Uncovering the origin of *Z*-configured double bonds in polyketides: intermediate *E*-double bond formation during borrelidin biosynthesis

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Supporting information

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Chemistry methods and materials

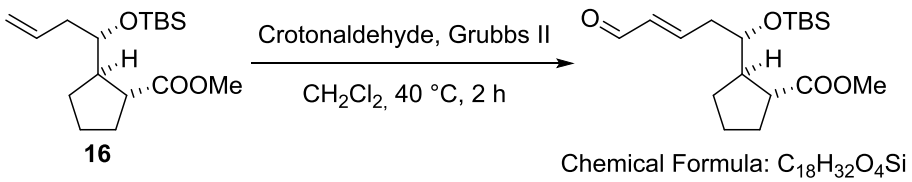
All reactions were performed in oven dried glassware under an atmosphere of argon gas unless otherwise stated. Dry solvents were purchased from Sigma-Aldrich and Acros or taken out of a solvent system from M. Braun. Dry reagents were ordered from Sigma-Aldrich, Fluka, Acros, ABCR and Roth. NMR spectra were recorded with Bruker DRX-500, DPX-400 and AVANCE-400 with the residual solvent signal as internal standard.[1] The solvents are given with the data. Multiplicities are described using the following abbreviations: s = singlet, d = doublet, t = triplet, q = quartet, m = multiplet, br = broad. ^{13}C NMR spectra are reported as values in ppm relative to the residual solvent signal as internal standards.[1] The multiplicities are elucidated using the distortionless enhancement by polarisation transfer (DEPT) spectral editing technique, with secondary pulses at 90° and 135°. Multiplicities are reported using the following abbreviations: q (quarternary carbon), t (tertiary carbon = methine), s (secondary carbon = methylene), p = (primary carbon = methyl). High resolution mass spectra are obtained with a Micromass LCT via loop-mode injection from a Waters (Alliance 2695) HPLC system. Alternatively a Micromass Q-TOF in combination with a Waters Aquity Ultraperformance LC system is employed. Ionisation is achieved by ESI or APCI. Modes of ionisation, calculated and found mass are given. Reversed phase-HPLC-applications were performed with membrane-filtrated and double distilled water as well as commercial available HPLC-grade solvents (methanol or acetonitrile), which have been degased with ultrasound. Preparative HPLC was operated at a Merck Hitachi LaChrome HPLC (Pump L-7150, Interface D-7000, Diode Array Detector L-7450). The following stationary phases were used: (C18-SP) Trentec Reprosil-Pur 120 C18 AQ 5µm, 250 mm * Ø 8 mm, corresponding precolumn cartridge, 40 mm * Ø 8 mm; (CN-SP) Trentec Reprosil 100 CN 5µm, 250 nm * Ø 8 mm, corresponding precolumn cartridge, 40 mm * Ø 8 mm. Alternatively preparative HPLC was performed with a Varian HPLC (Pumps Prepstar Model 218, Variable wavelength detector Prostar (λ = 248 nm) with parallel mass detection (Micromass Type ZMD ESI-Quad-Spectrometer) under use of a C18-$P_{[B]}$ stationary phase. Solvents, columns, operating procedures and retention times (t_R) are given with the corresponding experimental and analytical data. (Abbreviations: PE= petroleum ether; EtOAc = ethyl acetate).

Biochemistry methods and materials

All chemicals and antibiotics were purchased from Sigma-Aldrich and Roth. *E. coli* BL21(DE3)-CodonPlus-RP was purchased from Invitrogen. The expression constructs based on the pET-28a(+) vector and were prepared as stated previously.[2] Cell disruption was conducted by French Press R 125 from American Instrument Company. His-bind nickel chelate chromatography resin was purchased from Novagen. Sartorius Vivaspin 20 ultrafiltration units (10,000 MW Cut-off), Millipore Amicon® ultra centrifugal filters (30,000 MW Cut-off) and PD-10 desalting columns from GE Healthcare were used for protein concentration and buffer exchange respectively.

Synthetic procedures[4]

Methyl (1R,2R)-2-((S,E)-1-((tert-butyldimethylsilyl)oxy)-5-oxopent-3-en-1-yl)cyclopentane-1-carboxylate



16

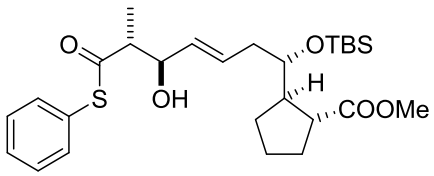
Chemical Formula: $C_{18}H_{32}O_4Si$
Molecular Weight: 340.53

200 mg (640 µmol, 1.0 equiv.) **16** and 1.30 mL (15.8 mmol, 25 equiv.) crotonaldehyde were solved in 40 mL CH_2Cl_2. 27 mg (32 µmol, 5 mol%) Grubbs II catalyst were added and it was heated to 40 °C under reflux for 2 h. All volatiles were removed under reduced pressure and the crude product was purified by flash chromatography on silica gel (petroleum ether:EtOAc (9:1)). After drying in vacuo, 192 mg (0.57 mmol) of a brown oil were obtained (88% crude yield).

The α,β-unsaturated aldehyde showed a strong tendency to quickly oxidize to the carboxylic acid. Therefore, the crude product of the metathesis reaction was routinely analyzed by ^{1}H NMR spectroscopy and TLC analysis. This material was subjected into the following reactions only after passing the crude product over a short filter column of silica gel. The amount that was inserted into the following reactions was calculated basing on the assumption of quantitative conversion in this step.

R_f = 0.33 (PE/EtOAc 9:1); **^{1}H NMR** (200 MHz, $CDCl_3$) δ 9.51 (d, *J* = 7.9 Hz, 1 H, *H*CO), 6.89 (dt, J_1 = 7.3 Hz, J_2 = 15.6 Hz, 1 H, HC(O)C*H*), 6.05-6.18 (m, 1 H, HC(O)CHC*H*), 3.76-3.86 (m, 1 H, C*H*OTBS), 3.65 (s, 3 H, COOCH_3), 2.67-2.78 (m, 1 H, C*H*COOCH$_3$), 2.39-2.55 (m, 3 H, CH_2CH_2CH, CH_2CHOTBS), 1.57-1.97 (m, 6 H, CH_2CH_2CH, CH_2CHCOOCH$_3$), 0.88 (s, 9 H, OSi(CH$_3$)$_2$C(CH_3)$_3$), 0.07 (s, 3 H, OSi(CH_3)$_2$C(CH$_3$)$_3$), 0.06 (s, 3 H, OSi(CH_3)$_2$C(CH$_3$)$_3$).

(1*R*,2*R*)-methyl 2-{(1*S*,5*R*,6*R*,*E*)-1-[(*tert*-butyldimethylsilyl)oxy]-5-hydroxy-6-methyl-7-oxo-7-(phenylthio)hept-3-en-1-yl}cyclopentanecarboxylate



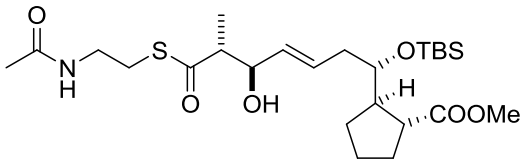
Chemical Formula: $C_{27}H_{42}O_5SSi$
Molecular Weight: 506.77

613 µL (0.61 mmol, 3.6 equiv., 1 M in hexane) chlorodicyclohexylborane were dissolved in 7 mL Et_2O and cooled to -78 °C. 81 µL (0.75 mmol, 4.4 equiv.) dimethylethylamine and 70 mg (0.42 mmol, 2.5 equiv.) thiophenolpropionate were added dropwise and the reaction was stirred for 2 h at 0 °C. The reaction mixture was cooled to -78 °C and 60 mg (0.17 mmol, 1 equiv.) of the unsaturated aldehyde were added. The reaction was stirred for 1 h at -78 °C and then stored in a freezer at -20 °C for 18 h. 2 mL methanol, 2 mL phosphate buffer (pH 7) and 2 mL H_2O_2 (35%) were added to the solution and the mixture was stirred for 1 h at ambient temperature. The aqueous layer was three times extracted with

CH_2Cl_2 and the combined organic layers were dried over $MgSO_4$. The solvent was removed *in vacuo* and the crude product was purified by flash chromatography on silica gel (petroleum ether:EtOAc (100:1) then petroleum ether:EtOAc (50:1) and petroleum ether:EtOAc (20:1)). After drying *in vacuo*, 50 mg (98.8 µmol) of a colourless oil, which consisted of an inseparable, approximately 1:1 mixture of both *anti*-aldol products, were obtained (57% yield over two steps).

R_f = 0.17 (PE/EtOAc 9:1); **^{1}H NMR** (400 MHz, $CDCl_3$) δ 7.42 (s, 5 H, C*H*(ar)), 5.68-5.77 (m, 1 H, CH_3CH=CH), 5.42-5.42 (m, 1 H, CH_3CH=C*H*), 4.26 (dd, J_1 = 7.5 Hz, J_2 = 7.5 Hz, 1 H, C*H*OH), 3.64-3.69 (m, 1 H, C*H*OTBS), 3.66 (s, 3 H, OCH_3), 2.87 (dq, J_1 = 7.5 Hz, J_2 = 7.0 Hz, 1 H, CH_3C*H*), 2.73-2.79 (m, 1 H, CH_2CH_2C*H*), 2.44-2.51 (m, 1 H, C*H*$COOCH_3$), 2.22-2.26 (m, 1 H, CH=CHCH_2), 1.83-1.92 (m, 1 H, C$H_2$$CHCOOCH_3$), 1.71-1.82 (m, 1 H, C$H_2$$CHCOOCH_3$, C$H_2$$CH_2$CH), 1.59-1.66 (m, 2 H, CH_2CH_2CH), 1.35-1.44 (m, 1 H, C$H_2$$CH_2$CH), 1.24 (d, J = 7.0 Hz, 3 H, CH_3CH), 0.88 (s, 9 H, $OSi(CH_3)_2C(CH_3)_3$), 0.06 (s, 3 H, $OSi(CH_3)_2C(CH_3)_3$), 0.05 (s, 3 H, $OSi(CH_3)_2C(CH_3)_3$); **^{13}C NMR** (100 MHz, $CDCl_3$) δ 201.3 (q, 1 C, S*C*O), 177.7 (q, 1 C, *C*$OOCH_3$), 134.4 (t, 2 C, Ar*C*), 132.3 (t, 1 C, *C*H=$CHCH_2$), 130.2 (t, 1 C, CH=*C*HCH_2), 129.4 (t, 2 C, Ar*C*), 129.2 (t, 1 C, Ar*C*), 127.5 (q, 1 C, Ar*C*), 75.1 (t, 1 C, *C*HOH), 74.4 (t, 1 C, *C*HOTBS), 53.7 (t, 1 C, CH_3*C*H), 51.7 (p, 1 C, $COOCH_3$), 47.2 (t, 1 C, *C*H$COOCH_3$), 44.4 (t, 1 C, CH_2CH_2*C*H), 38.9 (s, 1 C, CH=CH*C*H_2), 32.1 (s, 1 C, *C*$H_2CHCOOCH_3$), 30.1 (s, 1 C, CH_2*C*H_2CH), 26.1 (s, 1 C, *C*H_2CH_2CH), 25.9 (p, 3 C, $OSi(CH_3)_2C(CH_3)_3$), 18.0 (q, 1 C, $OSi(CH_3)_2C(CH_3)_3$), 15.0 (p, 1 C, *C*H_3CH), -4.1 (p, 1 C, $OSi(CH_3)_2C(CH_3)_3$), -4.7 (p, 1 C, $OSi(CH_3)_2C(CH_3)_3$); **HRMS (ESI)** *m/z* calculated for $C_{27}H_{42}O_5SSiNa$ $[M+Na]^+$: 529.2420, found: 529.2422.

(1*R*,2*R*)-methyl 2-[(5*S*,9*R*,10*R*,*E*)-9-hydroxy-2,2,3,3,10-pentanmethyl-11,16-dioxo-4-oxa-12-thia-15-aza-3-silaheptadec-7-en-5-yl]cyclopentanecarboxylate



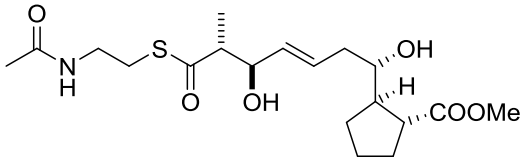
Chemical Formula: $C_{25}H_{45}NO_6SSi$
Molecular Weight: 515.78

10 mg (20 µmol, 1.0 equiv.) of the aldol product, 16 µL (91 µmol, 4.6 equiv.) DIPEA and 20 µL (188 µmol, 9.4 equiv.) HSNAC were dissolved in 1 mL DMF and stirred for 18 h at room temperature. 5 mL brine were added and the mixture was stirred for further 5 min. The mixture was three times extracted with Et_2O, the organic layers were washed with brine and dried over $MgSO_4$. The solvent was removed *in vacuo* and the crude product was purified by HPLC (HPLC (C18-ISIS) (H_2O:MeOH = 80:20 {10 min}, gradient H_2O:MeOH = 80:20 → 45:55 {30 min}, gradient H_2O:MeOH = 45:55 → 20:80 {25 min}, gradient H_2O:MeOH = 20:80 → 10:90 {30 min}, gradient H_2O:MeOH = 10:90 → 0:100 {5 min}, 2.5 mL/min → 3.5 mL/min). After drying *in vacuo*, 7.2 mg (14 µmol, t_R = 72.5 min) of a colourless oil were obtained (70% yield).

^{1}H NMR (500 MHz, $CDCl_3$) δ 5.93-6.01 (m, 1 H, N*H*), 5.69-5.79 (m, 1 H, CH=C*H*CH_2), 5.37-5.47 (m, 1 H, C*H*=$CHCH_2$), 4.22-4.26 (m, 1 H, C*H*OH), 3.67-3.71 (m, 1 H, C*H*OTBS), 3.68 (d, J = 5.6 Hz, 1 H, OMe), 3.42-3.56 (m, 2 H, NHCH_2), 3.04-3.14 (m, 2 H, CH_2S), 2.75-2.81 (m, 2 H, $CHCH_3$, C*H*COOH),

2.45-2.51 (m, 1 H, C*H*), 2.20-2.30 (m, 1 H, OH), 1.98 (s, 3 H, CHCH_3), 1.88-1.95 (m, 1 H, 1x CH_2CHCOOH), 1.73-1.82 (m, 2 H, 1x CH_2CHCOOH, 1x CH_2CH), 1.63-1.68 (m, 2 H, CH_2CH$_2$CH), 1.37-1.45 (m, 1 H, 1x CH_2CH), 1.16 (d, J = 7.0 Hz, 3 H, CH_3CO), 0.90 (s, 9 H, OSi(CH$_3$)$_2$C(CH_3)$_3$), 0.08 (dd, J_1 = 2.8 Hz, J_2 = 1.7 Hz, 6 H, OSi(CH_3)$_2$C(CH$_3$)$_3$); **^{13}C NMR** (125 MHz, $CDCl_3$) δ 203.4 (q, 1 C, S*C*O), 177.8 (q, 1 C, *C*OOCH$_3$), 170.4 (q, 1 C, CH$_3$*C*ONH), 132.4 (t, 1 C, *C*H=CHCH$_2$), 130.5 (t, 1 C, CH=*C*HCH$_2$), 75.4 (t, 1 C, *C*HOTBS), 74.2 (t, 1 C, *C*HOH), 54.1 (t, 1 C, *C*HCH$_3$), 51.6 (p, 1 C, OMe), 47.2 (t, 1 C, *C*HCOOH), 44.5 (t, 1 C, *C*H), 39.4 (s, 1 C, NH*C*H$_2$), 38.8 (s, 1 C, CH=CH*C*H$_2$), 32.1 (s, 1 C, *C*H$_2$CHCOOH), 30.2 (s, 1 C, CH$_2$*C*H$_2$CH), 26.1 (s, 1 C, *C*H$_2$S), 25.8 (p, 3 C, OSi(CH$_3$)$_2$C(*C*H$_3$)$_3$), 23.3 (s, 1 C, *C*H$_2$CH$_2$CH), 18.0 (q, 1 C, OSi(CH$_3$)$_2$*C*(CH$_3$)$_3$), 15.0 (p, 1 C, CH*C*H$_3$), -4.1 (p, 2 C, OSi(*C*H$_3$)$_2$C(CH$_3$)$_3$); **HRMS (ESI)** *m/z* calculated for $C_{25}H_{46}NO_6SSi$ $[M+H]^+$: 516.2815, found: 516.2820.

(1*R*,2*R*)-methyl 2-{(1*S*,5*R*,6*R*,*E*)-7-[(2-acetamidoethyl)thio)-1,5-dihydroxy-6-methyl-7-oxo hept-3-en-1-yl}cyclopentancarboxylate

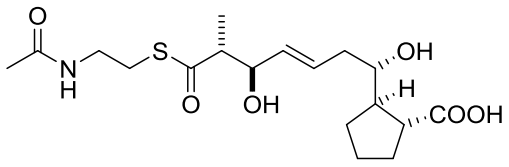


Chemical Formula: $C_{19}H_{31}NO_6S$
Molecular Weight: 401.52

30 mg (58 µmol, 1.0 equiv.) of the *bis*-protected SNAc ester were dissolved in a mixture of THF:HCOOH:H_2O (6:3:1, 2 mL) and stirred for 48 h at ambient temperature. 5 mL saturated $NaHCO_3$ solution were added and the aqueous layer was three times extracted with EtOAc. The organic layers were dried over $MgSO_4$ and the solvent was removed under reduced pressure. Free TBS side products were removed by azeotropic distillation with toluene and the crude product was used in the next step without further purification.

HRMS (ESI) *m/z* calculated for $C_{19}H_{31}NO_6S$ $[M+H]^+$: 402.1950, found: 402.1936.

(1*R*,2*R*)-2-{(1*S*,5*R*,6*R*,*E*)-7-[(2-acetamidoethyl)thio)-1,5-dihydroxy-6-methyl-7-oxohept-3-en-1-yl}cyclopentanecarboxylic acid (**17a** and **17b**)



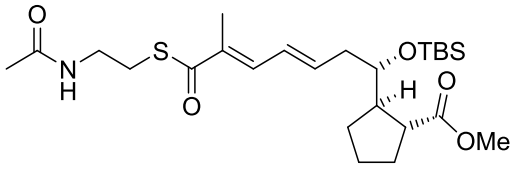
Chemical Formula: $C_{18}H_{29}NO_6S$
Molecular Weight: 387.49

The crude material (50 µmol) was dissolved in 2 mL phosphate buffer (pH 8) and 100 units of porcine liver esterase were added. It was stirred for 5 d at room temperature during which the course of the reaction was monitored by LC-MS. The reaction mixture was three times extracted with EtOAc as well as EtOAc/*i*PrOH (3:1, 1.5 mL) and the solvent was removed *in vacuo*. The crude product was purified by

preparative HPLC (C18-ISIS) (H_2O:MeCN = 80:20 {10 min}, gradient H_2O:MeCN = 80:20 → 0:100 {80 min}, H_2O:MeCN = 0:100 {10 min}, 15 mL/min). After drying *in vacuo*, 18 mg (46.5 µmol, t_R = 40.0 min) of the colourless oil, which contains a mixture of **17a** and **17b**, were obtained with full conversion and an isolated yield of 81% over two steps.

^{1}H NMR (400 MHz, CD_2Cl_2) δ 6.07 (bs, 1 H, NH), 5.72-5.81 (m, 1 H, CH=C*H*CH_2), 5.65 (dd, J_1 = 15.4 Hz, J_2 = 6.5 Hz, 1 H, C*H*=$CHCH_2$), 4.24-4.31 (m, 1 H, C*H*OH), 3.53-3.60 (m, 1 H, CH_2C*H*OH), 3.38-3.48 (m, 2 H, NHC*H*$_2$), 3.04-3.11 (m, 1 H, 1x C*H*$_2$S), 2.93-3.01 (m, 1 H, 1x C*H*$_2$S), 2.84-2.90 (m, 1 H, C*H*CH_3), 2.75-2.81 (m, 1 H, C*H*COOH), 2.44-2.51 (m, 1 H, 1x CH=CHC*H*$_2$), 2.05-2.24 (m, 3 H, 1x CH=CHC*H*$_2$, C*H*, 1x C*H*$_2$CH), 1.97 (s, 3 H, C*H*$_3$CO), 1.80-1.92 (m, 2 H, C*H*$_2$CHCOOH), 1.58-1.74 (m, 2 H, C*H*$_2$$CH_2$CH), 1.26-1.34 (m, 1 H, CH_2C*H*$_2$CH), 1.22 (d, J = 7.2 Hz, 3 H, CHC*H*$_3$); **^{13}C NMR** (100 MHz, CD_2Cl_2) δ 203.0 (q, 1 C, S*C*O), 176.6 (q, 1 C, *C*OOH), 171.2 (q, 1 C, CH_3*C*ONH) , 134.2 (t, 1 C, *C*H=$CHCH_2$), 128.4 (t, 1 C, CH=*C*HCH_2), 75.9 (t, 1 C, CH_2*C*HOH), 74.8 (t, 1 C, *C*HOH), 54.2 (t, 1 C, *C*HCH_3), 48.9 (t, 1 C, *C*H), 48.2 (t, 1 C, *C*HCOOH), 39.1 (s, 1 C, NH*C*H_2), 38.9 (s, 1 C, CH=CH*C*H_2), 30.6 (s, 1 C, *C*H_2CHCOOH), 29.3 (s, 1 C, CH_2*C*H_2CH), 28.8 (s, 1 C, CH_2*C*H_2S), 25.7 (s, 1 C, *C*$H_2$$CH_2$CH), 22.9 (p, 1 C, *C*$H_3$CO), 14.0 (p, 1 C, CH*C*$H_3$); **HRMS (ESI)** *m/z* calculated for $C_{18}H_{30}NO_6S$ [M+H]+: 388.1794, found: 388.1794.

(1R,2R)-methyl 2-((S,7E,9E)-2,2,3,3,10-pentamethyl-11,16-dioxo-4-oxa-12-thia-15-aza-3-silaheptadeca-7,9-dien-5-yl)cyclopentanecarboxylate



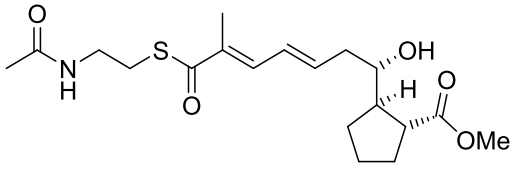
Chemical Formula: $C_{25}H_{43}NO_5SSi$
Molecular Weight: 497.77

5 mg (14.2 µmol, 1 equiv.) of **16** were subjected to metathesis reaction as described before. The product from this reaction was dissolved in 1 mL of CH_2Cl_2 and 7.2 mg (16.5 µmol, 1.2 equiv.) of **21** were added. The solution was stirred at 50 °C overnight and the solvent was removed under reduced pressure. The crude material was purified by flash chromatography on silica gel (petroleum ether:EtOAc (1:1)). After drying *in vacuo*, 6.4 mg (12.5 µmol) of a colourless oil were obtained (88% yield over two steps).

