

Supplementary material for

Carotenoid glycoside isolated and identified from cyanobacterium *Cylindrospermopsis raciborskii*

Veronika Nagy^{1*}, Attila Agócs¹, József Deli^{1,2}, Gergely Gulyás-Fekete², Tünde-Zita Illyés³, Tibor Kurtán³, Erika Turcsi¹, Szabolcs Béni⁴, Miklós Dékány⁵, Andreas Ballot⁶, Gábor Vasas⁷

¹ *University of Pécs, Department of Biochemistry and Medical Chemistry, Szigeti út 12, H-7624 Pécs, Hungary*

² *University of Pécs, Department of Pharmacognosy, Rókus u. 2, H-7624, Pécs, Hungary*

³ *University of Debrecen, Department of Organic Chemistry, POB 400, H-4002, Debrecen, Hungary*

⁴ *Semmelweis University, Department of Pharmacognosy, Üllői út 26, H-1085, Budapest, Hungary*

⁵ *Gedeon Richter Plc, Spectroscopic Research, Gyömrői út 19-21., H-1103, Budapest, Hungary*

⁶ *Norwegian Institute for Water Research, Gaustadalléen 21, NO-0349, Oslo, Norway*

⁷ *University of Debrecen, Department of Botany, 4032 Egyetem sq. 1, Debrecen, Hungary*

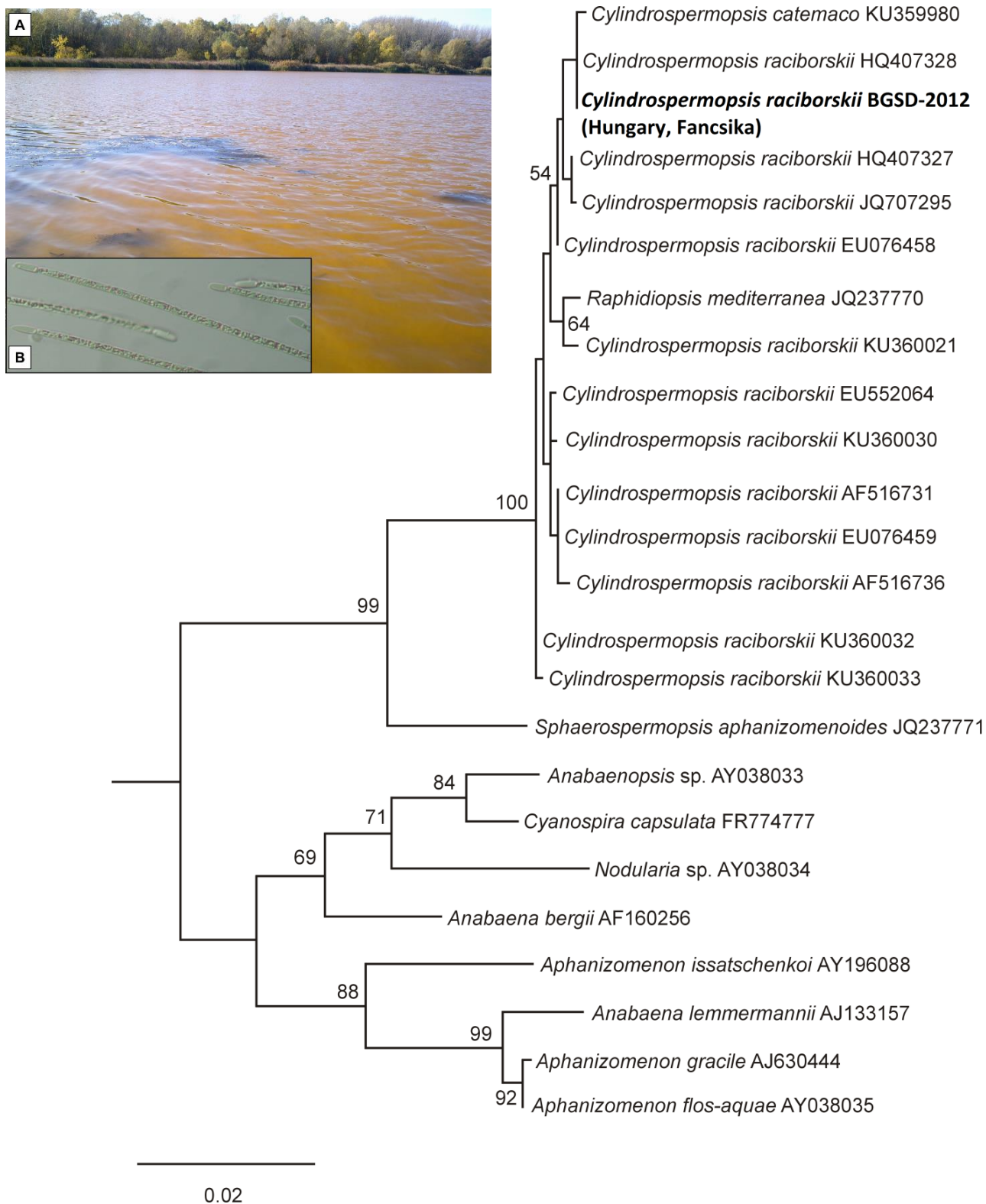


Fig. S1. Maximum likelihood tree determined on the basis of partial 16S rRNA gene sequences of 24 Nostocales strains. Outgroup = *Gloeobacter violaceus* (AF132790) (not shown). Strains from this study are marked in bold. Bootstrap values above 50 are included. The scale bar indicates 2% sequence divergence. Inset shows (A): Discoloration of the water turning orange caused by cyano-bacterial blooming (323104 filaments/mL) (B). The bloom-forming species identified as *C. raciborskii* by 16S rRNA gene sequences and its characteristic morphological feature (terminal heterocyst).

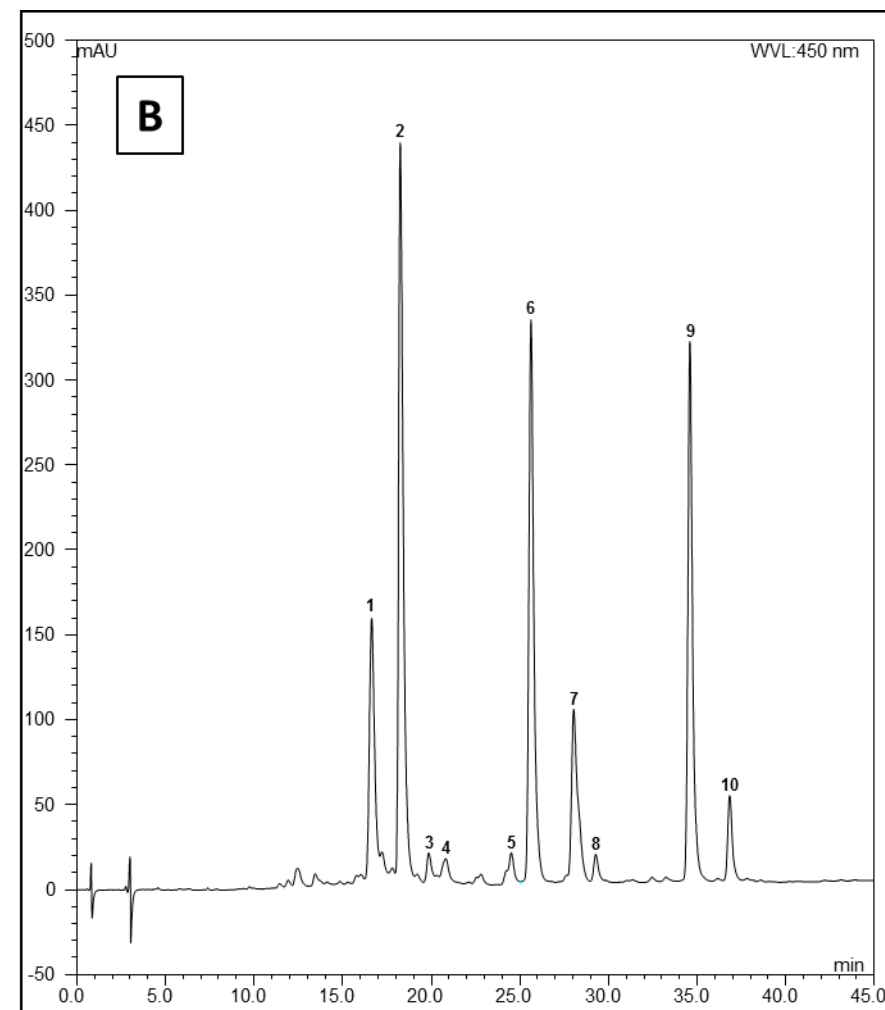
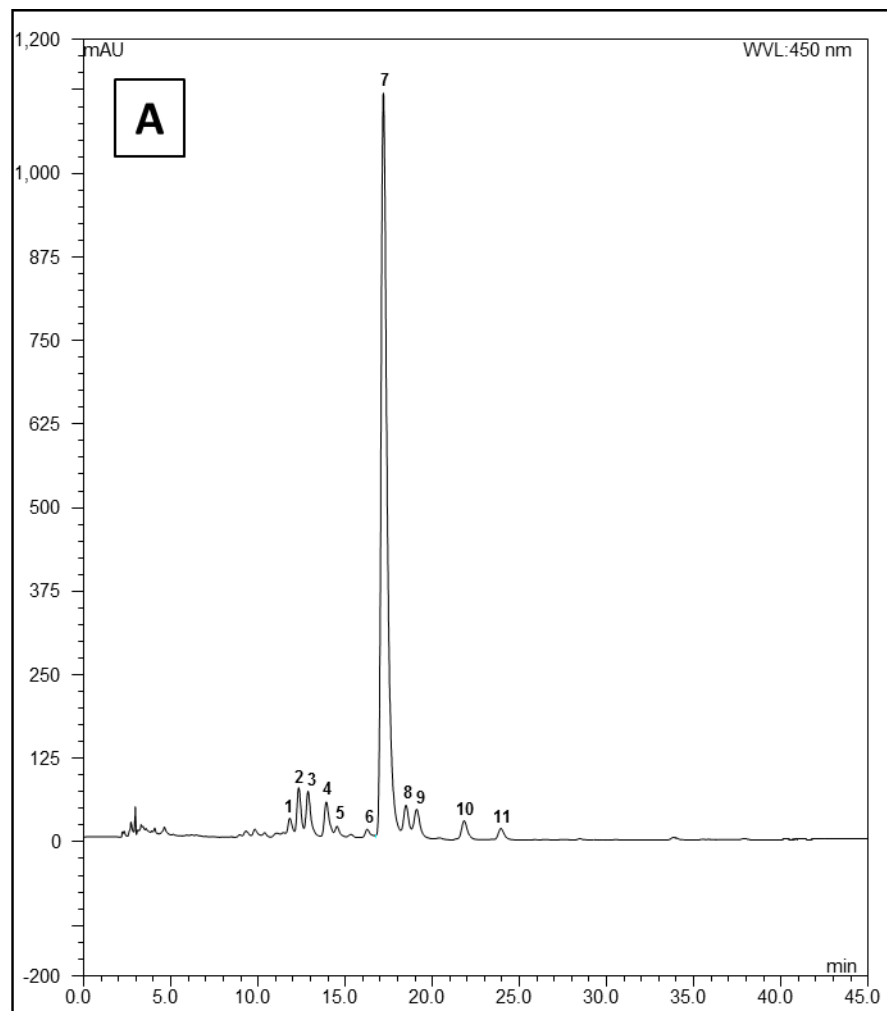


