Supporting Information

Chemoenzymatic Synthesis of Cholesteryl-6-*O*-tetradecanoyl-α-D-glucopyranoside: A Product of Host Cholesterol Efflux Promoted by *Helicobacter pylori*

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Contents

General Experimental. All reactions were conducted under a dry argon atmosphere. Solvents (chloroform ($CHCl_3$) 99.8%, benzene 99.8%, tetrahydrofuran (THF) 99.9%, and pyridine (pyr., 99.8%) were purchased as anhydrous extra dry solvents and used without further purification and stored under argon. Trimethylsilyl iodide (TMSI) and trimethylsilyl chloride (TMSCl) were stored at -15 °C under desiccated atmosphere in a sealed jar of drierite. Cholesterol (95%) and tetrabutyl ammonium iodide (TBAI) were both stored at 80 °C under vacuum for at least 48 h prior to use. Novozym 435 and myristic vinyl ester were stored at –15 °C. All other chemical reagents were commercial grade and used without further purification. Glass-backed TLC plates (Silica Gel 60 with a 254 nm fluorescent indicator) were used without further manipulation and stored over desiccant. Developed TLC plates were visualized with ammonium molybdate/cerium (IV) sulfate stain and heat provided by a hotplate. Silica gel flash column chromatography was performed using flash silica gel (32-63 μm) and employed a solvent polarity correlated with TLC mobility. NMR experiments were conducted on 600 and 800 MHz instruments and chemical shifts are reported relative to the appropriate deuterated solvent peak and are reported in parts per million (ppm). Melting points were taken on an automated melting point apparatus. Optical rotation was analyzed using 598 nm sodium lamp and a 100 mm cell. Samples were analyzed either by electrospray ionization mass spectrometry in positive mode using flow-injection analysis or by MALDI-TOF, with a matrix of commercial grade α-cyano-4-hydroxycinnamic acid. The microwave-assisted reaction was conducted in a commercial microwave reactor on standard, closed vessel mode setting at a temperature of 110 °C for 2 h with a monitoring setting of infrared.

Cholesteryl-6-*O*-tetradecanoyl-α-D-glucopyranoside (1). Compound **3** (50 mg, 0.07 mmol) generated from the per-*O*-trimethylsilylation of **2** was placed into a flame dried, argon purged round bottom and azeotroped three times with anhydrous benzene, after which it was diluted with anhydrous chloroform (1.0 mL, M = 0.10). The solution was then transferred to an oven dried argon purged microwave reaction vessel equipped with a stir bar and cooled to –5 – 0 °C. Once at the appropriate temperature, TMSI (1.2 equiv, 15 μL, 0.09 mmol) was added and the reaction mixture was allowed to stir over night (14 h) at –5 – 0 °C. Then chloroform was removed under reduced pressure and the *in situ* generated compound **4** was azeotroped three times with anhydrous benzene. The resulting yellow solid was then diluted with anhydrous benzene (1.0 mL) and TBAI (1.5 equiv, 40 mg, 0.10 mmol), cholesterol (2.0 equiv, 60 mg, 0.15 mmol), and Hünig's base (DIPEA, 2.0 equiv, 25 μL, 0.15 mmol) were added and the reaction mixture was subjected to microwave radiation for 2 h at 110 ˚C. The resulting solution was then decanted to a round bottom and the solvent was removed via reduced pressure. TBAI was triturated from the solution using a 1:1 ratio of cold ethyl acetate and hexanes (40 mL) and removed by filtration. The mother liquor was dried under reduced pressure to afford orange oil. The oil was dissolved in methanol (3.0 mL), Dowex 50WX8-200 acidic resin (200 mg) was added and the reaction was stirred for a period of 2 h at rt. The solution was decanted and the resin was removed by filtration and rinsed three times with methanol and then twice with chloroform. The organic solutions were combined with the decanted solution into a separate round bottom and the solvent was removed under reduced pressure to give a yellow solid. The yellow colored material was dissolved in a small amount of chloroform and was purified first by flash chromatography system using

silica gel as the stationary phase and a gradient mobile phase of 100% hexanes to 4:1 hexanes:ethyl acetate followed by a 9:1 ethyl acetate:methanol mobile phase. This purification procedure resulted in the isolation of 23 mg of cholesterol 38% and 35 mg of product. The product was further purified by normal phase high performance liquid chromatography using a 250 x 10 mm column and a gradient mobile phase starting with 100% ethyl acetate with gradually increasing polarity to a 9:1 ethyl acetate:methanol over a 20 min time period at a flow rate of 4 mL per min. HPLC ultimately gave 25 mg (33 μmol, 48%) of compound **1**, as a clear oil. R_f = 0.75 (ethyl acetate:methanol 9:1). $[\alpha]_D^{24}$ +21.9 (*c* 0.70, $CHCl_3$). ^{1}H NMR (800 MH_Z, $CDCl_3$): δ 0.67 (s, 3H), 0.86 (d, *J* = 6.6 Hz, 3H), 0.87 (d, *J* = 6.6 Hz, 3H), 0.88 (t, *J* = 7.2 Hz, 3H), 0.91 (d, *J* = 6.6 Hz, 3H), 0.97-0.99 (m, 1H), 1.00 (s, 3H), 1.02-1.17 (m, 7H), 1.23-1.27 (m, 22H), 1.28-1.38 (m, 7H), 1.42-1.64 (m, 9H), 1.80-1.89 (m, 3H), 1.96-2.02 (m, 2H), 2.31-2.37 (m, 4H), 3.32 (app. t, *J* = 9.4 Hz, 1H, H4'), 3.47 (dd, *J* = 4.3, 9.2 Hz, 1H, H2'), 3.47-3.50 (m, 1H), 3.72 (app. t, *J* = 9.2 Hz, 1H, H3'), 3.85 (ddd, *J* = 2.1, 4.6, 10.0 Hz, 1H, H5'), 4.23 (dd, *J* = 4.6, 12.2 Hz, 1H, H6'), 4.52 (dd, *J* = 4.6, 12.2 Hz, 1H, H6"), 5.02 (d, *J* = 4.0 Hz, 1H, H1'), 5.34-5.35 (m, 1 H). ^{13}C NMR (200 MHz, $CDCl_3$): δ 175.0, 140.4, 122.4, 97.0 (C1'), 78.6, 74.7 (C3'), 72.2 (C2'), 70.2 (C4'), 70.0 (C5'), 63.1(C6'), 56.8, 56.2, 50.2, 42.4, 40.2, 39.8, 39.6, 37.1, 36.8, 36.3, 35.9, 34.4, 32.08, 32.05, 32.0, 29.9, 29.9, 29.83, 29.81, 29.7, 29.5, 29.4, 29.3, 28.4, 28.2, 28.1, 25.1, 24.4, 24.0, 23.0, 22.8, 22.7, 21.2, 19.5, 18.8, 14.3, 12.0. HRMS (MALDI) calcd for: $C_{47}H_{82}O_7$, 781.5958 $[M + Na]^+$. Found: 781.6122.

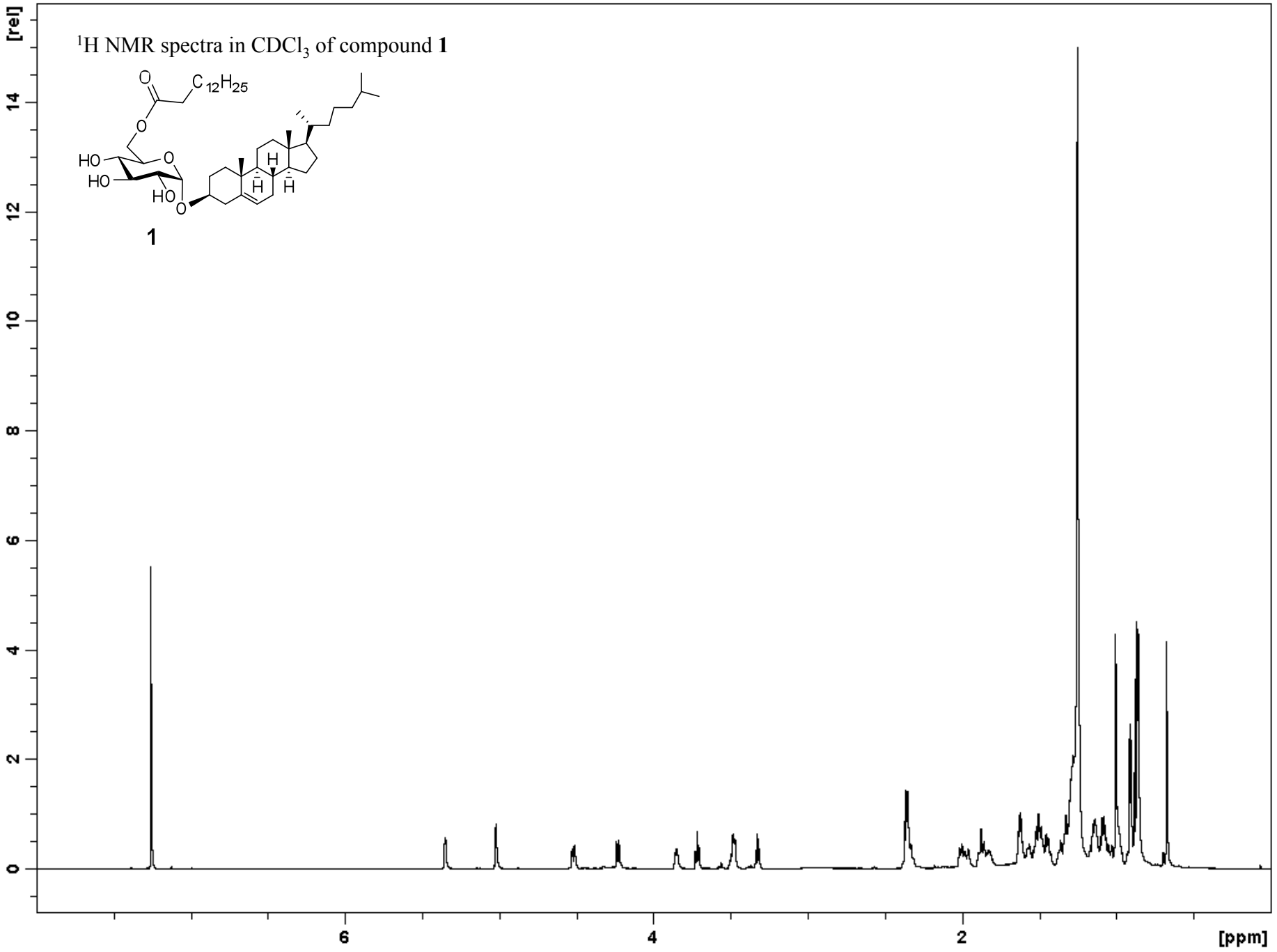
6-*O*-tetradecanoyl-α-D-glucopyranoside (2). α-D-Glucose (0.25 g, 1.4 mmol) was dried in a vacuum oven at 80 ˚C for at least 48 h prior to use. Glucose was then placed into an oven dried 6-dram glass vial equipped with a phenolic screw cap. To the vial was added anhydrous pyridine (2.0 mL) and the mixture was sonicated to solubilize glucose. Then anhydrous tetrahydrofuran (THF) (8.0 mL) was added to give a concentration of M = 0.14. Novozym 435 (50 mg) was then added along with the myristic vinyl ester (3.0 equiv., 1.25 mL, 4.2 mmol) and the vial was placed into a microplate thermoshaker at 40 ˚C and a mix setting of 3. The solution was shaken for 48 h and the solution was then decanted into a round bottom flask and the enzyme was removed by filtration and rinsed twice with methanol and twice with chloroform. The organic solutions were combined with the decanted solution and concentrated to afford a white solid. The solid is purified via trituration with anhydrous diethyl ether (100 mL). The resulting white solid was collected via suction filtration, to yield compound **2** quantitatively (530 mg, 1.4 mmol). Mp = 129.1-132.5 ˚C. R_f = 0.72 (ethyl acetate:methanol 9:1). ^{1}H NMR (800 MH_Z, d-DMSO): δ 0.84 (t, *J* = 7.0 Hz, 3H), 1.23-1.26 (m, 20H), 1.49 (p, *J* = 7.2 Hz, 2H), 2.26 (dt, *J* = 4.2, 7.2 Hz, 2H), 3.02 (ddd, *J* = 5.7, 9.8, 9.8 Hz, 1H, H4), 3.11 (ddd, *J* = 4.2, 6.7, 9.3 Hz, 1H, H2), 3.41 (ddd, *J* = 4.8, 9.2, 9.2 Hz, 1H, H3), 3.75 (ddd, *J* = 1.8, 6.2, 9.2 Hz, 1H, H5), 3.97 (dd, *J* = 6.2, 11.8 Hz, 1H, H6), 4.25 (dd, *J* = 1.8, 11.8 Hz, 1H, H6'), 4.51 (d, *J* = 6.7 Hz, 1H, 2OH), 4.73 (d, *J* = 4.6 Hz, 1H, 3OH), 4.88 (app. t, *J* = 4.2 Hz, 1H, H1), 5.02 (d, *J* = 5.6 Hz, 1H, 4OH), 6.32 (d, *J* = 4.7 Hz, 1H, 1OH). ^{13}C NMR (200 MHz, $CDCl_3$): δ 173.5, 92.9 (C1), 73.5 (C3), 72.8 (C2), 71.1 (C4), 69.7 (C5), 64.5 (C6), 34.0, 31.9, 29.7, 29.6, 29.6, 29.6, 29.5, 29.3, 29.3, 29.0, 25.1, 22.7, 14.6. HRMS (MALDI) calcd for $C_{20}H_{38}O_7$ $[M + Na]^+$, 413.2515. Found: 413.2274.

