Electronic Supplementary Material (ESI) for Organic & Biomolecular Chemistry.

Supporting Information

Side-chain deuterated cholesterol probe as a molecular probe to determine membrane order and cholesterol partitioning

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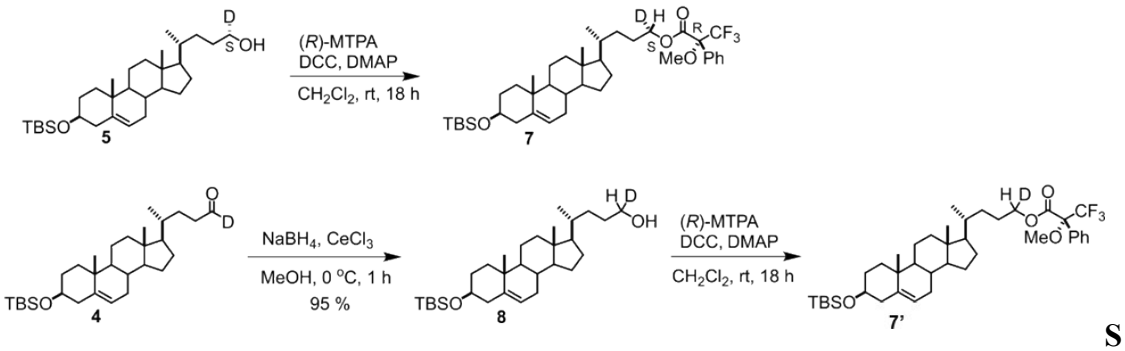
Determination of the stereochemistry and optical purity of the intermediate 5 and 24dCho

(*R*)-MTPA ester **7** was prepared and the stereochemistry at C24 was determined by comparison of the ^{1}H NMR spectra with the racemic mixture **7'** (Fig.S1). **7'** was prepared from aldehyde **4** via a reduction with $NaBH_4$ and $CeCl_3$ to give alcohol **8** in 95% yield. Then, MTPA ester was introduced to give **7'** as a diastereomeric mixture (Scheme S1).

^{1}H NMR spectra of **7** and its diastereomeric mixture **7'**, focusing on the H24 signal, are shown in Figure S1. The (*R*)-MTPA group more shields the proton on the *S* isomer than the *R* isomer due to the fixed phenyl group of the MTPA ester. Similar difference of the chemical shifts had been reported in the (*R*)-MTPA ester of (*S*)-1-*d*-1-octanol.[S1] Thus, the stereochemistry of C24 was determined in *S* configuration. Furthermore, the other stereoisomer was not observed in the ^{1}H NMR spectrum of **7**, thus the purity could be more than 95% d.e.

The stereochemisty at C24 of 24dCho was again confirmed by comparison of ^{1}H-^{13}C HSQC spectra between 24dCho and native Cho (Fig.S2). In f2 dimension (^{1}H), the signal at pro-chiral C24-H(pro*R*) of Cho was 1.09 ppm, while C24-H(pro*S*) was 1.11 ppm (Fig.S2A).[S2] On the other hand, the signal from the remaining proton at C24 of 24dCho was found at 1.07 ppm with a triplet in f1 dimension because of the geminal ^{13}C-^{2}H coupling ($^{1}J_{C\text{-}D}$ = 17.1 Hz) (Fig.S2B). The disagreement of the chemical shifts can be accounted by deuterium induced isotope (DIS) shifts. DIS of ^{1}H affected from the germinal ^{2}H can be estimated within the range between −0.01 and −0.02 ppm.[S3] Thus, the stereochemistry at C24 was confirmed as *S* because the C-H correlation corresponding tp the C24-H(pro*R*) was appeared in the spectrum.



Scheme S1. Synthesis of the (*R*)-MTPA ester **7** and the diastereomeric mixture **7'** from aldehyde **4**.

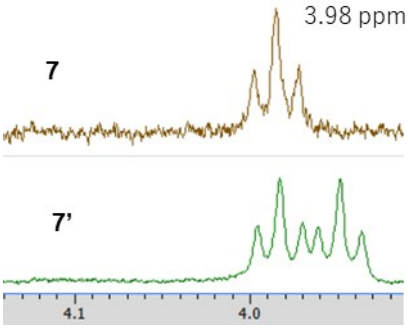


Figure S1. ^{1}H NMR spectra at C24-H of the synthetic (*R*)-MTPA esters of **7** and its diastereomeric mixture **7'**.

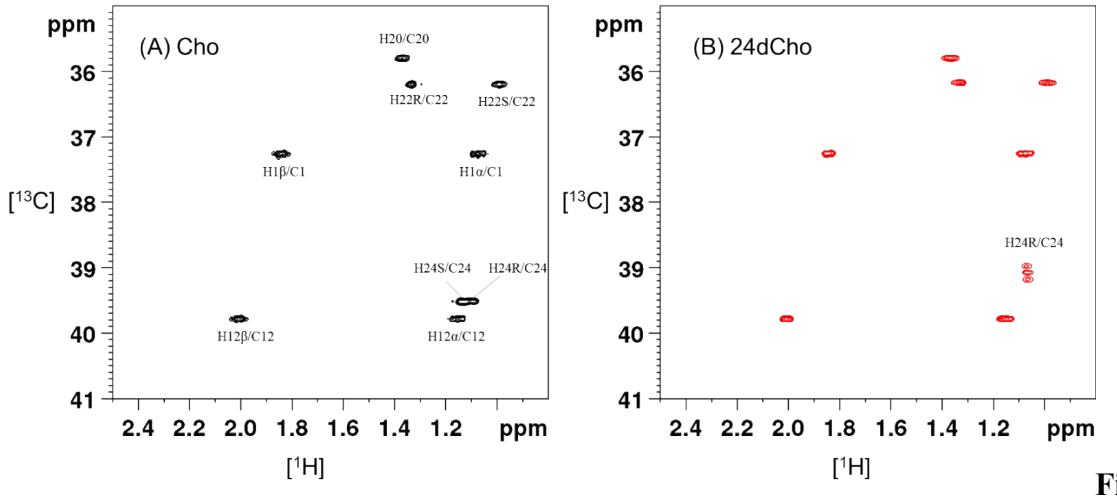


Figure S2. ^{1}H-^{13}C HSQC spectra of Cho (A) and 24dCho (B) in $CDCl_3$. Only one HSQC correlation was observed from the C24-H of 24dCho. The spectra were collected with 700 MHz spectrometer (Bruker AVANCE700).

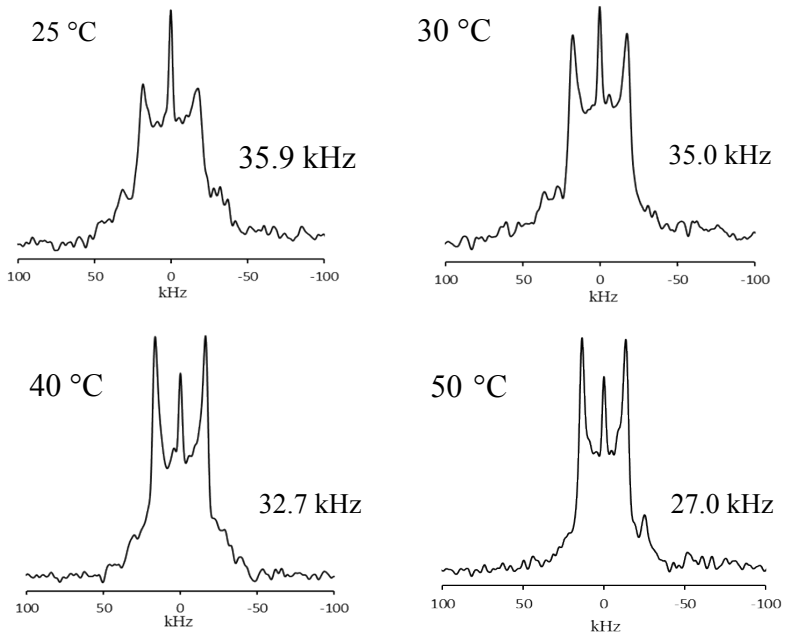


Figure S3. ^{2}H NMR spectra of 24dCho in PSM/24dCho 8:2 membrane.

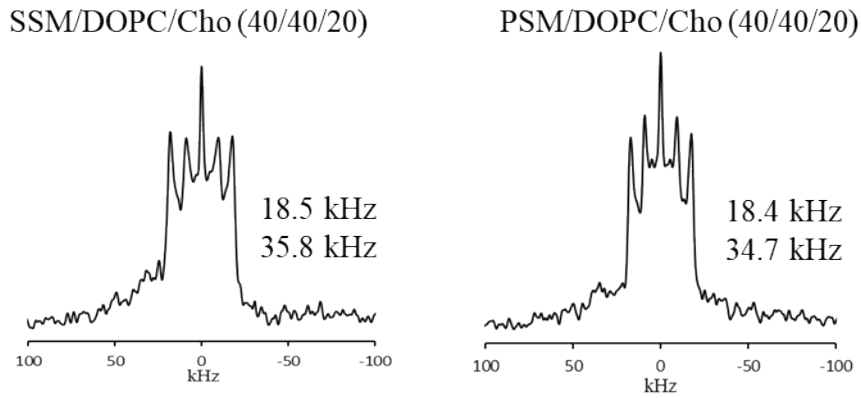


Figure S4. ^{2}H NMR spectra of 24dCho in SSM/DOPC/Cho 40:40:20 membrane and PSM/DOPC/Cho 40:40:20 membrane at 30 °C.

