Asymmetric Total Synthesis of (−)-(3*R*)-Inthomycin C

Sandra Balcells,[a] Maxwell B. Haughey,[a] Johannes C. L. Walker,[a] Laia Josa-Culleré,[a¥] Christopher Towers[b] and Timothy J. Donohoe[a*]

[a]*Department of Chemistry, University of Oxford, Chemistry Research Laboratory, 12 Mansfield Road, Oxford, OX1 3TA, United Kingdom*

[b]*Department of Oncology, University of Oxford, Old Road Campus Research Building, Roosevelt Drive, Oxford, OX3 7DQ, United Kingdom*

**timothy.donohoe@chem.ox.ac.uk*

[¥] Author to whom correspondence regarding the biological assays should be addressed.

Supporting Information

Table of contents **Page**

1 General Information

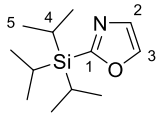
All solvents and reagents requiring purification were purified using standard laboratory techniques[1] apart from CH_2Cl_2, Et_2O, tetrahydrofuran (THF) and MeOH, which were dried by filtration through an activated alumina purification column. All non-aqueous reactions were performed in flame-dried glassware under an argon atmosphere. Thin layer chromatography (TLC) analyses were performed using Merck TLC Silica Gel 60 F254 precoated aluminium-backed plates, visualised under UV light (λ_{max} = 254 nm) and/or stained with aq. potassium permanganate solution. Flash column chromatography (FCC) was performed using Merck Geduran® Silica Gel 60 (40–63 μm). ^{1}H NMR spectra were recorded at 400 MHz or 500 MHz and ^{13}C NMR spectra were recorded at 101 MHz or 126 MHz. Chemical shifts (δ) are reported relative to residual solvent peaks and are quoted in parts per million (ppm) to the nearest 0.01 ppm for ^{1}H NMR and 0.1 ppm for ^{13}C NMR and ^{19}F NMR. Chemical shift multiplicities are reported as: s–singlet, d–doublet, t–triplet, q–quartet, quint.–quintet, sext.–sextet, spt.– septet, m–multiplet, br.–broad, or combinations thereof. Coupling constants (*J*) are quoted to the nearest 0.1 Hz in all instances. High resolution mass spectra (HRMS) under the conditions of positive electrospray ionisation (ESI^+) were recorded on a Thermo Exactive orbitrap spectrometer, and under the conditions of positive chemical ionization (CI^+) recorded on a Waters GTC spectrometer. Fourier transform infrared (FTIR) spectra were obtained from evaporated films using a Bruker Tensor 27 spectrometer; absorption maxima (ν_{max}) are quoted in wavenumbers (cm^{-1}). Melting points (m.p.) were obtained using a Leica VMTG heated-stage microscope and are quoted uncorrected. Specific optical rotations were recorded on a Schmidt and Haemsch Unipol 2000 polarimeter (λ = 589.4 nm). Compounds are named using CambridgeSoft ChemDraw software and, in some cases, atom numbering shown is not always consistent with this name. This is for clarity in assignment and consistency between classes of molecule.

Phosphate Buffered Silica Preparation[2]

A pH 7 buffer solution was prepared by dissolving Na_2HPO_4 (3.46 g) and $NaH_2PO_4.2H_2O$ (2.43 g) in H_2O (400 mL). The solution was mixed with Merck Geduran® Silica Gel 60 (40–63 μm) in a ratio of 50 mL of buffer solution per 5 g of silica. The resulting slurry was dried overnight at 80 °C.

2 Experimental Procedures

2-(Triisopropylsilyl)-oxazole (11)



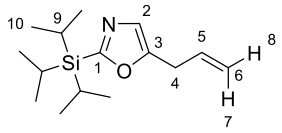
^{n}BuLi (34.2 mL, 2.38 M in hexanes, 79.6 mmol) was added dropwise to a solution of oxazole (**10**) (5.04 g, 73.0 mmol) in THF (103 mL) at –30 °C over 2.5 h. The reaction mixture was stirred for 20 min before addition of TIPSOTf (21.4 mL, 79.6 mmol) over 1.5 h. The solution was stirred for 45 min at –30 °C and for 3.5 h at room temperature before being quenched with sat. aq. NH_4Cl (100 mL). The reaction mixture was extracted with EtOAc (3 × 100 mL) and the combined organic phase washed with sat. aq. NaCl, dried over Na_2SO_4 and concentrated *in vacuo.* FCC (pentane/Et_2O = 97:3) afforded TIPS oxazole **11** as a yellow oil (14.8 g, 91%).

^{1}H NMR (400 MHz, $CDCl_3$) δ = 7.81 (d, *J* = 0.8 Hz, 1H, C(3)*H*), 7.20 (d, *J* = 0.8 Hz, 1H, C(2)*H*), 1.41 (spt, *J* = 7.5 Hz, 3H, 3×C(4)*H*) and 1.12 ppm (d, *J* = 7.5 Hz, 18H, 6×C(5)H_3).

^{13}C NMR (101 MHz, $CDCl_3$) δ = 168.8 (*C*(1)), 140.6 (*C*(3)), 126.7 (*C*(2)), 18.5 (6×*C*(5)) and 11.1 ppm (3×*C*(4)).

Analytical data are in accordance with those previously reported for this compound.[3]

5-Allyl-2-(triisopropylsilyl)oxazole (4)



^{n}BuLi (10.5 mL, 2.33 M in hexanes, 24.4 mmol) was added dropwise over 1 h to a solution of TIPS oxazole **11** (5.09 g, 22.6 mmol) in THF (37 mL) at –78 °C. The reaction mixture was stirred for 30 min at –78 °C and a solution of LiCl (828 mg, 19.5 mmol) and CuCN (875 mg, 9.77 mmol) in THF (74 mL) was added dropwise. [N.B. CuCN and LiCl solution requires sonication to ensure complete dissolution prior to addition to lithiated oxazole. LiCl was also dried prior to use by heating at 130 °C overnight under high vacuum]. After a further 2 h, freshly distilled allyl bromide (2.88 mL, 33.3 mmol) was added dropwise over 30 min and the reaction stirred at room temperature for 2 h.[1] The reaction was quenched with sat. aq. NH_4Cl (100 mL) and extracted with Et_2O (3 × 100 mL). The combined organic phase was washed with sat. aq. NaCl, dried over Na_2SO_4 and concentrated *in vacuo*. FCC (pentane/Et_2O = 95:5) afforded an inseparable 18:1 mixture of allyl oxazole **4** and TIPS oxazole **11** as a colourless oil (5.67 g, 95%). Ratio determined by ^{1}H NMR; δ = 6.84 (t, *J* = 1.1 Hz, 1H, C(2)*H* major) and δ = 7.21 ppm (d, *J* = 0.8 Hz, C(2)*H* minor). [N.B. Yield corrected to 90% to account for inseparable TIPS oxazole **11** impurity. Ratios of allyl oxazole **4** and TIPS oxazole **11** produced in the reaction varied typically between 11:1 and 18:1.]

^{1}H NMR (400 MHz, $CDCl_3$) δ = 6.84 (t, *J* = 1.1 Hz, 1H, C(2)*H*), 5.92 (ddt, *J* = 17.4, 9.7, 6.4 Hz, 1H, C(5)*H*), 5.19 – 5.08 (m, 2H, C(6)*H*(7), C(6)*H*(8)), 3.45 (dq, *J* = 6.4, 1.4 Hz, 2H, C(4)H_2), 1.38 (spt, *J* = 7.5 Hz, 3H, 3×C(9)*H*) and 1.12 ppm (d, *J* = 7.4 Hz, 18H, 6×C(10)H_3).

^{13}C NMR (101 MHz, $CDCl_3$) δ = 167.9 (*C*(1)), 152.6 (*C*(3)), 133.1 (*C*(5)), 123.0 (*C*(2)), 117.5 (*C*(6)), 30.2 (*C*(4)), 18.5 (6×*C*(10)) and 11.1 ppm (3×*C*(9)).

Analytical data are in accordance with those previously reported for this compound.[4]

(*E*)-1,1-Dimethoxybut-2-ene (6)

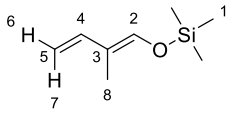
Trimethyl orthoformate (12.0 mL, 72.3 mmol), NH_4NO_3 (289 mg, 3.62 mmol), and MeOH (3.22 mL, 79.5 mmol) were added sequentially to crotonaldehyde (**9**) (5.1 g, 72.3 mmol). The reaction mixture was stirred for 16 h at room temperature before addition of Na_2CO_3 (350 mg, 3.33 mmol) and filtration. Concentration of the filtrate *in vacuo* followed by FCC (pentane/Et_2O = 90:10) through a small pad of silica afforded an inseparable 6.2:1 mixture of acetal **6** and trimethyl orthoformate as a colourless oil (7.44 g, 89%). Ratio determined by ^{1}H NMR; δ = 4.69 (d, *J* = 5.5 Hz, 1H, C(2)*H* major) and δ = 4.96 ppm (s, 1H, C(2')*H* minor).

^{1}H NMR (400 MHz, $CDCl_3$) δ = 5.89 – 5.76 (m, 1H, C(4)*H*), 5.53 – 5.42 (m, 1H, C(3)*H*), 4.69 (d, *J* = 5.5 Hz, 1H, C(2)*H*), 3.33 – 3.29 (m, 6H, 2×C(1)H_3) and 1.76 – 1.69 ppm (m, 3H, C(5)H_3).

^{13}C NMR (101 MHz, $CDCl_3$) δ = 130.6 (*C*(4)), 127.9 (*C*(3)), 103.6 (*C*(2)), 52.8 (2×*C*(1)) and 17.8 ppm (*C*(5)).

Analytical data are in accordance with those previously reported for this compound.[5]

(*E*)-Trimethyl((2-methylbuta-1,3-dien-1-yl)oxy)silane (7)



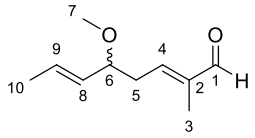
To a solution of tiglic aldehyde (**8**) (5.00 g, 59.4 mmol) in CH_2Cl_2 (74.1 mL) at 0 °C was added triethylamine (12.4 mL, 89.2 mmol). TMSOTf (11.8 mL, 65.3 mmol) was added dropwise over 20 minutes and the solution stirred at 0 °C for 4 h before warming to room temperature and stirring for a further 1 h. The reaction mixture was cooled to 0 °C, washed sequentially with sat. aq. $NaHCO_3$ (50 mL), sat. aq. NH_4Cl (50 mL) and sat. aq. $NaHCO_3$ (50 mL). [N.B. all aqueous solutions were cooled to 0 °C prior to use]. The combined organic phase was dried over Na_2SO_4, filtered and concentrated *in vacuo*. Distillation of the residue (bp 38-39 °C, 12 mbar) afforded silyl enol ether **7** as a colourless oil (9.09 g, 98%).

^{1}H NMR (400 MHz, $CDCl_3$) δ = 6.40 (s, 1H, C(2)*H*), 6.29 (dd, *J* = 17.2, 10.7 Hz, 1H, C(4)*H*), 4.99 (dd, *J* = 17.2, 0.7 Hz, 1H, C(5)*H*(7)), 4.84 (dd, *J* = 10.8, 0.8 Hz, 1H, C(5)*H*(6)), 1.71 (d, *J* = 1.3 Hz, 3H, C(8)H_3) and 0.21 ppm (s, 9H, 3×C(1)H_3).

^{13}C NMR (101 MHz, $CDCl_3$) δ = 141.4 (*C*(2)), 137.1 (*C*(4)), 119.0 (*C*(3)), 108.4 (*C*(5)), 8.9 (*C*(8)) and –0.3 ppm (3×*C*(1)).

Analytical data are in accordance with those previously reported for this compound.[6,7]

(2*E*,6*E*)-5-Methoxy-2-methylocta-2,6-dienal (5)



Acetal **6** (5.00 g, 43.0 mmol) in CH_2Cl_2 (464 mL) and Et_2O (52 mL) was cooled to –78 °C. Silyl enol ether **7** (10.1 g, 64.6 mmol) and $BF_3{\cdot}OEt_2$ (6.38 mL, 51.7 mmol) were added dropwise. The reaction was stirred for 5 h at –78 °C before addition of 1:1 MeOH/Et_3N (200 mL). The solution was diluted with sat. aq. $NaHCO_3$ (150 mL) and extracted with CH_2Cl_2 (3 × 300 mL). The combined organic phase was washed with sat. aq. NaCl (200 mL), dried over Na_2SO_4 and concentrated *in vacuo*. FCC (pentane/Et_2O = 90:10) afforded aldehyde **5** as a yellow oil (5.90 g, 81%).

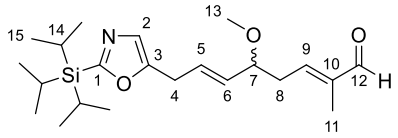
^{1}H NMR (400 MHz, $CDCl_3$) δ = 9.40 (s, 1H, C(1)*H*O), 6.56-6.49 (m, 1H, C(4)*H*), 5.69 (dq, *J* = 15.4, 6.5 Hz, 1H, C(9)*H*), 5.37-5.26 (m, 1H, C(8)*H*), 3.65 (dt, *J* = 7.9, 6.3 Hz, 1H, C(6)*H*), 3.25 (s, 3H, C(7)H_3), 2.65 – 2.46 (m, 2H, C(5)H_2) and 1.76 – 1.69 ppm (m, 6H, C(3)H_3, C(10)H_3).

^{13}C NMR (101 MHz, $CDCl_3$) δ = 195.3 (*C*(1)), 150.4 (*C*(4)), 140.6 (*C*(2)), 130.7 (*C*(8)), 130.1 (*C*(9)), 81.0 (*C*(6)), 56.1 (*C*(7)), 35.4 (*C*(5)), 17.8 (*C*(10)) and 9.5 ppm (*C*(3)).

HRMS (ESI^+) Found $[M+Na]^+$ = 191.1041; $C_{10}H_{16}NaO_2^+$ requires 191.1043.

FTIR ν_{max} (thin film): 2821, 1686, 1645, 1449, 1207, 1094, 969 and 869 cm^{-1}.

(2*E*,6*E*)-5-Methoxy-2-methyl-8-(2-(triisopropylsilyl)oxazole-5-yl)octa-2,6-dienal (12)



To a microwave vial was added Grubbs second generation catalyst (101 mg, 0.119 mmol) followed by aldehyde **5** (984 mg, 5.86 mmol) in degassed CH_2Cl_2 (2.37 mL). An 11.4:1 mixture of allyl oxazole **4** and TIPS oxazole **11** (314 mg, 1.09 mmol) in degassed CH_2Cl_2 (2.37 mL) was added dropwise to the reaction mixture over a period of 7 h at 40 °C. The reaction was heated at 40 °C for a further 44 h. Concentration *in vacuo* followed by FCC (pentane/Et_2O = 70:30) afforded aldehyde **12** as a yellow oil (242 mg, 57%). [N.B. yield calculated based on 1.09 mmol allyl TIPS oxazole **4** present in the 11.4:1 mixture.]

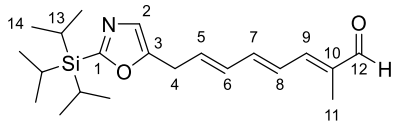
^{1}H NMR (400 MHz, $CDCl_3$) δ = 9.40 (s, 1H, C(12)*H*O), 6.84 (s, 1H, C(2)*H*), 6.53 (tq, *J* = 7.1, 1.4 Hz, 1H, C(9)*H*), 5.86 – 5.75 (m, 1H, C(5)*H*), 5.46 (ddt, *J* = 15.4, 8.0, 1.5 Hz, 1H, C(6)*H*), 3.73 (dt, *J* = 7.5, 6.0 Hz, 1H, C(7)*H*), 3.48 (d, *J* = 6.5 Hz, 2H, C(4)H_2), 3.27 (s, 3H, C(13)H_3), 2.65 – 2.49 (m, 2H, C(8)H_2), 1.74 (s, 3H, C(11)H_3), 1.44 – 1.31 (m, 3H, 3×C(14)*H*) and 1.11 ppm (d, *J* = 7.5 Hz, 18H, 6×C(15)H_3).

^{13}C NMR (101 MHz, $CDCl_3$) δ = 195.2 (*C*(12)), 168.2 (*C*(1)), 152.1 (*C*(3)), 149.9 (*C*(9)), 140.8 (*C*(10)), 132.5 (*C*(6)), 129.2 (*C*(5)), 123.1 (*C*(2)), 80.6 (*C*(7)), 56.4 (*C*(13)), 35.4 (*C*(8)), 28.7 (*C*(4)), 18.5 (6×*C*(15)), 11.1 (3×*C*(14)) and 9.6 ppm (*C*(11)).

HRMS (ESI$^+$) Found $[M+Na]^+$ = 414.2419; $C_{22}H_{37}NNaO_3Si^+$ requires 414.2435.

FTIR ν_{max} (thin film): 2944, 2867, 1688, 1645, 1465, 1101, 973, 920, 884, 733, 676 and 657 cm^{-1}.

(2*E*,4*E*,6*E*)-2-Methyl-8-(2-(triisopropylsilyl)oxazol-5-yl)octa-2,4,6-trienal (2)



To aldehyde **12** (222 mg, 568 μmol) in CH_2Cl_2 (6.4 mL) at 0 °C was added Et_3N (223 μL, 1.60 mmol). After 5 min TBSOTf (234 μL, 1.02 mmol) was added dropwise and the reaction warmed to room temperature over 16 h. $Sc(OTf)_3$ (629 mg, 1.28 mmol) was added, and after stirring for 5 h the reaction was quenched with sat. aq. $NaHCO_3$ (10 mL). The aqueous layer was extracted with CH_2Cl_2 (3 × 15 mL), and the combined organic phase washed with sat. aq. NaCl (10 mL), dried over Na_2SO_4 and concentrated *in vacuo*. FCC (pentane/Et_2O = 70:30) afforded a 8:1 mixture of geometrical isomers of triene **12** as a yellow oil (152 mg, 74%). Ratio determined by ^{1}H NMR; δ = 3.58 (d, *J* = 6.6 Hz, 2H, C(4)$\underline{H_2}$ major) and δ = 3.68 ppm (d, *J* = 7.7 Hz, C(4)$\underline{H_2}$ minor). Although complete separation of the two isomers could not be achieved, it was found that the undesired (*Z*,*E*,*E*) isomer eluted first from FCC (pentane/Et_2O = 70:30). Therefore, the purity of this compound was increased at this stage by cutting fractions to obtain up to a maximum of 22:1 in favour of the major (*E*,*E*,*E*) isomer. Ratios around 14:1 could reliably be obtained and material at 14.3:1 was subsequently carried forward in the synthesis.

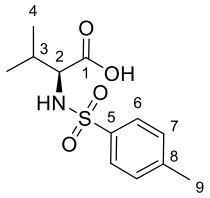
^{1}H NMR (400 MHz, $CDCl_3$) δ = 9.45 (s, 1H, C(12)$\underline{H}$O), 6.91 – 6.83 (m, 2H, C(9)$\underline{H}$, C(2)$\underline{H}$), 6.70-6.54 (m, 2H, C(7)$\underline{H}$, C(8)$\underline{H}$), 6.29 (ddt, *J* = 15.2, 10.0, 1.5 Hz, 1H, C(6)$\underline{H}$), 6.06 (dt, *J* = 15.1, 6.7 Hz, 1H, C(5)$\underline{H}$), 3.58 (d, *J* = 6.6 Hz, 2H, C(4)$\underline{H_2}$), 1.85 (d, *J* = 1.2 Hz, 3H, C(11)$\underline{H_3}$), 1.46 – 1.33 (m, 3H, 3×C(13)$\underline{H}$) and 1.13 ppm (d, *J* = 7.4 Hz, 18H, 6×C(14)$\underline{H_3}$).

^{13}C NMR (101 MHz, $CDCl_3$) δ = 194.8 (C(12)), 168.3 (C(1)), 151.6 (C(3)), 148.4 (C(9)), 140.6 (C(7)), 137.8 (C(10)), 133.9 (C(5)), 132.5 (C(6)), 126.9 (C(8)), 123.3 (C(2)), 29.3 (C(4)), 18.5 (6×C(14)), 11.1 (3×C(13)) and 9.7 ppm (C(11)).

HRMS (ESI+) Found [M+H]+ = 360.2344; $C_{21}H_{34}NO_2Si^+$ requires 360.2353.

FTIR νmax (thin film): 2945, 2867, 2360, 1680, 1613, 1465, 1197, 999, 884, 836 and 678 cm^{-1}.

***N*-Ts-L-valine**[8]



To a stirred solution of L-valine (2.00 g, 17.1 mmol) in aq. 1 M NaOH (35.9 mL, 35.9 mmol) at 0 °C was added dropwise *para*-toluenesulfonyl chloride (3.25 g, 17.1 mmol) in THF (17 mL). The reaction mixture was warmed to room temperature and stirred for 16 h. The mixture was concentrated *in vacuo*, washed with $CHCl_3$ (6 mL) and the aqueous layer acidified to pH = 2 with aq. 1 M HCl. The aqueous layer was extracted with EtOAc (3 × 100 mL) and the combined organic phase washed with sat. aq. NaCl (30 mL), dried over Na_2SO_4, filtered and concentrated *in vacuo* to afford the title compound as a colourless solid (3.27 g, 71%).

m.p. 147-150 °C [Lit.: 150-153 °C].[9]

^{1}H NMR (400 MHz, $CDCl_3$) δ = 7.71 (d, *J* = 8.3 Hz, 2H, 2×C(6)*H*), 7.27 (d, *J* = 8.5 Hz, 2H, 2×C(7)*H*), 5.20 (d, *J* = 9.9 Hz, 1H, C(2)N*H*), 3.78 (dd, *J* = 9.9, 4.6 Hz, 1H, C(2)*H*), 2.40 (s, 3H, C(9)H_3), 2.16 – 2.03 (m, 1H, C(3)*H*), 0.95 (d, *J* = 6.8 Hz, 3H, (C(4)H_3)$_A$(C(4)H_3)$_B$) and 0.86 ppm (d, *J* = 6.9 Hz, 3H, (C(4)H_3)$_A$(C(4)H_3)$_B$).

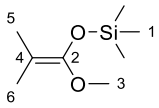
^{13}C NMR (101 MHz, $CDCl_3$) δ = 176.4 (*C*(1)), 144.0 (*C*(5)), 136.7 (*C*(8)), 129.8 (2×*C*(7)), 127.4 (2×*C*(6)), 60.7 (*C*(2)), 31.5 (*C*(3)), 21.7 (*C*(9)), 19.1 (*C*(4)$_A$) and 17.3 ppm (*C*(4)$_B$).

$[\alpha]_D^{25}$ +23 (*c* 1.0, $CHCl_3$).

[Lit. **$[\alpha]_D^{25}$** +17.1 (*c* 2.2, $CHCl_3$).][9]

Analytical data are in accordance with those previously reported for this compound.[9,10]

((1-Methoxy-2-methylprop-1-en-1-yl)oxy)trimethylsilane (3)



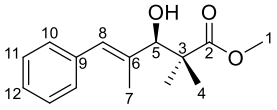
To a solution of diisopropylamine (17.8 mL, 127 mmol) in THF (98 mL) at 0 °C was added dropwise ^{n}BuLi (55.9 mL, 2.10 M in hexanes, 117 mmol). The reaction mixture was stirred for 30 min before addition of methyl isobutyrate (10.0 g, 97.9 mmol) at 0 °C. After 1 h, freshly distilled TMSCl (24.9 mL, 196 mmol) was added and the solution was stirred at 0 °C for a further 4 h. The crude reaction mixture was filtered, washed with Et_2O (15 mL) and concentrated *in vacuo*. Distillation of the residue (bp 38 °C, 12 mbar) afforded silyl enol ether **3** as a colourless oil (11.7 g, 69%).

^{1}H NMR (400 MHz, $CDCl_3$) δ = 3.49 (s, 3H, C(3)$\underline{H}_3$), 1.57 (s, 3H), 1.51 (s, 3H), (C(5)$\underline{H}_3$), (C(6)$\underline{H}_3$) and 0.20 ppm (s, 3H, 3×C(1)$\underline{H}_3$).

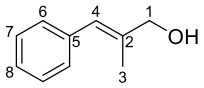
^{13}C NMR (101 MHz, $CDCl_3$) δ = 149.5 ($\underline{C}$(2)), 91.0 ($\underline{C}$(4)), 56.7 ($\underline{C}$(3)), 17.0, 16.3, ($\underline{C}$(5)), ($\underline{C}$(6)) and 0.2 ppm (3×$\underline{C}$(1)).

Analytical data are in accordance with those previously reported for this compound.[11,12]

Methyl (*R*,*E*)-3-hydroxy-2,2,4-trimethyl-5-phenylpent-4-enoate (14) and (*E*)-2-Methyl-3-phenylprop-2-en-1-ol (16)



Aldol product **14**



Alcohol **16**

To a solution *N*-Ts-L-valine (204 mg, 752 μmol) in CH_2Cl_2 (2.9 mL) at 0 °C was added dropwise 1 M BH_3·THF (684 μL, 684 μmol). After 20 min at 0 °C the reaction mixture was warmed to room temperature for 30 min before cooling to –78 °C. Freshly distilled silyl enol ether **3** (72 mg, 410 μmol) was added and the mixture stirred for 5 min before syringe pump addition of α-methyl-*trans*-cinnamaldehyde (50 mg, 340 μmol) in CH_2Cl_2 (0.58 mL) over a period of 3 h. At the end of the addition the syringe was rinsed with CH_2Cl_2 (0.2 mL) and the reaction stirred at –78 °C for a further 2 h before addition of a phosphate buffer solution (3.4 mL, pH = 6.9). The reaction mixture was extracted with CH_2Cl_2 (3 × 30 mL) and the combined organic phase washed with sat. aq. NaCl (10 mL), dried over Na_2SO_4 and concentrated *in vacuo*. The crude product was redissolved in THF (3.4 mL) and treated with aq. 1 M HCl (510 μL, 510 μmol) at room temperature. After 1 h, sat. aq. $NaHCO_3$ was added dropwise until pH = 7. The mixture was concentrated *in vacuo* and extracted with CH_2Cl_2 (3 × 30 mL). The combined organic phase was washed with sat. aq. NaCl (10 mL), dried over Na_2SO_4 and concentrated *in vacuo*. Purification by FCC (pentane/EtOAc = 90:10 to 80:20) afforded aldol product **14** as a colourless oil (69 mg, 81%), er = 93:7, determined by chiral HPLC (Daicel Chiralpak IB, ^{n}hexane/^{i}PrOH = 95:5, 275 nm, 1 mL min^{-1}).

Alcohol **16** was also obtained as a by-product of the reaction as a colourless oil (7 mg, 14%).

Aldol product **14**

^{1}H NMR (400 MHz, $CDCl_3$) δ = 7.37 – 7.30 (m, 2H, 2×C(11)H), 7.29 – 7.25 (m, 2H, 2×C(10)H), 7.22 (tt, *J* = 7.1, 1.4 Hz, 1H, C(12)H), 6.47 (s, 1H, C(8)H), 4.27 (dd, *J* = 5.8, 0.9 Hz, 1H, C(5)H), 3.73 (s, 3H, C(1)H_3), 3.27 (d, *J* = 5.9 Hz, 1H, C(5)OH), 1.84 (d, *J* = 1.4 Hz, 3H, C(7)H_3), 1.30 (s, 3H, (C(4)H_3)$_A$(C(4)H_3)$_B$) and 1.24 ppm (s, 3H, (C(4)H_3)$_A$(C(4)H_3)$_B$).

^{13}C NMR (101 MHz, $CDCl_3$) δ = 178.4 (C(2)), 137.4 (C(9)), 137.2 (C(6)), 129.3 (C(8)), 129.2 (2×C(10)), 128.2 (2×C(11)), 126.7 (C(12)), 83.1 (C(5)), 52.3 (C(1)), 46.9 (C(3)), 24.1 (C(4)$_A$), 21.2 (C(4)$_B$) and 14.9 ppm (C(7)).

$[\alpha]_D^{25}$ +67 (*c* 1.0, $CHCl_3$).

Analytical data are in accordance with those previously reported for this compound.[13]

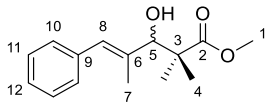
Alcohol **16**

^{1}H NMR (400 MHz, $CDCl_3$) δ = 7.38 – 7.31 (m, 2H, 2×C(7)*H*), 7.31 – 7.27 (m, 2H, 2×C(6)*H*), 7.23 (tt, *J* = 7.2, 1.3 Hz, 1H, C(8)*H*), 6.53 (s, 1H, C(4)*H*), 4.20 (d, *J* = 4.8 Hz, 2H, C(1)H_2), 1.91 ppm (d, *J* = 1.3 Hz, 3H, C(3)H_3), 1.64 ppm (t, *J* = 4.8 Hz, 1H, C(1)O*H*).

^{13}C NMR (101 MHz, $CDCl_3$) δ = 137.8, 137.7, (*C*(2)), (*C*(5)), 129.0 (*C*(6)), 128.3 (2×*C*(7)), 126.6 (*C*(8)), 125.2 (*C*(4)), 69.1 (*C*(1)) and 15.4 ppm (*C*(3)).

Analytical data are in accordance with those previously reported for this compound.[14,15]

Methyl (*E*)-3-hydroxy-2,2,4-trimethyl-5-phenylpent-4-enoate



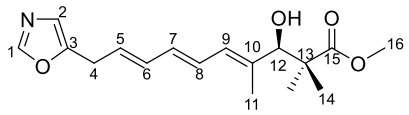
To a solution of diisopropylamine (72.0 µL, 0.513 mmol) in THF (0.860 mL) was added ^{n}BuLi (238 µL, 2.16 M in hexanes, 0.513 mmol) dropwise at −78 °C. The solution was allowed to stir at −78 °C for 30 min and then at 0 °C for 15 min. Methyl isobutyrate (59.0 µL, 0.513 mmol) was added dropwise at −78 °C and the mixture was allowed to stir at −78 °C for 30 min and then at −10 °C for 10 min. A solution of α-methyl-*trans*-cinnamaldehyde (25.0 mg, 0.171 mmol) in THF (0.86 mL) was added dropwise at −78 °C. The solution was allowed to stir for 45 min at −78 °C and then at −10 °C for 30 min. The reaction was quenched with sat. aq. NH_4Cl (5 mL), diluted with sat. aq. NaCl (10 mL), and the aqueous layer extracted with Et_2O (3 × 10 mL). The organic layers were combined, dried over anhydrous Na_2SO_4, filtered and evaporated to dryness. The crude residue was purified by flash column chromatography (pentane/EtOAc = 70:30) to afford the title compound as a pale yellow oil (50.3 mg, 93%).