6-*O*-Myristoyl-1,2,3,4-*O*-tetrakis(trimethylsilyl)-α-D-glucopyranoside (3). Compound **2** (400 mg, 1.0 mmol) was dried over night in the vacuum oven at a temperature of 80 °C prior to per-*O*-trimethylsilylation. Anhydrous pyridine (10.5 mL, M = 0.10) was added followed by slow addition of TMSCl (8.0 equiv., 0.9 mL, 8.2 mmol). After addition of TMSCl, the reaction was stirred over night (~15 h). The solvent was then removed via reduced pressure to give a yellow-white gel. The gel was dissolved in ethyl acetate (20 mL) and washed with a saturated solution of bicarbonate (20 mL). The aqueous solution was washed twice and the organic layers were combined and dried with sodium sulfate. The solvent was then removed and the substance was azeotroped with benzene three times followed by chloroform addition and evaporation another three times. The resulting compound **3** was produced in a quantitative and used without further purification (696 mg, 1.0 mmol). R_f = 0.52 (hexanes:ethyl acetate 9:1). ^{1}H NMR (600 MH_Z, C_6D_6): δ 0.10 (s, 9H), 0.15 (s, 9H), 0.26 (s, 9H), 0.28 (s, 9H), 0.93 (t, *J* = 6.9 Hz, 3H), 1.22-1.32 (m, 20H), 1.61 (p, *J* = 7.4 Hz, 2H), 2.25 (t, *J* = 7.4 Hz, 2H), 3.57 (dd, *J* = 3.1, 9.2 Hz, 1H, H2), 3.79 (dd, *J* = 8.8, 9.5 Hz, 1H, H4), 4.13 (app. t, *J* = 8.8 Hz, 1H, H3), 4.19 (ddd, *J* = 2.2, 4.6, 9.5 Hz, 1H, H5), 4.31 (dd, *J* = 4.6, 11.8 Hz, 1H, H6), 4.74 (dd, *J* = 2.2, 11.8 Hz, 1H, H6'), 5.17 (d, *J* = 3.1 Hz, 1H, H1). ^{13}C NMR (150 MHz, C_6D_6): δ 173.4, 94.6 (C1), 74.9 (C3), 74.8 (C2), 73.2 (C4), 71.0 (C5), 63.9 (C6), 34.7, 32.7, 30.5, 30.5, 30.5, 30.5, 30.3, 30.2, 30.1, 29.8, 25.6, 23.5, 14.8, 1.9, 1.4, 0.7, 0.5. HRMS (ESI) calcd for: $C_{32}H_{70}O_7Si_4$ $[M + Na]^+$, 701.4096. Found: 701.4100.

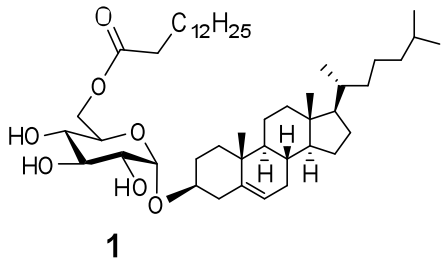
Iodo-6-*O*-myristoyl-2,3,4-*O*-tris(trimethylsilyl)-α-D-glucopyranoside (4). Compound **3** (50 mg, 0.07 mmol) was placed into a flame dried, argon purged round bottom and azeotroped three times with anhydrous benzene, after which it was diluted with deuterated chloroform (0.5 mL, M = 0.14). The solution was then transferred to a NMR tube that was dried over night in a vacuum desiccator. The 800 MHz proton NMR probe was first cooled to 273 K. A proton NMR of compound **3** was obtained and then the instrument was tuned, locked and shimmed again, prior to the collection of the next set of proton spectra. The sample was ejected and TMSI (1.2 Equiv., 15 μL, 0.09 mmol) was added to the NMR tube, which was shaken before returning to the probe. A series of proton spectra were collected (see supporting information). The time of the first spectrum was derived from a stopwatch, which was started when TMSI was added and stopped after the end of the first proton spectra. Subsequent reaction times were obtained from the FID time stamp of each proton spectra given by the NMR instrument. After 12 h, the reaction with TMSI did not progress any further and the product was characterized *in situ*. Rf = 0.0 (4:1 hexanes:ethyl acetate). ^{1}H NMR (600 MH$_Z$, C_6D_6): δ 0.00 (s, 9H), 0.20 (s, 9H), 0.26 (s, 9H), 0.91-0.94 (m, 3H), 1.26-1.30 (m, 20H), 1.57-1.60 (m, 2H), 2.19-2.22 (m, 2H), 2.49-2.51 (m, 1H, H2), 3.81 (app. t, *J* = 9.0 Hz, 1H, H4), 3.96-4.03 (m, 2H, H3, H5), 4.16 (dd, *J* = 4.6, 10.8 Hz, 1H, H6), 4.56 (dd, *J* = 1.8, 10.8 Hz, 1H, H6'), 6.73 (d, *J* = 3.0 Hz, 1H, H1). ^{13}C NMR (150 MHz, C_6D_6): δ 173.0, 84.7 (C1), 78.2 (C5), 78.1 (C4), 73.3 (C2), 71.0 (C3), 62.5 (C6), 34.6, 32.7, 30.6, 30.5, 30.5, 30.5, 30.3, 30.2, 30.1, 29.8, 25.6, 23.5, 14.8, 1.8, 1.3, 0.6.

Cholesteryl-6-*O*-tetradecanoyl-β-D-glucopyranoside (5). Compound **3** (100.0 mg, 0.147 mmol) was placed into a flame dried, argon purged round bottom and azeotroped three times with anhydrous benzene, upon which it was diluted with anhydrous dichloromethane (1.0 mL, M = 0.10). The solution was then equipped with a stir bar and cooled to –5 ˚C-0 ˚C. Once the appropriate temperature was achieved, TMSI (1.2 Equiv., 25 μL, 0.18 mmol) was added and the mixture was stirred over night (14 h) remaining at –5 ˚C-0 ˚C. After 14 h, the dichloromethane was removed under reduced pressure and the *in situ* generated compound **4** was azeotroped three times with anhydrous benzene. Compound **4** was then placed under vacuum to insure complete dryness for ~1 h. In a separate flame dried round bottom flask, cholesterol (0.5 equiv, 30 mg, 0.07 mmol), 4Å molecular sieves (130 mg), and silver triflate (AgOTf) (1.1 equiv, 42 mg, 0.16 mmol) were allowed to stir for 30 min in anhydrous dichloromethane under argon in the dark. This mixture was then cooled to –78 ˚C, and compound **4** was diluted with anhydrous dichloromethane (1 mL) and transferred via cannulation into the cholesterol mixture. Upon complete addition of **4**, the reaction was stirred at -78 ˚C for 6 h and then allowed to gradually warm up to rt for another 6 h, after which the solution was filtered through celite and rinsed with methanol. The crude material was diluted with a small portion of chloroform and was purified first by flash chromatography using silica gel as the stationary phase and a gradient mobile phase of 100% hexanes to 4:1 hexanes:ethyl acetate followed by a 9:1 ethyl acetate:methanol mobile phase. This resulted in the isolation of 7 mg of cholesterol 23% and 41 mg of product. The product was further purified by normal phase high performance liquid chromatography (HPLC) using a normal phase silica (250 x 10 mm) column with a gradient mobile phase starting with

100% ethyl acetate with gradually increasing polarity to 9:1 ethyl acetate:methanol over a time period of 20 min at a flow rate of 4 mL per min. This gave 5 mg (0.007 mmol, 9%) of compound **1** and 30 mg (0.04 mmol) of compound **2**, both as a clear oil. Compound **2** was generated in a yield of 54%. R_f = 0.78 (ethyl acetate:methanol 9:1) $[\alpha]_D^{24}$ -31.3 (*c* 1.00, $CHCl_3$). ^{1}H NMR (600 MH_Z, $CDCl_3$): δ 0.67 (s, 3H), 0.85 (d, *J* = 6.6 Hz, 3H), 0.86 (d, *J* = 6.6 Hz, 3H), 0.88 (t, *J* = 7.2 Hz, 3H), 0.91 (d, *J* = 6.6 Hz, 3H), 0.95-0.98 (m, 1H), 1.00 (s, 3H), 1.03-1.18 (m, 7H), 1.25-1.32 (m, 28H), 1.42-1.62 (m, 10H), 1.79-1.86 (m, 2H), 1.93-2.02 (m, 3H), 2.24-2.29 (m, 1H), 2.35 (t, *J* = 7.7 Hz, 2H), 2.34-2.37 (m, 1H), 3.36 (app. t, *J* = 8.2 Hz, 1H, H2), 3.38 (app. t, *J* = 9.0 Hz, 1H, H4), 3.45 (ddd, *J* = 1.6, 4.9, 9.0 Hz, 1H, H5), 3.52-3.59 (m, 1H), 3.58 (app. t, *J* = 9.0 Hz, 1H, H3), 4.26 (dd, *J* = 1.6, 12.2 Hz, 1H, H6), 4.38 (d, *J* = 7.8 Hz, 1H, H1), 4.48 (dd, *J* = 4.9, 12.2 Hz, 1H, H6'), 5.36-5.37 (m, 1H). ^{13}C NMR (150 MHz, $CDCl_3$): δ 175.0, 140.4, 122.3, 101.3 (C1), 79.7, 76.0 (C3), 74.1 (C5), 73.7 (C2), 70.1 (C4), 63.3 (C6), 56.9, 56.3, 50.3, 42.4, 39.9, 39.6, 39.0, 37.4, 36.8, 36.3, 35.9, 34.4, 32.08, 32.06, 32.0, 29.9, 29.9, 29.8, 29.8, 29.8, 29.7, 29.5, 29.5, 29.3, 28.4, 28.2, 25.1, 24.4, 24.0, 23.0, 22.8, 22.7, 21.2, 19.5, 18.8, 14.3, 12.0. HRMS (MALDI) calcd for $C_{47}H_{82}O_7$ $[M + Na]^+$, 781.5958 found 781.6122.



^{13}C NMR spectra in $CDCl_3$ of compound **1**



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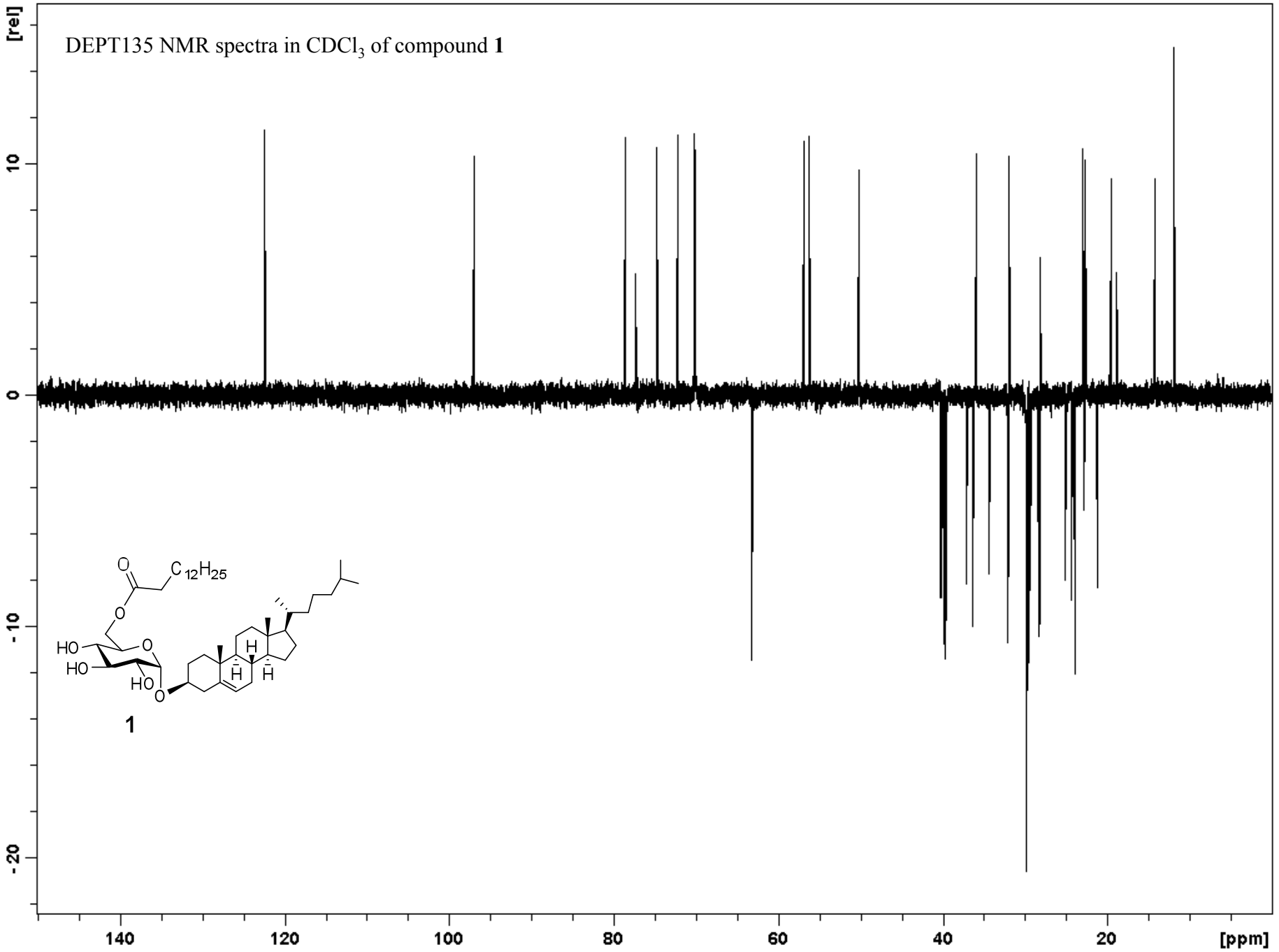
DEPT135 NMR spectra in $CDCl_3$ of compound **1**



