Supplementary Information

Using Nature's polyenes as templates: Studies of synthetic xanthomonadin analogues and realising their potential as antioxidants

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General chemistry experimental

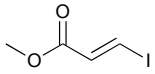
All reactions were carried out in oven-dried glassware with magnetic stirring unless otherwise stated. All chemicals were purchased from commercial suppliers and used without further purification. Where petroleum ether is stated, this refers to petroleum ether bp 40-60 °C. Anhydrous acetonitrile was obtained by distillation of HPLC grade acetonitrile over calcium hydride. Anhydrous THF was obtained by distillation of HPLC grade THF over sodium metal, with a benzophenone indicator. Solvents were degassed, unless stated otherwise, by sparging with argon for 20 minutes. For all palladium cross-coupling reactions, cross-shaped stirrer bars were used for the most efficient stirring. Monitoring of reactions was achieved using TLC and/or ^{1}H NMR. TLC was performed using silica plates. The silica plates were polyester-backed silica TLC plates with 0.2 mm silica gel and fluorescent indicator. Spots were visualised using an ultraviolet (UV) lamp and $KMnO_4$ dip, visualising in both long wave and short wave UV. NMR experiments were carried out on either a Bruker Avance-400 or a Varian VNMRS-700 spectrometer in deuterated chloroform (CDCl3-d), deuterated

methanol (MeOD-d4), or deuterated dimethyl sulfoxide (DMSO-d_6). Chemical shifts are reported in ppm relative to tetramethylsilane (TMS) reference. Experiments undertaken included ^{1}H, ^{11}B, ^{19}F, ^{13}C, COSY, PSYCHE, HSQC and HMBC NMR. Celite/silica filtration used Celite® S and technical grade silica gel: pore size 60 Å, 230-400 mesh particle size, 40-63 µm particle size. The term 'evaporated' refers to the removal of solvent in vacuo. Silica gel chromatography used technical grade silica gel: pore size 60 Å, 230-400 mesh particle size, 40-63 µm particle size. Electrospray ionisation (ESI) mass spectroscopy was undertaken using a LTQ FT (ThermoFinnigan) high resolution, accurate mass LC ES MS/MS or a Thermo Scientific LTQ Orbitrap XL. Samples were made up as 1 mg per mL solutions in acetonitrile. GC/MS EI was undertaken using a Waters GCT Premier. Atmospheric solids analysis probe (ASAP) mass spectroscopy was undertaken using LCT Premier XE (Waters) high resolution, accurate mass ultra performance liquid chromatography (UPLC) ASAP or a Thermo Scientific LTQ Orbitrap XL. Samples were either made up as 1 mg per mL solutions in acetonitrile or run as solids. Infra-red (IR) spectroscopy was undertaken using a Perkin Elmer-1600 FTIR, using both liquid and solid samples. Melting point measurements were undertaken using a Gallenkamp melting point apparatus.

UV-Vis measurements were carried out on a Varian Cary 100 Bio UV-Visible Spectrophotometer. Fluorescence measurements were carried out on a Jasco FP-6200 Spectrofluorometer. Both UV-Vis and fluorescence measurements were carried out using quartz cuvettes, with samples dissolved in either spectrophotometric grade diethyl ether, or spectrophotometric grade chloroform.

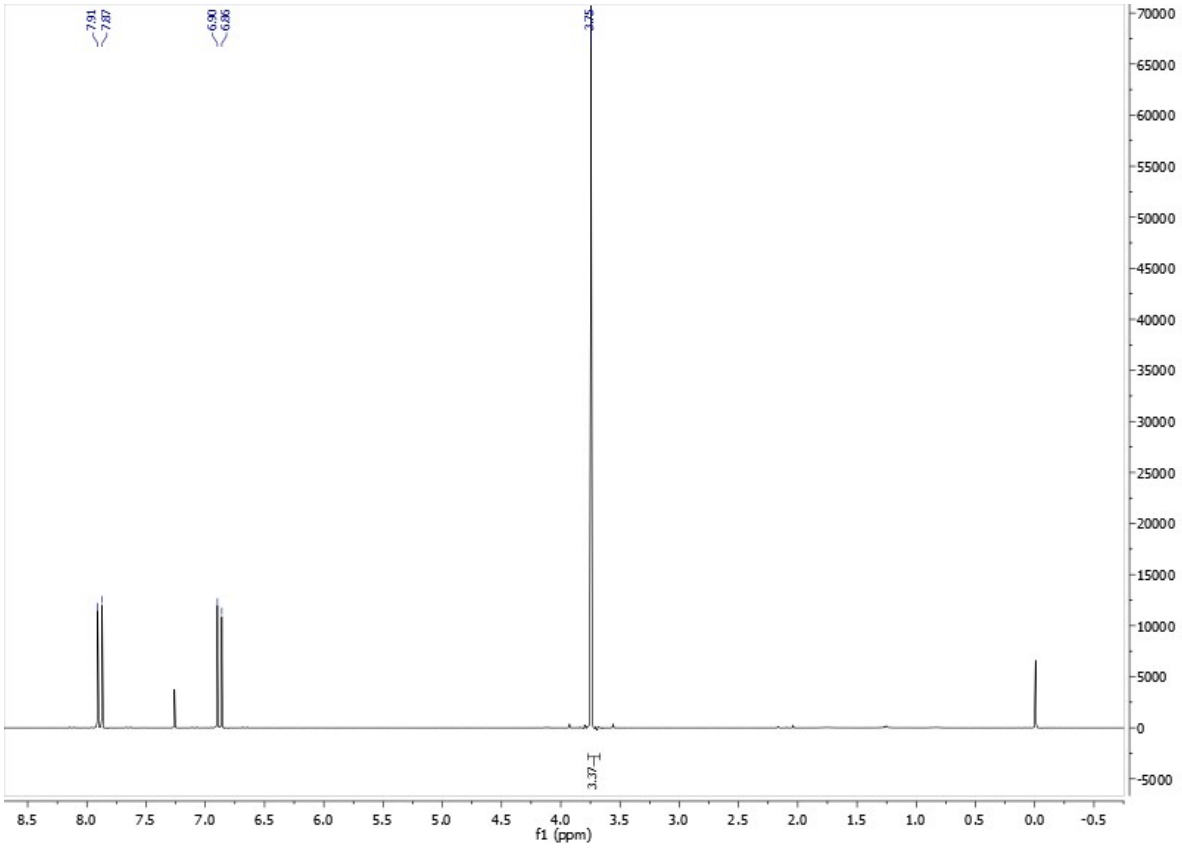
Chemical experimental

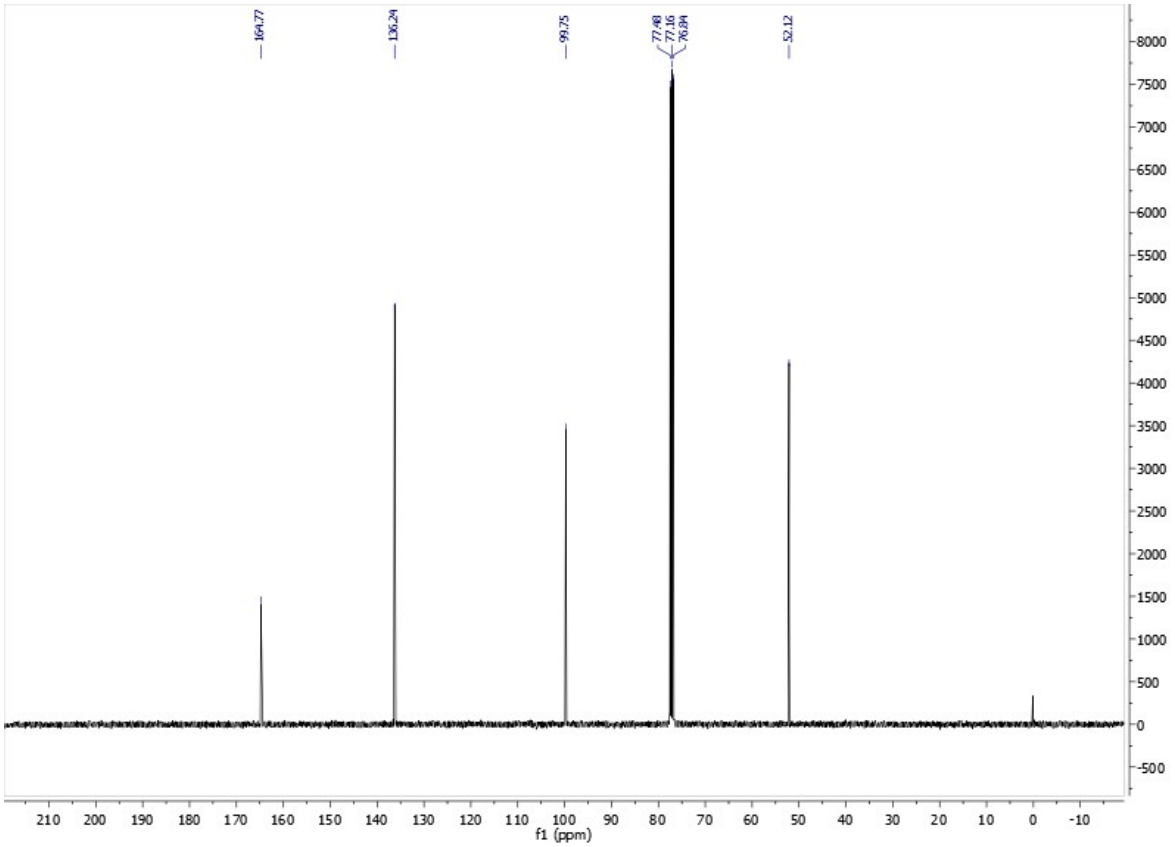
Methyl (2E)-3-iodoprop-2-enoate **7**



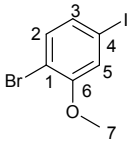
A solution of propiolic acid **6** (5.00 g, 71.0 mmol) in aq. HI (57%, 25 mL) was heated under reflux for 0.5 hours, after which time the reaction was cooled to 0 ^{o}C. The resulting crystals were then filtered, washed with water (3 x 20 mL) and air-dried to afford (*E*)-3-iodoprenoic acid (14.2 g) as an off-white solid. The stereochemistry as the (*E*)-isomer was confirmed by X-ray crystallography following slow evaporation from EtOAc. (*E*)-3-Iodopropenoic acid (14.0 g, 70.7 mmol) was dissolved in MeOH (20 mL) and conc. H_2SO_4 (1 mL) was added. The reaction mixture heated under reflux for 24 hours, after which the solvent was removed *in vacuo.* The resulting crude product was dissolved in Et_2O (100 mL), washed with H_2O (50 mL), sat. $NaHCO_3$ (2 x 50 mL), sat. $Na_2S_2O_3$ (2 x 50 mL) and brine (50 mL). The organic

extracts were then dried over $MgSO_4$ and evaporated to yield methyl (2*E*)-3-iodoprop-2-enoate **7** as an off-white solid (12.9 g, 86% over two steps): M.p. 48.5-51.3 ^oC; ^1H NMR (400 MHz, $CDCl_3$) δ 3.75 (3H, s), 6.88 (1H, d, *J* 14.8 Hz), 7.89 (1H, d, *J* 14.8 Hz). All other spectral data are consistent with those reported previously.[1]



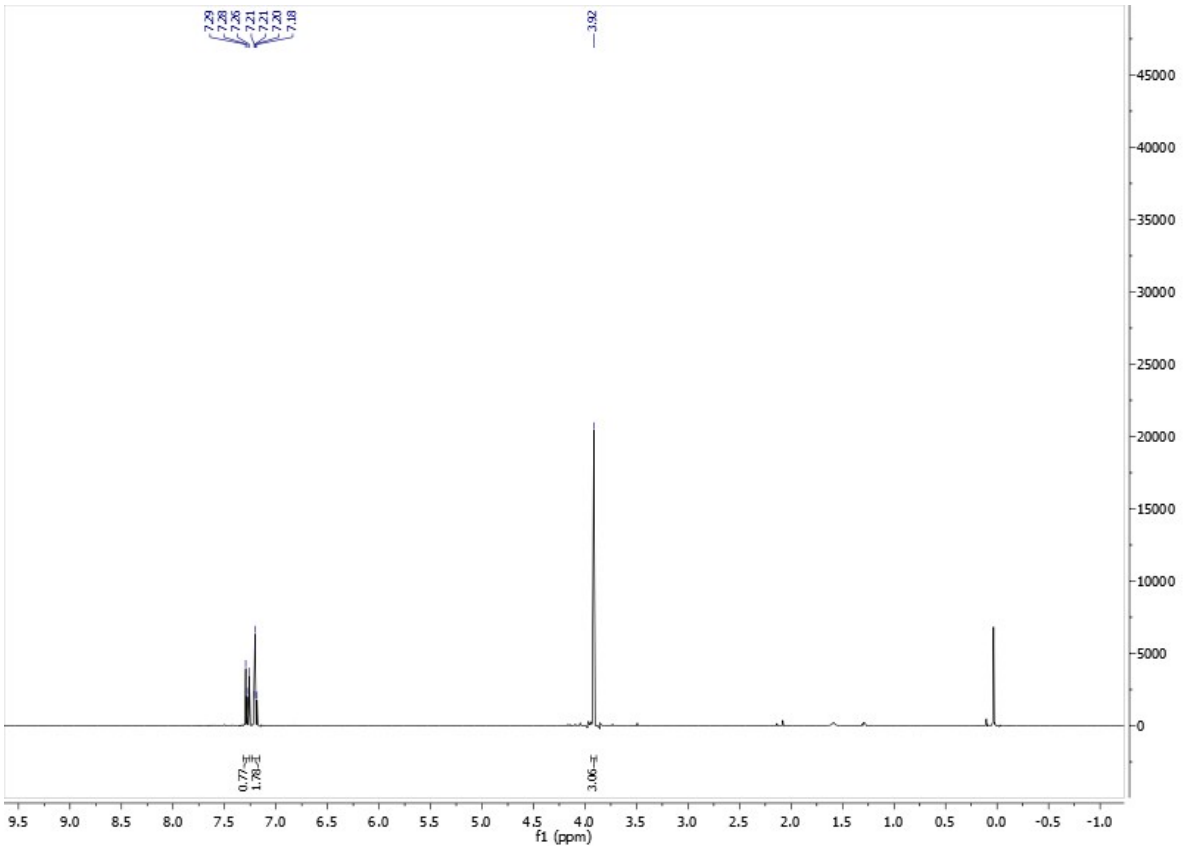


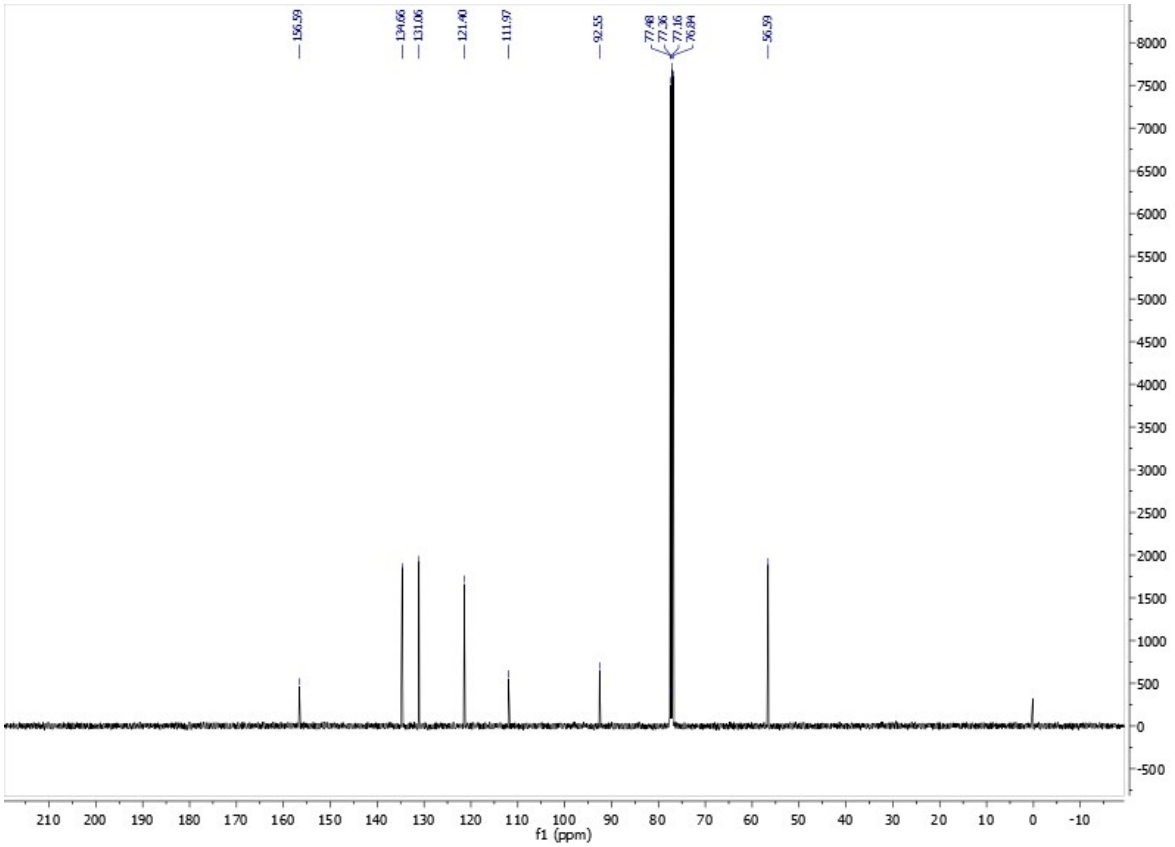
1-Bromo-4-iodo-2-methoxybenzene **16**



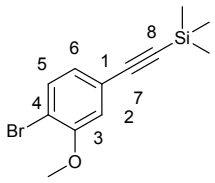
4-Bromo-3-methoxyaniline (5.00 g, 24.8 mmol) was stirred in aqueous HCl (37%, 250 mL) at 80 ˚C to ensure complete dissolution. This was then cooled to 0 ˚C and a cold solution of $NaNO_2$ (2.22 g, 32.2 mmol) in H_2O (125 mL) was added dropwise, keeping the temperature constant. The reaction mixture was stirred at 0 ˚C for 1 hour and a cold solution of KI (12.5 g, 74.3 mmol) was carefully added dropwise at 0 ˚C over a period of 1 hour. The resulting dark brown solution was stirred and allowed to reach room temperature overnight. The reaction mixture was diluted with EtOAc (250 mL) and the layers separated. The aqueous layer was extracted using EtOAc (2 x 250 mL). The organic layers were combined and washed sequentially with sat. $NaHCO_3$ (125 mL) and H_2O (125 mL) until neutral pH. The organic layers were then washed with 5% $Na_2S_2O_3$ (125 mL) and saturated brine (125 mL), dried over $MgSO_4$

