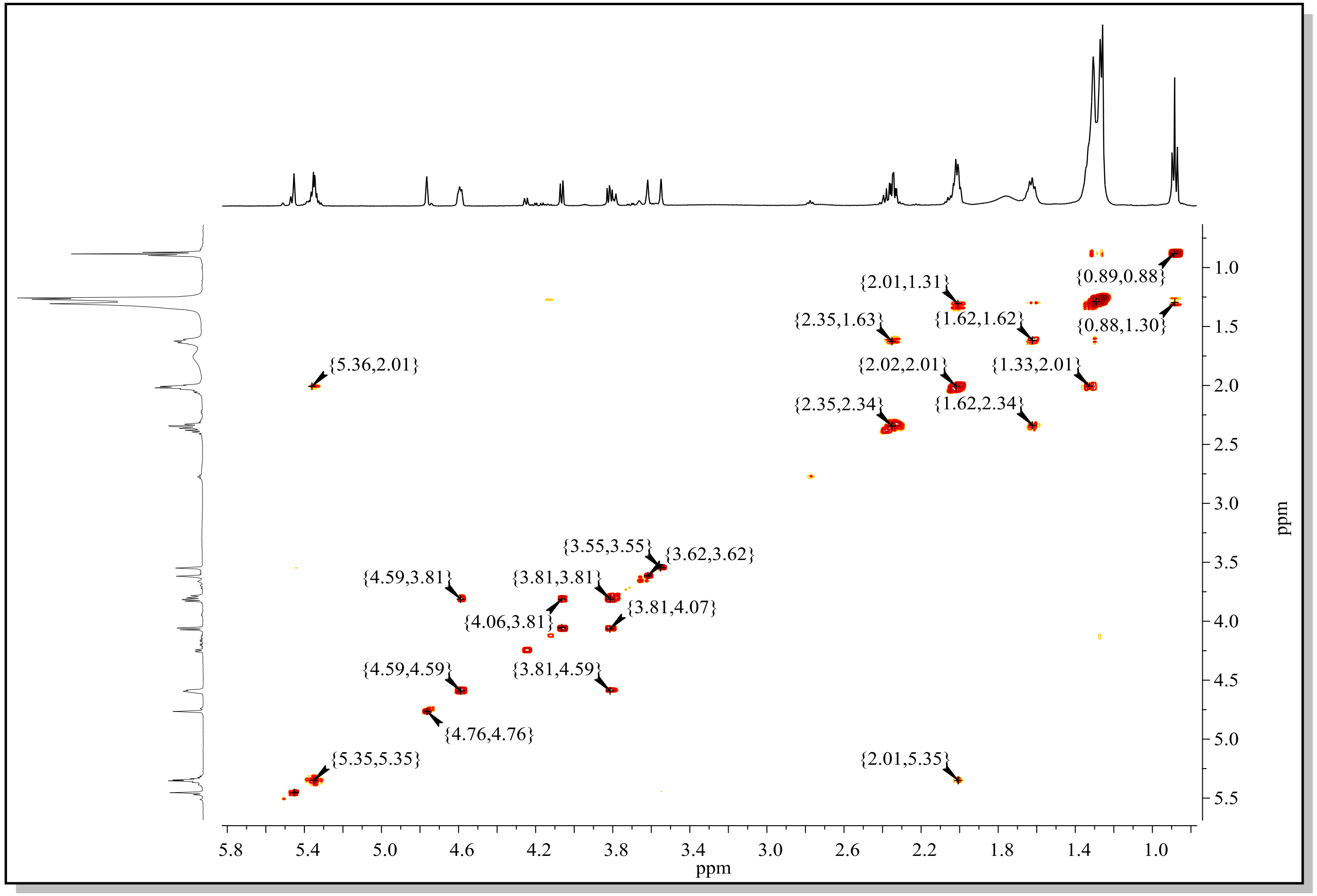


Figure 16: [1]H, [1]H COSY for 2-O-Oleoyl-1,6-anhydroglucopyranose **(CDCl$_3$).**