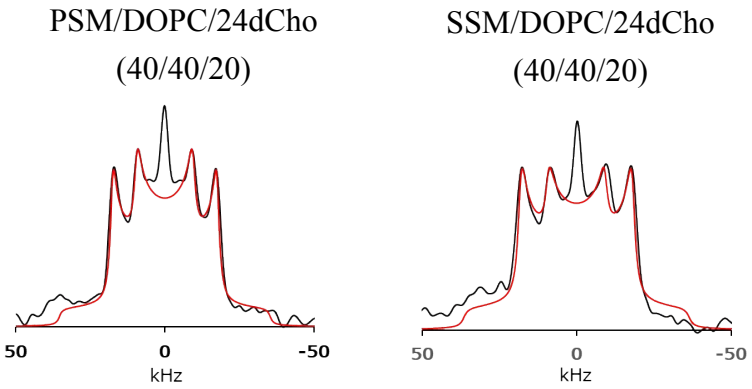


Figure S5. The ^{2}H NMR spectra and theoretical fitting curves of the SM/DOPC/24dCho 40:40:20 membranes at 30 °C. The experimental spectra were shown with the black traces and the fittings were shown with the red traces.

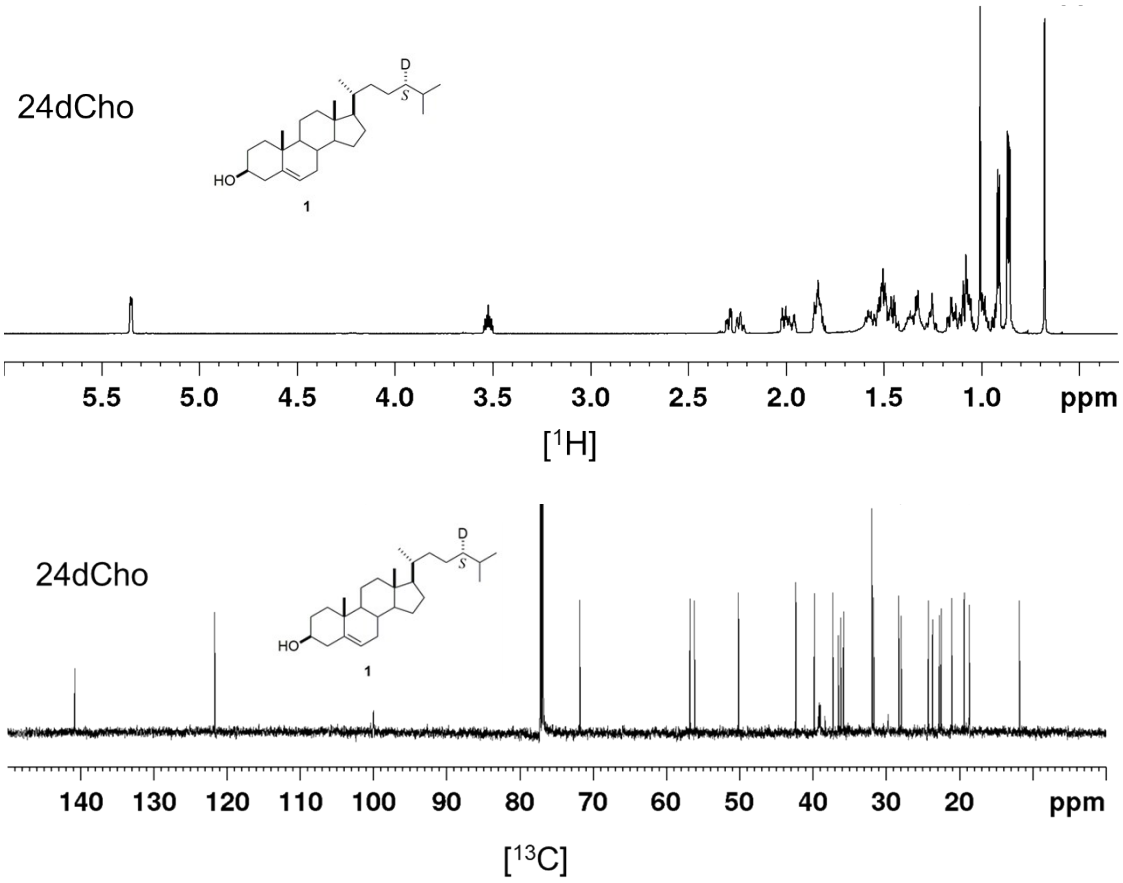


Figure S6. ^{1}H and ^{13}C NMR spectra of 24dCho in $CDCl_3$. The spectra were collected using 700 MHz spectrometer.

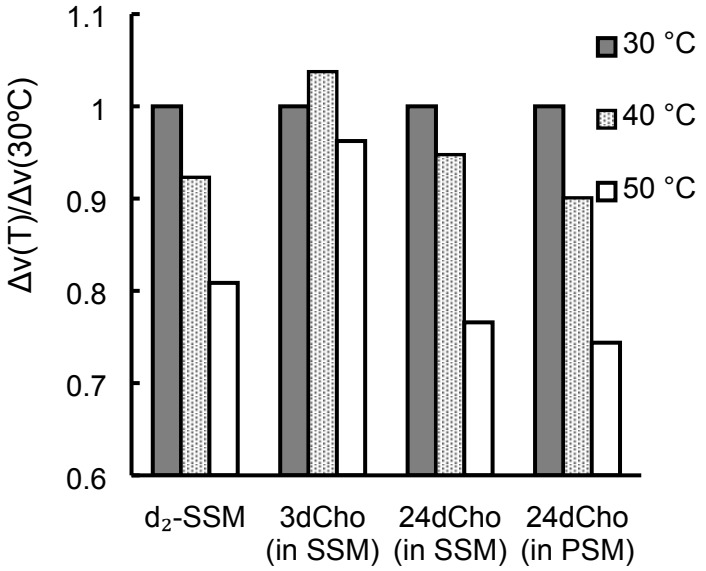


Figure S7. The relative Δ*v* values of d_2-SSM, 3dCho, and 24dCho in SSM/Cho (8:2) or PSM/Cho (8:2) membranes by changing temperature (*T*) at 30, 40 and 50 °C. The y-axis values were normalized by that at 30 °C. The Δν values are shown in Table 1.

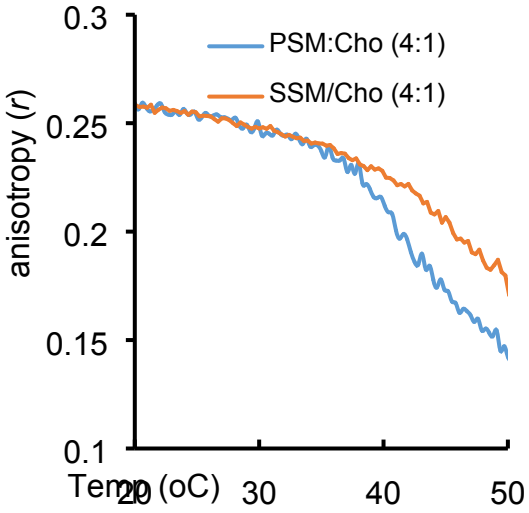
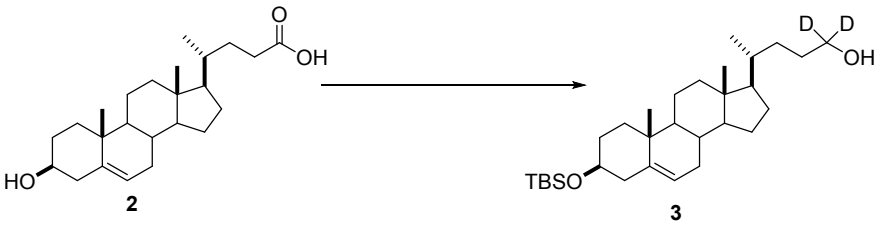


Figure S8. DPH anisotropy of the binary and ternary SM membranes whose lipid compositions were used for ^{2}H NMR measurements; PSM/Cho (8:2) and SSM/Cho (8:2). MLV was prepared with 200 nmol lipid plus 1 nmol DPH. The data was taken using JASCO FP-6500 spectrometer with 2 mL sample in a quartz cell. DPH; diphenylhexatriene

Synthetic Procedures

24-d_2-(3β)-3-O-*tert*-Butyldimethylsilyl-cholest-5-ene-3,24-diol (3) [4]

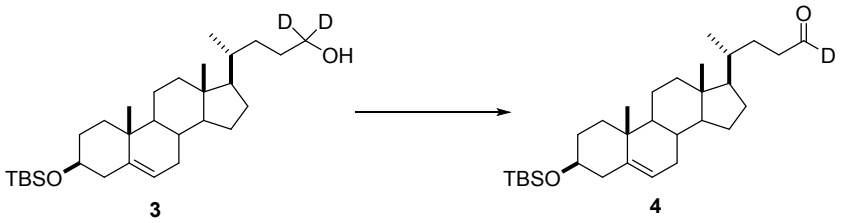


3β-Hydroxy-Δ5-cholenic acid

To a solution of the commercial 3-β-hydroxy cholenic acid **2** (244 mg, 0.65 mmol) in DMF (5.5 mL) was added TBSCl (313 mg, 2.08 mmol), imidazole (359 mg, 5.27 mmol), and DMAP (171 mg, 1.40 mmol). The reaction mixture was stirred overnight at room temperature, and then quenched with water. The aqueous phase was extracted with EtOAc (×3), and the combined organic layers were washed with water (×2) and brine (×2), and dried over Na_2SO_4. After filtration, the organic layer was concentrated and dried under high vacuum to obtain the crude material (312.5 mg). A part of the crude material (150 mg) was dissolved in dry THF (4 mL) and cooled on ice-water bath for 5 min. $LiAlD_4$ (63 mg, 1.50 mmol) was added to the solution on ice-water bath. The reaction mixture was stirred overnight at 0 °C to room temperature, and quenched with the sodium potassium tartrate solution. After additional stirring for 5 h, the aqueous layer was extracted with EtOAc (×3). The combined organic phase was washed with water (×2) and brine (×2), and dried over Na_2SO_4. After filtration, the organic phase was evaporated and purified with SiO_2 chromatography (Hex/EtOAc = 5:1) to give **3**[S4] (96.1 mg, 0.197 mmol, 66%, 2 steps). ^{1}H NMR (500 MHz, $CDCl_3$) δ 5.31 (1H, m, H6), 3.47 (1H, m, H3), 2.25 (2H, m, H4), 0.99 (3H, s, H19), 0.93 (3H, d, J = 6.5 Hz, H21), 0.88 (9H, s, TBS-tBu),