and the solvent evaporated to afford a dark brown oil, from with the desired product spontaneously crystallised to give 1-bromo-4-iodo-2-methoxybenzene as a dark brown solid (7.8 g, 100%): M.p. 53.1-55.6 ^{o}C; ^{1}H NMR (400 MHz, $CDCl_3$) δ 3.88 (3H, s), 7.14-7.18 (2H, m), 7.22-7.25 (1H, m). All other spectral data were consistent with those reported previously.[1]



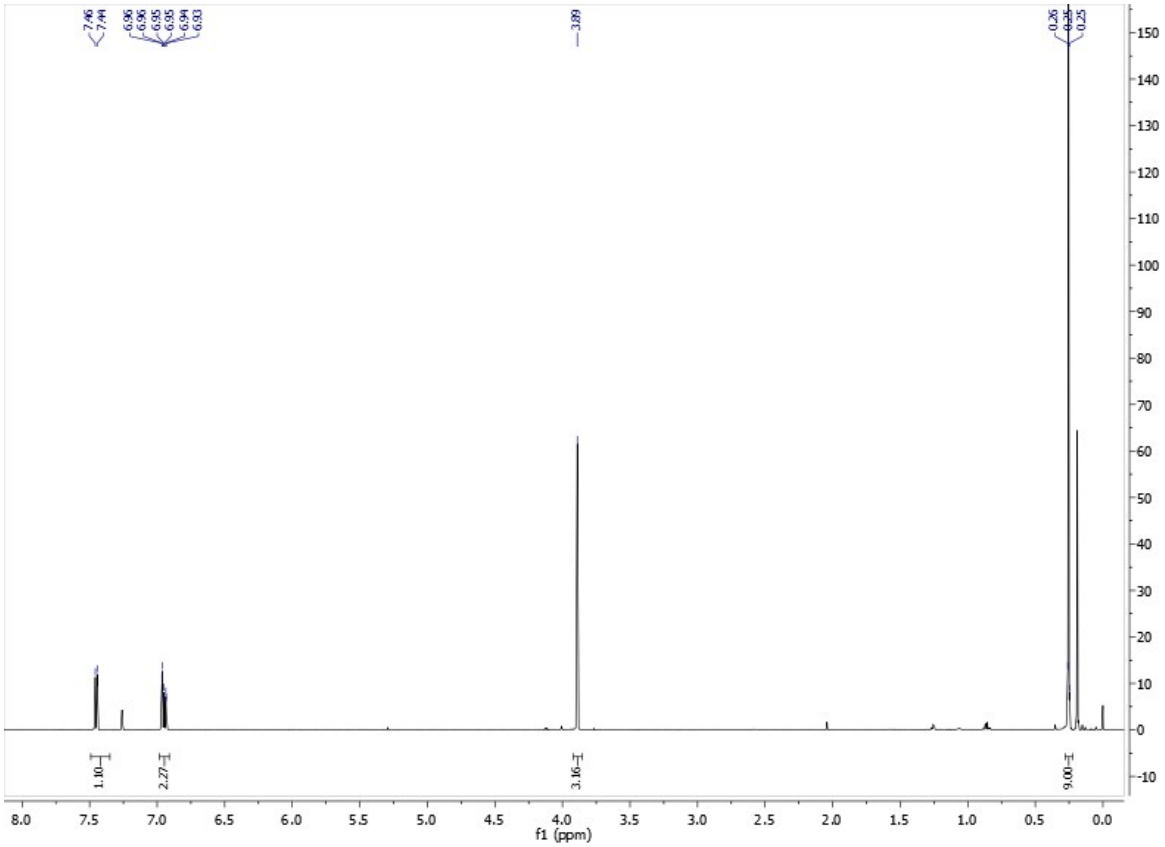


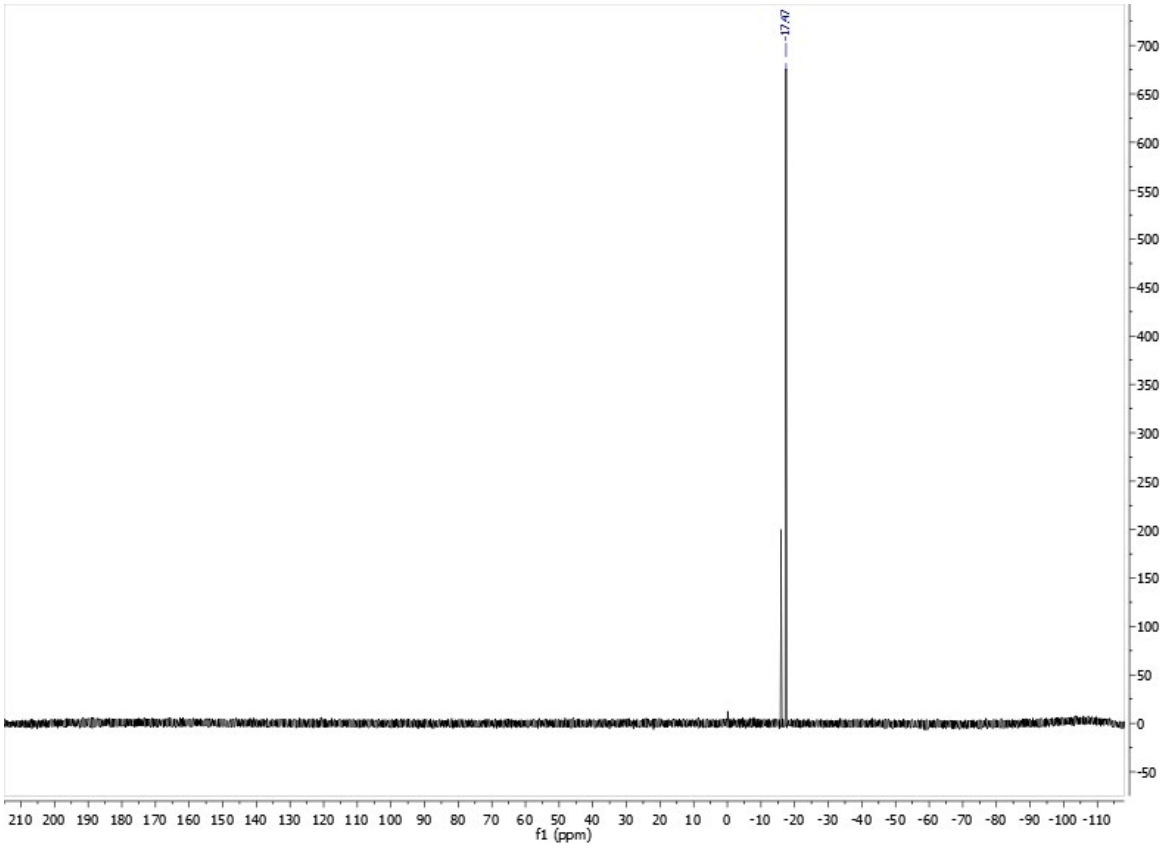
((4-Bromo-3-methoxyphenyl)ethynyl)trimethylsilane **18**

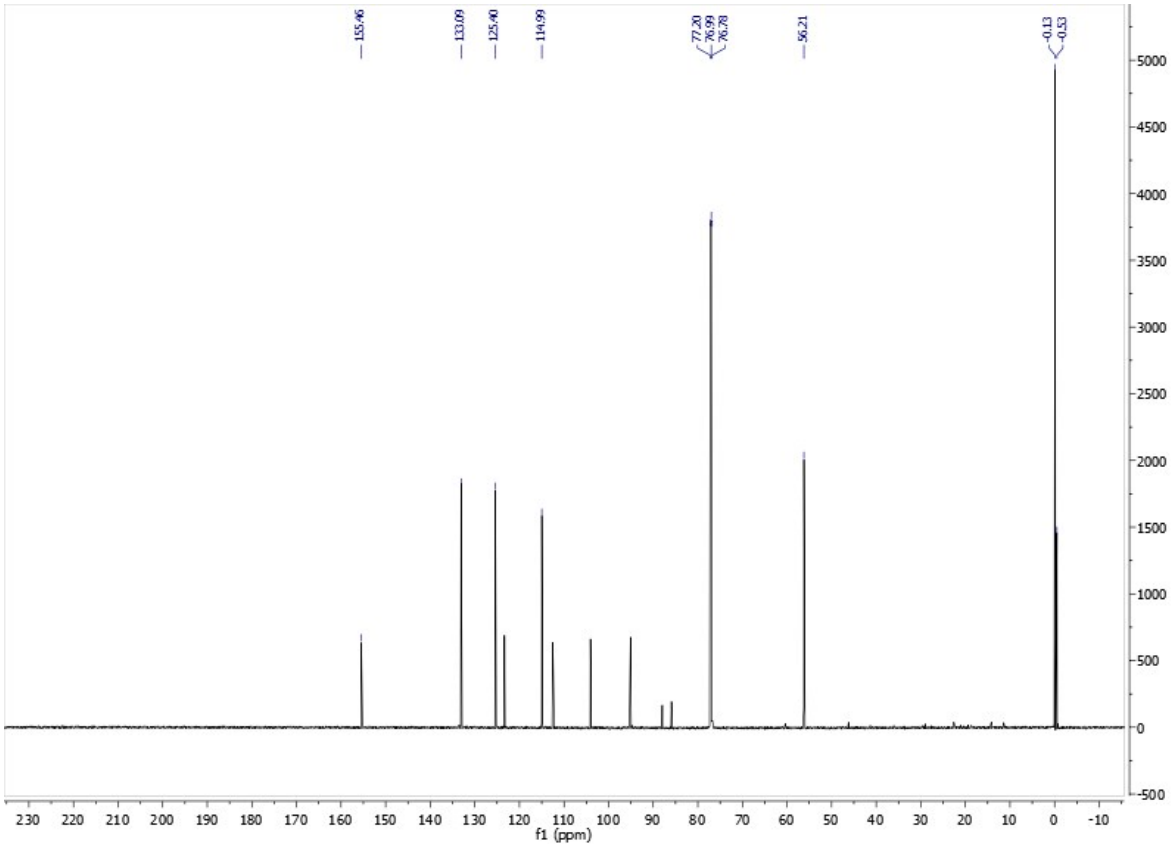


1-Bromo-4-iodo-2-methoxybenzene (7.71 g, 24.8 mmol), $Pd(PPh_3)_2Cl_2$ (0.173 g, 0.242 mmol) and CuI (47 mg, 0.242 mmol) were added to a dry flask. After purging the flask with argon for 5 minutes, dry, degassed Et_3N (139 mL) was added to the tube under argon, followed by TMS acetylene (4.0 mL, 29.8 mmol). The reaction was stirred at room temperature in the dark for 16 hours. The solvent was then evaporated and the residue was passed through a silica gel column, eluent 5% EtOAc in petroleum ether to give an orange oil (7.11 g, 100%): ^{1}H NMR (600 MHz, $CDCl_3$) δ 0.25 (9H, s), 3.89 (3H, s), 6.92-6.98 (2H, m), 7.45 (1H, d, *J* 8.1 Hz); ^{29}Si NMR (139 MHz, $CDCl_3$) δ -17.47 (s); ^{13}C NMR (151 MHz, $CDCl_3$) δ -0.2, 56.2, 95.0, 104.0, 112.5, 115.0, 123.3, 125.4, 133.1, 155.4; IR (ν_{max}, cm^{-1}) *inter alia* 2157 (w),

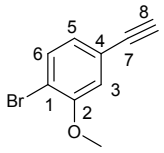
2959.0 (m); LRMS (ASAP) 282.0; HRMS (ASAP) [$C_{12}H_{15}OSi^{79}Br$] calculated 282.0076, found 282.0083.



