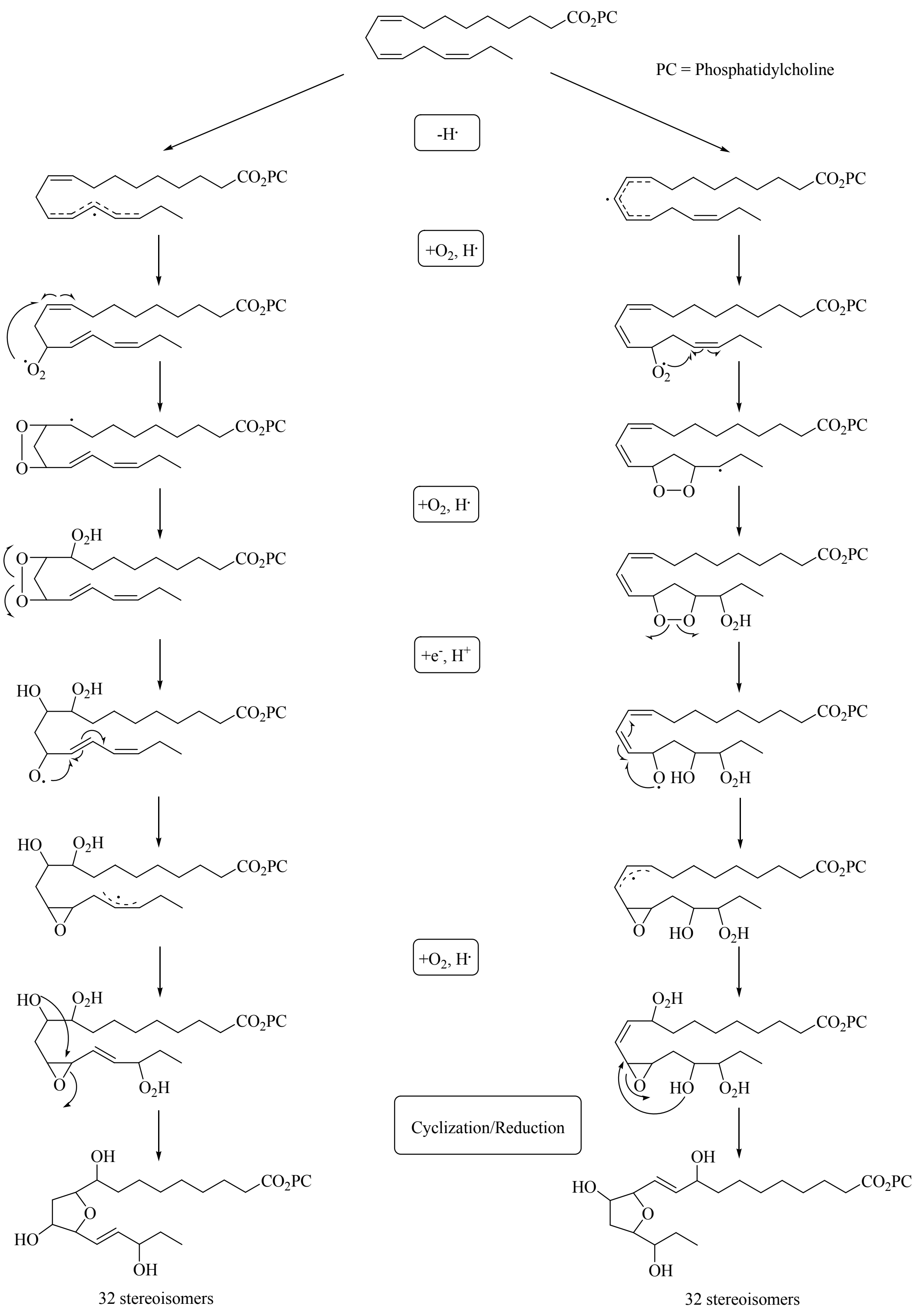
Supporting Information

Synthesis and Discovery of Phytofurans: Metabolites of α-Linolenic Acid Peroxidation

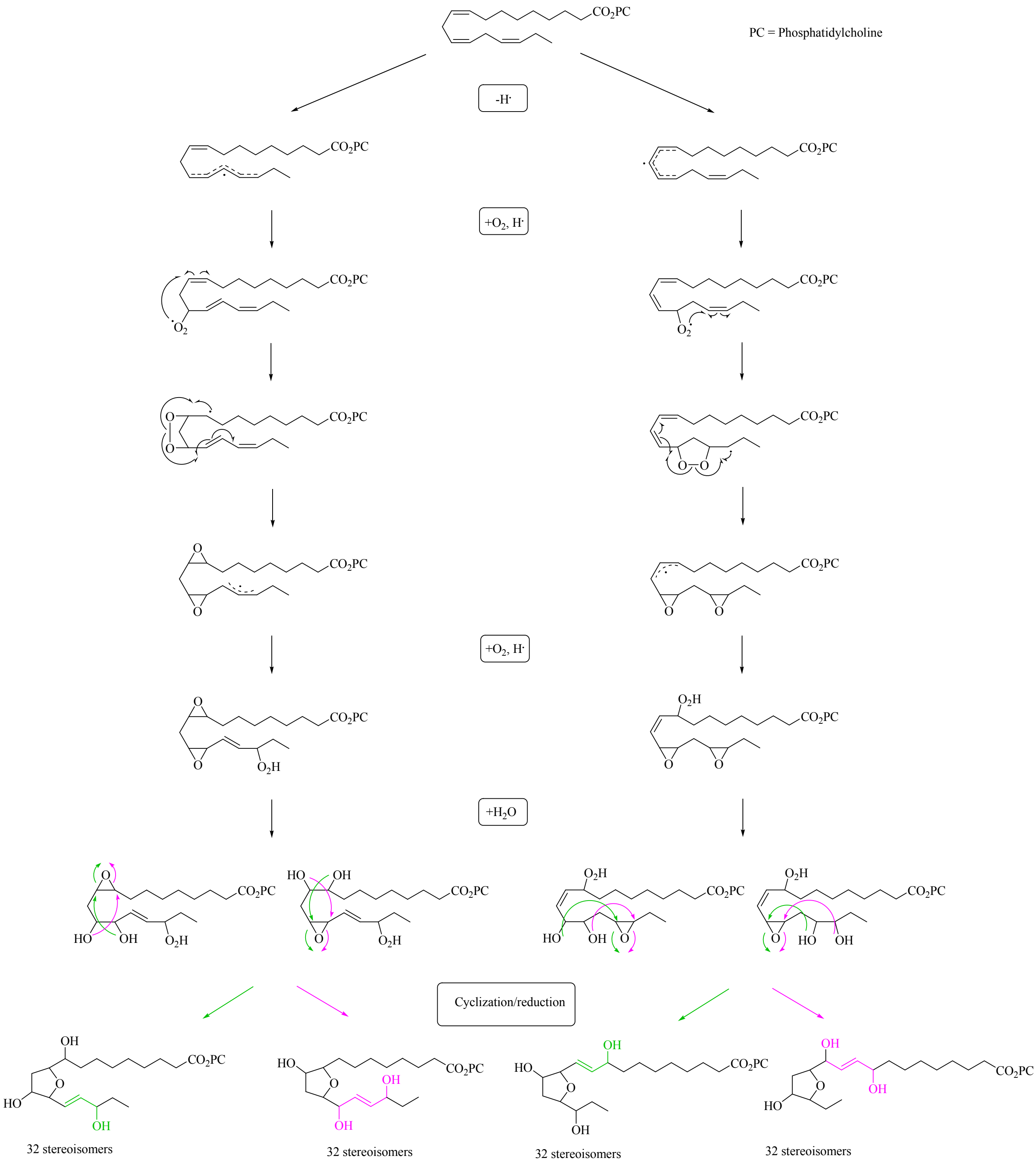
*Claire Cuyamendous, Kim Sum Leung, Thierry Durand, Jetty Chung-Yung Lee *, Camille Oger * and Jean-Marie Galano**

Table of Contents

1. The biosynthesis of Phytofurans (PhytoFs) based on the Isofuran pathways by Fessel and coworkers,[1] and Jahn and co-workers[2].



Scheme 1: Biosynthesis mechanism of alkenyl PhytoFs by reductive endoperoxide cleavage; 3-exo cyclization, and epoxide ring opening



Scheme 2: Alternative mechanism based on 1,3-S_Hi reaction, 3-exo cyclization, ring epoxide opening

2. General Information

All reactions requiring anhydrous conditions were conducted in oven-dried (120 °C) glassware with magnetic stirring under an atmosphere of nitrogen, unless mentioned otherwise. Syringes and needles for the transfer of reagents were dried at 120 °C and allowed to cool in a desiccators with $CaCl_2$ before use. Anhydrous THF and DCM were obtained from the Innovative Technology PS-Micro solvent purification system. Other solvents and reagents were used as obtained from the supplier unless otherwise noted. The reactions were monitored by TLC, using plates pre-coated Silica Gel 60 (Merck). Visualization of reaction components was achieved with 254 nm, and treatment with acidic *p*-anisaldehyde stain, followed by gentle heating. Organic layers were dried using $MgSO_4$ unless otherwise stated. Column chromatography was carried out on silica gel Kieselgel 60 (40-63 µm). Optical rotations ($[\alpha]_D^{20}$) were recorded on Perkin Elmer Polarimeter 341 (λ= 589 nm, 20 °C, concentration c in mg/mL, $CHCl_3$ or MeOH). Infrared spectra were obtained using a Perkin-Elmer Spectrum One spectrophotometer. They were reported as wavenumber (cm^{-1}) of significant peaks. Mass spectra were obtained by positive or negative electrospray ionization and electronic impact methods. Unless otherwise stated, ^1H NMR and ^{13}C NMR spectra were recorded at 303 K with 300 or 500 MHz on Bruker spectrometers. For the ^1H NMR, the peak due to residual $CHCl_3$ was used as the internal reference (fixed at 7.26 ppm) or for MeOD: the peak due to residual MeOH was used as the internal reference (fixed at 3.31 ppm) or for D_2O: the peak due to residual H_2O was used as the internal reference (fixed at 4.79 ppm). The ^1H NMR spectra are reported as follows: chemical shift in parts per million (multiplicity, coupling constant(s) *J* (Hz), relative integral, assignment) where multiplicity is defined as: br = broad, m = multiplet, s = singlet, d = doublet, t = triplet, or combination thereof). Selected ^{13}C NMR spectra were conducted using a *J* modulated sequence and in $CDCl_3$ the central peak of the $CDCl_3$ triplet was used as an internal reference (77.16 ppm), MeOD the central peak of the MeOD multiplet was used as an internal reference (49.00 ppm). The assignments of NMR spectra were assisted by homonuclear (^1H-^1H) and heteronuclear (^1H-^{13}C) correlation spectroscopy (COSY45, HMQC, HMBC) and are reported as follows: CH_3, CH_2, CH, and Cq (for quaternary carbon atoms).

3. Experimental procedures

3.1 Chemical synthesis

Preparation of hepta-2,5-diyne-1,7-diol 4:

To a solution of but-2-yne-1,4-diol (86 g, 1 mol, 1 eq) in 100 mL of dry benzene was added dry pyridine (87 mL, 1 mol, 1 eq). Dry thionyle chloride (80 mL, 1 mol, 1 eq) was added dropwise using syringe pump (over 3h). The temperature was maintained between 10-20 °C with cold bath. The mixture was stirred overnight at rt. The reaction was quenched with dropwise addition by syringe pump of 135 mL of water. After separation, the aqueous layer was extracted with ether (3 x 100 mL). The ether extracts were combined with the original benzene layer. The combined organic layers were stirred with saturated $NaHCO_3$ (150 mL) and solid $NaHCO_3$ (pH=8) for 20 min. After separation, the organic layers were dried over $MgSO_4$, filtered and the solvent was removed under vacuum. The product was purified by distillation under vacuum to give 4-chlorobut-2-yne-1-ol (48.50 g, 46%).

R_f = 0.69 (Cyclohexane/Et_2O: 3/7)
^{1}H NMR (300 MHz, $CDCl_3$) δ 4.31 (s, 2H, CH_2OH), 4.17 (s, 2H, CH_2Cl), 2.06 (br, 1H, OH)
^{13}C NMR (75 MHz, $CDCl_3$) δ 84.8 (1C, CH), 80.6 (1C, CH), 51.0 (1C, CH_2OH), 30.4 (1C, CH_2Cl)
EI: 69.2 [M-Cl], 87.2 [M-OH]
IR (ν_{max} cm^{-1}): 3324, 1430, 1263, 1142, 1009, 691

To a suspension of dry K_2CO_3 (17.82 g, 0.13 mol, 1.5 eq) in 60 mL of dry DMF, were successively added, dry NaI (25.8 g, 0.17 mol, 2 eq), dry CuI (32.7 g, 0.17 mol, 2 eq) and prop-2-yne-1-ol (7.02 mL, 0.12 mol, 1.4 eq). The mixture was stirred at rt for 1h. A solution of 4-chlorobut-2-yne-1-ol (9 g, 0.09 mol, 1 eq) in 40 mL of dry DMF was added by cannulation. The mixture was stirred overnight at rt. The reaction was quenched with saturated NH_4Cl (180 mL) and the pH was adjusted to 10 with $NH_{3(aq)}$. The product was extracted with EtOAc (9 x 150 mL). The combined organic layers were dried over $MgSO_4$, filtered and the solvent was evaporated under vacuum. The crude product was purified on silica gel (pentane/EtOAc : 50/50 to 0/100) to give hepta-2,5-diyne-1,7-diol **4** as a yellow solid (8.6 g, 81%).

R_f = 0.46 (Cyclohexane/EtOAc: 1/7)
^{1}H NMR (300 MHz, MeOD) δ 4.20-4.10 (m, 4H, CH_2OH), 3.30-3.20 (m, 2H, CH_2)
^{13}C NMR (75 MHz, MeOD) δ 79.9 (2C, Cq), 79.8 (2C, Cq), 50.8 (2C, CH_2OH), 9.9 (1C, CH_2)
EI: 106.2 [M-H_2O]
IR (ν_{max} cm^{-1}): 3245, 2918, 1362, 1312, 1145, 1011
Mp: 84.6-85.7 °C

Preparation of (2S,3S,5S,6S)-2,3:5,6-diepoxyheptane-1,7-diol 3:

To a solution of $LiAlH_4$ (100 mL, 2.4 M/THF, 240 mmol, 4.9 eq) in 100 mL of THF at -5 °C, 2-methoxyethanol (32 mL, 406 mmol, 8.4 eq) was added drop wise, using syringe pump over 1 h. After cooling to -40 °C a solution of the diynediol **4** (6 g, 48.4 mmol, 1 eq) in 45 mL of THF was added dropwise, using syringe pump, over 1h. The mixture was stirred at 0 °C for 1h and at rt for 15 h. After the installation of mechanic stirrer, the mixture was cooled at 0 °C and hydrolyzed by a slow addition (1.5h) of 30 mL of water. The pasty mixture was filtered on fritted glass and the residue was washed with ethanol (3 x 500 mL). The filtrates were concentrated under vacuum. A flash column chromatography of the residue, eluting with pentane/Et_2O (50/50 to 0/100), gave the (*E,E*)-hepta-2,5-diene-1,7-diol (3.44 g, 55%).

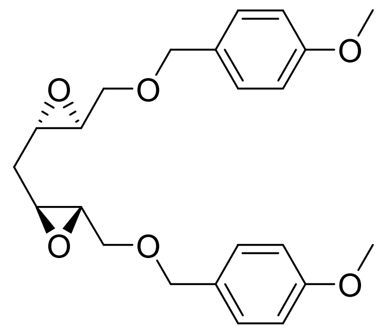
R_f = 0.19 (Et_2O:100%)
^{1}H NMR (300 MHz, MeOD) δ 5.80-5.50 (m, 4H, CH), 4.01 (d, *J* = 4.6, 4H, CH_2OH), 2.79 (t, *J* = 5.1, 2H, CH_2)
^{13}C NMR (75 MHz, MeOD) δ 131.3 (2C, CH), 130.7 (2C, CH), 63.4 (2C, CH_2OH), 35.7 (1C, CH_2)
EI: 81.2 [M-2H_2O]
IR (ν_{max} cm^{-1}): 3293, 2864, 1667, 1425, 1089, 967

To a suspension of powdered molecular sieves (4Å, 1.5g) in 30 mL of dry DCM and 10 mL of dry $CHCl_3$ at-10 °C, distilled (2*R*,3*R*)-(+)-diethyl tartrate (221 µL, 1.29 mmol, 0.15 eq), distilled titanium tetraisopropoxide (253 µL, 0.86 mmol, 0.1 eq) and a 5 M solution of *tert*-butyl hydroperoxide in decane (5.14 mL, 25.7 mmol, 3 eq) were successively added. After stirring for 30 min at -10 °C, the mixture was cooled to -30 °C. A solution of the (*E,E*)-heptadienediol (1.10 g, 8.59 mmol, 1 eq) in 10 mL of $CHCl_3$ was added. The mixture was stirred for 1 h at -30 °C and stored in a deep freeze for 48 h at -20 °C. A solution of citric acid monohydrate (192 mg, 0.91 mmol) in 3 mL of acetone and 20 mL of diethyl ether was added at this temperature. After reaching rt, the mixture was filtered and the residue was washed with 30 mL of THF and 80 mL of CH_3CN. The filtrate was concentrated in vacuo. The residue was washed with 80 mL of water to extract the product. The solution was concentrated by freeze drying to give (2S,3S,5S,6S)-2,3:5,6-diepoxyheptane-1,7-diol **3** (1.05 g, 76%, ee 90% determined below) as a white powder.

R_f = 0.23 (DCM/MeOH: 9/1)
^{1}H NMR (500 MHz, MeOD) δ 3.76 (dd, *J* = 12.5, 3.1, 2H, CH_2OH), 3.53 (dd, *J* = 12.5, 5.1, 2H, CH_2OH), 3.03(td, *J* = 5.7, 2.2, 2H, CHO), 2.95-2.90 (m, 2H, CHO), 1.80(t, *J* = 5.7, 2H, CH_2)
^{13}C NMR (155 MHz, MeOD) δ 62.8 (2C, CH_2OH), 59.6 (2C, CH), 54.0 (2C, CH), 35.4 (1C, CH_2)
ES+ : 183.1 $[M+Na]^+$, 343.1 $[M+M+Na]^+$
HRMS (ESI+): calculated for $C_7H_{12}O_4Na$ $[M+Na]^+$ 183.0633, found 183.0633
IR (ν_{max} cm^{-1}): 3120, 2873, 2338, 2169, 2028, 1479, 1334, 1254, 1018, 983, 956, 901, 875, 854, 715
M_p = 153.1-154.6 °C
$[\alpha]_D^{20}$(H_2O) = -48. 1 (c 4.33); lit:[3] $[\alpha]_D^{20}$(H_2O) = -49.8 *(c 0.64,).*

Determination of the enantiomeric excess

a) **Preparation of bis(4-methoxybenzyl) derivative**



To a solution bisepoxy diol **3** (50 mg, 0.31 mmol, 1 eq) in 3 mL of DMF, 4-methoxybenzyl chloride (93 µL, 0.69 mmol, 2.2 eq) and NaH (60% in grease, 30 mg, 0.75 mmol, 2.4 eq) were added. After 20 min at 100 °C, the reaction quenched with water (10 mL) and extracted with Et_2O (3 x 6 mL). The organics layers were washed with brine (10 mL), dried over $MgSO_4$, filtered and concentrated under vacuum. Fast column chromatography (pentane/EtOAc: 60/40) afforded bis(4-methoxybenzyl) derivative (34 mg, 30%).

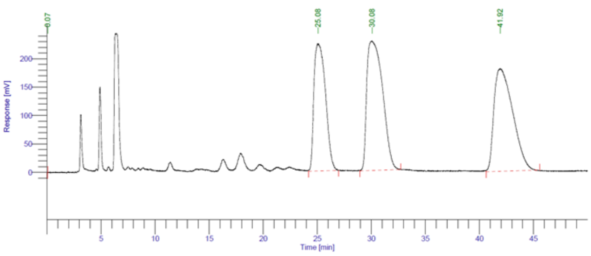
R_f = 0.41(Cyclohexane/EtOAc: 9/1)
^{1}H NMR (500 MHz, $CDCl_3$) δ 7.28-7.23 (m, 4H, CH_{Ph}), 6.87 (d, J = 7. 5, 4H, CH_{Ph}), 4.49 (q_{app}, J = 11.6, 4H, CH_2O), 3.8 (s, 6H, CH_3O), 3.70(d, J = 11.3, 2H, CH_2O), 3.47 (dd, J = 5.2, 11.5, 2H, CH_2O), 3.05-2.90 (m, 4H, CHO), 1.80 (t, J = 5.5, 2H, CH_2)
^{13}C NMR (125 MHz, $CDCl_3$) δ 159.4 (2C, Cq), 130.0 (2C, Cq), 129.6 (4C, CH), 113.9 (4C, CH), 73.1 (2C, CH_2O), 69.8 (2C, CH_2O), 56.9 (2C, CHO), 55.4 (2C, CH_3O), 53.0 (2C, CHO), 34.6 (1C, CH_2)
ES+ : 401.20 $[M+H]^+$, 423.18 $[M+Na]^+$
HRMS (ESI+): calculated for $C_{23}H_{29}O_6$ $[M+H]^+$ 401.1964, found 401.1967

b) Preparation of the racemic reference

Racemic bis(4-methoxybenzyl) derivative was prepared following the above procedure from a 1:1 mixture of (d,l)-racemic and meso 2,3:5,6-diepoxyheptane-1,7-diol prepared from corresponding (*E,E*)-hepta-2,5-diene-1,7-diol and mCPBA.

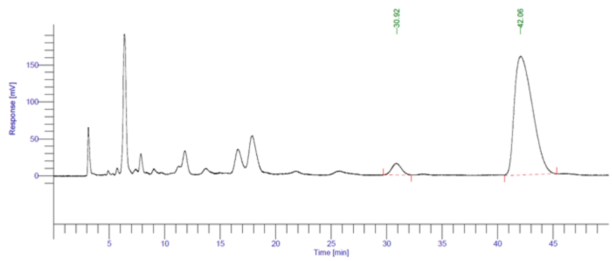
c) HPLC analysis of bis(4-methoxybenzyl) derivative for ee determination

HPLC Perkin Helmer series 200
Chiral column Chiralcel OD 0.46 cm x 25 cm
Diode Array Detector (λ=210 nm)
Flow: 1.0 mL/min;
Eluent: Hexane/*i*-PrOH: 85/15
Bis(4-methoxybenzyl) derivative from (2R,3R,5R,6R)-2,3:5,6-diepoxyheptane-1,7-diol tr= 30.92 min.
Bis(4-methoxybenzyl) derivative from (2S,3S,5S,6S)-2,3:5,6-diepoxyheptane-1,7-diol tr= 41.92 min.



Racemic/Meso-bis(4-methoxybenzyl) derivative bisepoxy diol

Peak #	Component Name	Time [min]	Height [uV]	Area [%]	Norm. Area [%]	BL	RT relatif	Rel. RT	
1		0.068	586.39	0.00	0.00	*BB	1.00	1.00	0.07
2		25.080	224191.96	27.04	27.04	*MM	367.93	367.93	25.08
3		30.081	227950.22	37.58	37.58	*MM	441.30	441.30	30.08
4		41.917	180595.13	35.38	35.38	*MM	614.93	614.93	41.92
			633323.69	100.00	100.00				97.15



(2S,3S,5S,6S)- bis(4-methoxybenzyl) derivative bisepoxy diol

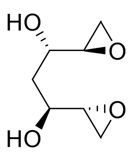
Peak #	Component Name	Time [min]	Height [uV]	Area [%]	Norm. Area [%]	BL	RT relatif	Rel. RT	
1		30.917	15673.57	5.08	5.08	*MM	1.00	1.00	30.92
2		42.064	160608.67	94.92	94.92	*MM	1.36	1.36	42.06
			176282.24	100.00	100.00				72.98

Preparation of tetraol 2:

To a solution of bisepoxy diol **3** (100 mg, 0.63 mmol, 1 eq) in 5 mL of water, a solution of KOH (175 mg, 3.12 mmol, 5 eq) in 5 mL of distilled water was added. The mixture was heated at 80 °C for 2 h. The mixture was neutralized by a solution of 1 M HCl. The solution was concentrated by freeze-drying. Column chromatography (DCM/MeOH: 95/5 to 80/20) afforded furanic tetraol **2** in mixture of two diastereoisomers (dr = 4/1; 72 mg, 65%) as a white solid.

R_f = 0.2 (DCM/MeOH: 8/2)
^{1}H NMR (500 MHz, D_2O) δ 4.20-4.12 (m, 1H, CHOH), 4.12-4.00 (m, 1H, CHO + *diast*, 0.4H, CHO), 3.76-3.80 (m, 1H, CHO + *diast*, 0.2H, CHO), 3.71-3.64 (m, 1H, CHOH + *diast*, 0.3H, CHOH), 3.60-3.50 (m, 2H, CH_2OH + *diast*, 0.2H, CHOH), 3.50-3.48 (*diast*, m, 0.4H, CH_2OH), 3.45-3.35 (m, 2H, CH_2OH + *diast*, 0.5H, CHOH), 2.25-2.10 (*diast*, m, 0.2H, CH_2), 2.00-1.90 (m, 1H, CH_2), 1.85-1.70 (m, 1H, CH_2 + *diast*, m, 0.3H, CH_2)
^{13}C NMR (125 MHz, D_2O) δ 88.1 (1C, CH), 87.3 (*diast*, 1C, CH), 80.4 (1C, CH), 80.0 (*diast*, 1C, CH), 74.6 (*diast*, 1C, CH), 74.4 (1C, CH), 73.8 (1C, CH), 72.8 (*diast*, 1C, CH), 64.6 (*diast*, 1C, CH_2OH), 64.4 (1C, CH_2OH), 63.7 (1C, CH_2OH), 63.2 (*diast*, 1C, CH_2OH), 36.1 (1C, CH_2 + *diast*)
ES+ : 201.1 $[M+Na]^+$
HRMS (ESI+): calculated for $C_7H_{14}O_5Na$ $[M+Na]^+$ 201.0739, found 201.0742
IR (ν_{max} cm^{-1}): 3347, 2947, 2885, 1453, 1075, 1026, 925

Column chromatography of the above reaction also afforded bisepoxy diol **B** resulting of double-Payne rearrangement of **3**:

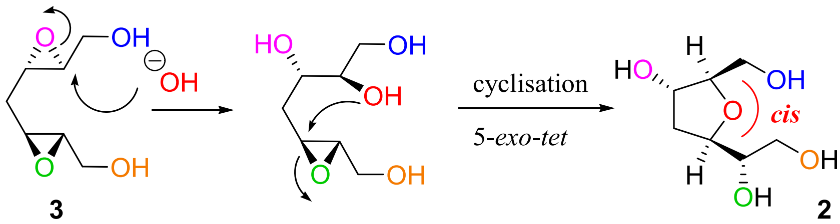


Bisepoxy diol **B** isolated (7 mg, 7%)
R_f = 0.46(DCM/MeOH: 9/1)

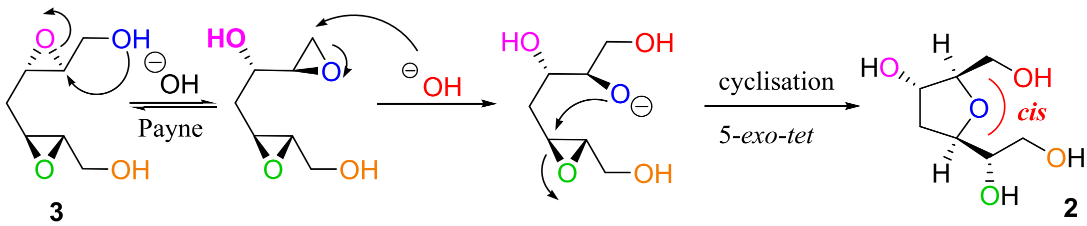
^{1}H NMR (500 MHz, MeOD) δ 4.48 (s, 2H, CHOH), 3.89 (t, *J* = 6.8, 2H, CHO), 3.78 (dd, *J* = 10.9, 7.2, 2H, CH_2O), 3.71 (dd, *J* = 10.9, 6.2, 2H, CH_2O), 2.12 (s, 2H, CH_2)
^{13}C NMR (150 MHz, MeOD) δ 86.2 (2C, CHO), 78.0 (2C, CHOH), 60.6 (2C, CH_2O), 39.0 (1C, CH_2)
IR (ν_{max} cm^{-1}): 3347, 2947, 2885, 1649, 1453, 1210, 1075, 1026, 925

It is important to note that bisepoxy diol **B** in the same condition as above do not furnish any THF ring but only recovered SM and very polar compounds (probably by opening of the epoxides) already observed for the reaction of 3 to give 2.