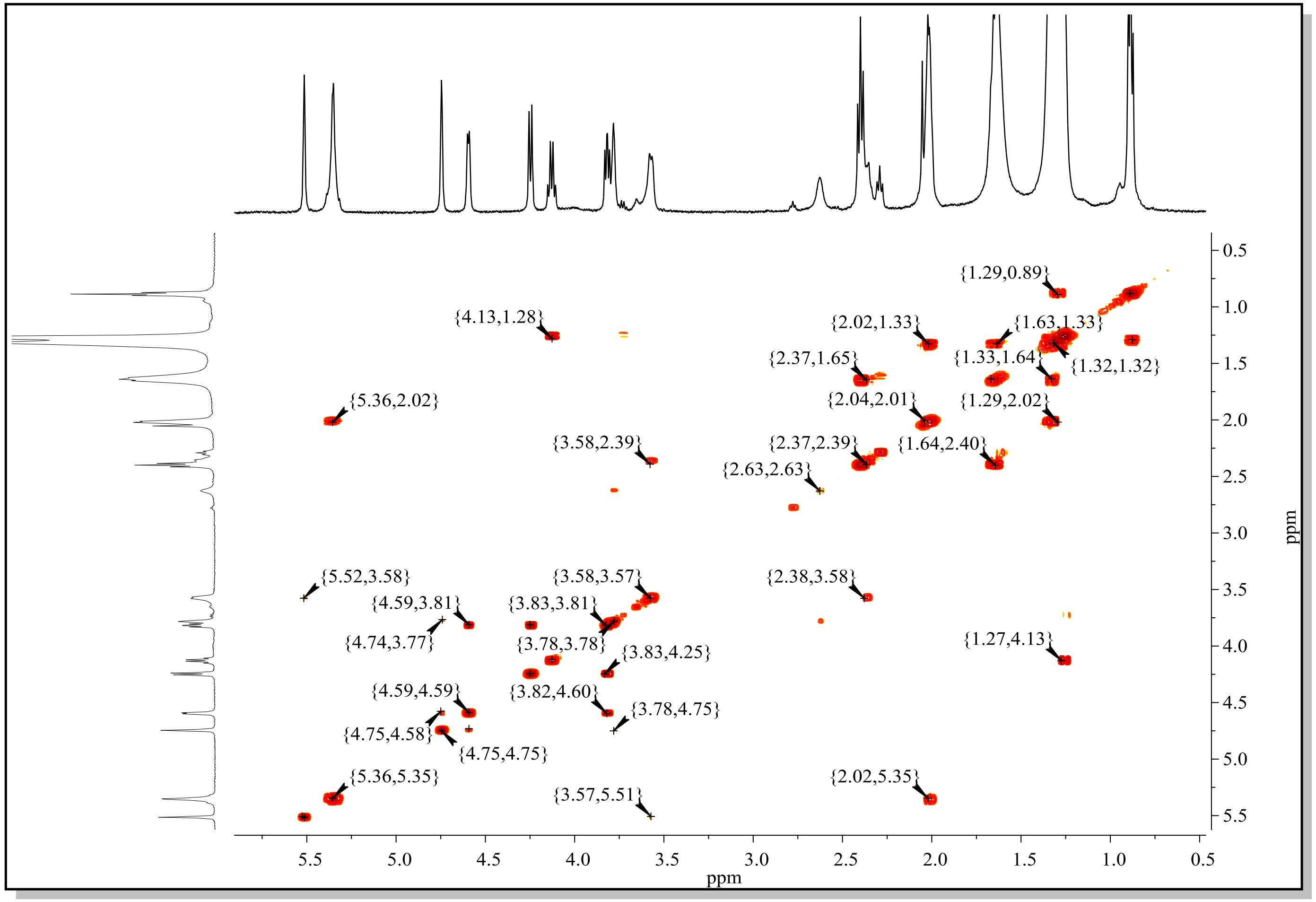


Figure 17: [1]H, [1]H COSY for 4-O-Oleoyl-1,6-anhydroglucopyranose **($CDCl_3$).**

