Precious Metal Complexes of Bis(pyridyl)allenes: Synthesis, Catalytic and Medicinal Applications

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1. General information

All reagents and solvents used in the described syntheses were of analytical grade and purchased from commercial suppliers, including all Grignard reagents (Merck, Thermo Fisher Scientific, Fluorochem); used without further purification, unless otherwise stated. Dry solvents (anhydrous ≥ 99.8%) were obtained directly from commercial sources. $ZnBr_2$ was dried with a heat gun in *vacuo* until it became visibly dry. Preparation of compounds **1a**-**b**, **3b** and **4b** was previously described by our group,[1] but have also been included here for clarity. Enynes **8** and **19** and allene **10** used in catalytic screening were prepared according to reported methods.[2,3] Accurate weights were obtained with a Denver Instrument SI-234 balance (230 g x 0.1 mg) for synthetic experiments and with a Mettler Toledo XS205DU (81/120 g x 0.01/0.1 mg) for the preparation of stock solutions. For thin layer chromatography (TLC) technique, commercially available aluminium sheets pre-coated with silica gel (0.20 mm with fluorescent indicator UV254, Grace GM BH & Co) were used. Column chromatography was performed using silica gel 60, 0.032-0.063 mm (230-450 mesh, Alfa Aesar). Ratios of the solvents used as eluents are given in brackets. For compounds isolated as a part of the mixture, the given yield was calculated for the compound in question only, provided that other components of the mixture were known. Non-conventional heating by microwave irradiation was performed in a Biotage Initiator$^+$ Microwave system equipment with normal absorption level, with the exception of reactions carried out in 1,4-dioxane where low level of absorption was applied. The ^{1}H nuclear magnetic resonance (NMR) and ^{13}C NMR spectra were recorded using a Bruker AscendTM 500 or a Bruker UltrashieldTM Plus 400 spectrometers (solvent given in brackets). Chemical shifts (δ) are given in parts per million (ppm) and the values of coupling constant *J* are given in Hertz (Hz). Abbreviations for multiplicities are as follows: s – singlet, d – doublet, t – triplet, q – quartet, m – multiplet, brs – signal broadened. Spectra were recorded at room temperature unless stated otherwise. 2D NMR experiments: correlation spectroscopy (COSY) and heteronuclear single quantum coherence spectroscopy (HSQC) were performed to assign proton and carbon peaks, respectively. In some cases, they were supported with nuclear Overhauser effect spectroscopy (NOESY) and heteronuclear multiple bond coherence spectroscopy (HMBC) experiments to facilitate structure elucidation. High resolution mass spectra were carried out using electro spray ionisation (ESI), electron impact (EI), nano spray ionisation (NSI) and atmospheric solid analysis probe ASAP techniques at the University of Sussex (UK) and National Mass Spectrometry Facility in Swansea (UK). ESI measurements were carried out on APEX II Bruker Daltonic Fourier transformer (FTMS) using Apollo ESI source. EI ionisation was performed using AutoSpec Fisons instrument. NSI measurements were carried out on LTQ Orbitrap XL spectrometer. ASAP measurements were carried out Xevo G2-S spectrometer. Microanalysis was performed with Thermo Flash 2000 Elemental

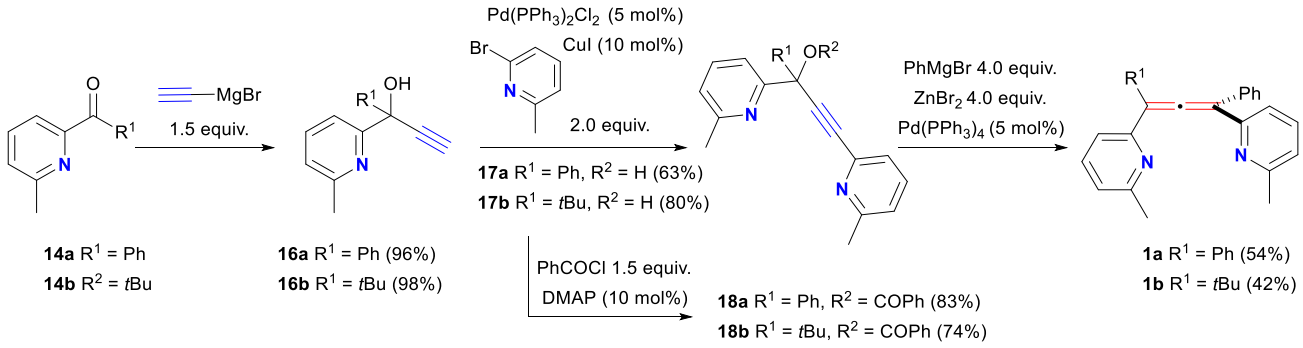
Analyser configured for for %CHN, at the London Metropolitan University (UK). For the plate assays, absorbance and fluorescence measurements were carried out using a CLARIOstar® (BMG Labtech) microplate reader at rt. Cyclic voltammetry was performed using an Autolab PGSTAT302N potentiostat/galvanostat (Metrohm) with a three-electrode system. The glassy carbon working electrode was polished using alumina. The counter electrode was Pt, and Ag or Ag/AgCl was used as a reference electrode with ferrocene internal standard (Fc/Fc^+) at 0.43 V *vs.* Ag/AgCl. The electrochemical cell was purged with Ar prior and after addition of the analyte solution.

Ethical statement: The DNA and the anticancer experiments use synthetic DNA sequences and cultured human cell lines that do not have any associated ethic approvals. The antimicrobial work carried out by the CO-ADD was performed in strict accordance with The Australian Code for the Responsible Conduct of Research (2018). Human blood was sourced from the Australian Red Cross Blood Service with informed consent, and its use in haemolysis assays was approved by The University of Queensland Institutional Human Research Ethics Committee, Approval Number 2014000031.

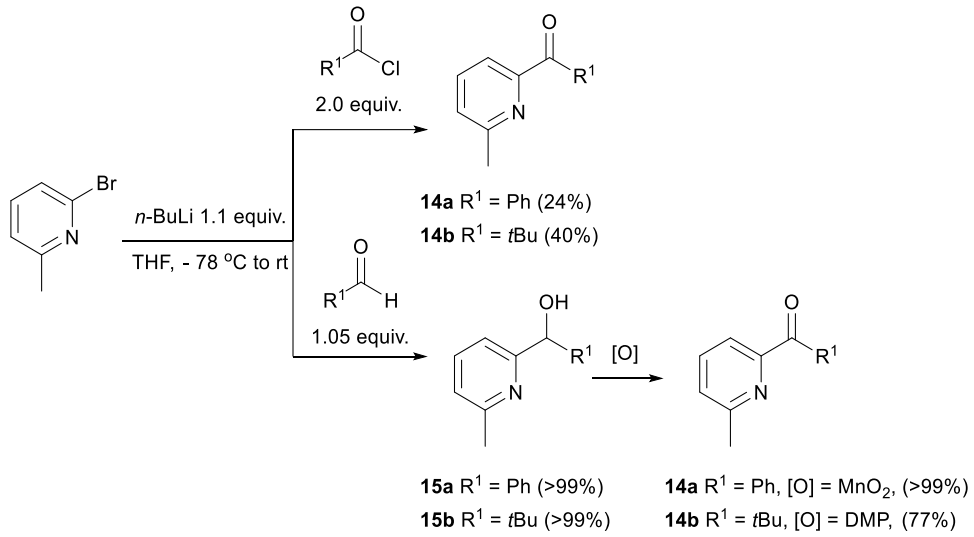
2. Synthetic details

2.1. Synthesis of bis(pyridyl)allenes

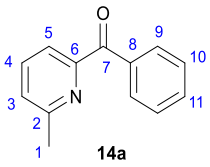
- General synthetic strategy



- Synthesis of ketones **14a**-**b**



Compound 14a



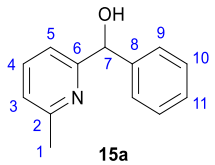
Procedure a:[4] To a solution of 2-bromo-6-methylpyridine (1.032 g, 6.00 mmol, 1.0 equiv.) in 10 mL of anhydrous THF, *n*-BuLi solution in hexanes (2.96 mL, 6.60 mmol, 1.1 equiv.) was added dropwise at - 78 °C under inert atmosphere. The resulting deep red solution was stirred at - 78 °C for 1 h. Subsequently it was transferred *via* cannula to the solution of benzoyl chloride (1.692 g, 12.00 mmol, 2.0 equiv.) in 13 mL of THF pre-cooled to -78 °C. The resulting solution was stirred at -78 °C for another 1 h, then was slowly warmed to rt and left stirring overnight. The solution changed colour to yellow. The solution was then quenched with water and the resulting mixture was poured into 20 mL of 30% aqueous NaOH solution and stirred at rt for 1 h. The aqueous layer was separated and extracted with Et_2O and the combined organic layers were washed with water, brine, dried over $MgSO_4$, filtered and concentrated in *vacuo*. The crude reaction mixture was purified by two consecutive chromatography columns using silica gel (Pet/AcOEt 94:6) to yield compound **14a** (283 mg, 24%) as a yellow oil.

Procedure b:[5] To a solution of alcohol **15a** (3.033 g, 15.24 mmol, 1.0 equiv.) in 76 mL of dichloromethane, activated MnO_2 (5.437 g, 62.49 mmol, 4.1 equiv.) was added and the resulting suspension was stirred at rt for 24 h (monitored by TLC). The reaction mixture was filtered through a pad of Celite and concentrated in *vacuo* to yield pure ketone **14a** (3.002 g, > 99%) as a yellow oil.

^{1}H NMR (400 MHz, $CDCl_3$) δ 8.12 – 8.07 (m, 2H, *10*), 7.80 – 7.73 (m, 2H, H_{Py}), 7.58 (t, *J* = 7.5 Hz, 1H, *11*), 7.47 (t, *J* = 7.5 Hz, 2H, *9*), 7.34 (dd, *J* = 6.8, 1.9 Hz, 1H, H_{Py}), 2.63 (s, 3H, *1*); **^{13}C NMR** (101 MHz, $CDCl_3$) δ 194.1 (*7*), 157.9 (C_{Py}), 154.9 (C_{Py}), 137.2 (*4*), 136.4 (*8*), 133.0 (*11*), 131.3 (2 x C_{Ph}-H), 128.2 (2 x C_{Ph}-H), 125.9 (C_{Py}-H), 121.8 (C_{Py}-H), 24.7 (*1*).

The characterisation data is in agreement with previously reported.[6]

Compound 15a[4]



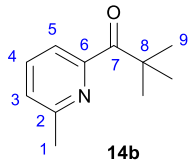
15a

To a solution of 2-bromo-6-methylpyridine (516 mg, 3.00 mmol, 1.0 equiv.) in 4.5 mL of anhydrous THF, *n*-BuLi solution in hexanes (1.48 mL, 3.30 mmol, 1.1 equiv.) was added dropwise at -7 8 °C under inert atmosphere and stirred for another 20 min. Benzaldehyde (0.32 mL, 3.2 mmol, 1.05 equiv.) was added dropwise at - 78 °C and the solution was allowed to warm to rt and stirring continued for 3 h. $NH_4Cl_{(aq)}$ was added and the aqueous layer was separated and extracted with dichloromethane. Combined organic layers were dried over $MgSO_4$, filtered and concentrated in *vacuo*. The crude was purified by colum chromatography using silica gel (AcOEt/Pet 2:3) to yield alcohol **15a** (597 mg, > 99%) as a yellow semisolid.

^{1}H NMR (500 MHz, $CDCl_3$) δ 7.49 (t, *J* = 7.7 Hz, 1H, *4*), 7.40 – 7.36 (m, 2H, H_{Ph}), 7.36 – 7.31 (m, 2H, H_{Ph}), 7.30 – 7.25 (m, 1H, *11*), 7.04 (d, *J* = 7.7 Hz, 1H, H_{Py}), 6.90 (d, *J* = 7.7 Hz, 1H, H_{Py}), 5.70 (brs, 2H, *7,*-O*H*), 2.60 (s, 3H, *1*); **^{13}C NMR** (126 MHz, $CDCl_3$) δ 160.0 (C_{Py}), 156.7 (C_{Py}), 143.5 (*8*), 137.3 (*4*), 128.6 (2 x C_{Ph}-H), 127.9 (*11*), 127.3 (2 x C_{Ph}-H), 122.0 (C_{Py}-H), 118.5 (C_{Py}-H), 74.6 (*7*), 24.3 (*1*).

The characterisation data is in agreement with previously reported.[6]

Compound 14b



14b

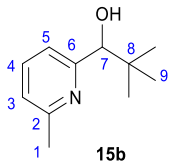
Procedure a:[4] To a solution of 2-bromo-6-methylpyridine (1.032 g, 6.00 mmol, 1.0 equiv.) in 10.2 mL of anhydrous THF, *n*-BuLi solution in hexanes (2.96 mL, 6.60 mmol, 1.1 equiv.) was added dropwise at - 78 °C under inert atmosphere. The resulting deep red solution was stirred

at - 78 °C for 1 h. Subsequently it was transferred *via* cannula to a solution of pivaloyl chloride (1.447 g, 12.00 mmol, 2.0 equiv.) in 2 mL of THF pre-cooled to -78 °C. The resulting solution was stirred at -78 °C for another 2 h, the colour changed to orange. The solution was slowly warmed to rt and left stirring overnight. Then, 5 mL of water was added, and the resulting mixture was poured into 50 mL of 40% aqueous NaOH solution and stirred at rt for 3.5 h. The aqueous layer was separated and extracted with Et_2O and the combined organic layers were washed with brine, dried over $MgSO_4$, filtered and concentrated in *vacuo*. The crude reaction mixture was purified by column chromatography using silica gel (Pet/AcOEt 50:1) to yield compound **14b** (424 mg, 40%) as a pale yellow oil slightly contaminated with pivaloyl chloride.

Procedure b: To a solution of alcohol **15b** (2.452 g, 13.70 mmol, 1.0 equiv.) in 108 mL dichloromethane, the Dess-Martin periodinane (6.970 g, 16.40 mmol, 1.2 equiv.) was added at 0 °C. The reaction mixture was slowly warmed to rt. After a total of 4 h, aqueous $NaHCO_3$ was added. The aqueous layer was separated and the organic layer was washed with $NaHCO_{3(aq)}$, brine, dried over $MgSO_4$, filtered and concentrated in *vacuo*. Crude product was purified by column chormatography using silica gel (Pet/AcOEt 9:1) to yield compound **14b** (1.864 g, 77%) as a yellow oil.

^{1}H NMR (500 MHz, $CDCl_3$) δ 7.69 – 7.62 (m, 2H, H_{Py}), 7.22 (dd, J = 7.0, 1.7 Hz, 1H, H_{Py}), 2.58 (s, 3H, *1*), 1.45 (s, 9H, *9*); **^{13}C NMR** (126 MHz, $CDCl_3$) δ 207.3 (*7*), 156.7 (C_{Py}), 154.4 (C_{Py}), 136.9 (C_{Py}-H), 125.4 (C_{Py}-H), 120.6 (C_{Py}-H), 44.4 (*8*), 27.8 (*9*), 24.5 (*1*); **HRMS** (ESI) ($C_{11}H_{16}NO$) $[M+H]^+$: cacld: 178.1226; found: 178.1225.

Compound 15b[4]



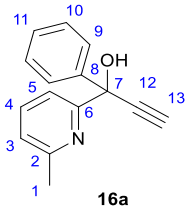
15b

To a solution of 2-bromo-6-methylpyridine (2.236 g, 13.00 mmol, 1.0 equiv.) in 19.4 mL of anhydrous THF, *n*-BuLi solution in hexanes (5.96 mL, 14.30 mmol, 1.1 equiv.) was added dropwise at - 78 °C under inert atmosphere and stirred for another 20 min. Pivalaldehyde (1.48 mL, 13.65 mmol, 1.05 equiv.) was added dropwise at - 78 ºC and the solution was allowed to warm to rt and stirring continued for 3.5 h. $NH_4Cl_{(aq)}$ was added and the aqueous layer was separated and extracted with dichloromethane. The combined organic layers were dried over $MgSO_4$, filtered and concentrated in *vacuo* to yield pure alcohol **15b** (2.327 g, > 99%) as a yellow solid.

^{1}H NMR (400 MHz, $CDCl_3$) δ 7.49 (t, *J* = 7.7 Hz 1H, *4*), 7.01 (d, *J* = 7.7 Hz, 1H, *5*), 6.96 (d, *J* = 7.7 Hz, 1H, *3*), 4.61 (d, *J* = 7.1 Hz, 1H, -O*H*), 4.29 (d, *J* = 7.1 Hz, 1H, *7*), 2.52 (s, 3H, *1*), 0.90 (s, 9H, *9*); **^{13}C NMR** (101 MHz, $CDCl_3$) δ 159.0 (*6*), 156.7 (*2*), 135.8 (*4*), 121.8 (*3*), 119.8 (*5*), 80.0 (*7*), 36.3 (*8*), 26.0 (*9*), 24.4 (*1*); **HRMS** (ESI) ($C_{11}H_{17}NONa$) $[M+Na]^+$: cacld: 202.1202; found: 202.1201.

The characterisation data is in agreement with previously reported.[7]

Compound 16a[8]

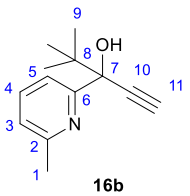


16a

To a solution of ketone **14a** (1.576 g, 8.00 mmol, 1.0 equiv.) in anhydrous THF (32.0 mL), a commercially available solution of ethynylmagnesium bromide (24.0 mL, 0.5 M in THF, 1.5 equiv.) was added at 0 °C under inert atmosphere. The reaction mixture was warmed to rt and then heated at reflux overnight. After cooling to rt, the reaction was quenched with aqueous NH_4Cl. The aqueous layer was separated and extracted with Et_2O. The combined organic layers were washed with brine, dried over $MgSO_4$, filtered and concentrated in *vacuo* to afford alcohol **16a** (1.705 g, 96%) as a black oil that was used without further purification.

^{1}H NMR (400 MHz, $CDCl_3$) δ 7.70 – 7.64 (m, 2H, *9*), 7.56 (t, *J* = 7.7 Hz, 1H, *4*), 7.38 – 7.32 (m, 2H, *10*), 7.30 – 7.25 (m, 1H, *11*), 7.19 (d, *J* = 7.7 Hz, 1H, H_{Py}), 7.09 (d, *J* = 7.7 Hz, 1H, H_{Py}), 6.95 (brs, 1H, -O*H*), 2.77 (s, 1H, *13*), 2.60 (s, 3H, *1*); **^{13}C NMR** (101 MHz, $CDCl_3$) δ 159.7 (C_{Py}), 156.1 (C_{Py}), 143.8 (*8*), 138.0 (*4*), 128.4 (2 x C_{Ph}-H), 128.0 (*11*), 126.5 (2 x C_{Ph}-H), 122.6 (C_{Py}-H), 118.7 (C_{Py}-H), 86.0 (*12*), 74.5 (*13*), 72.8 (*7*), 24.2 (*1*); **HRMS** (ESI) ($C_{15}H_{14}NO$) $[M+H]^+$: cacld: 224.1070; found: 224.1069.

Compound 16b[8]

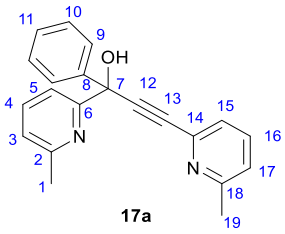


16b

To a solution of ketone **14b** (1.876 g, 10.60 mmol, 1.0 equiv.) in anhydrous THF (42.5 mL), a commercially available solution of ethynylmagnesium bromide (31.8 mL, 0.5 M in THF, 1.5 equiv.) was added at 0 °C under inert atmosphere. The reaction mixture was warmed to rt and then heated at reflux for 3 h. After cooling to rt, the reaction was quenched with aqueous NH_4Cl. The aqueous layer was separated and extracted with dichloromethane. Combined organic layers were washed with brine, dried over $MgSO_4$, filtered and concentrated in *vacuo* to afford alcohol **16b** (2.119 g, 98%) as a brown solid that was used without further purification.

^{1}H NMR (400 MHz, $CDCl_3$) δ 7.60 (t, *J* = 7.7 Hz, 1H, *4*), 7.45 (d, *J* = 7.7 Hz, 1H, H_{Py}), 7.09 (d, *J* = 7.7 Hz, 1H, H_{Py}), 6.14 (brs, 1H, -O*H*), 2.54 (s, 3H, *1*), 2.51 (s, 1H, *11*), 0.99 (s, 9H, *9*); **^{13}C NMR** (101 MHz, $CDCl_3$) δ 157.8 (C_{Py}), 155.7 (C_{Py}), 136.6 (*4*), 122.3 (C_{Py}-H), 120.1 (C_{Py}-H), 86.1 (*10*), 77.1 (*7*), 72.8 (*11*), 40.2 (*8*), 25.4 (*9*), 24.2 (*1*); **HRMS** (ESI) ($C_{13}H_{18}NO$) $[M+H]^+$: cacld: 204.1383; found: 204.1384.

Compound 17a

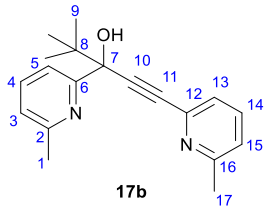


17a

Alkyne **16a** (1.474 g, 6.61 mmol, 1.0 equiv.), $Pd(PPh_3)_2Cl_2$ (232 mg, 0.33 mmol, 0.05 equiv.) and CuI (126 mg, 0.66 mmol, 0.1 equiv.) were placed under inert atmosphere. Triethylamine (16.1 mL) was added followed by 2-bromo-6-methylpyridine (1.5 mL, 13.2 mmol, 2.0 equiv.). The reaction was stirred at rt for 5 h, the reaction mixture turned black. The mixture was filtered through a pad of Celite, washed with dichloromethane and concentrated in *vacuo*. The crude product was purified by column chromatography (Pet/AcOEt/Et_3N 60/39/1) to yield alcohol **17a** (1.476 g, 63%) as a yellow solid.

^{1}H NMR (500 MHz, $CDCl_3$) δ 7.76 – 7.73 (m, 2H, H_{Ar}), 7.55 (q, *J* = 7.6 Hz, 2H, H_{Ar}), 7.37 – 7.34 (m, 3H, H_{Ph}), 7.31 – 7.27 (m, 2H, H_{Py}), 7.11 (d, *J* = 7.6 Hz, 1H, H_{Py}), 7.08 (d, *J* = 7.6 Hz, 1H, H_{Py}) 7.05 (s, 1H, -*OH*), 2.61 (s, 3H, *1/19*), 2.56 (s, 3H, 1/*19*); **^{13}C NMR** (126 MHz, $CDCl_3$) δ 159.6 (C_{Py}), 158.9 (C_{Py}), 156.0 (C_{Py}), 144.0 (C_{Py}), 142.2 (C_{Ph}), 138.1 (C_{Py}-H), 136.5 (C_{Py}-H), 128.4 (2 x C_{Ph}-H), 128.0 (*11*), 126.7 (2 x C_{Ph}-H), 125.0 (C_{Py}-H), 123.1 (C_{Py}-H), 122.5 (C_{Py}-H), 119.2 (C_{Py}-H), 91.0 (*12*), 85.5 (*13*), 73.3 (*7*), 24.6 (*1*), 24.2 (*19*); **HRMS** (ASAP) ($C_{21}H_{19}N_2O$) $[M+H]^+$: cacld: 315.1497; found: 315.1494.

Compound 17b



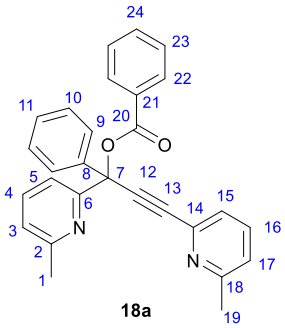
Alkyne **16b** (2.122 g, 10.45 mmol, 1.0 equiv.), $Pd(PPh_3)_2Cl_2$ (367 mg, 0.52 mmol, 0.05 equiv.) and CuI (200 mg, 1.05 mmol, 0.1 equiv.) were placed under inert atmosphere. 25.5 mL of triethylamine was added followed by 2-bromo-6-methylpyridine (3.699 g, 20.90 mmol, 2.0 equiv.). The reaction was stirred at rt for 5 h, the reaction mixture turned black. The mixture was filtered through a pad of Celite, washed with dichloromethane and concentrated in *vacuo*. The crude product was purified by colum chromatography (AcOEt/Pet 1:4 to 1:1) to yield alcohol **17b** (2.450 g, 80%) as a yellow solid.

^{1}H NMR (500 MHz, $CDCl_3$) δ 7.61 (m, 2H, *H*$_{Py}$), 7.56 – 7.48 (m, 1H, *H*$_{Py}$), 7.32 (d, *J* = 7.5 Hz, 1H, *H*$_{Py}$), 7.13 – 7.05 (m, 2H, *H*$_{Py}$), 6.25 (brs, 1H, -O*H*), 2.57 (brs, 3H, *1/17*), 2.56 (s, 3H, *1/17*), 1.07 (s, 9H, *9*); **^{13}C NMR** (126 MHz, $CDCl_3$) δ 158.8 (*C*$_{Py}$), 157.7 (*C*$_{Py}$), 155.5 (*C*$_{Py}$), 142.6 (*C*$_{Py}$), 136.7 (*4*/*14*), 136.3 (*4*/*14*), 124.9 (*C*$_{Py}$-H), 122.7 (*C*$_{Py}$-H), 122.3 (*C*$_{Py}$-H), 120.5 (*C*$_{Py}$-H), 91.5 (*10*), 84.2 (*11*), 77.5 (*7*), 40.7 (*8*), 25.6 (*9*), 24.6 (*1*/*17*), 24.2 (*1*/*17*); **HRMS** (ESI) ($C_{19}H_{23}N_2O$) $[M+H]^+$: cacld: 295.1805; found: 295.1807.

General procedure A for esterification of propargyl alcohols[9]

An oven-dried round bottom flask was charged with DMAP (0.1 equiv.) and triethylamine (1.5 equiv.) and purged with N_2. A solution of propargyl alcohol in anhydrous dichloromethane (0.2 M, 1.0 equiv.) was added, followed by benzoyl chloride (1.5 equiv.) added at 0 °C. The reaction mixture was warmed to rt; the progress of the reaction was monitored by TLC analysis. Upon completion water was added, the aqueous layer was separated and extracted with dichloromethane, the combined organic layers were washed with $NaHCO_3$, brine, dried over $MgSO_4$, filtered and concentrated in *vacuo.* Purification by column chromatography with the indicated mixture of Pet/AcOEt as the eluent yielded benzoate esters **18a**-**b**.

Compound 18a

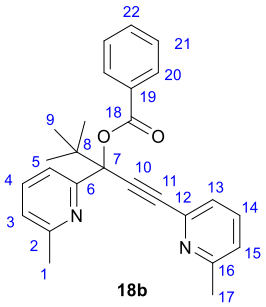


18a

Synthesised according to **General procedure A** from alcohol **17a** (945 mg, 3.01 mmol). Stirred at rt overnight. Column chromatography (Pet/AcOEt 4:1) yielded ester **18a** (1.047 g, 83%) as a yellow semisolid.

^{1}H NMR (500 MHz, $CDCl_3$) δ 8.17 – 8.14 (m, 2H, H_{Ph}), 7.83 – 7.80 (m, 2H, H_{Ph}), 7.76 (d, *J* = 7.8 Hz, 1H, H_{Py}), 7.60 – 7.55 (m, 2H, H_{Ar}), 7.53 (t, *J* = 7.8 Hz, 1H, H_{Py}), 7.46 (t, *J* = 7.7 Hz, 2H, H_{Ar}), 7.39 (d, *J* = 7.7 Hz, 1H, H_{Py}), 7.37 – 7.33 (m, 2H, H_{Ar}), 7.31 – 7.26 (m, 1H, H_{Ar}), 7.10 (d, *J* = 7.7 Hz, 1H, H_{Py}), 7.01 (d, *J* = 7.7 Hz, 1H, H_{Py}), 2.55 (s, 3H, *1/19*), 2.45 (s, 3H, *1/19*); **^{13}C NMR** (126 MHz, $CDCl_3$) δ 164.4 (*20*), 158.9 (C_{Py}), 158.6 (C_{Py}), 158.3 (C_{Py}), 142.2 (C_{Py}), 141.3 (C_{Ph}), 136.9 (C_{Py}-H), 136.3 (C_{Py}-H), 133.1 (C_{Ph}-H), 130.9 (C_{Ph}), 130.0 (C_{Ph}-H_2), 128.4 (C_{Ph}-H_2), 128.4 (C_{Ph}-H_2), 128.2 (C_{Ph}-H), 126.9 (C_{Ph}-H_2), 125.3 (C_{Py}-H), 123.1 (C_{Py}-H), 122.3 (C_{Py}-H), 118.2 (C_{Py}-H), 88.7 (*12*), 87.5 (*13*), 80.4 (*7*), 24.8 (*1/19*), 24.6 (*1/19*); **HRMS** (ESI) ($C_{28}H_{22}N_2O_2Na$) $[M+Na]^+$: cacld: 441.1573; found: 441.1574.

Compound 18b

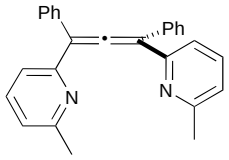


18b

Synthesised according to **General procedure A** from alcohol **17b** (50 mg, 0.17 mmol). Stirred at rt for 2 h 40 min. Column chromatography (Pet/AcOEt/Et_3N 0.73/0.25/0.02) yielded ester **18b** (50 mg, 74%) as a yellow semisolid contaminated with **17b** (**18b**:**17b** 3:1). The reaction is not easily scaleable, it is best to do it at a 1.0-1.5 mmol scale.

^{1}H NMR (500 MHz, $CDCl_3$) δ 8.11 – 8.08 (m, 2H, H_{Ph}), 7.58 – 7.49 (m, 4H, H_{Ar}), 7.45 (t, *J* = 7.7 Hz, 2H, H_{Ph}), 7.34 (d, *J* = 7.7 Hz, 1H, H_{Py}), 7.08 (d, *J* = 7.7 Hz, 1H, H_{Py}), 7.00 (dd, *J* = 7.0, 1.2 Hz, 1H, H_{Py}), 2.56 (s, 3H, *1/17*), 2.43 (s, 3H, *1/17*), 1.25 (s, 9H, *9*); **^{13}C NMR** (126 MHz, $CDCl_3$) δ 164.5 (*18*), 156.9 (C_{Py}), 155.7 (C_{Py}), 135.8 (C_{Py}-H), 132.8 (C_{Ph}-H), 131.4 (C_{Py}-H), 129.9 (2 x C_{Ph}-H), 128.4 (2 x C_{Ph}-H), 128.3 (C_{Ph}), 125.5 (C_{Py}-H), 123.2 (C_{Py}-H), 121.9 (C_{Py}-H), 119.9 (C_{Py}-H), 84.6 (*7*), 41.0 (*8*), 26.1 (*9*), 25.7 (*1/17*), 24.7 (*1/17*). Two C_{sp} and two C_q not detected; **HRMS** (ESI) ($C_{26}H_{27}N_2O_2$) $[M+H]^+$: cacld: 399.2067; found: 399.2061.

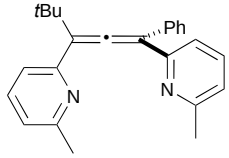
Allene 1a[1]



To a suspension of $ZnBr_2$ in anhydrous THF (0.8 M, 4.0 eq.), a phenylmagnesium bromide solution in Et_2O (4.0 eq.) was added dropwise at 0 ºC under inert atmosphere. Stirring at 0 ºC continued for 30 min. $Pd(PPh_3)_4$ (62 mg, 0.054 mmol, 0.05 eq.) and the propargyl benzoate **18a** (447 mg, 1.07 mmol, 1.0 eq.) in anhydrous THF (0.25 M solution of ester) were added at 0 ºC. Reaction mixture was stirred at 0 ºC for 3h. Water was added, aqueous layer was separated and extracted with Et2O, combined organic layers were dried over $MgSO_4$ and concentrated in vacuo. Column chromatography (Pet/AcOEt/NEt_3 85/14/1) yielded allene **1a** (215 mg, 54%) as a yellow oil.
The characterisation data is in agreement with previously reported.[10]

^{1}H NMR (500 MHz, $CDCl_3$) δ 7.60 – 7.54 (m, 6H), 7.41 (d, *J* = 7.7 Hz, 2H), 7.37 – 7.33 (m, 4H), 7.30 – 7.26 (m, 2H), 7.07 (d, *J* = 7.7 Hz, 2H), 2.58 (s, 6H); **^{13}C NMR** (126 MHz, $CDCl_3$) δ 212.7, 158.4, 154.8, 136.9, 135.1, 128.7, 128.5, 127.7, 121.9, 121.5, 114.0, 24.8; **MS (EI)** ($C_{27}H_{21}N_2$) $[M-H]^+$: cacld: 373; found: 373.

Allene 1b[1]



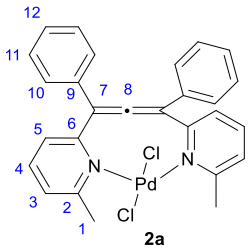
To a suspension of $ZnBr_2$ in anhydrous THF (0.8 M, 4.0 eq.), a phenylmagnesium bromide solution in Et_2O (4.0 eq.) was added dropwise at 0 ºC under inert atmosphere. Stirring at 0 ºC continued for 15 min, followed by 45 min at rt. $Pd(PPh_3)_4$ (145 mg, 0.125 mmol, 0.05 eq.) and

the propargyl benzoate **18b** (996 mg, 2.5 mmol, 1.0 eq.) in anhydrous THF (0.25 M solution of ester) were added at 0 ºC and stirring at this temperature continued for 15 min. Reaction mixture was brought to rt and stirred overnight. Water was added and the precipitate formed was filtered off. Aqueous layer of the filtrate was separated and extracted with Et_2O, combined organic layers were dried over $MgSO_4$ and concentrated in vacuo. Column chromatography (Pet/AcOEt/NEt_3 93/6/1) yielded allene **1b** (264 mg, 30%) as a yellow oil.
The characterisation data is in agreement with previously reported.[10]

^{1}H NMR (400 MHz, $CDCl_3$) δ 7.55 (t, *J* = 7.7 Hz, 1H), 7.49 (m, 2H), 7.43 (t, *J* = 7.7 Hz, 1H), 7.35 – 7.27 (m, 4H), 7.26 – 7.21 (m, 1H), 7.04 (d, *J* = 7.6 Hz, 1H), 6.95 (d, *J* = 7.6 Hz, 1H), 2.56 (s, 6H), 1.42 (s, 9H); **^{13}C NMR** (101 MHz, $CDCl_3$) δ 209.0, 158.1, 157.3, 155.7, 155.6, 136.8, 136.3, 135.8, 128.3, 128.2, 127.1, 121.5, 121.4, 120.9, 120.9, 120.2, 112.1, 36.3, 30.3, 24.8, 24.7; **HRMS (ESI)** ($C_{25}H_{26}N_2$) $[M+Na]^+$: cacld: 377.1988 ; found: 377.1990.

2.2. Synthesis of metal complexes

Compound 2a



2a

$Pd(CH_3CN)_2Cl_2$ (7 mg, 0.03 mmol, 1.0 equiv.) was added to a solution of allene **1a** (10 mg, 0.027 mmol, 1.0 equiv.) in dichloromethane (0.5 mL) at 12 °C. The reaction mixture changed colour from yellow to red over the course of 40 min. After that time the solution was filtered through a cotton wool plug to remove solid impurities, washed with more dichloromethane and concentrated in *vacuo* to afford **2a** (14 mg, > 99%) as a red solid.

^{1}H NMR (400 MHz, $CDCl_3$) δ 7.66 – 7.61 (m, 4H, *10*), 7.56 (t, *J* = 7.6 Hz, 2H, *4*), 7.46 – 7.38 (m, 6H, *11*, *12*), 7.19 (dd, *J* = 7.6, 0.7 Hz, 2H, *3/5*), 7.00 (d, *J* = 7.6 Hz, 2H, *3/5*), 3.17 (s, 6H, *1*); **^{13}C NMR** (101 MHz, $CDCl_3$) δ 204.1 (*8*), 160.8 (C_{Py}), 160.3 (C_{Py}), 138.4 (*4*), 136.4 (*9*), 129.7 (2 x C_{Ph}-H), 129.0 (2 x C_{Ph}-H), 128.9 (*12*), 124.7 (C_{Py}-H), 123.8 (C_{Py}-H), 117.9 (*7*), 26.9 (*1*); **HRMS** (ESI) ($C_{27}H_{21}N_2{}^{106}Pd$) $[M\text{-}Cl_2\text{-}H]^+$: cacld: 479.0734; found: 479.0777; **Anal.** Calcd. for $C_{27}H_{25}N_2PdCl_2O_{1.5}$ (**2a**+1.5H_2O): C 56.15, H 4.37, N 4.85; found: C 56.54, H 4.43; N 4.45.

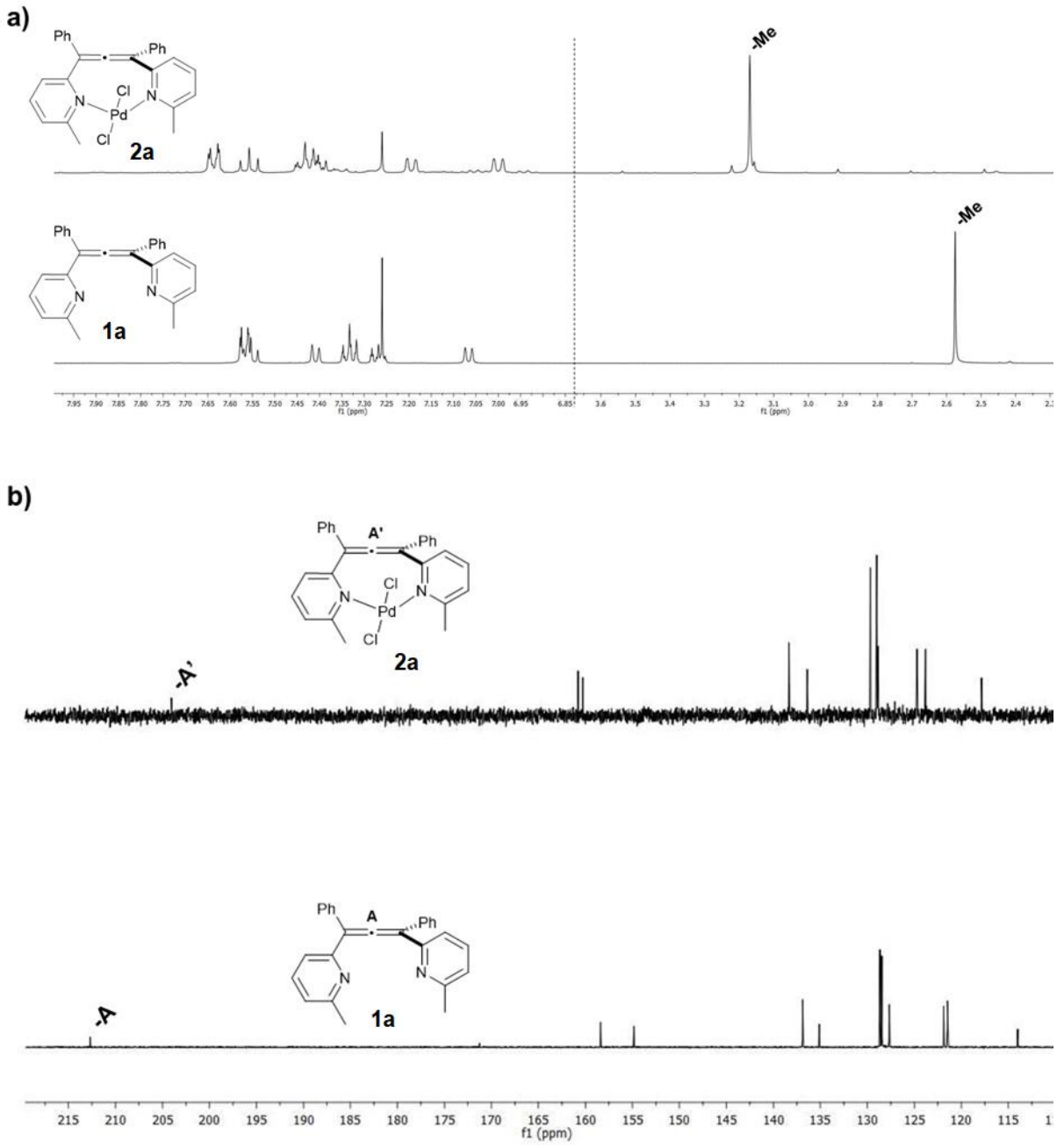
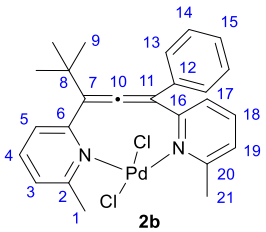


Figure 1S. Comparison of NMR spectra of the free ligand **1a** and its Pd complex **2a**: a) ^{1}H NMR expansion of regions at 2.3-3.6 ppm and 6.9-7.9 ppm; b) ^{13}C NMR expansion of region at 110-215 ppm.