Justification of the Payne rearrangement reaction over a direct opening of the epoxide.



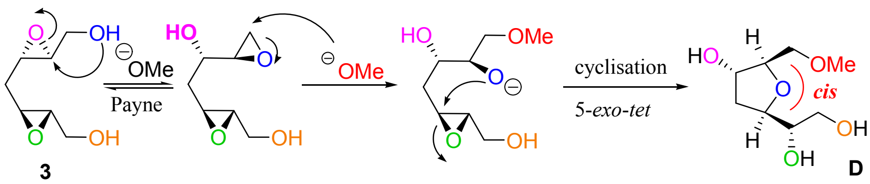
Direct opening of 3

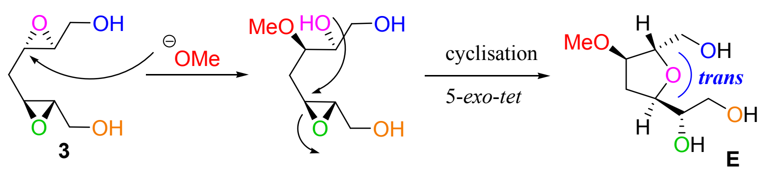


Payne reaction of 3 to give 2

A direct opening of **3** on the C2 atom follow by cyclization will give the same stereoisomer **2** which can also be obtained via a mono Payne rearrangement/opening of epoxide and cyclization. The following experiment in MeONa/MeOH condition reported almost the same ratio of THF ring products which permitted to assign the position of the reactive MeO group similarly as the OH group should have reacted.

Preparation of methoxy-tetraol D and E.

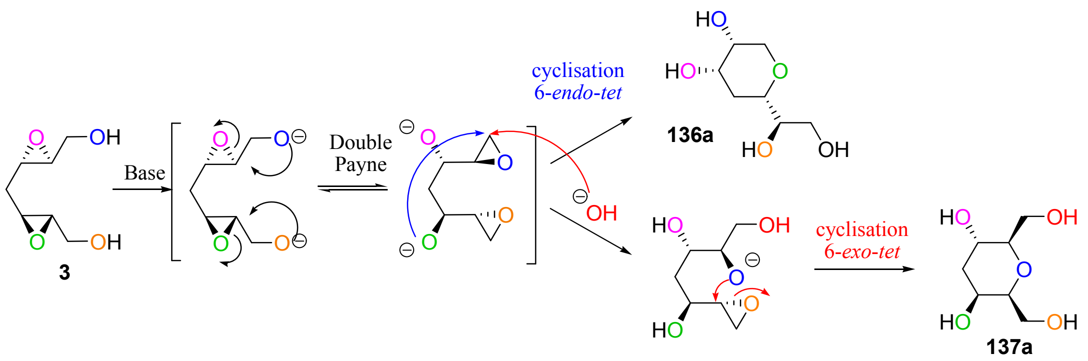


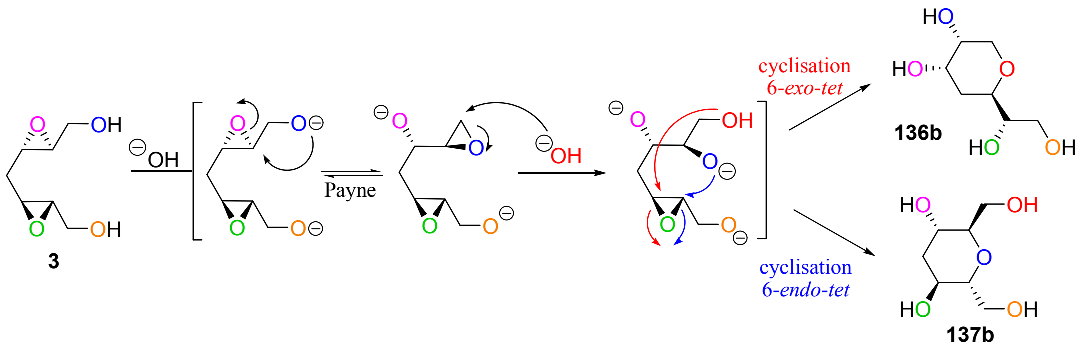


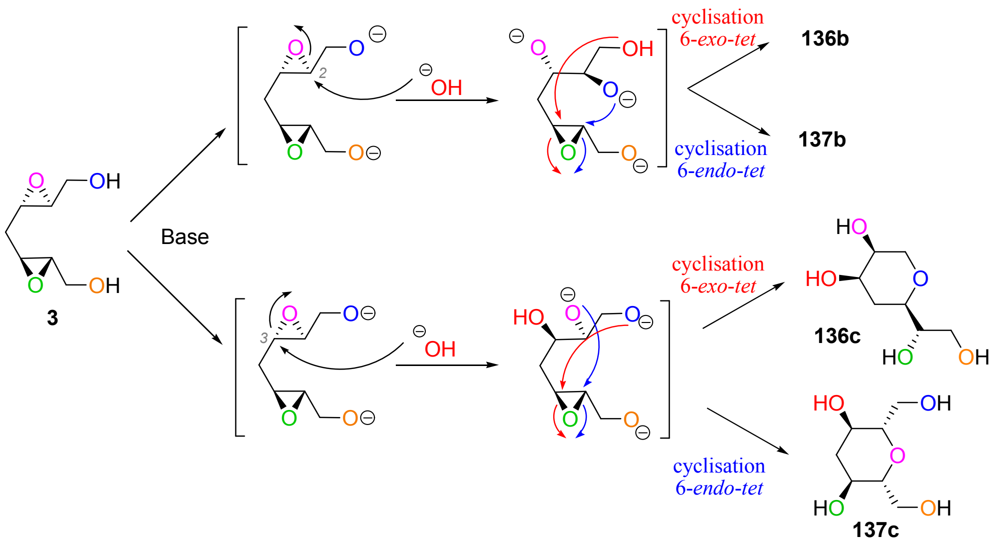
The bisepoxy diol **3** (100 mg, 0.63 mmol, 1 eq) was dissolved in 4 mL of NaOMe freshly prepared (0.2M in MeOH). The mixture was heated at 80°C for 16 h. The solvent was evaporated and the crude product was purified by column chromatography (DCM/MeOH: 95/5 to 80/20) to afford a mixture of furanic methoxy cycle **D** and **E** in a 7/3 ratio (69 mg, 58%). Relative configurations of **D** and **E** were assigned based on the position of the MeO group following a Payne rearrangement for **D** and a direct opening of **3** for **E**.

R_f = 0.4 (DCM/MeOH: 8/2)
^{1}H NMR (500 MHz, $CDCl_3$) δ 4.69 (br, 1H, OH),
4.33-4.30 (m, 1H, $CHOH_{(cycle)}$), 4.27-4.23 (m, 1H, CH), 4.14-4.10 (*minor*, m, 1H, CH), 4.04-4.01 (*minor*, m, 1H, CH), 3.94-3.92 (m, 1H, CH), 3.86-3.83 (m, 1H, CHOH + *minor*, 1H, CHOMe), 3.79-3.76 (*minor*, m, 1H, CHOH + *minor*, 1H, CH_2OH), 3.71-3.61 (m, 1H, CH_2OH + *minor*, 1H, CH_2OH), 3.60-3.52 (m, 1H, CH_2OH + *minor*, 1H, CH_2OH), 3.51-3.44 (m, 2H, CH_2OMe + *minor*, 1H, CH_2OH), 3.38 (s, 3H, CH_3), 3.34 (*minor*, s, 3H, CH_3), 1.13 (br, 2H, OH), 2.27-2.23 (*minor*, m, 1H, CH_2), 2.17-2.10 (m, 1H, CH_2), 2.07-2.02 (*minor*, m, 1H, CH_2), 1.90-1.85 (m, 1H, CH_2)
^{13}C NMR (125 MHz, $CDCl_3$) δ 84.8 (1C, CH), 84.0 (*minor*, 1C, CH), 82.1 (*minor*, 1C, CHOMe), 79.9 (1C, CH), 79.2 (*minor*, 1C, CH), 74.0 (1C, $CHOH_{(cycle)}$), 73.4 (1C, CH_2OMe), 73.3 (*minor*, 1C, CHOH), 73.1 (1C, CHOH), 63.7 (*minor*, 1C, CH_2OH), 63.6 (1C, CH_2OH), 63.1 (*minor*, 1C, CH_2OH), 59.4 (1C, OCH_3), 57.3 (*minor*, 1C, OCH_3), 35.7 (1C, CH_2), 33.1 (*minor*, 1C, CH_2)
ES+ : 2015.09 $[M+Na]^+$
HRMS (ESI+): calculated for $C_8H_{16}O_5Na$ $[M+Na]^+$ 215.0895, found 2015.0897

Possible formation of pyran derivatives







Formation of pyran derivatives such as above was a possibility but clearly refuted by the non-observation after acetonide formation of a bisprotected diol derivatives (for 136 a-c potential compounds). For compounds 137a-c the symmetrical nature of such compounds would have stood up by NMR.

Preparation of acetonide 5:

To a solution of the furanic tetraol **2** (639 mg, 5.59 mmol, 1 eq) in 30 mL of acetone, 2,2-dimethoxypropane (490 µL, 3.95 mmol, 1,1 eq) and para-toluenesulfonic acid (68 mg, 0.36 mmol, 0.1 eq) were added. The mixture was stirred at reflux for 3 h. Solid $NaHCO_3$ was added and the solvent was concentrated under reduced pressure prior to purification by column chromatography (DCM/MeOH: 98/2 to 94/6), to yield the acetonide **5** with its diastereoisomer (614 mg, 79%).

R_f = 0.12 (DCM/MeOH: 9.5/0.5)

Mixture of diastereoisomers :

^{1}H NMR (500 MHz, $CDCl_3 + D_2O$) δ 4.45-4.40 (m, 1H, CHOH), 4.38-4.32 (*diast*, m, 0.2H, CHOH), 4.32-4.28 (m, 1H, CH), 4.27-4.16 (m, 1H, CH + *diast*, 0.4H, CH), 4.12-4.06 (m, 1H, CH_2O + *diast*, 0.2H, CH_2O), 4.05-4.00 (*diast*, m, 0.3H, CH), 3.93-3.91 (m, 1H, CH), 3.75 (dd, J = 11.9, 3.2, 1H, CH_2OH), 3.69 (dd, J = 8.5, 6.0, 1H, CH_2O), 3.65-3.62 (*diast*, m, 0.3H, CH_2O), 3.63-3.59 (m, 1.2H uncluded (dd, J = 11.9, 3.5, 1H, CH_2OH + *diast*, m, 0.2H, CH_2OH)), 3.55-3.50 (*diast*, m, 0.2H, CH_2OH), 2.50-2.25 (br, 2H, OH + *diast*, m, 1H, CH_2), 2.20-2.10 (m, 1H, CH_2), 1.99-1.90 (m, 1H, CH_2 + *diast*, 1H, CH_2), 1.47 (*diast*, s, 0.7H, CH_3), 1.43 (s, 3H, CH_3), 1.37 (*diast*, s, 0.7H, CH_3), 1.35 (s, 3H, CH_3)

^{13}C NMR (125 MHz, $CDCl_3 + D_2O$) δ 109.8 (1C, Cq + *diast*), 87.6 (*diast*, 1C, CH), 87.3 (1C, CH), 79.3 (1C, CH), 79.3 (*diast*, 1C, CH), 77.6 (*diast*, 1C, CH), 77.1 (1C, CH), 74.1 (1C, CHOH), 72.9 (*diast*, 1C, CHOH), 66.7 (*diast*, 1C, CH_2), 66.6 (1C, CH_2), 63.3 (1C, CH_2), 63.1 (*diast*, 1C, CH_2), 36.3 (1C, CH_2), 35.3 (*diast*, 1C, CH_2), 26.3 (*diast*, 1C, CH_3), 26.2 (1C, CH_3), 25.1 (1C, CH_3), 25.0 (*diast*, 1C, CH_3)

To 30 mg of the mixture of diastereoisomeric acetonides, 3 mL of pentane was added. The solution was warmed at 40 °C and DCM was added until complete dissolution of the solid. Pentane was added until the solution become turbid (2 drops). The solution was allowed to reach rt and the solvent was slowly evaporated for 2 days. The crystals obtained were washed with cold cyclohexane then cold Et_2O to remove the non crystallized diastereoisomer. The washed crystals were dissolved in Et_2O (1 mL) and the solvent was slowly evaporated in 2 days to give pure crystals of the major acetonide **5** (10 mg).

Single cristal :

^{1}H NMR (500 MHz, $CDCl_3 + D_2O$) δ Cq is mising, 4.45-4.40 (m, 1H, CHOH), 4.35-4.30 (m, 1H, CH), 4.30-4.20 (m, 1H, CH), 4.10 (dd, J = 8.5, 6.9, 1H, CH_2O), 3.98-3.90 (m, 1H, CH), 3.76 (dd, J = 12, 3.3, 1H, CH_2OH), 3.69 (dd, J = 8.5, 6.1, 1H, CH_2O), 3.59 (dd, J = 11.9, 3.4, 1H, CH_2OH), 2.22-2.10 (m,1H, CH_2), 1.99-1.90 (m,1H, CH_2), 1.44 (s, 3H, CH_3), 1.36 (s, 3H, CH_3)

^{13}C NMR (125 MHz, $CDCl_3 + D_2O$) δ 88.6 (1C, CH), 80.6 (1C, CH), 78.3 (1C, CH), 75.3 (1C, CHOH), 67.9 (1C, CH_2), 64.9 (1C, CH_2), 37.5 (1C, CH_2), 27.4 (1C, CH_3), 26.3 (1C, CH_3)

ES+: 241.11 $[M+Na]^+$

HRMS (ESI+): calculated for $C_{10}H_{18}O_5Na$ $[M+Na]^+$ 241.1052, found 241.1055

IR (ν_{max} cm^{-1}): 3297, 2987, 2904, 2879, 1373, 1207, 1052, 995, 850

$[\alpha]_D^{20}$($CHCl_3$)= + 33.1 (c = 5.23)

X-ray diffraction:

Crystal evaluation and data collection were done at the ID29 beamline of the European Synchrotron Radiation Facility in Grenoble, France, with monochromatic X-rays (λ = 0.72932 Å) and a Pilatus-6M detector. Data reduction was performed using the XDS package.[4] The structure was solved using the *ab initio* iterative charge flipping method with parameters described elsewhere,[5] with use of the SUPERFLIP program.[6] The structure was then refined using full-matrix least-squares procedures as implemented in CRYSTALS[7] on all independent reflections with $I > 2\sigma(I)$. The hydrogen atoms were refined with riding constraints, except the two hydrogens involved in hydrogen bonding, which were refined with geometric restraints. The absolute configuration of the crystal structure was arbitrarily assigned, since the used wavelength does not permit to refine the Flack parameter for a structure that contains only carbon, hydrogen and oxygen atoms.

Crystal data for acetonide **5**: Formula= $C_{10}H_{18}O_5$, Moiety Formula= $C_{10}H_{18}O_5$, T = 100 K, M_r = 218.24 g.mol^{-1}, crystal size = 0.030x0.050x0.110 mm^3, monoclinic, space group $C2$, a = 8.8970(13), b = 6.7680(15), c = 18.304(2) Å, α = 90°, β = 90.365(6)°, γ = 90°, V = 1102.2(3) Å^3, Z = 4, ρ_{calcd}= 1.315 gcm^{-3}, μ= 0.105 mm^{-1}, θ_{max}= 31.361°, 10485 reflections measured, 1593 unique, 1589 with $I>2\sigma(I)$, R_{int}=0.059, 143 refined parameters, $R_1(I>2\sigma(I))$ =0.05210, $wR_2(I>2\sigma(I))$ =0.0491 R_1(all data) =0.0521, wR_2 (all data) =0.0491, GOF = 1.0434, Δρ(min/max)= -0.55/ 0.42 eÅ^{-3}.
CCDC-1403506 contains the supplementary crystallographic data for this paper. These data can be obtained free of charge via www.ccdc.cam. ac.uk/conts/retrieving.html (or from the Cambridge Crystallographic Data Centre, 12, Union Road, Cambridge CB21EZ, UK; fax: (+44)1223-336-033; or deposit@ccdc.cam.ac.uk).

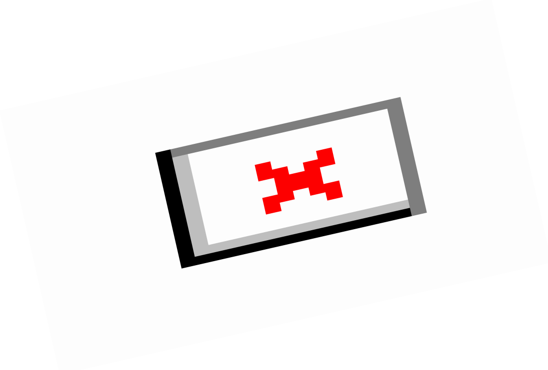


Figure 1 : Ortep representation of the structure of molecule acetonide 5 with ellipsoids at the 50% probability level

Preparation of mono protected TBS-ether 6:

To a solution of the previously prepared acetonide **5** and its diastereoisomer (600 mg, 2.77 mmol, 1 eq) in DCM (30 mL) at 0 °C were successively added TBSCl (1.67 g, 11.09 mmol, 4 eq), imidazole (1.51 g, 22.19 mmol, 8 eq) and DMAP (33 mg, 0.27 mmol, 0.1 eq), and the reaction was allowed to stir at rt overnight. A saturated NH_4Cl aqueous solution (ca. 20 mL) was added, the organic phase was separated and the aqueous phase extracted with DCM (3 x 15 mL). The combined organic phases were washed with $NaCl_{(Sat)}$ (20 mL), dried over $MgSO_4$ and concentrated under vacuum. The crude product was purified by column chromatography (pentane/EtOAc: 95:5) to afford the intermediate diTBS-acetonide (1.09 g, 88%) as a colourless oil.

R_f = 0.45 (Cyclohexane/ EtOAc: 90/10)
^{1}H NMR (500 MHz, $CDCl_3$) δ 4.35-4.34 (*diast*, m, 0.2H, CH), 4.32-4.30 (m, 1H, CH), 4.17-4.12 (*diast*, m, 0.2H, CH), 4.10-4.04 (m, 2H, CH_2O, CH + *diast*, 0.3H, CH_2O), 3.97-3.93 (m, 1H, CH + *diast*, 0.2H, CH), 3.87-3.83 (m, 1.2H, included (dd, J = 7.9, 5.9, 1H, CH_2O + *diast*, m, 0.2H, CH_2O)), 3.83-3.81 (*diast*, m, 0.2H, CH), 3.80-3.77 (m, 1H, CH), 3.62-3.55 (m, 1.2H, included (dd, J = 10.8, 4.1, 1H, CH_2O + *diast*, m, 0.2H, CH_2O), 3.52-3.48 (*diast*, m, 0.2H, CH_2O), 3.46 (dd, J = 10.8, 5.8, 1H, CH_2O), 2.30-2.20 (*diast*, m, 0.2H, CH_2), 1.96-1.89 (m, 1H, CH_2 + *diast*, 0.2H, CH_2), 1.88-1.83 (m, 1H, CH_2), 1.41 (s, 3H, CH_3), 1.39 (*diast*, s, 0.8H, CH_3), 1.34 (s, 3H, CH_3), 1.33 (*diast*, s, 0.8H, CH_3), 0.90-1.10 (m, 18H, $CH_{3(TBS)}$+ *diast*, 5H, $CH_{3(TBS)}$), 0.10-0.00 (m, 12H, CH_3Si + *diast*, 3H, CH_3Si)
^{13}C NMR (125 MHz, $CDCl_3$) δ 109.4 (1C, Cq), 109.2 (*diast*, 1C, Cq), 87.6 (1C, CH), 87.2 (*diast*, 1C, CH), 80.1 (*diast*, 1C, CH), 79.2 (1C, CH), 78.4 (1C, CH), 78.3 (*diast*, 1C, CH), 73.5 (1C, CH), 73.4

(*diast*, 1C, CH), 67.9 (*diast*, 1C, CH_2O), 67.5 (1C, CH_2O), 63.6 (1C, CH_2O), 63.5 (*diast*, 1C, CH_2O), 38.2 (1C, CH_2), 38.0 (*diast*, 1C, CH_2), 26.9 (*diast*, 1C, CH_3), 26.7 (1C, CH_3), 26.1 (3C, $CH_{3(TBS)}$), 26.0 (*diast*, 3C, $CH_{3(TBS)}$), 25.9 (3C, $CH_{3(TBS)}$), 25.8 (*diast*, 3C, $CH_{3(TBS)}$), 25.5 (1C, CH_3), 25.4 (*diast*, 1C, CH_3), 18.5 (1C, Cq + *diast*), 18.1 (1C, Cq + *diast*), -4.5 (1C, CH_3Si + *diast*), -4.6 (1C, CH_3Si + *diast*), -5.2 (1C, CH_3Si + *diast*), -5.3 (1C, CH_3Si + *diast*)
ES+: 447.30 $[M+H]^+$, 389.25 $[M+H-(CH_3)_2CO]^+$
HRMS (ESI+): calculated for $C_{22}H_{47}O_5Si_2$ $[M+H]^+$ 447.2962, found 447.2966
IR (ν_{max} cm^{-1}): 2954, 2930, 2858, 1473, 1463, 1380, 1370, 1252, 1214, 1064, 831, 774

To a solution of diTBS-acetonide (67 mg, 0.15 mmol, 1 eq) in EtOH 96% (3 mL), at 0 °C, PPTS (38 mg, 0.15 mmol, 1 eq) was added and the reaction was stirred at the same temperature for 4 days. At this temperature a saturated $NaHCO_3$ aqueous solution (ca. 2 mL) was added, the organic phase was separated and the aqueous phase extracted with EtOAc (3 x 2 mL). The combined organic phases were washed with water and $NaCl_{(Sat)}$, dried over $MgSO_4$ and concentrated under vacuum. The crude product was purified by column chromatography (pentane/EtOAc: 90:10 to 85:15) to afford the intermediate alcohol **6** (36 mg, 73%, 85 brsm) as a colourless oil, as well as starting material (10 mg).

$\mathbf{R_f}$ = 0.12 (Cyclohexane/EtOAc: 80:20)
^{1}H NMR (300 MHz, $CDCl_3$) δ 4.35-4.30 (m, 1H, CHO), 4.30-4.10 (m, 2H, CHO + *diast*, 0.5H, CH), 4.10-4.05 (m, 1H, CH_2O + *diast*, 0.4H, CH), 4.05-3.90 (*diast*, 0.2H, CH), 3.90-3.80 (m, 1H, CH + *diast*, 0.3H, CH_2O), 3.82-3.70 (*diast*, m, 0.2H, CH), 3.73-3.72 (m, 1H, CH_2O + *diast*, 0.4H, CH_2O), 3.70-3.68 (m, 1H, CH_2O), 3.56-3.50 (m, 1H, CH_2O + *diast*, 0.2H, CH_2O), 2.33-2.24 (*diast*, m, 0.2H, CH_2), 2.11-2.02 (m, 2H, CH_2, OH + *diast*, 0.2H, CH_2), 1.91-1.84 (m, 1H, CH_2), 1.43 (s, 3H, CH_3), 1.41 (*diast*, s, 0.9H, CH_3), 1.35 (s, 3H, CH_3), 1.34 (*diast*, s, 0.9H, CH_3), 0.95-0.80 (m, 9H, $CH_{3(TBS)}$ + *diast*, 3H, $CH_{3(TBS)}$), 0.10- -0.10 (m, 6H, CH_3Si + *diast*, 1.6H, CH_3Si)
^{13}C NMR (125 MHz, $CDCl_3$) δ 109.7 (1C, Cq), 87.7 (1C, CH), 85.9 (*diast*, 1C, CH), 79.4 (1C, CH), 79.3 (*diast*, 1C, CH), 78.2 (*diast*, 1C, CH), 77.4 (1C, CH), 74.0 (1C, CHOH), 72.6 (*diast*, 1C, CHOH), 67.5 (*diast*, 1C, CH_2), 66.7 (1C, CH_2), 63.4 (1C, CH_2), 62.4 (*diast*, 1C, CH_2), 38.3 (*diast*, 1C, CH_2), 36.8 (1C, CH_2), 26.9 (*diast*, 1C, CH_3), 26.2 (1C, CH_3), 25.9 (3C, $CH_{3(TBS)}$), 25.8 (*diast*, 3C, $CH_{3(TBS)}$), 25.4 (*diast*, 1C, CH_3), 25.1 (1C, CH_3), 18.1 (1C, Cq + *diast*), -4.5 (1C, CH_3Si + *diast*), -4.7 (1C, CH_3Si + *diast*)
ES+: 355.19 $[M+Na]^+$, 275.17 $[M+H-(CH_3)_2CO]^+$
HRMS (ESI+): calculated for $C_{16}H_{32}O_5NaSi$ $[M+Na]^+$ 355.1917, found 355.1916
IR (ν_{max} cm^{-1}): 3456, 2930, 2859, 1473, 1463, 1371, 1252, 1213, 1104, 1058, 832, 775

Preparation of Ester 7:

To a solution of **6** (200 mg, 0.60 mmol, 1 eq) in DCM (12 mL) and H_2O (24 µL) were added $NaHCO_3$ solid (152mg, 1.80 mmol, 3 eq) and DMP (5.12 mL, 2.4 mmol, 0.47 M in DCM, 4 eq) and the reaction was stirred at rt overnight. The reaction was quenched by adding a 10% $NaHCO_3/Na_2S_2O_3$ (10 mL, 1/1, v/v) aqueous solution, and stirred for 2 h. The aqueous phase was extracted with DCM (3 x 10 mL), washed with water, dried over $MgSO_4$ and concentrated under vacuum to afford the corresponding aldehyde, which is used for the next step without purification.