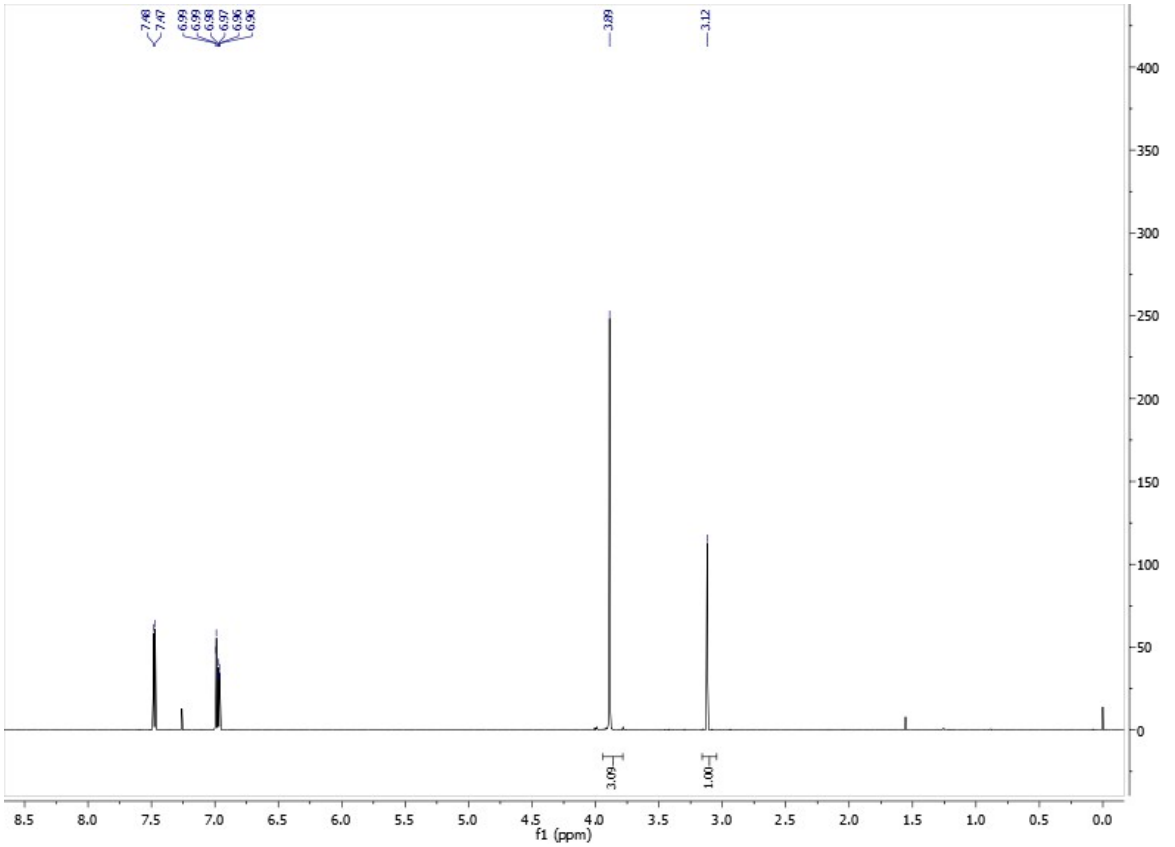




1-Bromo-4-ethynyl-2-methoxybenzene **19**



((4-Bromo-3-methoxyphenyl)ethynyl)trimethylsilane **18** (6.14 g, 21.8 mmol) was dissolved in THF (307 mL) and cooled to 0 ˚C under argon. TBAF (21.8 mL, 21.8 mmol) was then added dropwise at 0 ˚C. The reaction was warmed to room temperature, then stirred at this temperature for 3 days. This mixture was then evaporated to give a dark brown oil. The crude product was purified by silica gel chromatography, eluent 0-5% EtOAc in hexane to give desired product **19** as an orange solid (3.76 g, 86%): M.p. 37.4-38.8 ^{o}C; ^{1}H NMR (400 MHz, $CDCl_3$) δ 3.12 (1H, s), 3.89 (3H, s), 6.92-7.01 (2H, m), 7.48 (1H, d, *J* 8.1 Hz); ^{13}C NMR (176 MHz, $CDCl_3$): δ 56.2, 77.9, 82.8, 112.9, 115.2, 122.3, 125.5, 133.2, 155.6; IR (ν_{max}, cm^{-1}) *inter alia* 2051 (w), 2939 (w), 3258 (s); LRMS (ASAP) 210.0; HRMS (ASAP) [$C_9H_7O^{79}Br$] calculated 209.9680, found 209.9689.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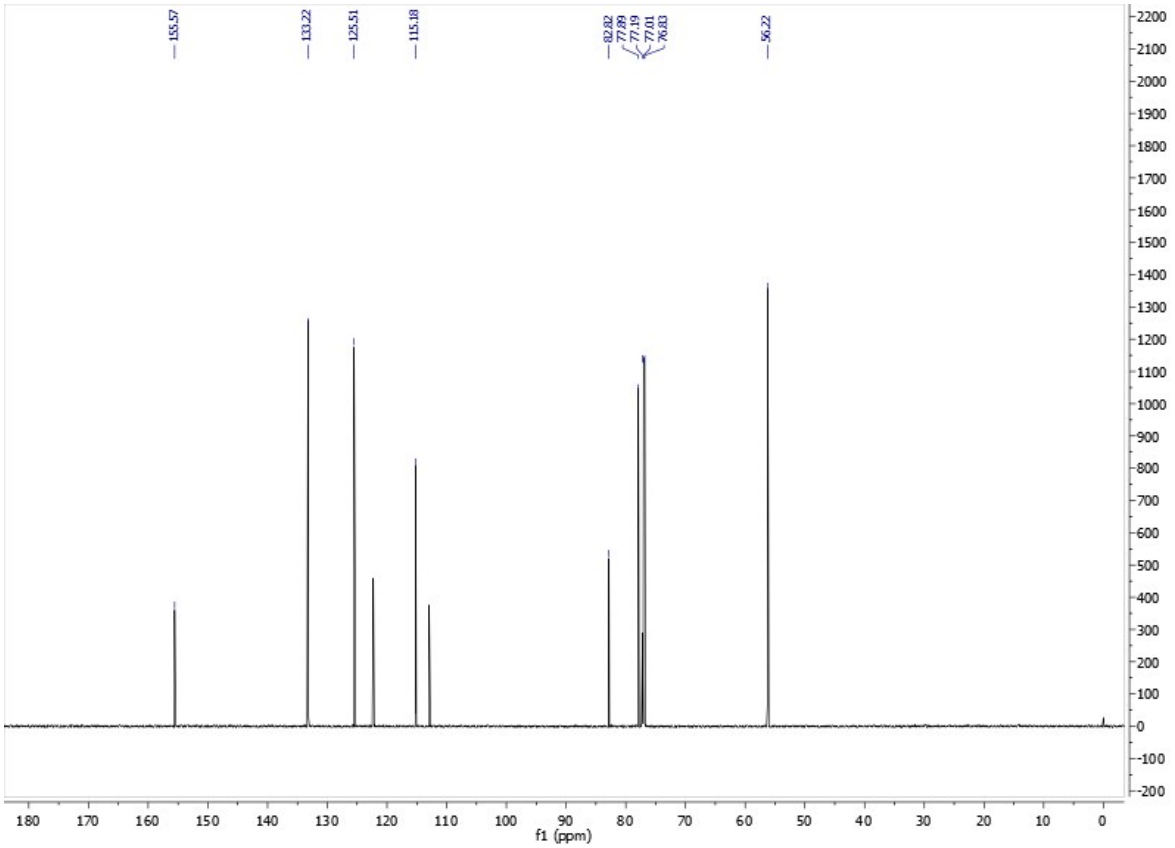
f1 (ppm)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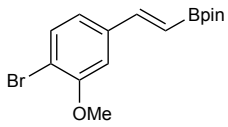
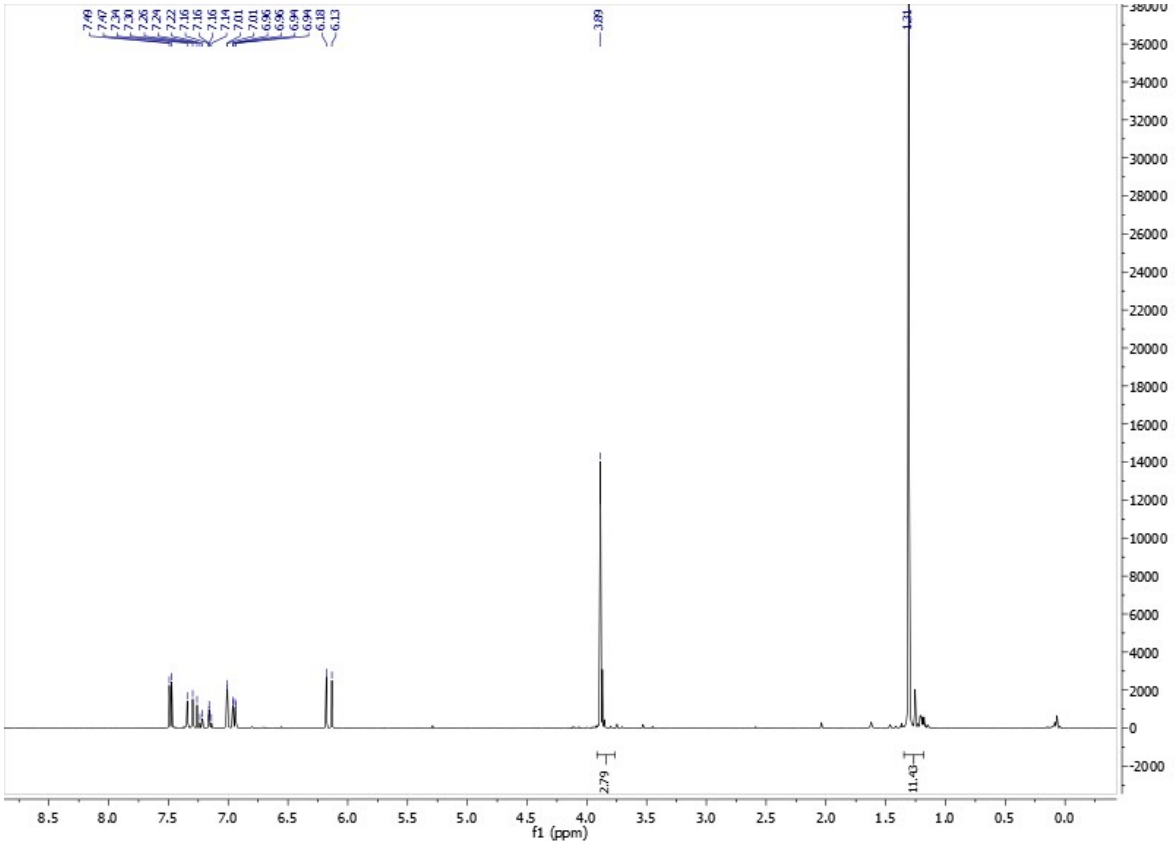
77.01
76.83
56.22
f1 (ppm)

2-[(E)-2-(4-Bromo-3-methoxyphenyl)ethenyl]-4,4,5,5-tetramethyl-1,3,2-dioxaborolane **20**



Copper(I) chloride (16 mg, 0.158 mmol), xantphos (92 mg, 0.158 mmol), sodium *tert*-butoxide (31 mg, 0.32 mmol) and 4,4,5,5-tetramethyl-2-(tetramethyl-1,3,2-dioxaborolan-2-yl)-1,3,2-dioxaborolane (1.33 g, 5.28 mmol) were added to a dry flask fitted with a Schlenk tap under argon. Dry THF (11 mL) was then added and the reaction stirred for 5 minutes. 1-bromo-4-ethynyl-2-methoxybenzene (1.11 g, 5.28 mmol) was then added and the reaction stirred for 5 minutes, then dry MeOH (0.42 mL) was added. The reaction was stirred at room temperature overnight. The reaction mixture was diluted with EtOAc (110 mL) and washed with H_2O (105 mL) and then brine (105 mL). The organics were dried over $MgSO_4$, filtered and evaporated to yield 2.40 g of a dark yellow oil. The crude product was purified by silica gel chromatography, eluent 0-5% EtOAc in hexane to yield desired product **20** as a yellow oil, which became a yellow solid on standing (1.40 g, 79%): M.p. 66.9-69.3 ^{o}C; ^{1}H NMR (400 MHz, $CDCl_3$) δ 1.31 (12H, s), 3.90 (3H, s), 6.16 (1H, d, *J* 18.4 Hz), 6.91-7.06 (2H, m), 7.33 (1H, d, *J* 18.4 Hz), 7.49 (1H, d, *J* 8.1 Hz); ^{11}B NMR (128 MHz, $CDCl_3$) δ 29.69; ^{13}C NMR (101 MHz, $CDCl_3$) δ 25.0, 56.2, 83.7, 110.1, 112.5, 120.8, 133.5, 138.4, 148.6, 156.1; IR (ν_{max}, cm^{-1}) *inter alia* 1548 (m), 1619.8 (m); LRMS (ESI) [M+H] = 339.0 (^{79}Br); HRMS (ESI) [$C_{15}H_{21}{}^{10}BO_3{}^{79}Br$] calculated 338.0803, found 338.0814.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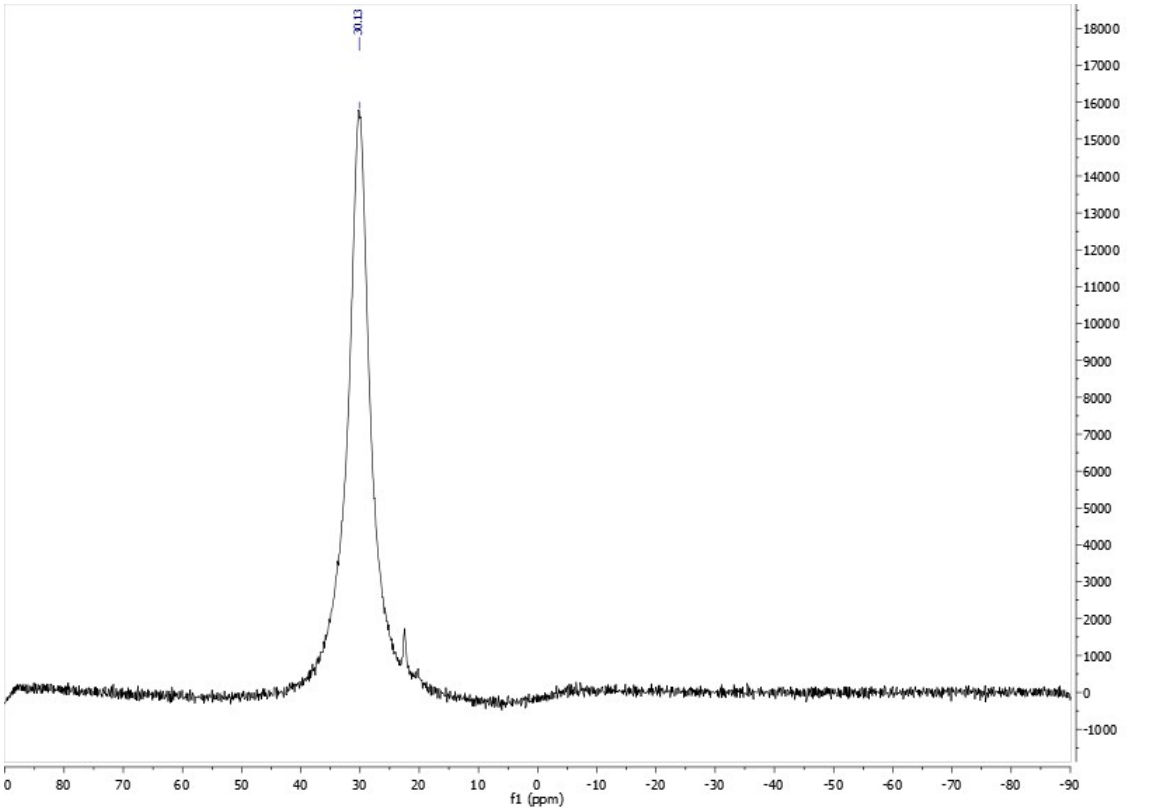
f1 (ppm)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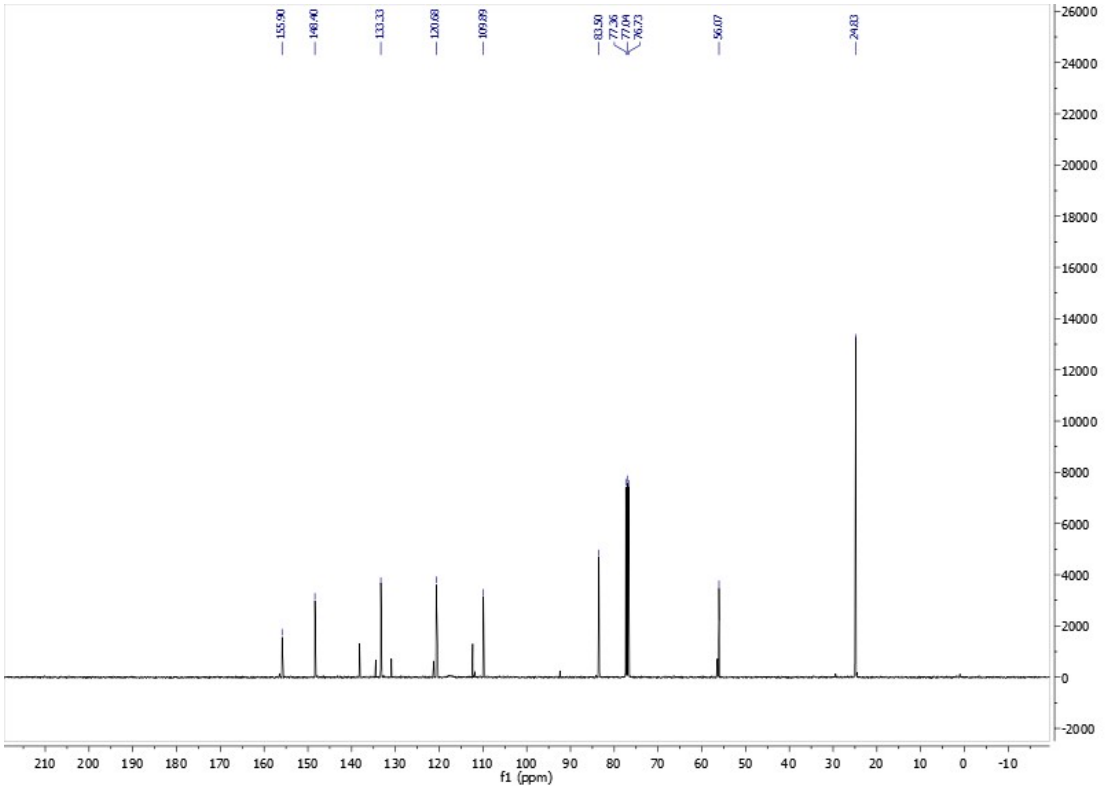
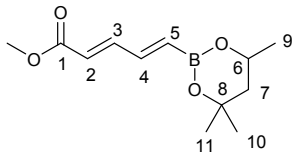
f1 (ppm)