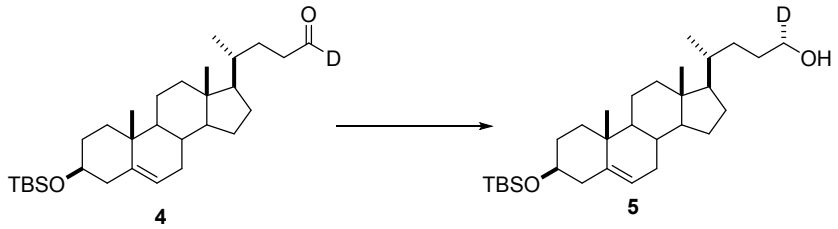
0.67 (3H, s, H18), 0.06 (6H, s, TBS-Me).

24-d_1-(3β)-3-O-*tert*-Butyldimethylsilyl-cholest-5-ene-3-oxy-24-al (4)



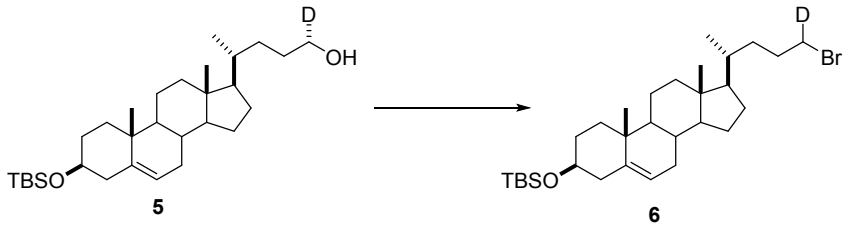
To a solution of the compound **4** (405 mg, 0.85 mmol) in CH_2Cl_2 (10 mL) was added NaOAc (25 mg, 0.31 mmol) and PCC (239 mg, 1.11 mmol) and stirred overnight at room temperature. Then, the solution was evaporated, and the residue was purified with SiO_2 chromatography (Hex/EtOAc = 2:1) to give **4** (215.6 mg, 0.455 mmol, 54%). The spectral data matches those reported in the literature for the non-deuterated compound.[S5] ^{1}H NMR (500 MHz, $CDCl_3$) δ 5.31 (1H, m, H6), 3.47 (1H, m, H3), 2.39 (2H, m, H23), 2.21 (2H, m, H4), 0.99 (3H, s, H19), 0.92 (3H, d, *J* = 6.5 Hz, H21), 0.88 (9H, s, TBS-tBu), 0.67 (3H, s, H18), 0.05 (6H, s, TBS-Me). ^{13}C NMR (125 MHz, $CDCl_3$) δ 202.9 (C24), 141.5 (C5), 121.1 (C6), 72.6 (C3), 56.8 (C14), 55.8 (C17), 50.2 (C9), 42.8 (C4), 42.3 (C13), 40.8 (C23), 39.8 (C12), 37.3 (C1), 36.6 (C10), 35.3 (C20), 32.1 (C22), 31.9 (C7,C8), 28.1, 27.9 (C16), 25.9 (3C, TBS-tBu), 24.2 (C15), 21.0 (C11), 19.4 (C19), 18.4 (C21), 18.3 (TBS), 11.9 (C18), −4.6 (2C, TBS-Me); HR-ESI-MS C30H52DO2Si $[M+H]^+$ calcd. 474.3878, found 474.3967.

24-(*S*)-d_1-(3β)-3-O-*tert*-Butyldimethylsilyl-cholest-5-ene-3,24-diol (5)



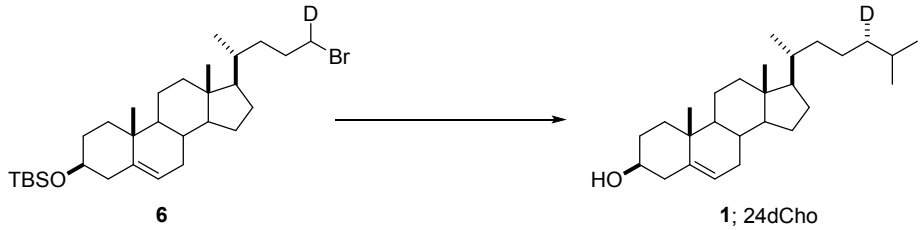
(*R*)-Alpine borane (0.5 M in THF, 1.82 mL, 0.91 mmol) was added to a solution of **4** (216 mg, 0.455 mmol) in dry THF (4 mL) and stirred 1 day at room temperature. Then, the mixture was quenched with acetaldehyde and the solvent was evaporated. The residue was dissolved in Et_2O (5 mL), cooled on ice-water bath, and then ethanolamine (0.1 mL) was added. After stirring for 1 h at room temperature, the mixture was diluted with water, and extracted with Et_2O (×3). The combined organic phase was washed with water (×2) and brine (×2), and dried over Na_2SO_4. After filtration, the residue was purified with SiO_2 chromatography (Hex/EtOAc = 10:1) to give **5** (207.9 mg, 0.437 mmol, 96%). The spectral data matches those reported for the di-deuterated **3**. ^{1}H NMR (500 MHz, $CDCl_3$) δ 5.31 (1H, m, H6), 3.65 (1H, m, H24), 3.47 (1H, m, H3), 2.21 (2H, m, H4), 0.99 (3H, s, H19), 0.93 (3H, d, *J* = 6.5 Hz, H21), 0.88 (9H, s, TBS-tBu), 0.67 (3H, s, H18), 0.05 (6H, s, TBS-Me); ^{13}C-NMR (125 MHz, $CDCl_3$) δ 141.6 (C5), 121.2 (C6), 72.6 (C3), 70.8 (C24), 56.8 (C14), 56.0 (C17), 50.2 (C9), 42.8 (C4), 42.3 (C13), 39.8 (C12), 37.4 (C1), 36.6 (C10), 35.6 (C20), 32.1 (C22), 31.9 (C2), 31.84 (C7), 31.81 (C8), 29.2 (C23), 28.2 (C16), 25.9 (3C, TBS-tBu), 24.3 (C15), 21.1 (C11), 19.4 (C19), 18.7 (C21), 18.2 (TBS), 11.8 (C18), -4.6 (2C, TBS-Me).; HR-ESI-MS C30H53DO2SiNa $[M+Na]^+$ calcd. 498.3854, found 498.3882.

24-(*S*)-d_1-(3β)-3-O-*tert*-Butyldimethylsilyl-cholest-5-ene-3-oxy-24-bromide (**6**)



To a solution of **5** (214 mg, 0.45 mmol) in CH_2Cl_2 (4.9 mL) was added Ph_3P (205 mg, 0.78 mmol), imidazole (53 mg, 0.78 mmol), and CBr_4 (226 mg, 0.68 mmol), and stirred for 3 h at room temperature. Upon addition of MeOH for quenching, the mixture was evaporated and purified with SiO_2 chromatography (Hex/EtOAc = 10:1) to give **6** (170 mg, 0.316 mmol, 70%). The spectral data matches those reported for non-deuterated compound.[S6] ^{1}H NMR (500 MHz, $CDCl_3$) δ 5.31 (1H, m, H6), 3.45 (1H, m, H3), 3.38 (1H, t, H24), 2.21 (2H, m, H4), 0.99 (3H, s, H19), 0.93 (3H, d, H21), 0.89 (9H, s, TBS-tBu), 0.68 (3H, s, H18), 0.05 (6H, s, TBS-Me); ^{13}C-NMR (125 MHz, $CDCl_3$) δ141.6 (C5), 121.1 (C6), 72.6 (C3), 56.8 (C14), 55.8 (C17), 50.2 (C9), 42.8 (C4), 42.3 (C13), 39.8 (C12), 37.4 (C1), 36.6 (C10), 35.2 (C20), 34.5 (C24), 32.1 (C22), 31.90 (C7), 31.88 (C8), 29.5 (C23), 28.2 (C16), 25.9 (3C, TBS-tBu), 24.2 (C15), 21.0 (C11), 19.4 (C19), 18.7 (C21), 18.2 (TBS), 11.8 (C18), −4.6 (2C, TBS-Me) ; HR-ESI-MS C30H53DOBrSi $[M+H]^+$ calcd.538.3190, found 538.3333.