R_f = 0.11 (PE/EtOAc 9:1); **$[\alpha]_D^{23}$** = +8.0 (*c* = 0.6, CH_2Cl_2); **^{1}H NMR** (500 MHz, Acetone-d_6) δ 7.17 (d, J = 11.1 Hz, 1 H, C=C*H*CH), 6.53 (dd, J_1 = 11.1 Hz, J_2 = 15.0 Hz, 1 H, C*H*=$CHCH_2$), 6.24-6.31 (m, 1 H,CH=C*H*CH_2), 3.86 (q, J = 5.7 Hz, 1 H, C*H*OTBS), 3.62 (s, 3 H, OMe), 3.32 (q, J = 6.5 Hz, 2 H, NHC*H*$_2$), 3.01-3.07 (m, 2 H, C*H*$_2$S), 2.75-2.85 (m, 1 H, C*H*COOMe), 2.43-2.53 (m, 3 H, CH, CH=CHC*H*$_2$), 1.96 (s, 3 H, C*H*$_3$CO), 1.86 (s, 3 H, CHC*H*$_3$), 1.82-1.86 (m, 2 H, C*H*$_2$CHCOOH), 1.68-1.82 (m, 1 H, 1x CH_2C*H*$_2$CH), 1.55-1.67 (m, 2 H, C*H*$_2$$CH_2$CH), 1.42-1.52 (m, 1H, 1x CH_2C*H*$_2$CH), 0.90 (s, 9 H, OSi(CH_3)$_2$C(C*H*$_3$)$_3$), 0.11 (2 s, 6 H, OSi(C*H*$_3$)$_2$C(CH_3)$_3$); **^{13}C NMR** (125 MHz, Acetone-d_6) δ 192.9 (1 C, q, S*C*O), 177.4 (1 C, q, *C*OOCH$_3$), 170.1 (1 C, q, CH_3*C*ONH), 141.4 (1 C, t, CH=*C*HCH_2), 138.0 (1 C, t, *C*HCH=CH), 134.0 (1 C, q, CH_3*C*) 128.9 (1 C, t, *C*H=$CHCH_2$), 75.2 (1 C, t, CH_2*C*HOTBS), 51.8 (1 C, p, COO*C*H$_3$), 48.7 (1 C, t, *C*H), 45.5 (1 C, t, *C*HCOOCH$_3$), 40.5 (1 C, s, *C*H_2CHOTBS), 39.7 (1 C, s, NH*C*H_2), 32.6 (1 C, s, *C*H_2CHCOOCH$_3$), 30.6 (1 C, s, *C*H_2S), 29.2 (1 C, s, CH_2*C*H_2CH), 26.7 (1 C, s,

CH_2CH_2CH), 26.3 (3 C, p, OSi(CH_3)$_2$C(*C*H_3)$_3$), 22.8 (1 C, p, *C*H_3C), 18.6 (1 C, q, OSi(CH_3)$_2$*C*(CH_3)$_3$), 12.7 (1 C, p, *C*H_3CONH), -3.9 (1 C, p, OSi(*C*H_3)$_2$C(CH_3)$_3$), -4.5 (1 C, p, OSi(*C*H_3)$_2$C(CH_3)$_3$); **HRMS (ESI)** *m/z* calculated for $C_{25}H_{44}NO_5SSi$ $[M+H]^+$: 498.2709, found: 498.2688.

(1R,2R)-methyl 2-((S,3E,5E)-7-((2-acetamidoethyl)thio)-1-hydroxy-6-methyl-7-oxohepta-3,5-dien-1-yl)cyclopentanecarboxylate



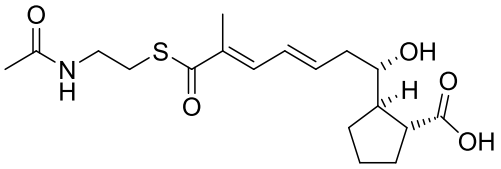
Chemical Formula: $C_{19}H_{29}NO_5S$
Molecular Weight: 383.50

3.2 mg (6.4 µmol, 1 equiv.) of the TBS-protected precursor were dissolved in 2 mL of THF : HCOOH : H_2O (6 : 3 : 1) and stirred at room temperature. After two days, the reaction was quenched by the addition of saturated $NaHCO_3$ solution. After extraction with EtOAc, the combined organic layers were dried over $MgSO_4$ and the solvent removed under reduced pressure. The crude product, which was obtained as a colourless oil, was analysed by ^{1}H NMR spectroscopy and directly subjected to esterase-catalysed deprotection.

The crude material from another entry was purified by HPLC for full spectroscopic characterisation (C18-P) (H_2O : MeOH = 90:10 {5 min}, gradient H_2O:MeOH = 90:10 → 45:55 {45 min}, gradient H_2O:MeOH = 45:55 → 0:100 {30 min}, H_2O:MeOH = 0:100 {20 min}, 2.25 mL/min).

$[\alpha]_D^{23}$ = -1.0 (*c* = 0.1, CH_2Cl_2); **^{1}H NMR** (500 MHz, acetone-d_6) δ 7.26 (bs, 1 H, N*H*), 7.16 (d, *J* = 11.7 Hz, 1 H, C=C*H*CH), 6.54 (dd, J_1 = 13.7 Hz, J_2 = 11.66 Hz, 1 H, C*H*=CHCH$_2$), 6.29-6.37 (m, 1 H, CH=C*H*CH$_2$), 3.61 (s, 3 H, OMe), 3.57-3.63 (m, 1 H,C*H*OH), 3.29-3.36 (m, 2 H, C*H*$_2$NH), 3.02-3.07 (m, 2 H, C*H*$_2$S), 2.75-2.78 (m, 1 H, C*H*COOCH$_3$), 2.42-2.52 (m, 1 H, 1x CH=CHC*H*$_2$), 2.29-2.39 (m, 2 H, 1x CH=CHC*H*$_2$, C*H*CHOH), 1.95 (s, 3 H, C*H*$_3$CO), 1.87-1.93 (m, 1 H, 1x C*H*$_2$CHCOOCH$_3$), 1.85 (s, 3 H, C*H*$_3$C=CH), 1.70-1.80 (m, 2 H, 1x C*H*$_2$CHCOOCH$_3$, 1x CH$_2$C*H*$_2$CH), 1.61-1.68 (m, 2 H, C*H*$_2$CH$_2$CH), 1.41-1.50 (m, 1 H, 1x CH$_2$C*H*$_2$CH); **^{13}C NMR** (125 MHz, acetone-d_6) δ 192.9 (1 C, q, S*C*O), 177.7 (1 C, q, *C*OOCH$_3$), 170.0 (1 C, q, *C*ONH), 142.7 (1 C, t, CH=*C*HCH$_2$), 138.3 (1 C, t, C*C*H=CH), 133.8 (1 C, q, *C*CH=CH), 128.4 (1 C, t, CH*C*H=CH), 74.5 (1 C, t, *C*HOH), 51.7 (1 C, p, COO*C*H$_3$), 50.2 (1 C, t, *C*HCHOH), 46.7 (1 C, t, *C*HCOOCH$_3$), 41.1 (1 C, s, CH=CH*C*H$_2$), 39.8 (1 C, s, *C*H$_2$NH), 32.2 (1 C, s, *C*H$_2$CHCOOCH$_3$), 30.5 (1 C, s, CH$_2$*C*H$_2$CH), 29.1 (1 C, s, *C*H$_2$S), 26.4 (1 C, s, *C*H$_2$CH$_2$CH), 22.9 (1 C, p, *C*H$_3$C), 12.7 (1 C, p, *C*H$_3$CO); **HRMS (ESI)** *m/z* calculated for $C_{19}H_{29}NO_5SNa$ $[M+Na]^+$: 406.1634, found: 406.1633.

(1R,2R)-2-((S,3E,5E)-7-((2-acetamidoethyl)thio)-1-hydroxy-6-methyl-7-oxohepta-3,5-dien-1-yl)cyclopentane-1-carboxylic acid (**18**)

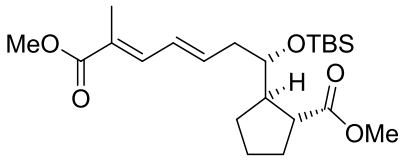


Chemical Formula: $C_{18}H_{27}NO_5S$
Molecular Weight: 369.48

The crude material from the TBS deprotection was dissolved in 1 mL phosphate buffer (pH 8) and 100 units of porcine liver esterase were added. It was stirred for 3 d at ambient temperature during which the course of the reaction was monitored by LC-MS. After conversion was complete, the crude material was purified by flash chromatography on C_{18}-reversed phase silica gel (H_2O then MeCN:H_2O (1:4)). After drying *in vacuo*, 1.6 mg (4.3 µmol) of the colourless oil **18** were obtained (67% yield over two steps). Compound **18** showed partial decomposition after chromatography.

^{1}H NMR (400 MHz, $CDCl_3$) δ 7.17 (d, J = 11.1 Hz, 1 H, C=C*H*CH), 6.48 (dd, J_1 = 11.1 Hz, J_2 = 15.0 Hz, 1 H, CHC*H*=CH), 6.17-6.27 (m, 1 H, CH=C*H*CH_2), 5.92-6.06 (m, 1 H, N*H*), 4.68 (s, 1 H, O*H*), 3.58-3.67 (m, 1 H,C*H*OH), 3.46 (dt, 2 H, J_1 = 6.4 Hz, J_2 = 5.9 Hz, CH_2NH), 3.09 (t, 2 H, J = 6.3 Hz, CH_2S), 2.77 (dt, 1 H, J_1 = 6.8 Hz, J_2 = 7.9 Hz, C*H*$COOCH_3$), 2.54-2.64 (m, 1 H, 1x CH=CHCH_2), 2.04-2.38 (m, 3 H, 1x CH=CHCH_2, C*H*CHOH, 1x CH_2CHCOOCH_3), 1.95-2.02 (2x s, 6 H, CH_3CO, CH=CCH_3), 1.80-1.94 (m, 2 H, 1x CH_2CHCOOCH_3, 1x CH_2CH_2CH), 1.61-1.73 (m, 2 H, C$H_2$$CH_2$CH), 1.27-1.33 (m, 1 H, 1x CH_2CH_2CH); **^{13}C NMR** (100 MHz, $CDCl_3$) δ 193.9 (1 C, q, S*C*O), 178.1 (1 C, q, *C*OOH), 170.7 (1 C, q, *C*ONH), 139.5 (1 C, t, CH=*C*HCH_2), 137.3 (1 C, t, C*C*H=CH), 133.9 (1 C, q, *C*CH=CH), 129.1 (1 C, t, CH*C*H=CH), 75.9 (1 C, t, *C*HOH), 49.2 (1 C, t, *C*HCHOH), 48.3 (1 C, t, *C*HCOOH), 40.5 (1 C, s, CH=CH*C*H_2), 40.0 (1 C, s, *C*H_2NH), 30.7 (1 C, s, CH_2*C*H_2CH), 29.9 (1 C, s, *C*H_2CHCOOH), 28.7 (1 C, s, *C*H_2S), 25.8 (1 C, s, *C*$H_2$$CH_2$CH), 23.4 (1 C, p, *C*$H_3$C), 12.9 (1 C, p, *C*$H_3$CO); **HRMS (ESI)** *m/z* calculated for $C_{18}H_{26}NO_5S$ $[M-H]^-$: 368.1532, found: 368.1523.

(1R,2R)-methyl 2-{(S,3E,5E)-1-[(*tert*-butyldimethylsilyl)oxy]-7-methoxy-6-methyl-7-oxo-hepta-3,5-diene-1-yl}cyclopentane carboxylate

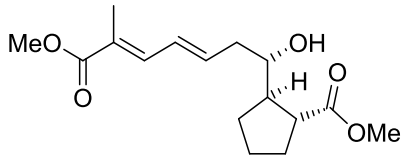


Chemical Formula: $C_{22}H_{38}O_5Si$
Molecular Weight: 410.63

8.8 mg (28 µmol, 1.0 equiv.) of olefin **16** were subjected to olefin cross metathesis with crotonaldehyde and second generation Grubbs catalyst and purified as described previously. The resulting unsaturated aldehyde was solved in 2 mL CH_2Cl_2 and 11 mg (31 µmol, 1.1 equiv) of **22** were added. The resulting solution was stirred at 50 °C for 21 h. The solvent was removed under reduced pressure and the crude product was purified by flash chromatography on C18-reversed phase silica gel (MeCN:H_2O (2:3)). After drying *in vacuo*, 7.4 mg (18 µmol) of a colourless oil, the *E,E*-configured methyl ester, were obtained (64% yield over two steps).

R_f = 0.4 (PE/EtOAc 9:1); **$[\alpha]_D^{23}$** = +5.8 (*c* = 0.7, CH_2Cl_2); **^{1}H NMR** (500 MHz, benzene-d_6) δ 7.46 (d, *J* = 11.4 Hz, 1 H, C=C*H*CH), 6.37 (dd, J_1 = 12.5 Hz, J_2 = 13.9 Hz, 1 H, C*H*=CHCH$_2$), 5.90-5.96 (m, 1 H, CH=C*H*CH$_2$), 3.53-3.57 (m, 1 H, C*H*OTBS), 3.44 (s, 3 H, OCH_3), 3.37 (s, 3 H, OCH_3), 2.81-2.87 (m, 1 H, C*H*COOCH$_3$), 2.58-2.64 (m, 1 H, CH$_2$CH$_2$C*H*), 2.29-2.38 (m, 1 H, CH_2CHOTBS), 2.21-2.26 (m, 1 H, CH_2CHOTBS), 1.99 (s, 3 H, CH_3CCH), 1.78-1.88 (m, 2 H, CH_2CHCOOCH$_3$), 1.62-1.70 (m, 1 H, 1x CH$_2$CH_2CH), 1.55-1.62 (m, 1 H, 1x CH_2CH$_2$CH), 1.47-1.54 (m, 1 H, CH_2CH$_2$CH), 1.27-1.32 (m, 1 H, CH$_2$CH_2CH), 0.94 (s, 9 H, OSi(CH$_3$)$_2$C(CH_3)$_3$), 0.04 (s, 3 H, OSi(CH_3)$_2$C(CH$_3$)$_3$), 0.01 (s, 3 H, OSi(CH_3)$_2$C(CH$_3$)$_3$); **^{13}C NMR** (125 MHz, benzene-d_6) δ 176.8 (q, 1 C, CH*C*OOCH$_3$), 168.5 (q, 1 C, C*C*OOCH$_3$), 138.6 (t, 1 C, CH=*C*HCH$_2$), 138.5 (t, 1 C, C*C*H=CH), 128.9 (t, 1 C, *C*H=CHCH$_2$), 126.2 (q, 1 C, CH$_3$*C*CH), 74.7 (t, 1 C, *C*HOTBS), 51.4 (p, 1 C, O*C*H$_3$), 51.3 (p, 1 C, O*C*H$_3$), 48.2 (t, 1 C, *C*HCOOCH$_3$), 44.8 (t, 1 C, CH$_2$CH$_2$*C*H), 40.3 (s, 1 C, *C*H$_2$CHOTBS), 32.3 (s, 1 C, *C*H$_2$CHCOOCH$_3$), 30.3 (s, 1 C, CH$_2$*C*H$_2$CH), 26.5 (s, 1 C, *C*H$_2$CH$_2$CH), 26.1 (p, 3 C, OSi(CH$_3$)$_2$C(*C*H$_3$)$_3$), 18.3 (q, 1 C, OSi(CH$_3$)$_2$*C*(CH$_3$)$_3$), 12.9 (p, 1 C, *C*H$_3$CCH), -3.9 (s, 1 C, OSi(*C*H$_3$)$_2$C(CH$_3$)$_3$), -4.4 (s, 1 C, OSi(*C*H$_3$)$_2$C(CH$_3$)$_3$); **HRMS (ESI)** *m/z* calculated for $C_{22}H_{39}O_5Si$ $[M+H]^+$: 411.2567, found: 411.2563.

(1*R*,2*R*)-methyl 2-{(*S*,3*E*,5*E*)-1-hydroxy-7-methoxy-6-methyl-7-oxohepta-3,5-dien-1-yl} cyclopentancarboxylat (**19**)

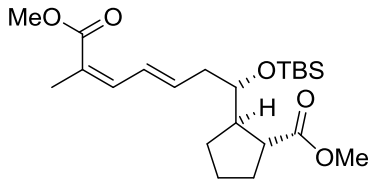


Chemical Formula: $C_{16}H_{24}O_5$
Molecular Weight: 296.36

7.4 mg (18 µmol, 1 equiv.) of the TBS-protected precursor were dissolved in 2 mL THF : HCOOH : H_2O (6 : 3 : 1) and stirred at room temperature. Monitoring of the reaction by LC-MS showed full conversion after two days and the reaction was quenched by the addition of saturated $NaHCO_3$ solution. After extraction with EtOAc, the combined organic layers were dried over $MgSO_4$ and the solvent removed under reduced pressure. The crude material was purified by flash chromatography on C_{18}-reversed phase silica gel (H_2O then MeCN:H_2O (1:4)). After drying in vacuo, 3.3 mg (11.1 µmol) of the colourless oil **19** were obtained (62% yield).

R_f = 0.21 (PE/EtOAc 9:1); **$[\alpha]_D^{23}$** = -1.0 (*c* = 0.1, CH_2Cl_2); **^{1}H NMR** (400 MHz, $CDCl_3$) δ 7.17 (d, *J* = 11.4 Hz, 1 H, C=C*H*=CH), 6.43 (dt, J_1 = 14.9 Hz, J_2 = 11.3 Hz, 1 H, C*H*=CHCH$_2$), 6.13 (dt, J_1 = 14.9 Hz, J_2 = 7.5 Hz, 1 H, CH=C*H*CH$_2$), 3.75 (s, 3 H, OCH_3), 3.70 (s, 3 H, OCH_3), 3.13-3.17 (dt, J_1 = 3.1 Hz, J_2 = 8.1 Hz, 1 H, C*H*OH), 2.71 (dt, J_1 = 8.2 Hz, J_2 = 8.2 Hz, 1 H, C*H*COOCH$_3$), 2.44-2.54 (m, 1 H, CHCH_2CHOTBS), 2.22-2.34 (m, 2 H, 1x CHCH_2CHOTBS, 1x CHC*H*CHOH), 1.93 (s, 3 H, CH_3CCH), 1.91-2.00 (m, 1 H, 1x CH_2CHCOOH), 1.81-1.91 (m, 2 H, 1x CH_2CHCOOH, 1x CH_2CH), 1.64-1.74 (m, 2 H, CH_2CH$_2$CH), 1.22-1.38 (m, 1 H, 1x CH_2CH); **^{13}C NMR** (100 MHz, $CDCl_3$) δ 177.7 (q, 1 C, CH*C*OOCH$_3$), 169.2 (q, 1 C, C*C*OOCH$_3$), 138.4 (t, 2 C, CH=*C*HCH$_2$, C*C*H=CH), 128.9 (t, 1 C, *C*H=CHCH$_2$), 126.0 (q, 1 C, CH$_3$*C*CH), 75.3 (t, 1 C, *C*HOH), 52.1 (p, 1 C, O*C*H$_3$), 51.9 (p, 1 C, O*C*H$_3$), 49.7 (t, 1 C, *C*HCOOCH$_3$), 47.8 (t, 1 C, CH$_2$CH$_2$*C*H), 40.4 (s, 1 C, CH*C*H$_2$CHOH), 31.1 (s, 1 C, *C*H$_2$CHCOOCH$_3$), 30.0 (s, 1 C, CH$_2$*C*H$_2$CH), 25.5 (s, 1 C, CH$_2$*C*H$_2$CH$_2$), 12.8 (p, 1 C, *C*H$_3$CCH); **HRMS (ESI)** *m/z* calculated for $C_{16}H_{24}O_5Na$ $[M+Na]^+$: 319.1521, found: 319.1525.

(1*R*,2*R*)-methyl 2-{(*S*,3*E*,5*Z*)-1-[(*tert*-butyldimethylsilyl)oxy]-7-methoxy-6-methyl-7-oxo-hepta-3,5-diene-1-yl}cyclopentane carboxylate

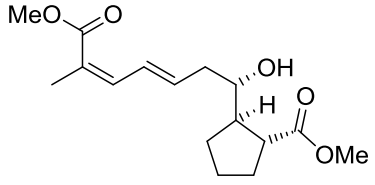


Chemical Formula: $C_{22}H_{38}O_5Si$
Molecular Weight: 410.63

190 mg (720 µmol, 12 equiv.) 18-crown-6 and 50 mg (360 µmol, 6 equiv.) K_2CO_3 were suspended in 2 mL toluene and stirred for 1 h at room temperature. The suspension was cooled to -20 °C and 20 mg (6 µmol, 1 equiv.) **23** were added. 25 mg (60 µmol, 1.0 equiv.) of the precursor aldehyde were added and the reaction was stirred for further 5 h at 0 °C. After the addition of 10 mL brine, the aqueous layer was three times extracted with Et_2O and the organic layers were dried over $MgSO_4$. The solvent was removed under reduced pressure and the crude product was purified by flash chromatography on silica gel (petroleum ether:EtOAc (50:1) then petroleum ether:EtOAc (20:1) and petroleum ether:EtOAc (10:1)). After drying *in vacuo*, 11 mg (28 µL) of a colourless oil were obtained (47% yield over two steps).

R_f = 0.6 (PE/EtOAc 9:1); **$[\alpha]_D^{23}$** = +14.4 (*c* = 0.8, CH_2Cl_2); **^{1}H NMR** (500 MHz, benzene-d_6) δ 7.64 (dd, J_1 = 12.5 Hz, J_2 = 13.8 Hz, 1 H, C*H*=CHCH$_2$), 6.27 (d, *J* = 10.9 Hz, 1 H, C=C*H*=CH), 5.93-6.01 (m, 1 H, CH=C*H*CH$_2$), 3.57-3.61 (m, 1 H, C*H*OTBS), 3.44 (s, 3 H, OCH_3), 3.40 (s, 3 H, OCH_3), 2.80-2.86 (m, 1 H, C*H*COOCH$_3$), 2.66-2.73 (m, 1 H, CH$_2$CH$_2$C*H*), 2.28-2.43 (m, 2 H, CH_2CHOTBS), 1.87 (s, 3 H, CH_3CCH), 1.77-1.86 (m, 2 H, CH_2CHCOOCH$_3$), 1.64-1.73 (m, 1 H, CH$_2$CH_2CH), 1.54-1.62 (m, 1 H, CH_2CH$_2$CH), 1.45-1.53 (m, 1 H, CH_2CH$_2$CH), 1.20-1.29 (m, 1 H, CH$_2$CH_2CH), 0.98 (s, 9 H, OSi(CH$_3$)$_2$C(CH_3)$_3$), 0.06 (s, 3 H, OSi(CH_3)$_2$C(CH$_3$)$_3$), 0.05 (s, 3 H, OSi(CH_3)$_2$C(CH$_3$)$_3$); **^{13}C NMR** (125 MHz, benzene-d_6) δ 176.9 (q, 1 C, CH*C*OOCH$_3$), 167.5 (q, 1 C, C*C*OOCH$_3$), 141.3 (t, 1 C, CH=*C*HCH$_2$), 137.5 (t, 1 C, C*C*H=CH), 130.9 (t, 1 C, *C*H=CHCH$_2$), 124.7 (q, 1 C, CH$_3$*C*CH), 75.2 (t, 1 C, *C*HOTBS), 51.3 (p, 1 C, O*C*H$_3$), 50.9 (p, 1 C, O*C*H$_3$), 48.2 (t, 1 C, *C*HCOOCH$_3$), 45.3 (t, 1 C, CH$_2$CH$_2$*C*H), 39.7 (s, 1 C, *C*H$_2$CHOTBS), 32.3 (s, 1 C, *C*H$_2$CHCOOCH$_3$), 30.2 (s, 1 C, CH$_2$*C*H$_2$CH), 26.4 (s, 1 C, *C*H$_2$CH$_2$CH), 26.2 (p, 3 C, OSi(CH$_3$)$_2$C(*C*H$_3$)$_3$), 20.9 (q, 1 C, OSi(CH$_3$)$_2$*C*(CH$_3$)$_3$), 18.3 (p, 1 C, *C*H$_3$CCH), -3.9 (s, 1 C, OSi(*C*H$_3$)$_2$C(CH$_3$)$_3$), -4.5 (s, 1 C, OSi(*C*H$_3$)$_2$C(CH$_3$)$_3$); **HRMS (ESI)** *m/z* calculated for $C_{22}H_{38}O_5NaSi$ $[M+Na]^+$: 433.2386, found: 433.2387.