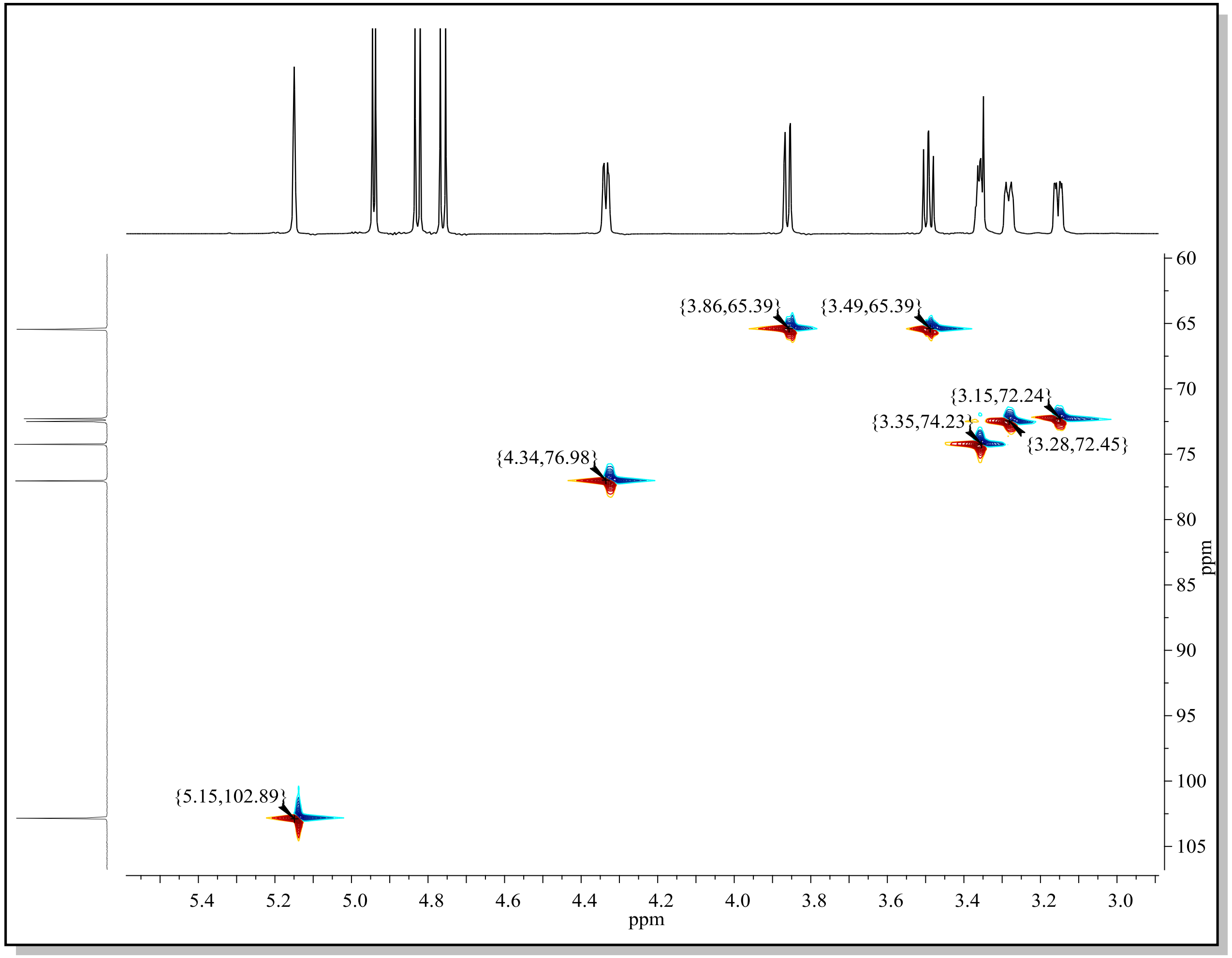


Figure 18: HSQC for levoglucosan (**DMSO**).