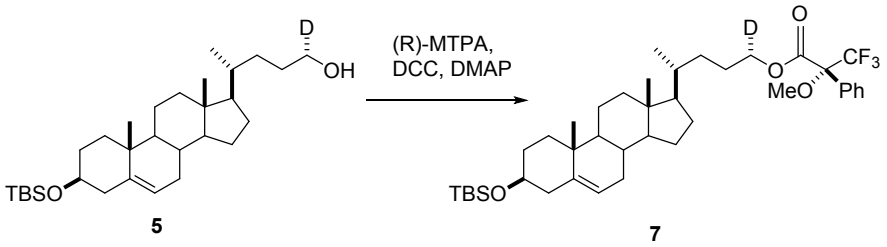
24-(*S*)-d_1-cholesterol (1)



The bromide **6** (170 mg, 0.32 mmol) was dissolved in dry THF (3 mL) and cooled on ice-water bath. Then, the mixed solution of Li_2CuCl_4 (0.1 M in THF, 0.47 mL, 0.047 mmol)

and *i*-PrMgBr (1 M in THF, 1.9 mL, 1.9 mmol) was dropwised to the reaction mixture on ice-water bath. After completion of the addition, the mixture was stirred for 3 h at room temperature. Then, the reaction mixture was quenched with NH_4Cl/Et_2O=1/1 solution, and the aqueous phase was extracted with Et_2O (×2). The combined organic phase was washed with brine (×2) and dried over Na_2SO_4. After filtration, the solution was evaporated and purified with short SiO_2 column (Hex/EtOAc = 100:1) to give a crude material. The crude was then dissolved in THF (4.5 mL) and the TBAF solution (1 M in THF, 2.87 mL, 2.87 mmol) was added. The mixture was stirred overnight at room temperature and then diluted with EtOAc. The organic phase was washed with 1 M HCl (×2) and brine (×2), and dried over Na_2SO_4. After filtration, the mixture was purified with SiO_2 chromatography (Hex:EtOAc = 6:1) to give 24dCho **1** (102 mg, 0.26 mmol, 82%). The spectral data matches those commercial Cho. ^{1}H NMR (400 MHz, $CDCl_3$) δ 5.35 (1H, m, H6), 3.52 (1H, m, H3), 2.25 (2H, m, H4), 1.99 (2H, m, H7, H12), 1.83 (3H, m, H1, H2, H16), 1.61-1.41 (6H), 1.40-1.03 (10H), 1.01 (3H, s, H19), 0.99 (2H), 0.95 (1H), 0.91 (3H, d, H21), 0.86 (6H, dd, H26, H27), 0.68 (3H, s, H18). ^{13}C NMR (175 MHz, $CDCl_3$) δ 140.7, 121.7, 71.8, 56.8, 56.1, 50.1, 42.3, 39.8, 39.1 (dd, $^1J_{C\text{-}D}$ = 18.9 Hz), 37.2, 36.5, 36.2, 35.8, 31.9, 31.7, 28.2, 27.9, 24.3, 23.7, 22.8, 22.5, 21.1, 19.4, 18.7, 11.9. HR-ESI-MS C27H47DO2 $[M+H_2O]^+$ calcd. 405.3712, found 405.3782.

24-(*S*)-d_1-(3β)-3-O-*tert*-Butyldimethylsilyl-cholest-5-ene-3,24-diol (*R*)-MTPA ester (7)



To a solution of **5** (5.5 mg, 0.012 mmol) in CH_2Cl_2 (0.50 mL) was added DCC (26.4 mg, 0.13 mmol), DMAP (15.6 mg, 0.13 mmol), and (*R*)-MTPA (24.0 mg, 0.10 mmol), and stirred overnight at room temperature. Then, the mixture was diluted with EtOAc, and the organic phase was washed with 1 M HCl and brine, and dried over Na_2SO_4. After filtration, the solvent was evaporated and the residue was purified with SiO_2 chromatography (Hex:EtOAc = 20:1) to give ester **7** (6.4 mg, 0.094 mmol, 78%). ^{1}H NMR (500 MHz, $CDCl_3$) δ 7.67-6.94 (5H, m, Ph), 5.36 (1H, m, H6), 3.99 (1H, t, H24), 3.60 (1H, m, H3), 0.99-0.92 (12H, m, H19, TBS-tBu), 0.77 (3H, d, H21), 0.54 (3H, s, H18), 0.07 (6H, d TBS-Me).

References

[S1] D. C. Kapeller, L. Brecker F. Hammerschmidt, *Chem. E. J.* **2007**, *13*, 9582-9588.