(1*R*,2*R*)-methyl 2-{(*S*,3*E*,5*Z*)-1-hydroxy-7-methoxy-6-methyl-7-oxohepta-3,5-dien-1-yl} cyclopentancarboxylat (**20**)



Chemical Formula: $C_{16}H_{24}O_5$
Molecular Weight: 296.36

2 mg (7 µmol, 1 equiv.) of the TBS-protected precursor were dissolved in 1 mL of THF:HCOOH:H_2O (6:3:1) and stirred at room temperature. Monitoring of the reaction by LC-MS showed full conversion after two days and the reaction was quenched by the addition of saturated $NaHCO_3$ solution. After extraction with EtOAc, the combined organic layers were dried over $MgSO_4$ and the solvent removed under reduced pressure. The crude material was purified by HPLC (C18-$P_{[B]}$) (H_2O:MeOH = 80:20 {5 min}, gradient H_2O:MeOH = 80:20 → 0:100 {95 min}, 4 mL/min → 5 mL/min). After drying *in vacuo*, 1 mg (4 µmol, t_R=72.3 min) of the colourless oil **20** was obtained (57% yield).

$[\alpha]_D^{23}$ = +1.0 (*c* = 0.1, CH_2Cl_2); **^{1}H NMR** (500 MHz, benzene-d_6) δ 7.14-7.20 (m, 1 H, C*H*=CHCH$_2$), 6.42 (d, *J* = 11.2 Hz, 1 H, C=C*H*=CH), 5.94-6.01 (m, 1 H, CH=C*H*CH$_2$), 3.76 (s, 3 H, OC*H*$_3$), 3.69 (s, 3 H, OC*H*$_3$), 3.64-3.67 (m, 1 H, C*H*OH), 3.48-3.54 (m, 2 H, C*H*$_2$CHOTBS), 2.66-2.72 (m, 1 H, C*H*COOCH$_3$), 1.95 (s, 3 H, C*H*$_3$CCH), 1.17-1.89 (m, 6 H, 3x C*H*$_2$(cyclopentane)); **HRMS (ESI)** *m/z* calculated for $C_{16}H_{24}O_5Na$ $[M+Na]^+$: 319.1521, found: 319.1516.

Protein expression and purification

Plasmids pET28a(+)-BorDH2, pET28a(+)-BorDH3 and pET28a(+)-BorDH3-H48A were available from a previous study.[2] For protein expression, chemically competent *E. coli* BL21(DE3)-CodonPlus-RP cells were transformed with the corresponding plasmids. Single colonies were used to inoculate 50-100 mL 2TY medium (16 g tryptone (Duchefa), 10 g yeast extract (Sigma), 5 g NaCl in 1 L). Cultures were grown to an OD_{600} of 0.6 at 37 °C, protein production was induced with 0.1 mM IPTG and shaken at room temperature for 22 hours. Cells were harvested by centrifugation (4,500 rpm, 4 °C, 30 min) and stored at -20 °C.

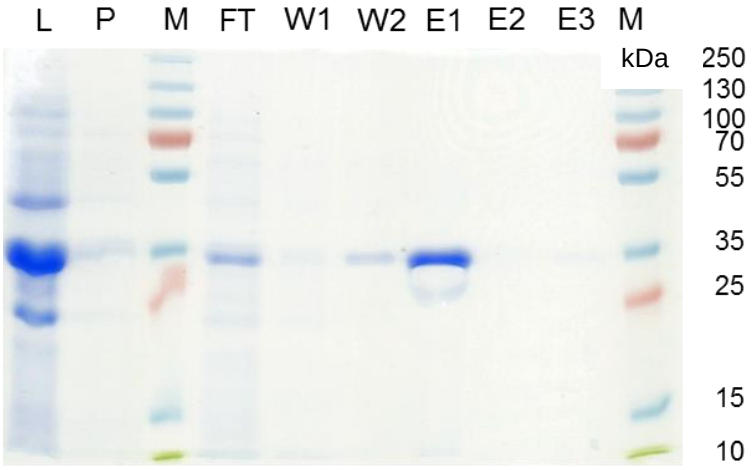


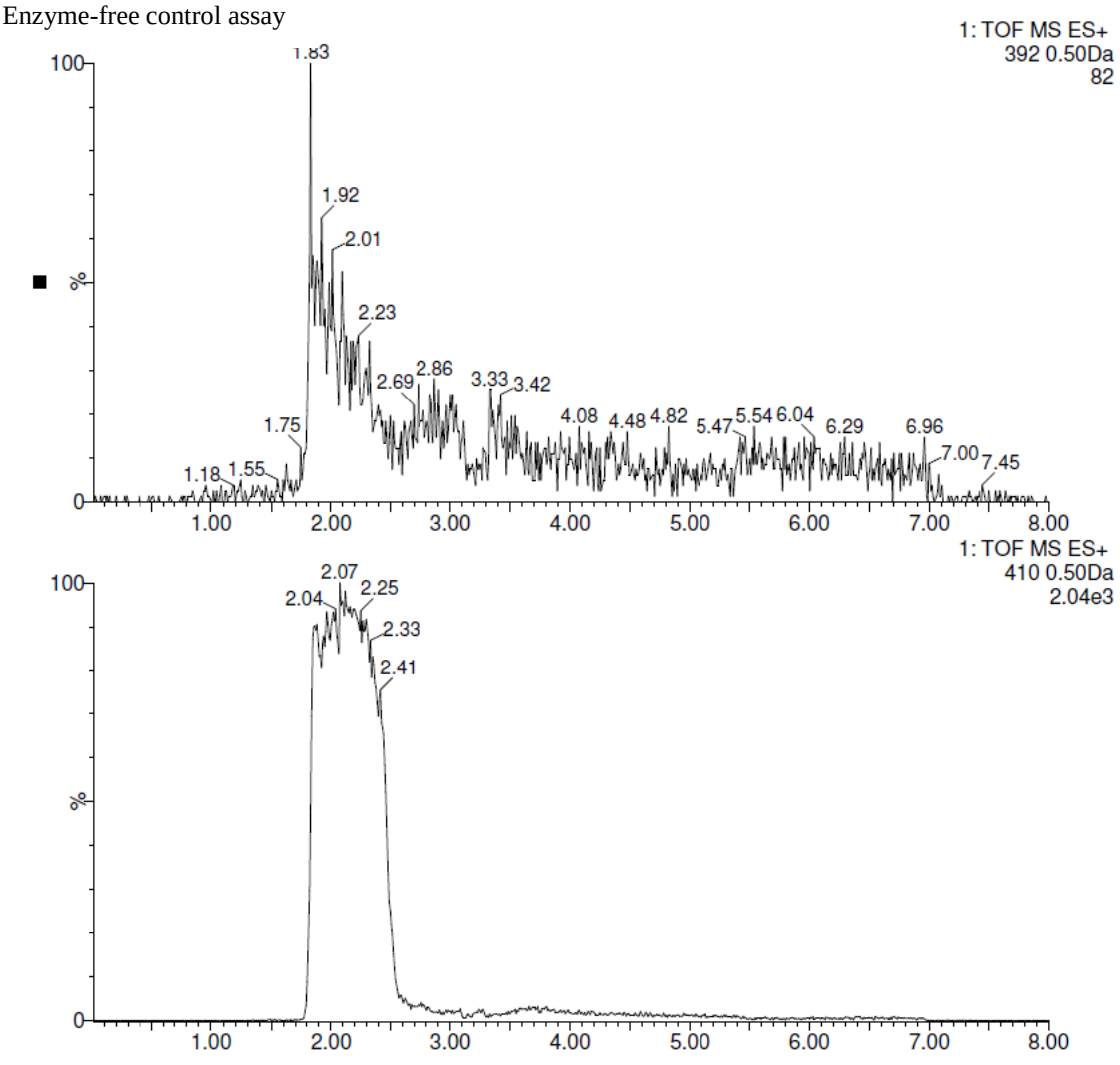
Figure S1: SDS-PAGE after protein purification by affinity chromatography; L: lysate, p: pellet, FT: flow through, W1-W2: wash steps, E1-E3: elution steps, M: Marker (PageRuler Plus Prestained Protein Ladder).

For purification, approximately 2 g cells were resuspended in 20 mL Binding Buffer (40 mM Tris-HCl, 100 mM NaCl, pH 7.8) and broken by cell disruption in a French Press. After centrifugation at 8,500 rpm for 45 min the resulting crude extract was passed through a Ni-NTA column (1-2 mL bed volume), the column was washed sequentially with each 5 column volumes of binding buffer containing 0 mM imidazole and 20 mM imidazole. The target protein was eluted with each 5 column volumes of 100 mM, 200 mM and 500 mM imidazole in Binding Buffer (Figure S1). Finally the eluate was concentrated, the buffer was replaced by Storage Buffer (25 mM Hepes, 150 mM NaCl, pH 6.8) and immediately used for activity assays. Alternatively, the protein solution was supplemented with 50% glycerol and stored at -20 °C.

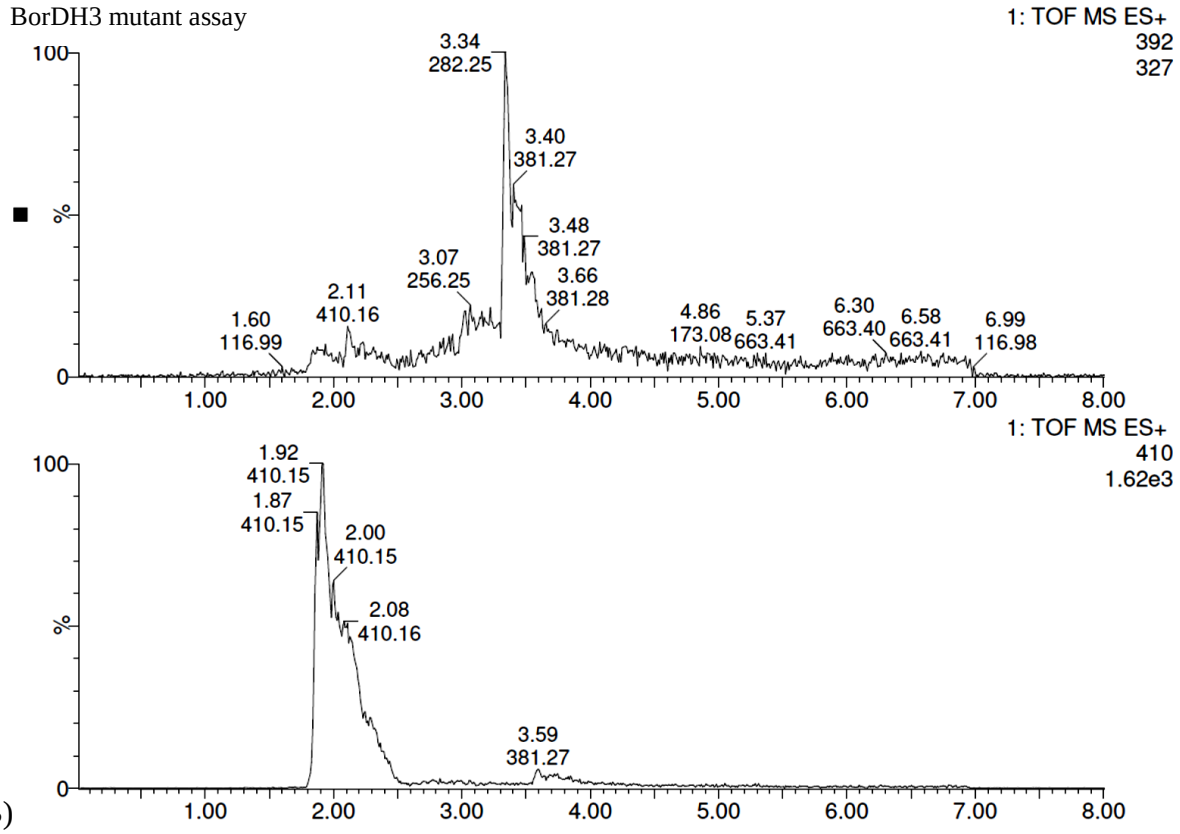
Enzyme activity assays

Activity assays were carried out in a total volume of 100 µL containing 12 mM of **17a/b** and up to 3 mg/mL of the corresponding BorDH enzyme in 25 mM HEPES (pH 7.5, 100 mM NaCl). Reactions were incubated at 37 °C for 16 h and extracted with 3 x 1 mL of ethyl acetate. After evaporation of the solvent, the sample was resuspended in 50 µL of methanol and analysed by UPLC-MS. All traces were compared to authentic standards. Reactions without enzyme or active site mutants served as controls.

A)



B)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%
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256.25
3.66
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410.16
1.60
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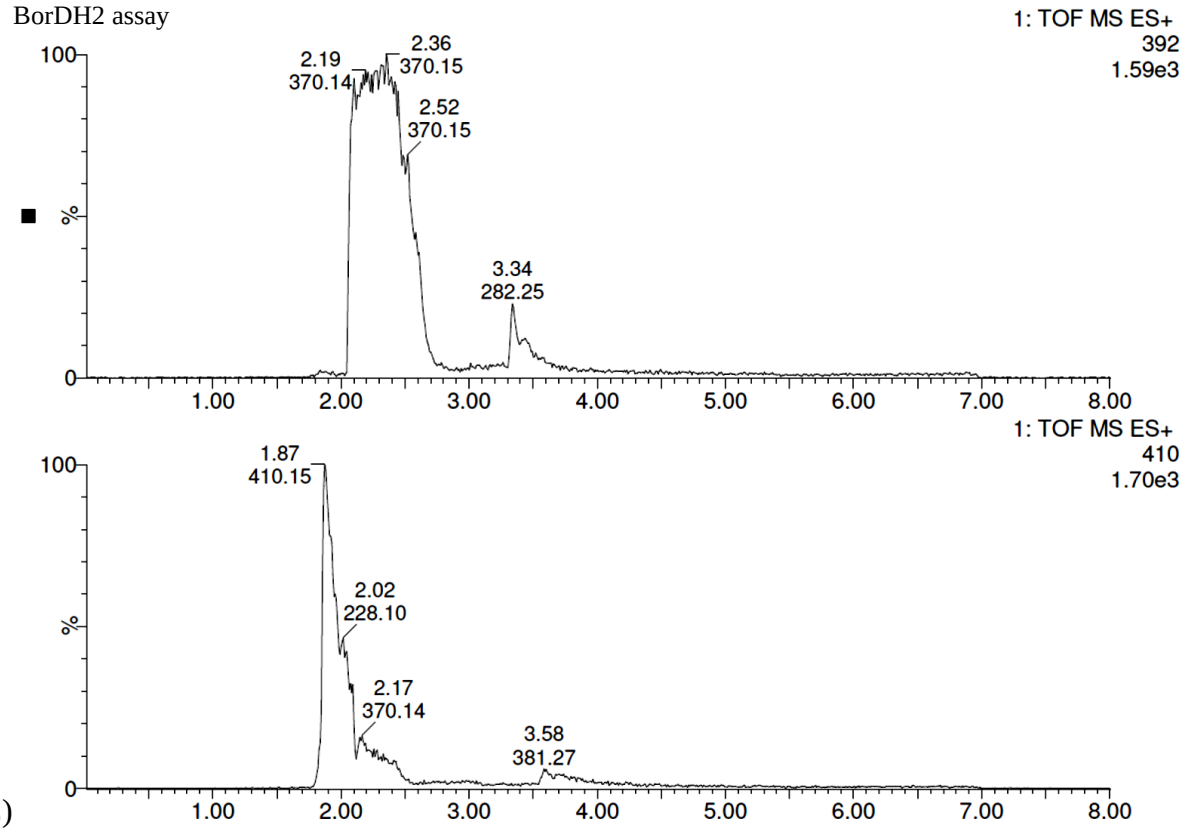
5.37
663.41
6.30
663.40
6.58
663.41
6.99
116.98
1.00
2.00
3.00
4.00
5.00
6.00
7.00
8.00
1: TOF MS ES+
410
1.62e3
100
%
0
1.92
410.15
1.87
410.15
2.00
410.15
2.08
410.16
3.59
381.27
1.00
2.00
3.00
4.00
5.00
6.00
7.00
8.00

C)


BorDH2 assay
1: TOF MS ES+
392
1.59e3
100
%
0
2.19
370.14
2.36
370.15
2.52
370.15
3.34
282.25
1.00
2.00
3.00
4.00
5.00
6.00
7.00
8.00
1: TOF MS ES+
410
1.70e3
100
%
0
1.87
410.15
2.02
228.10
2.17
370.14
3.58
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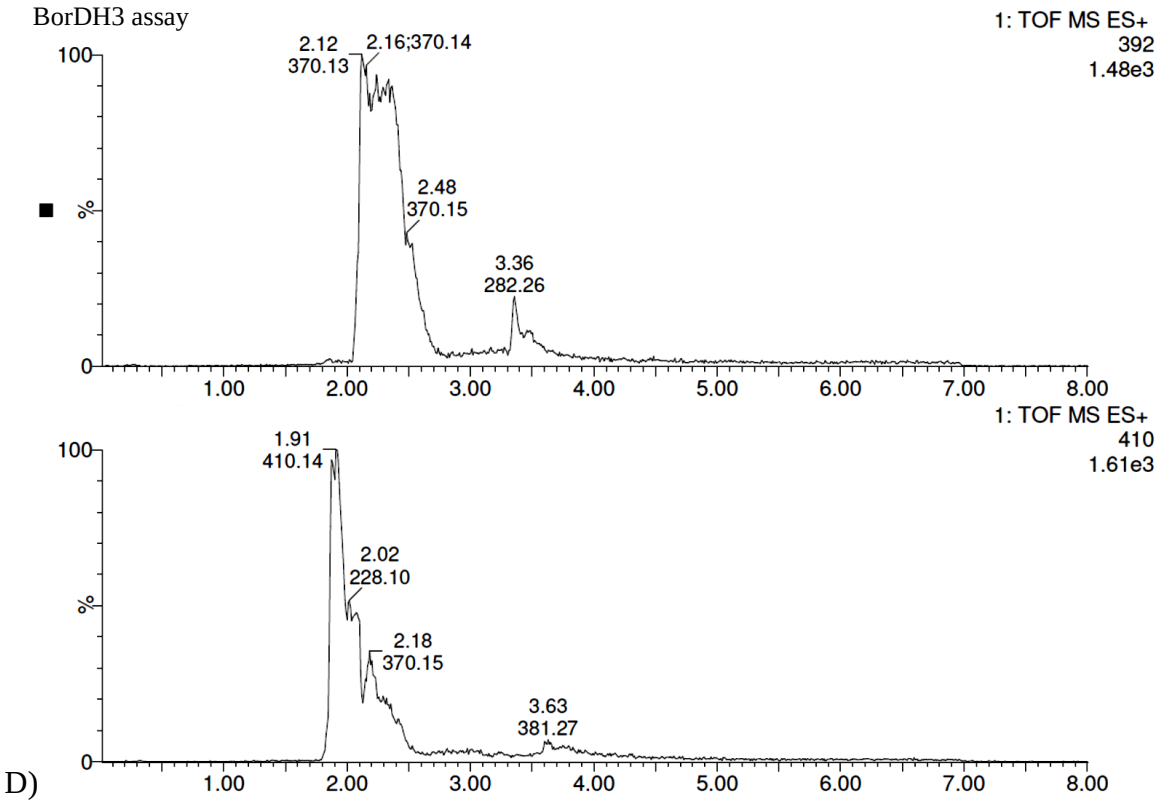
1.00
2.00
3.00
4.00
5.00
6.00
7.00
8.00

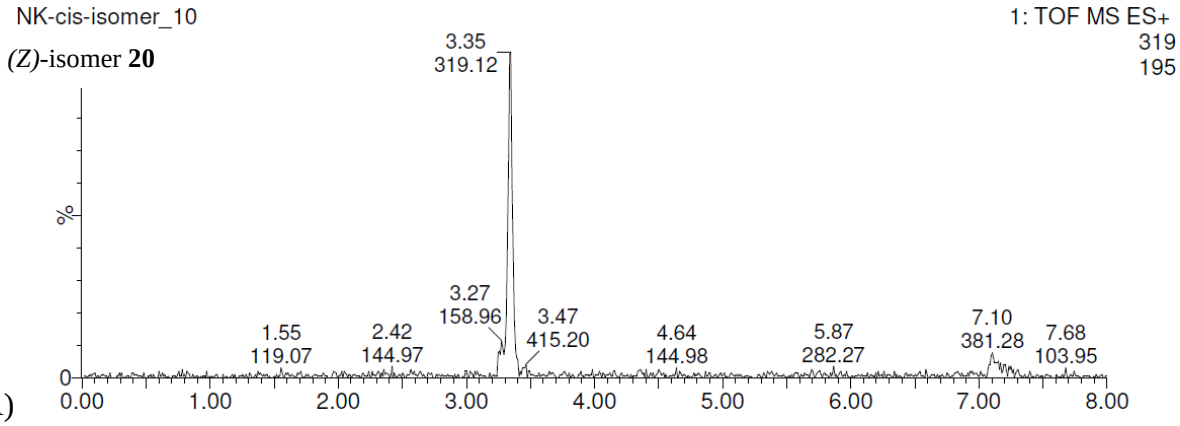


D)

Figure S2: UPLC-MS analysis of the conversion assays with substrate **17a/b**. a) Enzyme-free overnight incubation of **17a/b**; b) overnight incubation of **17a/b** with BorDH3-H49A; c) overnight incubation of **17a/b** with BorDH2; d) overnight incubation of **17a/b** with BorDH3; $[M+Na]^+$ (**17a/b**) = 410, $[M+Na]^+$ (**18**) = 392; x-axis: retention time, y-axis: relative intensity.

Product derivatization and configuration analysis

For determination of the product configuration, the assay products were transformed into the corresponding *bis*-methylesters. Therefore, the crude mixtures were treated with 50 µL of 0.5 M NaOH in 50 µL THF and 50 µL H_2O for 30 min. After acidification with 2 M HCl to pH 6, the solvent was removed under reduced pressure. Methylation occurred by treatment with 20 µL of trimethylsilyldiazomethane (10% in hexane) in methanol/toluene for 15 min. The derivatization products were compared to synthetic standards by UPLC-MS.



