

## Elucidating cylindrospermopsin toxicity via synthetic analogues: An in vitro approach

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## SUPPLEMENTARY MATERIAL

### Elucidating cylindrospermopsin toxicity via synthetic analogues: An *in vitro* approach

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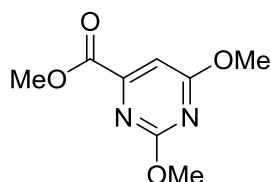
### Experimental

All glassware used was acetone washed and dried with N<sub>2</sub> or in a vacuum oven. Reagents and starting materials were from commercial suppliers and used as supplied. Diethyl ether (DE), tetrahydrofuran (THF) and dichloromethane (DCM) were dried with a Pure Solv MD-3 solvent purification system. Dry DMF and methanol were purchased from the Aldrich chemical company. Petroleum ether (PE) refers to the fraction distilled between boiling range of 40-60 °C. Column chromatography was carried out on silica gel (particle size 40-63 µm) and TLCs were conducted on pre-coated Kieselgel 60 F254 (Art. 5554; Merck) using HPLC grade acetic acid (AA) chloroform (CFM), dichloromethane (DCM), diethyl ether (DE), ethyl acetate (EA), hexane (HX) and methanol (ME). Compounds were visualized using UV, iodine or stained using polyphosphomolybdic acid (PMA) in EtOH or vanillin in EtOH/H<sub>2</sub>SO<sub>4</sub>, with heating. IR spectra were recorded on a Perkin-Elmer 1600 series FTIR instrument as KBr discs, thin films, Nujol mulls, or chloroform solution on NaCl plates with absorption frequencies reported in wavenumbers (cm<sup>-1</sup>). NMR spectra were obtained on a Bruker AC400 or on a Bruker Avance-500 spectrometer. Chemical shifts for spectra are reported in δ values (ppm) relative to the residual solvent peak in each case. Electron Ionisation (EI) and Chemical Ionisation (CI) mass spectra were recorded on an Agilent Tech. 6890N spectrometer or an

XCalibur MAT900 XLT spectrometer and ESI were recorded on a LTQ Orbitrap XL at the EPSRC Mass Spectrometry Service (Swansea, United Kingdom).

Column chromatography was carried out on silica gel (particle size 40-63  $\mu\text{m}$ ) and TLCs were conducted on precoated Kieselgel 60 F254 (Art. 5554; Merck) using HPLC grade chloroform (CFM), dichloromethane (DCM), diethyl ether (DE), ethyl acetate (EA), hexane (HX), toluene (TL) and methanol (ME). Petroleum ether (PE) refers to the fraction distilled between boiling range of 40-60  $^{\circ}\text{C}$ . Compounds were visualised using UV, iodine or stained using polyphosphomolybdic acid (PMA) in EtOH or vanillin in EtOH/H<sub>2</sub>SO<sub>4</sub>, with heating. All non-aqueous reactions were conducted in oven-dried apparatus under a static atmosphere of argon. DE, THF and DCM were dried on a Pure Solv MD-3 solvent purification system. Anhydrous ME and DMF was purchased from Sigma-Aldrich. Chemical shifts are reported in  $\delta$  values (ppm) relative to the solvent signals as an internal standard. Proton and carbon NMR spectra were recorded on a Bruker AC400 or a Bruker Avance-500 spectrometer. Mass spectra data were obtained at the EPSRC Mass Spectrometry Service Centre at the University of Wales, Swansea. Infrared spectra were recorded on a Bruker ALPHA FTIR spectrometer.

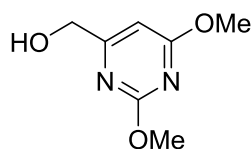
#### **Methyl 2,6-dimethoxypyrimidine-4-carboxylate (Xie et al. 2000)**



A stirred suspension of orotic acid monohydrate (10.2 g, 58.60 mmol) in phosphorous (V) oxychloride (80 mL) was heated at 120  $^{\circ}\text{C}$  for 16 h under an inert atmosphere. Upon cooling to rt, phosphorus pentachloride PCl<sub>5</sub> (25.25 g, 117.00 mmol) was added and the resulting red solution heated for a further 16 h at 120  $^{\circ}\text{C}$ . The reaction mixture was then cooled to rt and concentrated under reduced pressure, the resulting oil was cooled to 0  $^{\circ}\text{C}$  and anhydrous ME (50 mL) added dropwise over 2 h. After stirring to rt over 16 h, the reaction was quenched by the drop-wise addition of water (50 mL) and diluted with further water (200 mL), the mixture was then extracted with DCM (3 x 150 mL) and dried over anhydrous MgSO<sub>4</sub>. After evaporation, purification was achieved by flash column chromatography on silica gel eluting in EA/PE (25:75) giving the title compound as a white solid (8.43 g, 42.54 mmol, 76%). Data

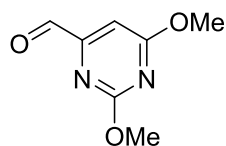
was in agreement with the literature (Xie et al. 2000). **R<sub>f</sub>** 0.25 (25% EA/PE); **M.p.** 106-108 °C (lit. 108-109 °C; Gershon 1962);  $\nu_{\max}$  3107, 3013, 2962, 1720, 1598, 1563, 1483, 1455, 1432, 1391, 1347, 1267, 1202;  $\delta_{\text{H}}$  3.96 (3H, s, CH<sub>3</sub>), 4.02 (3H, s, CH<sub>3</sub>), 4.06 (3H, s, CH<sub>3</sub>) 7.06 (1H, s, CH);  $\delta_{\text{C}}$  53.1, 54.4, 55.2, 103.2, 156.8, 164.5, 172.9.

**2,6-dimethoxypyrimidin-4-yl)methanol** (Xie et al. 2000)



To a solution of the previous methyl ester (5.47 g, 27.6 mmol) and LiBH<sub>4</sub> (0.72 g, 33.05 mmol) in THF (80 mL) was added EtOH (120 mL) and the mixture stirred at room temperature. Reaction progress was monitored *via* TLC and after 2 h the reaction was quenched by the drop-wise addition of HCl (1M, 30 mL). The volume of the mixture was then reduced to ca. 40 mL and water (50 mL) added and the mixture extracted with EA (3 x 80 mL). The combined organic extracts were then washed with cold water (60 mL) and brine (60 mL), dried over anhydrous MgSO<sub>4</sub> and the solvent removed under reduced pressure. Purification was achieved by dissolving the residue obtained in DE (60 mL) and passing it through a short plug of silica gel ca. 1 cm, which after evaporation of the solvent gave the title compound as a white solid (3.58 g, 21.04 mmol, 76%). Data was in agreement with the literature [42]. **R<sub>f</sub>** 0.23 (40% EA/PE); **M.p.** 105-106 °C (lit.<sup>1</sup> 108-109 °C);  $\nu_{\max}$  3387, 3217, 2992, 2950, 2886, 1604, 1564, 1483, 1470, 1453, 1397, 1382, 1354, 1205;  $\delta_{\text{H}}$  3.97 (3H, s, CH<sub>3</sub>), 4.01 (3H, s, CH<sub>3</sub>), 4.60 (2H, s, CH<sub>2</sub>), 6.35 (1H, s, CH);  $\delta_{\text{C}}$  54.0, 54.9, 63.5, 97.3, 165.0, 170.1, 172.1

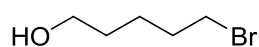
**2,6-dimethoxypyrimidine-4-carbaldehyde 16** (Xie et al. 2000)



