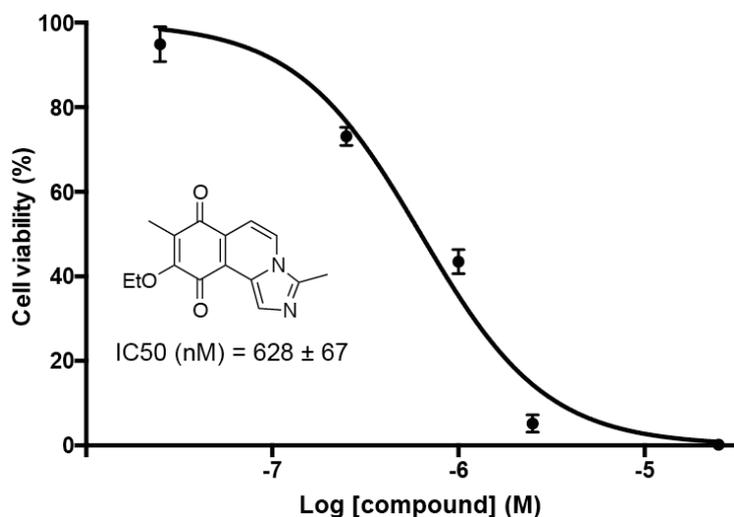


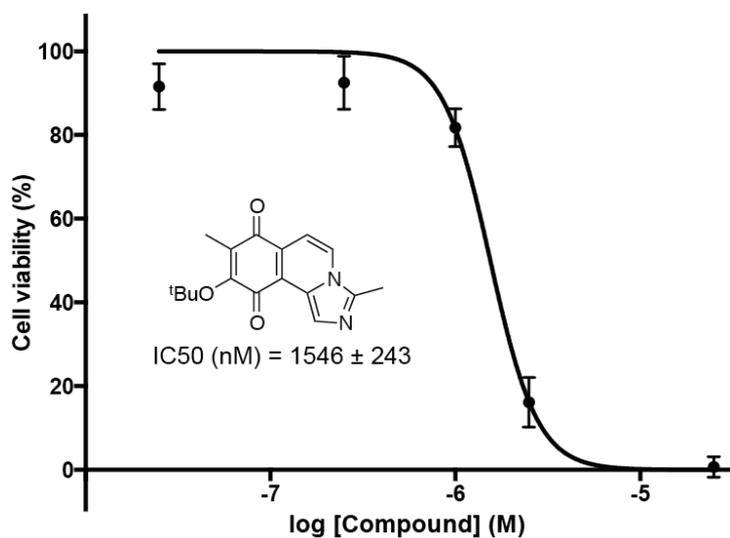
## Triggering apoptosis in cancer cells with an analogue of cribrastatin 6 that elevates intracellular ROS.

D. J. Asby, M. G. Radigois, D. C. Wilson, F. Cuda, C. L. L. Chai, A. Chen, A. S. Bienemann, M. E. Light, D. C. Harrowven, and A. Tavassoli.

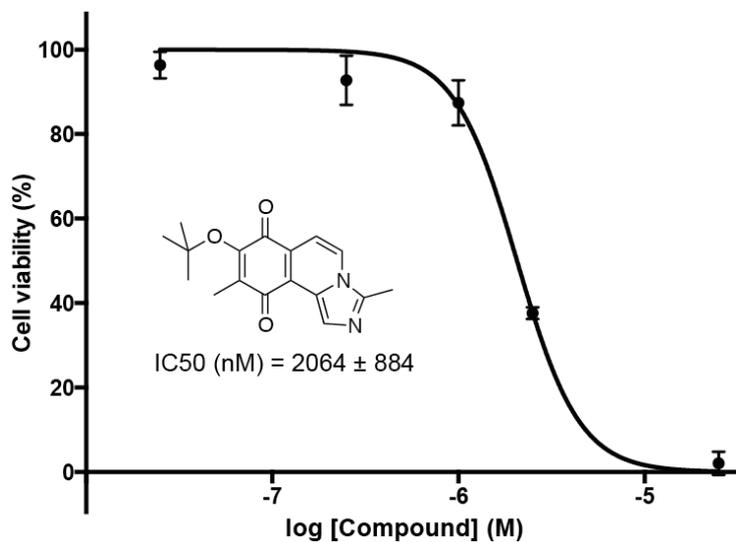
### Supplementary data



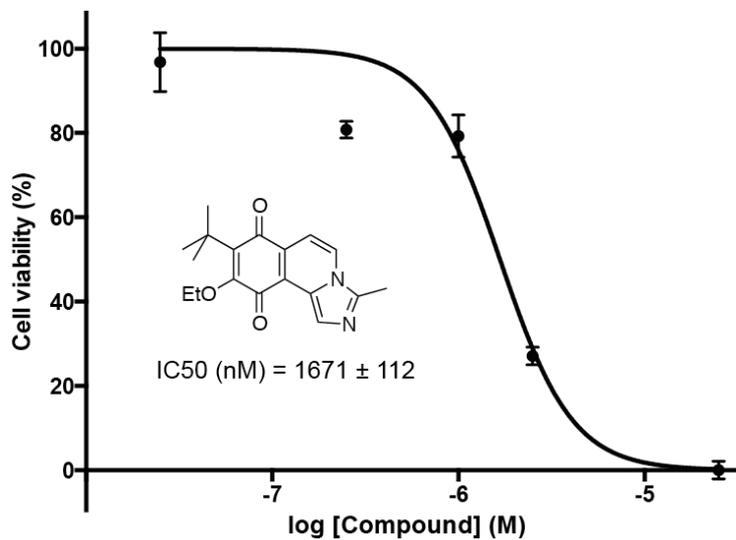
Supplementary Figure 1. The effect of cribrastatin 6 on the viability of MCF7 cells.



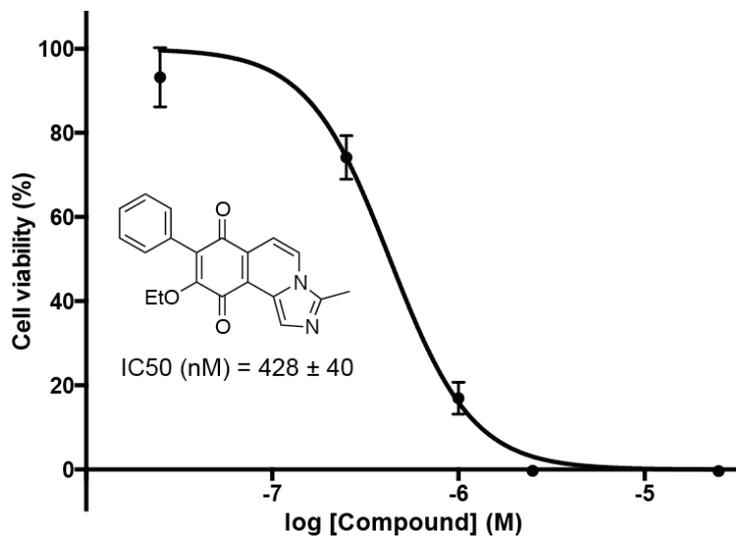
Supplementary Figure 2. The effect of analogue A on the viability of MCF7 cells.



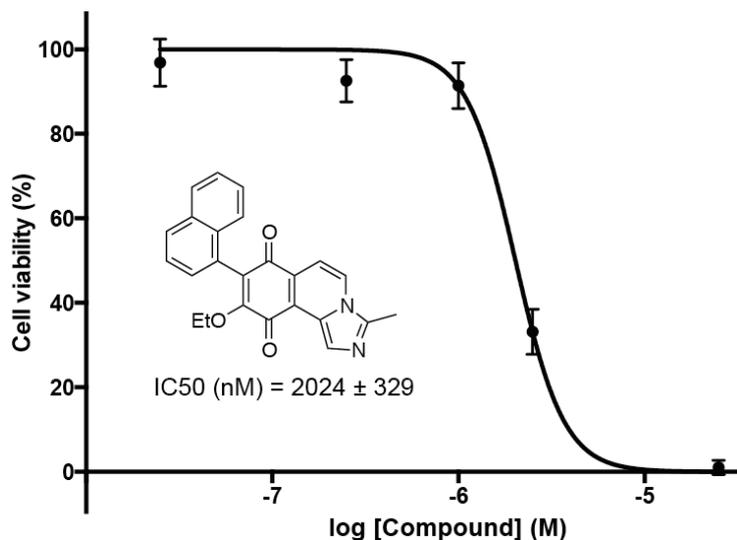
**Supplementary Figure 3.** The effect of analogue B on the viability of MCF7 cells.



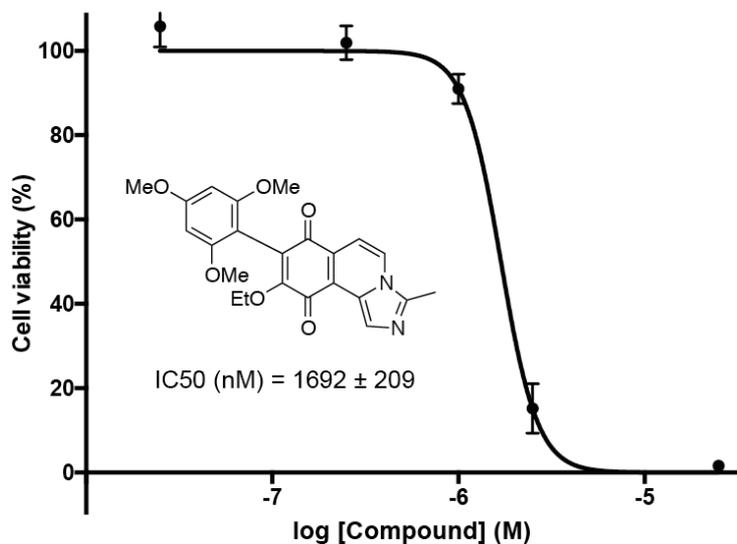
**Supplementary Figure 4.** The effect of analogue C on the viability of MCF7 cells.



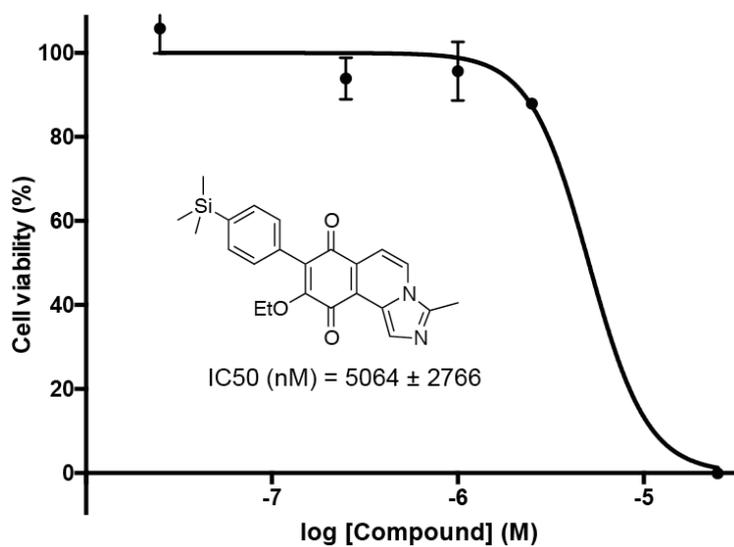
**Supplementary Figure 5.** The effect of analogue D (8PC6) on the viability of MCF7 cells.



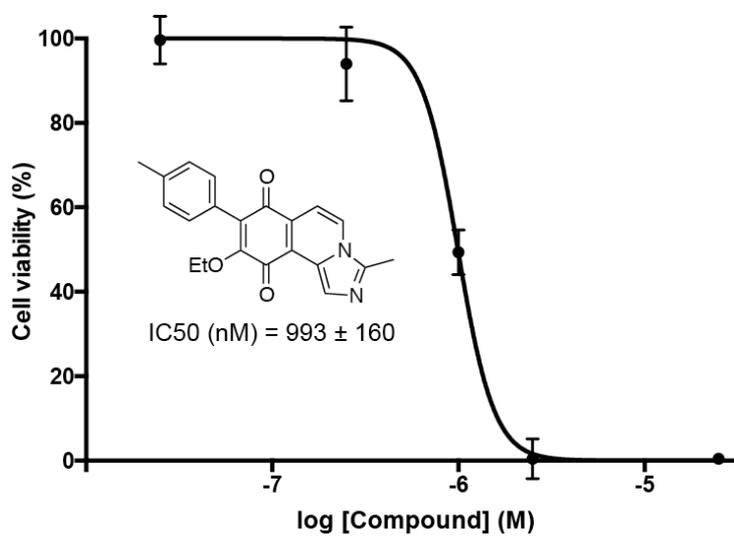
**Supplementary Figure 6.** The effect of analogue E on the viability of MCF7 cells.



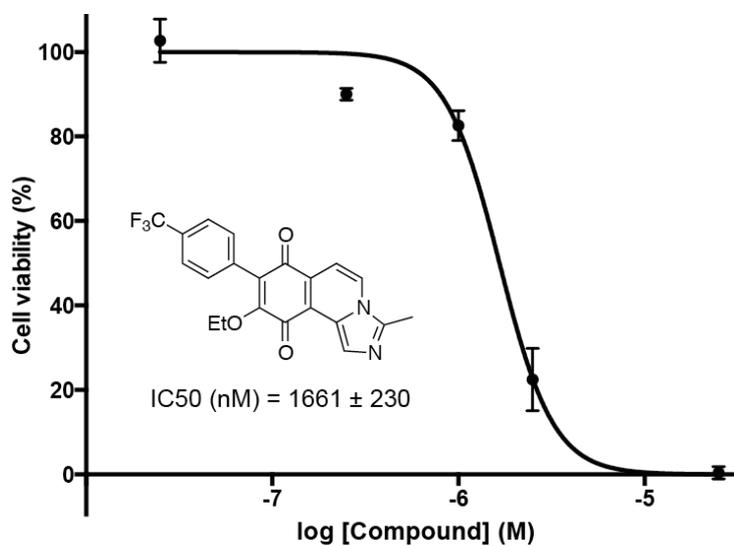
**Supplementary Figure 7.** The effect of analogue F on the viability of MCF7 cells.



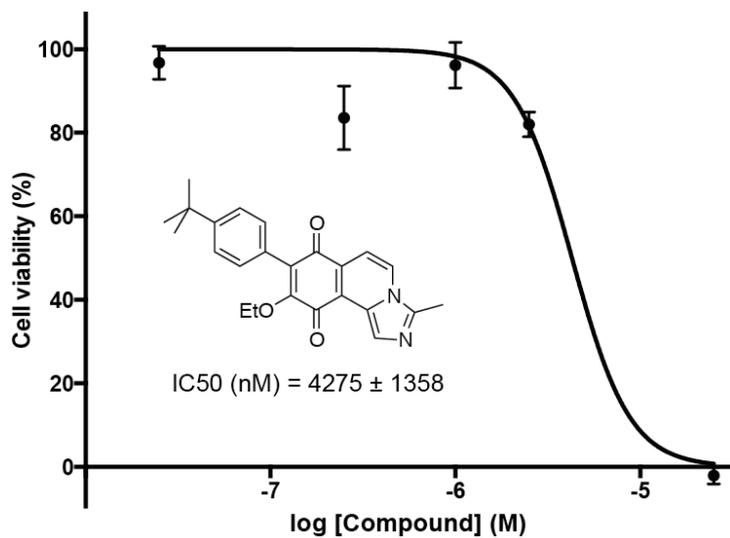
**Supplementary Figure 8.** The effect of analogue G on the viability of MCF7 cells.



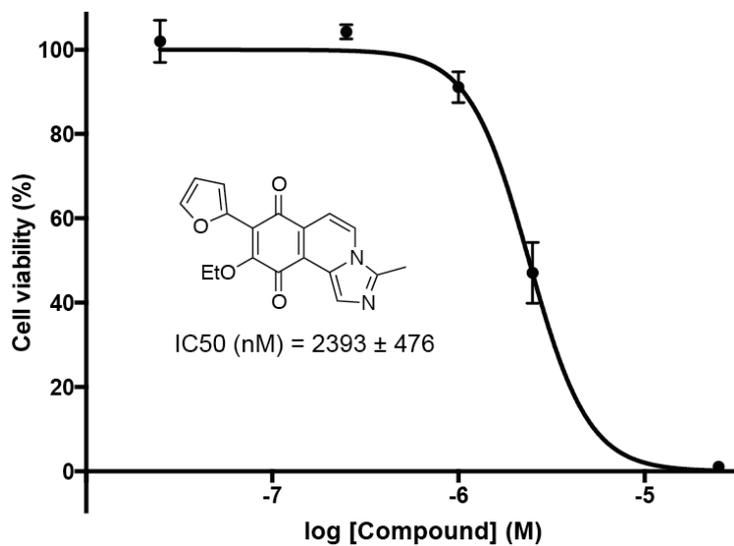
**Supplementary Figure 9.** The effect of analogue H on the viability of MCF7 cells.



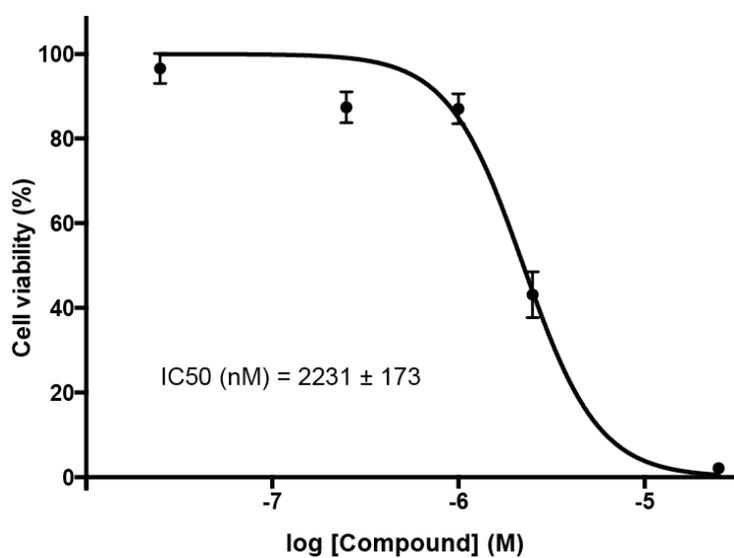
**Supplementary Figure 10.** The effect of analogue I on the viability of MCF7 cells.



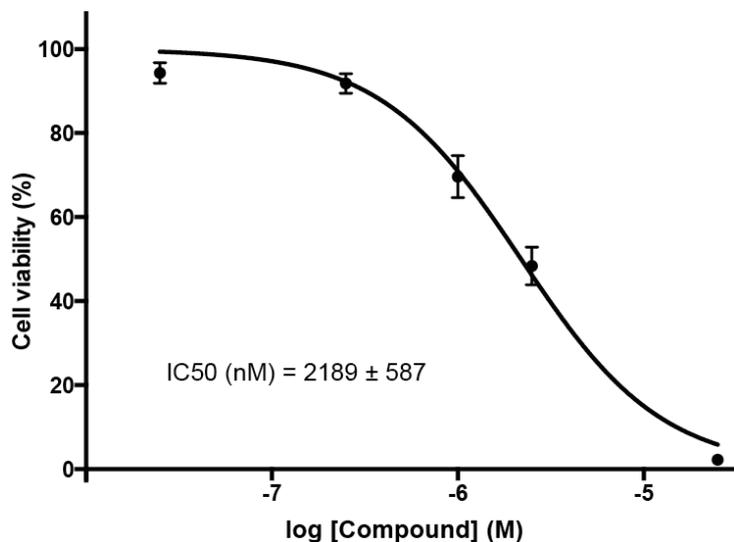
**Supplementary Figure 11.** The effect of analogue J on the viability of MCF7 cells.



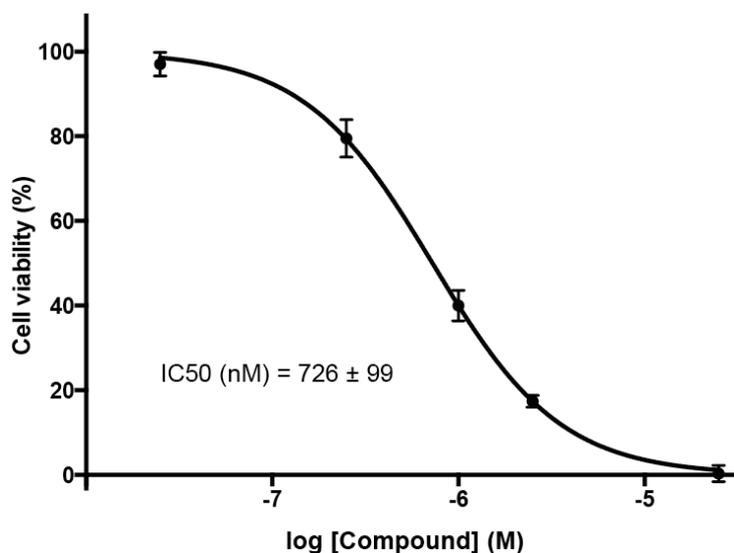
**Supplementary Figure 12.** The effect of analogue K on the viability of MCF7 cells.



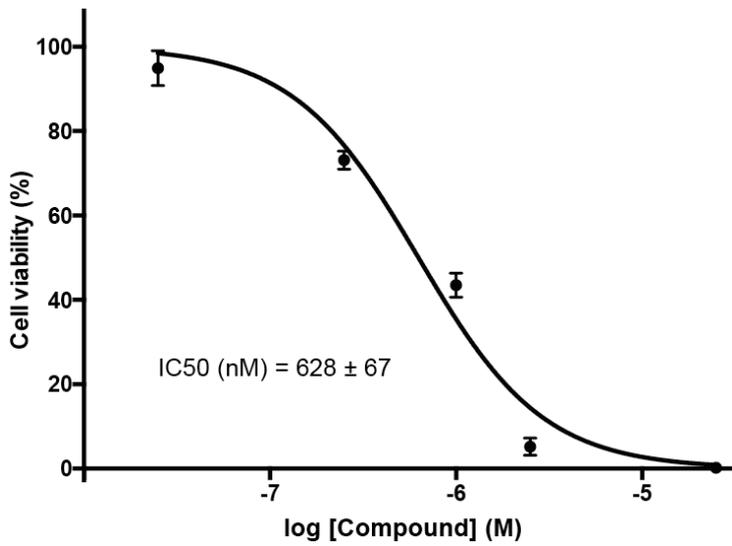
**Supplementary Figure 13.** The effect of cribrostatin 6 on the viability of MRC-5 cells.



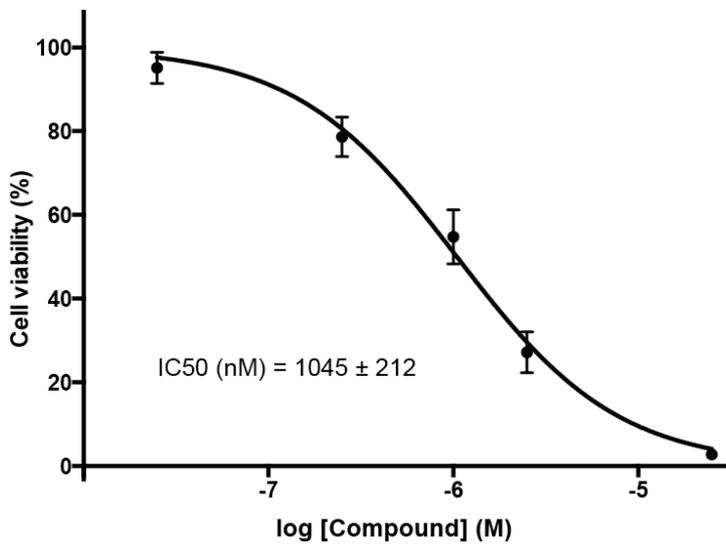
**Supplementary Figure 14.** The effect of cribrostatin 6 on the viability of CCD841 cells.



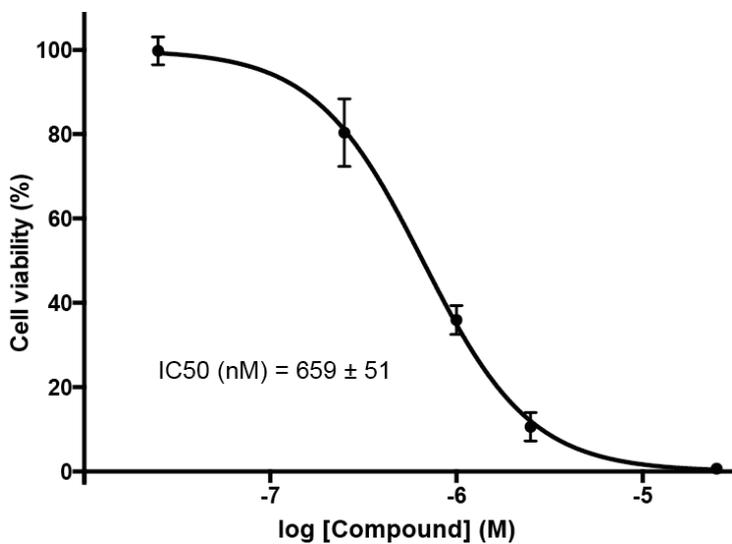
**Supplementary Figure 15.** The effect of cribrostatin 6 on the viability of MDA-MB-231 cells.



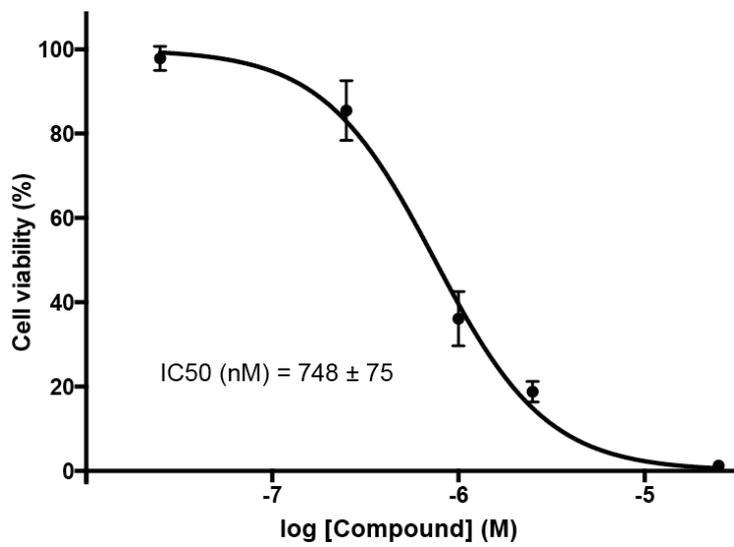
**Supplementary Figure 16.** The effect of cribrostatin 6 on the viability of MCF7 cells.



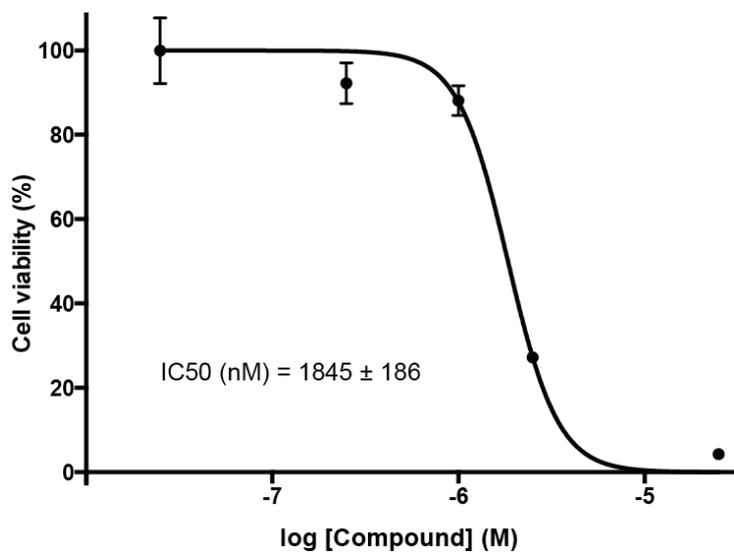
**Supplementary Figure 17.** The effect of cribrostatin 6 on the viability of PANC-1 cells.



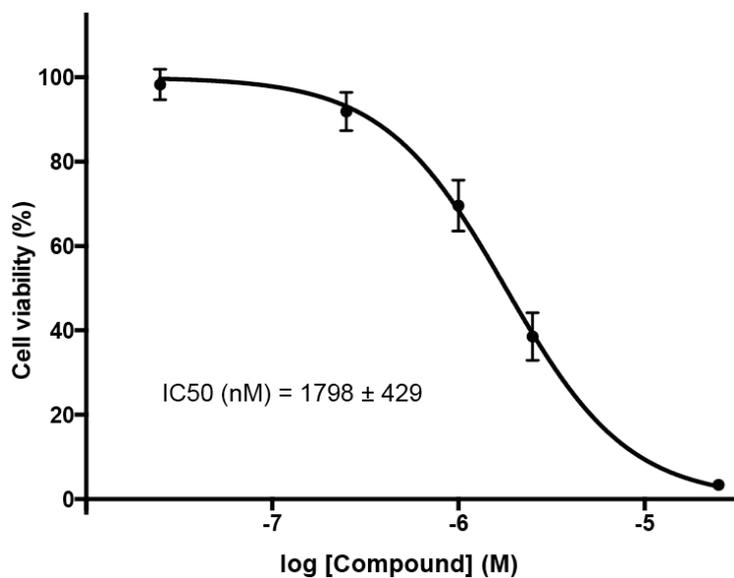
**Supplementary Figure 18.** The effect of cribrostatin 6 on the viability of HeLa cells.



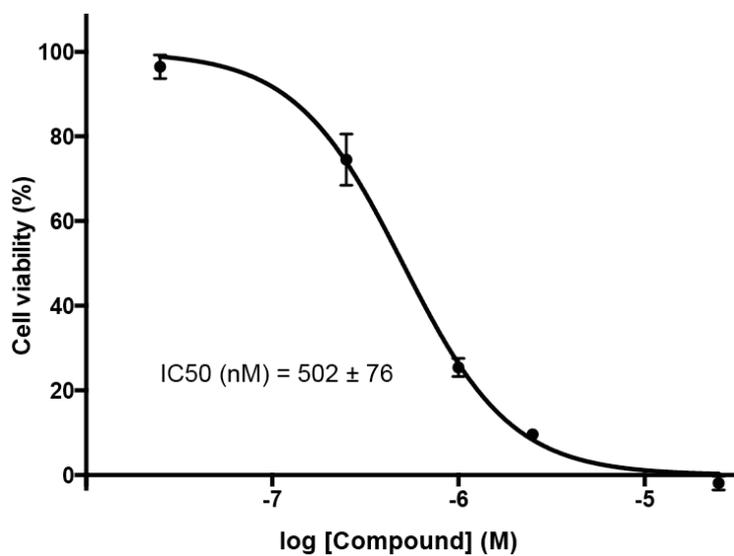
**Supplementary Figure 19.** The effect of cribrastatin 6 on the viability of HCT 116 cells.



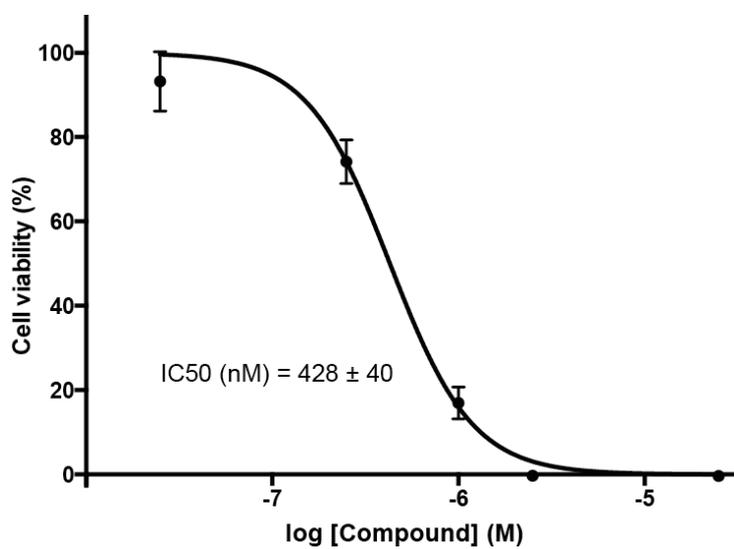
**Supplementary Figure 20.** The effect of 8PC6 on the viability of MRC-5 cells.