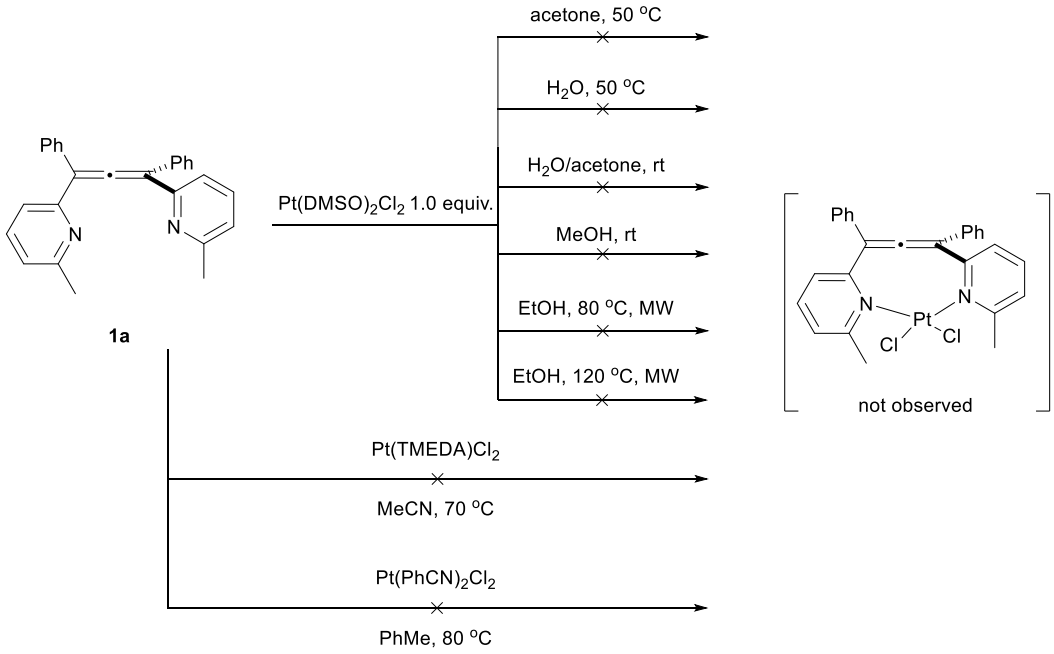
Compound 2b



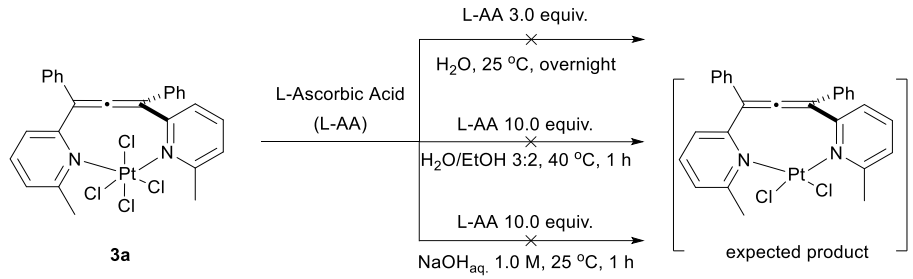
$Pd(CH_3CN)_2Cl_2$ (15 mg, 0.056 mmol, 1.0 equiv.) was added to a solution of allene **1b** (20 mg, 0.056 mmol, 1.0 equiv.) in dichloromethane (0.8 mL) at 12 °C. The reaction mixture changed colour from yellow to red over the course of 40 min. After that time the solution was filtered through a cotton wool plug to remove solid impurities, washed with more dichloromethane and concentrated in *vacuo* to afford **2b** (30 mg, > 99%) as a red solid. Red crystals of **2b** suitable for X-ray crystallography were obtained from vapour diffusion of dichloromethane/MeOH solution and *n*-Hex at 4 °C (see Section 4).

^{1}H NMR (400 MHz, $CDCl_3$) δ 7.63 (t, *J* = 7.8 Hz, 1H, *4/18*), 7.57 (t, *J* = 7.8 Hz, 1H, *4/18*), 7.50 – 7.46 (m, 2H, *13*), 7.44 (d, *J* = 7.8 Hz, 1H, H_{Py}), 7.40 – 7.31 (m, 3H, *14*, *15*), 7.20 (dd, *J* = 7.8, 0.8 Hz, 1H, H_{Py}), 7.16 (d, *J* = 7.8, 1H, H_{Py}), 7.03 (d, *J* = 7.8, Hz, 1H, H_{Py}), 3.17 (s, 3H, *1/21*), 3.13 (s, 3H, *1/21*), 1.47 (s, 9H, *9*); **^{13}C NMR** (126 MHz, $CDCl_3$) δ 202.7 (*10*), 161.0 (C_{Py}), 160.8 (C_{Py}), 160.4 (C_{Py}), 158.4 (C_{Py}), 138.1 (*4/18*), 138.0 (*4/18*), 137.0 (*12*), 129.5 (C_{Ph}-H_2), 128.9 (C_{Ph}-H_2), 128.5 (*15*), 124.5 (C_{Py}-H), 124.2 (C_{Py}-H), 123.5 (C_{Py}-H), 123.4 (C_{Py}-H), 121.9 (*7/11*), 118.2 (*7/11*), 37.0 (*8*), 30.9 (*9*), 27.0 (*1/21*), 27.0 (*1/21*); **HRMS** (ESI) ($C_{25}H_{26}N_2{}^{106}Pd^{35}Cl$) [M-Cl]$^+$: cacld: 495.0814; found: 495.0824.

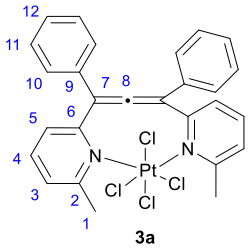
Attempts to access Pt(II)-bis(pyridyl)allene complexes from Pt(II) precursors



Attempts to access Pt(II)-bis(pyridyl)allene complexes from Pt(IV) precursors – chemical reduction



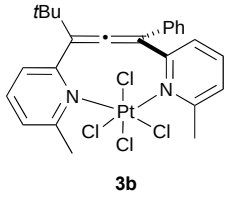
Compound 3a[11]



To a solution of $H_2PtCl_6{\cdot}6H_2O$ (41 mg, 0.08 mmol, 1.0 equiv.) in MeOH (1.9 mL) solution of **1a** (30 mg, 0.08 mmol) in MeOH (1.9 mL) was added at rt. Upon addition, a precipitate started to form instantaneously. After 40 min of stirring at rt the solid was filtered off, washed with MeOH and dried in *vacuo* to afford **3a** (53 mg, 92%) as a pale orange solid.

^{1}H NMR (400 MHz, DMSO-d_6) δ 8.22 (t, *J* = 7.7 Hz, 2H, *4*), 7.75 (d, *J* = 7.7 Hz, 2H, *3/5*), 7.68 (d, *J* = 7.7 Hz, 2H, *3/5*), 7.50 – 7.45 (m, 8H, *10*, *11*), 7.45 – 7.40 (m, 2H, *12*), 2.65 (s, 6H, *1*); **^{13}C NMR** (101 MHz, DMSO-d_6) δ 210.3 (*8*), 156.8 (*4*), 149.1 (C_{Py}), 143.1 (C_{Py}), 132.6 (*9*), 129.3 (2 x C_{Ph}-H), 129.1 (*12*), 127.8 (2 x C_{Ph}-H), 125.8 (C_{Py}-H), 123.7 (C_{Py}-H), 111.3 (*7*), 21.7 (*1*); **HRMS** (ESI) ($C_{27}H_{23}{}^{35}Cl_3N_2{}^{195}Pt$) $[M\text{-}Cl+H]^+$: cacld: 675.0575; found: 675.0565; **Anal.** Calcd. for $C_{29.5}H_{27}Cl_9N_2Pt$ (**3a**+2.5CH_2Cl_2): C 38.52, H 2.96, N 3.05; found: C 38.55, H 3.45; N 3.25.

Compound 3b[1]



To a solution of $H_2PtCl_6.6H_2O$ (29 mg, 0.056 mmol, 1.0 equiv.) in MeOH (1.2 ml) solution of **1b** (20 mg, 0.056 mmol) in MeOH (1.2 ml) was added. Upon addition a precipitate started to form

instantaneously. After 40 min of stirring at rt the solid was filtered off, washed with MeOH and dried under vacuum to afford **3b** (36 mg, 93%) as a pale orange solid.

^{1}H NMR (400 MHz, DMSO) δ 8.38 (t, *J* = 7.9 Hz, 1H), 8.18 (t, *J* = 7.8 Hz, 1H), 7.79 (dd, *J* = 12.9, 7.9 Hz, 2H), 7.70 (d, *J* = 7.8 Hz, 1H), 7.64 (d, *J* = 7.8 Hz, 1H), 7.49 – 7.43 (m, 2H), 7.41 – 7.36 (m, 1H), 7.34 (dd, *J* = 5.3, 3.2 Hz, 2H), 2.68 (s, 3H), 2.66 (s, 3H), 1.29 (s, 9H); **^{13}C NMR** (126 MHz, DMSO, 75 ^{o}C) δ 206.3, 156.3, 156.1, 151.3, 150.9, 141.3, 139.7, 133.7, 128.5, 127.6, 127.1, 124.0, 123.2, 122.2, 122.1, 118.3, 109.1, 35.7, 29.3, 22.2, 21.7; **HRMS (ESI)** ($C_{25}H_{27}N_{21}{}^{94}Pt^{35}Cl_4$) $[M+H]^+$: cacld: 692.0727; found: 692.0707; Anal. Calcd. for $C_{28.5}H_{33}N_2PtCl_{11}$ (**3b**+3.5CH_2Cl_2): C 34.80, H 3.38, N 2.85; found: C 34.90, H 3.61; N 3.13.

Cyclic voltammograms of 3b

We observed three reduction peaks below -1.0 V on the first voltammogram of **3b** recorded in the direction of positive E values (Figure 2S, a). We assigned the cathodic peak **A** at -1.1 V to a Pt(IV)/Pt(II) reduction event, whereas peaks **B**-**C** in more negative potential range perhaps arose from the reductions of the allene ligand fragment. When the experiment was run in the opposite direction we could also observe an oxidation event. We determined by narrowing the range of potential window to **A** peak only (Figure 2S, b), that the new anodic oxidation peak **A'** at 0.06 V was associated with **A** (Figure 2S, c). Initial Pt(IV)/Pt(II) reduction was probably followed by partial re-oxidation of resulting Pt(II) species. However, such re-oxidation would not lead to the original Pt(IV) complex, because in that case we would see a more symmetric voltammogram, corresponding to a classic two electron reversible process. What is more, the irreversible Pt(IV)/Pt(II) reduction is usually combined with a loss of two axial chloride ligands (electrochemical-chemical process) and therefore regeneration of the initial analyte is not likely. The corresponding values for **A** and **A'** peaks for complex **3a** under the same experimental conditions were -1.0 V and 0.19 V, respectively.

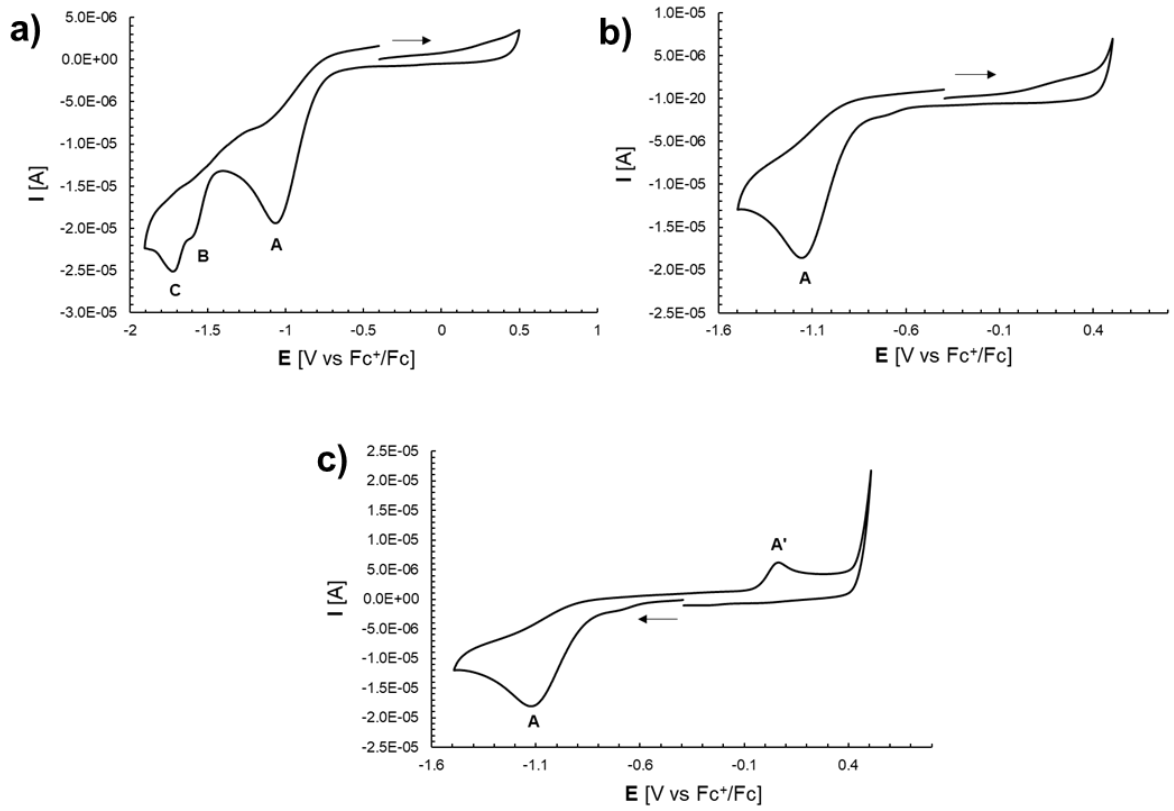
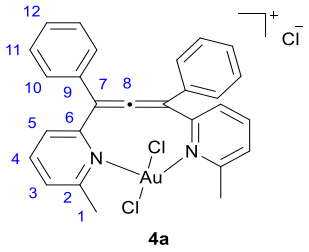


Figure 2S. Voltammograms of a solution of **3b** at 1.6 mM. in 0.1 M (*n*-Bu_4N)(PF_6) DMSO solution with a scan rate of 100 mV/s at rt at a glassy carbon working electrode: a-b) recorded in a direction of positive E values; c) recorded in a direction of negative E values. Potentials referenced to ferrocene internal standard.

Compound 4a



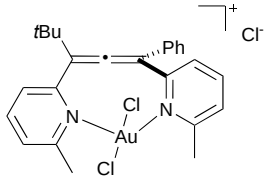
4a

A solution of allene **1a** (30 mg, 0.08 mmol, 1.0 equiv.) in EtOH (0.35 mL) was added to a solution of $HAuCl_4 \cdot H_2O$ (27 mg, 0.08 mmol, 1.0 equiv.) in EtOH (0.35 mL) at rt followed by water (0.14 mL) and $NaPF_6$ (27 mg, 0.16 mmol, 2.0 equiv.). After a few minutes an orange precipitate started to form. After 1 h 45 min the solid was filtered off, washed with EtOH, dried in *vacuo* to yield **4a** (23 mg, 43%) as a dark orange solid.

^{1}H NMR (400 MHz, DMSO-d_6) δ 8.06 (t, *J* = 7.7 Hz, 2H, *4*), 7.63 (d, *J* = 7.7 Hz, 2H, *3/5*), 7.54 (d, *J* = 7.7 Hz, 2H, *3/5*), 7.50 – 7.44 (m, 8H, *10*, *11*), 7.45 – 7.38 (m, 2H, *12*), 2.61 (s, 6H, *1*);

^{13}C NMR (101 MHz, DMSO-d_6) δ 211.1 (*8*), 157.3 (C_{Ar}), 150.7 (C_{Ar}), 141.2 (C_{Py}-H), 133.4 (*9*), 129.1 (*10*/*11*), 128.7 (*12*), 127.9 (*10*/*11*), 124.8 (C_{Py}-H), 122.7 (C_{Py}-H), 112.0 (*7*), 22.5 (*1*); **HRMS** (NSI) ($C_{27}H_{22}N_2Au^{35}Cl_2$) [M-Cl]$^+$: cacld: 641.0820; found: 641.0816; ($C_{27}H_{22}N_2Au$) [M-3Cl]$^+$: cacld: 571.1443; found: 571.1429; **Anal.** Calcd. for $C_{29}H_{28}N_2AuCl_3O$ (**4a**+1.0C_2H_5OH): C 48.19, H 3.91, N 3.88; found: C 48.57, H 3.53; N 4.11.

Compound 4b



4b

To a solution of $HAuCl_4$.xH2O (15.4 mg, 0.045 mmol, 1.0 equiv.) in methanol (0.5 ml) solution of **1b** (16 mg, 0.045 mmol, 1.0 equiv.) in 0.5 ml of methanol was added. The mixture was heated at reflux. After 30 min yellow precipitate started to form. After a total of 130 min, yellow solid was isolated by filtration, washed with MeOH and dried under vacuum to yield **5b** as 0.17:1 mixture of **5b'** and **5b''** (6 mg, 20%) as a yellow solid. The remaining filtrate was concentrated to yield **4b** (18 mg, 60%) as a yellow solid. Complex **3b** was previously characterised.[1]

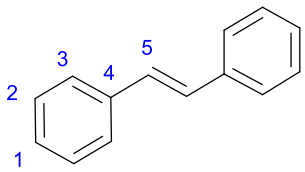
Complex 4b:

^{1}H NMR (500 MHz, MeOD) δ 8.17 (t, *J* = 7.9 Hz, 1H), 8.11 (t, *J* = 7.9 Hz, 1H), 7.68 (d, *J* = 7.8 Hz, 1H), 7.64 (d, *J* = 7.8 Hz, 1H), 7.59 (d, *J* = 7.8 Hz, 2H), 7.47 – 7.43 (m, 2H), 7.42 – 7.35 (m, 3H), 2.75 (s, 3H), 2.74 (s, 3H), 1.35 (s, 9H); **^{13}C NMR** (101 MHz, MeOD) δ 208.4, 158.4, 158.1, 156.6, 153.0, 152.5, 144.0, 143.2, 134.5, 130.3, 130.0, 129.0, 126.7, 126.3, 124.5, 120.1, 111.5, 37.5, 30.1, 22.3, 22.1; **HRMS (ESI)** ($C_{25}H_{27}Cl_3N_2Au$) [M+H]+: Cacld: 657.0900; found: 657.0950; **Anal. Calcd**. for $C_{25.5}H_{27.5}Cl_{4.5}N_2Au$ (**4b**+0.5$CHCl_3$): C 42.73, H 3.87, N 3.91; found: C 43.01, H 3.82, N 3.93.

2.3. General procedure for the Mizoroki-Heck coupling[12]

The corresponding Pd catalyst (0.005 mmol, 0.02 equiv.) and $HCOONH_4$ (0.025 mmol, 0.1 equiv.) were placed under N_2. Anhydrous DMF (0.25 mL) was added, followed by aryl halide (0.25 mmol, 1.0 equiv.), *i*Pr_2EtN (48mg, 0.38 mmol, 1.5 equiv.) and styrene (31 mg, 0.30 mmol, 1.2 equiv.). The reaction was heated at 80 °C for 16-19 h (overnight) and then cooled to rt, diluted with AcOEt and extracted with ice water (x 5). The organic phase was washed with brine, dried over $MgSO_4$, filtered and concentrated in *vacuo* to yield **7** without further purification.

Compound 7



7

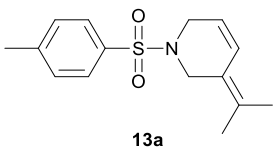
^{1}H NMR (400 MHz, $CDCl_3$) δ 7.53 – 7.51 (m, 4H, *3*), 7.39 – 7.33 (m, 4H, *2*), 7.29 – 7.24 (m, 2H, *1*), 7.12 (s, 2H, *5*); **^{13}C NMR** (101 MHz, $CDCl_3$) δ 137.5 (*4*), 128.8 (*5*), 128.8 (*2*), 127.8 (*1*), 126.7 (*3*).

The characterisation data is in agreement with previously reported.[13]

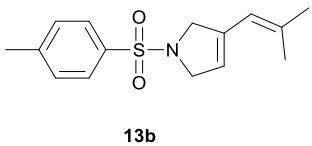
2.4. General procedure for metal-catalysed cyclisation of enynes 8 and 19

The catalyst (2-3 mol%) and $AgNTf_2$ (where applicable) were placed under N_2 and the corresponding solvent (0.5 mL) was added. The mixture was stirred at 25 °C for 5 min. The corresponding enyne (0.1 mmol, 1.0 equiv.) in the same solvent (1.0 mL) was added. Stirring continued for indicated amount of time at indicated temperature. The reaction mixture was filtered through a pad of Celite and concentrated in *vacuo*. The residue was analysed by ^{1}H NMR to determine the ratio of isomeric products.

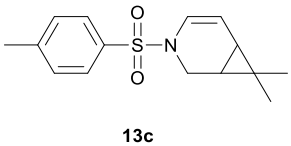
Compounds 9a:**9b**:**9c**:**9d** 1:0.08:0.08:0.01



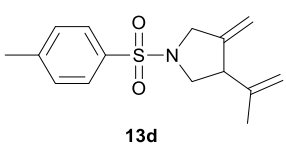
13a



13b



13c



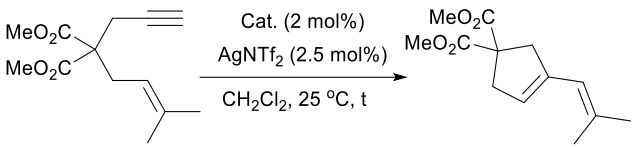
13d

^{1}H NMR (400 MHz, $CDCl_3$) δ 7.74 – 7.70 (m, 2H, **9b**), 7.67 – 7.62 (m, 2H, **9a**), 7.33 – 7.30 (m, 2H, **9b**,**c**), 7.28 – 7.25 (m, 2H, **9a**), 6.59 (d, *J* = 8.4 Hz, 1H, **9c**), 6.34 (dt, *J* = 10.3, 2.1 Hz, 1H,

9a), 5.61 (s, 1H, **9b**), 5.52 (dt, *J* = 10.3, 3.6 Hz, 1H, **9a**), 5.38 (s, 1H, **9b**), 5.06 (dd, *J* = 8.4, 4.8 Hz, 1H, **9c**), 5.01 (d, *J* = 2.1 Hz, 1H, **9d**), 4.87 – 4.85 (m, 1H, **9d**), 4.84 – 4.82 (m, 1H, **9d**), 4.80 (m, 1H, **9d**), 4.23 (d, *J* = 2.7 Hz, 2H, **9b**), 4.13 (s, 2H, **9b**), 3.90 (s, 2H, **9a**), 3.76 (s, 2H, **9a**), 3.48 (dd, *J* = 12.2, 1.5 Hz, 1H, **9c**), 3.36 (dd, *J* = 12.2, 6.0 Hz, 1H, **9c**), 2.42 (s, 3H, **9b**,**c**), 2.41 (s, 3H, **9a**), 1.76 (s, 3H, **9a**), 1.74 (s, 3H, **9b**), 1.66 (s, 3H, **9a**), 1.05 (s, 3H, **9c**), 1.02 (d, *J* = 1.1 Hz, 1H, **9c**), 0.97 (dd, *J* = 8.8, 4.8 Hz, 1H, **9c**), 0.73 (s, 3H, **9c**).

The characterisation data is in agreement with previously reported.[14–16]

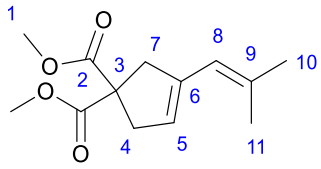
Table 1S. Cycloisomerisation of **19** with allene-derived catalysts.



19 **20a**

Entry	Cat.	$AgNTf_2$	t (h)	19	20a	Conv. (%)	Yield (%)
2	**2a**	-	1	1.0	-	0	-
3	**3a**	-	1	1.0	-	0	-
4	**2a**	+	1	1.0	-	0	-
5	**3a**	+	1	1.0	-	0	-
6	**2b**	+	1	1.0	-	0	-
7	**4a**	-	1	0.15	1.0	87	80
8	**4b**	-	1	0.9	1.0	53	53

Compound 20a



20a

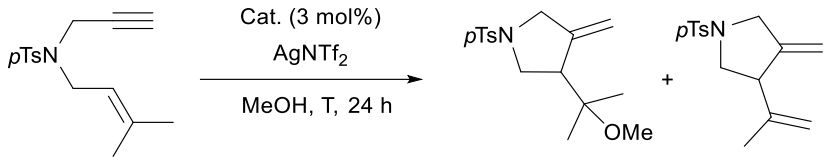
^{1}H NMR (400 MHz, $CDCl_3$) δ 5.72 (s, 1H, *8*), 5.37 (s, 1H, *5*), 3.72 (s, 6H, *1*), 3.18 (d, *J* = 1.7 Hz, 2H, *4*), 3.03 (s, 2H, *7*), 1.81 (s, 3H, *10/11*), 1.77 (s, 3H, *10/11*); **^{13}C NMR** (101 MHz, $CDCl_3$) δ 172.7 (*2*), 138.8 (*9*), 135.8 (*6*), 124.5 (*8*), 120.7 (*5*), 59.4 (*3*), 52.9 (*1*), 43.4 (*4*), 40.4 (*7*), 27.4 (*10*), 19.9 (*11*).

The characterisation data is in agreement with previously reported.[17]

2.5. General procedure for metal-catalysed alkoxycylisation of enynes 8 and 19

The catalyst (amount indicated) and $AgNTf_2$ were placed under N_2, and MeOH (0.5 mL) was added. The mixture was stirred for 5 min. The corresponding enyne (0.1 mmol, 1.0 equiv.) in MeOH (1.0 mL) was added. Stirring at indicated temperature continued for 16-24 h. The reaction mixture was filtered through a pad of Celite and concentrated in *vacuo*. The residue was analysed by ^1H NMR to determine ratio of isomeric products.

Table 2S. Alkoxycyclisation of **8** with allene-derived catalysts.

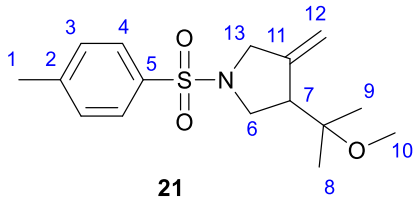


8 **21** **9d**

Entry	Cat.	$AgNTf_2$	T (°C)	8	21	9d	Conv. (%)	Yield (%)
1	**2a**	6.5	50	1.0	0.04	-	4	3
2	**2b**	6.5	50	1.0	-	-	0	-
3	**3a**	6.5	50	0.65	1.0	0.13	75	57[a]
4	**3b**	6.5	50	1.0	0.79	0.11	47	50[a]
5	**4a**	4.0	50	1.0	0,13	-	12	10
6	**4b**	4.0	50	1.0	0.2	-	17	19

[a] Plus 7% of **9d**.

Compound 21



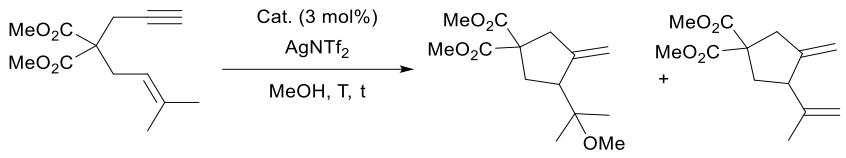
21

In 1:0.8 mixture of **21**:**8**.

^1H NMR (400 MHz, $CDCl_3$) δ 7.75 – 7.67 (m, 2H, *4*), 7.32 (d, *J* = 8.0 Hz, 2H, *3*), 5.03 (s, 2H, *12*), 3.77 (s, 2H, *13*), 3.39 (dd, *J* = 10.1, 4.4 Hz, 1H, *6*), 3.26 (m, 1H, *6*), 3.09 (s, 3H, *10*), 2.82 – 2.76 (m, 1H, *7*), 2.42 (s, 3H, *1*), 1.11 (s, 3H, *8/9*), 1.00 (s, 3H, *8/9*).

The characterisation data is in agreement with previously reported.[18]

Table 3S. Alkoxycyclisation of **19** with allene-derived catalysts.

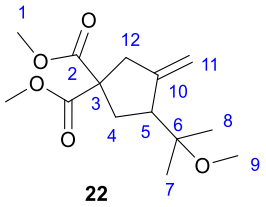


19 **22** **20b**

Entry	Cat.	$AgNTf_2$	T (°C)	t (h)	19	22	20b	Conv. (%)	Yield (%)
1	**2a**	6.5	35	24	1.0	0.3	-	23	26
2	**2b**	6.5	35	24	1.0	0.22	-	18	16
3	**3a**	6.5	35	24	1.0	0.6	0.26	46	68[a]
4	**3b**	6.5	35	24	0.75	1.0	0.55	67	77[a]
5	**4a**	4.0	35	24	-	1.0	-	>99	>99
6	**4b**	4.0	35	24	-	1.0	-	>99	96

[a] Total of **22** and **20b**.

Compound 22

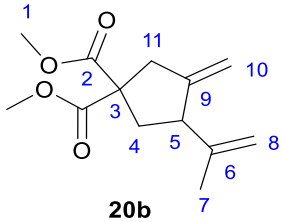


22

^{1}H NMR (400 MHz, $CDCl_3$) δ 5.04 – 5.01 (m, 1H, *11*), 4.98 – 4.95 (m, 1H, *11*), 3.72 (s, 3H, *1*), 3.71 (s, 3H, *1*), 3.18 (s, 3H, *9*), 2.93 – 2.80 (m, 3H, *12, 5*), 2.54 (ddd, *J* = 13.5, 8.5, 1.7 Hz, 1H, *4*), 2.00 (dd, *J* = 13.5, 9.3 Hz, 1H, *4*), 1.17 (s, 3H, *7*), 1.11 (s, 3H, *8*); **^{13}C NMR** (101 MHz, $CDCl_3$) δ 172.2 (*2*), 172.1 (*2*), 148.3 (*10*), 110.7 (*11*), 76.9 (*6*), 58.7 (*3*), 52.9 (*1*), 52.8 (*1*), 49.2 (*5*), 49.1 (*9*), 43.5 (*12*), 36.1 (*4*), 22.8 (*9*), 22.3 (*8*).

The characterisation data is in agreement with previously reported.[19]

Compound 20b



20b

In 1:0.33:1 mixture of **20b**:**20a**:**19**.

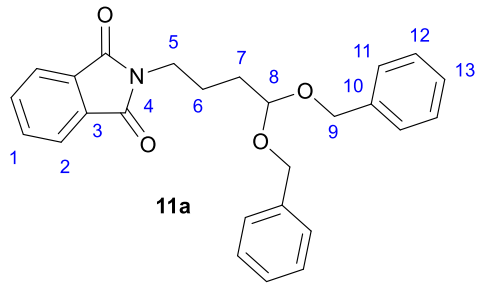
^{1}H NMR (400 MHz, $CDCl_3$) δ 5.01 (d, *J* = 2.0 Hz, 1H, *10*), 4.83 (d, *J* = 1.1 Hz, 2H, *8*), 4.79 (dd, *J* = 4.4, 2.0 Hz, 1H, *10*), 3.73 (s, 6H, *1*), 3.31 – 3.23 (m, 1H, *5*), 3.04 (d, *J* = 1.5 Hz, 1H, *11*), 2.96 – 2.87 (m, 1H, *11*), 2.52 (ddd, *J* = 13.0, 7.8, 1.5 Hz, 1H, *4*), 2.12 (dd, *J* = 13.0, 11.3 Hz, 1H, *4*), 1.64 (s, 3H, *7*).

The characterisation data is in agreement with previously reported.[20]

2.6. General procedure for metal-catalysed addition to allenes

The catalyst (0.05 equiv.) and $AgNTf_2$ (amount indicated) were placed under N_2, and dichloromethane (0.3 mL) was added. The reaction mixture was stirred at 25 °C for 5 min. A solution of allene **10** (25 mg, 0.13 mmol, 1.0 equiv.) in dichloromethane (0.35 mL) was added, followed by benzyl alcohol (0.13 mL, 1.3 mmol, 10.0 equiv.). The reaction was stirred at 25 °C for the indicated amount of time, then filtered through a pad of Celite and concentrated in *vacuo*. The ratio of products and conversions were calculated based on the ^{1}H NMR of the crude reaction mixture or the products were isolated by column chromatography (PhMe/AcOEt 19:1).

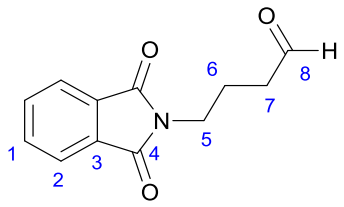
Compound 11a



11a

^{1}H NMR (500 MHz, $CDCl_3$) δ 7.84 (dd, *J* = 5.4, 3.0 Hz, 2H, *2*), 7.71 (dd, *J* = 5.4, 3.0 Hz, 2H, *1*), 7.34 (d, *J* = 4.4 Hz, 8H, *11, 12*), 7.31 – 7.26 (m, 2H, *13*), 4.78 (t, *J* = 4.5 Hz, 1H, *8*), 4.66 (d, *J* = 11.7 Hz, 2H, *9*), 4.56 (d, *J* = 11.7 Hz, 2H, *9*), 3.72 (t, *J* = 6.5 Hz, 2H, *5*), 1.85 – 1.78 (m, 4H, *6,7*); **^{13}C NMR** (126 MHz, $CDCl_3$) δ 168.5 (*4*), 138.2 (*10*), 134.0 (*1*), 132.2 (*3*), 128.5 (2 x C_{Ph}-H), 127.9 (2 x C_{Ph}-H), 127.7 (*13*), 123.3 (*2*), 101.8 (*8*), 67.5 (*9*), 37.8 (*5*), 30.9 (*7*), 24.1 (*6*); **HRMS** (NSI) ($C_{26}H_{29}N_2O_4$) $[M+NH_4]^+$: cacld: 433.2122; found: 433.2116; a fragment from C(8)-O bond cleavage was also observed: ($C_{19}H_{18}NO_3$) $[M-OBn]^+$: cacld: 308.1281; found: 308.1283.

Compound 12a



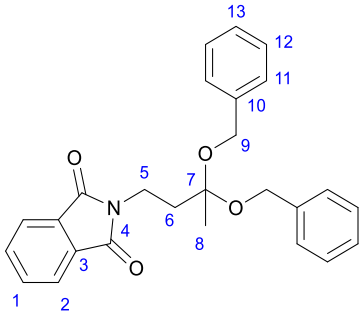
12a

In 36.5:1.13:1.0 mixture of **BnOH**:**11a**:**12a**.

^{1}H NMR (500 MHz, $CDCl_3$) δ 9.77 (s, 1H, -CO*H*), 7.84 (m, 2H, *2*), 7.75 – 7.69 (m, 2H, *1*), 3.74 (t, *J* = 7.0 Hz, 1H, *5*), 2.54 (t, *J* = 7.0 Hz, 1H, *7*), 2.02 (p, *J* = 7.0 Hz, 1H, *6*).

The characterisation data is in agreement with previously reported.[21]

Compound 11b

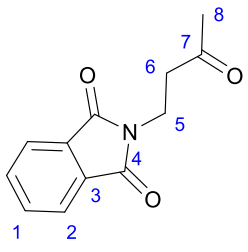


11b

Product **11b** not stable enough for full characterisation and hydrolyses to **12b**.

^{1}H NMR (500 MHz, $CDCl_3$) δ 7.87 – 7.82 (m, 2H, *2*), 7.74 – 7.69 (m, 2H, *1*), 7.40 – 7.34 (m, 8H, *11, 12*), 7.30 (m, 2H, *13*), 4.71 (s, 4H, *9*), 3.96 (t, *J* = 7.3 Hz, 2H, *5*), 2.88 (t, *J* = 7.3 Hz, 2H, *6*), 1.25 (s, 3H, *8*).

Compound 12b

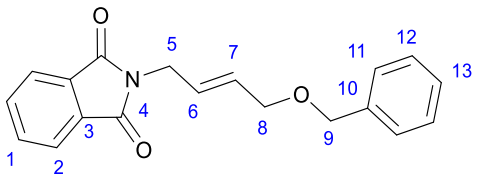


12b

^{1}H NMR (500 MHz, $CDCl_3$) δ 7.86 – 7.82 (m, 2H, *2*), 7.74 – 7.69 (m, 2H, *1*), 3.96 (t, *J* = 7.4 Hz, 2H, *5*), 2.88 (t, *J* = 7.4 Hz, 2H, *6*), 2.19 (s, 3H, *8*).