A)

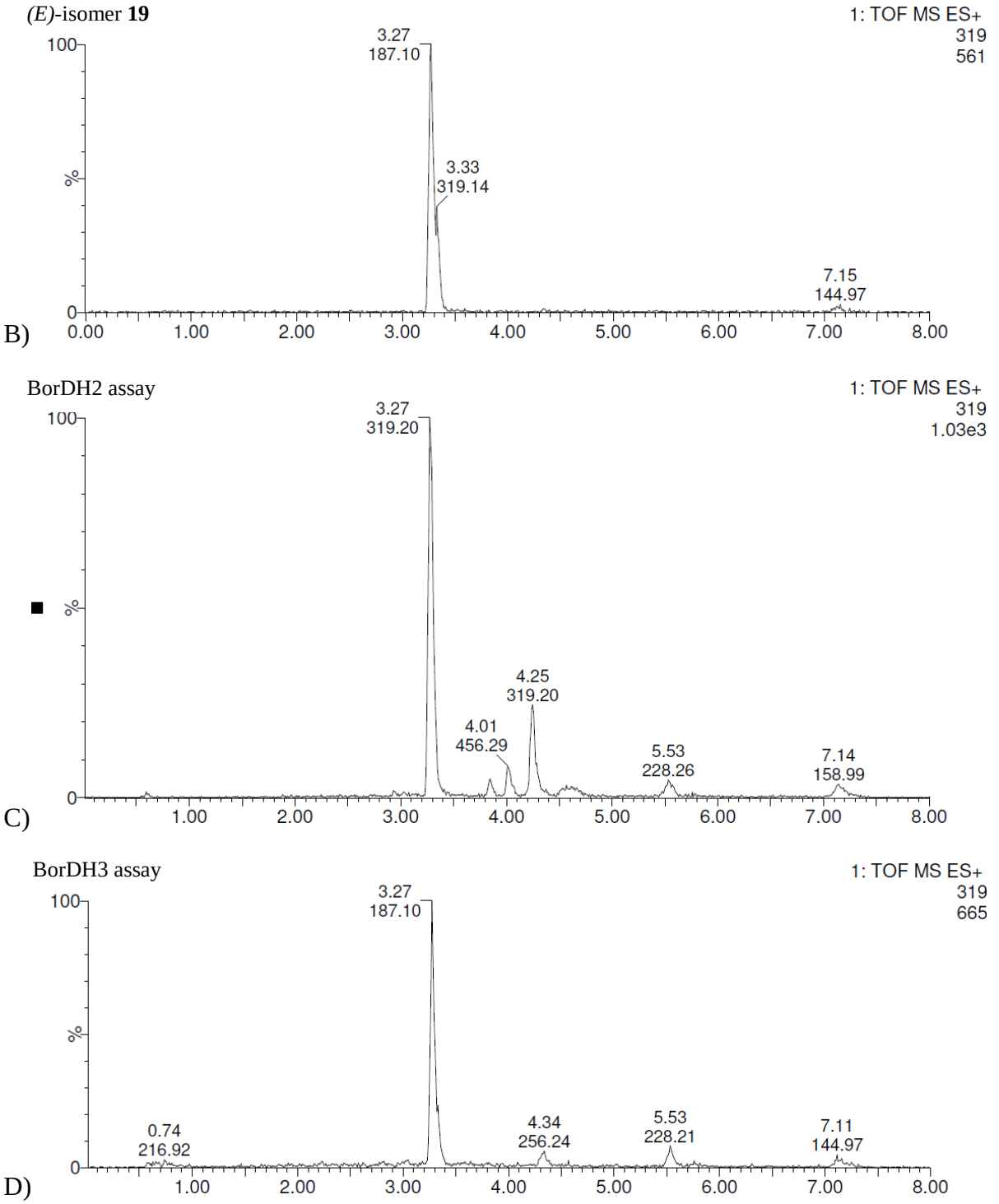
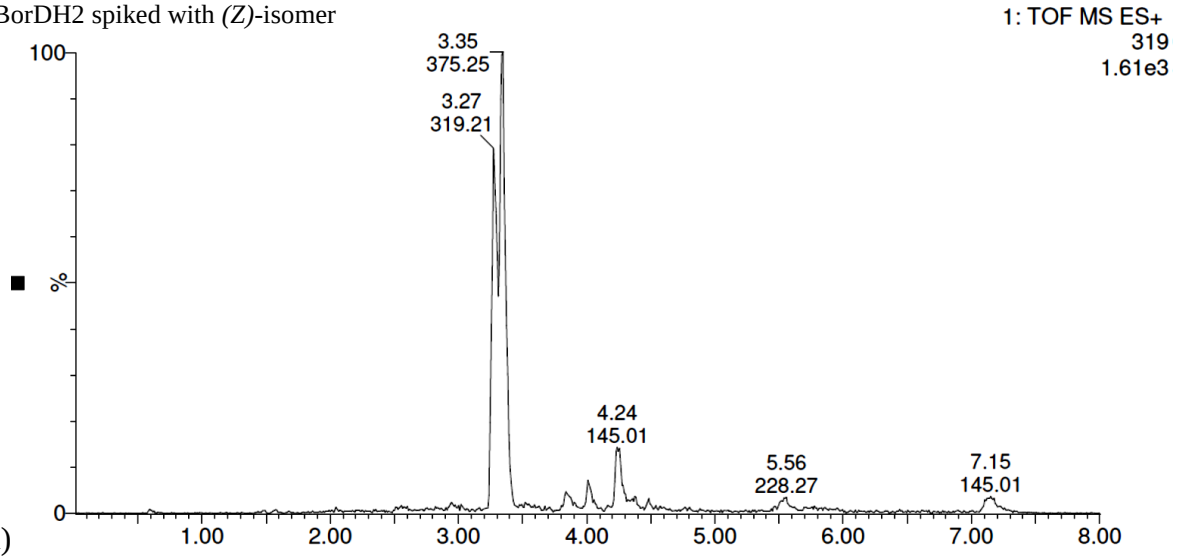


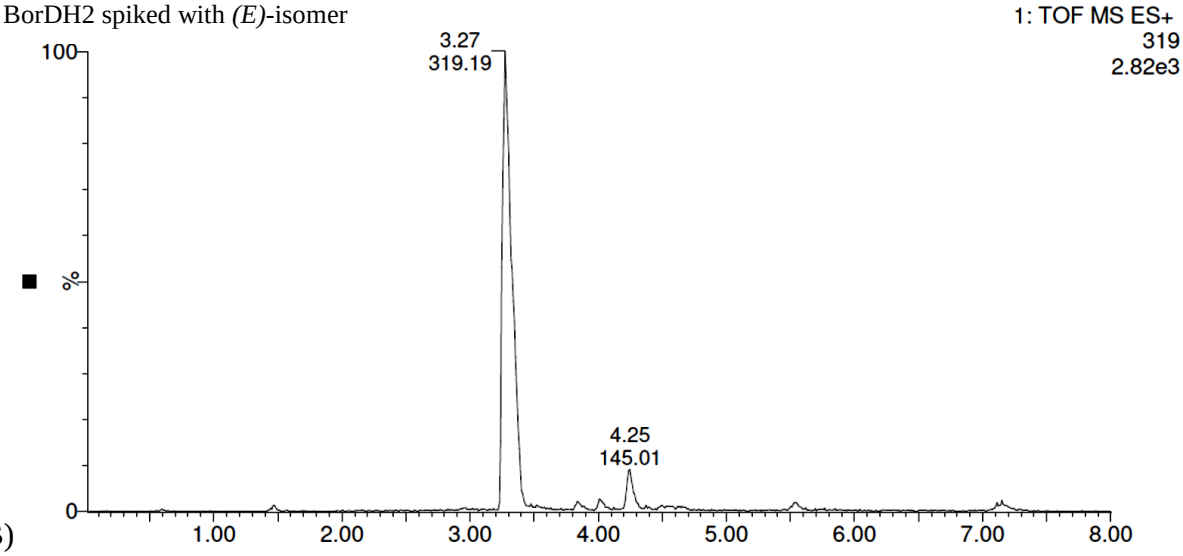
Figure S3: UPLC-MS analysis of derivatized assay products and comparison to reference molecules. A) *(Z)*-isomer, reference *bis*-methylester **20**; B) *(E)*-isomer, reference *bis*-methylester **19**; C) derivatized dehydration product of BorDH2; D) derivatized dehydration product of BorDH3; $[M+Na]^+$ (**19**/**20**) = 319; x-axis: retention time, y-axis: relative intensity.

BorDH2 spiked with *(Z)*-isomer



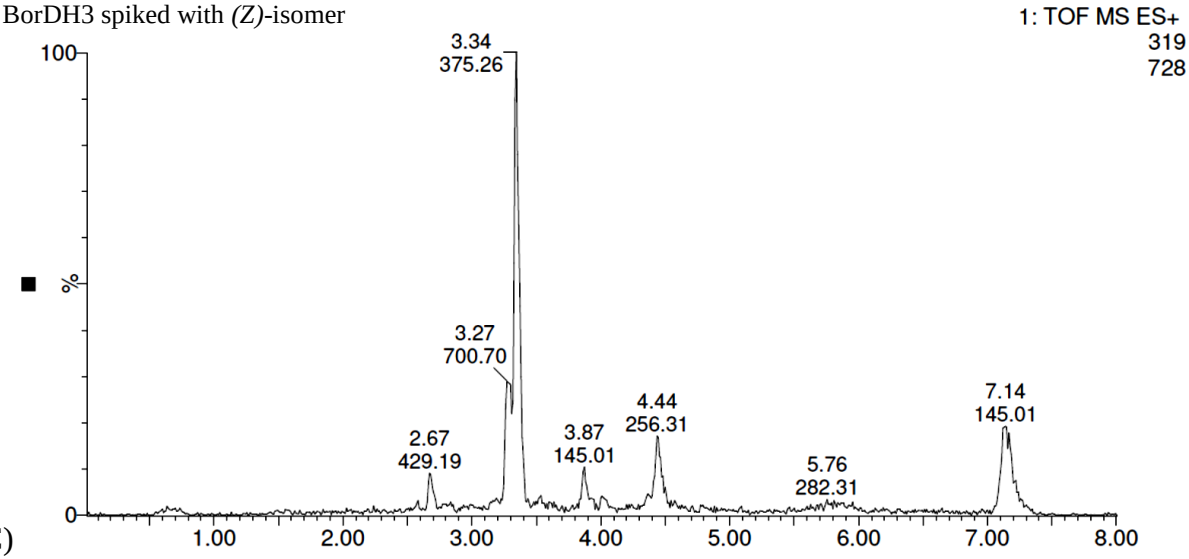
A)

BorDH2 spiked with *(E)*-isomer

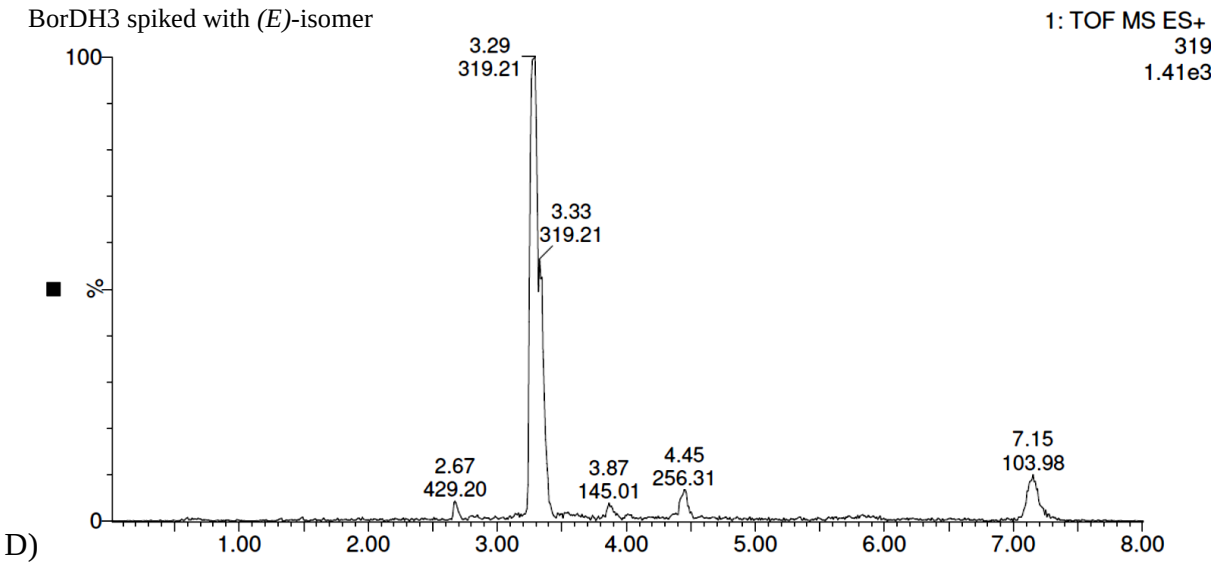


B)

BorDH3 spiked with *(Z)*-isomer



C)

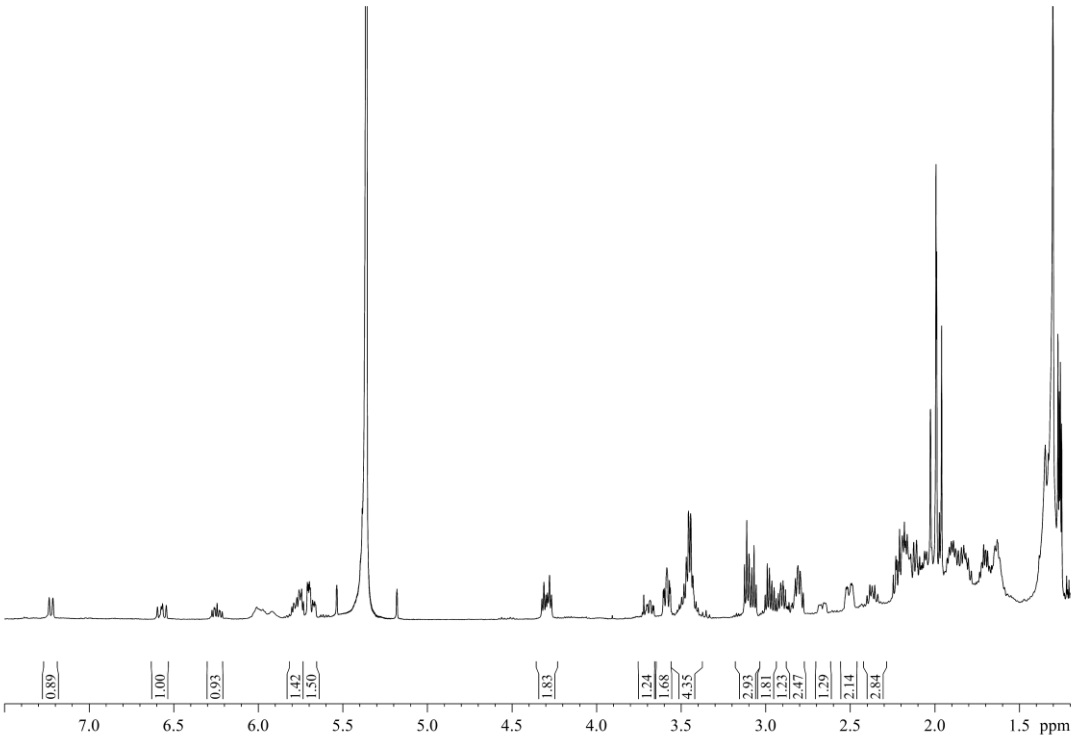


D)

Figure S4: UPLC-MS runs of derivatized assay products, which were spiked with the respective isomers **19** or **20** before analysis. A) BorDH2 assayed with **17a/b**, spiked with **20**; B) BorDH2 assayed with **17a/b**, spiked with **19**; C) BorDH3 assayed with **17a/b**, spiked with **20**; D) BorDH3 assayed with **17a/b**, spiked with **19**; $[M+Na]^+$ (**19**/**20**) = 319; x-axis: retention time, y-axis: relative intensity.

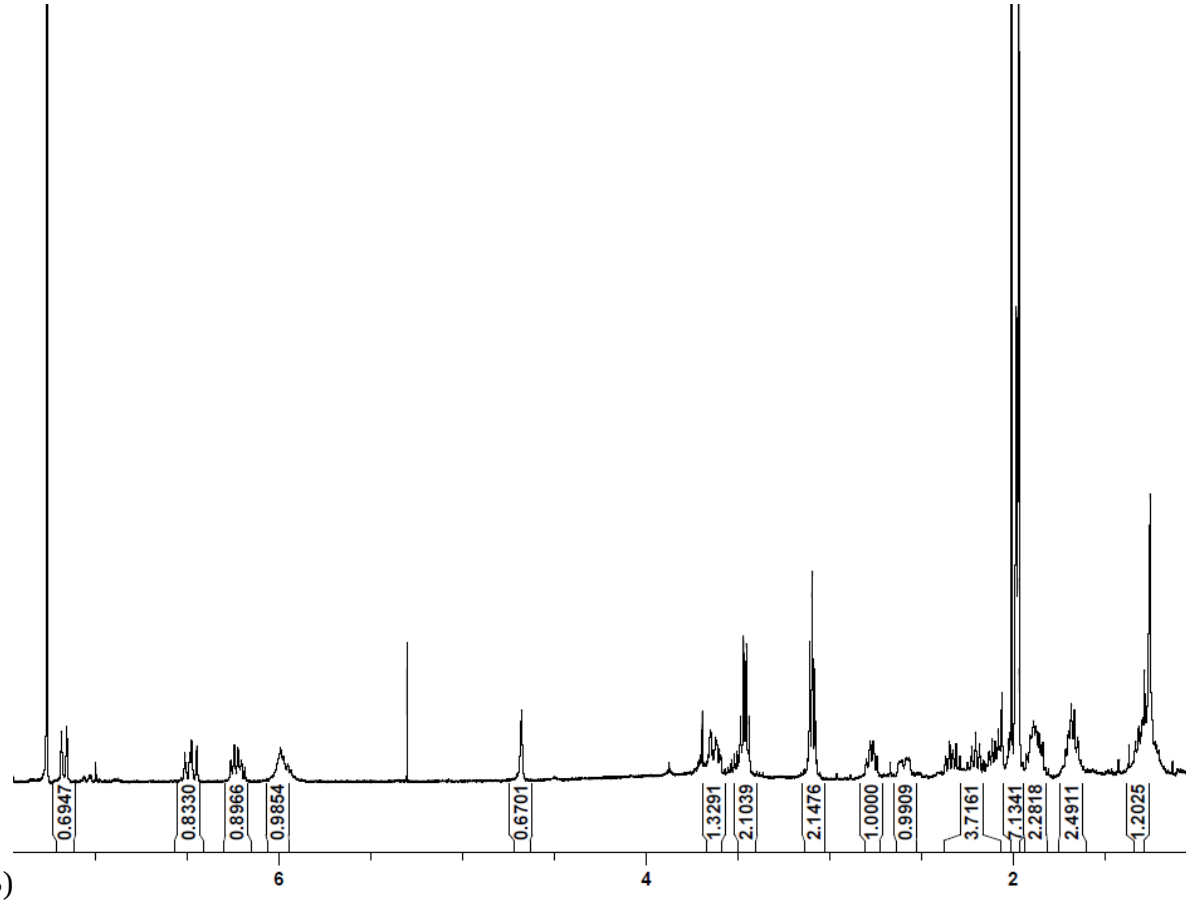
NMR analysis of the large-scale BorDH3 assay

10 mg **17a/b** were incubated with 6 mg/mL BorDH3 in 25 mM HEPES (pH7.5, 100 mM NaCl) at 37 °C for 16 h (end volume 1 mL). The sample was 3 times extracted with EtOAc and the solvent was removed *in vacuo*. The sample was dissolved in CD_2Cl_2 and the resulting NMR spectrum was compared to the spectrum of synthetic **18**. The chemical shifts of the olefinic protons H3, H4 and H5 correlate to (2*E*,4*E*)-configuration of the diene (Figure S5).

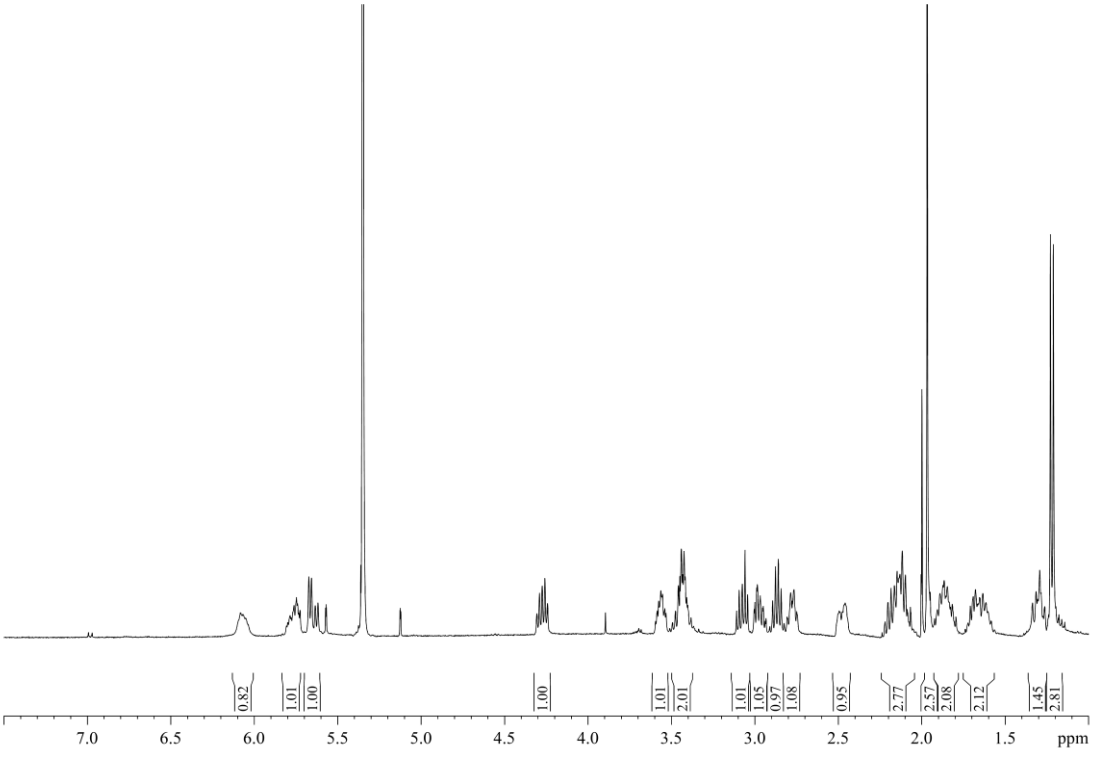


A)

B)


0.6947
0.8330
0.8966
0.9854
0.6701
1.3291
2.1039
2.1476
1.0000
0.9909
3.7161
7.1341
2.2818
2.4911
1.2025
6
4
2

C)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1.01
1.00
1.00
1.01
2.01
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1.05
0.97
1.08
0.95
2.77
2.57
2.08
2.12
1.45
2.81
7.0
6.5
6.0
5.5
5.0
4.5
4.0
3.5
3.0
2.5
2.0
1.5
ppm

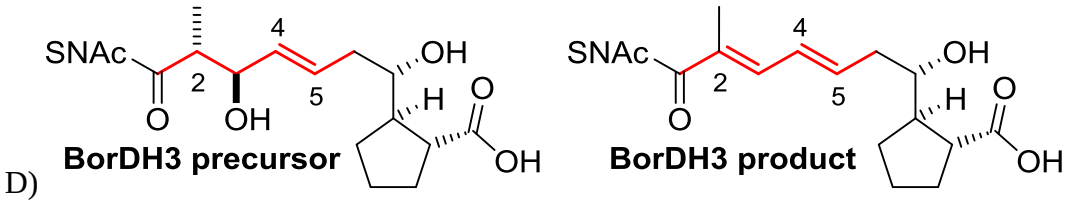
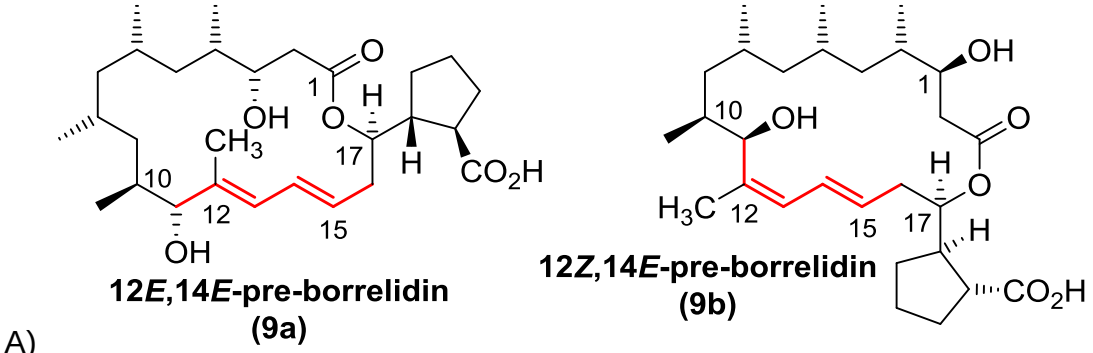


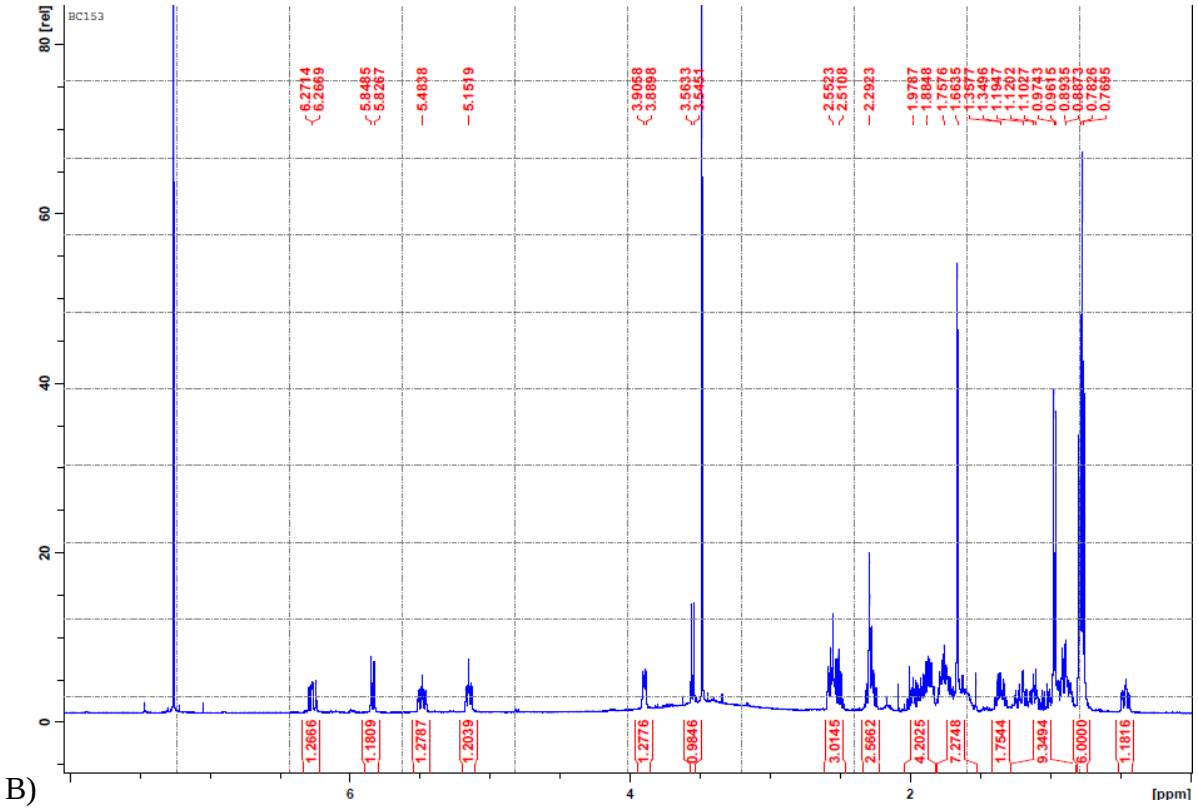
Figure S5: A) ^{1}H NMR spectrum of an incubation of **17a/b** with BorDH3 (CD_2Cl_2, 500 MHz). B) ^{1}H NMR spectrum of synthetic **18** ($CDCl_3$, 400 MHz); C) ^{1}H NMR spectrum of synthetic **17a/b** (CD_2Cl_2, 400 MHz); D) Structures of the BorDH3 precursor and the BorDH3 product.