To a -78 °C cold suspension of the phosphonium salt (1.2 g, 2.4 mmol, 4 eq) in THF (30 mL) was slowly added NaHMDS (1.11 mL, 2.2 mmol, 2 M in THF, 3.7 eq). The solution coloured in orange. After 1 h stirring, a solution of the previously prepared aldehyde in THF (20 mL) was slowly added at -

78 °C by cannulation, and the reaction was allowed to reach rt, then left to stir for 24 h. The reaction was quenched by addition of water (20 mL) and Et_2O (20 mL), extracted with Et_2O (3 x 10 mL), washed with brine, dried over $MgSO_4$ and concentrated under vacuum. The crude product was purified by chromatography column (pentane/EtOAc: 90/10) to afford the corresponding alkene THF derivative (211 mg, 75%) as colourless oil.

R_f = 0.45 (Cyclohexane/EtOAc: 5/5)
^{1}H NMR (500 MHz, $CDCl_3$) δ 5.60-5.51 (m, 1H, CH=), 5.29-5.22 (m, 1H, CH=), 4.49-4.39 (*diast*, m, 0.03H, CH + dd, *J* = 9.6, 4.3, 1H, CH), 4.15-4.02 (m, 4H, CH_2O, CH + *diast*, 0.6H, CH_2O, CH), 4.01-3.97 (m, 2H, CH + *diast*, 0.3H, CH), 3.89-3.79 (m, 1.15H, included (*diast*, dd, *J* = 8.1, 5.3, 0.15H, CH_2O + dd, *J* = 8.3, 5.6, 1H, CH_2O)), 2.31-2.24 (m, 2.5H, included (t, *J* = 7.5, 2H, CH_2CO + *diast*, m, 0.5H, CH_2)), 2.18-2.11 (m, 1H, CH_2 + *diast*, 0.3H, CH_2), 2.11-1.92 (m, 3H, CH_2 + *diast*, 0.3H, CH_2), 1.68-1.57 (m, 2H, CH_2 + *diast*, 0.3H, CH_2), 1.43-1.27 (m, 10H, CH_3, CH_2 + *diast*, 1.5H, CH_3, CH_2), 1.25 (t, *J* = 7.1, 3H, CH_3 + *diast*, 0.6H, CH_3), 0.90-0.84 (m, 9H, $CH_{3(TBS)}$ + *diast*, 1.7H, $CH_{3(TBS)}$), 0.10- -0.05 (m, 6H, CH_3Si + *diast*, 1H, CH_3Si)
^{13}C NMR (75 MHz, $CDCl_3$) δ 173.9 (1C, Cq + *diast*), 134.3 (*diast*, 1C, CH=), 134.2 (1C, CH=), 128.5 (1C, CH=), 129.3 (*diast*, 1C, CH=), 109.5 (1C, Cq + *diast*), 82.2 (1C, CH), 81.2 (*diast*, 1C, CH), 78.9 (1C, CH), 78.6 (*diast*, 1C, CH), 78.5 (*diast*, 1C, CH), 78.4 (1C, CH), 77.7 (*diast*, 1C, CH), 77.5 (1C, CH), 67.9 (*diast*, 1C, CH_2O), 67.6 (1C, CH_2O), 60.5 (*diast*, 1C, CH_2O), 60.3 (1C, CH_2O), 38.3 (*diast*, 1C, CH_2), 38.2 (1C, CH_2), 34.4 (1C, CH_2COO), 29.5 (1C, CH_2), 29.5 (*diast*, 1C, CH_2), 29.0 (1C, CH_2), 28.9 (*diast*, 1C, CH_2), 27.9 (1C, CH_2), 27.8 (*diast*, 1C, CH_2), 26.9 (*diast*, 1C, CH_3), 26.7 (1C, CH_3), 25.9 (3C, $CH_{3(TBS)}$), 25.8 (*diast*, 3C, $CH_{3(TBS)}$), 25.4 (1C, CH_3), 25.4 (*diast*, 1C, CH_3), 25.0 (*diast*, 1C, CH_2), 25.0 (1C, CH_2), 18.2 (1C, $Cq_{(TBS)}$ + *diast*), 14.4 (1C, CH_3), 14.3 (*diast*, 1C, CH_3), -4.6 (1C, CH_3Si + *diast*), -4.7 (1C, CH_3Si + *diast)*
ES+: 471.31 $[M+H]^+$, 488.34 $[M+NH_4]^+$
HRMS (ESI+): calculated for $C_{25}H_{47}O_6Si$ $[M+H]^+$ 471.3142, found 471.3141
IR (ν_{max} cm^{-1}): 2931, 2858, 1736, 1463, 1370, 1252, 1211, 1150, 1110, 1061, 834, 776

To a solution of the previously synthesized alkene THF derivative (200 mg, 0.3 mmol, 1 eq) in 20 mL of EtOH, Pd/C 5% (50 mg, 120 mg/mmol) was added. Under H_2 atmosphere, the suspension was stirred at rt overnight. The mixture was filtered over a Celite® pad and rinsed with EtOH. The solvents were removed under reduced pressure and the crude reaction mixture was purified by flash chromatography (pentane/EtOAc: 90/10) to obtain ester **7** as colourless oil (175 mg, 87%).

R_f = 0.21 (Cyclohexane/EtOAc: 9/1)
^{1}H NMR (500 MHz, $CDCl_3$) δ 4.15-4.09 (m, 2.4H, included (q, *J* = 7.2, 2H, CH_2O + *diast*, 0.4H, CH_2O)), 4.09-4.04 (m, 1.1H, included (dd, *J* = 8.3, 6.4, 1H, CH_2O + *diast*, m, 0.1H, CH_2O)), 4.04-3.98 (m, 1H, CH + *diast*, 0.3H, CH), 3.98-3.90 (m, 2H, CH + *diast*, 1H, CH), 3.90-3.87 (*diast*, m, 0.17H, CH), 3.86-3.79 (m, 1.17H, included (dd, *J* = 8.3, 5.9, 1H, CH_2O + *diast*, m, 0.17H, CH_2O)), 3.77-3.71 (*diast*, m, 0.3H, CH), 3.70-3.63 (*diast*, m, 0.16H, CH), 3.63-3.58 (m, 1H, CH), 2.29-2.23 (m, 2.6H, included (t, *J* = 7.5, 2H, CH_2CO + *diast*, m, 0.6H, CH_2CO)), 1.89 (dd, *J* = 7.2, 5.0, 2H, CH_2) 1.87-1.81 (*diast*, m, 0.4H, CH_2), 1.65-1.55 (m, 2H, CH_2 + *diast*, 0.6H, CH_2), 1.45-1.18 (m, 22.9H included (s, 3H, CH_3 + s, 3H, CH_3 + m, 10H, CH_2 + *diast*, 3.9H, CH_3, CH_2 + t, *J* = 7.2, 3H, CH_3)), 1.00-0.80 (m, 9H, $CH_{3(TBS)}$ + *diast*, 1.8H, $CH_{3(TBS)}$), 0.10- -0.10 (m, 6H, CH_3Si + *diast*, 1.1H, CH_3Si)
^{13}C NMR (125 MHz, $CDCl_3$) δ 174.0 (1C, Cq + *diast*), 109.4 (1C, Cq + *diast*), 86.6 (1C, CH), 85.7 (*diast*, 1C, CH), 78.6 (1C, CH), 78.5 (1C, CH), 78.4 (*diast*, 1C, CH), 78.4 (*diast*, 1C, CH), 76.6 (*diast*,

1C, CH), 76.4 (1C, CH), 67.8 (*diast*, 1C, CH_2O), 67.5 (1C, CH_2O), 65.9 (*diast*, 1C, CH_2O), 60.3 (1C, CH_2O), 38.1 (1C, CH_2), 38.1 (*diast*, 1C, CH_2), 34.6 (1C, CH_2COO), 34.0 (1C, CH_2), 33.6 (*diast*, 1C, CH_2), 29.6 (*diast*, 1C, CH_2), 29.5 (1C, CH_2), 29.3 (1C, CH_2), 29.2 (*diast*, 1C, CH_2), 29.2 (1C, CH_2), 26.9 (*diast*, 1C, CH_3), 26.7 (1C, CH_3), 26.1 (1C, CH_2), 26.0 (*diast*, 1C, CH_2), 25.9 (1C, CH_3), 25.8 (*diast*, 1C, CH_3), 25.5 (3C, $CH_{3(TBS)}$), 25.1 (*diast*, 3C, $CH_{3(TBS)}$), 25.0 (1C, CH_2 + *diast*), 18.1 (1C, $Cq_{(TBS)}$ + *diast*), 14.4 (1C, CH_3 + *diast*), -4.4 (1C, CH_3Si + *diast*), -4.6 (1C, CH_3Si + *diast*)
ES+: 473.33 $[M+H]^+$, 490.36 $[M+NH_4]^+$
HRMS (ESI+): calculated for $C_{25}H_{49}O_6Si$ $[M+H]^+$ 473.3298, found 473.3299
IR (ν_{max} cm^{-1}): 2930, 2858, 1737, 1464, 1370, 1251, 10212, 1179, 1156, 1108, 1062, 834, 775

Preparation of 1-methyl-1-cyclopropyl hydroxyl derivative 8:

To a solution of ester **7** (85 mg, 0.18 mmol, 1 eq) in 3 mL of DCM was added freshly distilled *i*-Pr_2NEt (61 µL, 0.36 mmol, 1 eq) and was cooled to 0 °C. Then, freshly distilled TMSOTf (55 µL, 0.31mmol, 1.7 eq) was added dropwise and the mixture was warmed to rt and heated over reflux overnight Then, the mixture was diluted with hexane, filtered through a plug of neutral alumina (activity III; 6% of water), rinsed with hexane/EtOAc: 90/10, and concentrated under reduced pressure.

To the resulting enol ether, in 2 mL of Et_2O, was added Et_2Zn (539 µL, 0.54 mmol, 1 M in decane, 3 eq) and distilled CH_2I_2 (73 µL, 0.90 mmol, 5 eq) over 10 min. The mixture was stirred overnight at rt. The reaction mixture was diluted with 10 mL of 1 N NaOH, extracted with Et_2O (3 x 7 mL), dried, and concentrated under reduced pressure.

The crude oil was treated with 2 mL of MeOH and 7 mg of K_2CO_3 for 15 min at rt to remove the remaining TMS group. The mixture was quenched with saturated NH_4Cl, extracted with Et_2O (3 x 2 mL), dried over $MgSO_4$ and concentrated under reduced pressure. Purification by flash chromatography (pentane/EtOAc: 90/10) gave 1-methyl-1-cyclopropyl (MCP) hydroxyl derivative **8** free from its diastereoisomer (54 mg, 62%) as a colorless oil.

$\mathbf{R_f}$ = 0.22 (Cyclohexane/EtOAc: 80/20)
^{1}H NMR (300 MHz, $CDCl_3$) δ 4.17-4.00 (m, 3H, included (1H, CH + q, *J* = 7.1, 2H, CH_2OCO)), 3.94-3.86 (m, 1H, CH), 3.71-3.66 (m, 2H, CH_2OH), 3.66-3.55 (m, 2H, CH), 2.40 (br, 1H, OH), 2.27 (t, *J* = 7.1, 2H, CH_2CO_2), 1.87-1.79 (m, 2H, CH_2), 1.64-1.54 (m, 3H, CH_2), 1.45-1.20 (m, 15H, included (s, 3H, CH_3 + m, 9H, CH_2 + t, *J* = 7.1, 3H, CH_3)), 0.90-0.83 (m, 11H, included (s, 9H, $CH_{3(TBS)}$ + m, 2H, $CH_{2(MCP)}$)), 0.47-0.36 (m, 2H, $CH_{2(MCP)}$), 0.10- -0.03 (m, 6H, CH_3Si)
^{13}C NMR (75 MHz, $CDCl_3$) δ 174.0 (1C, Cq), 86.8 (1C, CH), 79.5 (1C, CH), 78.6 (1C, CH), 76.4 (1C, CH), 63.8 (1C, CH_2OH), 60.3 (1C, CH_2OCO), 58.4 (1C, $Cq_{(MCP)}$), 38.2 (1C, CH_2), 34.5 (1C, CH_2), 34.2 (1C, CH_2), 29.6 (1C, CH_2), 29.3 (1C, CH_2), 29.2 (1C, CH_2), 26.2 (1C, CH_2), 25.0 (3C, $CH_{3(TBS)}$), 25.1 (1C, CH_2), 22.7 (1C, $CH_{3(MCP)}$), 18.1 (1C, $Cq_{(TBS)}$), 14.5 (1C, $CH_{2(MCP)}$), 14.4 (1C, CH_3), 13.5 (1C, $CH_{2(MCP)}$), -4.4 (1C, CH_3Si), -4.6 (1C, CH_3Si)
ES+ : 509.33 $[M+Na]^+$
HRMS (ESI+): calculated for $C_{26}H_{50}O_6SiNa$ $[M+Na]^+$ 509.3274, found 509.3275
IR (ν_{max} cm^{-1}): 3446, 2929, 2857, 1737, 1464, 1384, 1255, 1179, 1109, 1047, 834, 775
$[\alpha]_D^{20}$($CHCl_3$)= +26.4 (c=10)

Preparation of enone 9:

To a solution of alcohol **8** (54 mg, 0.11 mmol, 1 eq) in DCM (2 mL) and 2 drops of water was added, at 0 °C, the DMP (353 µL, 0.17 mmol, 0.47 M in DCM, 1.5 eq). After stirring for 1.5 h, the reaction was quenched by adding a 10% $NaHCO_3/Na_2S_2O_3$ (2 mL, 1/1, v/v) aqueous solution, and stirred for 30 min. The aqueous phase was extracted with DCM (3 x 1 mL), washed with brine, dried over $MgSO_4$ and concentrated under vacuum to afford the crude aldehyde.

To a solution of the crude aldehyde in 1 mL of DCM was added the 1-(triphenylphosphoranylidene)-butan-2-one (96 mg, 0.28 mmol, 2.6 eq). After stirring at rt for 64 h, the reaction was quenched by addition of water (1 mL), extracted with DCM (3 x 1 mL), dried over $MgSO_4$ and concentrated under vacuum. The crude product was purified by chromatography column (pentane/EtOAc: 90/10) to afford enone **9** (30 mg, 51%) as a colourless oil.

R_f = 0.44 (Cyclohexane/EtOAc: 80/20)
^{1}H NMR (500 MHz, $CDCl_3$) δ 6.76 (dd, *J* = 16.1, 5.6, 1H, CH=), 6.30 (dd, *J* = 16.1, 1.5, 1H, CH=), 4.23-4.19 (m, 1H, CH), 4.11 (q, *J* = 7.2, 2H, CH_2OCO), 4.09-4.02 (m, 1H, CH), 3.87-3.82 (m, 1H, CH), 3.63-3.53 (m, 1H, CH), 2.58 (q, *J* = 7.3, 2H, CH_2CO), 2.27 (t, *J* = 7.5, 2H, CH_2CO_2), 1.86-1.78 (m, 1H, CH_2), 1.64-1.54 (m, 3H, CH_2 + 1H, CH_2), 1.40-1.28 (m, 9H, CH_2 + s, 3H, CH_3), 1.24 (t, *J* = 7.2, 3H, CH_3), 1.09 (t, *J* = 7.3, 3H, CH_3), 0.95-0.90 (m, 1H, $CH_{2(MCP)}$), 0.85 (s, 9H, $CH_{3(TBS)}$), 0.83-0.76 (m, 1H, $CH_{2(MCP)}$), 0.42-0.31 (m, 2H, $CH_{2(MCP)}$), 0.02 (m, 6H, CH_3Si)
^{13}C NMR (125 MHz, $CDCl_3$) δ 200.9 (1C, Cq), 174.0 (1C, Cq), 145.3 (1C, CH=), 130.0 (1C, CH=), 86.6 (1C, CH), 79.4 (1C, CH), 78.8 (1C, CH), 76.2 (1C, CH), 60.3 (1C, CH_2OCO), 59.7 (1C, $Cq_{(MCP)}$), 35.5 (1C, CH_2), 34.5 (1C, CH_2CO), 34.0 (1C, CH_2), 33.9 (1C, CH_2CO), 29.6 (1C, CH_2), 29.3 (1C, CH_2), 29.2 (1C, CH_2), 26.2 (1C, CH_2), 25.9 (3C, $CH_{3(TBS)}$), 25.1 (1C, CH_2), 22.6 (1C, $CH_{3(MCP)}$), 18.1 (1C, $Cq_{(TBS)}$), 14.4 (1C, $CH_{2(MCP)}$), 14.4 (1C, CH_3), 13.4 (1C, $CH_{2(MCP)}$), 8.1 (1C, CH_3), -4.4 (1C, CH_3Si), -4.6 (1C, CH_3Si)
ES+ : 539.38 $[M+H]^+$, 556.40 $[M+NH_4]^+$, 467.32 $[(M+H)- C_4H_7O]^+$
HRMS (ESI+): calculated for $C_{30}H_{55}O_6Si$ $[M+H]^+$ 539.3768, found 539.3769
IR (ν_{max} cm^{-1}): 2931, 2857, 1735, 1702, 1679, 1463, 1374, 1254, 1190, 1108, 1041, 834, 775

$[\alpha]_D^{20}$($CHCl_3$)= +26.9 (c = 10)

Preparation of enone 10:

To a solution of the methylcyclopropyl protected alcohol **9** (30 mg, 0.06 mmol, 1 eq) in 0.5 mL of THF and 50 µL of water was added NBS (12 mg, 0.07 mmol, 1.2 eq). After stirring 1 h at rt, the mixture was quenched with saturated $Na_2S_2O_3$, extracted with Et_2O (3 x 0.5 mL) washed with brine, dried over $MgSO_4$, and concentrated under vacuum. The crude product was purified by chromatography column (pentane/EtOAc: 80/20) to afford the allylic alcohol **10** (23 mg, 85%) as colourless oil.

R_f = 0.26 (Cyclohexane/EtOAc: 80/20)
^{1}H NMR (300 MHz, $CDCl_3$) δ 6.70 (dd, *J* = 15.9, 4.1, 1H, CH=), 6.42 (dd, *J* = 15.9, 1.9, 1H, CH=), 4.60-4.50 (m, 1H, CH), 4.27-4.17 (m, 1H, CH), 4.11 (q, *J* = 7.1, 2H, CH_2OCO), 3.95-3.87 (m, 1H, CH), 3.73-3.67 (m, 1H, CH), 2.58 (q, *J* = 7.3, 2H, CH_2CO), 2.35 (d, *J* = 2.2, 1H, OH), 2.28 (t, *J* = 7.4, 2H, CH_2CO_2), 1.97-1.86 (m, 1H, CH_2), 1.65-1.49 (m, 3H, CH_2), 1.49-1.20 (m, 13H, included (10H, CH_2 + t, *J* = 7.1, 3H, CH_3)), 1.10 (t, *J* = 7.3, 3H, CH_3), 0.90-0.80 (m, 9H, $CH_{3(TBS)}$), 0.10- -0.10 (m, 6H, CH_3Si)

^{13}C NMR (75 MHz, $CDCl_3$) δ 200.7 (1C, Cq), 174.0 (1C, Cq), 145.3 (1C, CH=), 129.1 (1C, CH=), 87.0 (1C, CH), 79.9 (1C, CH), 76.6 (1C, CH), 71.1 (1C, CH), 60.3 (1C, CH_2OCO), 34.5 (1C, CH_2), 34.4 (1C, CH_2CO), 34.2 (1C, CH_2), 34.0 (1C, CH_2CO), 29.5 (1C, CH_2), 29.3 (1C, CH_2), 29.2 (1C, CH_2), 26.1 (1C, CH_2), 25.9 (3C, $CH_{3(TBS)}$), 25.1 (1C, CH_2), 18.1 (1C, $Cq_{(TBS)}$), 14.4 (1C, CH_3), 8.1 (1C, CH_3), -4.4 (1C, CH_3Si), -4.6 (1C, CH_3Si)
ES+ : 485.33 $[M+H]^+$, 507.31 $[M+Na]^+$
HRMS (ESI+): calculated for $C_{26}H_{49}O_6Si$ $[M+H]^+$ 485.3298, found 485.3304
IR (ν_{max} cm^{-1}): 3443, 2932, 2855, 2361, 1737, 1676, 1460, 1371, 1256, 1194, 1113, 1059, 836, 777
$[\alpha]_D^{20}$($CHCl_3$)= +30.1 (c = 9)

Preparation of *ent*-16-(*RS*)-13-*epi*-ST-Δ^{14}-9-PhytoF 1:

To a solution of enone **10** (18 mg, 0.04 mmol, 1 eq) in MeOH (1.8 mL) was added $CeCl_3.7H_2O$ (14 mg, 0.04 mmol, 1.01 eq), the reaction was cooled to 0 °C and $NaBH_4$ (1 mg, 0.03 mmol, 0.7 eq) was added. After 1 h stirring at rt., water (ca. 1 mL) was added and the aqueous phase was extracted with EtOAc (3 x 1 mL). The combined organic phases were washed with brine, dried over $MgSO_4$ and concentrated under vacuum. The crude product was purified by flash chromatography (pentane/ EtOAc: 80/20) to give the enediol compound (16 mg, 89%) as colourless oil.

R_f = 0.35 (Cyclohexane/ EtOAc: 50/50)
^{1}H NMR (500 MHz, $CDCl_3$) δ 5.80 (ddd, *J* = 17.1, 11.3, 6.3, 1H, CH= in addition with a shift of 1.4Hz linked to the *epimer*), 5.60 (ddd, *J* = 15.6, 5.7, 3.6, 1H, CH= in addition with a shift of 1.2Hz linked to the *epimer*), 4.37-4.30 (m, 1H, CH), 4.16-4.09 (m, 1H, CH + q, *J* = 7.5, 2H, CH_2OCO), 4.09-4.02 (m, 1H, CH), 3.93-3.87 (m, 1H, CH), 3.71-3.64 (m, 1H, CH), 2.27 (t, *J* = 7.50, 2H, CH_2CO_2), 2.21 (br, 1H, OH), 2.00-1.92 (m, 1H, CH_2), 1.64-1.50 (m, 5H, CH_2), 1.50-1.30 (m, 10H, CH_2 + t, *J* = 7.15, 3H, CH_3), 0.93-0.90 (td, *J* = 7.45, 3.15, 3H, CH_3), 0.90-0.80 (m, 9H, CH_3), 0.10- -0.10 (m, 6H, CH_3Si)
^{13}C NMR (125 MHz, $CDCl_3$) δ 174.0 (1C, Cq), 135.2 (1C, CH=), 135.1 (*epi*, 1C, CH=), 128.6 (1C, CH=), 128.5 (*epi*, 1C, CH=), 86.8 (1C, CH), 80.7 (1C, CH), 80.6 (*epi*, 1C, CH), 76.6 (1C, CH), 76.6 (*epi*, 1C, CH), 73.8 (1C, CH), 73.8 (*epi*, 1C, CH), 71.8 (1C, CH), 71.7 (*epi*, 1C, CH), 60.3 (1C, CH_2OCO), 34.5 (1C, CH_2CO), 34.2 (1C, CH_2), 34.1 (*epi*, 1C, CH_2), 34.0 (1C, CH_2), 34.0 (*epi*, 1C, CH_2), 30.2 (1C, CH_2), 30.1 (*epi*, 1C, CH_2), 29.5 (1C, CH_2), 29.3 (1C, CH_2), 29.2 (1C, CH_2), 26.1 (1C, CH_2), 25.9 (3C, $CH_{3(TBS)}$), 25.1 (1C, CH_2), 18.1 (1C, $Cq_{(TBS)}$), 14.4 (1C, CH_3), 9.8 (1C, CH_3), -4.4 (1C, CH_3Si), -4.6 (1C, CH_3Si)
ES+: 509.33 $[M+Na]^+$, 504.34 $[M+NH_4]^+$
HRMS (ESI+): calculated for $C_{26}H_{50}O_6SiNa$ $[M+Na]^+$ 509.3274, found 509.3275
IR (ν_{max} cm^{-1}): 3416, 2931, 1737, 1463, 1250, 1112, 1056, 969, 837, 776
$[\alpha]_D^{20}$($CHCl_3$)= +30.6 (c = 7.85)

A solution of TBAF (1 M/THF, 241 µL, 0.24 mmol, 7.6 eq) was added to the solution of enediol (15.5 mg, 0.03 mmol) in 0.5 mL of THF. The reaction was allowed to stir at rt for 3 h. $CaCO_3$ (46 mg), DOWEX-50W resin (16 mg) and MeOH (1 mL) were added, the mixture was allowed to stir at rt for 1 h and was filtrated through a pad of Celite® and concentrated under vacuum. The crude product was obtained and the triol, in mixture with transesterified methylated product (7/3 Ethyl/Methyl ratio) (11.3 mg) was directly put in the next step.

R_f = 0.47 (DCM/MeOH: 9/1)

To a solution the triol in THF (1 mL) and water (1 mL), $LiOH.H_2O$ (7 mg, 0.17 mmol, 6 eq) was added and the reaction was allowed to stir at rt overnight. At 0 °C, the reaction was quenched by slow addition of an aqueous solution of HCl (1 M), until acidic pH and the aqueous phase was extracted with EtOAc (4 x 2 mL), washed with brine, dried and concentrated under vacuum. The crude product was purified by column chromatography (EtOAc/MeOH: 97/3) to afford the *ent*-16-(*RS*)-13-*epi*-ST-Δ^{14}-9-PhytoF **1** (8 mg, 76% in 2 steps) as colourless oil.

R_f = 0.12 (DCM/MeOH: 9/1)
^{1}H NMR (500 MHz, MeOD) δ 5.79-5.60 (m, 2H, CH=), 4.18-4.00 (m, 2H, CHO), 4.00-3.91 (m, 2H, CHO), 3.69-3.62 (m, 1H, CHO), 2.28 (t, J = 7.4, 2H, CH_2CO), 2.03-1.95 (m, 1H, CH_2), 1.83-1.76 (m, 1H, CH_2), 1.65-1.41 (m, 7H, CH_2), 1.35 (br, 7H, CH_2), 0.92 (td, , J = 7.4, 2.1, 3H, CH_3)
^{13}C NMR (125 MHz, MeOD) δ 177.8 (1C, Cq), 136.0 (1C, CH=), 135.8 (*epi*, CH=), 131.1 (1C, CH=), 131.1 (*epi*, CH=), 87.8 (*epi*, CH), 87.7 (1C, CH), 82.3 (*epi*, CH), 82.3 (1C, CH), 76.6 (1C, CH), 74.7 (*epi*, CHOH), 74.6 (1C, CHOH), 74.5 (*epi*, CHOH), 74.4 (1C, CHOH), 36.6 (1C, CH_2), 36.4 (*epi*, CH_2), 35.1 (1C, CH_2), 35.0 (1C, CH_2), 31.1 (1C, CH_2), 31.1 (*epi*, CH_2), 30.6 (1C, CH_2), 30.4 (1C, CH_2), 30.4 (*epi*, CH_2), 30.2 (1C, CH_2), 27.1 (1C, CH_2), 26.1 (1C, CH_2), 10.2 (1C, CH_3), 10.2 (*epi*, CH_3)
ES- : 343.21 $[M-H]^-$
HRMS (ESI-): calculated for $C_{18}H_{31}O_6$ $[M-H]^-$ 343.2121, found 343.2117
$[\alpha]_D^{20}$(MeOH)= + 16.5 (c = 4)

3.2 Quantitation

Extraction of lipids from nut or seed samples

Raw, unprocessed, organic nut (Noberasco, Savona, Italy) and seed (Organic Gardens Co., Melbourne, Australia) samples (4 g), imported from local food market were obtained and finely grounded using electronic grinder (Kenwood AT320A). The ground sample was then extracted in a Soxhlet extractor with 100 mL n-hexane/ diethyl ether (80:20, v/v) for 6 hours [8]. The extracted oil was dried completely and purged using nitrogen gas and stored at -80°C until analysis.