The characterisation data is in agreement with previously reported.[22]

Compound 13

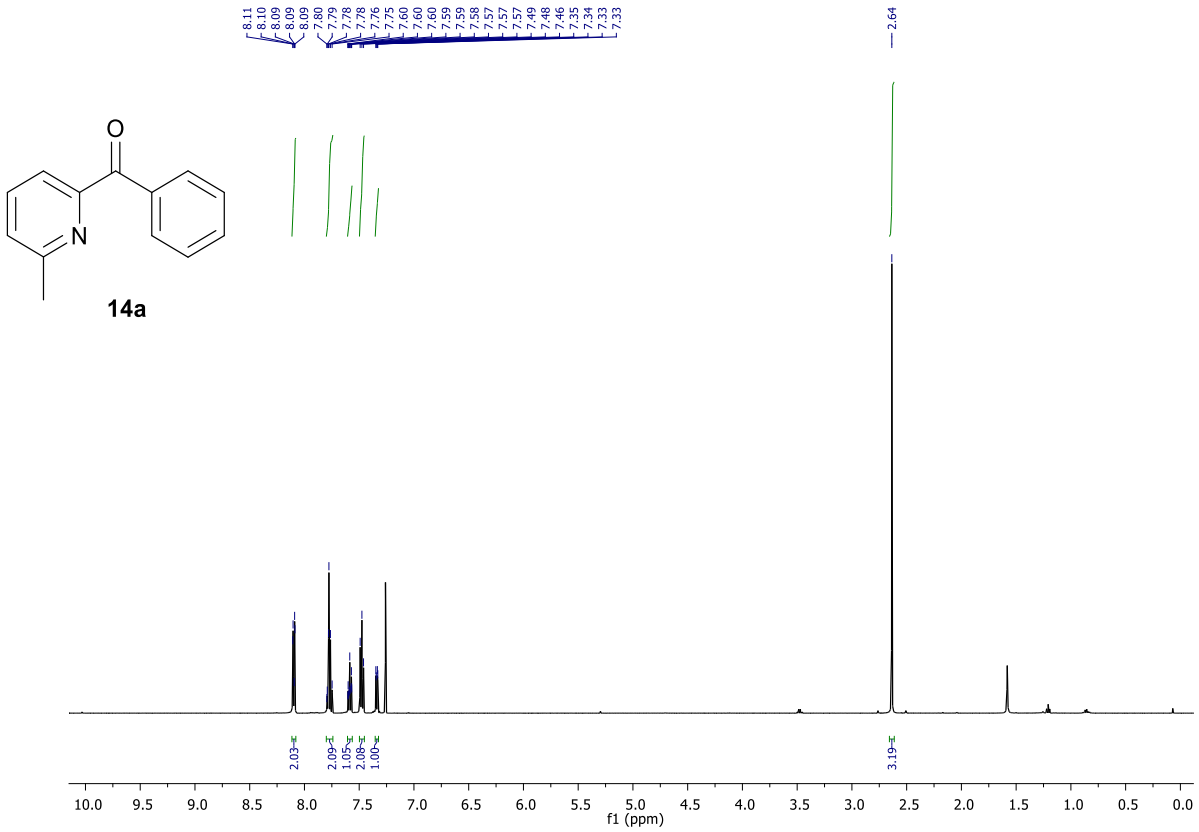


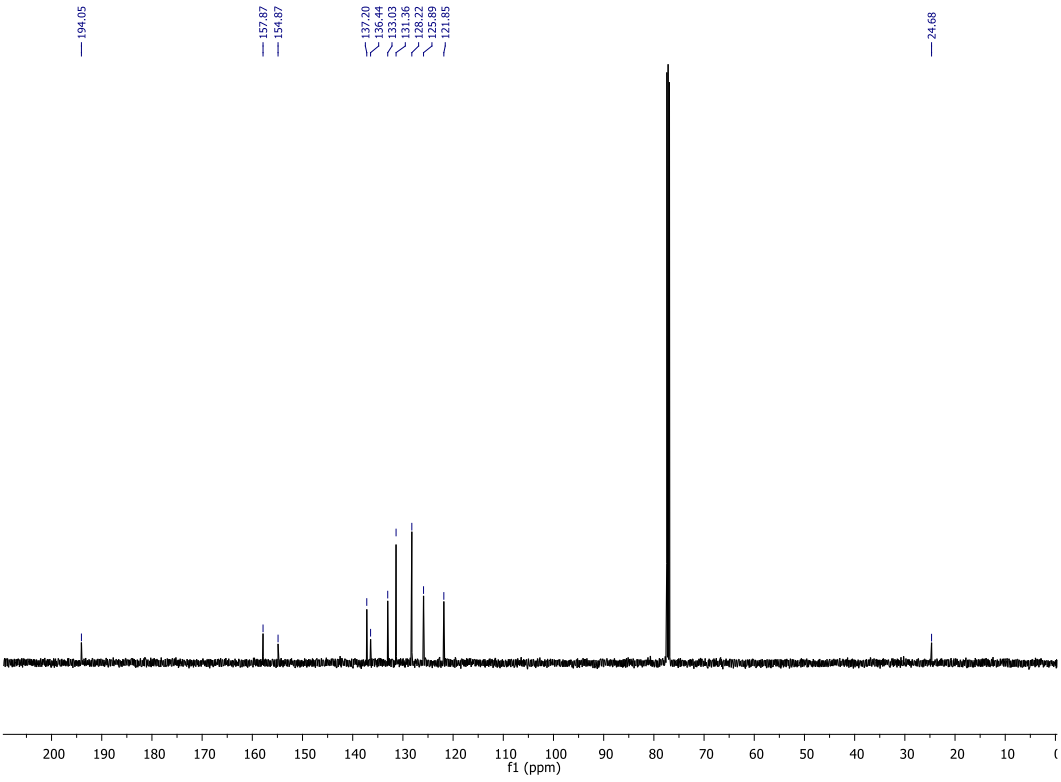
13

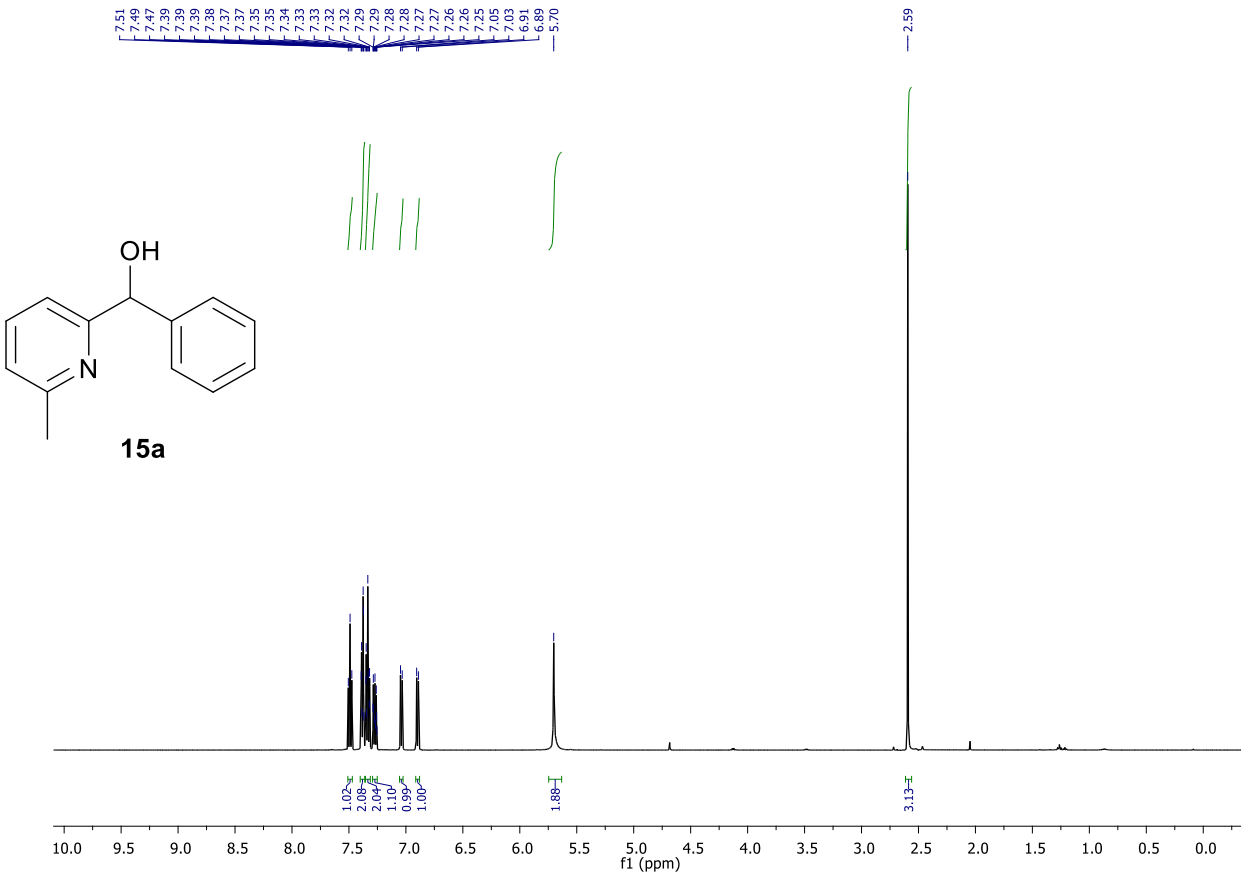
^{1}H NMR (400 MHz, $CDCl_3$) δ 7.87 – 7.81 (m, 2H, *2*), 7.73 – 7.68 (m, 2H, *1*), 7.38 – 7.29 (m, 4H, *11, 12*), 7.28 – 7.22 (m, 1H, *13*), 5.89 – 5.76 (m, 2H, *6, 7*), 4.48 (s, 2H, *9*), 4.34 – 4.27 (m, 2H, *5*), 4.00 (m, 2H, *8*); **^{13}C NMR** (101 MHz, $CDCl_3$) δ 168.0 (*4*), 138.2 (*10*), 134.1 (*1*), 132.2 (*7*), 130.4 (*3*), 128.5 (2 x C_{Ph}-H), 127.8 (2 x C_{Ph}-H), 127.7 (*13*), 126.2 (*6*), 123.4 (*2*), 72.3 (*9*), 69.8 (*8*), 39.1 (*5*); **HRMS** (ASAP) ($C_{19}H_{18}NO_3$) $[M+H]^+$: cacld: 308.1287; found: 308.1288.

The characterisation data reported for the (*Z*)-isomer of **13** was not in agreement, hence the structure of (*E*)-isomer proposed.[22]

3. Spectral data






7.51
7.49
7.47
7.39
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7.39
7.38
7.37
7.37
7.35
7.35
7.34
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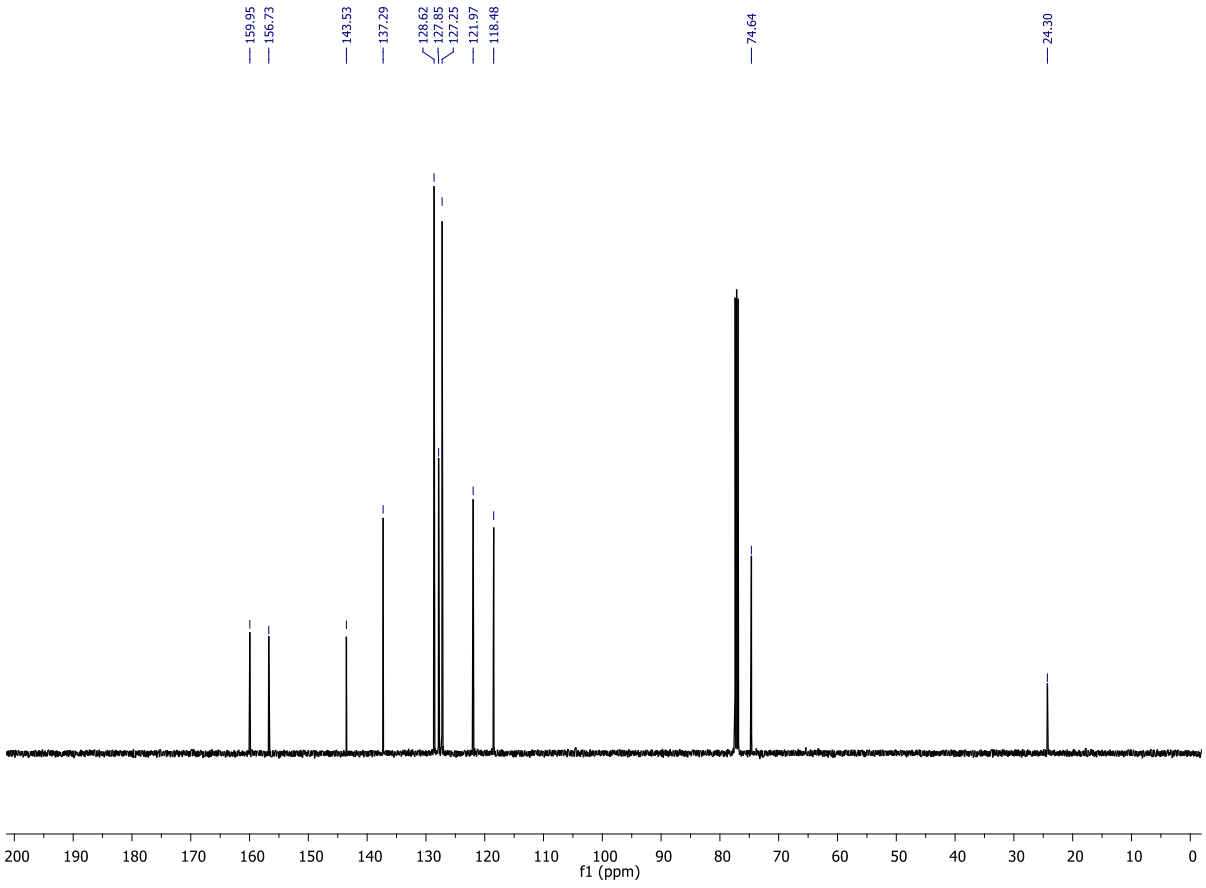
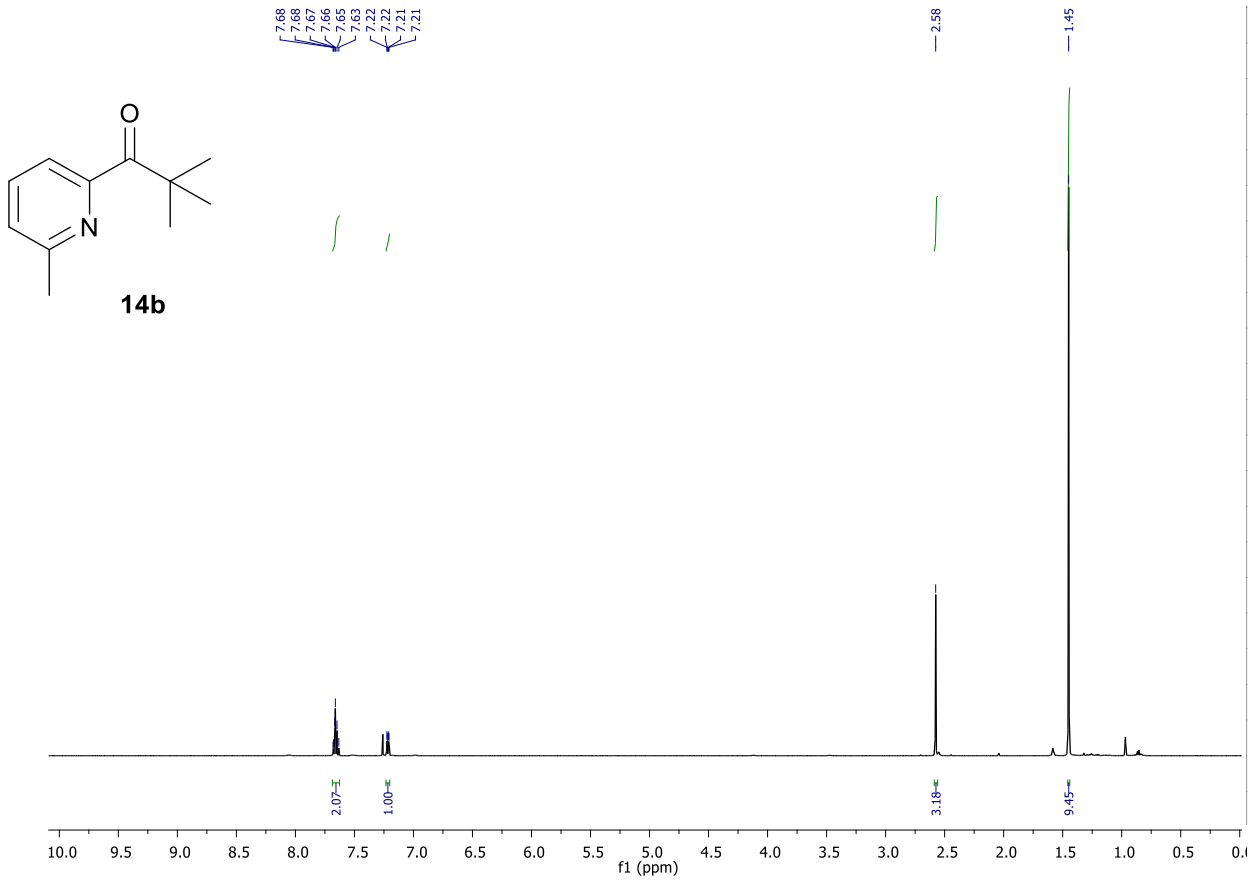
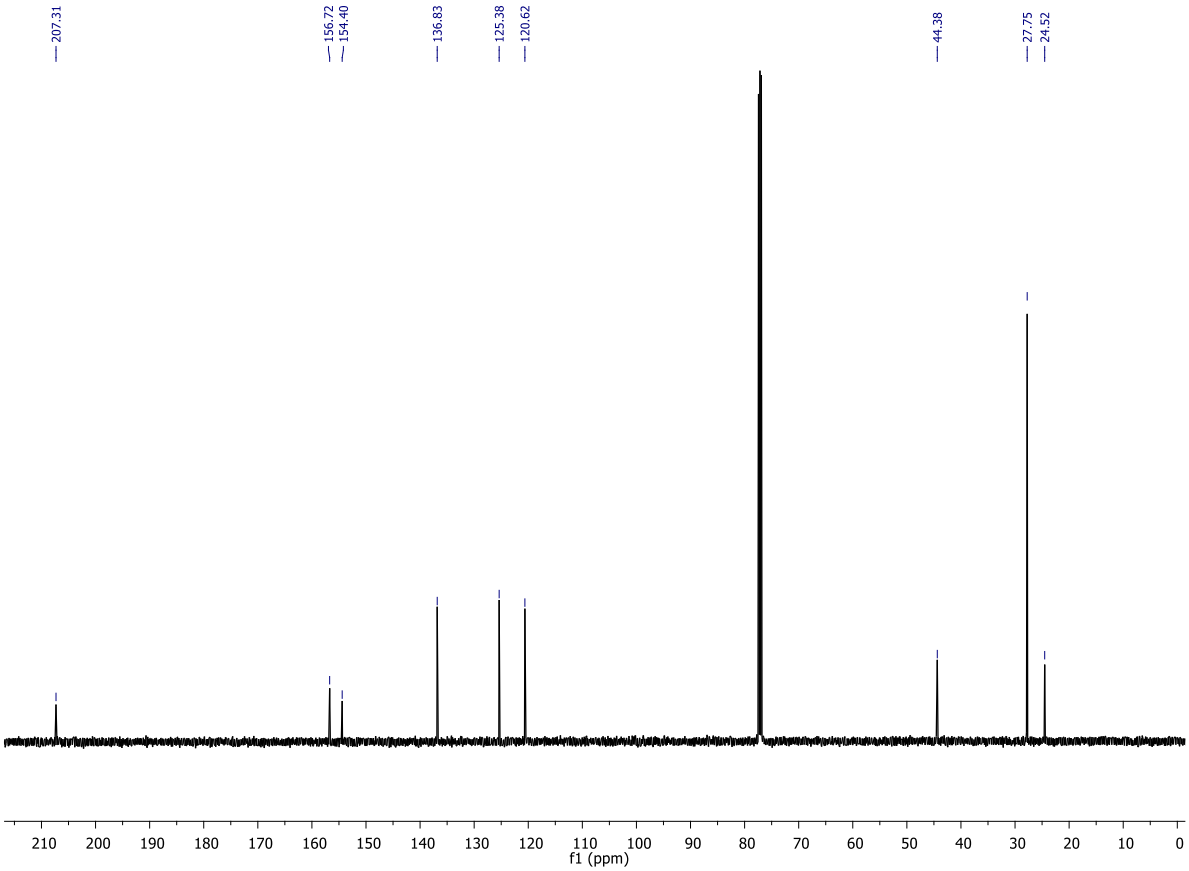
7.32
7.32
7.29
7.29
7.28
7.28
7.27
7.27
7.26
7.26
7.25
7.05
7.03
6.91
6.89
5.70
2.59
OH
N
15a
1.02
2.08
2.04
1.10
0.99
1.00
1.88
3.13
10.0 9.5 9.0 8.5 8.0 7.5 7.0 6.5 6.0 5.5 5.0 4.5 4.0 3.5 3.0 2.5 2.0 1.5 1.0 0.5 0.0
f1 (ppm)


159.95
156.73
143.53
137.29
128.62
127.85
127.25
121.97
118.48
74.64
24.30
200 190 180 170 160 150 140 130 120 110 100 90 80 70 60 50 40 30 20 10 0
f1 (ppm)


7.68
7.68
7.67
7.66
7.65
7.63
7.22
7.22
7.21
7.21
2.58
1.45
O
N
14b
2.07
1.00
3.18
9.45
f1 (ppm)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156.72
154.40
136.83
125.38
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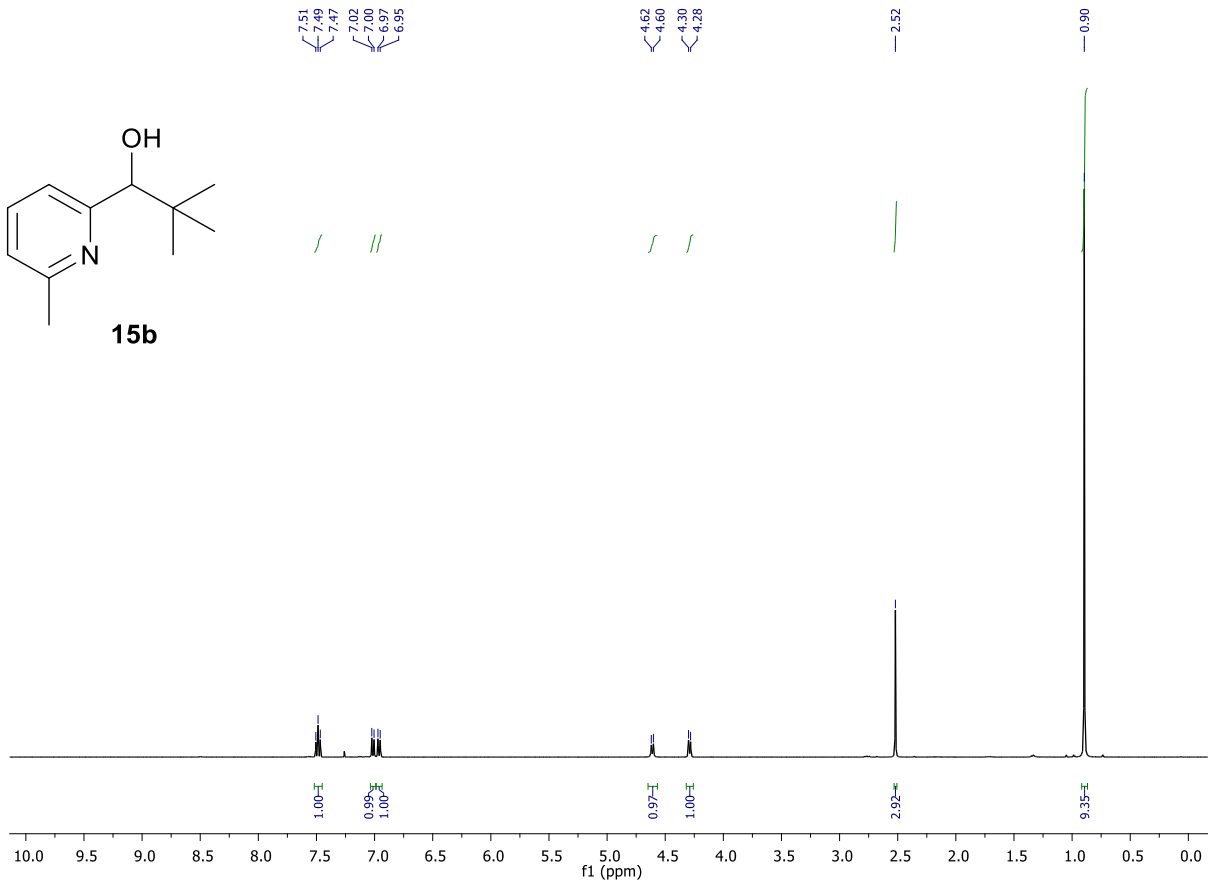
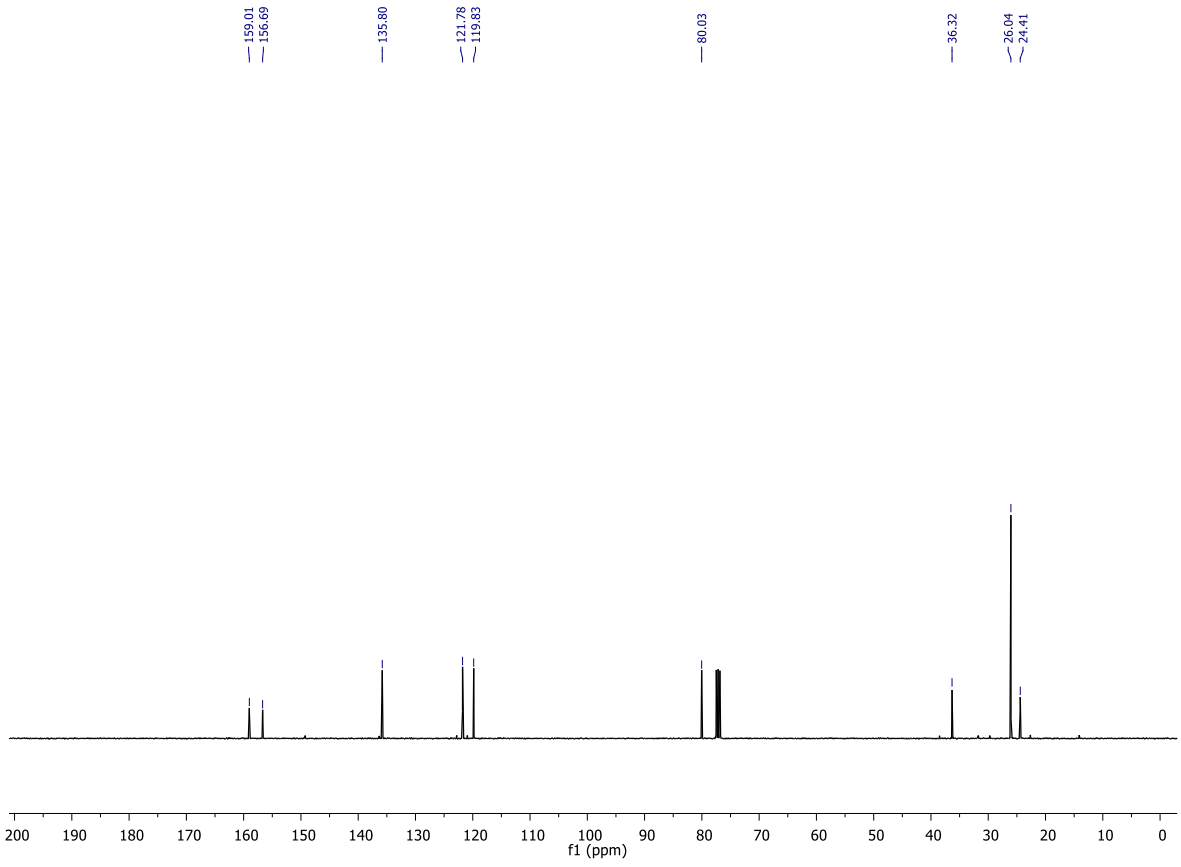
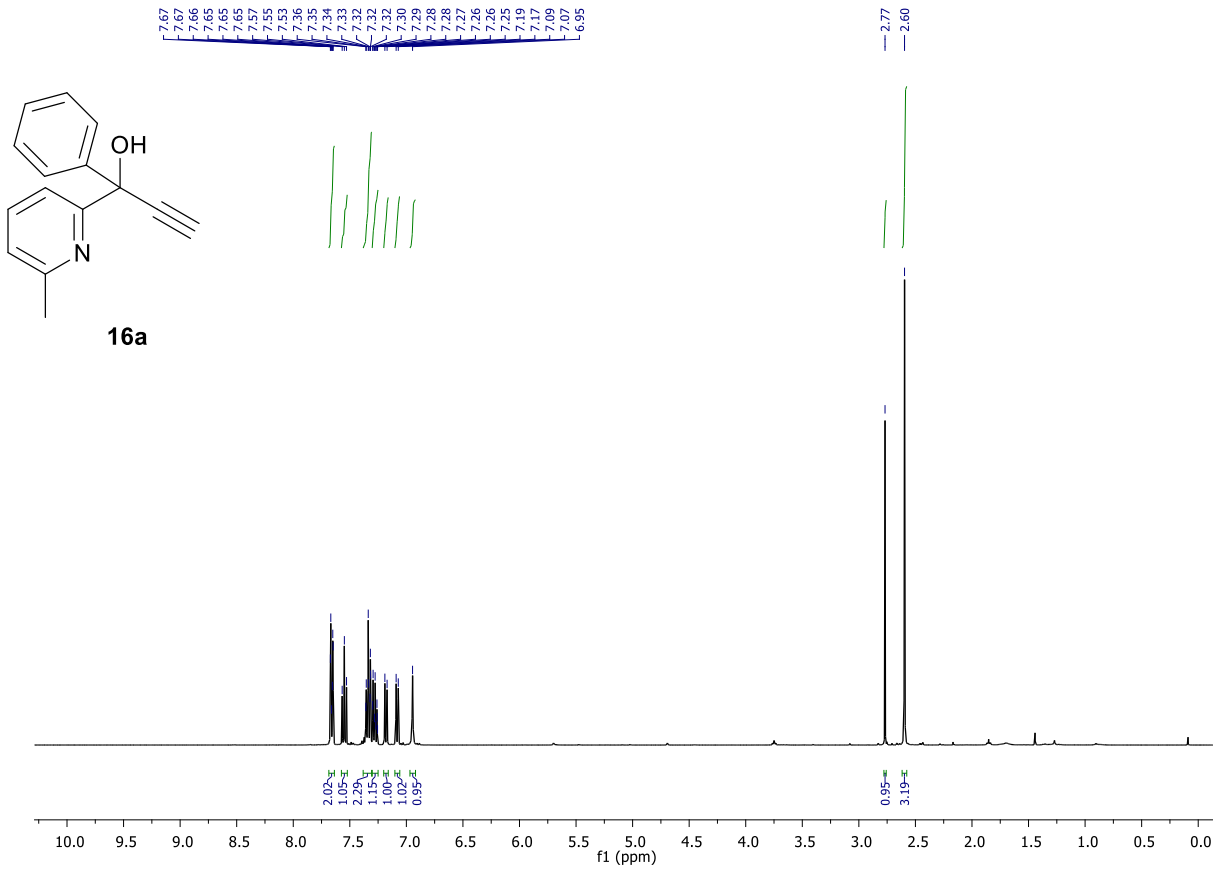
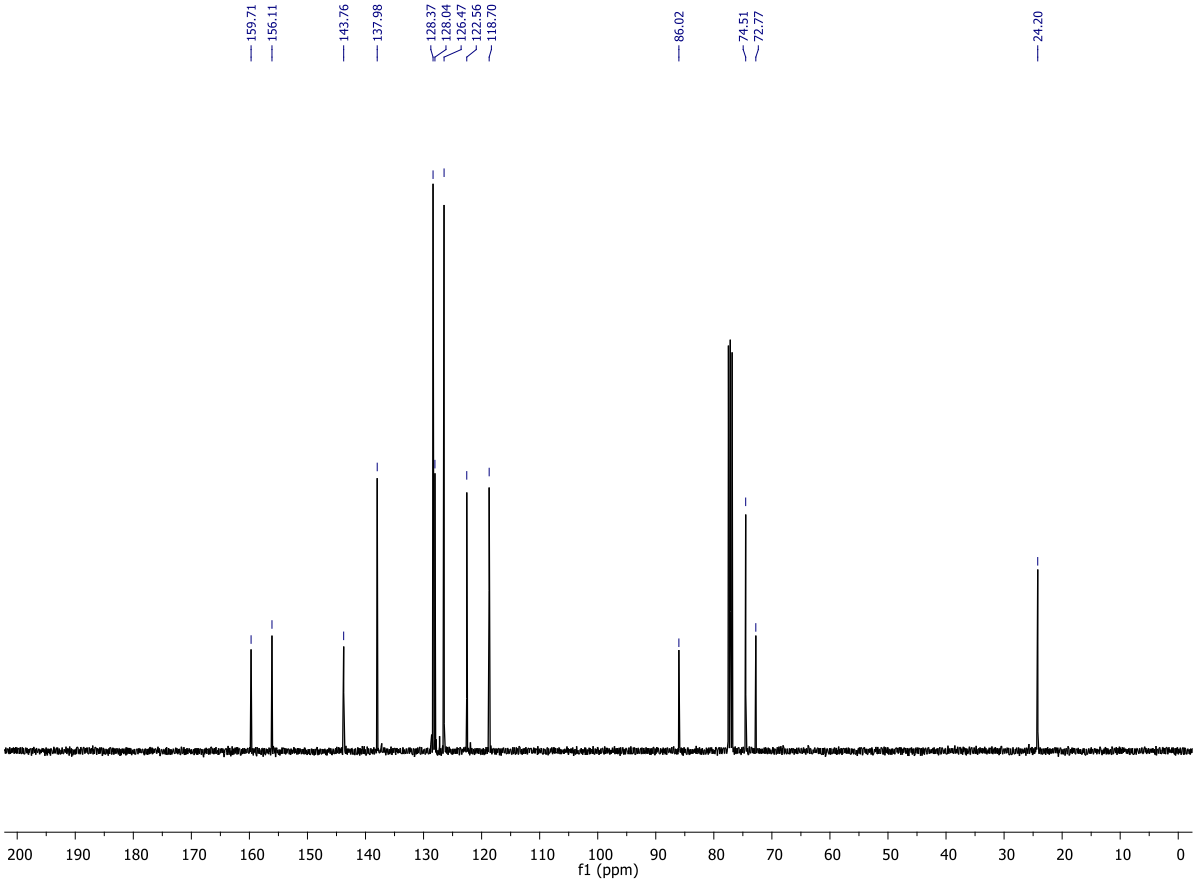
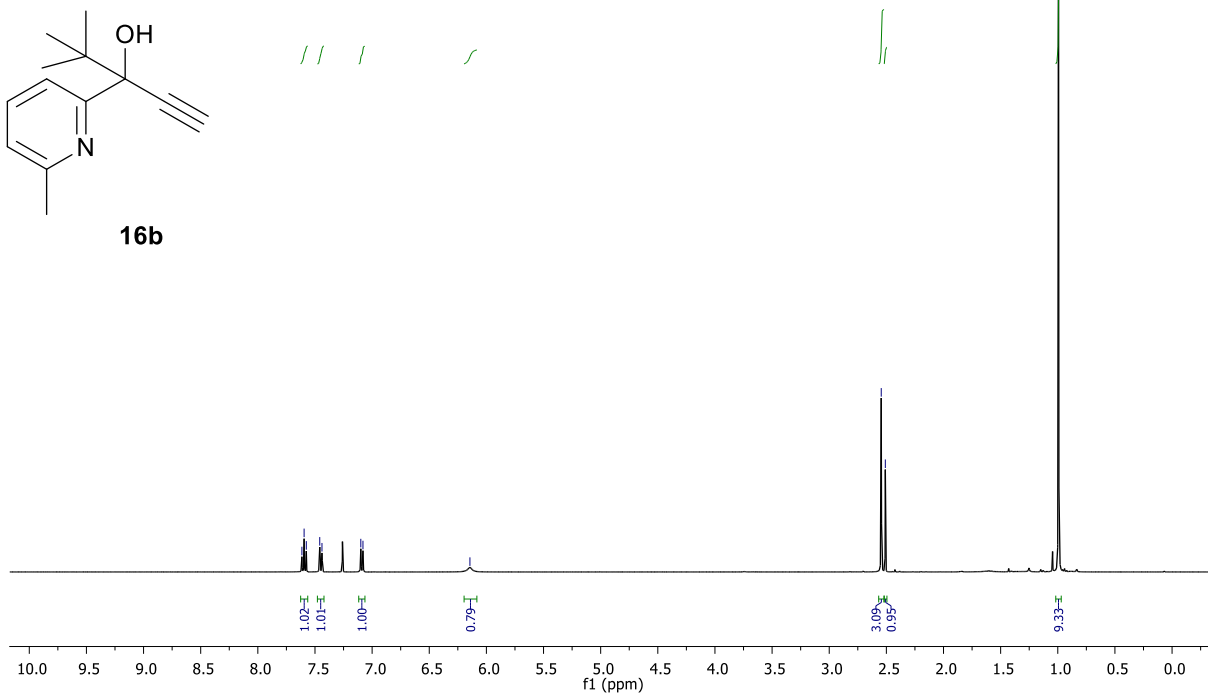
44.38
27.75
24.52
f1 (ppm)


7.51
7.49
7.47
7.02
7.00
6.97
6.95
4.62
4.60
4.30
4.28
2.52
0.90
OH
N
15b
1.00
0.99
1.00
0.97
1.00
2.92
9.35
f1 (ppm)

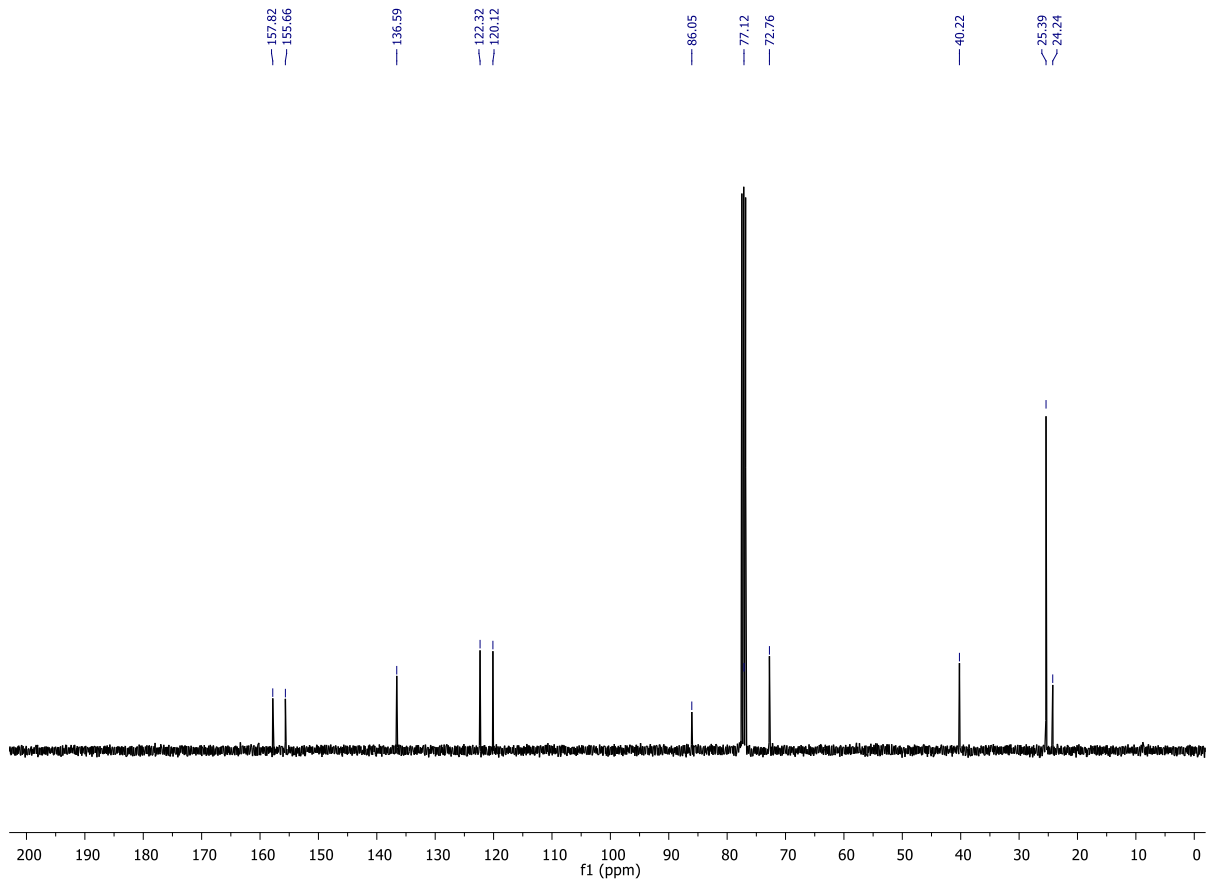

159.01
156.69
135.80
121.78
119.83
80.03
36.32
26.04
24.41
f1 (ppm)