^{1}H NMR ($CDCl_3$, 400 MHz): δ = 7.36 – 7.30 (m, 2 H, 2×C(10)*H*), 7.29 – 7.25 (m, 2 H, 2×C(11)*H*), 7.25 – 7.20 (m, 1 H, C(12)*H*), 6.47 (s, 1 H, C(8)*H*), 4.27 (d, *J* = 5.6 Hz, 1 H, C(5)*H*), 3.73 (s, 3 H, C(1)H_3), 3.24 (d, *J* = 5.8 Hz, 1 H, C(5)O*H*), 1.84 (d, *J* = 1.3 Hz, 3 H, C(7)*H*), 1.30 (s, 3 H, (C(4)H_3)$_A$(C(4)H_3)$_B$), 1.24 ppm (s, 3 H, (C(4)H_3)$_A$(C(4)H_3)$_B$).

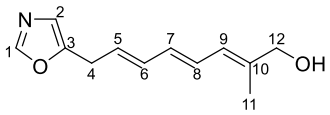
^{13}C NMR ($CDCl_3$, 101 MHz): δ = 178.4 (*C*(2)), 137.4 (*C*(9)), 137.2 (*C*(6)), 129.3 (*C*(8)), 129.2 (2×*C*(11)), 128.2 (2×*C*(10)), 126.7 (*C*(12)), 83.1 (*C*(5)), 52.3 (*C*(1)), 47.0 (*C*(3)), 24.0 (*C*(4)$_A$), 21.2 (*C*(4)$_B$), 14.9 ppm (*C*(7)).

Analytical data are in accordance with those previously reported for this compound.[13]

Methyl (*R*,4*E*,6*E*,8*E*)-3-hydroxy-2,2,4-trimethyl-10-(oxazol-5-yl)deca-4,6,8-trienoate (17) and (2*E*,4*E*,6*E*)-2-Methyl-8-(oxazol-5-yl)octa-2,4,6-trien-1-ol (19)



Aldol product **17**



Alcohol **19**

To a solution *N*-Ts-L-valine (598 mg, 2.21 mmol) in CH_2Cl_2 (8.35 mL) at 0 °C was added dropwise 1 M BH_3·THF (2.01 mL, 2.01 mmol). After 20 min at 0 °C the reaction mixture was warmed to room temperature for 30 min before cooling to –78 °C. Freshly distilled silyl enol ether **3** (210 mg, 1.20 mmol) was added and the mixture stirred for 5 min before syringe pump addition of a 14.3:1 mixture of geometrical isomers of triene **2** (360 mg, 1.00 mmol) in CH_2Cl_2 (1.7 mL) over a period of 3 h. At the end of the addition the syringe was rinsed with CH_2Cl_2 (0.3 mL) and the reaction stirred at –78 °C for a further 2 h before addition of a phosphate buffer solution (10 mL, pH = 6.9). The reaction mixture was extracted with CH_2Cl_2 (3 × 100 mL) and the combined organic phase washed with sat. aq. NaCl (30 mL), dried over Na_2SO_4 and concentrated *in vacuo*. The crude product was redissolved in THF (10 mL) and treated with aq. 1 M HCl (1.5 mL, 1.5 mmol) at room temperature. After 1 h, sat. aq. $NaHCO_3$ was added dropwise until pH = 7. The mixture was concentrated *in vacuo* and extracted with CH_2Cl_2 (3 × 100 mL). The combined organic phase was washed with sat. aq. NaCl (30 mL), dried over Na_2SO_4 and concentrated *in vacuo*. Purification by FCC (pentane/EtOAc = 70:30 to 0:100) afforded a 13.3:1 mixture of geometrical isomers of aldol product **17** as a yellow oil (194 mg, 63%), er = 94:6, determined by chiral HPLC (Daicel Chiralpak IA, ^{n}hexane/^{i}PrOH = 85:15, 284 nm, 1 mL min^{-1}). Ratio determined by ^{1}H NMR; δ = 3.47 (d, *J* = 6.8 Hz, 2H, C(4)$\underline{H}_2$ major) and δ = 3.57 ppm (d, *J* = 7.7 Hz, C(4)$\underline{H}_2$ minor).

An 11.1:1 mixture of geometrical isomers of alcohol **19** was also obtained as a by-product of the reaction as a yellow oil (43 mg, 21%). Ratio determined by ^{1}H NMR; δ = 3.46 (d, *J* = 6.9 Hz, 2H, C(4)$\underline{H}_2$ major) and δ = 3.55 ppm (d, *J* = 7.6 Hz, C(4)$\underline{H}_2$ minor).

Aldol product **17**

^{1}H NMR (400 MHz, $CDCl_3$) δ = 7.78 (s, 1H, C(1)$\underline{H}$), 6.79 (s, 1H, C(2)$\underline{H}$), 6.44 – 6.32 (m, 1H, C(8)$\underline{H}$), 6.28 – 6.15 (m, 2H, C(6)$\underline{H}$, C(7)$\underline{H}$), 6.02 (d, *J* = 11.1 Hz, 1H, C(9)$\underline{H}$), 5.75 (dt, *J* = 13.9, 6.8 Hz, 1H, C(5)$\underline{H}$), 4.17 (d, *J* = 5.1 Hz, 1H, C(12)$\underline{H}$), 3.70 (s, 3H, C(16)$\underline{H}_3$), 3.48 (d, *J* = 6.8 Hz, 2H, C(4)$\underline{H}_2$), 3.05 (d, *J* = 5.5 Hz, 1H, C(12)O$\underline{H}$), 1.74 (s, 3H, C(11)$\underline{H}_3$), 1.20 (s, 3H, (C(14)$\underline{H}_3$)$_A$(C(14)H$_3$)$_B$) and 1.15 ppm (s, 3H, (C(14)H$_3$)$_A$(C(14)$\underline{H}_3$)$_B$).

^{13}C NMR (101 MHz, $CDCl_3$) δ = 178.2 (*C*(15)), 150.9 (*C*(3)), 150.5 (*C*(1)), 137.5 (*C*(10)), 133.6, 132.3, (*C*(6)), (*C*(7)), 128.6 (*C*(9)), 128.3 (*C*(8)), 127.4 (*C*(5)), 122.7 (*C*(2)), 82.3 (*C*(12)), 52.2 (*C*(16)), 47.2 (*C*(13)), 29.0 (*C*(4)), 23.8 (*C*$(14)_A$), 20.9 (*C*$(14)_B$) and 14.1 ppm (*C*(11)).

$[\alpha]_D^{25}$ +1.5 (*c* 1.0, $CHCl_3$).

Analytical data are in accordance with those previously reported for this compound.[13,16–19]

Alcohol **19**

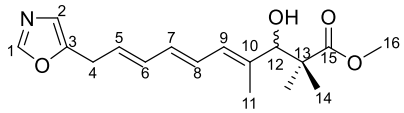
^{1}H NMR (400 MHz, $CDCl_3$) δ = 7.77 (s, 1H, C(1)*H*), 6.77 (s, 1H, C(2)*H*), 6.45 – 6.33 (m, 1H, C(8)*H*), 6.27 – 6.12 (m, 2H, C(6)*H*, C(7)*H*), 6.07 (d, *J* = 11.3 Hz, 1H, C(9)*H*), 5.71 (dt, *J* = 13.8, 6.7 Hz, 1H, C(5)*H*), 4.06 (s, 2H, C(12)H_2), 3.46 (d, *J* = 6.9 Hz, 2H, C(4)H_2), 2.31 (br s, 1H, C(12)O*H*) and 1.77 ppm (s, 3H, C(11)H_3).

^{13}C NMR (101 MHz, $CDCl_3$) δ = 151.0 (*C*(3)), 150.5 (*C*(1)), 138.5 (*C*(10)), 133.7, 131.6, (*C*(6)), (*C*(7)), 128.5 (*C*(8)), 126.9 (*C*(5)), 124.5 (*C*(9)), 122.5 (*C*(2)), 68.2 (*C*(12)), 28.9 (*C*(4)) and 14.3 ppm (*C*(11)).

HRMS (CI^+) Found $[M+H]^+$ = 206.1173; $C_{12}H_{16}NO_2$ requires 206.1176.

FTIR ν_{max} (thin film): 3352, 3130, 3025, 2912, 2857, 1726, 1656, 1511, 1424, 1212, 989, 965, 825 and 647 cm^{-1}.

Methyl (4*E*,6*E*,8*E*)-3-hydroxy-2,2,4-trimethyl-10-(oxazol-5-yl)deca-4,6,8-trienoate



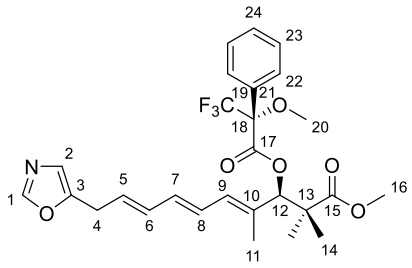
To a solution of diisopropylamine (32 µL, 230 µmol) in THF (0.38 mL) at –78 °C was added dropwise ^{n}BuLi (96 µL, 2.33 M in hexanes, 230 µmol). The solution was stirred for 30 min at –78 °C then warmed to 0 °C. After 15 min the solution was cooled to –78 °C and methyl isobutylate (26 µL, 230 µmol) was added. After 1 h the reaction mixture was warmed to –10 °C and stirred for 30 min before addition of a 12.5:1 mixture of geometrical isomers of triene **2** (27 mg, 75 µmol) in THF (0.5 mL) at –78 °C. After 45 min the reaction mixture was warmed to –10 °C and stirred for 2 h. The reaction was quenched with sat. aq. NH_4Cl (2 mL), diluted with sat. aq. NaCl (10 mL) and extracted with Et_2O (3 × 30 mL). The combined organic phase was dried over Na_2SO_4 and concentrated *in vacuo*. The crude product was redissolved in THF (2 mL) and treated with aq. 1 M HCl (113 µL, 113 µmol) at room temperature. After 30 min, sat. aq. $NaHCO_3$ was added until pH = 7. The mixture was concentrated *in vacuo* and the crude mixture was extracted with Et_2O (3 × 20 mL). The combined organic phase was washed with sat. aq. NaCl, dried over Na_2SO_4 and concentrated *in vacuo*. FCC (pentane/EtOAc = 70:30) afforded an 11.8:1 racemic mixture of geometrical isomers of the title compound as a yellow oil (21.5 mg, 94%). Ratio determined by ^{1}H NMR; δ = 3.48 (d, *J* = 6.6 Hz, 2H, C(4)$\underline{H_2}$ major) and δ = 3.57 ppm (d, *J* = 7.7 Hz, C(4)$\underline{H_2}$ minor).

^{1}H NMR ($CDCl_3$, 400 MHz): δ = 7.78 (s, 1H, C(1)$\underline{H}$), 6.79 (s, 1H, C(2)$\underline{H}$), 6.45 – 6.31 (m, 1H, C(8)$\underline{H}$), 6.29 – 6.15 (m, 2H, C(6)$\underline{H}$, C(7)$\underline{H}$), 6.02 (d, *J*=11.0 Hz, 1H, C(9)$\underline{H}$), 5.74 (spt, *J* = 7.0 Hz, 1H, C(5)$\underline{H}$), 4.17 (d, *J* = 5.5 Hz, 1H, C(12)$\underline{H}$), 3.70 (s, 3H, C(16)$\underline{H_3}$), 3.48 (d, *J* = 6.6 Hz, 2H, C(4)$\underline{H_2}$), 3.12 – 3.06 (m, 1H, C(12)O$\underline{H}$), 1.73 (d, *J* = 1.0 Hz, 3H, C(11)$\underline{H_3}$), 1.20 (s, 3H, (C(14)$\underline{H_3}$)$_A$(C(14)H$_3$)$_B$) and 1.15 ppm (s, 3H, (C(14)H$_3$)$_A$(C(14)$\underline{H_3}$)$_B$).

^{13}C NMR ($CDCl_3$, 101 MHz): δ = 178.3 ($\underline{C}$(15)), 150.9 ($\underline{C}$(3)), 150.5 ($\underline{C}$(1)), 137.4 ($\underline{C}$(10)), 133.6, 132.3 ($\underline{C}$(6)), ($\underline{C}$(7)), 128.6 ($\underline{C}$(9)), 128.2 ($\underline{C}$(8)), 127.4 ($\underline{C}$(5)), 122.7 ($\underline{C}$(2)), 82.3 ($\underline{C}$(12)), 52.3 ($\underline{C}$(16)), 47.2 ($\underline{C}$(13)), 29.0 ($\underline{C}$(4)), 23.8 ($\underline{C}$(14)$_A$), 20.9 ($\underline{C}$(14)$_B$) and 14.1 ppm ($\underline{C}$(11)).

Analytical data are in accordance with those previously reported for this compound.[13,16,17]

Methyl (*R*,4*E*,6*E*,8*E*)-2,2,4-trimethyl-10-(oxazol-5-yl)-3-(((*R*)-3,3,3-trifluoro-2-methoxy-2-phenylpropanoyl)oxy)deca-4,6,8-trienoate[20]



To a stirred solution of (94:6 er) aldol product **17** (5.5 mg, 18 μmol), in CH_2Cl_2 (1 mL) was added (*R*)-(+)-*α*-methoxy-*α*-(trifluoromethyl)phenylacetic acid (42 mg, 180 μmol) followed by DCC (37 mg, 180 μmol) and DMAP (22 mg, 180 μmol) at room temperature. After 48 h additional DCC (19 mg, 90 μmol) and DMAP (11 mg, 90 μmol) were added. The reaction was stirred for 120 h before filtration through a small plug of cotton wool and concentration *in vacuo*. The crude product was redissolved in Et_2O and filtered through cotton wool at –78 °C. Purification twice by multiple elution preparative TLC (cyclohexane:EtOAc = 70:30) afforded the title compound as a pale yellow oil (6.8 mg, 72%).

^{1}H NMR (400 MHz, $CDCl_3$) δ = 7.79 (s, 1H, C(1)*H*), 7.50 – 7.43 (m, 2H, 2×C(23)*H*), 7.40 – 7.33 (m, 3H, 2×C(22)*H*, C(24)*H*), 6.81 (s, 1H, C(2)*H*), 6.32 (dd, *J* = 14.8, 11.1 Hz, 1H, C(8)*H*), 6.22 (dd, *J* = 15.0, 10.6 Hz, 1H, C(6)*H*), 6.10 (dd, *J* = 14.9, 10.7 Hz, 1H, C(7)*H*), 5.91 (d, *J* = 11.1 Hz, 1H, C(9)*H*), 5.77 (dt, *J* = 14.4, 6.8 Hz, 1H, C(5)*H*), 5.61 (s, 1H, C(12)*H*), 3.63 (s, 3H, C(16)H_3), 3.52 – 3.46 (m, 5H, C(20)H_3, C(4)H_2), 1.74 (s, 3H, C(11)H_3), 1.22 (s, 3H, (C(14)H_3)$_A$(C(14)H_3)$_B$) and 1.15 ppm (s, 3H, (C(14)H_3)$_A$(C(14)H_3)$_B$).

^{13}C NMR (126 MHz, $CDCl_3$) δ = 176.0 (*C*(15)), 165.4 (*C*(17)), 150.8 (*C*(3)), 150.6 (*C*(1)), 133.5 (*C*(7)), 133.4 (*C*(6)), 132.5, 132.2, (*C*(10)), (*C*(21)), 130.2 (*C*(9)), 129.6, (2×*C*(22)), 128.5 (*C*(24)) 128.2 (*C*(5)), 127.6 (2×*C*(23)), 127.5 (*C*(8)), 123.5 (q, *J* = 288.0 Hz, *C*(19)), 122.8 (*C*(2)), 84.7 (q, *J* = 27.7 Hz, *C*(18)), 84.2 (*C*(12)), 55.7 (*C*(20)), 52.3 (*C*(16)), 47.2 (*C*(13)), 29.0 (*C*(4)), 22.8 (*C*(14)$_A$), 20.2 (*C*(14)$_B$) and 15.9 ppm (*C*(11)).

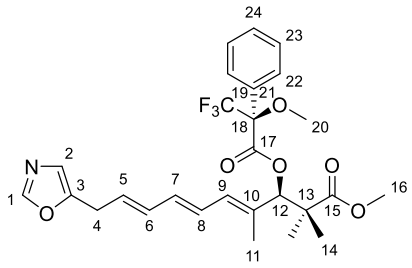
^{19}F NMR (471 MHz, $CDCl_3$) δ = –71.0 ppm (C(19)F_3).

HRMS (CI^+) Found $[M+H]^+$ = 522.2094; $C_{27}H_{31}F_3NO_6$ requires 522.2098.

FTIR ν_{max} (thin film): 3030, 2990, 2952, 2925, 2851, 1747, 1510, 1253, 1131, 1016, 991, 722 and 648 $cm^{-1.}$

$[\alpha]_D^{25}$ +30 (*c* 0.3, $CHCl_3$).

Methyl (*R*,4*E*,6*E*,8*E*)-2,2,4-trimethyl-10-(oxazol-5-yl)-3-(((*S*)-3,3,3-trifluoro-2-methoxy-2-phenylpropanoyl)oxy)deca-4,6,8-trienoate[20]



To a stirred solution of (94:6 er) aldol product **17** (5.9 mg, 19.3 μmol) in CH_2Cl_2 (1 mL) was added (*S*)-(–)-*α*-methoxy-*α*-(trifluoromethyl)phenylacetic acid (22.6 mg, 96.6 μmol) followed by DCC (19.9 mg, 96.6 μmol) and DMAP (11.8 mg, 96.6 μmol) at room temperature. After 144 h additional (*S*)-(–)-*α*-methoxy-*α*-(trifluoromethyl)phenylacetic acid (22.6 mg, 96.6 μmol), DCC (19.9 mg, 96.6 μmol) and DMAP (11.8 mg, 96.6 μmol) were added. After a further 48 h, DCC (19.9 mg, 96.6 μmol) and DMAP (11.8 mg, 96.6 μmol) were added. The reaction was stirred for 96 h before filtration through a small plug of cotton wool and concentration *in vacuo*. The crude product was redissolved in Et_2O and filtered through cotton wool at –78 °C. Purification twice by multiple elution preparative TLC (cyclohexane:EtOAc = 70:30) afforded the title compound as a pale yellow oil (6.8 mg, 67%).

^{1}H NMR (400 MHz, $CDCl_3$) δ = 7.80 (s, 1H, C(1)*H*), 7.50 – 7.44 (m, 2H, 2×C(23)*H*), 7.41 – 7.34 (m, 3H, 2×C(22)*H*, C(24)*H*), 6.81 (s, 1H, C(2)*H*), 6.35 (dd, *J* = 14.2, 11.1 Hz, 1H, C(8)*H*), 6.27 – 6.12 (m, 2H, C(6)*H*, C(7)*H*), 6.02 (d, *J* = 11.4 Hz, 1H, C(9)*H*), 5.79 (dt, *J* = 14.0, 6.9 Hz, 1H, C(5)*H*), 5.66 (s, 1H, C(12)*H*), 3.60 (s, 3H, C(16)H_3), 3.50 (d, *J* = 6.8 Hz, 2H, C(4)H_2), 3.47 (s, 3H, C(20)H_3), 1.79 (s, 3H, C(11)H_3), 1.19 (s, 3H, (C(14)H_3)$_A$(C(14)H_3)$_B$) and 1.14 ppm (s, 3H, (C(14)H_3)$_A$(C(14)H_3)$_B$).

^{13}C NMR (126 MHz, $CDCl_3$) δ = 175.7 (*C*(15)), 165.5 (*C*(17)), 150.8 (*C*(3)), 150.6 (*C*(1)), 133.6, 133.4, (*C*(6)), (*C*(7)), 132.5, 132.1, (*C*(10)), (*C*(21)), 130.5 (*C*(9)), 129.7 (2×*C*(22)), 128.6 (*C*(24)), 128.4 (*C*(5)), 127.8 (2×*C*(23)), 127.5 (*C*(8)), 123.6 (q, *J* = 288.0 Hz, *C*(19)), 122.8 (*C*(2)), 84.7 (q, *J* = 27.7 Hz, *C*(18)), 84.1 (*C*(12)), 55.4 (*C*(20)), 52.2 (*C*(16)), 47.3 (*C*(13)), 29.0 (*C*(4), 22.5 (*C*(14)$_A$), 20.6 (*C*(14)$_B$) and 15.8 ppm (*C*(11)).

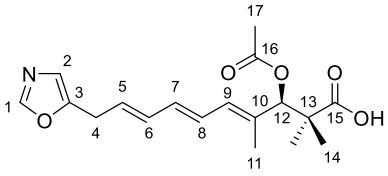
^{19}F NMR (471 MHz, $CDCl_3$) δ = –71.0 ppm (C(19)F_3).

HRMS (ESI^+) Found $[M+Na]^+$ = 544.1914; $C_{27}H_{30}F_3NNaO_6$ requires 544.1917.

FTIR ν_{max} (thin film): 3029, 2989, 2952, 2850, 1744, 1510, 1249, 1169, 1131, 1014, 990, 966, 699 and 647 cm^{-1}.

$[\alpha]_D^{25}$ –7.2 (*c* 0.3, $CHCl_3$).

(*R*,4*E*,6*E*,8*E*)-3-Acetoxy-2,2,4-trimethyl-10-(oxazol-5-yl)deca-4,6,8-trienoic acid (18)[13]



To a 13.3:1 mixture of geometrical isomers of aldol product **17** (195 mg, 638 μmol) in a 3:1:1 solution of THF/MeOH/H_2O (15.9 mL) at 0 °C was added LiOH.H_2O (77.6 mg, 1.85 mmol). The reaction mixture was stirred at room temperature for 12 h before acidifying to pH = 3-4 with 1 M HCl and extraction with EtOAc (3 × 100 mL). The combined organic phase was washed with sat. aq. NaCl (50 mL), dried over Na_2SO_4 and concentrated *in vacuo*. The crude product was redissolved in dry pyridine (14.9 mL, 184 mmol) and cooled to 0 °C. Freshly distilled Ac_2O (7.34 mL, 77.6 mmol) was added dropwise and the reaction mixture warmed to room temperature over 16 h.[1] A solution of $NaHCO_3$ (682 mg, 8.12 mmol) in MeOH (8 mL) was added and the reaction mixture stirred for a further 2 h. Distilled water (50 mL) was added, and the reaction mixture extracted with EtOAc (3 × 100 mL). The combined organic phase was washed with sat. aq. NaCl, dried over Na_2SO_4 and concentrated *in vacuo*. The crude product was azeotroped with benzene (4 × 25 mL) and dried under high vacuum (0.1 mbar) for 1 h. FCC ($CHCl_3$/MeOH = 95:5) afforded a 13.3:1 mixture of geometrical isomers of acid **18** (172 mg, 81%) in addition to a 25:1 mixture of geometrical isomers of acid **18** (13 mg, 6%) as an orange oil. Ratio determined by ^{1}H NMR; δ = 3.48 (d, *J* = 6.8 Hz, 2H, C(4)$\underline{H}_2$ major) and δ = 3.58 ppm (d, *J* = 7.5 Hz, C(4)$\underline{H}_2$ minor).

^{1}H NMR (400 MHz, $CDCl_3$) δ = 7.83 (s, 1H, C(1)$\underline{H}$), 6.81 (s, 1H, C(2)$\underline{H}$), 6.41 – 6.29 (m, 1H, C(8)$\underline{H}$), 6.26 – 6.16 (m, 2H, C(6)$\underline{H}$, C(7)$\underline{H}$), 6.06 (d, *J* = 10.8 Hz, 1H, C(9)$\underline{H}$), 5.80 – 5.68 (m, 1H, C(5)$\underline{H}$), 5.43 (s, 1H, C(12)$\underline{H}$), 3.48 (d, *J* = 6.8 Hz, 2H, C(4)$\underline{H}_2$), 2.05 (s, 3H, C(17)$\underline{H}_3$), 1.79 (s, 3H, C(11)$\underline{H}_3$), 1.23 (s, 3H, (C(14)$\underline{H}_3$)$_A$(C(14)H$_3$)$_B$) and 1.18 ppm (s, 3H, (C(14)H$_3$)$_A$(C(14)$\underline{H}_3$)$_B$).

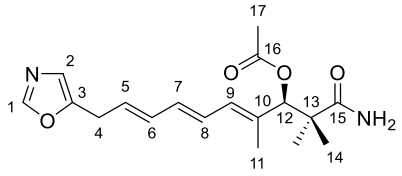
^{13}C NMR (101 MHz, $CDCl_3$) δ = 180.8 ($\underline{C}$(15)), 169.8 ($\underline{C}$(16)), 151.0 ($\underline{C}$(3)), 150.7 ($\underline{C}$(1)), 133.8 ($\underline{C}$(10)), 133.6, 132.9, ($\underline{C}$(6)), ($\underline{C}$(7)), 129.3 ($\underline{C}$(9)), 128.0 ($\underline{C}$(8)), 127.7 ($\underline{C}$(5)), 122.5 ($\underline{C}$(2)), 81.5 ($\underline{C}$(12)), 47.1 ($\underline{C}$(13)), 29.0 ($\underline{C}$(4), 22.5 ($\underline{C}$(14)$_A$), 21.1, ($\underline{C}$(17), 20.7 ($\underline{C}$(14)$_B$) and 15.4 ppm ($\underline{C}$(11)).

$[\alpha]_D^{25}$ +8.3 (*c* 1.0, $CHCl_3$).

[Lit. **$[\alpha]_D^{20}$** +12.55 (*c* 1.1, $CHCl_3$).][13]

Analytical data are in accordance with those previously reported for this compound.[13]

(*R*,4*E*,6*E*,8*E*)-1-Amino-2,2,4-trimethyl-10-(oxazol-5-yl)-1-oxodeca-4,6,8-trien-3-yl acetate[13]



A 13.3:1 mixture of geometrical isomers of acid **18** (34.0 mg, 102 μmol) was azeotroped with benzene (3 × 3 mL) in a 5 mL flask with stirrer bar and dried under high vacuum (0.1 mbar) for 1 h. Freshly distilled CH_2Cl_2 (0.85 mL) was added and the solution cooled to 0 °C. Freshly distilled oxalyl chloride (15.5 μL, 184 μmol) was added dropwise followed by 1 drop of freshly distilled DMF. After stirring for 1 h at 0 °C the reaction was warmed to room temperature and stirred for a further 2 h before addition of NH_4OH (28.0-30.0% NH_3, 4 mL). After 16 h the reaction mixture was diluted with distilled water (10 mL) and extracted with CH_2Cl_2 (3 × 100 mL). The combined organic phase was washed with sat. aq. NaCl (20 mL), dried over Na_2SO_4 and concentrated *in vacuo*. FCC ($CHCl_3$/MeOH = 98:2) afforded an 11.8:1 mixture of geometrical isomers of the title compound as a yellow oil (22.1 mg, 65%). In addition, an 11.1:1 mixture of geometrical isomers of inthomycin C (**1**) was obtained as a yellow oil (5.9 mg, 20%). Ratio determined by ^{1}H NMR; δ = 3.48 (d, *J* = 6.7 Hz, 2H, C(4)$\underline{H}_2$ major) and δ = 3.58 ppm (d, *J* = 7.6 Hz, C(4)$\underline{H}_2$ minor).

^{1}H NMR (400 MHz, $CDCl_3$) δ = 7.78 (s, 1H, C(1)$\underline{H}$), 6.79 (s, 1H, C(2)$\underline{H}$), 6.40 – 6.29 (m, 1H, C(8)$\underline{H}$), 6.27 – 6.16 (m, 2H, C(6)$\underline{H}$, C(7)$\underline{H}$), 6.06 (d, *J* = 11.1 Hz, 1H, C(9)$\underline{H}$), 5.90 (br s, 1H, C(15)N$\underline{H}$), 5.81 – 5.71 (m, 1H, C(5)$\underline{H}$), 5.66 (br s, 1H, C(15)N$\underline{H}$), 5.30 (s, 1H, C(12)$\underline{H}$), 3.48 (d, *J* = 6.7 Hz, 2H, C(4)$\underline{H}_2$), 2.08 (s, 3H, C(17)$\underline{H}_3$), 1.79 (s, 3H, C(11)$\underline{H}_3$), 1.19 (s, 3H, (C(14)$\underline{H}_3$)$_A$(C(14)H$_3$)$_B$) and 1.19 ppm (s, 3H, (C(14)H$_3$)$_A$(C(14)$\underline{H}_3$)$_B$).

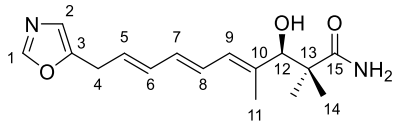
^{13}C NMR (101 MHz, $CDCl_3$) δ = 178.0 ($\underline{C}$(15)), 169.5 ($\underline{C}$(16)), 150.9 ($\underline{C}$(3)), 150.5 ($\underline{C}$(1)), 133.9 ($\underline{C}$(10)), 133.5, 133.1, ($\underline{C}$(6)), ($\underline{C}$(7)), 129.6 ($\underline{C}$(9)), 127.9 ($\underline{C}$(5)), 127.8 ($\underline{C}$(8)), 122.7 ($\underline{C}$(2)), 82.7 ($\underline{C}$(12)), 46.3 ($\underline{C}$(13)), 29.0 ($\underline{C}$(4)), 23.7 ($\underline{C}$(14)$_A$), 21.7 ($\underline{C}$(14)$_B$), 21.2 ($\underline{C}$(17)) and 15.1 ppm ($\underline{C}$(11)).

$[\alpha]_D^{25}$ –2.1 (*c* 1.0, $CHCl_3$).