Figure S2. HPLC chromatograms of the partitioned extract: hypophase (A), and epiphase (B).

(A): peak 7: major carotenoid glycoside (78.1 % purity)

(B): peaks 1,2: chlorophylls, peak 5: β -cryptoxanthin, peak 6: echinenone, peak 7: (9/9'*Z*)-echinenone, peak 9: β -carotene, peak 10: (9*Z*)- β -carotene

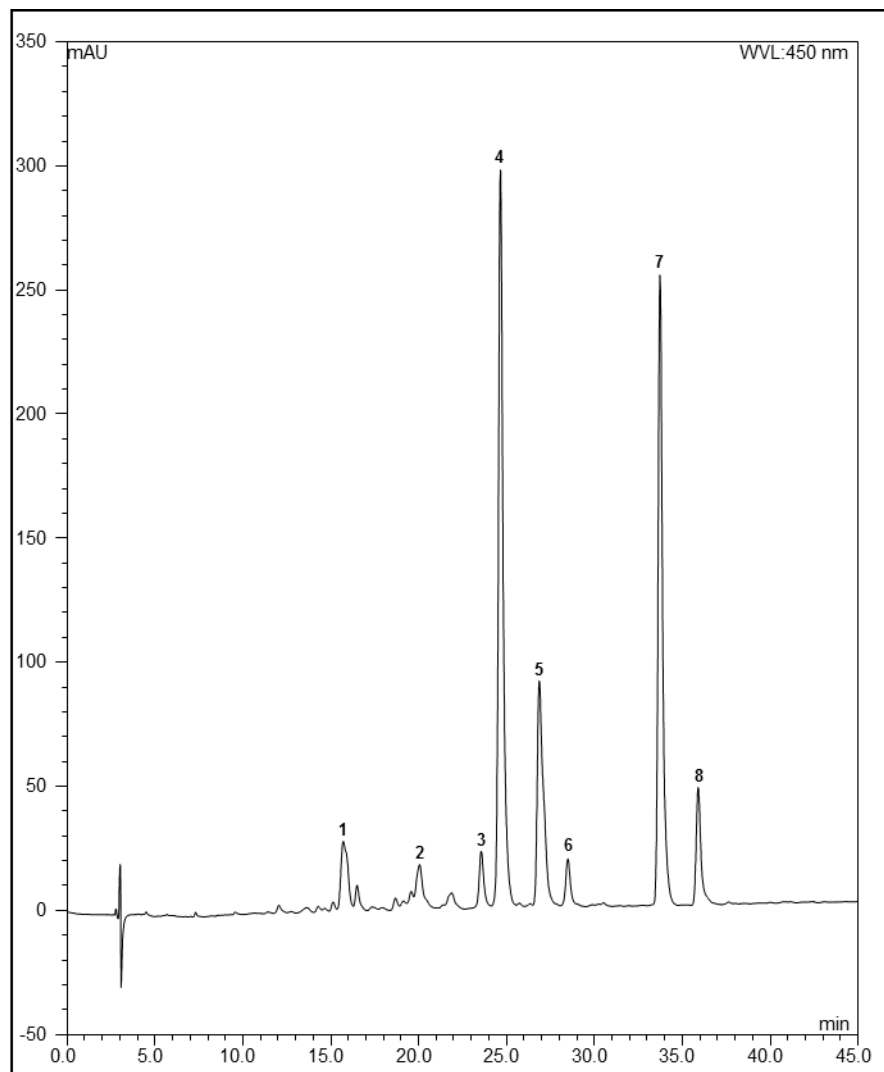


Figure S3. HPLC chromatogram of the saponified epiphase.

peak 3: β -cryptoxanthin, peak 4: echinenone, peak 5: (9/9'-Z)-echinenone, peak 7: β -carotene, (9Z)- β -carotene

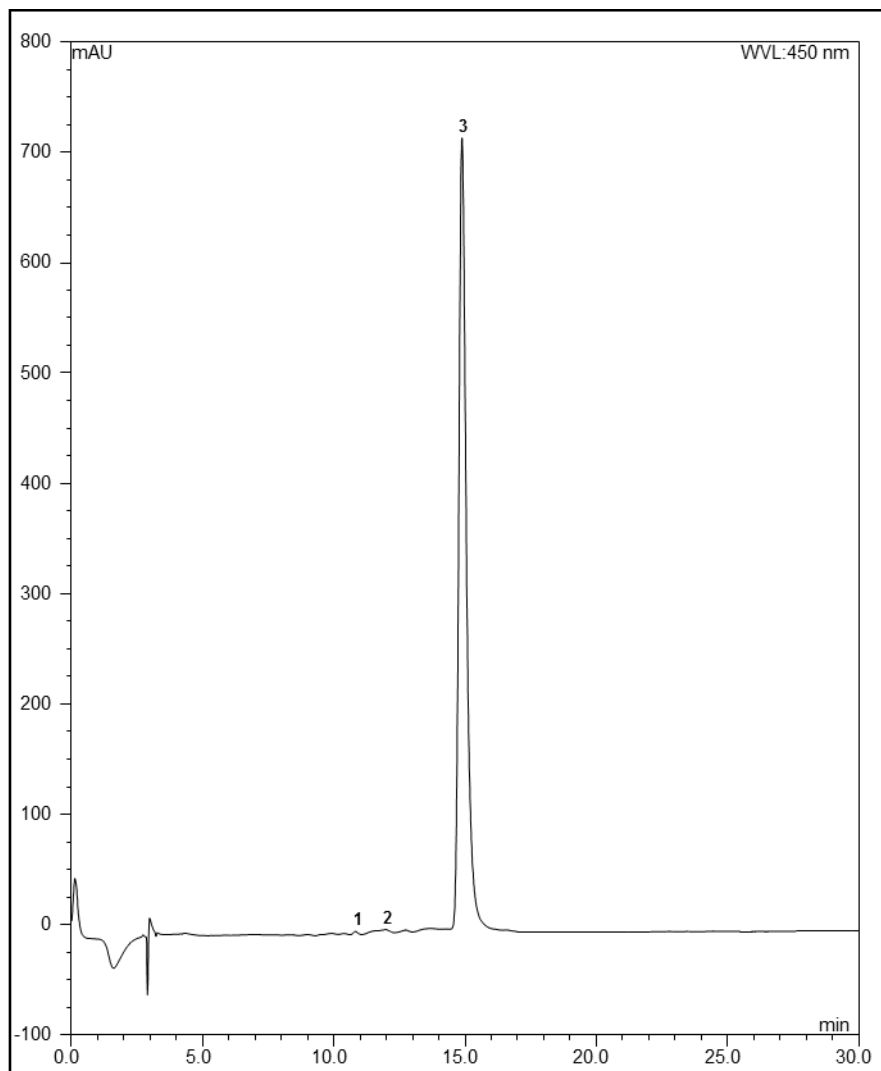


Figure S4. HPLC chromatogram of the crystalline glycoside (peak 3, 99.9% purity) gained by OCC chromatography of the hypophase.