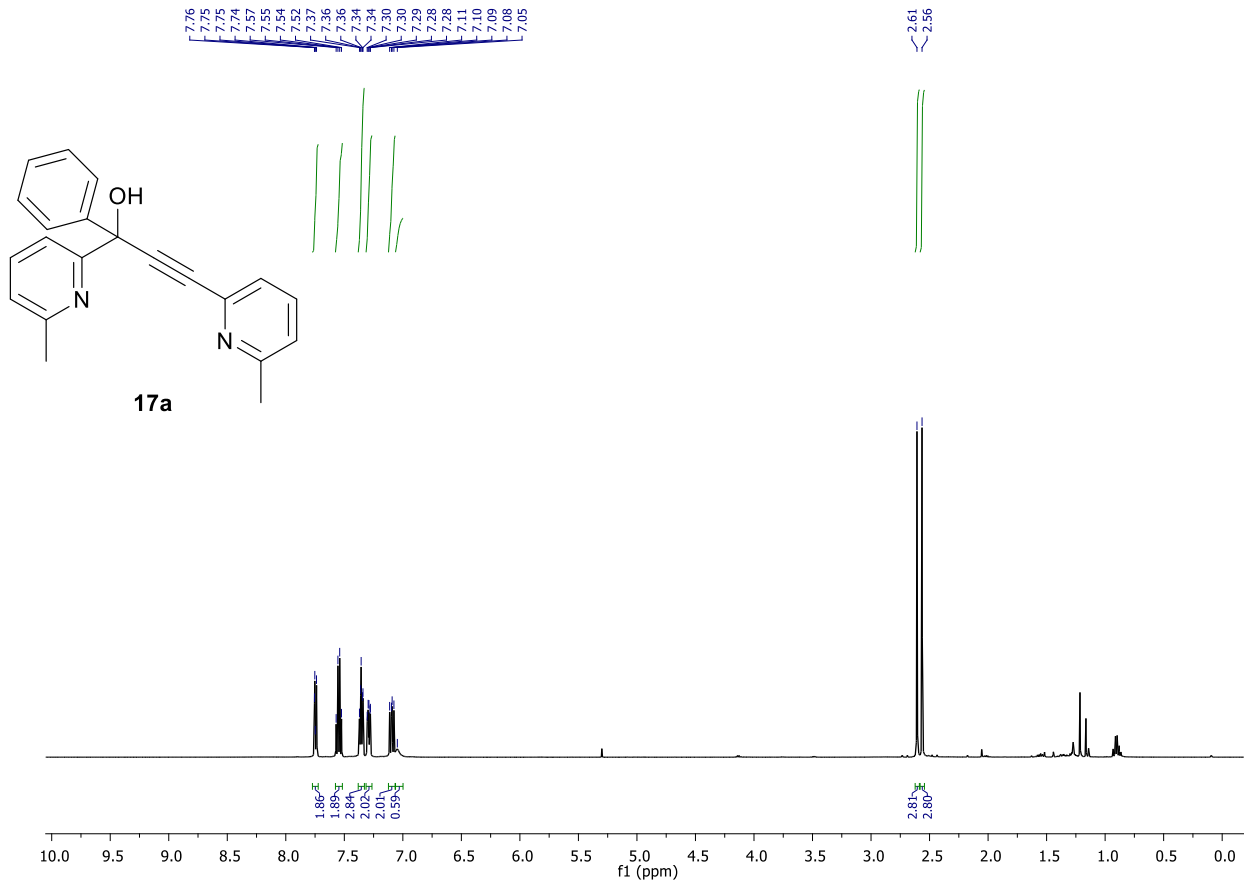

OH
N
16a
f1 (ppm)

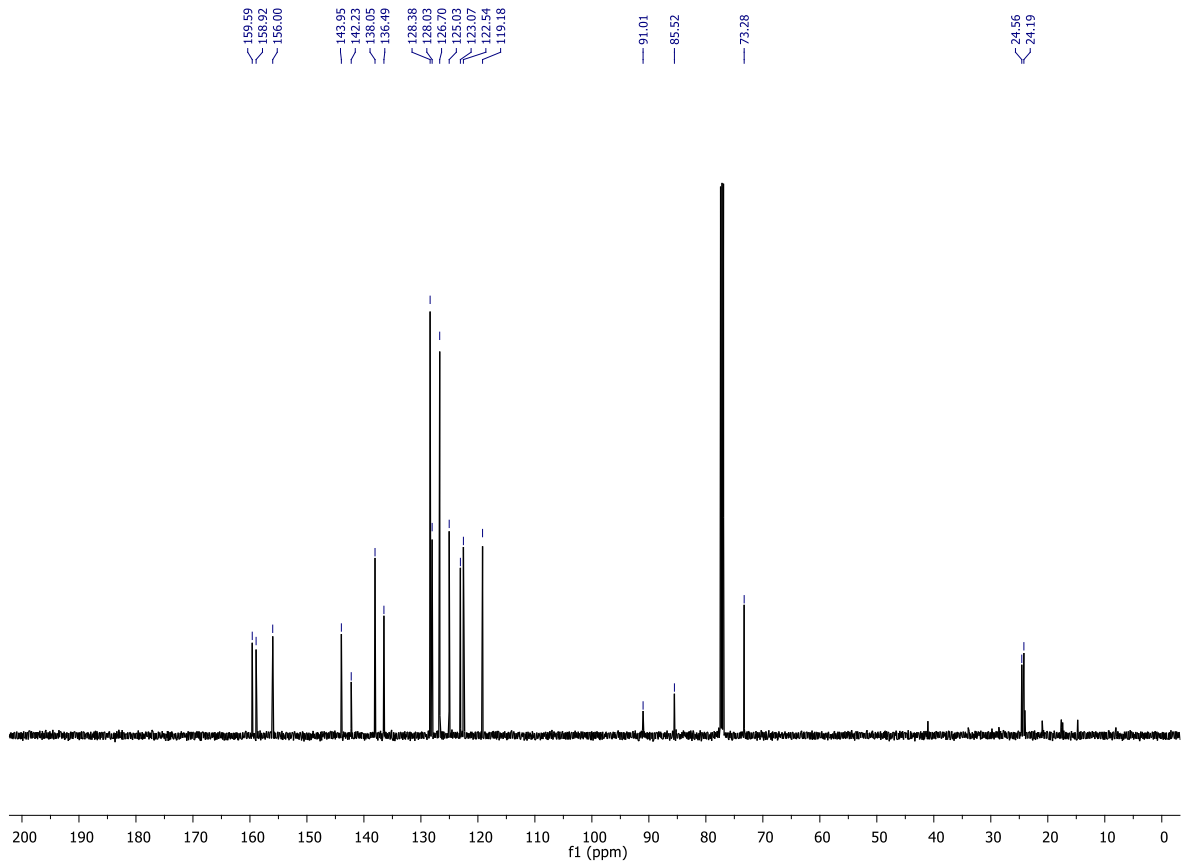

f1 (ppm)

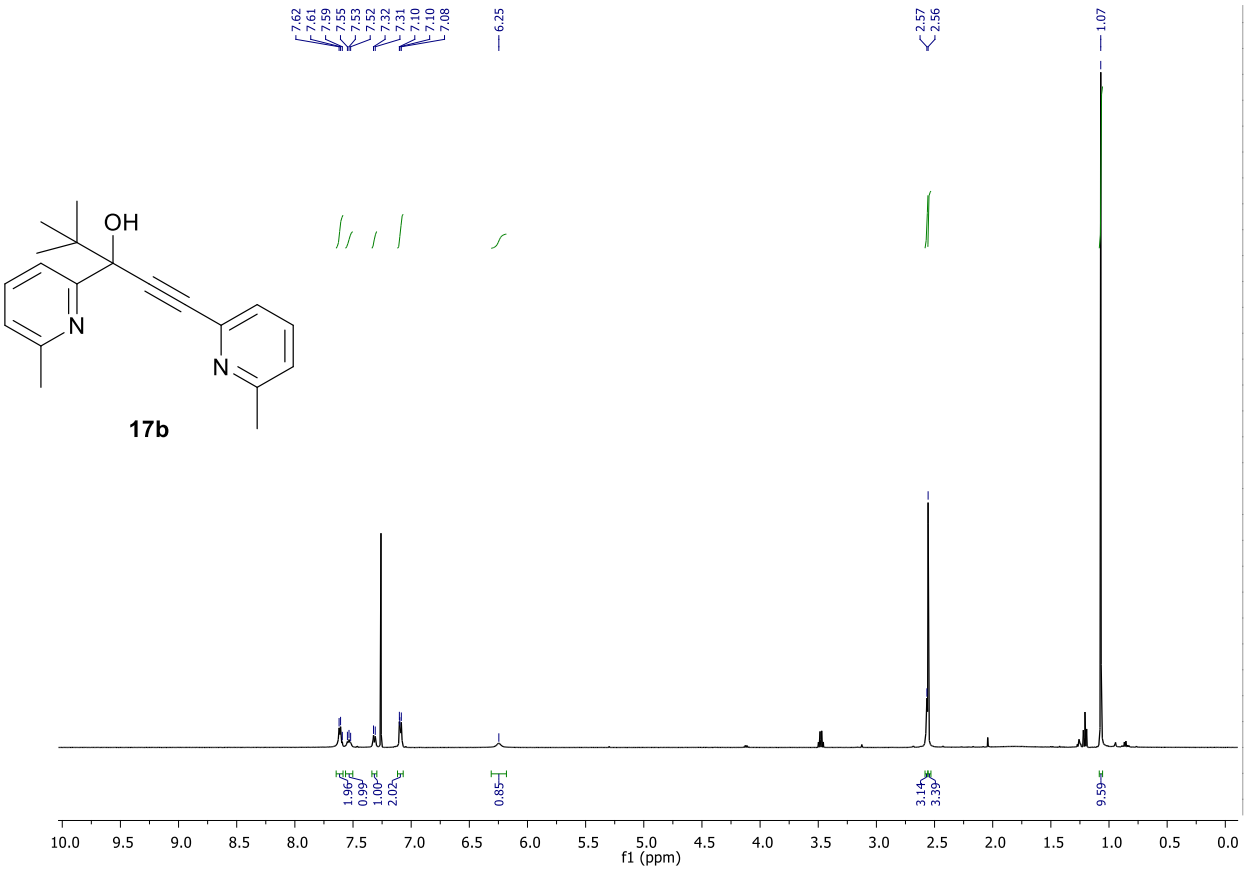

7.61
7.60
7.58
7.46
7.44
7.10
7.08
6.14
2.54
2.51
0.99
OH
N
1.02
1.01
1.00
0.79
3.09
0.95
9.33
f1 (ppm)

16b

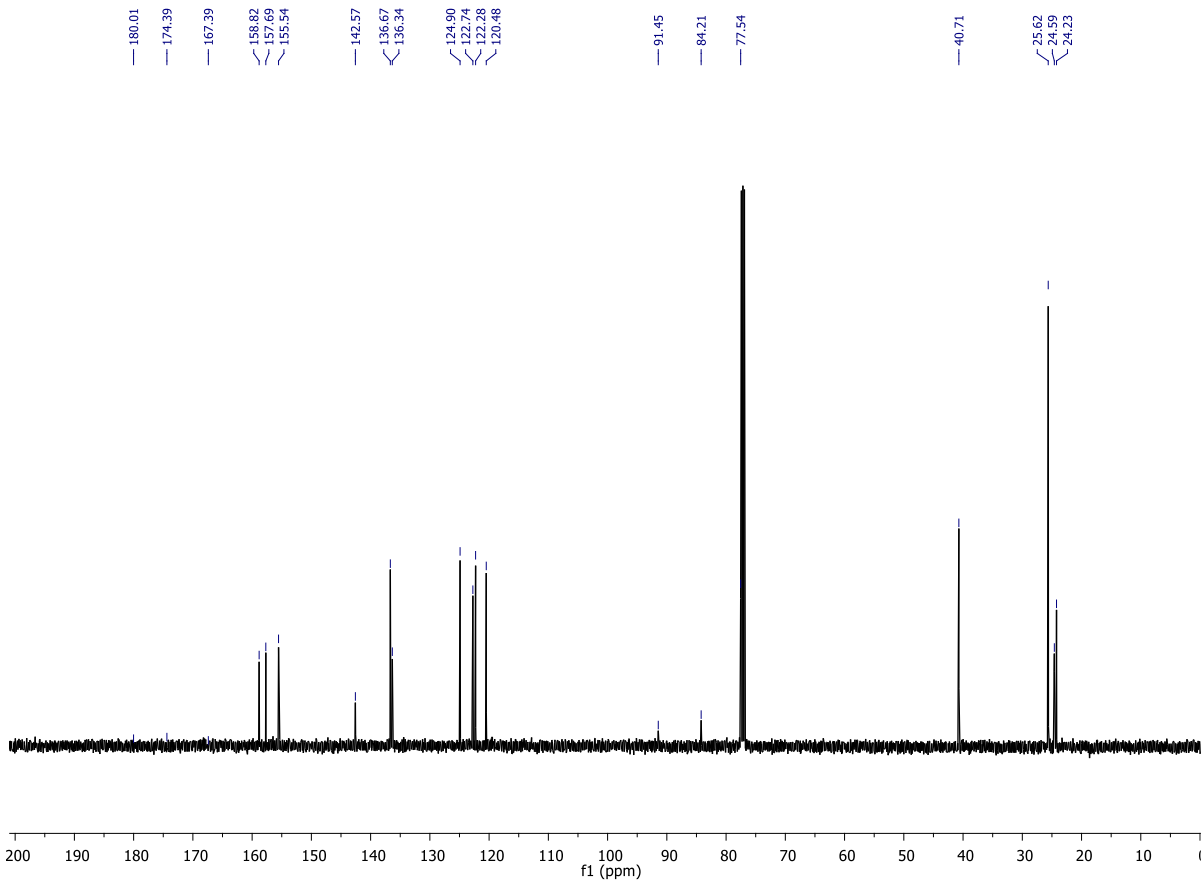

157.82
155.66
136.59
122.32
120.12
86.05
77.12
72.76
40.22
25.39
24.24
f1 (ppm)

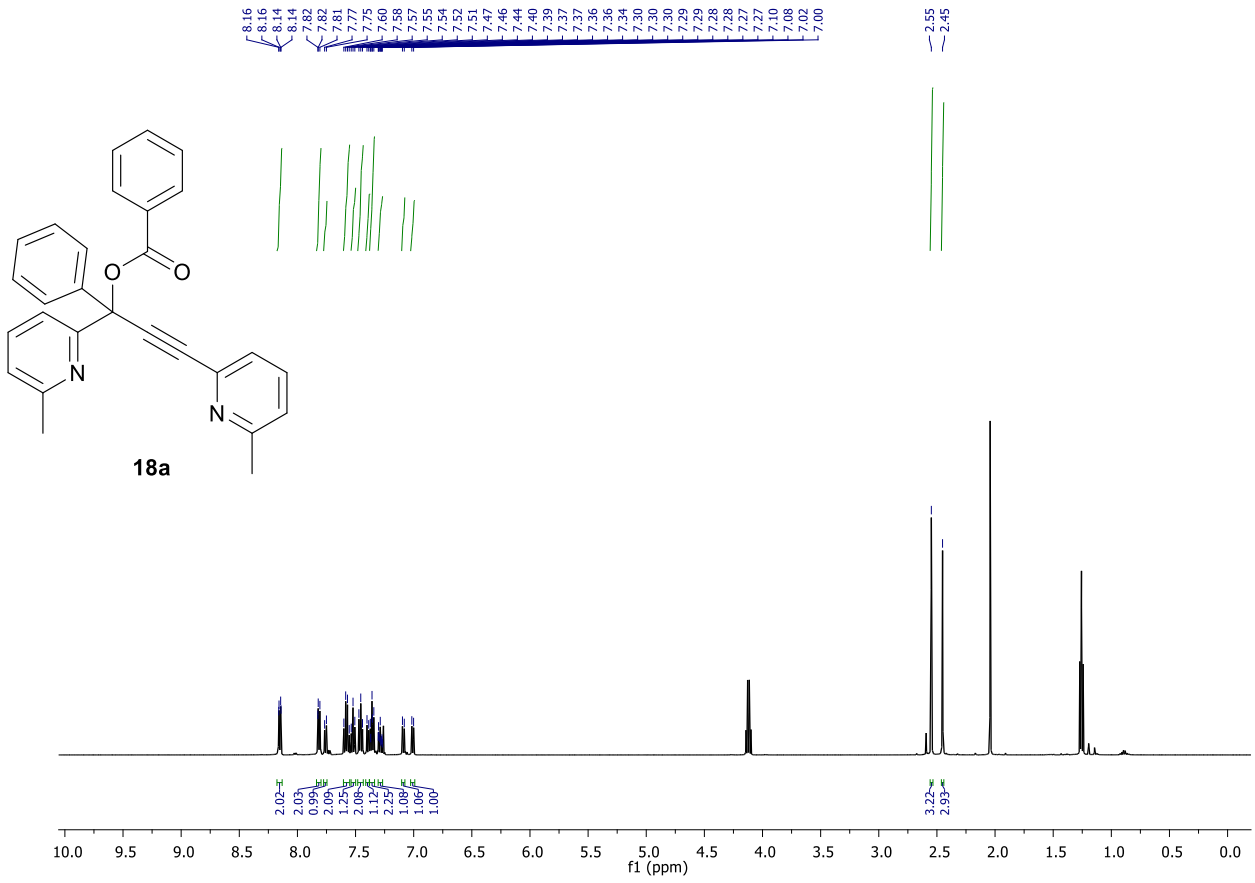

7.76
7.75
7.75
7.74
7.57
7.55
7.54
7.52
7.37
7.36
7.36
7.34
7.34
7.30
7.30
7.29
7.28
7.28
7.11
7.10
7.09
7.08
7.05
2.61
2.56
OH
N
N
17a
1.86
1.89
2.84
2.02
2.01
0.59
2.81
2.80
10.0
9.5
9.0
8.5
8.0
7.5
7.0
6.5
6.0
5.5
5.0
4.5
4.0
3.5
3.0
2.5
2.0
1.5
1.0
0.5
0.0
f1 (ppm)

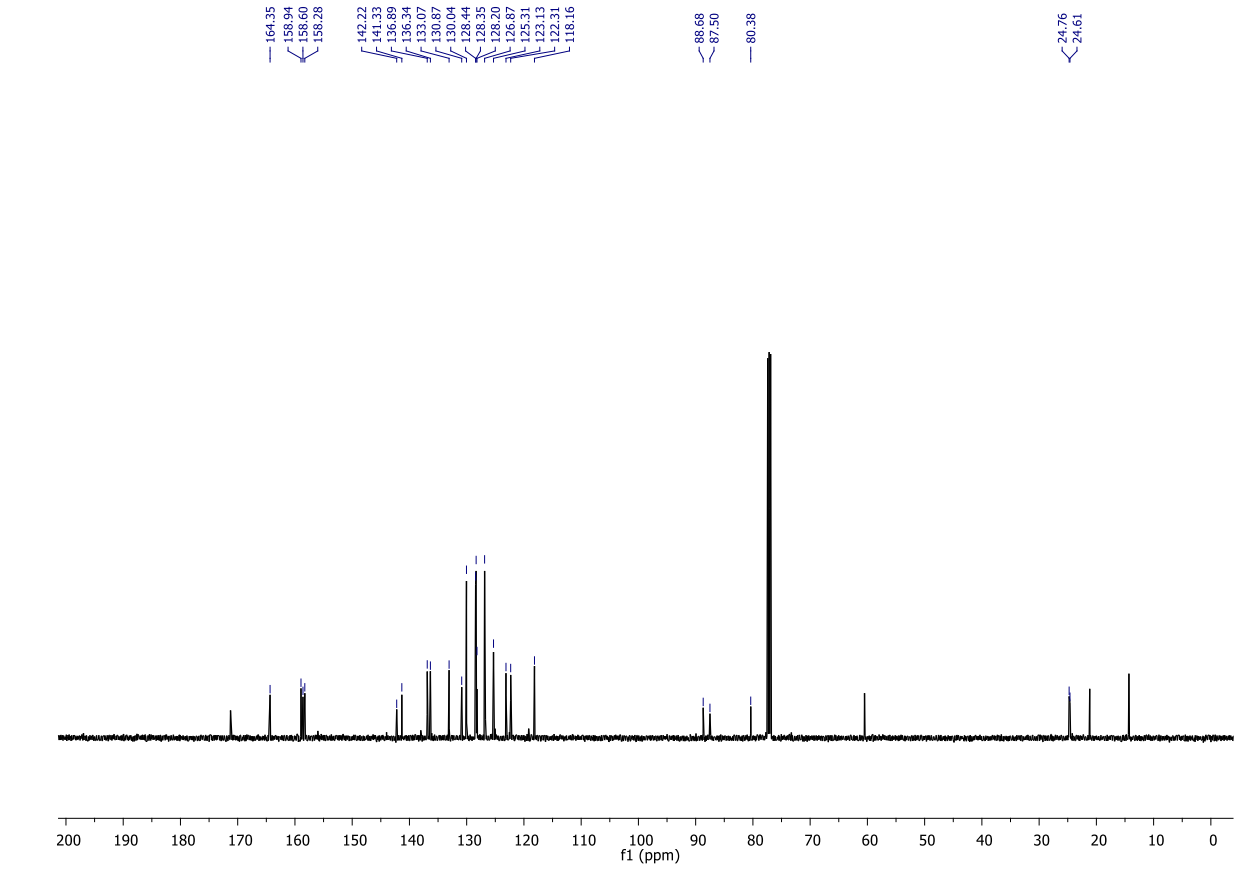

159.59
158.92
156.00
143.95
142.23
138.05
136.49
128.38
128.03
126.70
125.03
123.07
122.54
119.18
91.01
85.52
73.28
24.56
24.19
200
190
180
170
160
150
140
130
120
110
100
90
80
70
60
50
40
30
20
10
0
f1 (ppm)

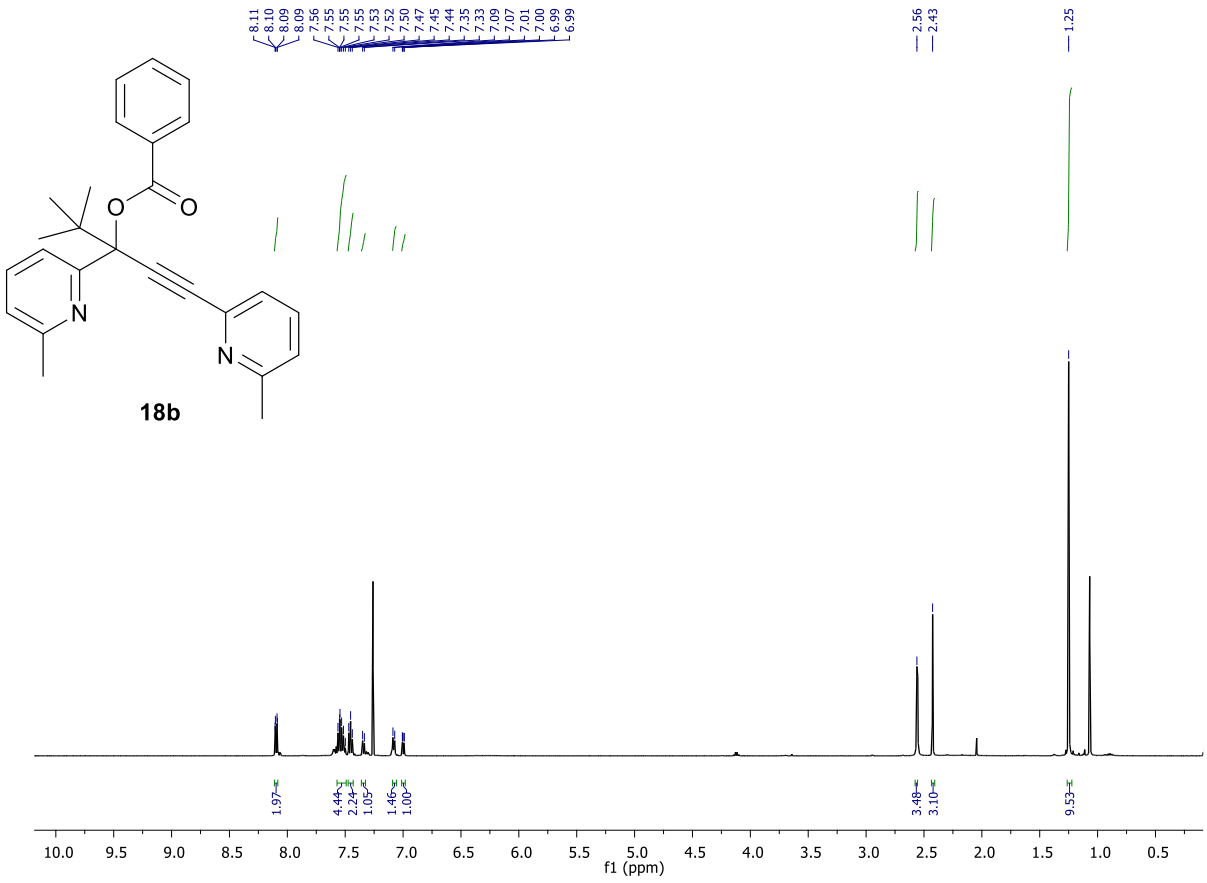

7.62
7.61
7.59
7.55
7.53
7.52
7.32
7.31
7.10
7.10
7.08
6.25
2.57
2.56
1.07
OH
N
N
1.96
0.99
1.00
2.02
0.85
3.14
3.39
9.59
f1 (ppm)

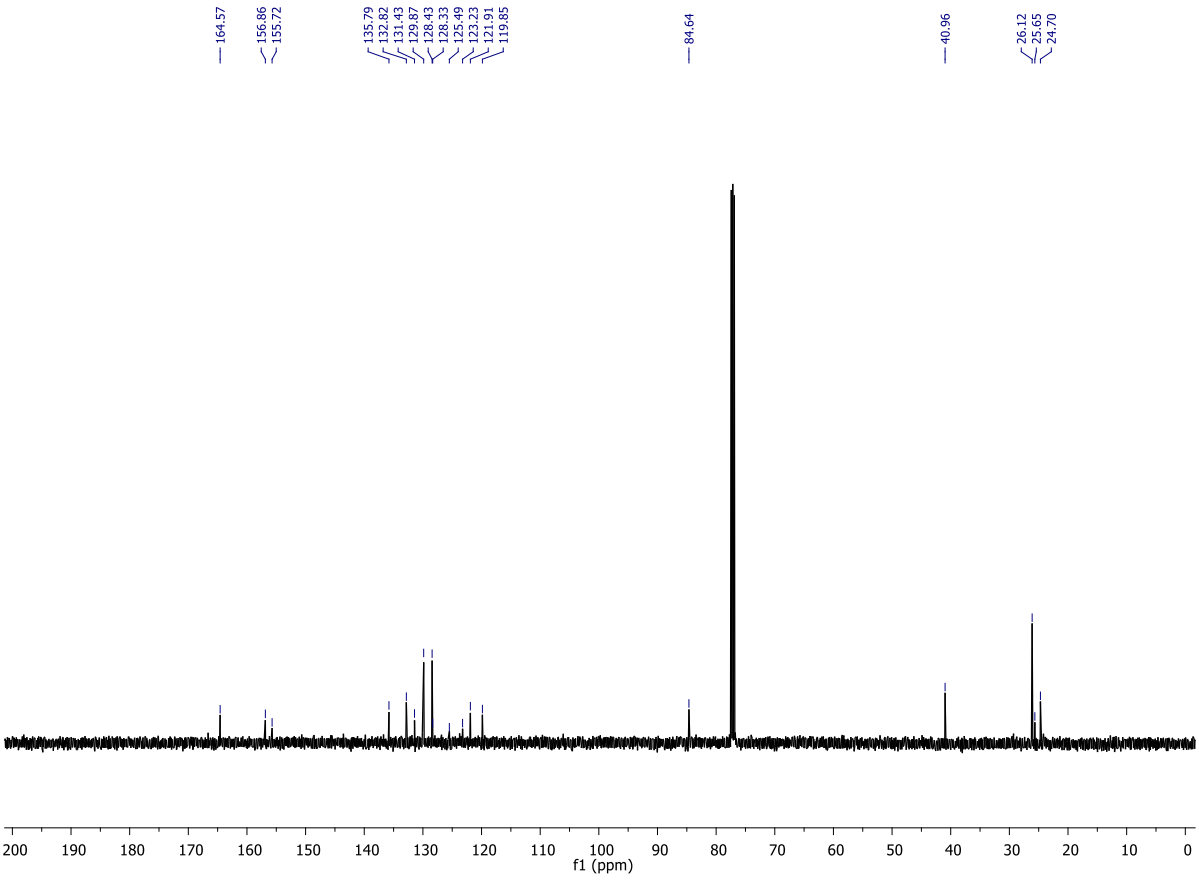
17b

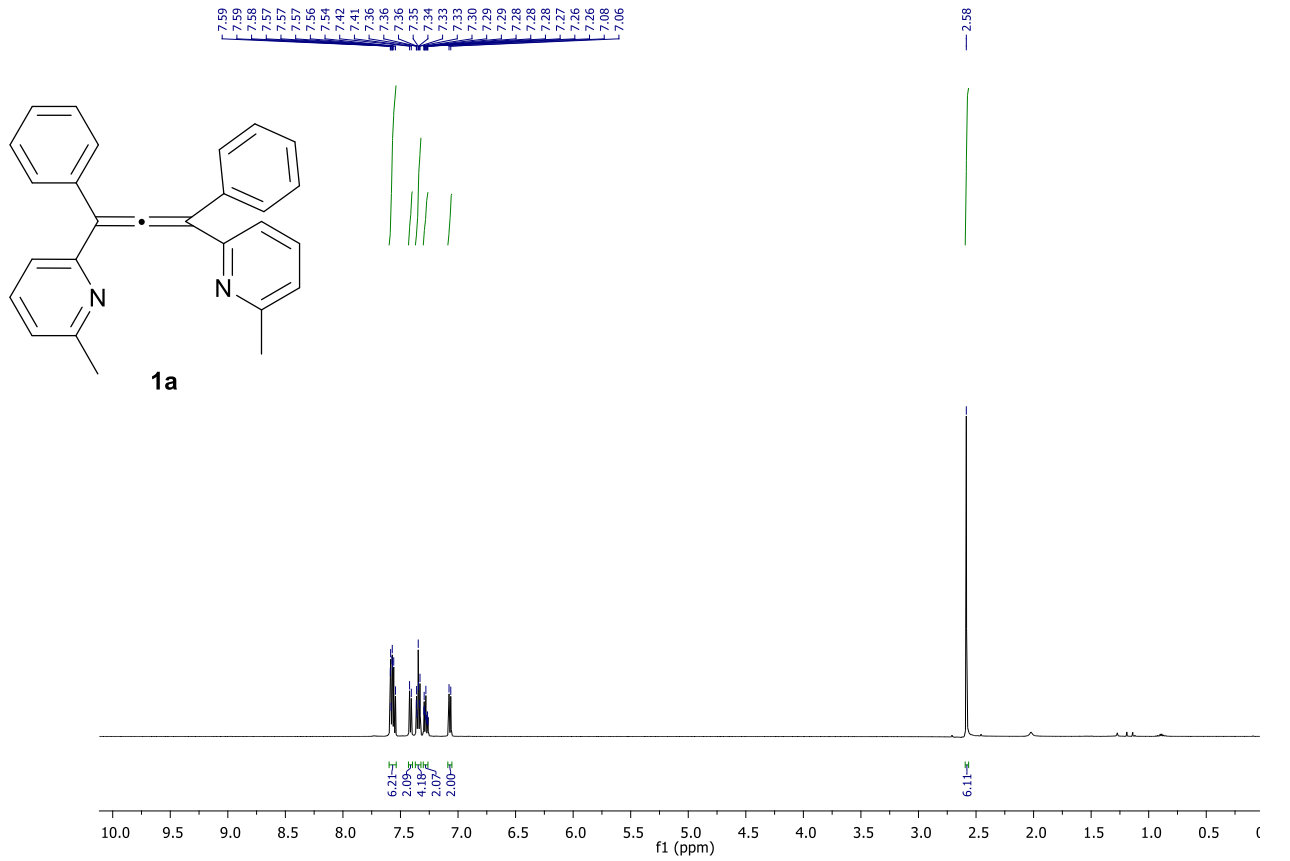

180.01
174.39
167.39
158.82
157.69
155.54
142.57
136.67
136.34
124.90
122.74
122.28
120.48
91.45
84.21
77.54
40.71
25.62
24.59
24.23
f1 (ppm)

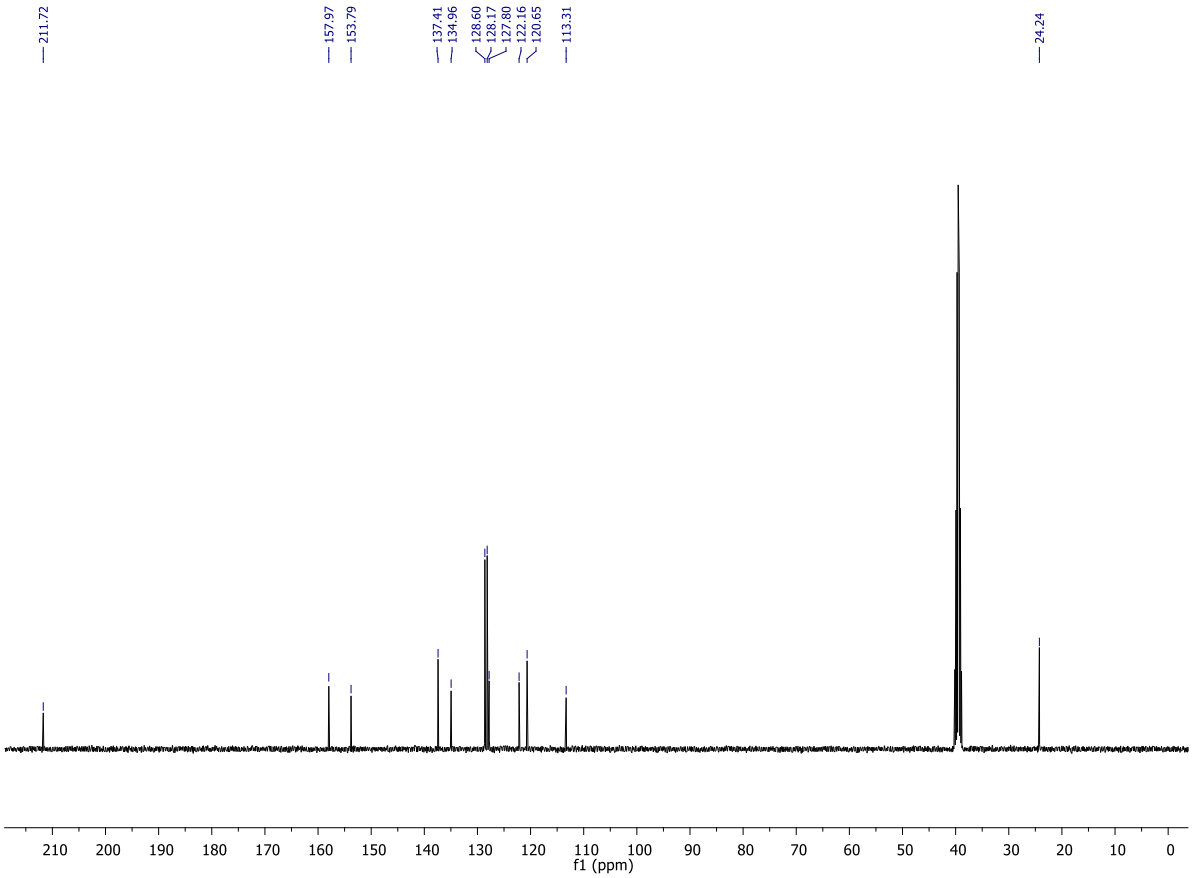

8.16
8.16
8.14
8.14
7.82
7.82
7.81
7.77
7.75
7.60
7.58
7.57
7.55
7.54
7.52
7.51
7.47
7.46
7.44
7.40
7.39
7.37
7.37
7.36
7.36
7.34
7.30
7.30
7.30
7.29
7.29
7.28
7.28
7.27
7.27
7.10
7.08
7.02
7.00
2.55
2.45
O
O
N
N
18a
2.02
2.03
0.99
2.09
1.25
2.08
1.12
2.25
1.08
1.06
1.00
3.22
2.93
10.0 9.5 9.0 8.5 8.0 7.5 7.0 6.5 6.0 5.5 5.0 4.5 4.0 3.5 3.0 2.5 2.0 1.5 1.0 0.5 0.0
f1 (ppm)

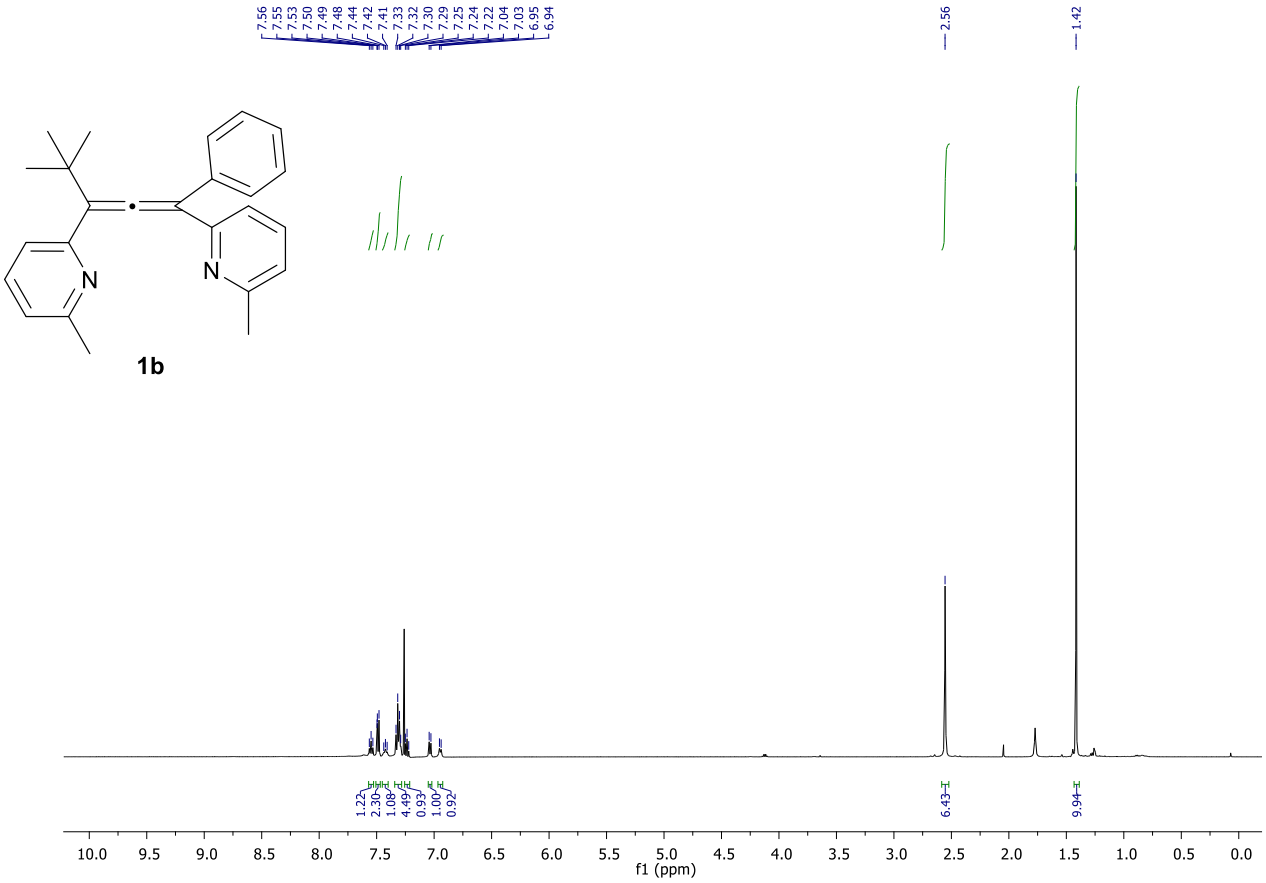

164.35
158.94
158.60
158.28
142.22
141.33
136.89
136.34
133.07
130.87
130.04
128.44
128.35
128.20
126.87
125.31
123.13
122.31
118.16
88.68
87.50
80.38
24.76
24.61
200 190 180 170 160 150 140 130 120 110 100 90 80 70 60 50 40 30 20 10 0
f1 (ppm)