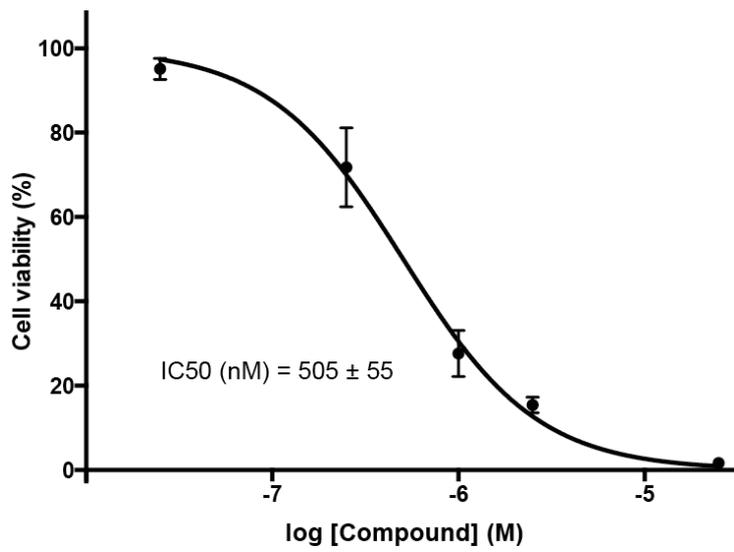
**Supplementary Figure 21.** The effect of 8PC6 on the viability of CCD841 cells.



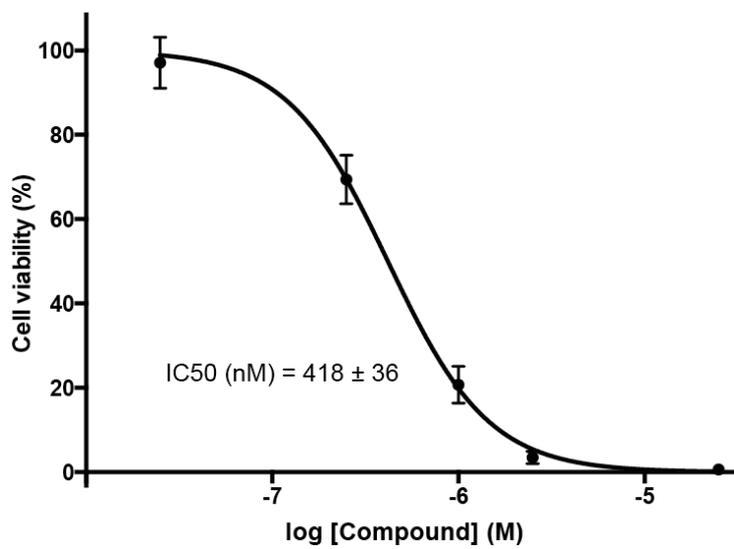
**Supplementary Figure 22.** The effect of 8PC6 on the viability of MDA-MB-231 cells.



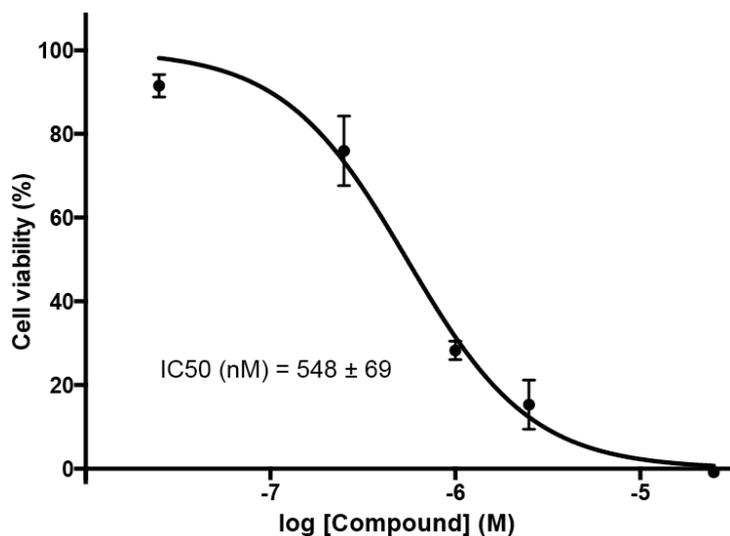
**Supplementary Figure 23.** The effect of 8PC6 on the viability of MCF7 cells.



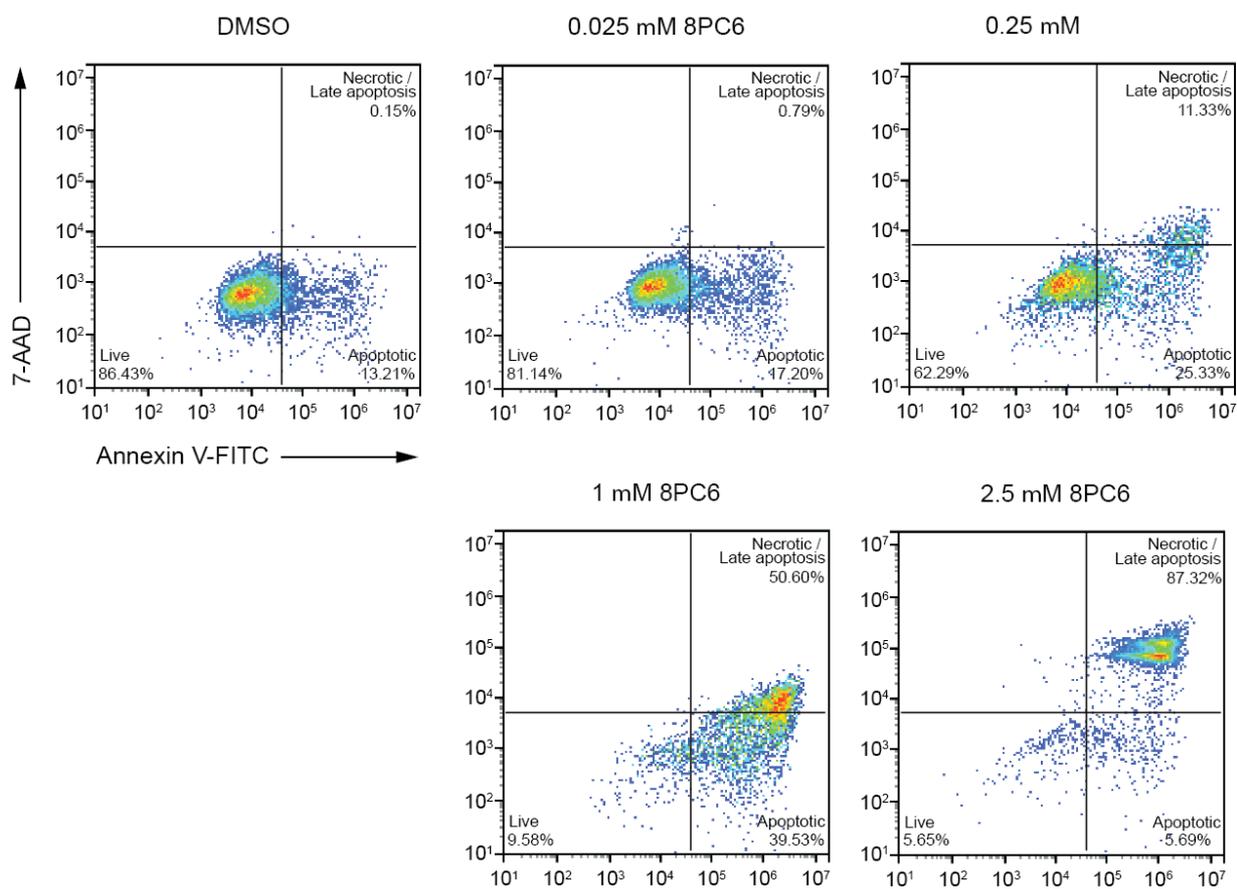
**Supplementary Figure 24.** The effect of 8PC6 on the viability of PANC-1 cells.



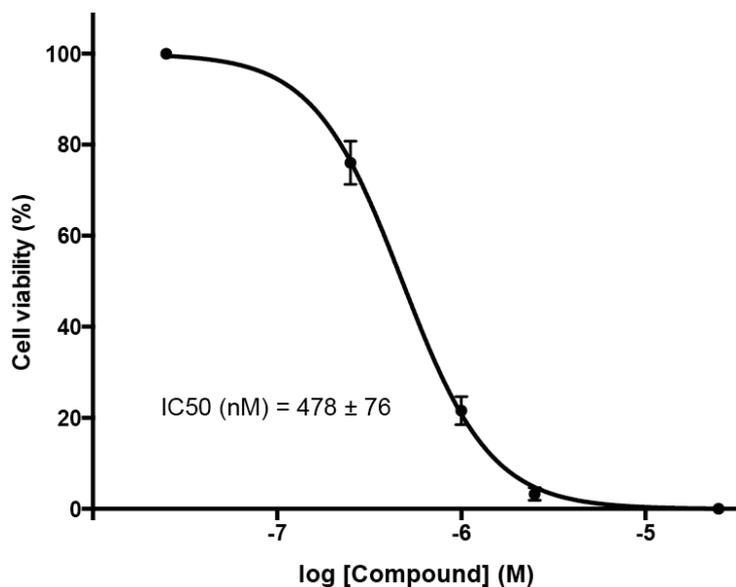
**Supplementary Figure 25.** The effect of 8PC6 on the viability of HeLa cells.



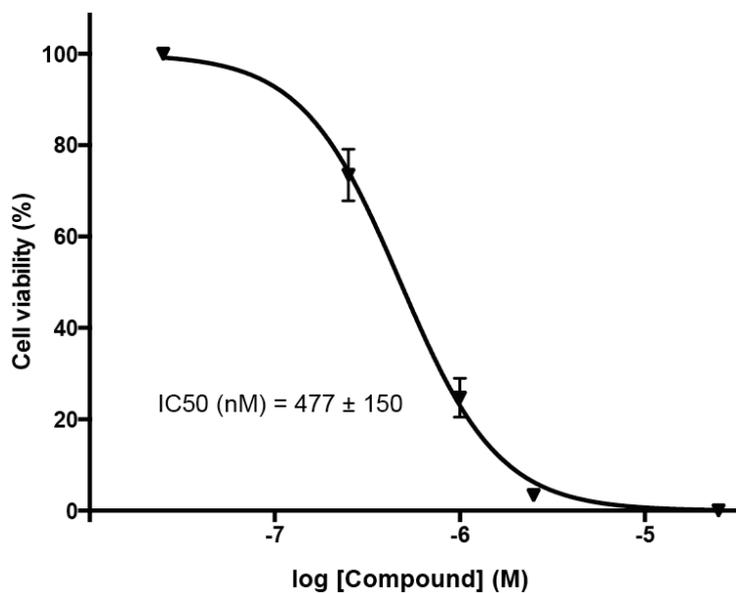
**Supplementary Figure 26.** The effect of 8PC6 on the viability of HCT 116 cells.



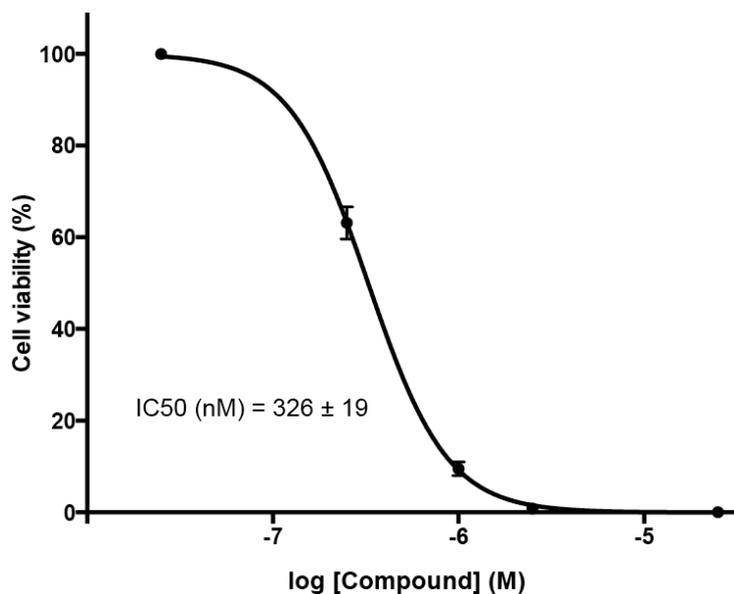
**Supplementary Figure 27.** Representative flow cytometry scatter plots.



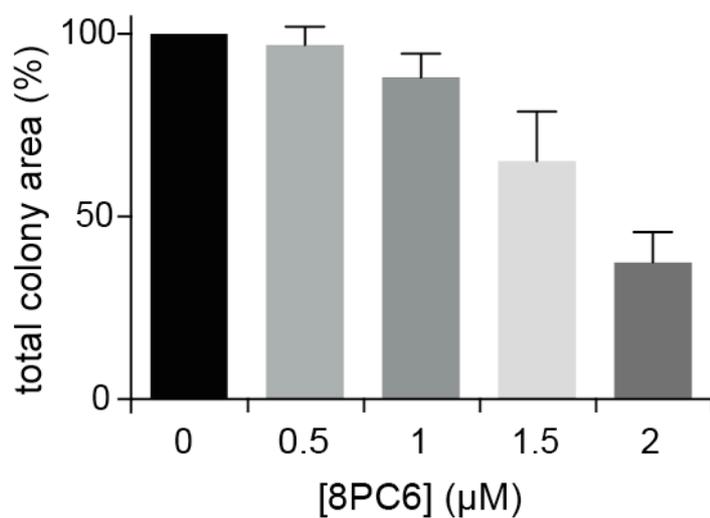
**Supplementary Figure 28.** The effect of 8PC6 on the viability of MCF7 cells not transfected with siRNA.



**Supplementary Figure 29.** The effect of 8PC6 on the viability of MCF7 cells transfected with scrambled siRNA.



**Supplementary Figure 30.** The effect of 8PC6 on the viability of MCF7 cells transfected with TIGAR siRNA.



**Supplementary Figure 31.** Quantification of the effect of 8PC6 in MCF7 clonogenic assays using the ColonyArea ImageJ plugin.

### **Supplementary Materials and methods**

Moisture sensitive reactions were carried out in oven-dried glassware cooled to RT under an inert atmosphere of argon. Thermolyses were carried out in stainless steel tubing (1mm internal diameter, capacity 10 mL) using a Vapourtec R4/R2+ continuous flow device unless otherwise stated. Solvents were purchased from Fisher Scientific except 1,4-dioxane which was purchased from Rathburn. Dry solvents were prepared by distillation over sodium wire (THF, toluene) or CaH<sub>2</sub> (DCM, MeCN). TLC was performed using Merck DC-Alufolien 60 F254 0.2 mm aluminium-backed plates. Once run, plates were first visualised under a UV lamp then using a Hanessian stain (cerium molybdate). Column chromatography was conducted using silica (60 Å, 230-400 mesh size) purchased from Sigma-Aldrich and packed as a slurry. HPLC purification was conducted on all test samples using a silica semi-preparative column connected to a refractive index detector.

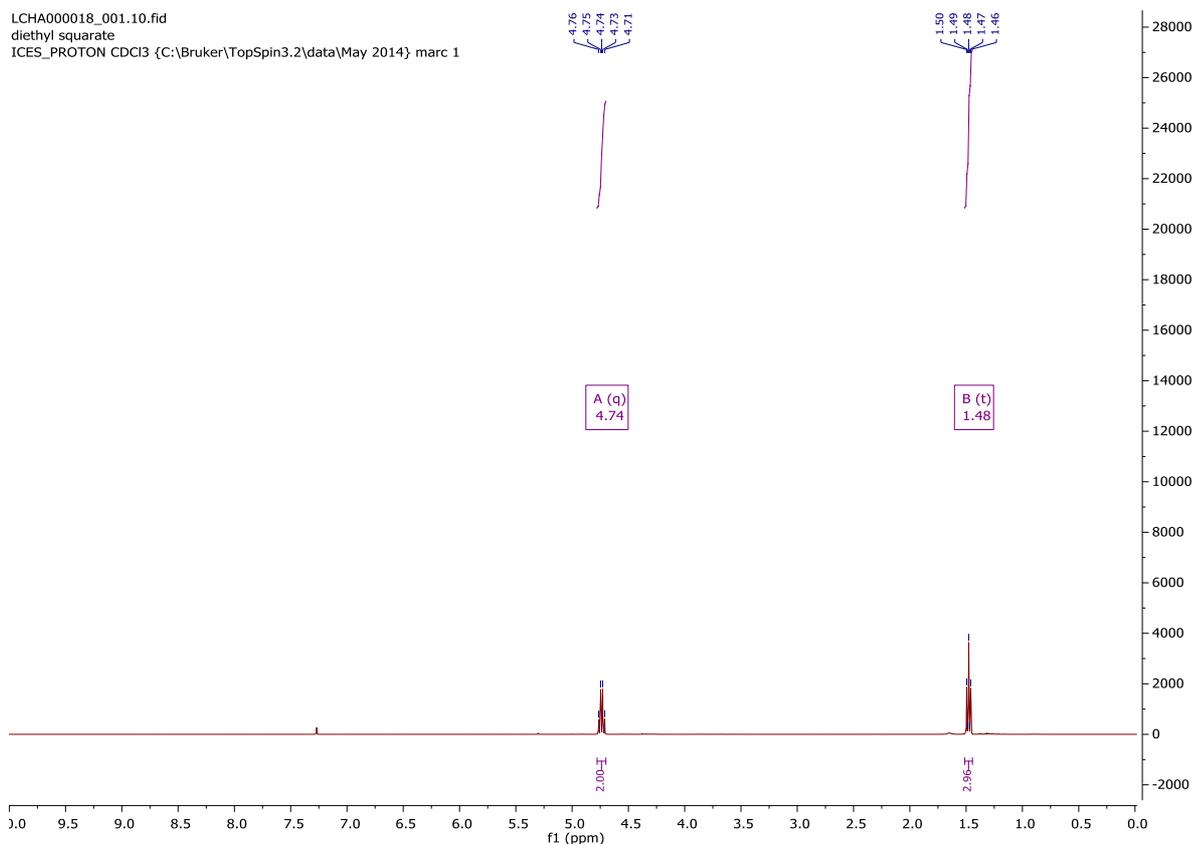
Analytical data was acquired using the following equipment. Nuclear magnetic resonance spectroscopy was obtained using Bruker AV300 (300/75 MHz), AVII400 or AVIIIHD400 (400/100 MHz) NMR spectrometers with samples prepared in CDCl<sub>3</sub> unless stated otherwise. Infrared spectroscopy was performed using a Thermo Nicolet 380 FT-IR spectrometer. Mass spectrometry analyses were performed using electron ionisation (EI) and positive mode electrospray ionisation (ESI+). EI data were generated from a Finnigan Trace 2000 series GC-MS using a Zebron ZB5-MS column while ESI+ analyses were performed using a Waters HPLC system connected to a ZMD mass spectrometer using a C18 column. High resolution mass spectra were recorded on either a Bruker Apex TF-ICR mass spectrometer equipped with a 4.7 T actively shielded superconducting magnet and Apollo ESI ion source or a Bruker maXis ESI-ToF spectrometer coupled to a Dionex Ultimate 3000 HPLC and Apollo ESI ion source. High resolution mass spectra were recorded by Dr G. J. Langley or J. Herniman (Chemistry, Southampton) and are reported to four decimal places. Melting points were recorded using an Electrothermal 1A9100 series digital melting point apparatus and are uncorrected. Detailed synthetic methodology and spectra are given in the Synthetic Protocols section of Supplementary Results.

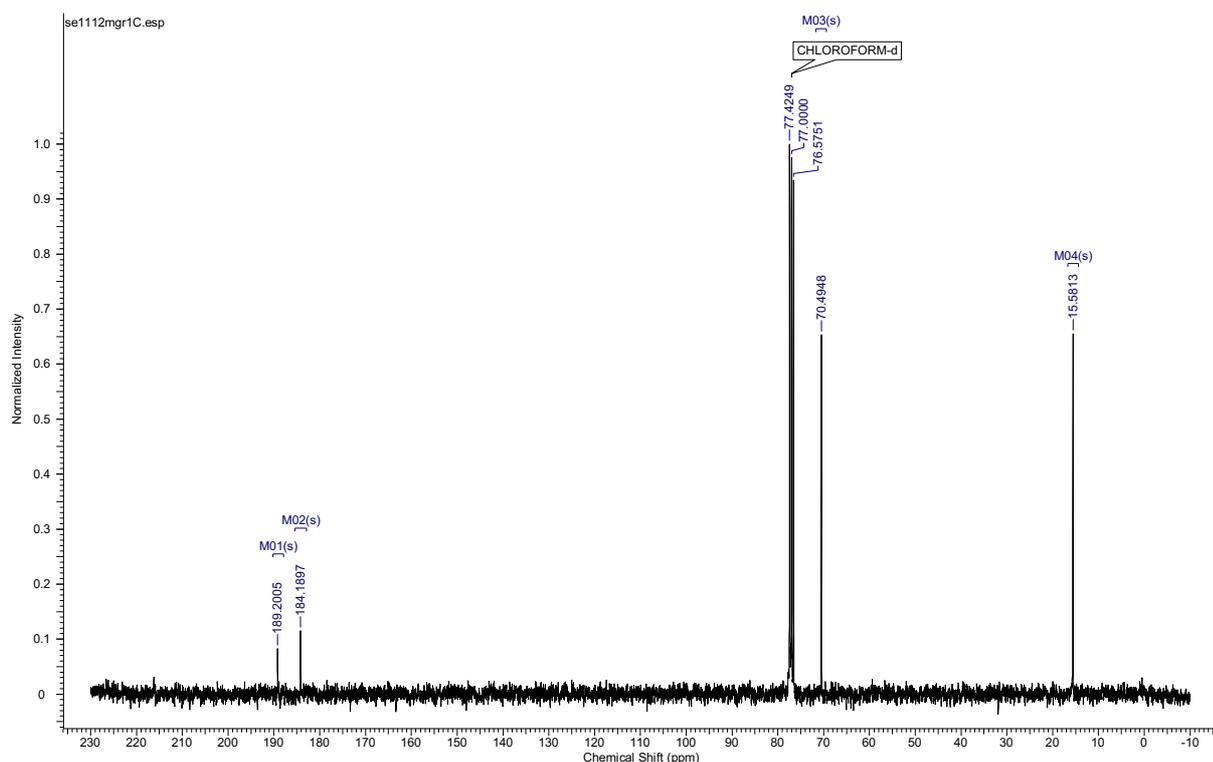
All cell culture reagents were purchased from Life Technologies unless otherwise stated. Cell lines were sourced from the American Type Culture Collection (ATCC) or Sigma Aldrich and not grown continually for more than 3 months. All cells were cultured at 37°C in a humidified 5% CO<sub>2</sub> atmosphere, according to the supplier's instructions. MCF-7, CCD 841 CoN (CCD841), MDA-MB-231, Panc-1 and HeLa cells were routinely maintained in DMEM (high glucose) containing 10% fetal bovine serum (FBS) and 1% penicillin/streptomycin; HCT116 cells in McCoy's 5A medium supplemented with 10% FBS and 1% penicillin/streptomycin; and MRC-5 cells in DMEM (high glucose) containing 10% fetal bovine serum (FBS), 1% MEM non-essential amino acids and 1% penicillin/streptomycin. Biological reagents were purchased from Fisher Scientific. All other

chemicals were obtained from Sigma-Aldrich unless stated otherwise. All microscope images were captured using a Zeiss Axiovert.A1 microscope with an AxioCam MRm, and ZEN imaging software (Zeiss) used to process images. Data was analysed with Graphpad Prism and is expressed as the mean  $\pm$  SEM unless otherwise indicated.

### **Synthetic Protocols and spectroscopic data**

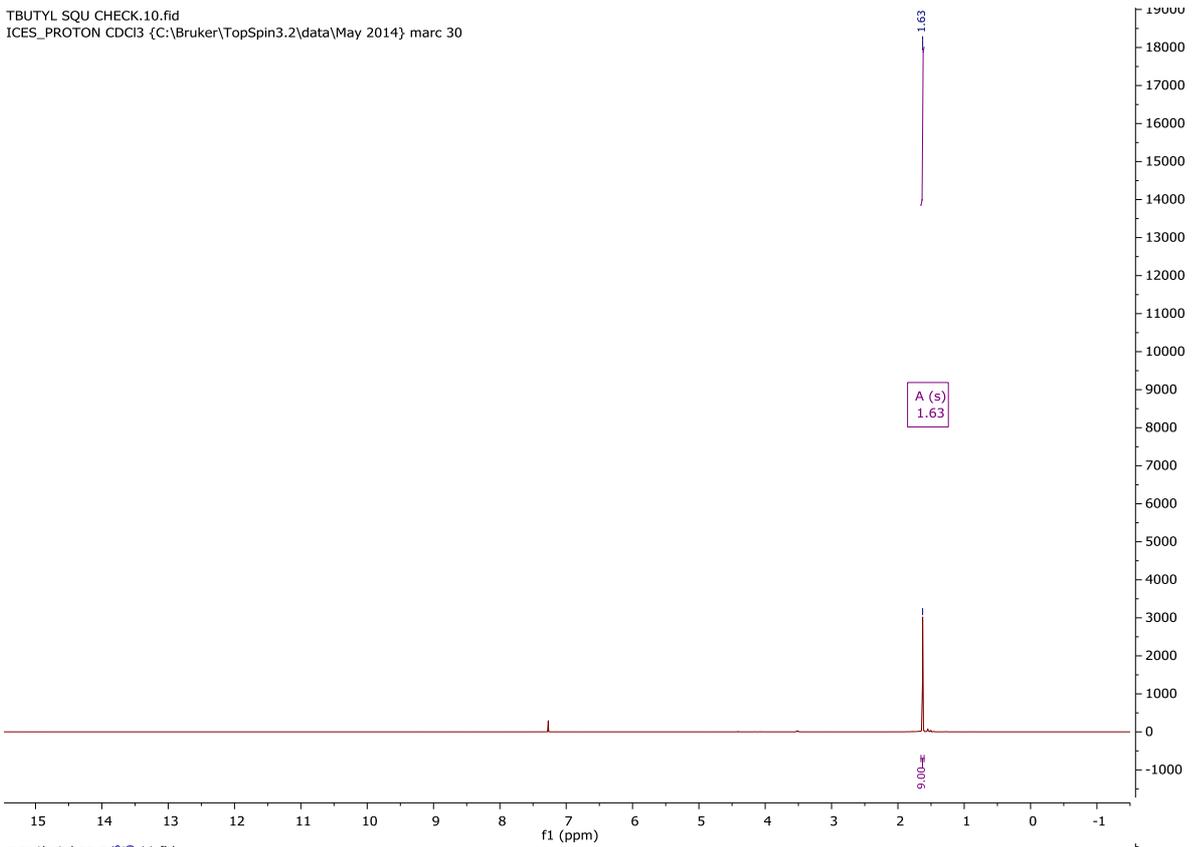
**3,4-Diethoxycyclobut-3-ene-1,2-dione, 6b.** Adapting a procedure by Moore *et al.*<sup>27</sup> To a solution of squaric acid **5** (13.7 g, 120 mmol) in ethanol (120 mL) was added triethyl orthoformate (50 mL, 301 mmol). The reaction mixture was heated at reflux for 7 h, then concentrated to ca. two thirds volume by short path distillation before being heated at reflux again for 15 h. Concentration *in vacuo* and purification by column chromatography (0–50% EtOAc/petrol) afforded the title compound (17.5 g, 103 mmol, 85%) as a clear oil. IR:  $\nu$  2985 (w), 2360 (w), 1811 (m), 1729 (s), 1590 (s), 1480 (m), 1420 (s), 1381 (s), 1327 (s), 1076 (s), 1022 (s); <sup>1</sup>H NMR (300 MHz, CDCl<sub>3</sub>):  $\delta$  = 4.74 (4 H, q,  $J$  = 7.3 Hz, 2 x CH<sub>2</sub>CH<sub>3</sub>), 1.48 (6H, t,  $J$  = 7.1 Hz, 2 x CH<sub>2</sub>CH<sub>3</sub>) ppm; <sup>13</sup>C NMR (75 MHz, CDCl<sub>3</sub>):  $\delta$  = 189.2 (C), 184.2 (C), 70.5 (CH<sub>2</sub>), 15.6 (CH<sub>3</sub>) ppm; MS (EI)  $m/z$  (%): 170 ([M]<sup>+</sup>). Spectroscopic data were in agreement with literature values.<sup>28</sup>



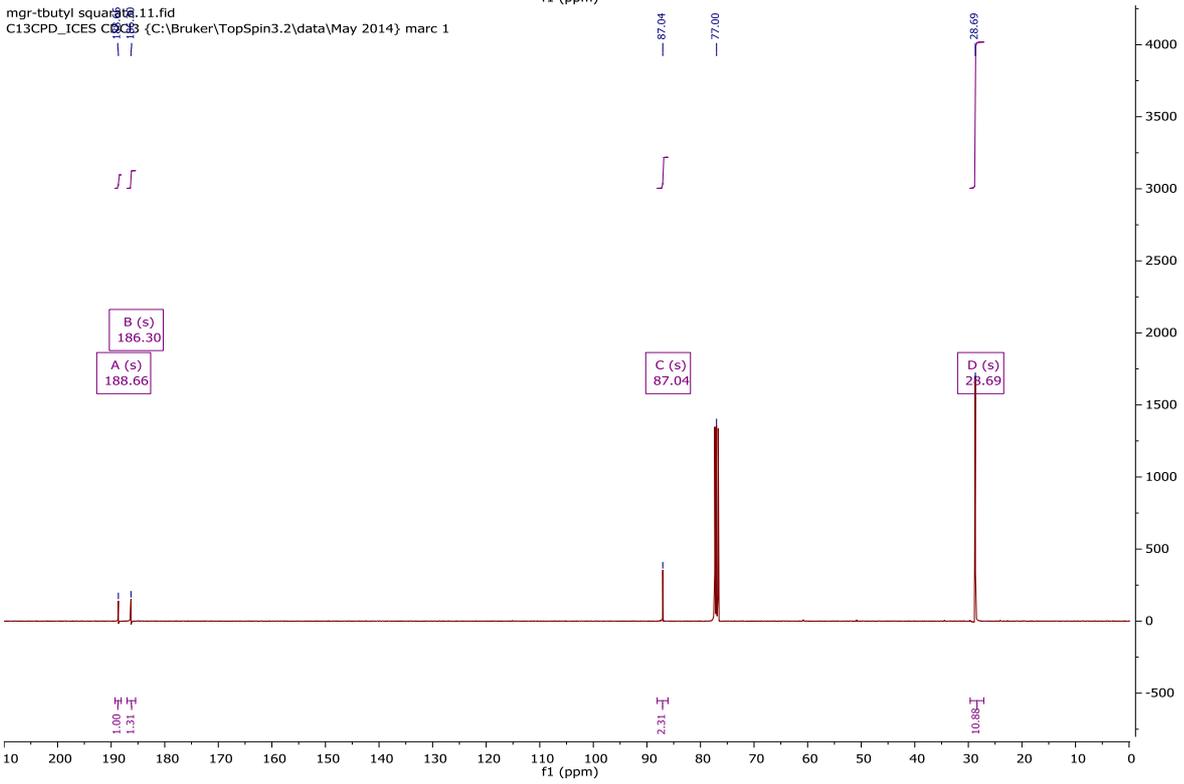


**3,4-Di-*tert*-butoxycyclobut-3-ene-1,2-dione, 6c.** Adapting a procedure by Moore *et al.*<sup>27</sup> Squaric acid **5** (2.53 g, 22.2 mmol) and *t*BuOH (88.8 mL) were heated at reflux for 30 min then trimethyl orthoformate (35.0 mL, 330 mmol) was added over 30 min. MeOH was then removed from the solution by short path distillation then, after a further 16.5 h at reflux, the solution was concentrated *in vacuo*. Purification by column chromatography (1–10% EtOAc/hexane) afforded firstly the title compound (2.77 g, 12.4 mmol, 55%) as a white solid; MP: 99–101 °C [Lit: 106–108 °C EA/hexane]<sup>29</sup>; IR:  $\nu$  2979 (w), 2927 (w), 1804 (w), 1773 (w), 1725 (w), 1574 (s), 1144 (s), 1069 (m); <sup>1</sup>H NMR:  $\delta$  = (300 MHz, CDCl<sub>3</sub>):  $\delta$  1.63 (18 H, s, 2 x C(CH<sub>3</sub>)<sub>3</sub>) ppm; <sup>13</sup>C NMR:  $\delta$  = (75 MHz, CDCl<sub>3</sub>):  $\delta$  = 188.7 (C), 186.3 (C), 87.0 (C), 28.7 (CH<sub>3</sub>) ppm; MS (EI) *m/z*: 226 ([M]<sup>+</sup>). Spectroscopic data were in agreement with literature values.<sup>29</sup> Secondly 3-*tert*-butoxy-4-methoxycyclobut-3-ene-1,2-dione (0.50 g, 2.71 mmol, 12%) as a white solid MP: 96.5–97 °C [Lit: 89–90 °C]<sup>27</sup>; IR:  $\nu$  2957 (w), 2925 (w), 2855 (w), 1721 (s), 1585 (s), 1457 (s), 1383 (s), 1374 (s), 1150 (s), 1092 (s), 1008 (m), 821 (s), 753 (s); <sup>1</sup>H NMR (300 MHz, CDCl<sub>3</sub>):  $\delta$  = 4.41 (3 H, s, OCH<sub>3</sub>), 1.61 (9 H, s, C(CH<sub>3</sub>)<sub>3</sub>) ppm; <sup>13</sup>C NMR (75 MHz, CDCl<sub>3</sub>):  $\delta$  = 189.5 (C), 188.5 (C), 185.7 (C), 184.8 (C), 87.4 (C), 60.8 (CH<sub>3</sub>), 28.6 (CH<sub>3</sub>) ppm; MS (EI) *m/z* (%): 184 ([M]<sup>+</sup>). Spectroscopic data were in agreement with literature values.<sup>27</sup>