[Lit. $[\alpha]_D^{20}$ –20.7 (*c* 0.2, $CHCl_3$).][13]

Analytical data are in accordance with those previously reported for this compound.[13]

(–)-(3*R*)-Inthomycin C (1)[13]



To an 11.8:1 mixture of geometrical isomers of (*R*,4*E*,6*E*,8*E*)-1-Amino-2,2,4-trimethyl-10-(oxazol-5-yl)-1-oxodeca-4,6,8-trien-3-yl acetate (18.0 mg, 54.2 μmol) in a 3:1:1 solution of THF/MeOH/H_2O (0.6 mL) at 0 °C was added LiOH·H_2O (4.8 mg, 110 μmol). The reaction mixture was stirred at 0 °C for 1 h and room temperature for 2 h. Additional LiOH·H_2O (2.3 mg, 54 μmol) was added at 0 °C and the solution stirred at room temperature for 2 h before concentration *in vacuo*. Distilled water (10 mL) was added and the solution acidified to pH = 4 with 1 M HCl. The aqueous layer was extracted with EtOAc (3 × 50 mL), and the combined organic phase washed with sat. aq. NaCl (15 mL), dried over Na_2SO_4 and concentrated *in vacuo*. FCC ($CHCl_3$/MeOH = 98:2) afforded an 11.1:1 mixture of geometrical isomers of (–)-(3*R*)-inthomycin C (**1**) as a yellow oil (13.7 mg, 87%). Ratio determined by ^{1}H NMR; δ = 3.48 (d, *J* = 7.0 Hz, 2H, C(4)$\underline{H_2}$ major) and δ = 3.58 ppm (d, *J* = 7.7 Hz, C(4)$\underline{H_2}$ minor).

[N.B. For convenience we have calculated the yield of (–)-(3*R*)-inthomycin C (**1**) from acid **18** over 2 steps as (65 × 0.87) + 20 = 77%]

^{1}H NMR (500 MHz, $CDCl_3$) δ = 7.78 (s, 1H, C(1)$\underline{H}$), 6.79 (s, 1H, C(2)$\underline{H}$), 6.43 – 6.33 (m, 1H, C(8)$\underline{H}$), 6.30 – 6.18 (m, 3H, C(15)N$\underline{H}$, C(6)$\underline{H}$, C(7)$\underline{H}$), 6.01 (d, *J* = 11.1 Hz, 1H, C(9)$\underline{H}$), 5.75 (dt, *J* = 13.9, 6.9 Hz, 1H, C(5)$\underline{H}$), 5.59 (br s, 1H, C(15)N$\underline{H}$), 4.01 (d, *J* = 4.5 Hz, 1H, C(12)$\underline{H}$), 3.91 (d, *J* = 5.0 Hz, 1H, C(12)O$\underline{H}$), 3.48 (d, *J* = 7.0 Hz, 2H, C(4)$\underline{H_2}$), 1.78 (s, 3H, C(11)$\underline{H_3}$), 1.29 (s, 3H, (C(14)$\underline{H_3}$)$_A$(C(14)H$_3$)$_B$) and 1.10 ppm (s, 3H, (C(14)H$_3$)$_A$(C(14)$\underline{H_3}$)$_B$).

^{13}C NMR (126 MHz, $CDCl_3$) δ = 180.9 ($\underline{C}$(15)), 150.9 ($\underline{C}$(3)), 150.6 ($\underline{C}$(1)), 138.1 ($\underline{C}$(10)), 133.6, 132.5, ($\underline{C}$(6)), ($\underline{C}$(7)), 128.9 ($\underline{C}$(9)), 128.2 ($\underline{C}$(8)), 127.5 ($\underline{C}$(5)), 122.7 ($\underline{C}$(2)), 83.9 ($\underline{C}$(12)), 45.1 ($\underline{C}$(13)), 29.0 ($\underline{C}$(4)), 25.8 ($\underline{C}$(14)$_A$), 21.8 ($\underline{C}$(14)$_B$) and 13.5 ppm ($\underline{C}$(11)).

^{1}H NMR (500 MHz, Acetone-d_6) δ = 7.99 (s, 1H, C(1)$\underline{H}$), 6.93 (s, 1H, C(15)N$\underline{H}$), 6.83 (s, 1H, C(2)$\underline{H}$), 6.48 (dd, *J* = 14.4, 11.4 Hz, 1H, C(8)$\underline{H}$), 6.35 – 6.19 (m, 3H, C(6)$\underline{H}$, C(7)$\underline{H}$, C(15)N$\underline{H}$), 6.04 (d, *J* = 11.2 Hz, 1H, C(9)$\underline{H}$), 5.79 (dt, *J* = 14.6, 6.9 Hz, 1H, C(5)$\underline{H}$), 5.05 (d, *J* = 4.9 Hz, 1H, C(12)O$\underline{H}$), 4.02 (d, *J* = 5.0 Hz, 1H, C(12)$\underline{H}$), 3.52 (d, *J* = 6.8 Hz, 2H, C(4)$\underline{H_2}$), 1.77 (s, 3H, C(11)$\underline{H_3}$), 1.21 (s, 3H, (C(14)$\underline{H_3}$)$_A$(C(14)H$_3$)$_B$) and 1.07 ppm (s, 3H, (C(14)H$_3$)$_A$(C(14)$\underline{H_3}$)$_B$).

^{13}C NMR (126 MHz, Acetone-d_6) δ = 180.6 ($\underline{C}$(15)), 151.8 ($\underline{C}$(3)), 151.6 ($\underline{C}$(1)), 140.1 ($\underline{C}$(10)), 134.2 ($\underline{C}$(6)), 132.7 ($\underline{C}$(7)), 129.1 ($\underline{C}$(8)), 128.7 ($\underline{C}$(9)), 128.3 ($\underline{C}$(5)), 123.2 ($\underline{C}$(2)), 83.9 ($\underline{C}$(12)), 45.7 ($\underline{C}$(13)), 29.2 ($\underline{C}$(4)), 25.6 ($\underline{C}$(14)$_A$), 22.5 ($\underline{C}$(14)$_B$) and 13.4 ppm ($\underline{C}$(11)).

HRMS (ESI$^+$) Found [M+Na]$^+$ = 313.1522; $C_{16}H_{22}N_2NaO_3$ requires 313.1523.

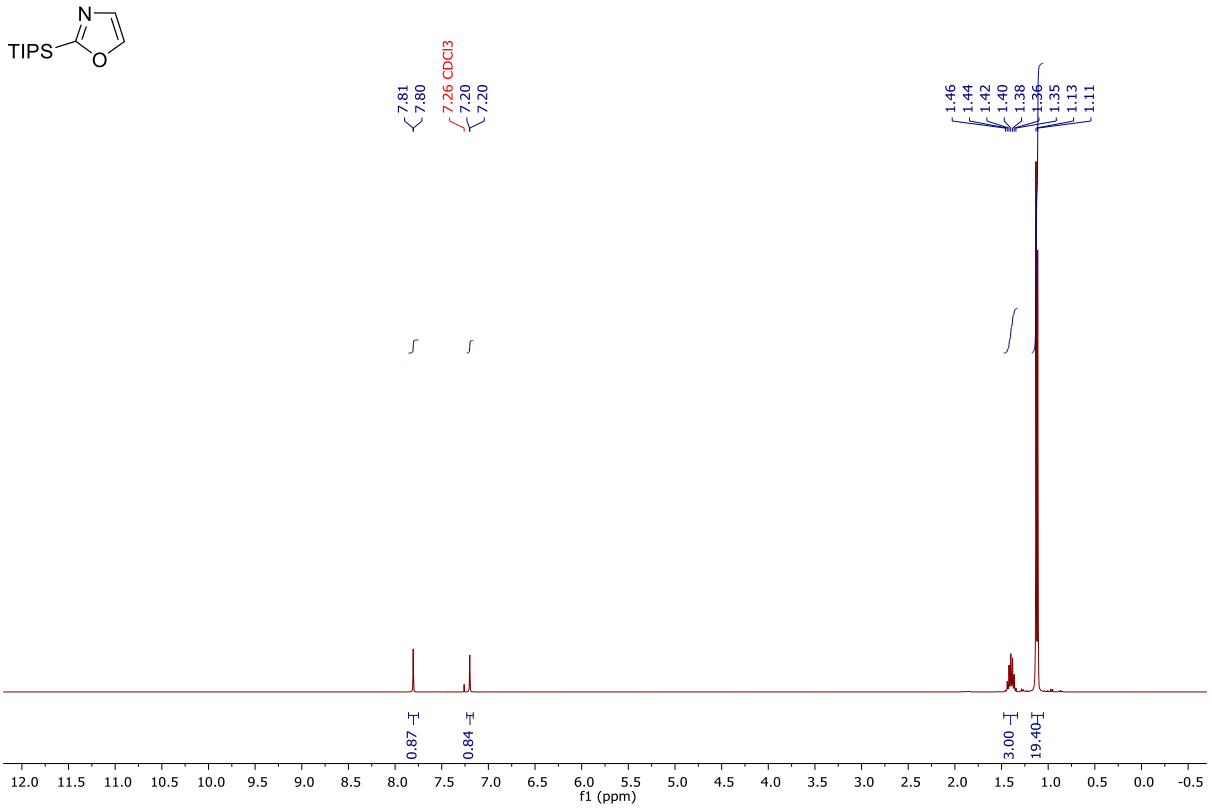
FTIR ν_{max} (thin film): 3339, 3026, 2974, 2925, 2871, 1655, 1601, 1510, 1472, 1363, 1287, 1108, 1042, 989, 825 and 647 cm^{-1}.

$\mathbf{[\alpha]_D^{25}}$ –8.2 (*c* 1.0, $CHCl_3$).

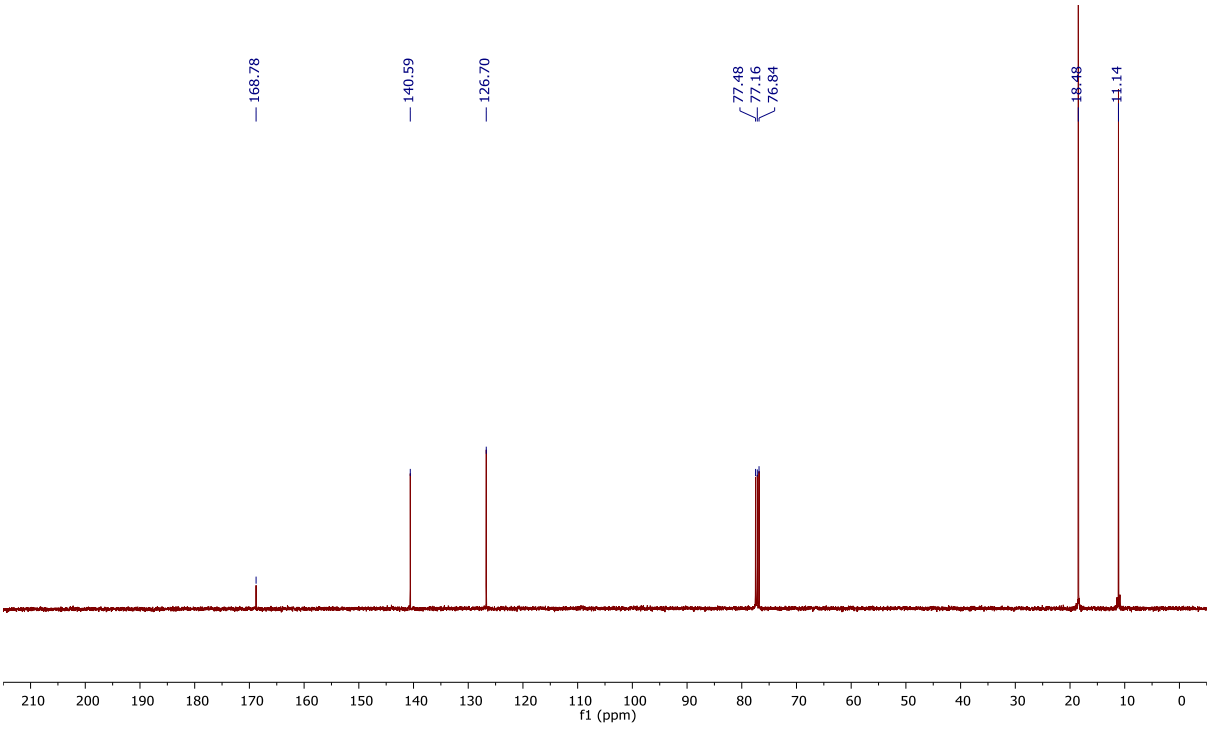
Analytical data are in accordance with those previously reported for this compound.[13,16–19]

3 NMR Spectra

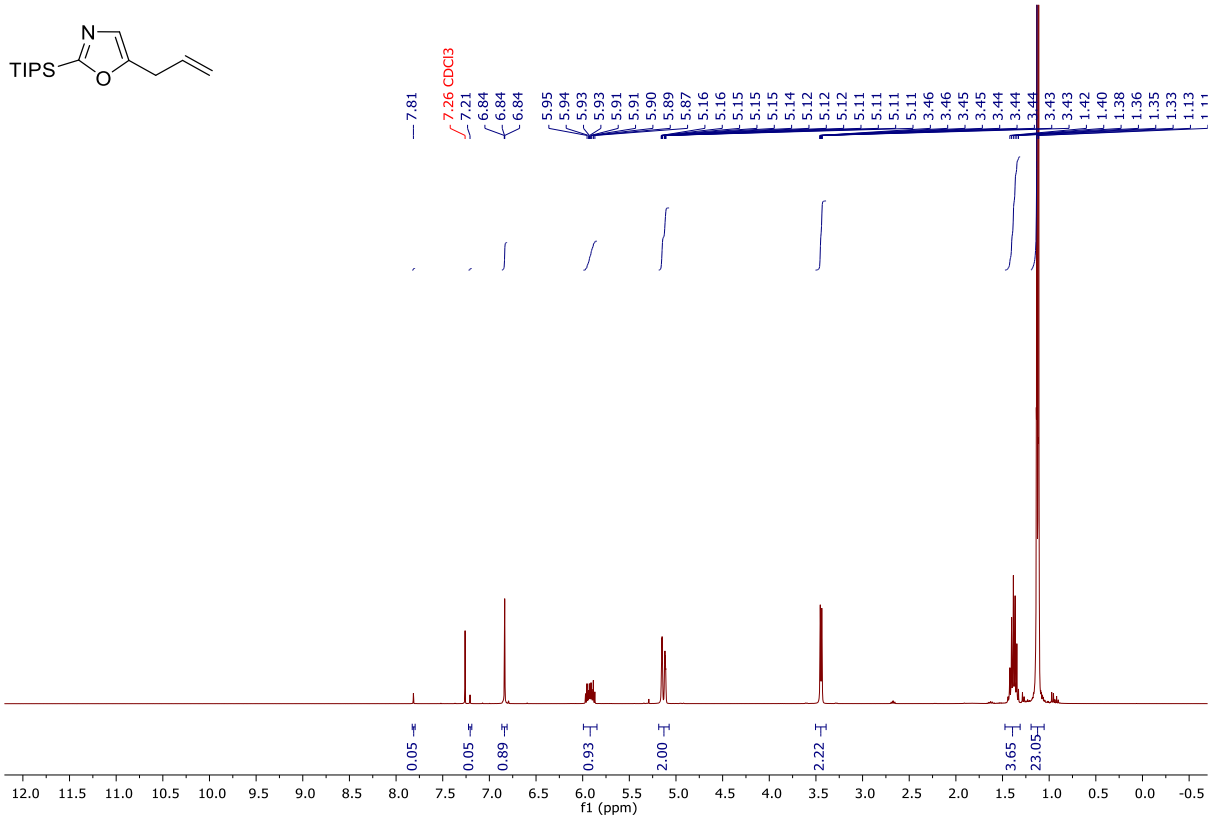
^{1}H NMR (400 MHz, $CDCl_3$): 2-(Triisopropylsilyl)oxazole (11)



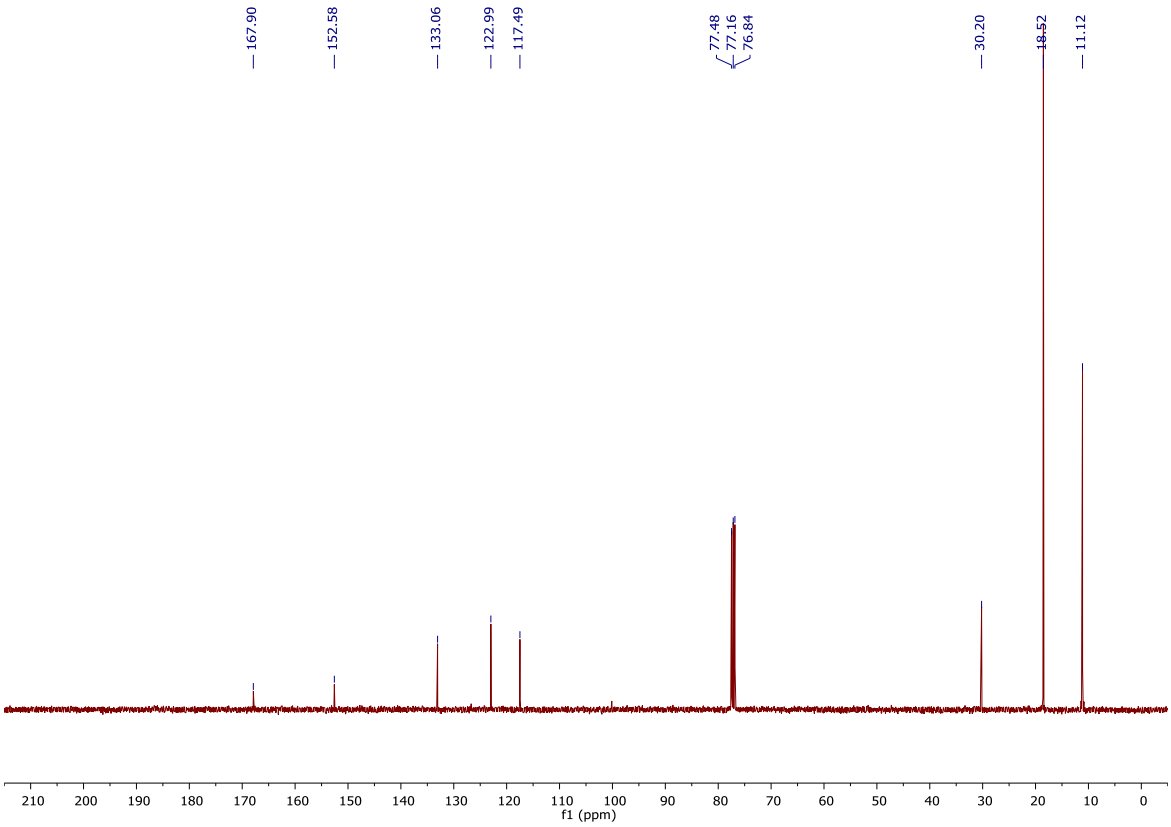
^{13}C NMR (101 MHz, $CDCl_3$):



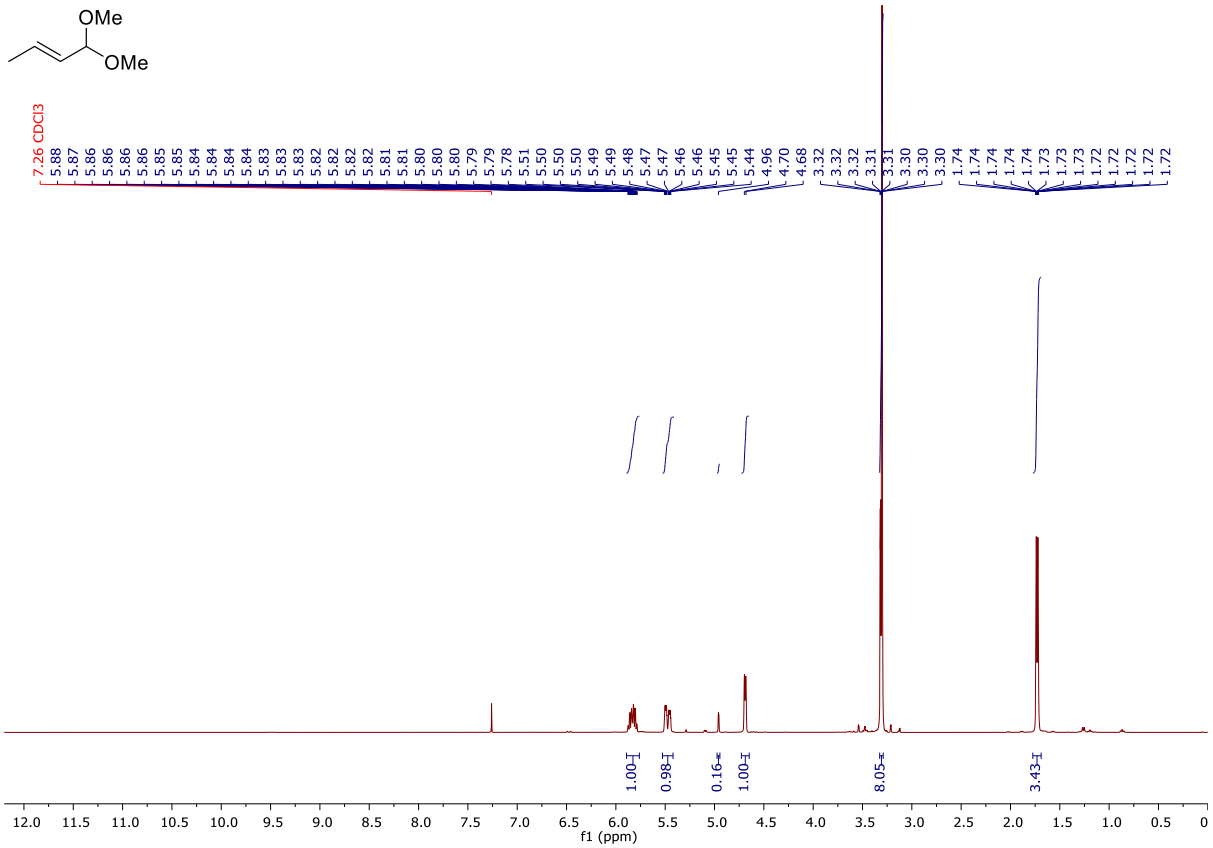
^{1}H NMR (400 MHz, $CDCl_3$): 5-allyl-2-(Triisopropylsilyl)oxazole (4)



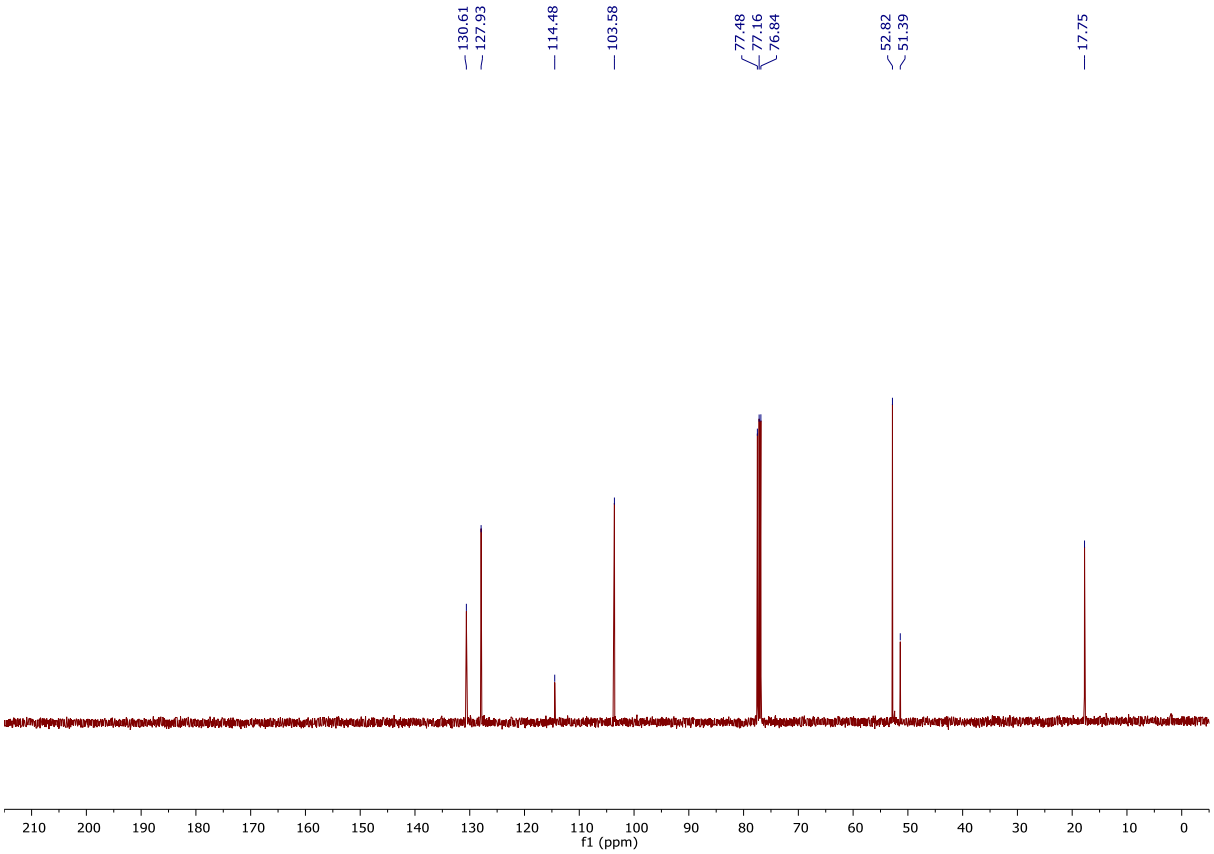
^{13}C NMR (101 MHz, $CDCl_3$):



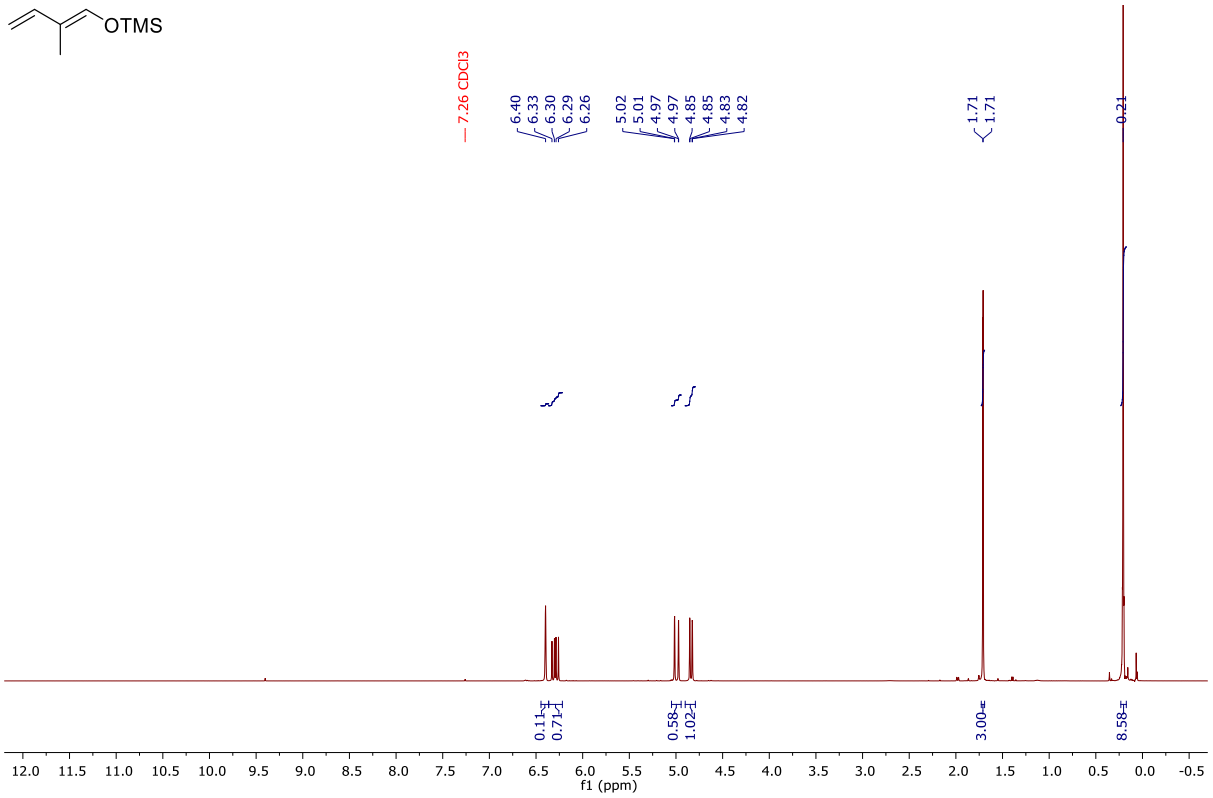
^{1}H NMR (400 MHz, $CDCl_3$): (*E*)-1,1-Dimethoxybut-2-ene (6)