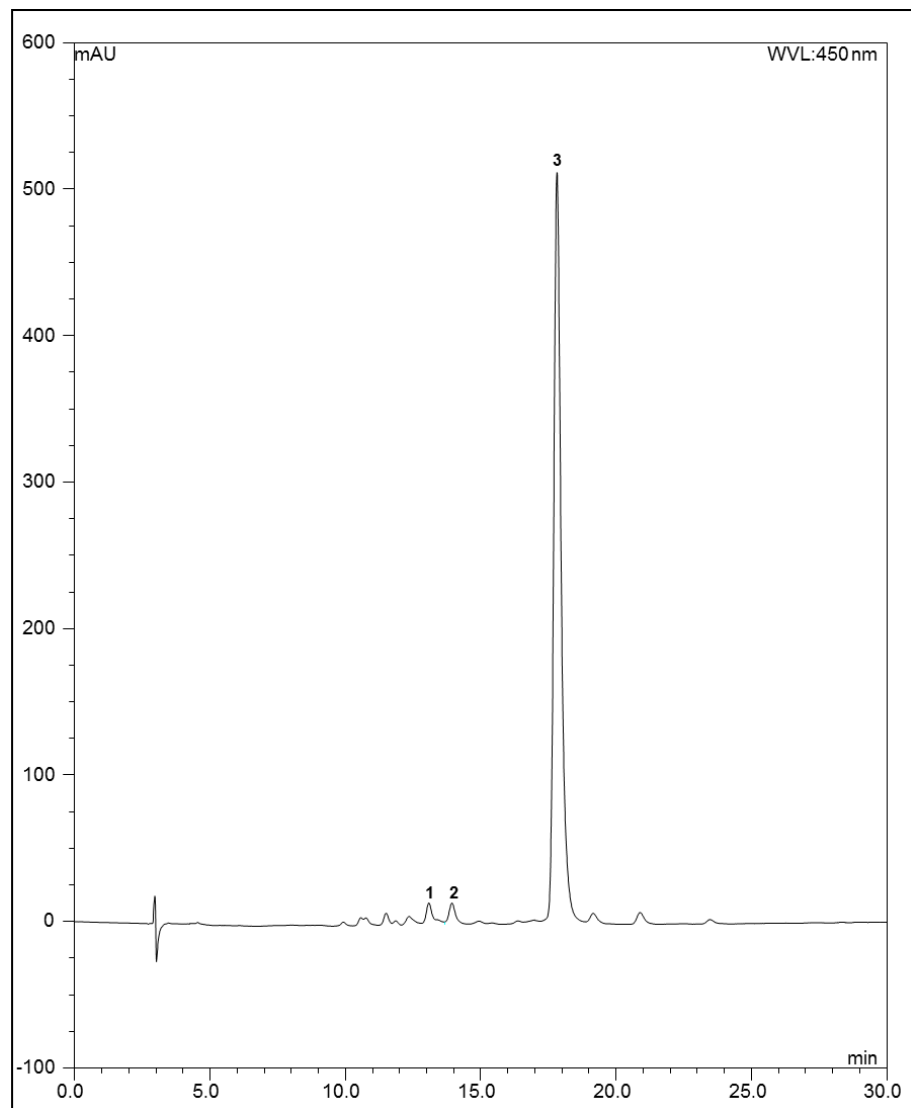


Figure S5. HPLC chromatogram of the crystalline acetylated glycoside (peak 3, 95.2% purity).

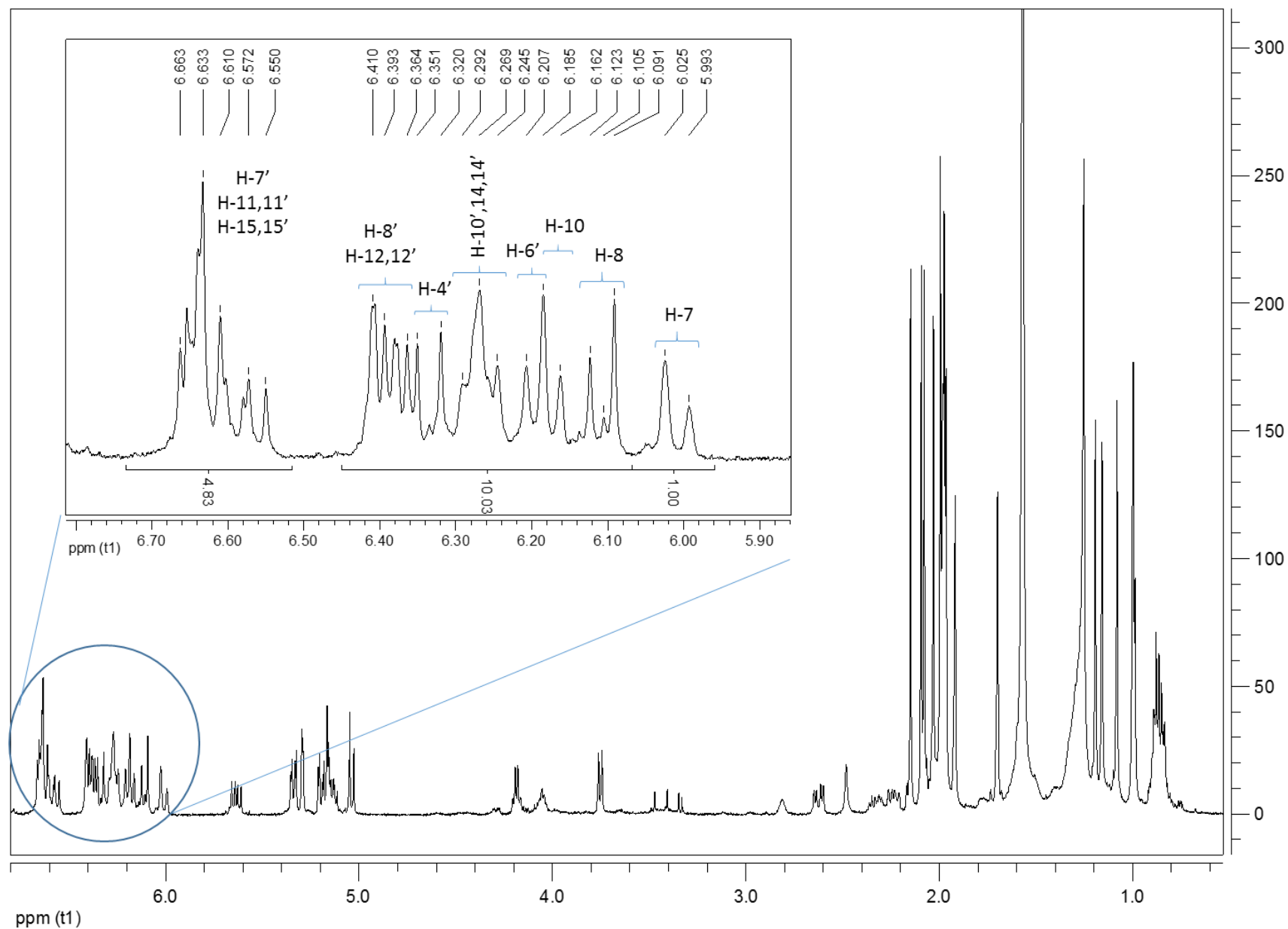


Figure S6.a. ^1H NMR spectrum of the acetylated glycoside in CDCl_3 .