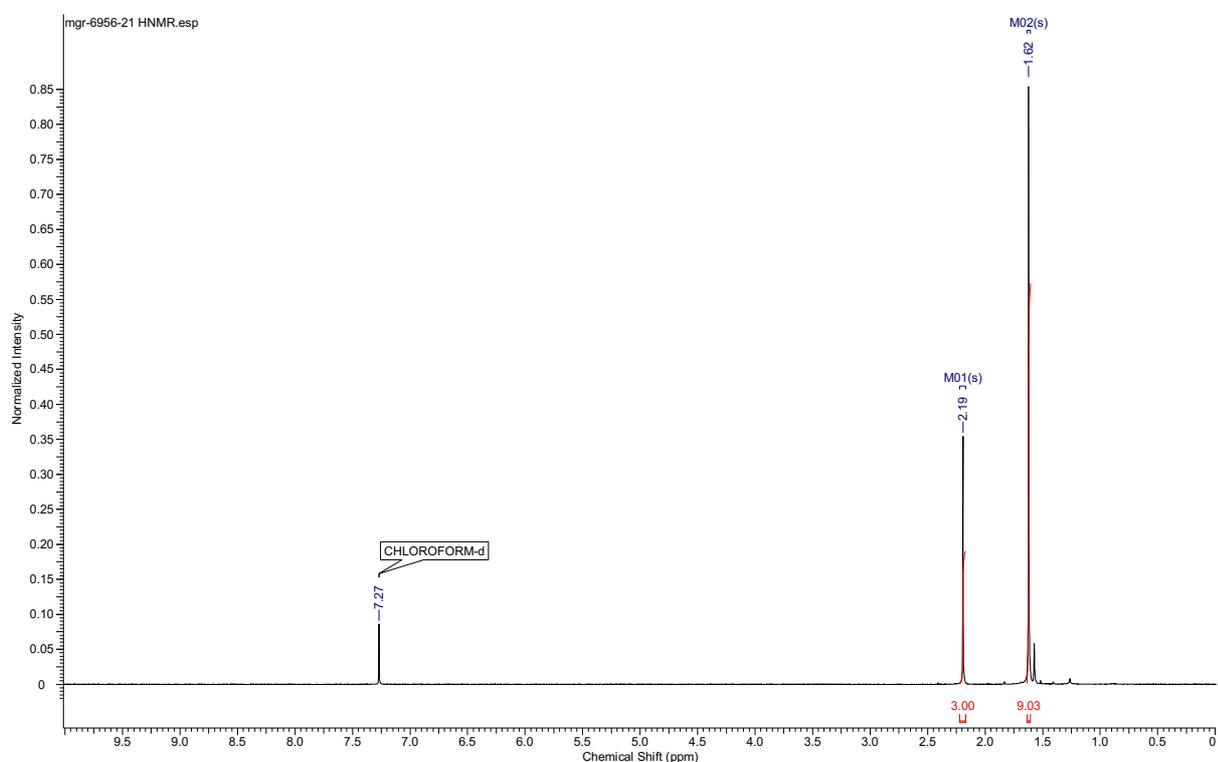
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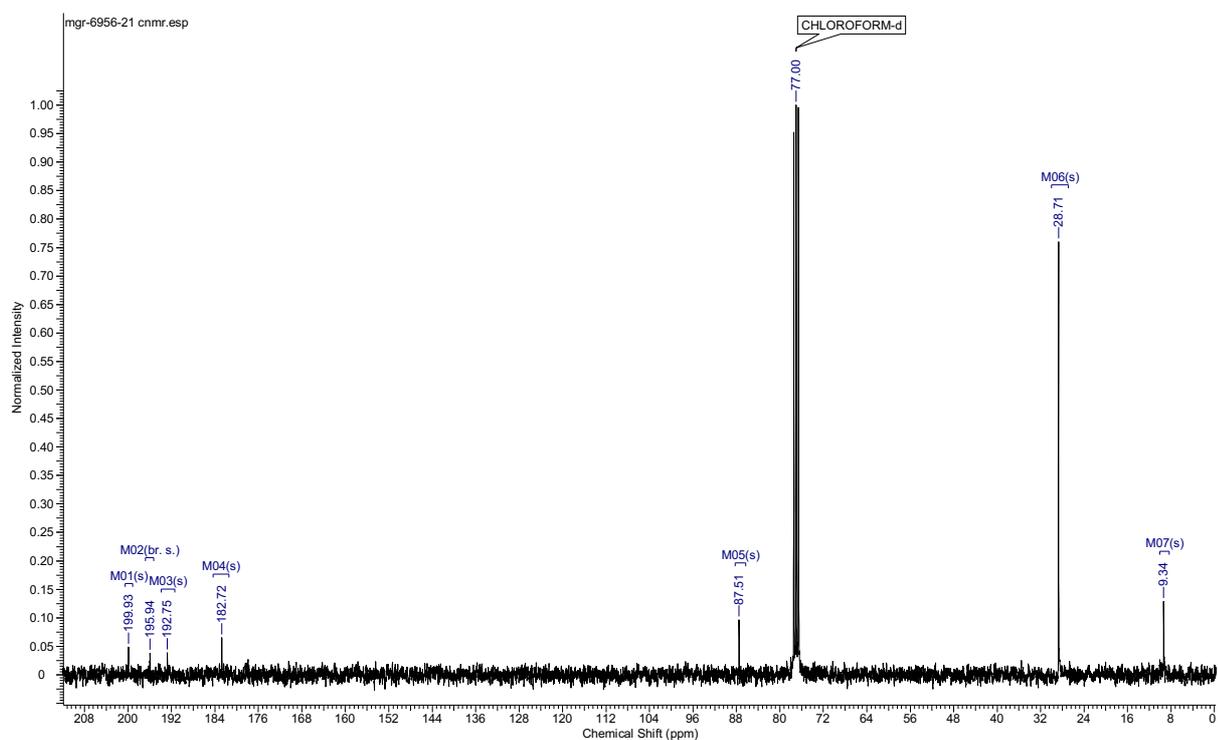


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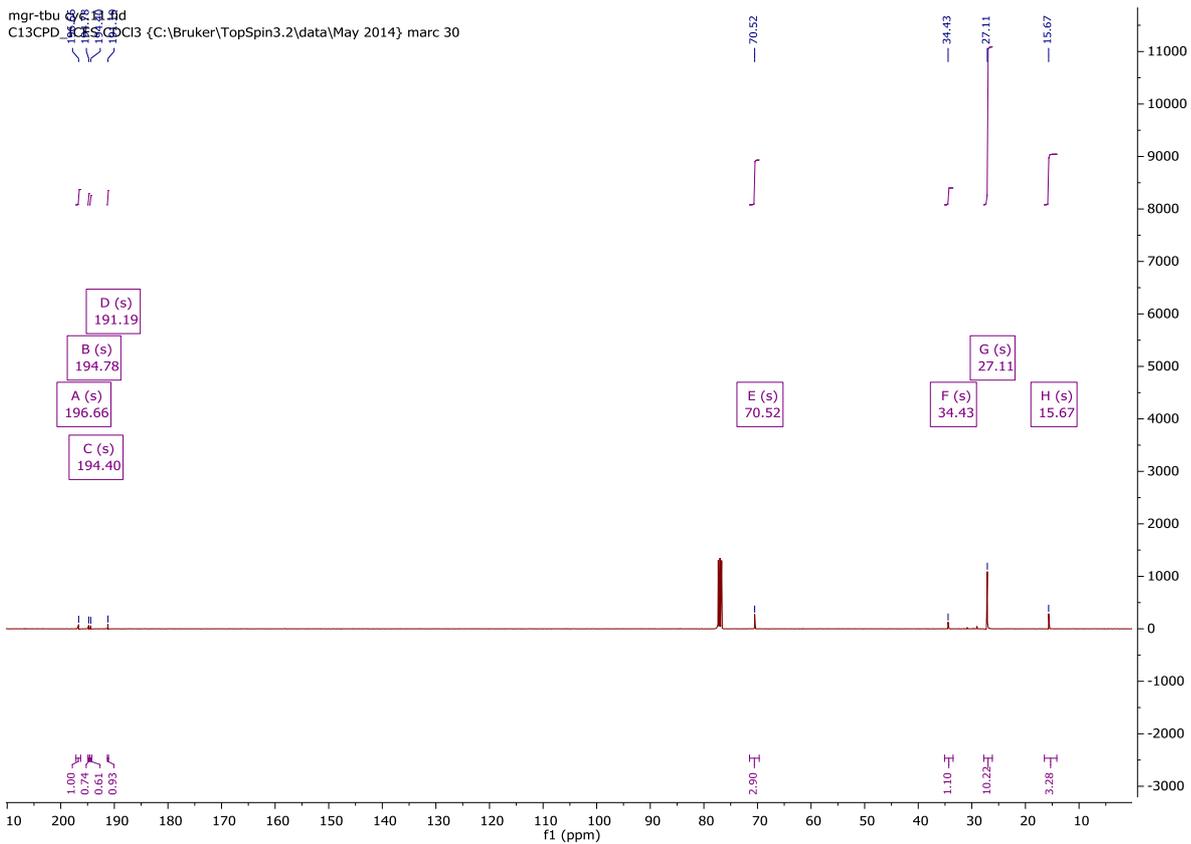
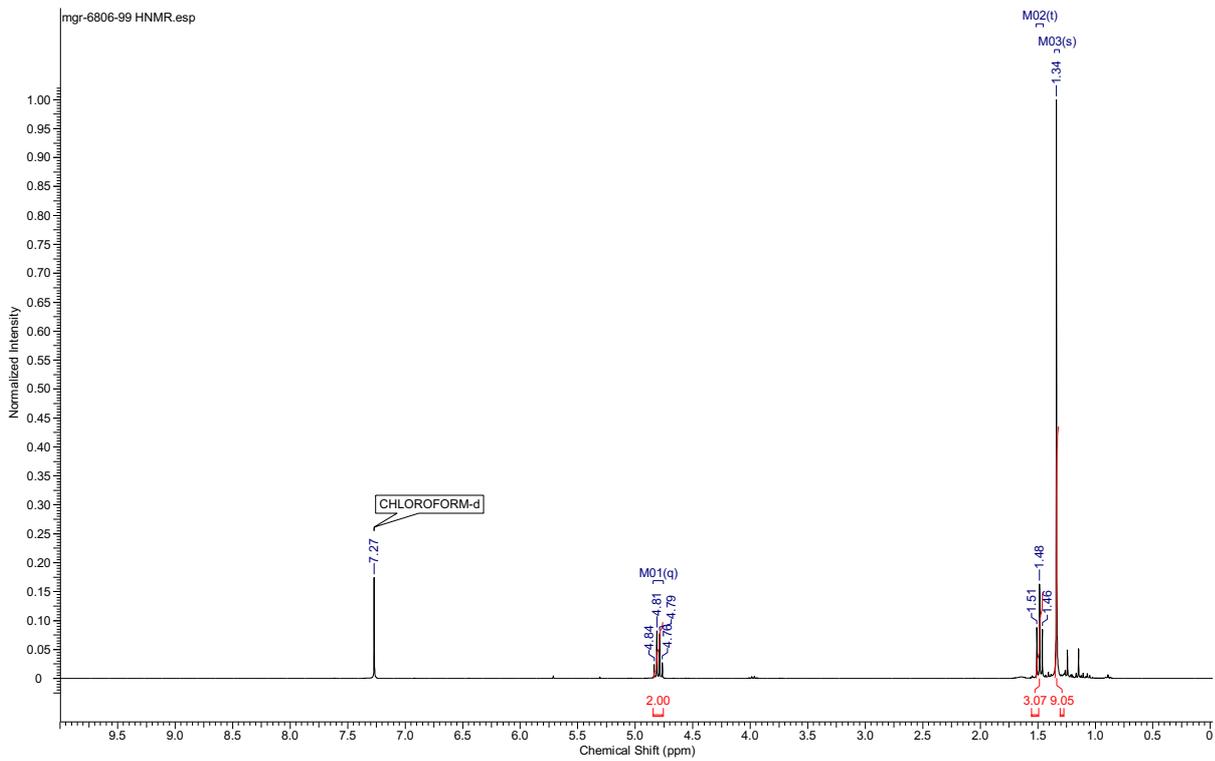


**3-(*tert*-butoxy)-4-methylcyclobut-3-ene-1,2-dione, 8a/b.** Adapting a procedure by Moore *et al.*<sup>30</sup> To a solution of di-*tert*-butyl squarate **6c** (1.00 g, 4.42 mmol) in THF (40 mL) at  $-78\text{ }^{\circ}\text{C}$  was added MeLi (1.84 M in Et<sub>2</sub>O, 2.60 mL, 4.84 mmol) in THF (2.5 mL) over 1 min. After 1 h, TFAA (0.74 mL, 5.30 mmol) was added over 1 min followed after 10 min by sat. NaHCO<sub>3</sub> (30 mL). The resulting mixture was warmed to RT then the aqueous phase was separated and extracted with Et<sub>2</sub>O (3 x 30 mL). The organic phases were combined, washed with brine (30 mL), dried over MgSO<sub>4</sub>, filtered and concentrated *in vacuo* and purified by column chromatography (30-100% EtOAc/cyclohexane) to afford the title compound (0.22 g, 1.31 mmol, 30%) as an off-white solid; MP: 72–74  $^{\circ}\text{C}$  [Lit: 72–73  $^{\circ}\text{C}$ ]<sup>31</sup>; IR:  $\nu$  1799 (s), 1748 (s), 1584 (s), 1393 (s), 1375 (s), 1348 (s), 1153 (s); <sup>1</sup>H NMR (300 MHz, CDCl<sub>3</sub>):  $\delta$  = 2.19 (3 H, s, CH<sub>3</sub>), 1.62 (9 H, s, C(CH<sub>3</sub>)<sub>3</sub>) ppm; <sup>13</sup>C NMR (75 MHz, CDCl<sub>3</sub>):  $\delta$  = 199.9 (C), 195.9 (C), 192.8 (C), 182.7 (C), 87.5 (C), 28.7 (CH<sub>3</sub>), 9.3 (CH<sub>3</sub>) ppm; MS (EI) *m/z*: 168 ([M]<sup>+</sup>). Spectroscopic data were in agreement with literature values.<sup>31</sup>

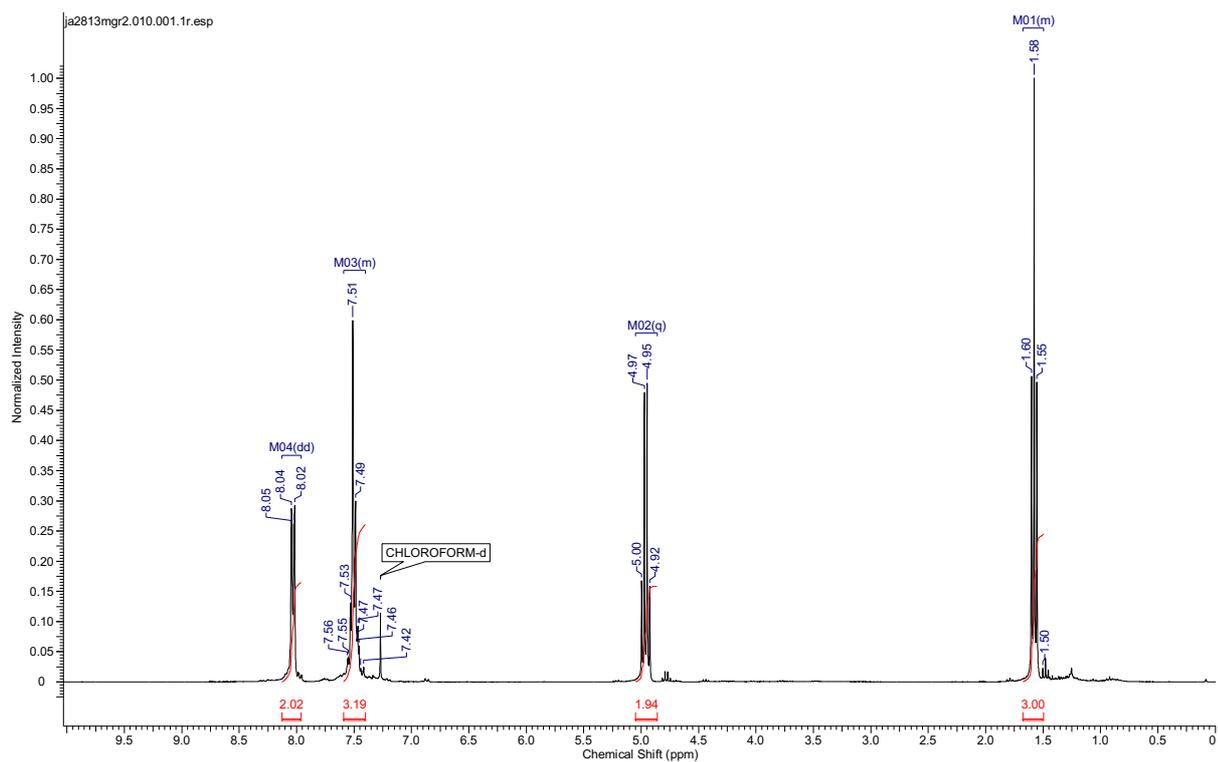


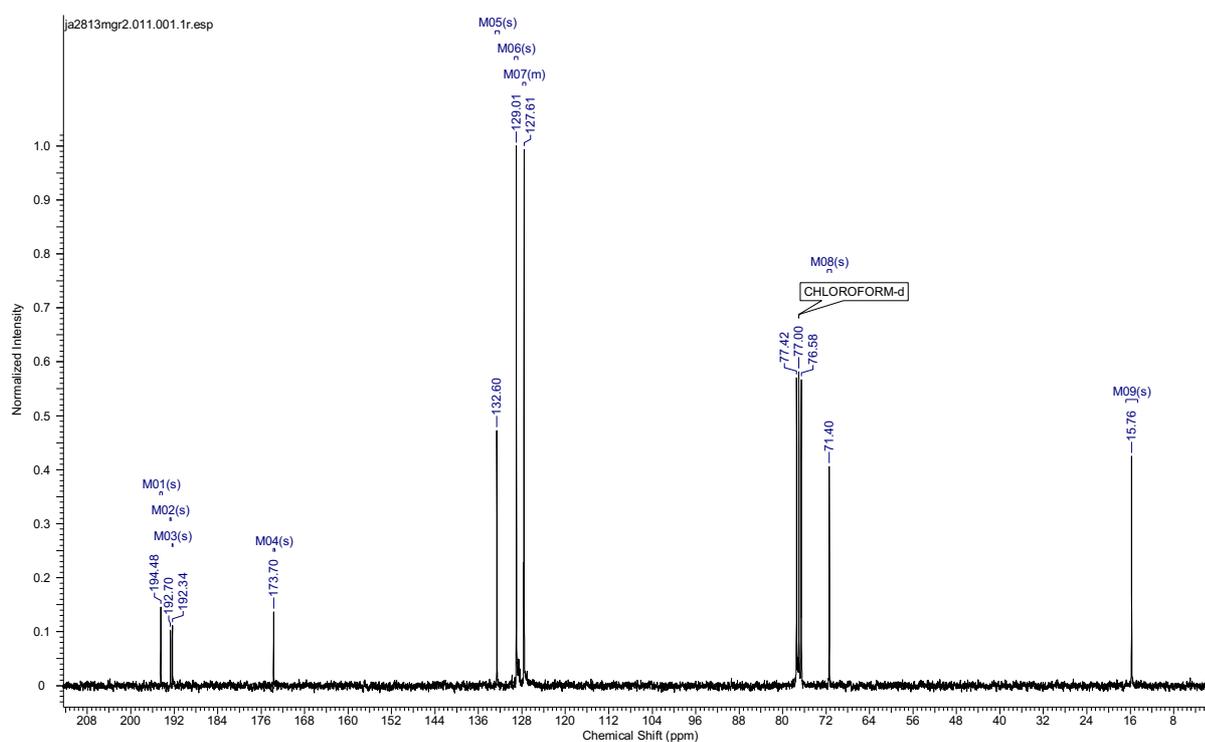


**3-(*tert*-Butyl)-4-ethoxycyclobut-3-ene-1,2-dione, **8c**.** Adapting a procedure by Moore *et al.*<sup>30</sup> To a solution of <sup>t</sup>BuLi (2.01 M in heptane, 1.81 mL, 3.63 mmol) in THF (10 mL) at  $-78$  °C was added diethyl squarate **6b** (0.56 g, 3.30 mmol) in THF solution (30 mL) over 35 min. After 1 h, TFAA (0.55 mL, 3.96 mmol) was added dropwise over 1 min followed after 10 min by sat. NaHCO<sub>3</sub> (30 mL). The resulting mixture was warmed to RT and extracted with Et<sub>2</sub>O (3 x 30 mL). The organic phases were combined, washed with brine (30 mL), dried over MgSO<sub>4</sub>, filtered and concentrated *in vacuo* and purified by column chromatography (30–100% EtOAc/petrol) to afford the title compound (0.24 g, 1.32 mmol, 40%) as a yellow oil; IR:  $\nu$  2970 (w), 2871 (w), 2362 (w), 1791 (s), 1752 (s), 1586 (s), 1380 (m), 1344 (s), 1019 (m), 790 (m); <sup>1</sup>H NMR (300 MHz, CDCl<sub>3</sub>):  $\delta$  = 4.80 (2 H, q,  $J$  = 7.2 Hz, CH<sub>2</sub>CH<sub>3</sub>), 1.48 (3 H, t,  $J$  = 7.1 Hz, CH<sub>2</sub>CH<sub>3</sub>), 1.34 (9 H, s, C(CH<sub>3</sub>)<sub>3</sub>) ppm; <sup>13</sup>C NMR (101 MHz, CDCl<sub>3</sub>):  $\delta$  = 196.7 (C), 194.8 (C), 194.4 (C), 191.2 (C), 70.5 (CH<sub>2</sub>), 27.1 (CH<sub>3</sub>), 15.7 (CH<sub>3</sub>) ppm; MS (ESI+)  $m/z$  (%): 183 ([M+H]<sup>+</sup>).

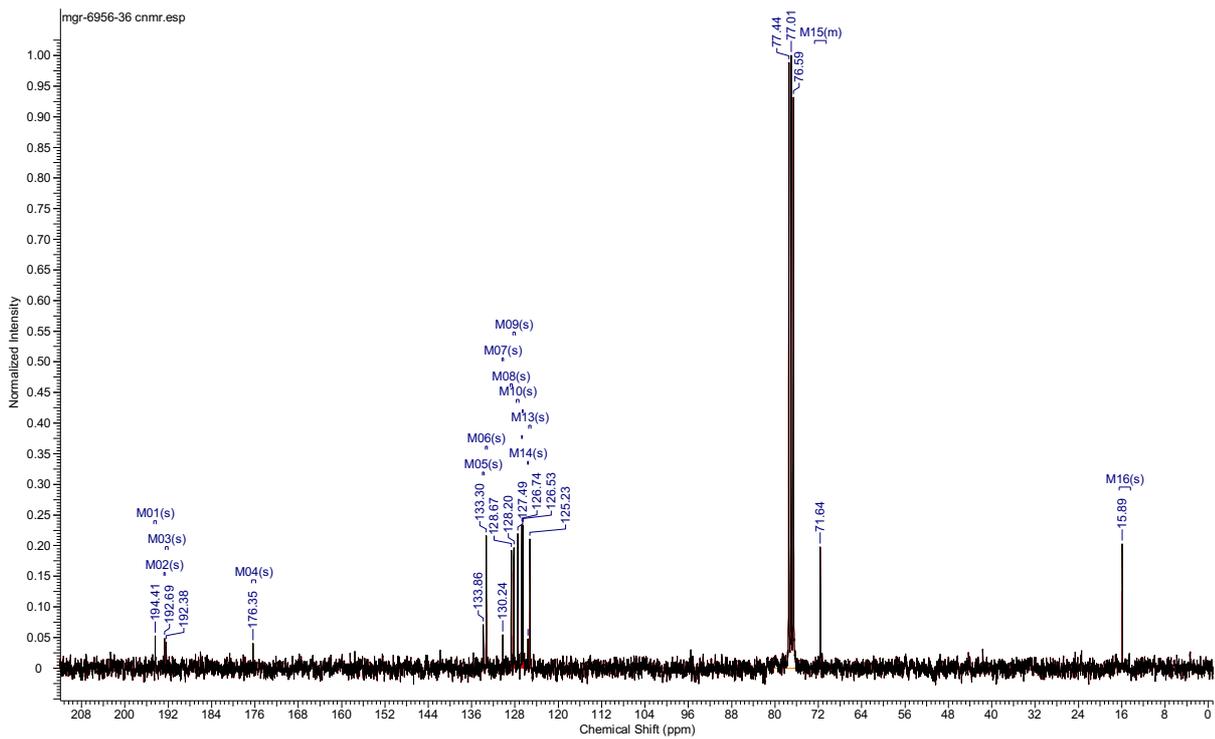
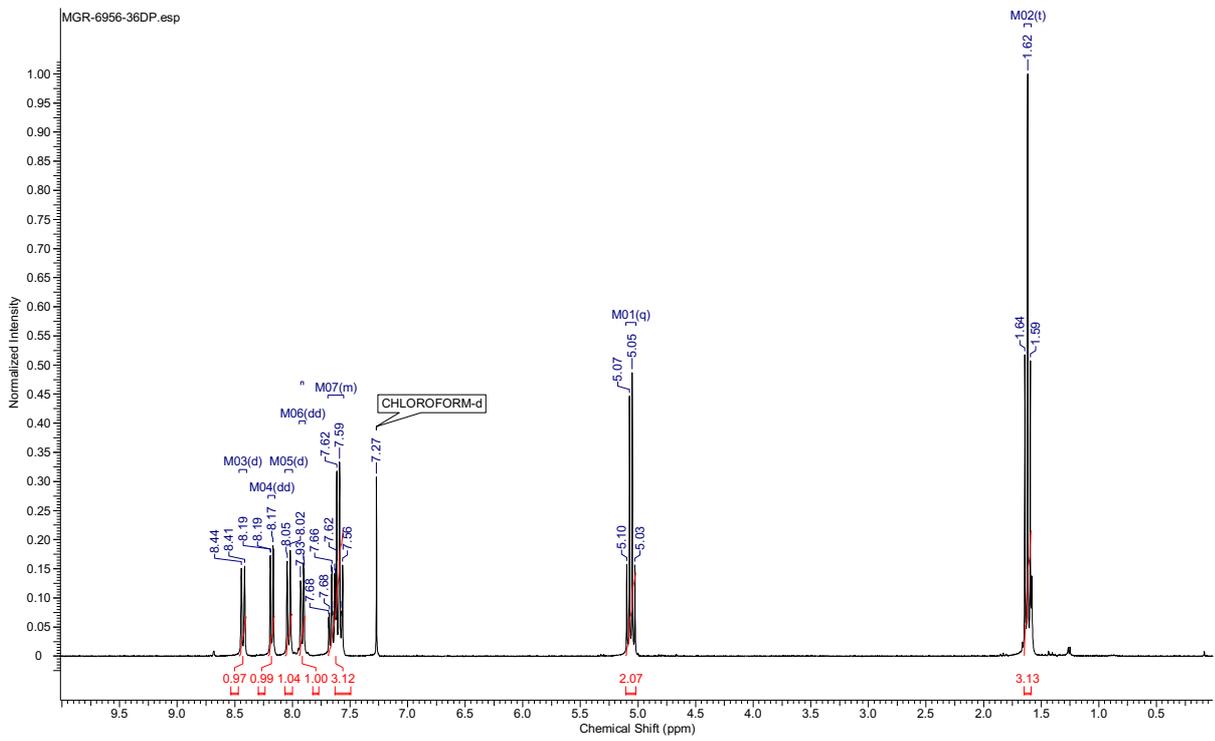


**3-Ethoxy-4-phenylcyclobut-3-ene-1,2-dione, 8d.** Adapting a procedure by Moore *et al.*<sup>30</sup> To a solution of diethyl squarate **6b** (0.50g, 2.95 mmol) in THF (30 mL) at  $-78\text{ }^{\circ}\text{C}$  was added a solution of PhLi (0.91 M in Bu<sub>2</sub>O, 3.5 mL, 3.2 mmol) in THF (10 mL) dropwise over 10 min. After 1 h, TFAA (0.5 mL, 3.5 mmol) was added dropwise over 1 min followed by sat. NaHCO<sub>3</sub> (15 mL) 10 min later. The resultant mixture was warmed to RT and the aqueous phase separated and extracted with Et<sub>2</sub>O (3 x 20 mL). The organic phases were combined, washed with sat. NaHCO<sub>3</sub> (20 mL) and brine (20 mL), dried over MgSO<sub>4</sub>, filtered and concentrated *in vacuo* and purified by flash chromatography (30-100% EtOAc/petrol) to afford the title compound (0.31g, 1.53 mmol, 52%) as a fine yellow solid; MP: 128.0-128.5  $^{\circ}\text{C}$  [Lit 130-131  $^{\circ}\text{C}$ ]<sup>32</sup>; IR:  $\nu$  3058 (w), 2360 (w), 1778 (m), 1740 (m), 1583 (m), 1495 (w), 1469 (w), 1450 (w), 1384 (m), 1346 (m), 1319 (w), 1177 (w), 1103 (w), 1023 (m), 994 (m), 912 (w), 848 (w), 793 (w), 770 (m), 743 (m), 694 (m); <sup>1</sup>H NMR (300 MHz, CDCl<sub>3</sub>):  $\delta$  = 8.03 (2 H, dd,  $J$  = 7.9, 1.6 Hz, ArH), 7.56 - 7.44 (3 H, m, ArH), 4.96 (2 H, q,  $J$  = 7.3 Hz, CH<sub>2</sub>CH<sub>3</sub>), 1.58 (3 H, t,  $J$  = 7.1 Hz, CH<sub>2</sub>CH<sub>3</sub>) ppm; <sup>13</sup>C NMR (75 MHz, CDCl<sub>3</sub>):  $\delta$  = 194.5 (C), 192.7 (C), 192.3 (C), 173.7 (C), 132.6 (CH), 129.0 (CH), 127.7 (C), 127.6 (CH), 71.4 (CH<sub>2</sub>), 15.8 (CH<sub>3</sub>) ppm; LRMS (EI)  $m/z$ ; 202 ([M]<sup>+</sup>). Spectroscopic data were in agreement with literature values.<sup>32</sup>