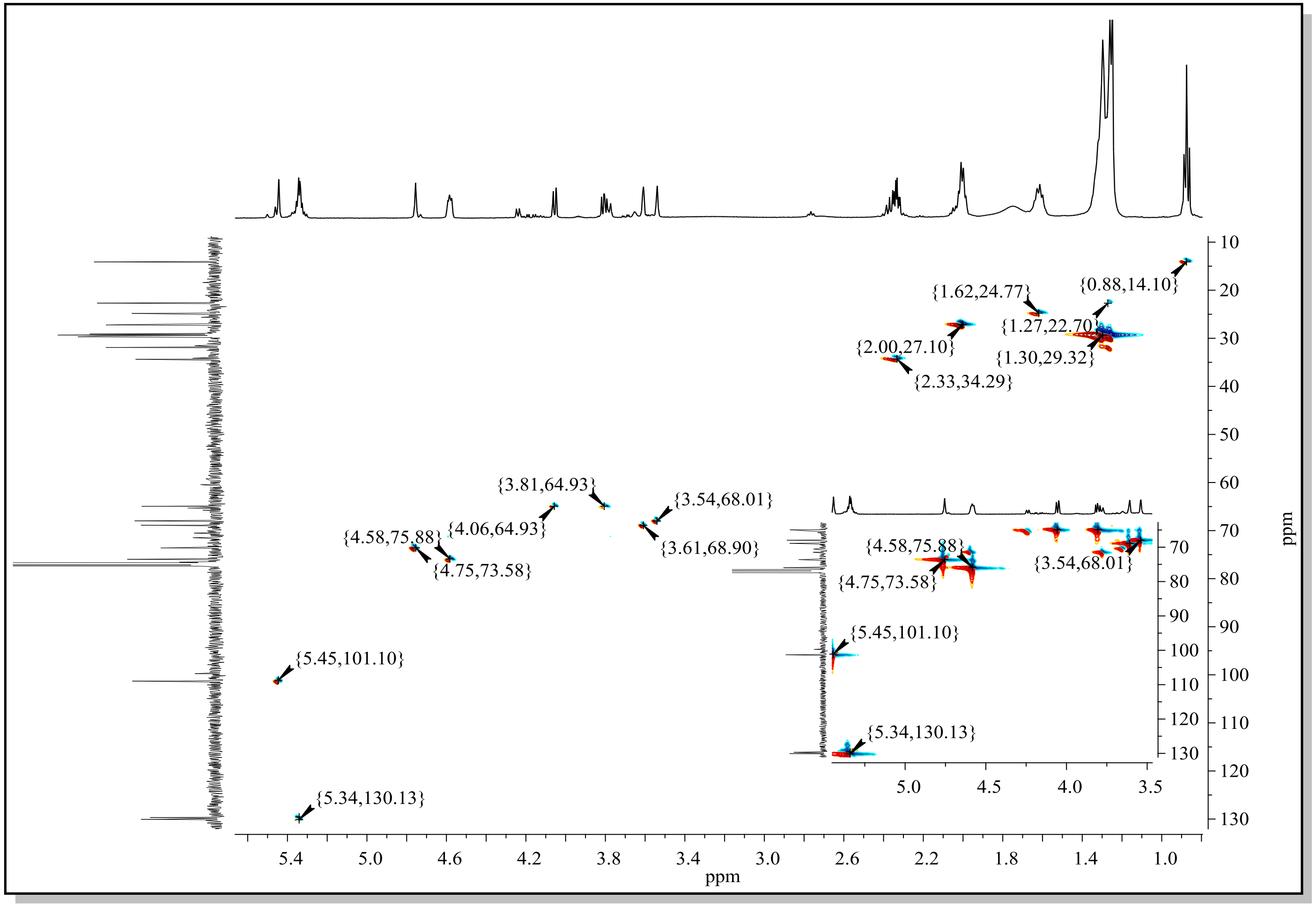


Figure 19: HSQC for 2-O-Oleoyl-1,6-anhydroglucopyranose **($CDCl_3$).**

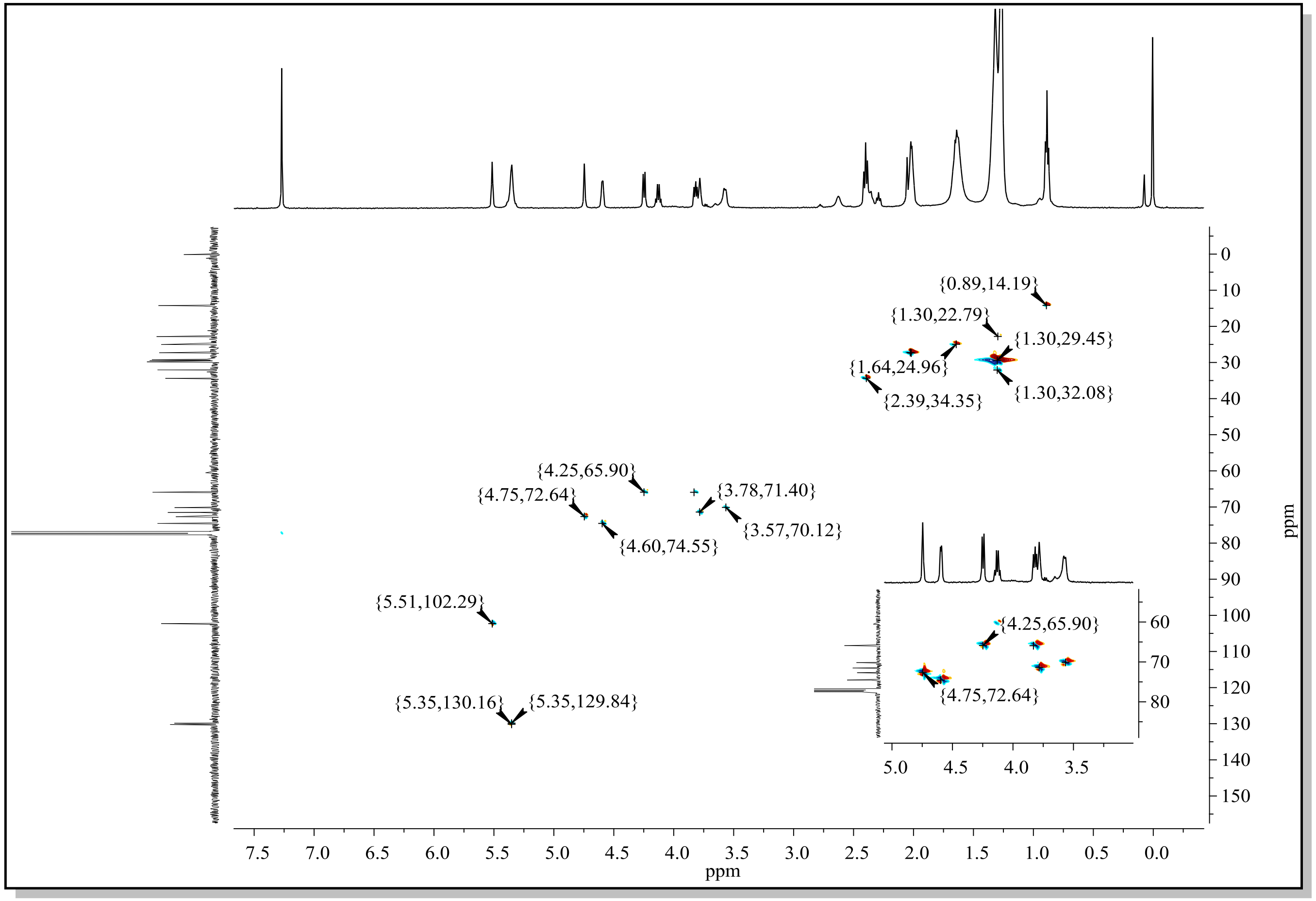


Figura 20: HSQC for 4-O-Oleoyl-1,6-anhydroglucopyranose **($CDCl_3$).