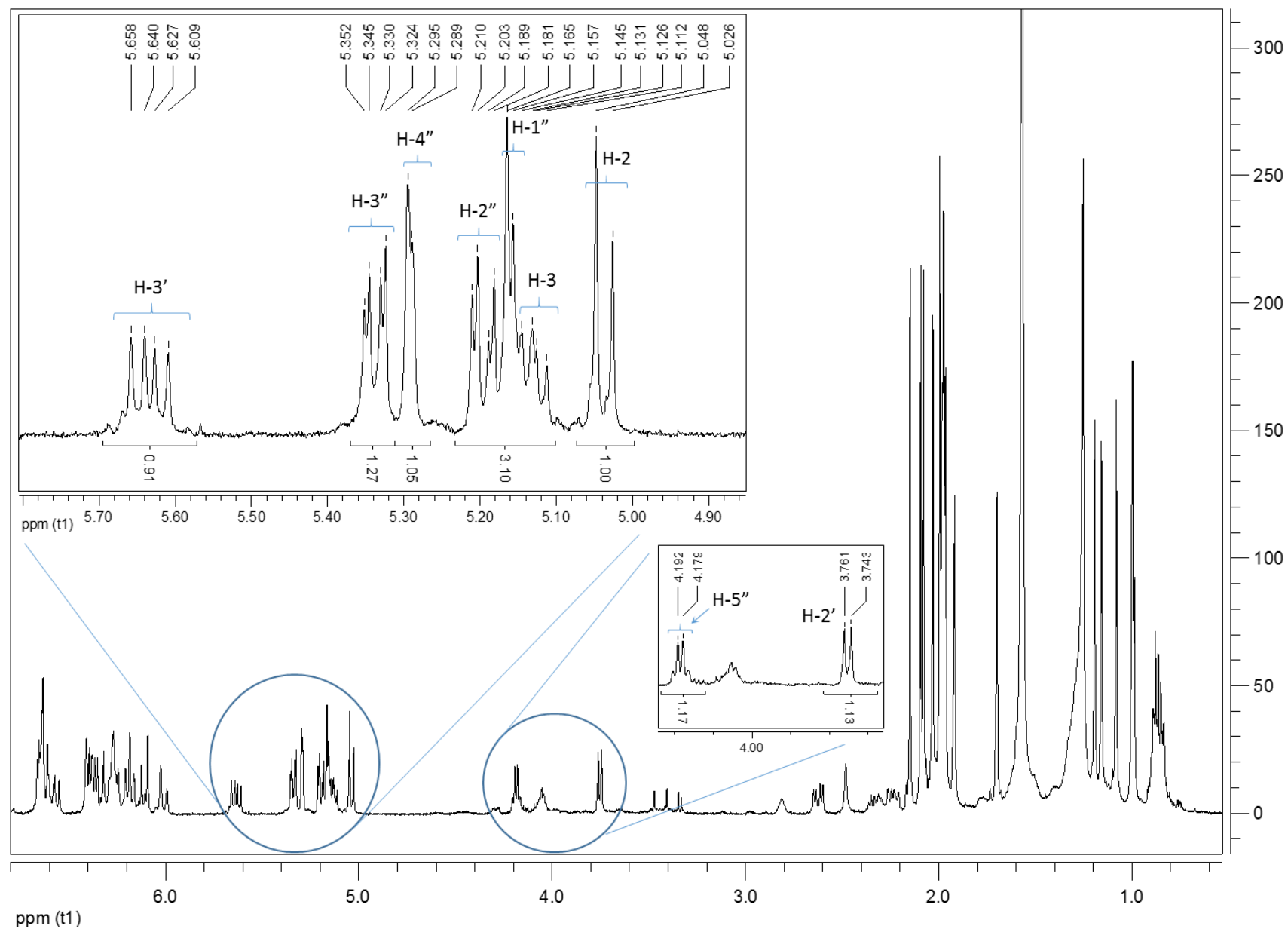


Figure S6.b. ^1H NMR spectrum of the acetylated glycoside in CDCl_3 .

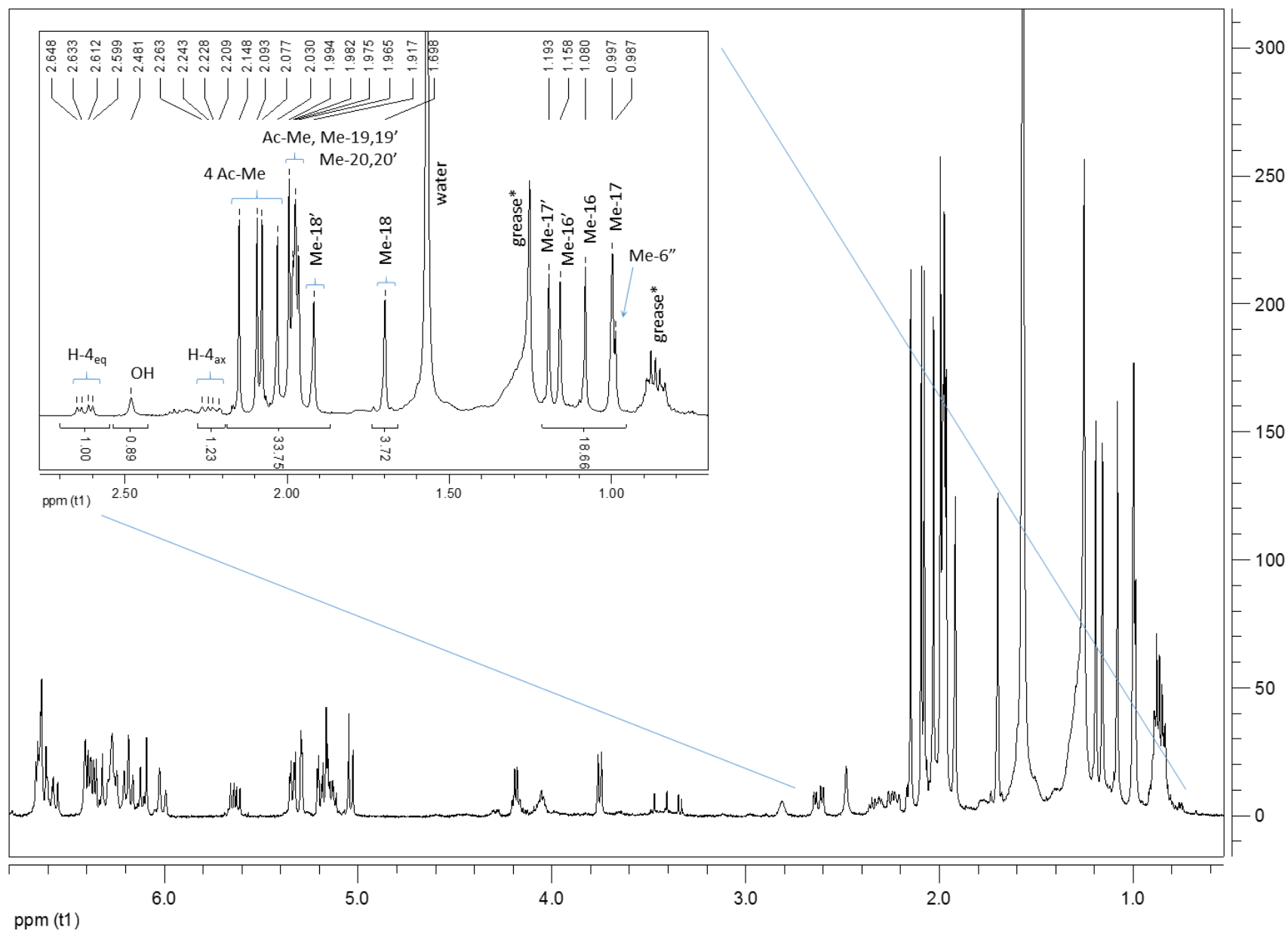


Figure S6.c. ^1H NMR spectrum of the acetylated glycoside in CDCl_3 . (*grease: long chain fatty acids)