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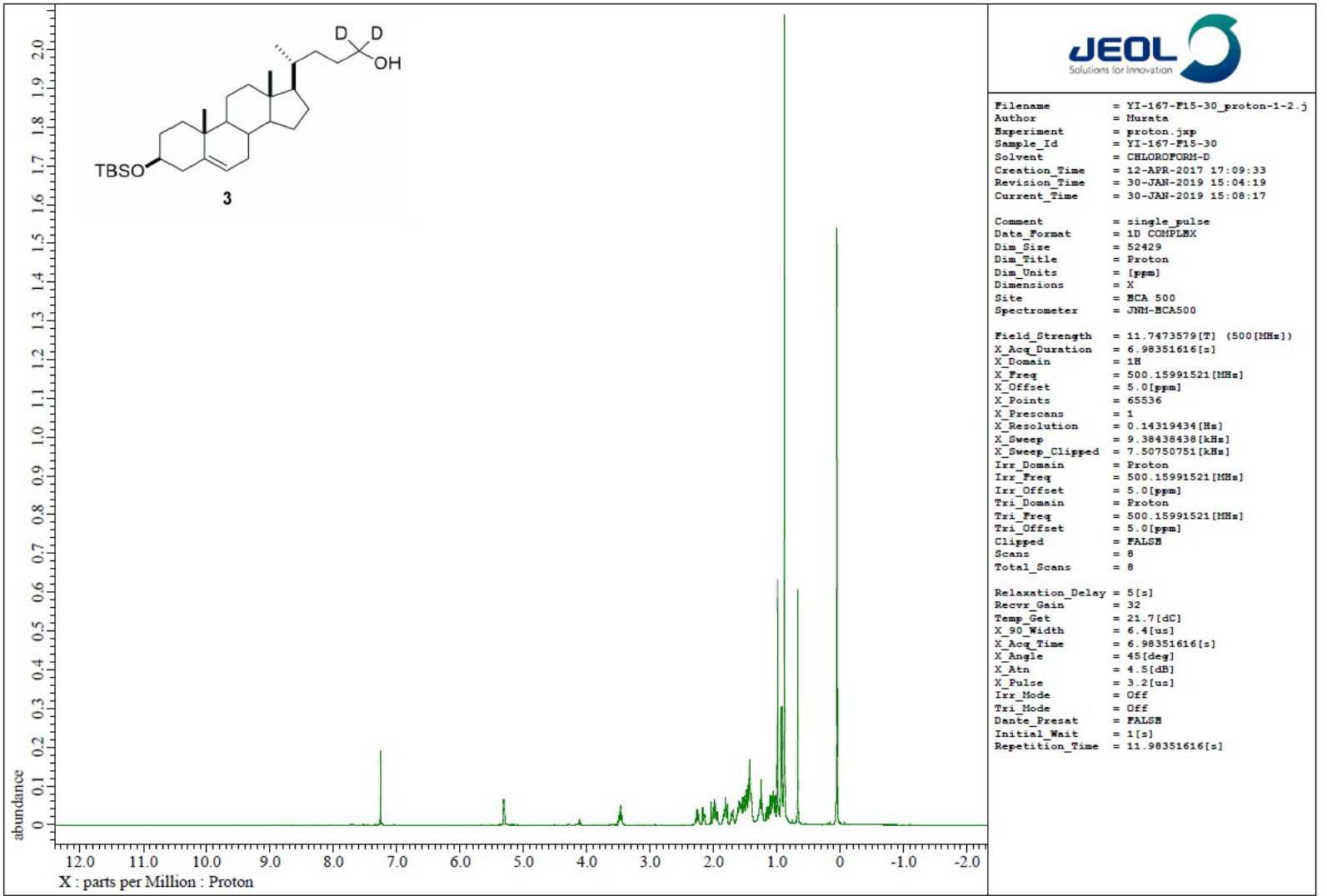
[S6] J. B. Johnston, A. A. Singh, A. A. Clary, C. K. Chen, P. Y. Hayes, S. Chow, J. J. De Voss P. R. Ortiz de Montellano, *Bioorg. Med. Chem.* **2012**, *20*, 4064-4081.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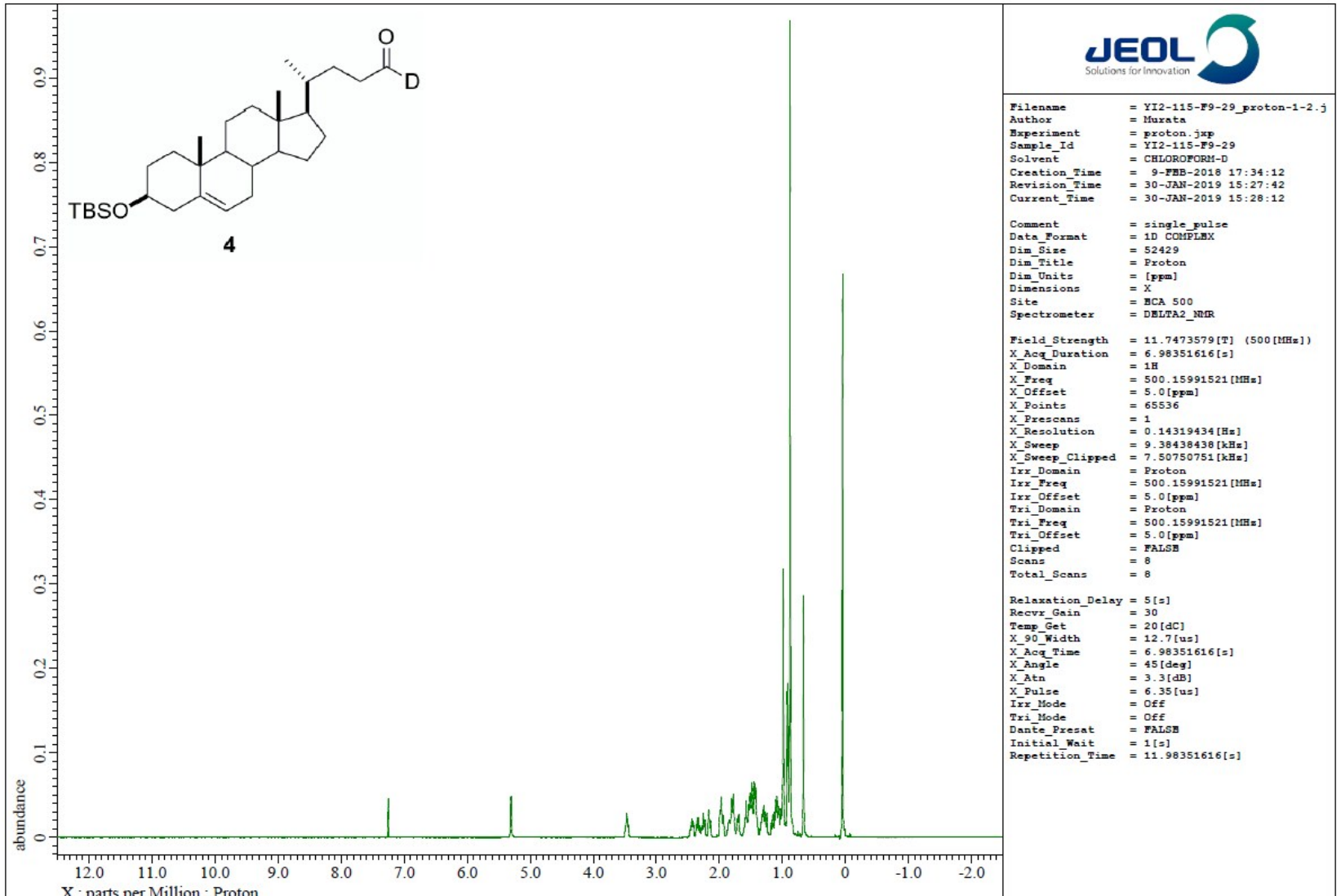
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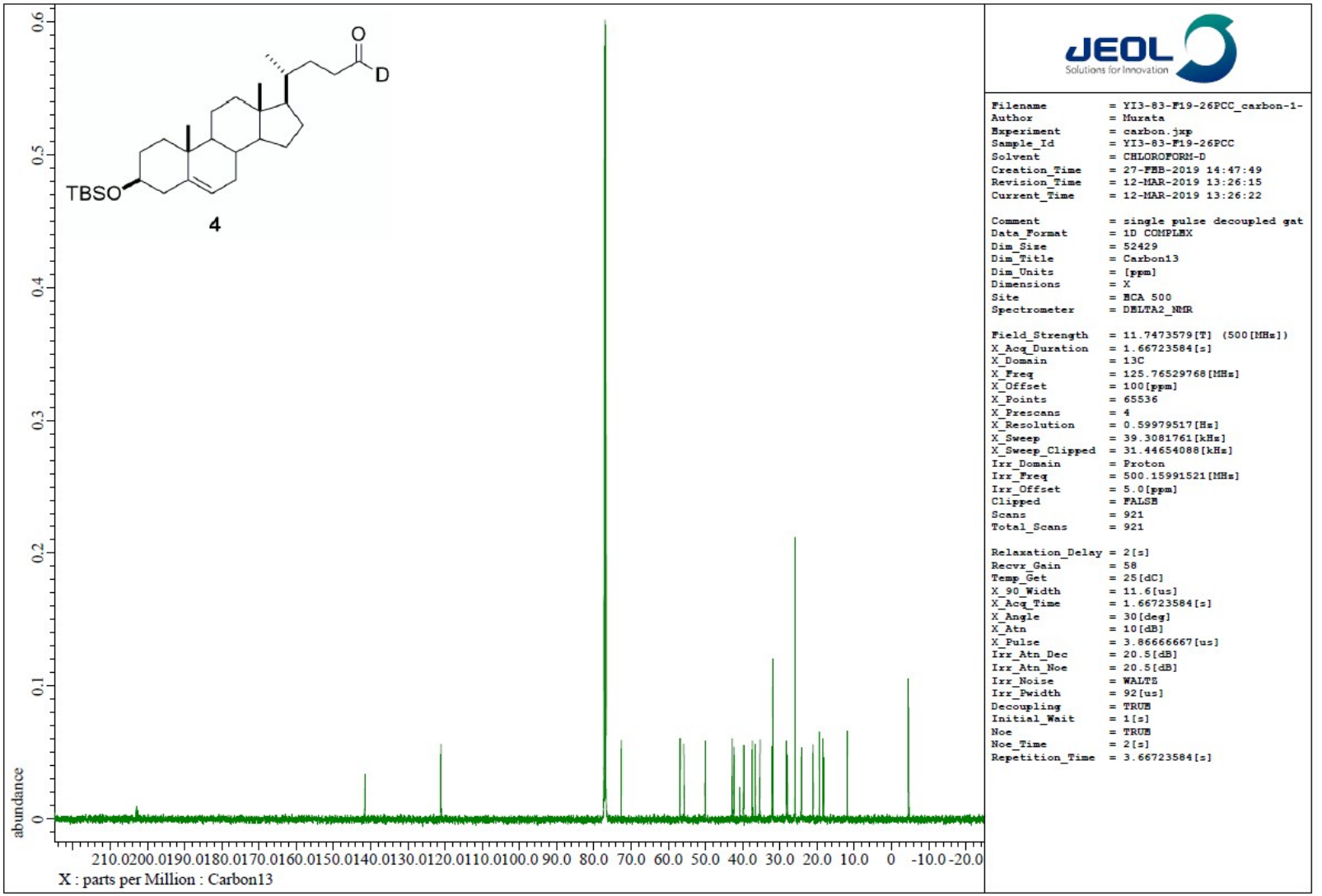
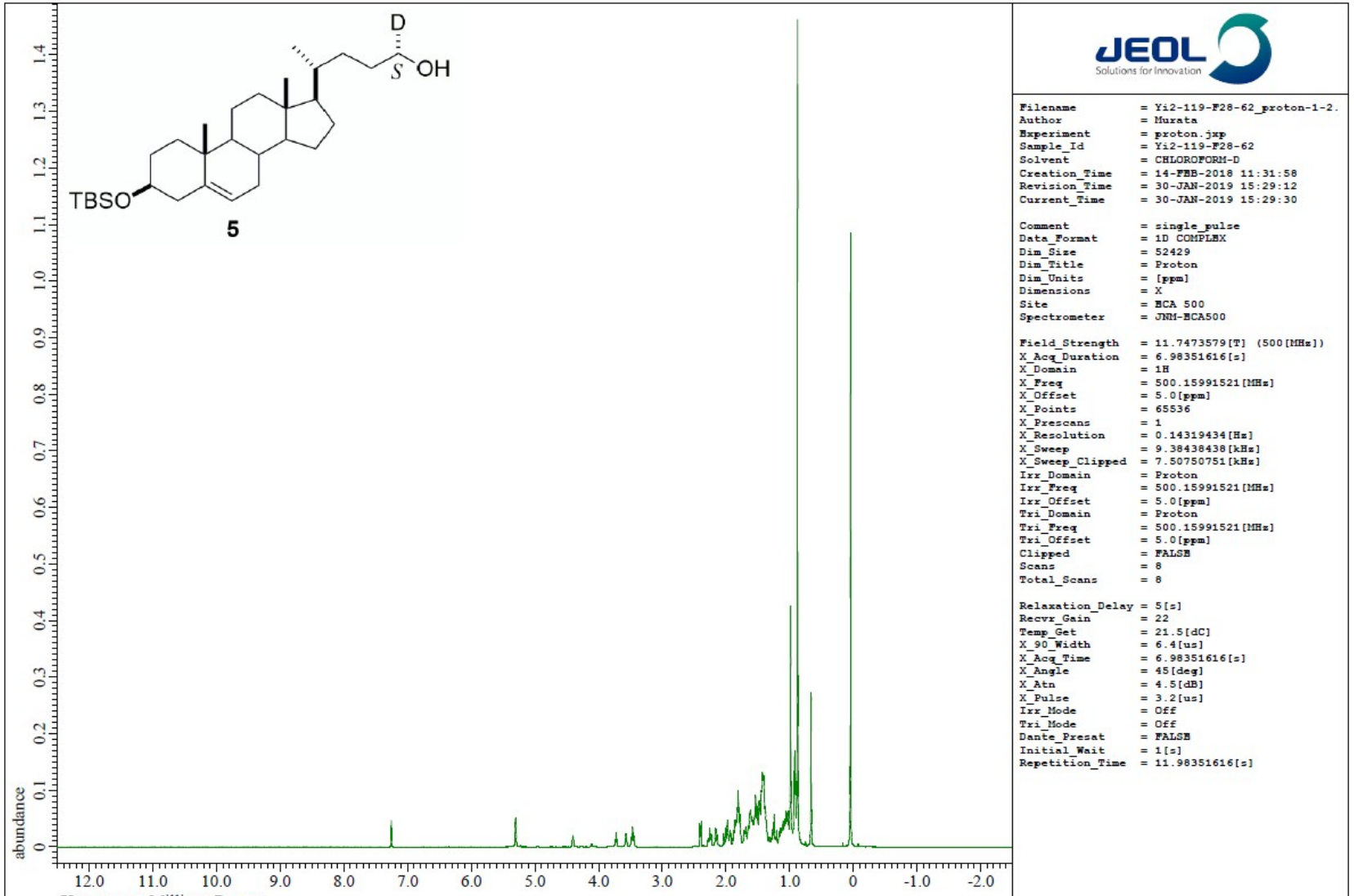
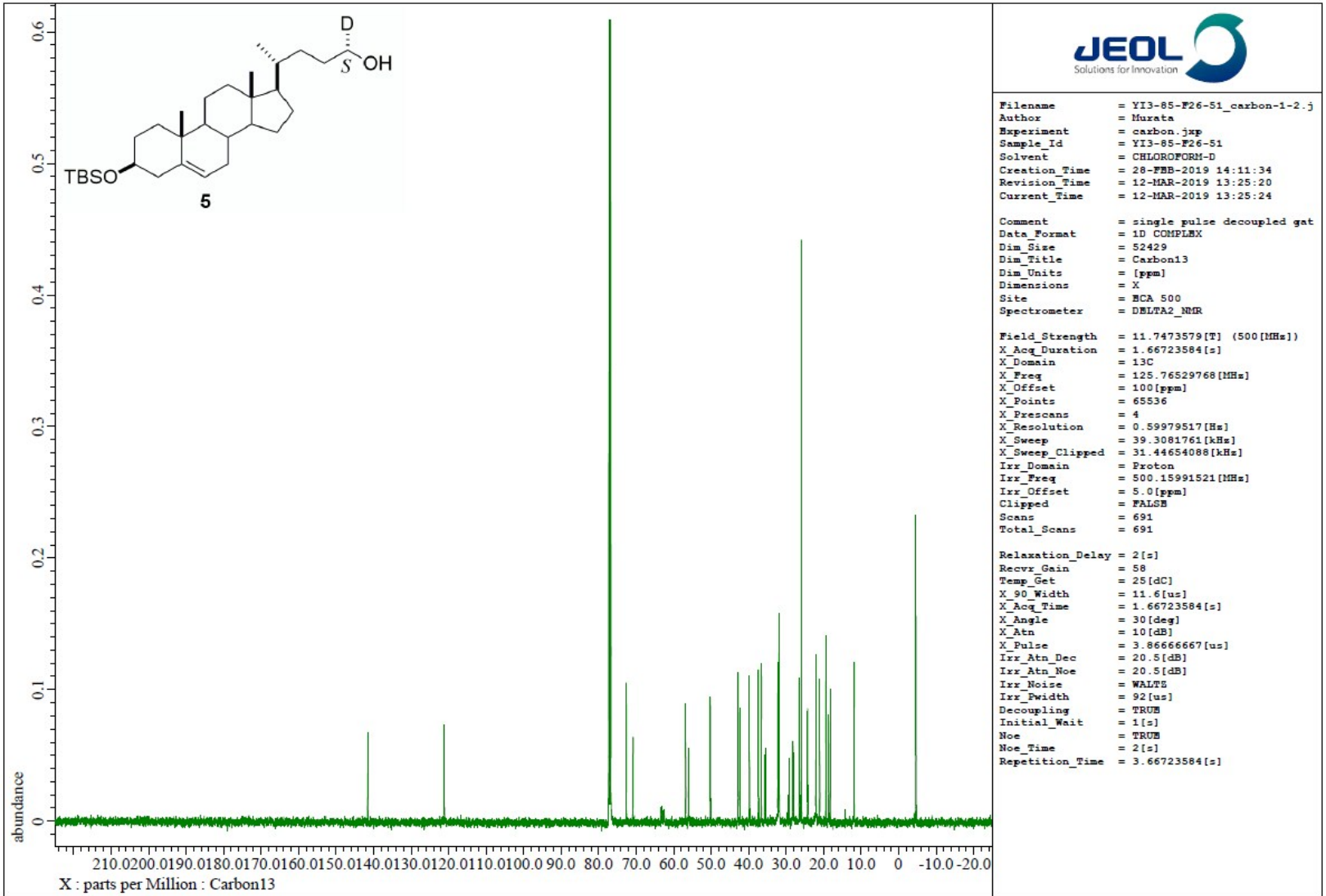
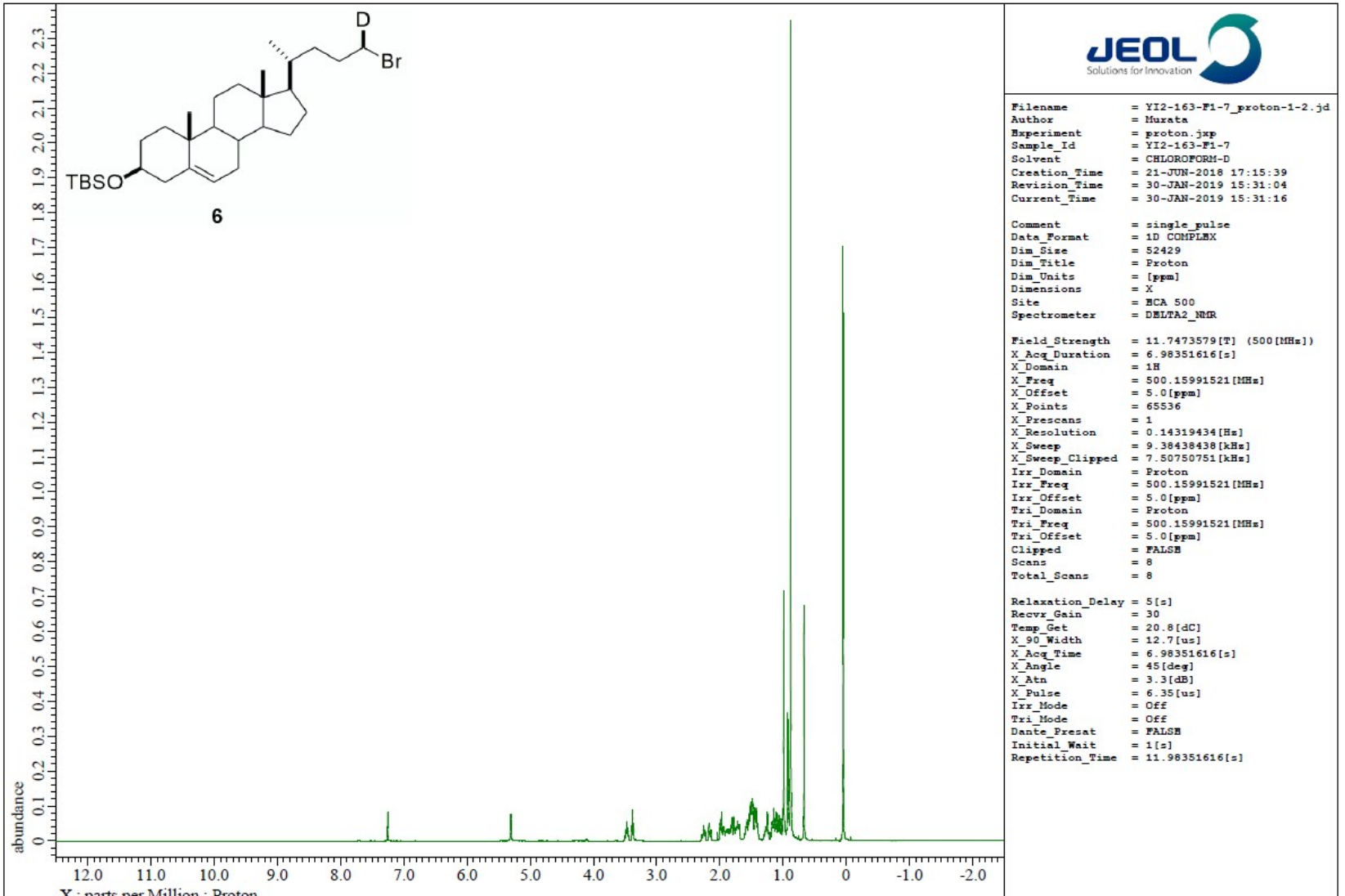
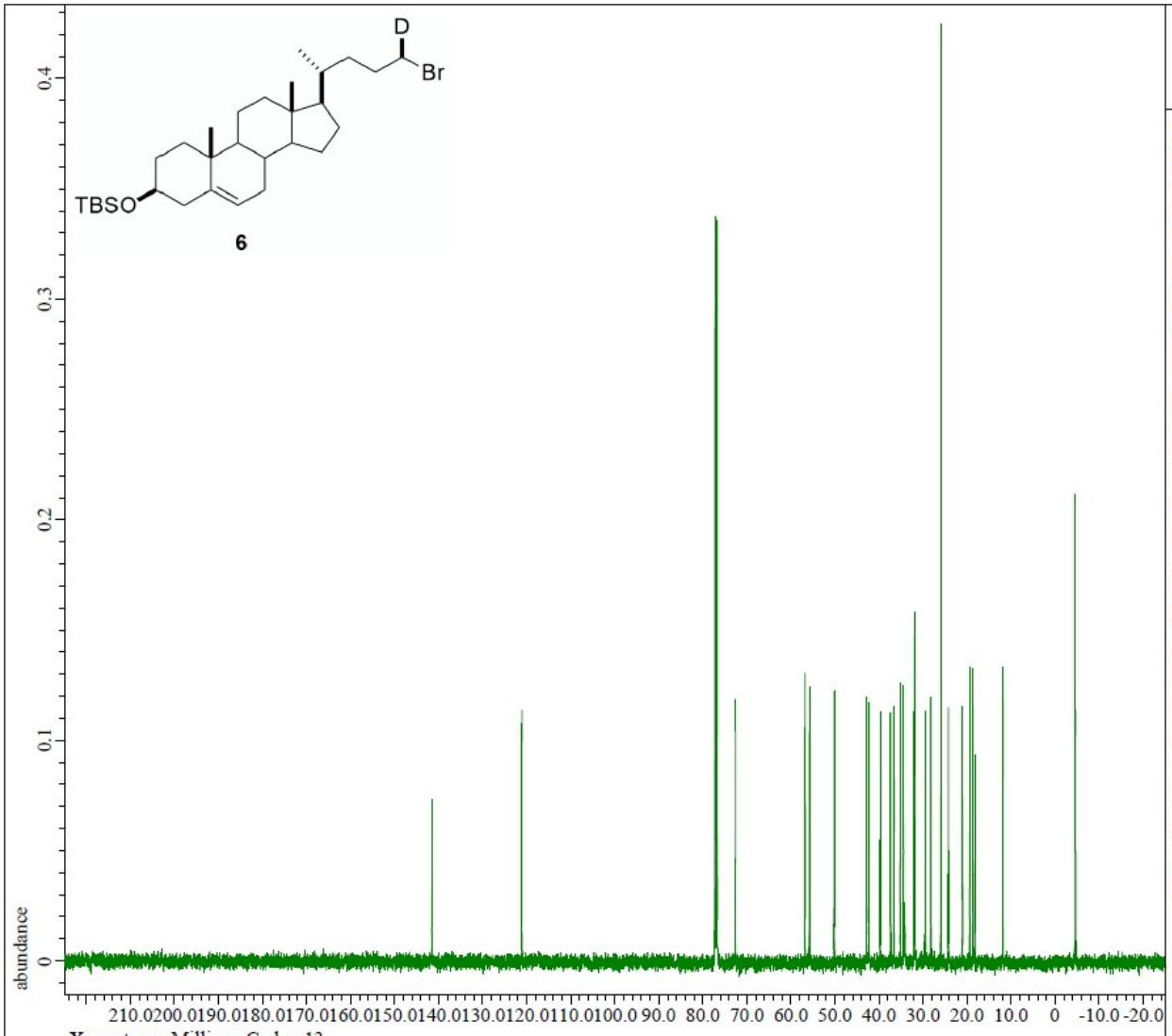
Irr_Offset = 5.0[ppm]
Tri_Domain = Proton
Tri_Freq = 500.15991521[MHz]
Tri_Offset = 5.0[ppm]
Clipped = FALSE
Scans = 8
Total_Scans = 8
Relaxation_Delay = 5[s]
Recvr_Gain = 30
Temp_Get = 20[dC]
X_90_Width = 12.7[us]
X_Acq_Time = 6.98351616[s]
X_Angle = 45[deg]
X_Atn = 3.3[dB]
X_Pulse = 6.35[us]
Irr_Mode = Off
Tri_Mode = Off
Dante_Presat = FALSE
Initial_Wait = 1[s]
Repetition_Time = 11.98351616[s]