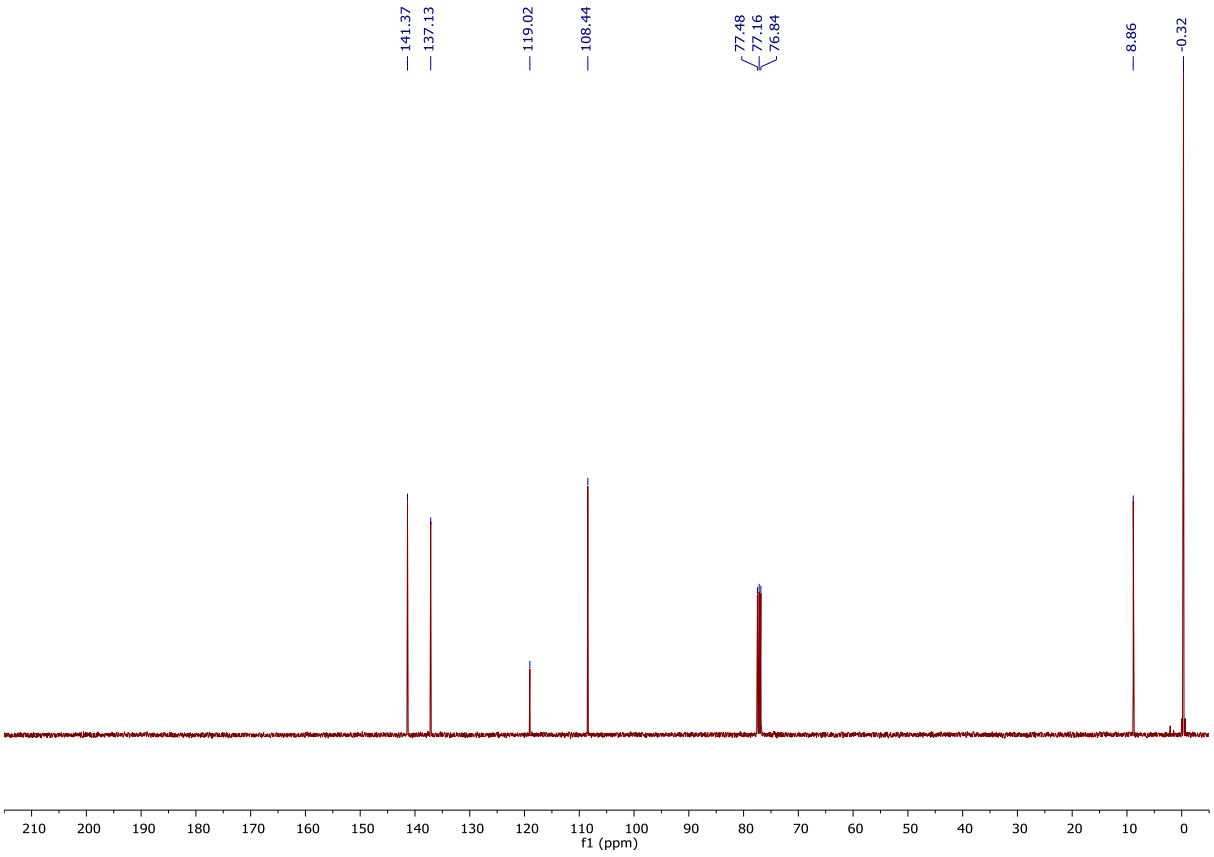
^{13}C NMR (101 MHz, $CDCl_3$):



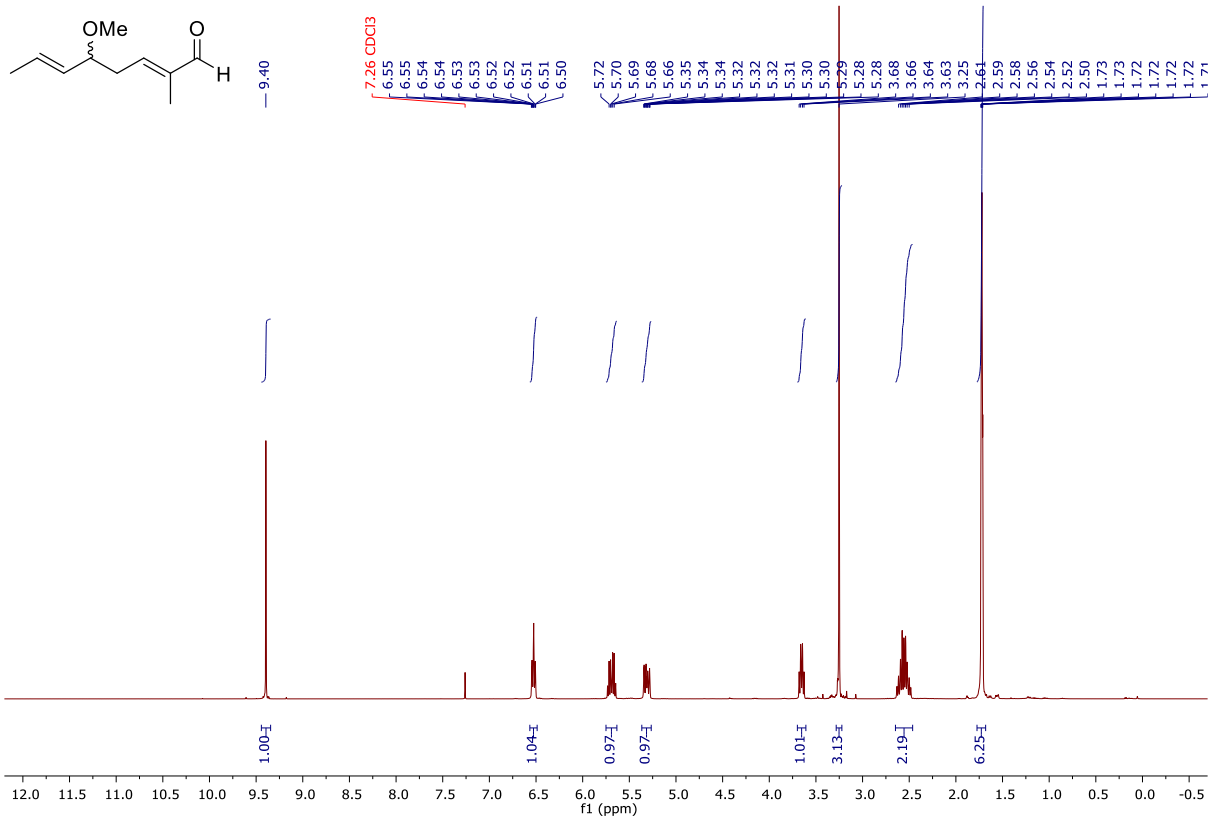
^{1}H NMR (400 MHz, $CDCl_3$): (*E*)-Trimethyl((2-methylbuta-1,3-dien-1-yl)oxy)silane (7)



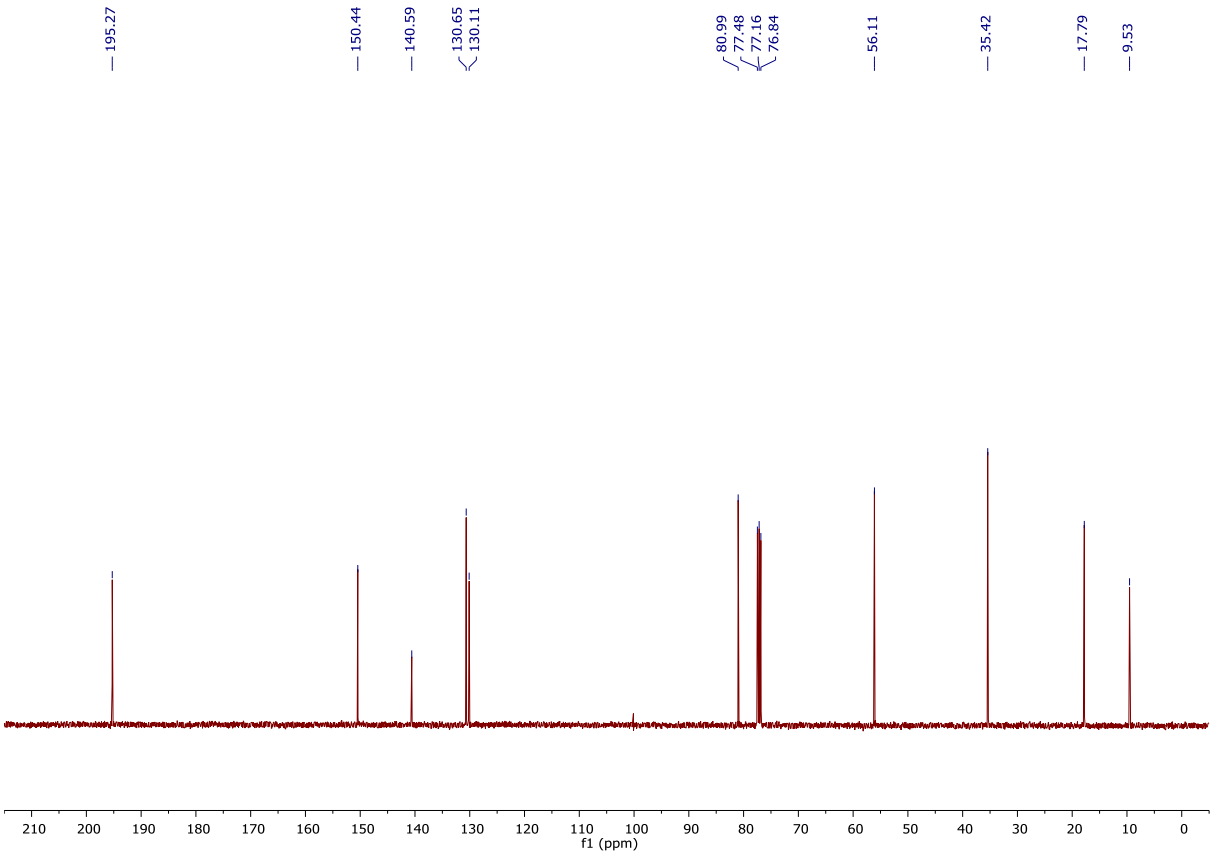
^{13}C NMR (101 MHz, $CDCl_3$):



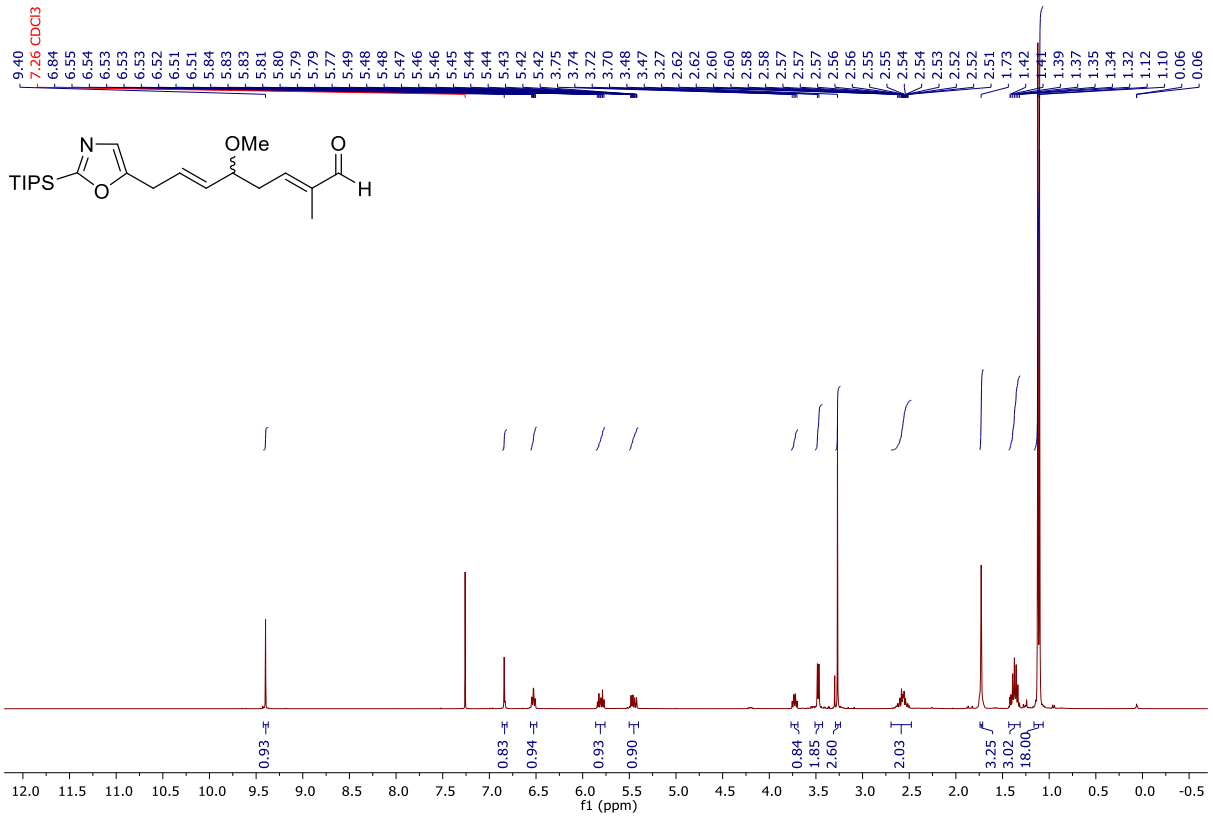
^{1}H NMR (400 MHz, $CDCl_3$): (2*E*,6*E*)-5-Methoxy-2-methylocta-2,6-dienal (5)



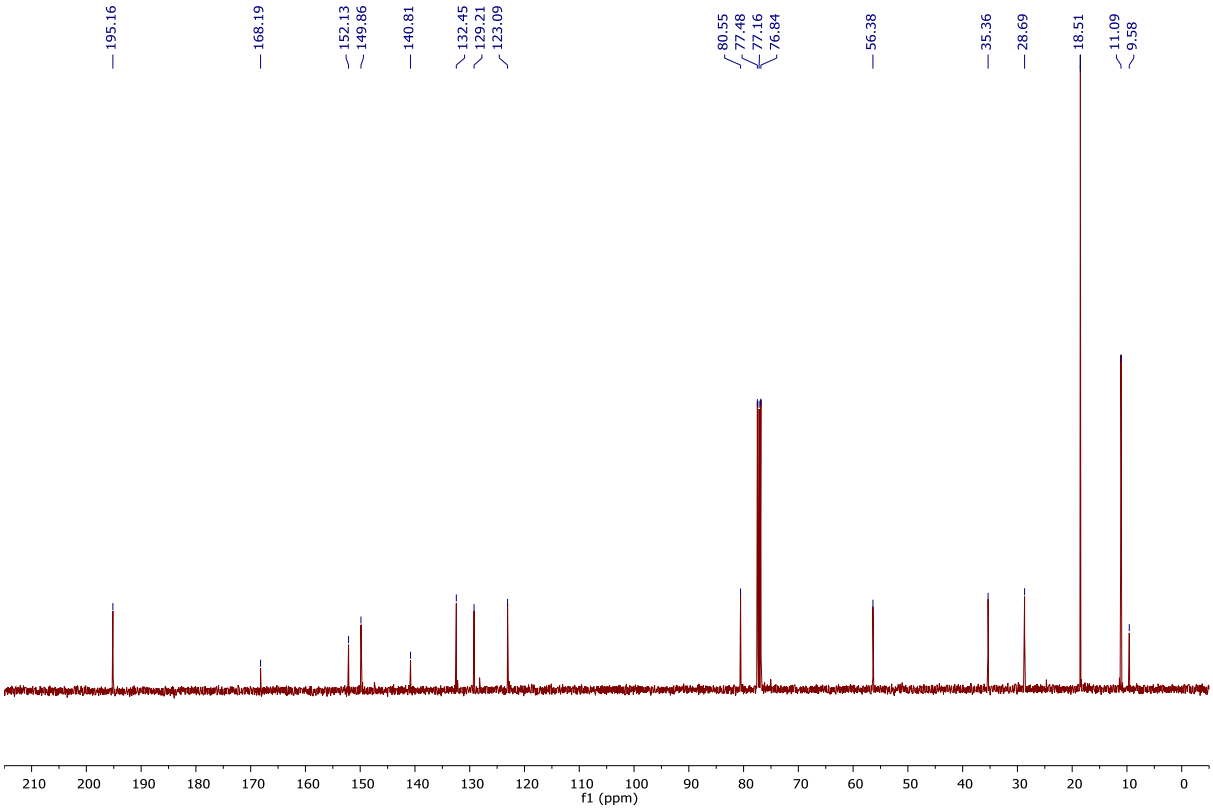
^{13}C NMR (101 MHz, $CDCl_3$):



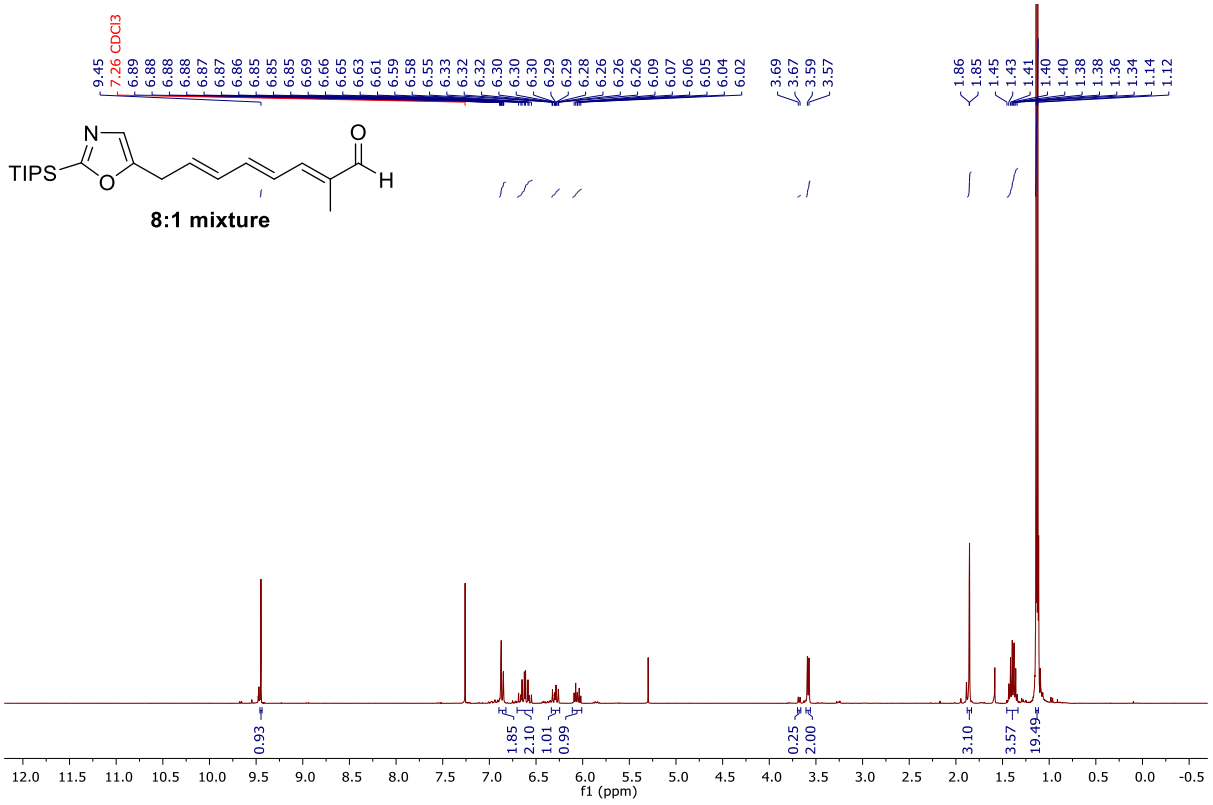
^{1}H NMR (400 MHz, $CDCl_3$): (2*E*,6*E*)-5-Methoxy-2-methyl-8-(2-(triisopropylsilyl)oxazol-5-yl)octa-2,6-dienal (12)



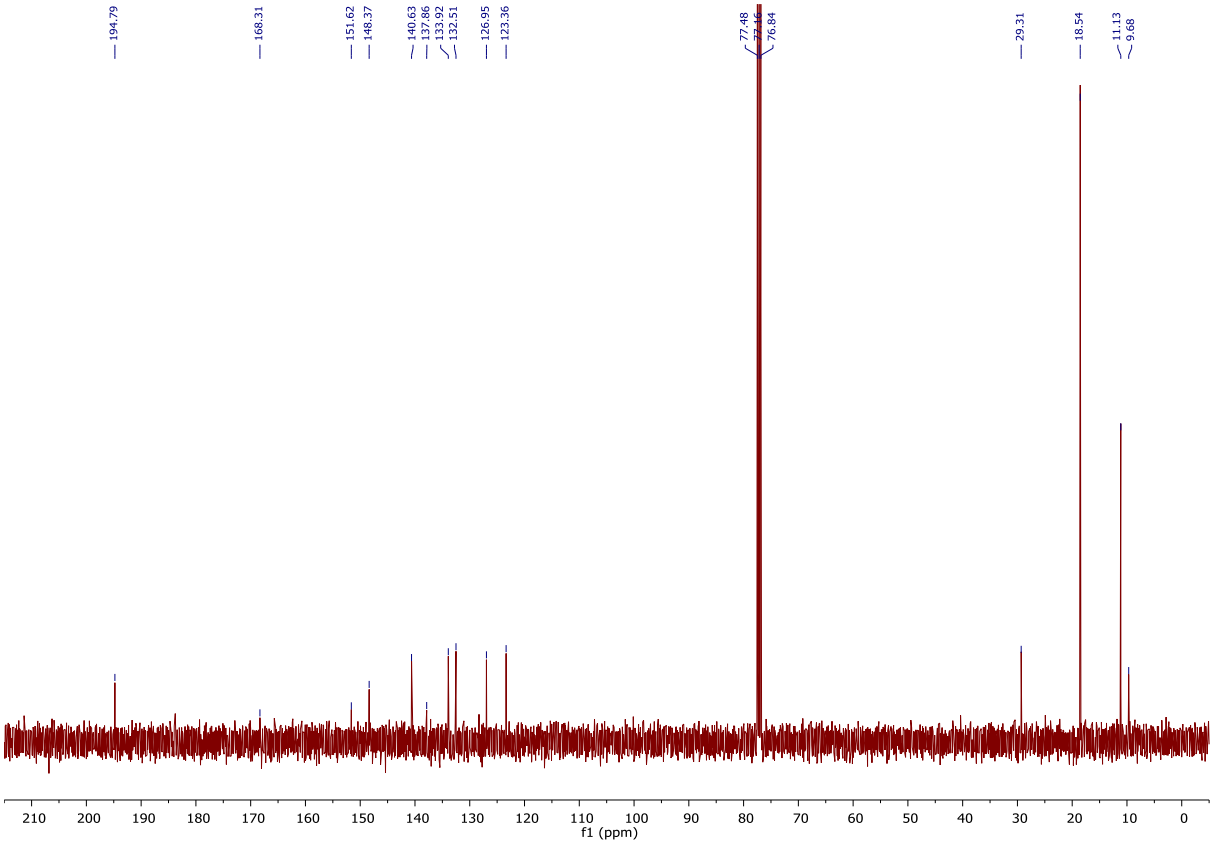
^{13}C NMR (101 MHz, $CDCl_3$):



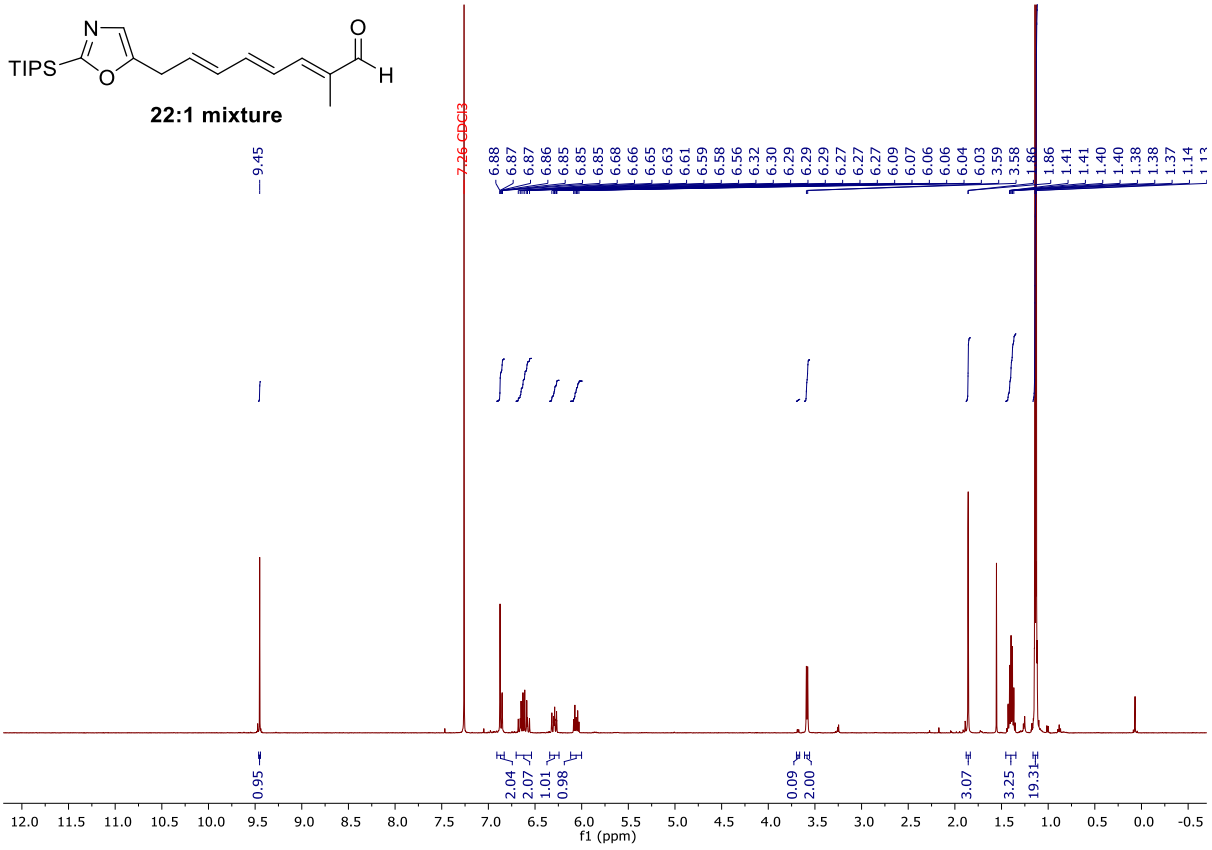
^{1}H NMR (400 MHz, $CDCl_3$): (4*E*,6*E*,8*E*)-2-Methyl-8-(2-(triisopropylsilyl)oxazol-5-yl)octa-2,4,6-trienal (2)



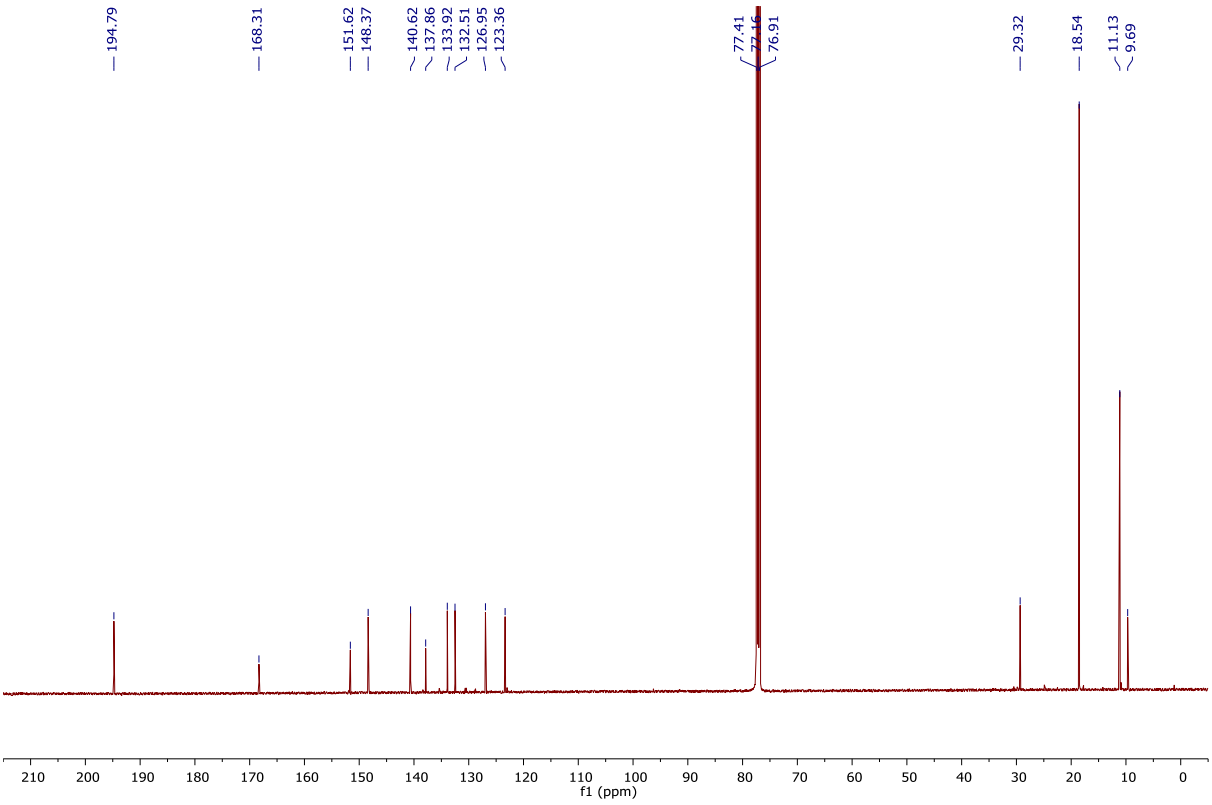
^{13}C NMR (101 MHz, $CDCl_3$):



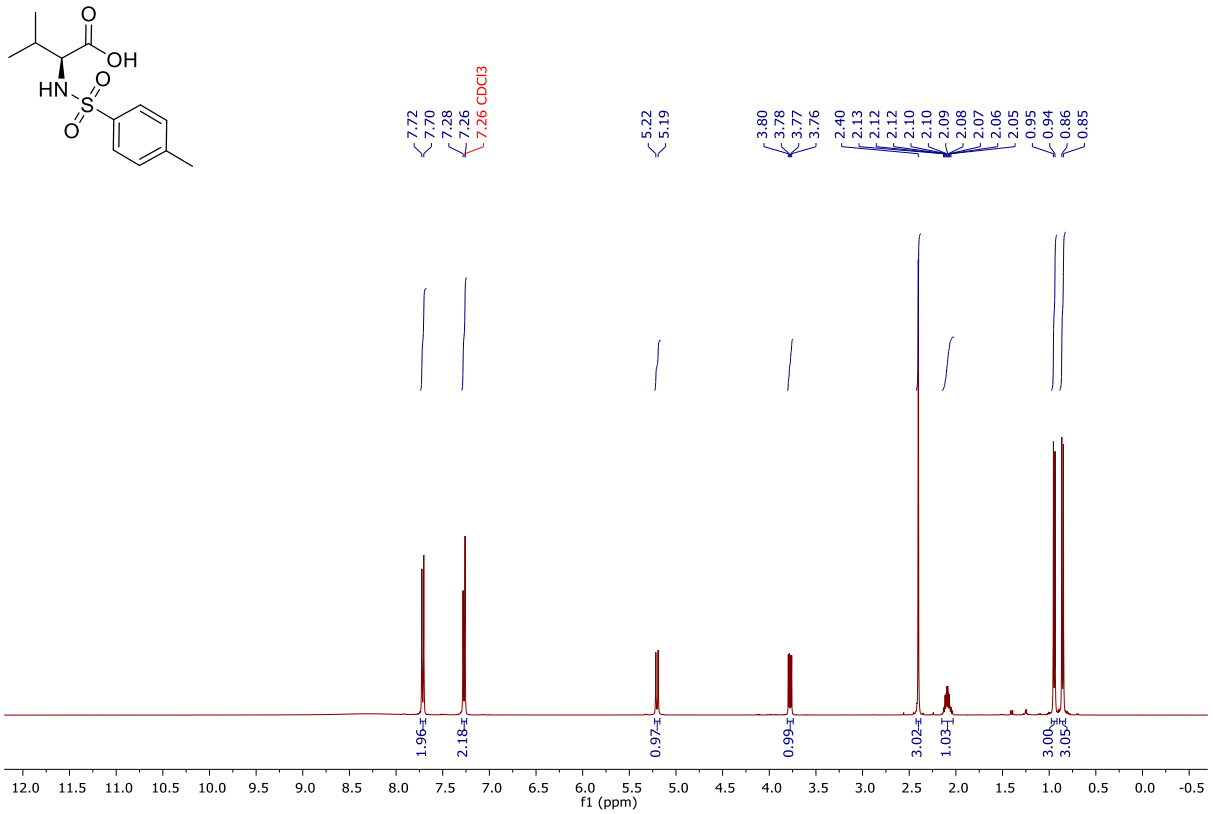
^{1}H NMR (500 MHz, $CDCl_3$): (4*E*,6*E*,8*E*)-2-Methyl-8-(2-(triisopropylsilyl)oxazol-5-yl)octa-2,4,6-trienal (2)