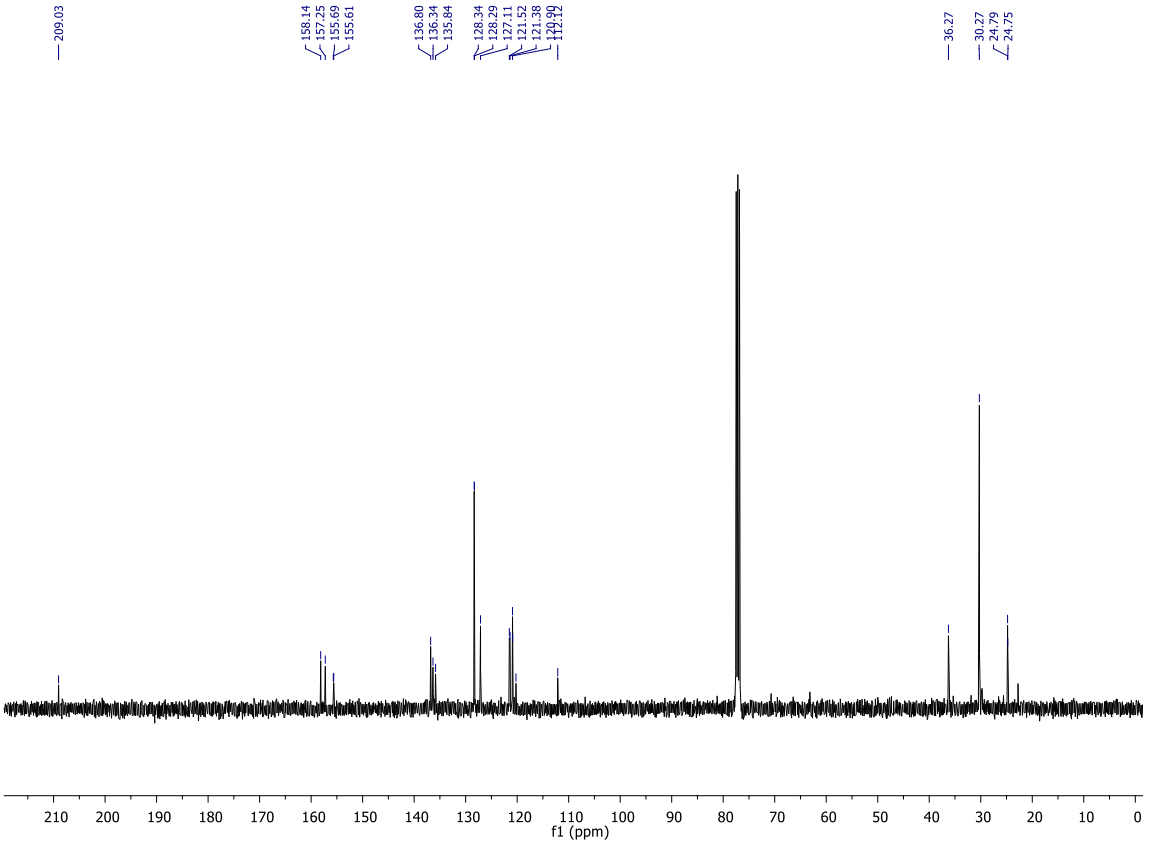

8.11
8.10
8.09
8.09
7.56
7.55
7.55
7.55
7.53
7.52
7.50
7.47
7.45
7.44
7.35
7.33
7.09
7.07
7.01
7.00
6.99
6.99
2.56
2.43
1.25
O
O
N
N
18b
1.97
4.44
2.24
1.05
1.46
1.00
3.48
3.10
9.53
10.0 9.5 9.0 8.5 8.0 7.5 7.0 6.5 6.0 5.5 5.0 4.5 4.0 3.5 3.0 2.5 2.0 1.5 1.0 0.5
f1 (ppm)

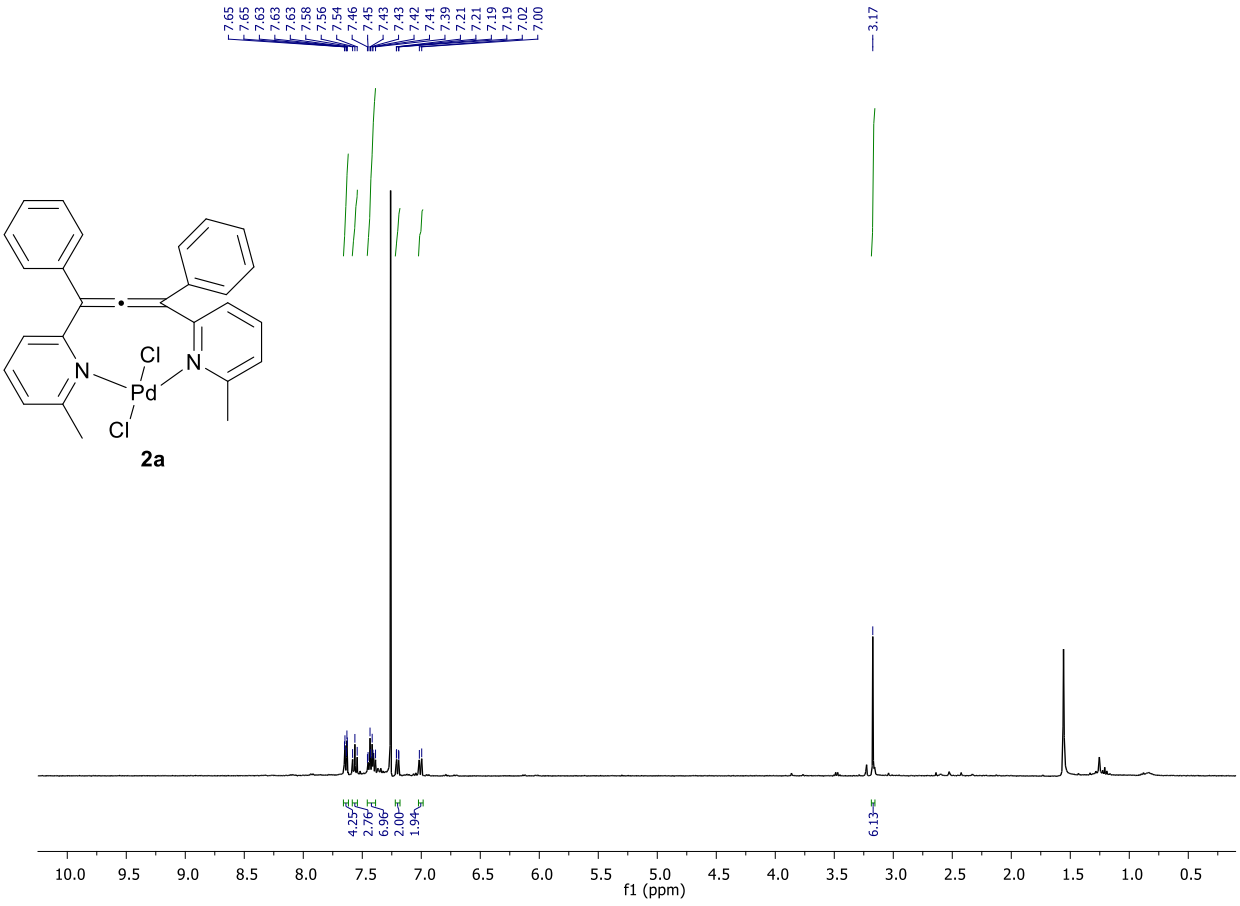

164.57
156.86
155.72
135.79
132.82
131.43
129.87
128.43
128.33
125.49
123.23
121.91
119.85
84.64
40.96
26.12
25.65
24.70
200 190 180 170 160 150 140 130 120 110 100 90 80 70 60 50 40 30 20 10 0
f1 (ppm)

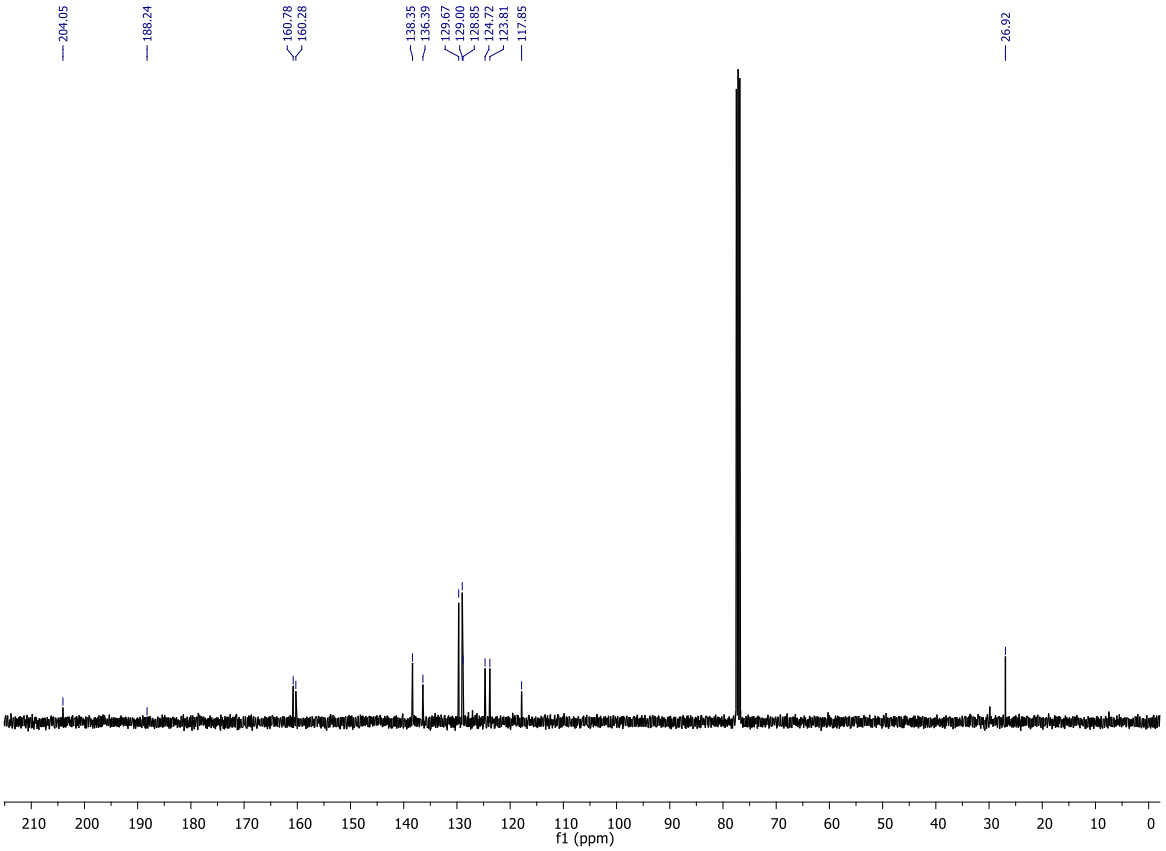

7.59
7.59
7.58
7.57
7.57
7.57
7.56
7.54
7.42
7.41
7.36
7.36
7.36
7.35
7.34
7.33
7.33
7.30
7.29
7.29
7.28
7.28
7.28
7.27
7.26
7.26
7.08
7.06
2.58
N
N
1a
6.21
2.09
4.18
2.07
2.00
6.11
10.0
9.5
9.0
8.5
8.0
7.5
7.0
6.5
6.0
5.5
5.0
4.5
4.0
3.5
3.0
2.5
2.0
1.5
1.0
0.5
f1 (ppm)

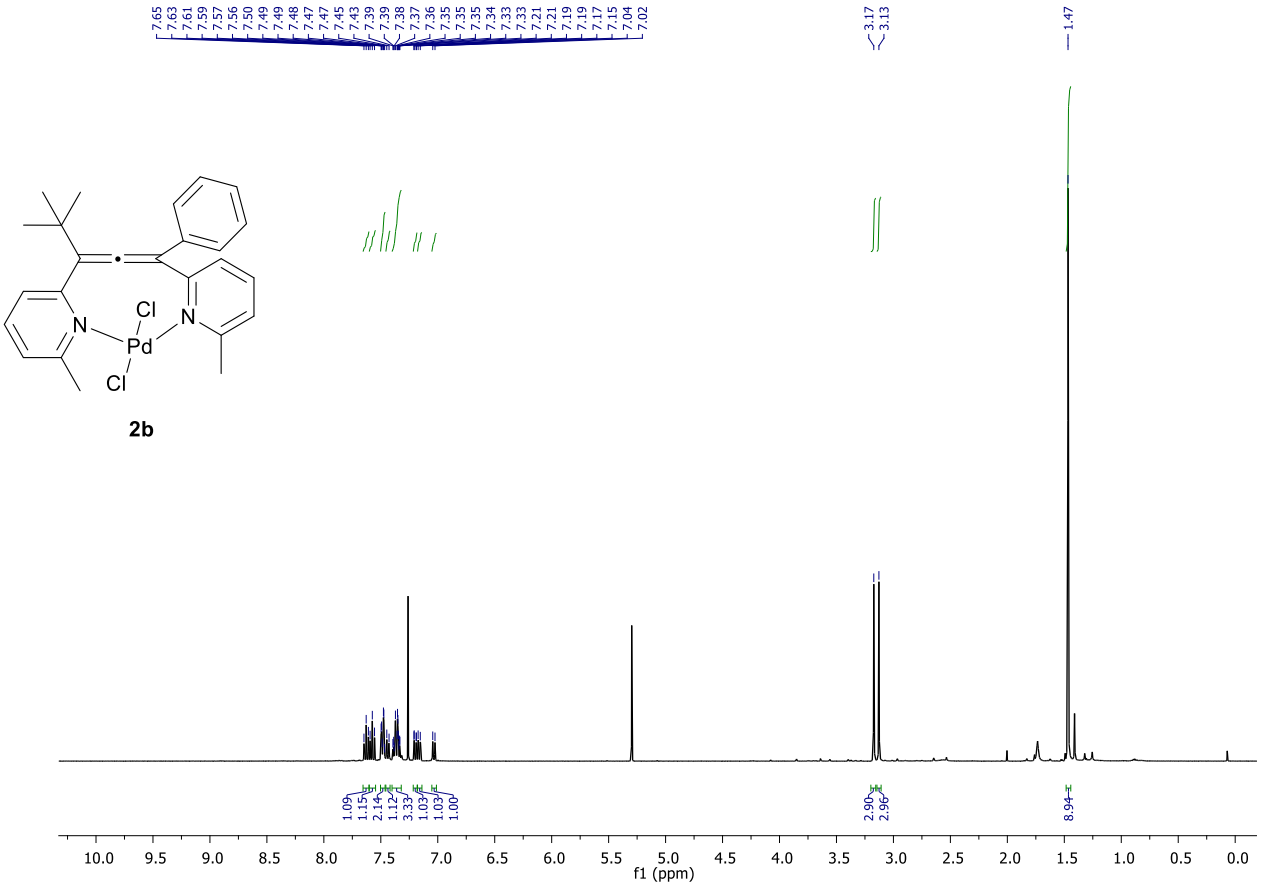

211.72
157.97
153.79
137.41
134.96
128.60
128.17
127.80
122.16
120.65
113.31
24.24
210
200
190
180
170
160
150
140
130
120
110
100
90
80
70
60
50
40
30
20
10
0
f1 (ppm)


7.56
7.55
7.53
7.50
7.49
7.48
7.44
7.42
7.41
7.33
7.32
7.30
7.29
7.25
7.24
7.22
7.04
7.03
6.95
6.94
2.56
1.42
N
N
1b
1.22
2.30
1.08
4.49
0.93
1.00
0.92
6.43
9.94
f1 (ppm)

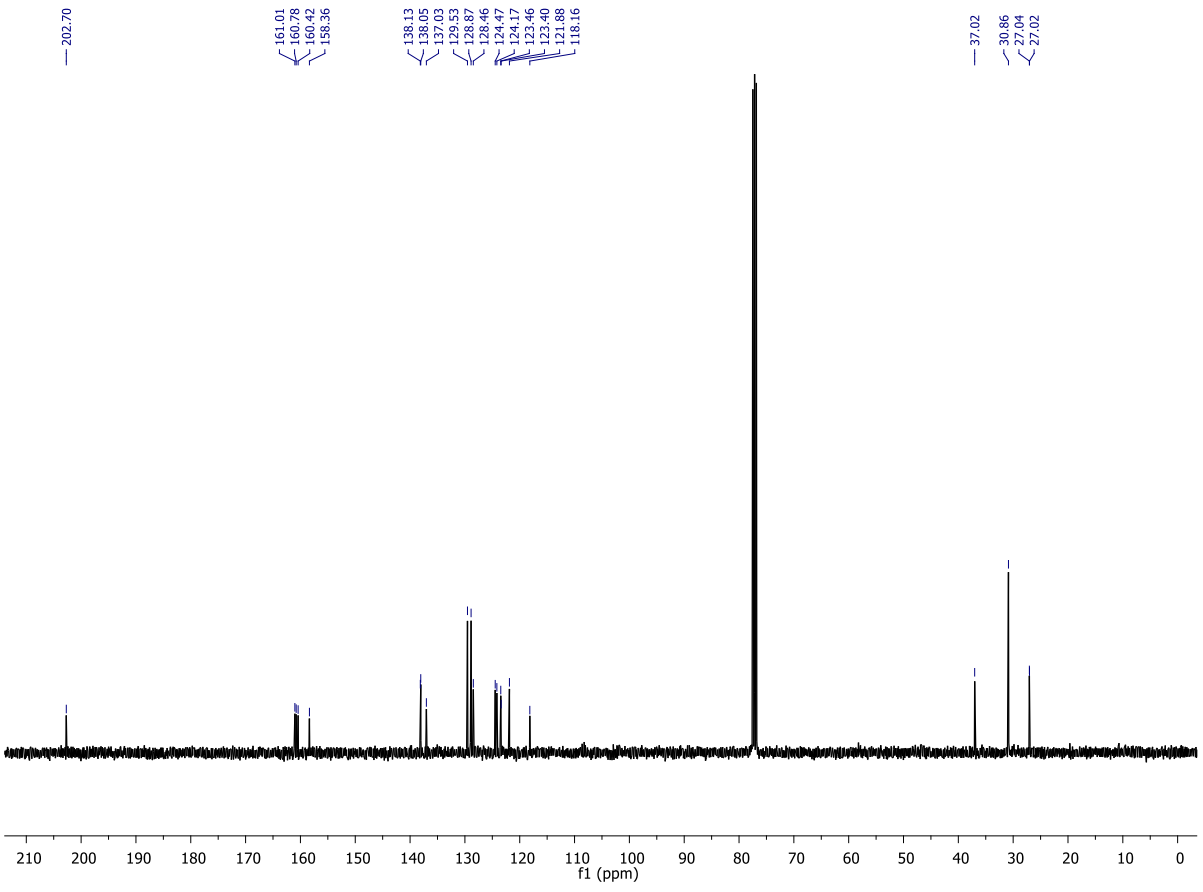

209.03
158.14
157.25
155.69
155.61
136.80
136.34
135.84
128.34
128.29
127.11
121.52
121.38
120.90
112.12
36.27
30.27
24.79
24.75
f1 (ppm)

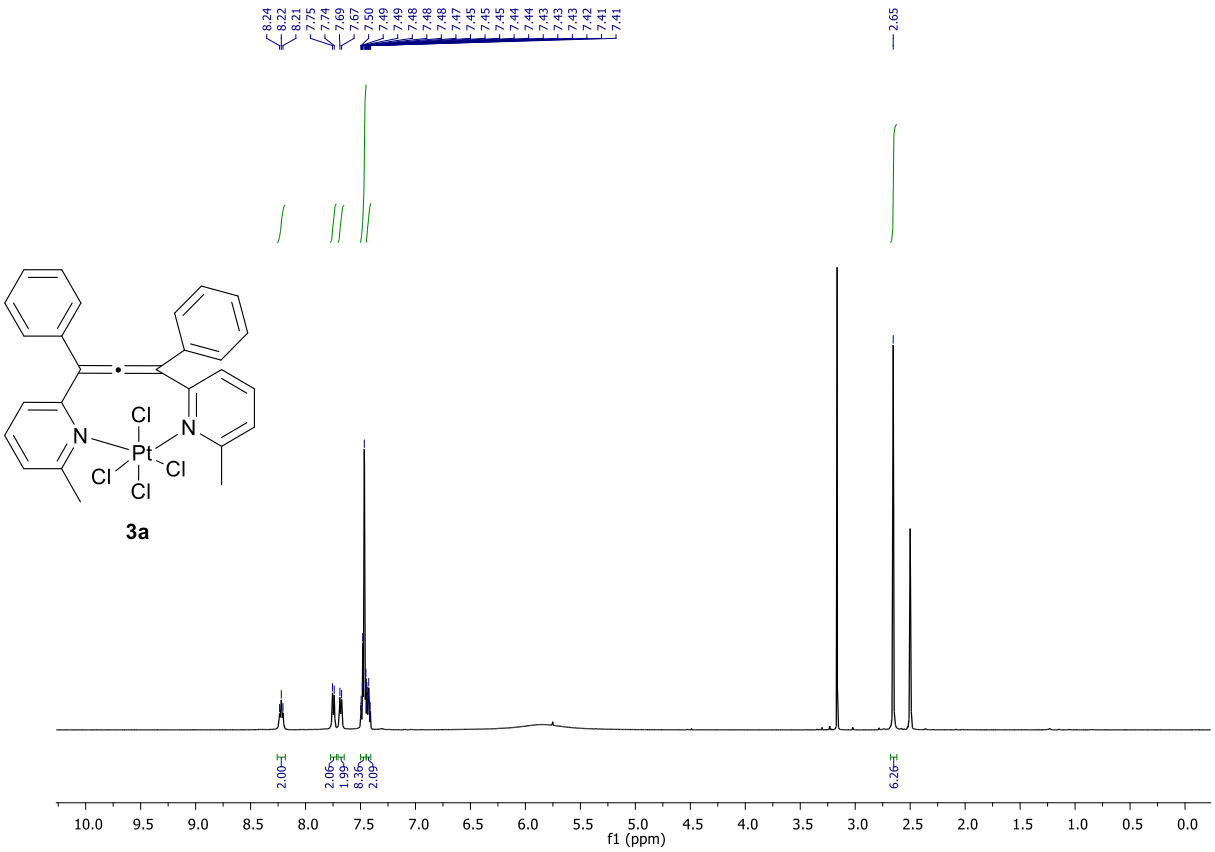

7.65
7.65
7.63
7.63
7.63
7.58
7.56
7.54
7.46
7.45
7.43
7.43
7.42
7.41
7.39
7.21
7.21
7.19
7.19
7.02
7.00
3.17
Cl
N
N
Pd
Cl
2a
4.25
2.76
6.96
2.00
1.94
6.13
10.0
9.5
9.0
8.5
8.0
7.5
7.0
6.5
6.0
5.5
5.0
4.5
4.0
3.5
3.0
2.5
2.0
1.5
1.0
0.5
f1 (ppm)


204.05
188.24
160.78
160.28
138.35
136.39
129.67
129.00
128.85
124.72
123.81
117.85
26.92
210
200
190
180
170
160
150
140
130
120
110
100
90
80
70
60
50
40
30
20
10
0
f1 (ppm)

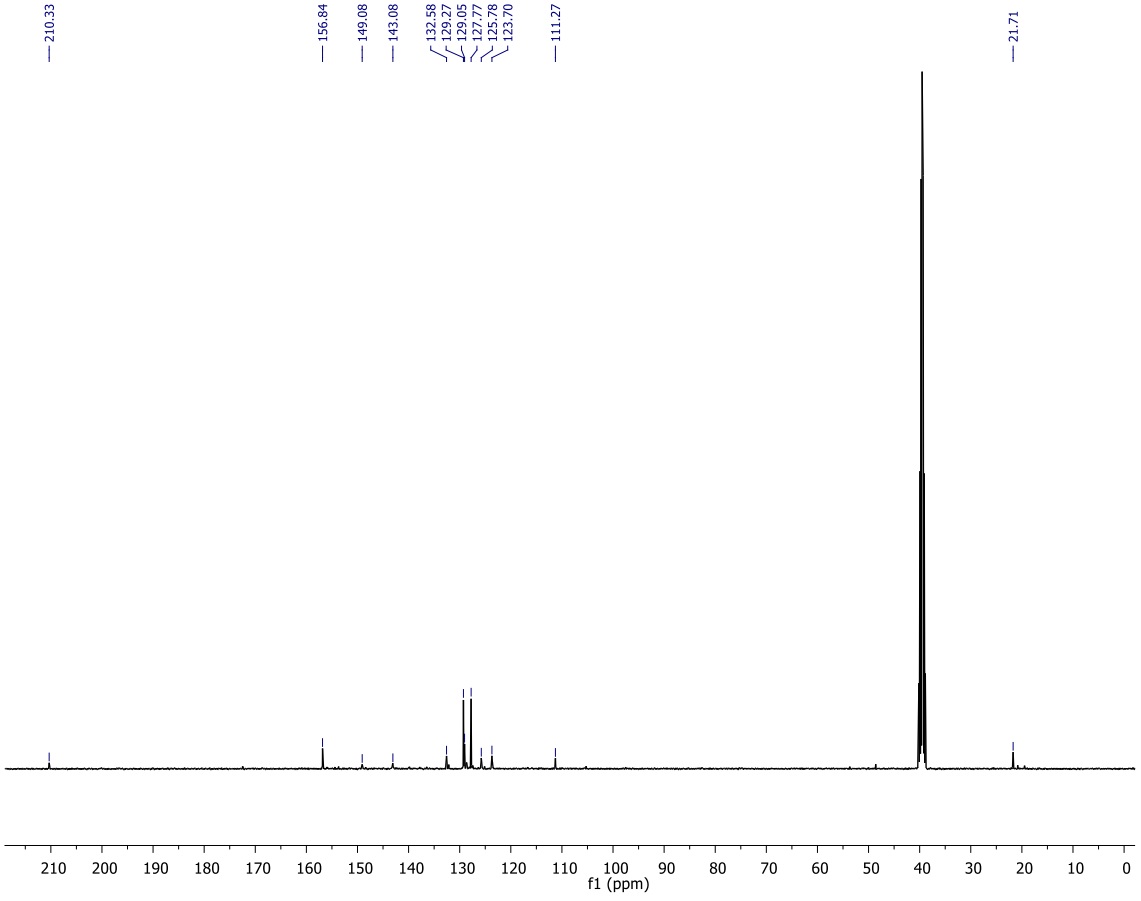

7.65
7.63
7.61
7.59
7.57
7.56
7.50
7.49
7.49
7.48
7.47
7.47
7.45
7.43
7.39
7.39
7.38
7.37
7.36
7.35
7.35
7.35
7.34
7.33
7.33
7.21
7.21
7.19
7.19
7.17
7.15
7.04
7.02
3.17
3.13
1.47
N
Cl
N
Pd
Cl
1.09
1.15
2.14
1.12
3.33
1.03
1.03
1.00
2.90
2.96
8.94
10.0 9.5 9.0 8.5 8.0 7.5 7.0 6.5 6.0 5.5 5.0 4.5 4.0 3.5 3.0 2.5 2.0 1.5 1.0 0.5 0.0
f1 (ppm)

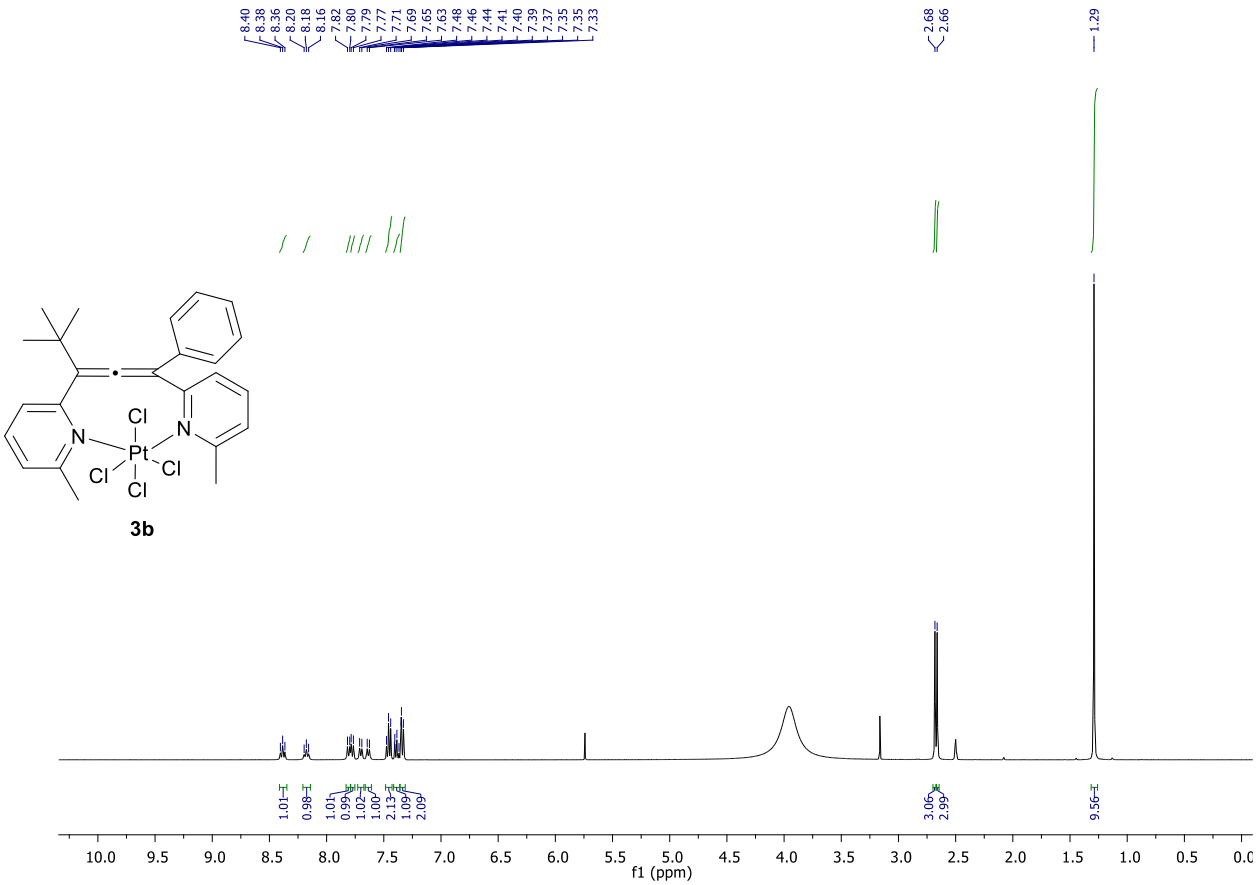
2b


202.70
161.01
160.78
160.42
158.36
138.13
138.05
137.03
129.53
128.87
128.46
124.47
124.17
123.46
123.40
121.88
118.16
37.02
30.86
27.04
27.02
210 200 190 180 170 160 150 140 130 120 110 100 90 80 70 60 50 40 30 20 10 0
f1 (ppm)

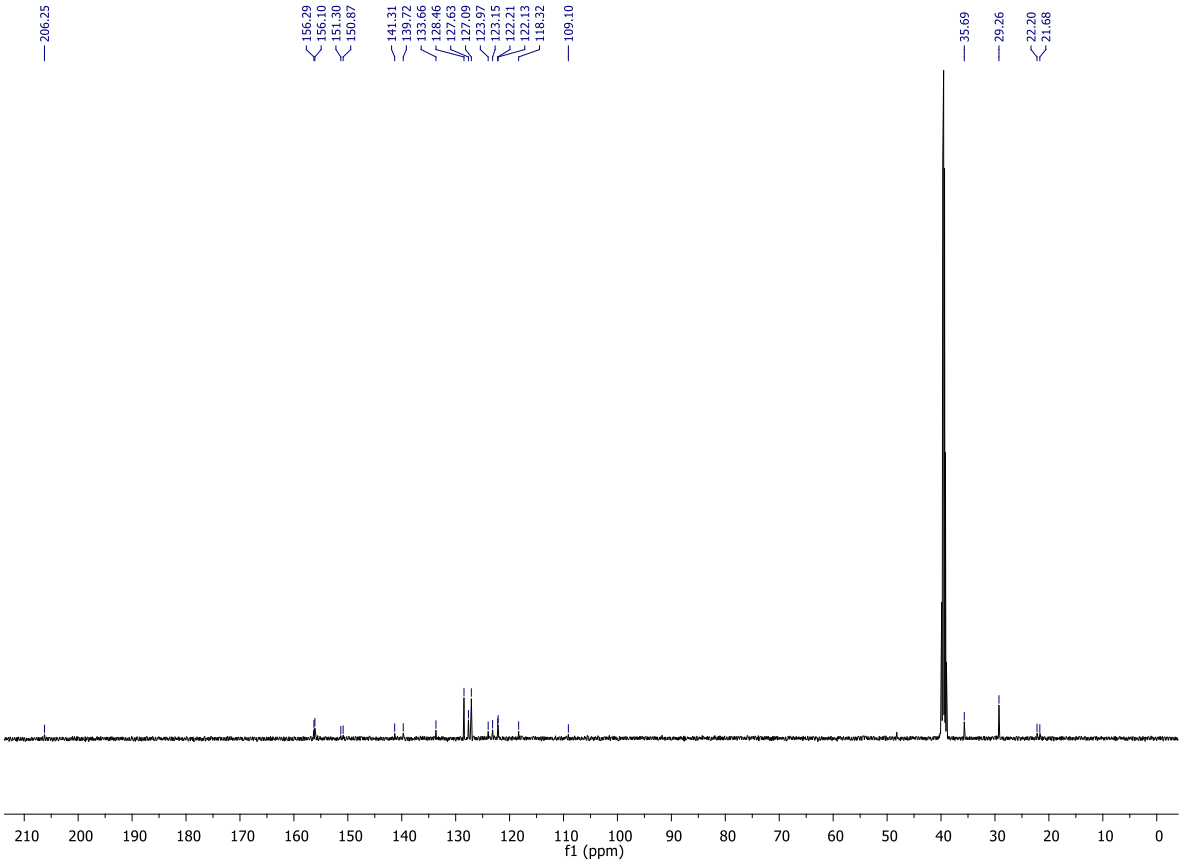

8.24
8.22
8.21
7.75
7.74
7.69
7.67
7.50
7.49
7.49
7.48
7.48
7.48
7.47
7.45
7.45
7.45
7.44
7.44
7.43
7.43
7.42
7.41
7.41
2.65
Cl
N
N
Pt
Cl
Cl
Cl
2.00
2.06
1.99
8.36
2.09
6.26
10.0 9.5 9.0 8.5 8.0 7.5 7.0 6.5 6.0 5.5 5.0 4.5 4.0 3.5 3.0 2.5 2.0 1.5 1.0 0.5 0.0
f1 (ppm)

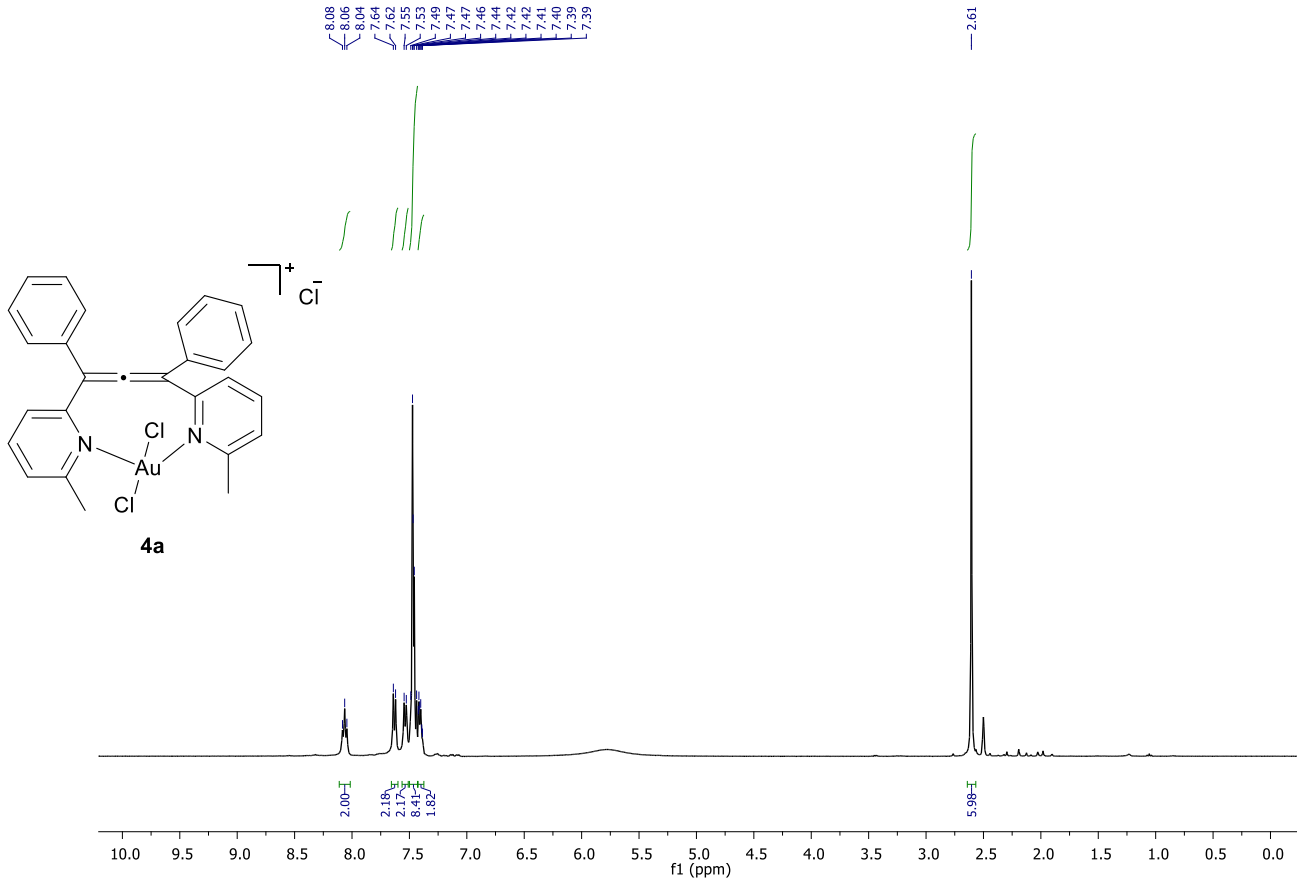
3a


210.33
156.84
149.08
143.08
132.58
129.27
129.05
127.77
125.78
123.70
111.27
21.71
210 200 190 180 170 160 150 140 130 120 110 100 90 80 70 60 50 40 30 20 10 0
f1 (ppm)