TBSO
O
D
4
abundance
X : parts per Million : Carbon13
JEOL
Solutions for Innovation
Filename = YI3-83-F19-26PCC_carbon-1-
Author = Murata
Experiment = carbon.jxp
Sample_Id = YI3-83-F19-26PCC
Solvent = CHLOROFORM-D
Creation_Time = 27-FEB-2019 14:47:49
Revision_Time = 12-MAR-2019 13:26:15
Current_Time = 12-MAR-2019 13:26:22
Comment = single pulse decoupled gat
Data_Format = 1D COMPLEX
Dim_Size = 52429
Dim_Title = Carbon13
Dim_Units = [ppm]
Dimensions = X
Site = RCA 500
Spectrometer = DELTA2_NMR
Field_Strength = 11.7473579[T] (500[MHz])
X_Acq_Duration = 1.66723584[s]
X_Domain = 13C
X_Freq = 125.76529768[MHz]
X_Offset = 100[ppm]
X_Points = 65536
X_Prescans = 4
X_Resolution = 0.59979517[Hz]
X_Sweep = 39.3081761[kHz]
X_Sweep_Clipped = 31.44654088[kHz]
Irr_Domain = Proton
Irr_Freq = 500.15991521[MHz]
Irr_Offset = 5.0[ppm]
Clipped = FALSE
Scans = 921
Total_Scans = 921
Relaxation_Delay = 2[s]
Recvr_Gain = 58
Temp_Get = 25[dC]
X_90_Width = 11.6[us]
X_Acq_Time = 1.66723584[s]
X_Angle = 30[deg]
X_Atn = 10[dB]
X_Pulse = 3.86666667[us]
Irr_Atn_Dec = 20.5[dB]
Irr_Atn_Noe = 20.5[dB]
Irr_Noise = WALTZ
Irr_Pwidth = 92[us]
Decoupling = TRUE
Initial_Wait = 1[s]
Noe = TRUE
Noe_Time = 2[s]
Repetition_Time = 3.66723584[s]