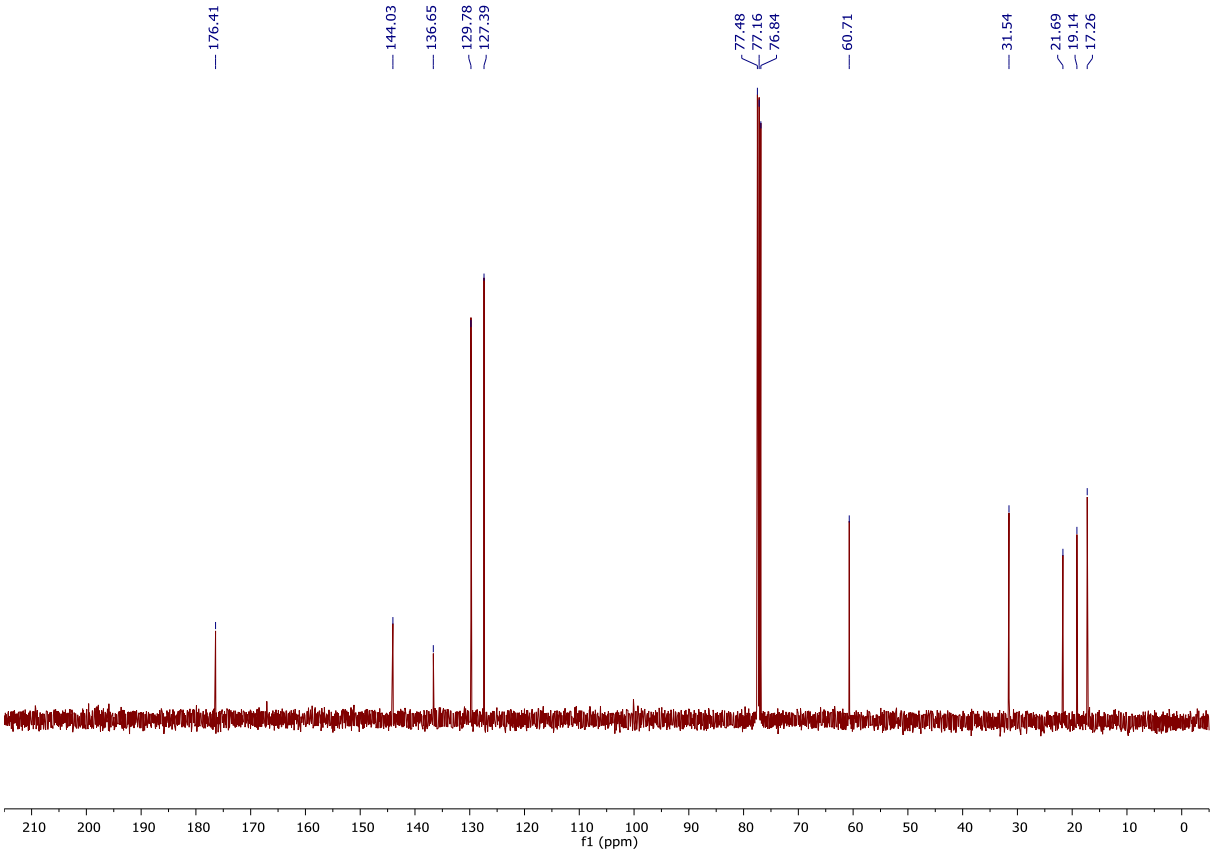
^{13}C NMR (126 MHz, $CDCl_3$):



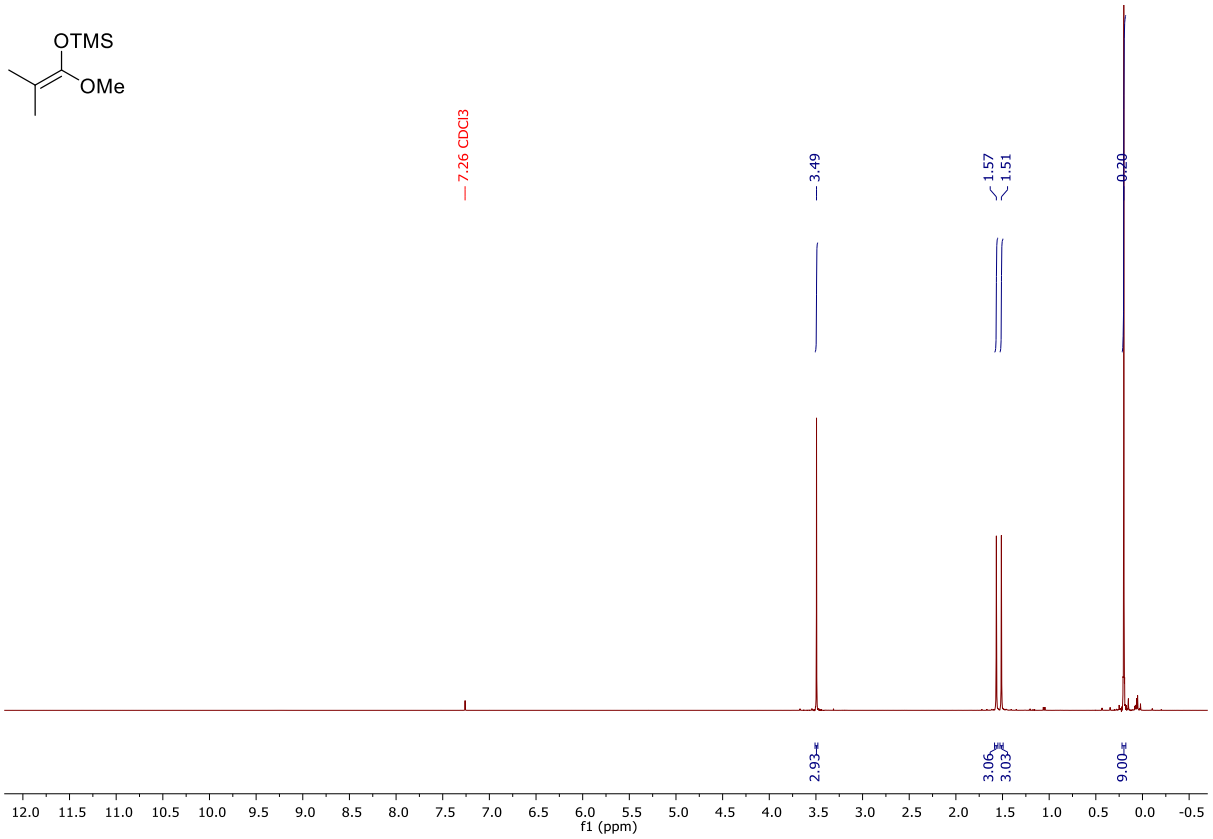
^{1}H NMR (400 MHz, $CDCl_3$): Tosyl-*L*-valine



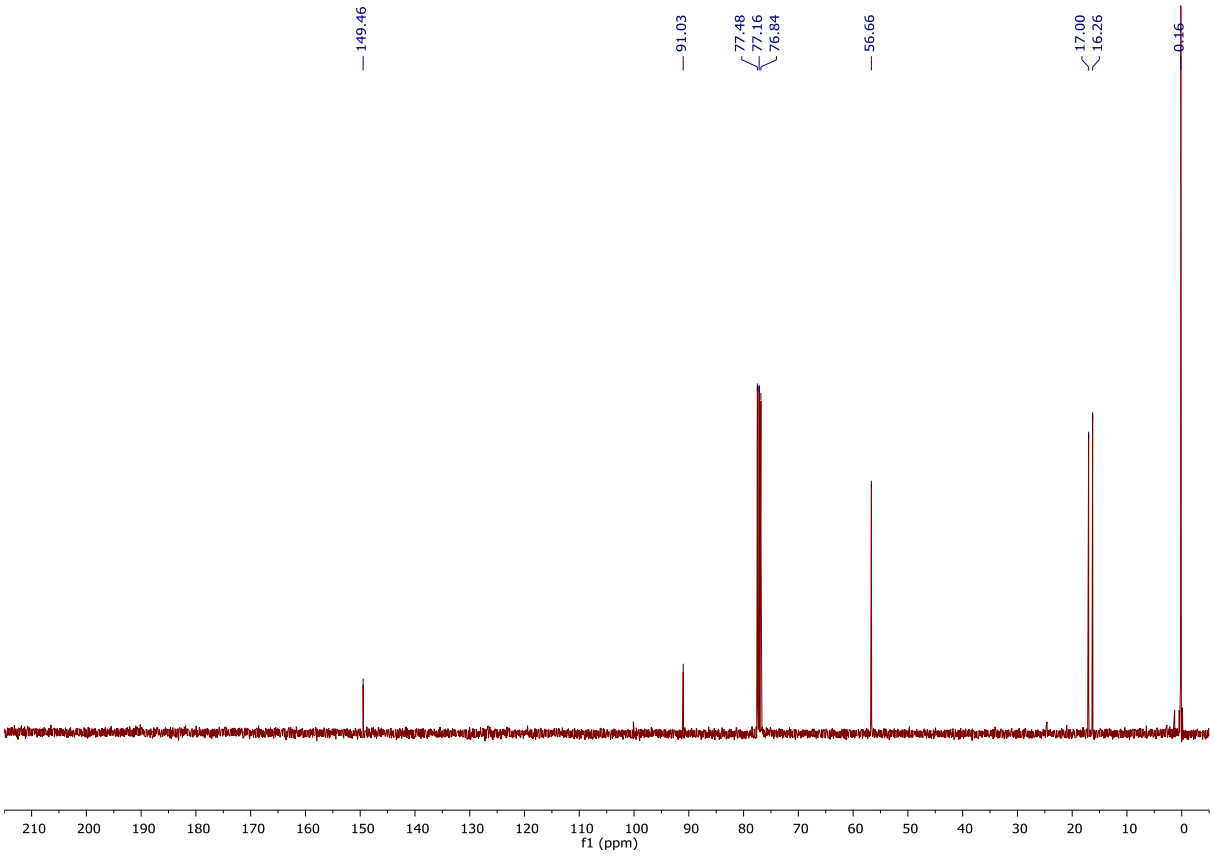
^{13}C NMR (101 MHz, $CDCl_3$):



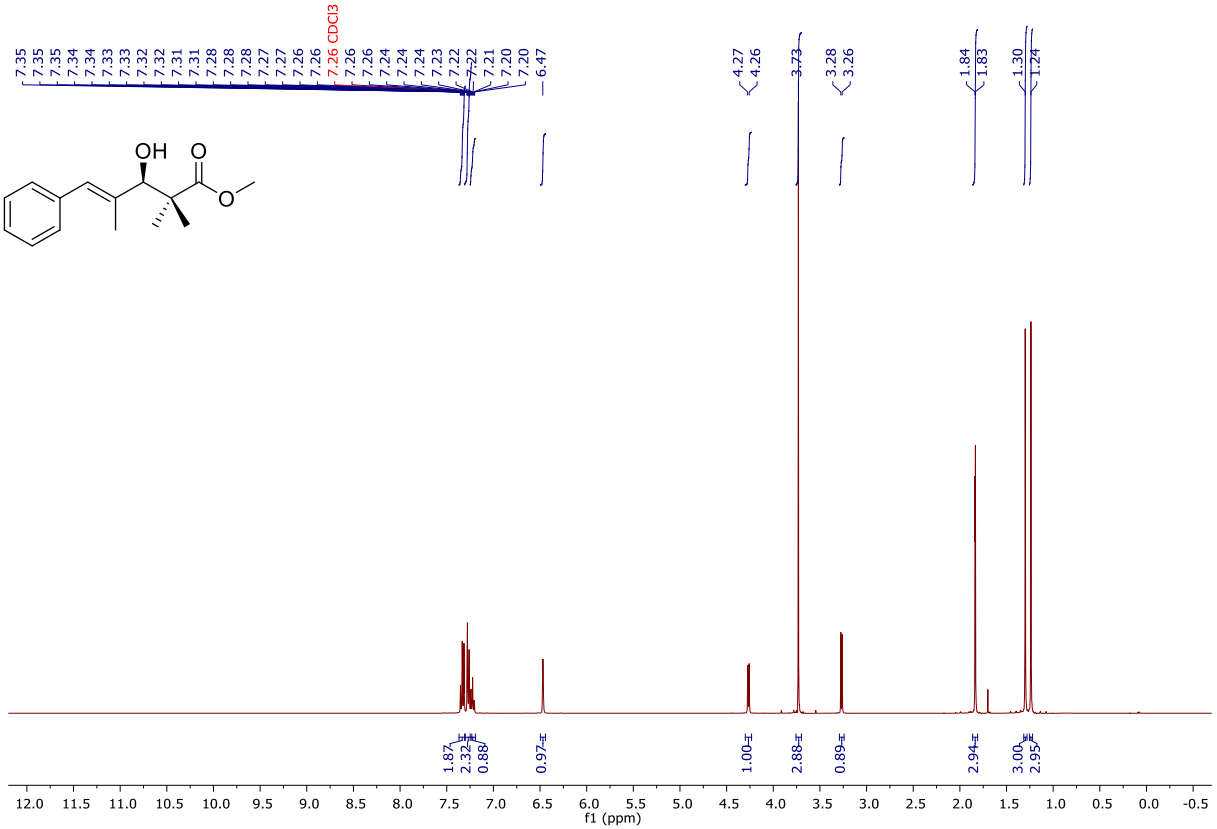
^{1}H NMR (400 MHz, $CDCl_3$): ((1-Methoxy-2-methylprop-1-en-1-yl)oxy)trimethylsilane (3)



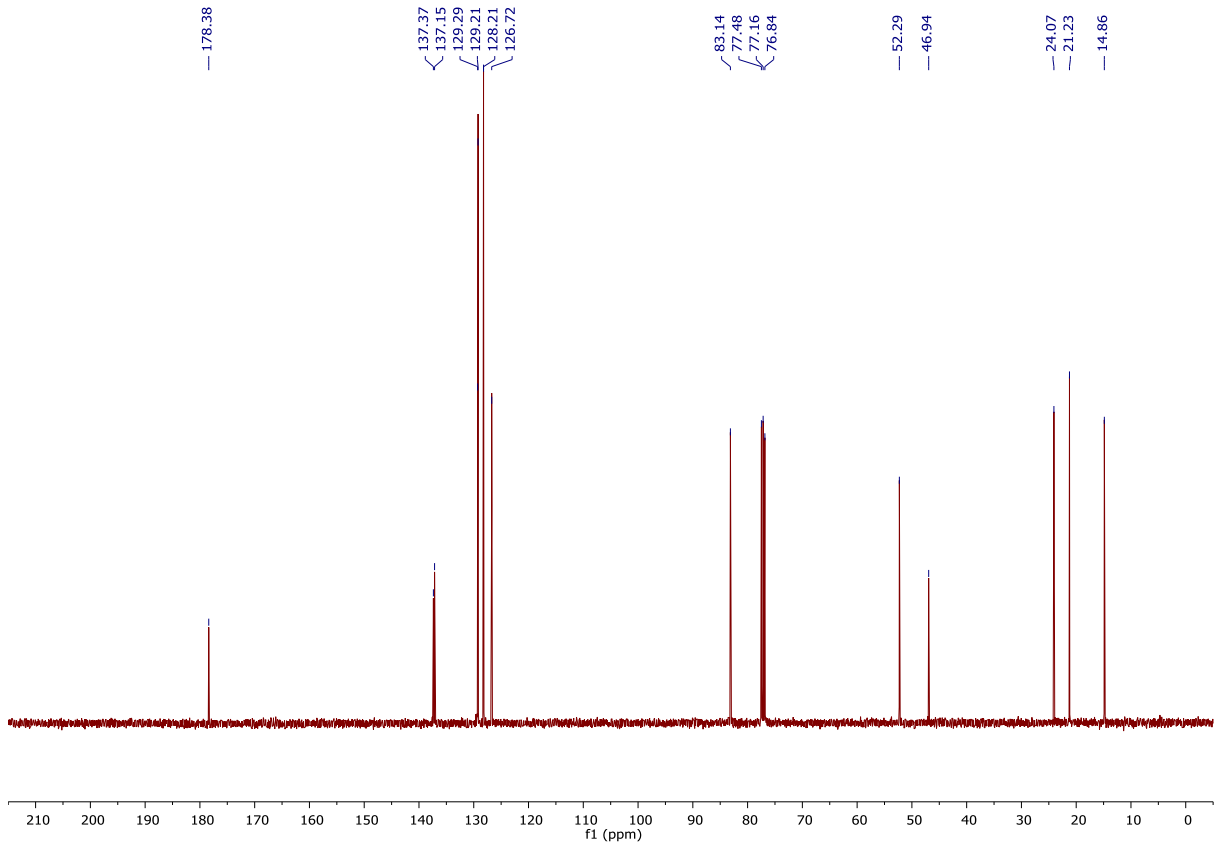
^{13}C NMR (101 MHz, $CDCl_3$):



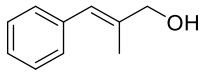
^{1}H NMR (400 MHz, $CDCl_3$): Methyl (*R*,*E*)-3-hydroxy-2,2,4-trimethyl-5-phenylpent-4-enoate (14)

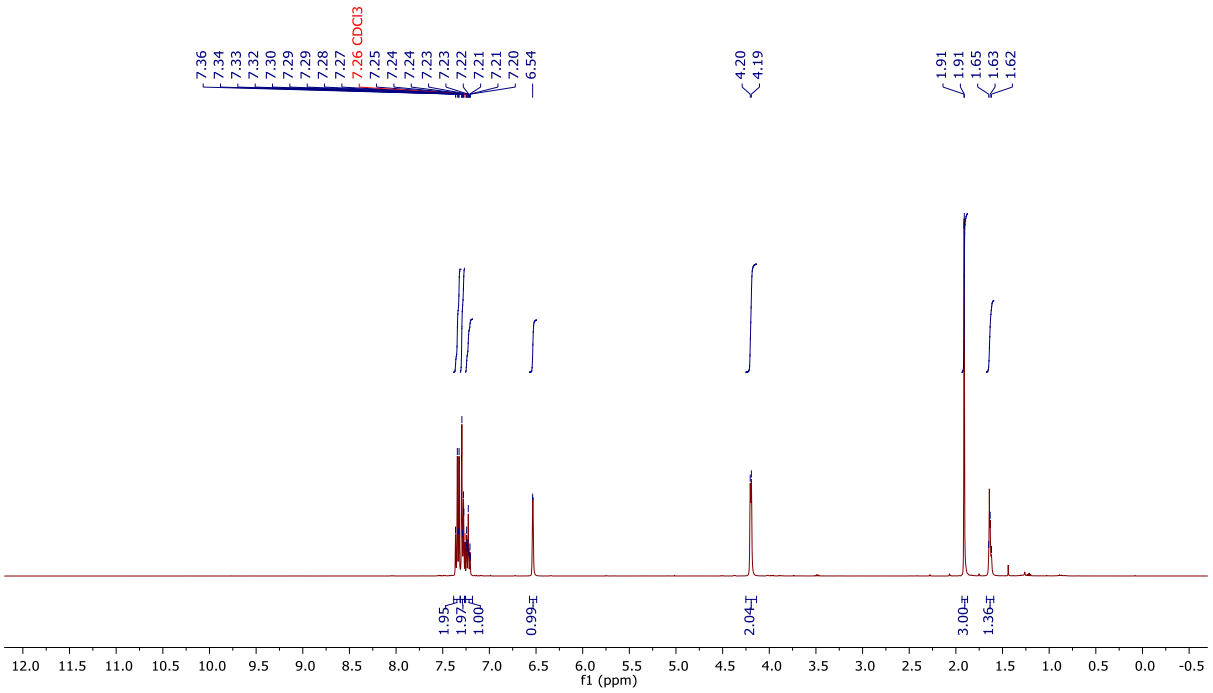


^{13}C NMR (101 MHz, $CDCl_3$):

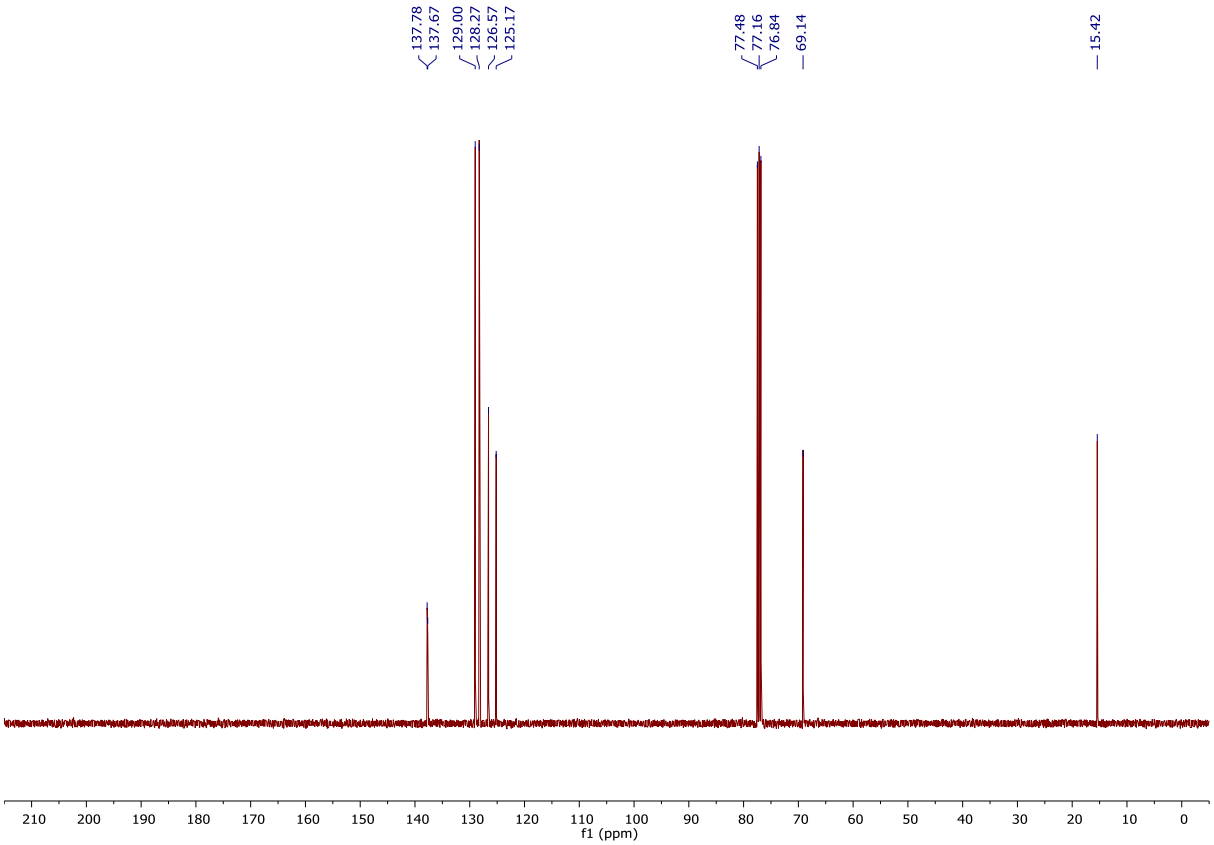


^{1}H NMR (400 MHz, $CDCl_3$): (*E*)-2-Methyl-3-phenylprop-2-en-1-ol (16)

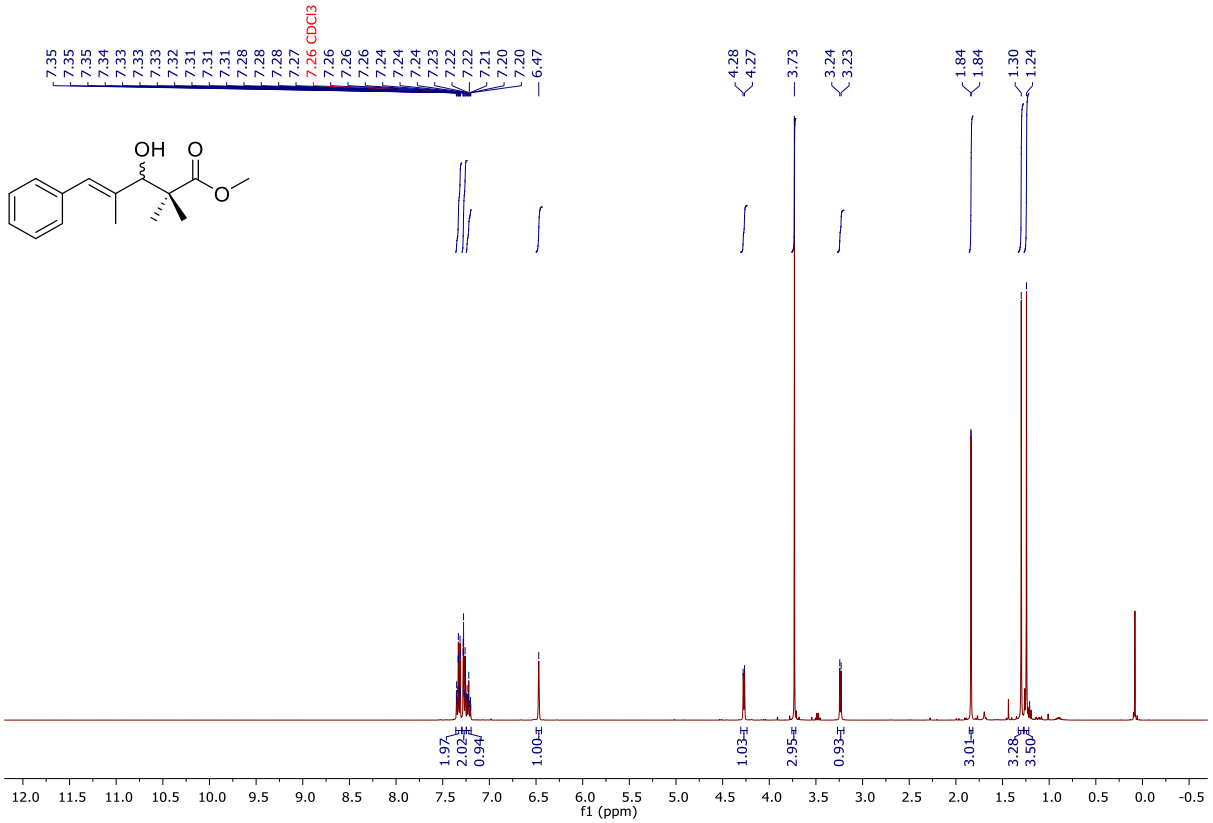




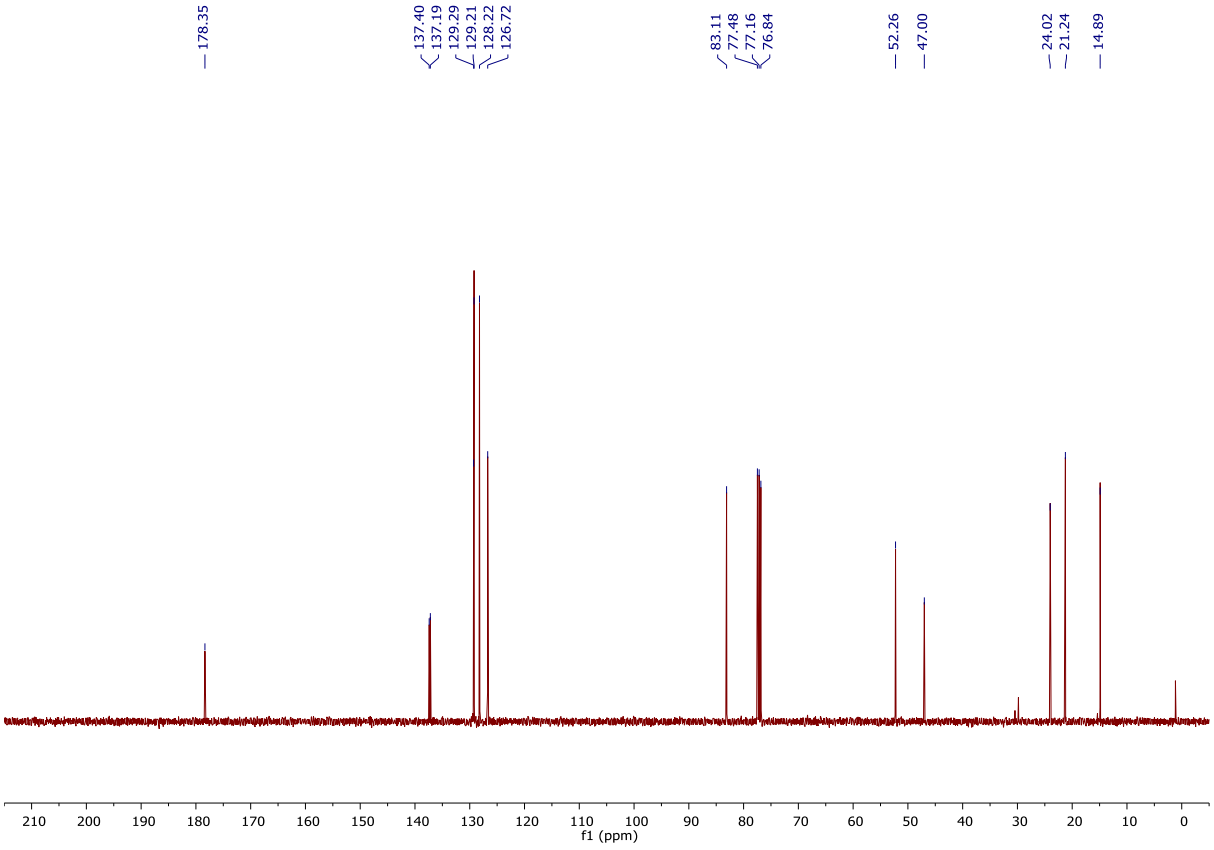
^{13}C NMR (101 MHz, $CDCl_3$):