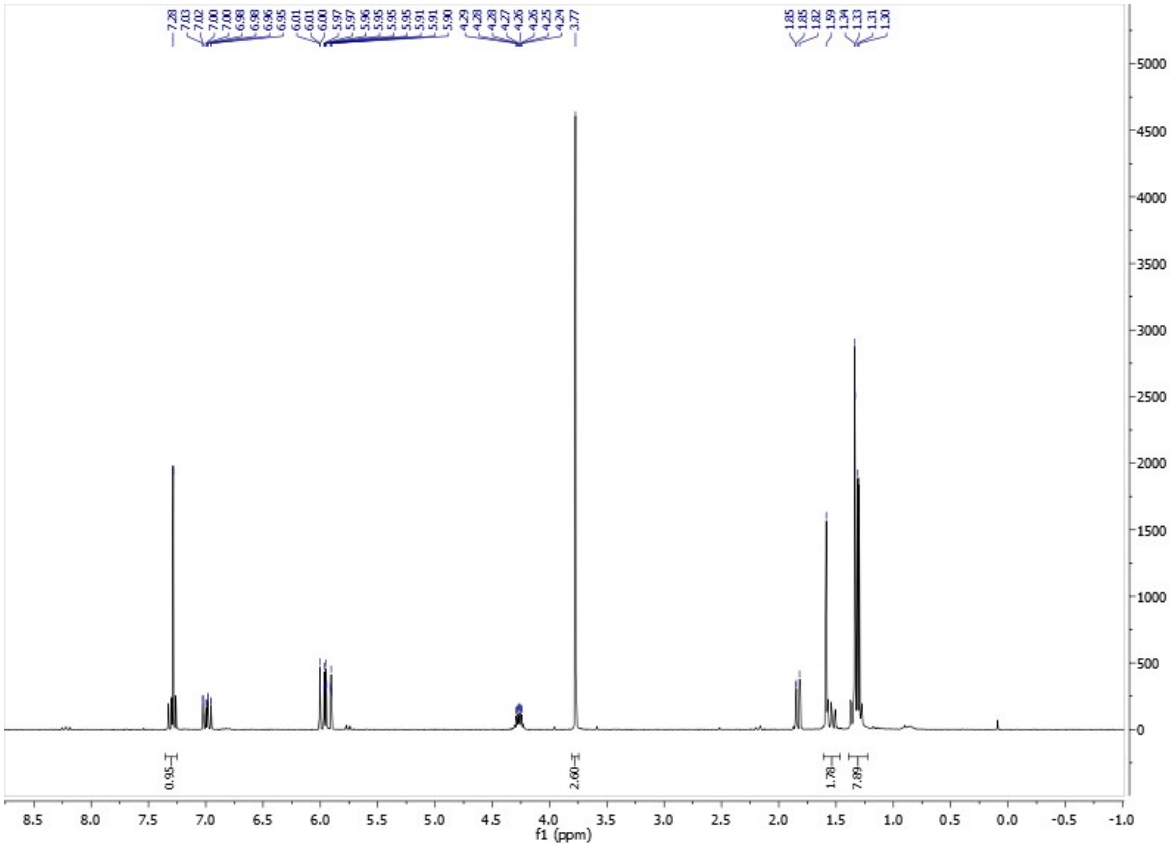
(2E,4E)-5-(4,4,6-Trimethyl-[1,3,2-dioxaborinan-2-yl]-penta-2,4-dienoic acid methyl ester **9**



Method 1: To a dry Schlenk flask was added $Pd(OAc)_2$ (36 mg, 0.160 mmol), P(*o*-tol)$_3$ (0.10 g, 0.330 mmol) and AgOAc (0.601 g, 3.60 mmol). The flask was purged with argon, and dry, degassed MeCN (10 mL) was added. 4,4,6-Trimethyl-2-vinyl-1,3,2-dioxaborinane (0.655 mL, 3.80 mmol) was then added, followed by methyl (2*E*)-3-iodoprop-2-enoate (0.704 g, 3.32 mmol). The vessel was purged further with argon, and the reaction mixture was then heated to 50 °C with vigorous stirring for 2 days. The mixture was allowed to cool, then diluted with Et_2O (280 mL) and passed through a short Celite/silica plug. The organic extracts were washed with 5% HCl (40 mL), H_2O (80 mL) and brine (80 mL), dried over $MgSO_4$ and evaporated to yield 0.980 g of crude product as an orange oil. The crude product was purified by silica gel chromatography, eluent 10% EtOAc in hexane elution to yield (2*E*,4*E*)-5-(4,4,6-

trimethyl-[1,3,2-dioxaborinan-2-yl]-penta-2,4-dienoic acid methyl ester **9** as a yellow oil (0.404 g, 51%): ^{1}H NMR (400 MHz, $CDCl_3$) δ 1.35-1.24 (9H, m), 1.5-1.47 (1H, m,), 1.81 (1H, dd, *J* 14.0, 2.9 Hz), 3.75 (3H, s), 4.24 (1H, dqd, *J* 12.3, 6.2, 2.9 Hz), 5.99-5.86 (2H, m), 6.97 (1H, ddd, *J* 17.3, 11.0, 0.7 Hz), 7.33-7.21 (1H, m); ^{11}B NMR (128 MHz, $CDCl_3$) δ 26; ^{13}C NMR (101 MH, $CDCl_3$): δ 23.6, 28.6, 31.7, 46.4, 52.1, 65.5, 71.7, 123.2, 143.9, 146.5, 167.9; IR (ν_{max}, cm^{-1}) *inter alia* 2974 (w) 1720 (s); LCMS (ESI+) 239.2 ; HRMS (ESI+) calculated $[C_{12}H_{19}BO_4+H]^+$ 238.1470, found 238.1491.

Method 2: To a dry flask was added methyl (2*E*)-3-iodoprop-2-enoate (2.82 g, 13.3 mmol), $Pd(OAc)_2$ (0.150 g, 0.67 mmol), P(*o*-tol)$_3$ (0.408 g, 1.34 mmol) and AgOAc (2.41 g, 14.4 mmol). The flask was purged with argon, and dry, degassed MeCN (80 mL) was added. 4,4,6-Trimethyl-2-vinyl-1,3,2-dioxaborinane (2.6 mL, 15.2 mmol) was then added, the vessel was purged further with argon, and the reaction mixture was then heated to 50 °C with vigorous stirring for 23 hours. The mixture was allowed to cool, then diluted with Et_2O (200 mL) and passed through a short Celite/silica plug. The organic extracts were washed with NH_4Cl (200 mL), H_2O (200 mL) and brine (200 mL), dried over $MgSO_4$, filtered and evaporated to give crude product as a yellow oil (2.65g, 83%). The compound was taken on to the next stage without any further purification or characterisation.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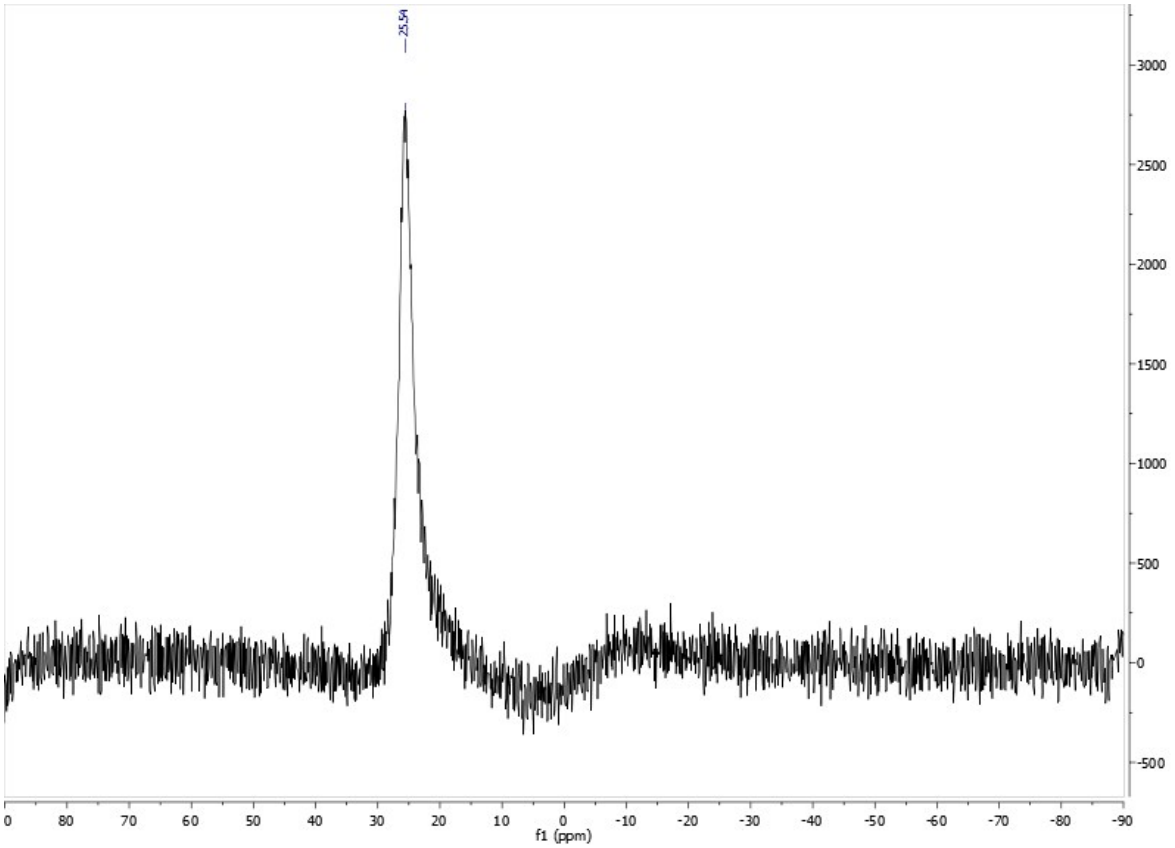
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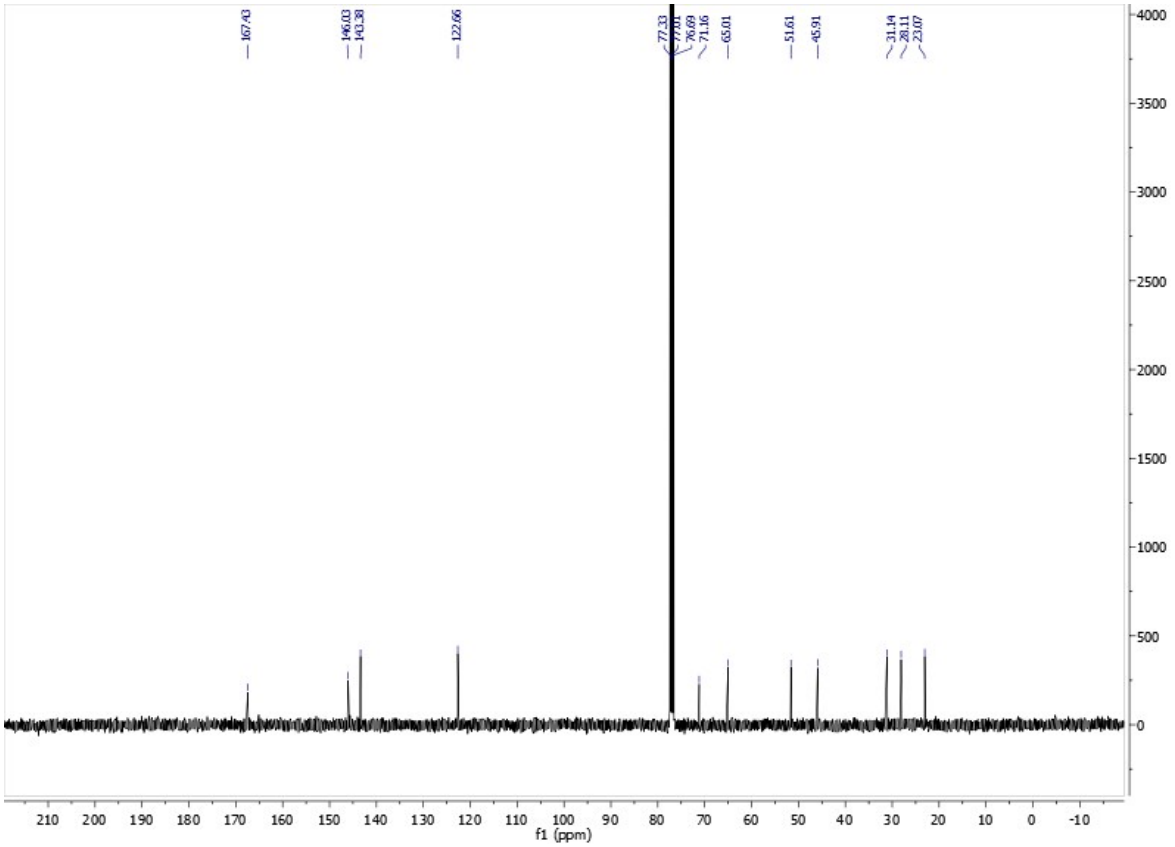
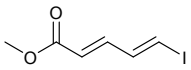
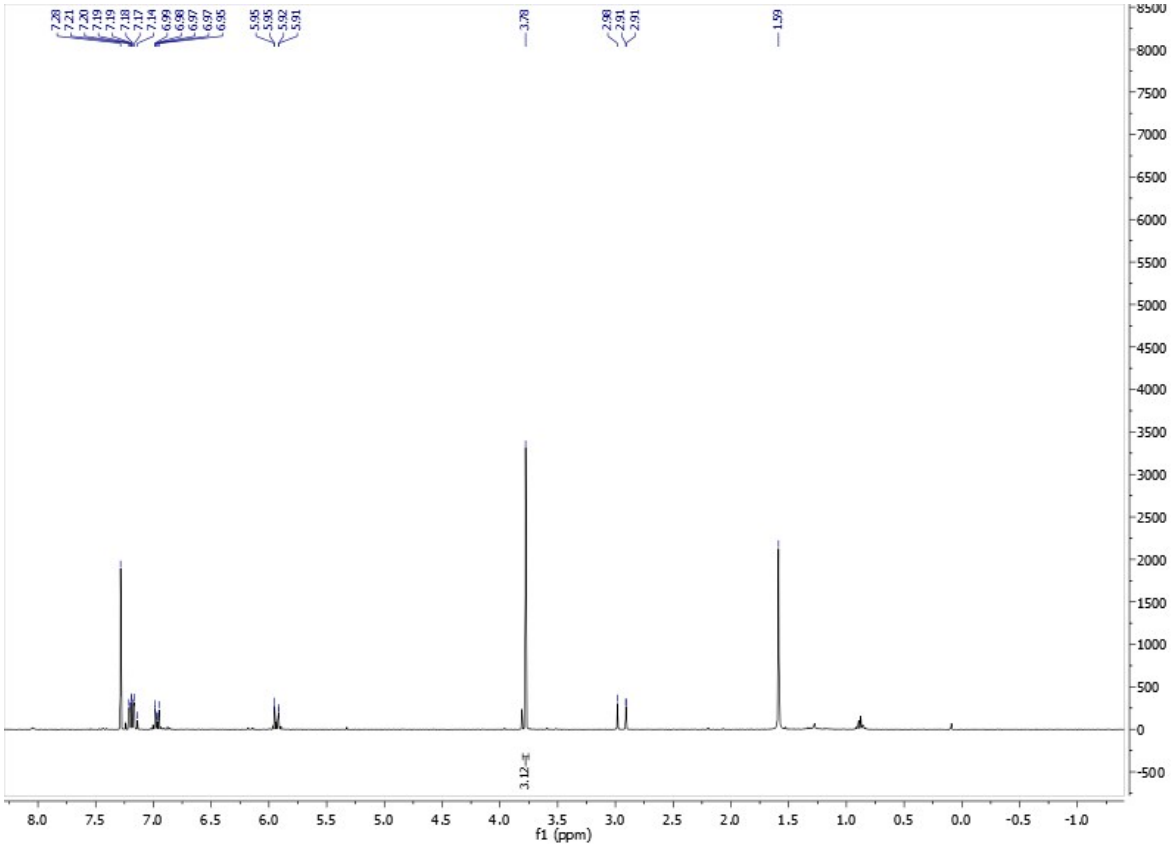