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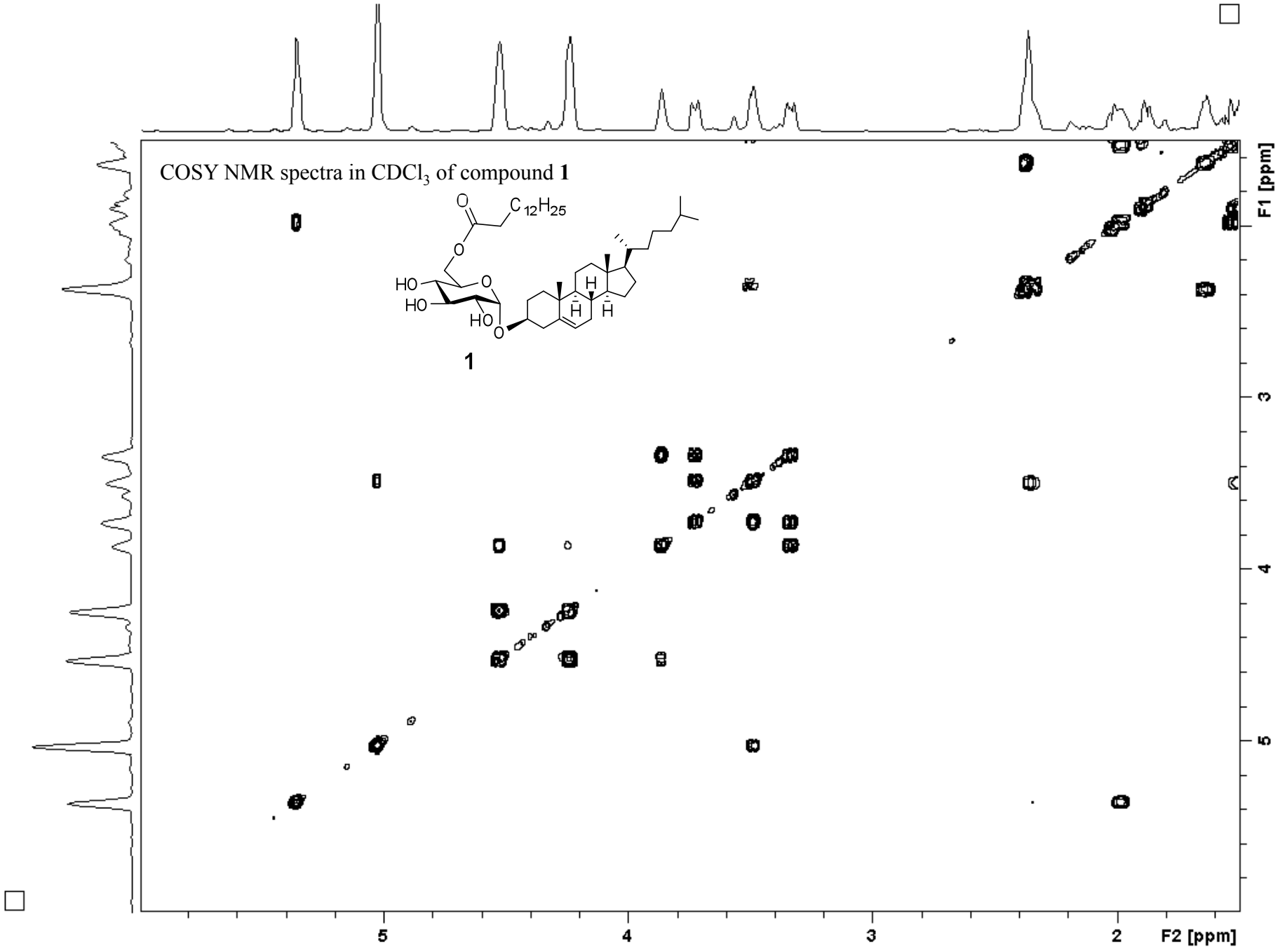
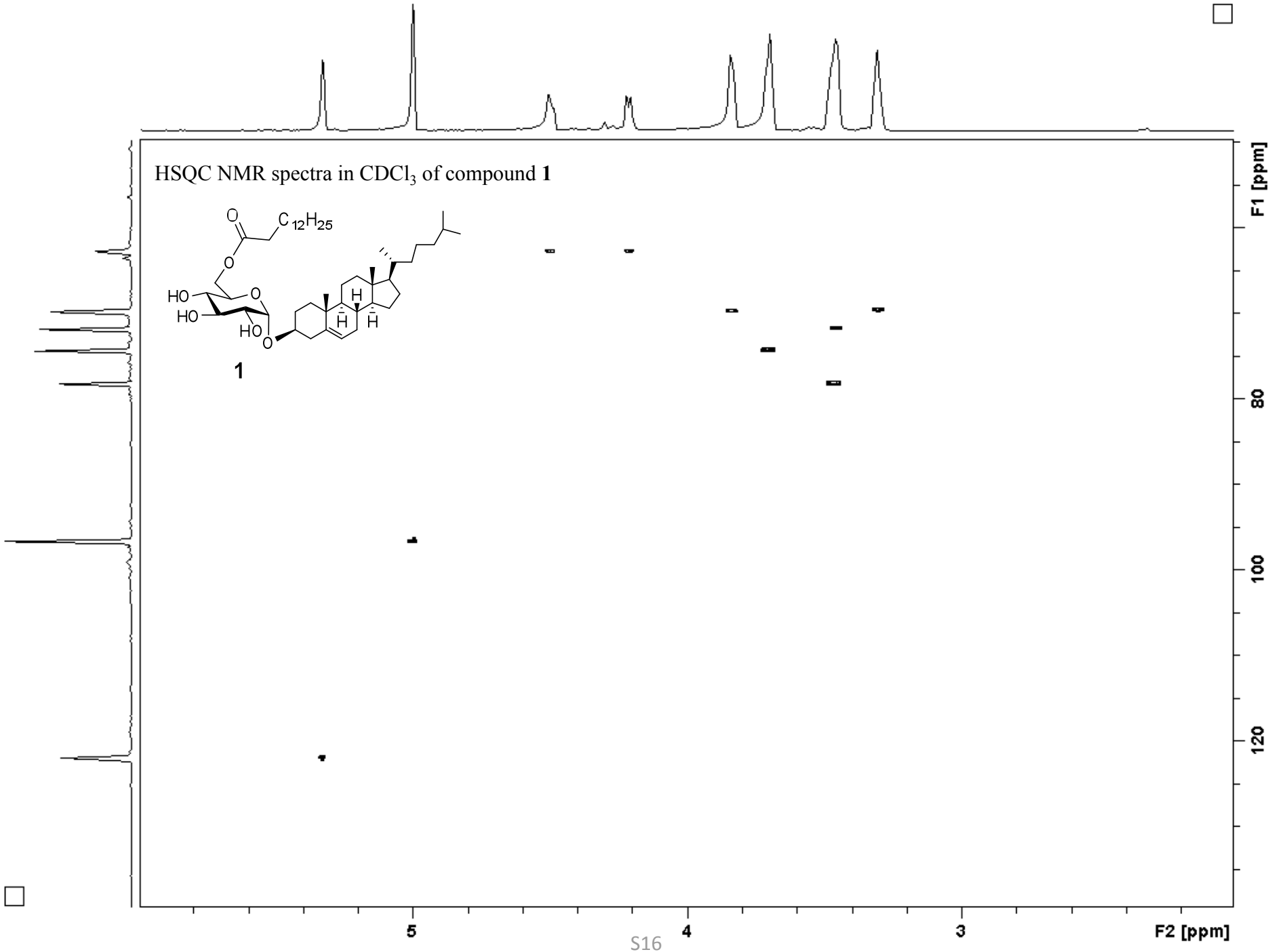
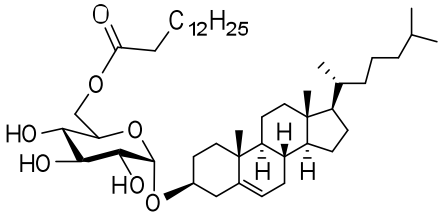




HRMS MALDI-TOF spectra of compound **1**



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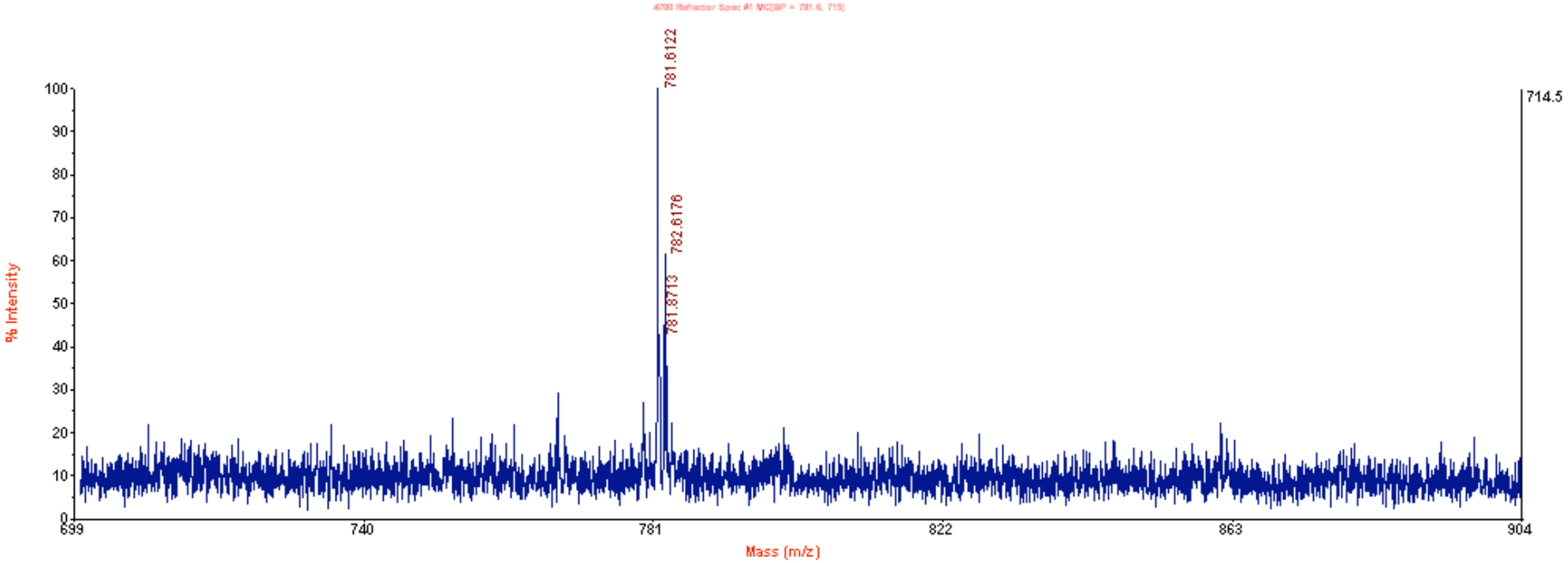

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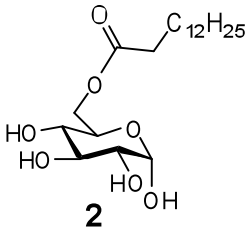

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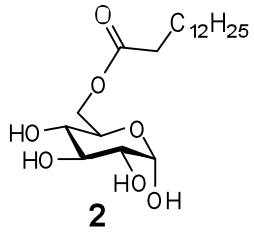
^{1}H NOEPresat NMR spectra in DMSO of compound **2**



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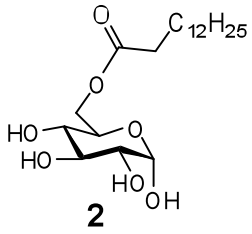