To a solution of the previous alcohol (3.02 g, 17.75 mmol) in DCM (140 mL) was added Dess-Martin periodinane (7.68 g, 21.04 mmol) and the mixture was stirred at room temperature. Reaction progress was monitored *via* TLC and after 1 h the reaction mixture was washed with 10% Na<sub>2</sub>S<sub>2</sub>O<sub>3</sub> (180 mL), NaHCO<sub>3</sub> (sat. 160 mL) and the combined aqueous

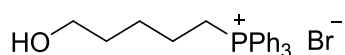
washings extracted with DCM (150 mL). The combined organic extracts were then washed with water (150 mL) and brine (150 mL), dried over anhydrous  $\text{MgSO}_4$  and the solvent removed under reduced pressure. Purification was achieved by flash column chromatography on silica gel eluting in EA/PE (20:80) gave **16** as an off-white solid (2.16 g, 12.85 mmol, 72%). Data was in agreement with the literature [42].  $R_f$  0.23 (20% EA/PE); **M.p.** 109-112 °C (lit. 107 °C [44]);  $\nu_{\text{max}}$  3387, 3102, 2960, 2877, 2844, 1713, 1600, 1579, 1561, 1485, 1460, 1447, 1393, 1365, 1350, 1255, 1205;  $\delta_{\text{H}}$  4.03 (3H, s,  $\text{CH}_3$ ), 4.08 (3H, s,  $\text{CH}_3$ ), 6.90 (1H, s, CH), 9.90 (1H, CH);  $\delta_{\text{C}}$  54.6, 55.3, 97.5, 192.2 (3 x C not observed).

### 5-bromopentan-1-ol (Chong et al. 2000)



To a mixture of 1,5-pentanediol (30 g, 205 mmol) and TL (600 mL) was added HBr (48% aq., 27 mL, 240 mmol). The mixture was stirred vigorously and heated at reflux for 36 h, after which time HBr (48% aq., 10 mL, 90 mmol) was added and the reaction mixture heated for a further 36 h. The reaction mixture was then cooled to room temperature, the phases separated and the organic extract diluted with DE (200 mL). The organic extract was then washed with NaOH (1M, 100 mL) and brine (100 mL), dried over anhydrous  $\text{MgSO}_4$  and the solvent removed under reduced pressure. After evaporation, purification was achieved by flash column chromatography on silica gel using EA/PE (15:85 to 20:80). Fractions eluting in EA/PE (15:85) gave the title compound as a pale yellow oil (7.19 g, 43.04 mmol, 21%). Data was in agreement with the literature (Chong et al. 2000).  $R_f$  0.48 (80% EA/PE);  $\nu_{\text{max}}$  3346, 2937, 2864, 1720, 1640, 1455, 1432, 1369, 1270, 1246, 1200;  $\delta_{\text{H}}$  1.46-1.61 (4H, m, 2 x  $\text{CH}_2$ ), 1.79 (1H, br s, OH), 1.84-1.91 (2H, m,  $\text{CH}_2$ ), 3.40 (2H, t,  $J$  6.8 Hz,  $\text{CH}_2$ ), 3.63 (2H, t,  $J$  6.4 Hz,  $\text{CH}_2$ );  $\delta_{\text{C}}$  24.3, 31.6, 32.4, 33.7, 62.3.