**

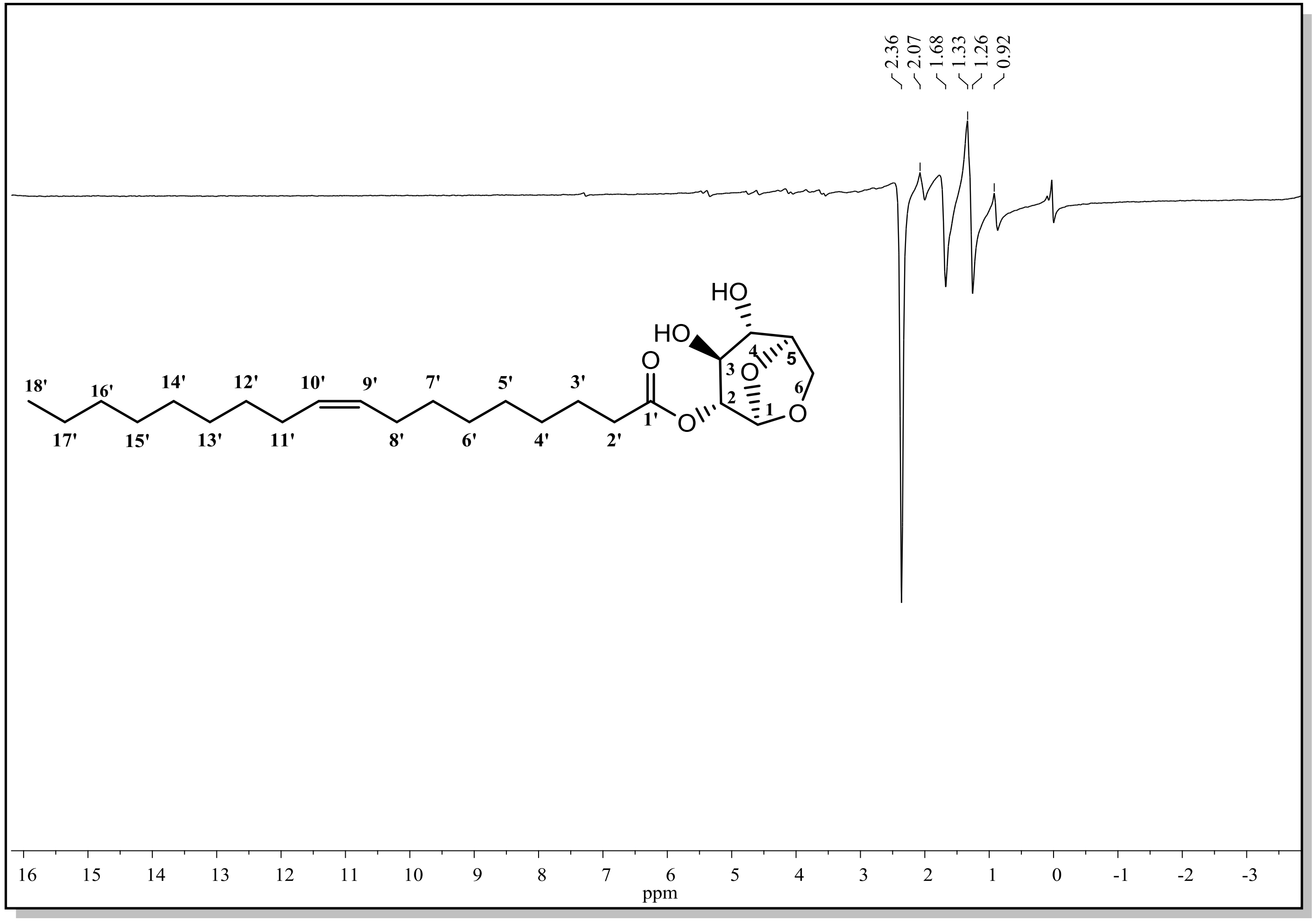


Figura 21: n.0.e difference spectra for irradiation in the H-2′ compound 2-O-Oleoyl-1,6-anhydroglucopyranose **(CDCl$_3$).**