D
S
OH
TBSO
5
abundance
12.0
11.0
10.0
9.0
8.0
7.0
6.0
5.0
4.0
3.0
2.0
1.0
0
-1.0
-2.0
JEOL
Solutions for Innovation
Filename = Yi2-119-F28-62_proton-1-2.
Author = Murata
Experiment = proton.jxp
Sample_Id = Yi2-119-F28-62
Solvent = CHLOROFORM-D
Creation_Time = 14-FEB-2018 11:31:58
Revision_Time = 30-JAN-2019 15:29:12
Current_Time = 30-JAN-2019 15:29:30
Comment = single_pulse
Data_Format = 1D COMPLEX
Dim_Size = 52429
Dim_Title = Proton
Dim_Units = [ppm]
Dimensions = X
Site = NCA 500
Spectrometer = JNM-NCA500
Field_Strength = 11.7473579[T] (500[MHz])
X_Acq_Duration = 6.98351616[s]
X_Domain = 1H
X_Freq = 500.15991521[MHz]
X_Offset = 5.0[ppm]
X_Points = 65536
X_Prescans = 1
X_Resolution = 0.14319434[Hz]
X_Sweep = 9.38438438[kHz]
X_Sweep_Clipped = 7.50750751[kHz]
Irr_Domain = Proton
Irr_Freq = 500.15991521[MHz]
Irr_Offset = 5.0[ppm]
Tri_Domain = Proton
Tri_Freq = 500.15991521[MHz]
Tri_Offset = 5.0[ppm]
Clipped = FALSE
Scans = 8
Total_Scans = 8
Relaxation_Delay = 5[s]
Recvr_Gain = 22
Temp_Get = 21.5[dC]
X_90_Width = 6.4[us]
X_Acq_Time = 6.98351616[s]
X_Angle = 45[deg]
X_Atn = 4.5[dB]
X_Pulse = 3.2[us]
Irr_Mode = Off
Tri_Mode = Off
Dante_Presat = FALSE
Initial_Wait = 1[s]
Repetition_Time = 11.98351616[s]


D
S
OH
TBSO
5
abundance
0
0.1
0.2
0.3
0.4
0.5
0.6
210.0 200.0 190.0 180.0 170.0 160.0 150.0 140.0 130.0 120.0 110.0 100.0 90.0 80.0 70.0 60.0 50.0 40.0 30.0 20.0 10.0 0 -10.0 -20.0
X : parts per Million : Carbon13
JEOL
Solutions for Innovation
Filename = YI3-85-F26-51_carbon-1-2.j
Author = Murata
Experiment = carbon.jxp
Sample_Id = YI3-85-F26-51
Solvent = CHLOROFORM-D
Creation_Time = 28-FEB-2019 14:11:34
Revision_Time = 12-MAR-2019 13:25:20
Current_Time = 12-MAR-2019 13:25:24
Comment = single pulse decoupled gat
Data_Format = 1D COMPLEX
Dim_Size = 52429
Dim_Title = Carbon13
Dim_Units = [ppm]
Dimensions = X
Site = RCA 500
Spectrometer = DELTA2_NMR
Field_Strength = 11.7473579[T] (500[MHz])
X_Acq_Duration = 1.66723584[s]
X_Domain = 13C
X_Freq = 125.76529768[MHz]
X_Offset = 100[ppm]
X_Points = 65536
X_Prescans = 4
X_Resolution = 0.59979517[Hz]
X_Sweep = 39.3081761[kHz]
X_Sweep_Clipped = 31.44654088[kHz]
Irr_Domain = Proton
Irr_Freq = 500.15991521[MHz]
Irr_Offset = 5.0[ppm]
Clipped = FALSE
Scans = 691
Total_Scans = 691
Relaxation_Delay = 2[s]
Recvr_Gain = 58
Temp_Get = 25[dC]
X_90_Width = 11.6[us]
X_Acq_Time = 1.66723584[s]
X_Angle = 30[deg]
X_Atn = 10[dB]
X_Pulse = 3.86666667[us]
Irr_Atn_Dec = 20.5[dB]
Irr_Atn_Noe = 20.5[dB]
Irr_Noise = WALTS
Irr_Pwidth = 92[us]
Decoupling = TRUE
Initial_Wait = 1[s]
Noe = TRUE
Noe_Time = 2[s]
Repetition_Time = 3.66723584[s]