### (5-hydroxypentyl)triphenylphosphonium bromide **17** (Meyers and Collington 1974; Lei and Atkinson 2000)

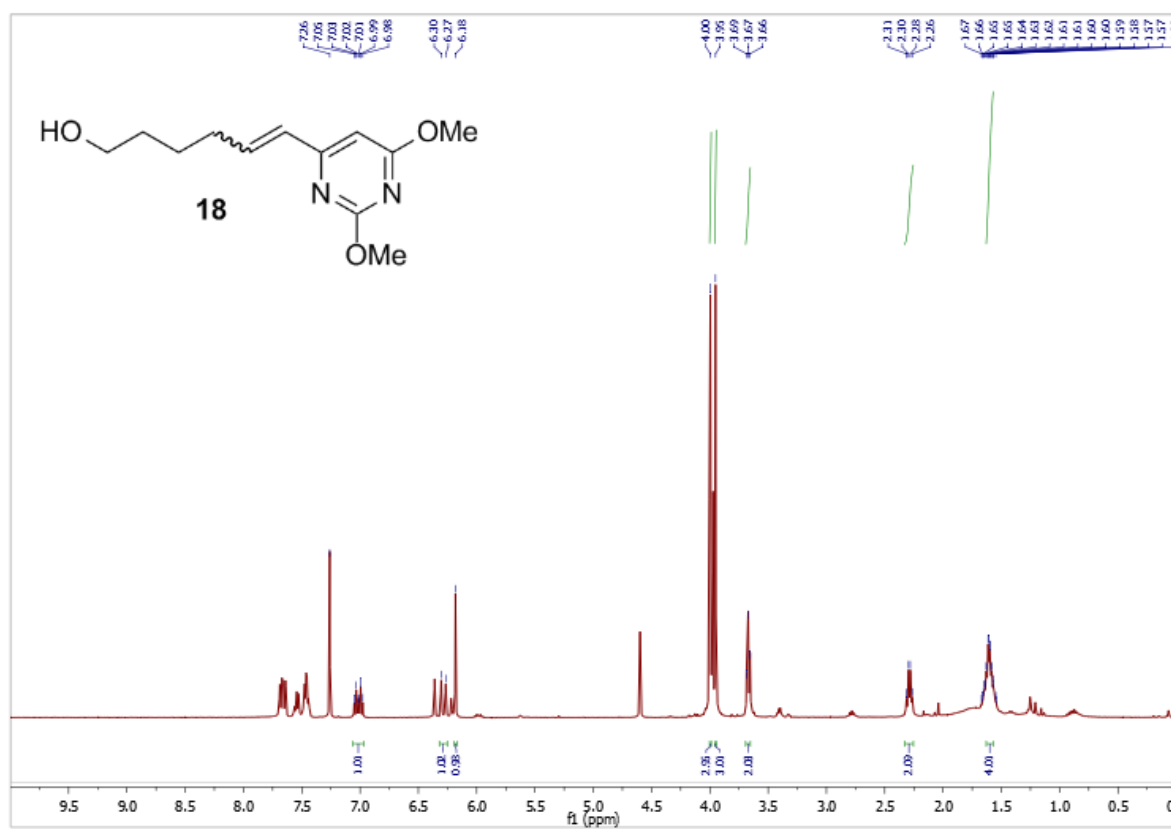


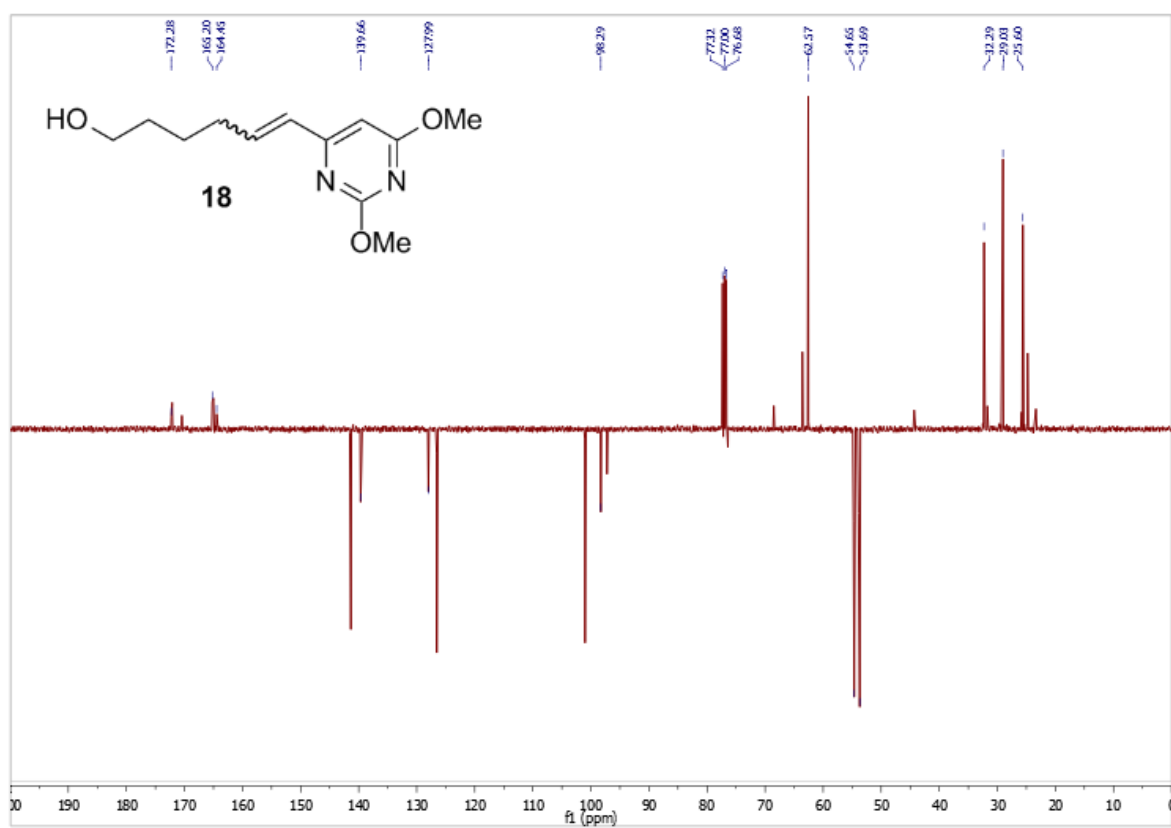
To a stirred solution of 5-bromo-1-pentanol (1.7 g, 10.18 mmol) in absolute EtOH (65 mL) was added triphenylphosphine (2.67 g, 10.18 mmol) and  $\text{K}_2\text{CO}_3$  (0.1 eqv, 0.14 g) and the mixture heated at reflux for 16 h. The reaction mixture was then evaporated to dryness and the residue suspended in CFM (30 mL), filtered, and the solvent removed under reduced pressure.

The reaction mixture was then heated to 100 °C in TL (30 mL) for 2 h with vigorous stirring. Upon cooling phosphonium salt **17** crystallised and the TL was decanted to give the title compound as an off-white solid (3.06 g, 8.76 mmol, 86%). Data was in agreement with the literature (Xie et al. 2000). **M.p.** 181-183 °C (lit. 190-191 °C; (Meyers and Collington 1974; Lei and Atkinson 2000);  $\nu_{max}$  3310, 3046, 3008, 2932, 2800, 1617, 1585, 1483, 1468, 1434, 1410, 1381, 1317, 1237, 1200;  $\delta_H$  1.65-1.83 (6H, m, 3 x CH<sub>2</sub>), 2.17-2.27 (1H, br s, OH), 3.65 (2H, t, *J* 5.0 Hz, CH<sub>2</sub>), 3.70-3.80 (2H, m, CH<sub>2</sub>), 7.64-7.86 (15H, m, 3 x C<sub>6</sub>H<sub>5</sub>);  $\delta_C$  22.1, 23.0, 26.9, 31.0, 61.7, 118.0, 118.9, 130.4, 130.5, 133.7, 133.8, 134.9, 135.0.

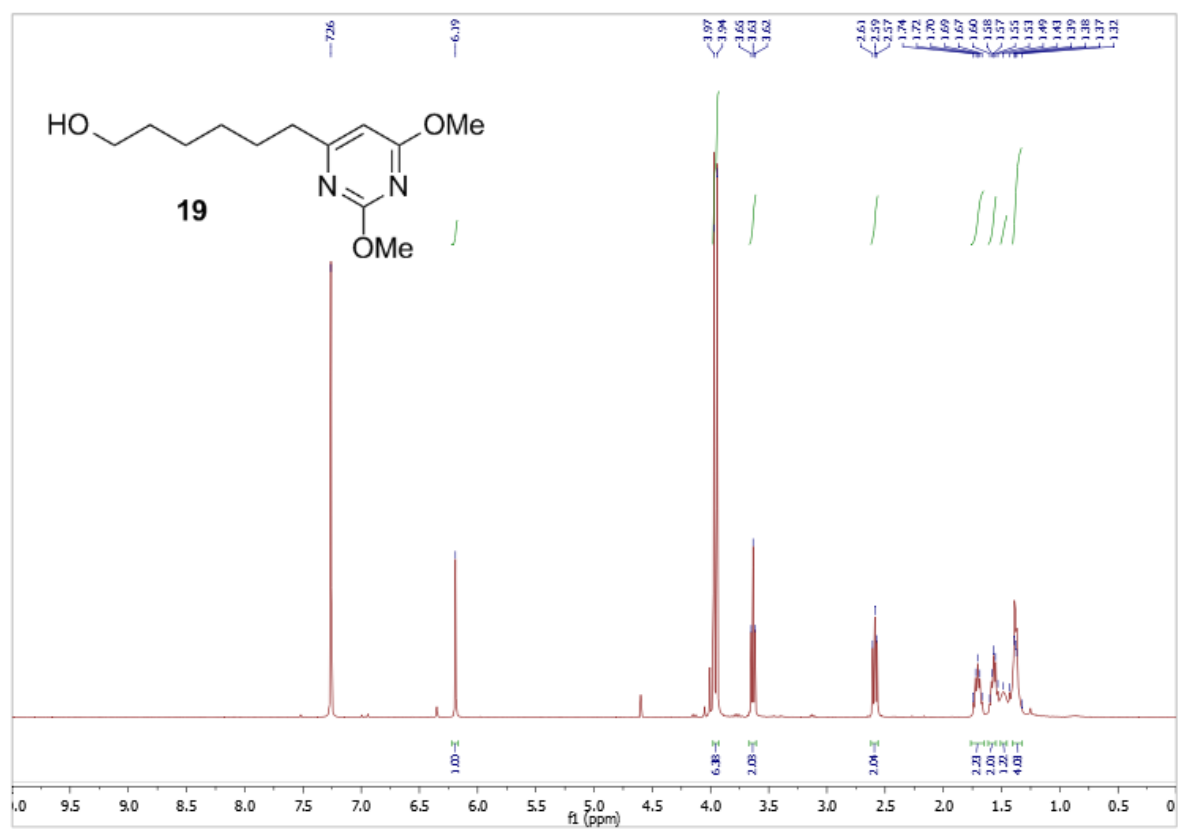
$^1\text{H}$  and  $^{13}\text{C}$  data for compounds 13-15 18, 19, 20 and 22 in reaction sequence order.