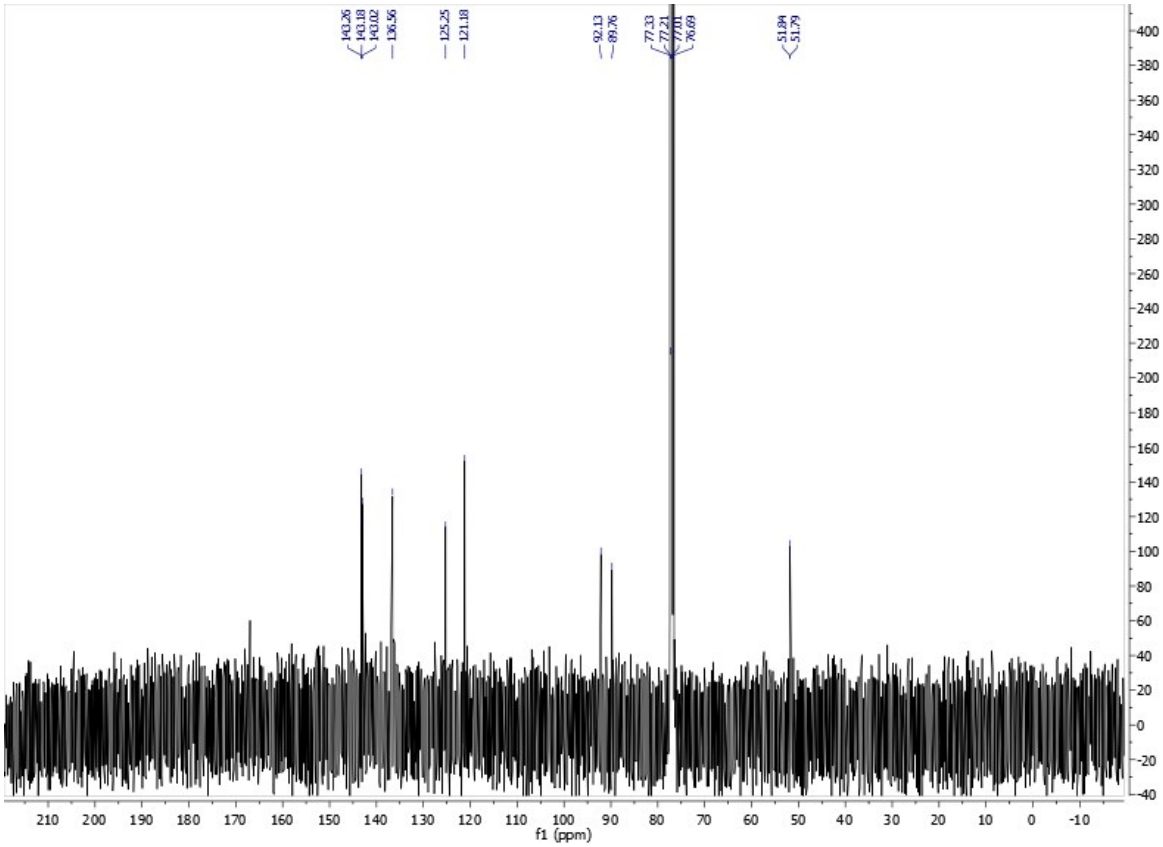
Methyl (2E,4E)-5-iodopenta-2,4-dienoate **10**



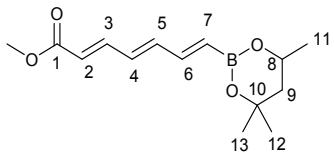
Method 1: NaOMe (4.2 mL, 2.10 mmol, 0.5 M solution in MeOH) was added dropwise to a solution of (2*E*,4*E*)-5-(4,4,6-trimethyl-[1,3,2-dioxaborinan-2-yl]-penta-2,4-dienoic acid methyl ester **9** (0.404 g, 1.70 mmol) in THF (6.0 mL) cooled to -78 °C for 1 hour 50 minutes. The reaction mixture was allowed to warm to room temperature whilst stirring. The mixture was diluted with Et_2O (60 mL) and washed with 5% $Na_2S_2O_3$ (2 x 20 mL), H_2O (20 mL) and brine (20 mL). The organic extracts were dried over $MgSO_4$, filtered and evaporated to yield a pale orange solid. The crude material was purified by silica gel chromatography, eluent 5% EtOAc in petroleum ether at 0 °C to yield methyl (*2E,4E*)-5-iodopenta-2,4-dienoate **10** as a white solid (0.337 g, 83%): ^{1}H NMR (400 MHz, $CDCl_3$) δ 3.75 (3H, s), 5.84-5.98 (1H, m), 6.89-6.98 (1H, m), 7.12-7.22 (2H, m); ^{13}C NMR (101 MHz, $CDCl_3$) δ 51.6, 89.6, 92.0, 121.0, 125.1, 136.4, 142.9. The compound was taken on to the next stage without any further characterisation.

Method 2: NaOMe (28 mL, 14.2 mmol, 0.5 M solution in MeOH) was added dropwise to a solution of (2*E*,4*E*)-5-(4,4,6-trimethyl-[1,3,2-dioxaborinan-2-yl]-penta-2,4-dienoic acid methyl ester (2.84 g, 11.9 mmol) in THF (44 mL) cooled to -78 °C under argon in the absence of light. The mixture was stirred at this temperature for 1 hour 5 minutes and iodine monochloride (12 mL, 12.1 mmol, of a 1.0 M solution in DCM) was added dropwise. The mixture was stirred at -78 °C for 2 hours, then allowed to warm to room temperature whilst stirring. The mixture was diluted with Et_2O (356 mL) and washed with 5% $Na_2S_2O_3$ (2 x 142 mL), water (142 mL) and brine (142 mL). The organic extracts were dried over $MgSO_4$, filtered and evaporated to 3.2 g of a brown oil containing desired product (2.14 g, 76%). The compound was taken on to the next stage without any further purification or characterisation.





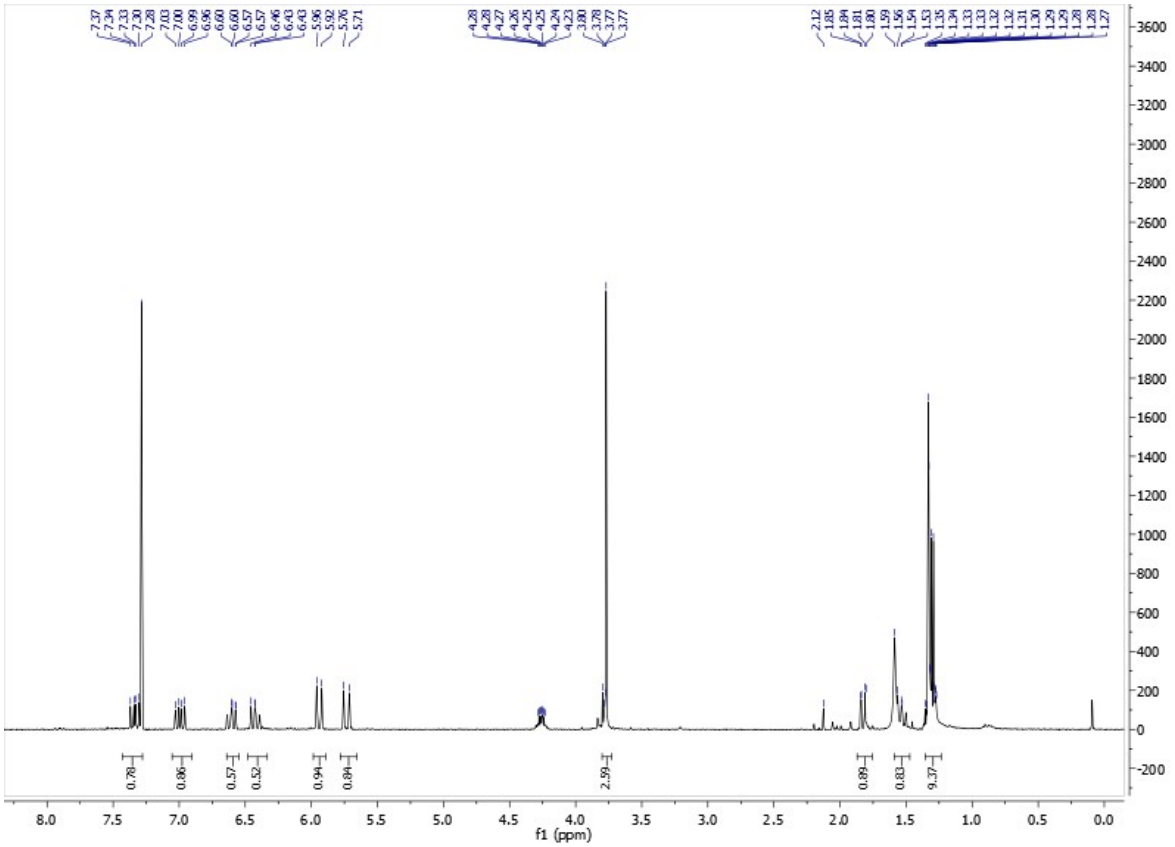
Methyl (2E,4E,6E)-7-(4,4,6-trimethyl-1,3,2-dioxaborinane-2-yl)hepta-2,4,6-trienoate



Method 1: To a dry Schlenk flask was added $Pd(OAc)_2$ (16 mg, 0.0710 mmol), P(*o*-tol)$_3$ (43 mg, 0.142 mmol) and AgOAc (0.284 g, 1.70 mmol), followed by a solution of (*2E,4E*)-5-iodopenta-2,4-dienoate **10** (0.337 g, 1.42 mmol) in dry, degassed MeCN (4.5 mL). 4,4,6-Trimethyl-2-vinyl-1,3,2-dioxaborinane (0.24 mL, 1.42 mmol) was then added and the reaction mixture was then heated to 50 °C with vigorous stirring for 2 days. The mixture was allowed to cool, then diluted with Et_2O (80 mL) and passed through a short Celite/silica plug. The organic extracts were washed with H_2O (40 mL) and brine (40 mL), dried over $MgSO_4$, filtered and evaporated to yield 0.333 g of crude product as an orange oil. The crude product was purified by silica gel chromatography, eluent 10% EtOAc in hexane, to yield methyl (*2E*,*4E*,*6E*)-7-(4,4,6-trimethyl-1,3,2-dioxaborinane-2-yl)hepta-2,4,6-trienoate as a pale yellow solid (78

mg, 21%): M.p. 80.1-82.1°C; ^{1}H NMR (400 MHz, $CDCl_3$) δ 1.36-1.23 (9H, m,), 1.56-1.47 (1H, m), 1.80 (1H, dd, *J* 13.9, 3.0 Hz), 3.75 (3H, s), 4.24 (1H, ddp, *J* 12.3, 6.2, 3.2 Hz), 5.71 (1H, d, *J* 17.4 Hz), 5.92 (1H, d, *J* 15.3 Hz), 6.46-6.33 (1H, m), 6.58 (1H, ddd, *J* 15.0, 10.7, 0.9 Hz), 6.97 (1H, dd, *J* 17.4, 10.7 Hz), 7.35-7.27 (1H, m); ^{11}B NMR (128 MHz, $CDCl_3$) δ 26; ^{13}C NMR (101 MHz, $CDCl_3$) δ 23.1, 28.1, 31.2, 46.0, 51.6, 64.9, 71.0, 121.5, 131.7, 142.4, 144.5, 145.2, 167.4; IR (ν_{max}, cm^{-1}) *inter alia* 2972 (w,), 2950 (w), 2924 (w) 1706; LCMS (ESI+) 264.1; HRMS (ESI+) calculated $[C_{14}H_{21}BO_4+H]^+$ 264.1620, found 264.1647.

Method 2: To a dry flask was added $Pd(OAc)_2$ (0.120 g, 0.525 mmol), P(*o*-tol)$_3$ (0.315 g, 1.50 mmol) and AgOAc (1.89 g, 11.3 mmol). The flask was purged with argon, and a solution of (*2E,4E*)-5-iodopenta-2,4-dienoate (2.50 g, 10.5 mmol) in dry, degassed MeCN (63 mL) was added. 4,4,6-Trimethyl-2-vinyl-1,3,2-dioxaborinane (2.2 mL, 12.6 mmol) was then added, the vessel was purged further with argon, and the reaction mixture was then heated to 50 °C with vigorous stirring for 19.5 hours. The mixture was allowed to cool, then diluted with Et_2O (180 mL) and passed through a short Celite/silica plug. The organic extracts were washed with NH_4Cl (180 mL), H_2O (180 mL) and brine (100 mL), dried over $MgSO_4$, filtered, evaporated to give crude product as a brown oil (1.7 g, 48% from starting acrylate). The compound was taken on to the next stage without any further purification or characterisation.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7.34
7.33
7.30
7.28
7.03
7.00
6.99
6.96
6.60
6.60
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6.46
6.43
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5.96
5.92
5.76
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1.54
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1.32
1.32
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1.29
1.29
1.28
1.28
1.27
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0.57
0.52
0.94
0.84
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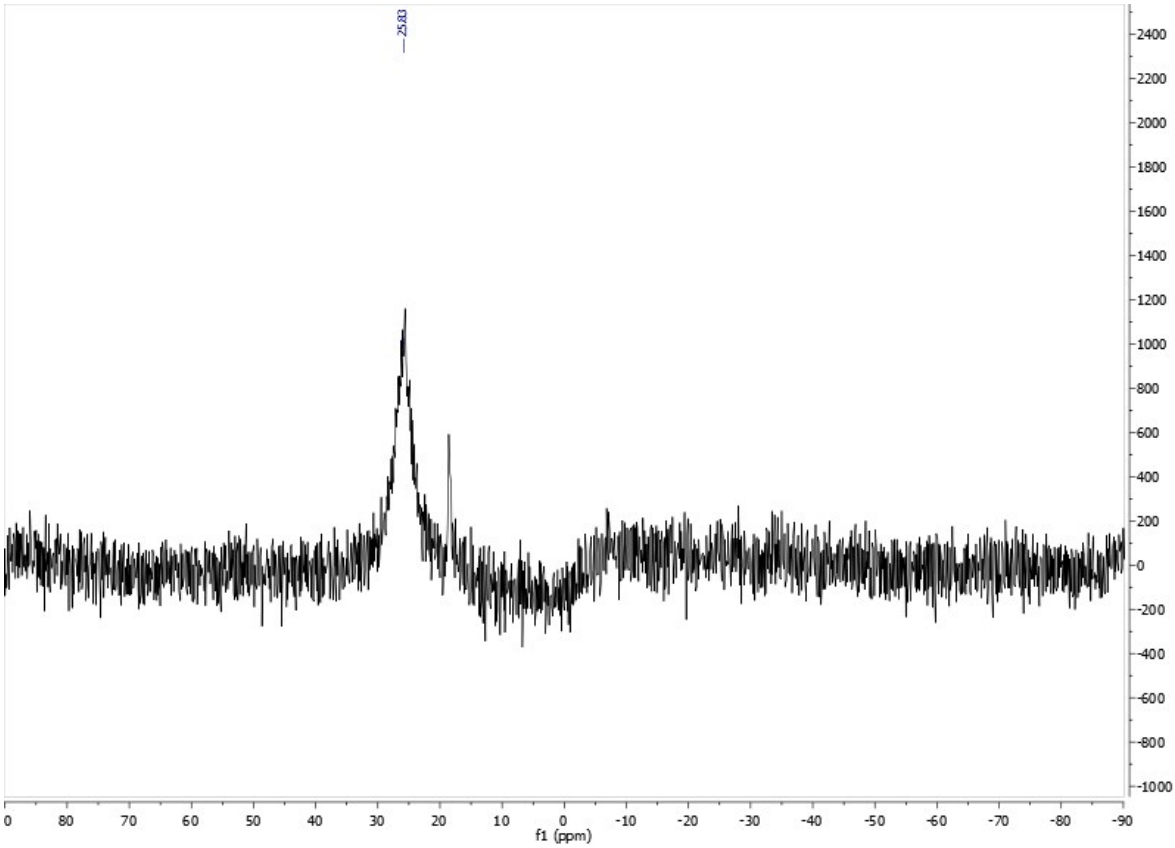
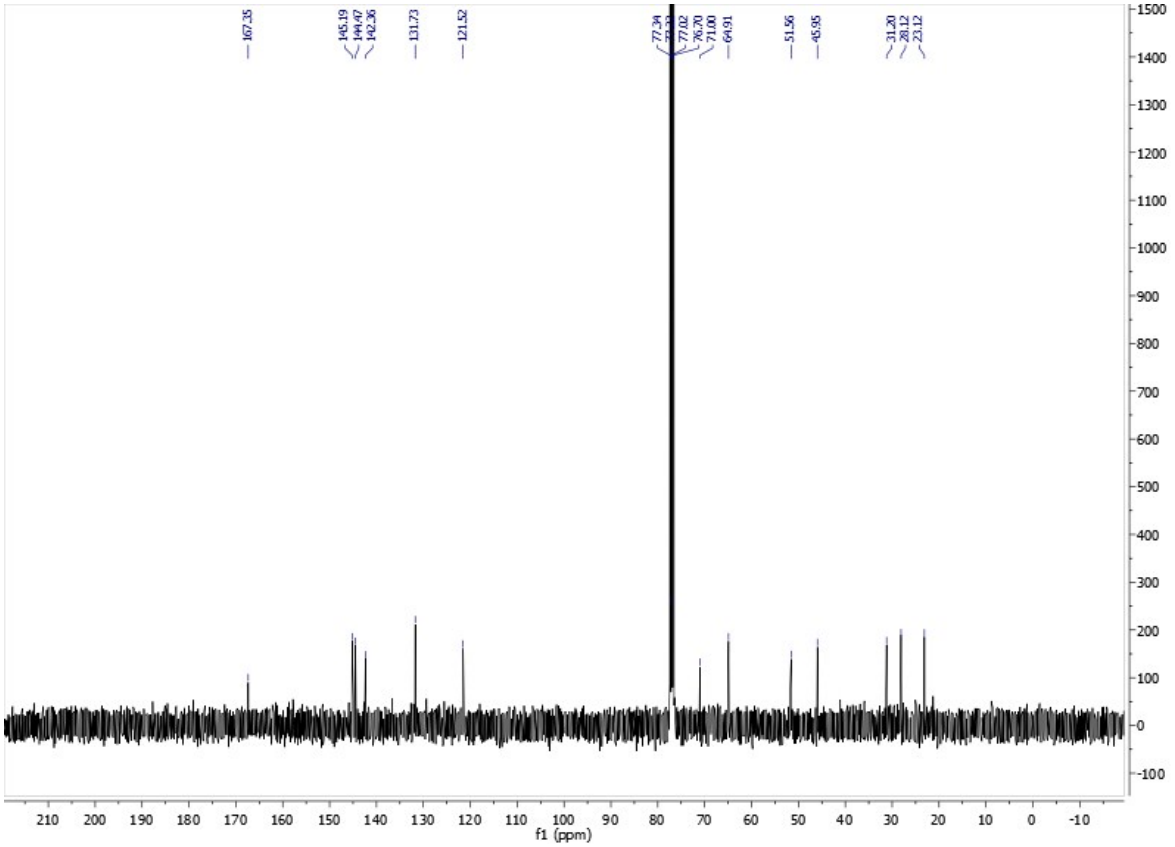
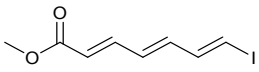
0.89
0.83
9.37
f1 (ppm)