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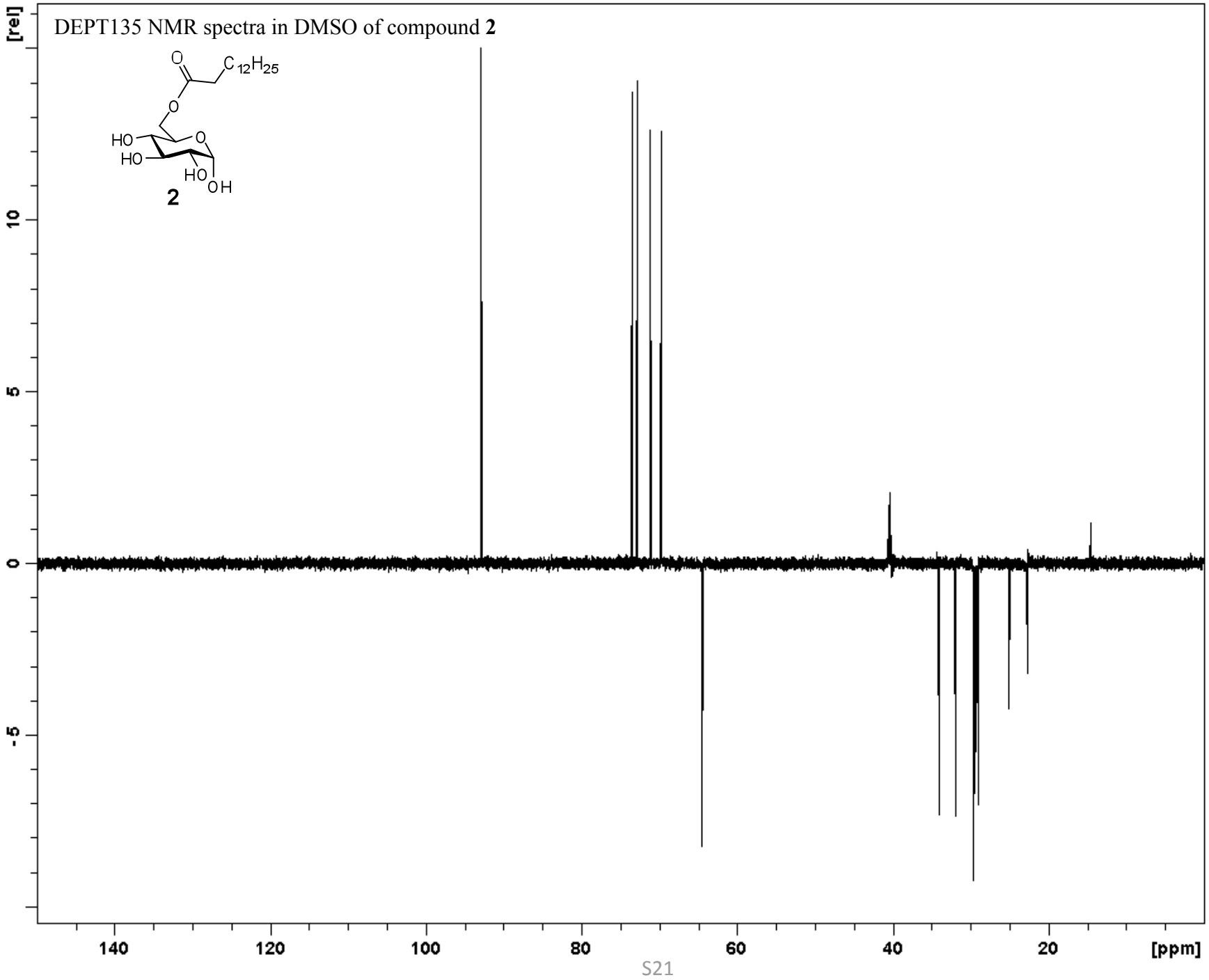
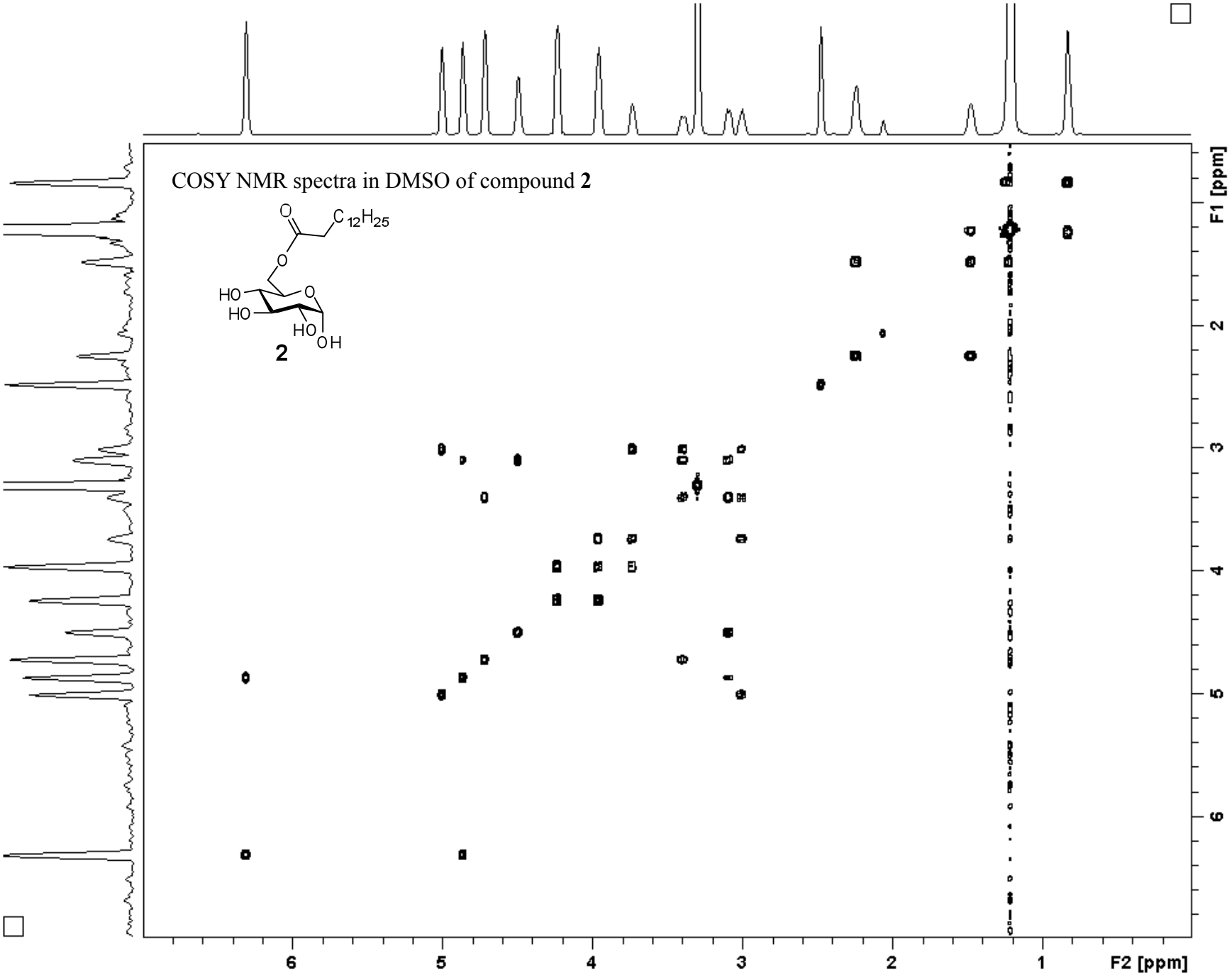
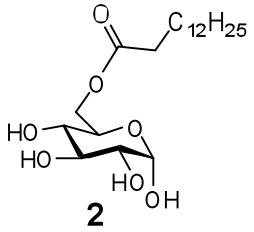




HSQC NMR spectra in DMSO of compound **2**



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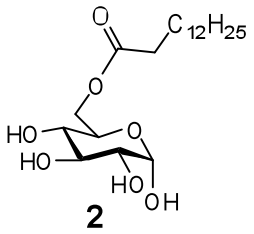
HMBC NMR spectra in DMSO of compound **2**



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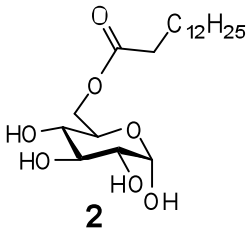
HRMS MALDI-TOF spectra of compound **2**



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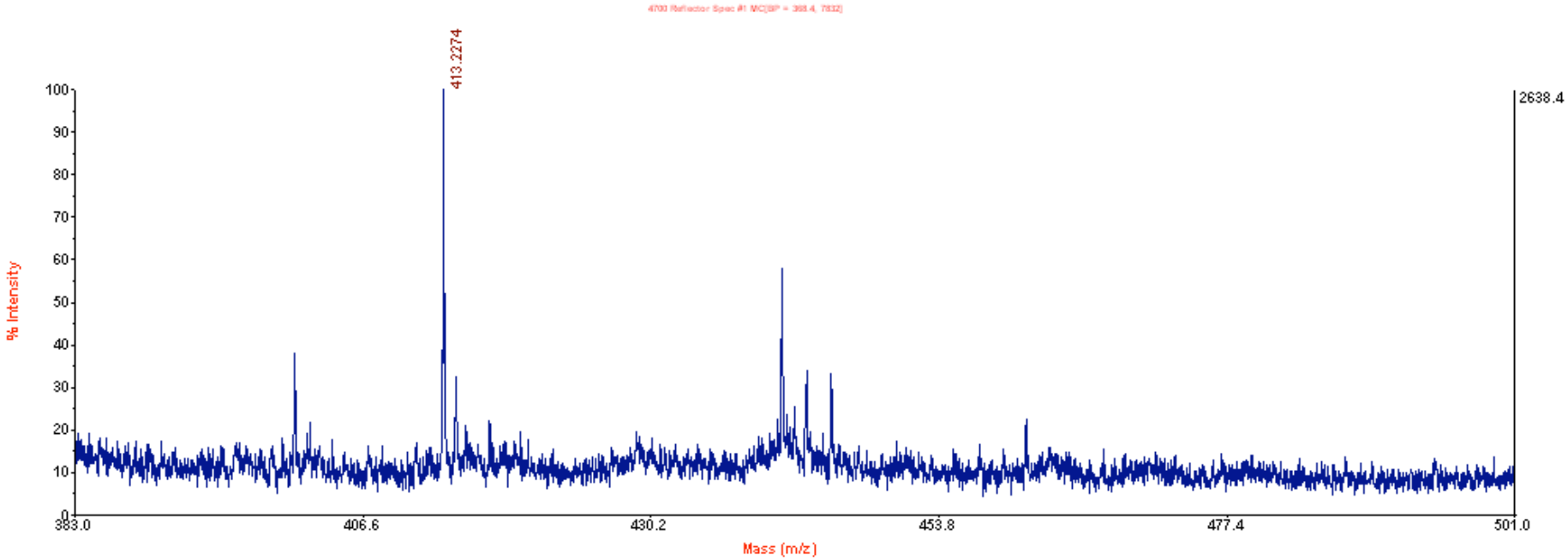
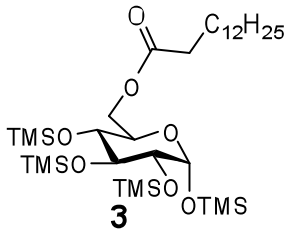
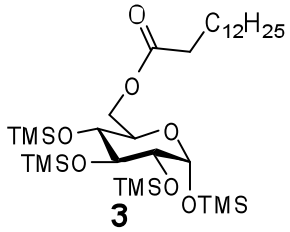