NMR analysis of pre-borrelidin

Pre-borrelidin was isolated from the mutant strain M19B9I1 as previously described.[3] The previous NMR assignments for pre-borrelidin were confirmed using ^{1}H, ^{13}C, DEPT, COSY, HSQC and HMBC experiments. The observed ^{3}J coupling constants are consistent with either of the (12*Z*,14*E*) or (12*E*,14*E*)-configurations as shown in Figure S6.



A)



B)

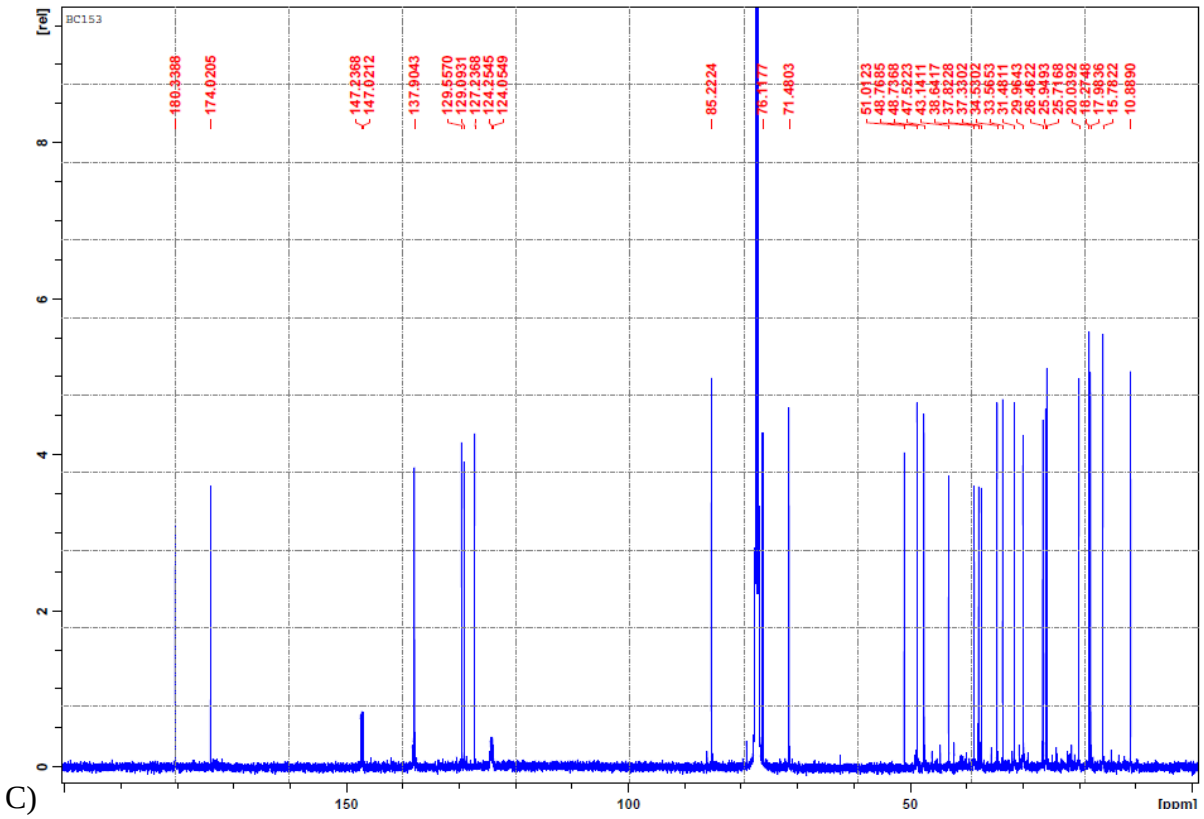


Figure S6: A) Structures of the potential configurational isomers of pre-borrelidin; B) ^{1}H NMR spectrum of pre-borrelidin **9a** ($CDCl_3$, 500 MHz); C) ^{13}C NMR spectrum of pre-borrelidin **9a** ($CDCl_3$, 125 MHz).

To determine the configuration of the C12-C13 double bond a NOESY experiment was performed and the data are summarised in Table S1 and Figure S7. In accordance with a 12*E* configuration, a strong correlation for the methyl group attached to C12 to both H14 and H11 is visible. In contrast there is no correlation between the methyl group attached to C12 and H13, which would be expected for 12*Z*.

Table S1: ^{1}H NMR spectrum of pre-borrelidin and listing of selected NMR data for pre-borrelidin ($CDCl_3$, 500 MHz).

Position	**Chemical shift (ppm)**	**J (Hz)**	**NOESY correlations**
H10	1.60	m	10-CH_3
H11	3.53	9 (d)	H13, 10-CH_3, 12-CH_3
H12	-	-	-
H13	5.84	11.0 (d)	H11, H14(w), H15
H14	6.28	1.8, 11.0, 14.9 (ddd)	H13(w), H16a, 12-CH_3
H15	5.48	3.5, 10.7, 14.9 (ddd)	H13, H16b, H17
H16a H16b	2.22 2.53	m	H14, H16b H15, H16a, H17
H17	5.10	2.9, 8.0, 11.3 (ddd)	H15, H16b, H19
10-CH_3	0.94	6.5 (d)	H9, H10, H11, 8-CH_3, 12-CH_3
12-CH_3	1.64	s	H11, H14, 10-CH_3

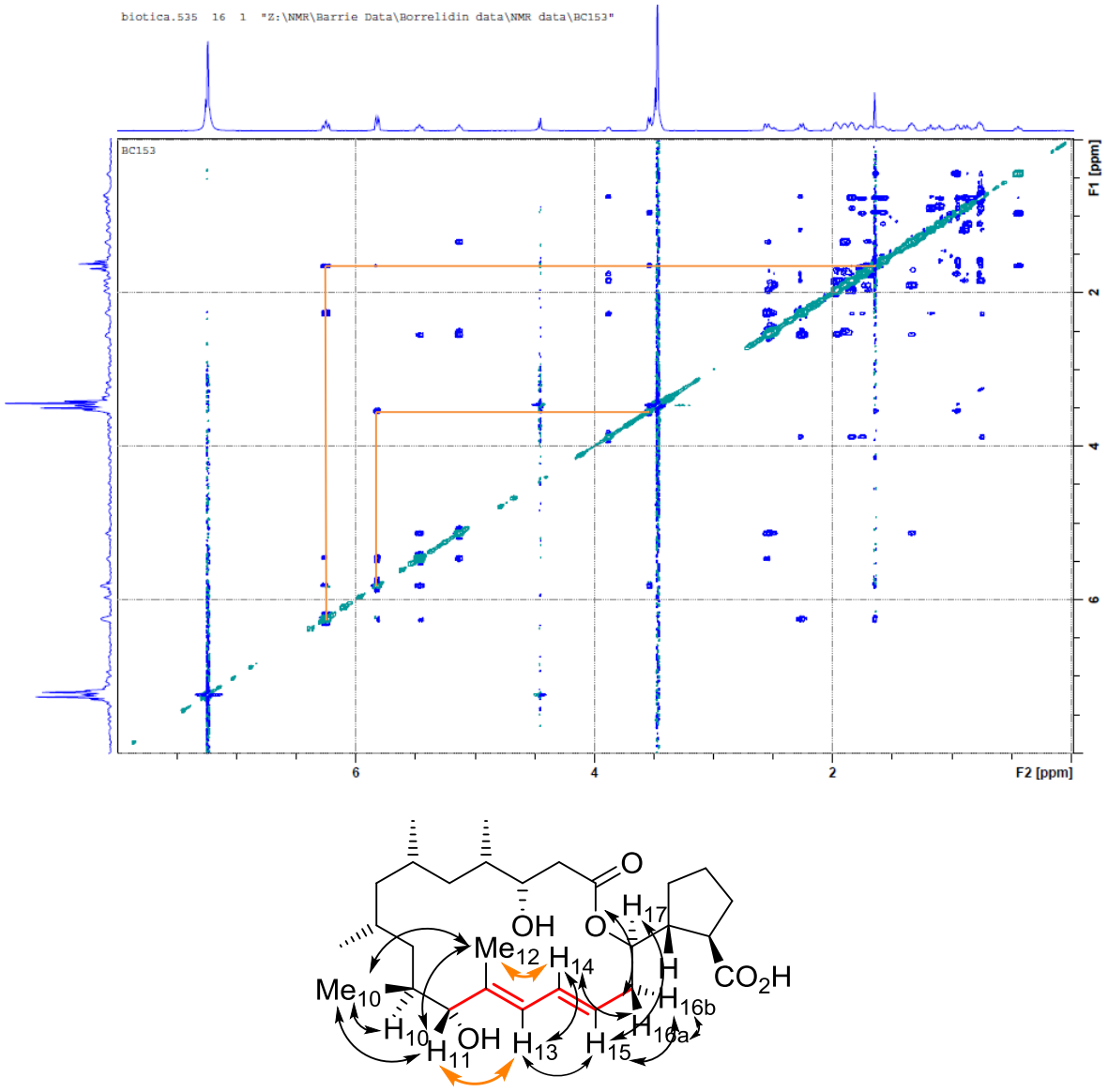


Figure S7: NOESY spectrum and correlations of pre-borrelidin ($CDCl_3$, 500 MHz). The correlations between 12-CH_3 and H14 as well as between H11 and H13 are highlighted in orange.

In a gradient NOE irradiating at 5.84 ppm (H13) strong correlations to H11 and H15 (and very weak signal to H14) and no correlation to the 12-CH_3 group were observed. These combined data firmly establish the geometry of pre-borrelidin as (12*E*,14*E*).

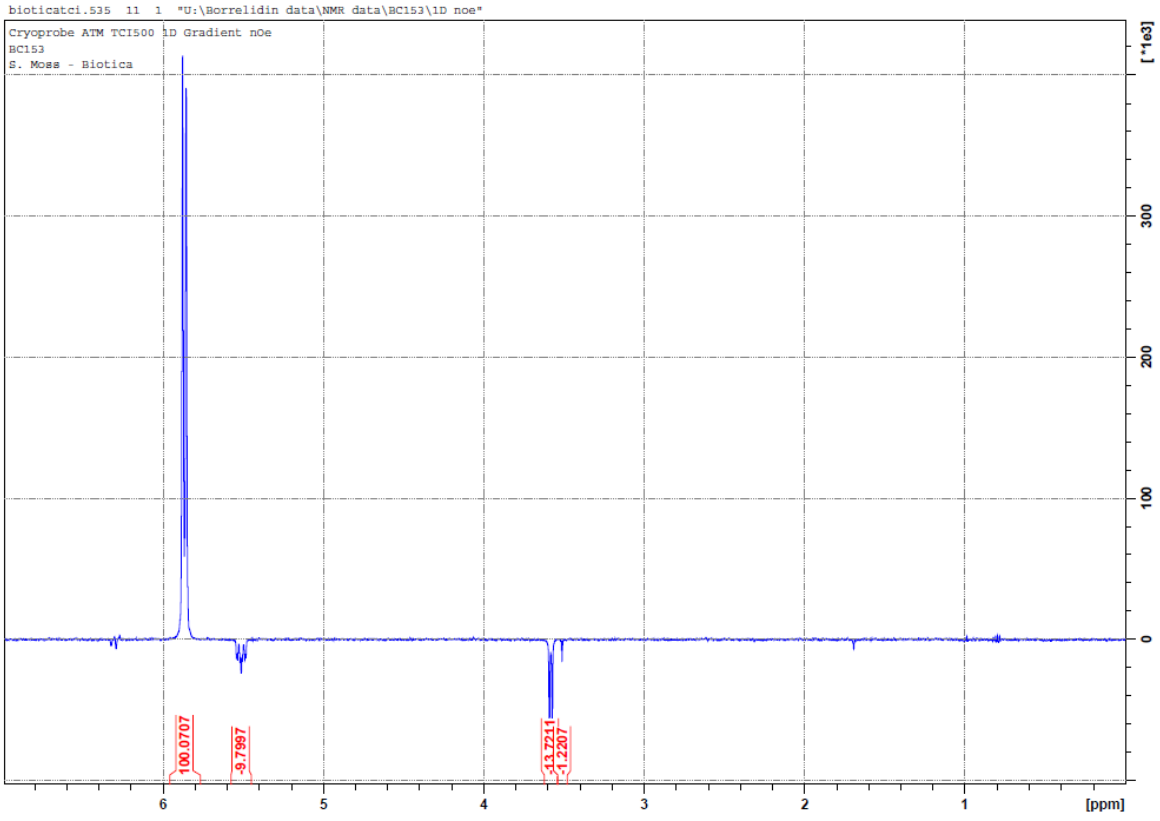


Figure S8: NOE correlation spectrum of pre-borrelidin ($CDCl_3$, 500 MHz). Irradiation on proton H13 led to a correlation to protons H11 and H15, which is in agreement to a 12*E*-configuration.

References supporting information

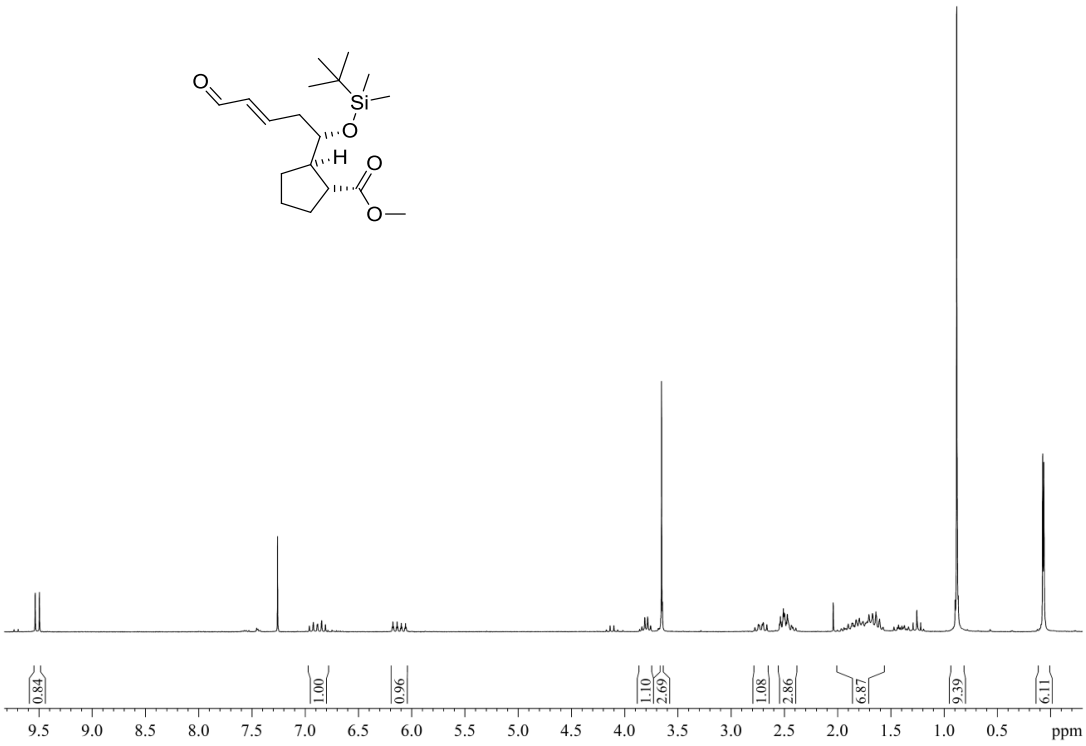
1. H. E. Gottlieb, V. Kotlyar, A. Nudelman, *J. Org. Chem.* **1997**, *62*, 7512.
2. O. Vergnolle, F. Hahn, A. Baerga-Ortiz, P. F. Leadlay, J. N. Andexer, *ChemBioChem* **2011**, *12*, 1011.
3. C. Olano, S. J. Moss, A. F. Braña, R. M. Sheridan, V. Math, A. J. Weston, C. Méndez, P. F. Leadlay, B. Wilkinson and J. A. Salas, *Mol. Microbiol.* **2004**, *6*, 1745.
4. F. Hahn, N. Kandziora, S. Friedrich, P. F. Leadlay, *Beilst. J. Org. Chem.* **2014**, in press.

Uncovering the origin of *Z*-configured double bonds in polyketides: intermediate *E*-double bond formation during borrelidin biosynthesis

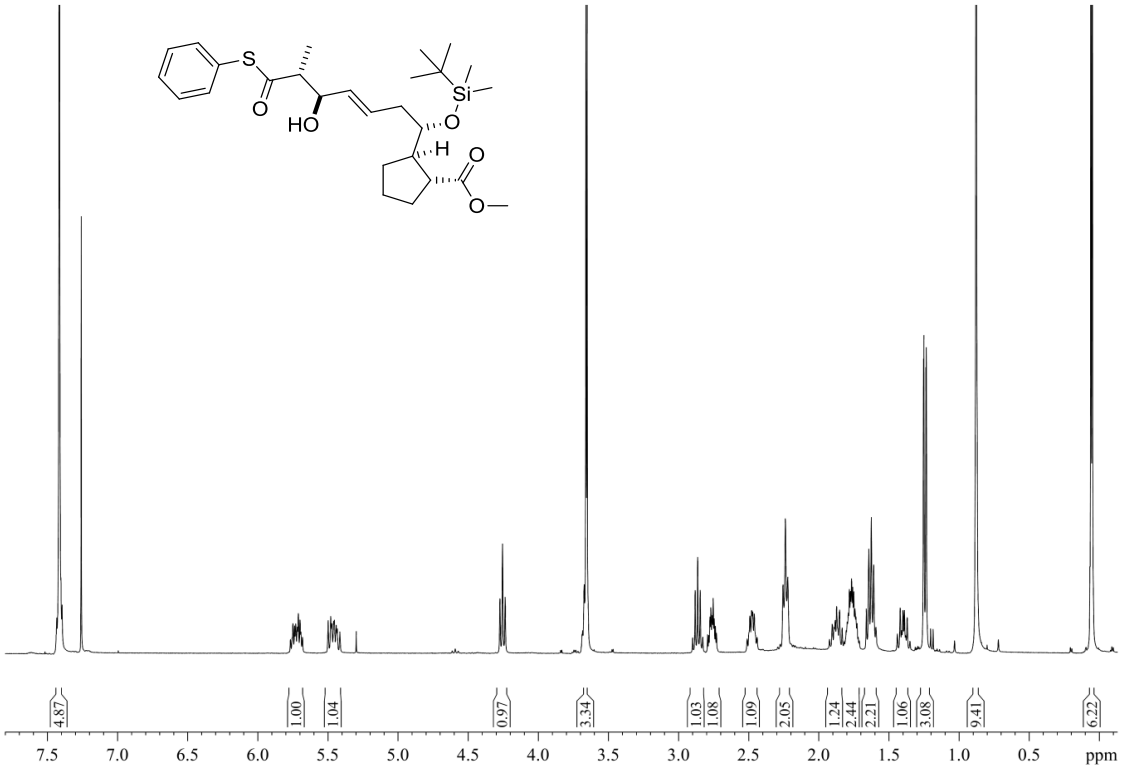
Nadine Kandziora, Jennifer Andexer, Steven J. Moss, Barrie Wilkinson, Peter F. Leadlay and Frank Hahn