D
Br
TBSO
6
abundance
X : parts per Million : Proton
Filename = YI2-163-F1-7_proton-1-2.jd
Author = Murata
Experiment = proton.jxp
Sample_Id = YI2-163-F1-7
Solvent = CHLOROFORM-D
Creation_Time = 21-JUN-2018 17:15:39
Revision_Time = 30-JAN-2019 15:31:04
Current_Time = 30-JAN-2019 15:31:16
Comment = single_pulse
Data_Format = 1D COMPLEX
Dim_Size = 52429
Dim_Title = Proton
Dim_Units = [ppm]
Dimensions = X
Site = ECA 500
Spectrometer = DELTA2_NMR
Field_Strength = 11.7473579[T] (500[MHz])
X_Acq_Duration = 6.98351616[s]
X_Domain = 1H
X_Freq = 500.15991521[MHz]
X_Offset = 5.0[ppm]
X_Points = 65536
X_Prescans = 1
X_Resolution = 0.14319434[Hz]
X_Sweep = 9.38438438[kHz]
X_Sweep_Clipped = 7.50750751[kHz]
Irr_Domain = Proton
Irr_Freq = 500.15991521[MHz]
Irr_Offset = 5.0[ppm]
Tri_Domain = Proton
Tri_Freq = 500.15991521[MHz]
Tri_Offset = 5.0[ppm]
Clipped = FALSE
Scans = 8
Total_Scans = 8
Relaxation_Delay = 5[s]
Recvr_Gain = 30
Temp_Get = 20.8[dC]
X_90_Width = 12.7[us]
X_Acq_Time = 6.98351616[s]
X_Angle = 45[deg]
X_Atn = 3.3[dB]
X_Pulse = 6.35[us]
Irr_Mode = Off
Tri_Mode = Off
Dante_Presat = FALSE
Initial_Wait = 1[s]
Repetition_Time = 11.98351616[s]



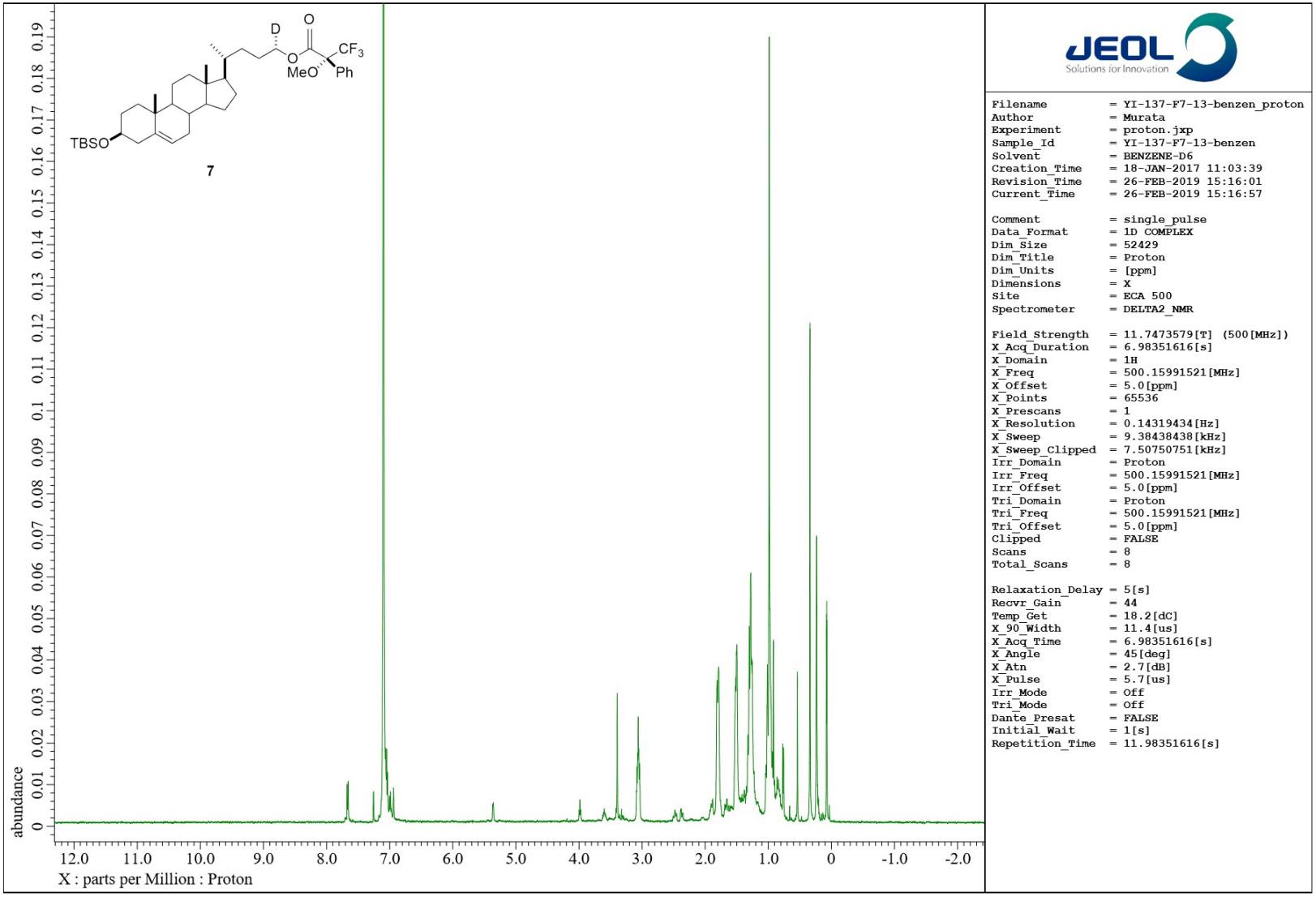
JEOL Solutions for Innovation

```
Filename          = YI3-87-F3-6_carbon-1-2.jdf
Author            = Murata
Experiment        = carbon.jxp
Sample_Id         = YI3-87-F3-6
Solvent           = CHLOROFORM-D
Creation_Time     = 27-FEB-2019 09:55:39
Revision_Time     = 12-MAR-2019 13:23:44
Current_Time      = 12-MAR-2019 13:23:50

Comment           = single pulse decoupled gat
Data_Format       = 1D COMPLEX
Dim_Size          = 52429
Dim_Title         = Carbon13
Dim_Units         = [ppm]
Dimensions        = X
Site              = ECA 500
Spectrometer      = JNM-ECA500

Field_Strength    = 11.7473579[T] (500[MHz])
X_Acq_Duration    = 1.66723584[s]
X_Domain          = 13C
X_Freq            = 125.76529768[MHz]
X_Offset          = 100[ppm]
X_Points          = 65536
X_Prescans        = 4
X_Resolution      = 0.59979517[Hz]
X_Sweep           = 39.3081761[kHz]
X_Sweep_Clipped   = 31.44654088[kHz]
Irr_Domain        = Proton
Irr_Freq          = 500.15991521[MHz]
Irr_Offset        = 5.0[ppm]
Clipped           = TRUE
Scans             = 551
Total_Scans       = 551

Relaxation_Delay  = 2[s]
Recvr_Gain        = 54
Temp_Get          = 25[dC]
X_90_Width        = 22[us]
X_Acq_Time        = 1.66723584[s]
X_Angle           = 30[deg]
X_Atn             = 7[dB]
X_Pulse           = 7.33333333[us]
Irr_Atn_Dec       = 27.65[dB]
Irr_Atn_Noe       = 27.65[dB]
Irr_Noise         = WALTZ
Irr_Pwidth        = 92[us]
Decoupling        = TRUE
Initial_Wait      = 1[s]
Noe               = TRUE
Noe_Time          = 2[s]
Repetition_Time   = 3.66723584[s]
```


TBSO
D
O
O
MeO
CF3
Ph
7
abundance
0 0.01 0.02 0.03 0.04 0.05 0.06 0.07 0.08 0.09 0.1 0.11 0.12 0.13 0.14 0.15 0.16 0.17 0.18 0.19
12.0 11.0 10.0 9.0 8.0 7.0 6.0 5.0 4.0 3.0 2.0 1.0 0 -1.0 -2.0
X : parts per Million : Proton
JEOL
Solutions for Innovation
Filename = YI-137-F7-13-benzen_proton
Author = Murata
Experiment = proton.jxp
Sample_Id = YI-137-F7-13-benzen
Solvent = BENZENE-D6
Creation_Time = 18-JAN-2017 11:03:39
Revision_Time = 26-FEB-2019 15:16:01
Current_Time = 26-FEB-2019 15:16:57
Comment = single_pulse
Data_Format = 1D COMPLEX
Dim_Size = 52429
Dim_Title = Proton
Dim_Units = [ppm]
Dimensions = X
Site = ECA 500
Spectrometer = DELTA2_NMR
Field_Strength = 11.7473579[T] (500[MHz])
X_Acq_Duration = 6.98351616[s]
X_Domain = 1H
X_Freq = 500.15991521[MHz]
X_Offset = 5.0[ppm]
X_Points = 65536
X_Prescans = 1
X_Resolution = 0.14319434[Hz]
X_Sweep = 9.38438438[kHz]
X_Sweep_Clipped = 7.50750751[kHz]
Irr_Domain = Proton
Irr_Freq = 500.15991521[MHz]
Irr_Offset = 5.0[ppm]
Tri_Domain = Proton
Tri_Freq = 500.15991521[MHz]
Tri_Offset = 5.0[ppm]
Clipped = FALSE
Scans = 8
Total_Scans = 8
Relaxation_Delay = 5[s]
Recvr_Gain = 44
Temp_Get = 18.2[dC]
X_90_Width = 11.4[us]
X_Acq_Time = 6.98351616[s]
X_Angle = 45[deg]
X_Atn = 2.7[dB]
X_Pulse = 5.7[us]
Irr_Mode = Off
Tri_Mode = Off
Dante_Presat = FALSE
Initial_Wait = 1[s]
Repetition_Time = 11.98351616[s]