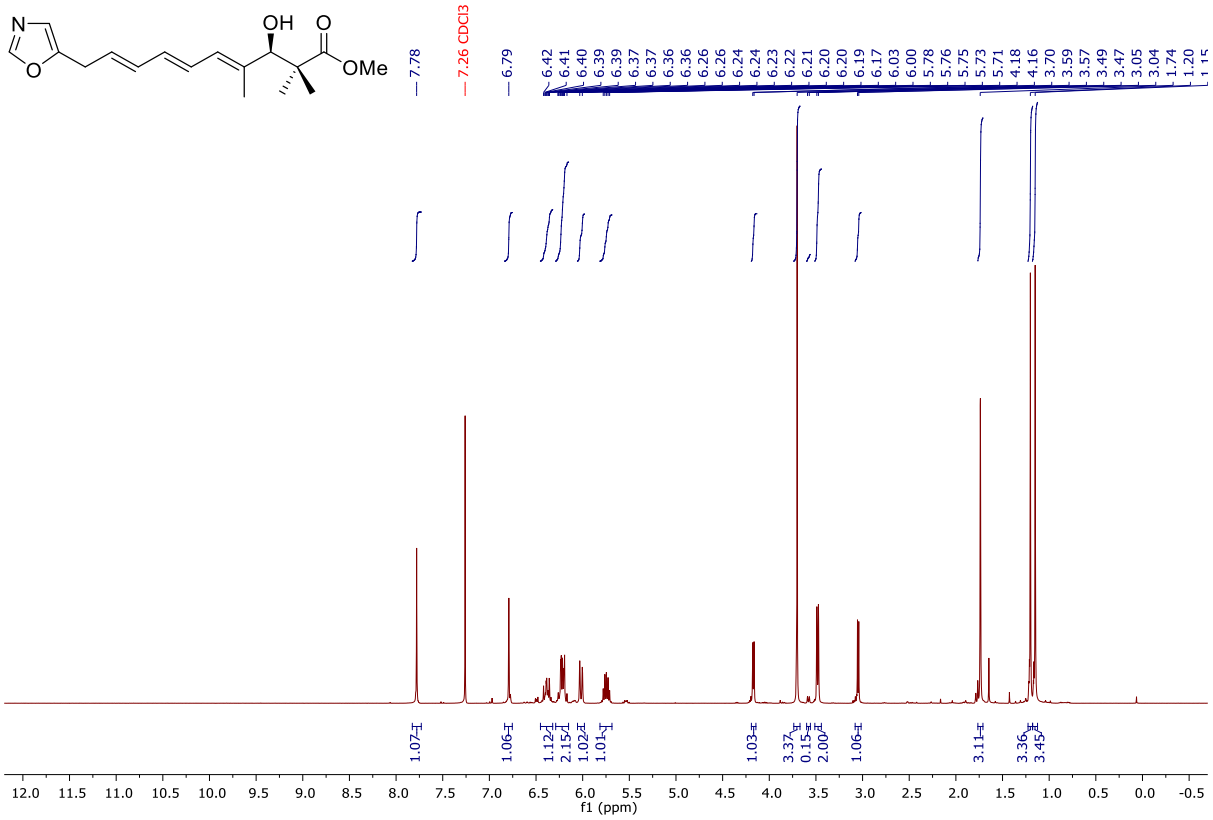
^{1}H NMR (400 MHz, $CDCl_3$): Methyl (*E*)-3-hydroxy-2,2,4-trimethyl-5-phenylpent-4-enoate



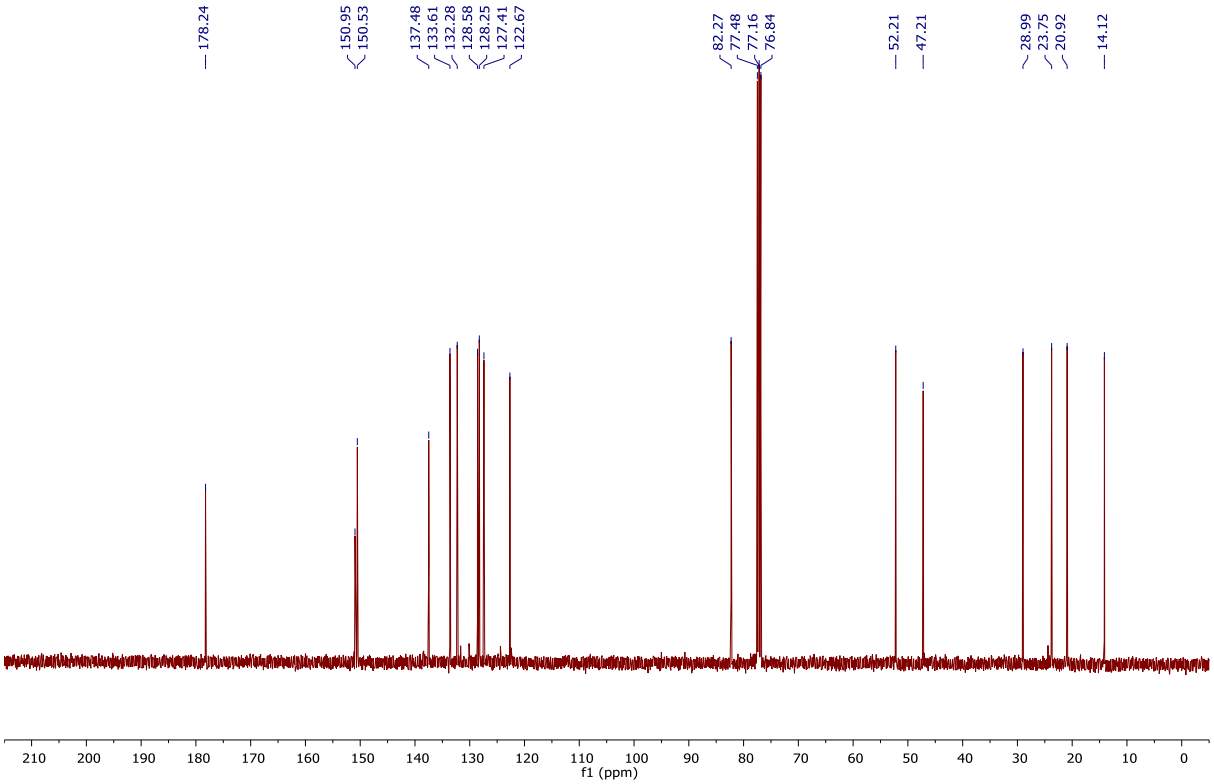
^{13}C NMR (101 MHz, $CDCl_3$):



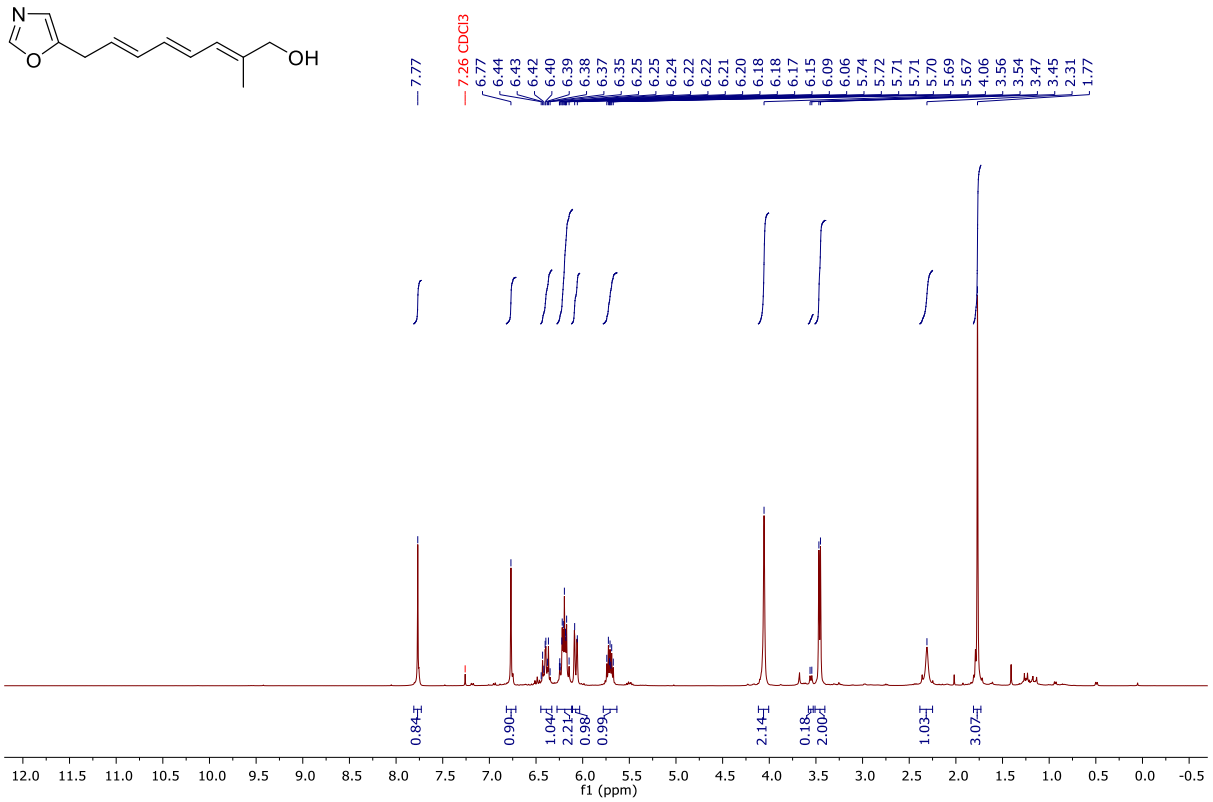
^{1}H NMR (400 MHz, $CDCl_3$): Methyl (*R*,4*E*,6*E*,8*E*)-3-hydroxy-2,2,4-trimethyl-10-(oxazol-5-yl)deca-4,6,8-trienoate (17)



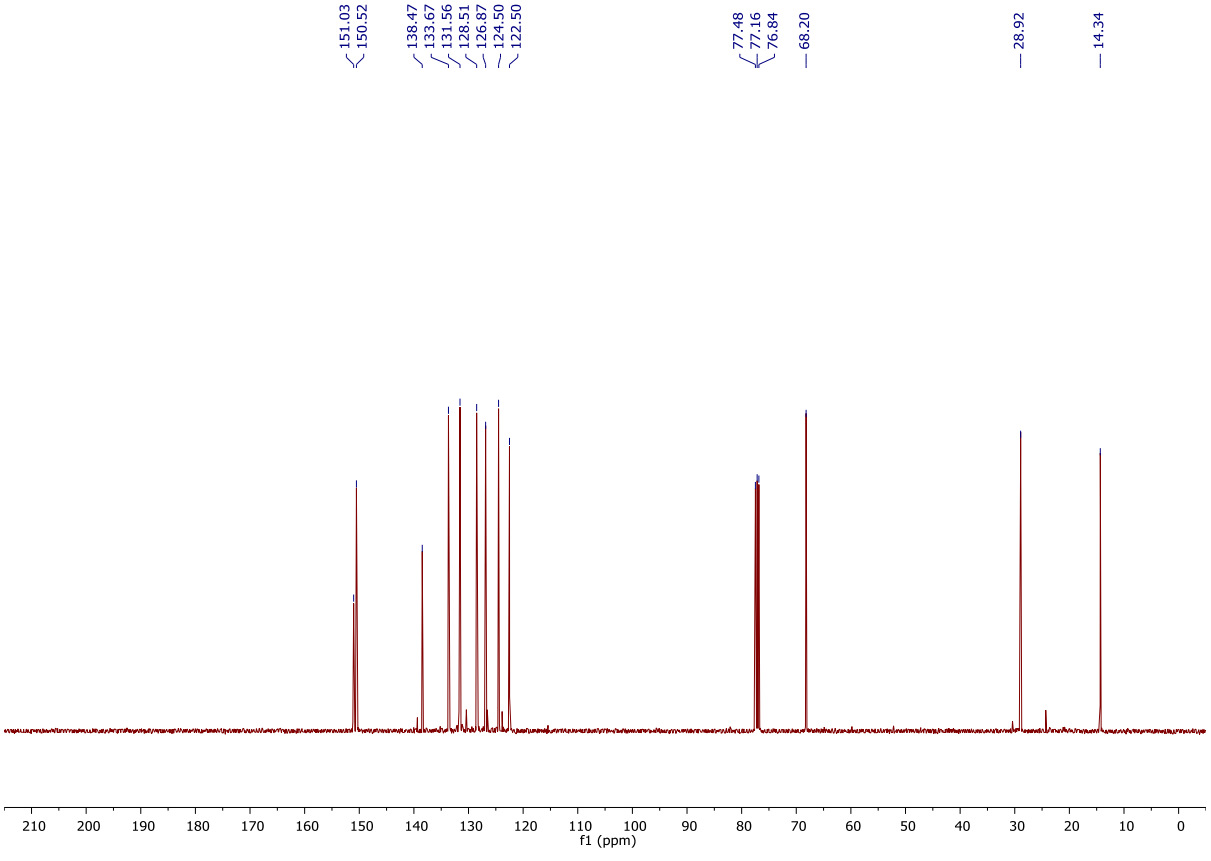
^{13}C NMR (101 MHz, $CDCl_3$):



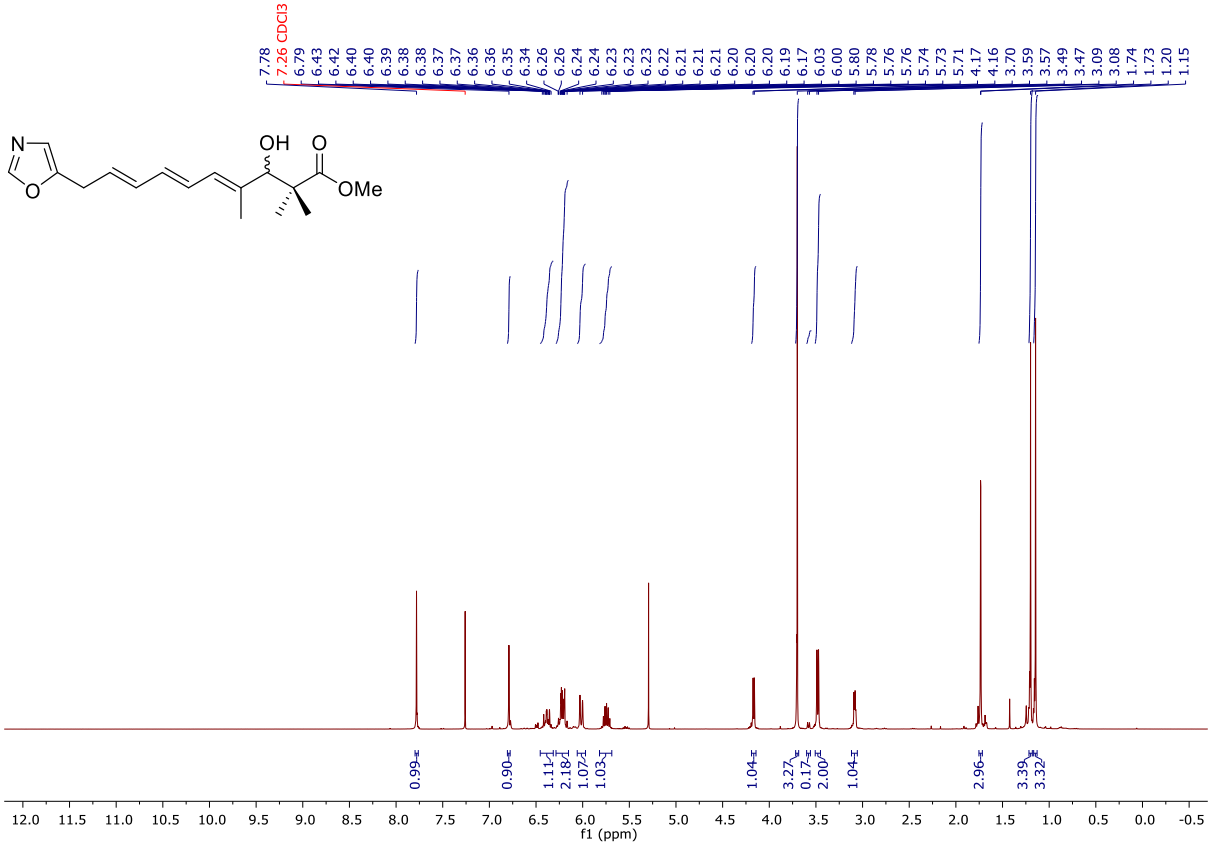
^{1}H NMR (400 MHz, $CDCl_3$): (2*E*,4*E*,6*E*)-2-Methyl-8-(oxazol-5-yl)octa-2,4,6-trien-1-ol



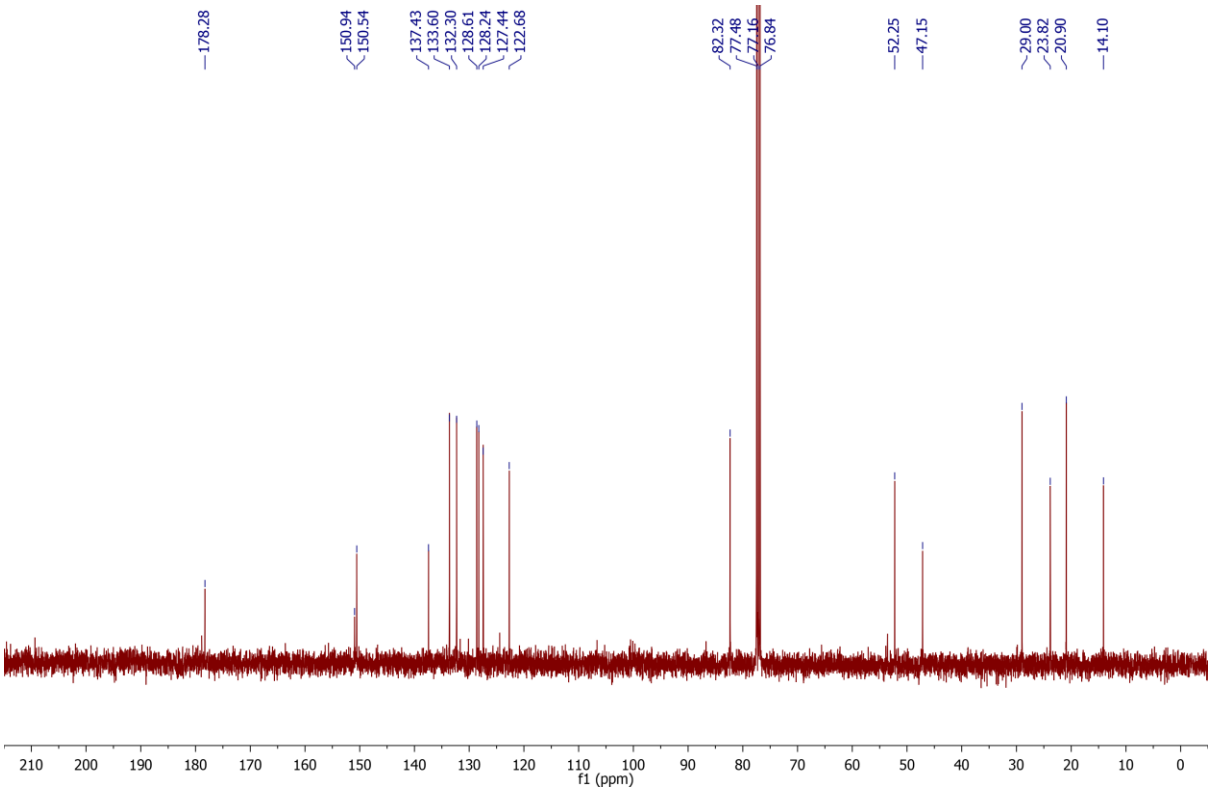
^{13}C NMR (101 MHz, $CDCl_3$):



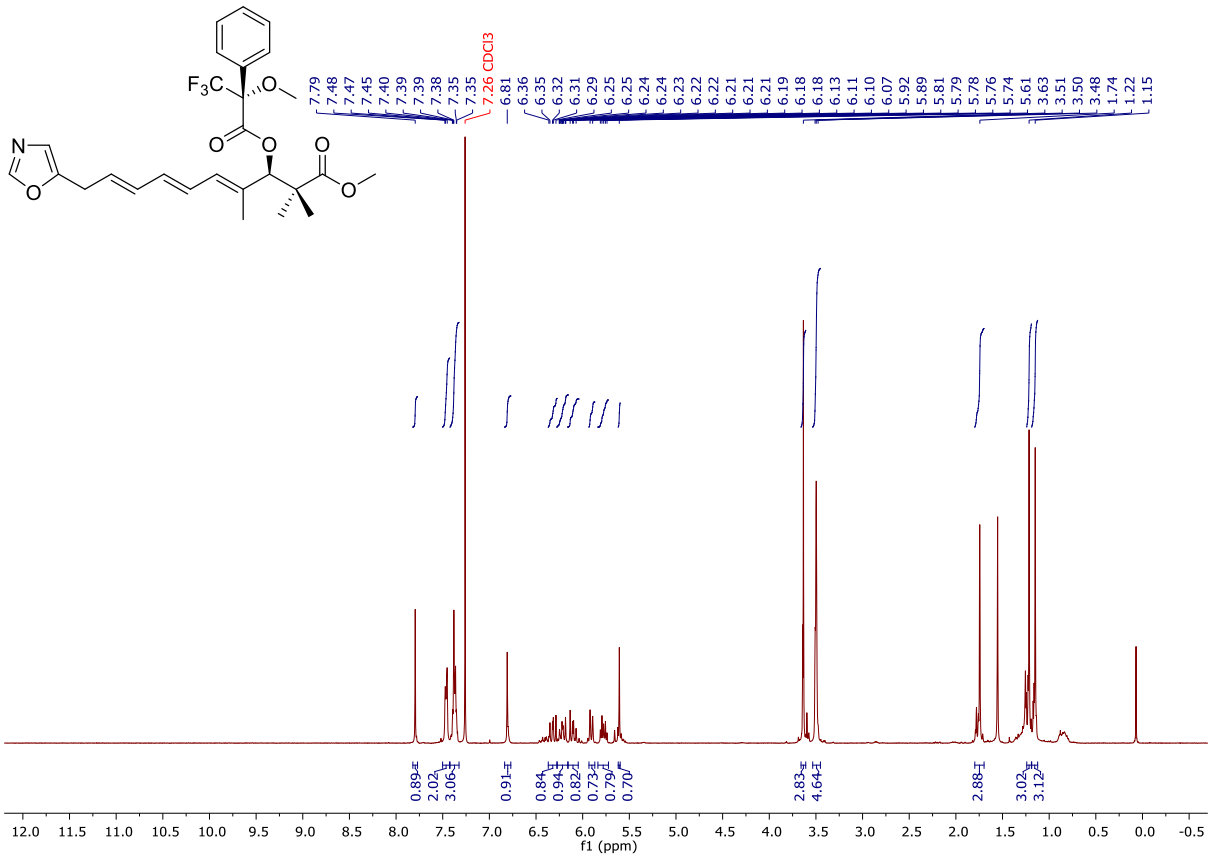
^{1}H NMR (400 MHz, $CDCl_3$): Methyl (4*E*,6*E*,8*E*)-3-hydroxy-2,2,4-trimethyl-10-(oxazol-5-yl)deca-4,6,8-trienoate



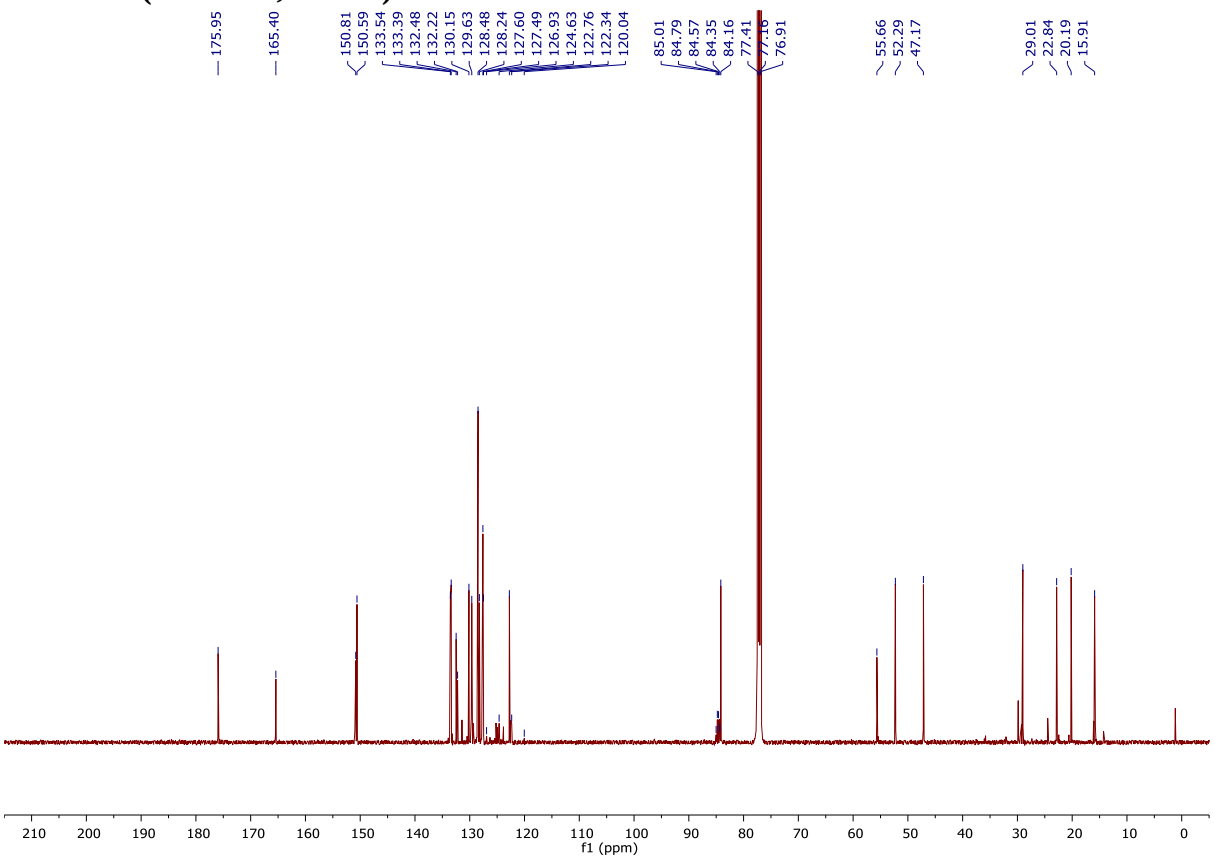
^{13}C NMR (101 MHz, $CDCl_3$):



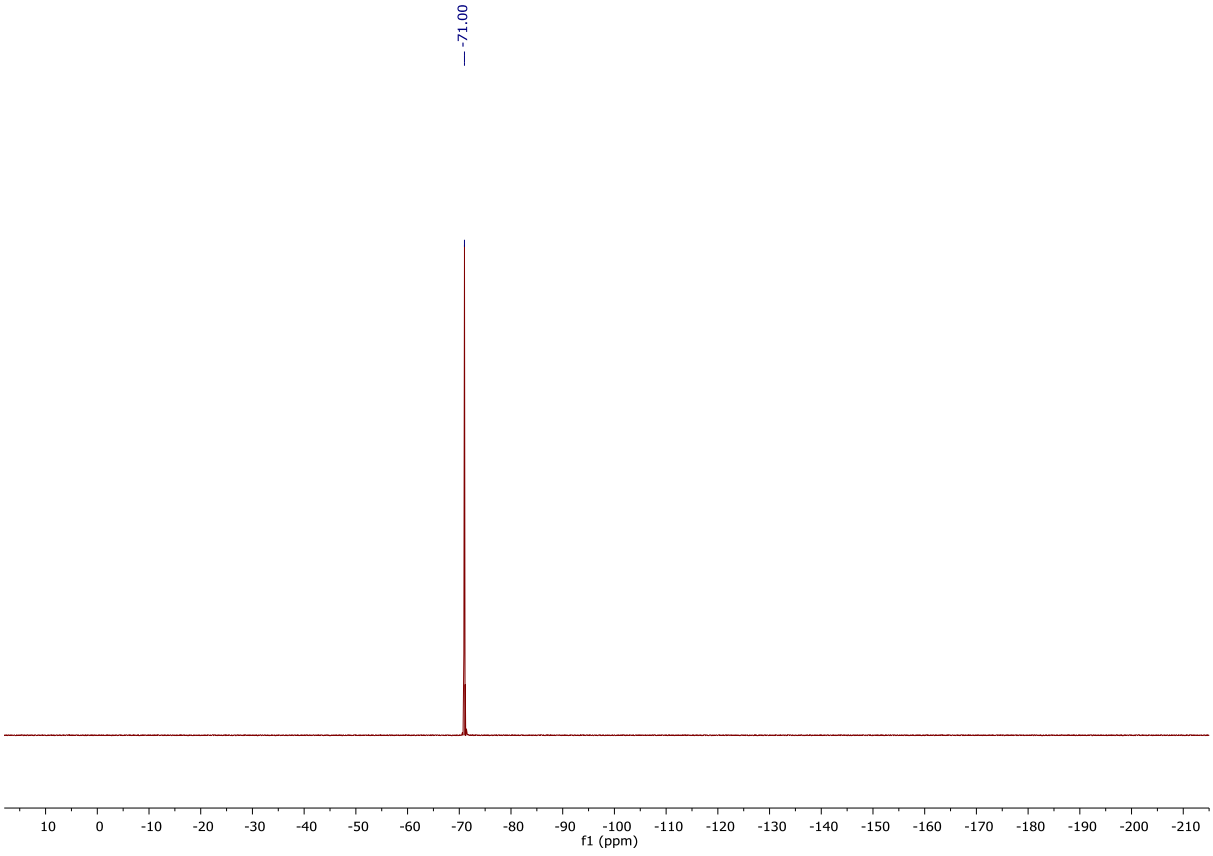
^{1}H NMR (400 MHz, $CDCl_3$): Methyl (*R*,4*E*,6*E*,8*E*)-2,2,4-trimethyl-10-(oxazol-5-yl)-3-(((*R*)-3,3,3-trifluoro-2-methoxy-2-phenylpropanoyl)oxy)deca-4,6,8-trienoate