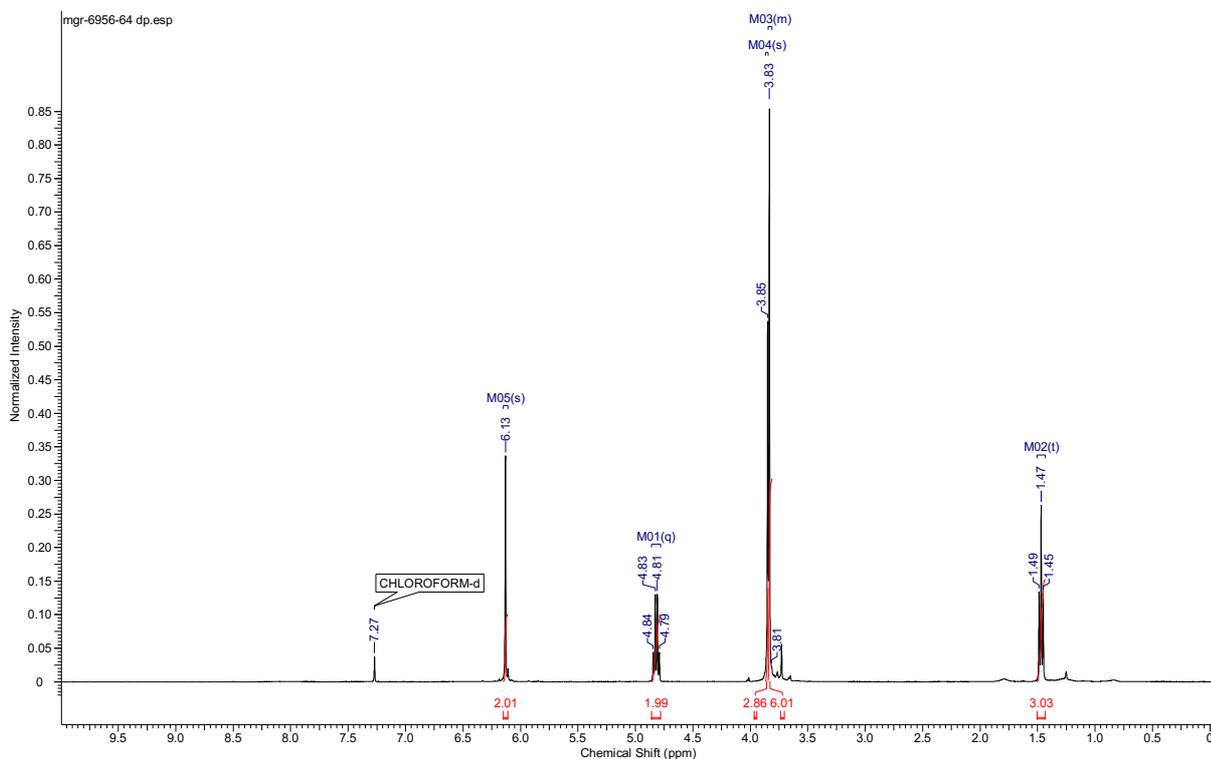


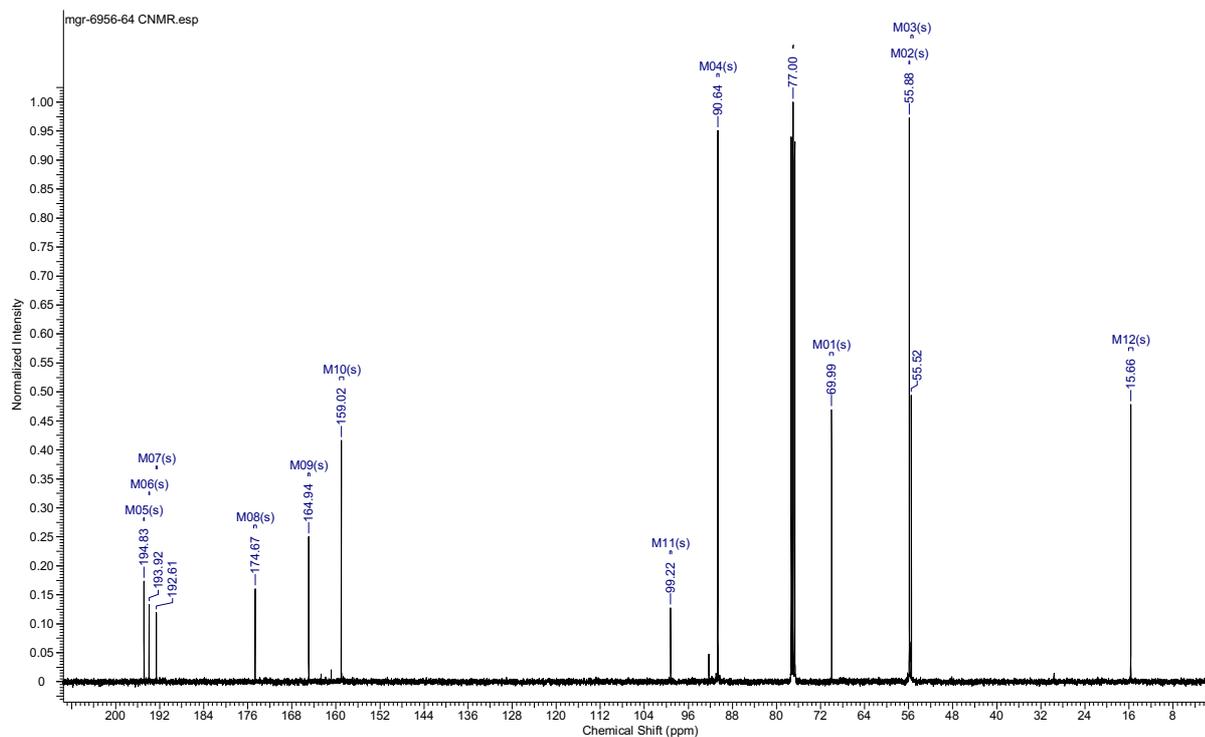


**3-Ethoxy-4-(naphthalen-1-yl)cyclobut-3-ene-1,2-dione, 8e.** Adapting a procedure by Moore *et al.*<sup>30</sup> To a solution of <sup>t</sup>BuLi (1.92 M in heptane, 2.1 mL, 4.03 mmol) in THF (25 mL) at  $-78$  °C was added 1-bromonaphthalene (0.28 mL, 2.00 mmol) over 1 min. After 45 min, diethyl squarate **6b** (0.33g, 1.92 mmol) in THF (45 mL) was added over 10 min followed after 1 h by TFAA (0.33 mL, 2.4 mmol). After a further 10 min sat. NaHCO<sub>3</sub> (30 mL) was added and the resulting mixture was warmed to RT. The aqueous phase separated and extracted with Et<sub>2</sub>O (3 x 40 mL) then the organic phases were combined, washed with brine (30 mL), dried over MgSO<sub>4</sub>, filtered and concentrated *in vacuo* and purified by column chromatography (0–50% EtOAc/petrol) to afford the title compound (0.23 g, 0.91 mmol, 47%) as a dark yellow solid; MP: 113–117 °C; IR: 3048 (w), 2983 (w), 2933 (w), 1777 (s), 1741 (s), 1566 (s), 1510 (m), 1414 (m), 1379 (m), 1335 (s), 812 (m), 796 (m), 743 (m), 774 (m); <sup>1</sup>H NMR (300 MHz, CDCl<sub>3</sub>):  $\delta$  = 8.43 (1 H, d,  $J$  = 8.4 Hz, ArH), 8.18 (1 H, dd,  $J$  = 7.3, 1.1 Hz, ArH), 8.03 (1 H, d,  $J$  = 8.1 Hz, ArH), 7.91 (1 H, dd,  $J$  = 7.7, 1.5 Hz, ArH), 7.56–7.69 (3 H, m, ArH), 5.06 (2 H, q,  $J$  = 7.0 Hz, CH<sub>2</sub>CH<sub>3</sub>), 1.62 (3 H, t,  $J$  = 7.1 Hz, CH<sub>2</sub>CH<sub>3</sub>) ppm; <sup>13</sup>C NMR (101 MHz, CDCl<sub>3</sub>):  $\delta$  = 194.4 (C), 192.7 (C), 192.4 (C), 176.4 (C), 133.9 (C), 133.3 (CH), 130.2 (C), 128.7 (CH), 128.2 (CH), 127.5 (CH), 126.7 (CH), 126.5 (CH), 125.6 (C), 125.2 (CH), 71.6 (CH<sub>2</sub>), 15.9 (CH<sub>3</sub>) ppm; MS (EI)  $m/z$  (%): 252 ([M]<sup>+</sup>); HRMS (ESI+)  $m/z$  Found 253.0854, C<sub>16</sub>H<sub>13</sub>O<sub>3</sub> [M+H]<sup>+</sup> requires 253.0859.

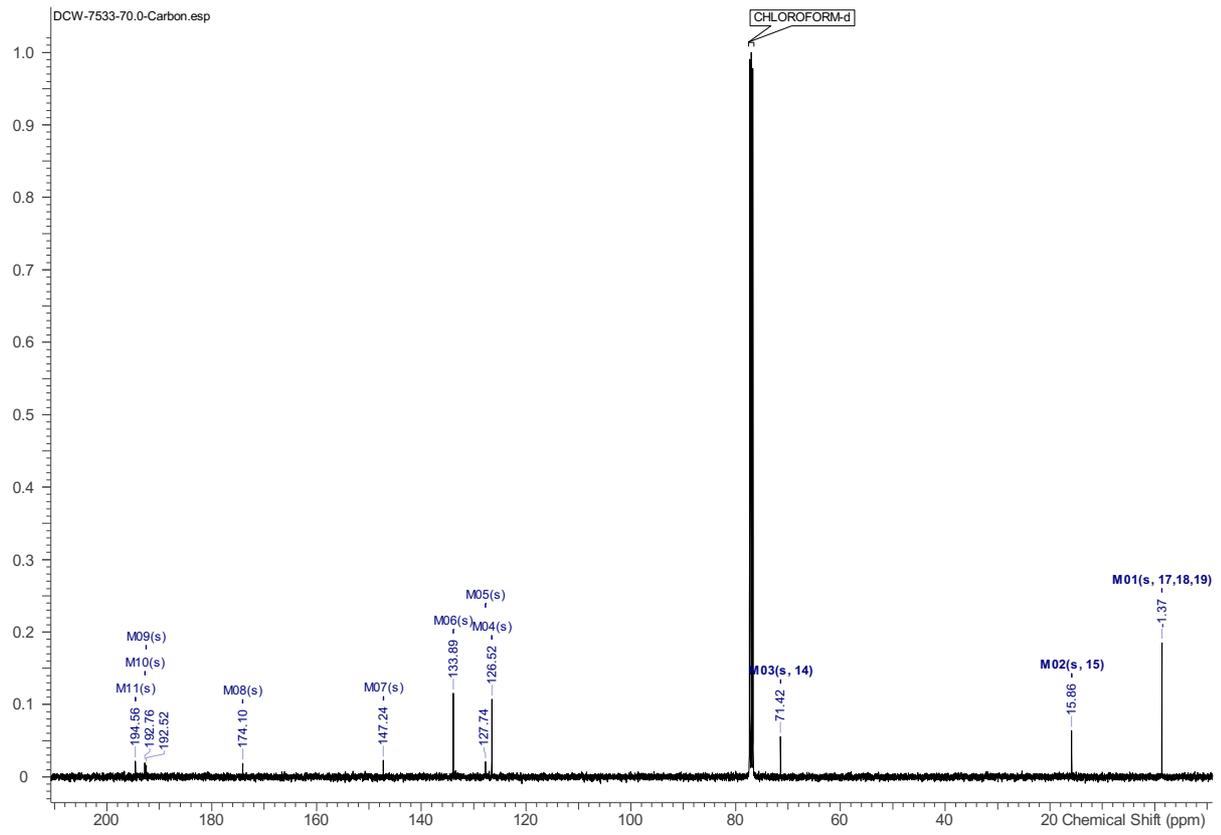
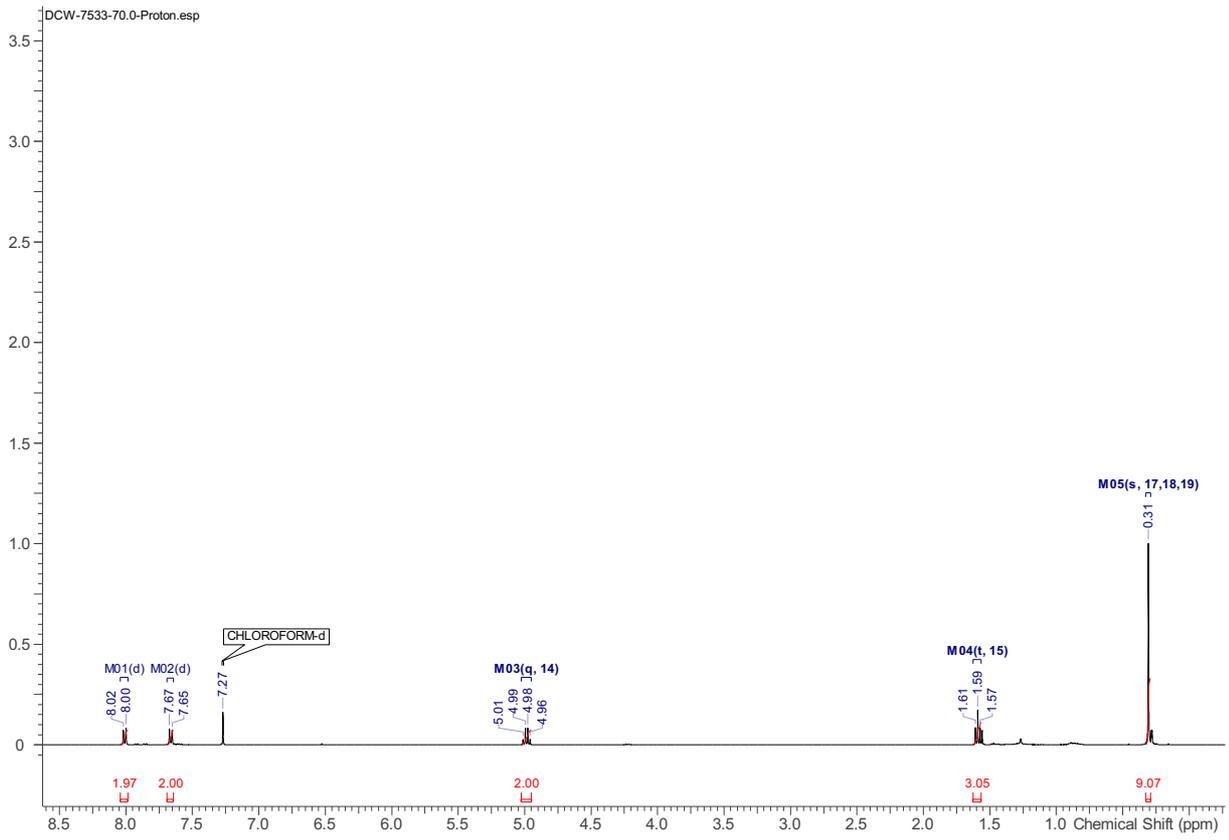


**3-Ethoxy-4-(2,4,6-trimethoxyphenyl)cyclobut-3-ene-1,2-dione, 8f.** Adapting a procedure by Moore *et al.*<sup>30</sup> To a solution of 1,3,5-trimethoxybenzene (0.44 g, 2.61 mmol) in Et<sub>2</sub>O (26 mL) at -78 °C was added a solution of <sup>n</sup>BuLi (2.39 M in hexane, 1.2 mL, 2.87 mmol). The solution was warmed to RT and after 4.5 h was added to a solution of diethyl squarate **6b** (0.38 g, 2.22 mmol) in Et<sub>2</sub>O (20 mL) at -78 °C (together with further Et<sub>2</sub>O (15 mL) that was used to rinse through residual organolithium). After 2 h, TFAA (0.36 mL, 2.62 mmol) was added over 1 min then the resultant mixture was warmed to RT over 10 min and sat. NaHCO<sub>3</sub> (15 mL) was added. The aqueous phase separated and extracted with Et<sub>2</sub>O (3 x 20 mL) then the organic phases were combined, washed with brine (20 mL), dried over MgSO<sub>4</sub>, filtered and concentrated *in vacuo* and purified by column chromatography (5–40% EtOAc/petrol) to afford the title compound (67.5 mg, 0.26 mmol, 10%) as a yellow solid; MP: 92–94 °C; IR: 2942 (w), 2844 (w), 1783 (m), 1735 (m), 1604 (s), 1571 (s), 1454 (m), 1400 (m), 1330 (s), 1230 (m), 1205 (s), 1133 (s), 1089 (m), 1025 (s), 948 (m), 921 (m), 814 (m), 797 (s); <sup>1</sup>H NMR (400 MHz, CDCl<sub>3</sub>): δ = 6.13 (2 H, s, ArH), 4.82 (2 H, q, *J* = 7.1 Hz, CH<sub>2</sub>CH<sub>3</sub>), 3.85 (3 H, s, OCH<sub>3</sub>), 3.83 (6 H, s, 2 × OCH<sub>3</sub>), 1.47 (3 H, t, *J* = 7.1 Hz, CH<sub>2</sub>CH<sub>3</sub>) ppm; <sup>13</sup>C NMR (101 MHz, CDCl<sub>3</sub>): δ = 194.8 (C), 193.9 (C), 192.6 (C), 174.7 (C), 164.9 (C), 159.0 (C), 99.2 (C), 90.6 (CH), 70.0 (CH<sub>2</sub>), 55.9 (CH<sub>3</sub>), 55.5 (CH<sub>3</sub>), 15.7 (CH<sub>3</sub>) ppm; MS (EI) *m/z*: 292 ([M]<sup>+</sup>); HRMS (ESI+) *m/z* (%): Found 293.1019, C<sub>15</sub>H<sub>17</sub>O<sub>3</sub> [M+H]<sup>+</sup> requires 293.1020.

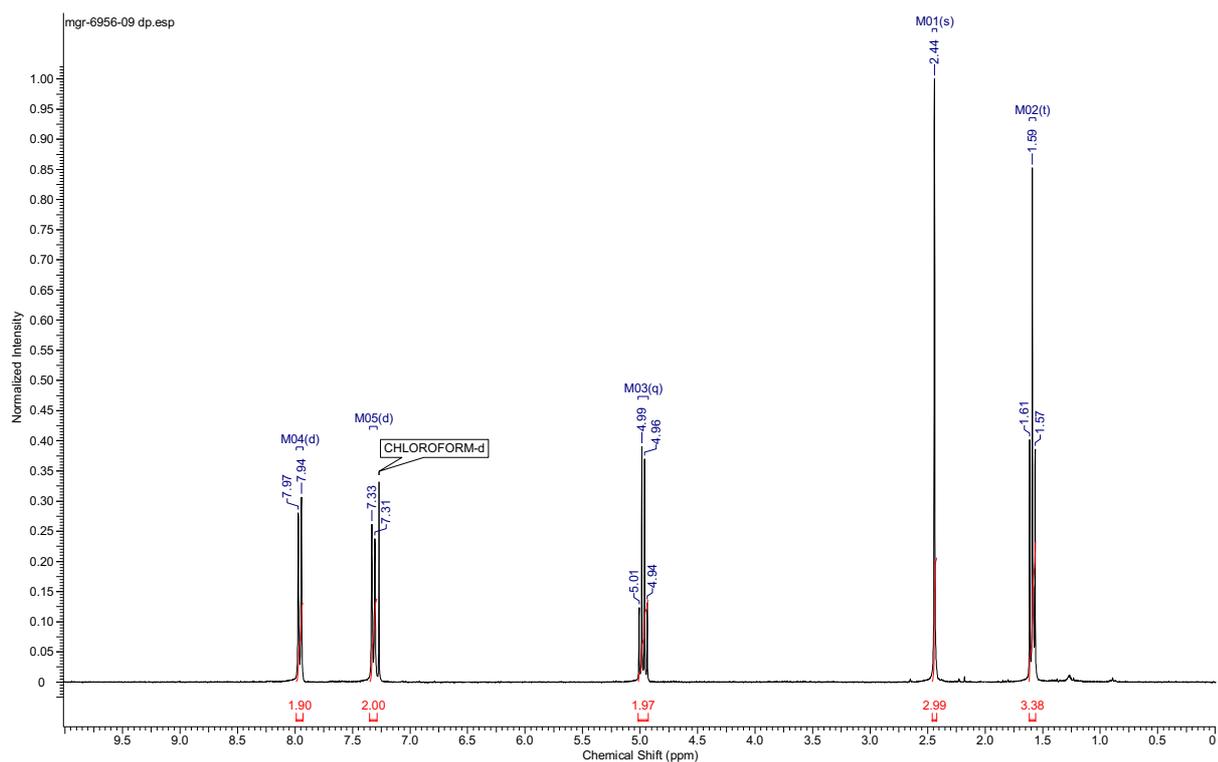


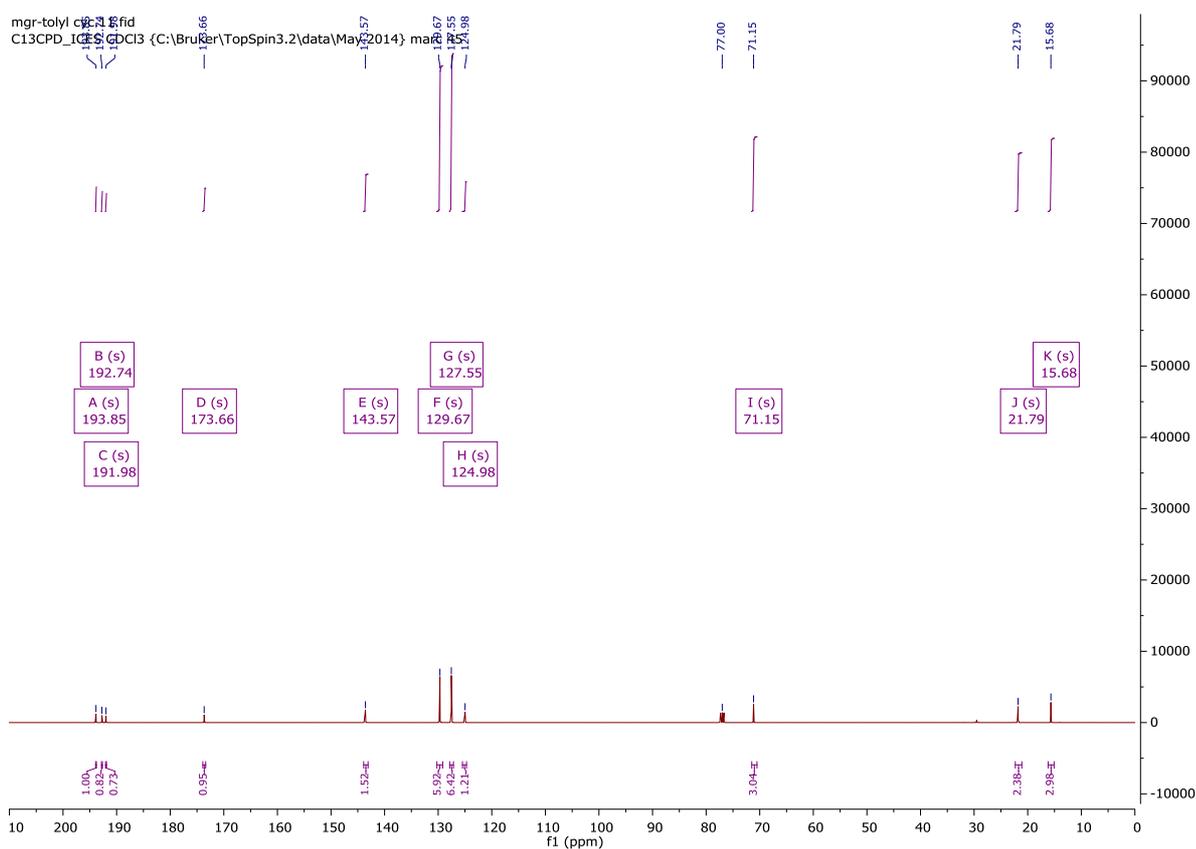


**3-Ethoxy-4-(4-(trimethylsilyl)phenyl)cyclobut-3-ene-1,2-dione, 8g.** Adapting a procedure by Moore *et al.*<sup>30</sup> To a solution of 1-bromo-4-(trimethylsilyl)benzene (0.72 mL, 3.68 mmol) in THF (20 mL) at  $-78$  °C was added  $n$ BuLi (2.44 M in hexane, 1.90 mL, 4.64 mmol). After 20 min a solution of diethyl squarate **6b** (600 mg, 3.53 mmol) in THF (20 mL) at  $-78$  °C was added over 20 min. After 1 h 40 min at  $-78$  °C, TFAA (0.64 mL, 4.60 mmol) was added followed after 50 min by sat.  $\text{NH}_4\text{Cl}$  (30 mL). The reaction mixture was then warmed to RT and sat.  $\text{NaHCO}_3$  (40 mL) was added. The aqueous phase was extracted with  $\text{CHCl}_3$  (4 x 30 mL), and the organic phases were combined, dried over  $\text{MgSO}_4$ , filtered and concentrated *in vacuo*. Purification by column chromatography (0-6% EtOAc/cyclohexane) afforded the title compound (385 mg, 1.40 mmol, 40%) as a dark yellow solid; MP: 66-67 °C; IR: 2956 (m), 2893 (w), 1782 (s), 1755 (s), 1687 (w), 1603 (s), 1590 (s), 1500 (m), 1472 (w), 1411 (s), 1383 (s), 1345 (s), 1249 (m), 1149 (m), 1102 (m), 1027 (m), 843 (s), 831 (s);  $^1\text{H}$  NMR (400 MHz,  $\text{CDCl}_3$ ):  $\delta$  = 8.01 (d,  $J$  = 8.0 Hz, 2H, 2xArH), 7.66 (d,  $J$  = 8.2 Hz, 2H, 2xArH), 4.99 (q,  $J$  = 7.1 Hz, 2H,  $\text{CH}_2\text{CH}_3$ ), 1.59 (t,  $J$  = 7.1 Hz, 3H,  $\text{CH}_2\text{CH}_3$ ), 0.31 (s, 9H,  $\text{Si}(\text{CH}_3)_3$ ) ppm;  $^{13}\text{C}$  NMR (101 MHz,  $\text{CDCl}_3$ ):  $\delta$  = 194.6 (C), 192.8 (C), 192.5 (C), 174.1 (C), 147.2 (C), 133.9 (CH) 127.7 (C), 126.5 (CH), 71.4 ( $\text{CH}_2$ ), 15.9 ( $\text{CH}_3$ ), -1.4 ( $\text{Si}(\text{CH}_3)_3$ ) ppm; LRMS (ESI+)  $m/z$ ; 275 ( $[\text{M}+\text{H}]^+$ ); HRMS (ESI+)  $m/z$  Found 297.0916,  $\text{C}_{15}\text{H}_{18}\text{O}_3\text{SiNa}$   $[\text{M}+\text{Na}]^+$  requires 297.0917.

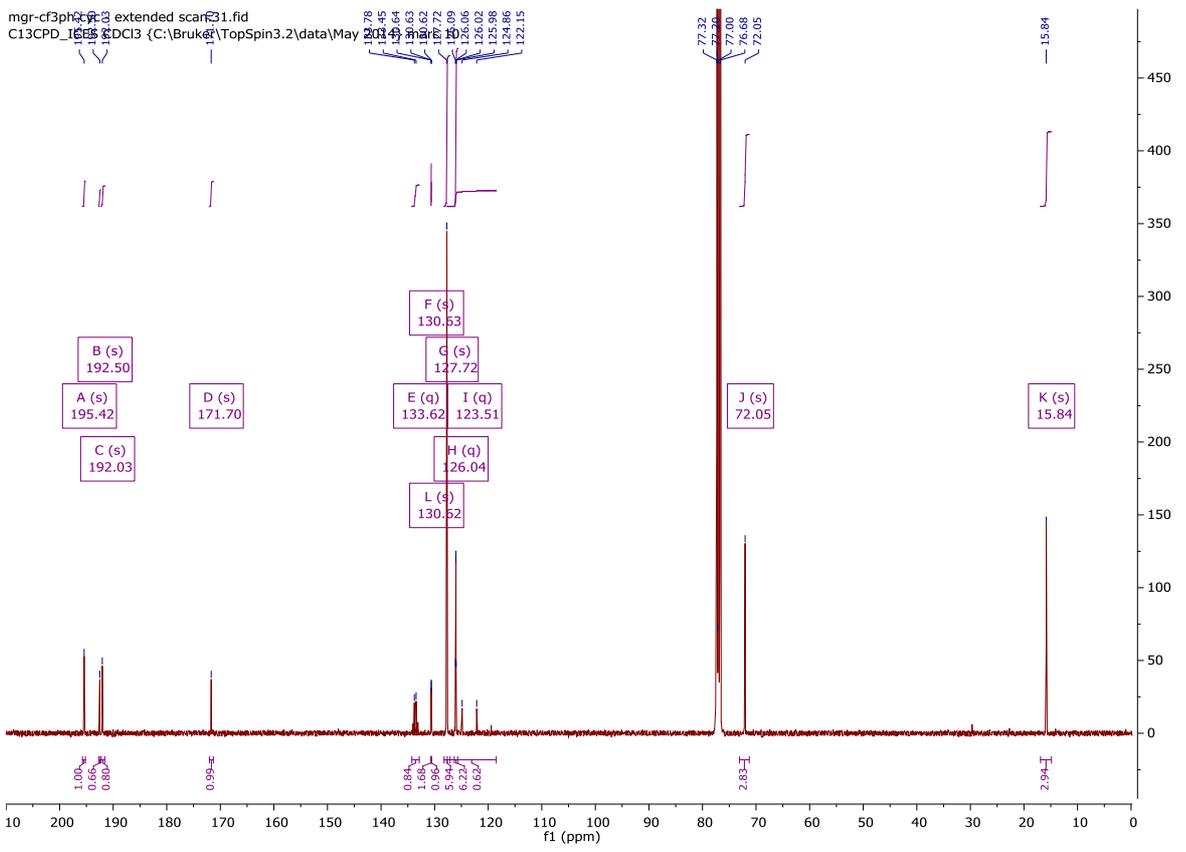
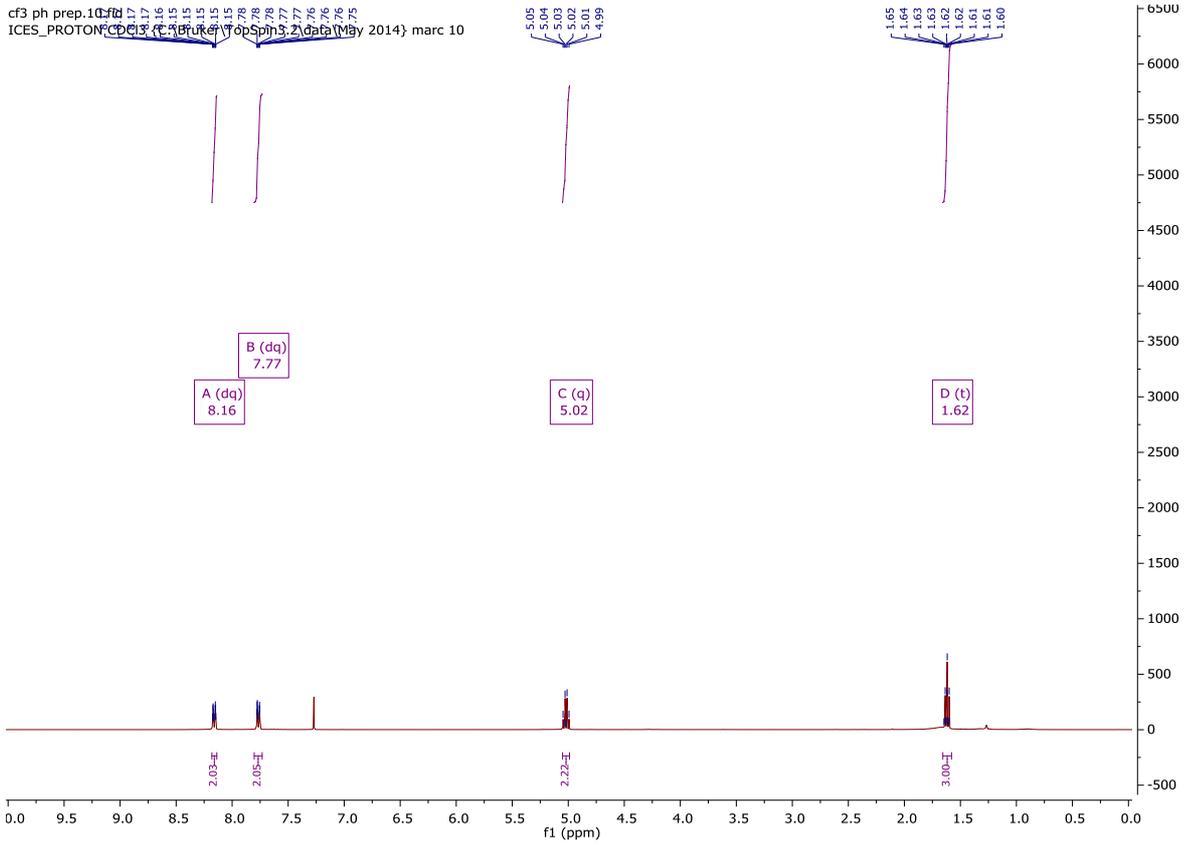


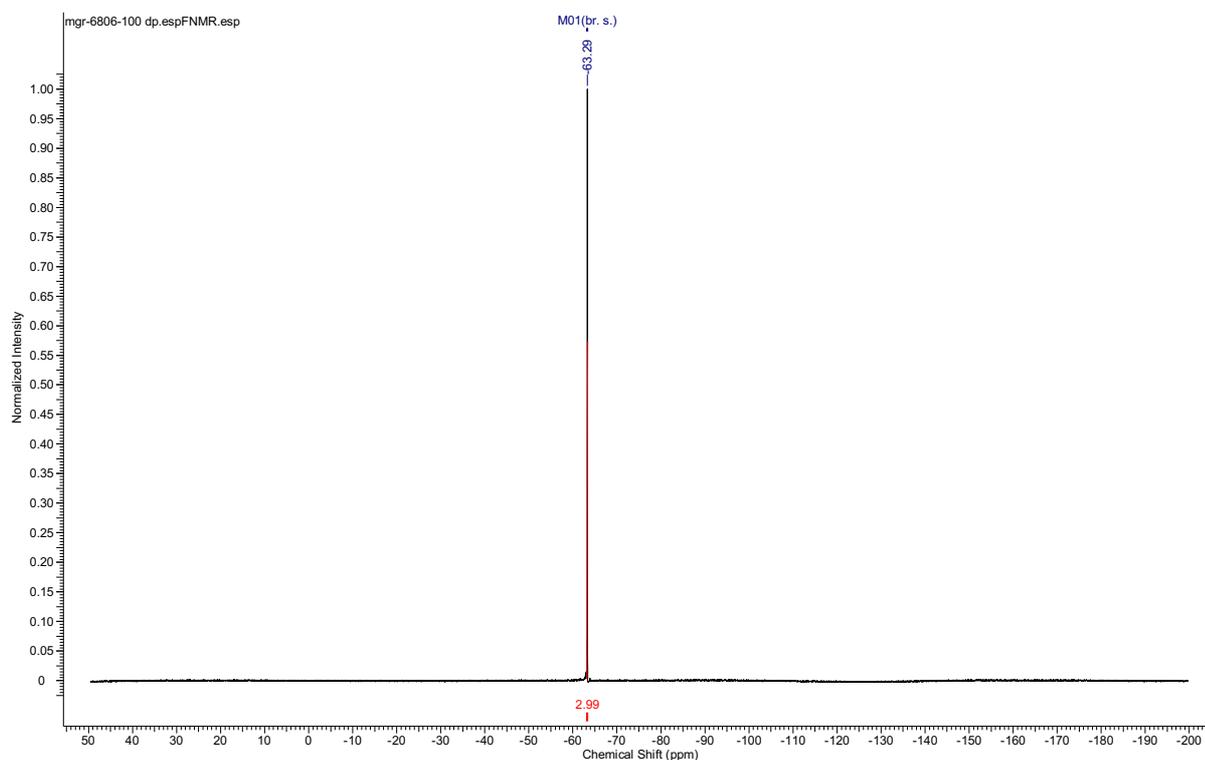
**3-Ethoxy-4-tolylcyclobut-3-ene-1,2-dione, 8h.** Adapting a procedure by Moore *et al.*<sup>30</sup> To a solution of 4-bromotoluene (0.26 g, 1.54 mmol) in THF (6 mL) at  $-78$  °C was added  $n$ BuLi (2.33 M in hexane, 0.78 mL, 1.82 mmol). After 0.5 h, diethyl squarate **6b** (0.22 g, 1.28 mmol) in THF (6 mL) was added. After 1 h, TFAA (0.24 mL, 1.69 mmol) was added dropwise over 2 min followed by sat. NaHCO<sub>3</sub> (15 mL) 10 min later. The resultant mixture was warmed to RT and the aqueous phase separated and extracted with Et<sub>2</sub>O (3 x 20 mL). The organic phases were combined, washed with sat. NaHCO<sub>3</sub> (20 mL) and brine (20 mL), dried over MgSO<sub>4</sub>, filtered and concentrated *in vacuo* and purified by flash chromatography (5-15% EtOAc/petrol) to afford the title compound (0.12g, 0.56 mmol, 44%) as a yellow solid; MP: 123-129 °C; IR:  $\nu$  2361 (s), 2340 (m), 2159 (m), 1777 (s), 1736 (s), 1587 (s), 1510 (m), 1386 (m), 1350 (s), 830 (m), 798 (m), 462 (s); <sup>1</sup>H NMR (300 MHz, CDCl<sub>3</sub>):  $\delta$  = 7.96 (2 H, d,  $J$  = 8.4 Hz, ArH), 7.32 (2 H, d,  $J$  = 8.1 Hz, ArH), 4.97 (2 H, q,  $J$  = 7.0 Hz, CH<sub>2</sub>CH<sub>3</sub>), 2.44 (3 H, s, CH<sub>3</sub>), 1.59 (4 H, t,  $J$  = 7.1 Hz, CH<sub>2</sub>CH<sub>3</sub>) ppm; <sup>13</sup>C NMR (101 MHz, CDCl<sub>3</sub>):  $\delta$  = 193.9 (C), 192.7 (C), 192.0 (C), 173.7 (C), 143.6 (C), 129.7 (CH), 127.6 (CH), 125.0 (C), 71.2 (CH<sub>2</sub>), 21.8 (CH<sub>3</sub>), 15.7 (CH<sub>3</sub>) ppm; LRMS (EI)  $m/z$ ; 216 ([M]<sup>+</sup>); HRMS (ESI+)  $m/z$  Found 217.0864, C<sub>13</sub>H<sub>13</sub>O<sub>3</sub> [M+H]<sup>+</sup> requires 217.0859.