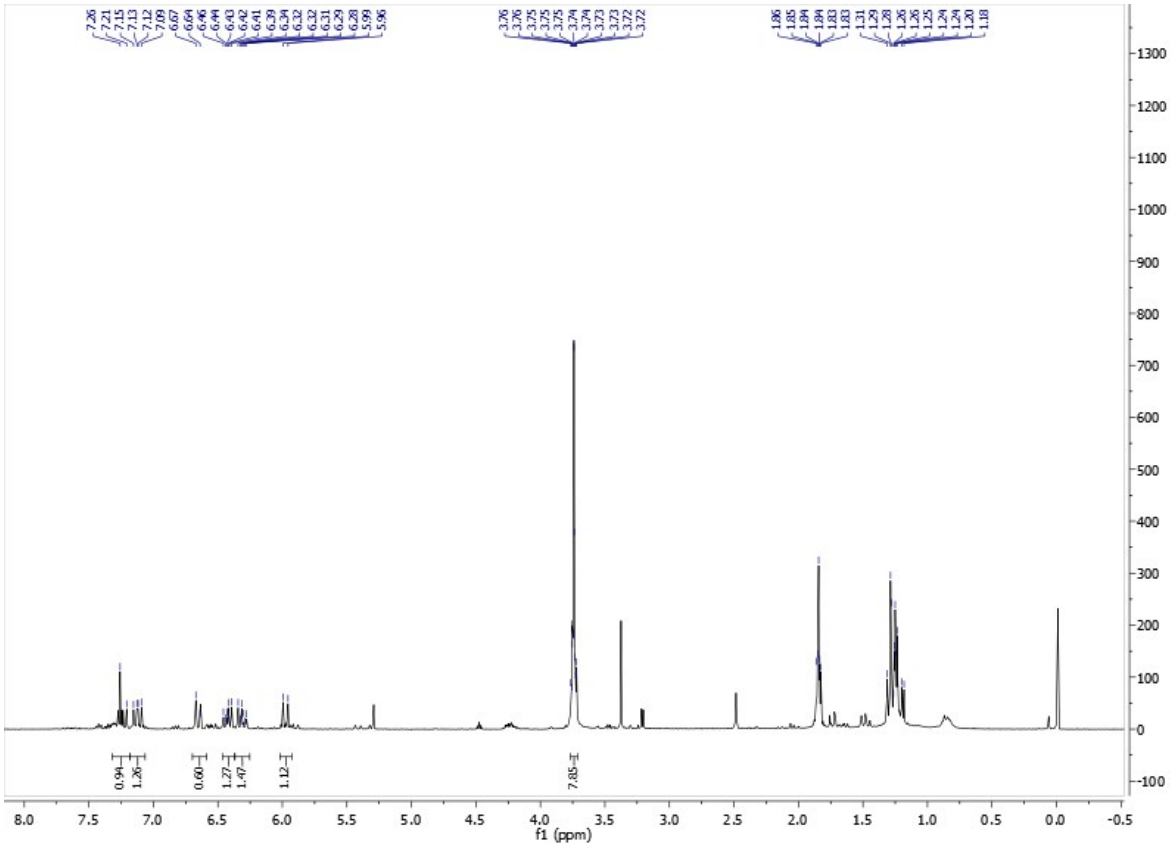

25.83
f1 (ppm)



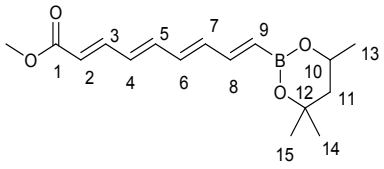
Methyl (2E,4E,6E)-7-iodohepta-2,4,6-trienoate



NaOMe (21 mL, 10.3 mmol, 0.5 M solution in MeOH) was added dropwise to a solution of methyl (2*E*,4*E*,6*E*)-7-(4,4,6-trimethyl-1,3,2-dioxaborinane-2-yl)hepta-2,4,6-trienoate (2.28 g, 8.6 mmol) in THF (32 mL) cooled to -78 °C under argon in the absence of light. The mixture was stirred at this temperature for 40 minutes and iodine monochloride (8.8 mL, 8.77 mmol, 1.0 M solution in DCM) was added dropwise. The mixture was stirred at -78 °C for 2 hours, then allowed to warm to room temperature whilst stirring. The mixture was diluted with Et_2O (258 mL) and washed with $Na_2S_2O_3$ (2 x 103 mL), water (103 mL) and brine (103 mL). The organic extracts were dried over $MgSO_4$, filtered and evaporated to yield 4.9 g of a red solid: ^{1}H NMR (400 MHz, $CDCl_3$) δ 3.74 (3H, s), 5.98 (1H, d, *J* 15.3 Hz), 6.24-6.47 (2H, m), 6.61-6.71 (1H, m), 7.08-7.18 (1H, m), 7.20-7.31 (1H, m). The compound was taken on to the next stage without any further purification or characterisation.



Methyl (2E,4E,6E,8E)-9-(4,4,6-trimethyl-1,3,2-dioxaborinan-2-yl)nona-2,4,6,8-tetraenoate



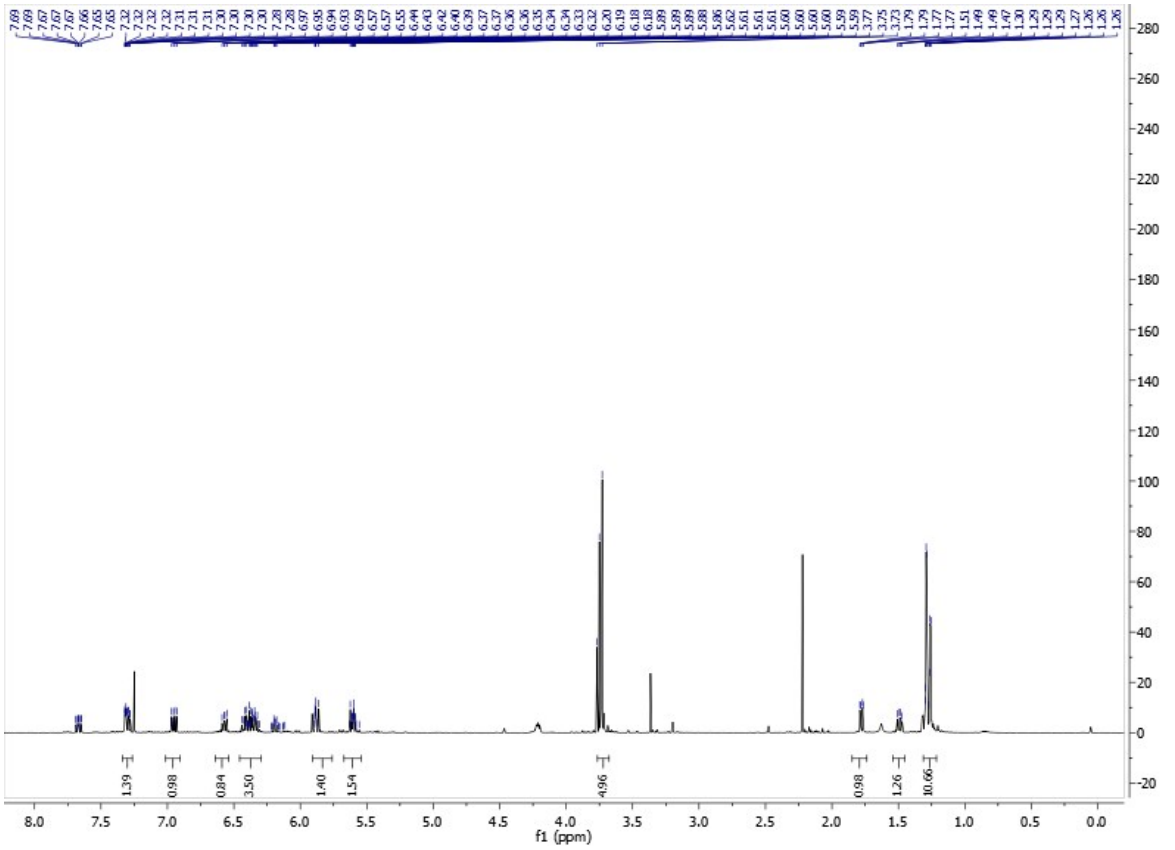
Method 1: To a dry flask was added $Pd(OAc)_2$ (82 mg, 0.357 mmol), P(*o*-tol)$_3$ (0.214 g, 0.714 mmol) and AgOAc (1.29 g, 7.71 mmol), followed by a solution of *methyl* (2*E*, 4*E*, 6*E*)-7-iodohepta-2,4,6-trienoate (1.70 g, 6.44 mmol) in dry, degassed MeCN (43 mL), which had been previously degassed by a bubbling argon needle. 4,4,6-trimethyl-2-vinyl-1,3,2-dioxaborinane (1.5 mL, 8.57 mmol) was then added and the reaction mixture was then heated to 50 °C with vigorous stirring for 21 hours. The mixture was allowed to cool, then diluted with Et_2O (150 mL) and passed through a short Celite/silica plug. The organic extracts were washed with H_2O (150 mL) and brine (150 mL), dried over $MgSO_4$, filtered and evaporated to yield 2.2 g of crude product as a brown oil. After drying the crude product on the high vacuum line for 2

days, 1.2 g of an orange solid was obtained. The compound was taken on to the next stage without any further purification or characterisation.

Method 2: To a dry flask was added $Pd(OAc)_2$ (98 mg, 0.44 mmol), P(*o*-tol)$_3$ (0.265 g, 0.875 mmol) and AgOAc (1.57 g, 9.42 mmol), followed by a solution of methyl (2*E*, 4*E*, 6*E*)-7-iodohepta-2,4,6-trienoate (2.28 g, 8.60 mmol) in dry, degassed MeCN (52 mL). 4,4,6-Trimethyl-2-vinyl-1,3,2-dioxaborinane (1.4 mL, 8.60 mmol) was then added and the reaction mixture was then heated to 30 °C with vigorous stirring for 15.5 hours. The mixture was allowed to cool, then diluted with Et_2O (131 mL) and passed through a short Celite/silica plug. The solvent was evaporated to yield 3.95 g of a crude viscous orange oil containing desired product (1.90 g, 76%). After drying 1.2 g of an orange solid was obtained. The compound was taken on to the next stage without any further purification or characterisation.

Method 3: To a dry flask was added $Pd(OAc)_2$ (0.134 g, 0.440 mmol), P(*o*-tol)$_3$ (0.364 g, 1.20 mmol) and AgOAc (2.16 g, 13.0 mmol), followed by a solution of methyl (2*E*, 4*E*, 6*E*)-7-iodohepta-2,4,6-trienoate (3.14 g, 11.9 mmol) in dry, degassed MeCN (72 mL). 4,4,6-Trimethyl-2-vinyl-1,3,2-dioxaborinane (2.0 mL, 11.9 mmol) was then added and the reaction mixture was then heated to 30 °C with vigorous stirring for 18 hours. The mixture was allowed to cool, then diluted with Et_2O (180 mL) and passed through a short Celite/silica plug to yield 4.5 g of a crude viscous orange oil. The crude product was purified by silica gel chromatography, eluent 5% EtOAc in petroleum ether, to give desired product as a bright yellow solid (0.9 g, 23% from iodoacrylate).