Sample preparation for LC-MS/MS analysis

The prepared samples (n=7) were thawed at room temperature. The lipid component of the samples was extracted by Folch method with slight modifications [9,10]. Briefly, 20 mL of ice-cold chloroform/ methanol (2:1, v/v) containing 0.01% butylated hydroxytoluene was added into each samples and agitated at room temperature for 15 min by an orbital shaker. Phase separation was introduced into the samples by adding 4 mL of 0.9% NaCl solution. Samples were mixed for 10-30 seconds and then centrifuged at 3,000 x g for 10 min at 4ºC. The lower organic phase was collected into a glass vial and dried under a stream of nitrogen gas. The dried Folch extract was hydrolyzed with potassium hydroxide in methanol (1:1) with heavy labeled isotopic isoprostane (derived from arachidonic acid) internal standard, 15-F_{2t}-IsoP-d_4 and α-linolenic acid-d_{14} (Cayman Chemicals, MI, USA). After overnight hydrolysis in room temperature and neutralized by the addition of hydrochloric acid, equal volume of formic acid (pH 4.5) was further added and the samples were purified by solid phase extraction (SPE) using 60 mg mixed anionic exchange cartridges (MAX Oasis, Waters, USA) [11]. In brief, the cartridges were washed and preconditioned with 2 mL methanol and formic acid (pH 4.5) respectively. After loading the sample, it was further cleaned with 2 mL 2% ammonium hydroxide and then 2 mL hexane. The analytes were eluted with 2 mL hexane/ethanol/acetic acid mix (70/29.95/0.05), immediately dried under nitrogen and reconstituted in methanol for liquid chromatography tandem mass spectrometry (LC-MS/MS) analysis. To a part of the samples (n=3) prepared, 5 ng of *ent*-16-(*RS*)-13-*epi*-ST-Δ^{14}-9-PhytoF was added to evaluate the relative retention time of the chromatogram with the pure standard. All solvents used in the analysis were high performance liquid chromatography (HPLC) grade.

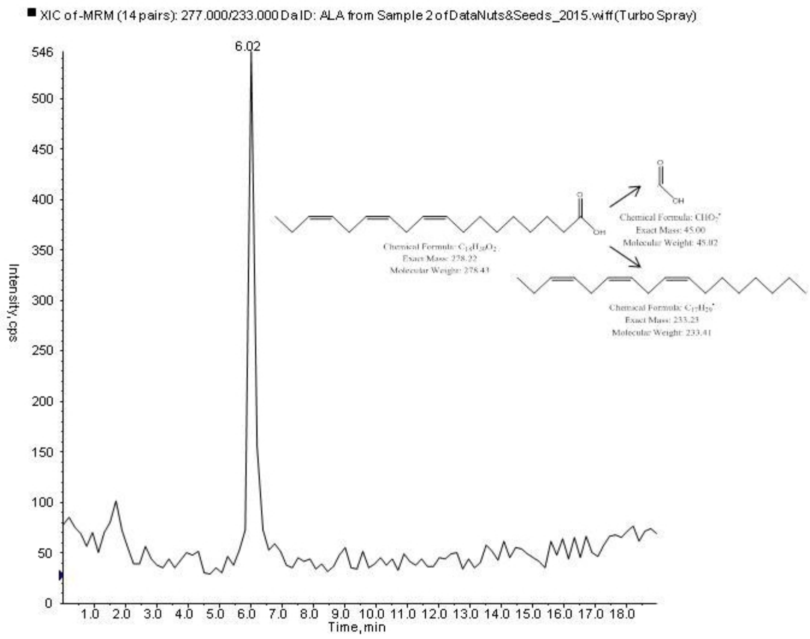
Liquid chromatography tandem mass spectrometry analysis

The α-linolenic acid and *ent*-16-(*RS*)-13-*epi*-ST-Δ^{14}-9-PhytoF were analyzed using LC-MS/MS system, 1290 Infinity LC (Agilent, USA) with Hillic C_{18} column (2.6 mm particle size, 150 x 3.0 mm, Phenomenex, CA USA) maintained at 30°C. The mobile phase consisted of 5 mM ammonium formate in 90 v/v % acetonitrile (A) and 5 mM ammonium formate in 50 v/v % acetonitrile (B) and set at a gradient elution from 100 % A for 2.5 min, followed by an increase to 100 % B from 2.5 to 10 min and then kept constant for 3 min. The flow rate was constant at 100 μl/min throughout the analysis. The triple quad mass spectrometer (Sciex Applied Biosystems, MA USA) was operated in a negative atmospheric pressure chemical ionization (APCI) mode. The spray voltage was set to -4200 V and nitrogen gas was used as the curtain gas. The analytes were detected by MS/MS using multiple reaction monitoring (MRM) and the transitions determined from synthesized *ent*-16-(*RS*)-13-*epi*-ST-Δ^{14}-9-PhytoF, and commercially available ALA, ALA and 15-F_{2t}-IsoP-d_4 (Cayman Chemicals, USA) were used (as shown in Table 1). A dwell time of 800 ms was used for each ion transition for a total scan time of 3.0 s. Quantitation of the compounds was achieved by relating the peak area with its corresponding deuterated internal standard peak ALA-d_{14}, and 15-F_{2t}-IsoP-d_4 was used to quantitate *ent*-16-(*RS*)-13-*epi*-ST-Δ^{14}-9-PhytoF. The response ratio of 15-F_{2t}-IsoP-d_4 to *ent*-16-(*RS*)-13-*epi*-ST-Δ^{14}-9-PhytoF (1:0.07) was adjusted accordingly in the calculation. The chromatogram and fragmentations of ALA and *ent*-16-(*RS*)-13-*epi*-ST-Δ^{14}-9-PhytoF are depicted in Figure 1. The relative retention time for the chromatogram of spiked samples and pure standard of *ent*-16-(*RS*)-13-*epi*-ST-Δ^{14}-9-PhytoF was 0.98 to 1.05. Concentrations of the *ent*-16-(*RS*)-13-*epi*-ST-Δ^{14}-9-PhytoF are expressed per gram of powdered nuts or seeds.

Statistical analysis

Statistical analysis was performed by GraphPad Prism version 6.0 for Macintosh (GraphPad Software, CA USA). All values are annotated as mean ± SD. One-way analysis of variance (ANOVA) and Tukey's multiple comparison tests were performed between the compounds measured in the nuts and seeds. Only significant level of $p<0.05$ was noted in the results.

(A) α-Linolenic acid



(B) *ent*-16-(*RS*)-13-*epi*-ST-Δ^{14}-9-PhytoF

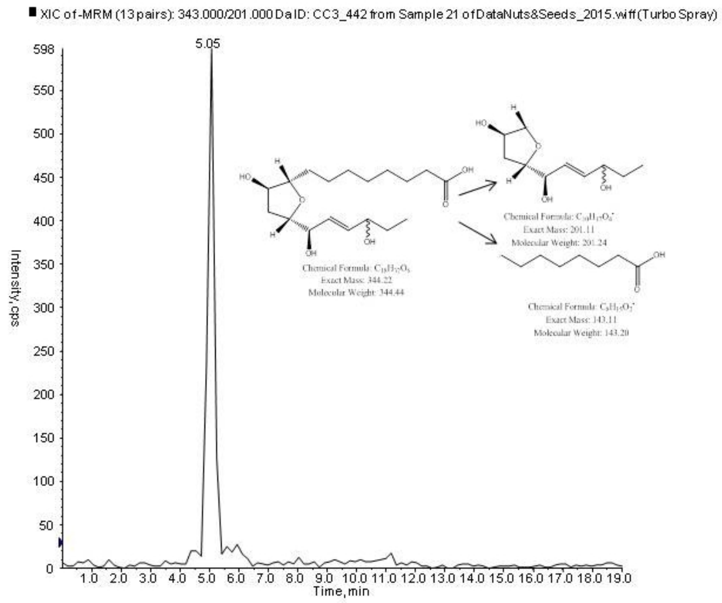


Figure 1. Mass ion transition of pure (A) α-Linolenic acid (*m/z* 277→233) and synthesized (B) *ent*-16-(*RS*)-13-*epi*-ST-Δ^{14}-9-PhytoF (*m/z* 343→201) standard. The precursor and fragmentation ions were used to detect and quantify α-Linolenic acid (ALA) and *ent*-16-(*RS*)-13-*epi*-ST-Δ^{14}-9-PhytoF by the LC-MS/MS in lipid extract of nuts and seeds. Typical chromatograms of ALA and *ent*-16-(*RS*)-13-*epi*-ST-Δ^{14}-9-PhytoF elucidated in lipid extract of flaxseed are depicted.

Table 1. Multiple reaction monitoring transition measured by the LC-MS/MS of the internal standards commercially available (α-Linolenic acid, α-Linolenic acid-d_{14}, 15-F_{2t}-IsoP-d_4) and 16(*R,S*)-13-*epi*-ST-Δ^{14}-9-PhytoF synthesized in the study.

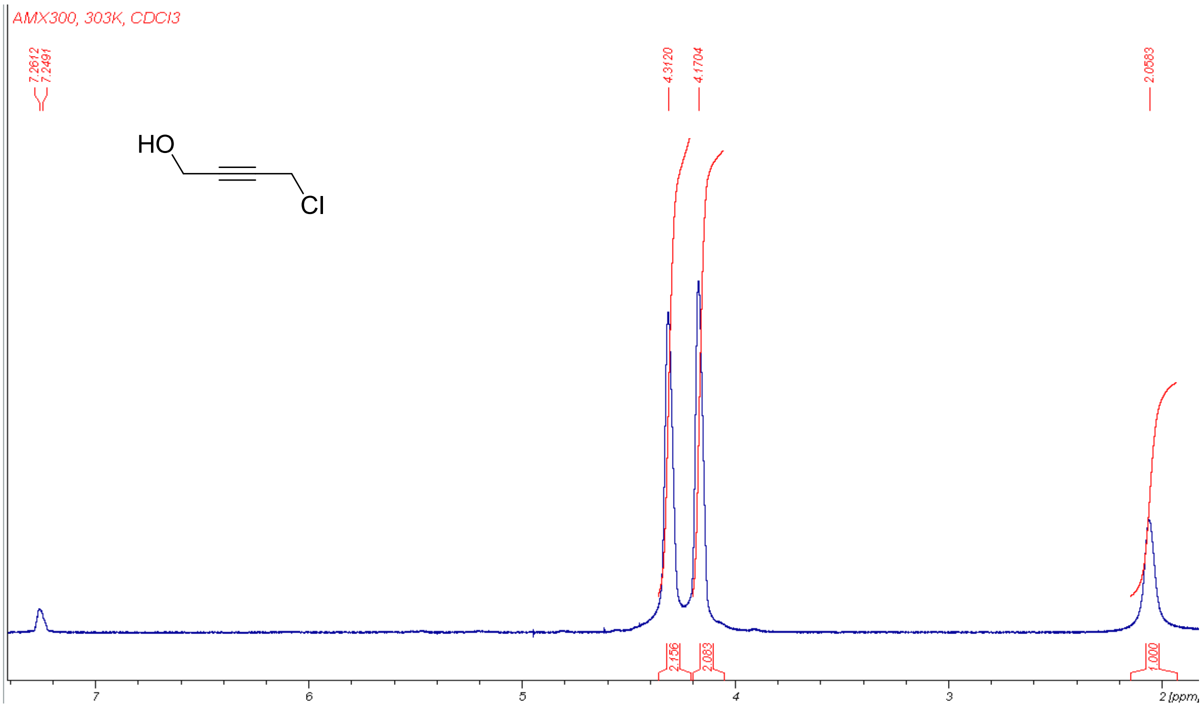
Analyte	*Q1 m/z*	*Q3 m/z*	*CE*	*DP*
α-Linolenic acid	277	233	-50	-16
α-Linolenic acid-d_{14}	291	247	-50	-26
15-F_{2t}-IsoP-d_4	357	197	-50	-25
16(*R,S*)-13-*epi*-ST-Δ^{14}-9-PhytoF	343	201	-55	-28

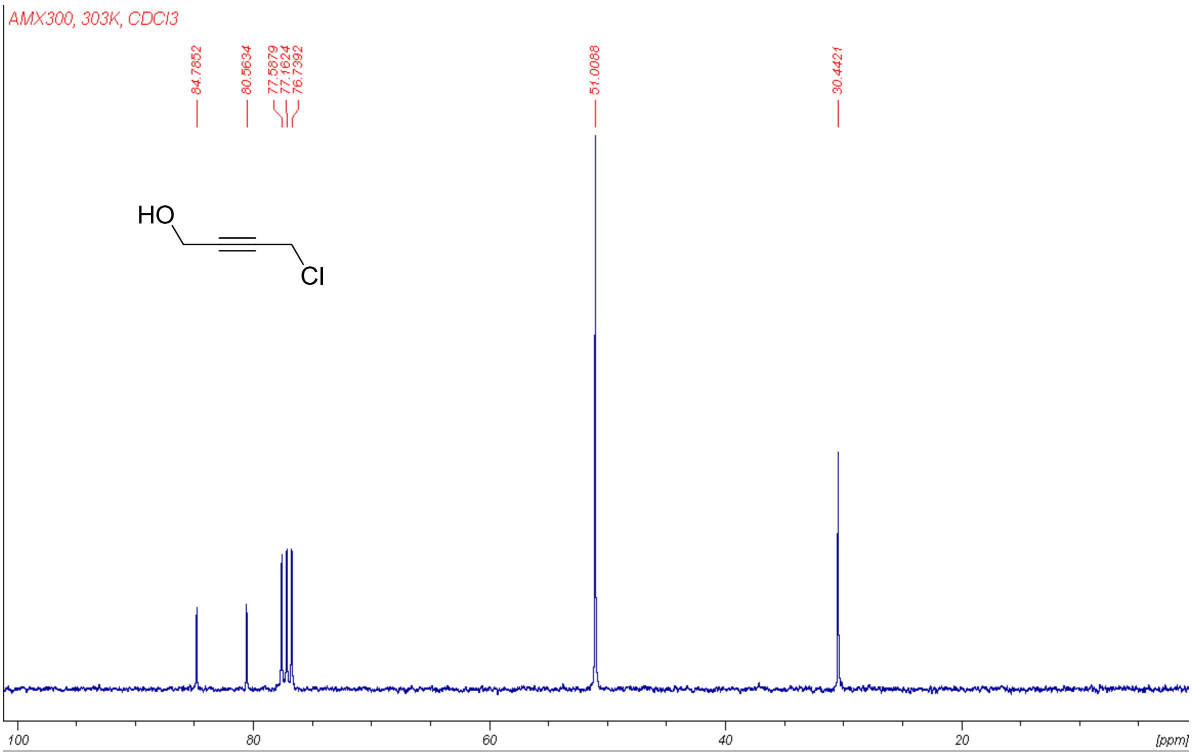
Q1 *m/z:*precursor ion; Q3 *m/z*: fragmented ion; DP: declustering potential; CE: collision energy

.

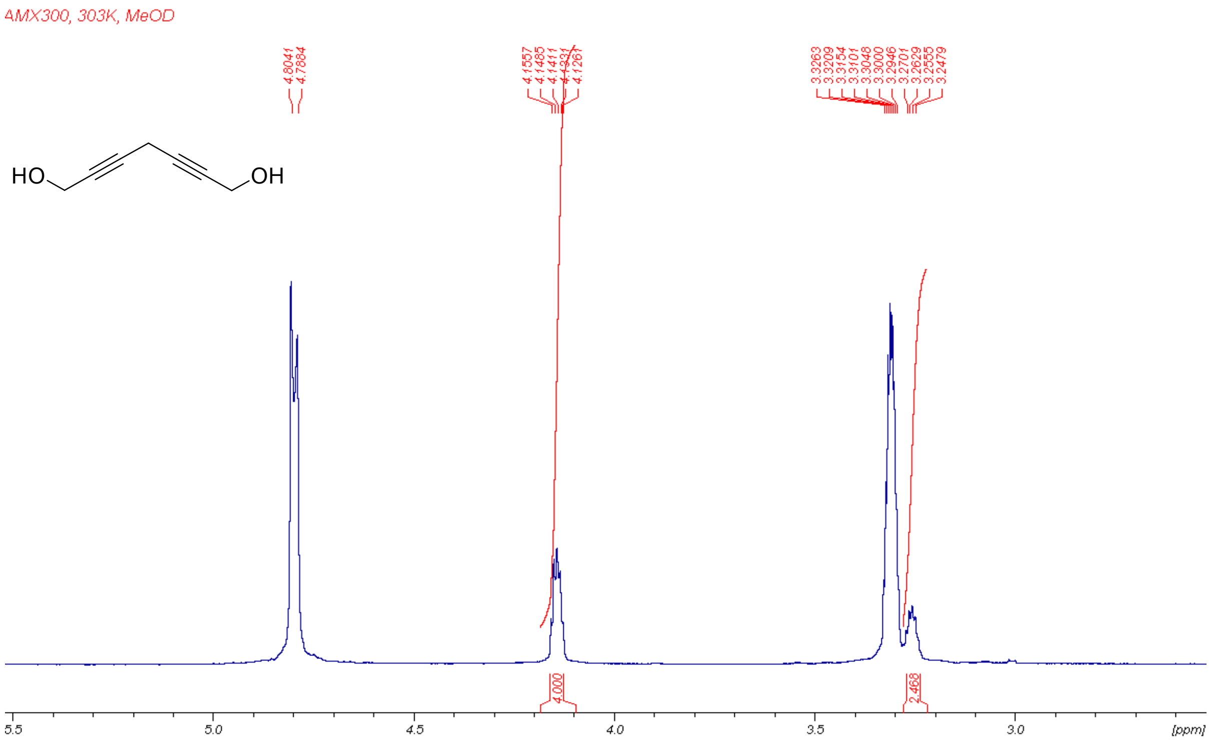
4. ¹H, ¹³C and selected 2D NMR spectra

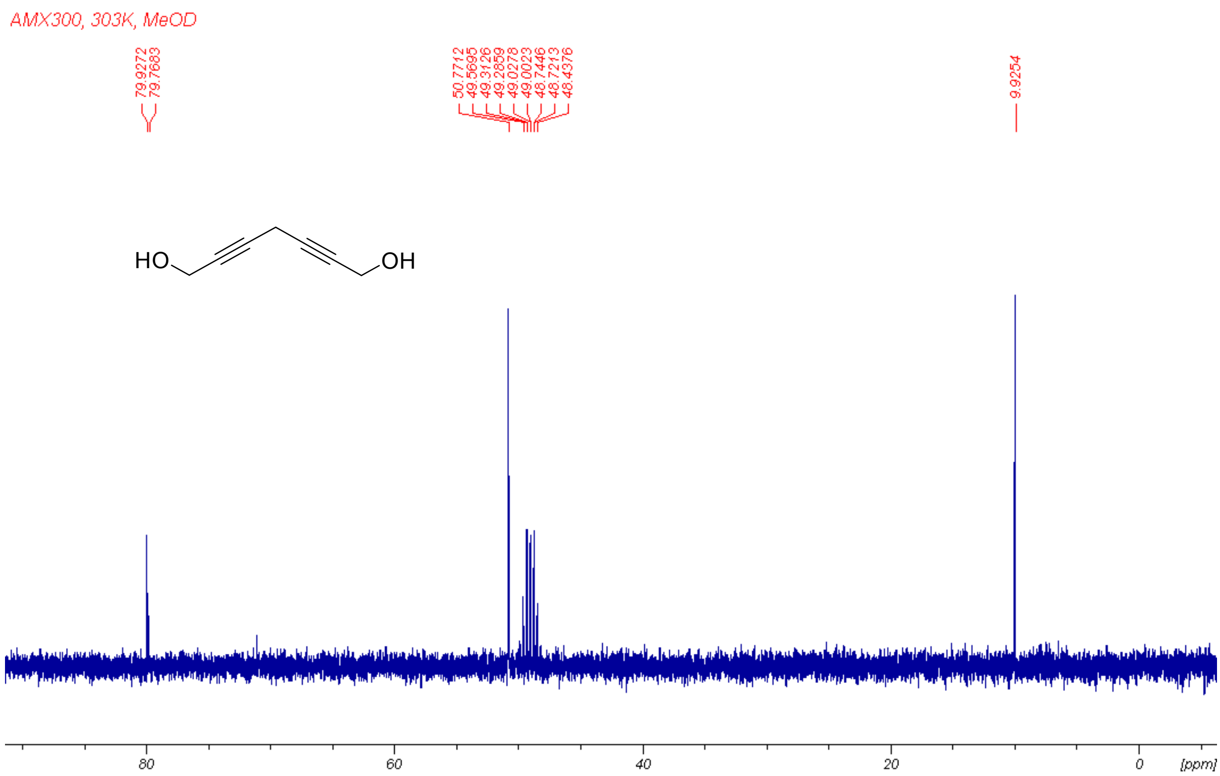
4-chlorobut-2-yn-1-ol:



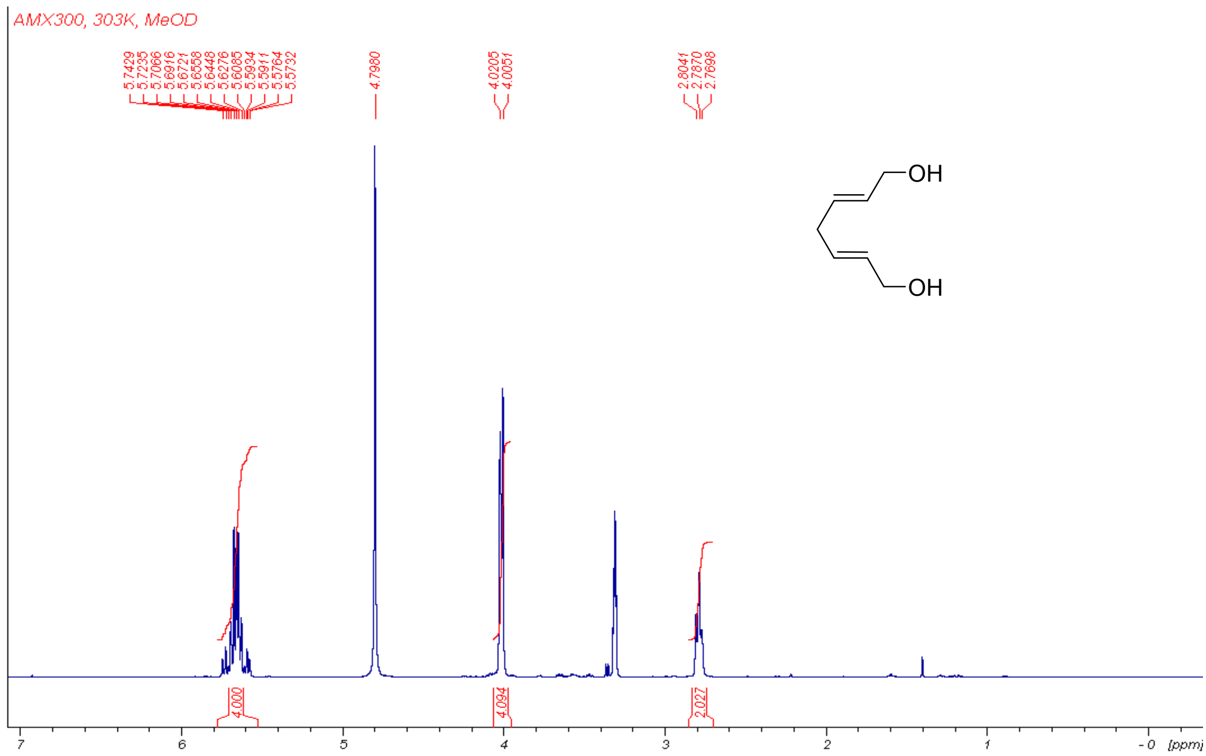


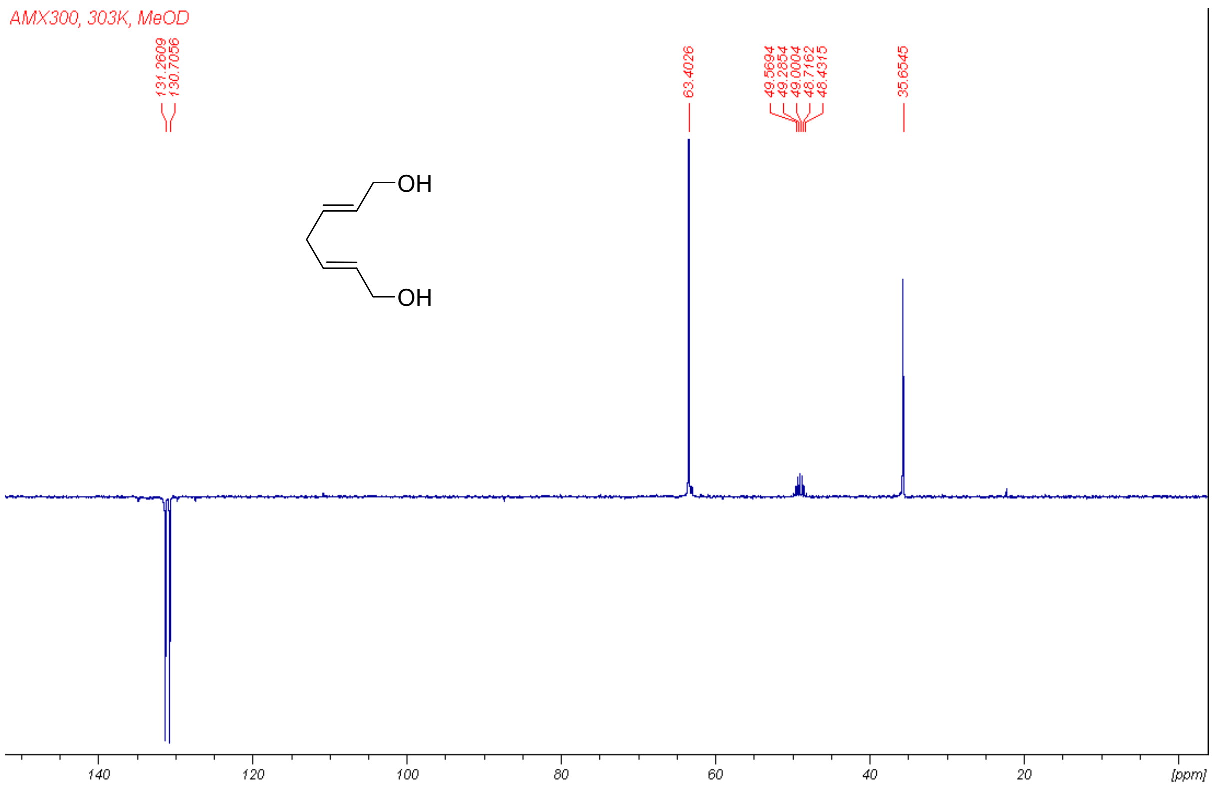
hepta-2,5-diyne-1,7-diol **4**:



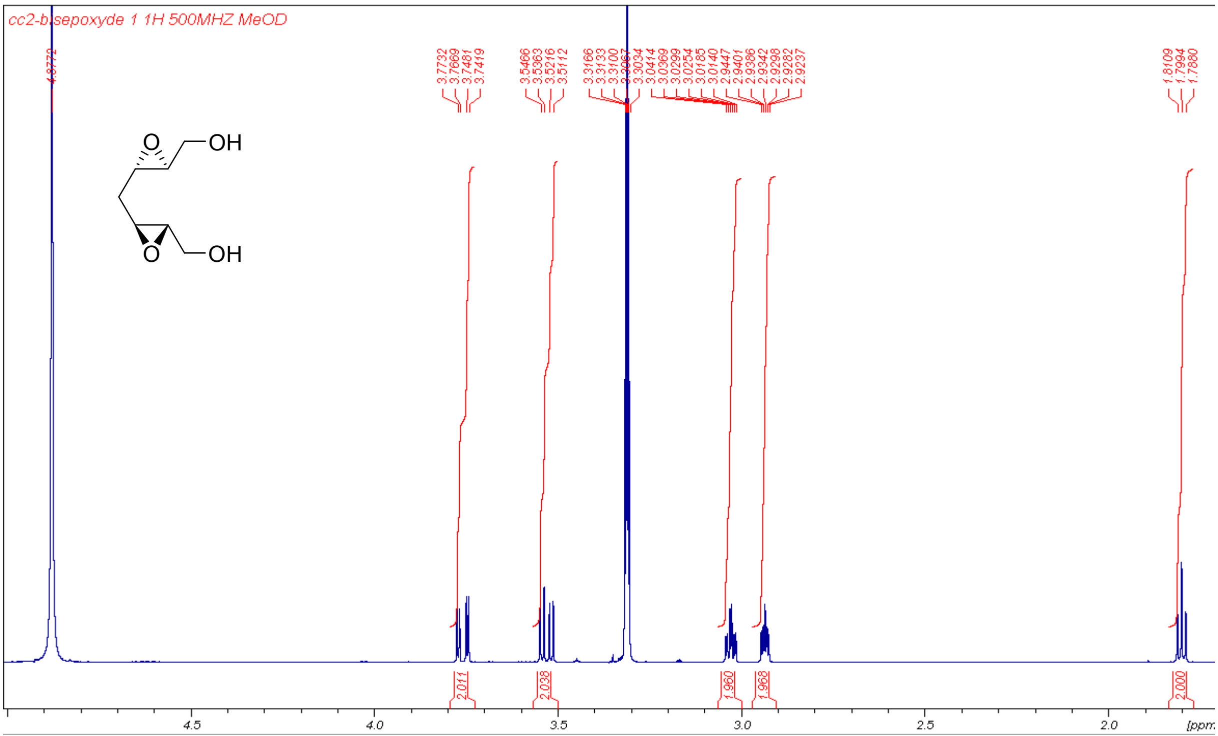


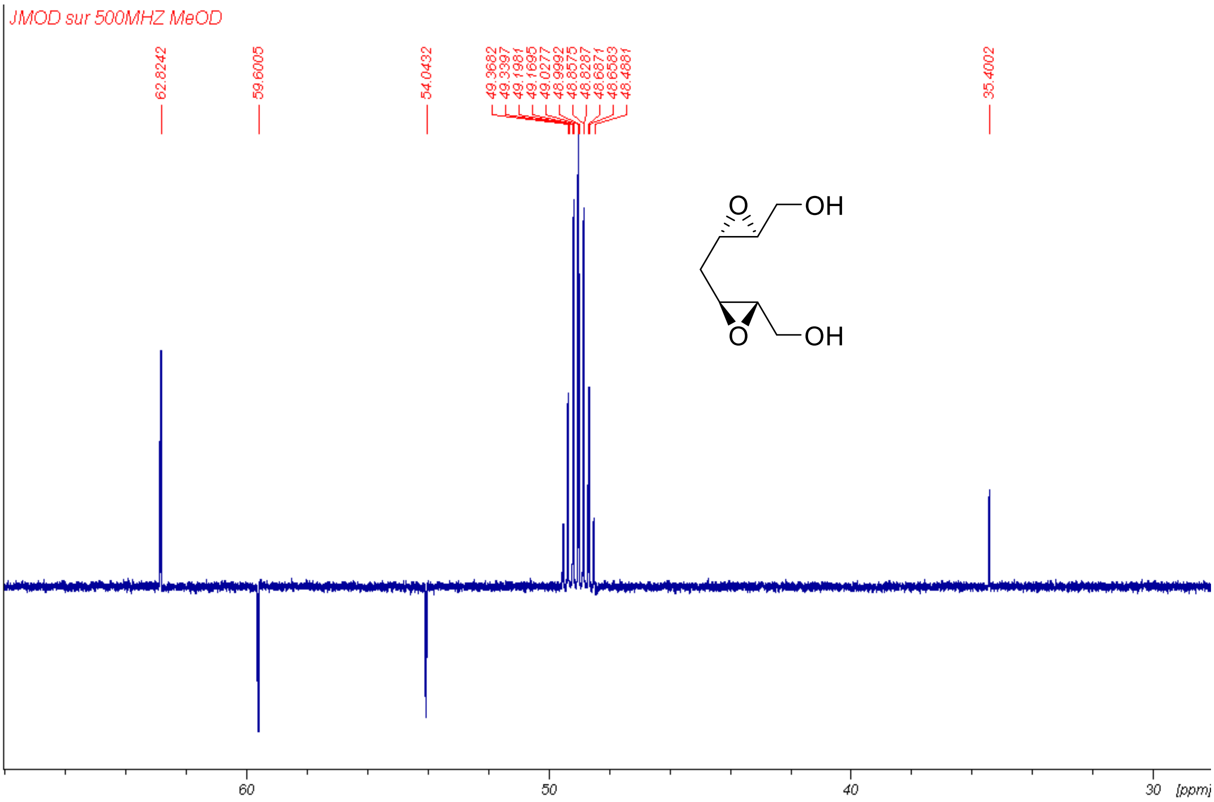
(*E,E*)-hepta-2,5-diene-1,7-diol:



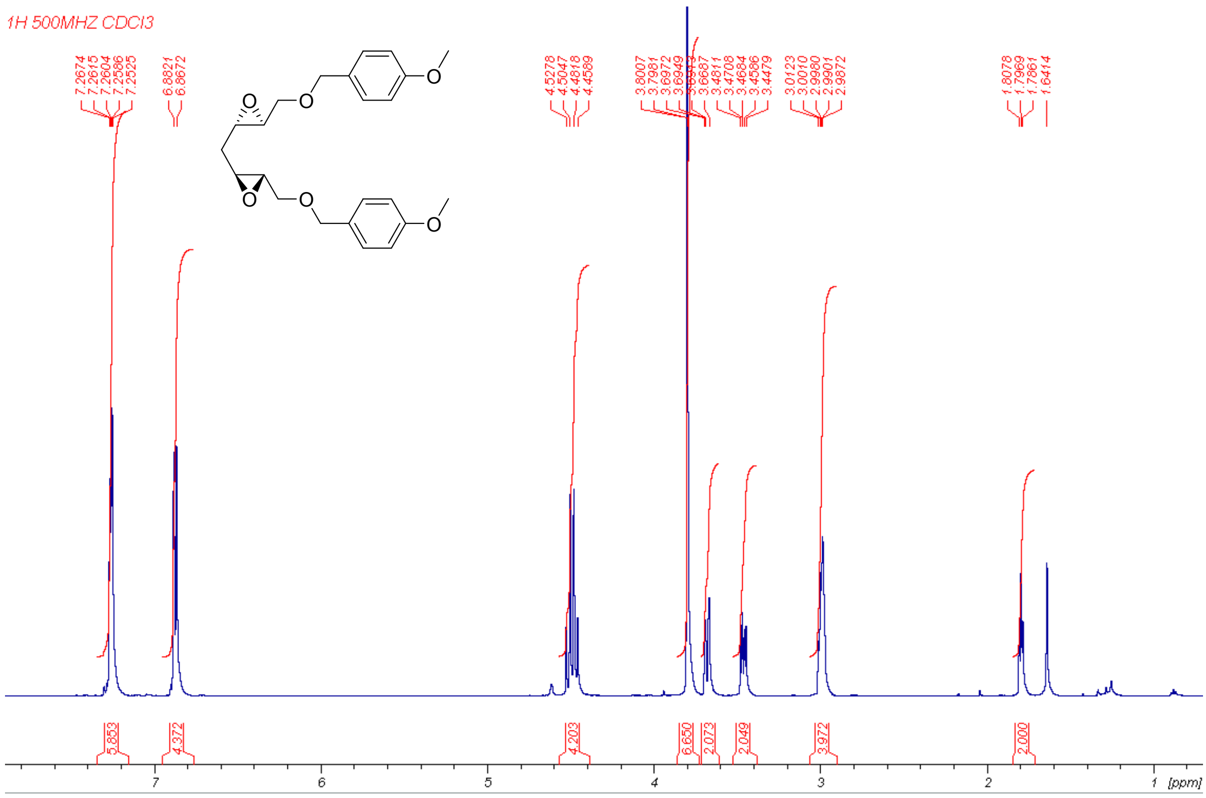


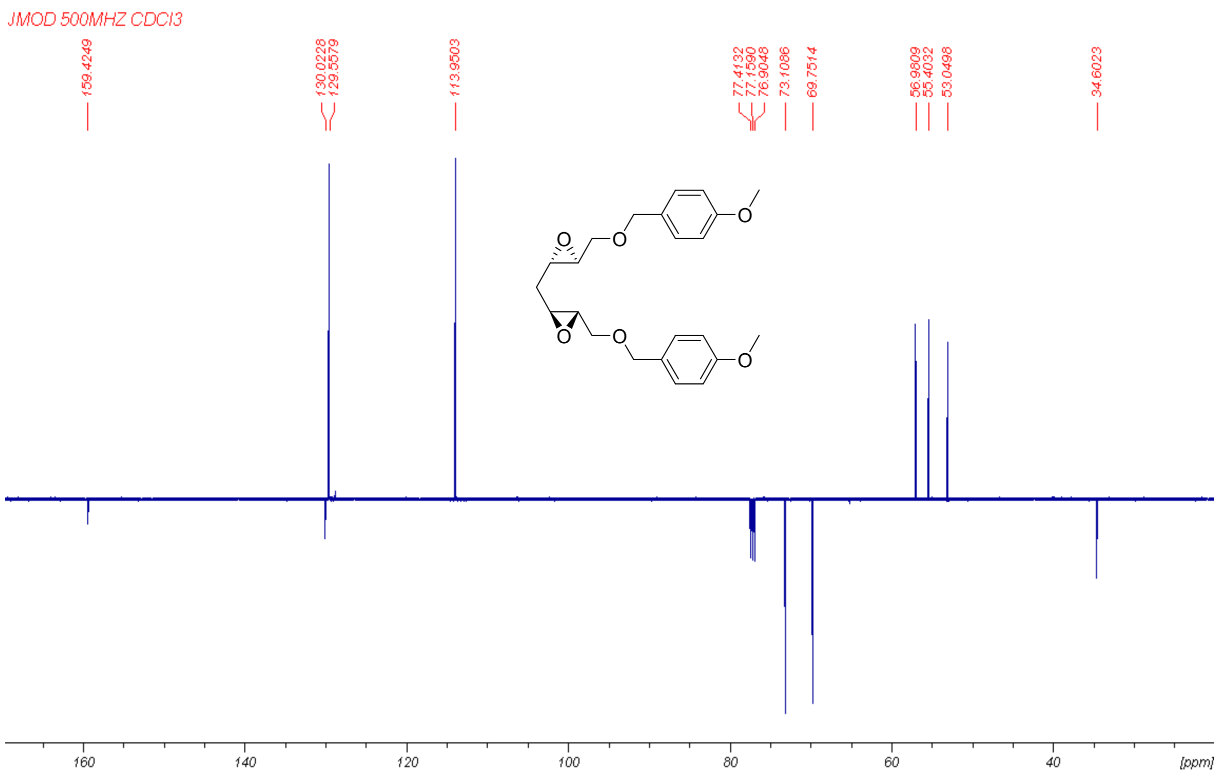
(2S,3S,5S,6S)-2,3:5,6-diepoxyheptane-1,7-diol **3**:



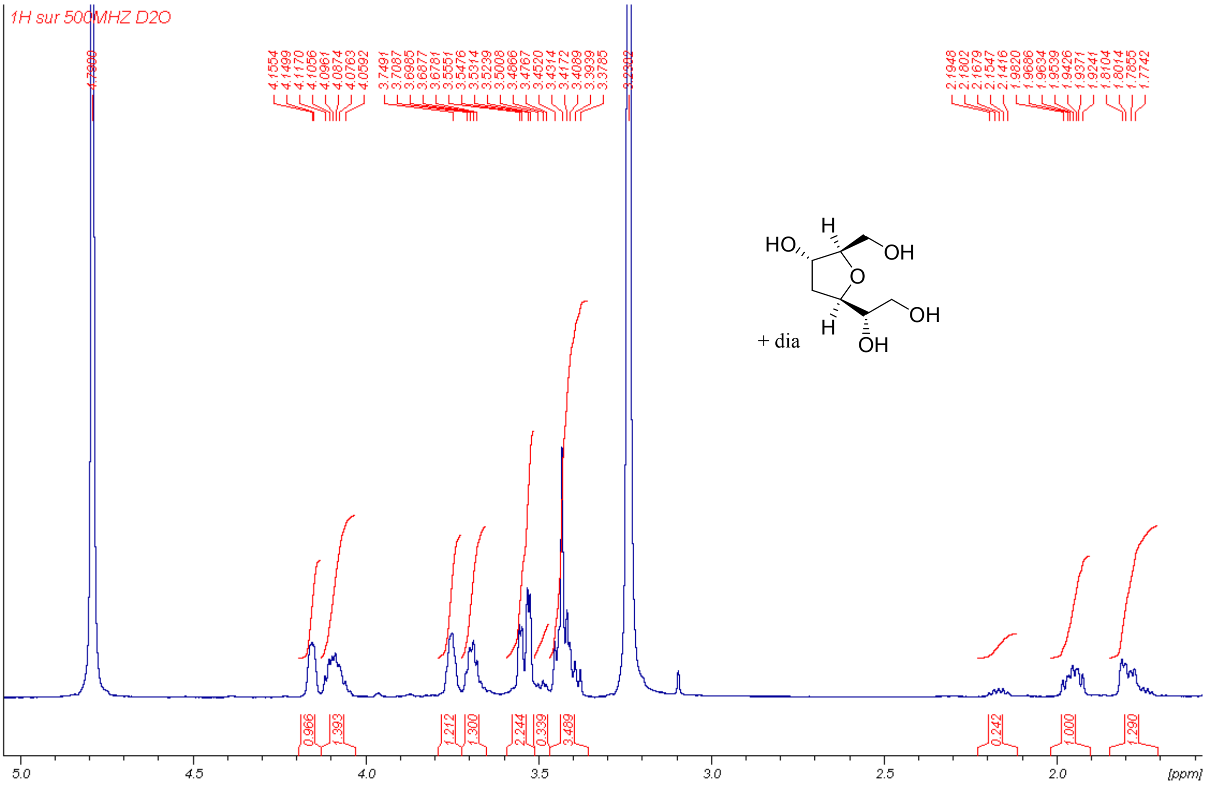


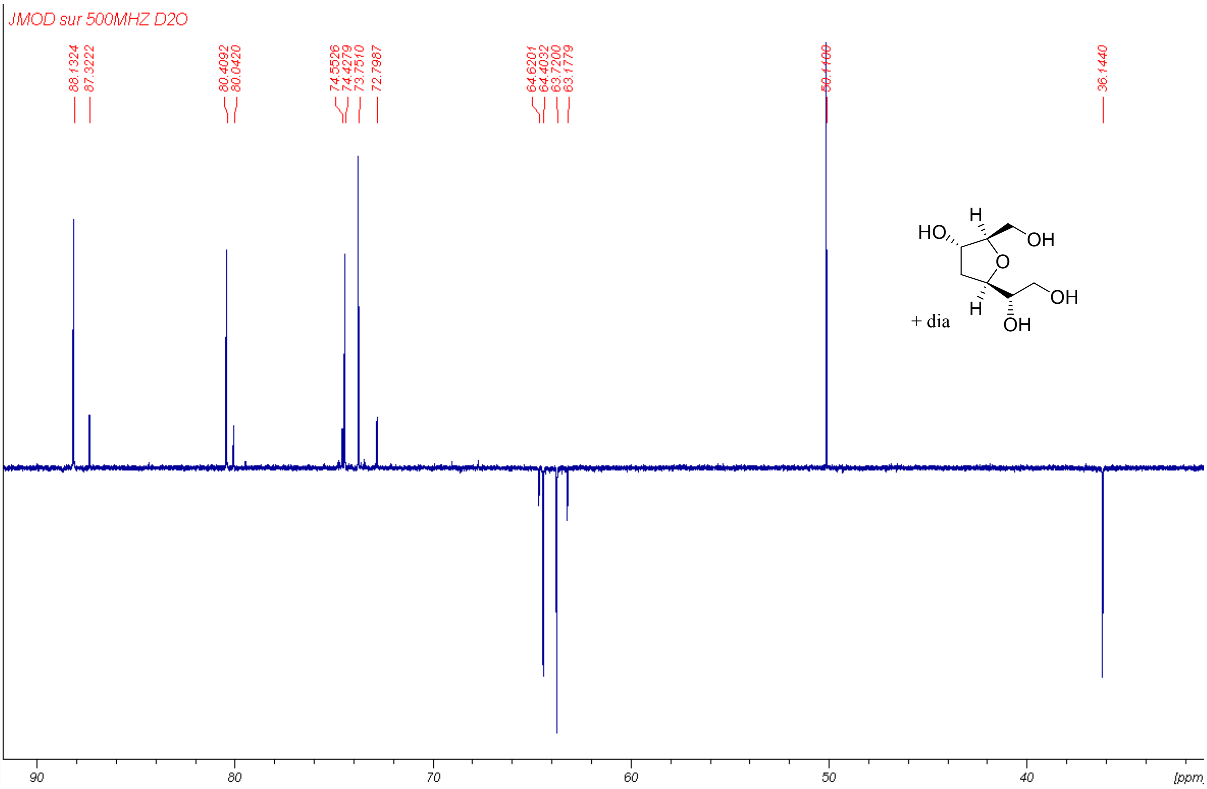
bis((2S,3S)-3-((4-methoxybenzyloxy)methyl)oxiran-2-yl)methane:



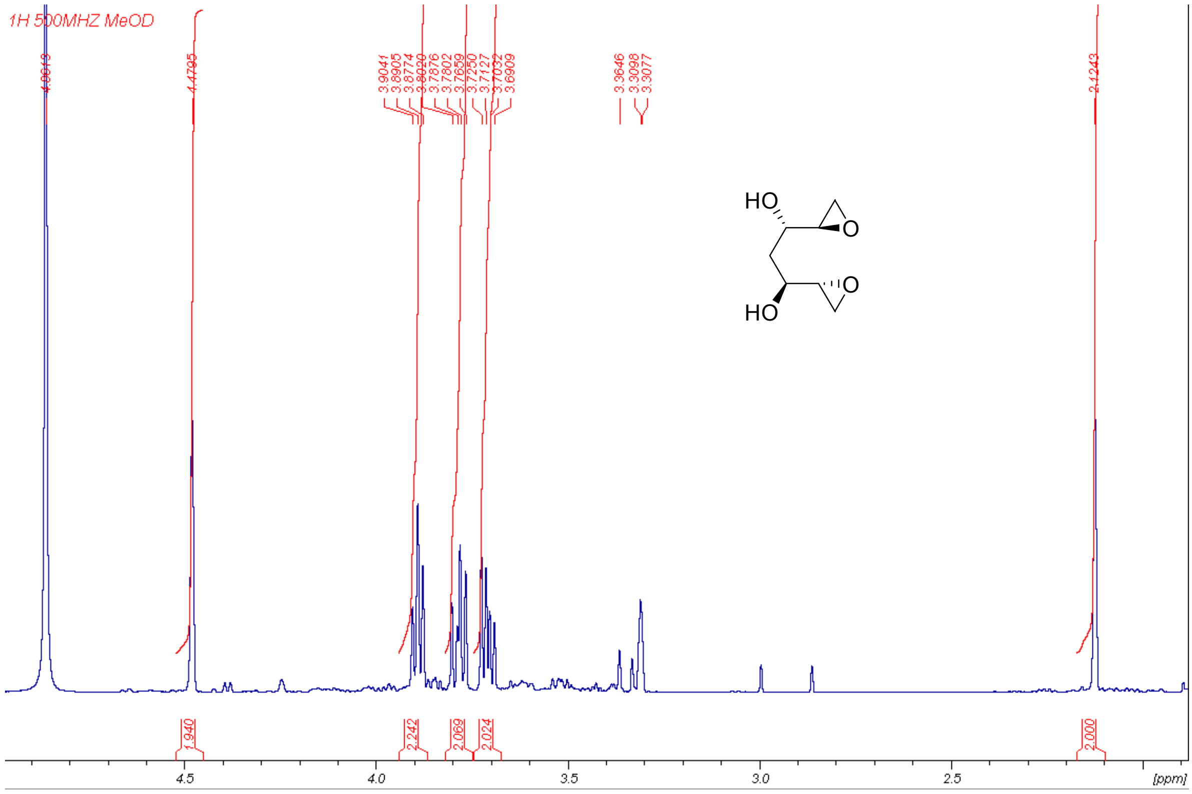


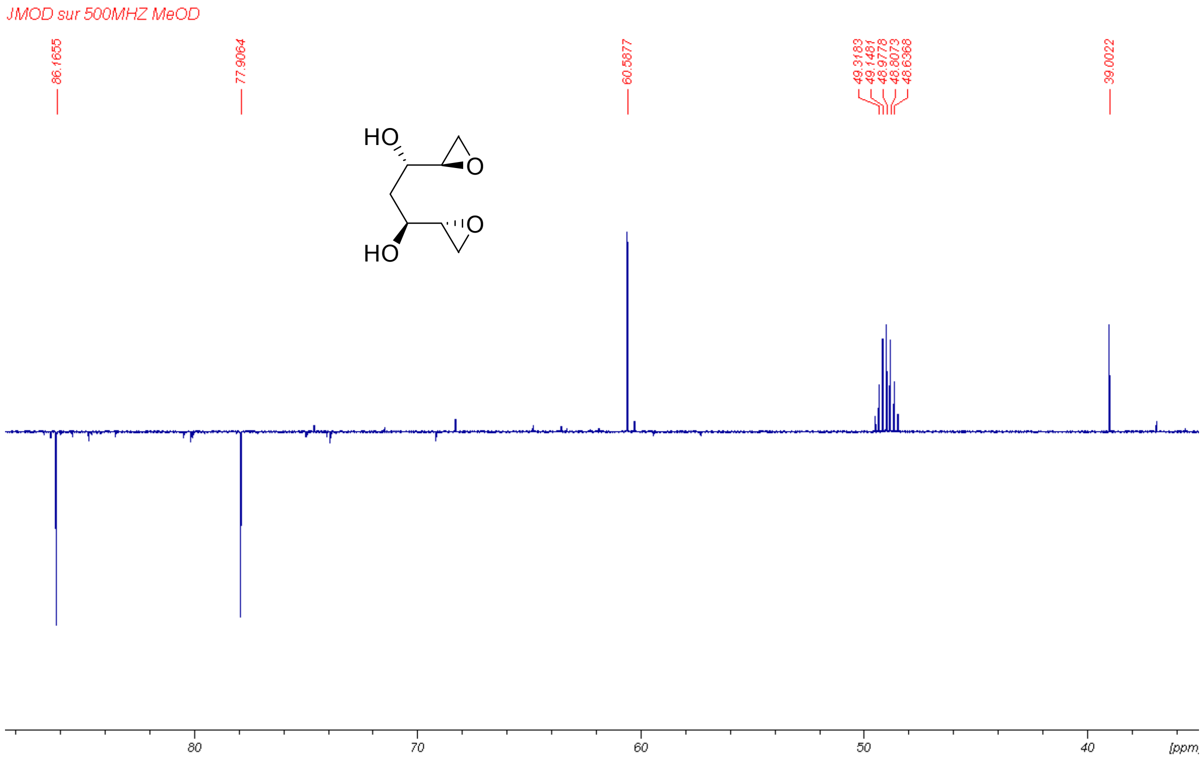
tetraol **2**:



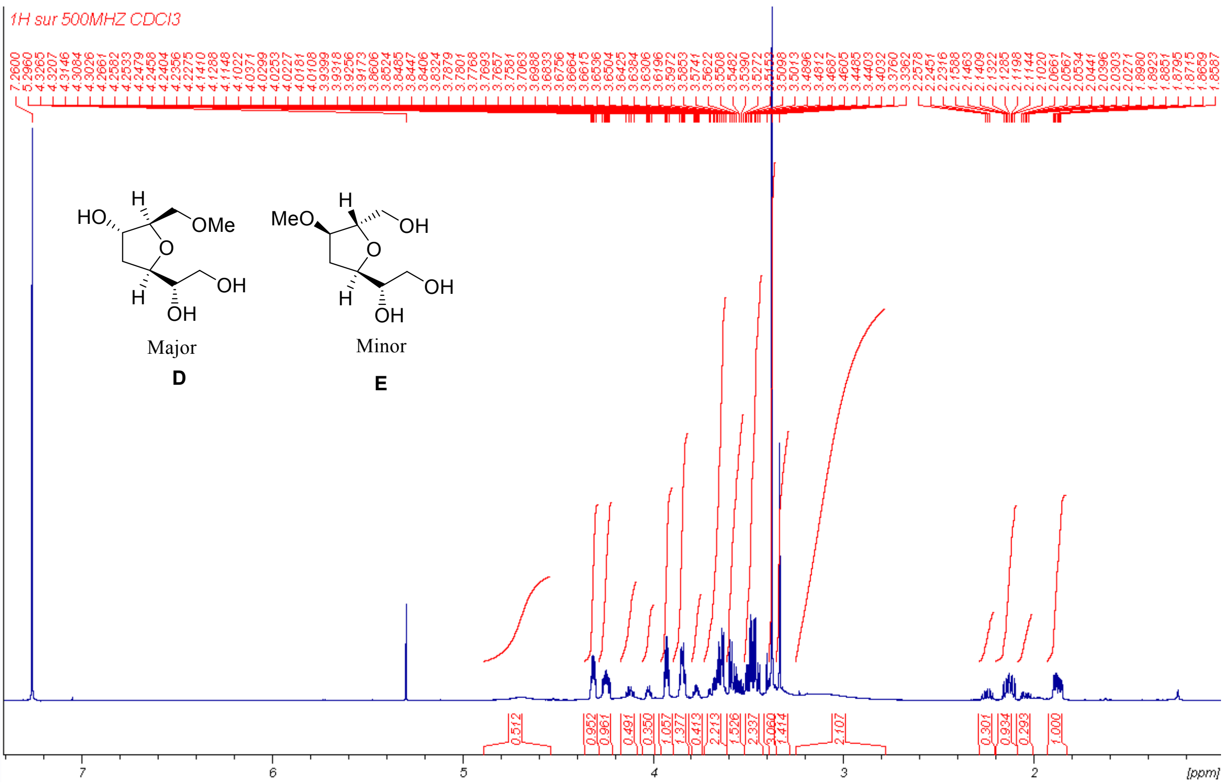


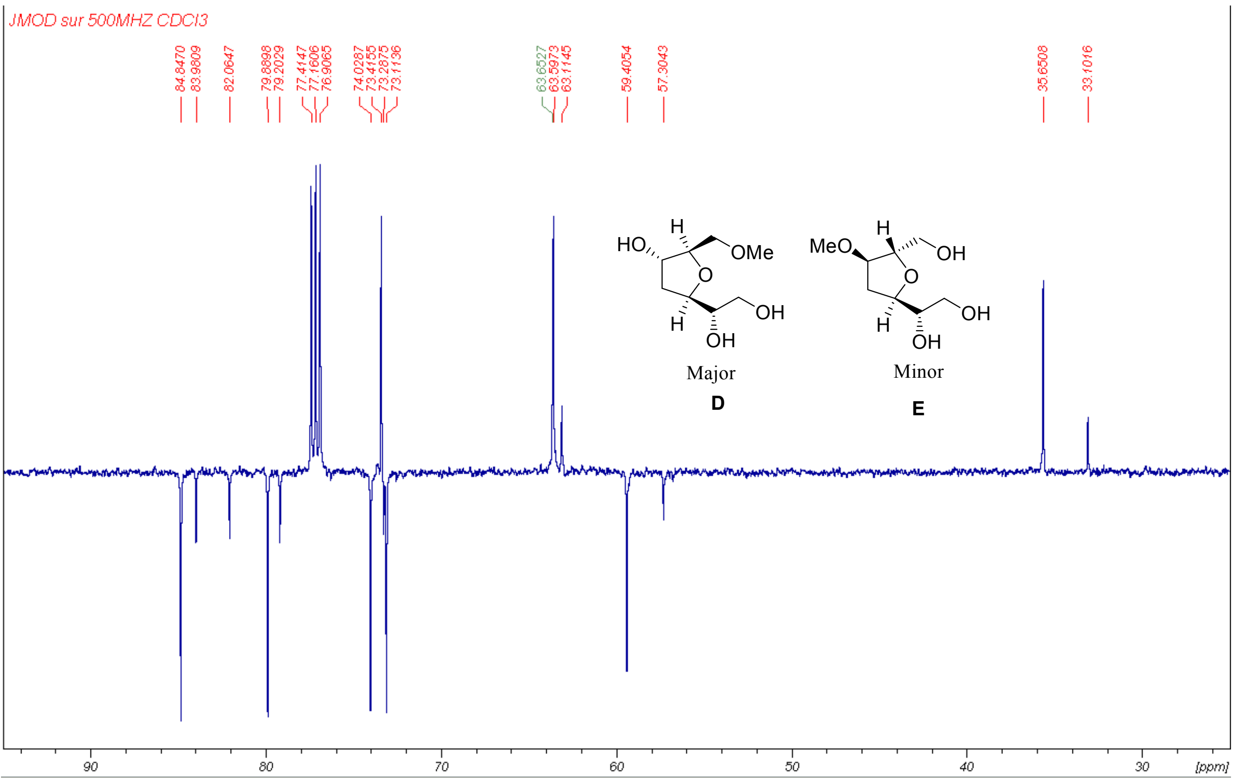
Bisepoxy diol resulting of double-Payne rearrangement **B**:

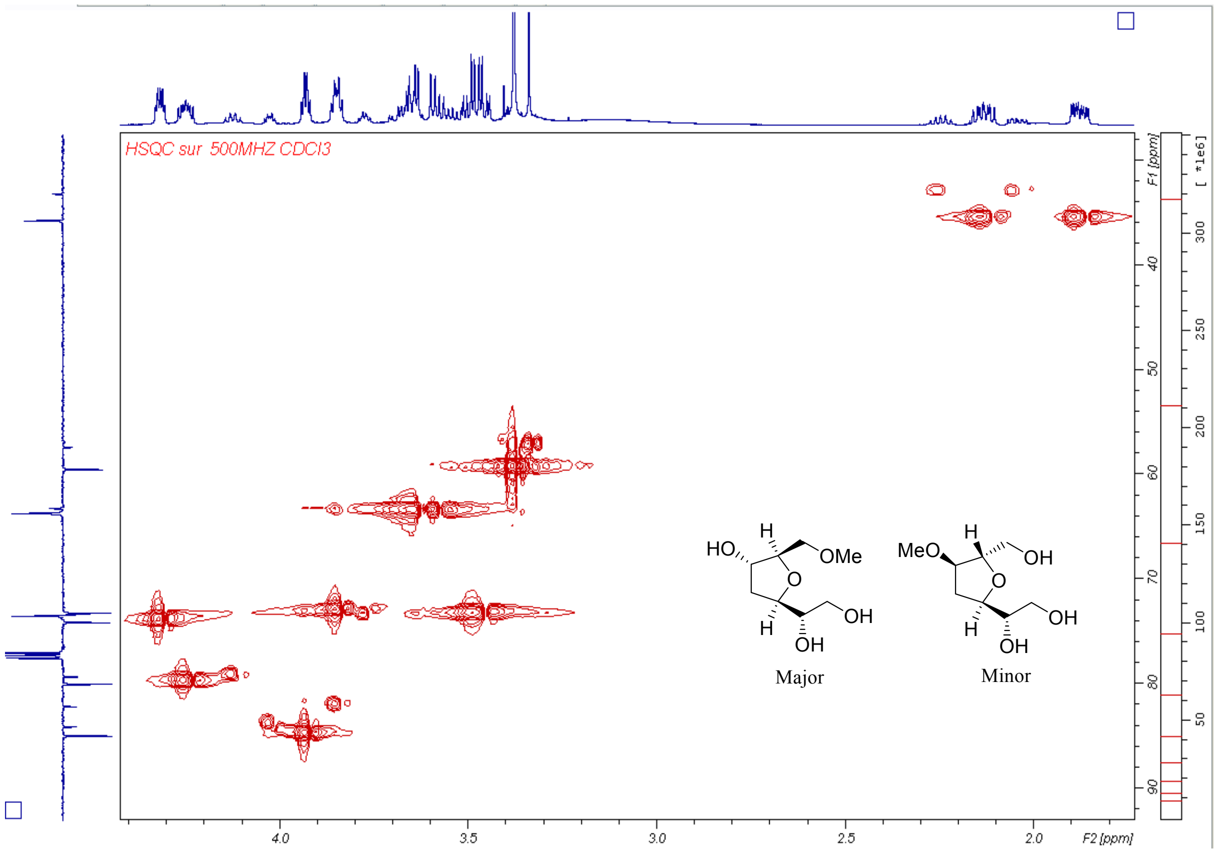


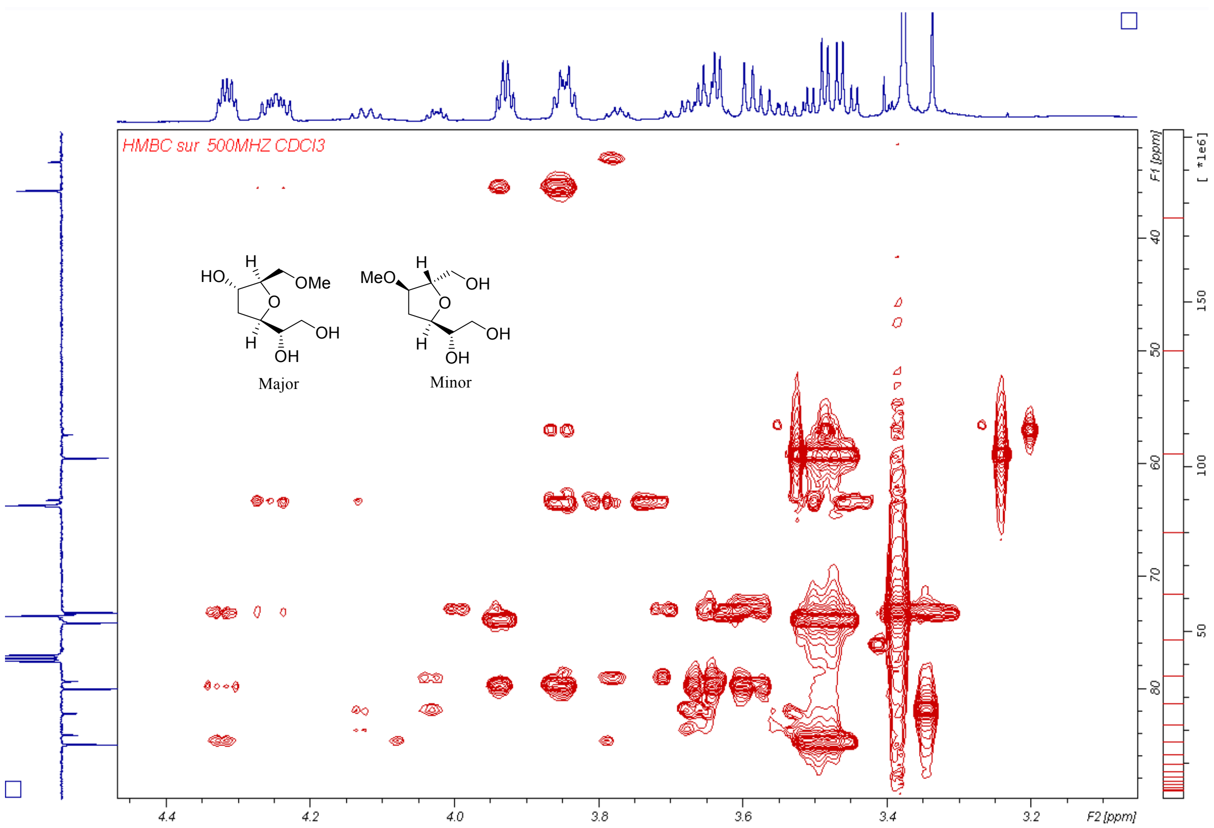


Methoxy-tetraol D and E:

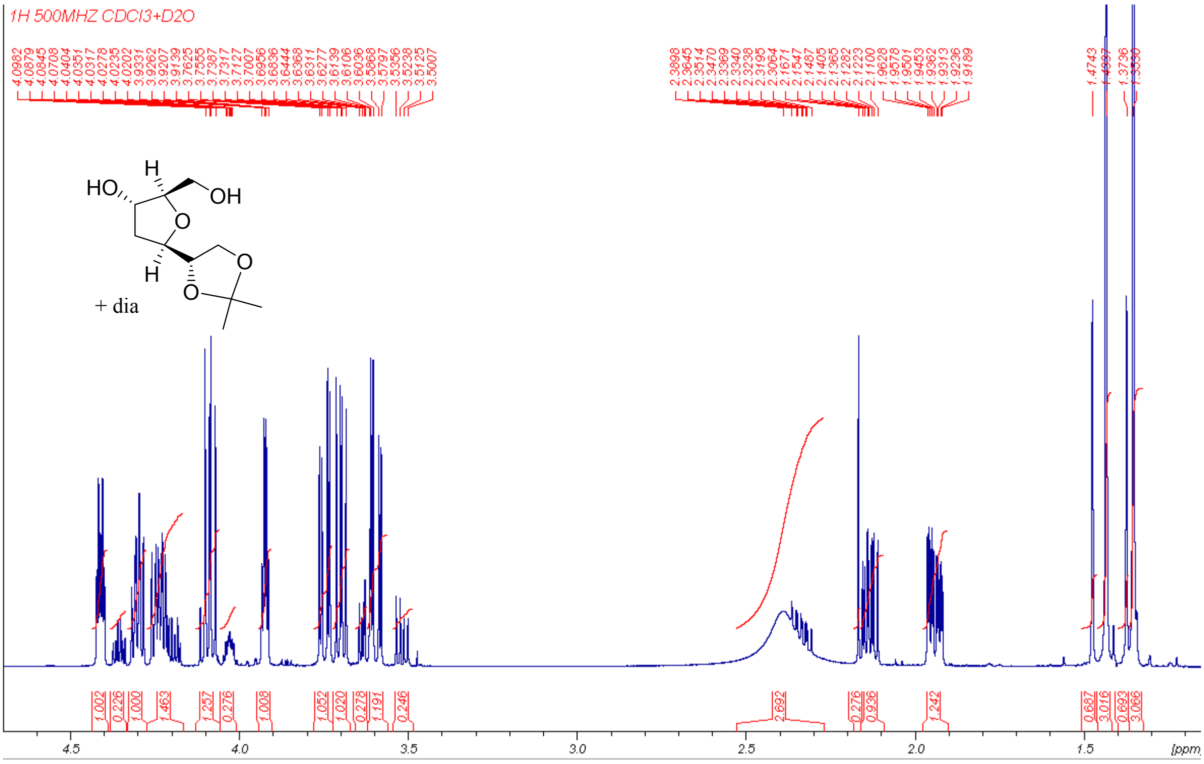


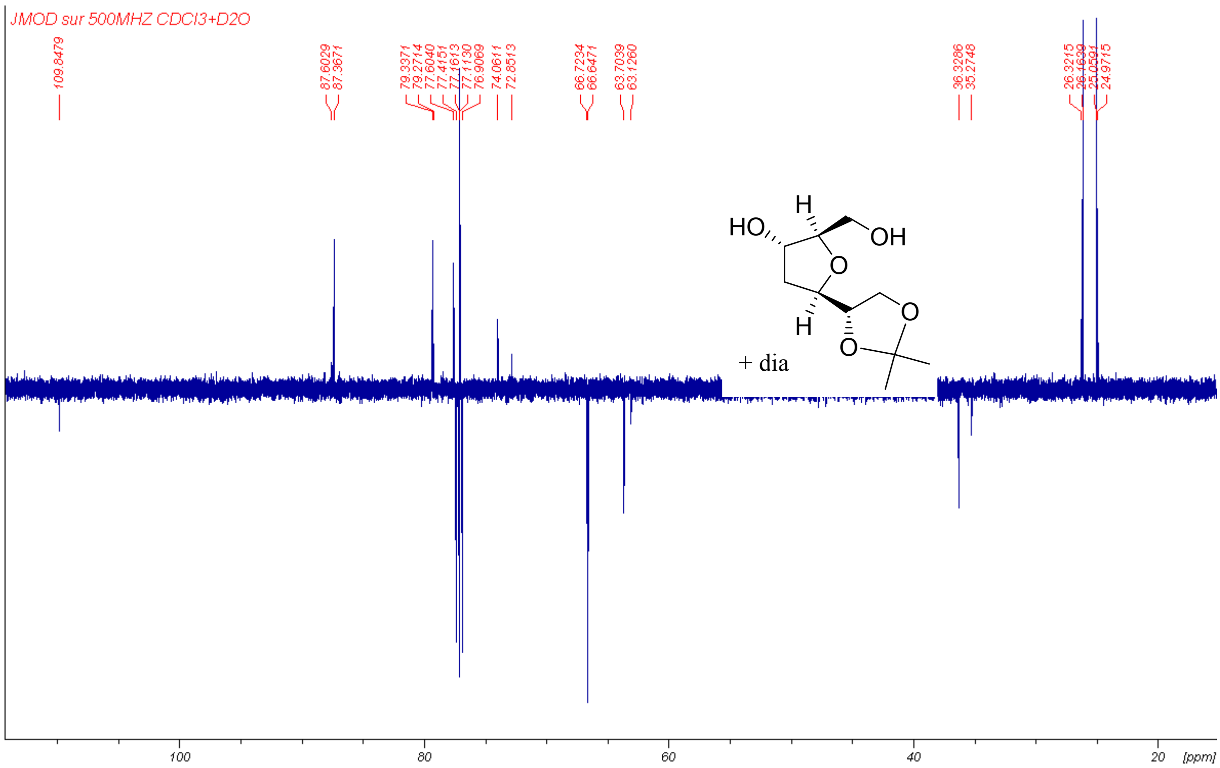



HSQC sur 500MHZ CDCl3
HO
OMe
OH
OH
Major
MeO
OH
OH
OH
Minor
F2 [ppm]
F1 [ppm]

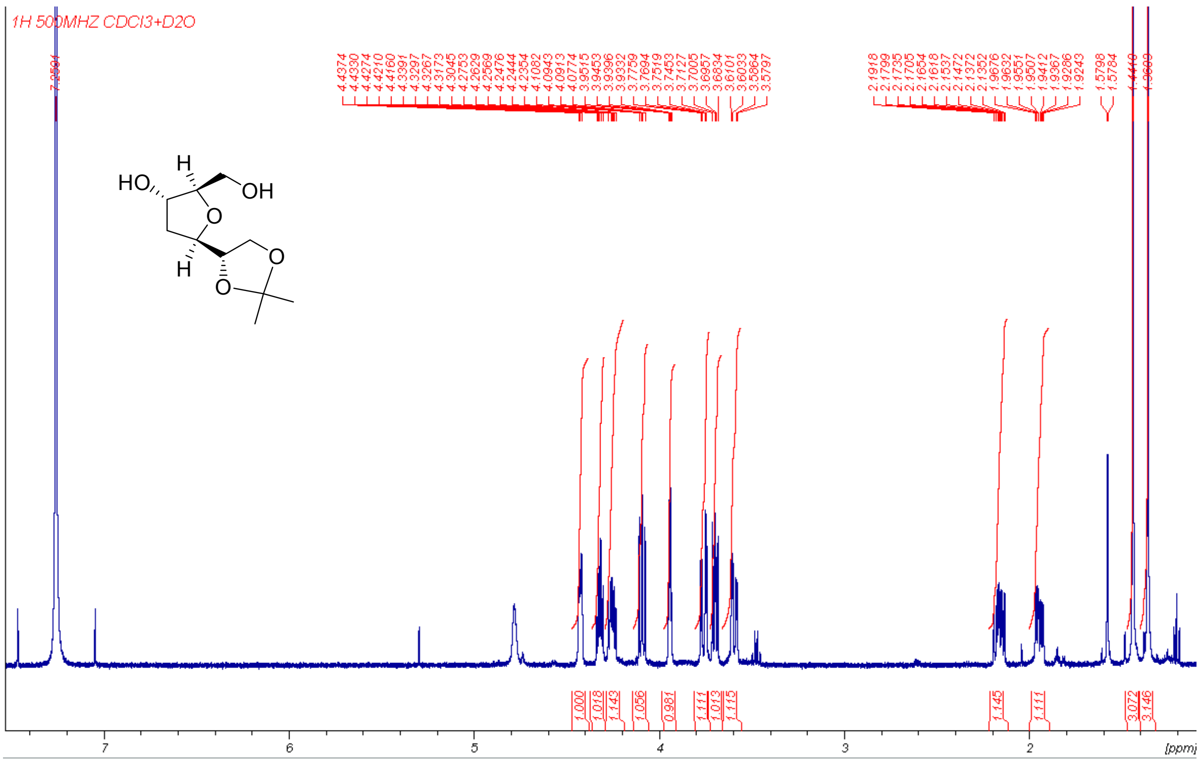

HMBC sur 500MHZ CDCl3
HO
OMe
OH
OH
Major
MeO
OH
OH
OH
Minor
F2 [ppm]
F1 [ppm]

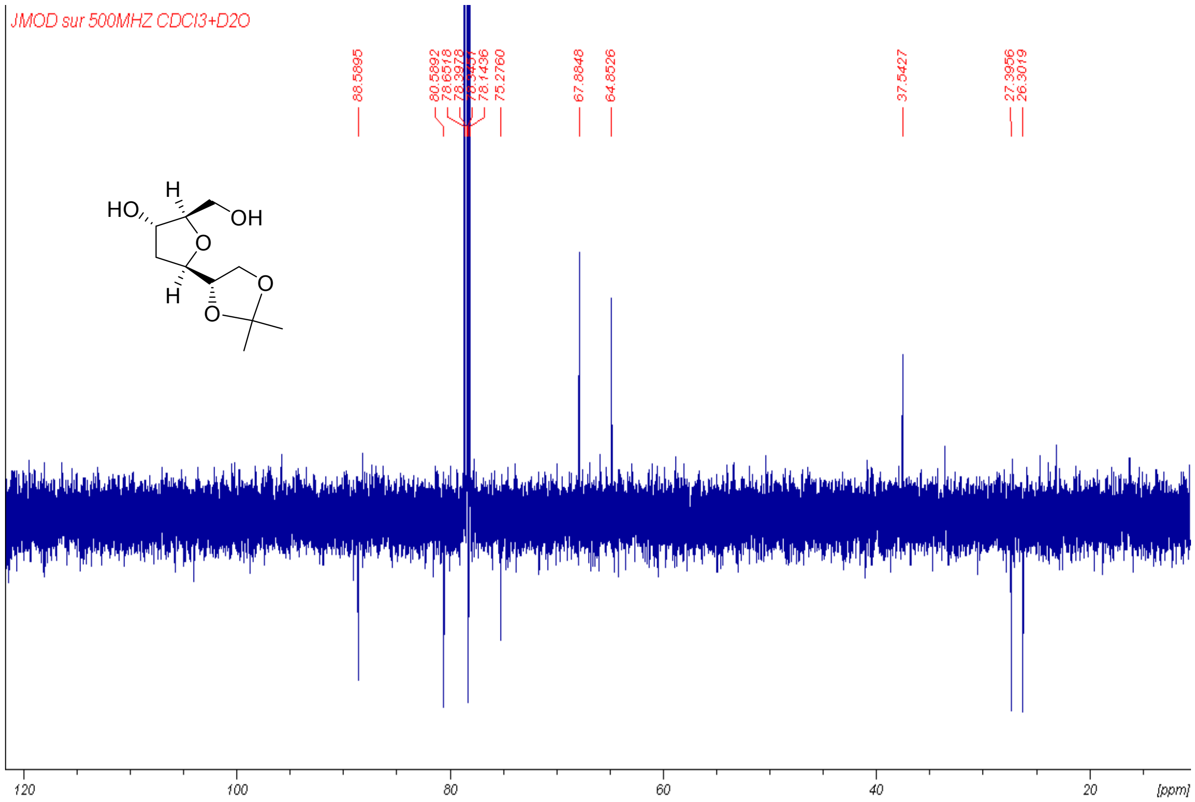
Acetonide **5**:


1H 500MHZ CDCl3+D2O
HO
H
OH
O
H
O
O
+ dia
ppm

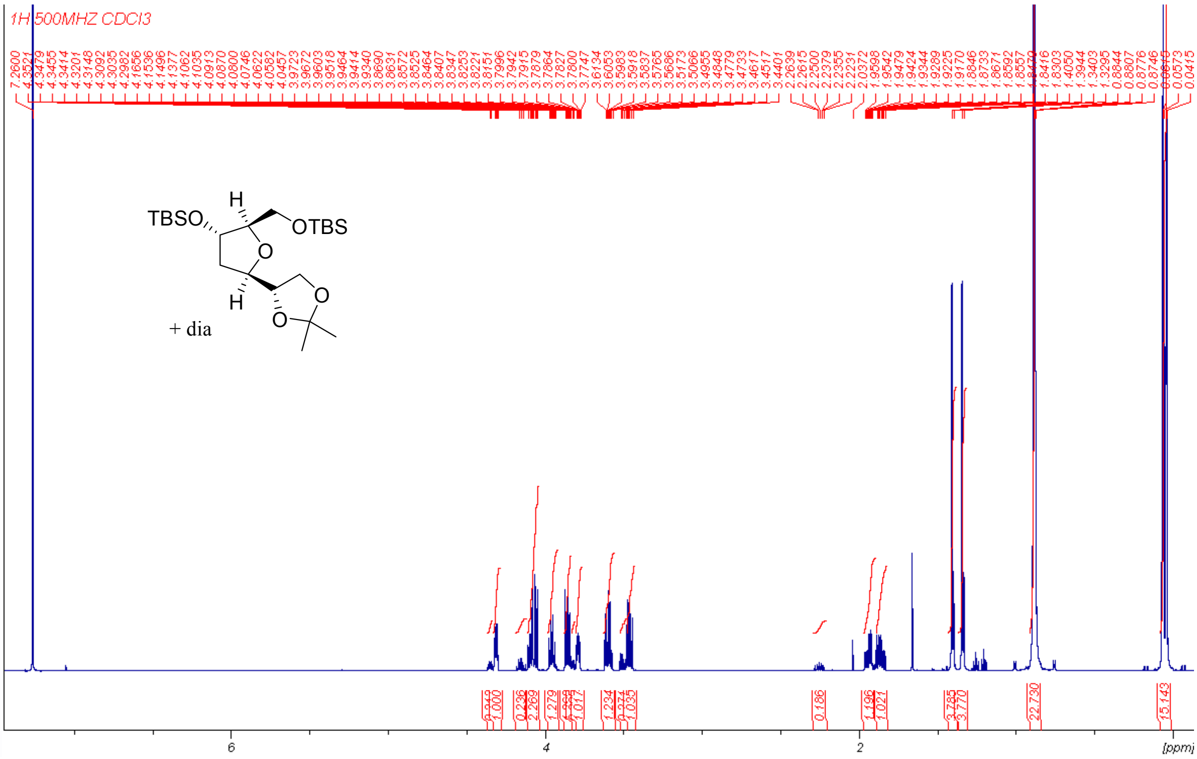

JMOD sur 500MHZ CDCl3+D2O
HO
H
OH
O
H
O
O
+ dia
ppm

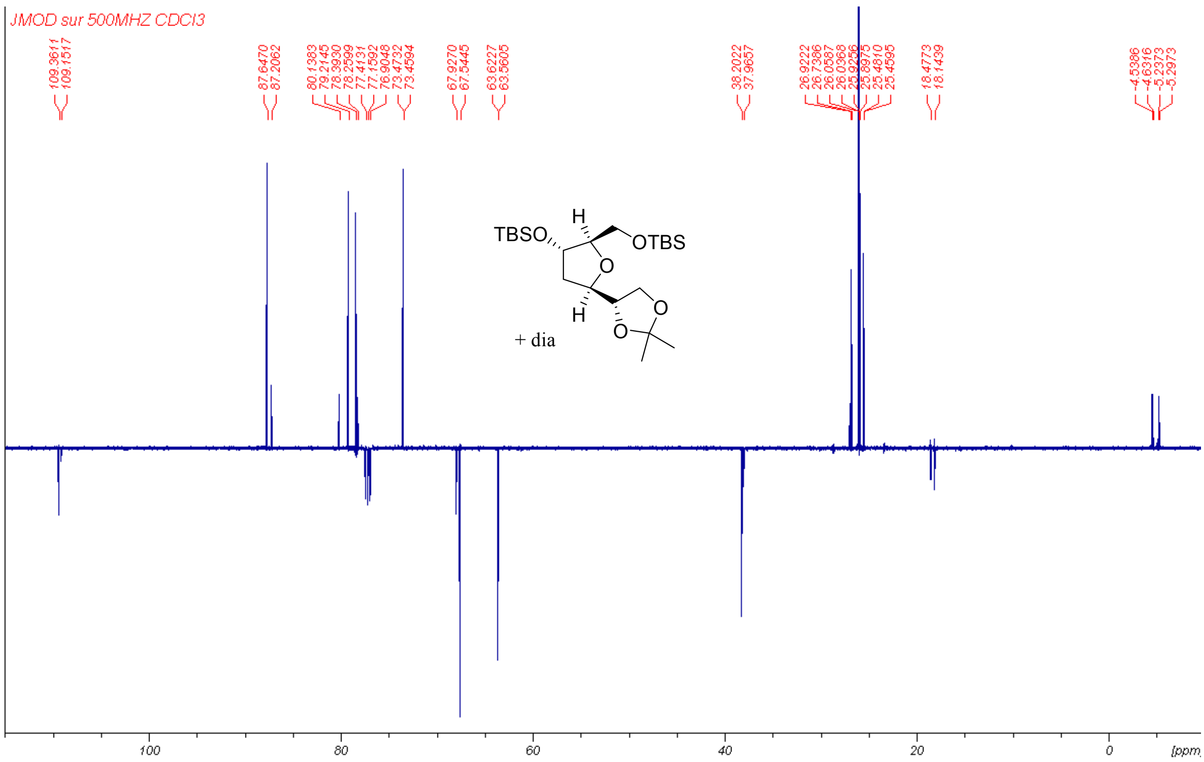
Crystals of acetonide **5**:



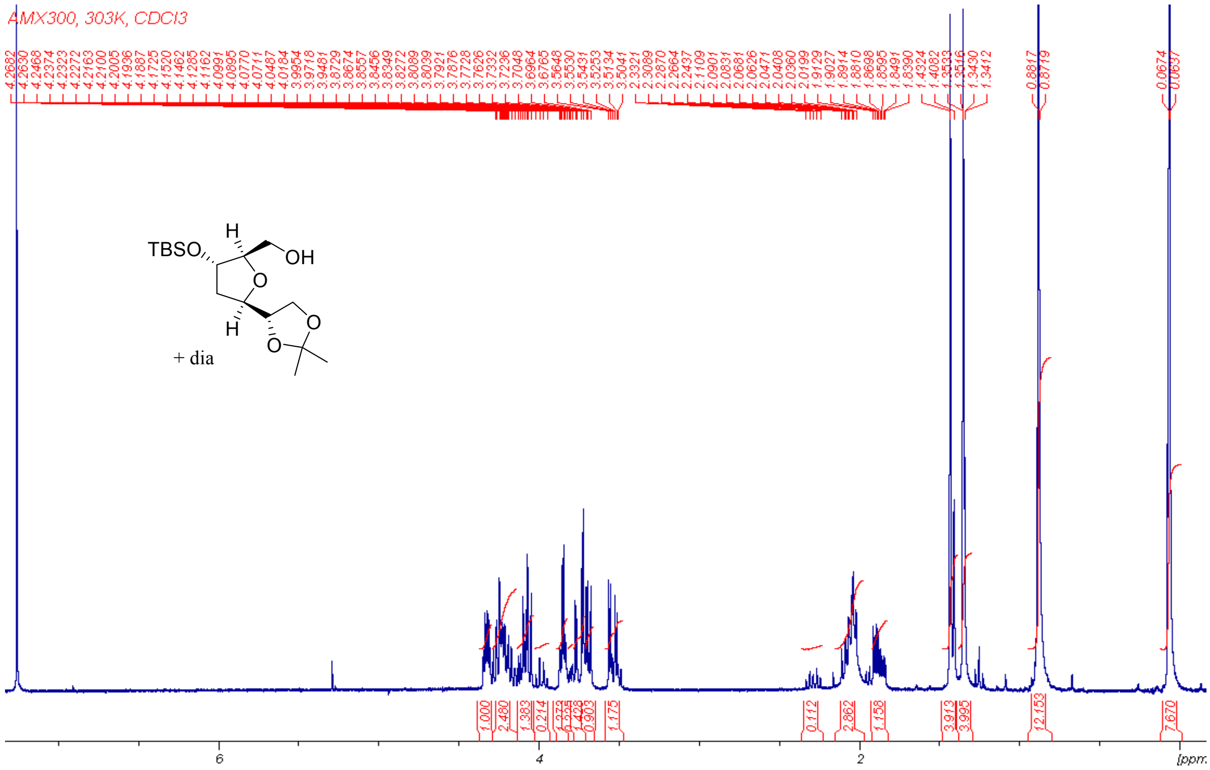


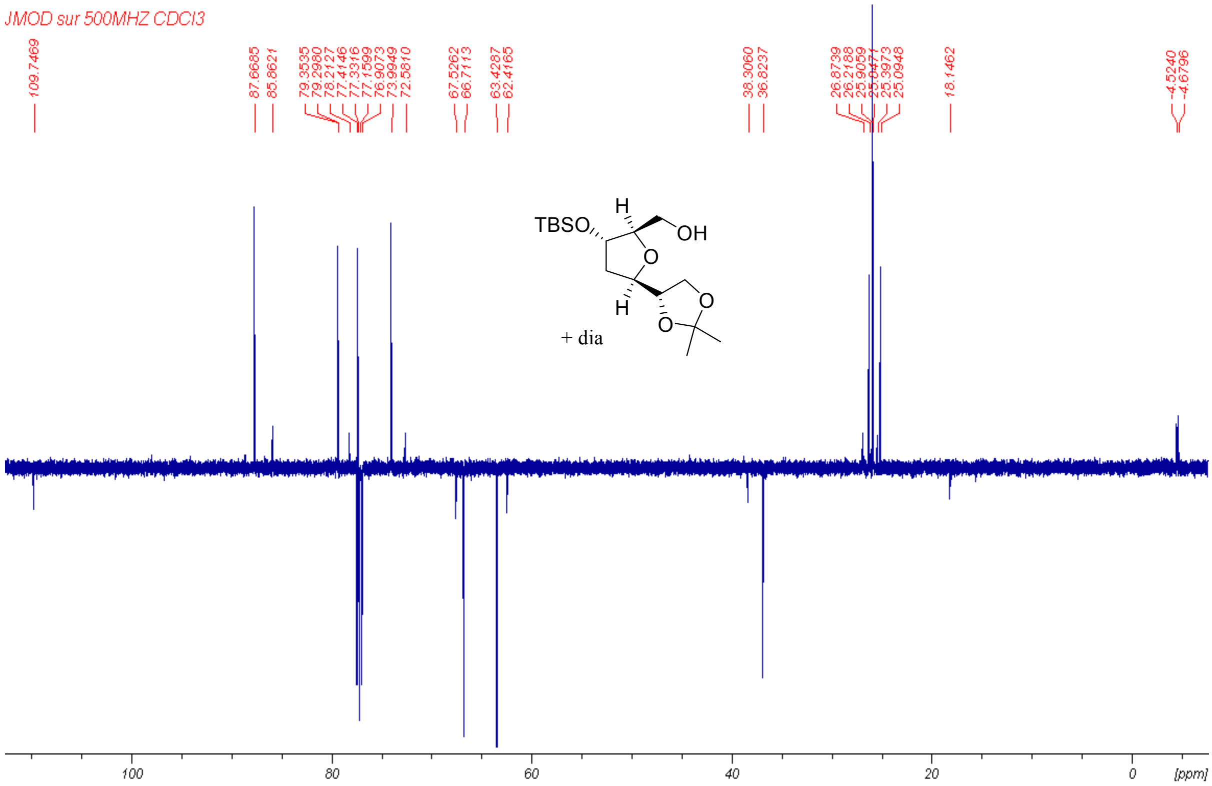
diTBS-acetonide:



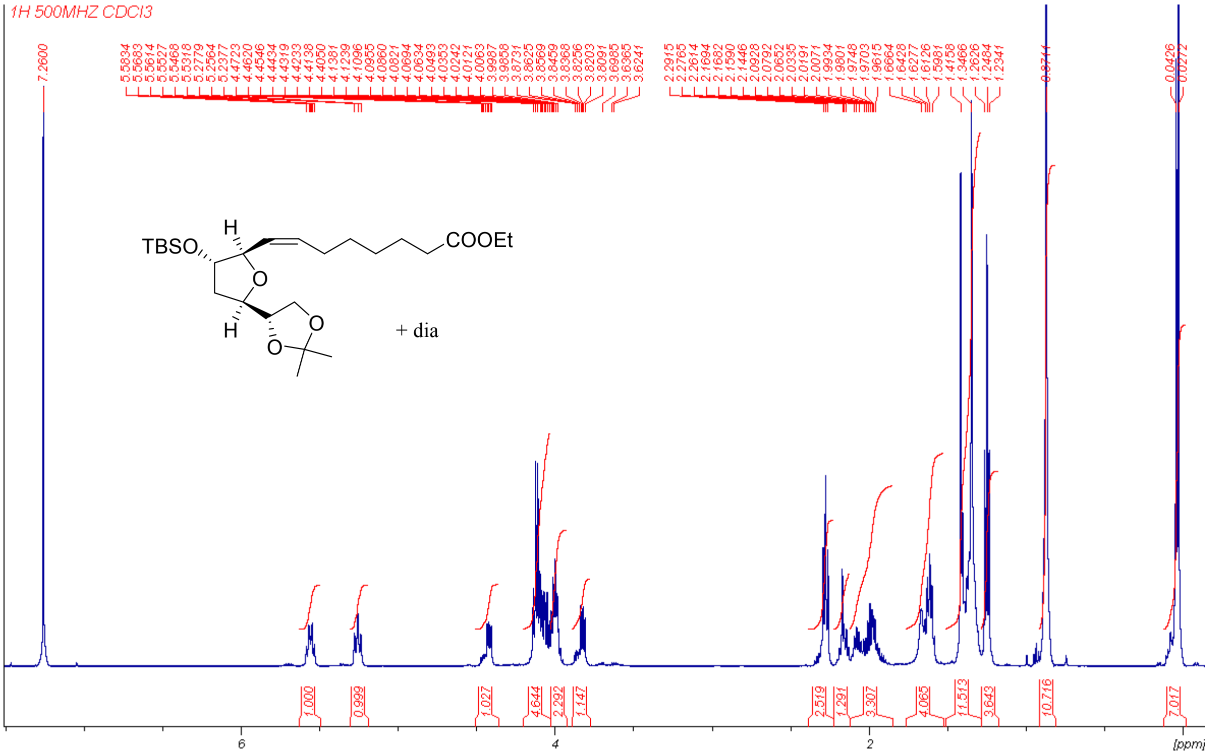


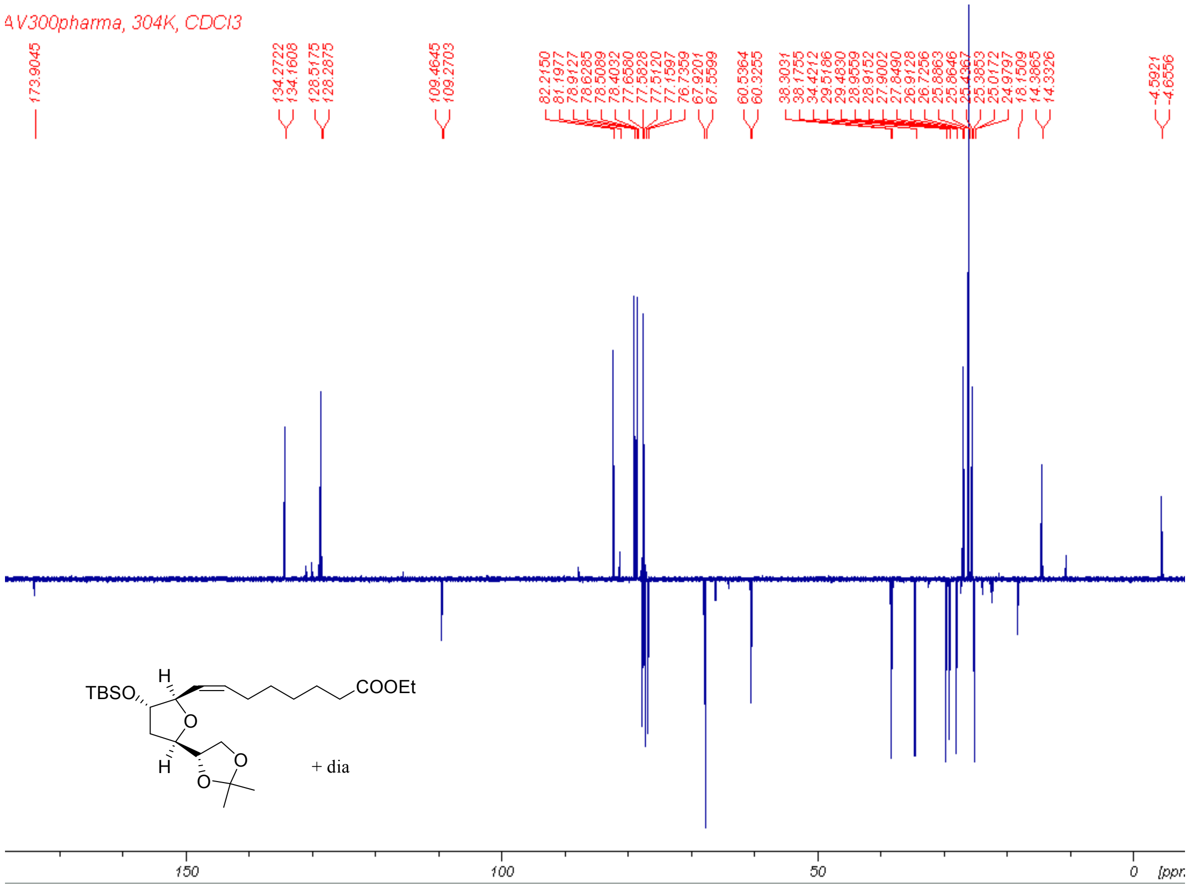
mono protected TBS-ether **6**:



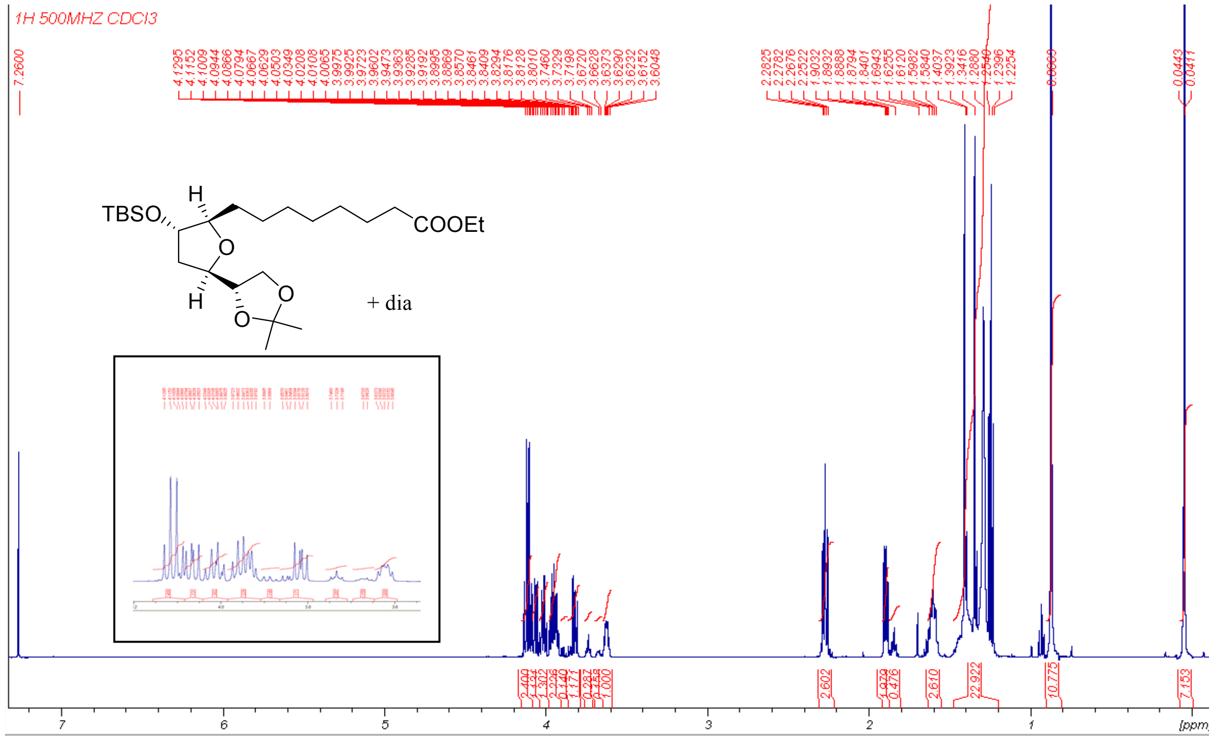


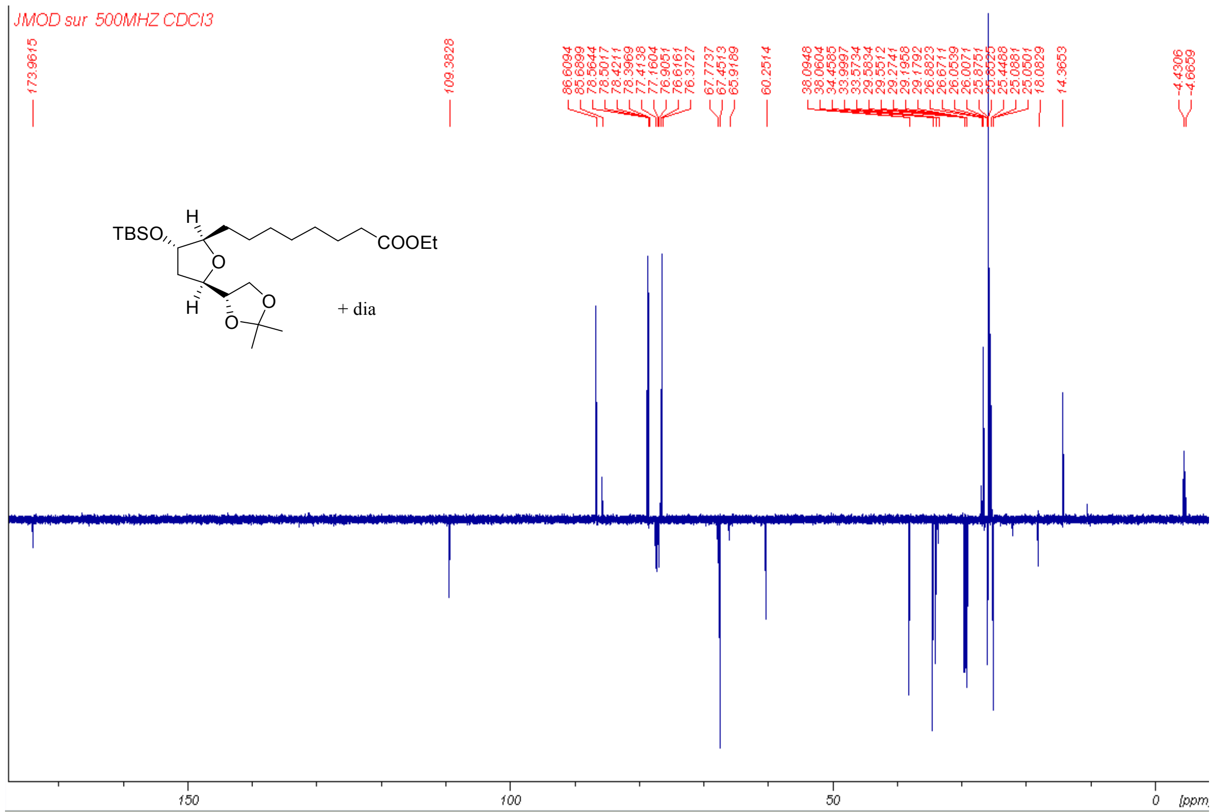
Alkene:


1H 500MHZ CDCl3
TBSO
COOEt
+ dia
[ppm]

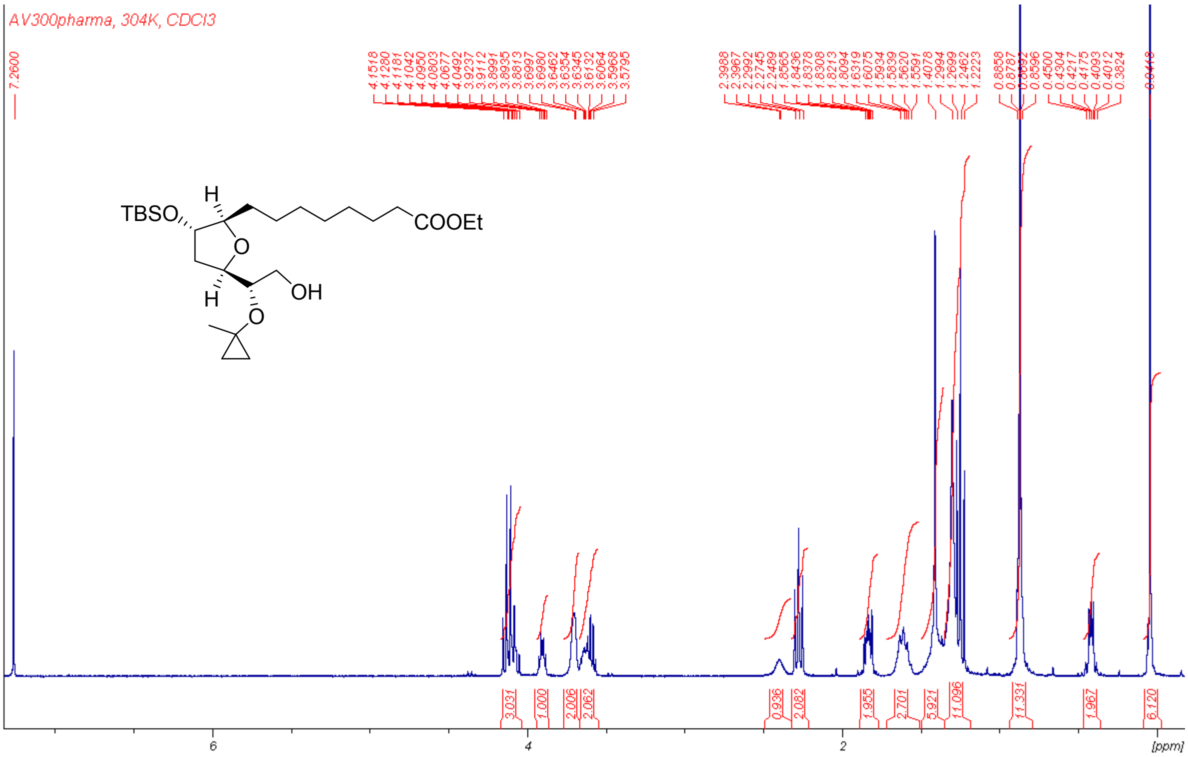

AV300pharma, 304K, CDCl3
TBSO
COOEt
+ dia

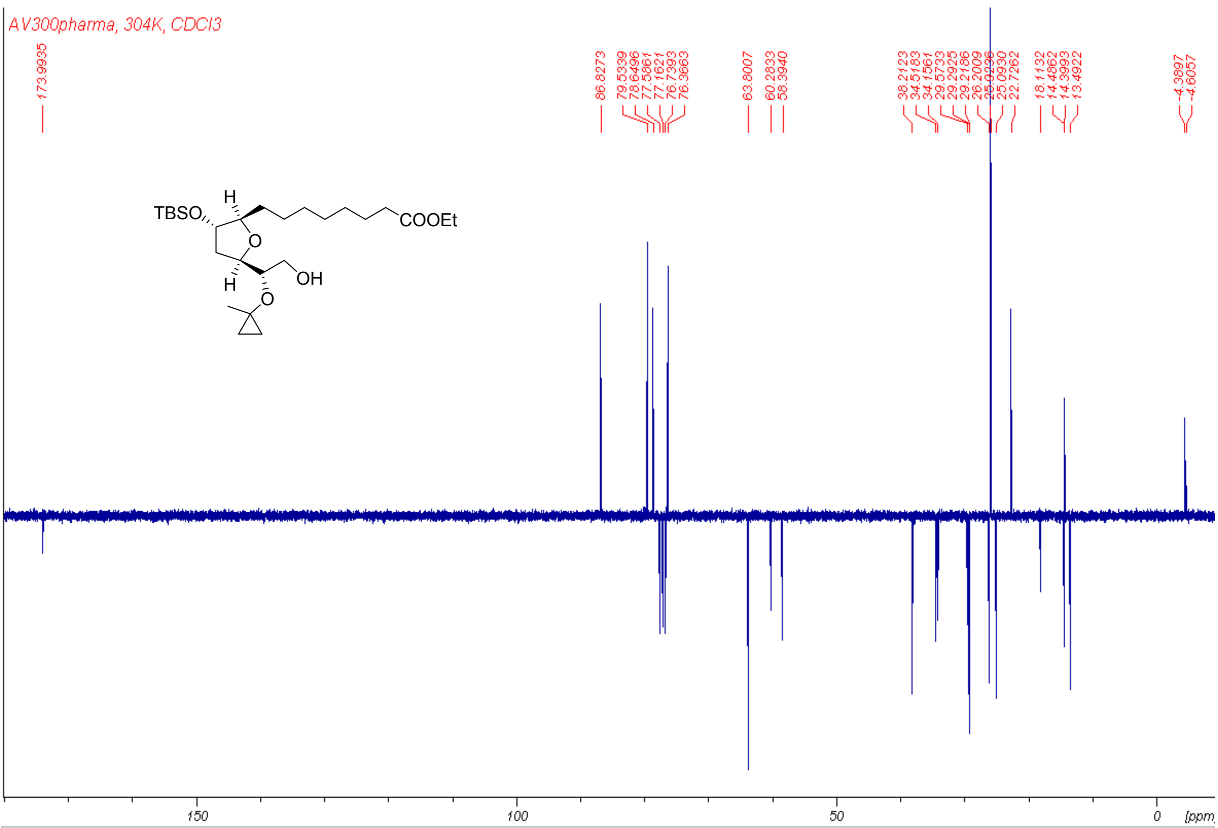
Acetonide **7**:



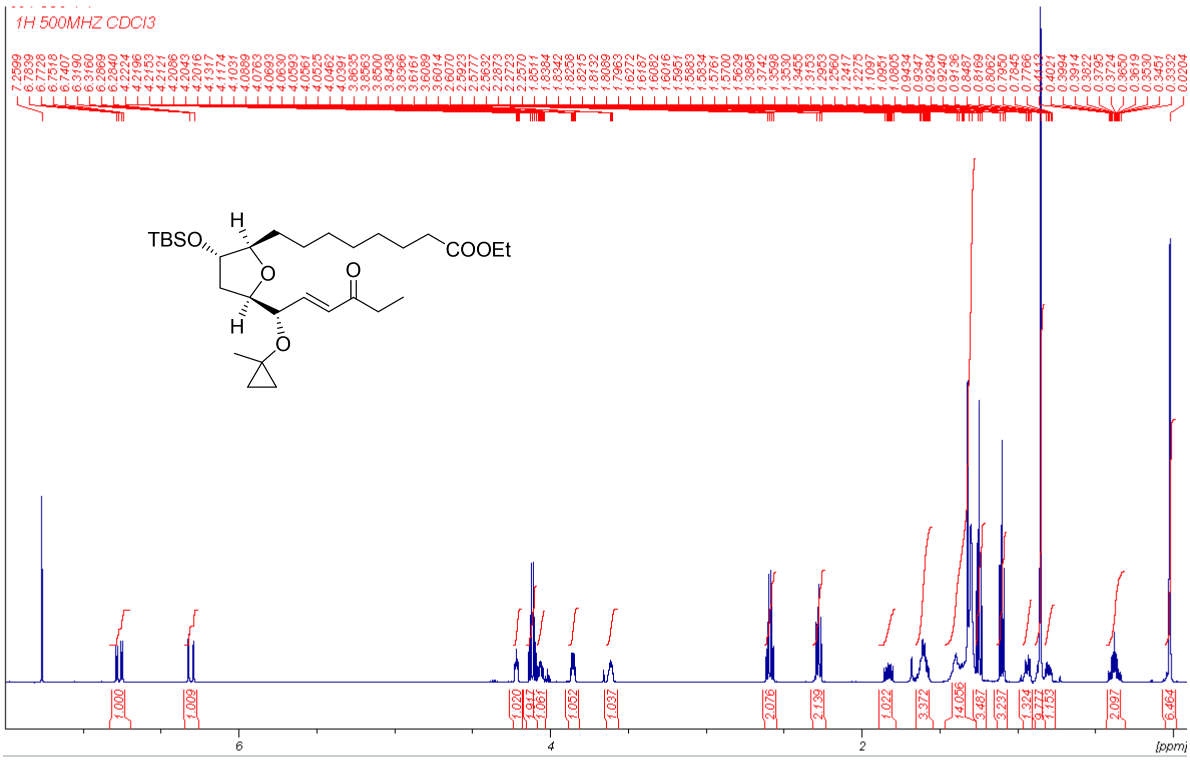


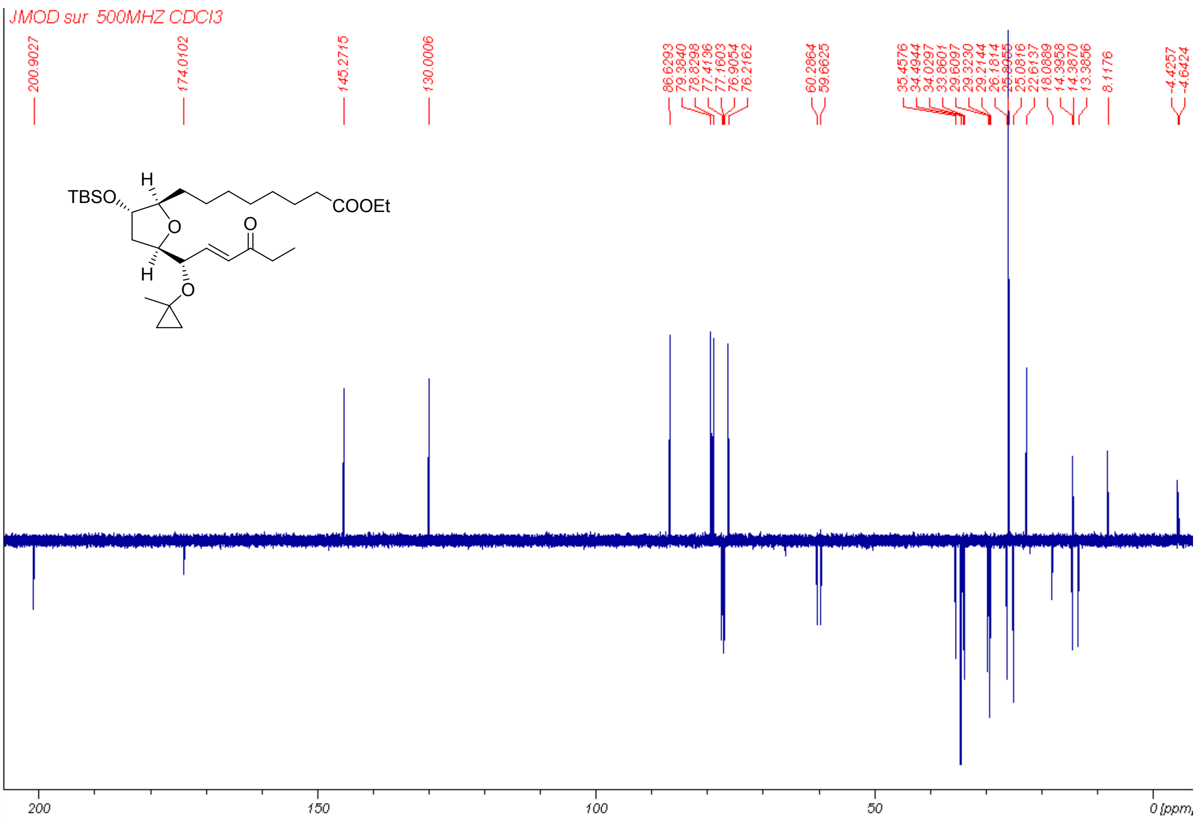
1-methyl-1-cyclopropyl hydroxyl derivative **8**:



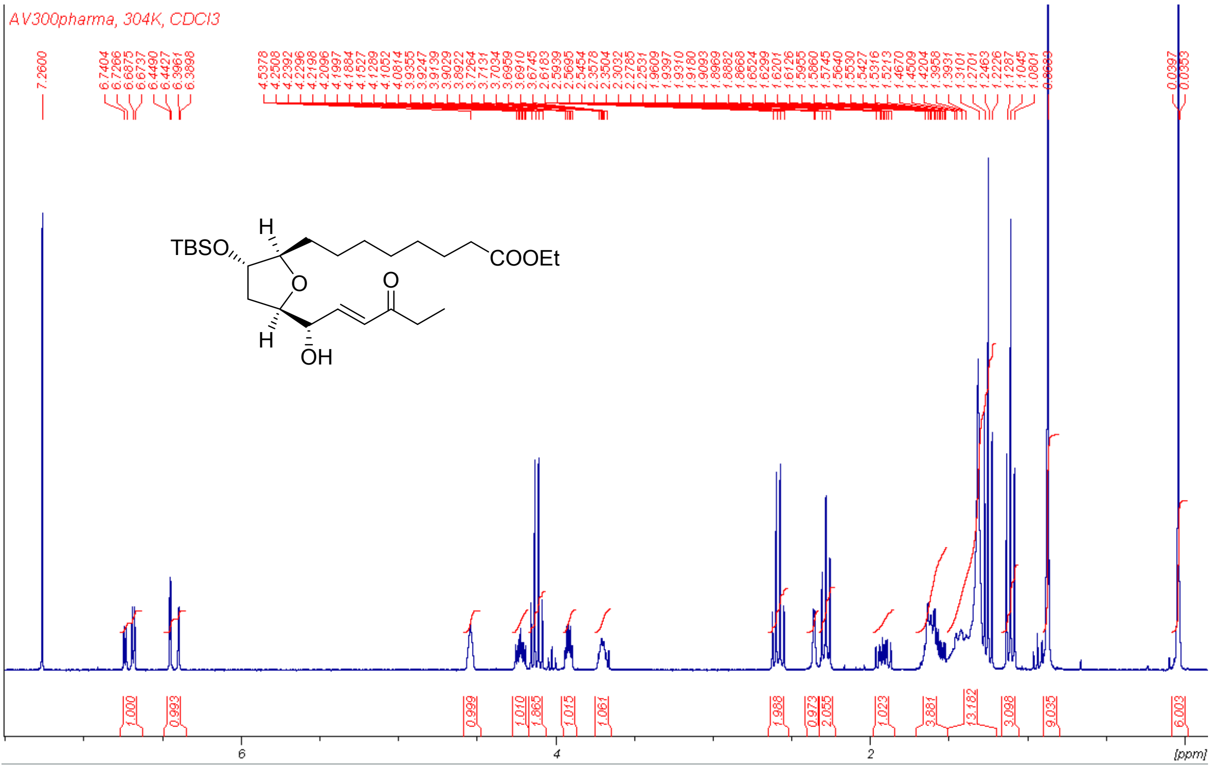


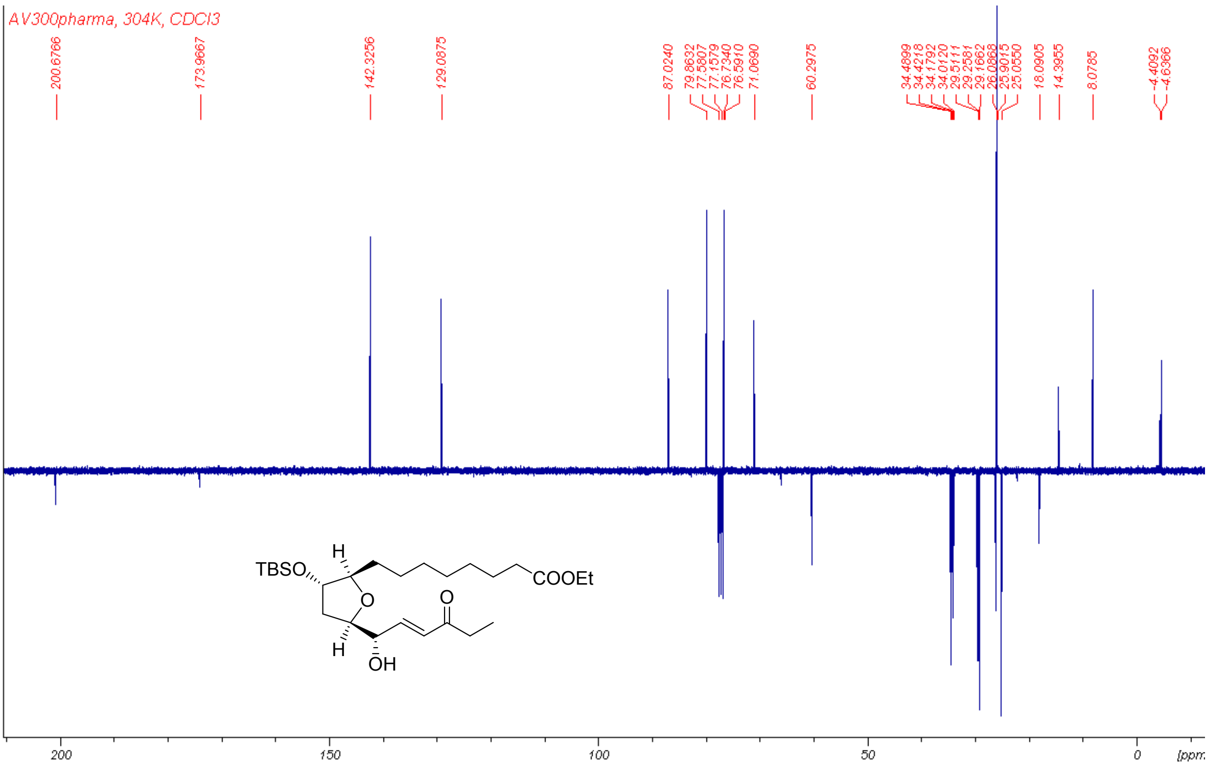
enone **9**:


1H 500MHZ CDCl3
TBSO
H
O
COOEt
O
H
O
ppm

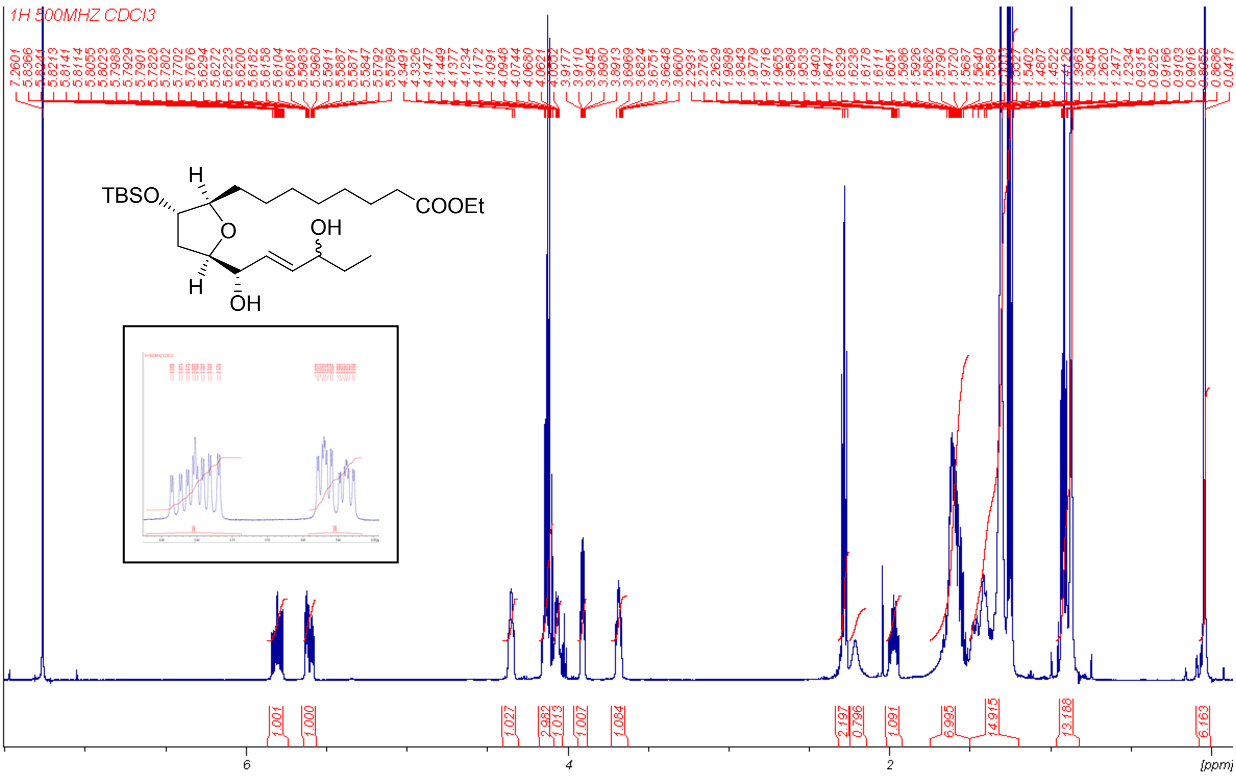

JMOD sur 500MHZ CDCl3
200.9027
174.0102
145.2715
130.0006
TBSO
H
O
COOEt
O
H
O
ppm

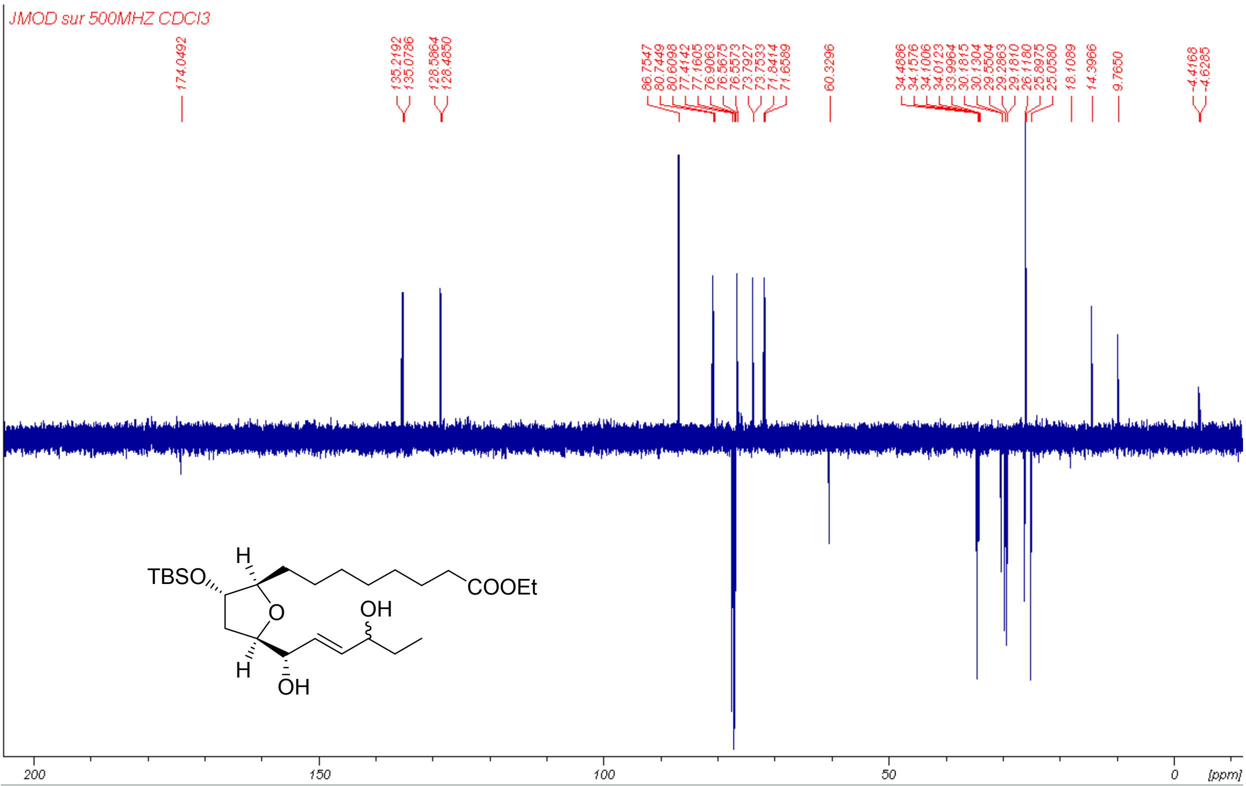
Enone **10**:


AV300pharma, 304K, CDCl3
TBSO
H
O
COOEt
O
H
OH
[ppm]

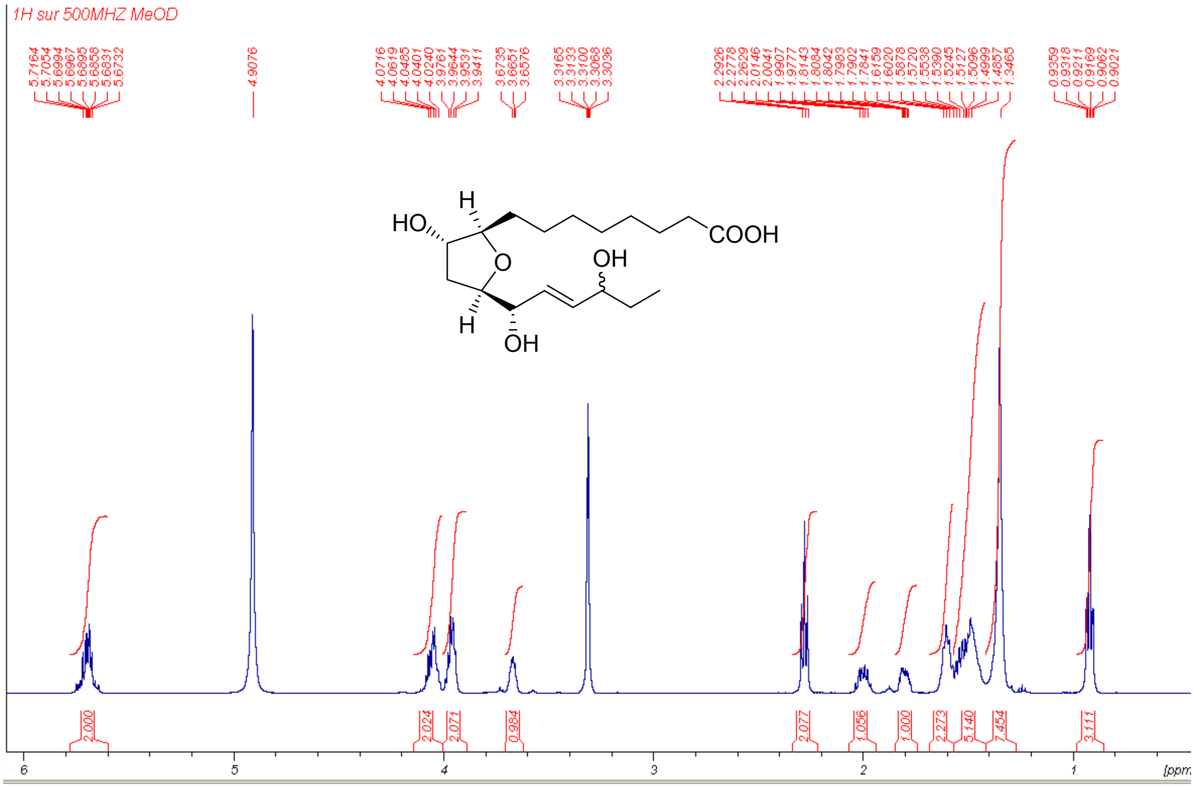

AV300pharma, 304K, CDCl3
TBSO
H
O
COOEt
O
H
OH
[ppm]

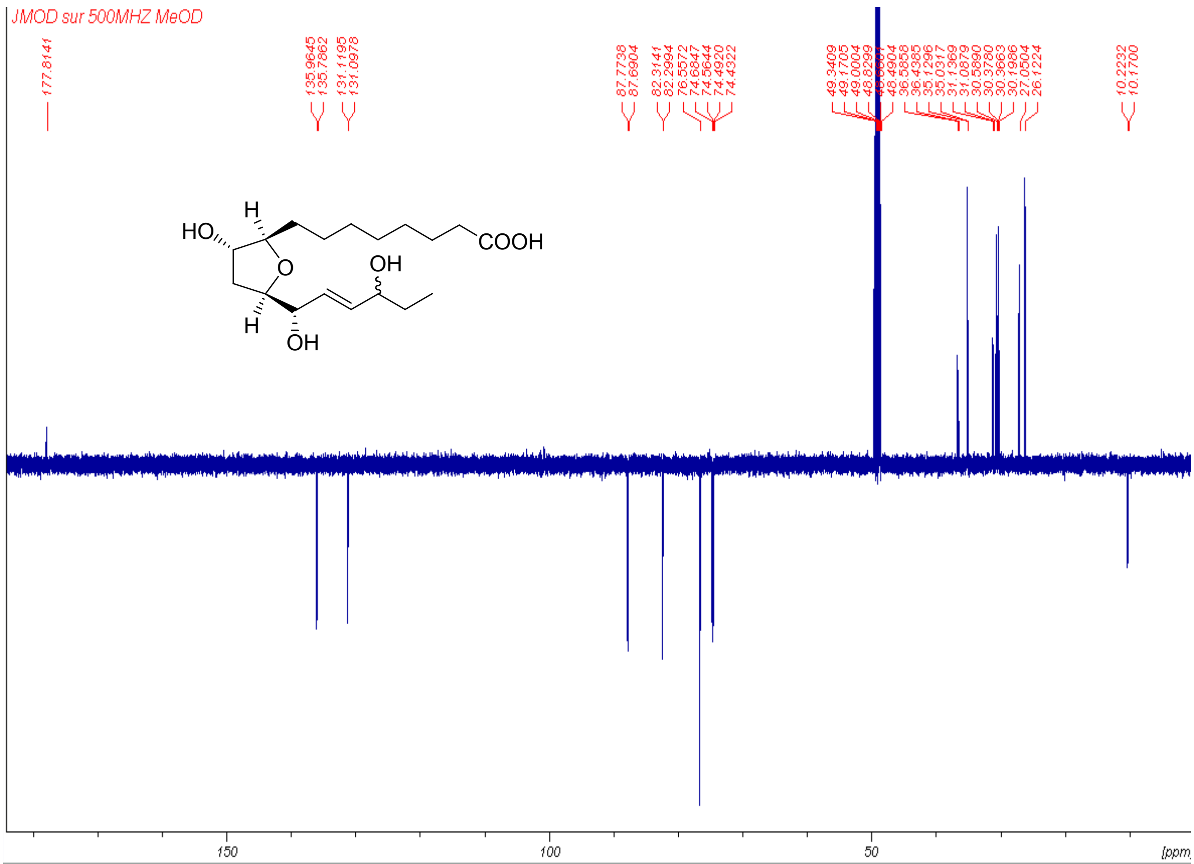
enediol**:**


1H 500MHZ CDCl3
TBSO
H
O
OH
COOEt
H
OH
1.001
1.000
1.027
2.982
1.013
1.007
1.084
2.197
0.796
1.091
6.995
14.915
13.188
6.163
6
4
2
[ppm]


JMOD sur 500MHZ CDCl3
174.0492
135.2192
135.0786
128.5864
128.4850
60.3296
18.1089
14.3966
9.7650
TBSO
H
O
OH
COOEt
H
OH
200
150
100
50
0
[ppm]

ent-16-(*RS*)-13-*epi*-ST-Δ^{14}-9-PhytoF **1**:





References

1 J. P. Fessel, N. A. Porter, K. P. Moore, J. R. Sheller and L. J. Roberts, *Proc. Natl. Acad. Sci.*, 2002, **99**, 16713–16718.

2 U. Jahn, J.-M. Galano and T. Durand, *Angew Chem Int Ed.*, 2008, **47**, 5894–5955.

3 R. W. Hoffmann, B. C. Kahrs, J. Schiffer and J. Fleischhauer, *J. Chem. Soc. Perkin Trans. 2*, 1996, 2407–2414.

4 W. Kabsch, *Acta Crystallogr. D Biol. Crystallogr.*, 2010, **66**, 125–132.

5 A. van der Lee, *J. Appl. Crystallogr.*, 2013, **46**, 1306–1315.

6 L. Palatinus and G. Chapuis, *J. Appl. Crystallogr.*, 2007, **40**, 786–790.

7 P. W. Betteridge, J. R. Carruthers, R. I. Cooper, K. Prout and D. J. Watkin, *J. Appl. Crystallogr.*, 2003, **36**, 1487–1487.

8 H. K. Mangold, *Fresenius Z. Für Anal. Chem.*, 1988, **332**, 679–684.

9 S. T. Girisha, K. Ravikumar, B. R. Mrunalini and V. Girish, *Asian J. Plant Sci. Res.*, 2014, **4**, 28–35.

10 K. S. Leung, X. Chen, W. Zhong, A. C. H. Yu and C.-Y. J. Lee, *Chem. Phys. Lipids*, 2014, **180**, 53–60.

11 C.-Y. J. Lee, R. C. S. Seet, S. H. Huang, L. H. Long and B. Halliwell, *Antioxid. Redox Signal.*, 2009, **11**, 407–420.