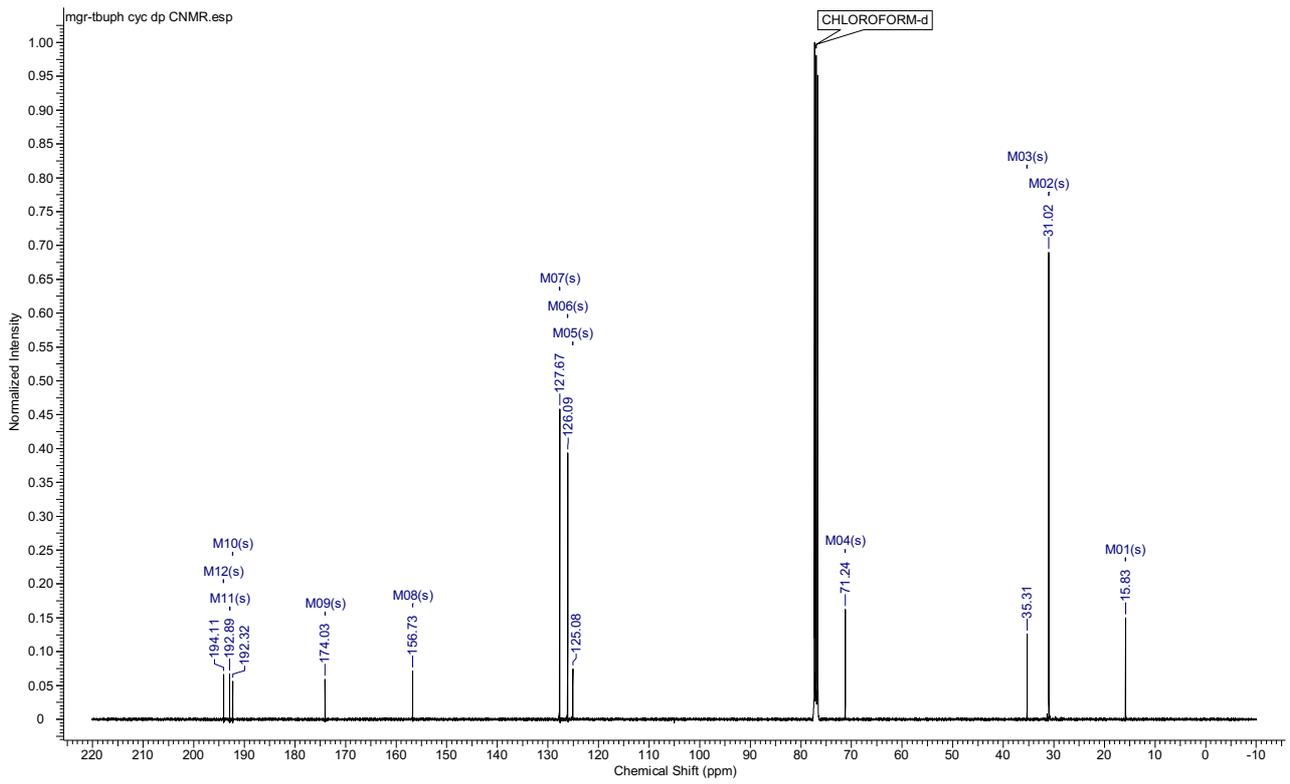
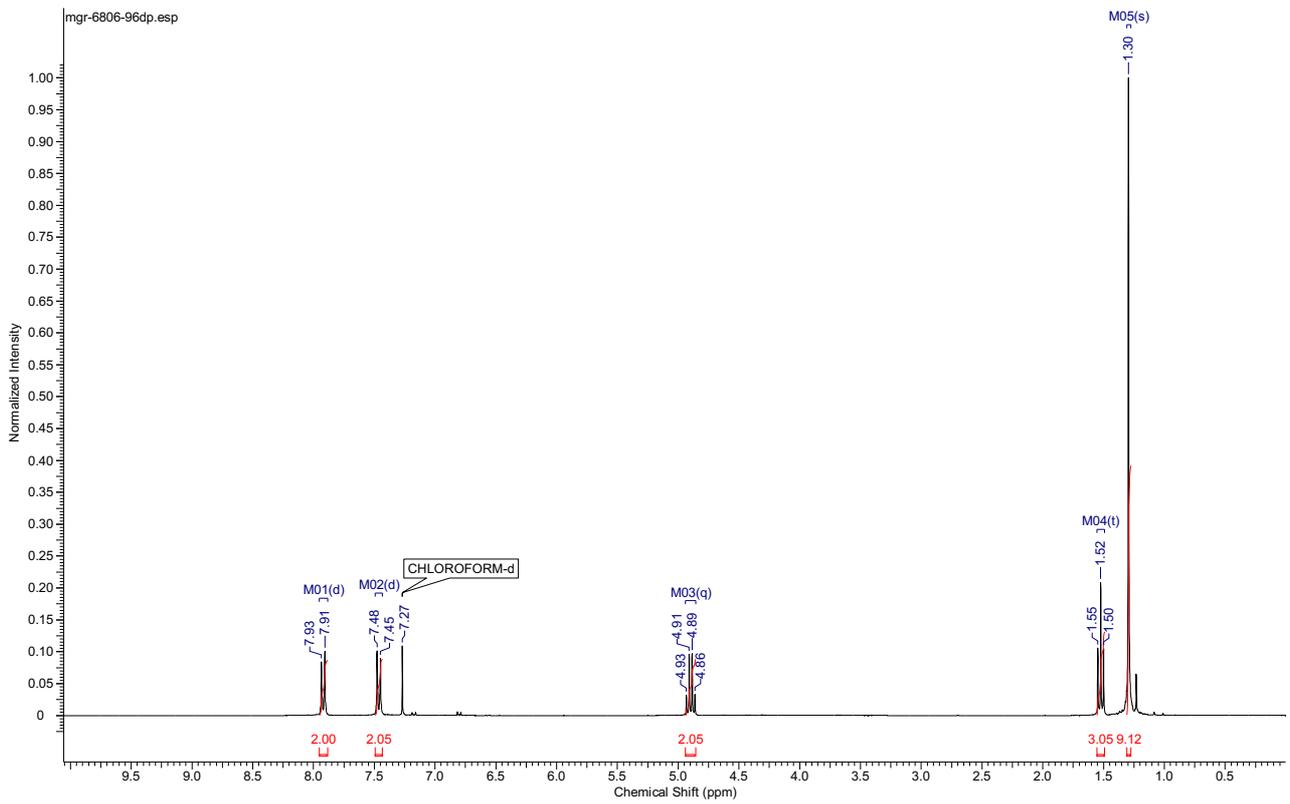


**3-Ethoxy-4-(4-(trifluoromethyl)phenyl)cyclobut-3-ene-1,2-dione, **8i**.** Adapting a procedure by Moore *et al.*<sup>30</sup> To a solution of <sup>t</sup>BuLi (2.00 M in heptane, 2.87 mL, 5.75 mmol) in THF (5 mL) at  $-78$  °C was added 4-bromobenzotrifluoride (0.65 g, 2.87 mmol) in THF (10 mL). After 80 min, a solution of diethyl squarate **6b** (0.48 g, 2.82 mmol) in THF (20 mL) was added over 12 min followed after 1 h by TFAA (0.48 mL, 3.4 mmol). After a further 10 min sat. NaHCO<sub>3</sub> (30 mL) was added and the resulting mixture was warmed to RT. The aqueous phase was separated and extracted with Et<sub>2</sub>O (3 x 15 mL) then the organic phases were combined, washed with brine (30 mL), dried over MgSO<sub>4</sub>, filtered and concentrated *in vacuo* and purified by column chromatography (0–10% EtOAc/petrol) to afford the title compound (0.18 g, 0.65 mmol, 23%) as a yellow solid; MP: 116–120 °C; IR: 2992 (w), 2942 (w), 1786 (m), 1736 (m), 1598 (m), 1409 (m), 1385 (m), 1384 (m), 1350 (m), 1317 (s), 1171 (m), 1118 (s), 1064 (s), 1018 (m), 990 (m), 855 (m); <sup>1</sup>H NMR (300 MHz, CDCl<sub>3</sub>):  $\delta$  = 8.16 (2 H, br. d,  $J$  = 7.9 Hz, ArH), 7.77 (2 H, br. d,  $J$  = 8.0 Hz, ArH), 5.02 (2 H, q,  $J$  = 7.3 Hz, CH<sub>2</sub>CH<sub>3</sub>), 1.62 (3 H, t,  $J$  = 7.1 Hz, CH<sub>2</sub>CH<sub>3</sub>) ppm; <sup>13</sup>C NMR (101 MHz, CDCl<sub>3</sub>):  $\delta$  = 195.4 (C), 192.5 (C), 192.0 (C), 171.7 (C), 133.6 (q, <sup>2</sup> $J_{C-F}$  = 33.2 Hz, C), 130.6 (q, <sup>4</sup> $J_{C-F}$  = 1.4 Hz, C), 127.7 (CH), 126.0 (q, <sup>3</sup> $J_{C-F}$  = 4.2 Hz, CH), 123.5 (q, <sup>1</sup> $J_{C-F}$  = 272.7 Hz, CF<sub>3</sub>), 72.0 (CH<sub>2</sub>), 15.8 (CH<sub>3</sub>) ppm; MS (EI)  $m/z$ : 270 ([M]<sup>+</sup>); HRMS (ESI+)  $m/z$  (%): Found 271.0577, C<sub>13</sub>H<sub>10</sub>F<sub>3</sub>O<sub>3</sub> [M+H]<sup>+</sup> requires 271.0577.

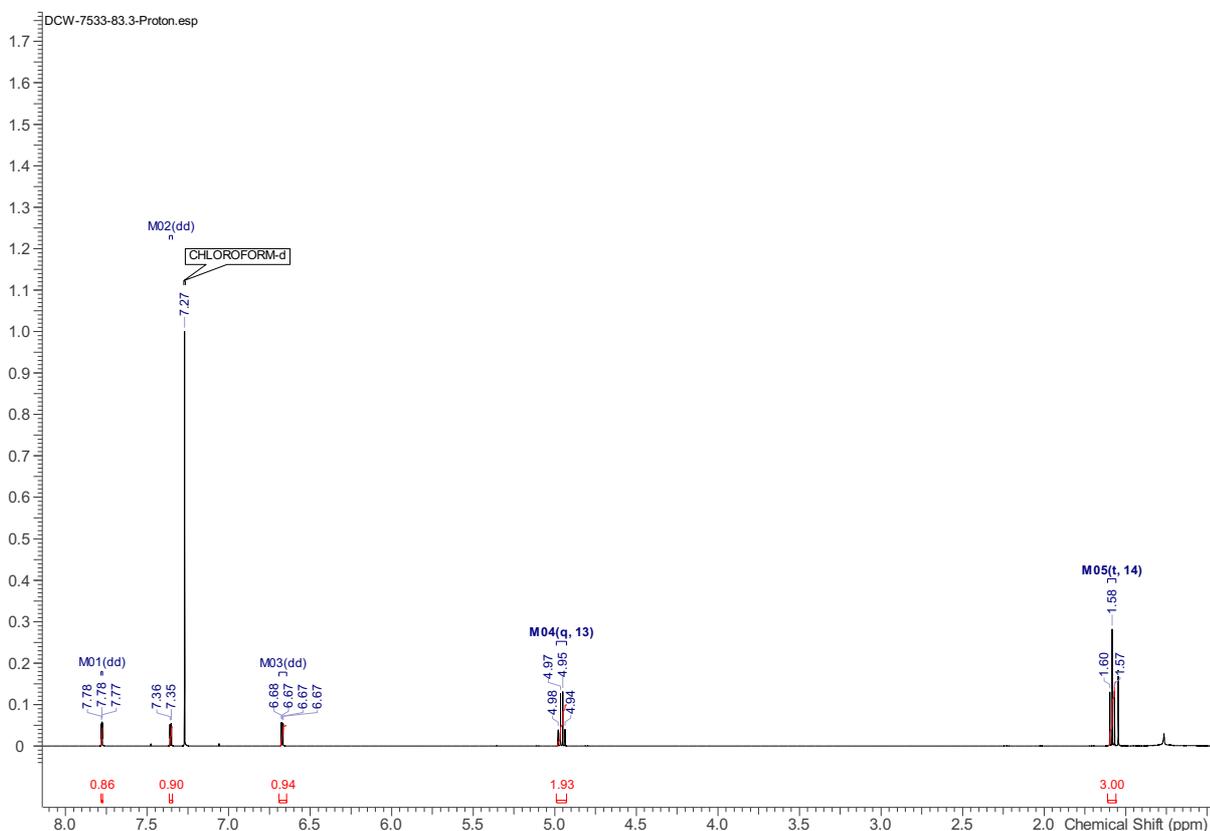


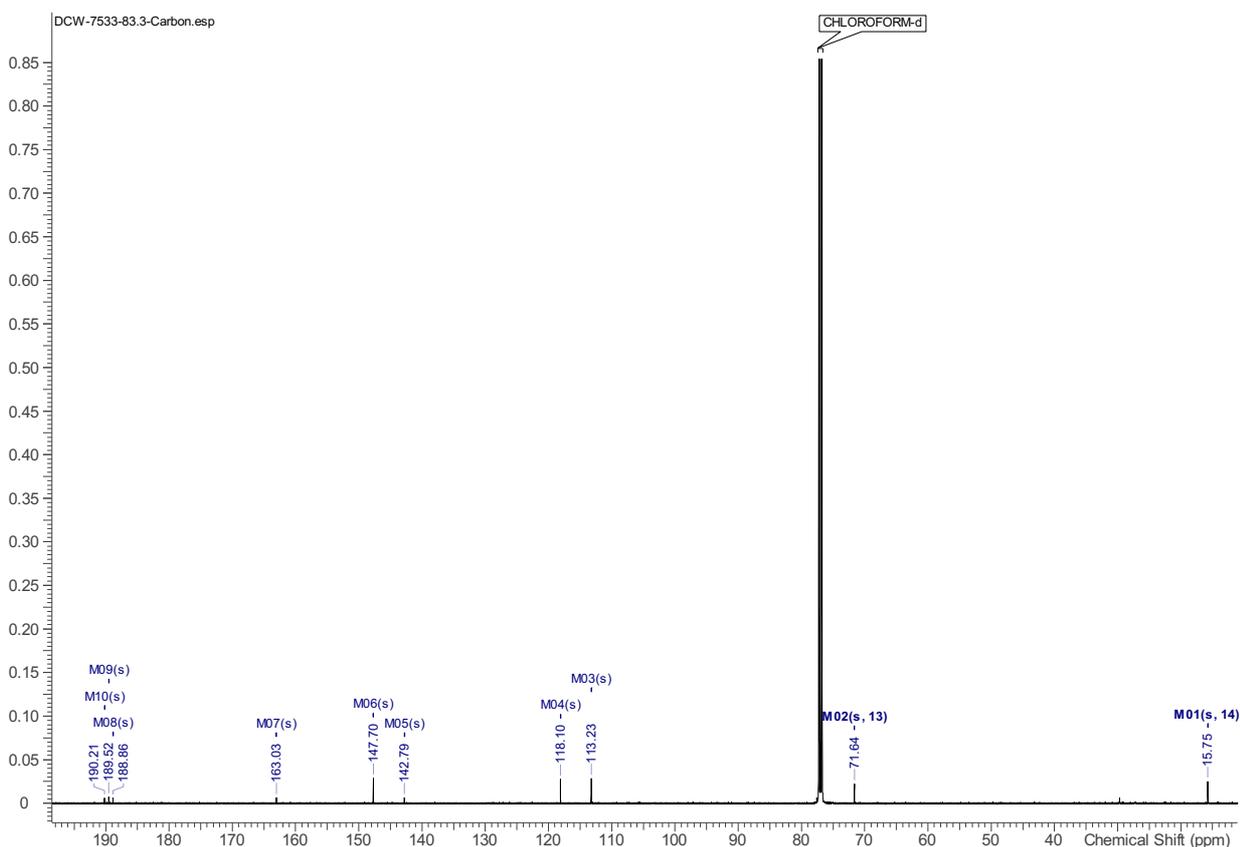


**3-(4-(*tert*-Butyl)phenyl)-4-ethoxycyclobut-3-ene-1,2-dione, 8j.** Adapting a procedure by Moore *et al.*<sup>30</sup> To a solution of 1-bromo-4-*tert*-butylbenzene (0.52 g, 2.43 mmol) in THF (10 mL) at  $-78\text{ }^{\circ}\text{C}$  was added  ${}^n\text{BuLi}$  (2.33 M in hexane, 1.3 mL, 3.03 mmol) over 1 min. After 40 min, a solution of diethyl squarate **6b** (0.34 g, 2.01 mmol) in THF (10 mL) was added followed after 1 h by TFAA (0.41 mL, 2.95 mmol). After a further 10 min sat.  $\text{NaHCO}_3$  (30 mL) was added and the resulting mixture was warmed to RT. The aqueous phase separated and extracted with  $\text{Et}_2\text{O}$  (3 x 15 mL) then the organic phases were combined, washed with brine (30 mL), dried over  $\text{MgSO}_4$ , filtered and concentrated *in vacuo* and purified by column chromatography (0–10% EtOAc/petrol) to afford the title compound (256 mg, 0.99 mmol, 49%) as a yellow solid; MP: 112–117  $^{\circ}\text{C}$ ; IR:  $\nu$  2965 (w), 2901 (w), 2868 (w), 1781 (s), 1742 (m), 1589 (s), 1405 (m), 1382 (m), 1343 (m), 1315 (m), 1027 (m), 908 (m), 845 (m), 728 (s);  ${}^1\text{H}$  NMR (300 MHz,  $\text{CDCl}_3$ ):  $\delta$  = 7.92 (2 H, d,  $J$  = 8.1 Hz, ArH), 7.46 (2 H, d,  $J$  = 8.4 Hz, ArH), 4.90 (2 H, q,  $J$  = 7.0 Hz,  $\text{CH}_2\text{CH}_3$ ), 1.52 (3 H, t,  $J$  = 7.1 Hz,  $\text{CH}_2\text{CH}_3$ ), 1.30 (9 H, s,  $\text{C}(\text{CH}_3)_3$ ) ppm;  ${}^{13}\text{C}$  NMR (101 MHz,  $\text{CDCl}_3$ ):  $\delta$  = 194.1 (C), 192.9 (C), 192.3 (C), 174.0 (C), 156.7 (C), 127.7 (CH), 126.1 (CH), 125.1 (C), 71.2 ( $\text{CH}_2$ ), 35.3 (C), 31.0 ( $\text{CH}_3$ ), 15.8 ( $\text{CH}_3$ ) ppm; MS (ESI+)  $m/z$  (%): 259 ( $[\text{M}+\text{H}]^+$ ); HRMS (ESI+)  $m/z$  Found 259.1329,  $\text{C}_{16}\text{H}_{19}\text{O}_3$   $[\text{M}+\text{H}]^+$  requires 259.1329.



**3-Ethoxy-4-(furan-2-yl)cyclobut-3-ene-1,2-dione, 8k.** Adapting a procedure by Moore *et al.*<sup>33</sup> To a solution of furan (1.60 mL, 21.7 mmol) in THF (35 mL) at  $-15\text{ }^{\circ}\text{C}$  was added  $n\text{BuLi}$  (2.44 M in hexanes, 2.40 mL, 5.89 mmol) dropwise over 30 min. The reaction mixture was warmed to  $0\text{ }^{\circ}\text{C}$  and after 2 h was added via cannula over 20 min to a solution of diethyl squarate **6b** (1.01 g, 5.96 mmol) in THF (150 mL) at  $-78\text{ }^{\circ}\text{C}$ . After 1 h sat.  $\text{NH}_4\text{Cl}$  (20 mL) was added and the reaction mixture was warmed to RT. The aqueous phase was separated and extracted with DCM (4 x 30 mL) then the organic phases were combined, dried over  $\text{MgSO}_4$ , filtered and concentrated *in vacuo*. The residue was dissolved in THF (30 mL), cooled to  $-78\text{ }^{\circ}\text{C}$  and TFAA (2.25 mL, 16.2 mmol) was added. After 2.5 h sat.  $\text{NH}_4\text{Cl}$  (20 mL) and sat.  $\text{NaHCO}_3$  (20 mL) were added and the reaction mixture was warmed to RT. The aqueous phase was separated and extracted with DCM (4 x 30 mL) then the organic phases were combined, dried over  $\text{MgSO}_4$ , filtered, concentrated *in vacuo* and purified by column chromatography (60–100%  $\text{CHCl}_3/\text{cyclohexane}$ ) to afford the title compound (334 mg, 1.22 mmol, 29%) as a yellow solid; MP:  $95\text{--}97\text{ }^{\circ}\text{C}$ ; IR: 2923 (s), 2853 (m), 1788 (s), 1748 (s), 1611 (s), 1541 (s), 1500 (m), 1475 (s), 1457 (s), 1341 (s), 1251 (m), 1013 (s);  $^1\text{H}$  NMR (500 MHz,  $\text{CDCl}_3$ ):  $\delta = 7.78$  (1 H, dd,  $J = 1.8, 0.7$  Hz, ArH), 7.36 (1 H, dd,  $J = 3.6, 0.7$  Hz, ArH), 6.67 (1 H, dd,  $J = 3.5, 1.7$  Hz, ArH), 4.96 (2 H, q,  $J = 7.1$  Hz,  $\text{OCH}_2$ ), 1.58 (3 H, t,  $J = 7.0$  Hz,  $\text{CH}_3$ ) ppm;  $^{13}\text{C}$  NMR (125 MHz,  $\text{CDCl}_3$ ):  $\delta = 190.2$  (C), 189.5 (C), 188.9 (C), 163.0 (C), 147.7 (CH), 142.8 (C), 118.1 (CH), 113.2 (CH), 71.6 ( $\text{CH}_2$ ), 15.8 ( $\text{CH}_3$ ) ppm; MS (ESI+)  $m/z$  (%): 193 ( $[\text{M}+\text{H}]^+$ ); HRMS (ESI+)  $m/z$  Found 215.0314,  $\text{C}_{10}\text{H}_8\text{O}_4\text{Na}$   $[\text{M}+\text{Na}]^+$  requires 215.0315.

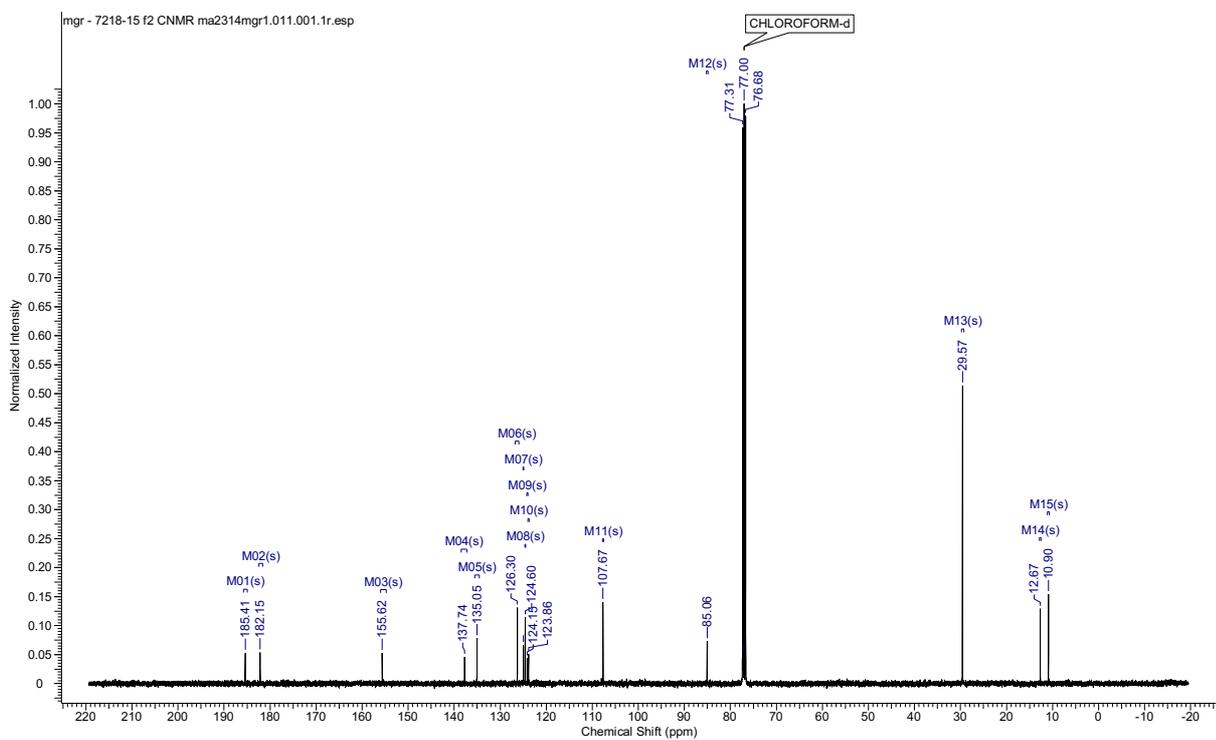
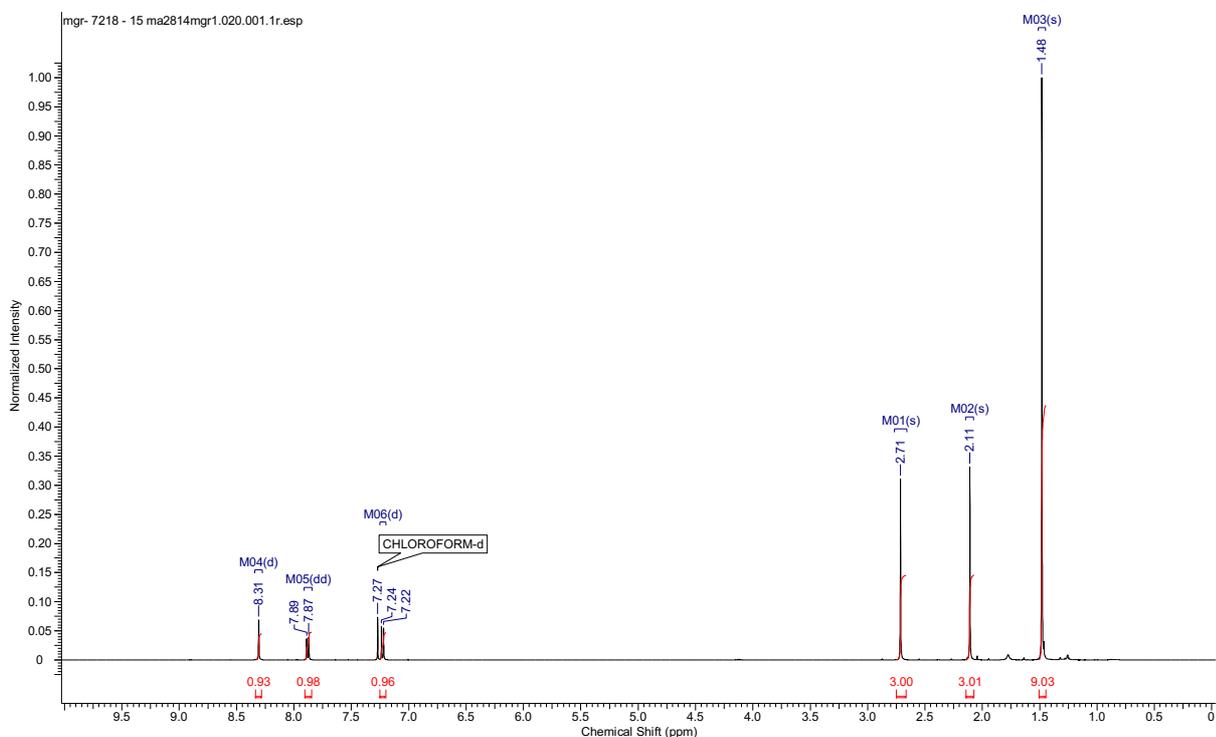