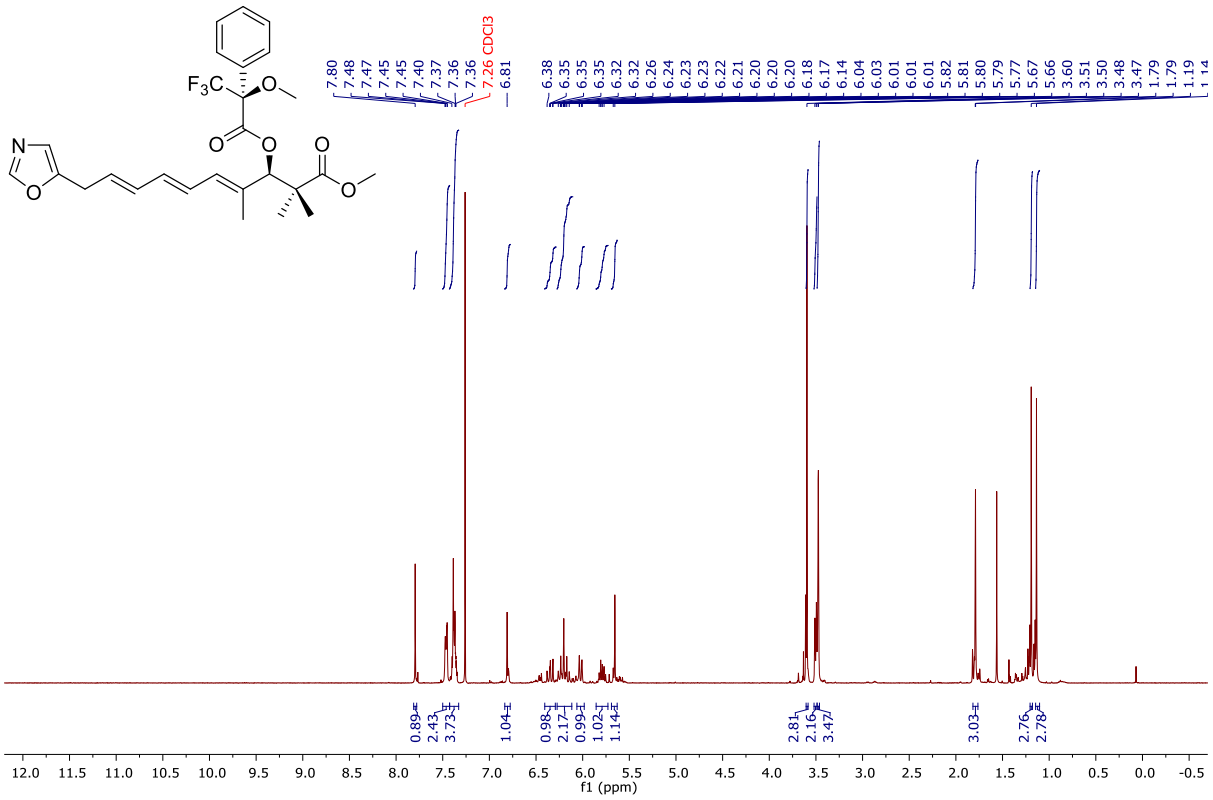
^{13}C NMR (126 MHz, $CDCl_3$):



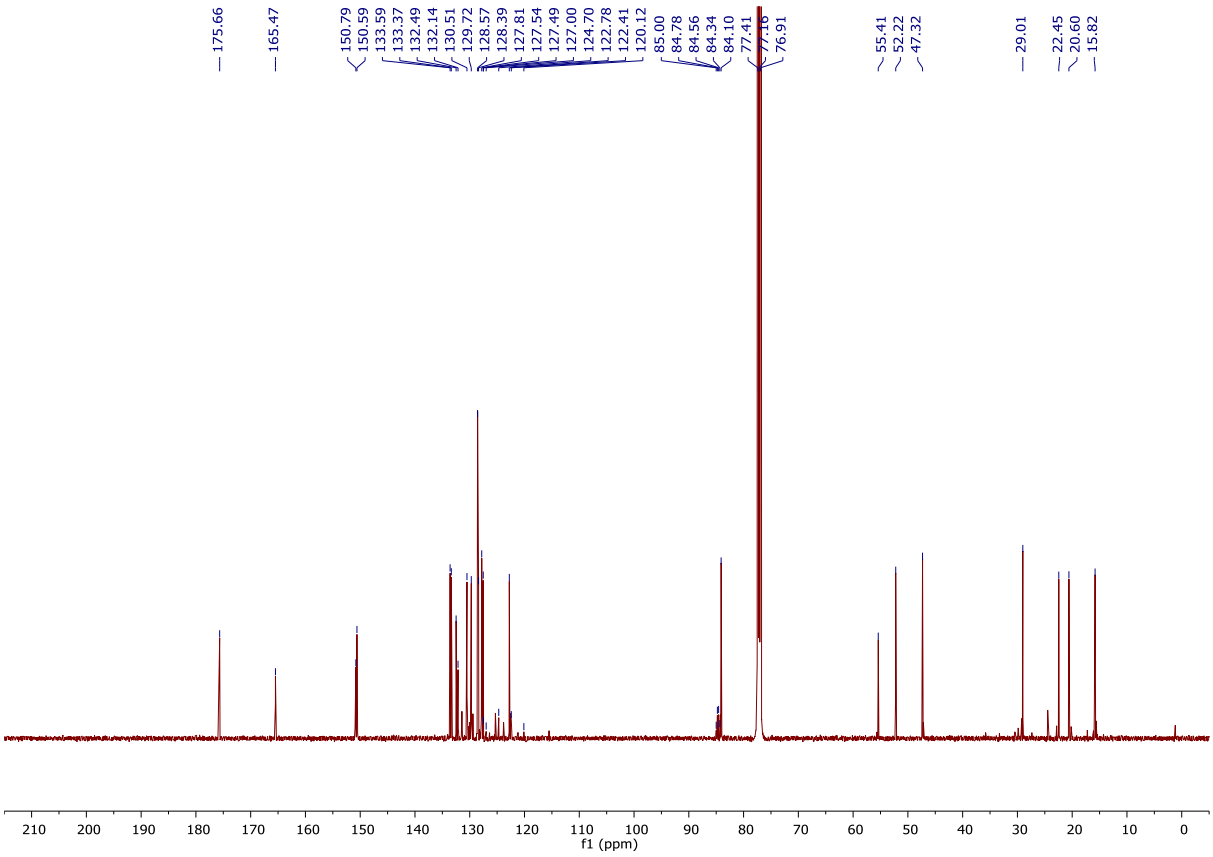
^{19}F NMR (471 MHz, $CDCl_3$):



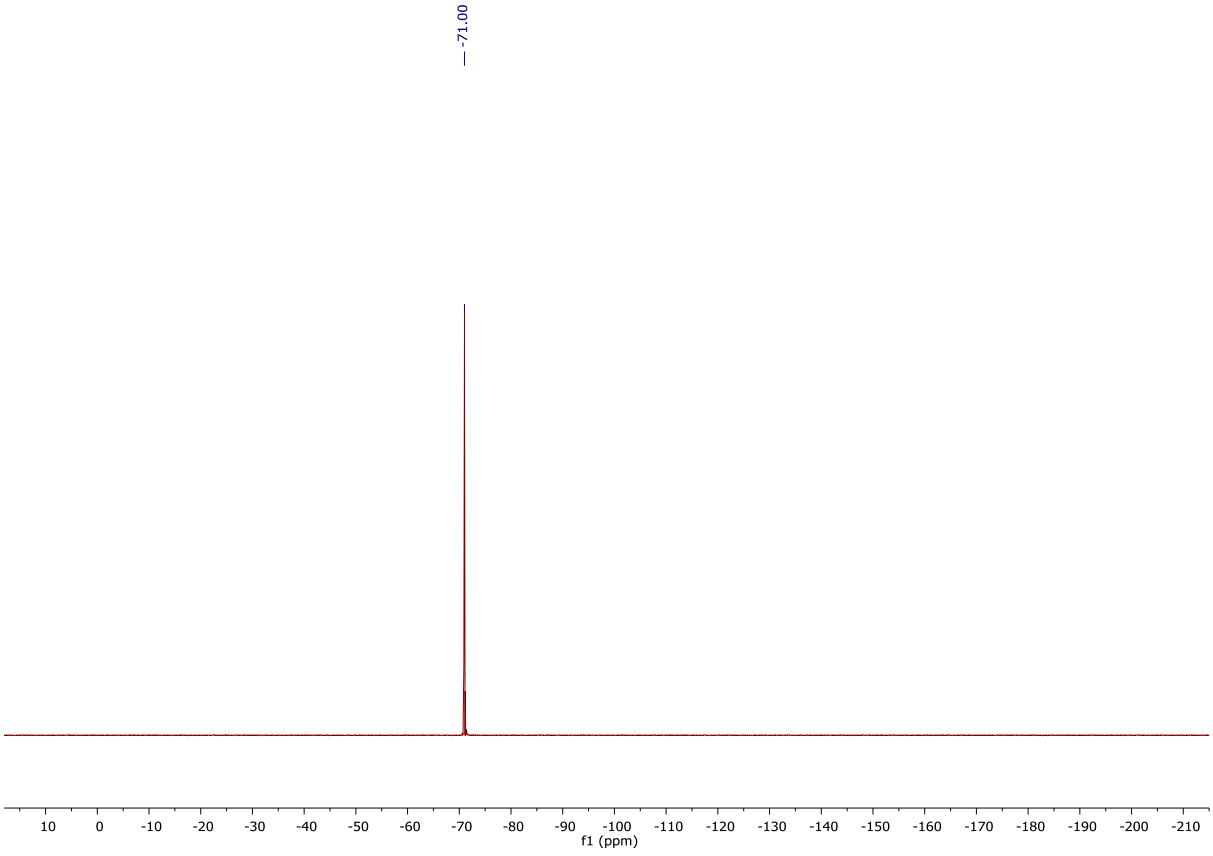
^{1}H NMR (400 MHz, $CDCl_3$): Methyl (*R*,4*E*,6*E*,8*E*)-2,2,4-trimethyl-10-(oxazol-5-yl)-3-(((*S*)-3,3,3-trifluoro-2-methoxy-2-phenylpropanoyl)oxy)deca-4,6,8-trienoate



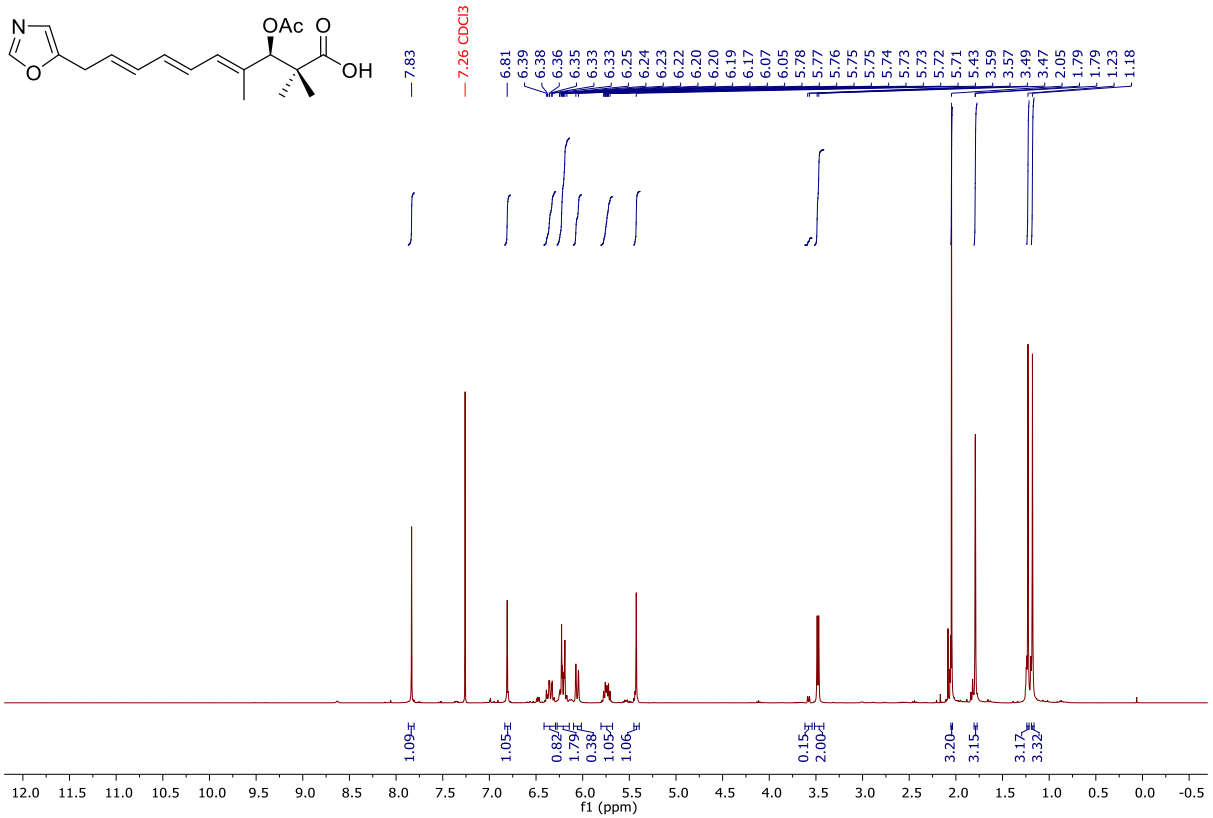
^{13}C NMR (126 MHz, $CDCl_3$):



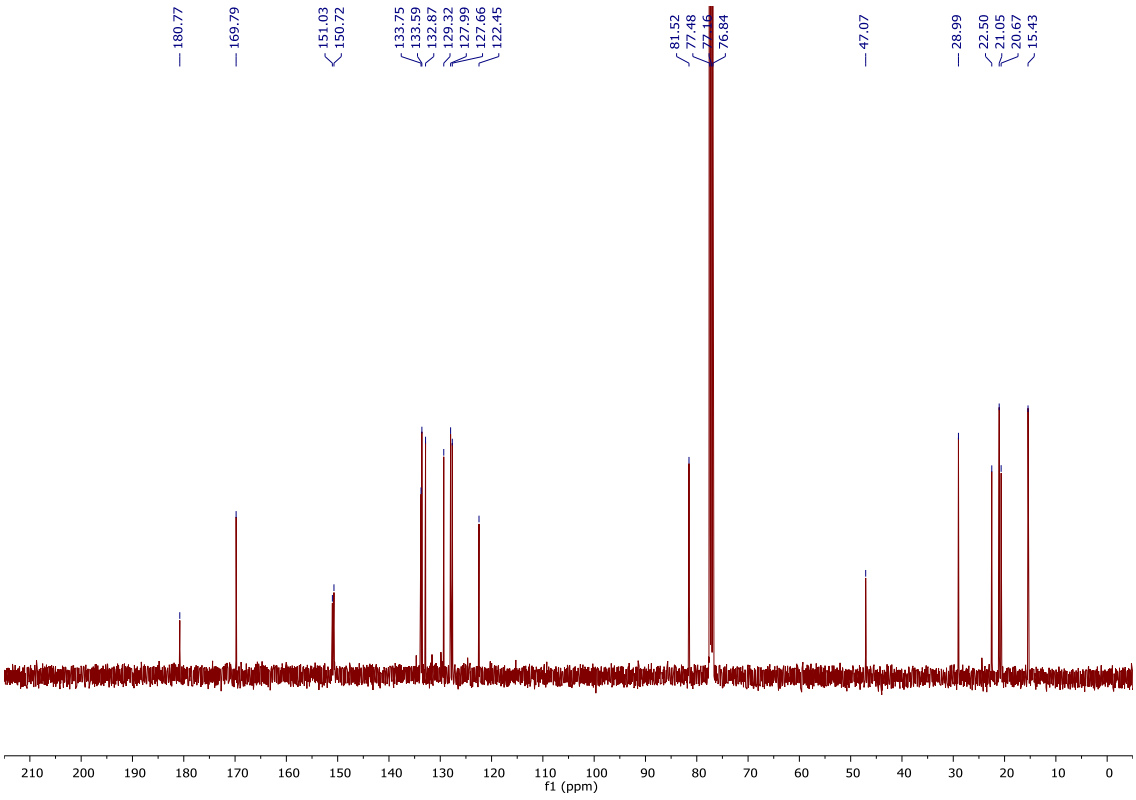
^{19}F NMR (471 MHz, $CDCl_3$):



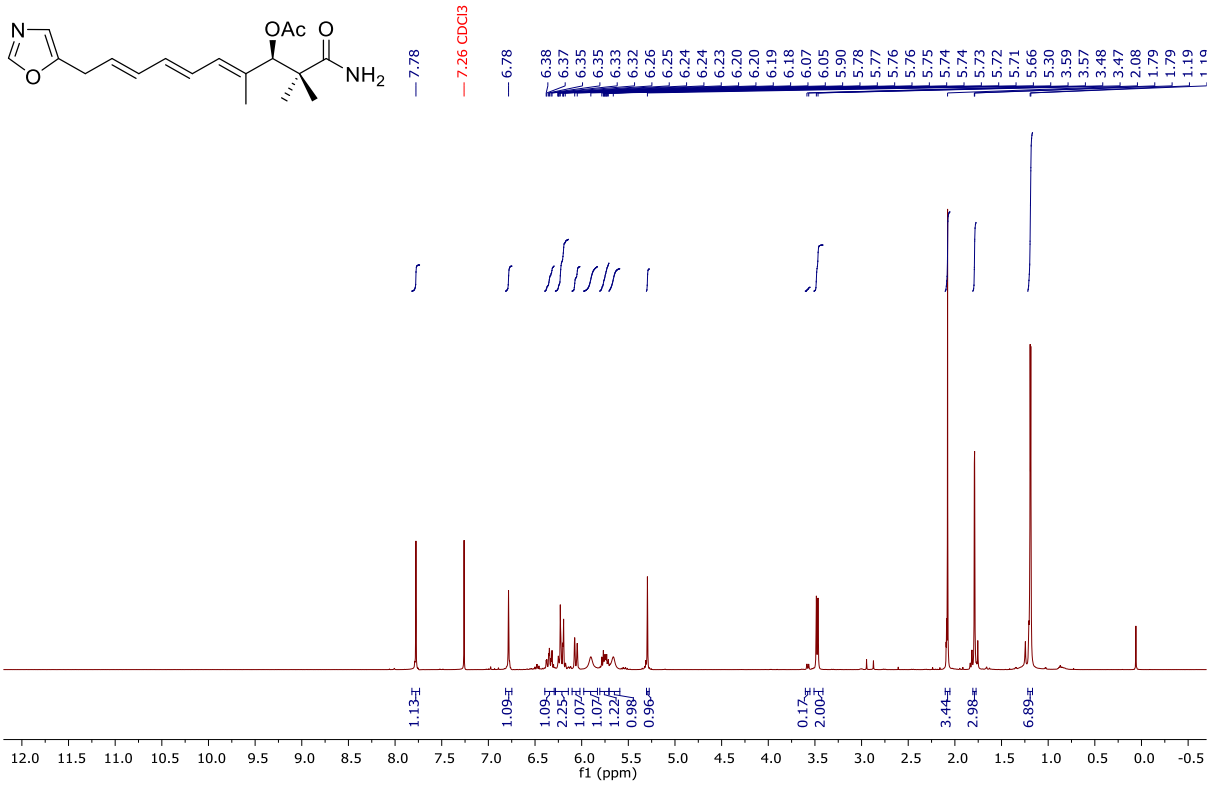
^{1}H NMR (400 MHz, $CDCl_3$): (*R*,4*E*,6*E*,8*E*)-3-Acetoxy-2,2,4-trimethyl-10-(oxazol- 5-yl)deca-4,6,8-trienoic acid (18)



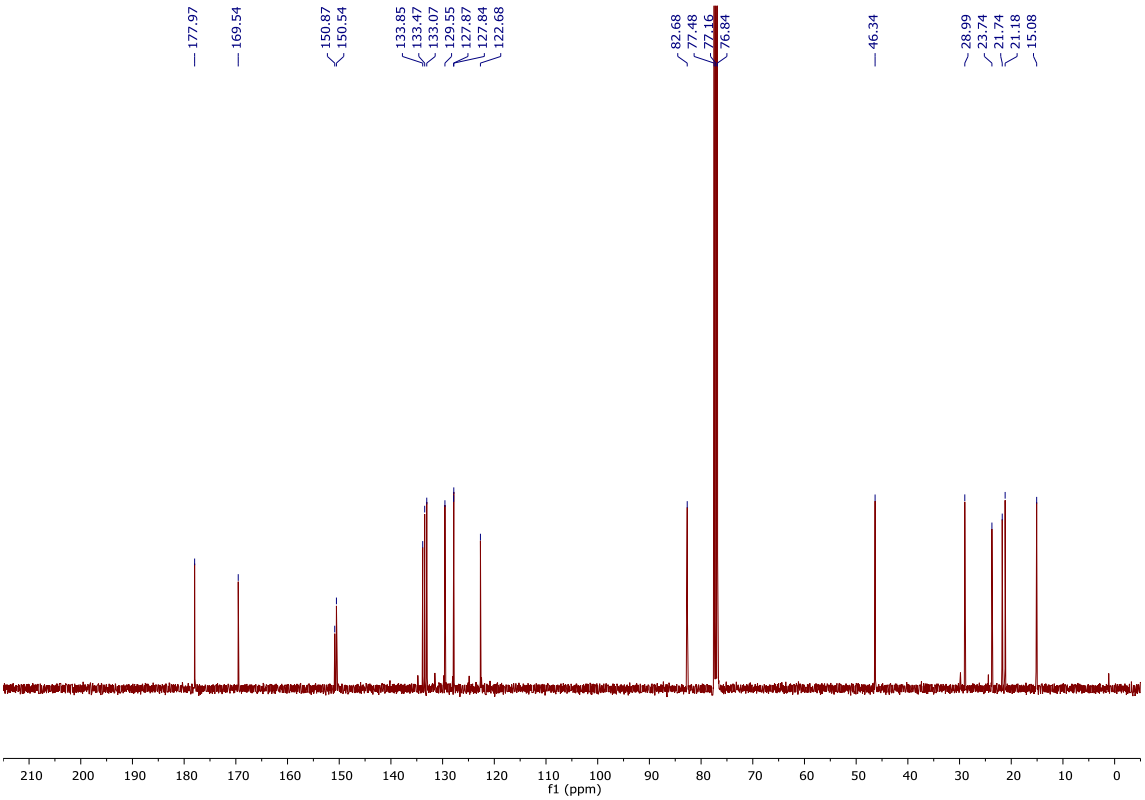
^{13}C NMR (101 MHz, $CDCl_3$):



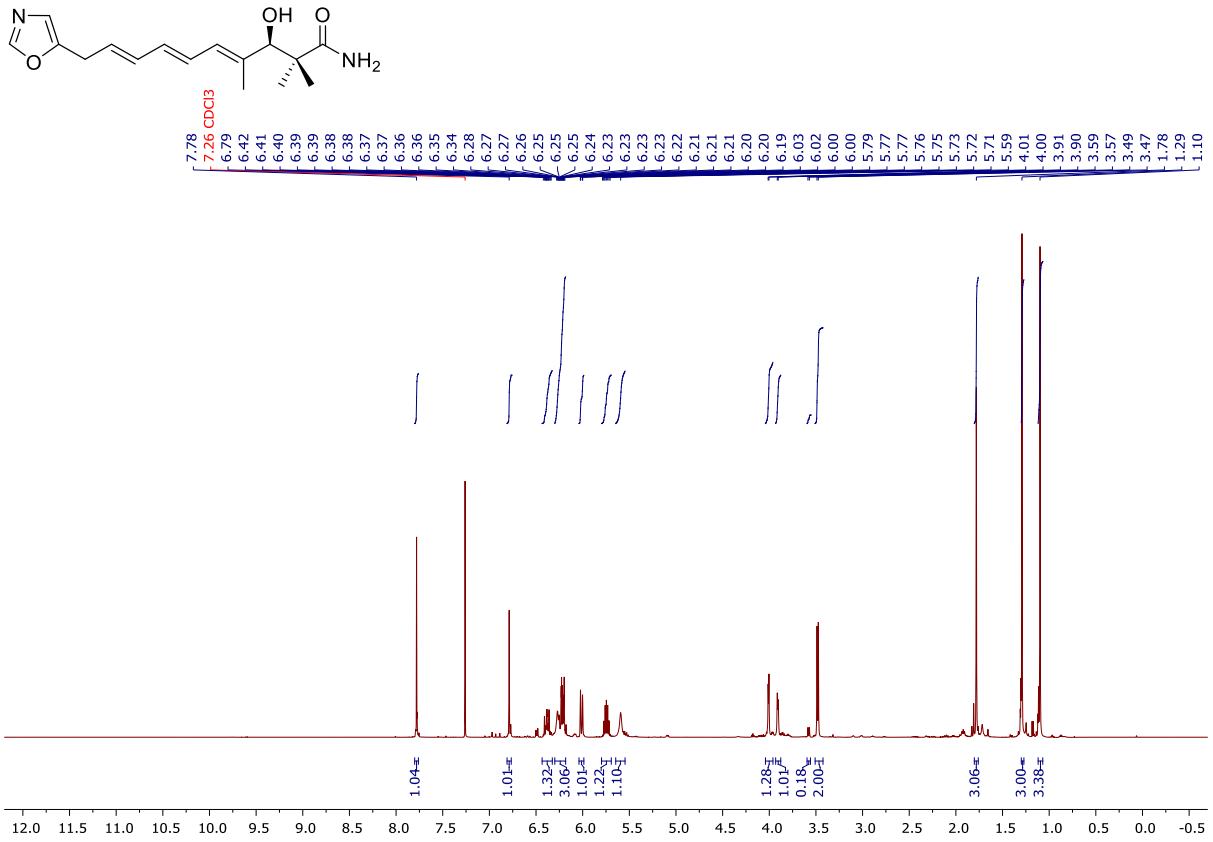
^{1}H NMR (400 MHz, $CDCl_3$): (*R*,4*E*,6*E*,8*E*)-1-Amino-2,2,4-trimethyl-10-(oxazol-5-yl)-1-oxodeca-4,6,8-trien-3-yl acetate



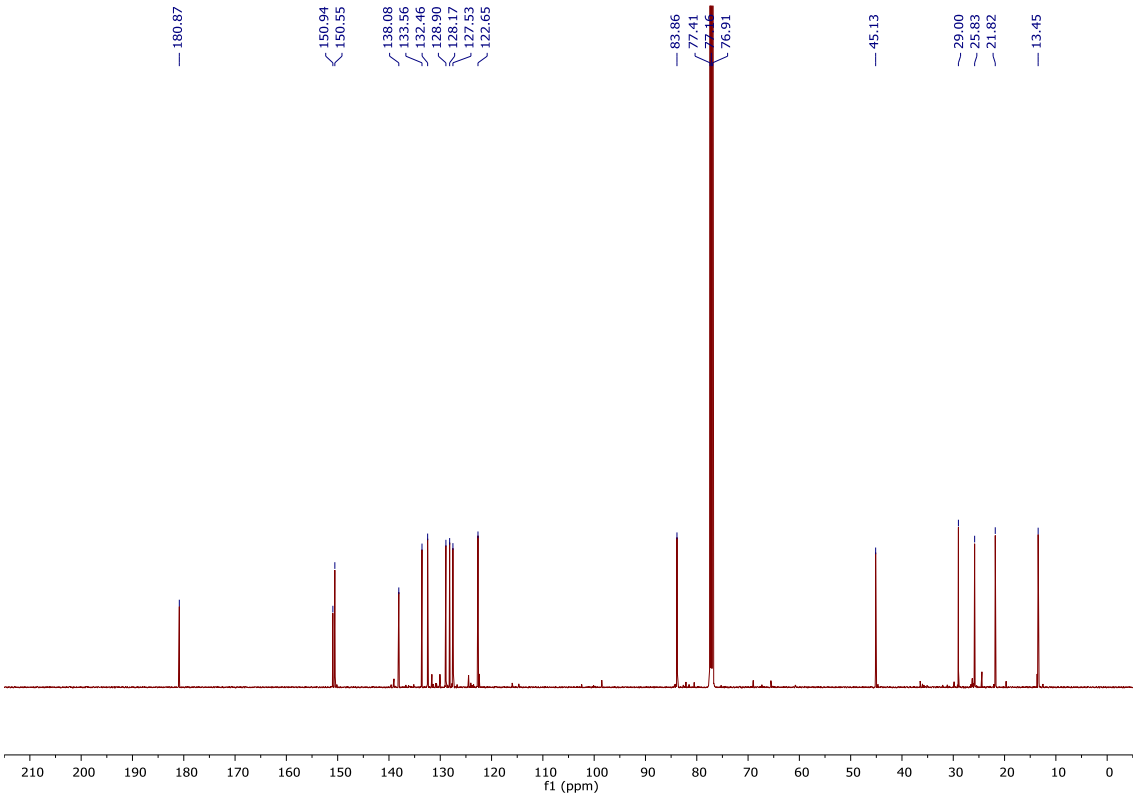
^{13}C NMR (101 MHz, $CDCl_3$):