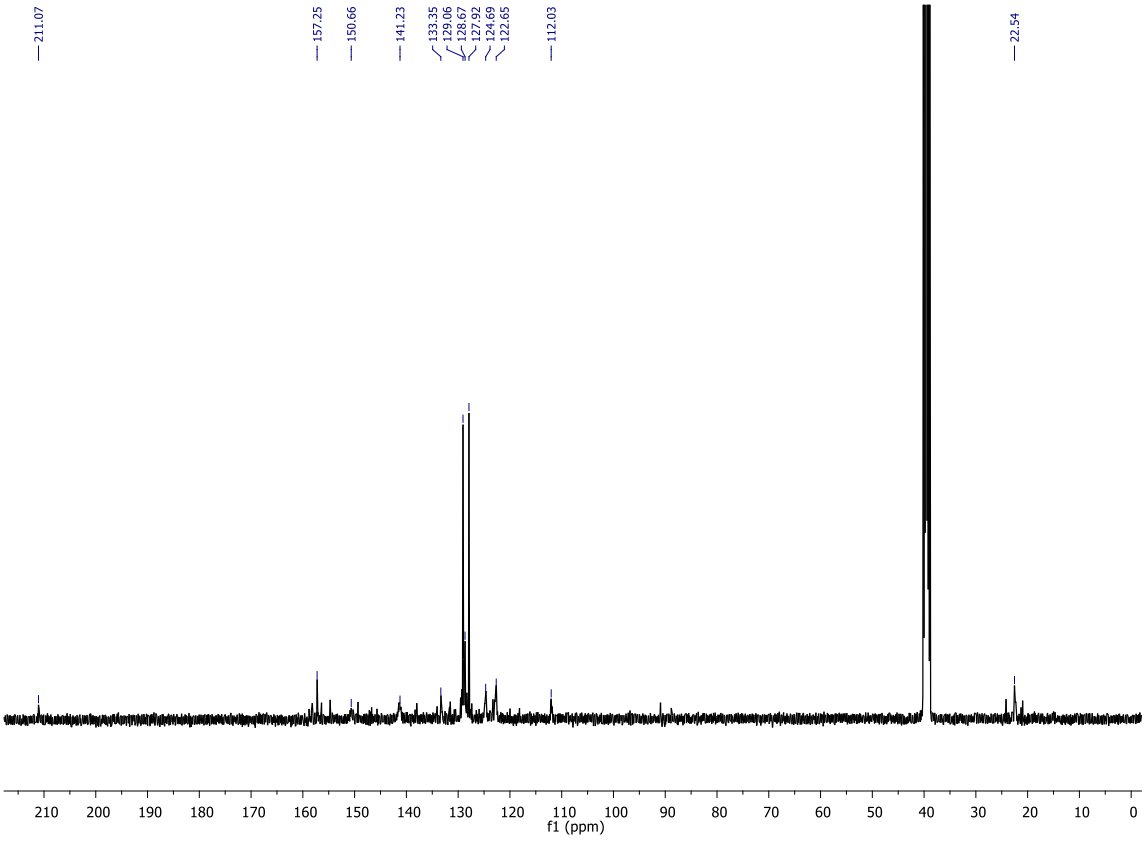

8.40
8.38
8.36
8.20
8.18
8.16
7.82
7.80
7.79
7.77
7.71
7.69
7.65
7.63
7.48
7.46
7.44
7.41
7.40
7.39
7.37
7.35
7.35
7.33
2.68
2.66
1.29
Cl
N
N
Pt
Cl
Cl
Cl
1.01
0.98
1.01
0.99
1.02
1.00
2.13
1.09
2.09
3.06
2.99
9.56
10.0
9.5
9.0
8.5
8.0
7.5
7.0
6.5
6.0
5.5
5.0
4.5
4.0
3.5
3.0
2.5
2.0
1.5
1.0
0.5
f1 (ppm)

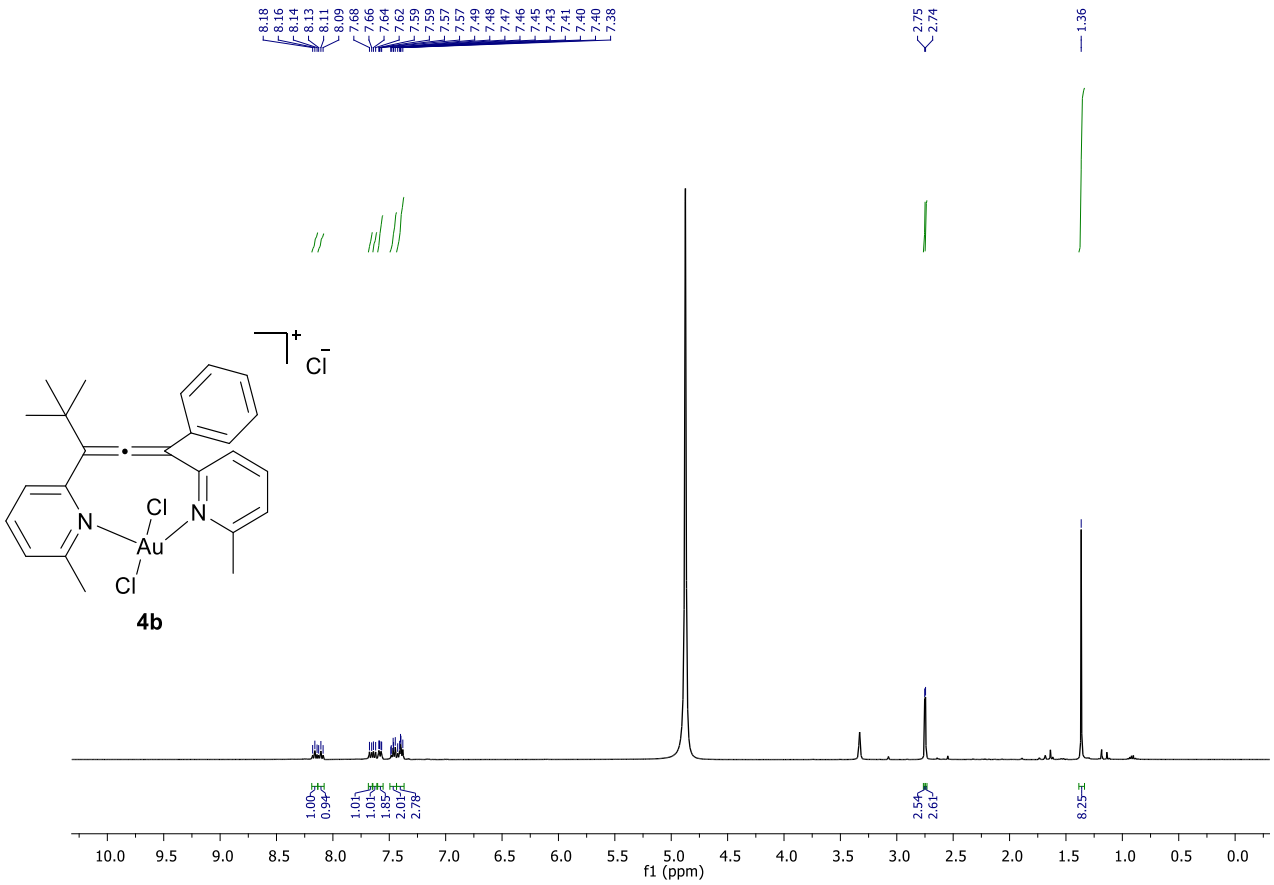
3b


206.25
156.29
156.10
151.30
150.87
141.31
139.72
133.66
128.46
127.63
127.09
123.97
123.15
122.21
122.13
118.32
109.10
35.69
29.26
22.20
21.68
210
200
190
180
170
160
150
140
130
120
110
100
90
80
70
60
50
40
30
20
10
0
f1 (ppm)


8.08
8.06
8.04
7.64
7.62
7.55
7.53
7.49
7.47
7.47
7.46
7.44
7.42
7.42
7.41
7.40
7.39
7.39
2.61
Cl
N
Cl
Au
N
Cl
2.00
2.18
2.17
8.41
1.82
5.98
10.0 9.5 9.0 8.5 8.0 7.5 7.0 6.5 6.0 5.5 5.0 4.5 4.0 3.5 3.0 2.5 2.0 1.5 1.0 0.5 0.0
f1 (ppm)

4a


211.07
157.25
150.66
141.23
133.35
129.06
128.67
127.92
124.69
122.65
112.03
22.54
210 200 190 180 170 160 150 140 130 120 110 100 90 80 70 60 50 40 30 20 10 0
f1 (ppm)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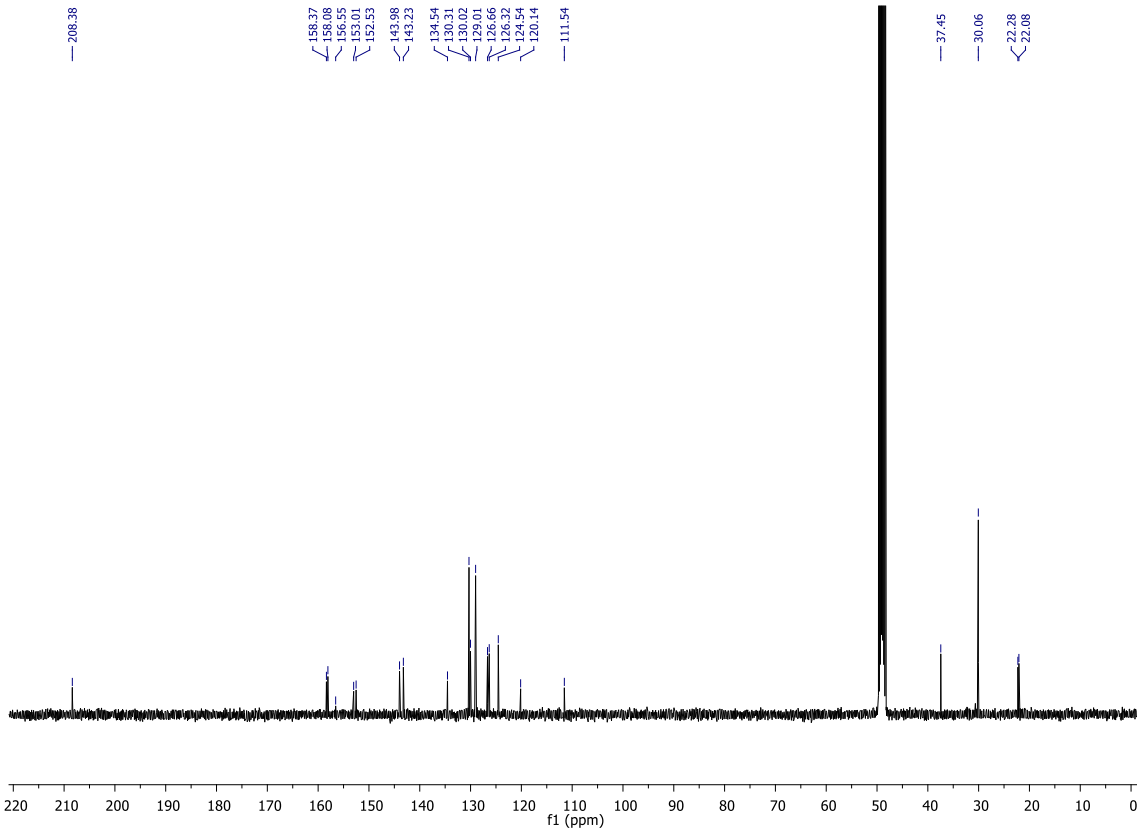
7.57
7.49
7.48
7.47
7.46
7.45
7.43
7.41
7.40
7.40
7.38
2.75
2.74
1.36
Cl
N
Cl
N
Au
Cl
4b
1.00
0.94
1.01
1.01
1.85
2.01
2.78
2.54
2.61
8.25
10.0 9.5 9.0 8.5 8.0 7.5 7.0 6.5 6.0 5.5 5.0 4.5 4.0 3.5 3.0 2.5 2.0 1.5 1.0 0.5 0.0
f1 (ppm)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143.23
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129.01
126.66
126.32
124.54
120.14
111.54
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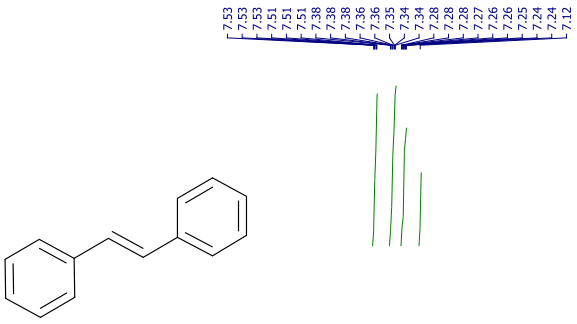
30.06
22.28
22.08
220 210 200 190 180 170 160 150 140 130 120 110 100 90 80 70 60 50 40 30 20 10 0
f1 (ppm)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7.38
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7.36
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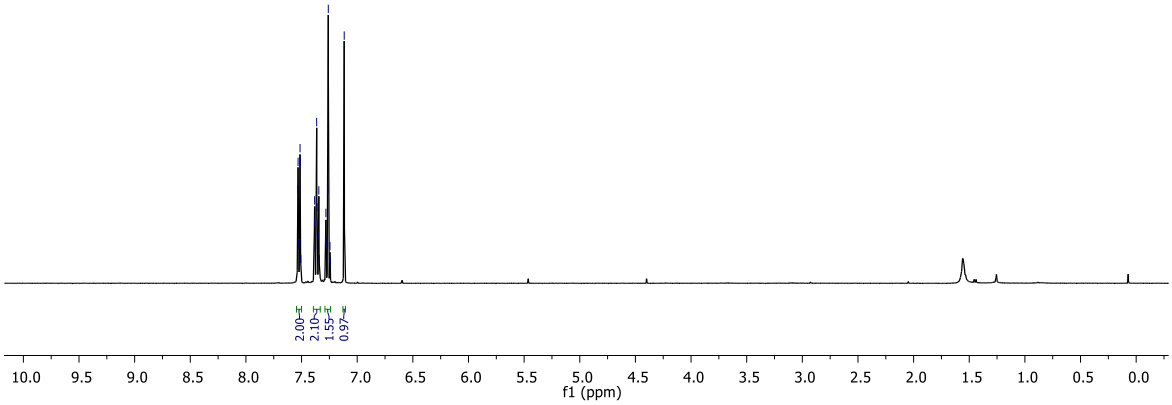
7.24
7.12

7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1.55
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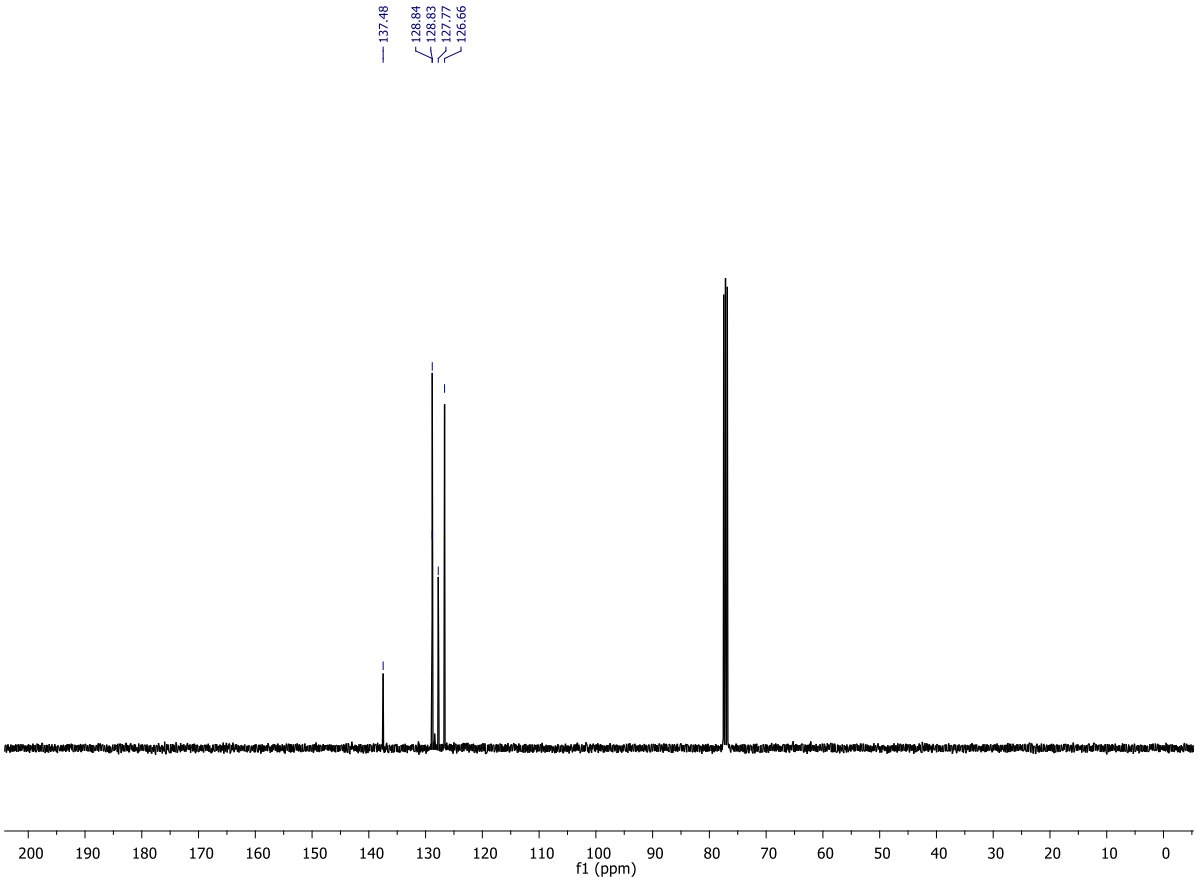
10.0 9.5 9.0 8.5 8.0 7.5 7.0 6.5 6.0 5.5 5.0 4.5 4.0 3.5 3.0 2.5 2.0 1.5 1.0 0.5 0.0
f1 (ppm)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128.84
128.83
127.77
126.66
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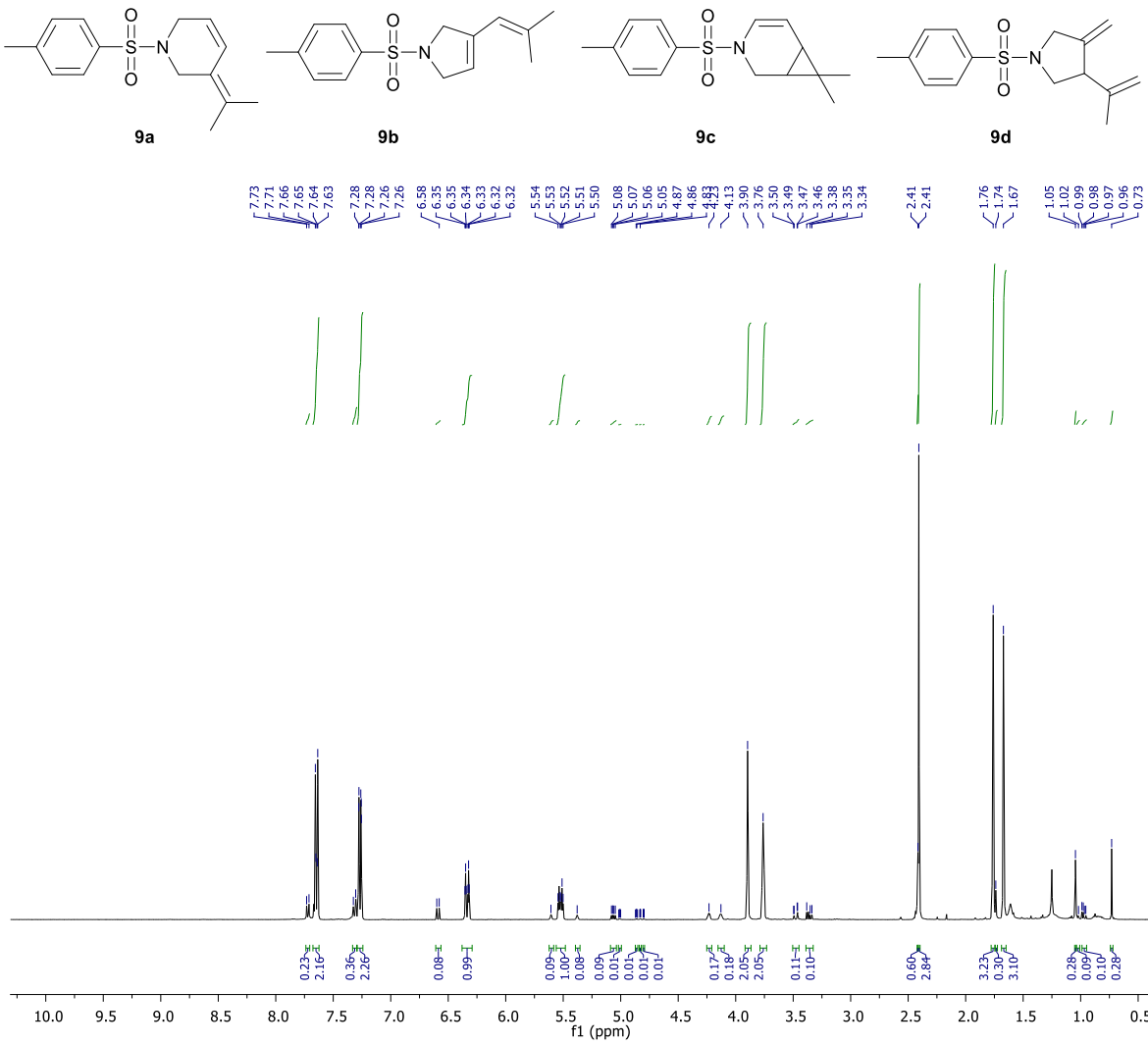
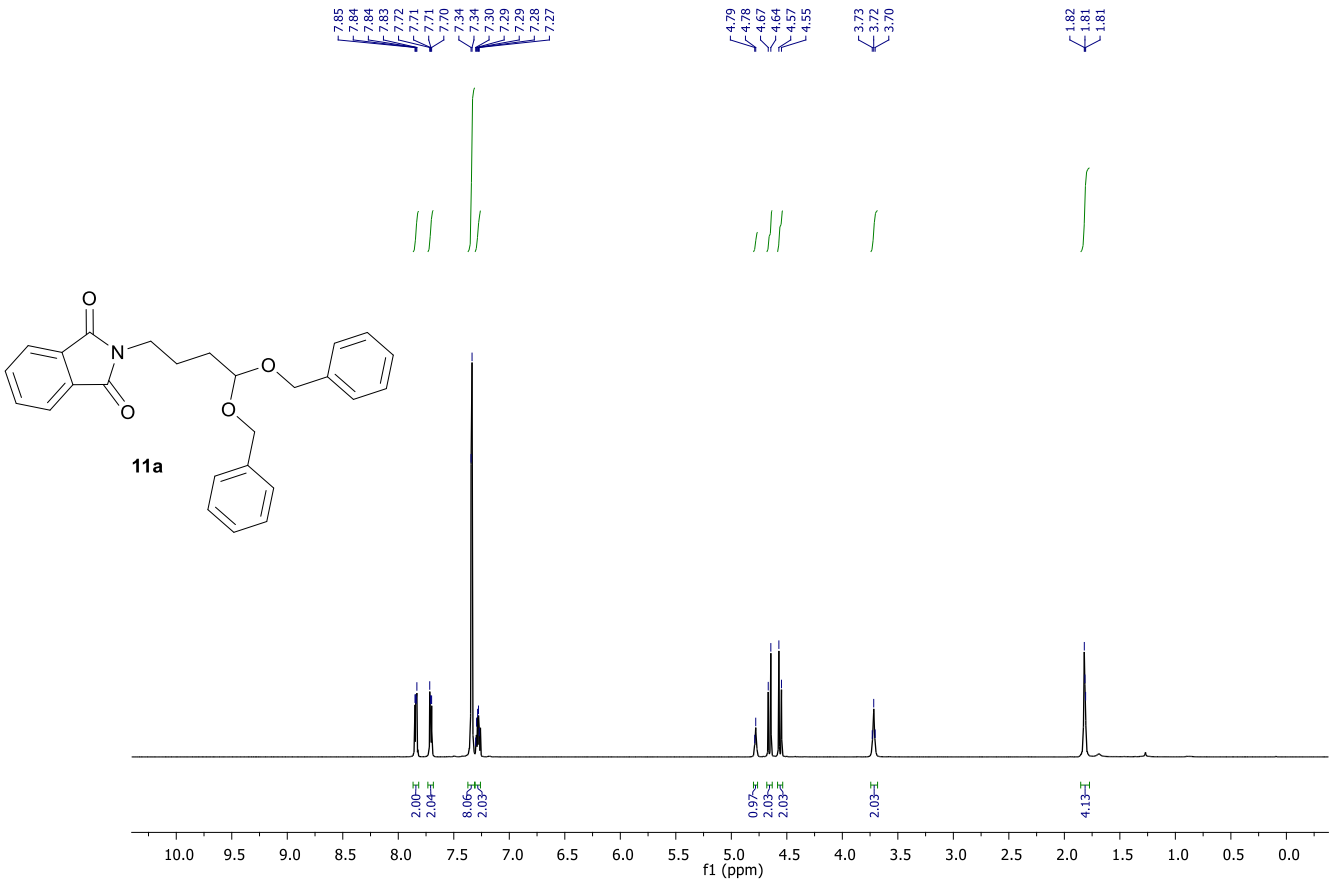
f1 (ppm)


9a
9b
9c
9d
f1 (ppm)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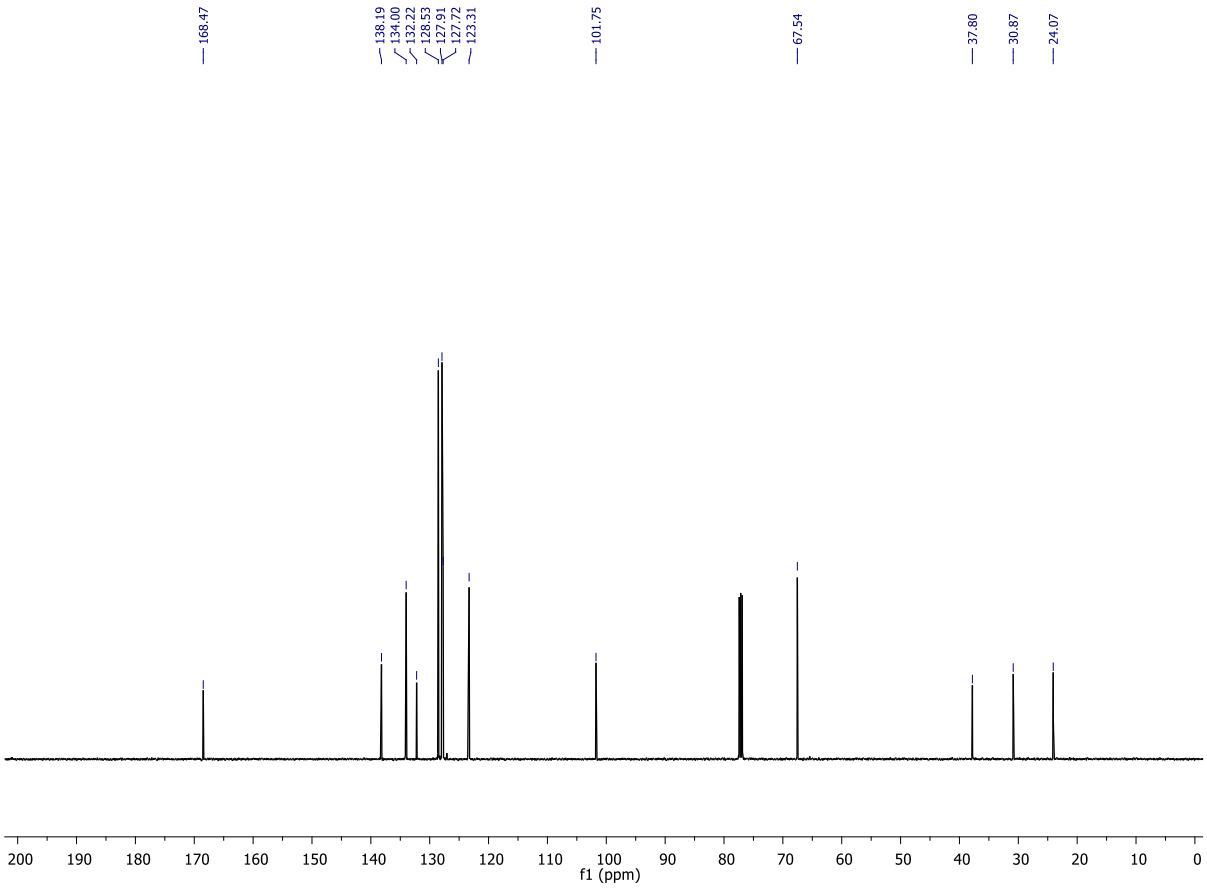
7.84
7.84
7.83
7.72
7.71
7.71
7.70
7.34
7.30
7.29
7.29
7.28
7.27
4.79
4.78
4.67
4.64
4.57
4.55
3.73
3.72
3.70
1.82
1.81
1.81
O
N
O
O
O
11a
2.00
2.04
8.06
2.03
0.97
2.03
2.03
2.03
4.13
10.0 9.5 9.0 8.5 8.0 7.5 7.0 6.5 6.0 5.5 5.0 4.5 4.0 3.5 3.0 2.5 2.0 1.5 1.0 0.5 0.0
f1 (ppm)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127.91
127.72
123.31
101.75
67.54
37.80
30.87
24.07
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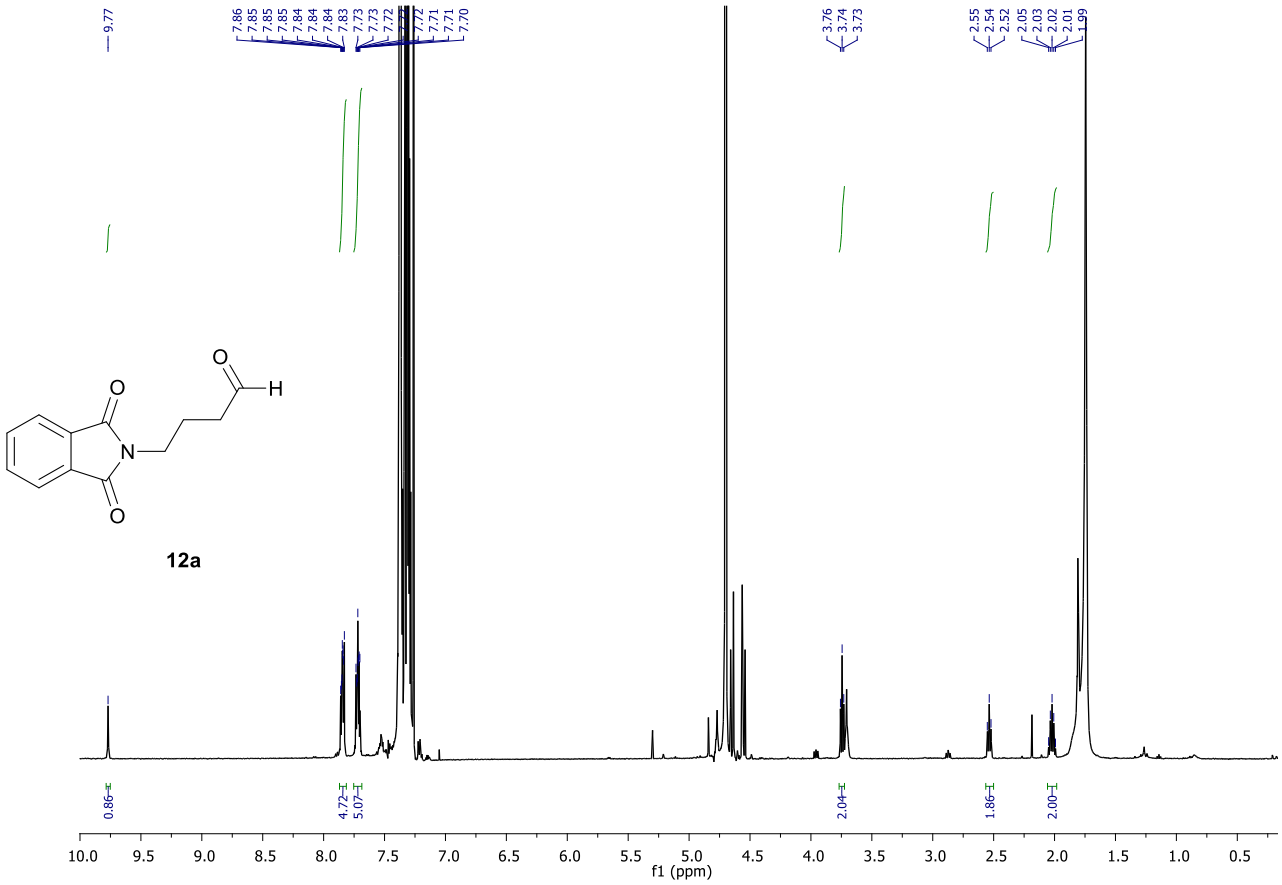
f1 (ppm)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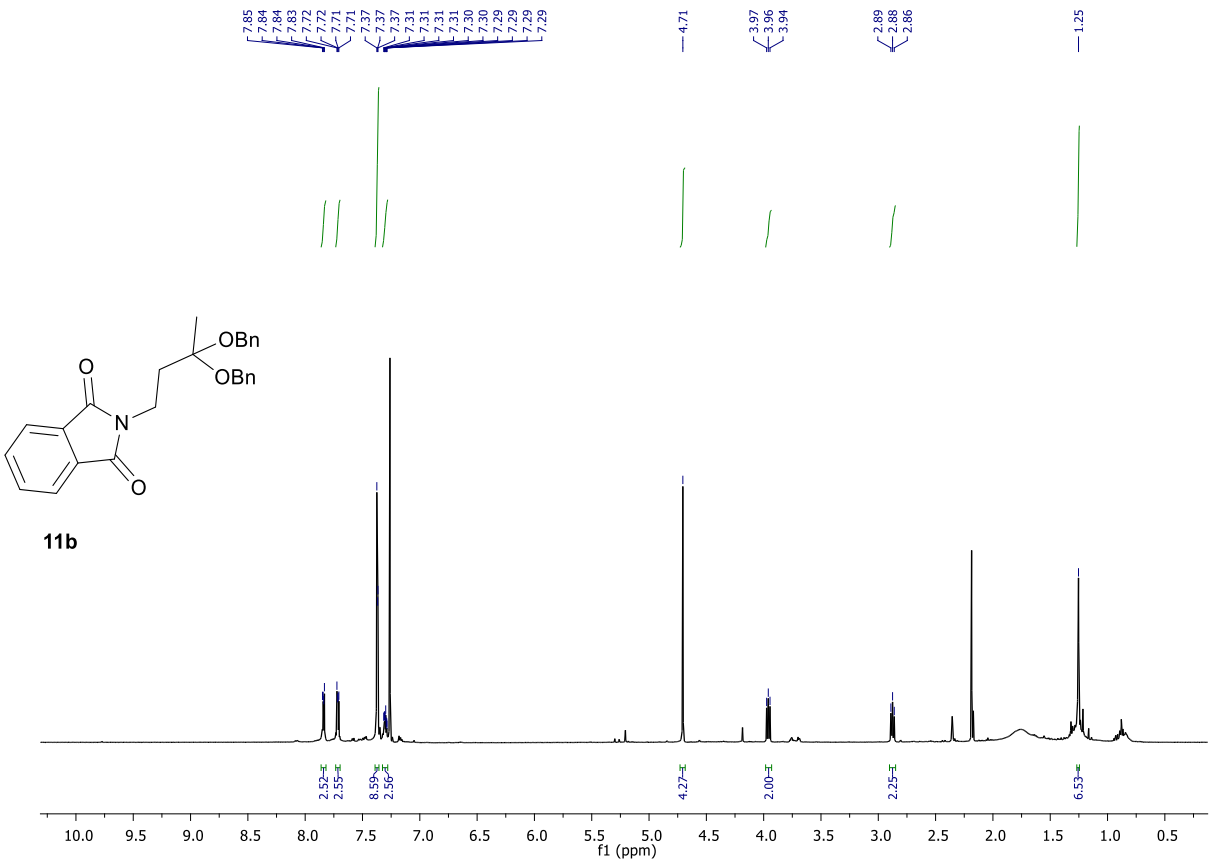
7.84
7.84
7.84
7.83
7.73
7.73
7.72
7.72
7.71
7.71
7.70
3.76
3.74
3.73
2.55
2.54
2.52
2.05
2.03
2.02
2.01
1.99
O
O
H
N
O
O
12a
0.86
4.72
5.07
2.04
1.86
2.00
10.0 9.5 9.0 8.5 8.0 7.5 7.0 6.5 6.0 5.5 5.0 4.5 4.0 3.5 3.0 2.5 2.0 1.5 1.0 0.5
f1 (ppm)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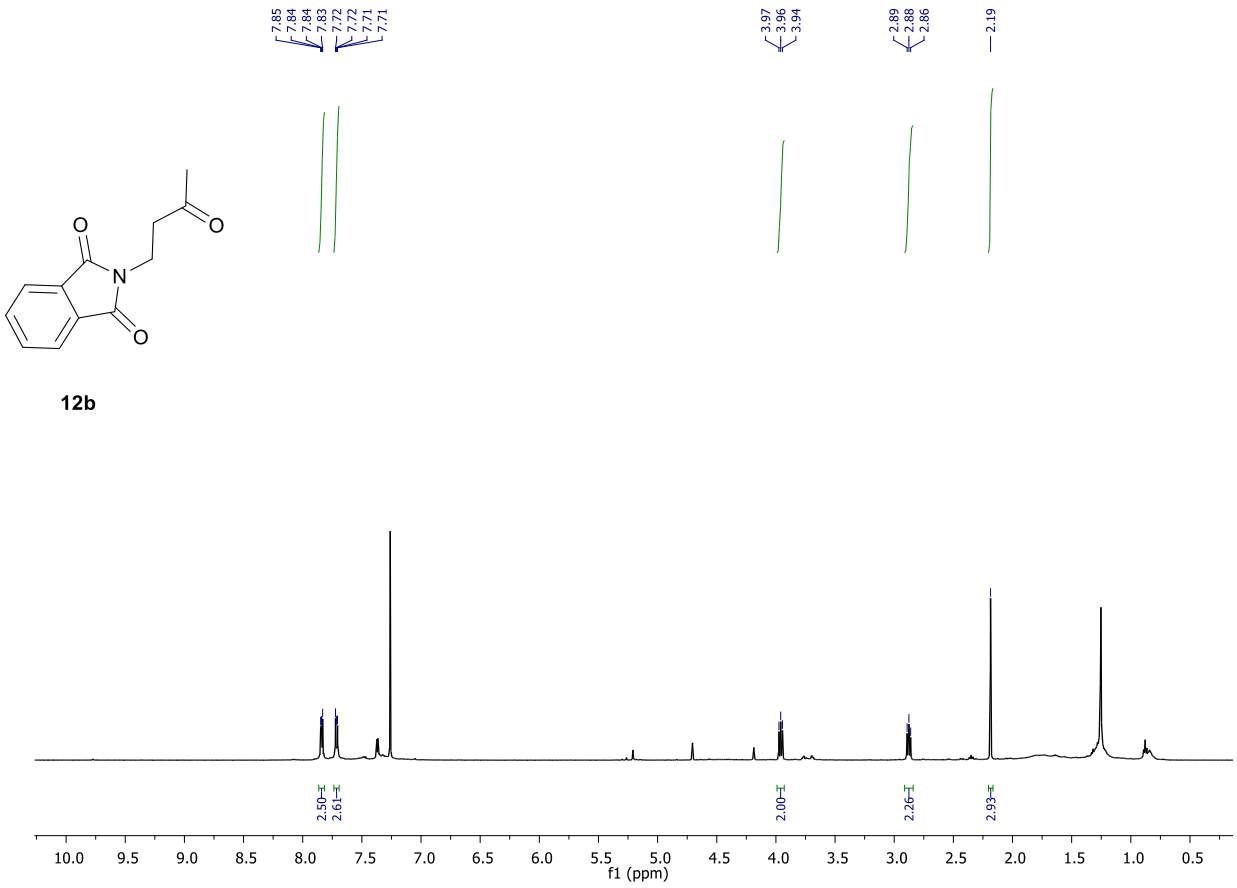
7.84
7.84
7.83
7.72
7.72
7.71
7.71
7.37
7.37
7.37
7.31
7.31
7.31
7.30
7.29
7.29
7.29
7.29
4.71
3.97
3.96
3.94
2.89
2.88
2.86
1.25
OBn
OBn
O
N
O
11b
2.52
2.55
8.59
2.56
4.27
2.00
2.25
6.53
10.0 9.5 9.0 8.5 8.0 7.5 7.0 6.5 6.0 5.5 5.0 4.5 4.0 3.5 3.0 2.5 2.0 1.5 1.0 0.5
f1 (ppm)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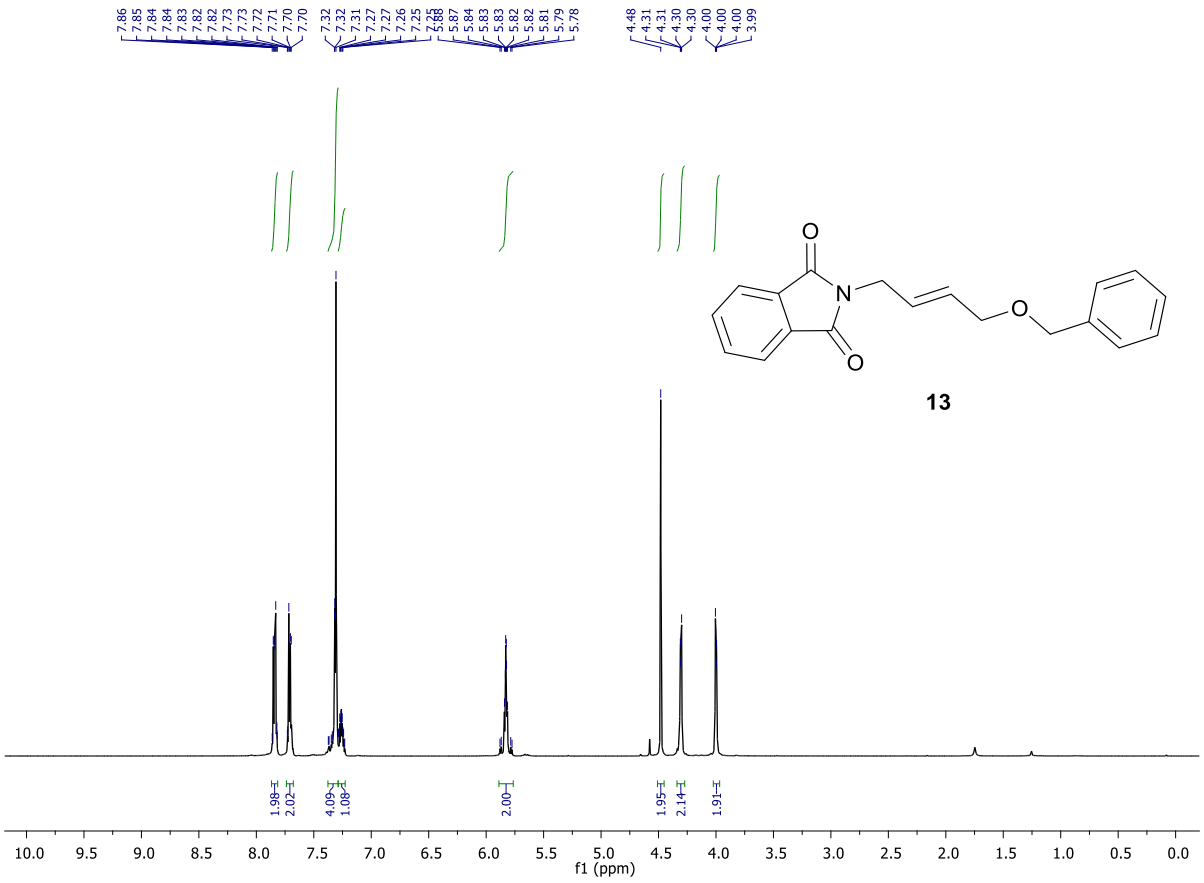
7.84
7.84
7.83
7.72
7.72
7.71
7.71
3.97
3.96
3.94
2.89
2.88
2.86
2.19
O
O
N
O
2.50
2.61
2.00
2.26
2.93
10.0
9.5
9.0
8.5
8.0
7.5
7.0
6.5
6.0
5.5
5.0
4.5
4.0
3.5
3.0
2.5
2.0
1.5
1.0
0.5
f1 (ppm)