^{1}H NMR spectra in $CDCl_3$ of compound **3**



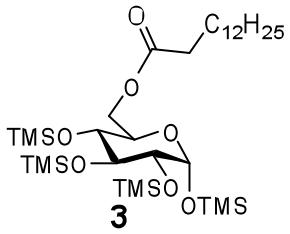
3

^{13}CNMR spectra in $CDCl_3$ of compound **3**



3

DEPT135 NMR spectra in $CDCl_3$ of compound **3**



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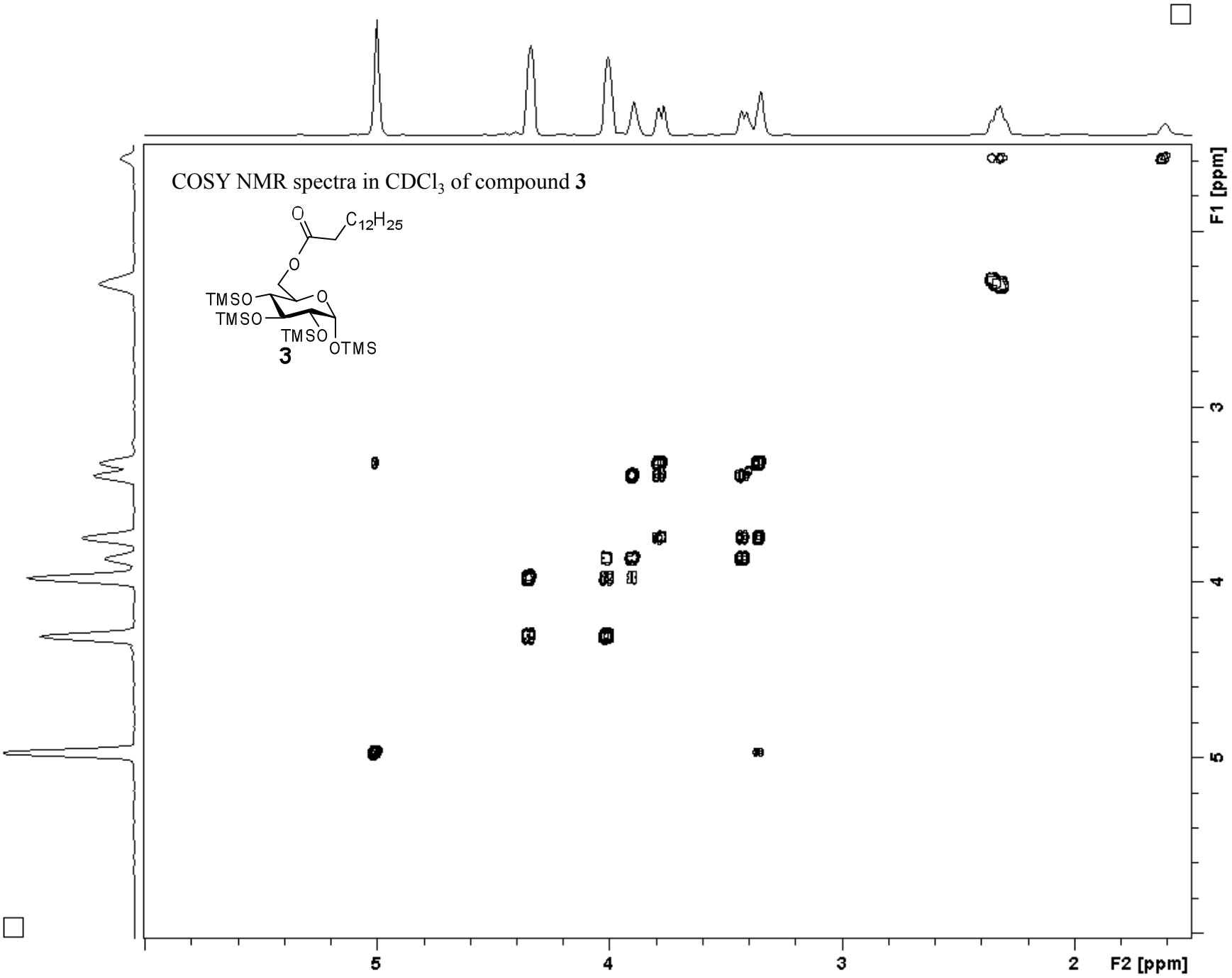
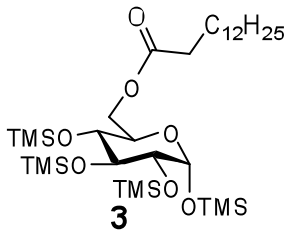
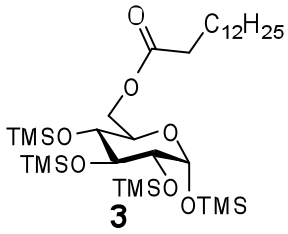


HSQC NMR spectra in $CDCl_3$ of compound **3**



3

^{1}H NMR spectra in C_6D_6 of compound **3**



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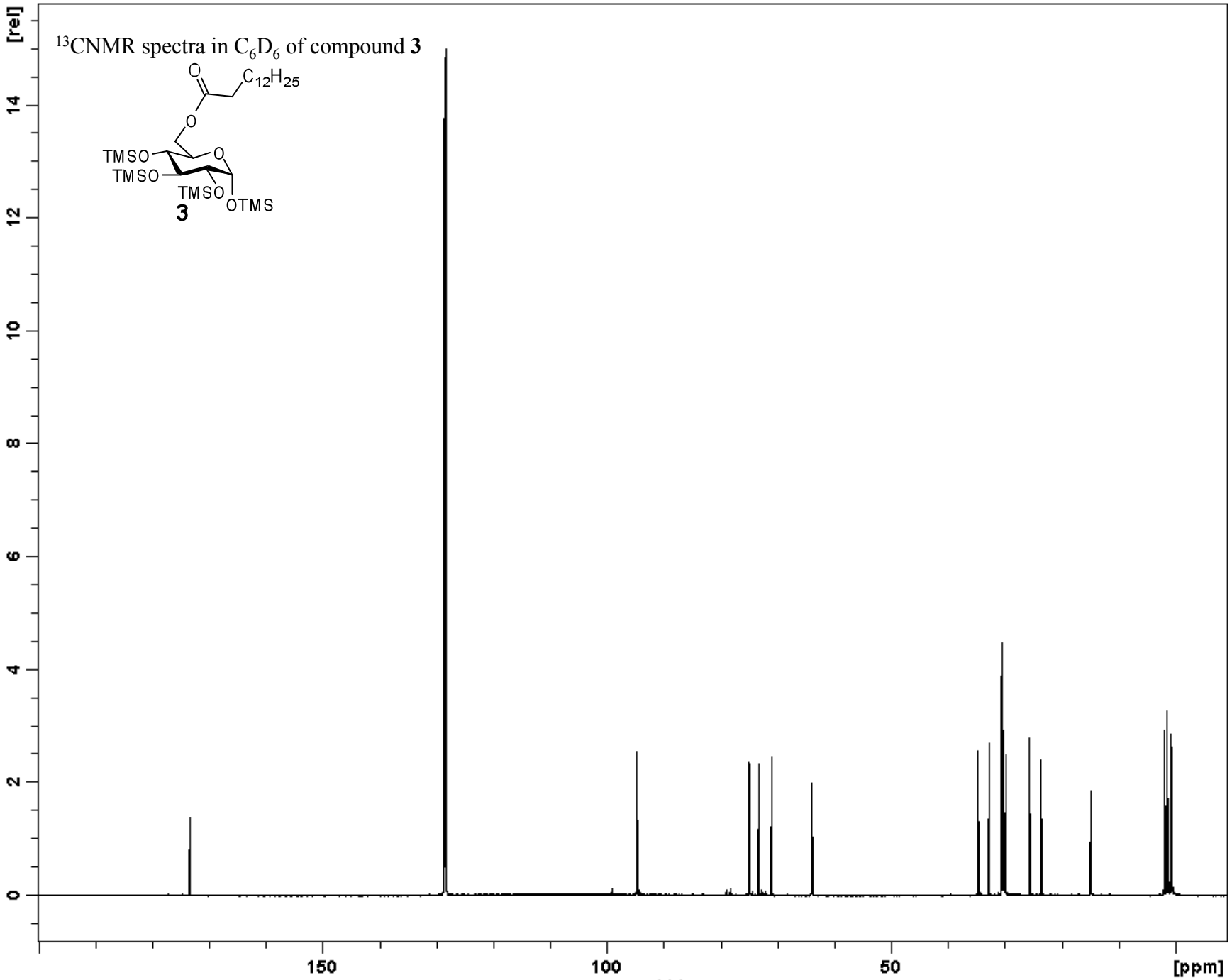
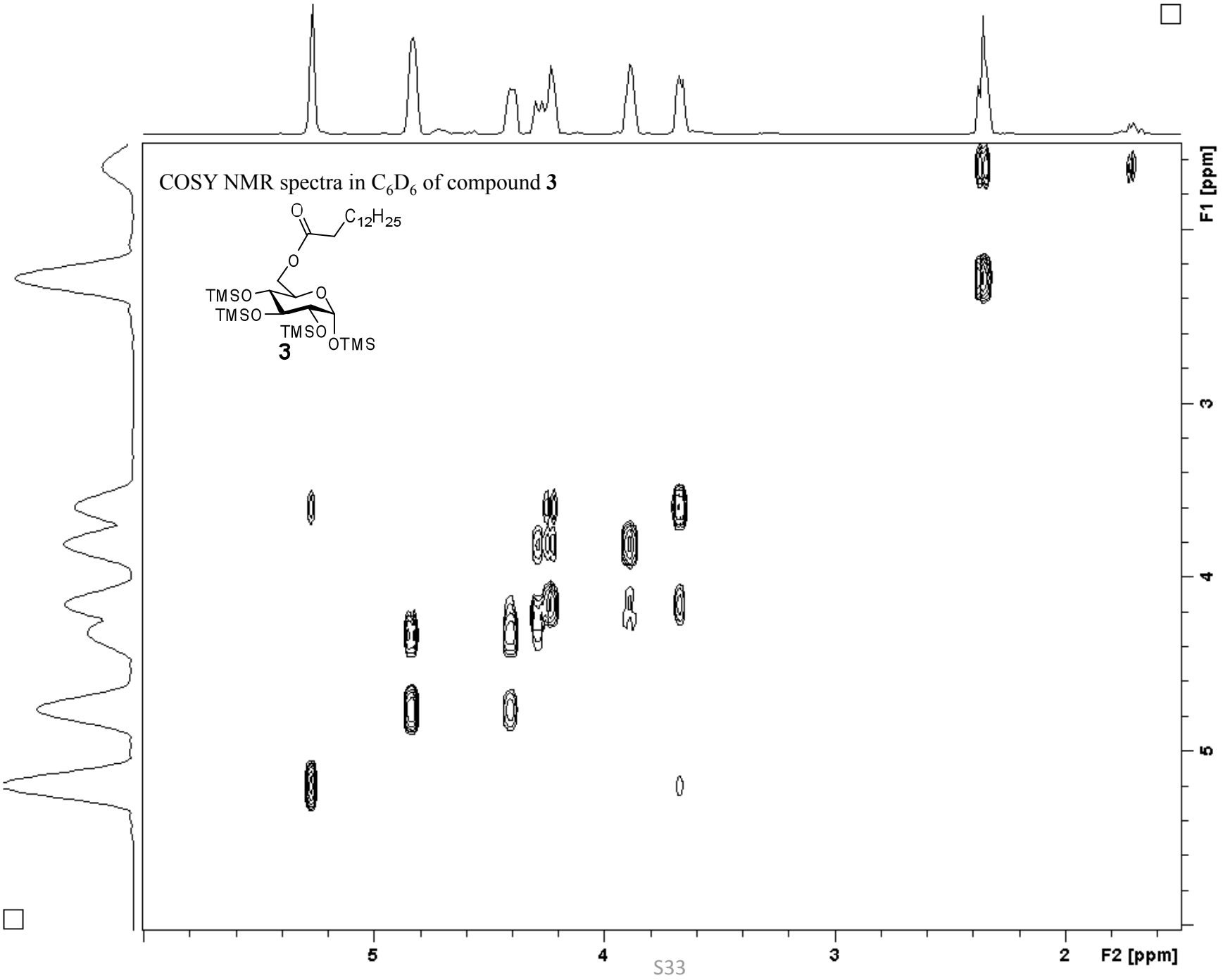
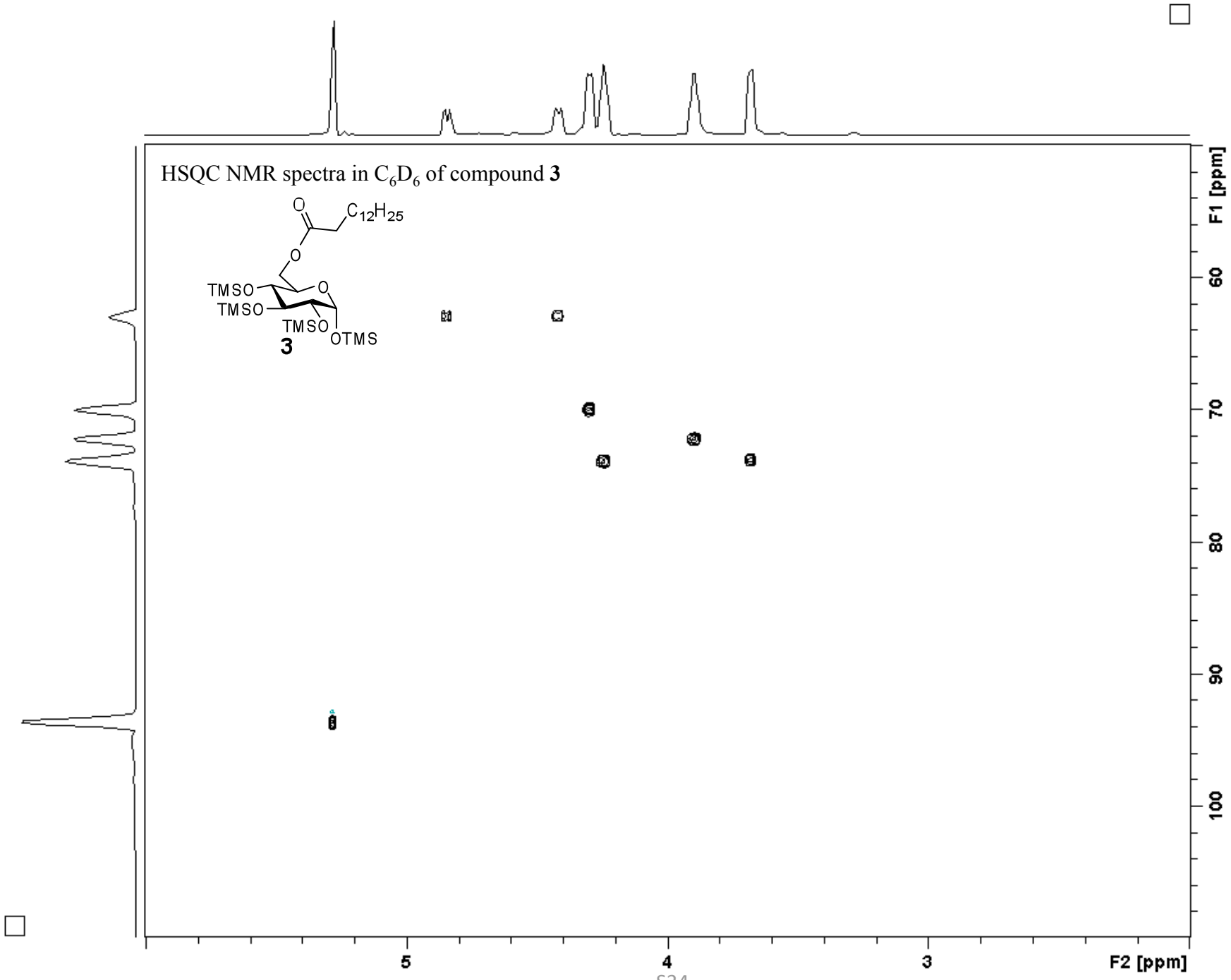
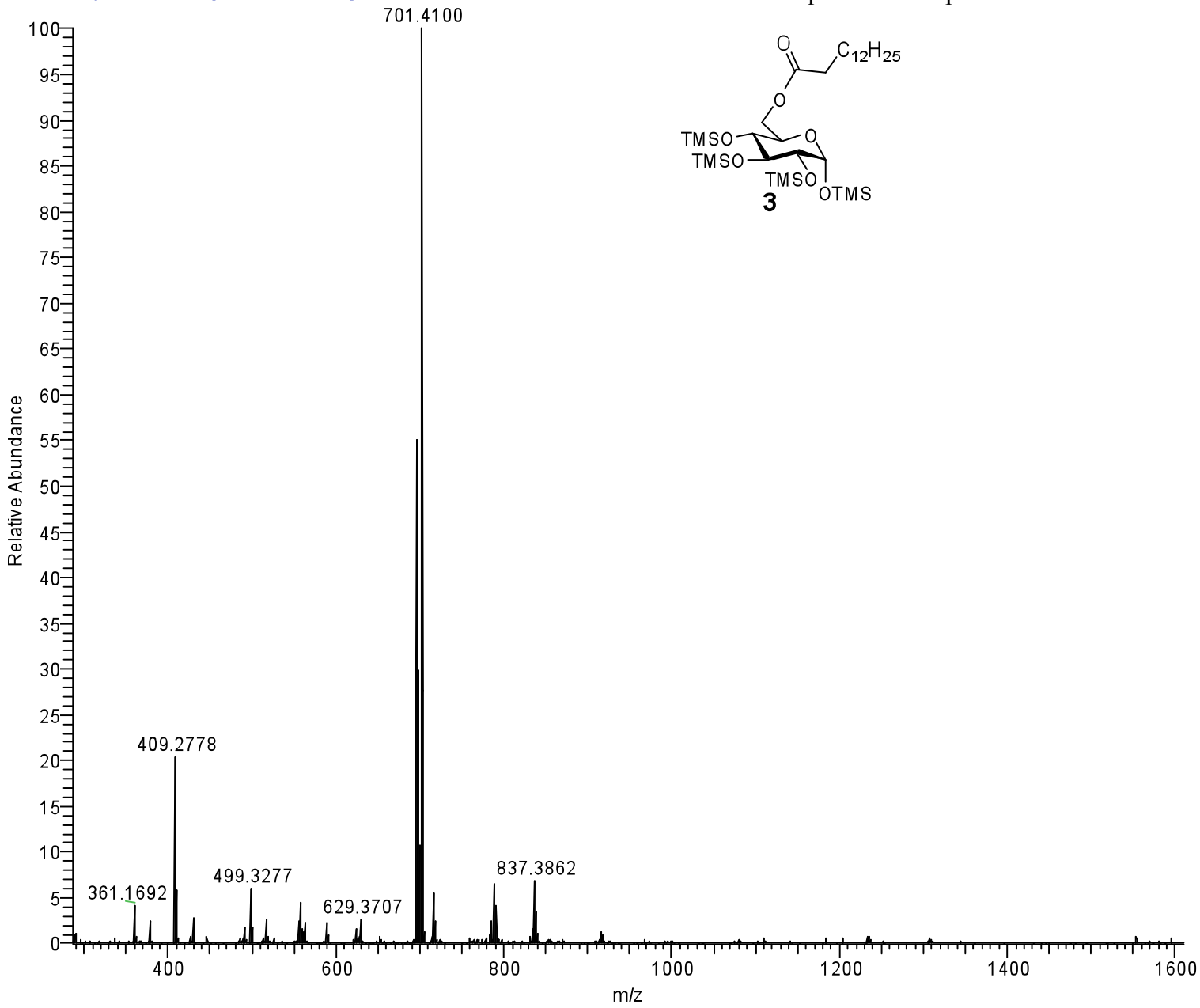