### Compound 18

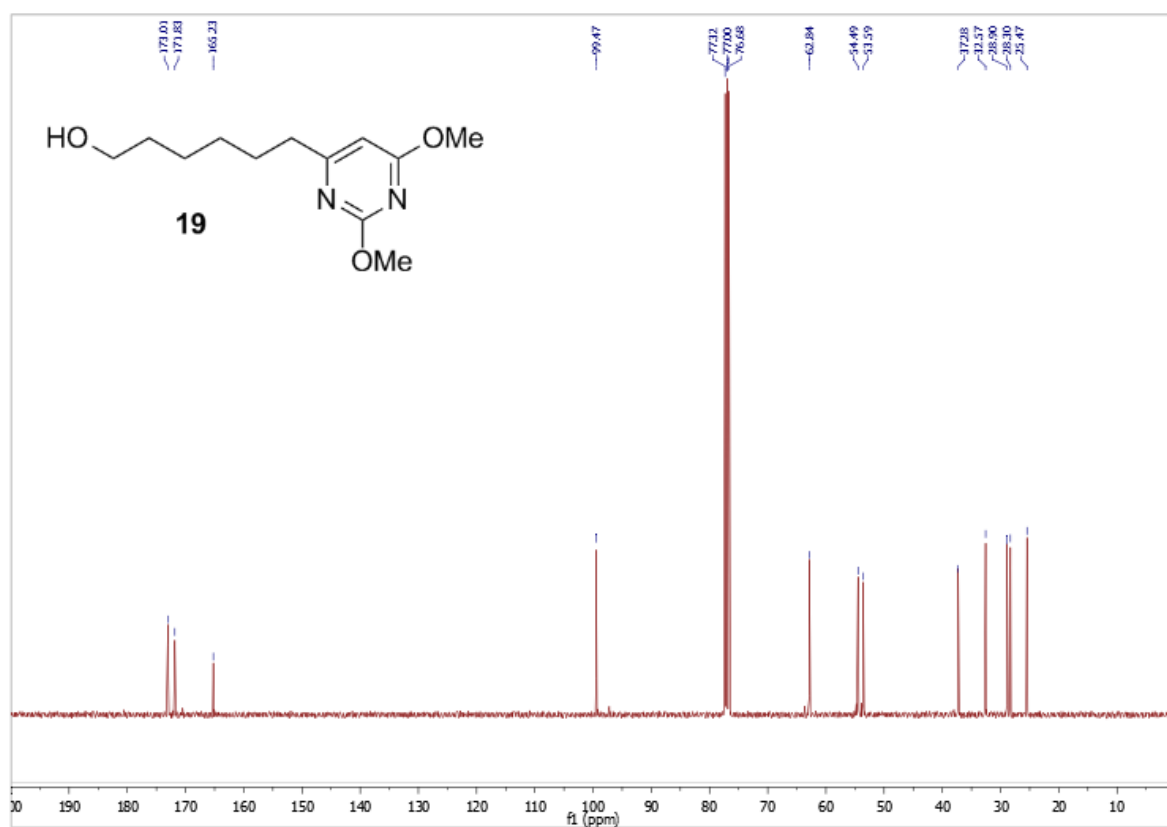




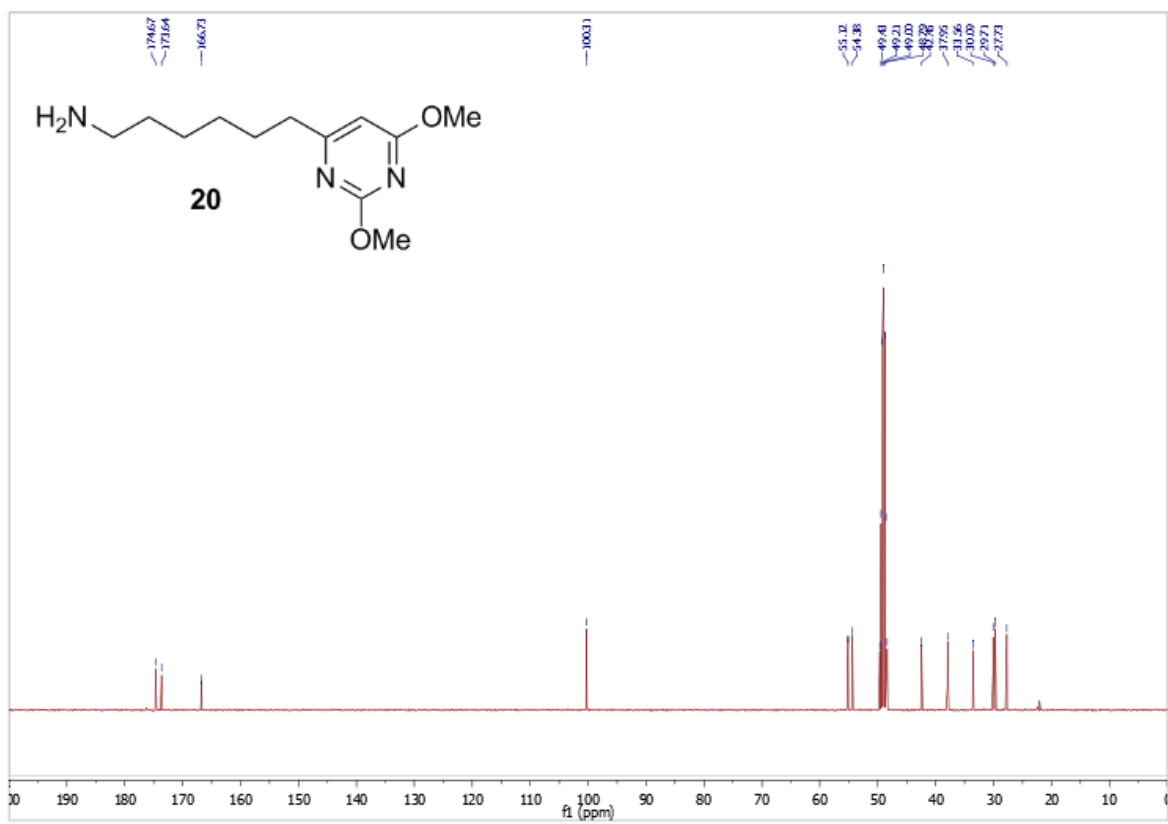
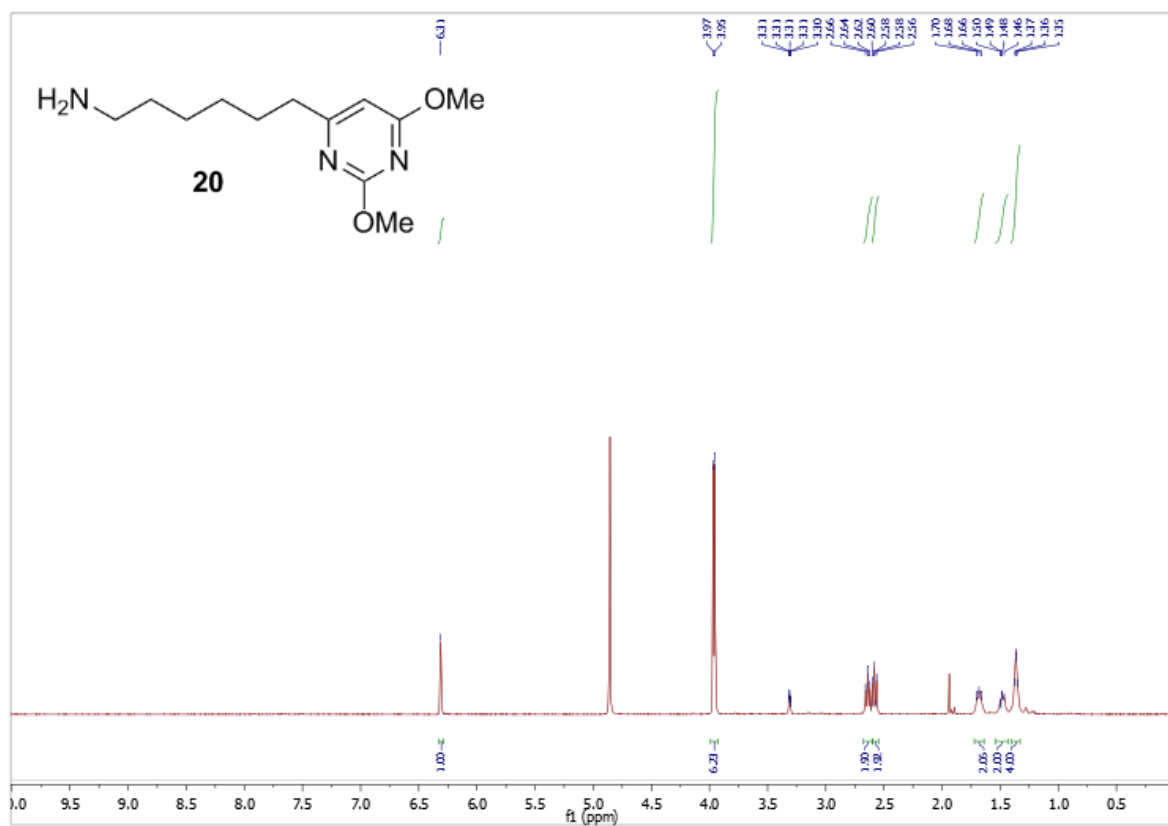
**Compound 19**