12b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7.84
7.84
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7.82
7.73
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7.72
7.71
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7.32
7.32
7.31
7.27
7.27
7.26
7.25
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5.84
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5.83
5.82
5.82
5.81
5.79
5.78
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4.00
3.99
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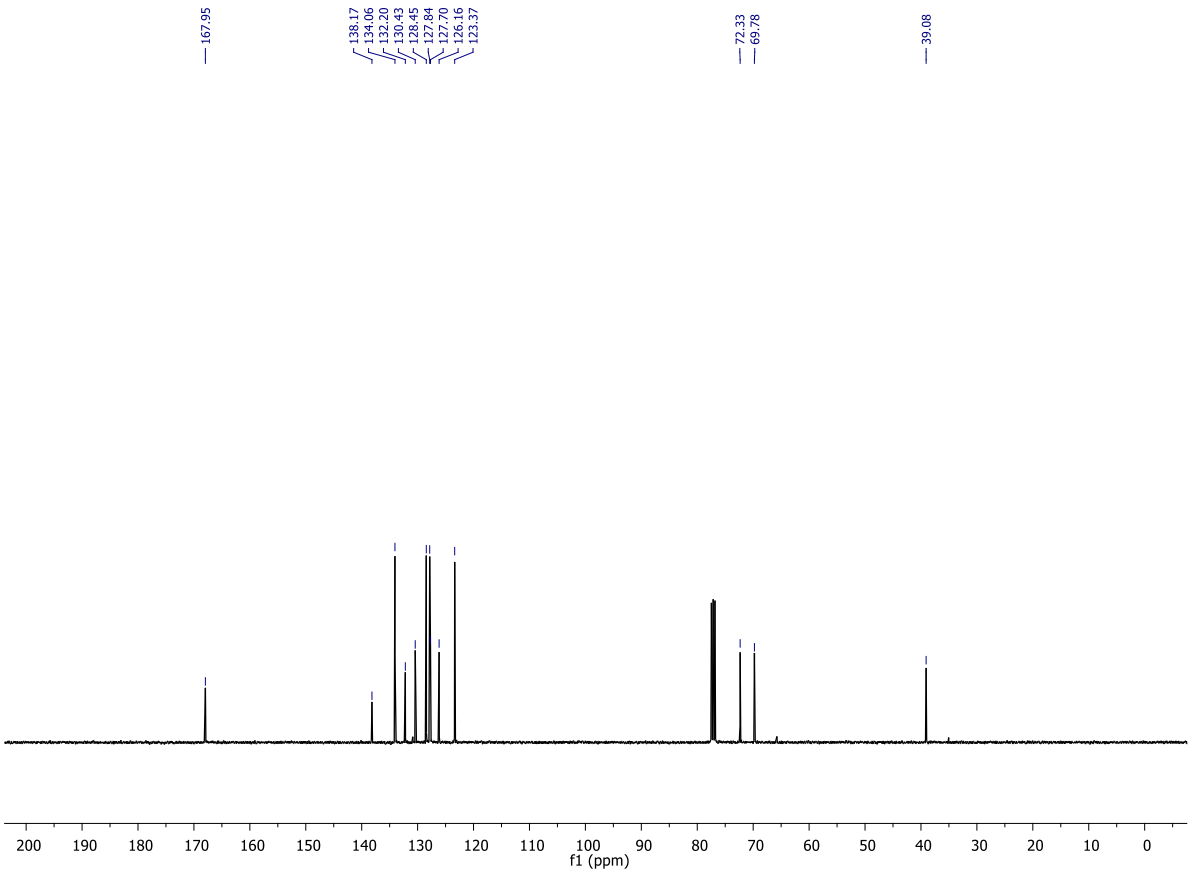
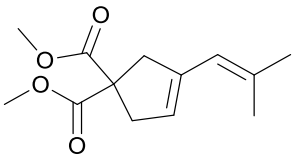
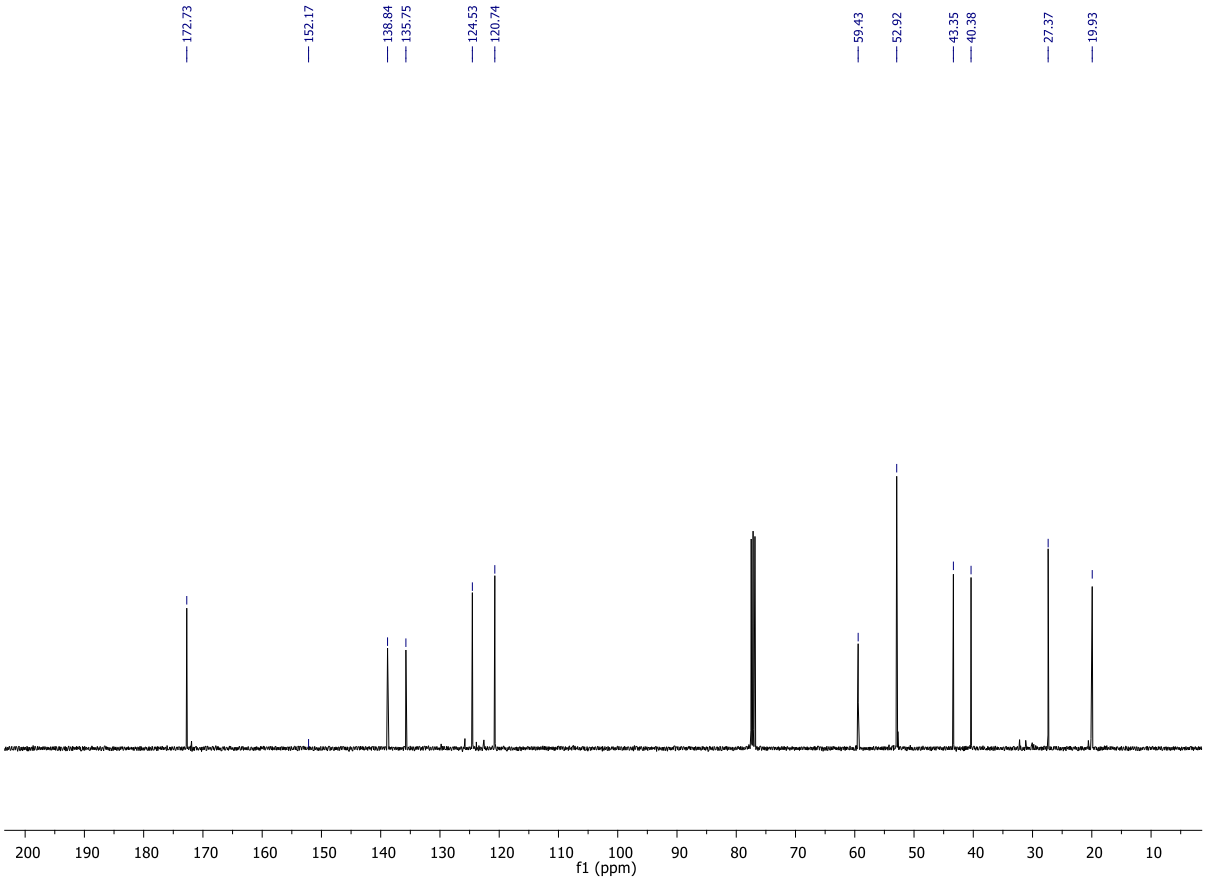
O
O
13
1.98
2.02
4.09
1.08
2.00
1.95
2.14
1.91
10.0 9.5 9.0 8.5 8.0 7.5 7.0 6.5 6.0 5.5 5.0 4.5 4.0 3.5 3.0 2.5 2.0 1.5 1.0 0.5 0.0
f1 (ppm)

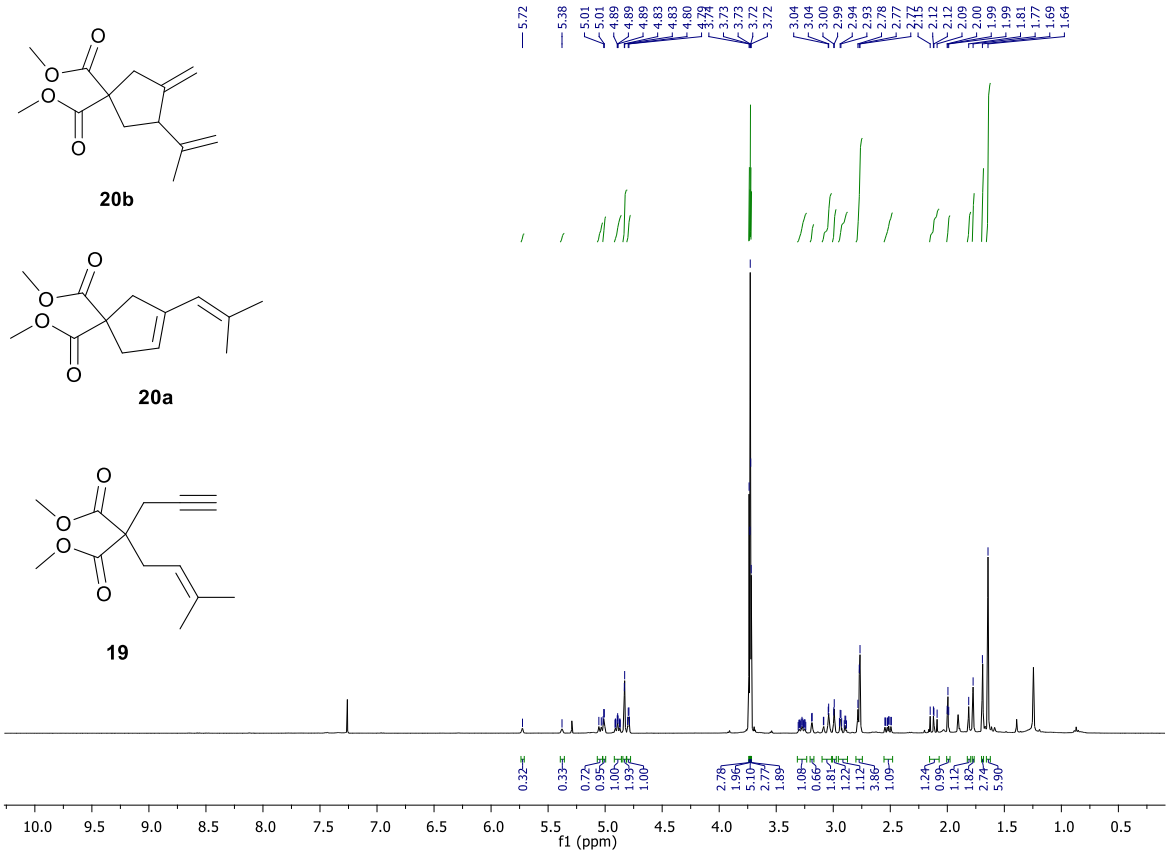

167.95
138.17
134.06
132.20
130.43
128.45
127.84
127.70
126.16
123.37
72.33
69.78
39.08
200 190 180 170 160 150 140 130 120 110 100 90 80 70 60 50 40 30 20 10 0
f1 (ppm)

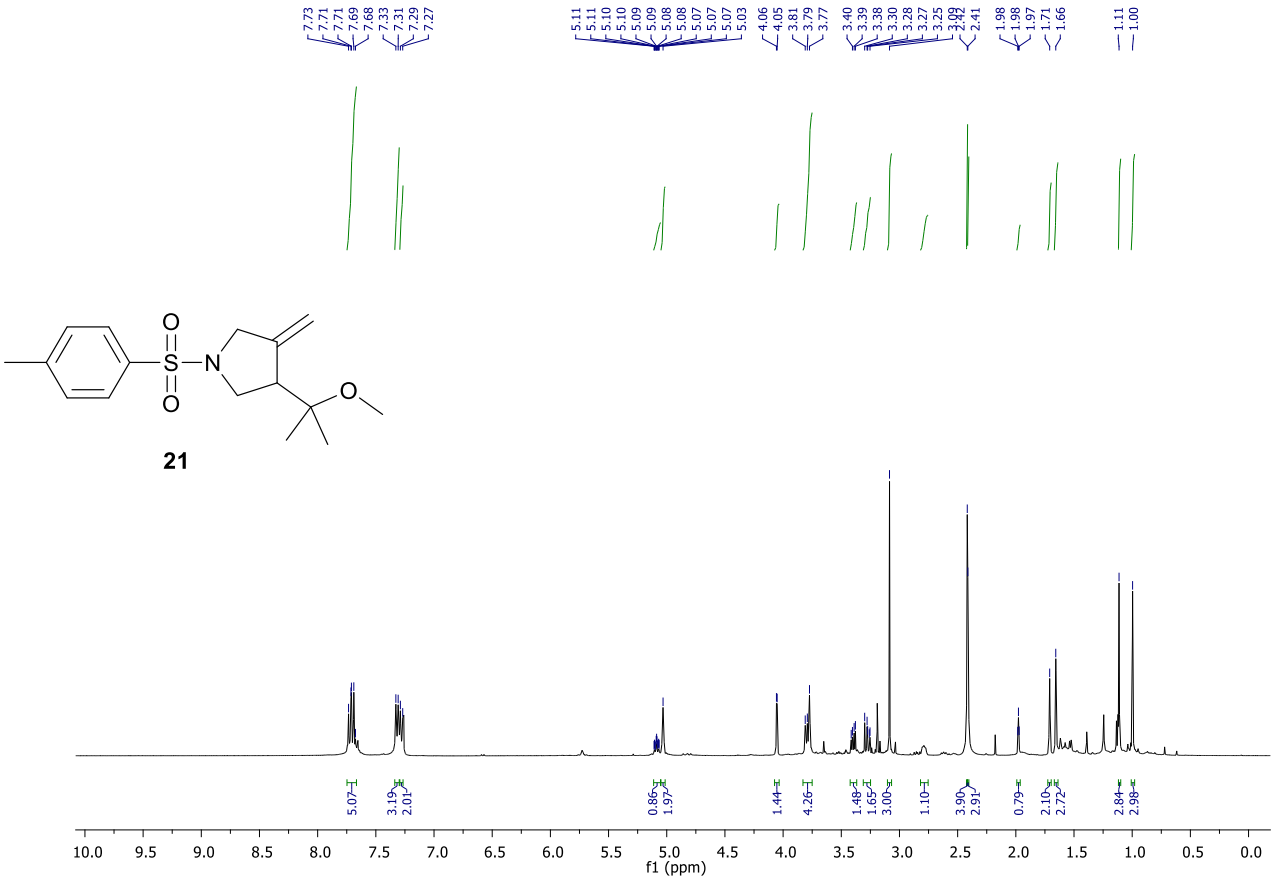


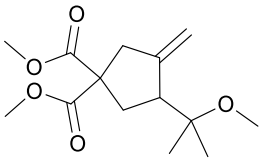
20a

5.72
5.37
3.72
3.18
3.18
3.03
1.81
1.77
0.99
1.00
6.09
2.03
2.02
2.90
3.42
f1 (ppm)


172.73
152.17
138.84
135.75
124.53
120.74
59.43
52.92
43.35
40.38
27.37
19.93
f1 (ppm)

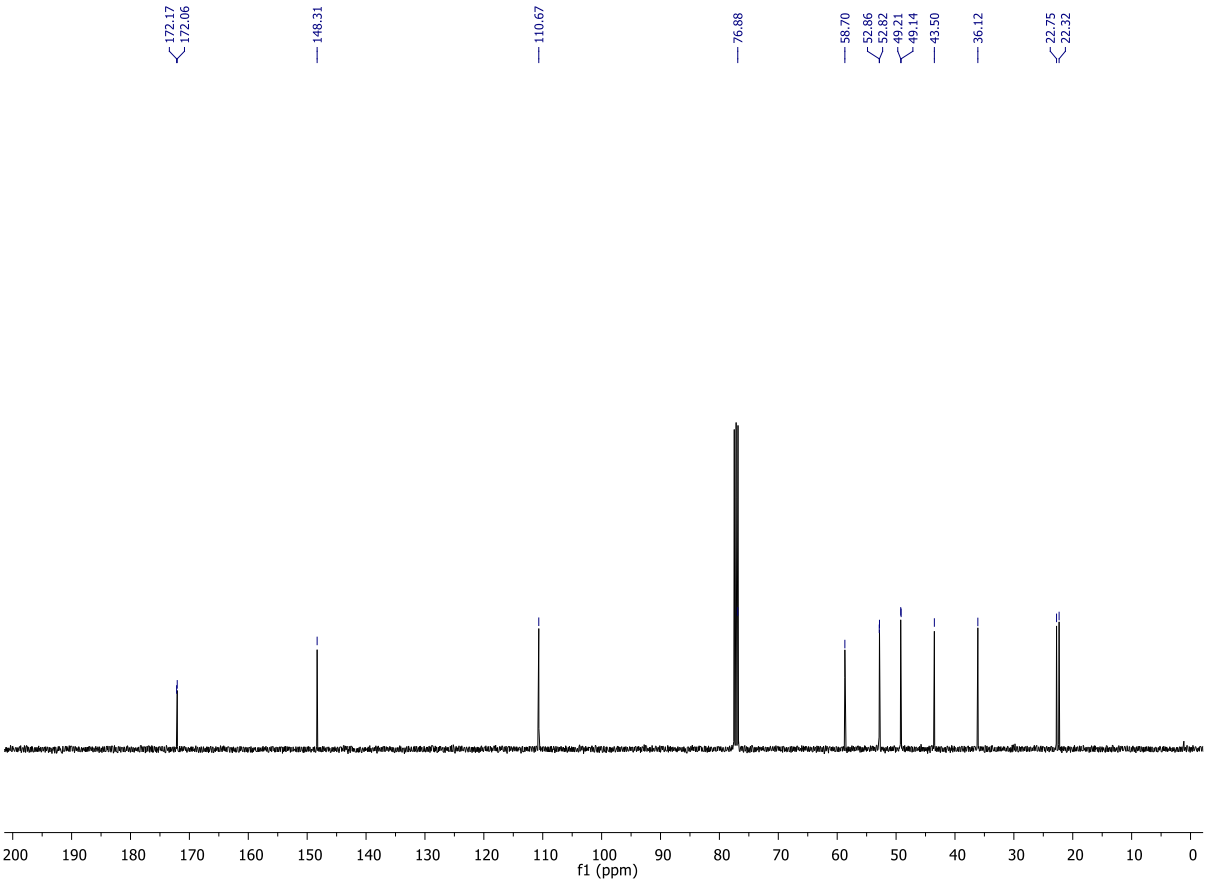

20b
20a
19
f1 (ppm)


21
f1 (ppm)



22

5.03
5.03
5.03
5.02
4.97
4.97
4.96
4.96
4.96
3.72
3.71
3.18
2.89
2.88
2.85
2.85
2.84
2.83
2.81
2.81
2.00
1.99
1.97
1.17
1.11
0.99
0.99
2.81
3.08
3.04
3.09
1.04
1.07
3.03
3.17
10.0 9.5 9.0 8.5 8.0 7.5 7.0 6.5 6.0 5.5 5.0 4.5 4.0 3.5 3.0 2.5 2.0 1.5 1.0 0.5 0.0
f1 (ppm)


172.17
172.06
148.31
110.67
76.88
58.70
52.86
52.82
49.21
49.14
43.50
36.12
22.75
22.32
200 190 180 170 160 150 140 130 120 110 100 90 80 70 60 50 40 30 20 10 0
f1 (ppm)

4. X-ray structure determination

Crystal of **2b** was mounted on a small loop and fixed in the cold nitrogen stream on the Rigaku Oxford Diffraction XtaLAB Synergy diffractometer, equipped with Mo-Kα radiation, HyPix detector and a mirror monochromator. Intensity data were measured by thin-slice ω-scans. Data was processed using the CrysAlisPro-CCD and -RED programs.[23] The structure was determined by the intrinsic phasing routines in the SHELXT program and refined by full-matrix least-squares methods, on F^2's, in SHELXL.[24,25] The non-hydrogen atoms were refined with anisotropic thermal parameters. Hydrogen atoms were included in idealised positions and their Uiso values were set to ride on the Ueq values of the parent carbon atoms. In the final difference map the highest peaks were near the Pd atom.

CCDC deposition number: 2094617

Empirical formula	$C_{25}H_{26}Cl_2N_2Pd$
Formula weight	531.78
Temperature/K	100.00(10)
Crystal system	Monoclinic
Space group	P 2_1/c
a [Å]	9.3560(3)
b [Å]	14.1423(4)
c [Å]	17.5530(5)
α [°]	90
β [°]	103.017(3)
γ [°]	90
Volume [Å^3]	2262.85(12)
Z	4
ρcalc [g/cm^3]	1.561
μ [mm^{-1}]	1.071
F(000)	1080.0
Crystal size [mm]	0.332 × 0.286 × 0.019
Radiation	Mo Kα (λ = 0.71073)
2Θ range for data collection [°]	4.468 to 61.994
Index ranges	-11 ≤ h ≤ 12, -19 ≤ k ≤ 17, -22 ≤ l ≤ 21
Reflections collected	26885
Independent reflections	5767 [Rint = 0.0316, Rsigma = 0.0217]
Data/restraints/parameters	5767/0/276
Goodness-of-fit on F^2	1.063

Final R indexes [I>=2σ (I)]	R_1 = 0.0240, wR_2 = 0.0567
Final R indexes [all data]	R_1 = 0.0262, wR_2 = 0.0574
Largest diff. peak/hole [e Å^{-3}]	0.65/-0.71

Table 4S. Fractional atomic coordinates (×10^4) and equivalent isotropic displacement parameters (Å^2×10^3). Ueq is defined as 1/3 of the trace of the orthogonalised Uij tensor.

Atom	*x*	*y*	*z*	U(eq)
Pd01	2358.9(2)	2603.0(2)	4526.2(2)	10.33(4)
Cl02	-9.8(5)	2741.9(3)	4728.0(2)	14.91(8)
Cl03	4543.6(5)	2330.6(3)	4146.6(3)	17.11(9)
N004	2503.2(16)	1287.3(10)	4995.4(8)	12.4(3)
N005	2634.6(16)	4035.3(10)	4458.0(8)	12.1(3)
C006	2968.6(18)	4450.7(12)	5180.5(10)	12.1(3)
C007	3497.5(18)	2986.8(12)	5923.6(9)	12.2(3)
C008	3413.6(19)	1259.0(12)	5718.1(10)	12.4(3)
C009	4121.9(19)	2148.2(12)	6072.3(9)	12.4(3)
C00A	2930.1(18)	3846.3(11)	5876.9(9)	11.4(3)
C00B	3024(2)	5501.5(13)	3864.6(11)	17.2(3)
C00C	5614.3(19)	2090.6(12)	6610.4(10)	12.7(3)
C00D	8228(2)	1834.1(13)	6807.1(11)	17.8(4)
C00E	1779(2)	503.9(12)	4673.9(10)	15.3(3)
C00F	2171.1(19)	4197.5(12)	6522.4(10)	12.9(3)
C00G	6826(2)	1816.6(12)	6321.6(10)	15.6(3)
C00H	3339.1(19)	5404.0(12)	5251.0(10)	14.6(3)
C00I	3362(2)	5933.6(13)	4587.9(11)	18.2(4)
C00J	7212(2)	2335.1(13)	7882.7(11)	18.2(4)
C00K	5813(2)	2348.2(12)	7397.0(10)	15.4(3)
C00L	2671.2(19)	4542.2(12)	3810.0(10)	14.0(3)
C00M	3314(2)	4695.5(13)	7169.9(10)	16.9(3)
C00N	8415(2)	2093.6(13)	7588.7(12)	19.9(4)
C00O	1545(2)	3338.5(13)	6877.0(11)	18.4(4)
C00P	3669(2)	409.6(12)	6126.1(11)	16.3(3)
C00Q	891(2)	4866.2(13)	6186.1(11)	18.3(4)
C00R	1964(2)	-344.5(13)	5080.2(11)	17.7(3)
C00S	2329(2)	4041.2(14)	3036.2(10)	19.2(4)
C00T	2925(2)	-400.8(12)	5802.4(11)	18.8(4)
C00U	835(2)	600.6(14)	3865.5(11)	20.6(4)

Table 5S. Anisotropic displacement parameters (Å^2×10^3). The anisotropic displacement factor exponent takes the form: -2π2[h2a*2U11+2hka*b*U12+…].

Atom	U_{11}	U_{22}	U_{33}	U_{23}	U_{13}	U_{12}
Pd01	12.37(7)	9.59(7)	8.66(7)	0.63(4)	1.57(5)	0.16(4)
Cl02	12.63(18)	15.2(2)	15.98(19)	0.16(13)	1.21(15)	-0.18(13)
Cl03	18.0(2)	19.1(2)	15.9(2)	0.99(14)	7.31(16)	2.91(15)
N004	14.9(7)	10.7(7)	11.7(7)	0.0(5)	3.5(6)	0.8(5)
N005	11.5(7)	11.5(7)	13.0(7)	1.9(5)	2.3(5)	0.1(5)
C006	9.6(7)	12.4(8)	13.9(8)	1.7(6)	1.5(6)	1.0(5)
C007	12.3(8)	16.0(8)	7.9(7)	-0.1(6)	1.7(6)	-1.3(6)
C008	12.7(8)	12.7(8)	12.6(8)	0.6(6)	4.2(6)	1.4(6)
C009	15.2(8)	12.8(8)	9.0(7)	0.5(5)	2.3(6)	0.6(6)
C00A	11.6(8)	11.1(8)	10.4(7)	-0.3(5)	0.1(6)	-0.9(5)
C00B	15.3(8)	16.9(9)	19.4(9)	7.7(6)	4.0(7)	-0.3(6)
C00C	14.7(8)	9.6(7)	13.1(8)	2.7(6)	1.3(6)	0.9(6)
C00D	13.9(8)	15.9(9)	24.2(9)	-0.3(6)	5.5(7)	0.8(6)

C00E	16.6(8)	13.4(8)	16.6(8)	-3.6(6)	5.5(7)	-0.1(6)
C00F	13.8(8)	13.0(8)	11.4(8)	-0.9(6)	1.9(6)	0.2(6)
C00G	19.4(9)	13.9(8)	13.6(8)	1.2(6)	3.9(7)	2.1(6)
C00H	14.7(8)	12.1(8)	16.3(8)	0.8(6)	1.7(7)	-0.3(6)
C00I	17.9(9)	11.2(8)	24.7(9)	5.2(6)	3.1(7)	-1.2(6)
C00J	22.3(9)	15.3(9)	14.3(8)	-1.2(6)	-1.7(7)	1.7(6)
C00K	17.7(8)	14.0(8)	14.5(8)	0.5(6)	3.5(7)	3.5(6)
C00L	12.3(8)	15.7(8)	13.6(8)	3.6(6)	2.4(6)	2.1(6)
C00M	20.0(9)	17.2(9)	12.0(8)	-3.7(6)	0.8(7)	-0.1(6)
C00N	15.7(9)	16.2(9)	24.3(9)	0.6(7)	-3.2(7)	0.1(6)
C00O	22.3(9)	19.1(9)	15.5(8)	-0.1(6)	8.1(7)	-3.2(7)
C00P	18.6(9)	13.9(8)	16.0(8)	3.5(6)	3.2(7)	3.0(6)
C00Q	16.1(8)	20.0(9)	18.7(9)	-0.2(6)	4.1(7)	5.1(6)
C00R	20.3(9)	12.3(8)	22.3(9)	-3.3(6)	8.6(7)	-1.3(6)
C00S	22.8(9)	21.7(9)	12.7(8)	3.6(6)	3.4(7)	0.6(7)
C00T	24.9(9)	9.7(8)	23.3(9)	3.2(6)	8.3(8)	1.8(6)
C00U	26.4(10)	17.9(9)	16.0(9)	-3.7(6)	1.6(7)	-3.1(7)

Table 6S. Bond lengths.

Atom	Atom	Length/Å	Atom	Atom	Length/Å
Pd01	Cl02	2.3300(4)	C00B	C00I	1.380(3)
Pd01	Cl03	2.3203(5)	C00B	C00L	1.395(2)
Pd01	N004	2.0269(14)	C00C	C00G	1.397(3)
Pd01	N005	2.0489(14)	C00C	C00K	1.399(2)
Pd01	C007	2.5038(16)	C00D	C00G	1.394(2)
N004	C008	1.360(2)	C00D	C00N	1.393(3)
N004	C00E	1.354(2)	C00E	C00R	1.387(3)
N005	C006	1.368(2)	C00E	C00U	1.499(2)
N005	C00L	1.351(2)	C00F	C00M	1.544(2)
C006	C00A	1.499(2)	C00F	C00O	1.540(2)
C006	C00H	1.391(2)	C00F	C00Q	1.536(2)
C007	C009	1.322(2)	C00H	C00I	1.388(2)
C007	C00A	1.321(2)	C00J	C00K	1.392(3)
C008	C009	1.490(2)	C00J	C00N	1.383(3)
C008	C00P	1.391(2)	C00L	C00S	1.501(2)
C009	C00C	1.501(2)	C00P	C00T	1.394(3)
C00A	C00F	1.548(2)	C00R	C00T	1.382(3)

Table 7S. Bond angles.

Atom	Atom	Atom	Angle/˚	Atom	Atom	Atom	Angle/˚
Cl02	Pd01	C007	92.41(4)	C007	C00A	C006	119.48(16)
Cl03	Pd01	Cl02	170.825(16)	C007	C00A	C00F	119.58(15)
Cl03	Pd01	C007	96.28(4)	C00I	C00B	C00L	119.70(17)
N004	Pd01	Cl02	89.67(4)	C00G	C00C	C009	120.17(15)
N004	Pd01	Cl03	88.97(4)	C00G	C00C	C00K	119.47(16)
N004	Pd01	N005	159.00(6)	C00K	C00C	C009	120.31(16)
N004	Pd01	C007	80.00(6)	C00N	C00D	C00G	119.95(18)
N005	Pd01	Cl02	93.56(4)	N004	C00E	C00R	120.07(16)
N005	Pd01	Cl03	90.96(4)	N004	C00E	C00U	116.92(16)
N005	Pd01	C007	79.13(6)	C00R	C00E	C00U	122.98(16)
C008	N004	Pd01	112.11(11)	C00M	C00F	C00A	109.21(14)
C00E	N004	Pd01	126.91(12)	C00O	C00F	C00A	108.78(14)
C00E	N004	C008	120.95(15)	C00O	C00F	C00M	109.02(14)
C006	N005	Pd01	112.07(11)	C00Q	C00F	C00A	111.28(14)
C00L	N005	Pd01	126.94(12)	C00Q	C00F	C00M	110.67(14)
C00L	N005	C006	120.56(15)	C00Q	C00F	C00O	107.83(15)
N005	C006	C00A	117.94(14)	C00D	C00G	C00C	120.13(17)

N005	C006	C00H	119.93(16)	C00I	C00H	C006	119.94(17)
C00H	C006	C00A	122.12(15)	C00B	C00I	C00H	119.27(16)
C009	C007	Pd01	94.04(11)	C00N	C00J	C00K	120.46(17)
C00A	C007	Pd01	93.57(11)	C00J	C00K	C00C	119.91(17)
C00A	C007	C009	172.31(17)	N005	C00L	C00B	120.59(16)
N004	C008	C009	119.37(14)	N005	C00L	C00S	118.07(15)
N004	C008	C00P	120.28(16)	C00B	C00L	C00S	121.35(16)
C00P	C008	C009	120.34(15)	C00J	C00N	C00D	120.02(17)
C007	C009	C008	122.65(15)	C008	C00P	C00T	119.22(16)
C007	C009	C00C	118.68(15)	C00T	C00R	C00E	120.12(17)
C008	C009	C00C	118.67(14)	C00R	C00T	C00P	119.25(16)
C006	C00A	C00F	120.87(14)				

Table 8S. Hydrogen atom coordinates (Å×10^4) and isotropic displacement parameters (Å^2×10^3).

Atom	*x*	*y*	*z*	U(eq)
H00B	3032.31	5848.6	3415.52	21
H00D	9037.56	1672.53	6609.24	21
H00G	6697.87	1622.29	5804.45	19
H00H	3571.38	5686.57	5741.83	18
H00I	3602.24	6572.46	4630.73	22
H00J	7337.62	2489.8	8408.59	22
H00K	5011.56	2527.94	7594.9	19
H00A	4083.98	4259.16	7388.06	25
H00C	2849.38	4910.6	7573.14	25
H00E	3720.83	5226.42	6951.07	25
H00N	9349.93	2104.47	7913.09	24
H00F	884.63	2999.54	6470.64	28
H00L	1026.08	3551.19	7258.79	28
H00M	2333.12	2929.31	7122.66	28
H00P	4327.58	383	6609.17	20
H00O	1259.76	5423.47	5983.72	27
H00Q	403.28	5041.04	6591.44	27
H00R	209.1	4552.2	5772.92	27
H00S	1439.4	-876.55	4866.19	21
H00T	3190.96	3719.98	2960.98	29
H00U	2018.28	4493.54	2624.42	29
H00V	1560.01	3589.59	3028.28	29
H00W	3074.64	-973.12	6069.52	23
H00X	168.86	1119.46	3852.38	31
H00Y	289.05	27.91	3724.31	31
H	1444.3	716.17	3502.17	31

5. Stability in solution

Samples of **3b** (0.4 mL, 0.036 mmol/mL) and **3a** (0.4 mL, 0.035 mmol/mL) in DMSO-d_6 were submitted to a series of ^{1}H NMR experiments at 2 h intervals for 48 h, then were collected once every 2-3 days for 13 days since the initial sample preparation.

The spectrum of the **3b** remained unchanged in the 13 day period (Figure 3S and 4S). There were no changes in the intensity of signals corresponding to the phenyl group (7.32-7.48 ppm) or the broadened pyridyl peaks (7.55-8.40 ppm). No new signals were observed.

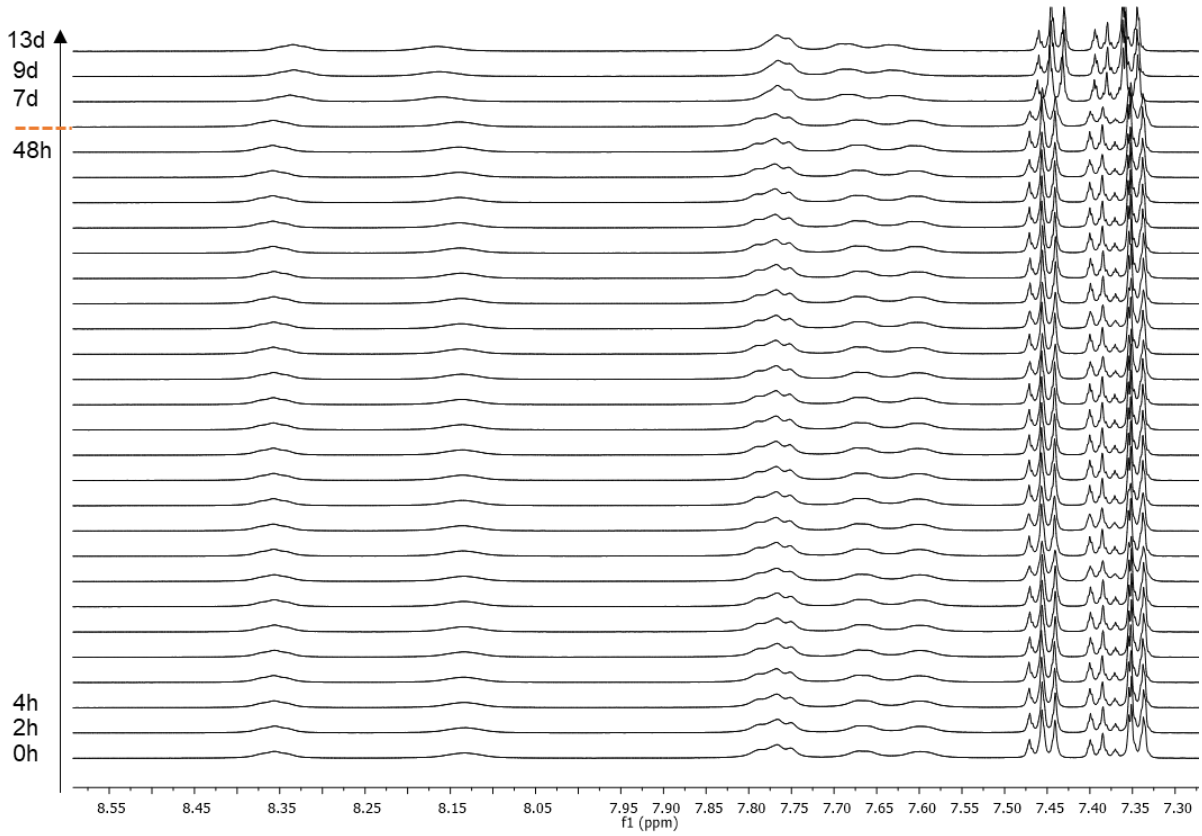


Figure 3S. Aromatic region of the ^{1}H NMR spectrum of **3b** recorded in the space of 13 days.