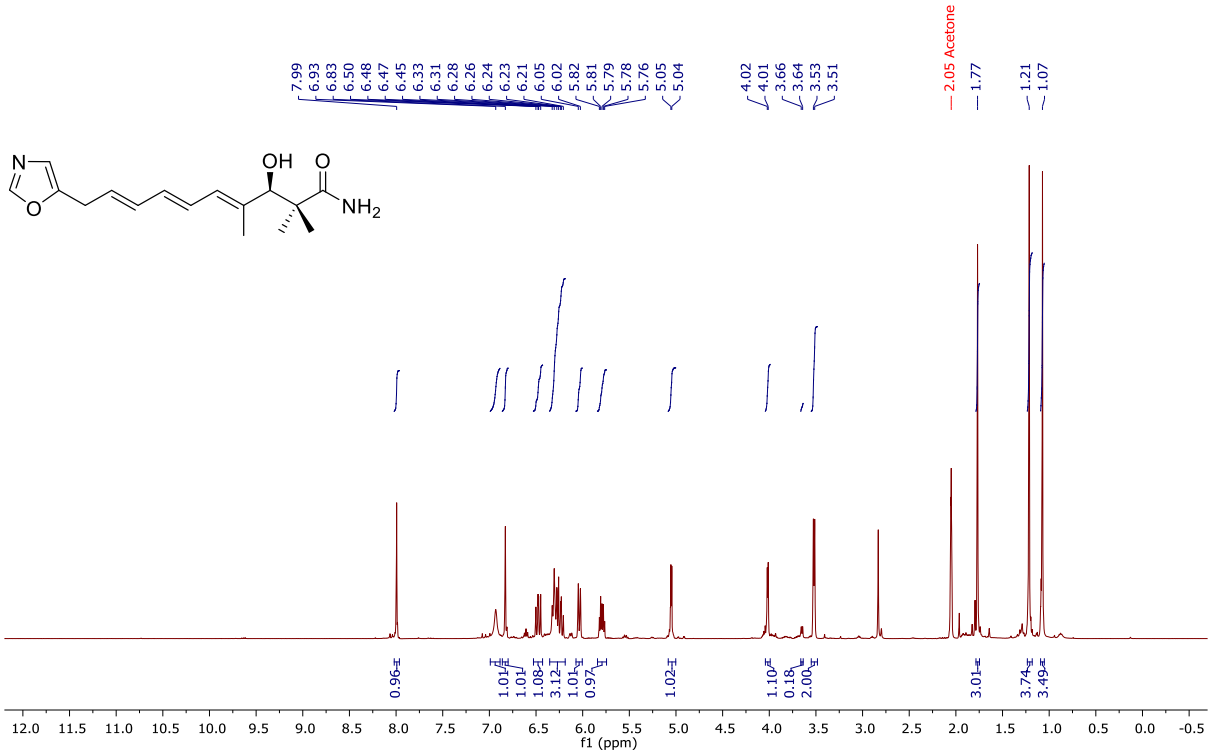
^{1}H NMR (500 MHz, $CDCl_3$): (–)-(3*R*)-inthomycin C (1)



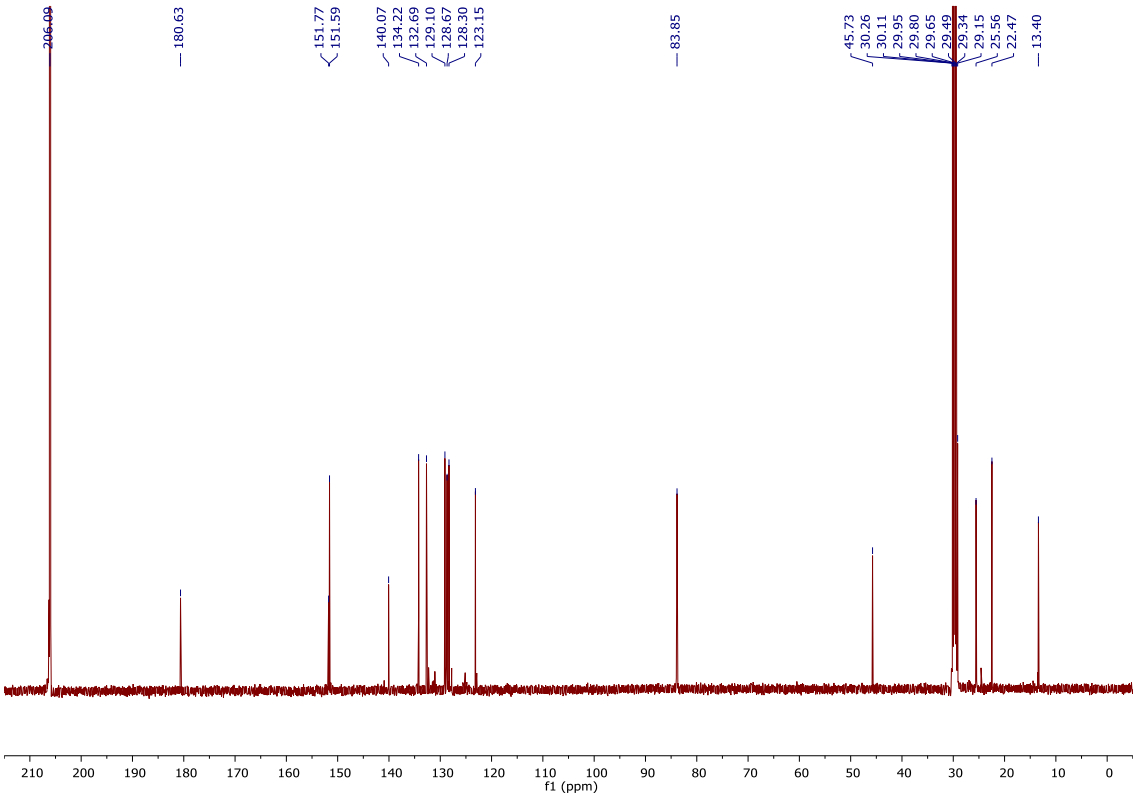
^{13}C NMR (126 MHz, $CDCl_3$):



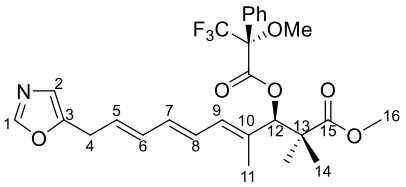
^{1}H NMR (500 MHz, Acetone-d_6): (–)-(3*R*)-inthomycin C (1)



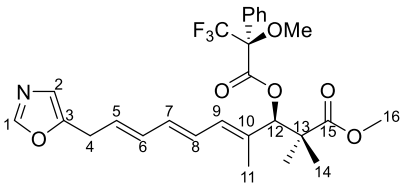
^{13}C NMR (126 MHz, Acetone-d_6):



4 Mosher Ester Analysis and Confirmation of (3*R*) Stereochemistry



(*R*)-MTPA ester

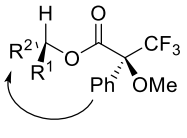


(*S*)-MTPA ester

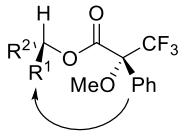
Entry	Proton	δ (*R*)-MTPA Ester/ppm	δ (*S*)-MTPA Ester/ppm	$\Delta\delta^{SR}$ ($\delta_S - \delta_R$) ppm	$\Delta\delta^{SR}$ ($\delta_S - \delta_R$) Hz (400 MHz)
1	C(1)<u>*H*</u>	7.79	7.80	+0.01	+4
2	C(2)<u>*H*</u>	6.81	6.81	0.00	0
3	C(5)<u>*H*</u>	5.77	5.79	+0.02	+8
4	C(8)<u>*H*</u>	6.32	6.35	+0.03	+12
5	C(9)<u>*H*</u>	5.91	6.02	+0.11	+44
6	C(11)<u>*H*</u>$_3$	1.74	1.79	+0.05	+20
7	C(12)<u>*H*</u>	5.61	5.66	+0.05	+20
8	(C(14)<u>*H*</u>$_3$)$_A$	1.22	1.19	–0.03	–12
9	(C(14)<u>*H*</u>$_3$)$_B$	1.15	1.14	–0.01	–4
10	C(16)<u>*H*</u>$_3$	3.63	3.60	–0.03	–12

Table 1 Chemical shifts of (*R*)/(*S*)-MTPA esters. N.B. ^{1}H resonances corresponding to C(4)<u>*H*</u>$_2$, C(6)<u>*H*</u> and C(7)<u>*H*</u> were observed to overlap with other signals and were therefore not included above

As described by Hoye, the Mosher method of stereochemical assignment can be explained by a conformational model in which the terminal -CF_3, carbinol hydrogen and the ester carbonyl are *syn*-coplanar. Due to neighbouring group anisotropy, the phenyl ring differentially shields the protons on substituents R^1 and R^2 (see below).[20]



(*S*)-MTPA ester

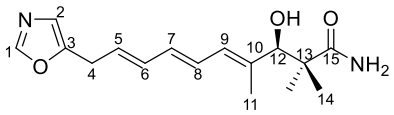


(*R*)-MTPA ester

Anisotropy in MTPA ester analysis[20]

This effect results in differences in chemical shift between the two diastereomers, and by calculating values of $\Delta\delta^{SR}$ ($\delta_S - \delta_R$ by convention) it is possible to assign the absolute configuration of the parent alcohol. Positive values of $\Delta\delta^{SR}$ correspond to protons from R^1, and negative values to R^2 protons.[20] Application of this model confirms the absolute configuration of the aldol product as (*R*) and corroborates the stereochemical model for the aldol reaction proposed by Kiyooka.

5 ^{13}C NMR Data of Isolated and Synthetic Inthomycin C (1)



inthomycin C (**1**)

Carbon	δ_C Isolated/ ppm	δ_C Synthetic/ ppm	$\Delta\delta_C$ (δ_C Synthetic – δ_C Isolated)/ppm
1	151.6	151.6	0
2	123.1	123.1	0
3	151.6	151.8	+0.2
4	Not observed	29.1	N/A
5*	128.2	128.3	+0.1
6	134.2	134.2	0
7	132.7	132.7	0
8*	129.1	129.1	0
9*	128.7	128.7	0
10	140.0	140.1	+0.1
11	13.4	13.4	0
12	83.7	83.8	+0.1
13	45.8	45.7	–0.1
14_A	25.5	25.6	+0.1
14_B	22.4	22.5	+0.1
15	180.8	180.6	–0.2

Table 2 ^{13}C NMR data of isolated and synthetic inthomycin C. *denotes reassignment of previous work by Henkel and Zeek[21] i.e. *C*(5) has been reassigned as *C*(9), *C*(8) as *C*(5) and *C*(9) as *C*(8) by this work. ^{13}C NMR (126 MHz, Acetone-d_6) referenced to δ_C = 29.8 ppm for both data sets

6 Specific Rotation Data Summary

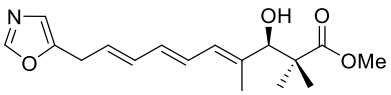
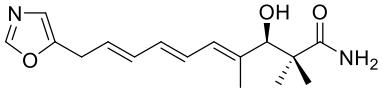
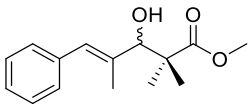
Author	 **17**	 (-)-(3*R*)-inthomycin C (**1**)
This Work	$[\alpha]_D$ +1.2 (*c* 1.0, $CHCl_3$) 89% ee 13.3:1 mixture of **17** and (4*E*,6*E*,8*Z*) isomer	$[\alpha]_D$ –8.2 (*c* 1.0, $CHCl_3$) 89% ee 11.1:1 mixture of **1** and (4*E*,6*E*,8*Z*) isomer
Hale[11]	$[\alpha]_D$ –0.43 (*c* 0.7, $CHCl_3$) 83% ee 17:1 mixture of **17** and unknown isomer	$[\alpha]_D$ –8.4 (*c* 1.0, $CHCl_3$) 83% ee 5.9:1 mixture of **1** and unknown isomer
Hatakeyama (Corrected Data)[10,32]	$[\alpha]_D$ +0.78 (*c* 1.39, $CHCl_3$) 98% ee	$[\alpha]_D$ –7.9 (*c* 0.33, $CHCl_3$) 98% ee
Ryu[9]	$[\alpha]_D$ +8.48 (*c* 0.9, $CHCl_3$) 93% ee	$[\alpha]_D$ –34.33 (*c* 0.1, $CHCl_3$) 93% ee
Taylor[8]	$[\alpha]_D$ +5.2 (*c* 1.55, $CHCl_3$) 76% ee	$[\alpha]_D$ +25.9 (*c* 0.27, $CHCl_3$) 76% ee contaminated with 20% tetramethyl urea

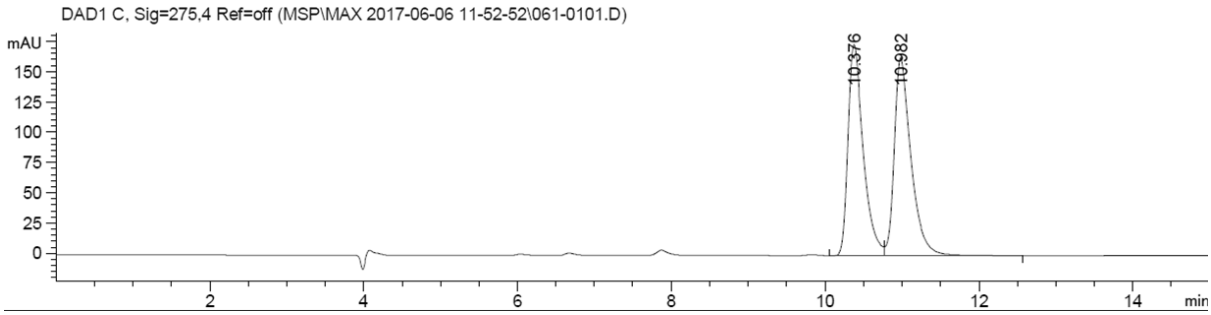
Table 3 Specific rotation data summary

7 HPLC Spectra

Chiral HPLC Conditions: (Daicel Chiralpak IB, ^{n}hexane/^{i}PrOH = 95:5, 1.0 mL min^{-1}, λ = 275 nm, 15 μL injection) τ_R (minor) = 10.4 min, τ_R (major) = 11.0 min.

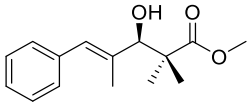
Racemic

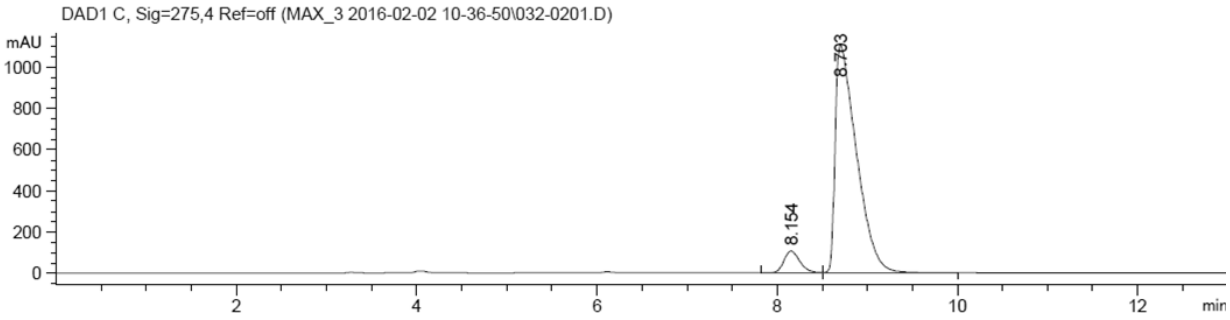




Peak Number	Retention Time/min	Area/mAU*min	Height/mAU	Relative Area/%
1	10.376	39.809	174.349	49.092
2	10.982	41.282	163.775	50.908
Total:		**81.091**	**338.124**	**100.00**

Asymmetric 93:7 er

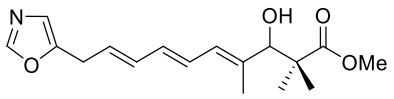


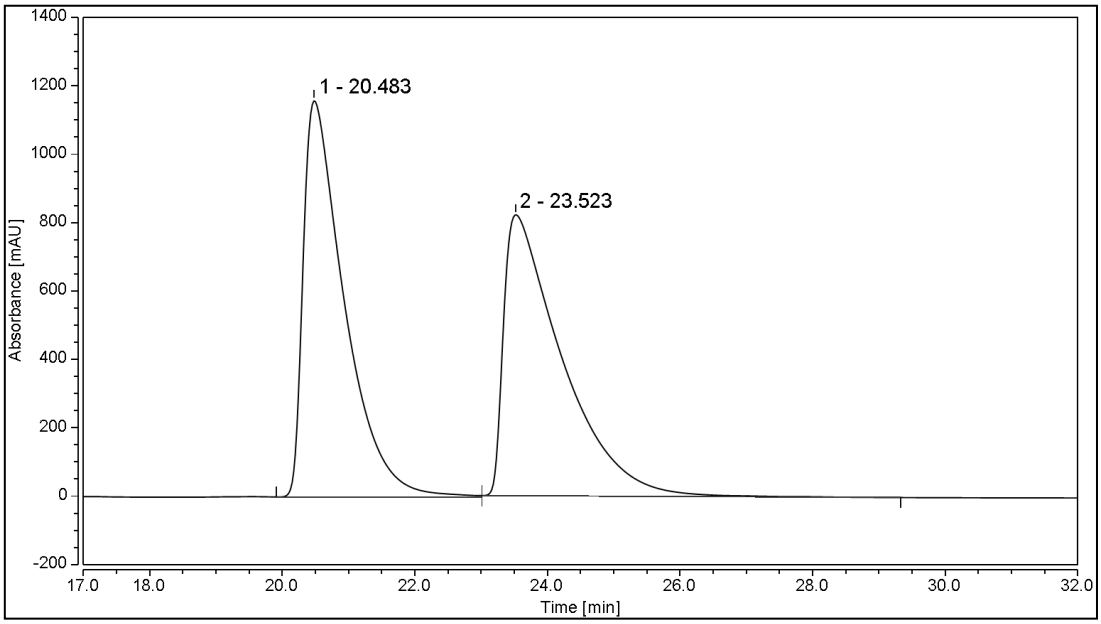


Peak Number	Retention Time/min	Area/mAU*min	Height/mAU	Relative Area/%
1	8.154	21.630	105.618	6.683
2	8.703	302.00	1107.369	93.317
Total:		**323.63**	**1212.987**	**100.00**

Chiral HPLC Conditions: (Daicel Chiralpak IA, ^{n}hexane/^{i}PrOH = 85:15, 1.0 mL min^{-1}, λ = 284 nm, 20 μL injection racemic, 50 μL asymmetric) τ_R (minor) = 20.5 min, τ_R (major) = 23.5 min.

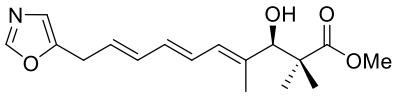
Racemic

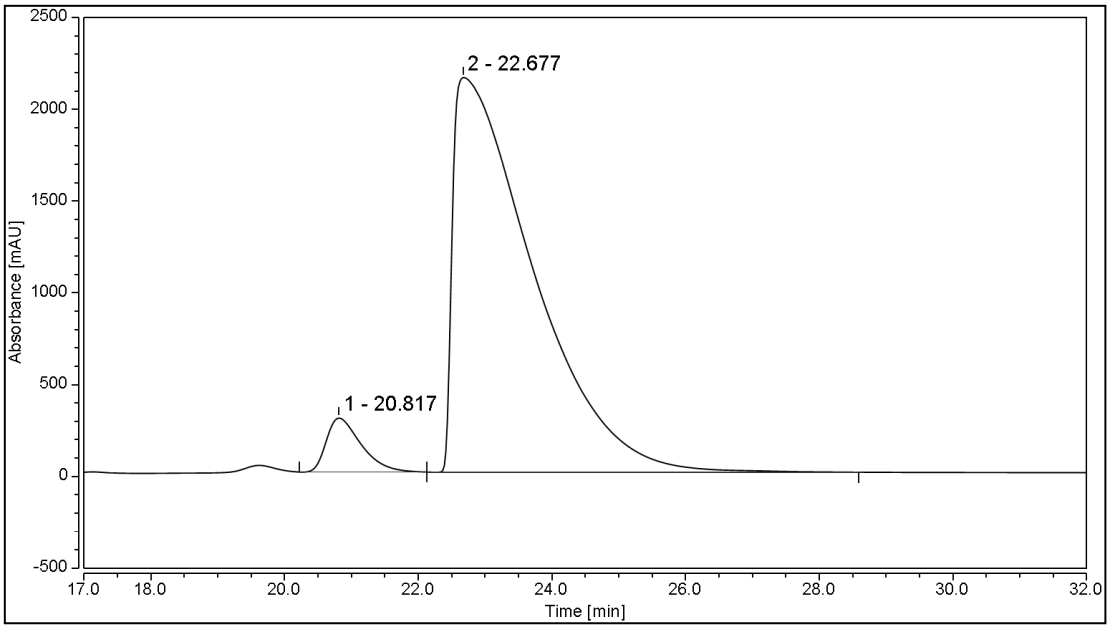




Peak Number	Retention Time/min	Area/mAU*min	Height/mAU	Relative Area/%
1	20.483	837.106	1160.071	50.00
2	23.523	837.042	822.079	50.00
Total:		**1674.148**	**1982.151**	**100.00**

Asymmetric 94:6 er





Peak Number	Retention Time/min	Area/mAU*min	Height/mAU	Relative Area/%
1	20.817	176.896	293.838	5.59
2	22.677	2987.132	2150.165	94.41
Total:		**3164.028**	**2444.004**	**100.00**

8 Biological Assay Details

Cell culture:

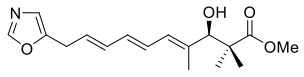
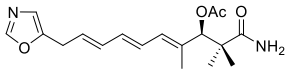
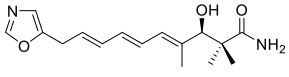
H460, HeLa and MCF7 cells were purchased from ATCC and cultured in DMEM containing 10% foetal calf serum (FCS) and 1% penicillin-streptavidin (PS), all purchased from Gibco, in a humidified atmosphere at 37°C and 5% CO_2. SKOV-3 cells were purchased from ATCC and cultured as above in RPMI containing 10% FCS and 1% PS. The KMS-12BM cell line was purchased from DMSZ and cultured in RPMI containing 10% FCS and 1% PS under the same conditions.

Reagents:

Stock solutions of the compounds were prepared at 10 mM in DMSO, stored at -15 °C and defrosted each time before use. Hoechst 33342 was purchased from Sigma, and propidium iodide from Biolegend.

Viability assays:

The effect of the compounds on cell viability was analysed by obtaining the total number of live and dead cells per well of a 96-well plate, using Hoechst 33342 and propidium iodide staining. Cells were seeded at densities of 5000 cells/well. Cells were incubated in the presence of compound for three cell cycles and then stained and counted using a Celigo imaging cytometer. The IC_{50} (the compound concentration at which cell viability is 50% of that of the control) value for each compound was determined from a plot of viable cells, expressed as a percentage of the control cell count, against log[compound] in Graphpad Prism v7, using the equation $y = 100/(1 + 10^{(m(\log IC50 - x))})$ in which m is the gradient of the slope. The compounds were tested in duplicates.

	Cytotoxicity IC_{50}				
Compound	HeLa	H460	MCF-7	KMS-12BM	SKOV-3
 **17**	>50 μM >50 μM	>50 μM >50 μM	>50 μM >50 μM	>50 μM >50 μM	>50 μM >50 μM
	>50 μM >50 μM	>50 μM >50 μM	>50 μM >50 μM	-	-
 (-)-(3*R*)-inthomycin C (**1**)	>50 μM >50 μM	>50 μM >50 μM	>50 μM >50 μM	-	-

Proteasome assay:

Cells were assayed for proteasome activity using the Proteasome-Glo™ Chymotrypsin-like Cell based assay kit (Promega). H460 cells were seeded at a density of $7.5x10^4$ cells/per well and incubated overnight. For the KMS-12BM cell line $1.5\ x10^5$ cells were seeded on the day of the experiment. The length of preincubation of cells with compound did not alter the extent of observed inhibition, so a 30 min incubation of compound at the required concentration was chosen for convenience. The assay was performed as described in the manufacturer's instructions with the modification that additional compound was added at the same time as reagent in order to maintain the correct compound concentration. Luminescence readings were taken using an OMEGA POLARstar plate reader. The luminescence emitted was proportional to proteasome activity and results were analysed relative to the control, obtained in the absence of compound. IC_{50} values were obtained from determining the concentration dependence of inhibition and fitting data to the equation $y = 100/(1 + 10^{(m(\log IC50 - x))})$ where y is the normalised response, x the concentration of compound and m is the gradient of the slope. For compounds in which the IC_{50} was greater than 50 μM (the maximum concentration that could be used in the assays) results were obtained at 50 μM compound concentration and expressed as a percentage of the control value.

To test for whether the action of the compound was reversible, cells were preincubated with compound and then the activity assay was performed in the presence of reagent alone with no additional compound. Thus, if the compound bound in a reversible manner, inhibition would be decreased or no longer be observed.

Results of proteasome inhibitory activity:

An IC_{50} value of 11 μM (n = 2) was obtained for proteasome inhibitory activity by compound **17** in H460 cells and 39 μM (n=2) in KMS-12BM cells (Table 4).

Compound	Cell line	
	H460	KMS-12BM
17	11.9 μM 9.8 μM	41.7 μM 22.4 μM

Table 4 IC_{50} values for the proteasome inhibitory activity of **17** in H460 cells.

Reversibility of inhibition of proteasome activity in H460 cells:

After either a 15 min or overnight incubation of H460 cells with **17**, reagent was added with and without **17**. In the latter, all proteasome inhibitory activity was lost indicating complete reversibility of the interaction (Figure 1).

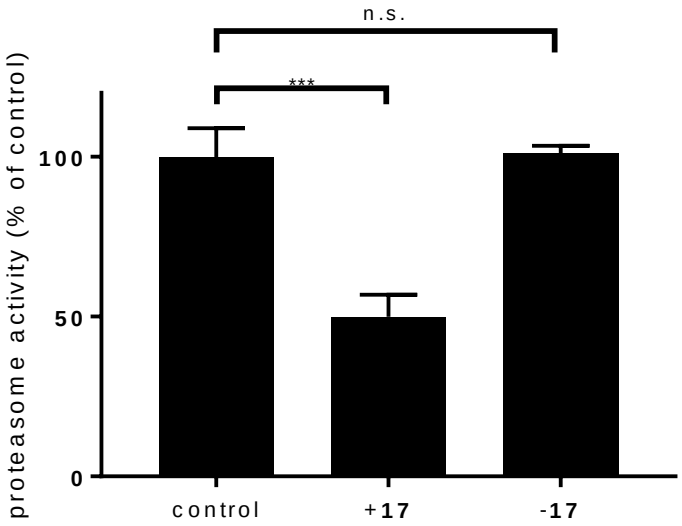


Figure 1 Results show the mean and standard deviation of triplicate readings after a 15min preincubation with 10 μM of **17** followed by addition of reagent with (+) or without (-) **17**; n.s. = not significant, *** $p = 0.0002$

9 References

1. Armarego, W.L.F. *Purification of Laboratory Chemicals* **2009**, Butterworth-Heinemann.
2. Schwarzenbach, R. *J. Chromatogr. A* **1985**, *334*, 35.
3. Miller, R. A.; Smith, R. M.; Karady, S.; Reamer, R. A. *Tetrahedron Lett*. **2002**, *43*, 935.
4. Miller, R. A.; Smith, R. M.; Marcune, B. *J. Org. Chem*. **2005**, *70*, 9074.
5. Childs, R. F.; Hagar, M. E. *Can. J. Chem*. **1980**, *58*, 1788.
6. Liu, G.S.; Dong, Q. L.; Yao, Y. S.; Yao, Z. J. *Org. Lett*. **2008**, *10*, 5393.
7. Gieseler, M. T.; Kalesse, M. *Org. Lett*. **2011**, *13*, 2430.
8. Imashiro, R.; Kuroda, T. *J. Org. Chem*. **2003**, *68*, 974.
9. Itsuno, S.; Watanabe, K.; Matsumoto, T.; Kuroda, S.; Yokoi, A.; El-Shehawy, A. *J. Chem. Soc. Perkin Trans. 1*. **1999**, *14*, 2011.
10. Li, T. T.; Liu, G. Q.; Wang, Y. M.; Cui, B.; Sun, H.; Li, Y. M., *Tetrahedron*, **2015**, *71*, 7003.
11. Paquette, L. A.; Parker, G. D.; Tei, T.; Dong, S. *J. Org. Chem.* **2007**, *72*, 7125.
12. Dong, S.; Parker, G. D.; Tei, T.; Paquette, L. A. *Org. Lett.* **2006**, *8*, 2429.
13. Senapati, B. K.; Gao, L.; Lee, S. I.; Hwang, G.S.; Ryu, D.H. *Org. Lett.* **2010**, *12*, 5088.
14. Lu, T.J.; Lin, C.K. *J. Org. Chem*. **2008**, *73*, 9527.
15. Rösler, S.; Obenauf, J.; Kempe, R. *J. Am. Chem. Soc.* **2015**, *137*, 7998.
16. Webb, M. R.; Addie, M. S.; Crawforth, C. M.; Dale, J. W.; Franci, X.; Pizzonero, M.; Donald, C.; Taylor, R. J. K. *Tetrahedron* **2008**, *64*, 4778.
17. Yoshino, M.; Eto, K.; Takahashi, K.; Ishihara, J.; Hatakeyama, S. *Org. Biomol. Chem.* **2012**, *10*, 8164.
18. Hale, K. J.; Grabski, M.; Manaviazar, S.; Maczka, M. *Org. Lett.* **2014**, *16*, 1164.
19. Hale, K. J.; Hatakeyama, S.; Urabe, F.; Ishihara, J.; Manaviazar, S.; Grabski, M.; Maczka, M. *Org. Lett*. **2014**, *16*, 3536.
20. Hoye, T. R.; Jeffrey, C. S.; Shao, F. *Nature Protocols* **2007**, *2*, 2451.
21. Henkel, T.; Zeeck, A. *Liebigs Ann Chem.* **1991**, 367.