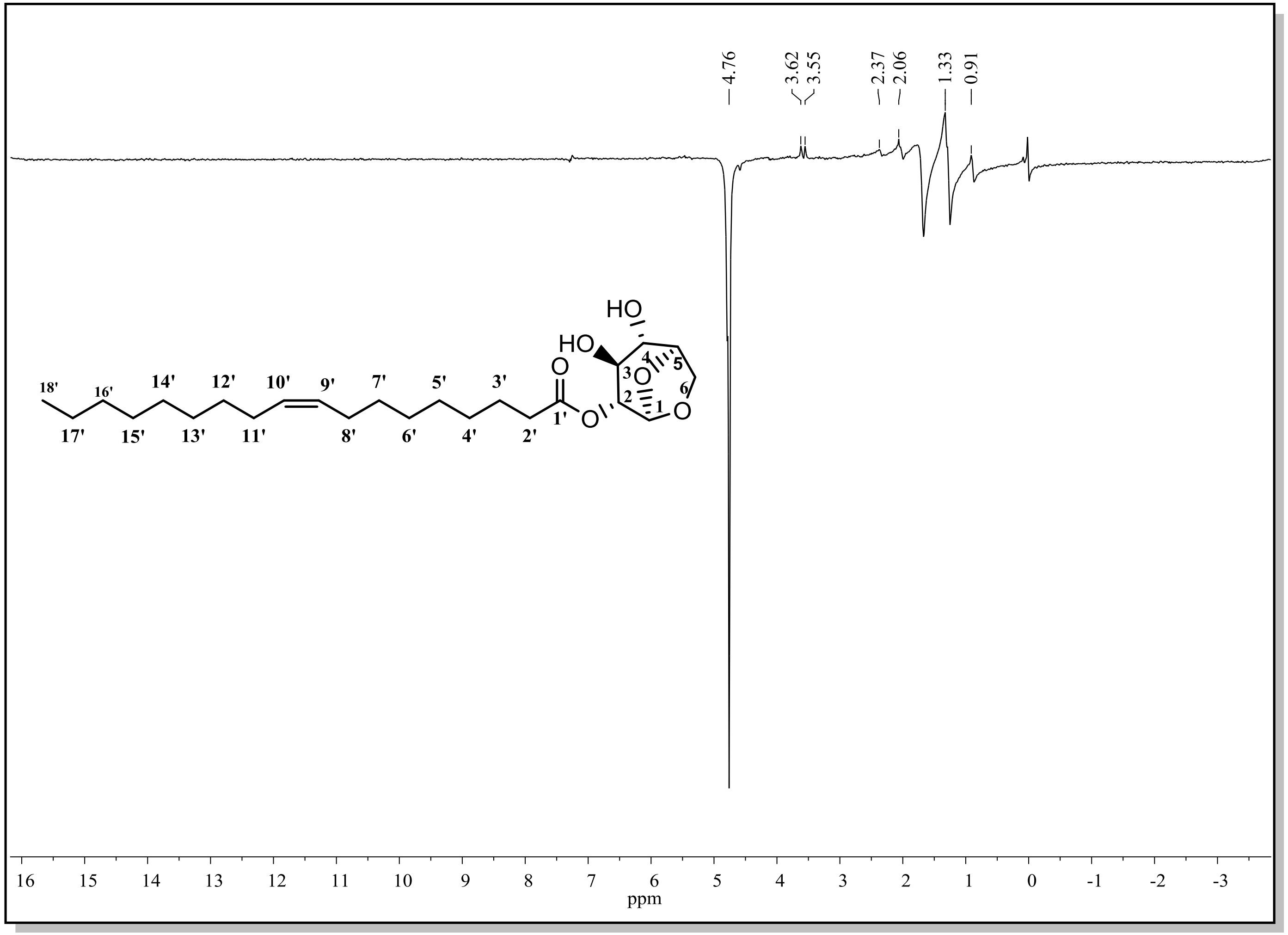


Figura 22: n.0.e difference spectra for irradiation in the H-2 compound 2-O-Oleoyl-1,6-anhydroglucopyranose **(CDCl₃).**