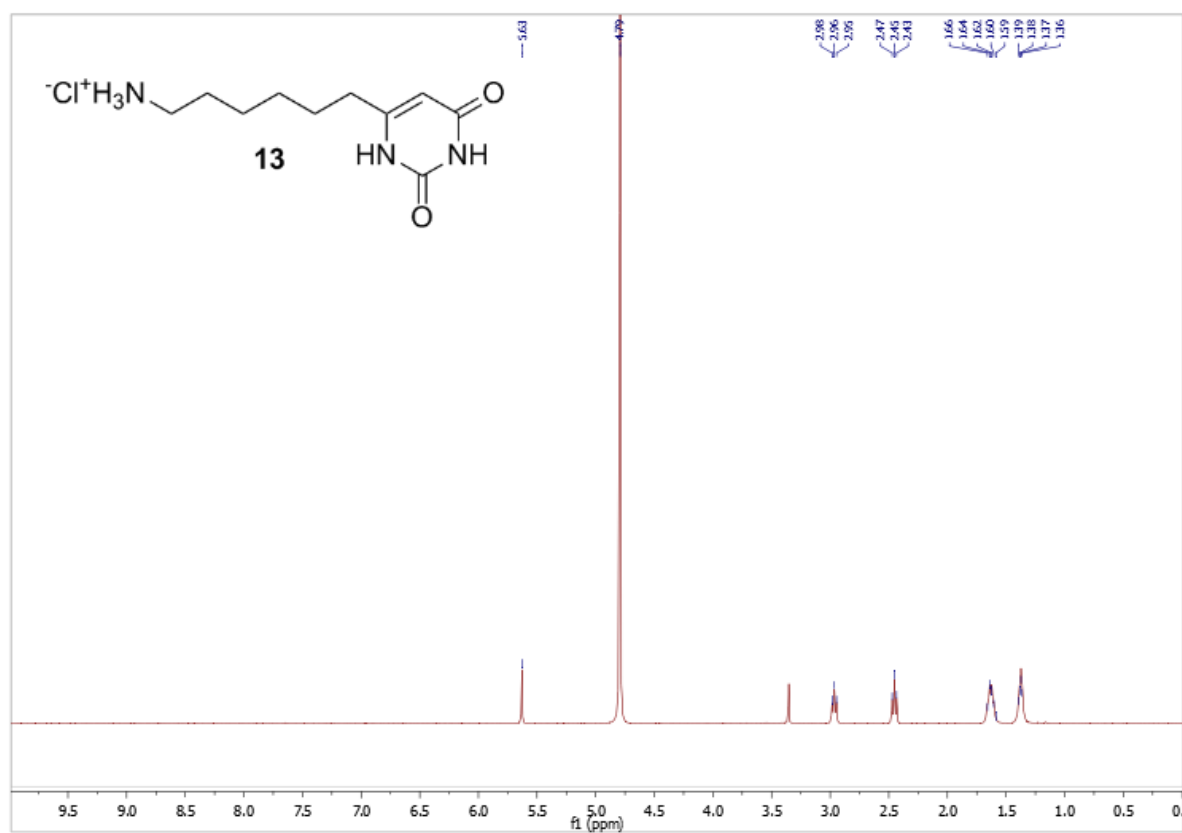


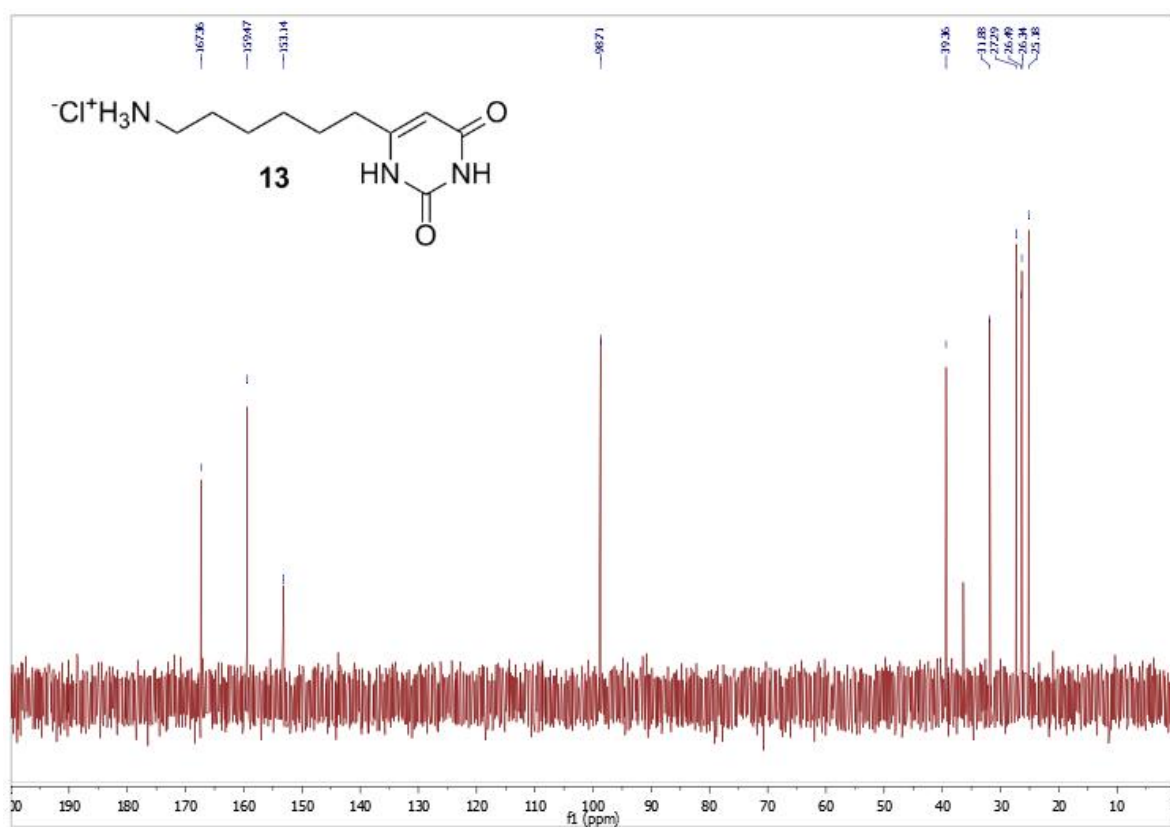