6Myristic1,2,3,4-OTMSGlucose #74 RT: 1.03 AV: 1 NL: 4.13E6
T: FTMS + p ESI Full ms [200.00-2000.00]

HRMS ESI-FTMS spectra of compound **3**



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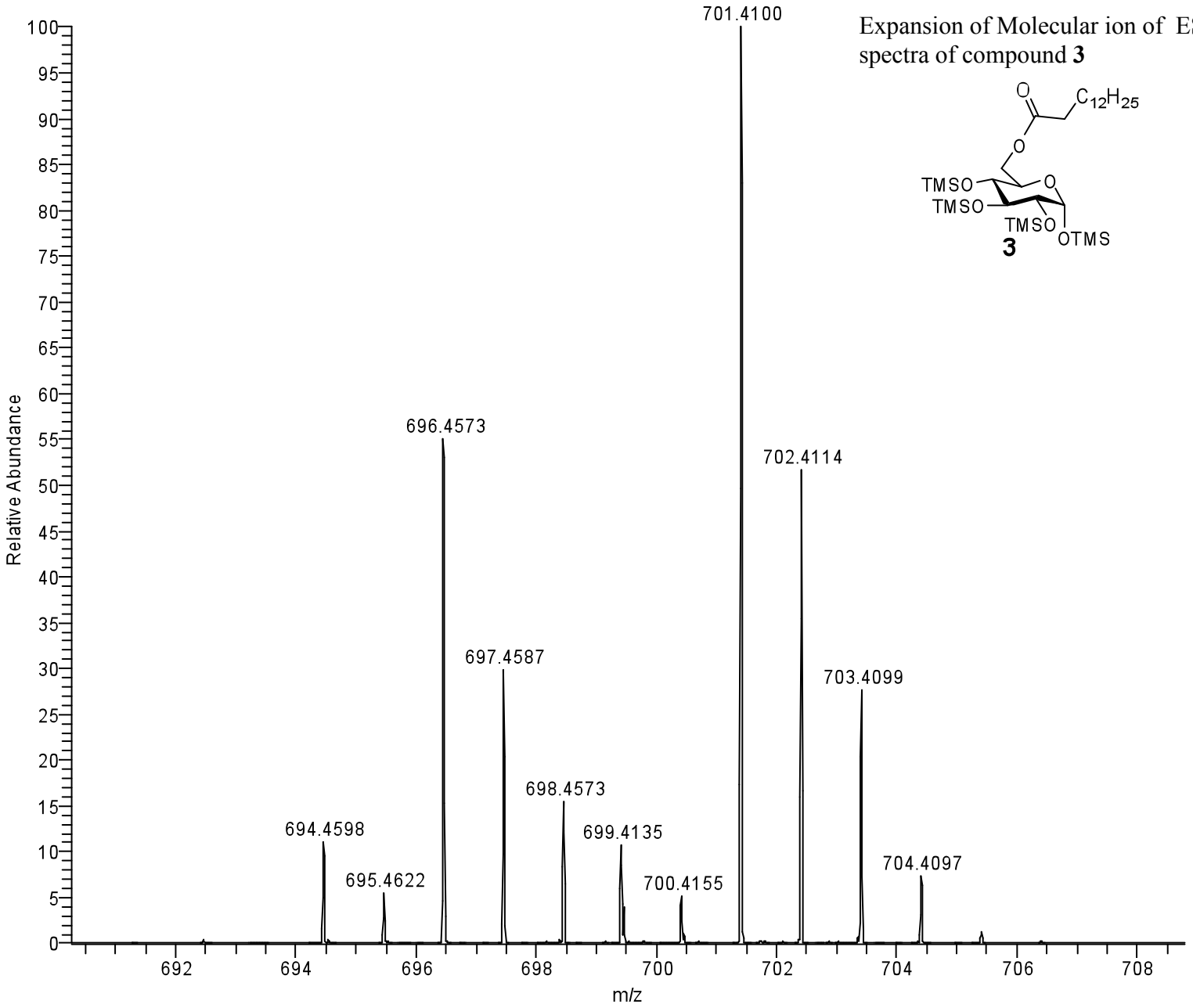
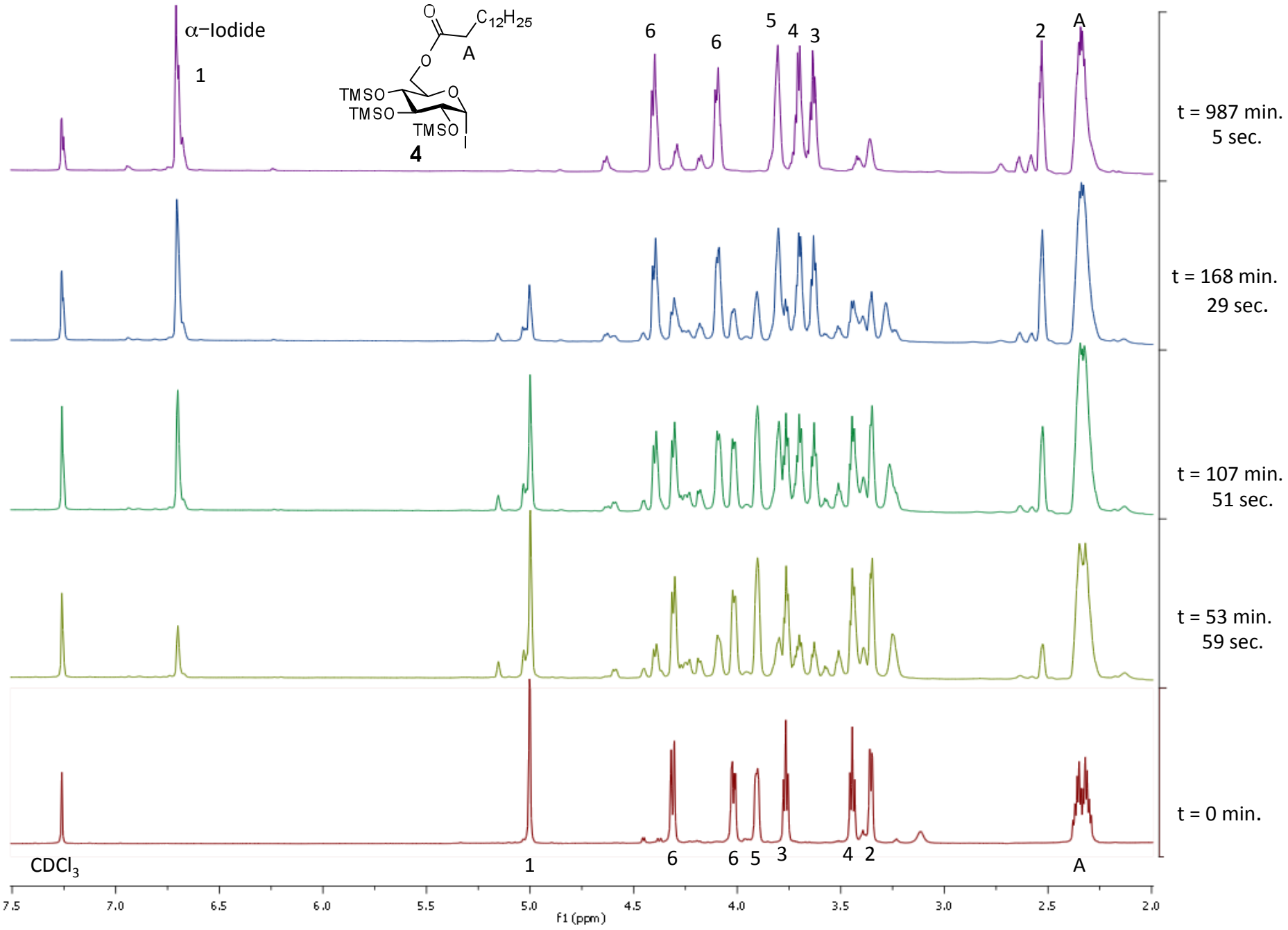
6Myristic1,2,3,4-OTMSGlucose #74 RT: 1.03 AV: 1 NL: 4.13E6
T: FTMS + p ESI Full ms [200.00-2000.00]