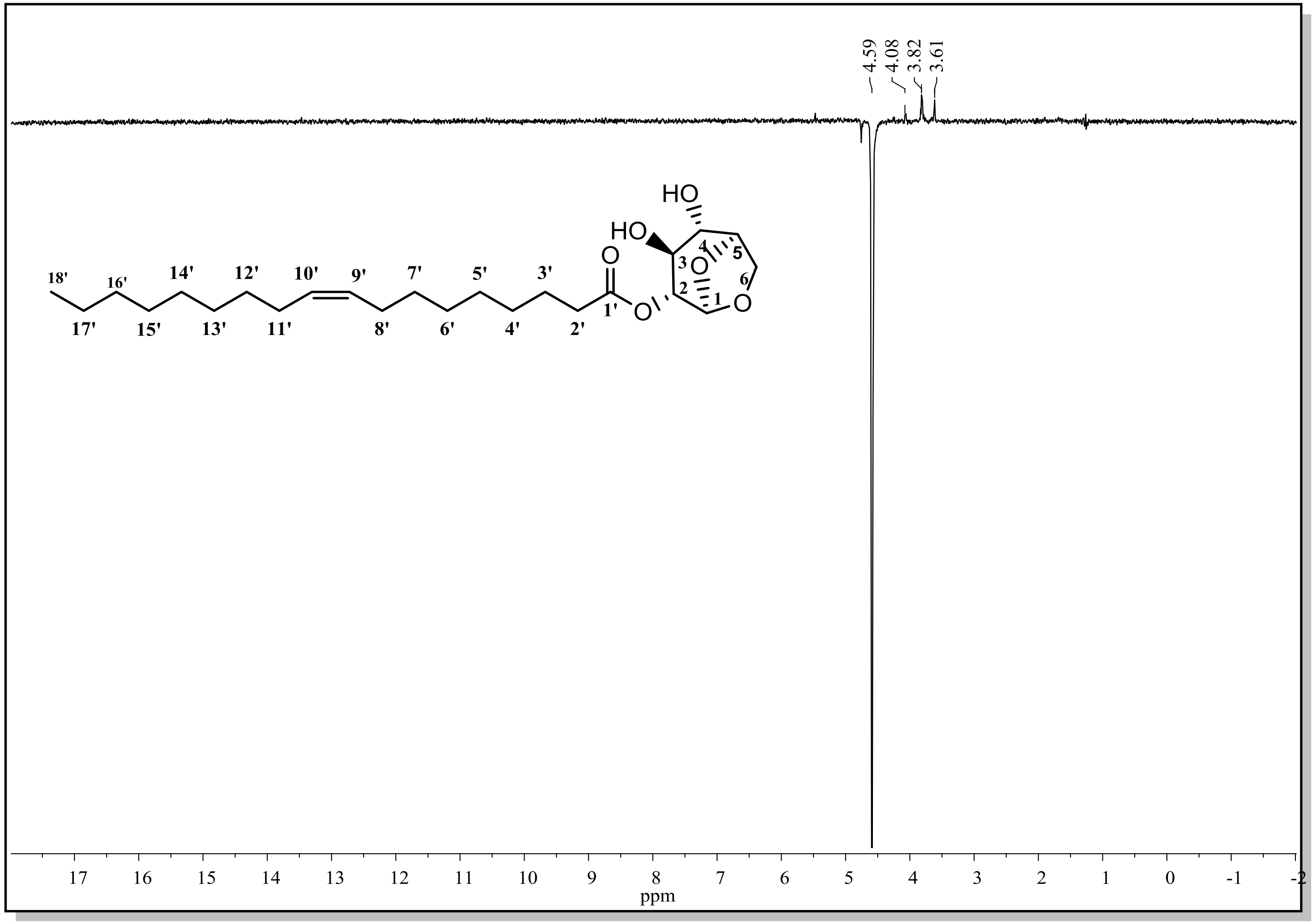


Figura 23: n.0.e difference spectra for irradiation in the H-5 compound 2-O-Oleoyl-1,6-anhydroglucopyranose **(CDCl₃).**