## Compound 20

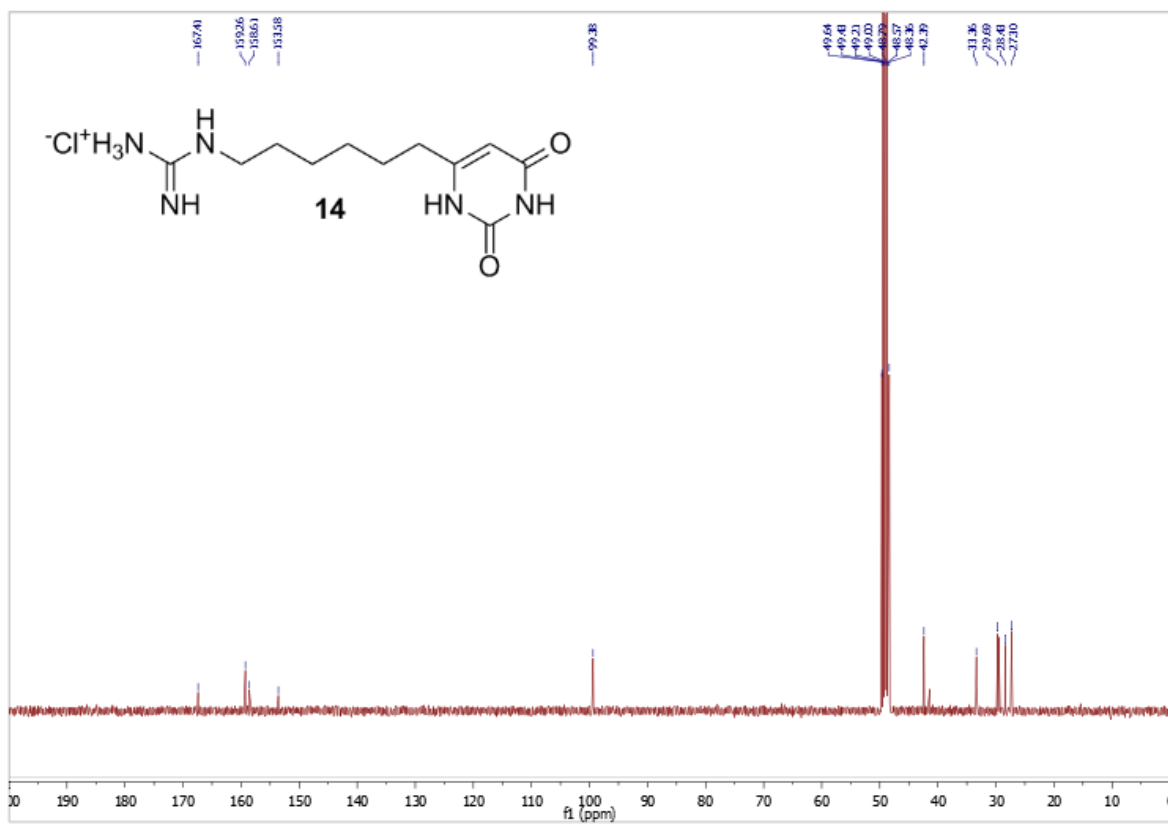
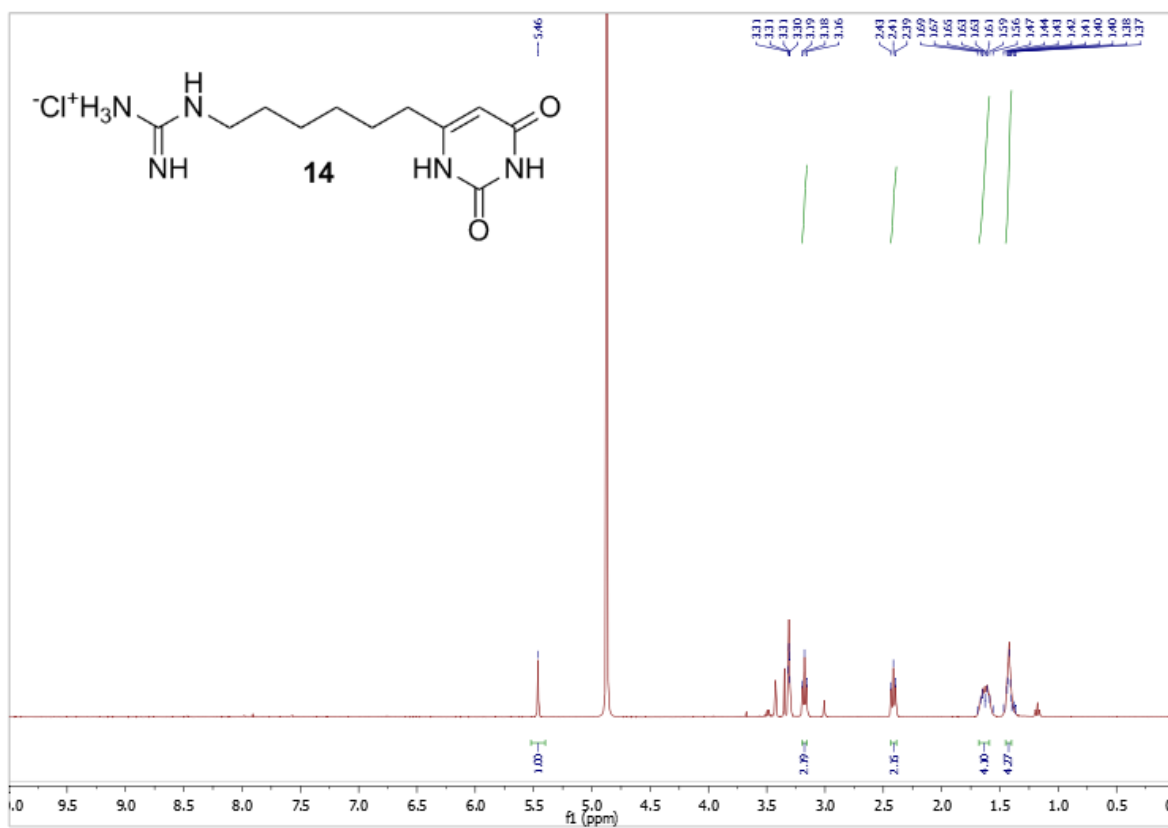