^{1}H NMR (700 MHz, $CDCl_3$) δ 1.23-1.33 (9H, m,), 1.45-1.56 (1H, m), 1.79 (1H, dd, *J* 13.9, 2.9 Hz), 3.74 (3H, s), 4.22 (1H, ddt, *J* 11.5, 6.1, 2.9 Hz), 5.59-5.65 (1H, m), 5.84-5.93 (1H, m), 6.27-6.46 (3H, m), 6.54-6.63 (1H, m), 6.91-7.00 (1H, m), 7.28-7.37 (1H, m); ^{11}B NMR (128 MHz, $CDCl_3$) δ 26; ^{13}C NMR (176 MHz, $CDCl_3$) δ 23.1, 28.1, 31.2, 45.9, 51.5, 64.8, 70.9, 110.1, 120.6, 130.9, 133.6, 138.9, 140.6, 144.4, 145.8, 167.4; LCMS (ASAP) [M+H]=291.2; HRMS (ASAP) calculated [$C_{16}H_{24}{}^{10}BO_4$] 290.1804, found 290.1777. The compound was taken on to the next stage without any further purification or characterisation.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10.66
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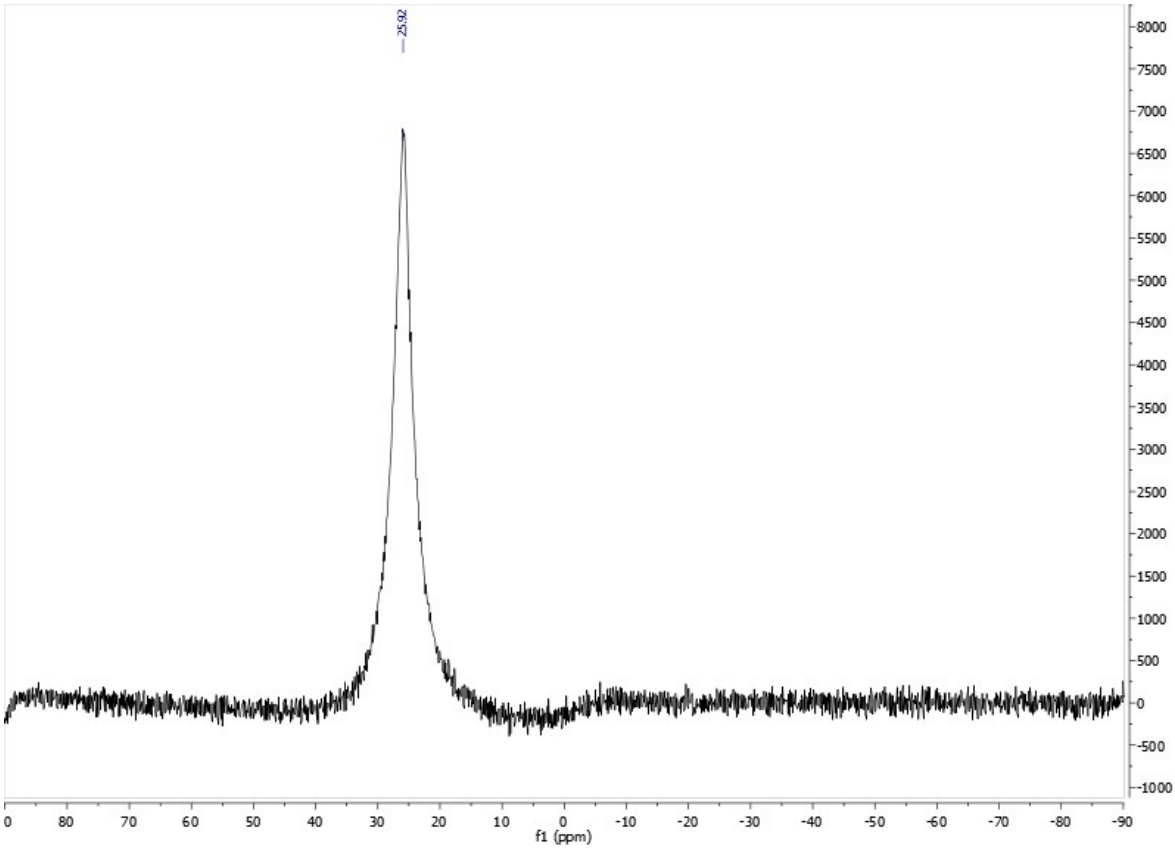
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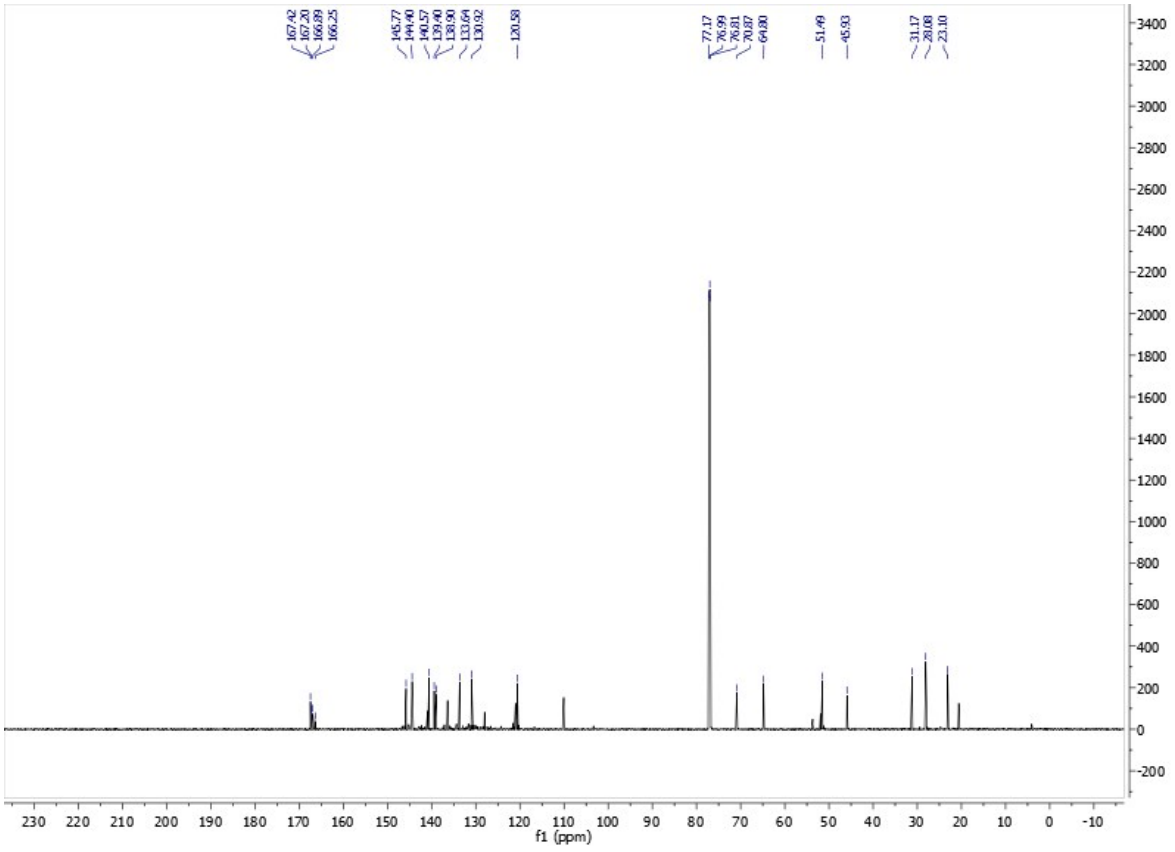
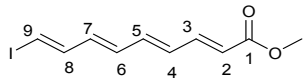
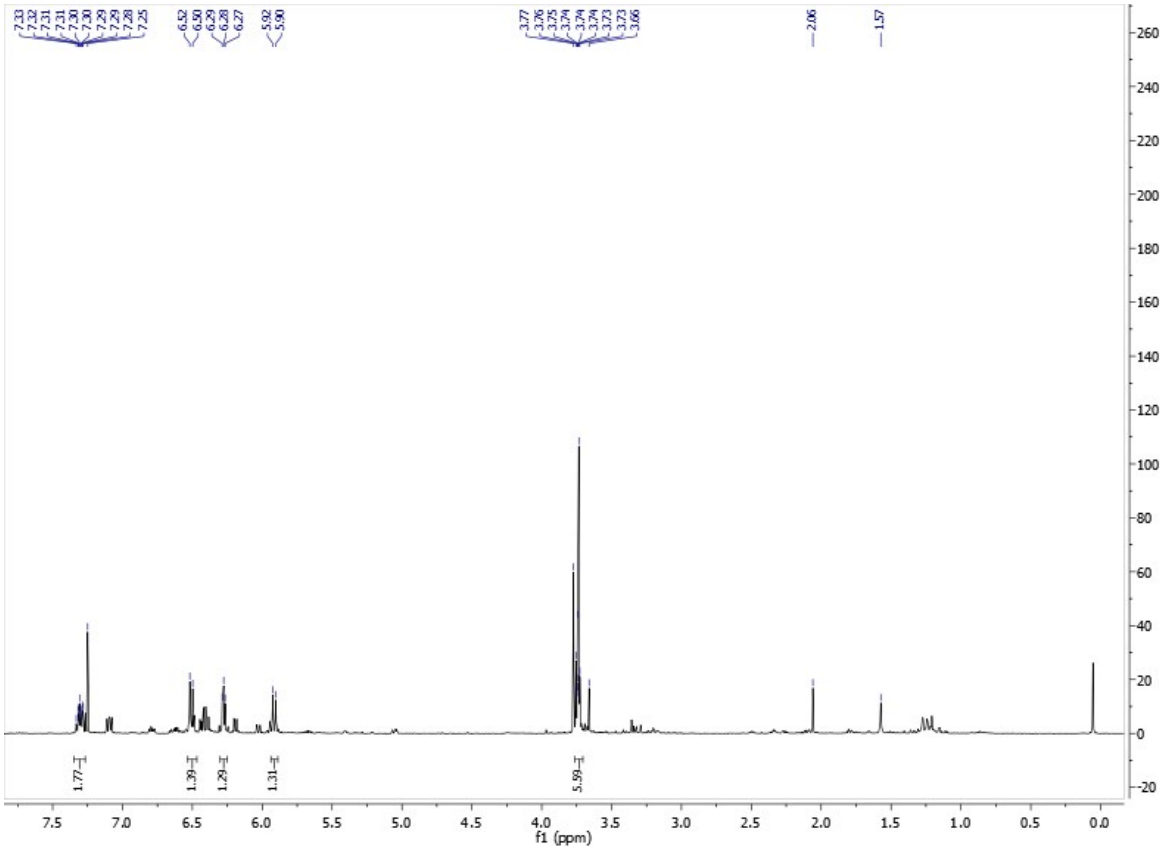


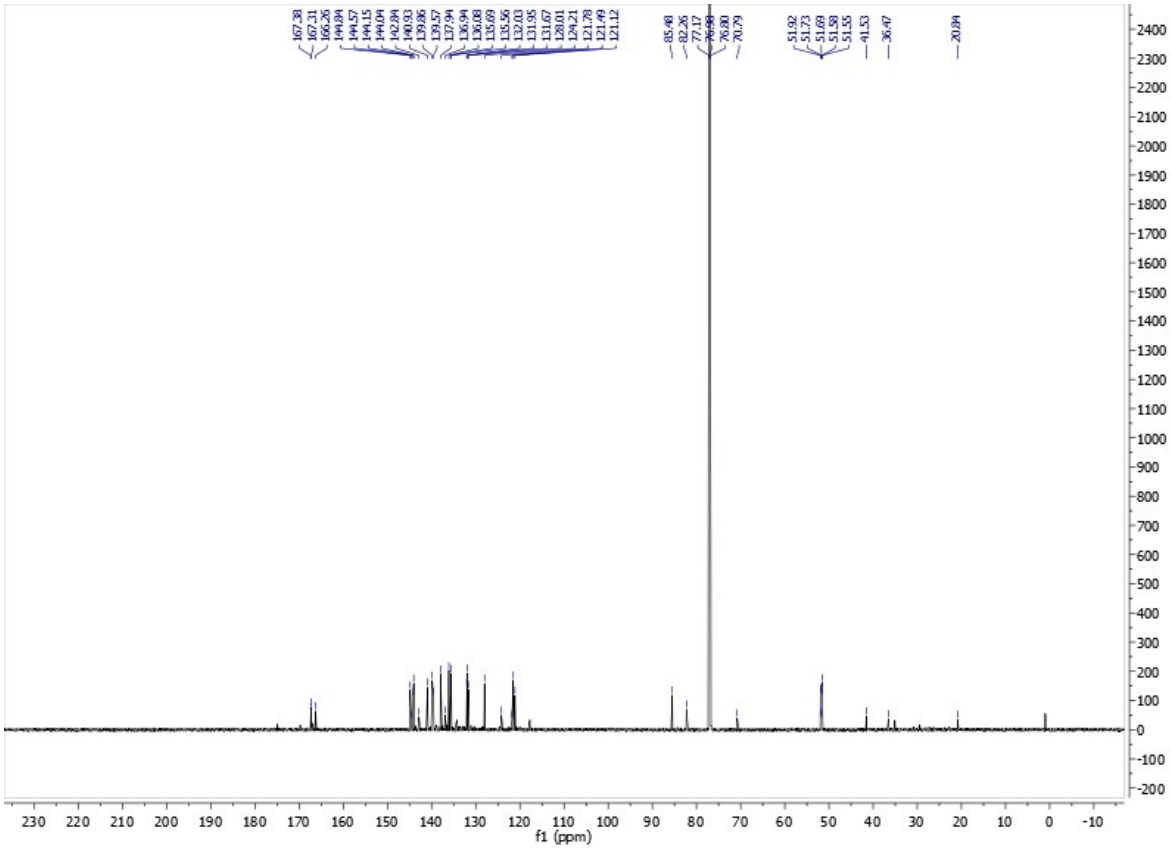
Methyl (2E,4E,6E,8E)-9-iodonona-2,4,6,8-tetraenoate **11**



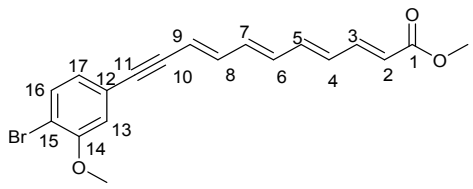
NaOMe (26 mL, 13.1 mmol, 0.5 M solution in MeOH) was added dropwise over 5 minutes to a solution of a crude mixture containing methyl (2*E*,4*E*,6*E*,8*E*)-9-(4,4,6-trimethyl-1,3,2-dioxaborinan-2-yl)nona-2,4,6,8-tetraenoate (3.20 g, 10.9 mmol) in dry THF (41 mL) cooled to -78 °C under argon in the absence of light. The mixture was stirred at this temperature for 50 minutes and iodine monochloride (2.10 g, 11.3 mmol) in dry DCM (11 mL) was added dropwise over 5 minutes. The mixture was stirred at -78 °C for 3 hours 40 minutes, then allowed to warm to room temperature. The mixture was diluted with Et_2O (329 mL) and washed with $Na_2S_2O_3$ (2 x 132 mL), H_2O (132 mL) and brine (132 mL). The organic extracts were dried over $MgSO_4$, filtered and evaporated to yield 3.3 g of a dark brown solid. The crude product was purified by silica gel chromatography at 0 °C, eluent 0-5 % EtOAc in petroleum ether to yield desired product as an unstable yellow solid (0.754 g, 20% from starting

iodoacrylate): ^{1}H NMR (700 MHz, $CDCl_3$) δ 3.75 (3H,s), 5.92 (1H, d, *J* 15.0 Hz), 6.29 (1H, dd, J 8.7, 6.1 Hz), 6.38-6.45 (1H, m), 6.47-6.55 (2H, m), 7.07-7.14 (1H, m), 7.27-7.34 (2H, m); ^{13}C NMR (176 MHz, $CDCl_3$) δ 51.7, 121.3, 131.9, 132.1, 135.7, 139.8, 141.1, 144.2, 145.0, 167.5; LRMS (ASAP) [M+H] = 291.0. HRMS (ASAP) [$C_{10}H_{12}O_2{}^{127}I$], calculated 290.9882, found 290.9896.