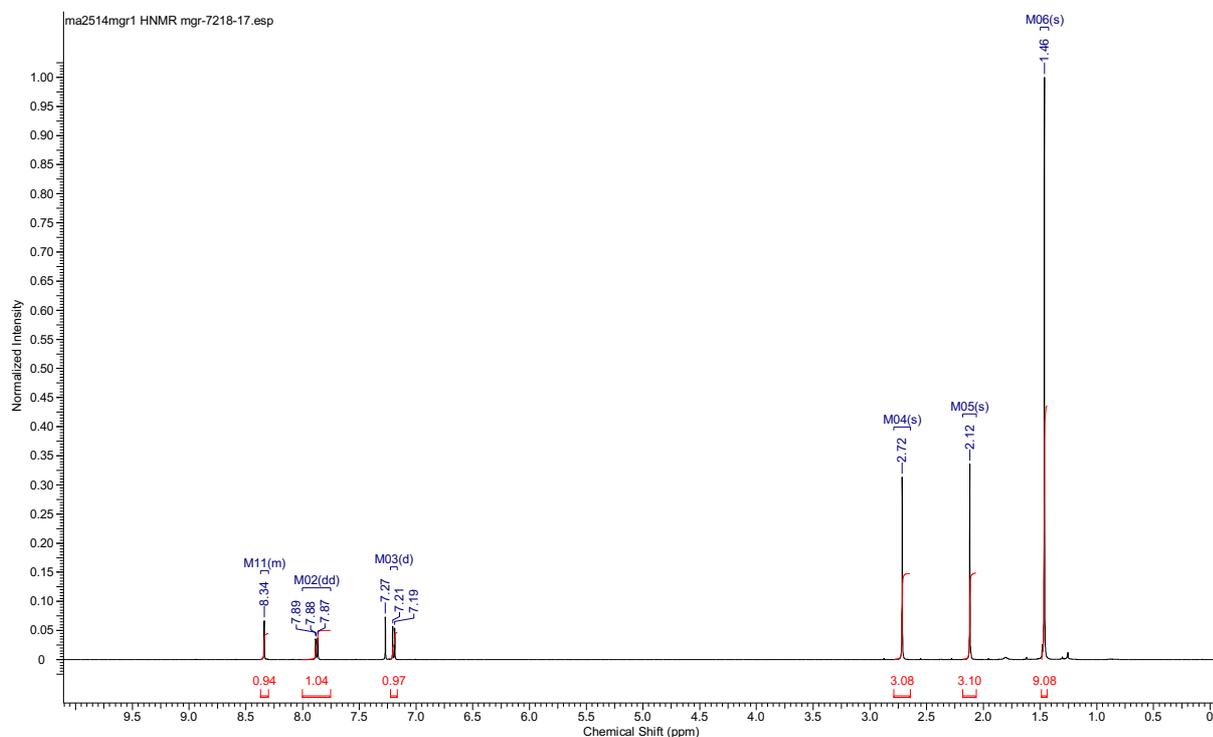
8-bromo-3-methylimidazo[5,1-a]pyridine and the following analogues **A-K** were prepared by adapting a procedure by Harrowven *et al.*<sup>20,22</sup>

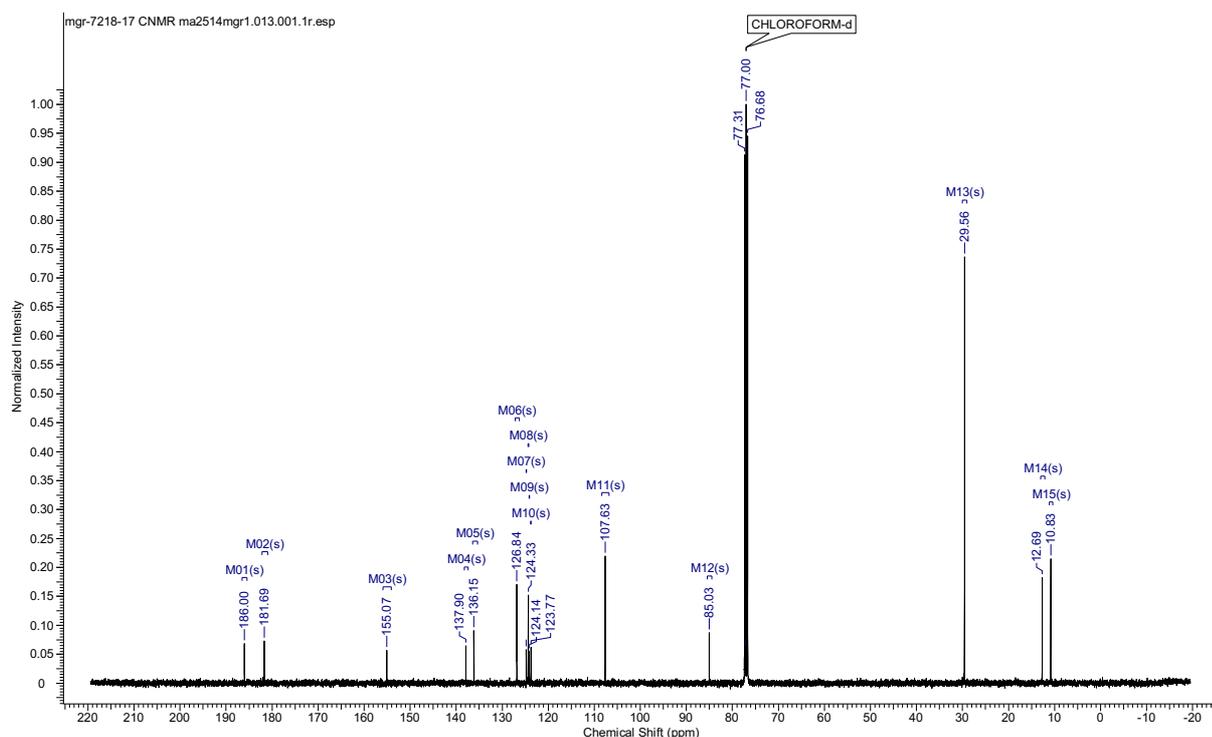
**8-(*tert*-Butoxy)-3,9-dimethylimidazo[5,1-a]isoquinoline-7,10-dione, Analogue A.** To a solution of *t*BuLi (1.55 M in Bu<sub>2</sub>O, 0.58 mL, 1.11 mmol) in THF (1 mL) at -78 °C was added 8-bromo-3-methylimidazo[5,1-a]pyridine (111 mg, 0.53 mmol) in THF (2 mL) dropwise over 1 min. After 30 min, the solution was then added via cannula to a solution of ytterbium triflate (299 mg, 0.48 mmol) in THF (5 mL) at -78 °C over 4 min. After 1 h, 3-*t*-butoxy-4-methylcyclobut-3-ene-1,2-dione (77.9 mg, 0.46 mmol) in THF (3 mL) was added dropwise over 1 min, followed after 1 h by sat. NaHCO<sub>3</sub> (10 mL). The resulting solution was warmed to RT, DCM (15 mL) was added then the aqueous phase was separated and extracted with DCM (2 × 15 mL). The organic phases were combined, washed with brine (2 × 15 mL), dried over MgSO<sub>4</sub>, filtered and concentrated *in vacuo* to a brown oil (36.7 mg, 0.12 mmol). Part of this crude material (21.3 mg) was dissolved in 1,4-dioxane (1.7 mL) and heated under continuous flow at 110 °C in stainless-steel tubing for a residence time of 1 h using a Vapourtec R4/R2+ device. The resulting solution was then stirred under air for 30 min, concentrated *in vacuo* and purified by column chromatography (0-10% MeOH/DCM with 1% aq. NH<sub>3</sub> solution) to give the title compound (12.2 mg, 0.07 mmol, 16%) as a dark blue solid MP: 161–164 °C; IR:  $\nu$  2977 (w), 2927 (w), 1658 (s), 1619 (m), 1603 (s), 1530 (m), 1369 (s), 1326 (m), 1287 (s), 1216 (m), 1149 (s), 1072 (m), 1013 (m), 840 (m), 793 (m), 727 (m); <sup>1</sup>H NMR (400 MHz, CDCl<sub>3</sub>):  $\delta$  = 8.34 (1 H, d, *J* = 0.9 Hz, ArH), 7.88 (1 H, dd, *J* = 7.3, 0.6 Hz, ArH), 7.20 (1 H, d, *J* = 7.3 Hz,

ArH), 2.72 (3 H, s, CH<sub>3</sub>), 2.12 (3 H, s, CH<sub>3</sub>), 1.46 (9 H, s, C(CH<sub>3</sub>)<sub>3</sub>) ppm; <sup>13</sup>C NMR (101 MHz, CDCl<sub>3</sub>): δ = 186.0 (C), 181.7 (C), 155.0 (C), 137.9 (C), 136.2 (C), 126.8 (CH), 124.8 (C), 124.3 (CH), 124.1 (C), 123.8 (C), 107.6 (CH), 85.0 (C), 29.6 (C(CH<sub>3</sub>)<sub>3</sub>), 12.7 (CH<sub>3</sub>), 10.8 (CH<sub>3</sub>) ppm; MS (ESI+) *m/z* (%): 299 [M+H]<sup>+</sup>; HRMS (ESI+) *m/z* Found 299.1392, C<sub>17</sub>H<sub>19</sub>N<sub>2</sub>O<sub>3</sub> [M+H]<sup>+</sup> requires 299.1390.

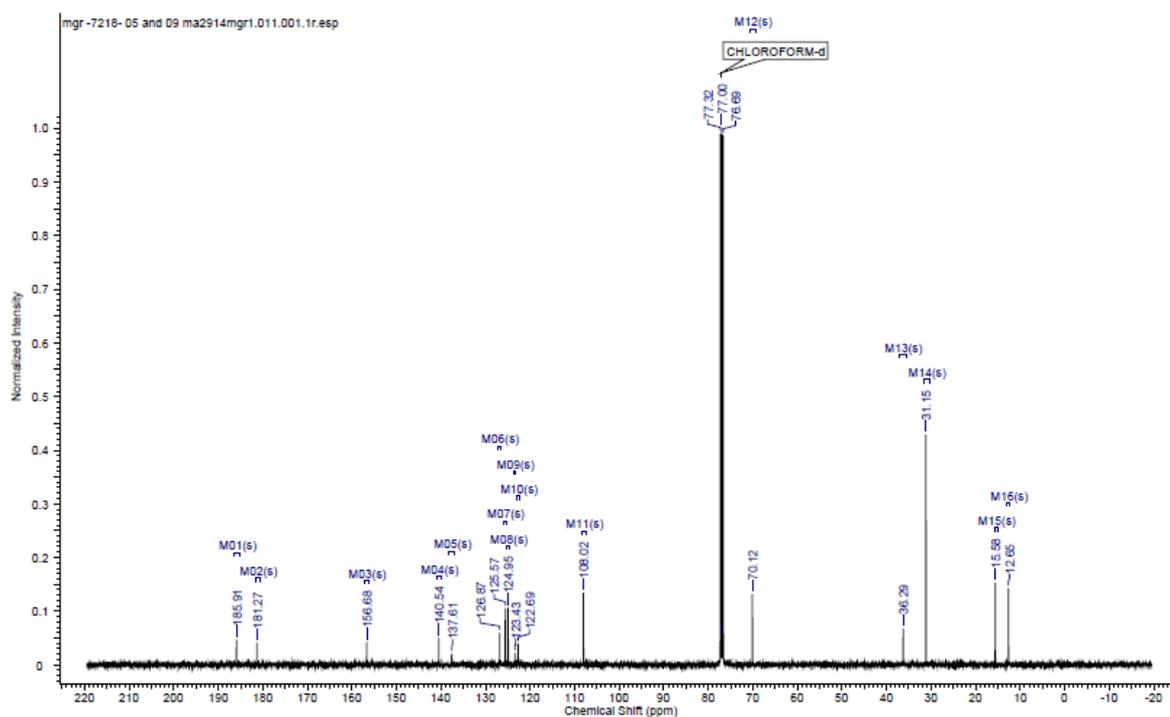
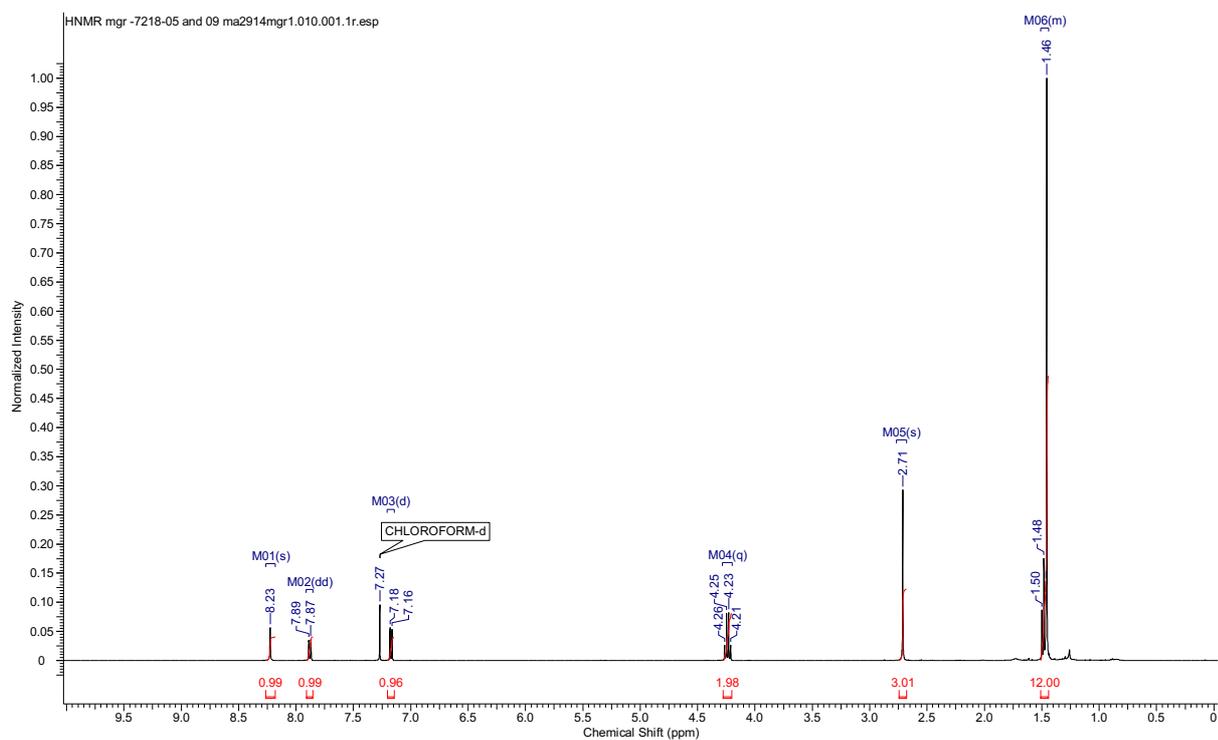


**9-(*tert*-Butoxy)-3,8-dimethylimidazo[5,1-*a*]isoquinoline-7,10-dione, Analogue B.** To a solution of <sup>t</sup>BuLi (1.55 M in Bu<sub>2</sub>O, 0.56 mL, 1.07 mmol) in THF (1 mL) at -78 °C was added 8-bromo-3-methylimidazo[5,1-*a*]pyridine (107 mg, 0.51 mmol) in THF (2 mL) dropwise over 1 min. After 30 min, 3-*t*-butoxy-4-methylcyclobut-3-ene-1,2-dione (83.1 mg, 0.49 mmol) in THF (3 mL) was added dropwise over 5 min, followed after 1 h by sat. NaHCO<sub>3</sub> (10 mL). The resulting solution was warmed to RT, DCM (15 mL) was added then the aqueous phase was separated and extracted with DCM (2 × 15 mL). The organic phases were combined, washed with brine (2 × 15 mL), dried over MgSO<sub>4</sub>, filtered and concentrated *in vacuo* to a brown oil (24.7 mg, 0.08 mmol). This crude material was then dissolved in 1,4-dioxane (1.6 mL) and heated under continuous flow at 110 °C in stainless-steel tubing for a residence time of 1 h using a Vapourtec R4/R2+ device. The resulting solution was stirred under air for 30 min then concentrated *in vacuo* and purified by column chromatography (0-10% MeOH/DCM with 1% aq. NH<sub>3</sub> solution) to give the title compound (11.6 mg, 0.04 mmol, 8%) as a dark blue solid MP: 187–190 °C; IR:  $\nu$  2977 (w), 2927 (w), 1668 (m), 1641 (s), 1621 (s), 1603 (m), 1530 (m), 1461 (m), 1367 (s), 1332 (m), 1304 (s), 1289 (s), 1141 (s), 1090 (s), 986 (m), 863 (s), 820 (m), 732 (m), 714 (m); <sup>1</sup>H NMR (400 MHz, CDCl<sub>3</sub>):  $\delta$  = 8.31 (1 H, d, *J* = 0.7 Hz, ArH), 7.88 (1 H, d, *J* = 7.3, 0.6 Hz, ArH), 7.23 (1 H, *J* = 7.3 Hz, ArH), 2.71 (3 H, s, CH<sub>3</sub>), 2.11 (3 H, s, CH<sub>2</sub>), 1.47 (9 H, s, C(CH<sub>3</sub>)<sub>3</sub>) ppm; <sup>13</sup>C NMR (101 MHz, CDCl<sub>3</sub>):  $\delta$  = 185.4 (C), 182.2 (C), 155.6 (C), 137.7 (C), 135.1 (C), 126.3 (CH), 125.0 (C), 124.6 (CH), 124.2 (C), 123.9 (C), 107.7 (CH), 85.1 (C), 29.6 (C(CH<sub>3</sub>)<sub>3</sub>), 12.7 (CH<sub>3</sub>), 10.9 (CH<sub>3</sub>) ppm; MS (ESI+) *m/z* (%): 299 [M+H]<sup>+</sup>; HRMS (ESI+) *m/z* Found 299.1389, C<sub>17</sub>H<sub>19</sub>N<sub>2</sub>O<sub>3</sub> [M+H]<sup>+</sup> requires 299.1390.



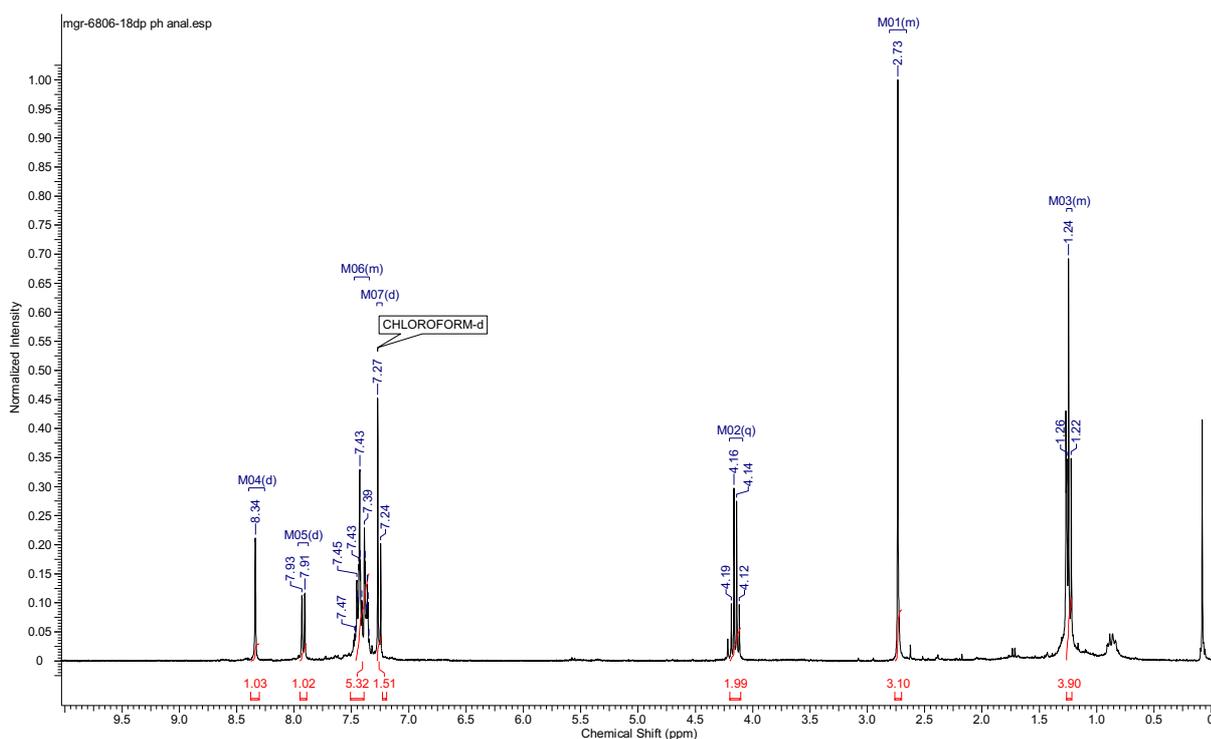


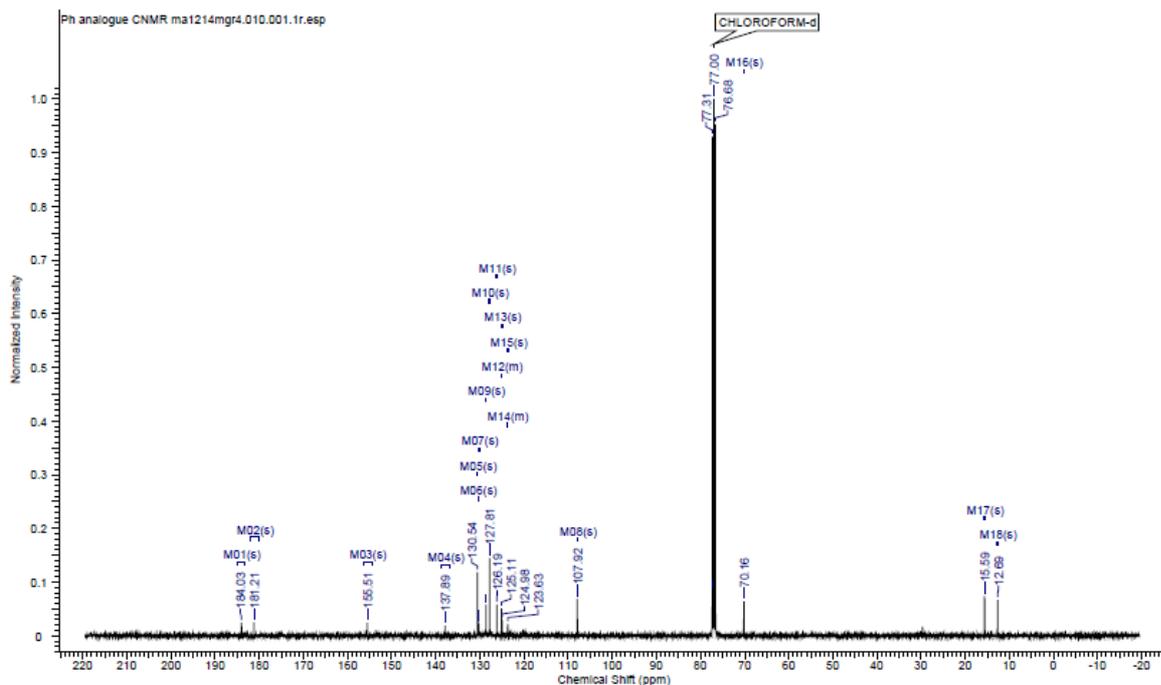
**8-(*tert*-Butyl)-9-ethoxy-3-methylimidazo[5,1-*a*]isoquinoline-7,10-dione, Analogue C.** To a solution of <sup>t</sup>BuLi (1.91 M in Bu<sub>2</sub>O, 0.55 mL, 1.05 mmol) in THF (1 mL) at –78 °C was added 8-bromo-3-methylimidazo[5,1-*a*]pyridine (106 mg, 0.50 mmol) in THF (2 mL) dropwise over 5 min. After 30 min, 4-*t*-butyl-3-ethoxycyclobut-3-ene-1,2-dione (88.5 mg, 0.49 mmol) in THF (3 mL) was added, dropwise over 5 min, followed after 1 h by sat. NaHCO<sub>3</sub> (10 mL). The resulting solution was warmed to RT, DCM (15 mL) was added then the aqueous phase was separated and extracted with DCM (2 × 15 mL). The organic phases were combined, washed with brine (2 × 15 mL), dried over MgSO<sub>4</sub>, filtered and concentrated *in vacuo* to an orange/brown solid (97.9 mg, 0.31 mmol). A sample of this crude material (77.6 mg) was then dissolved in 1,4-dioxane (6.6 mL) and heated under continuous flow at 110 °C in stainless-steel tubing for a residence time of 1 h using a Vapourtec R4/R2+ device. The resulting solution was stirred in air for 30 min then concentrated *in vacuo* and purified by column chromatography (0-10% MeOH/DCM with 1% aq. NH<sub>3</sub> solution) to give the title compound (28.2 mg, 0.09 mmol, 19%) as a burgundy red solid MP: 128–130 °C; IR:  $\nu$  2957 (m), 2925 (m), 1665 (s), 1642 (s), 1620 (s), 1538 (s), 1458 (m), 1362 (m), 1324 (m), 1284 (s), 1176 (s), 1068 (s), 877 (m), 835 (m), 816 (m), 745 (m), 664 (m); <sup>1</sup>H NMR (400 MHz, CDCl<sub>3</sub>):  $\delta$  = 8.23 (1 H, s, ArH), 7.88 (1 H, dd, *J* = 0.7, 7.4 Hz, ArH), 7.17 (1 H, d, *J* = 7.3 Hz, ArH), 4.24 (2 H, q, *J* = 7.1 Hz, CH<sub>2</sub>CH<sub>3</sub>), 2.71 (3H, s, CH<sub>3</sub>), 1.48 (3 H, t, *J* = 7.0 Hz, CH<sub>2</sub>CH<sub>3</sub>), 1.46 (9 H, s, C(CH<sub>3</sub>)<sub>3</sub>) ppm; <sup>13</sup>C NMR (101 MHz, CDCl<sub>3</sub>):  $\delta$  = 185.9 (C), 181.3 (C), 156.7 (C), 140.5 (C), 137.6 (C), 126.9 (C), 125.6 (CH), 125.0 (CH), 123.4 (C), 122.7 (C), 108.0 (CH), 70.1 (CH<sub>2</sub>), 36.3 (C), 31.2 (C(CH<sub>3</sub>)<sub>3</sub>), 15.6 (CH<sub>3</sub>), 12.7 (CH<sub>3</sub>) ppm; MS (ESI+) *m/z* (%): 313 [M+H]<sup>+</sup>; HRMS (ESI+) *m/z* Found 313.1543, C<sub>18</sub>H<sub>21</sub>N<sub>2</sub>O<sub>3</sub> [M+H]<sup>+</sup> requires 313.1547.



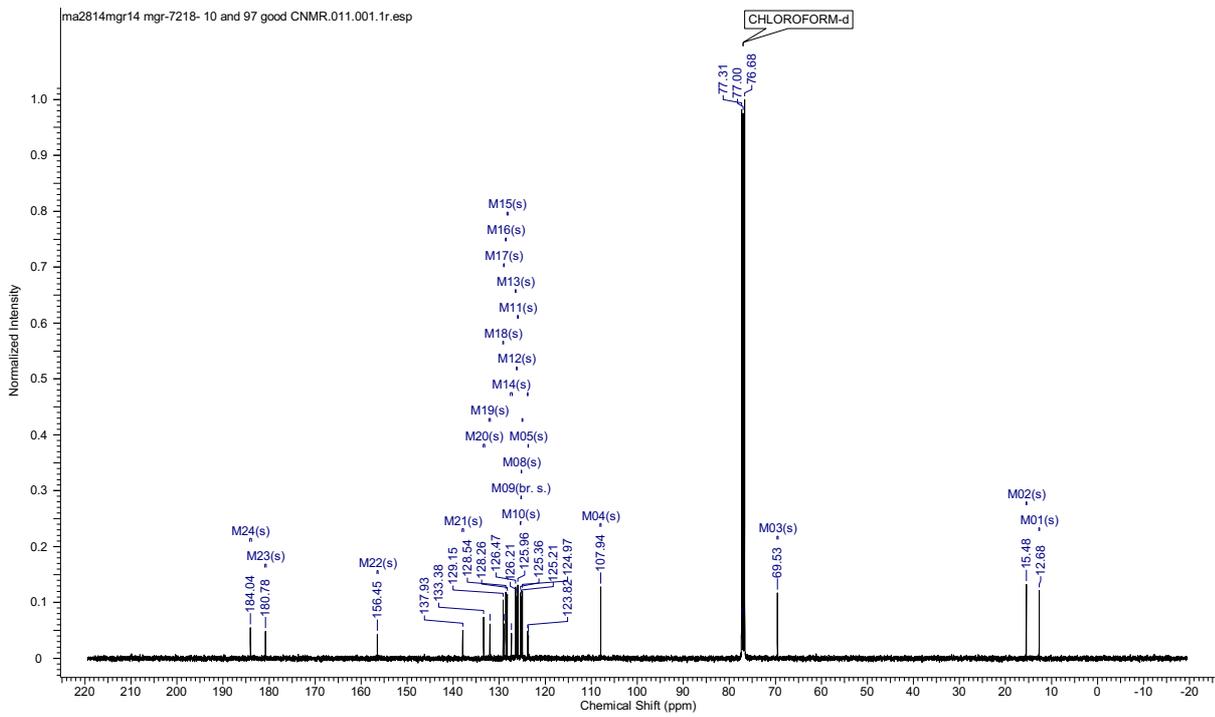
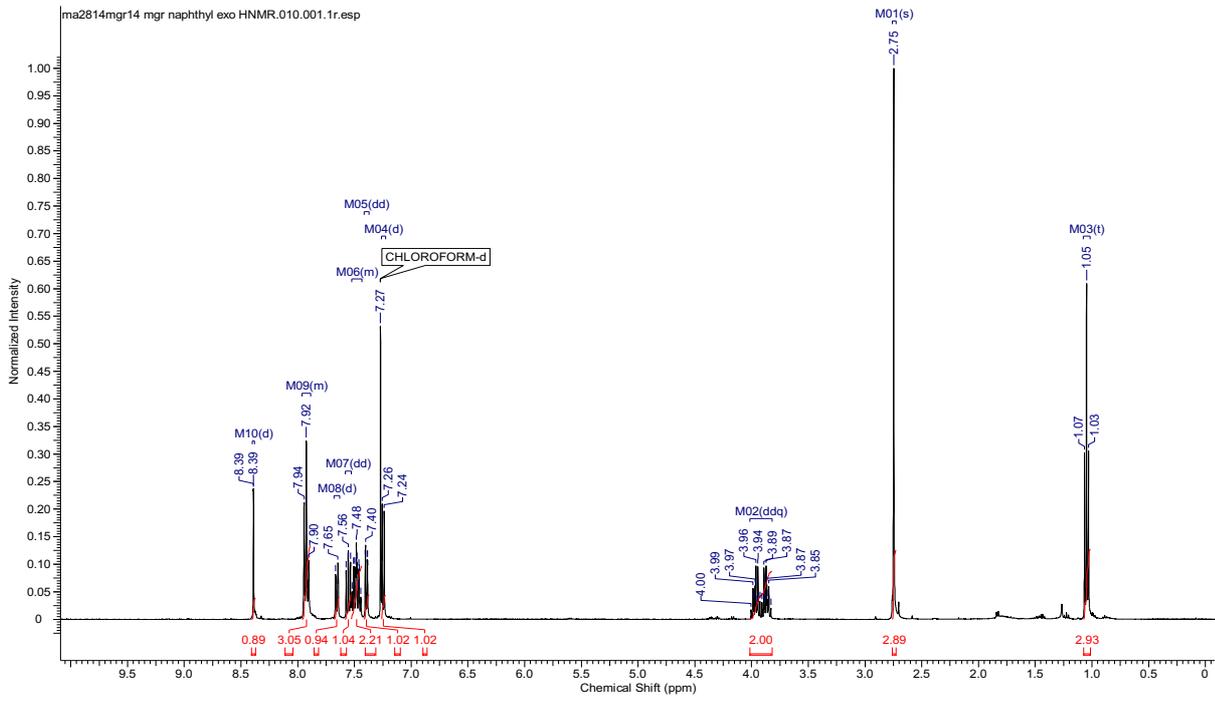
**9-Ethoxy-8-phenyl-3-methylimidazo[5,1-a]isoquinoline-7,10-dione, Analogue D.** To a solution of *t*BuLi (1.55 M in Bu<sub>2</sub>O, 0.62 mL, 0.96 mmol) in THF (2 mL) at -78 °C was added 8-bromo-3-methylimidazo[5,1-a]pyridine (96.4 mg, 0.46 mmol) in THF (2 mL) dropwise over 1 min. After 30 min, 3-ethoxy-4-phenylcyclobut-3-ene-1,2-dione (99.5 mg, 0.49 mmol) in THF (2 mL) was added dropwise over 1 min followed after 1 h by sat. NaHCO<sub>3</sub> (10 mL). The resulting solution was warmed to RT and DCM (15 mL) was added. The aqueous phase was separated and extracted with DCM (2 × 15 mL) then the organic phases were combined, washed with brine (2 × 15 mL),

dried over  $\text{MgSO}_4$ , filtered and concentrated *in vacuo*. The resulting brown oil (128 mg, 0.38 mmol) was dissolved in 1,4-dioxane (7.6 mL) then heated under continuous flow at 110 °C under flow in stainless-steel tubing for a residence time of 1 h using a Vapourtec R4/R2+ device. The resulting solution was stirred under air for 30 min, concentrated *in vacuo* then purified by column chromatography (0-100% EtOAc/DCM with 1% aq.  $\text{NH}_3$  solution) to give the title compound (28.0 mg, 0.08 mmol, 17%) as a burgundy red solid MP: 130 °C dec.; IR:  $\nu$  2983 (w), 2922 (w), 2851 (w), 1658 (s), 1615 (s), 1566 (m), 1440 (m), 1369 (m), 1273 (m), 1259 (m), 1242 (m), 1204 (m), 1171 (s), 1057 (s), 1016 (s), 783 (s), 745 (m), 507 (m);  $^1\text{H}$  NMR (300 MHz,  $\text{CDCl}_3$ ):  $\delta$  = 8.34 (1 H, s, ArH), 7.92 (1 H, d,  $J$  = 7.3 Hz, ArH), 7.46–7.35 (5 H, m, 5  $\times$  ArH), 7.26 (1 H, d,  $J$  = 7.3 Hz, ArH), 4.15 (2 H, q,  $J$  = 7.2 Hz,  $\text{CH}_2\text{CH}_3$ ), 2.73 (3 H, s,  $\text{CH}_3$ ) 1.25 (3 H, t,  $J$  = 7.0 Hz,  $\text{CH}_2\text{CH}_3$ ) ppm;  $^{13}\text{C}$  NMR (101 MHz,  $\text{CDCl}_3$ ):  $\delta$  = 184.0 (C), 181.2 (C), 155.5 (C), 137.9 (CH), 130.5 (CH), 130.4 (C), 130.2 (C), 128.6 (CH), 127.8 (CH), 126.2 (C), 125.1 (CH), 125.0 (C), 123.8 (C), 123.6 (C), 107.9 (CH), 70.2 ( $\text{CH}_2$ ), 15.6 ( $\text{CH}_3$ ), 12.7 ( $\text{CH}_3$ ) ppm; MS (EI)  $m/z$  (%): 332 ( $[\text{M}]^+$ ); HRMS (ESI+)  $m/z$  Found 333.1238,  $\text{C}_{20}\text{H}_{17}\text{N}_2\text{O}_3$   $[\text{M}+\text{H}]^+$  requires 333.1234.