## Compound 13

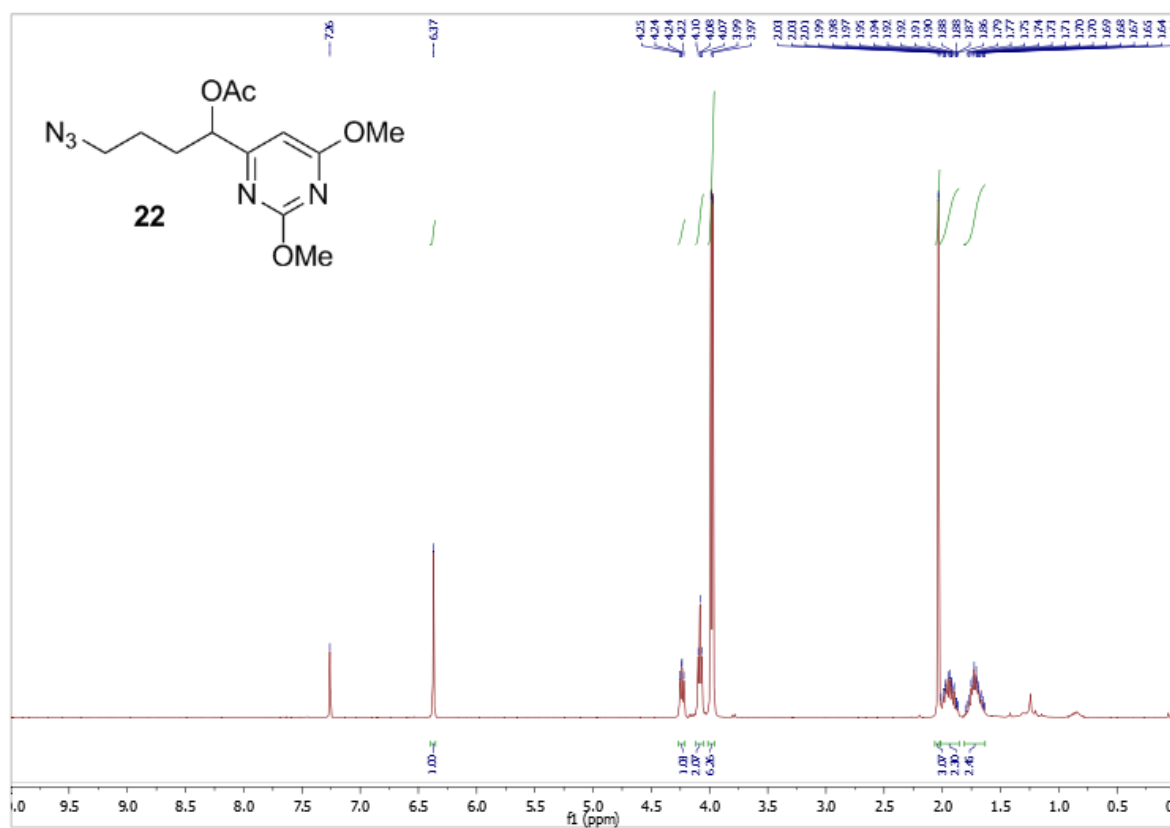


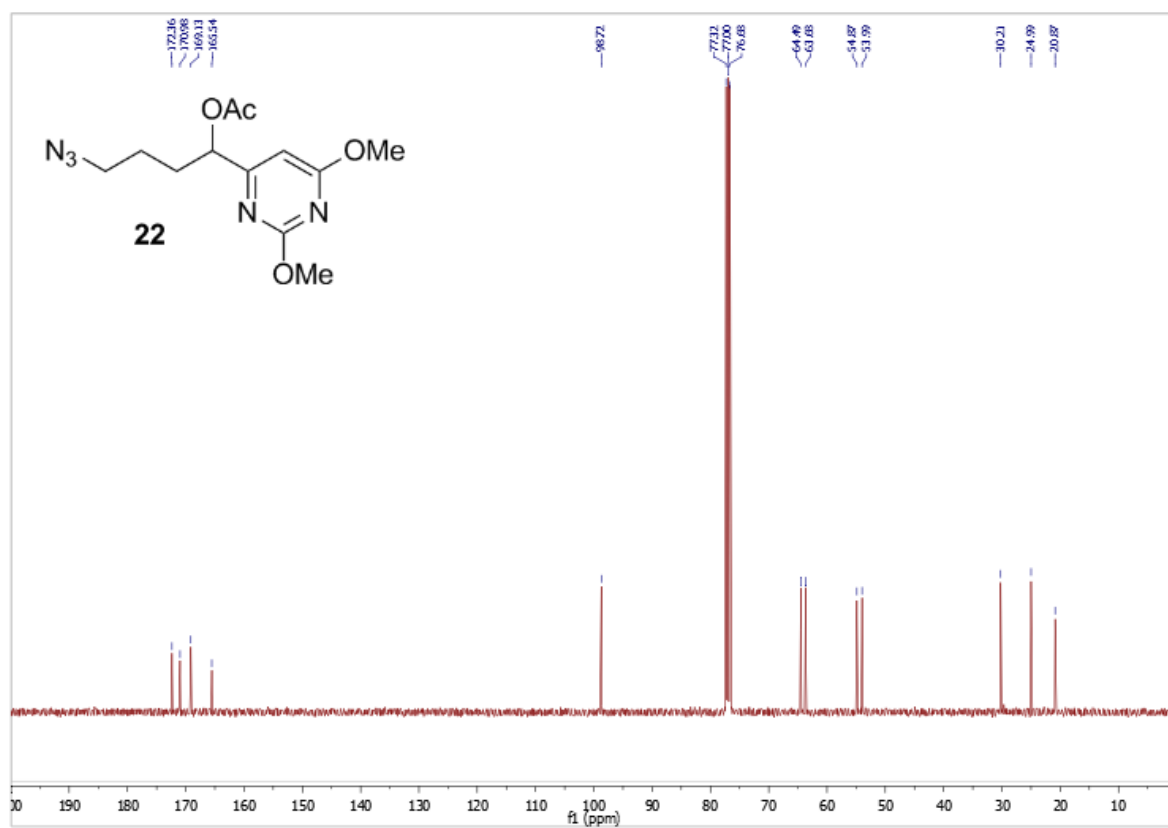


**Compound 14**



# Compound 22





**Compound 15**

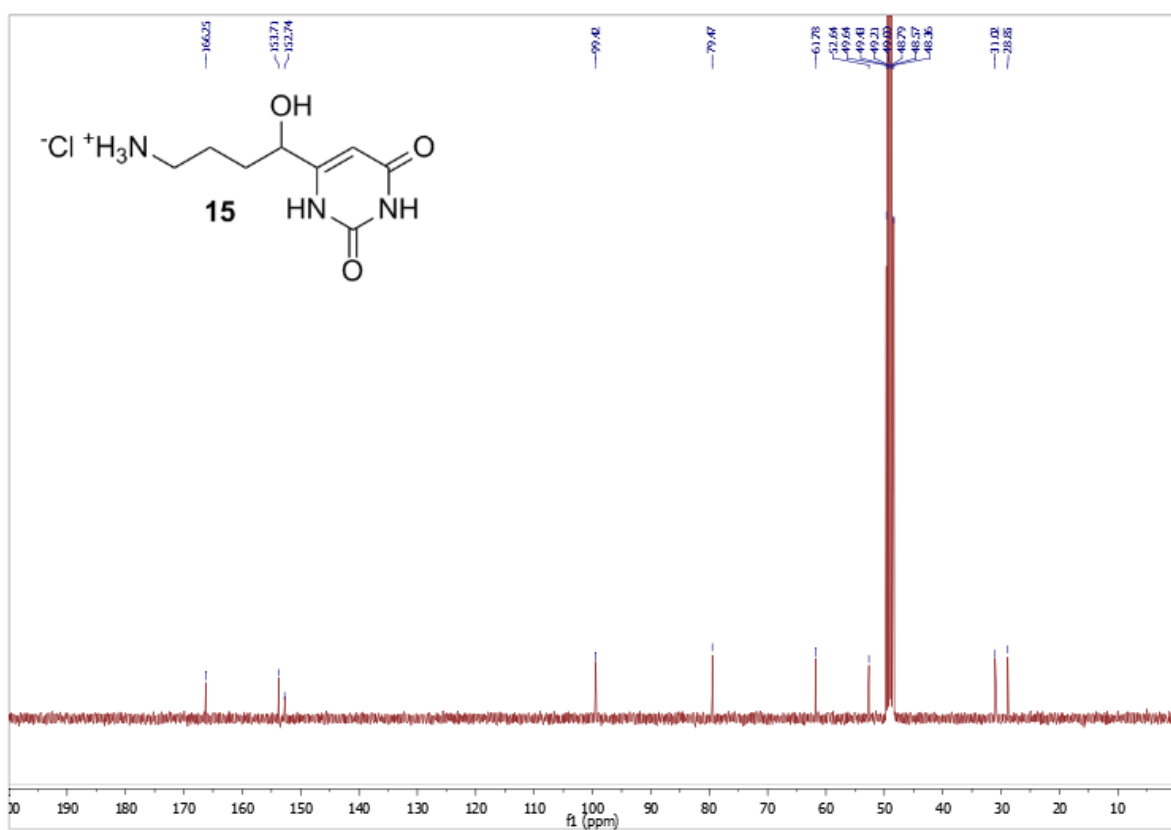
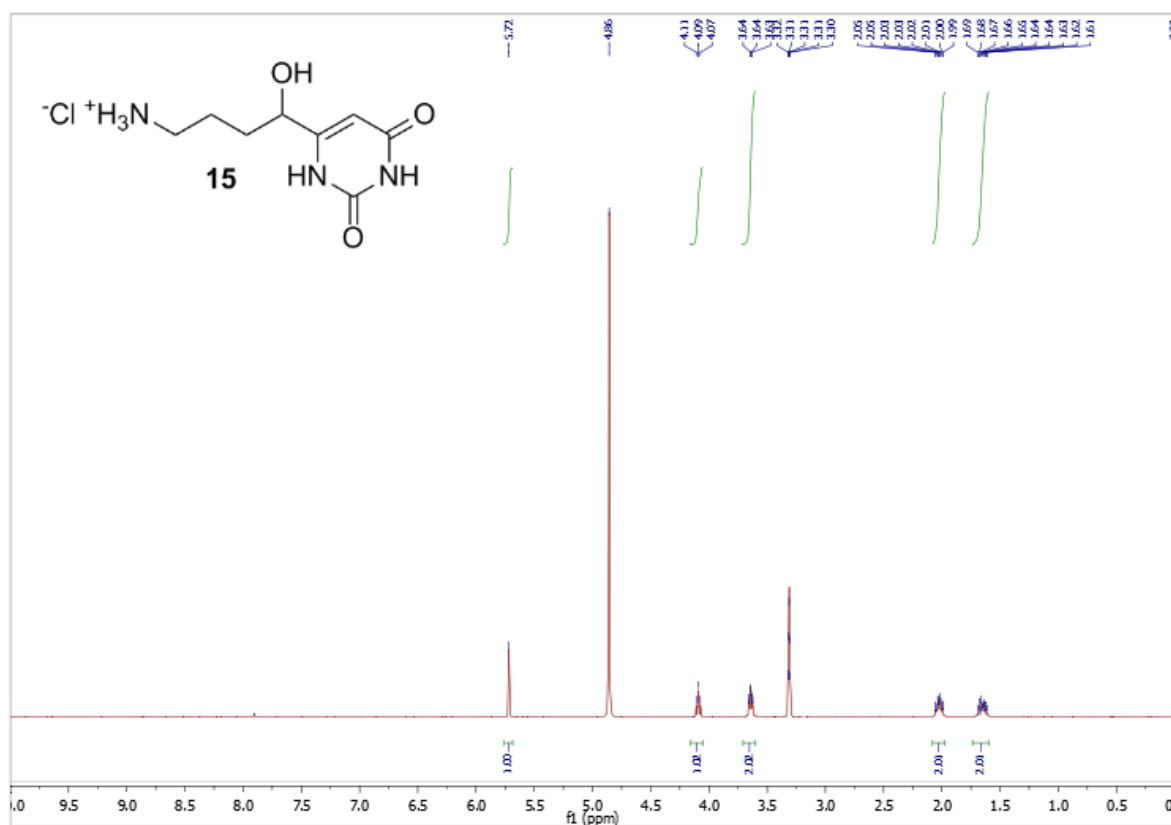




Table S1. **Crystallographic details on the collection of 14.2H<sub>2</sub>O.**

Identification code	<b>2017ncs0676 / DME-691</b>	
Empirical formula	C <sub>11</sub> H <sub>24</sub> ClN <sub>5</sub> O <sub>4</sub>	
Formula weight	325.80	
Temperature	100(2) K	
Wavelength	1.54178 Å	
Crystal system	Triclinic	
Space group	<i>P</i> −1	
Unit cell dimensions	<i>a</i> = 7.3617(3) Å	<i>α</i> = 77.351(3)°
	<i>b</i> = 8.0772(3) Å	<i>β</i> = 77.703(3)°
	<i>c</i> = 14.7036(5) Å	<i>γ</i> = 80.316(3)°
Volume	826.71(6) Å <sup>3</sup>	
<i>Z</i>	2	
Density (calculated)	1.309 Mg / m <sup>3</sup>	
Absorption coefficient	2.256 mm <sup>−1</sup>	
<i>F</i> (000)	348	
Crystal	Block; colourless	
Crystal size	0.200 × 0.150 × 0.050 mm <sup>3</sup>	
<i>θ</i> range for data collection	5.660 – 70.640°	
Index ranges	−8 ≤ <i>h</i> ≤ 8, −9 ≤ <i>k</i> ≤ 9, −17 ≤ <i>l</i> ≤ 17	
Reflections collected	22939	