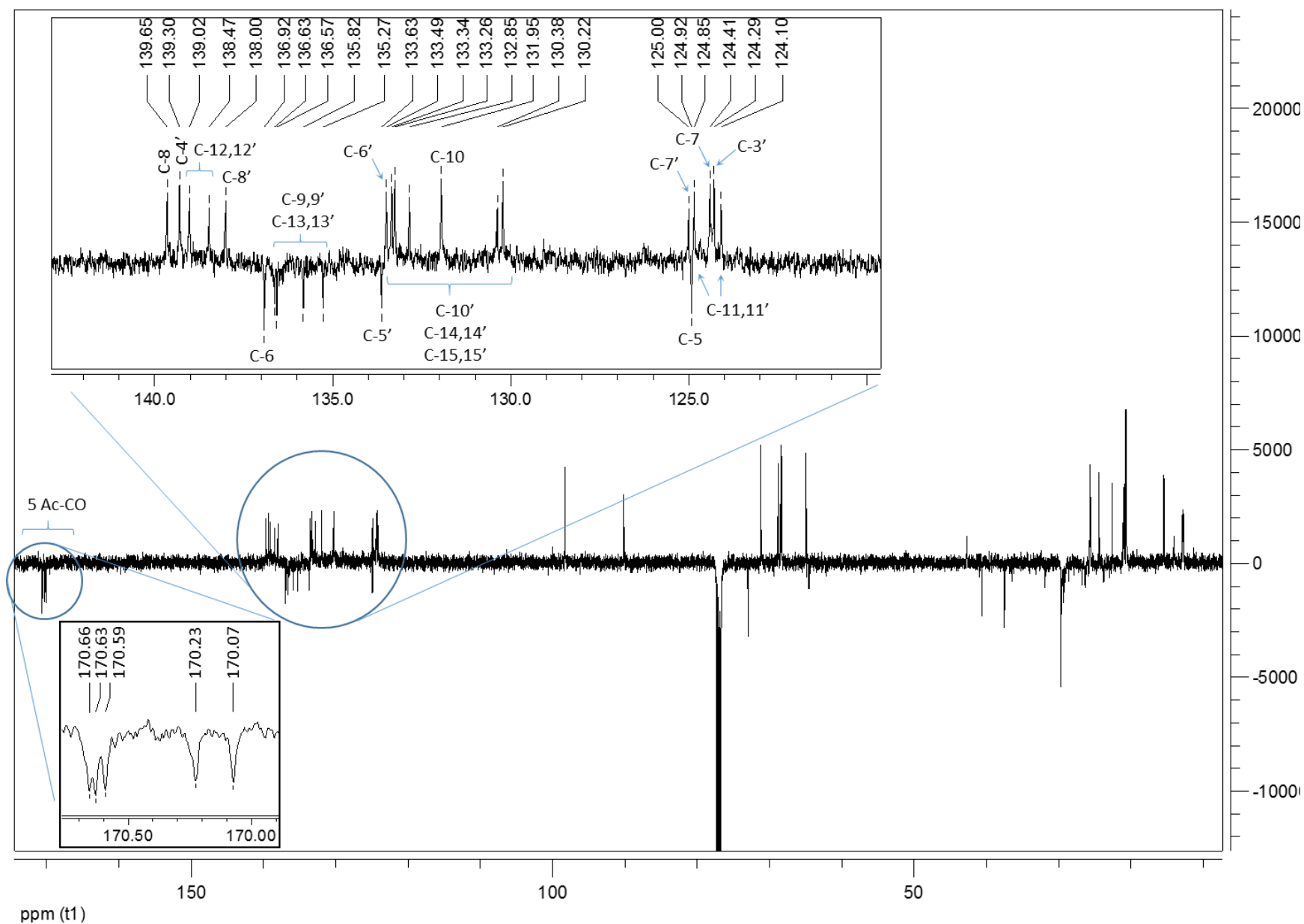


Figure S7.a. ^{13}C NMR spectrum of the acetylated glycoside in CDCl_3 .

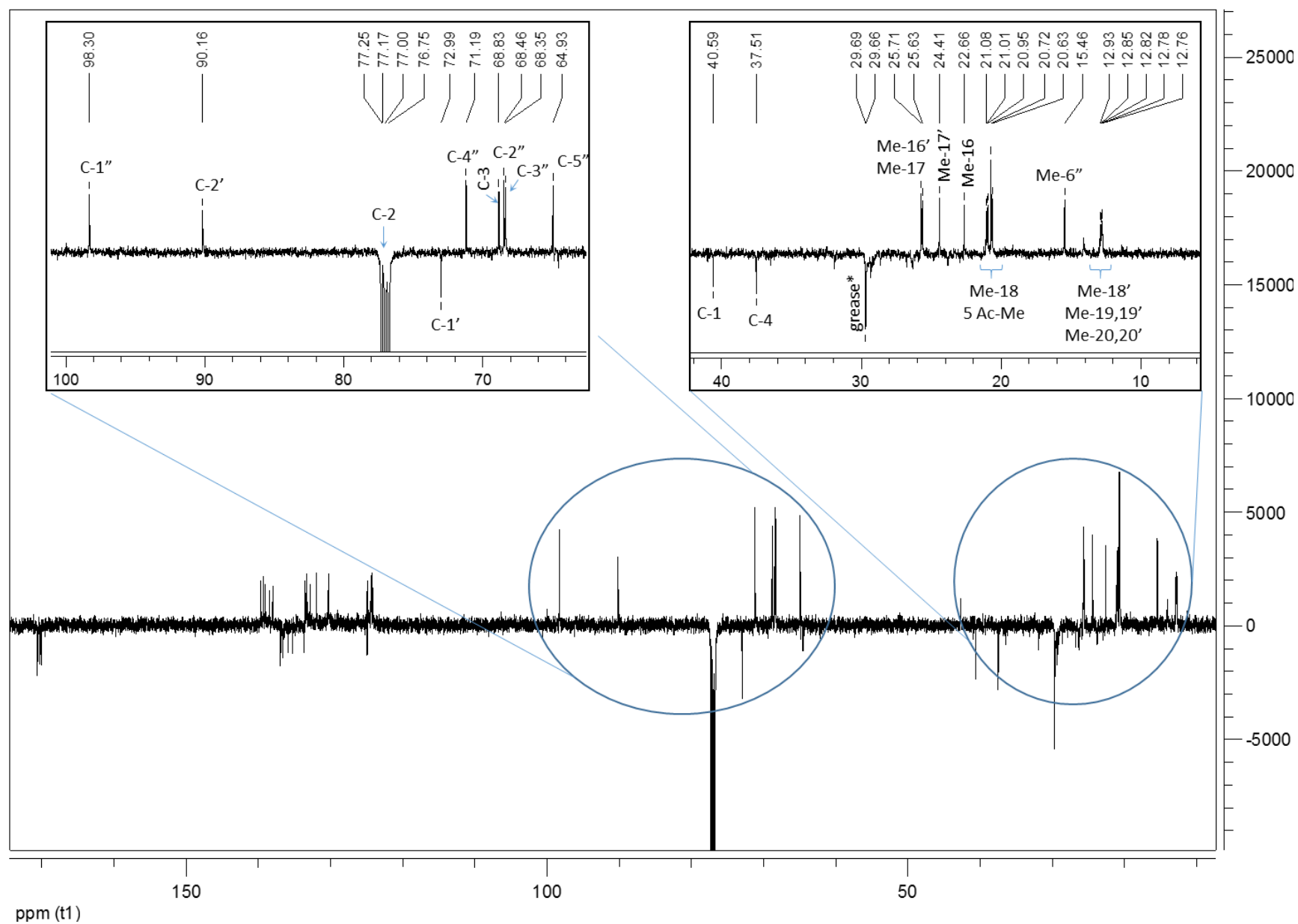


Figure S7.b. ^{13}C NMR spectrum of the acetylated glycoside in CDCl_3 . (*grease: long chain fatty acids)