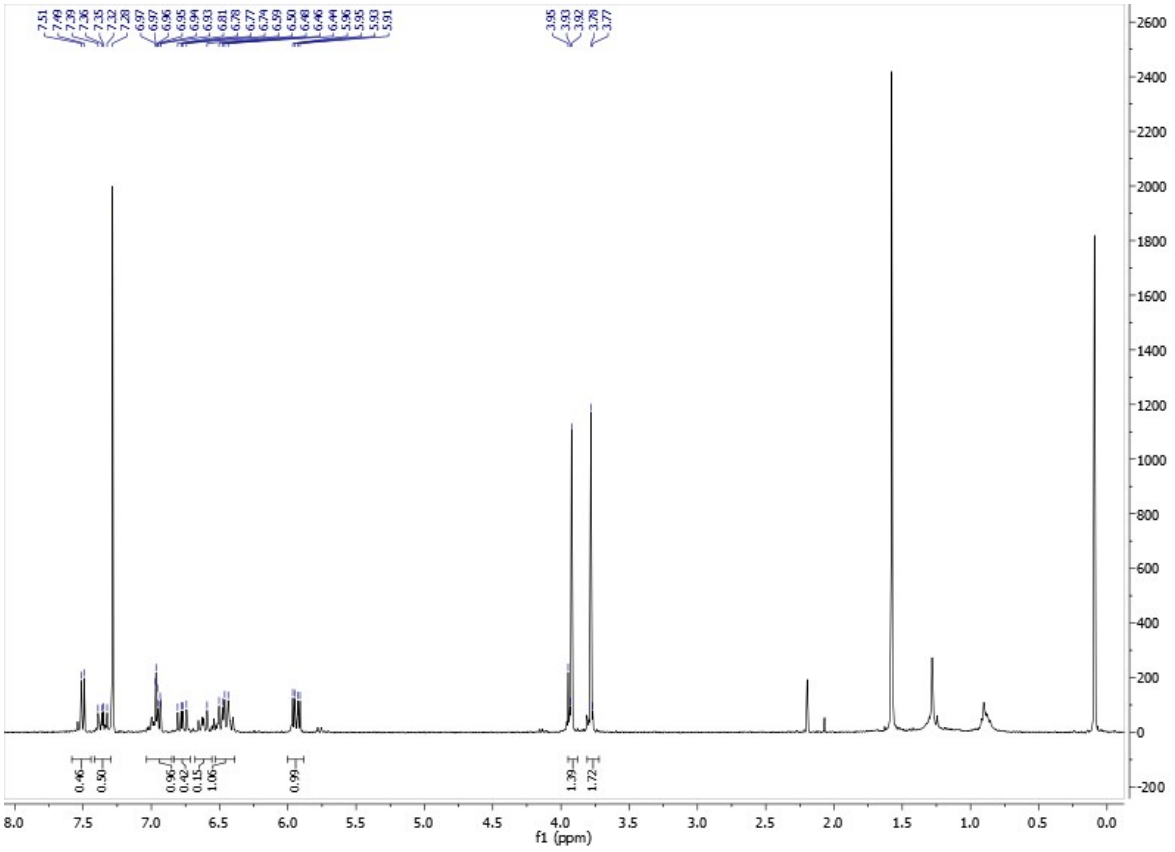


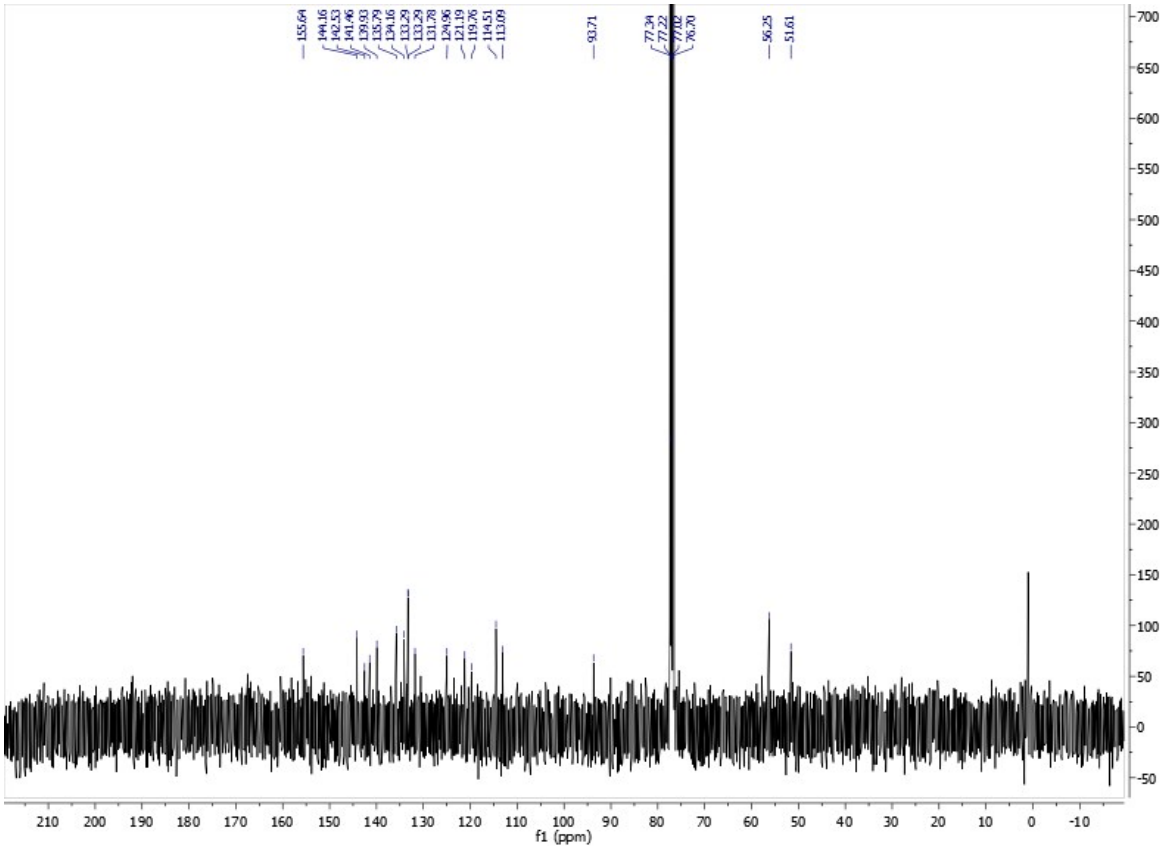
Methyl (2E,4E,6E,8E)-11-(4-bromo-3-methoxyphenyl)undeca-2,4,6,8-tetraen-10-ynoate **12**



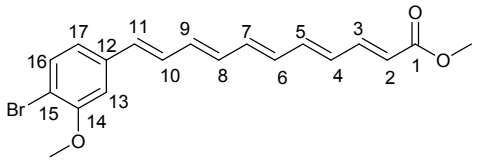
$Pd(PPh_3)_2Cl_2$ (1 mg, 0.002 mmol), copper (I) iodide (0.3 mg, 0.002 mmol), 1-bromo-4-ethynyl-2-methoxybenzene **11** (43 mg, 0.20 mmol) and methyl (*2E*,*4E*,*6E*,*8E*)-9-iodonona-2,4,6,8-tetraenoate (50 mg, 0.170 mmol) were added to a dry flask and the vessel purged with argon. Dry, degassed Et_3N (0.90 mL) was then added and the mixture was briefly degassed. The reaction mixture was then stirred at room temperature overnight. The solvent was removed to give a dark red residue, which was purified by silica gel chromatography at 0 ˚C, elution gradient 0-10% EtOAc in hexane to give desired product as a bright yellow amorphous solid (36 mg, 56%): ^1H

NMR (400 MHz, $CDCl_3$) δ 3.76 (s, 3H), 3.90 (s, 3H), 5.91 (dd, *J* 15.3, 6.1 Hz, 2H), 6.29-6.54 (m, 3H), 6.60 (dd, *J* 14.8, 10.5 Hz, 1H), 6.75 (dd, *J* 15.4, 10.4 Hz, 1H), 6.89-6.99 (m, 2H), 7.28-7.39 (m, 1H), 7.49 (dd, *J* 11.4, 8.3 Hz, 1H); ^{13}C NMR (101 MHz, $CDCl_3$) δ 51.6, 56.2, 90.0, 93.7, 112.4, 113.1, 114.5, 121.2, 123.4, 124.9, 131.7, 133.3, 134.1, 135.7, 139.9, 141.4, 144.1, 155.6, 167.4; IR (ν_{max}, cm^{-1}) *inter alia* 1706, 2185, 2956; LRMS (ASAP) [M+H] 373.0; HRMS (ASAP) [$C_{19}H_{17}O_3Br$] calculated 372.0361, found 372.0357; UV (Et_2O 5μM, nm) 370 (ε 148 400), 391 (ε 117 200); Fluorescence (Et_2O 100 nM, nm) 439.5, 467.5, 493.5.



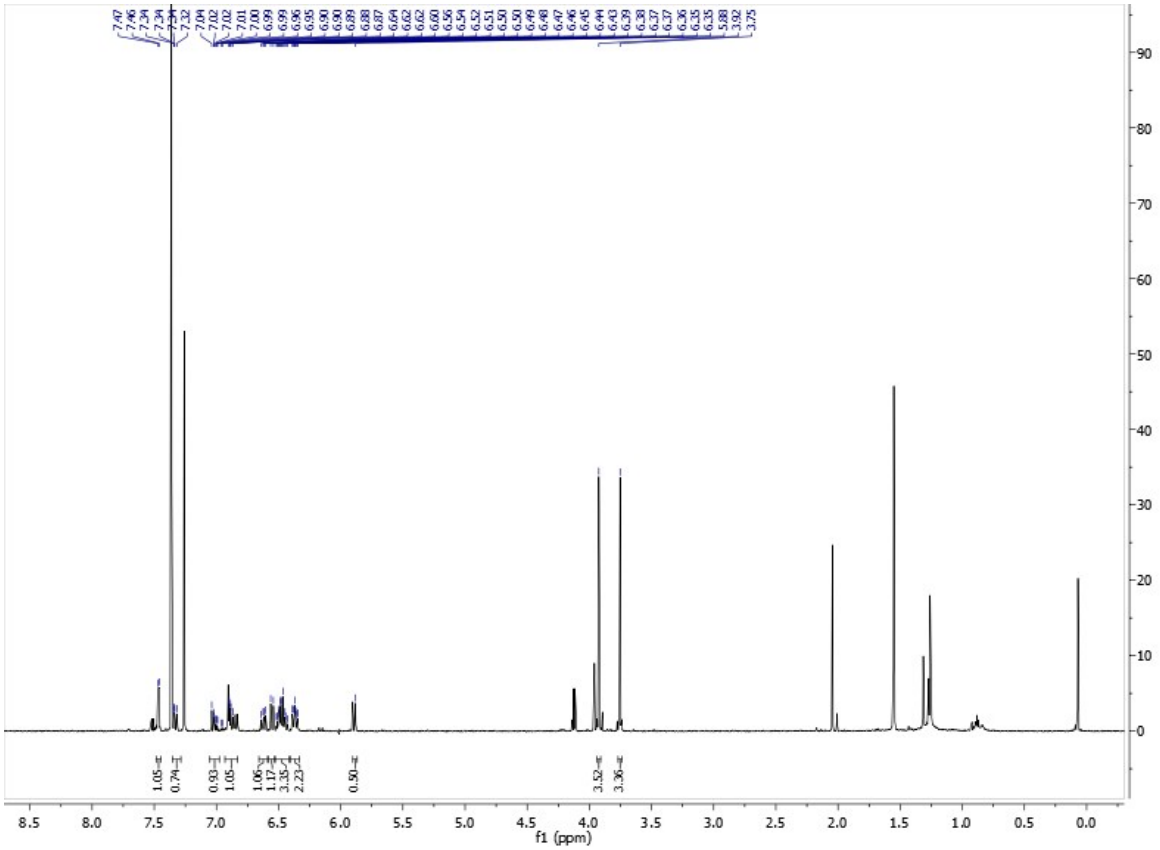


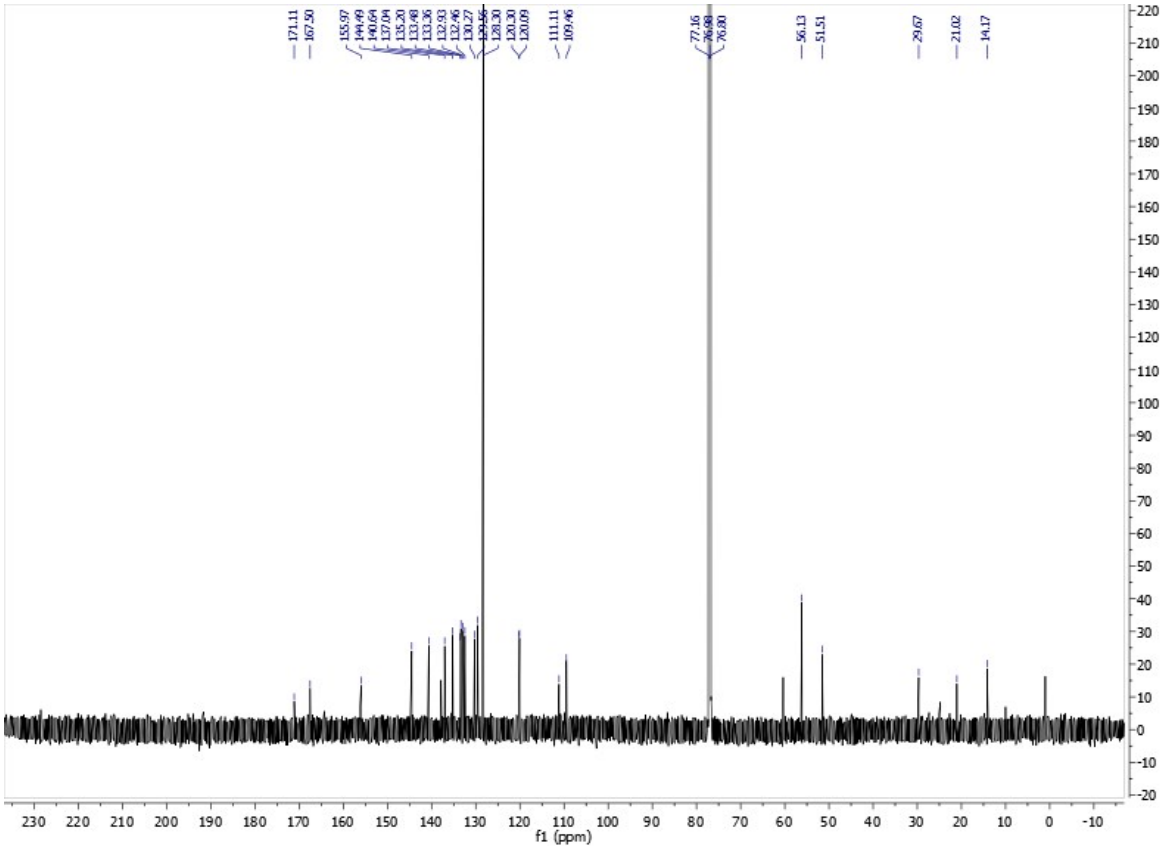
Methyl (2E,4E,6E,8E,10E)-11-(4-bromo-3-methoxyphenyl)undeca-2,4,6,8,10-pentaenoate **13**



2-[(*E*)-2-(4-Bromo-3-methoxyphenyl)ethenyl]-4,4,5,5-tetramethyl-1,3,2-dioxaborolane (26 mg, 0.0760 mmol), methyl (*2E*,*4E*,*6E*,*8E*)-9-iodonona-2,4,6,8-tetraenoate **11** (18 mg, 0.0610 mmol), $Pd(PPh_3)_4$ (7 mg, 0.0061 mmol) and Ag_2O (17 mg, 0.0760 mmol) were added to a dry flask and the flask purged with argon. Dry, degassed DME (0.46 mL) was then added and the reaction stirred at 40 °C for 17 hours. The reaction mixture was then diluted with EtOAc containing ~ 3ppm BHT (6.0 mL) and passed through a short plug of Celite/silica to give 55 mg of a green residue. The crude product was purified by silica gel chromatography at 0 ˚C, eluent benzene, to give desired product as a bright yellow solid (10 mg, 34%): M.p. 207.3-208.9 °C; ^{1}H NMR (700 MHz, $CDCl_3$) δ 3.75 (3H, s), 3.92 (3H, s), 5.89 (1H, d, *J* 15.2 Hz), 6.37 (2H, ddd,

J 15.1, 11.2, 4.3 Hz), 6.41-6.52 (3H, m), 6.55 (1H, d, *J* 15.5 Hz), 6.62 (1H, dd, *J* 14.7, 11.2 Hz), 6.80-6.85 (1H, m), 6.87-6.93 (2H, m), 7.29-7.35 (1H, m), 7.47 (1H, d, *J* 8.1 Hz); ^{13}C NMR (176 MHz, $CDCl_3$) δ 51.7, 56.3, 109.6, 111.3, 120.3, 120.5, 129.7, 130.5, 132.6, 133.1, 133.5, 133.7, 135.4, 137.2, 138.0, 140.8, 144.7, 156.2, 167.7; IR (ν_{max}, cm^{-1}) *inter alia* 1704 (m), 2913 (w), 2924 (w), 2938 (w), 2952 (w); LRMS (ASAP) M = 374.1; HRMS (ASAP) [$C_{19}H_{19}O_3Br$] calculated 374.0499, found 374.050; UV ($CHCl_3$ 5μM, nm) 397 (ε 67 000), 417 (ε 56 000); Fluorescence ($CHCl_3$ 100 nM, nm) 498, 527, 566, 594.





Biology methods and materials

Bacterial strains used

"Wild-type" *Escherichia coli* K-12 strain MG1655 (FR Blattner et al (1997) Science 277, 1453-62.

Xanthomonas species used were gifts from Dr Bart Cottyn (ILVO, Merelbeke, Belgium):

Xanthomonas arboricola GBBC 2191

Xanthomonas sp. Slc7 GBBC 2056

Xanthomonas maliensis GBBC 2113

Xanthomonas sp. Slc1 GBBC 2252

Growth of bacteria

Xanthomonas species were grown at 28 °C on Tryptone Soya Agar (TSA) plates (Oxoid Microbiology Products, Thermo Scientific).

E. coli was grown at 30-37 °C in Lysogeny Broth (LB) medium (J Sambrook et al (1989) Molecular cloning: a laboratory manual. Cold Spring Harbor Laboratory, Cold Spring Harbor, New York).

Xanthomonadin labelling of *E. coli*

An overnight *E. coli* culture was diluted x10 in fresh LB medium and grown for 1 hour with shaking at 35 °C. 2 ml of cell suspension was mixed with 100 µl xanthomonadin solution (100 mM in dimethylsulfoxide) and incubated for a further 2 hours with shaking at 35 °C. The cells were harvested by centrifugation and washed 3-4 times with fresh LB medium.

Confocal microscopy

Xanthomonas or *E. coli* cells were prepared for microscopy by spotting small drops of cell suspension onto a BG11-agar plate. BG11 (a minimal mineral medium)[2] was used to minimise background fluorescence. When the droplets of cell suspension had dried onto the agar surface, small blocks of agar were cut out and mounted beneath a glass cover slip in a custom-built microscope sample holder. Fluorescence micrographs were recorded with a Leica TCS SP5 laser scanning confocal microscope, using a 63X oil immersion objective lens (NA 1.4) and the pinhole set to give an optical section in the *z*-direction of about 0.8 µm. Excitation was with the 458 nm line of an Argon laser and the emission range was set to 465-505 nm. Simultaneous bright-field images were recorded to show the location of the cells.

ROS survival assay

E. coli cell suspensions with or without loading with xanthomonadin as described above were mixed with Rose Bengal solution to a final concentration of 30 µM. Cell aliquots were then exposed to high light (white light at approximately 600 µE m^{-2} s^{-1}) at room temperature for 20 min. 50 µl aliquots of cell suspension were then spread on LB agar plates and grown for 48 h in the dark at 24 °C before counting colonies. The dilutions used were chosen to give about 70-90 colonies per plate from untreated cell suspensions. Colonies were counted from 3 replicate plates.

References

1. Katrina S. Madden, K.S., Laroche, B., David, S., Batsanov, A. S., Thompson, D., Knowles, J. P., Whiting, A., Approaches to styrenyl suilding blocks for the synthesis of polyene xanthomonadin and its analogues. *Eur. J. Org. Chem.* (2018), DOI:10.1002/ejoc.201800540
2. Castenholz, R. W., *Methods in Enzymology, 167*, 68-93 (1988)