NMR spectra

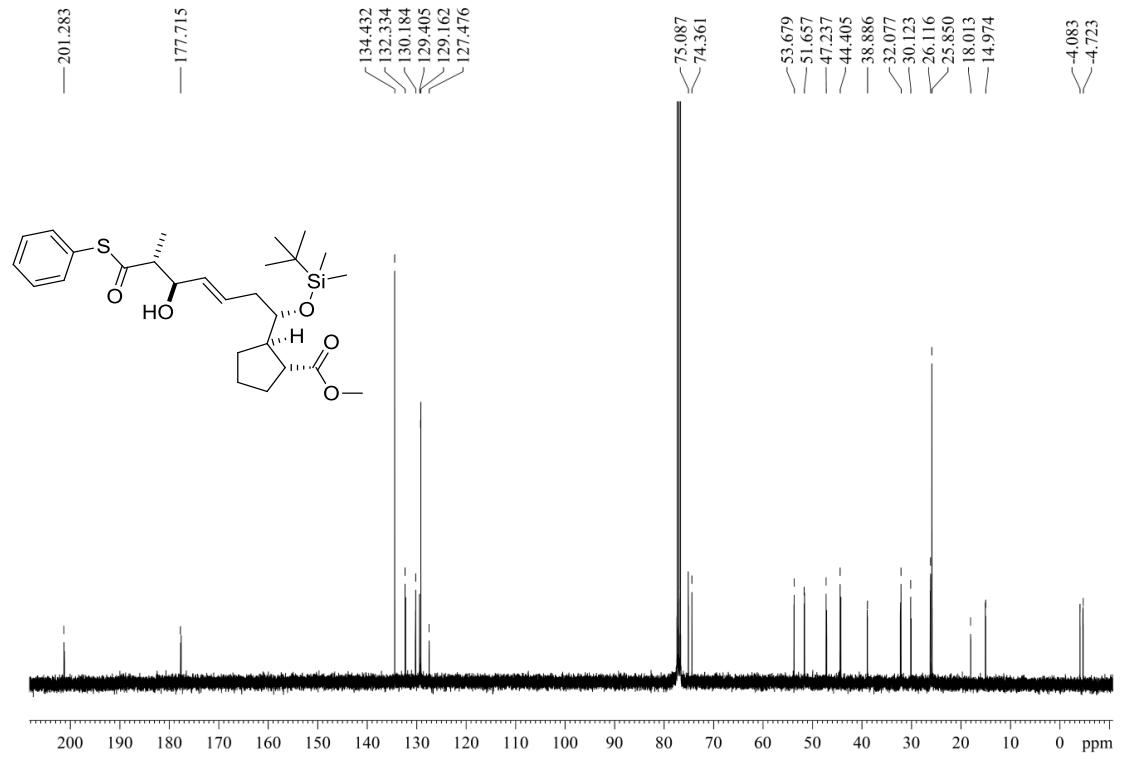
$CDCl_3$, 200 MHz



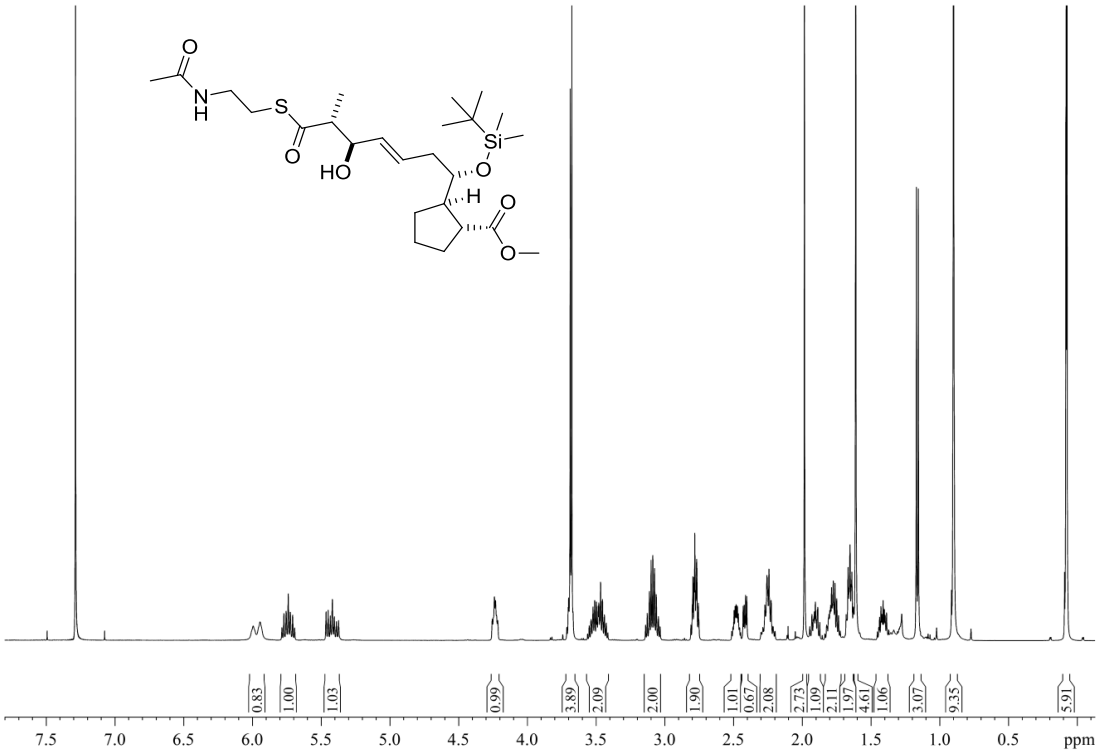
$CDCl_3$, 400 MHz



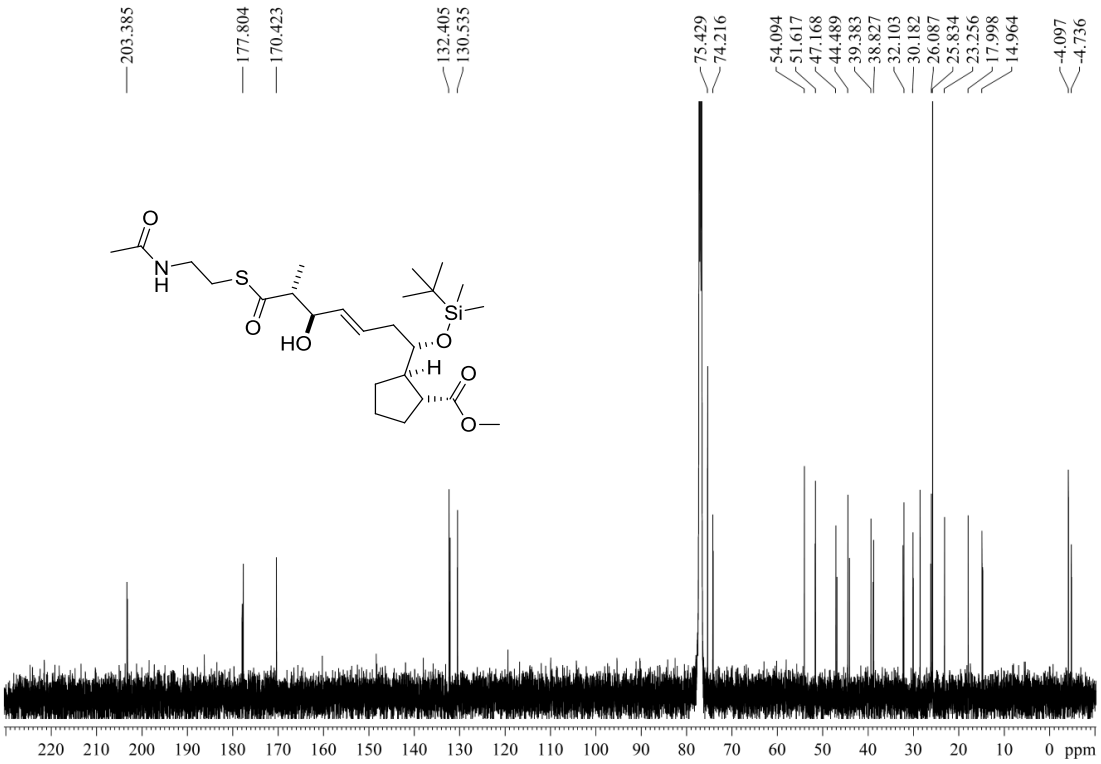
$CDCl_3$, 100 MHz



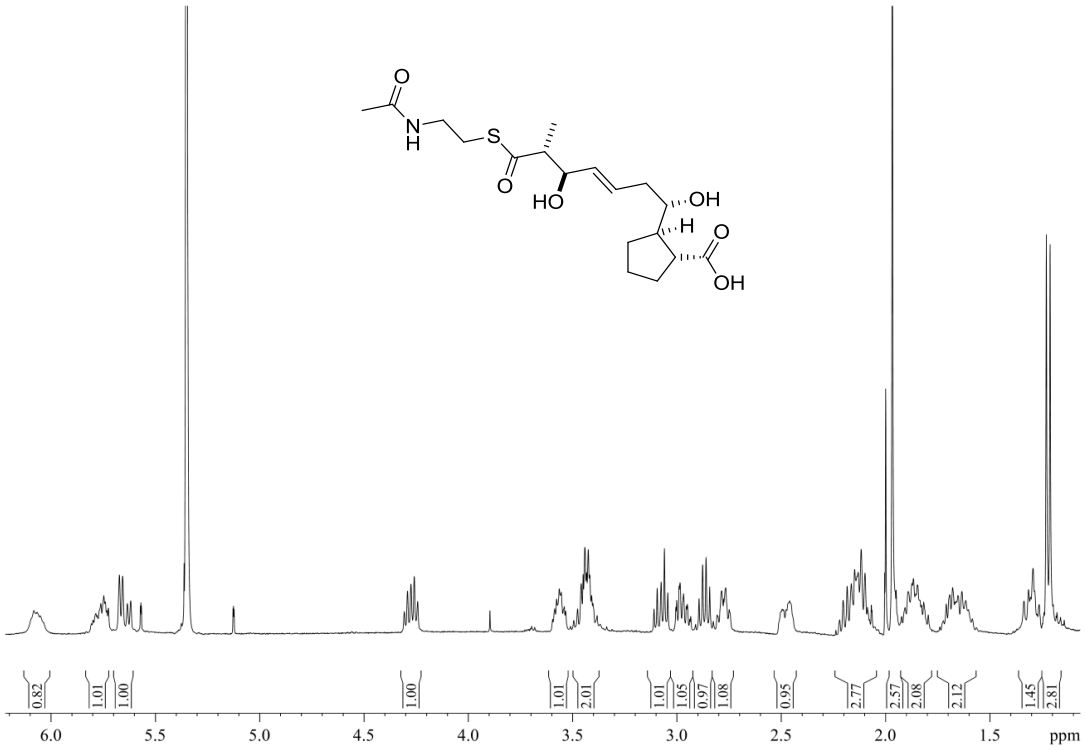
$CDCl_3$, 500 MHz



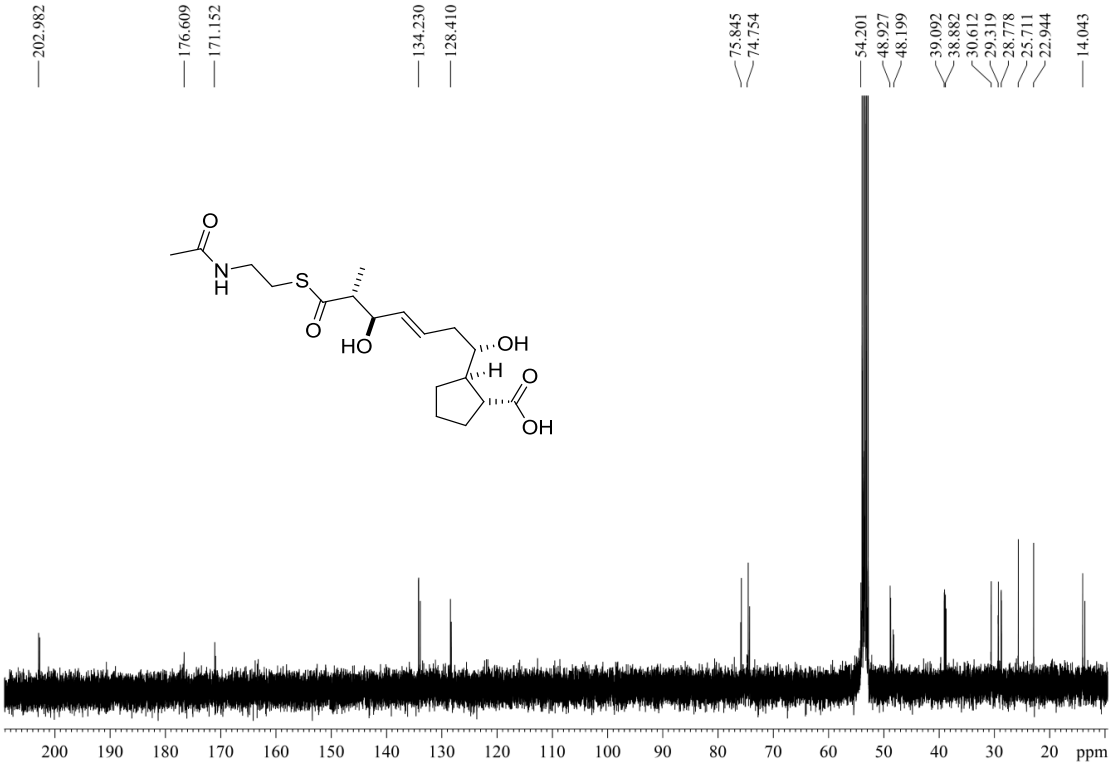
$CDCl_3$, 125 MHz



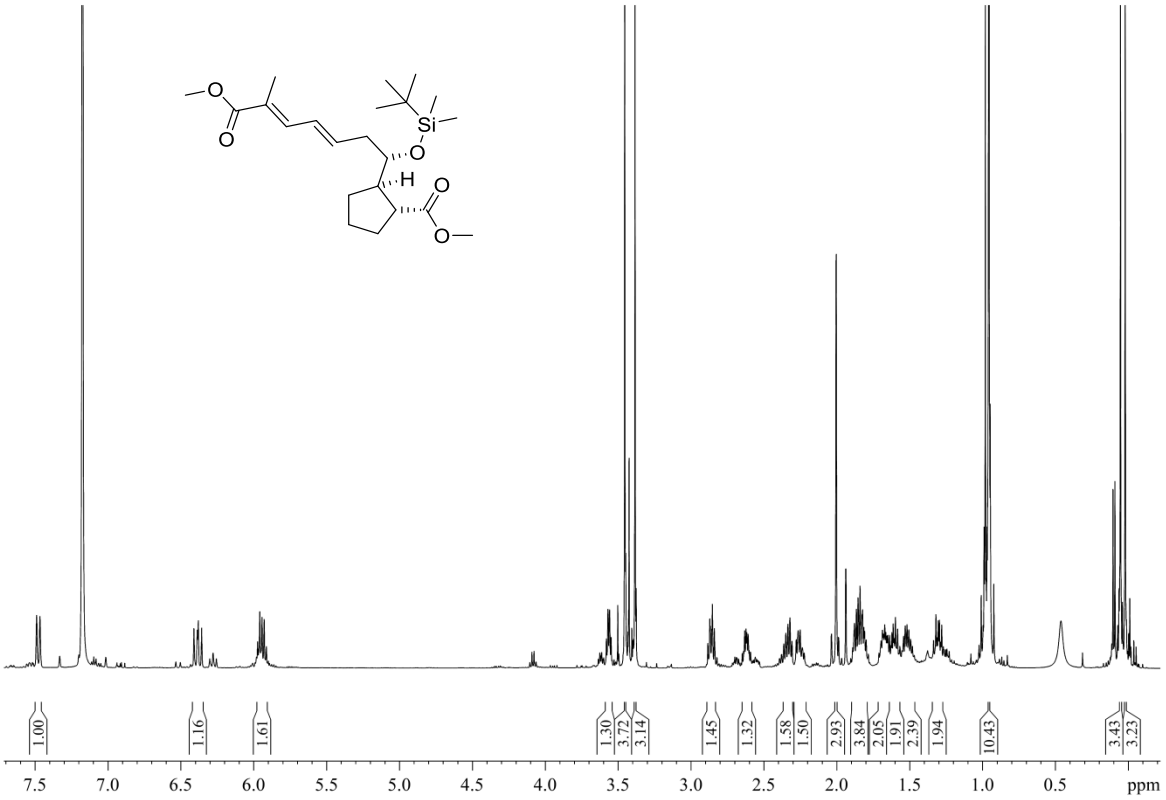
CD_2Cl_2, 400 MHz



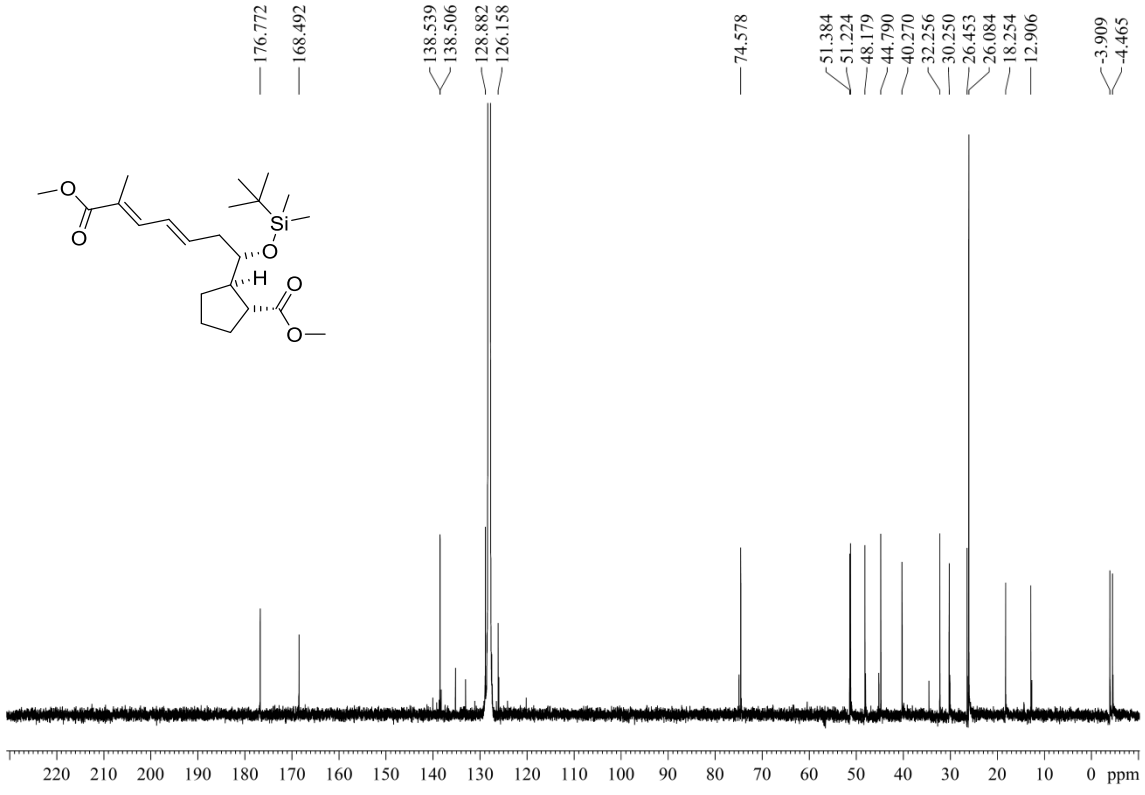
CD_2Cl_2, 100 MHz



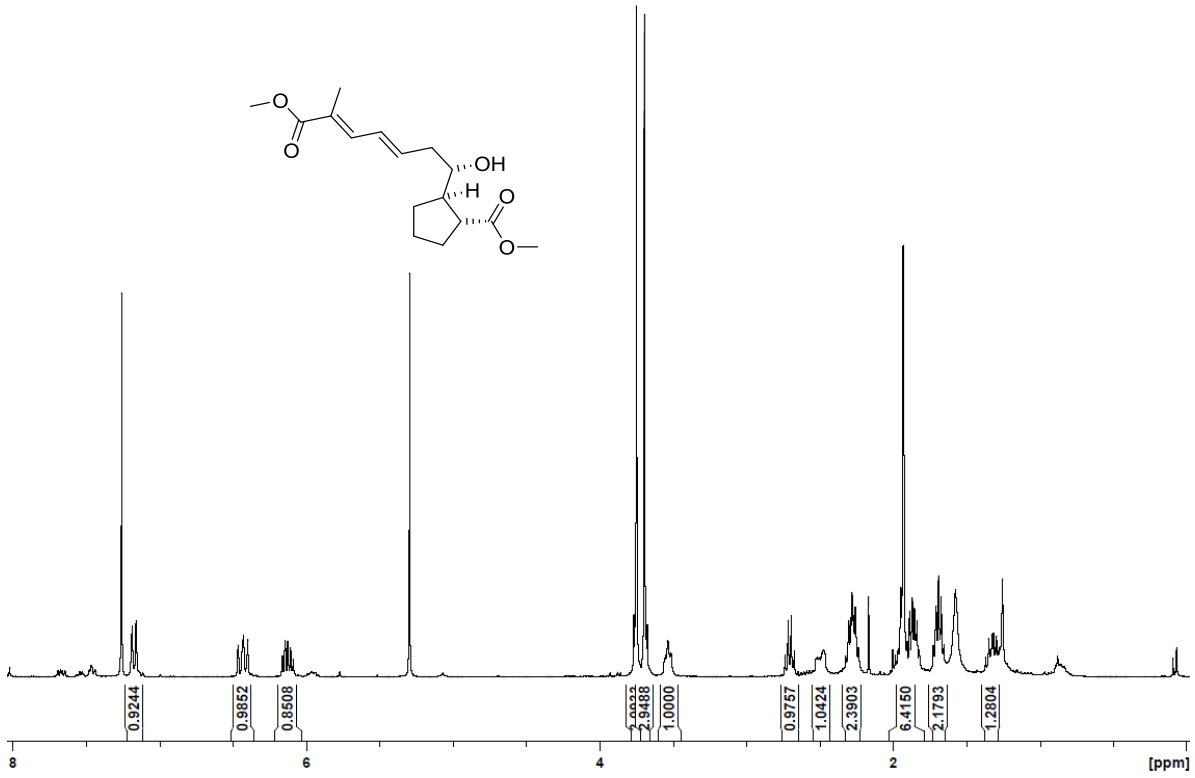
d6-benzene, 500 MHz



d6-benzene, 125 MHz



$CDCl_3$, 400 MHz



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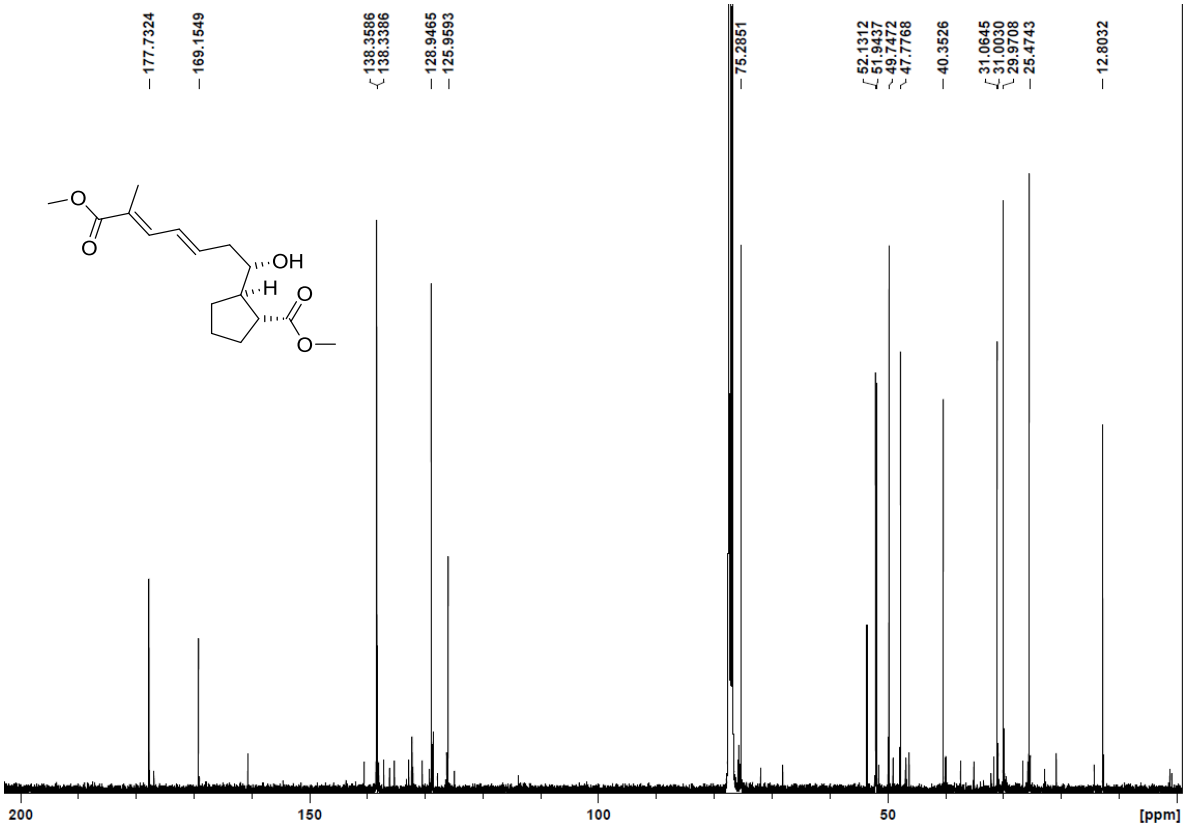