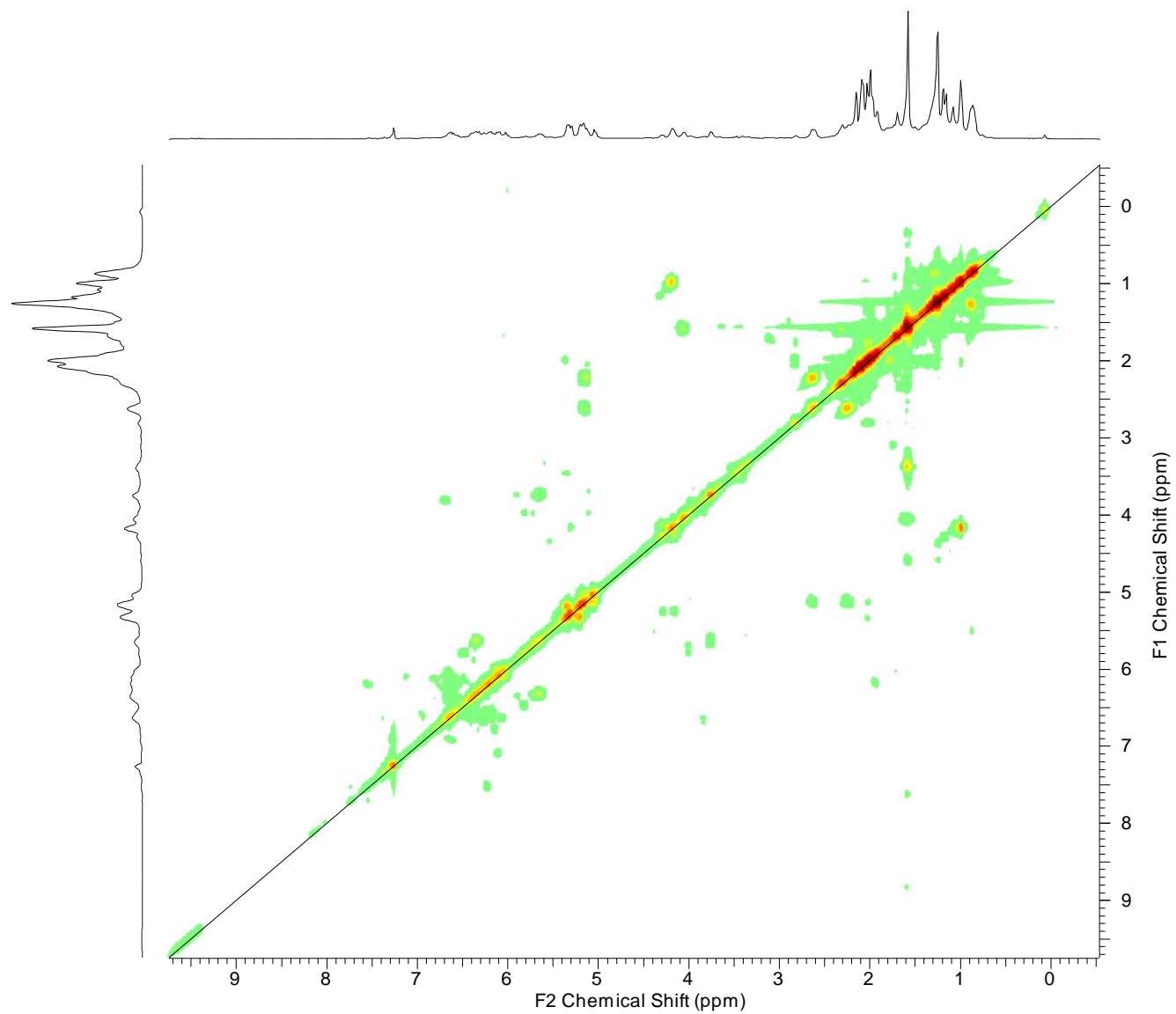


Figure S8. COSY NMR spectrum of the acetylated glycoside in CDCl_3 .

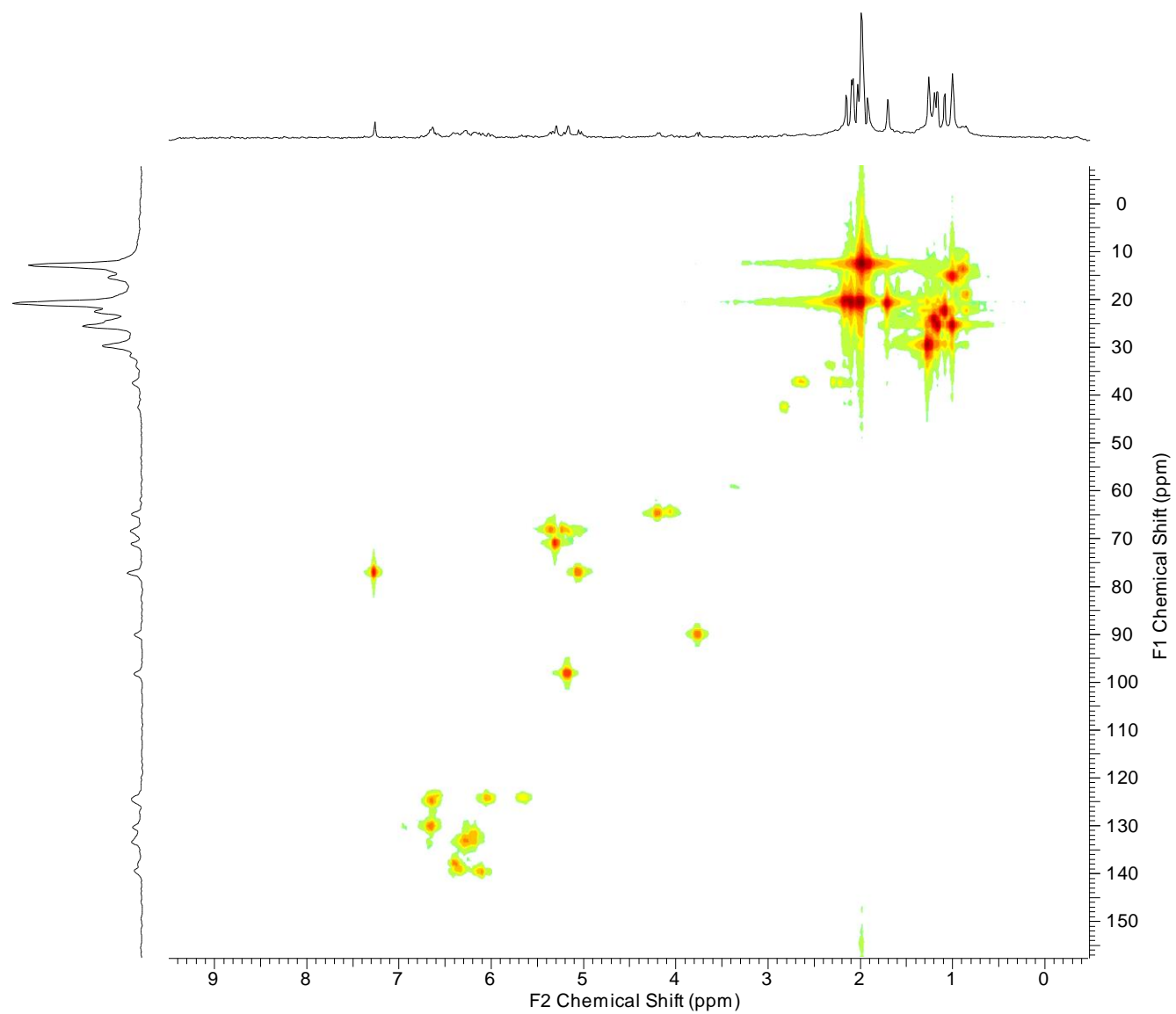


Figure S9. HSQC NMR spectrum of the acetylated glycoside in CDCl_3 .