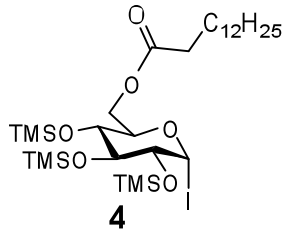


Expansion of Molecular ion of ESI-FTMS spectra of compound **3**

3

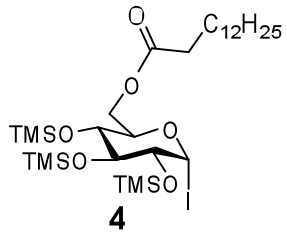


^{1}H NMR spectra in $CDCl_3$ of compound **4**

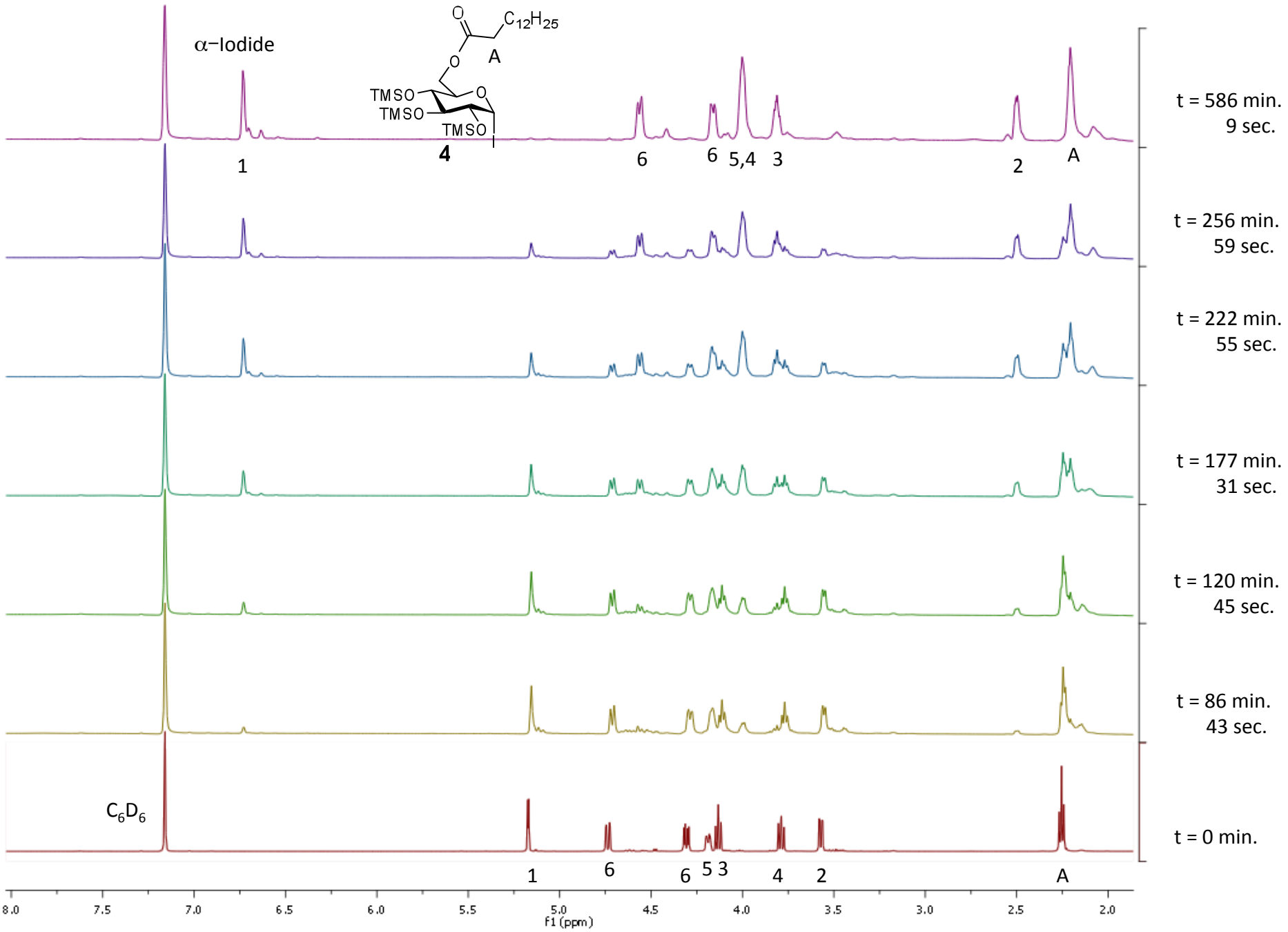


4

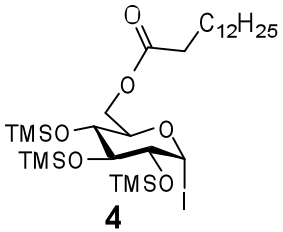
^{13}C NMR spectra in $CDCl_3$ of compound **4**



4

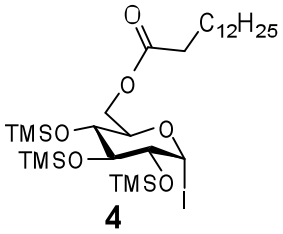


^{1}H NMR spectra in C_6D_6 of compound **4**



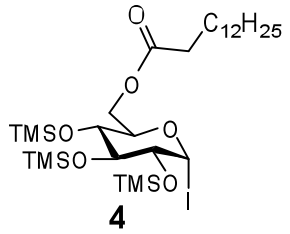
4

^{13}C NMR spectra in C_6D_6 of compound **4**



4

DEPT135 NMR spectra in C_6D_6 of compound **4**



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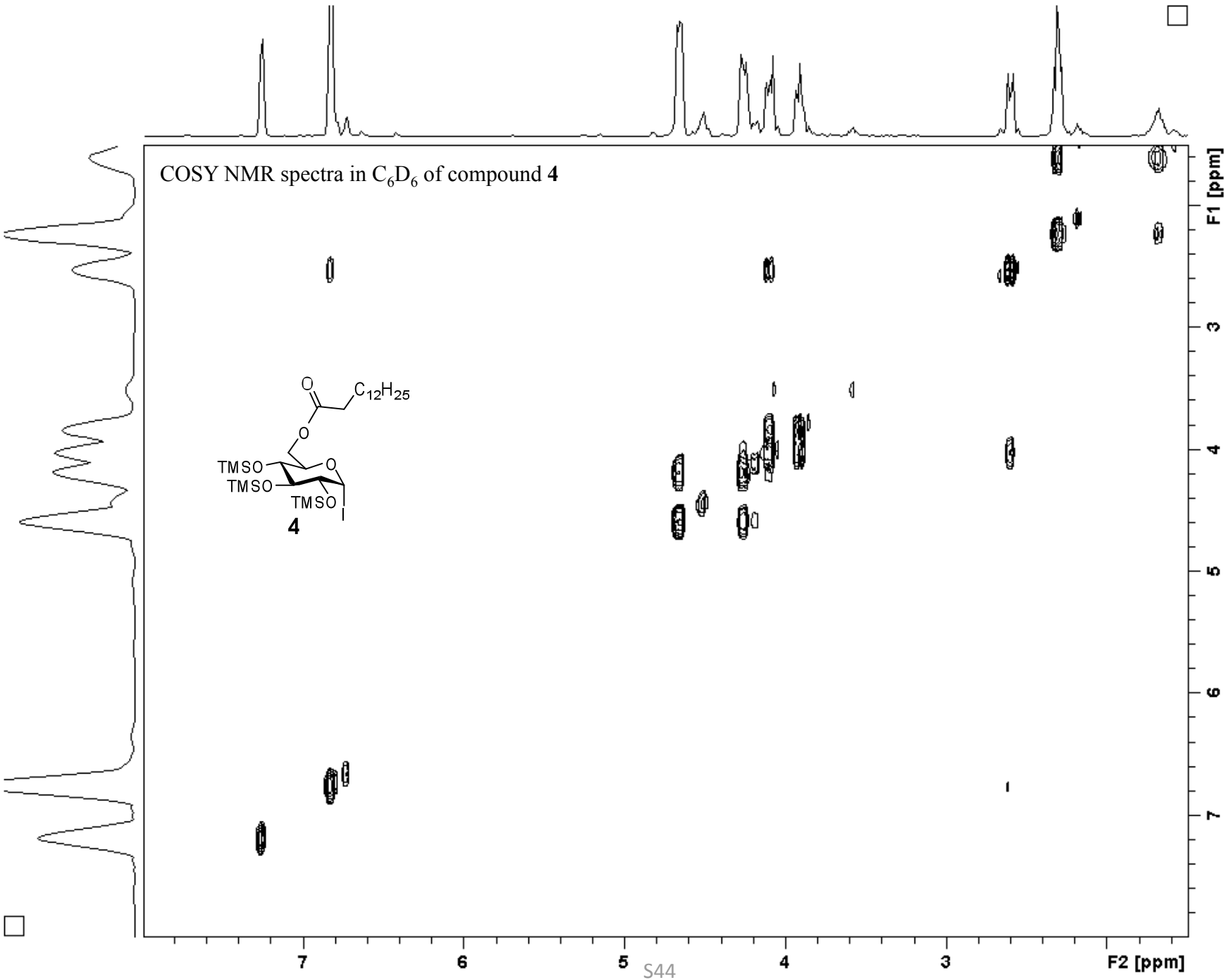
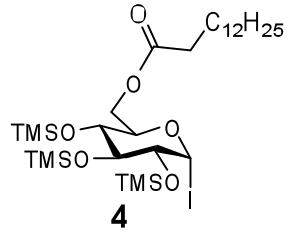


HSQC NMR spectra in C_6D_6 of compound **4**



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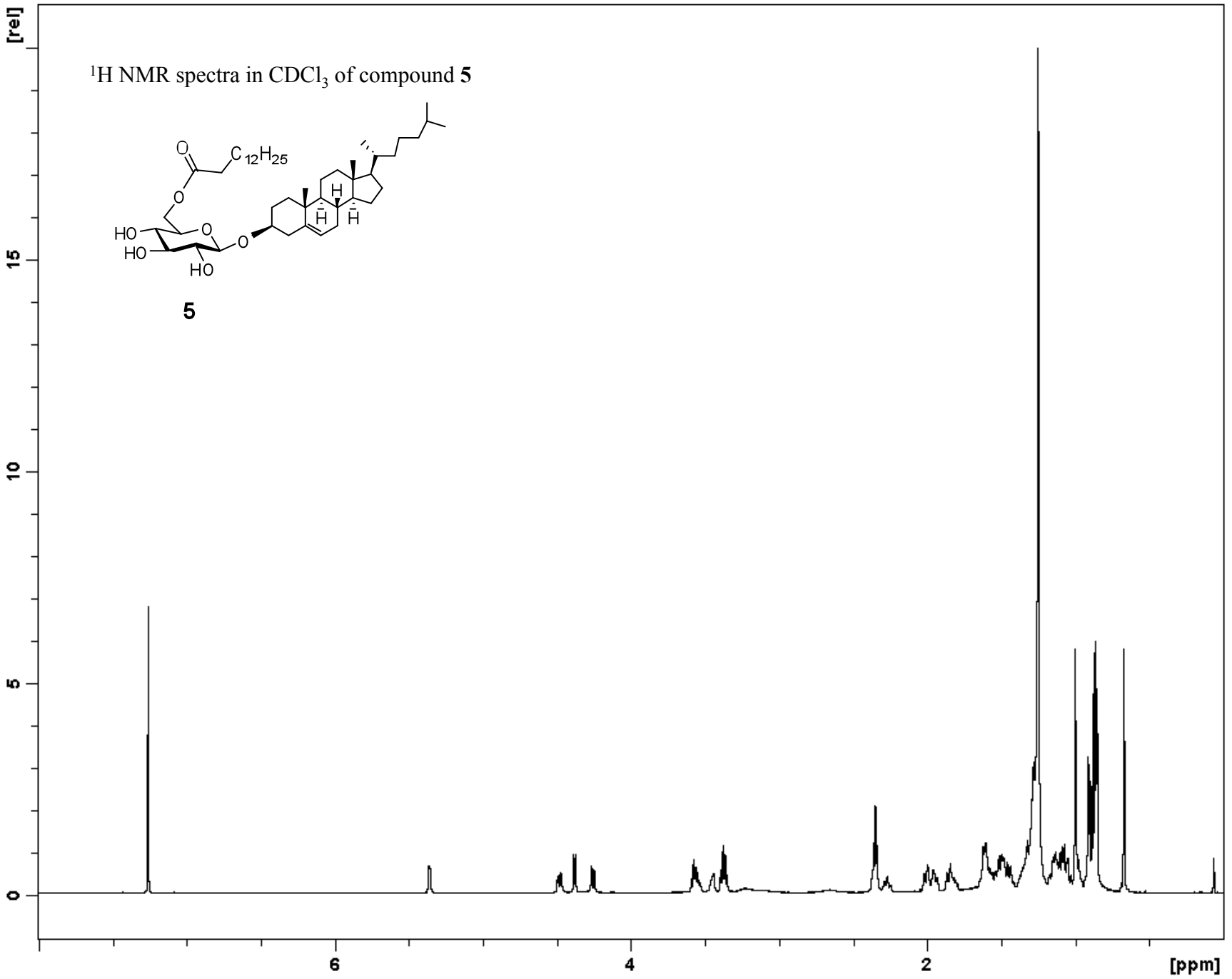
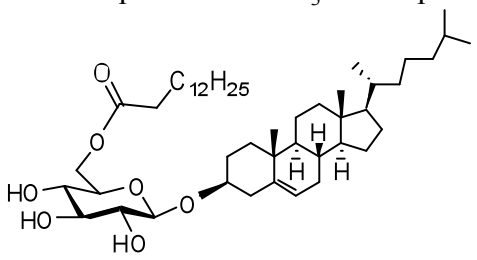