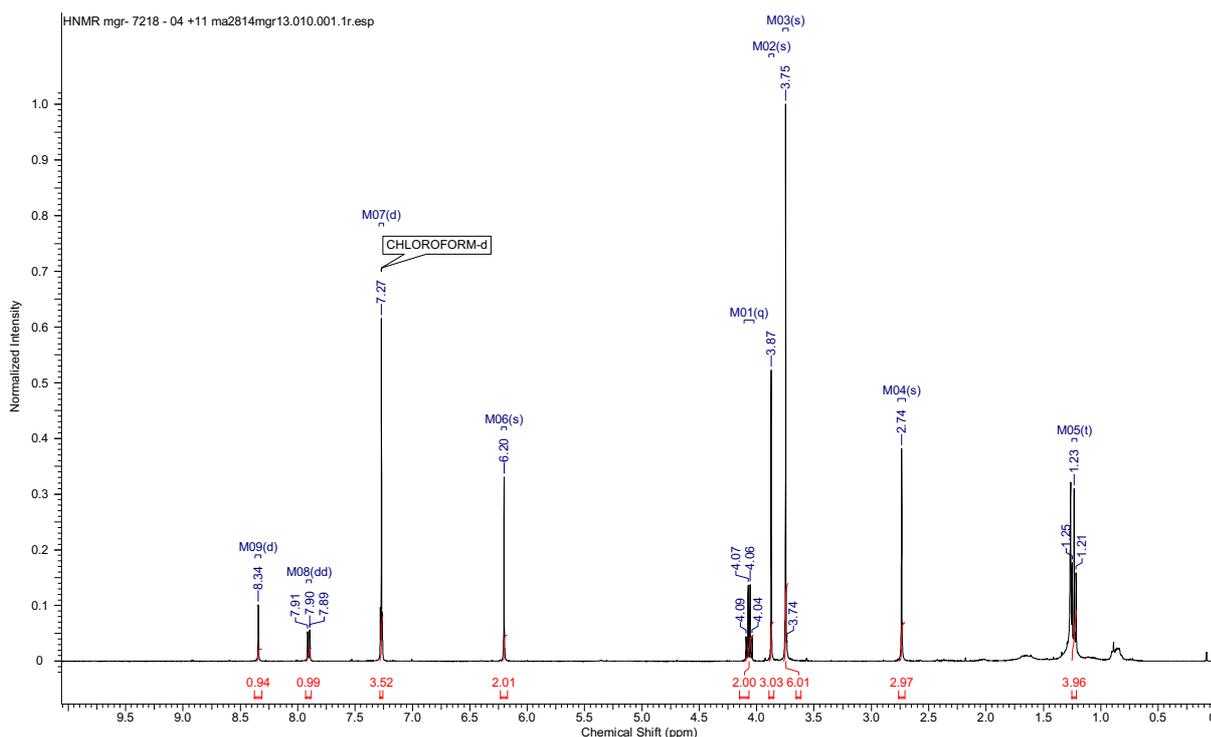


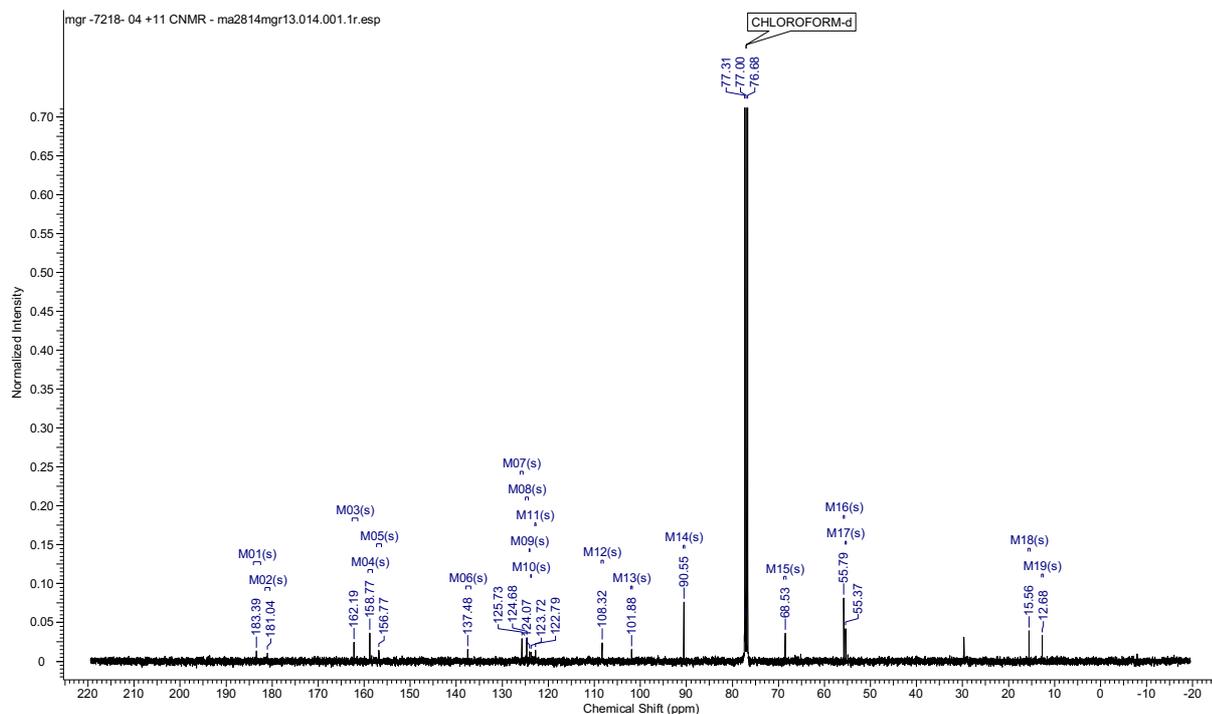
**9-Ethoxy-3-methyl-8-(naphthalen-1-yl)imidazo[5,1-a]isoquinoline-7,10-dione, Analogue E.** To a solution of <sup>t</sup>BuLi (1.92 M in Bu<sub>2</sub>O, 0.56 mL, 1.07 mmol) in THF (1 mL) at -78 °C was added 8-bromo-3-methylimidazo[5,1-a]pyridine (108 mg, 0.51 mmol) in THF (2 mL), dropwise over 1 min. After 30 min, 3-ethoxy-4-naphthylcyclobut-3-ene-1,2-dione (123 mg, 0.49 mmol) in THF (3 mL) was added dropwise over 2 min, followed after 1 h by sat. NaHCO<sub>3</sub> (10 mL). The resulting solution was warmed to RT, DCM (15 mL) was added then the aqueous phase was separated and extracted with DCM (2 × 15 mL). The organic phases were combined, washed with brine (2 × 15 mL), dried over MgSO<sub>4</sub>, filtered and concentrated *in vacuo* to afford a brown oil (112 mg, 0.29 mmol). Part of this crude material (73.2 mg) was dissolved in 1,4-dioxane (4.6 mL) then heated under continuous flow at 110 °C in stainless-steel tubing for a residence time of 1 h using a Vapourtec R4/R2+ device. The resulting solution was then stirred under air for 30 min, concentrated *in vacuo* and purified by column chromatography (0-10% MeOH/DCM with 1% aq. NH<sub>3</sub> solution) to give the title compound (53.0 mg, 0.14 mmol, 28%) as a dark grey solid MP: 95 °C dec.; IR: ν 3049 (w), 2981 (w), 2926 (w), 1667 (m), 1640 (m), 1618 (s), 1531 (m), 1459 (m), 1298 (s), 1177 (s), 1076 (m), 1060 (m), 910 (m), 800 (m), 779 (m), 732 (s); <sup>1</sup>H NMR (400 MHz, CDCl<sub>3</sub>): δ = 8.39 (1 H, d, *J* = 0.6 Hz ArH), 7.96–7.89 (3 H, m, 3 × ArH), 7.66 (1 H, d, *J* = 8.1 Hz, ArH), 7.55 (1 H, dd, *J* = 7.2, 8.1 Hz, ArH), 7.53–7.44 (2 H, m, 2 × ArH), 7.39 (1 H, dd, *J* = 1.0, 7.0 Hz, ArH), 7.25 (1 H, d, *J* = 7.3 Hz, ArH), 3.97 (1 H, dq, *J* = 7.2, 9.9 Hz, OCHH), 3.87 (1 H, dq, *J* = 7.0, 9.9 Hz, OCHH), 2.75 (3 H, s, CH<sub>3</sub>), 1.05 (3 H, t, *J* = 7.0 Hz, CH<sub>2</sub>CH<sub>3</sub>) ppm; <sup>13</sup>C NMR (101 MHz, CDCl<sub>3</sub>): δ = 184.0 (C), 180.8 (C), 156.5 (C), 137.9 (C), 133.4 (C), 132.0 (C), 129.2 (CH), 129.0 (C), 128.5 (CH), 128.3 (CH), 127.4 (C), 126.5 (CH), 126.2 (CH), 126.0 (CH), 125.4 (CH), 125.2 (CH), 125.2 (C), 125.0 (CH), 123.8 (C), 123.7 (C), 107.9 (CH), 69.5 (CH<sub>2</sub>), 15.5 (CH<sub>3</sub>), 12.8 (CH<sub>3</sub>) ppm; MS (ESI+) *m/z* 383 [M+H]<sup>+</sup>; HRMS (ESI+) *m/z* (%): Found 383.1387, C<sub>24</sub>H<sub>19</sub>N<sub>2</sub>O<sub>3</sub> [M+H]<sup>+</sup> requires 383.1390.



### 9-Ethoxy-3-methyl-8-(2,4,6-trimethoxyphenyl)imidazo[5,1-a]isoquinoline-7,10-dione,

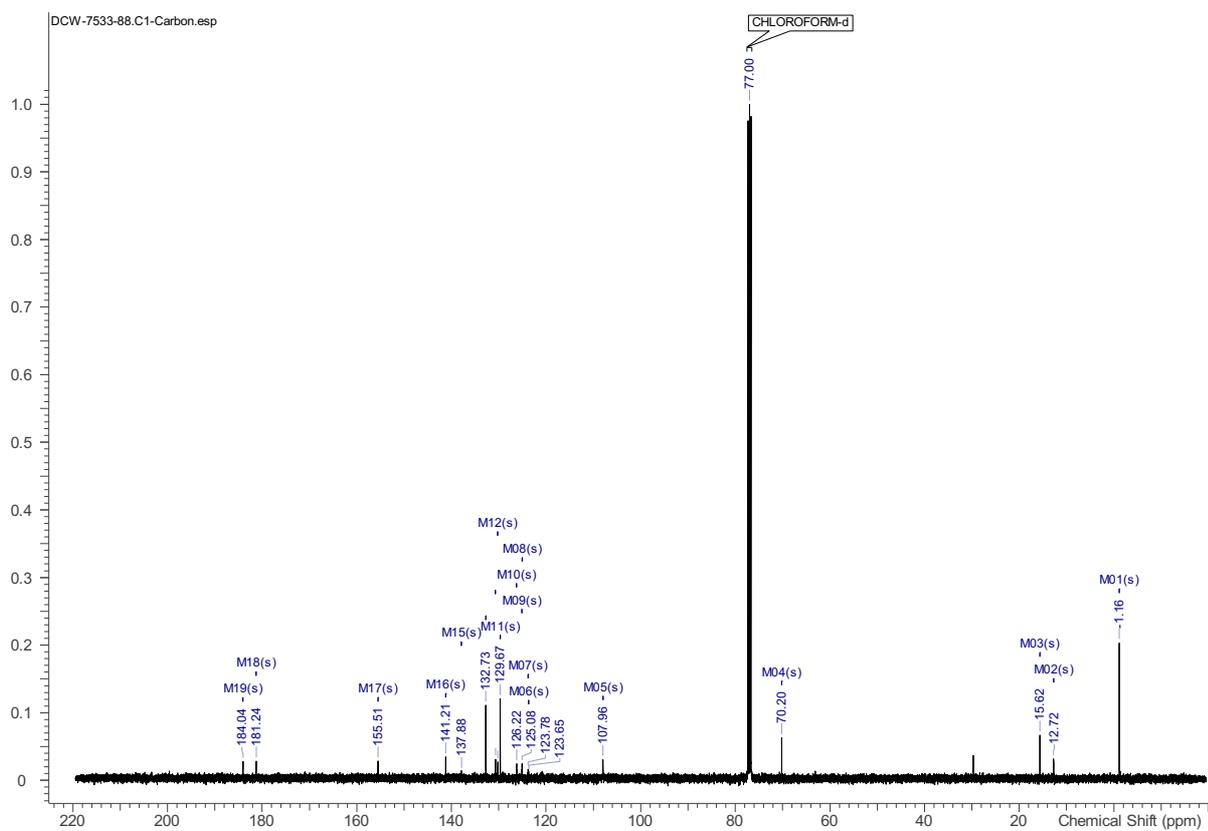
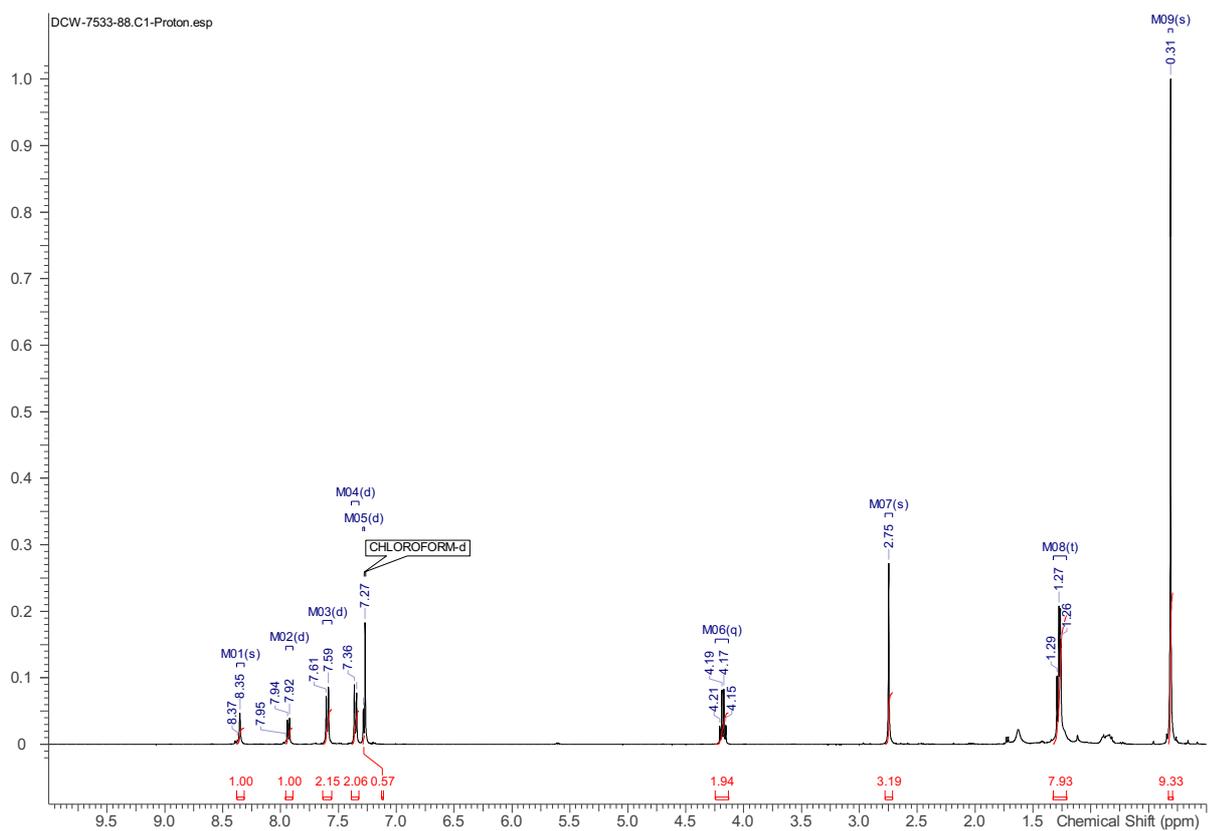
**Analogue F.** To a solution of <sup>t</sup>BuLi (1.92 M in Bu<sub>2</sub>O, 0.53 mL, 1.02 mmol) in THF (2 mL) at -78 °C was added 8-bromo-3-methylimidazo[5,1-a]pyridine (134 mg, 0.46 mmol) in THF (2 mL) dropwise over 3 min. After 45 min, 3-ethoxy-4-(2,4,6-trimethoxyphenyl)cyclobut-3-ene-1,2-dione (130 mg, 0.45 mmol) in THF (5 mL) was added dropwise over 5 min, followed after 1 h by sat. NaHCO<sub>3</sub> (10 mL). The resulting solution was warmed to RT, DCM (15 mL) added then the aqueous phase was separated and extracted with DCM (2 × 15 mL). The organic phases were combined, washed with brine (2 × 15 mL), dried over MgSO<sub>4</sub>, filtered and concentrated *in vacuo* to a brown oil (154 mg, 0.36 mmol). A sample of the crude material (67.4 mg) was then dissolved in 1,4-dioxane (3.7 mL) and heated under continuous flow at 110 °C in stainless-steel tubing for a residence time of 1 h using a Vapourtec R4/R2+ device. The resulting solution was then stirred under air for 30 min, concentrated *in vacuo* and purified by column chromatography (0-10% MeOH/DCM with 1% aq. NH<sub>3</sub> solution) to give the title compound (59.4 mg, 0.14 mmol, 32%) as a dark grey solid MP: 250–253 °C; IR:  $\nu$  2923 (m), 2850 (w), 1665 (m), 1610 (s), 1589 (s), 1460 (m), 1300 (s), 1228 (m), 1205 (m), 1178 (m), 1156 (s), 1127 (s), 1065 (m); <sup>1</sup>H NMR (400 MHz, CDCl<sub>3</sub>):  $\delta$  = 8.34 (1 H, s, ArH), 7.90 (1 H, dd, *J* = 0.7, 7.5 Hz, ArH), 7.27 (1 H, d, *J* = 7.5 Hz, ArH), 6.20 (2 H, s, ArH), 4.07 (2 H, q, *J* = 7.1 Hz, CH<sub>2</sub>CH<sub>3</sub>), 3.87 (3 H, s, OCH<sub>3</sub>), 3.75 (6 H, s, 2 × OCH<sub>3</sub>), 2.74 (3H, s, CH<sub>3</sub>), 1.23 (3 H, t, *J* = 7.0 Hz, CH<sub>2</sub>CH<sub>3</sub>) ppm; <sup>13</sup>C NMR (101 MHz, CDCl<sub>3</sub>):  $\delta$  = 183.4 (C), 181.0 (C), 162.2 (C), 158.8 (C), 156.8 (C), 137.5 (C), 125.7 (CH), 124.7 (CH), 124.1 (C), 123.7 (C), 122.8 (C), 108.3 (CH), 101.9 (C), 90.6 (CH), 68.5 (CH<sub>2</sub>), 55.8 (CH<sub>3</sub>), 55.4 (CH<sub>3</sub>), 15.6 (CH<sub>3</sub>), 12.7 (CH<sub>3</sub>) ppm with one C not observed; MS (ESI+) *m/z* (%): 423 [M+H]<sup>+</sup>; HRMS (ESI+) *m/z* Found 423.1553, C<sub>23</sub>H<sub>23</sub>N<sub>2</sub>O<sub>6</sub> [M+H]<sup>+</sup> requires 423.1551.



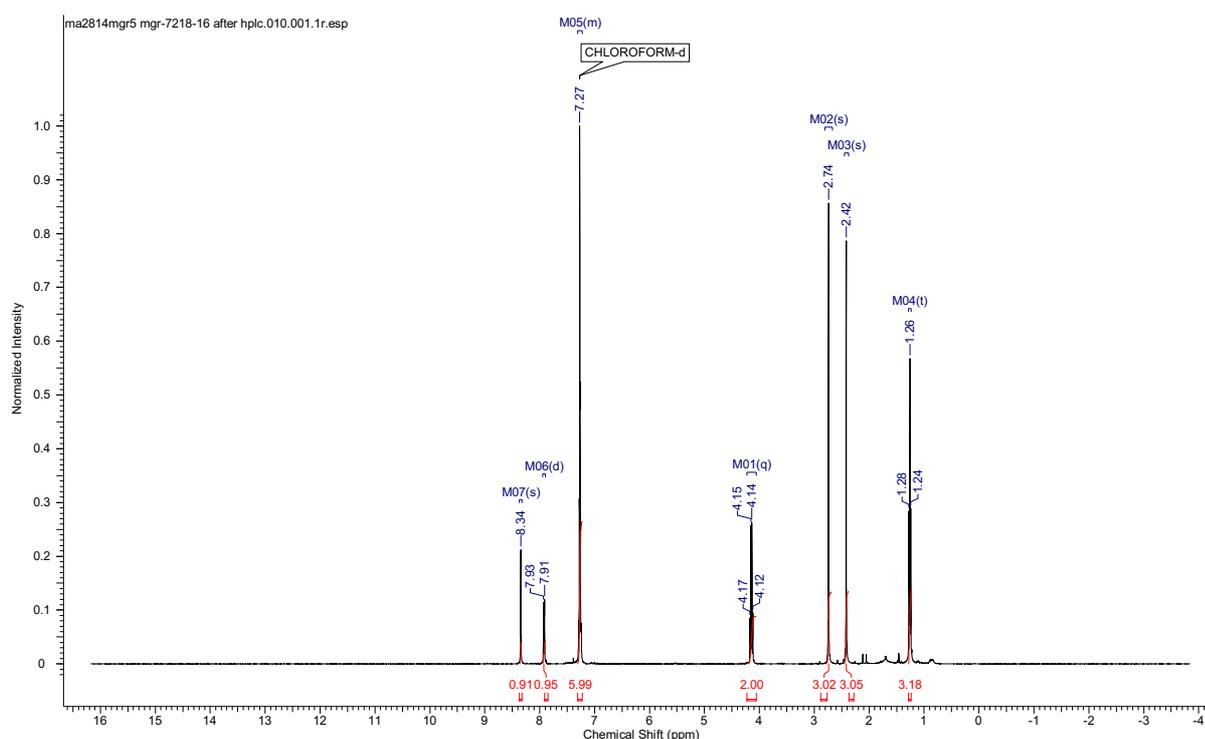


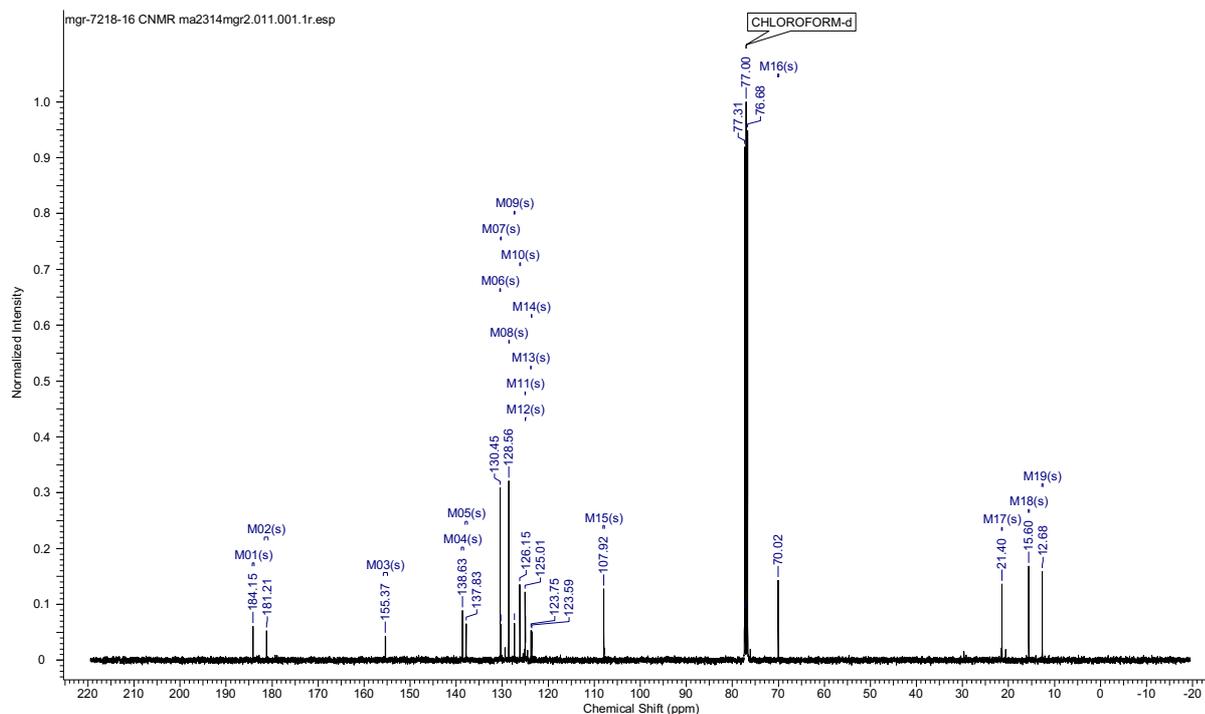
### 9-Ethoxy-8-(4-(trimethylsilyl)phenyl)-3-methylimidazo[5,1-a]isoquinoline-7,10-dione,

**Analogue G.** A solution  $n$ BuLi (2.44 M in hexane, 0.30 mL, 0.73 mmol) was added to a solution of 8-bromo-3-methylimidazo[5,1-a]pyridine (133 mg, 0.63 mmol) in THF (20 mL) at  $-78$  °C. After 20 min a solution of 3-ethoxy-4-(4-trimethylsilylphenyl)cyclobut-3-ene-1,2-dione (157 mg, 0.57 mmol) in THF (30 mL) was added over 40 min, followed after an additional 35 min by sat.  $\text{NH}_4\text{Cl}$  (20 mL). The reaction was allowed to warm to RT then the aqueous phase was extracted with  $\text{CHCl}_3$  (3 x 30 mL). The combined organic phases were dried over  $\text{MgSO}_4$ , filtered and concentrated *in vacuo* then dissolved in 1,4 dioxane (10 mL) and filtered. This solution was then heated under continuous flow at 110 °C in stainless-steel tubing for a residence time of 1 h using a Vapourtec R4/R2+ device, stirred in air overnight and purified by column chromatography (0-1% MeOH/ $\text{CHCl}_3$ ) and HPLC (20% acetone/DCM) to afford the title compound (9.1 mg, 0.02 mmol, 4%) as a burgundy solid MP: 111 °C dec.; IR:  $\nu$  2955 (s), 2924 (s), 2852 (w), 1668 (s), 1644 (m), 1618 (m), 1532 (m), 1458 (m), 1371 (m), 1299 (s), 1249 (s), 1177 (s), 1120 (w), 1099 (w), 1062 (m), 837 (s);  $^1\text{H}$  NMR (400 MHz,  $\text{CDCl}_3$ ):  $\delta$  = 8.35 (1 H, s, ArH), 7.93 (1 H, d,  $J$  = 7.3 Hz, ArH), 7.60 (2 H, d,  $J$  = 8.1 Hz, ArH), 7.35 (2 H, d,  $J$  = 8.0 Hz, ArH), 7.28 (1 H, d,  $J$  = 7.3 Hz, ArH), 4.18 (2 H, q,  $J$  = 7.0 Hz,  $\text{CH}_2\text{CH}_3$ ), 2.75 (3 H, s,  $\text{CH}_3$ ), 1.27 (3 H, t,  $J$  = 7.1 Hz,  $\text{CH}_2\text{CH}_3$ ), 0.31 (9 H, s,  $\text{Si}(\text{CH}_3)_3$ ) ppm;  $^{13}\text{C}$  NMR (101 MHz,  $\text{CDCl}_3$ ):  $\delta$  = 184.1 (C), 181.2 (C), 155.5 (C), 141.2 (C), 137.9 (C), 132.7 (CH), 130.7 (C), 130.2 (C), 129.7 (CH), 126.2 (CH), 125.1 (CH), 125.0 (C), 123.8 (C), 123.7 (C), 108.0 (CH), 70.2 ( $\text{CH}_2$ ), 15.6 ( $\text{CH}_3$ ), 12.7 ( $\text{CH}_3$ ),  $-1.15$  ( $\text{Si}(\text{CH}_3)_3$ ) ppm; MS (ESI+)  $m/z$  (%): 405 [ $\text{M}+\text{H}$ ] $^+$ ; HRMS (ESI+)  $m/z$  (%): Found 405.1640,  $\text{C}_{23}\text{H}_{25}\text{N}_2\text{O}_3\text{Si}$  [ $\text{M}+\text{H}$ ] $^+$  requires 405.1629.