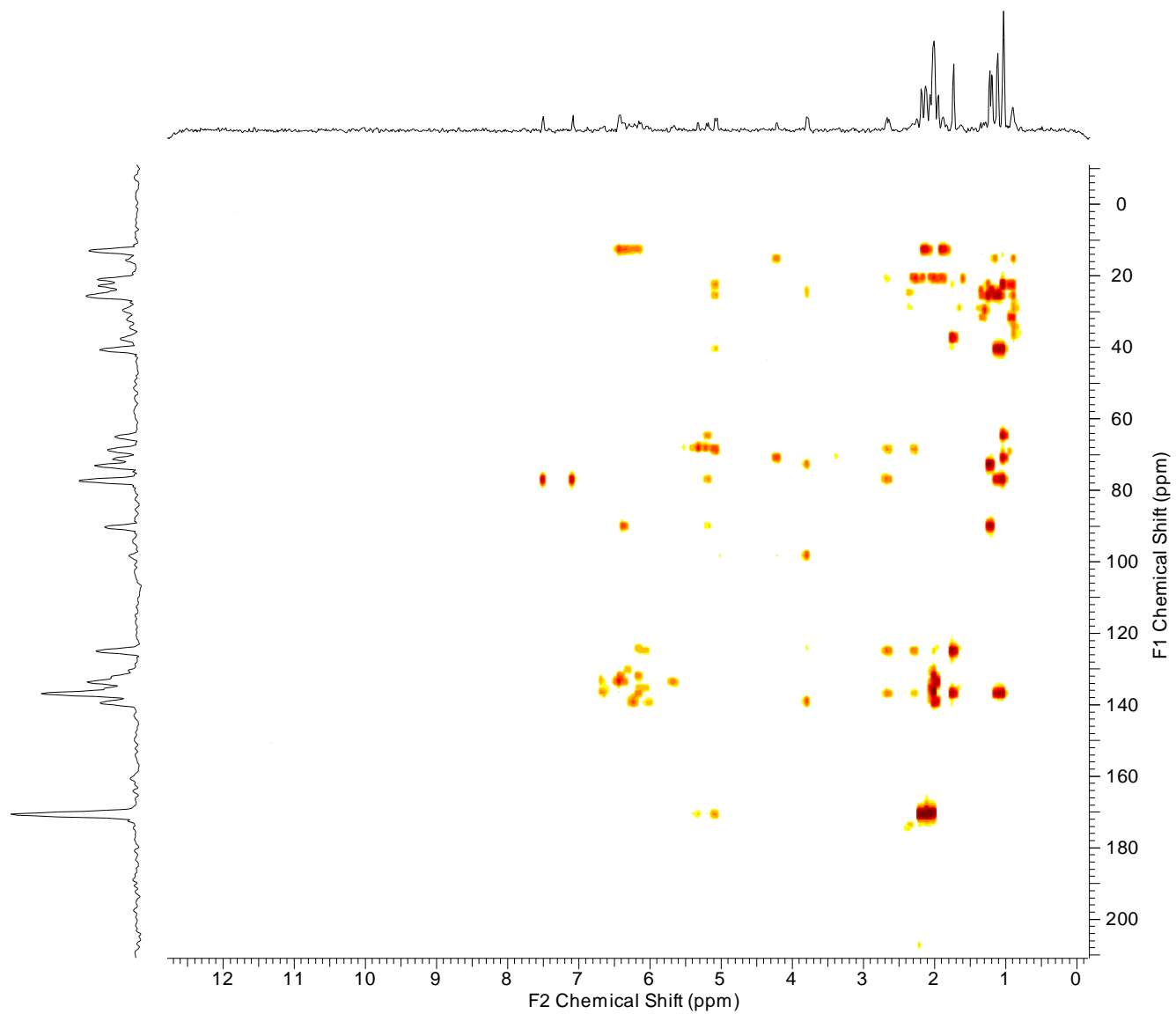
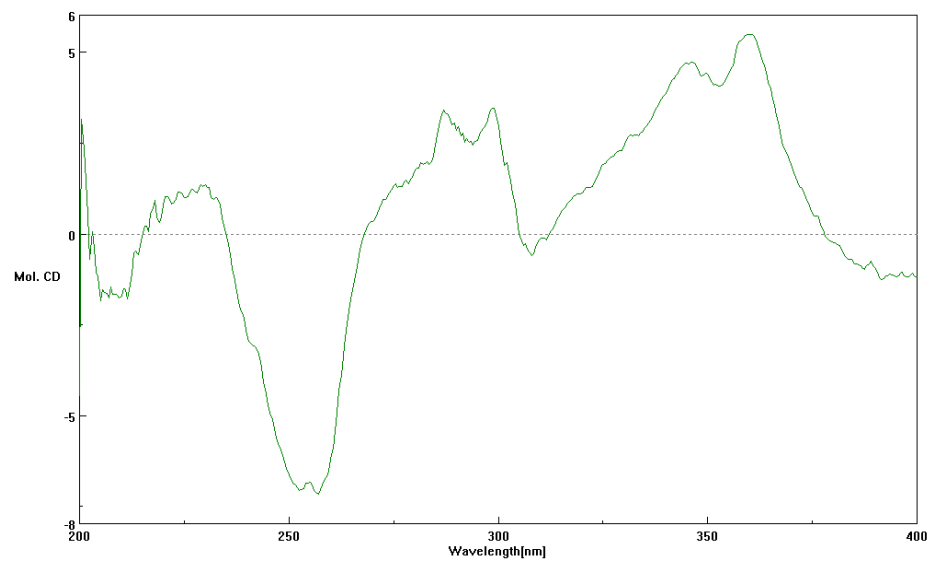
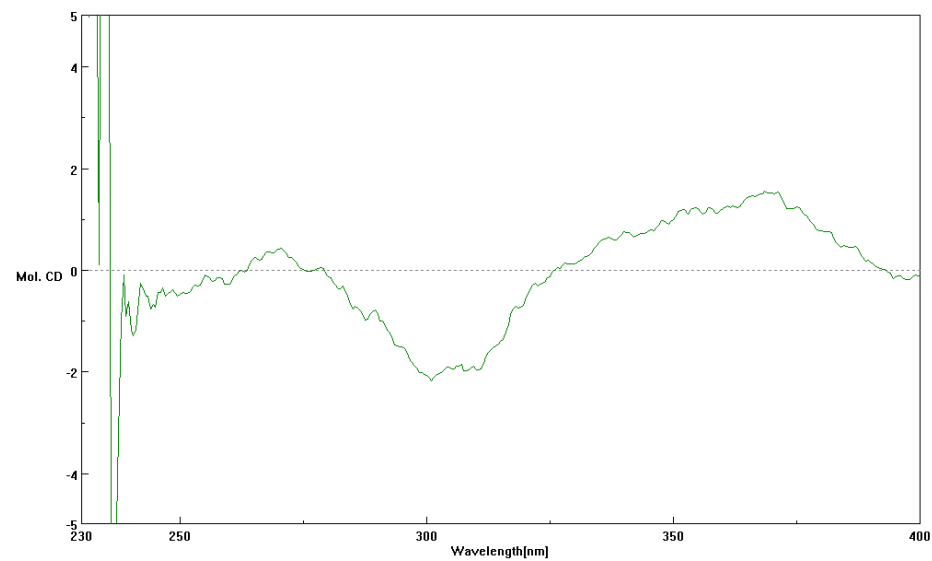


Figure S10. HMBC NMR spectrum of the acetylated glycoside in CDCl_3 .

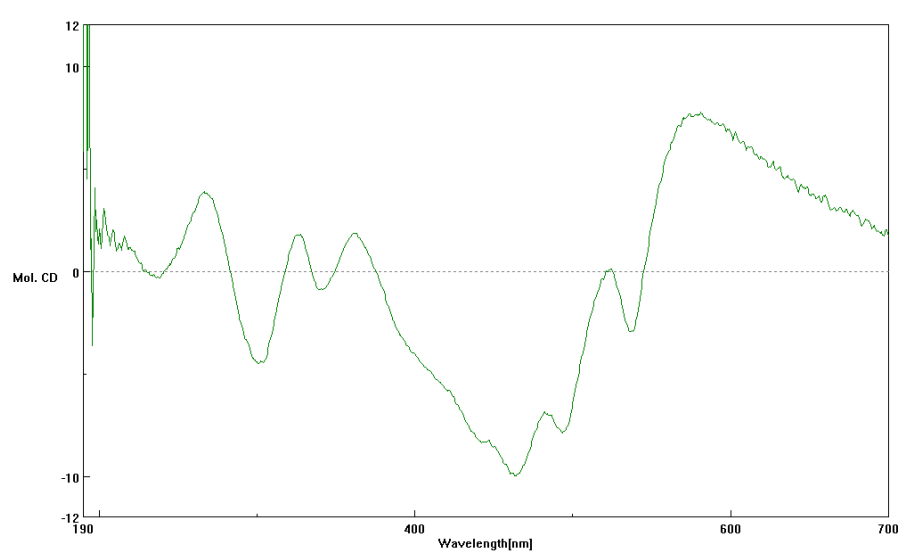
0.098 mg in 4 mL **MeOH**, c: 3.284E-5



0.098 mg 4 mL **CHCl₃**, c: 3,28E-5



0.098mg 4 mL **Hexan**, c: 3.28E-5



0.098 mg 4 mL **Acetonitrile**, c: 3.28E-5

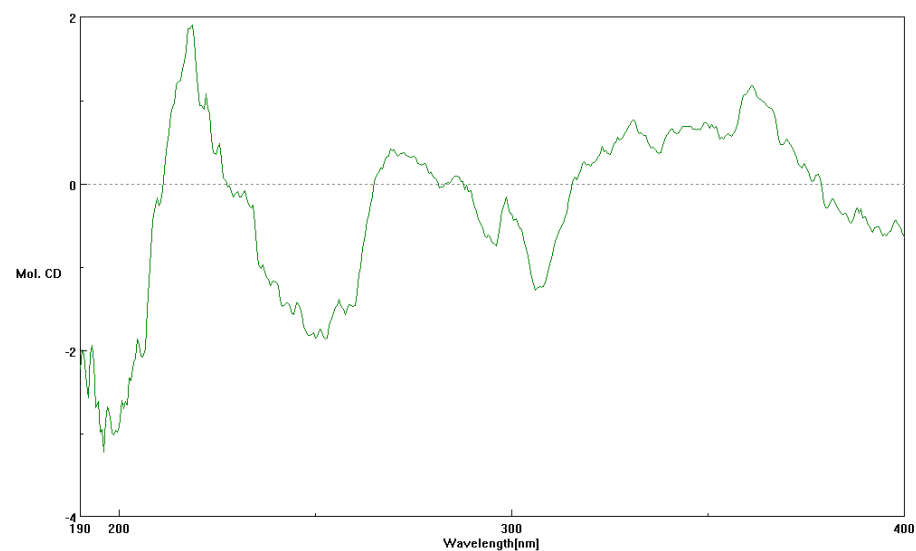


Figure S11. ECD spectrum of the acetylated glycoside in different solvents (cell width: 1 cm).