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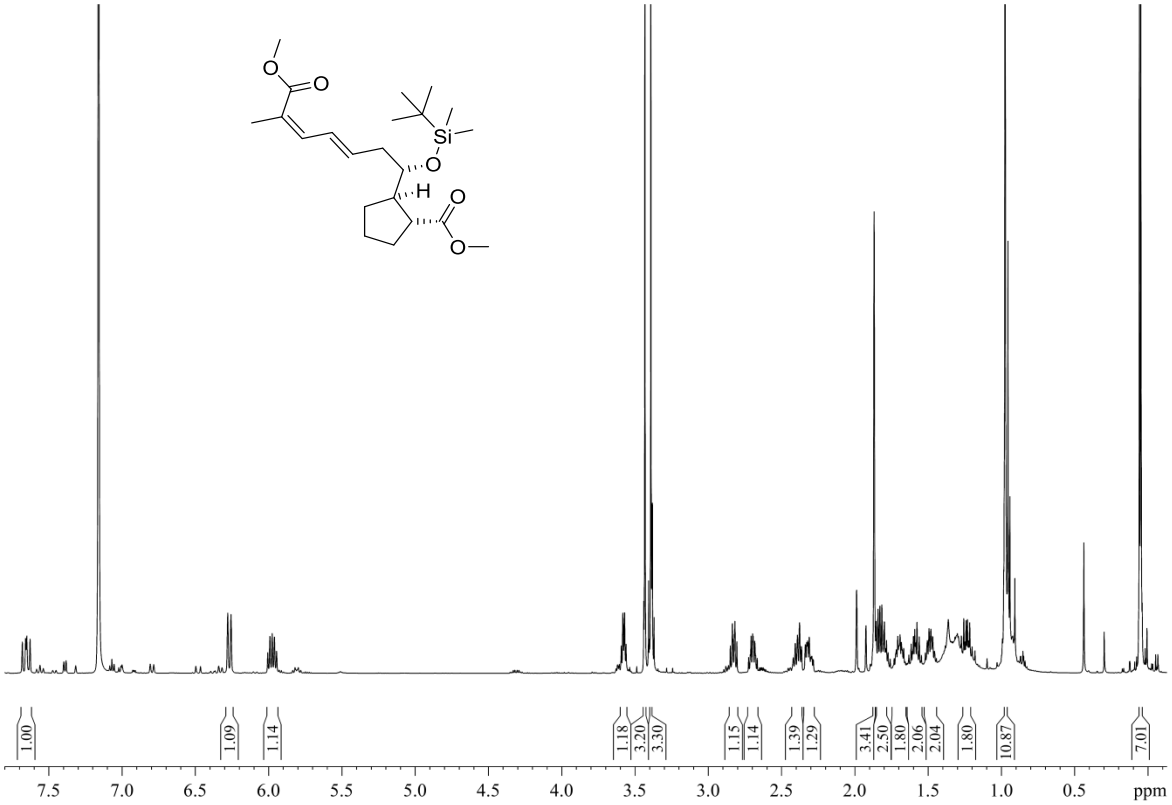
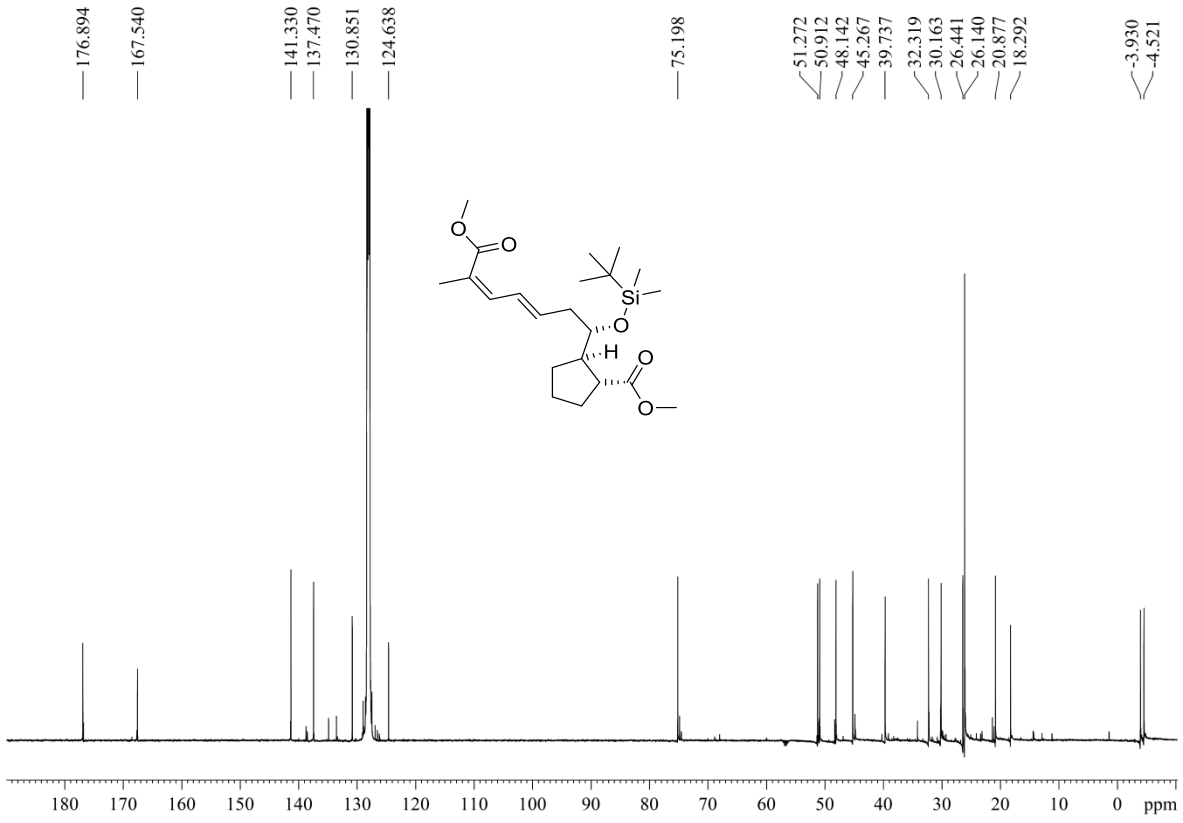
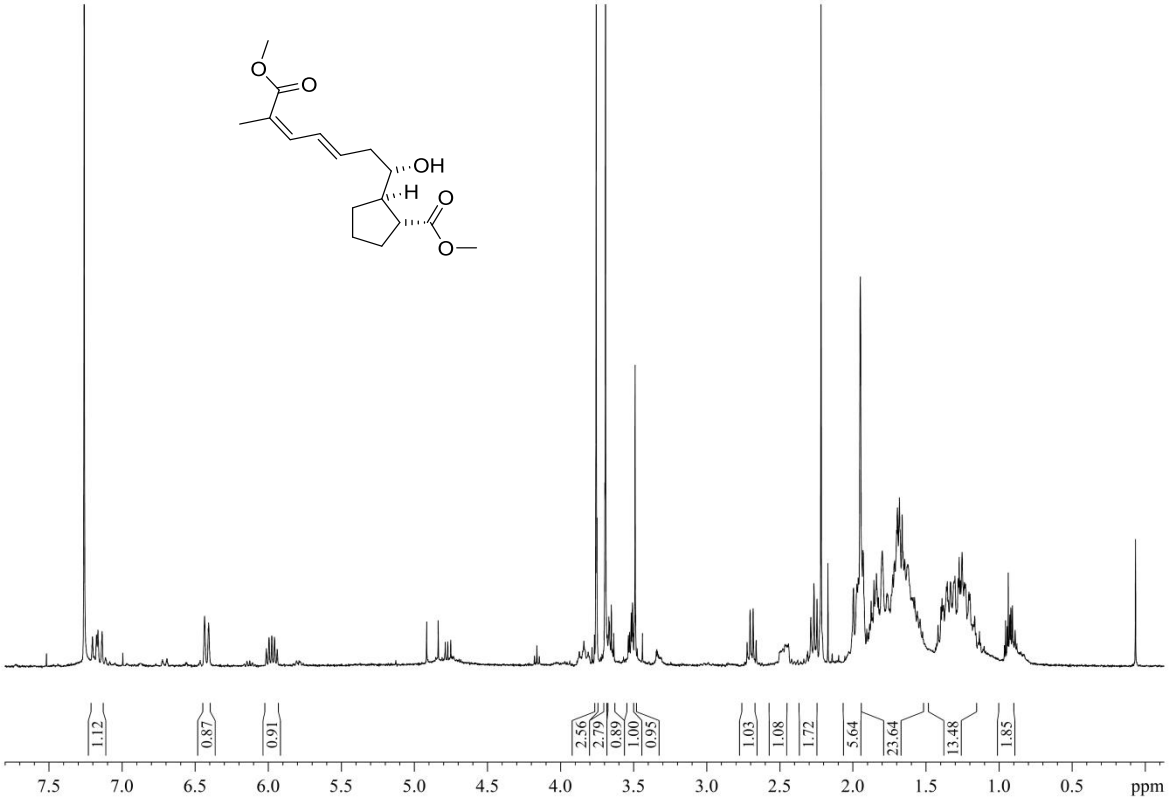


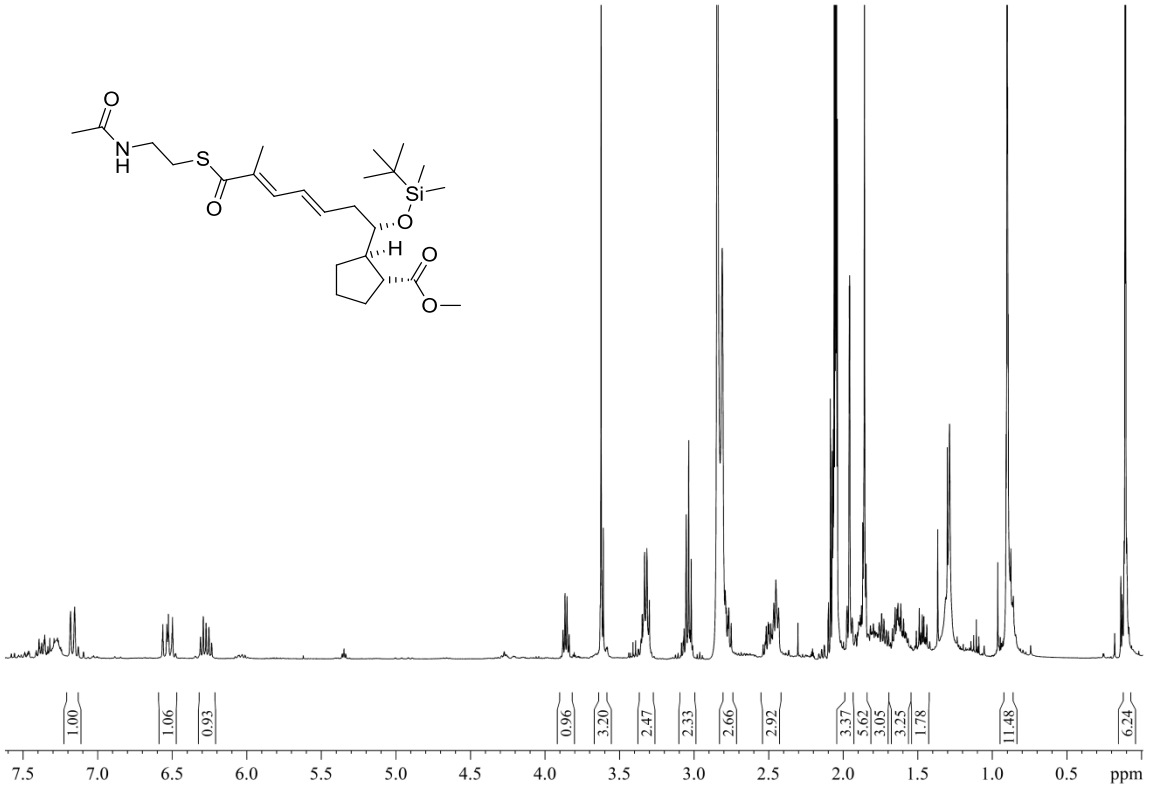
d6-benzene, 125 MHz



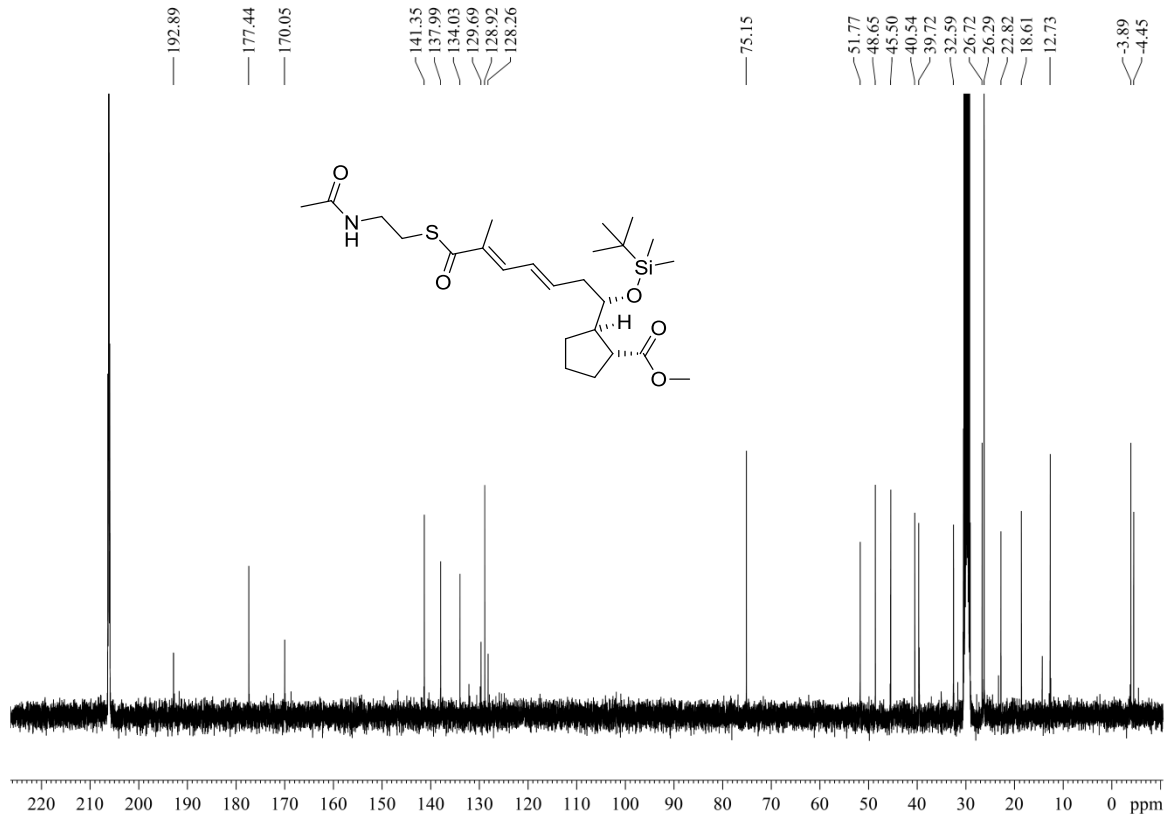
d_6-benzene, 500 MHz



d6-acetone, 500 MHz



d6-acetone, 125 MHz



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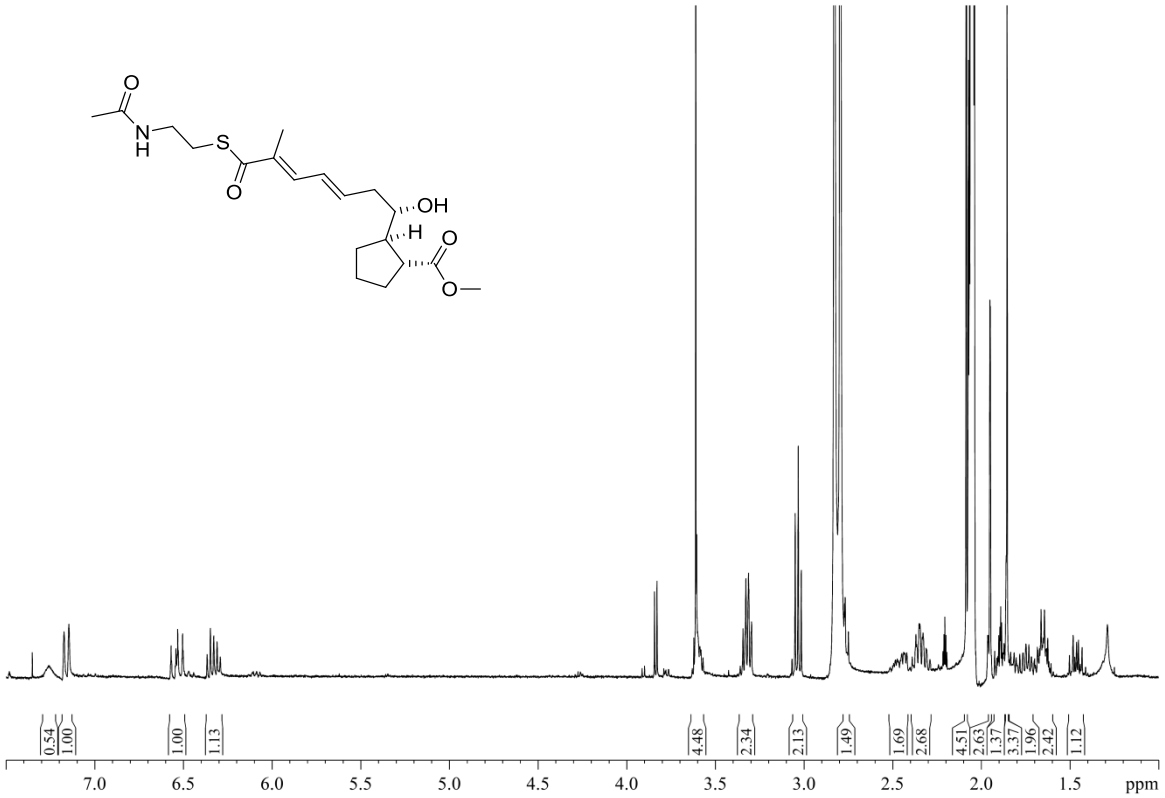
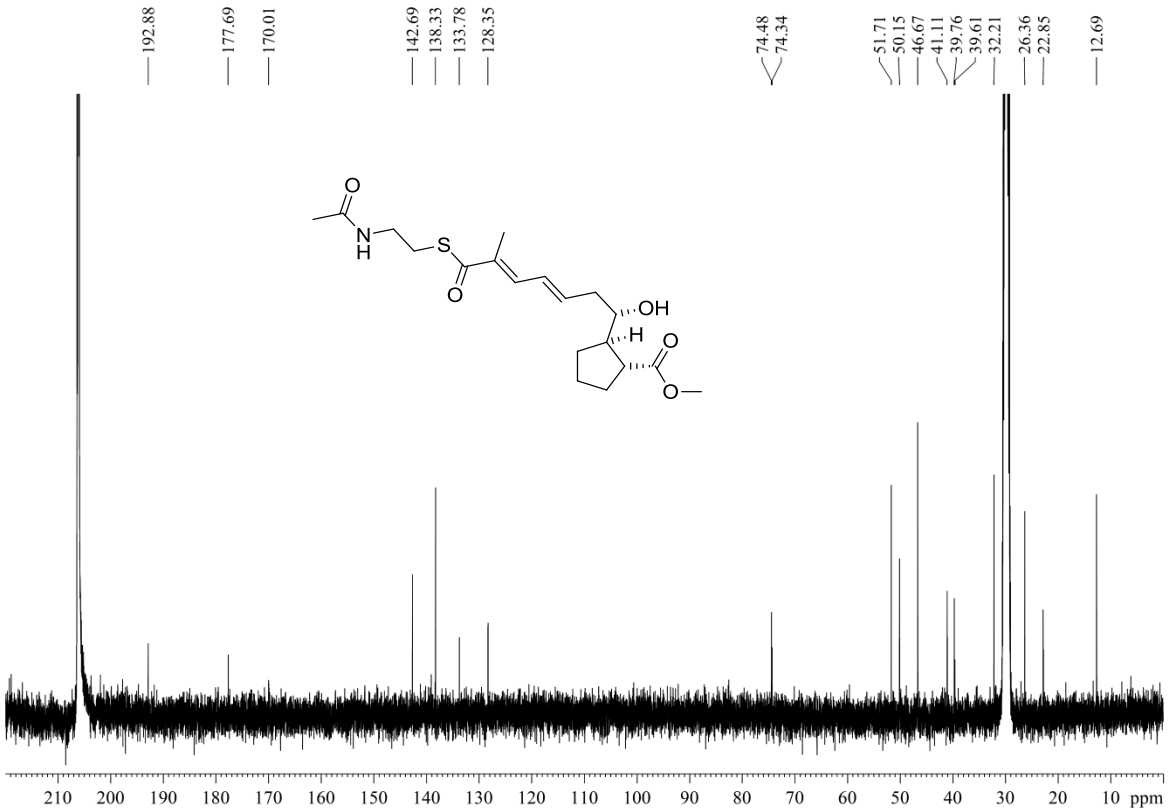
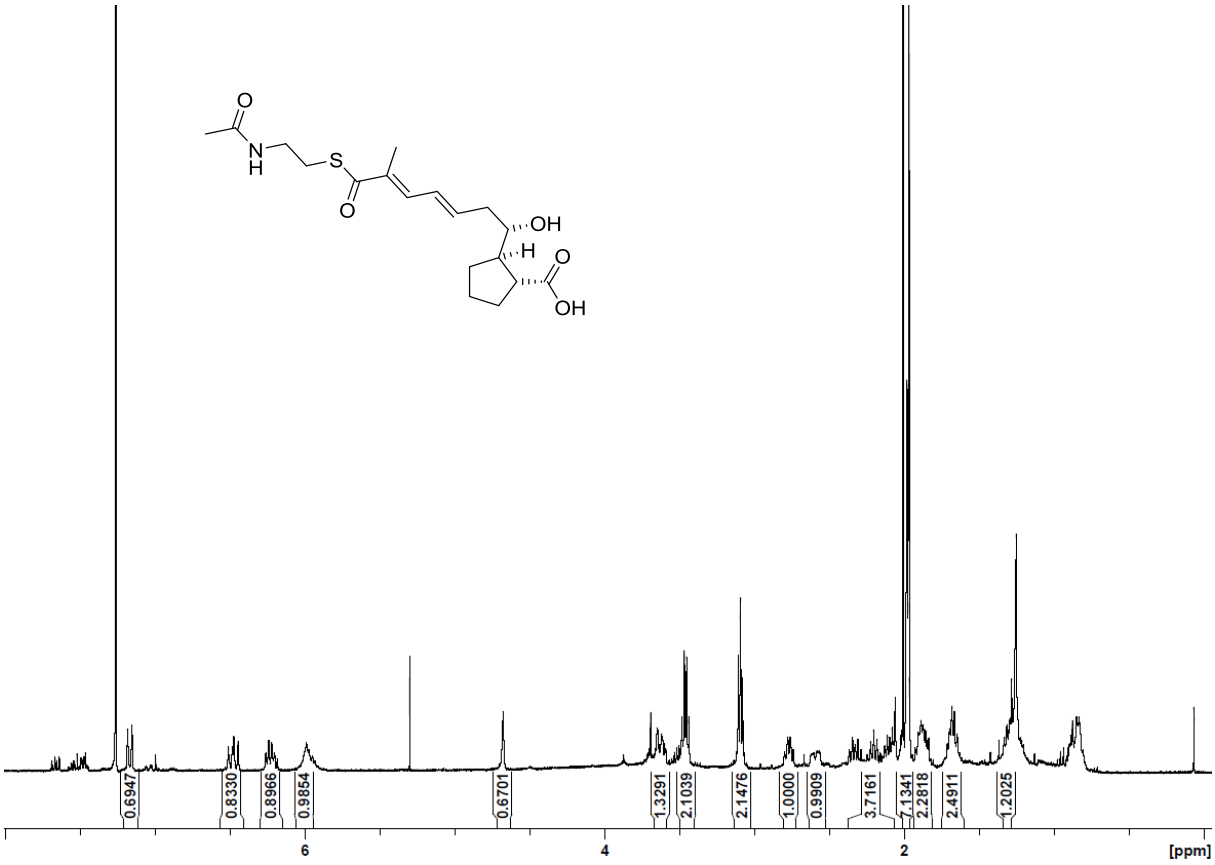