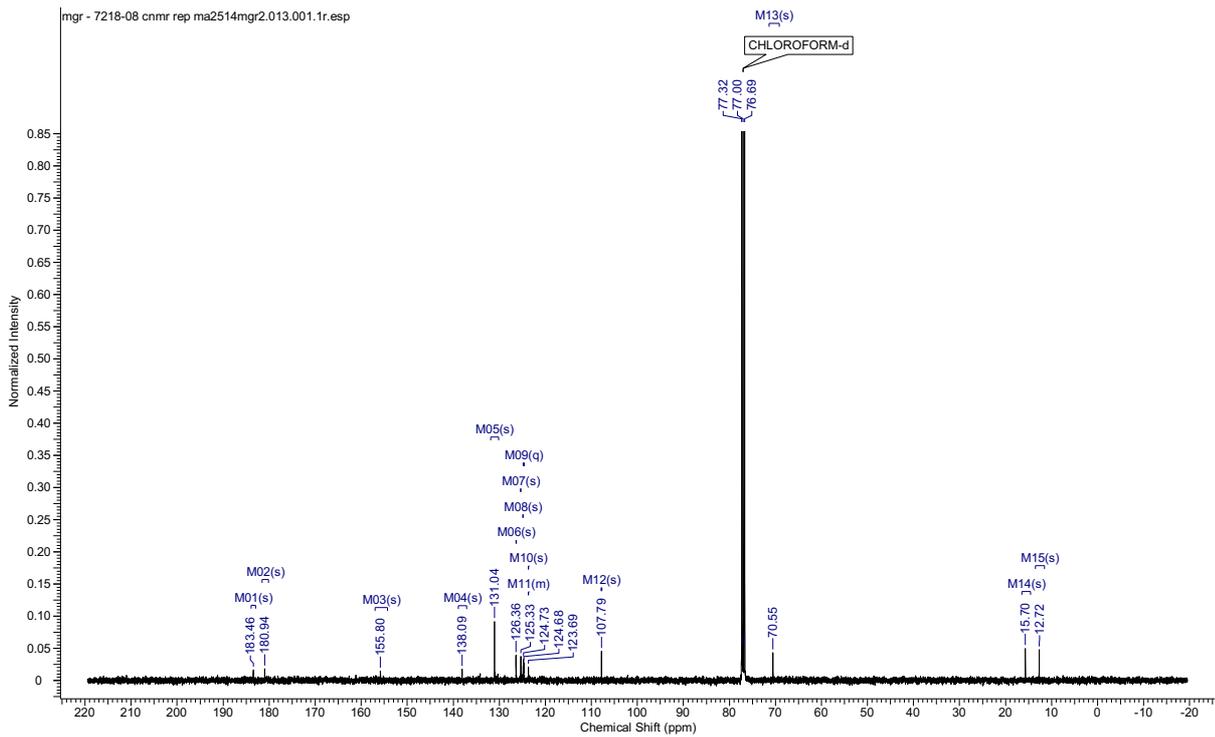
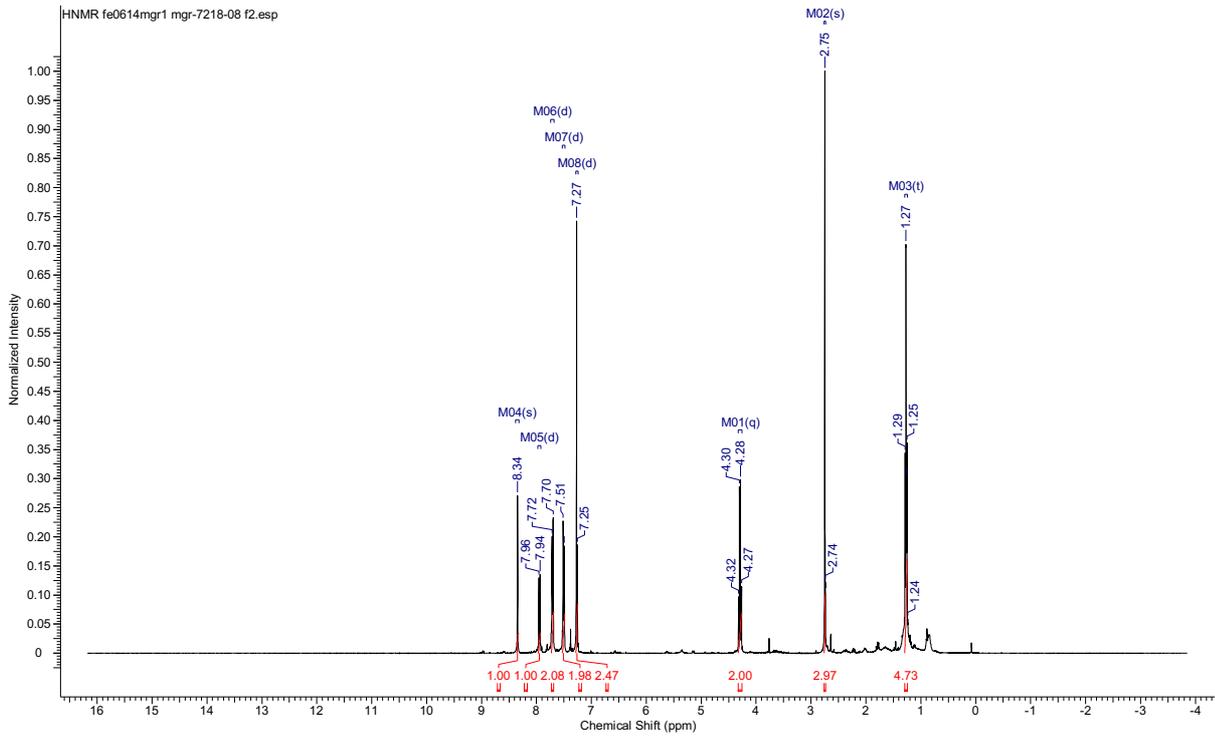
**9-Ethoxy-3-methyl-8-(*p*-tolyl)imidazo[5,1-*a*]isoquinoline-7,10-dione, Analogue H.** To a solution of <sup>t</sup>BuLi (1.92 M in Bu<sub>2</sub>O, 0.54 mL, 1.04 mmol) in THF (2 mL) at -78 °C was added 8-bromo-3-methylimidazo[5,1-*a*]pyridine (104 mg, 0.49 mmol) in THF (2 mL) dropwise over 1 min. After 30 min, 3-ethoxy-4-(4-methylphenyl)cyclobut-3-ene-1,2-dione (102 mg, 0.47 mmol) in THF (3 mL) was added dropwise over 4 min, followed after 1 h by sat. NaHCO<sub>3</sub> (10 mL). The resulting solution was warmed to RT, DCM (15 mL) was added then the aqueous phase was separated and extracted with DCM (2 × 15 mL). The organic phases were combined, washed with brine (2 × 15 mL), dried over MgSO<sub>4</sub>, filtered and concentrated *in vacuo* to a brown oil (101 mg, 0.29 mmol). The crude material was dissolved in 1,4-dioxane (3.5 mL) then heated under continuous flow at 110 °C in stainless-steel tubing for a residence time of 1 h using a Vapourtec R4/R2+ device. The resulting solution was then stirred in air for 30 min, concentrated *in vacuo* and purified by column chromatography (0-10% MeOH/DCM with 1% aq. NH<sub>3</sub> solution) to give the title compound (32.9 mg, 0.09 mmol, 20%) as a burgundy solid MP: 162–164 °C; IR: ν 2924 (w), 1664 (s), 1642 (m), 1616 (s), 1512 (m), 1458 (m), 1329 (m), 1300 (s), 1176 (s), 1061 (s), 810 (m), 743 (m); <sup>1</sup>H NMR (400 MHz, CDCl<sub>3</sub>): δ = 8.34 (1 H, s, ArH), 7.92 (1 H, d, *J* = 7.3 Hz, ArH), 7.31–7.23 (5 H, m, ArH), 4.14 (2 H, q, *J* = 7.0 Hz, CH<sub>2</sub>CH<sub>3</sub>), 2.74 (3 H, s, CH<sub>3</sub>), 2.42 (3 H, s, CH<sub>3</sub>), 1.26 (3 H, t, *J* = 7.0 Hz, CH<sub>2</sub>CH<sub>3</sub>) ppm; <sup>13</sup>C NMR (101 MHz, CDCl<sub>3</sub>): δ = 184.2 (C), 181.2 (C), 155.4 (C), 138.6 (C), 137.8 (C), 130.5 (CH), 130.3 (C), 128.6 (CH), 127.4 (C), 126.2 (CH), 125.0 (CH), 125.0 (C), 123.8 (C), 123.6 (C), 107.9 (CH), 70.0 (CH<sub>2</sub>), 21.4 (CH<sub>3</sub>), 15.6 (CH<sub>3</sub>), 12.7 (CH<sub>3</sub>) ppm; MS (ESI+) *m/z* (%): 347 [M+H]<sup>+</sup>; HRMS (ESI+) *m/z* Found 347.1398, C<sub>22</sub>H<sub>19</sub>N<sub>2</sub>O<sub>3</sub> [M+H]<sup>+</sup> requires 347.1390.

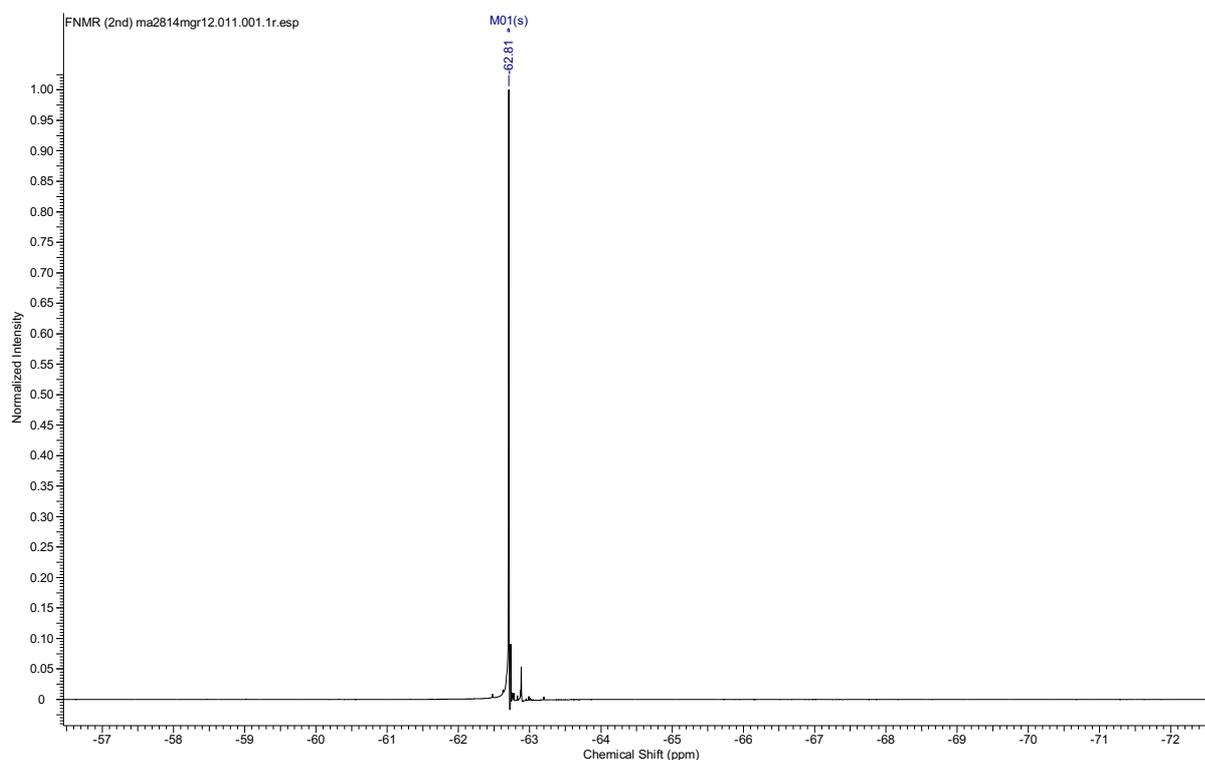




### 9-Ethoxy-3-methyl-8-(4-(trifluoromethyl)phenyl)imidazo[5,1-a]isoquinoline-7,10-dione,

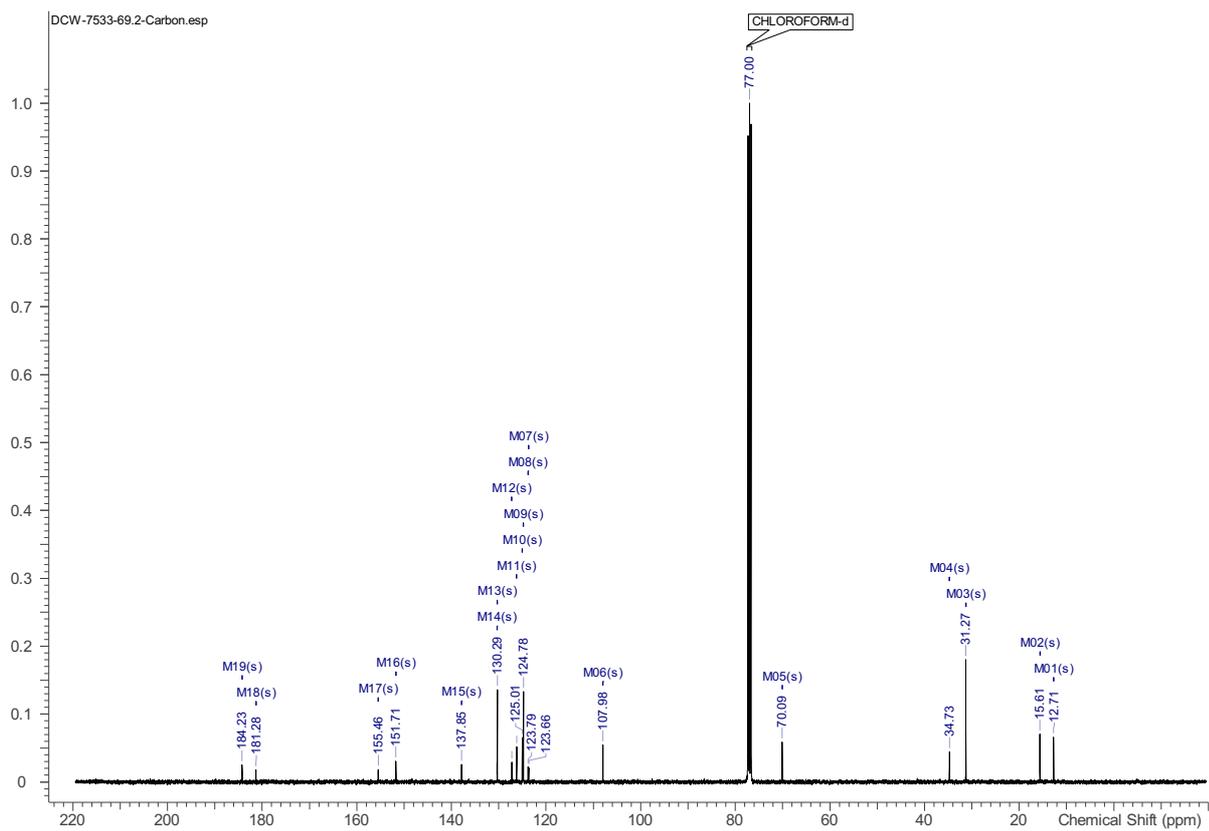
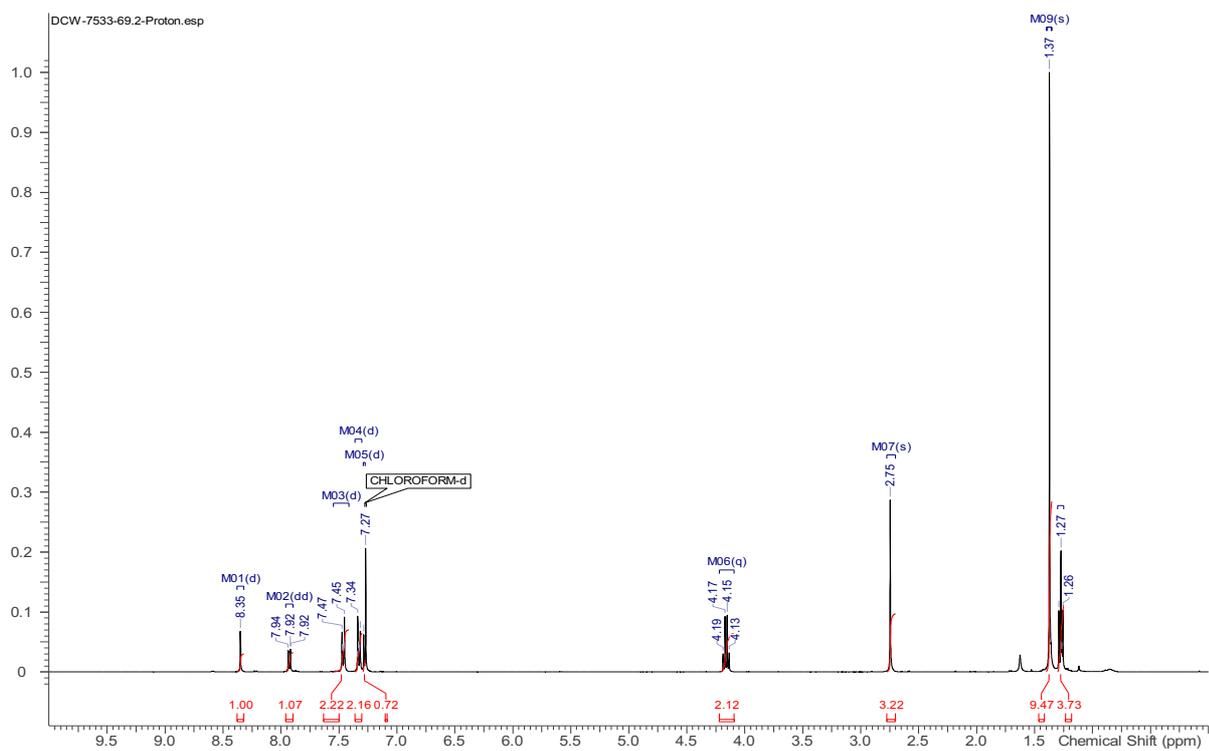
**Analogue I.** To a solution of <sup>t</sup>BuLi (1.92 M in Bu<sub>2</sub>O, 0.52 mL, 1.00 mmol) in THF (1 mL) at -78 °C was added 8-bromo-3-methylimidazo[5,1-a]pyridine (100 mg, 0.48 mmol) in THF (2 mL) dropwise over 1 min. After 30 min, 3-ethoxy-4-(4-trifluoromethylphenyl)cyclobut-3-ene-1,2-dione (121 mg, 0.45 mmol) in THF (3 mL) was added dropwise over 1 min, followed after 1 h by sat. NaHCO<sub>3</sub> (10 mL). The resulting solution was warmed to RT, DCM (15 mL) was added then the aqueous phase was separated and extracted with DCM (2 × 15 mL). The organic phases were combined, washed with brine (2 × 15 mL), dried over MgSO<sub>4</sub>, filtered and concentrated *in vacuo* to a brown oil (67.7 mg, 0.17 mmol). A sample of the crude material (56 mg) was then dissolved in 1,4-dioxane (3.5 mL) and heated under continuous flow at 110 °C in stainless-steel tubing for a residence time of 1 h using a Vapourtec R4/R2+ device. The resulting solution was then stirred under air for 30 min, concentrated *in vacuo* and purified by column chromatography (0-10% MeOH/DCM with 1% aq. NH<sub>3</sub> solution) to give the title compound (6.1 mg, 0.01 mmol, 4%) as a burgundy red solid MP: 144–149 °C; IR: ν 2926 (w), 2363 (w), 1667 (m), 1643 (m), 1617 (m), 1325 (s), 1168 (s), 1123 (s), 1060 (s), 1020 (w); <sup>1</sup>H NMR (400 MHz, CDCl<sub>3</sub>): δ = 8.34 (1 H, s, ArH), 7.95 (1 H, d, *J* = 7.3 Hz, ArH), 7.71 (2 H, d, *J* = 8.2 Hz, ArH), 7.50 (2 H, d, *J* = 8.1 Hz, ArH), 7.26 (1 H, d, *J* = 7.1 Hz, ArH), 4.29 (2 H, q, *J* = 7.1 Hz, CH<sub>2</sub>CH<sub>3</sub>), 2.74 (3 H, s, CH<sub>3</sub>), 1.27 (3 H, t, *J* = 7.0 Hz, CH<sub>2</sub>CH<sub>3</sub>) ppm; <sup>13</sup>C NMR (101 MHz, CDCl<sub>3</sub>): δ = 183.5 (C), 180.9 (C), 155.8 (C), 138.1 (C), 131.0 (CH), 126.4 (CH), 125.3 (CH), 124.9 (C), 124.7 (CH, q, *J*<sub>CF</sub> = 3.9 Hz), 123.7 (C), 123.7 (C), 107.8 (CH), 70.5 (CH<sub>2</sub>), 15.7 (CH<sub>3</sub>), 12.7 (CH<sub>3</sub>) ppm with four C not observed; <sup>19</sup>F NMR (376 MHz, CDCl<sub>3</sub>): δ = -62.8 (CF<sub>3</sub>) ppm; MS (ESI+) *m/z* (%): 401 [M+H]<sup>+</sup>; HRMS (ESI+) *m/z* Found 401.1115, C<sub>21</sub>H<sub>16</sub>F<sub>3</sub>N<sub>2</sub>O<sub>3</sub> [M+H]<sup>+</sup> requires 401.1108.



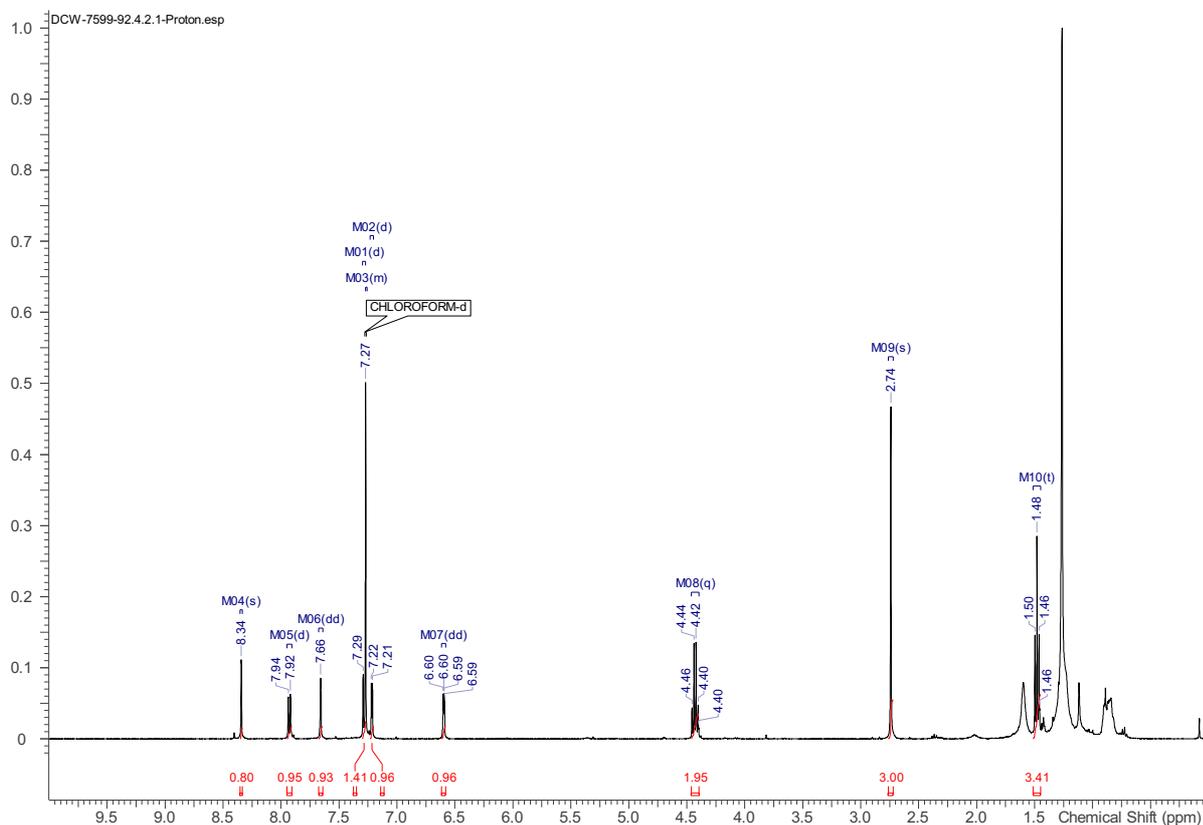


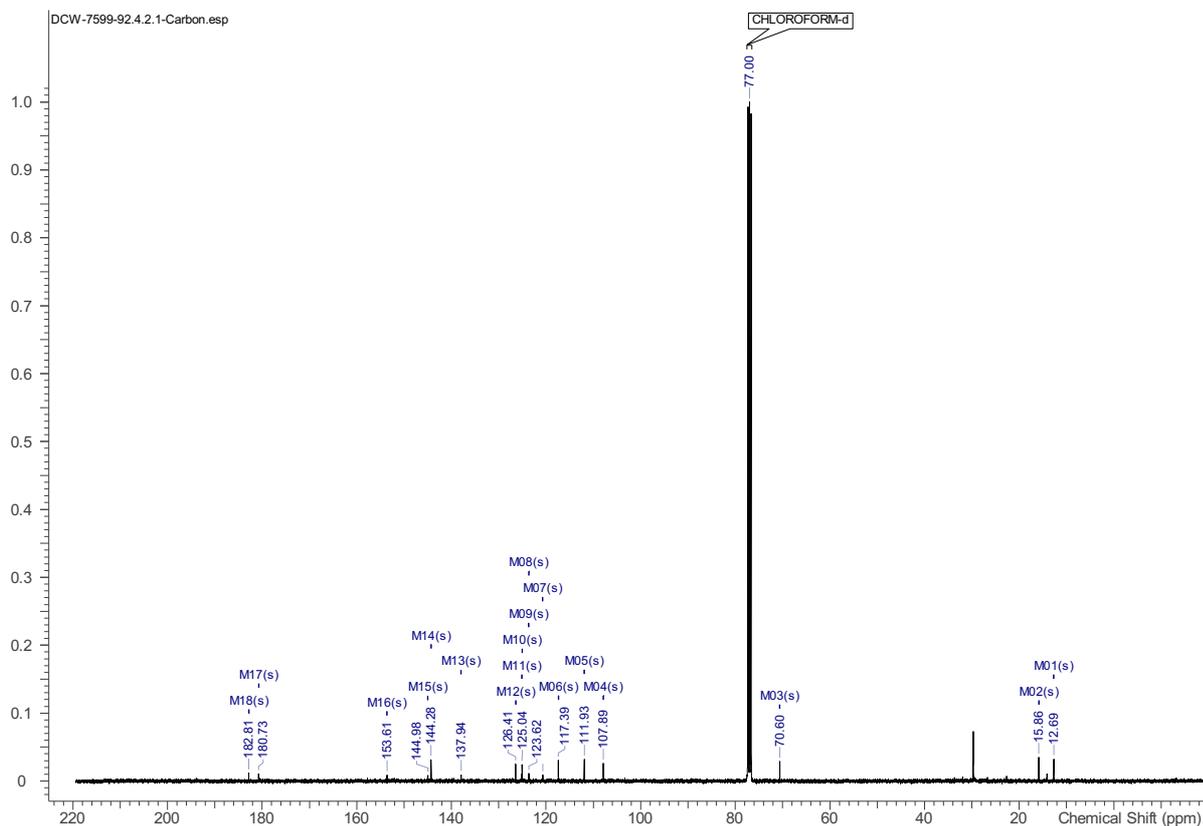
### 9-Ethoxy-8-(4-*t*-butylphenyl)-3-methylimidazo[5,1-*a*]isoquinoline-7,10-dione, Analogue J.

<sup>7</sup>BuLi (2.44 M in hexane, 0.70 mL, 1.71 mmol) was added to a solution of 8-bromo-3-methylimidazo[5,1-*a*]pyridine (416 mg, 1.97 mmol) in THF (30 mL) at  $-78\text{ }^{\circ}\text{C}$ . After 20 min a solution of 3-ethoxy-4-(4-*tert*-butylphenyl)cyclobut-3-ene-1,2-dione (339 mg, 1.31 mmol) in THF (20 mL) at  $-78\text{ }^{\circ}\text{C}$  was added over 1 h, followed after an additional 5 h by sat.  $\text{NH}_4\text{Cl}$  (20 mL). After warming to RT the aqueous phase was extracted with  $\text{CHCl}_3$  (4 x 30 mL). The combined organic phases were dried over  $\text{MgSO}_4$ , filtered and concentrated *in vacuo*, dissolved in 1,4 dioxane (20 mL) and filtered. A 10 mL aliquot of this solution was then heated under continuous flow at  $110\text{ }^{\circ}\text{C}$  in stainless-steel tubing for a residence time of 1 h by using a Vapourtec R4/R2+ device. Ceric ammonium nitrate (256 mg, 466  $\mu\text{mol}$ ) and silica (748 mg) were added to the resulting solution, which was stirred under air overnight. Purification by column chromatography (EtOAc) and HPLC (20% acetone/DCM) afford the title compound (20.7 mg, 0.06 mmol, 3%) as a burgundy solid MP:  $109\text{ }^{\circ}\text{C}$  dec.; IR:  $\nu$  2963 (s), 2867 (w), 1671 (s), 1645 (m), 1619 (s), 1532 (m), 1460 (m), 1363 (m), 1330 (m), 1301 (s), 1176 (s), 1063 (s); <sup>1</sup>H NMR (400 MHz,  $\text{CDCl}_3$ ):  $\delta$  = 8.35 (1 H, d,  $J$  = 0.8 Hz, ArH), 7.93 (1 H, dd,  $J$  = 7.5, 0.9 Hz, ArH), 7.46 (2 H, d,  $J$  = 8.7 Hz, ArH), 7.33 (2 H, d,  $J$  = 8.5 Hz, ArH), 7.28 (1 H, d,  $J$  = 7.5 Hz, ArH), 4.16 (2 H, q,  $J$  = 7.1 Hz,  $\text{CH}_2\text{CH}_3$ ), 2.75 (3 H, s,  $\text{CH}_3$ ), 1.37 (9 H, s,  $\text{C}(\text{CH}_3)_3$ ), 1.27 (3 H, t,  $J$  = 6.9 Hz,  $\text{CH}_2\text{CH}_3$ ) ppm; <sup>13</sup>C NMR (101 MHz,  $\text{CDCl}_3$ ):  $\delta$  = 184.2 (C), 181.3 (C), 155.5 (C), 151.7 (C), 137.9 (C), 130.3 (C), 130.3 (CH), 127.2 (C), 126.2 (CH), 125.0 (CH), 124.8 (CH), 123.8 (C), 123.7 (C), 108.0 (CH), 70.1 ( $\text{CH}_2$ ), 34.7 (C), 31.3 ( $\text{C}(\text{CH}_3)_3$ ), 15.6 ( $\text{CH}_3$ ), 12.7 ( $\text{CH}_3$ ) ppm with one C not observed; MS (ESI+)  $m/z$  (%): 389 [ $\text{M}+\text{H}$ ]<sup>+</sup>; HRMS (ESI+)  $m/z$  Found 389.1870,  $\text{C}_{24}\text{H}_{25}\text{N}_2\text{O}_3$  [ $\text{M}+\text{H}$ ]<sup>+</sup> requires 389.1860.



**9-Ethoxy-8-(furan-2-yl)-3-methylimidazo[5,1-a]isoquinoline-7,10-dione Analogue K.** <sup>n</sup>BuLi (2.44 M in hexane, 0.33 mL, 0.81 mmol) was added to a solution of 8-bromo-3-methylimidazo[5,1-a]pyridine (139 mg, 0.66 mmol) in THF (30 mL) at  $-78\text{ }^{\circ}\text{C}$ . After 15 min a solution of 3-ethoxy-4-(furan-2-yl)cyclobut-3-ene-1,2-dione (121 mg, 0.63 mmol) in THF (30 mL) at  $-78\text{ }^{\circ}\text{C}$  was added over 30 min, followed by sat.  $\text{NH}_4\text{Cl}$  (20 mL) after an additional 20 min. After warming to RT the aqueous phase was extracted with  $\text{CHCl}_3$  (4 x 30 mL). The combined organic phases were dried over  $\text{MgSO}_4$ , filtered and concentrated *in vacuo*. This intermediate material was dissolved in 1,4 dioxane (10 mL), filtered and subsequently heated under continuous flow at  $110\text{ }^{\circ}\text{C}$  in stainless-steel tubing for a residence time of 1 h by using a Vapourtec R4/R2+ device. The resulting solution was stirred in air overnight and purified by column chromatography (0-1% MeOH/ $\text{CHCl}_3$ ) and HPLC (20% acetone/DCM) to afford the title compound (10.2 mg, 0.03 mmol, 5%) as a burgundy solid MP:  $147\text{ }^{\circ}\text{C}$  dec.; IR:  $\nu$  2958 (w), 2921 (s), 2850 (m), 1662 (s), 1617 (m), 1531 (m), 1459 (m), 1329 (m), 1304 (s), 1180 (m), 1062 (m);  $^1\text{H}$  NMR (400 MHz,  $\text{CDCl}_3$ ):  $\delta$  = 8.34 (1 H, s, ArH), 7.93 (1 H, d,  $J$  = 7.3 Hz, ArH), 7.66 (1 H, dd,  $J$  = 1.6, 0.5 Hz, ArH), 7.28 (1 H, d,  $J$  = 7.3 Hz, ArH), 7.22 (1 H, d,  $J$  = 3.6 Hz, ArH), 6.60 (1 H, dd,  $J$  = 3.6, 1.8 Hz, ArH), 4.43 (2 H, q,  $J$  = 7.0 Hz,  $\text{CH}_2\text{CH}_3$ ), 2.74 (3 H, s,  $\text{CH}_3$ ), 1.48 (3 H, t,  $J$  = 7.1 Hz,  $\text{CH}_2\text{CH}_3$ ) ppm;  $^{13}\text{C}$  NMR (101 MHz,  $\text{CDCl}_3$ ):  $\delta$  = 182.8 (C), 180.7 (C), 153.6 (C), 145.0 (C), 144.3 (CH) 138.0 (C), 126.4 (CH), 125.1 (C), 125.0 (CH), 123.7 (C), 123.6 (C), 120.7 (C), 117.4 (CH), 111.9(CH), 107.9 (CH), 70.6 ( $\text{CH}_2$ ), 15.9 ( $\text{CH}_3$ ), 12.7 ( $\text{CH}_3$ ) ppm; LRMS (ESI+)  $m/z$  323  $[\text{M}+\text{H}]^+$ ; HRMS (ESI+)  $m/z$  Found 323.1036,  $\text{C}_{18}\text{H}_{15}\text{N}_2\text{O}_4$   $[\text{M}+\text{H}]^+$  323.1026.





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