Independent reflections	3086 [ <i>R</i> <sub>int</sub> = 0.0581]	
Completeness to <i>θ</i> = 67.684°	99.2 %	
Absorption correction	Semi-empirical from equivalents	
Max. and min. transmission	1.00000 and 0.57015	
Refinement method	Full-matrix least-squares on <i>F</i> <sup>2</sup>	
Data / restraints / parameters	3086 / 0 / 234	
Goodness-of-fit on <i>F</i> <sup>2</sup>	1.042	
Final <i>R</i> indices [ <i>F</i> <sup>2</sup> > 2σ( <i>F</i> <sup>2</sup> )]	<i>R</i> 1 = 0.0598, <i>wR</i> 2 = 0.1786	
<i>R</i> indices (all data)	<i>R</i> 1 = 0.0609, <i>wR</i> 2 = 0.1797	
Extinction coefficient	n/a	
Largest diff. peak and hole	0.843 and −0.464 e Å <sup>−3</sup>	

Diffractometer: Rigaku AFC11 quarter chi goniometer equipped with a Rigaku Hypix 6000 detector mounted at the window of 007 HF copper rotating anode generator with Varimax optics. (300 $\mu$ m focus). Cell determination, Data collection, Data reduction and cell refinement & Absorption correction: CrysAlisPro 1.171.39.31c (Rigaku Oxford Diffraction, 2017), Structure solution: SHELXT. Structure refinement: SHELXL-2014/7. (Sheldrick 2015) CCDC Number: 1894387.

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