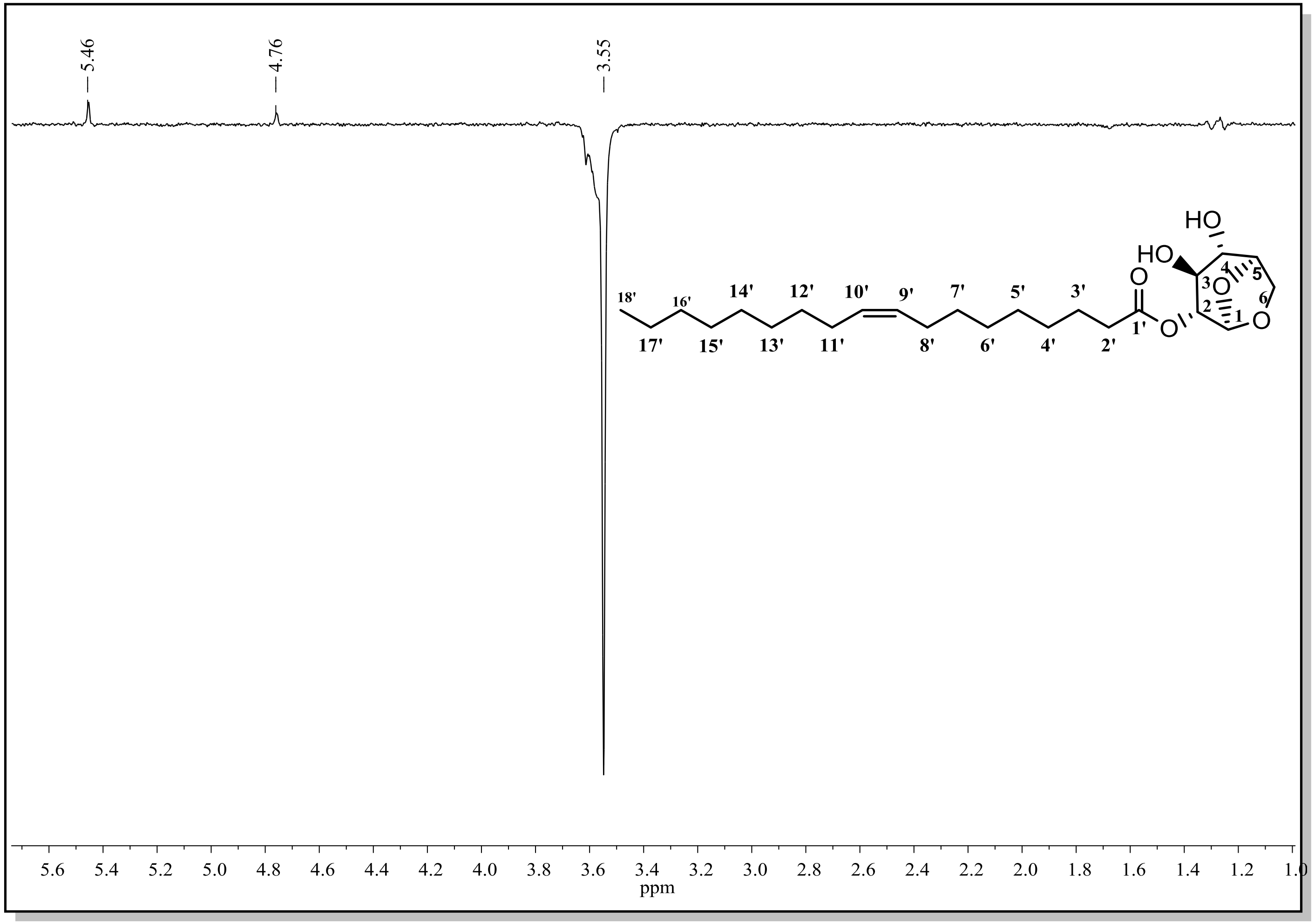


Figura 24: n.0.e difference spectra for irradiation in the H-3 compound 2-O-Oleoyl-1,6-anhydroglucopyranose **(CDCl₃).**

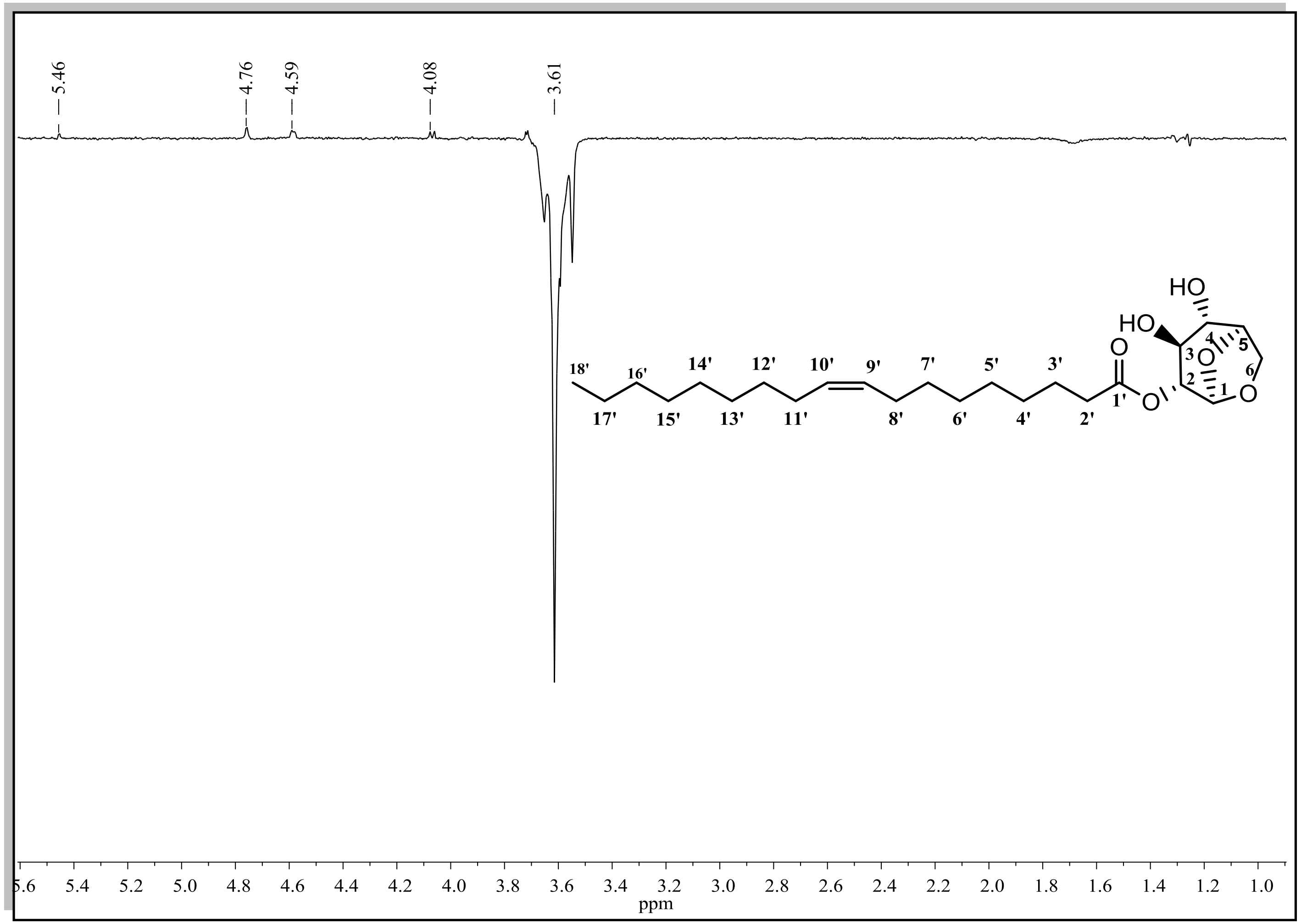


Figura 25: n.0.e difference spectra for irradiation in the H-4 compound 2-O-Oleoyl-1,6-anhydroglucopyranose **($CDCl_3$)**..