^{1}H NMR spectra in $CDCl_3$ of compound **5**

5

^{13}C NMR spectra in $CDCl_3$ of compound **5**



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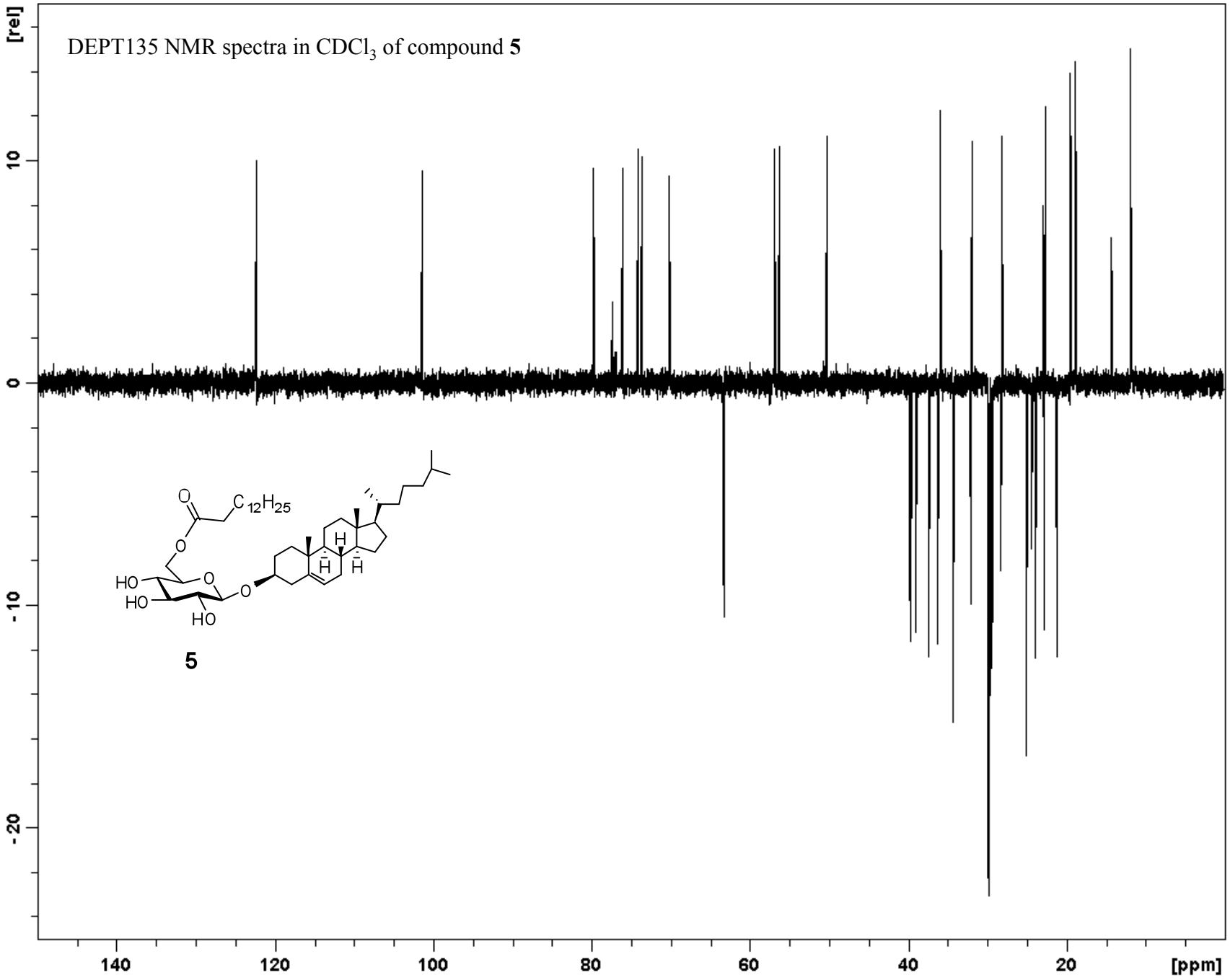
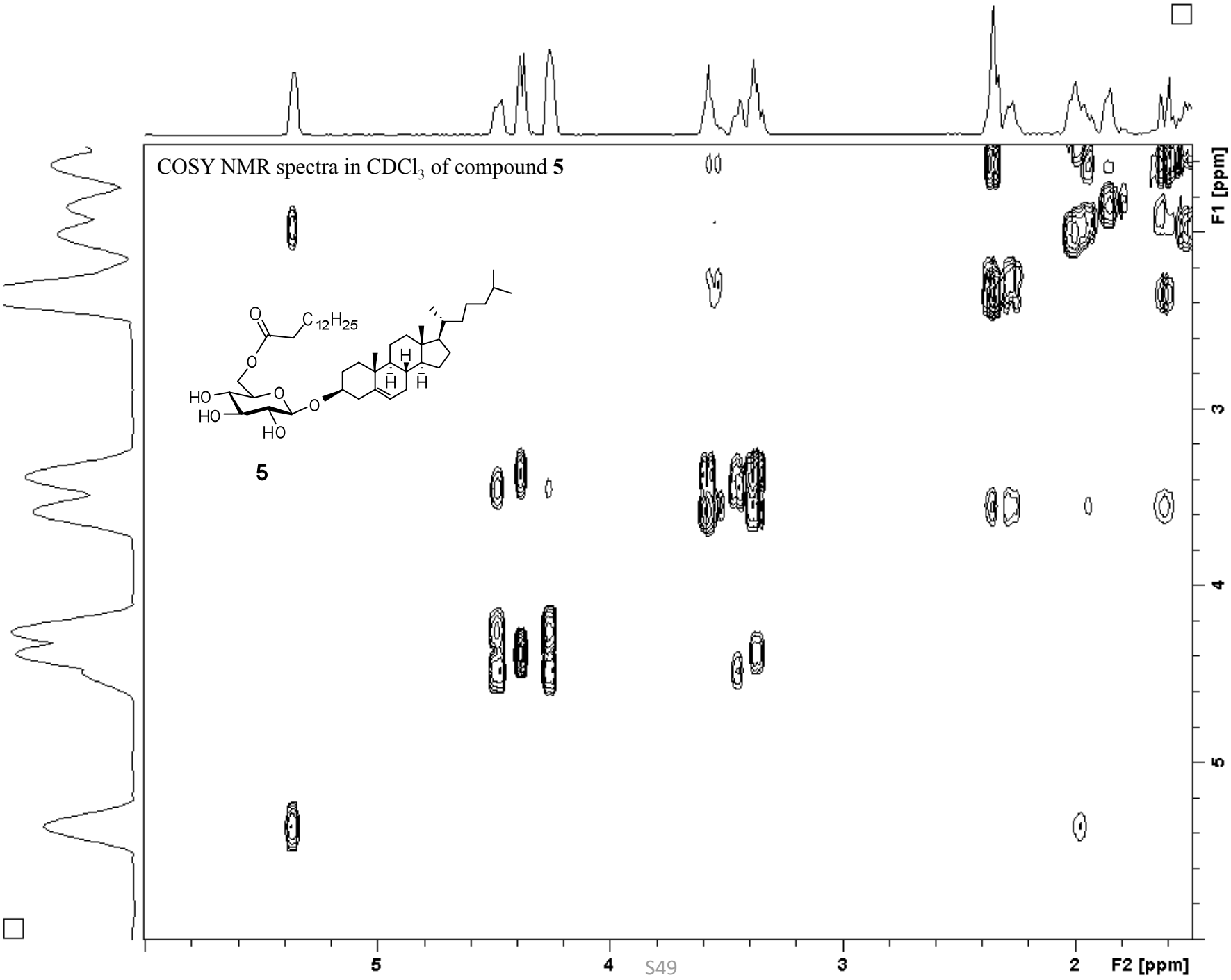
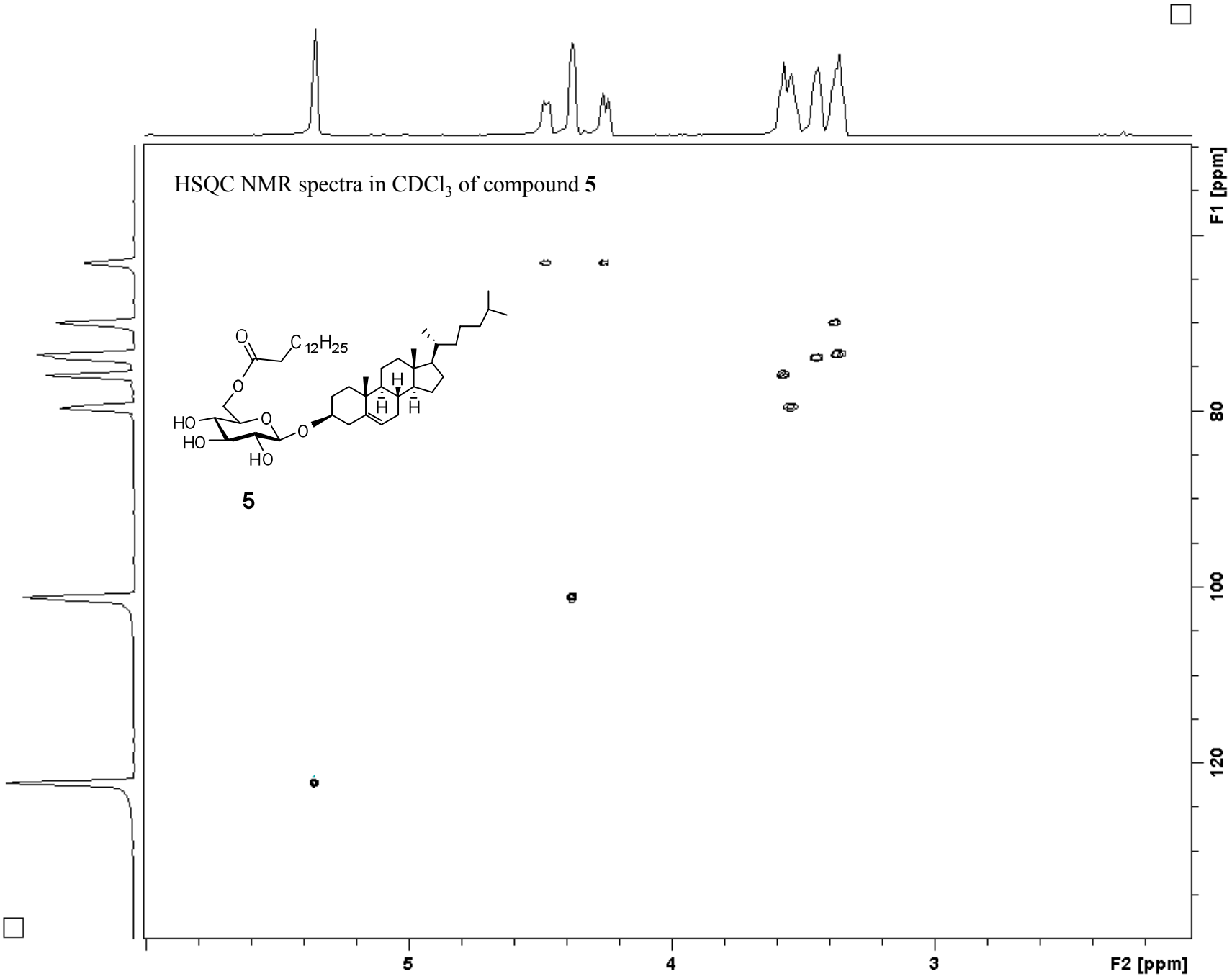
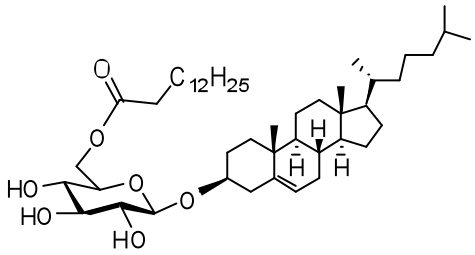






HRMS MALDI-TOF spectra of compound **5**



5