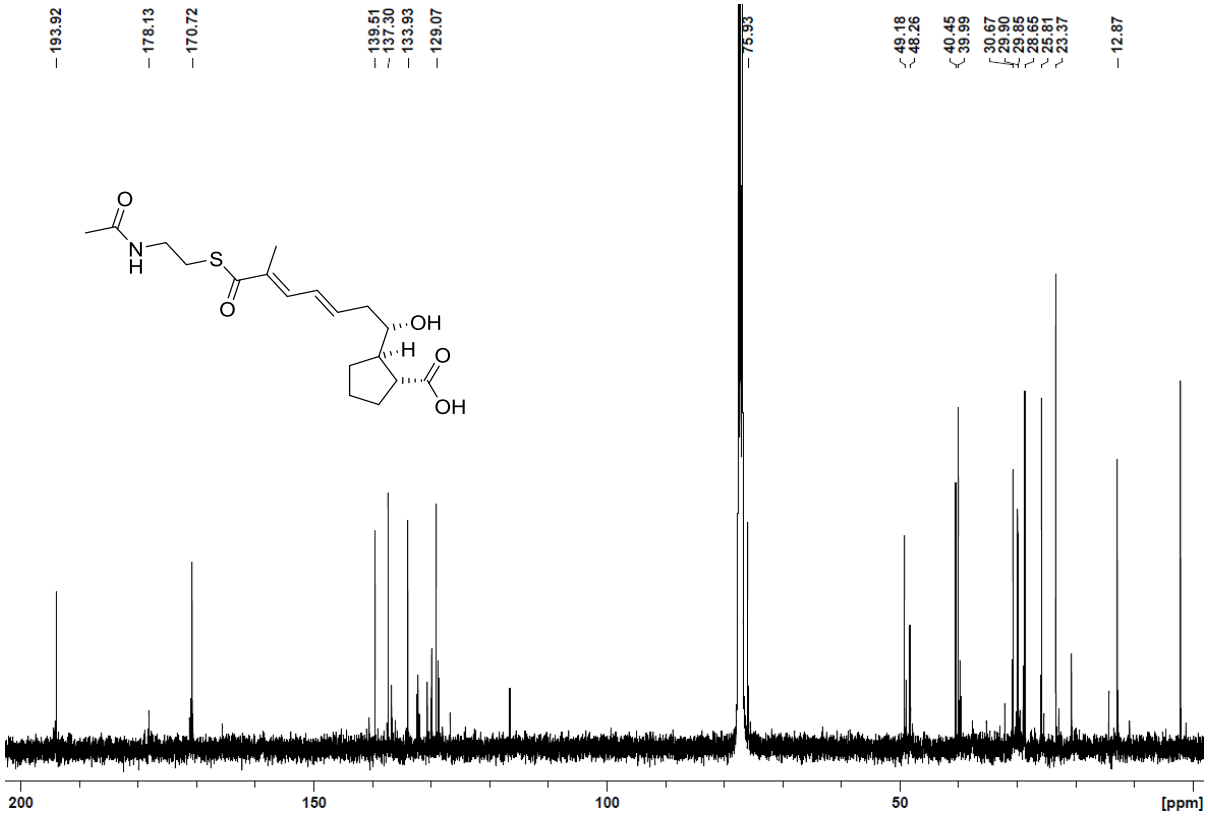
d6-acetone, 125 MHz



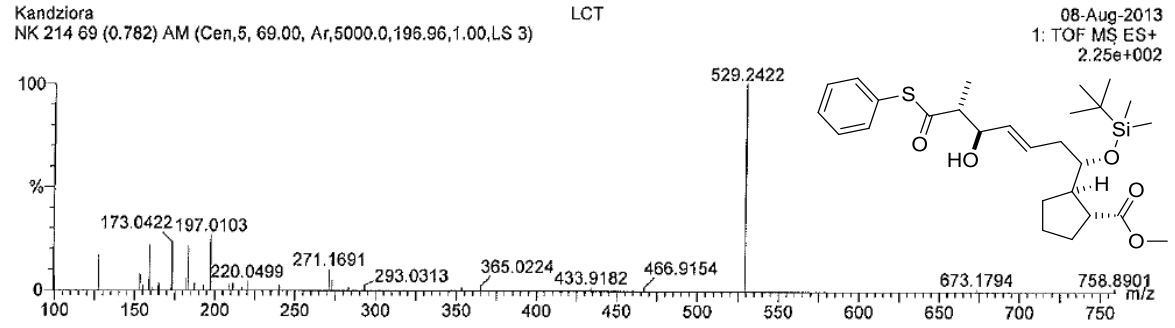
$CDCl_3$, 400 MHz



$CDCl_3$, 100 MHz



Mass spectra



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Maximum:		6.0	5.0	50.0		
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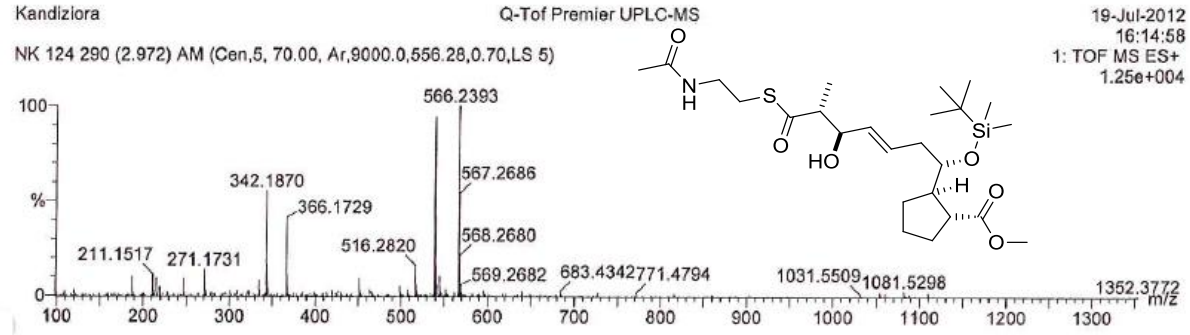
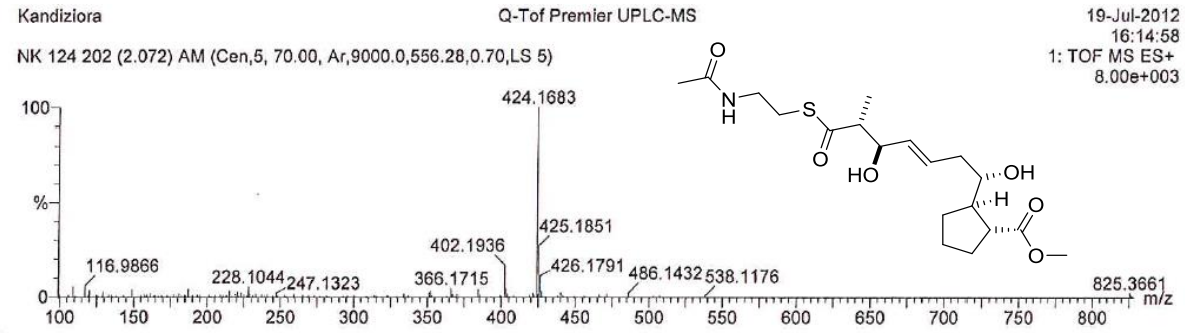
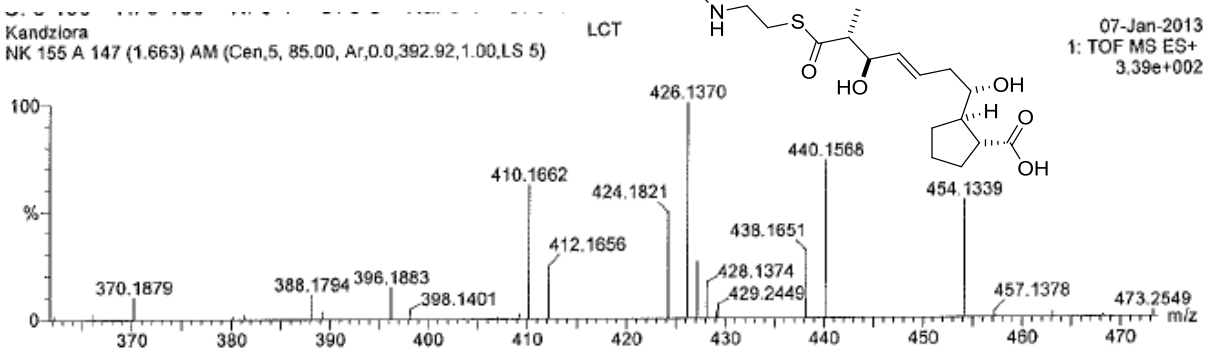
	529.2414	0.8	1.5	8.5	n/a	C27 H38 O9 Na
	529.2413	0.9	1.7	15.5	n/a	C33 H37 O4 S
	529.2410	1.2	2.3	15.5	n/a	C32 H37 O5 Si
	529.2438	-1.6	-3.0	11.5	n/a	C29 H37 O9
	529.2444	-2.2	-4.2	10.5	n/a	C29 H41 O5 S Si
	529.2445	-2.3	-4.3	3.5	n/a	C23 H42 O10 Na Si
	529.2447	-2.5	-4.7	3.5	n/a	C24 H42 O9 Na S



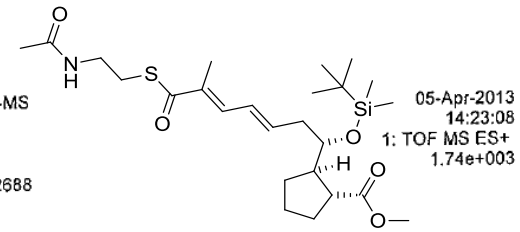
Minimum:			-1.5			
Maximum:		6.0	5.0	51.0		
Mass	Calc. Mass	mDa	PPM	DBE	i-FIT	Formula
516.2820	516.2815	0.5	1.0	4.5	0.1	C25 H46 N O6 S Si
	516.2826	-0.6	-1.2	3.5	0.4	C27 H50 N S4
	516.2824	-0.4	-0.8	3.5	0.6	C26 H50 N O S3 Si
	516.2817	0.3	0.6	4.5	3.5	C26 H46 N O5 S2
	516.2809	1.1	2.1	5.5	9.7	C25 H42 N O10
	516.2842	-2.2	-4.3	0.5	19.1	C22 H46 N O10 S



Minimum:				-1.5		
Maximum:		6.0	5.0	51.0		
Mass	Calc. Mass	mDa	PPM	DBE	i-FIT	Formula
402.1936	402.1917	1.9	4.7	9.5	0.2	C22 H28 N O6
	402.1950	-1.4	-3.5	4.5	0.8	C19 H32 N O6 S
	402.1925	1.1	2.7	8.5	2.2	C23 H32 N O S2

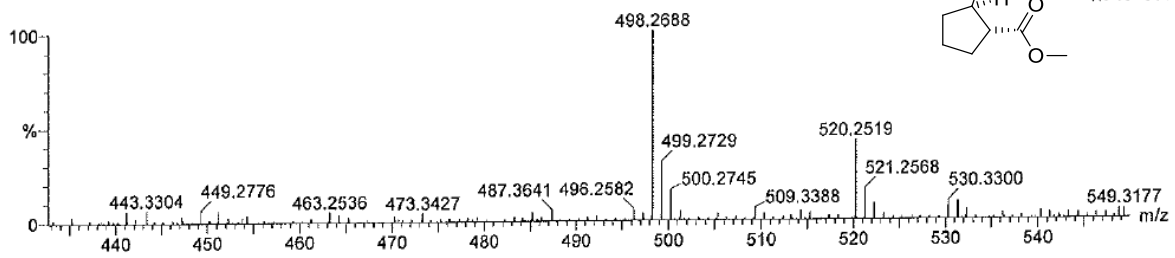


Minimum:				-1.5		
Maximum:		6.0	20.0	50.0		
Mass	Calc. Mass	mDa	PPM	DBE	i-FIT	Formula
388.1794	388.1794	0.0	0.0	4.5	n/a	C18 H30 N O6 S
	388.1770	2.4	6.2	1.5	n/a	C16 H31 N O6 Na S
	388.1760	3.4	8.8	9.5	n/a	C21 H26 N O6
	388.1736	5.8	14.9	6.5	n/a	C19 H27 N O6 Na
	388.1735	5.9	15.2	13.5	n/a	C25 H26 N O S



Kandziora Q-Tof Premier UPLC-MS 05-Apr-2013 14:23:08

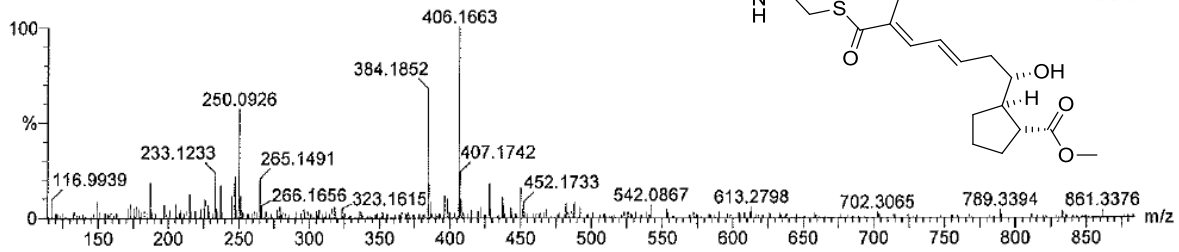
NK166_crude 299 (3.065) AM (Cen,4, 70.00, Ar,10000.0,556.28,0.70,LS 10) 1: TOF MS ES+ 1.74e+003



Minimum:				-1.5		
Maximum:		6.0	20.0	51.0		
Mass	Calc. Mass	mDa	PPM	DBE	i-FIT	Formula
498.2688	498.2678	1.0	2.0	10.5	2.4	C29 H40 N O4 S
	498.2676	1.2	2.4	10.5	8.5	C28 H40 N O5 Si
	498.2709	-2.1	-4.2	5.5	3.5	C25 H44 N O5 Si S
	498.2651	3.7	7.4	14.5	24.7	C32 H40 N Si S
	498.2644	4.4	8.8	15.5	7.3	C32 H36 N O4
	498.2617	7.1	14.2	19.5	35.5	C35 H36 N Si

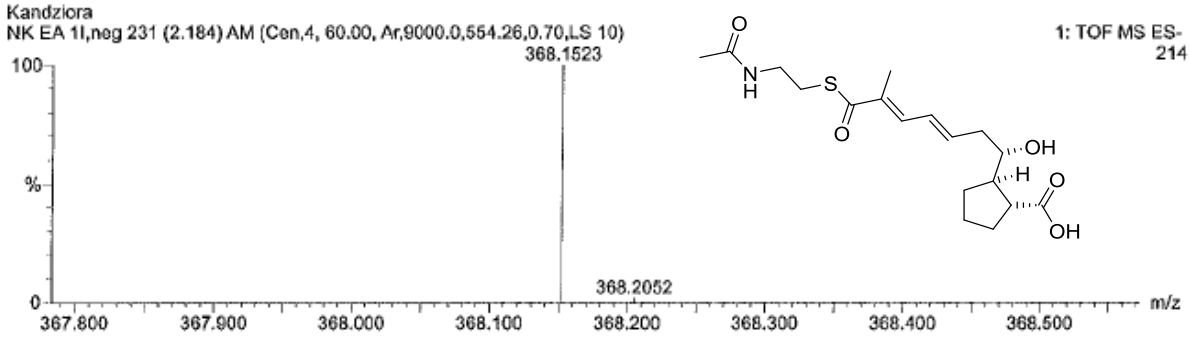
Kandziora Q-Tof Premier UPLC-MS 08-Aug-2013 14:29:20

NK 220 257 (2.898) AM (Cen,5, 90.00, Ar,10000.0,556.28,0.70,LS 5) 1: TOF MS ES+ 2.84e+003

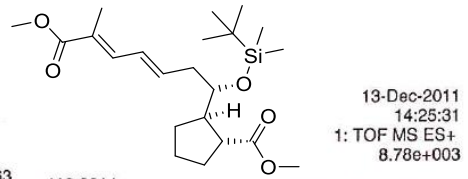


Minimum:				-1.5		
Maximum:		6.0	15.0	51.0		
Mass	Calc. Mass	mDa	PPM	DBE	i-FIT	Formula
406.1663	406.1664	-0.1	-0.2	5.5	2.0	C19 H29 N O5 Na S
	406.1654	0.9	2.2	13.5	38.4	C24 H24 N O5
	406.1688	-2.5	-6.2	8.5	2.0	C21 H28 N O5 S
	406.1689	-2.6	-6.4	1.5	61.9	C15 H29 N O10 Na
	406.1630	3.3	8.1	10.5	40.5	C22 H25 N O5 Na
	406.1629	3.4	8.4	17.5	35.6	C28 H24 N S
	406.1713	-5.0	-12.3	4.5	45.6	C17 H28 N O10
	406.1605	5.8	14.3	14.5	24.1	C26 H25 N Na S

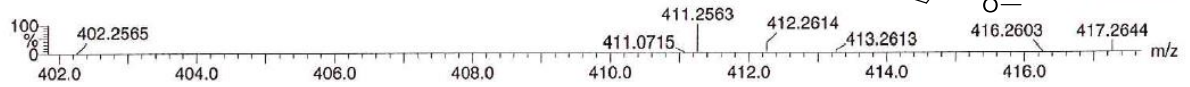
Kandziora
NK EA 1l,neg 231 (2.184) AM (Cen,4, 60.00, Ar,9000.0,554.26,0.70,LS 10)



Minimum:				-1.5							
Maximum:		20.0	10.0	50.0							
Mass	Calc. Mass	mDa	PPM	DBE	i-FIT	Formula					
368.1523	368.1586	-6.3	-17.1	8.5	5546118.0	C18	H23	N3	O4	Na	
	368.1474	4.9	13.3	8.5	5546118.5	C19	H23	N	O5	Na	
	368.1532	-0.9	-2.4	6.5	5546118.5	C18	H26	N	O5	S	✓
	368.1709	-18.6	-50.5	6.5	5546119.0	C18	H26	N	O7		
	368.1409	11.4	31.0	8.5	5546119.5	C18	H23	N3	O2	Na	S
	368.1359	16.4	44.5	12.5	5546119.5	C18	H18	N5	O4		
	368.1545	-2.2	-6.0	11.5	5546120.5	C19	H22	N5	O	S	
	368.1723	-20.0	-54.3	11.5	5546121.0	C19	H22	N5	O3		
	368.1354	16.9	45.9	6.5	5546121.5	C18	H26	N	O3	S2	
	368.1718	-19.5	-53.0	5.5	5546122.0	C19	H30	N	O2	S2	



Kandziora
Q-Tof Premier UPLC-MS
13-Dec-2011
14:25:31
NK 094 319 (3.259) AM (Cen,5, 99.00, Ar,8000.0,556.28,0.70,LS 2)
1: TOF MS ES+
8.78e+003



Minimum:				-1.5							
Maximum:		6.0	5.0	51.0							
Mass	Calc. Mass	mDa	PPM	DBE	i-FIT	Formula					
411.2563	411.2567	-0.4	-1.0	4.5	2.3	C22	H39	O5	Si		
	411.2571	-0.8	-1.9	3.5	306.9	C21	H43	O2	Si3		
	411.2547	1.6	3.9	0.5	257.0	C19	H44	O2	Na	Si3	
	411.2543	2.0	4.9	1.5	16.7	C20	H40	O5	Na	Si	

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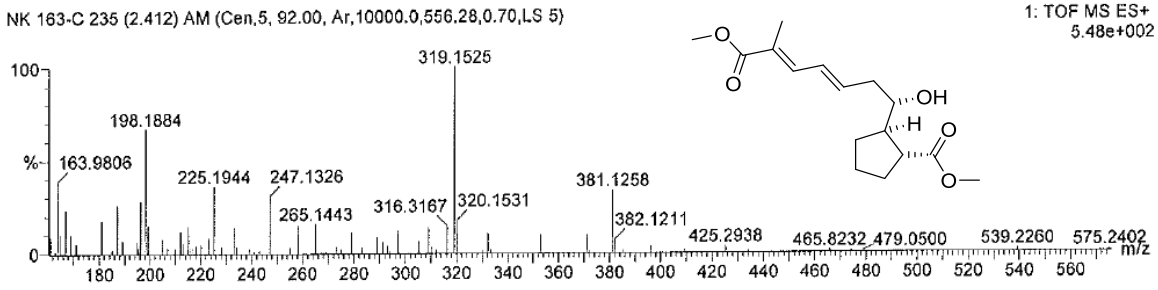
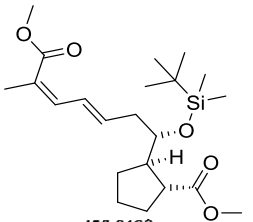
Q-Tof Premier UPLC-MS

20-Mar-2013
09:44:16
1: TOF MS ES+
5.48e+002

NK 163-C 235 (2.412) AM (Cen,5, 92.00, Ar,10000.0,556.28,0.70,LS 5)



Minimum:				-1.5		
Maximum:		6.0	20.0	51.0		
Mass	Calc. Mass	mDa	PPM	DBE	i-FIT	Formula
319.1525	319.1521	0.4	1.3	4.5	0.1	C16 H24 O5 Na
	319.1545	-2.0	-6.3	7.5	1.4	C18 H23 O5
	319.1487	3.8	11.9	16.5	12.7	C25 H19
	319.1463	6.2	19.4	13.5	8.9	C23 H20 Na

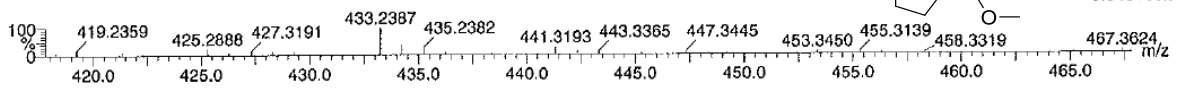


Kandziora

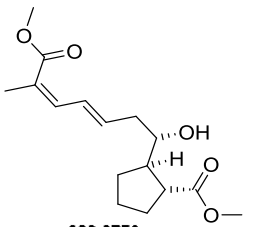
Q-Tof Premier UPLC-MS

15-Dec-2011
08:57:04
1: TOF MS ES+
9.64e+002

NK 101 341 (3.487) AM (Cen,5, 61.00, Ar,8000.0,556.28,0.70,LS 5)



Minimum:				-1.5		
Maximum:		6.0	5.0	51.0		
Mass	Calc. Mass	mDa	PPM	DBE	i-FIT	Formula
433.2387	433.2386	0.1	0.2	4.5	65.3	C22 H38 O5 Na Si
	433.2390	-0.3	-0.7	3.5	17.6	C21 H42 O2 Na Si3

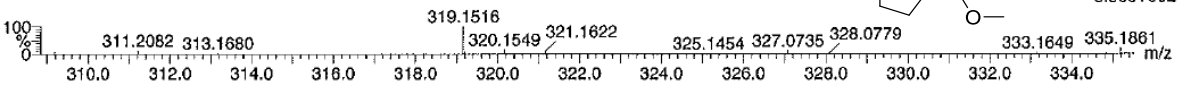


Kandziora

Q-Tof Premier UPLC-MS

13-Dec-2011
14:42:55
1: TOF MS ES+
6.56e+002

NK 101 265 (2.716) AM (Cen,5, 62.00, Ar,8000.0,556.28,0.70,LS 5)



Minimum:				-1.5		
Maximum:		6.0	5.0	51.0		
Mass	Calc. Mass	mDa	PPM	DBE	i-FIT	Formula
319.1516	319.1521	-0.5	-1.6	4.5	17.3	C16 H24 O5 Na