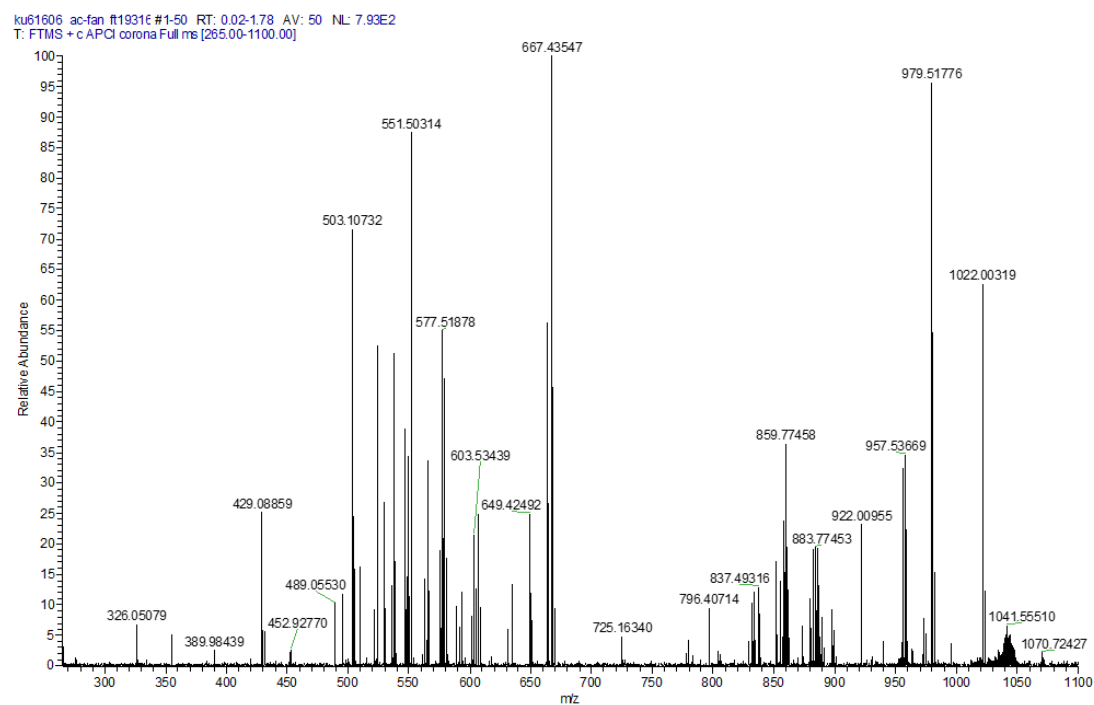
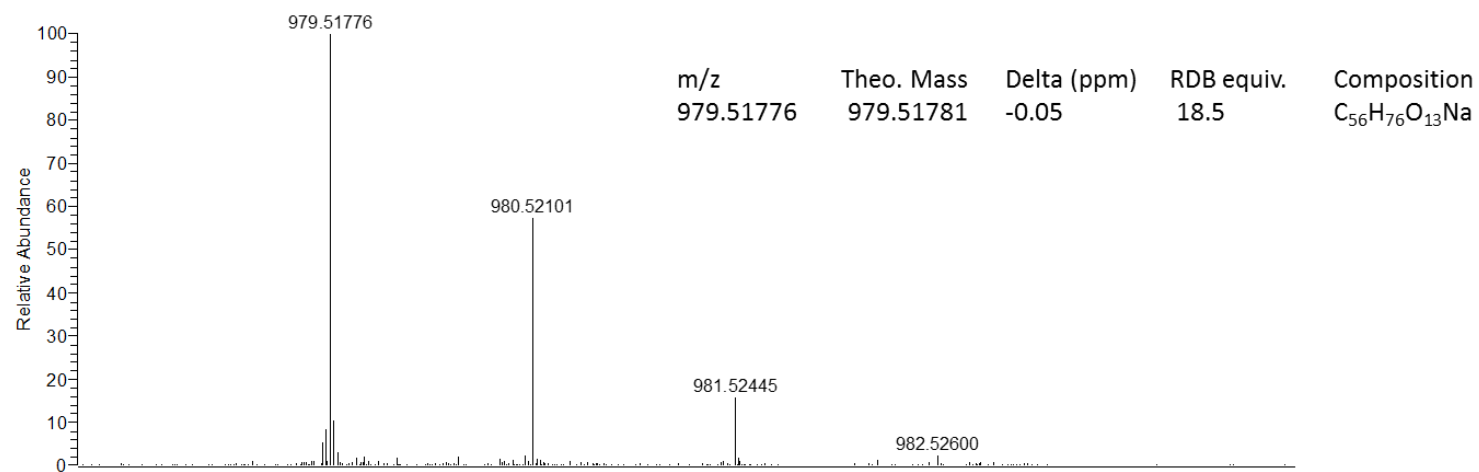
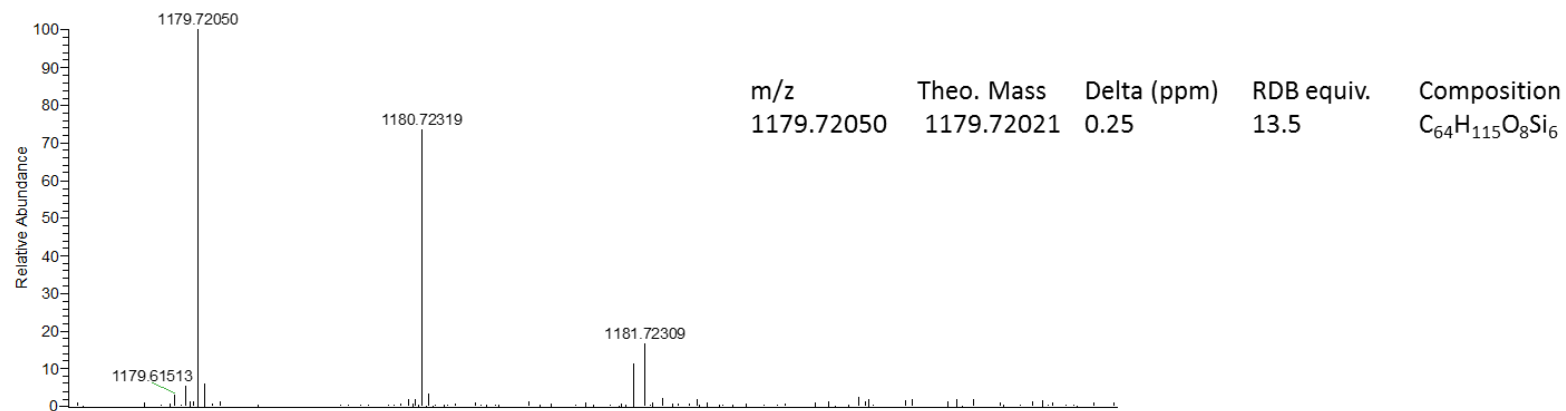


Figure S12. HRMS spectrum of the acetylated glycoside.



ku61607 sil-fan ft19319 #1-100 RT: 0.01-3.05 AV: 100 NL: 1.27E4
T: FTMS + c APCI corona Full ms [260.00-2000.00]

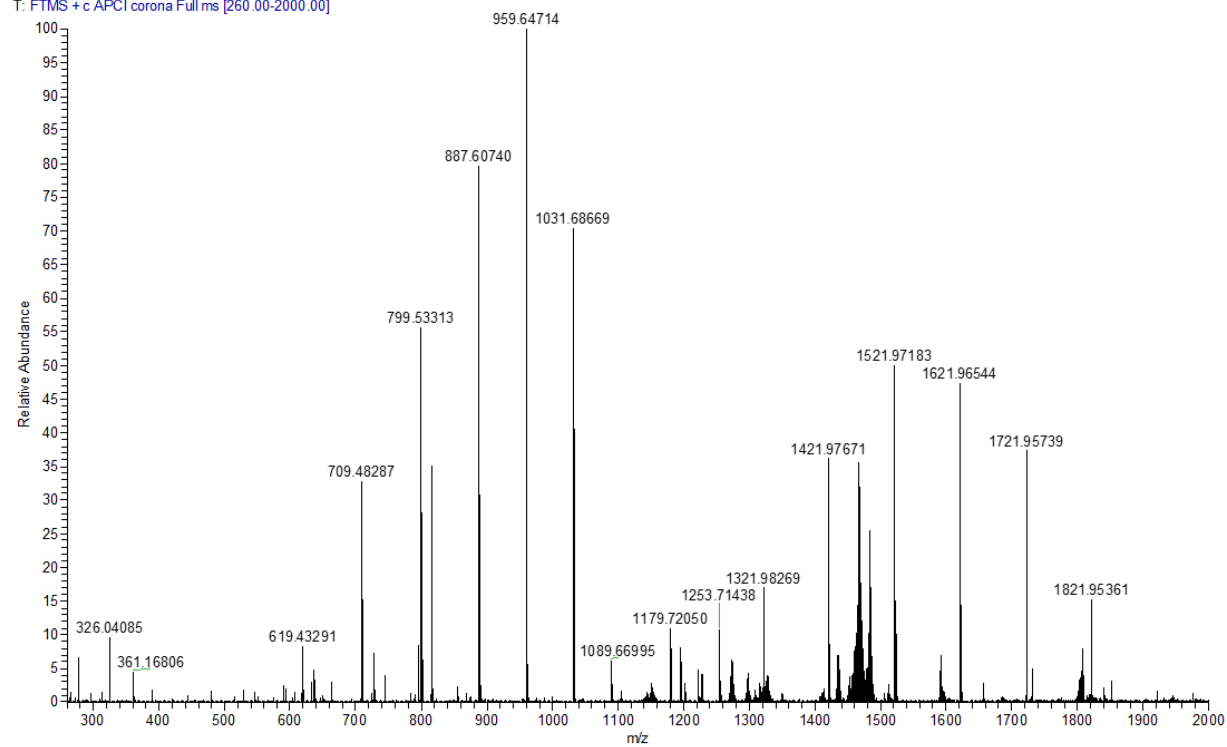


Figure S13. HRMS spectrum of the silylated glycoside.