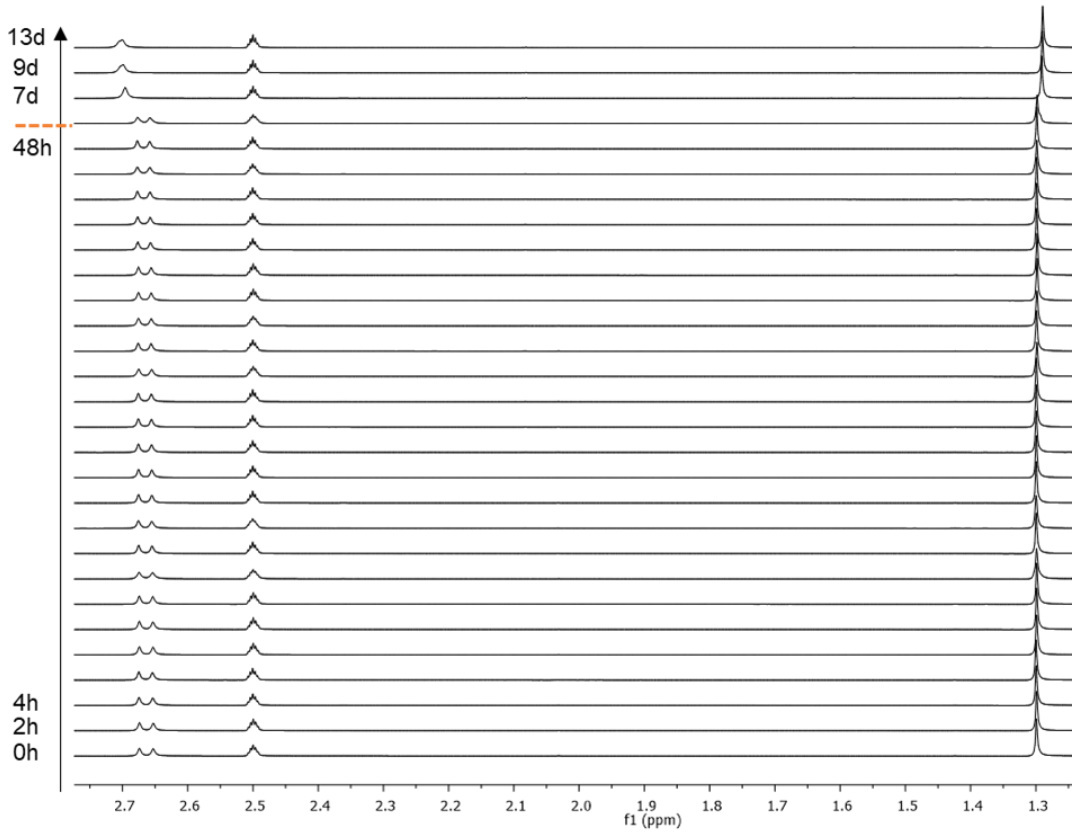


Figure 4S. Aliphatic region of the ^{1}H NMR spectrum of **3b** recorded in the space of 13 days.

Pt(IV)-Complex **3a** started to decompose/react in solution after a few hours from the preparation of the NMR sample (Figure 5S). At least two new unidentified species (**S1** and **S2**) started to appear on the spectrum after 6-8 h. Based on the integration of the triplet peaks corresponding to **3a**, **S1** and **S2** at 8.22 (corresponding to the pyridyl proton *meta* to the methyl group), 8.30 and 8.42 ppm, respectively, we could estimate the approximate share of **3a** in the mixture at different time intervals. Starting from 100% at the beginning, this share decreased to 67% after 12 h, through 54% after 24 h and 38% after 48 h. After 7 days the mixture did not contain **3a** at all.

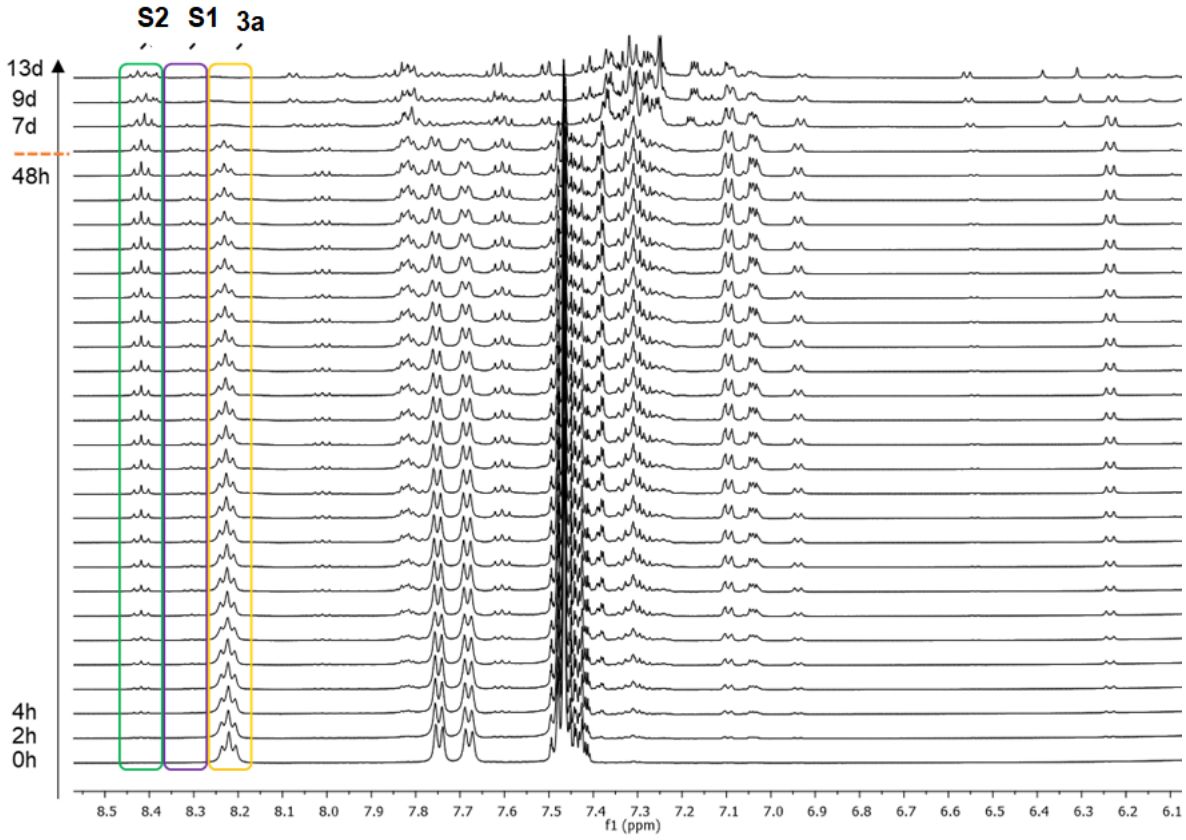


Figure 5S. Aromatic region of the ^{1}H NMR spectrum of **3a** recorded in the space of 13 days.

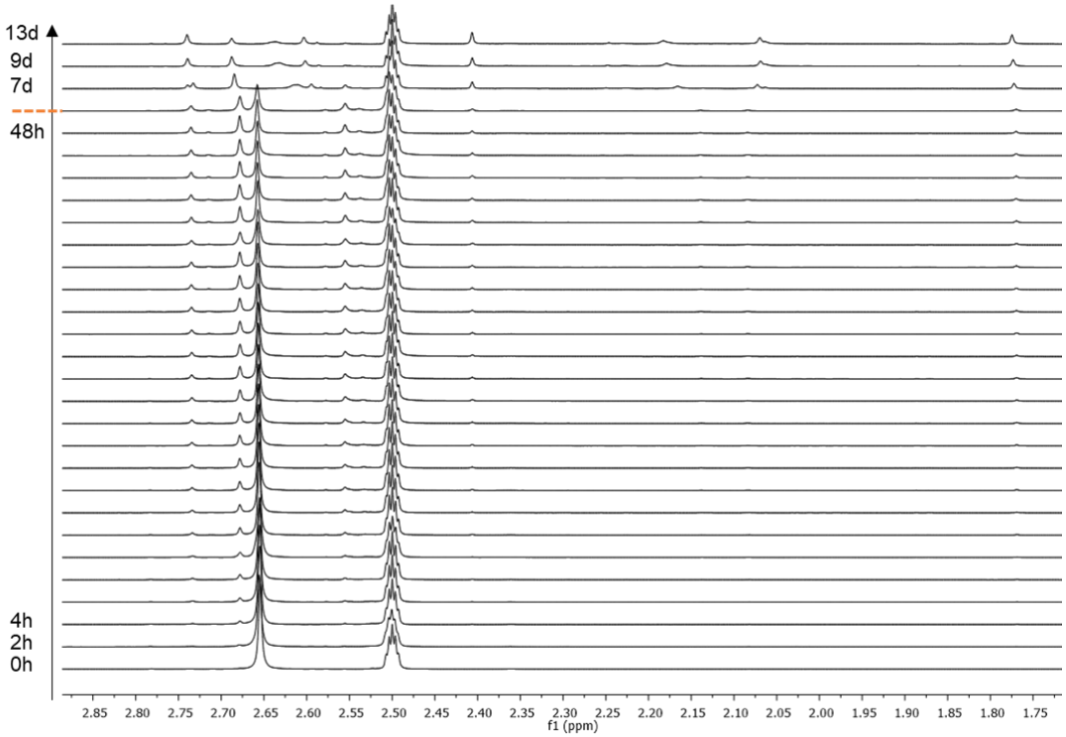


Figure 6S. Aliphatic region of the ^{1}H NMR spectrum of **3a** recorded in the space of 13 days.

6. Antimicrobial studies

Procedures from CO-ADD are presented as received. The results for ligands **1a**-**b** and complexes **2**-**4** are presented below (Table 9 and Table 10).

Primary Antimicrobial Screening

Bacterial and Fungal

Procedure and Materials

1.0 Summary

1.1 Study

Primary antimicrobial screening study by whole cell growth inhibition assays, using the provided samples at a single concentration, in duplicate (n=2). The inhibition of growth is measured against 5 bacteria: *Escherichia coli*, *Klebsiella pneumoniae, Acinetobacter baumannii, Pseudomonas aeruginosa* and *Staphylococcus aureus*, and 2 fungi: *Candida albicans* and *Cryptococcus neoformans*.

1.2 Assay Parameters

Test concentration		32 µg/mL or 20 µM ≤1% DMSO
QC		Duplicate (n=2) Control MIC: Pass
Plates		Non-Binding Surface, 384 well plate
Media	***Bacteria*** ***Fungi***	Cation-adjusted Mueller Hinton broth Yeast Nitrogen Base
Read Out	***Bacteria*** ***C. albicans*** ***C. neoformans***	OD_{600} OD_{530} Resazurin $OD_{600-570}$

1.3 Outcomes

Primary Screening outcomes are detailed in individual Project reports, personalised for each Project Submission for each CO-ADD user.

Please see your data sheet with file extension **P0XXX_PS_data.xlsx,** for example CO-ADD Project **P0100, P0100_PS_data.xlsx**

1.4 Comments

To confirm the inhibitory activity, the hit compound/s will be re-tested against the strains in a dose response assay to determine the minimum inhibitory concentration (MIC) of

the compounds. Furthermore, to further evaluate the antimicrobial potential of the compounds they will be assayed against a mammalian cell line to determine general cell toxicity.

In order to continue with Hit Confirmation assays, CO-ADD requests (as per the standard T&C's) that chemical structures of the compound/s (both active and inactive) be supplied after receipt of the primary screening report. All structural information will be kept confidential and only used internally by CO-ADD for the purpose of evaluating novelty of the chemistry to choose compounds for further validation. No publication will result without your written consent.

If possible, please provide structures as **smiles, sdf/sd** or **cdx** files. If you do not have this means, images may also be accepted. Once we have received your structures, we will schedule the dose response assay of the active compound.

If you have not already provided structures to CO-ADD for your full compound set, please do so within a reasonable timeframe after receiving this report, so as not to delay Hit Confirmation.

1.5 Publishing CO-ADD data

If you wish to publish data provided by CO-ADD, we kindly ask that you acknowledge CO-ADD appropriately with the following reference:

Helping Chemists Discover New Antibiotics

M.A. Blaskovich, J. Zuegg, A.G. Elliott, M.A. Cooper
ACS Infect. Dis., **2015**, 1(7), 285-287.

DOI: 10.1021/acsinfecdis.5b00044; PMID: 27622818

as well as an acknowledgement for the funding of CO-ADD:

"The antimicrobial screening performed by CO-ADD (The Community for Antimicrobial Drug Discovery) was funded by the Wellcome Trust (UK) and The University of Queensland (Australia)."

Please advise CO-ADD at your earliest convenience that you have used provided data for publication purposes. This information is extremely helpful in keeping track of the outputs from the CO-ADD initiative and supports the program in renewed funding

possibilities to continue CO-ADD as a free screening service available to the academic community.

CO-ADD also asks, that where possible you publish your data in an Open Access journals.

2.0 Methods

2.1 Sample preparation

Samples were provided by the collaborator and stored frozen at -20 □C. Samples were prepared in DMSO and water to a final testing concentration of 32 µg/mL or 20 µM (unless otherwise indicated in the data sheet), in 384-well, non-binding surface plate (NBS) for each bacterial/fungal strain, and in duplicate (n=2), and keeping the final DMSO concentration to a maximum of 1% DMSO. All the sample-preparation where done using liquid handling robots.

Compounds that showed solubility issues during stock solution preparation are detailed in the data sheet.

2.2 Antimicrobial Assay

2.2.1 Procedure

All bacteria were cultured in Cation-adjusted Mueller Hinton broth (**CAMHB**) at 37 □C overnight. A sample of each culture was then diluted 40-fold in fresh broth and incubated at 37 □C for 1.5-3 h. The resultant mid-log phase cultures were diluted (CFU/mL measured by OD_{600}), then added to each well of the compound containing plates, giving a cell density of 5□10^5 CFU/mL and a total volume of 50 µL. All the plates were covered and incubated at 37 °C for 18 h without shaking.

2.2.2 Analysis

Inhibition of bacterial growth was determined measuring absorbance at 600 nm (OD_{600}), using a Tecan M1000 Pro monochromator plate reader. The percentage of growth inhibition was calculated for each well, using the negative control (media only) and positive control (bacteria without inhibitors) on the same plate as references. The significance of the inhibition values was determined by modified Z-scores, calculated using the median and MAD of the samples (no controls) on the same plate. Samples

with inhibition value above 80% and Z-Score above 2.5 for either replicate (n=2 on different plates) were classed as actives. Samples with inhibition values between 50 - 80% and Z-Score above 2.5 for either replicate (n=2 on different plates) were classed as partial actives. Samples with inhibition values between 50 - 80% and Z-Score above 2.5 for either replicate (n=2 on different plates) were classed as partial actives.

2.3 Antifungal Assay

2.3.1 Procedure

Fungi strains were cultured for 3 days on Yeast Extract-Peptone Dextrose (**YPD**) agar at 30 °C. A yeast suspension of 1 x 10^6 to 5 x 10^6 CFU/mL (as determined by OD_{530}) was prepared from five colonies. The suspension was subsequently diluted and added to each well of the compound-containing plates giving a final cell density of fungi suspension of 2.5 x 10^3 CFU/mL and a total volume of 50 µL. All plates were covered and incubated at 35 □C for 24 h without shaking.

2.3.2 Analysis

Growth inhibition of *C. albicans* was determined measuring absorbance at 530 nm (OD_{530}), while the growth inhibition of *C. neoformans* was determined measuring the difference in absorbance between 600 and 570 nm ($OD_{600-570}$), after the addition of resazurin (0.001% final concentration) and incubation at 35 □C for additional 2 h. The absorbance was measured using a Biotek Synergy HTX plate reader. The percentage of growth inhibition was calculated for each well, using the negative control (media only) and positive control (fungi without inhibitors) on the same plate. The significance of the inhibition values was determined by modified Z-scores, calculated using the median and MAD of the samples (no controls) on the same plate. Samples with inhibition value above 80% and Z-Score above 2.5 for either replicate (n=2 on different plates) were classed as actives. Samples with inhibition values between 50 - 80% and Z-Score above 2.5 for either replicate (n=2 on different plates) were classed as partial actives.

2.4 Antibiotic standards preparation and Quality control

Colistin and Vancomycin were used as positive bacterial inhibitor standards for Gramnegative and Gram-positive bacteria, respectively. Fluconazole was used as a positive fungal inhibitor standard for *C. albicans and C. neoformans.*

The antibiotics were provided in 4 concentrations, with 2 above and 2 below its MIC value, and plated into the first 8 wells of column 23 of the 384-well NBS plates.

The quality control (QC) of the assays was determined by the antimicrobial controls and the Z'-factor (using positive and negative controls). Each plate was deemed to fulfil the quality criteria (pass QC), if the Z'-factor was above 0.4, and the antimicrobial standards showed full range of activity, with full growth inhibition at their highest concentration, and no growth inhibition at their lowest concentration.

3.0 Materials

3.1 Assay materials

Material	*Code*	*Brand*	*Cat No.*
Compound preparation plate [Polypropylene]	PP	Corning	3364
Assay Plates [Non-binding surface]	NBS 384w	Corning	3640
Growth media - bacteria	CAMHB	Bacto Laboratories	212322
Culture agar - fungi	YPD	Becton Dickinson	242720
Growth media - fungi	YNB	Becton Dickinson	233520
Resazurin		Sigma-Aldrich	R7017

3.2 Standards

Sample Name	*Sample ID*	*Full MW*	*Stock Conc. (mg/mL)*	*Solvent*	*Source*
Colistin - Sulfate	MCC_000094:02	1400.63	10.0	DMSO	Sigma; C4461
Vancomycin - HCL	MCC_000095:02	1485.71	10.0	DMSO	Sigma; 861987
Fluconazole	MCC_008383:01	306.27	2.56	DMSO	Sigma; F8929

3.3 Microbial Strains

ID	Batch	*Organism*	*Strain*	*Description*
GN_001	02	*Escherichia coli*	ATCC 25922	FDA control strain
GN_003	02	*Klebsiella pneumoniae*	ATCC 700603	MDR
GN_034	02	*Acinetobacter baumannii*	ATCC 19606	Type strain
GN_042	02	*Pseudomonas aeruginosa*	ATCC 27853	Quality control strain
GP_020	02	*Staphylococcus aureus*	ATCC 43300	MRSA
FG_001	01	*Candida albicans*	ATCC 90028	CLSI reference
FG_002	01	*Cryptococcus neoformans*	ATCC 208821	H99 - Type strain

4.0 Controls

All antibiotic and antifungal controls displayed inhibitory values within the expected range. For further information please contact the CO-ADD team at support@co-add.org.

Strain ID	*Species*	*Antibiotic*	*Pass/Fail*
GN_001:02	*E. coli*	Colistin	Pass
GN_003:02	*K. pneumoniae* (MDR)	Colistin	Pass
GN_034:02	*A. baumannii*	Colistin	Pass
GN_042:02	*P. aeruginosa*	Colistin	Pass
GP_020:02	*S. aureus* (MRSA)	Vancomycin	Pass
FG_001:01	*C. albicans*	Fluconazole	Pass
FG_002:01	*C. neoformans* (H99)	Fluconazole	Pass

4.1 Antimicrobial susceptibility of tested strains

Values are the average of ≥ 6 independent biological replicates. All values are within the expected range as per CLSI guidelines.

4.1.1 Antibiotic standards

MIC determined by BMD method, CA-MHB, Corning 3640 384 NBS plates		**GN_001:02** ***Escherichia coli*** FDA Control ATCC 25922	**GN_003:02** ***Klebsiella pneumophila*** ESBL ATCC 700603	**GN_034:02** ***Acinetobacter baumannii*** Type strain ATCC 19606	**GN_042:02** ***Pseudomonas aeruginosa*** QC strain ATCC 27853
Compound	Compound Type	MIC (µg/mL)			
Colistin - sulfate	Antibiotic	**0.125**	**0.25**	**0.25**	**0.25**

MIC determined by BMD method, CA-MHB, Corning 3640 384 NBS plates		**GP_020:02** ***Staphylococcus aureus*** MRSA ATCC 43300
Compound	Compound Type	MIC (µg/mL)
Vancomycin - HCl	Antibiotic	**1**

4.1.2 Antifungal standard

MIC determined by BMD method, YNB, Corning 3640 384 NBS plates		**FG_001:02** ***Candida albicans*** CLSI reference ATCC 90028	**FG_002:02** ***Cryptococcus neoformans H99*** Type strain ATCC 208821
Compound	Compound Type	MIC (µg/mL)	
Fluconazole	Antifungal	**0.125**	**8**

Hit-Confirmation

Antimicrobial screening, Cytotoxicity & Haemolysis

Procedure and Materials

1.0 Summary

1.1 Study

Hit Confirmation of active compounds by whole cell growth inhibition assays was conducted as an 8-point dose response to determine the Minimum Inhibitory Concentration (MIC), in duplicate (n=2). The inhibition of growth is measured against those microorganisms that showed susceptibility to the compounds tested in the Primary Screen.

Included in the Hit Confirmation were 5 bacteria: *Escherichia coli*, *Klebsiella pneumoniae, Acinetobacter baumannii, Pseudomonas aeruginosa* and *Staphylococcus aureus*, and 2 fungi *Candida albicans* and *Cryptococcus neoformans*.

In addition to determining MIC, active compounds were counter screened for cytotoxicity against a human embryonic kidney cell line, HEK293, by determining their CC_{50} value. The compounds were also screened for haemolysis of human red blood cells.

1.2 Assay Parameters

Assay Parameters	Bacteria	Fungi	HEK293	Haemolysis
Test concentration	32 - 0.25 µg/mL or 20 – 0.15 µM ≤0.5% DMSO	32 - 0.25 µg/mL or 20 – 0.15 µM ≤0.5% DMSO	32 - 0.25 µg/mL or 20 – 0.15 µM ≤0.5% DMSO	32 - 0.25 µg/mL or 20 – 0.15 µM ≤0.5% DMSO
QC	Duplicate (n=2) Control MIC: Pass	Duplicate (n=2) Control MIC: Pass	Duplicate (n=2) Control CC_{50}: Pass	Duplicate (n=2) Control HC_{10}: Pass
Plates	Non-Binding Surface (NBS), 384-well plate	Non-Binding Surface (NBS), 384-well plate	TC, 384-well black wall/clear bottom	Polypropylene 384-well and polystyrene 384well plates
Media	Cation-adjusted Mueller Hinton broth	Yeast Nitrogen Base	DMEM supplemented with 10% FBS	0.9% NaCl
Read Out	OD_{600}	OD_{630} Resazurin $OD_{600-570}$	Resazurin $F_{560/590}$	OD_{405}

1.3 Outcomes

Hit Confirmation outcomes are detailed in individual Project reports, personalised for each Project Submission for each CO-ADD user.

Please see your data sheet with file extension **P0XXX_HC_data.xlsx,** for example CO-ADD Project **P0100, P0100_HC_data.xlsx**

1.4 Structural Novelty

As per the T&C's of CO-ADD, structures for all submitted compounds for antimicrobial screening should be disclosed to CO-ADD following Primary Screening. Without structures for all submitted compounds, Hit Confirmation assays will not be triggered.

If you have not already done so, please **provide CO-ADD with the chemical structure** of the full sample set in this study (both for compounds showing activity and those that do not), which will allow CO-ADD to filter out future samples with the same, or highly similar structure. In addition, please **notify CO-ADD** if you agree to publish the data (*i.e.* structures and activity) in the public bioactive database ChEMBL (www.ebi.ac.uk/chembl/). CO-ADD aims to increase the public knowledge of antimicrobial research, including data about non-active compounds.

All confirmed hits, without cytotoxicity or haemolytic activity, will be considered for further HitValidation, after a detailed analysis of structure-activity relationship and antimicrobial novelty, within CO-ADD samples, as well as, within public antimicrobial activity databases, like ChEMBL (www.ebi.ac.uk/chembl/).

1.5 Publishing CO-ADD Data

If you wish to publish data provided by CO-ADD, we kindly ask that you acknowledge CO-ADD appropriately with the following reference:

Helping Chemists Discover New Antibiotics

M.A. Blaskovich, J. Zuegg, A.G. Elliott, M.A. Cooper
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DOI: 10.1021/acsinfecdis.5b00044; PMID: 27622818

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Please advise CO-ADD at your earliest convenience that you have used provided data for publication purposes. This information is extremely helpful in keeping track of the outputs from the CO-ADD initiative and supports the program in renewed funding possibilities to continue CO-ADD as a free screening service available to the academic community.

CO-ADD also asks, that where possible you publish your data in an Open Access journals.

2.0 Methods

2.1 Sample Preparation

Samples were provided by the collaborator and stored frozen at -20 °C. Samples were prepared in DMSO and water to a final testing concentration of 32 µg/mL or 20 µM (unless otherwise indicated in the data sheet) and serially diluted 1:2 fold for 8 times. Each sample concentration was prepared in 384-well plates, non-binding surface plate (**NBS;** Corning 3640) for each bacterial/fungal strain, tissue-culture treated (**TC-treated;** Corning 3712/3764) black for mammalian cell types and polypropylene 384-well (**PP**; Corning 3657) for haemolysis assays, all in duplicate (n=2), and keeping the final DMSO concentration to a maximum of 0.5%. All the sample preparation was done using liquid handling robots.

Compounds that showed notable solubility issues during stock solution preparation are detailed in the **Data sheet** for the individual Project.

2.2 Antibacterial Assay

2.2.1 Procedure

All bacteria were cultured in Cation-adjusted Mueller Hinton broth (**CAMHB**) at 37 °C overnight. A sample of each culture was then diluted 40-fold in fresh broth and incubated at 37 °C for 1.5-3 h. The resultant mid-log phase cultures were diluted (CFU/mL measured by OD_{600}), then added to each well of the compound containing

plates, giving a cell density of 5 x 10^5 CFU/mL and a total volume of 50 µL. All the plates were covered and incubated at 37 °C for 18 h without shaking.

2.2.2 Analysis

Inhibition of bacterial growth was determined measuring absorbance at 600 nm (OD_{600}), using a Tecan M1000 Pro monochromator plate reader. The percentage of growth inhibition was calculated for each well, using the negative control (media only) and positive control (bacteria without inhibitors) on the same plate as references.

The percentage of growth inhibition was calculated for each well, using the negative control (media only) and positive control (bacteria without inhibitors) on the same plate. The MIC was determined as the lowest concentration at which the growth was fully inhibited, defined by an inhibition ≥ 80%. In addition, the maximal percentage of growth inhibition is reported as D_{Max}, indicating any compounds with partial activity.

Hits were classified by MIC ≤ 16 µg/mL or MIC ≤ 10 µM in either replicate (n=2 on different plates).

2.3 Antifungal Assay

2.3.1 Procedure

Fungi strains were cultured for 3 days on Yeast Extract-Peptone Dextrose (**YPD**) agar at 30 °C. A yeast suspension of 1 x 10^6 to 5 x 10^6 CFU/mL (as determined by OD_{530}) was prepared from five colonies. The suspension was subsequently diluted and added to each well of the compound-containing plates giving a final cell density of fungi suspension of 2.5 x 10^3 CFU/mL and a total volume of 50 µL. All plates were covered and incubated at 35 °C for 36 h without shaking.

2.3.2 Analysis

Growth inhibition of *C. albicans* was determined measuring absorbance at 630 nm (OD_{630}), while the growth inhibition of *C. neoformans* was determined measuring the difference in absorbance between 600 and 570 nm ($OD_{600-570}$), after the addition of resazurin (0.001% final concentration) and incubation at 35 °C for 2 h. The absorbance was measured using a Biotek Multiflo Synergy HTX plate reader.

In both cases, the percentage of growth inhibition was calculated for each well, using the negative control (media only) and positive control (fungi without inhibitors) on the same plate. The MIC was determined as the lowest concentration at which the growth was fully inhibited, defined by an inhibition ≥ 80% for *C. albicans* and an inhibition ≥ 70% for *C. neoformans.* Due to a higher variance in growth and inhibition, a lower threshold was applied to the data for *C. neoformans*. In addition, the maximal percentage of growth inhibition is reported as D_{Max}, indicating any compounds with marginal activity.

Hits were classified by MIC ≤ 16 µg/mL or MIC ≤ 10 µM in either replicate (n=2 on different plates).

2.4 Cytotoxicity Assay

2.4.1 Procedure

HEK293 cells were counted manually in a Neubauer haemocytometer and then plated in the 384-well plates containing the compounds to give a density of 5000 cells/well in a final volume of 50 µL. **DMEM** supplemented with **10% FBS** was used as growth media and the cells were incubated together with the compounds for 20 h at 37 °C in 5% CO_2.

2.4.2 Analysis

Cytotoxicity (or cell viability) was measured by fluorescence, ex: 560/10 nm, em: 590/10 nm ($F_{560/590}$), after addition of 5 µL of 25 µg/mL resazurin (2.3 µg/mL final concentration) and after incubation for further 3 h at 37 °C in 5% CO_2. The fluorescence intensity was measured using a Tecan M1000 Pro monochromator plate reader, using automatic gain calculation.

CC_{50} (concentration at 50% cytotoxicity) were calculated by curve fitting the inhibition values *vs.* log(concentration) using a sigmoidal dose-response function, with variable fitting values for bottom, top and slope. In addition, the maximal percentage of cytotoxicity is reported as D_{Max}, indicating any compounds with partial cytotoxicity.

The curve fitting was implemented using Pipeline Pilot's dose-response component, resulting in similar values to curve fitting tools such as GraphPad's Prism and IDBS's XlFit. Any value with > indicate sample with no activity (low D_{Max} value) or samples with CC_{50} values above the maximum tested concentration (higher D_{Max} value).

Cytotoxic samples were classified by CC_{50} ≤ 32 µg/mL or CC_{50} ≤ 10 µM in either replicate (n=2 on different plates). In addition, samples were flagged as partial cytotoxic if D_{Max} ≥ 50%, even with CC_{50} > the maximum tested concentration.

2.5 Haemolysis Assay

2.5.1 Procedure

Human whole blood was washed three times with 3 volumes of 0.9% NaCl and then resuspended in same to a concentration of 0.5 x 10^8 cells/mL, as determined by manual cell count in a Neubauer haemocytometer. The washed cells were then added to the 384-well compound-containing plates for a final volume of 50 µL. After a 10 min shake on a plate shaker the plates were then incubated for 1 h at 37 °C. After incubation, the plates were centrifuged at 1000*g* for 10 min to pellet cells and debris, 25 µL of the supernatant was then transferred to a polystyrene 384-well assay plate.

2.5.2 Analysis

Haemolysis was determined by measuring the supernatant absorbance at 405 mm (OD_{405}). The absorbance was measured using a Tecan M1000 Pro monochromator plate reader.

HC_{10} and HC_{50} (concentration at 10% and 50% haemolysis, respectively) were calculated by curve fitting the inhibition values *vs*. log(concentration) using a sigmoidal dose-response function with variable fitting values for top, bottom and slope. In addition, the maximal percentage of haemolysis is reported as D_{Max}, indicating any compounds with partial haemolysis.

The curve fitting was implemented using Pipeline Pilot's dose-response component, resulting in similar values to curve fitting tools such as GraphPad's Prism and IDBS's XlFit. Any value with > indicate sample with no activity (low D_{Max} value) or samples with HC_{10} values above the maximum tested concentration (higher D_{Max} value).

Haemolysis samples were classified by HC_{10} ≤ 32 µg/mL or HC_{10} ≤ 10 µM in either replicate (n=2 on different plates). In addition, samples were flagged as partial haemolytic if D_{Max} ≥ 50%, even with HC_{10} > the maximum tested concentration.

2.6 Antibiotic, Cytotoxic and Haemolytic Standards Preparation and Quality Control

Colistin and Vancomycin were used as positive bacterial inhibitor standards for Gramnegative and Gram-positive bacteria, respectively. Fluconazole was used as a positive fungal inhibitor standard for *C. albicans and C. neoformans.* Tamoxifen was used as a positive cytotoxicity standard. Melittin was used as a positive haemolytic standard.

Each antibiotic standard was provided in 4 concentrations, with 2 above and 2 below its MIC or CC_{50} value, and plated into the first 8 wells of column 23 of the 384-well NBS plates. Tamoxifen and melittin was used in 8 concentrations in 2 fold serial dilutions with 50 µg/mL highest concentration.

The quality control (QC) of the assays was determined by Z'-Factor, calculated from the Negative (media only) and Positive Controls (bacterial, fungal or cell culture without inhibitor), and the Standards. Plates with a Z'-Factor of ≥ 0.4 and Standards active at the highest and inactive at the lowest concentration, were accepted for further data analysis.

3.0 Materials

3.1 Assay Materials

Material	*Code*	*Brand/Supplier*	*Cat No.*
Compound preparation plate, Polypropylene	PP	Corning	3364
Assay Plates – Antimicrobial Non-binding surface	NBS 384w	Corning	3640
Assay Plates – Cytotoxicity Tissue culture treated	Black/Clear bottom 384w	Corning	3712
Assay Plates - Haemolysis	PP-Haem	Corning	3657
Reading Plates - Haemolysis	Clear 384w	Corning	3680
Growth media - bacteria	CAMHB	Bacto Laboratories	212322
Culture agar - fungi	YPD	Becton Dickinson	242720
Growth media - fungi	YNB	Becton Dickinson	233520
Resazurin		Sigma-Aldrich	R7017
Dulbecco's Modified Eagle Medium	DMEM	Life Technologies	11995-073

Foetal Bovine Serum	FBS	Bovogen	FFBS-500
0.9% NaCl	Saline	Baxter	AHF7124

3.2 Standards

Sample Name	*Sample ID*	*Full MW*	*Stock Conc. (mg/mL)*	*Solvent*	*Source*
Colistin - Sulfate	MCC_000094:02	1400.63	10.0	DMSO	Sigma; C4461
Vancomycin - HCL	MCC_000095:02	1485.71	10.0	DMSO	Sigma; 861987
Fluconazole	MCC_008383:01	306.27	2.56	DMSO	Sigma; F8929
Tamoxifen	MCC_000096:01	371.50	10	DMSO	Sigma; T5648
Melittin	MCC_008868:02	2846.46	10	Water	Sigma: M2272

3.3 Microbial strains and cell lines

ID	Batch	*Organism*	*Strain*	*Description*
GN_001	02	*Escherichia coli*	ATCC 25922	FDA control strain
GN_003	02	*Klebsiella pneumoniae*	ATCC 700603	MDR
GN_034	02	*Acinetobacter baumannii*	ATCC 19606	Type strain
GN_042	02	*Pseudomonas aeruginosa*	ATCC 27853	Quality control strain
GP_020	02	*Staphylococcus aureus*	ATCC 43300	MRSA
FG_001	01	*Candida albicans*	ATCC 90028	CLSI reference
FG_002	01	*Cryptococci neoformans*	ATCC 208821	H99, Type strain
MA_007	02	*Homo sapiens* embryonic kidney cells	ATCC CRL-1573	HEK 293
HA_150	-	*Homo sapiens*	ARCBS 5400 00150	Whole blood

4.0 Controls

4.1 Antimicrobial susceptibility of tested strains

Values are the average of ≥ 6 independent biological replicates. All values are within the expected range as per CLSI guidelines.

4.1.1 Antibiotic standards

MIC determined by BMD method, CA-MHB, Corning 3640 384 NBS plates		**GN_001:02** ***Escherichia coli*** FDA Control ATCC 25922	**GN_003:02** ***Klebsiella pneumophila*** ESBL ATCC 700603	**GN_034:02** ***Acinetobacter baumannii*** Type strain ATCC 19606	**GN_042:02** ***Pseudomonas aeruginosa*** QC strain ATCC 27853
Compound	Compound Type	MIC (µg/mL)			
Colistin - sulfate	Antibiotic	**0.125**	**0.25**	**0.25**	**0.25**

MIC determined by BMD method, CA-MHB, Corning 3640 384 NBS plates		**GP_020:02** ***Staphylococcus aureus*** MRSA ATCC 43300
Compound	Compound Type	MIC (µg/mL)
Vancomycin - HCl	Antibiotic	**1**

4.1.2 Antifungal standard

MIC determined by BMD method, YNB, Corning 3640 384 NBS plates		**FG_001:02** ***Candida albicans*** CLSI reference ATCC 90028	**FG_002:02** ***Cryptococcus neoformans H99*** Type strain ATCC 208821
Compound	Compound Type	MIC (µg/mL)	
Fluconazole	Antifungal	**0.125**	**8**

4.2 Susceptibility profile of cell lines

Values are the average of > 6 independent biological replicates. CC_{50} is the concentration at 50% cytotoxicity.		MA_007 HEK293 ATCC CRL-1573	
		CC_{50} (µg/mL)	
Compound	Compound Type	Average	Stdev
Tamoxifen	PKC inhibitor	**9**	**2.2**

4.3 Susceptibility profile of human washed red cells

Values are the average of > 6 independent biological replicates. HC_{10} and HC_{50} are the concentrations at 10% and 50% haemolysis, respectively.		HA_150 Human Whole blood ARCBS 00150			
		HC_{10} (µg/mL)		HC_{50} (µg/mL)	
Compound	Compound Type	Average	Stdev	Average	Stdev
Melittin	Haemolytic peptide	**2.7**	**0.9**	**8.5**	**2.5**

4.4 Outcome

All standard compound controls displayed inhibitory values within the expected range for each assay type and each organism tested. For further information please contact the CO-ADD team at support@co-add.org.

Strain ID	*Species*	*Standard positive inhibitor control*	*Pass/Fail*
GN_001:02	*E. coli*	Colistin	Pass
GN_003:02	*K. pneumoniae* (MDR)	Colistin	Pass
GN_034:02	*A. baumannii*	Colistin	Pass
GN_042:02	*P. aeruginosa*	Colistin	Pass
GP_020:02	*S. aureus* (MRSA)	Vancomycin	Pass
FG_001:01	*C. albicans*	Fluconazole	Pass
FG_002:01	*C. neoformans* (H99)	Fluconazole	Pass
MA_007:02	*Homo sapiens* HEK293	Tamoxifen	Pass
HA_150	*Homo sapiens*	Melittin	Pass

Table 9S. Percentage of growth inhibition (± 10%) of all tested bacteria and fungi in the presence of allene-derived metal complexes and allene ligands in the initial screening at 32 µg/ml of the drug.

	Entry	Compound	G +ve	G -ve				Yeast	
			S. aureus (Sa)	*E. coli* (Ec)	*K. pneumoniae* (Kp)	*P. aeruginosa* (Pa)	*A. baumannii* (Ab)	*C. albicans* (Ca)	*C. neoformans var. grubii* (Cn)
complexes	1	**2a**	89.50	19.97	17.13	1.78	72.15	98.92	66.46
	2	**2b**	88.65	48.49	26.24	53.44	92.24	99.78	73.68
	3	**3a**	33.08	-6.04	6.61	2.90	8.76	71.29	-4.92
	4	**3b**	0.49	-2.56	17.84	17.75	21.87	100.22	0.49
	5	**4a**	91.03	87.82	41.8	90.52	89.96	100.22	67.40
	6	**4b**	96.95	96.12	97.27	98.97	95.9	101.48	72.16
ligands	7	**1a**	0.06	-3.91	4.43	19.85	6.66	2.30	-3.13
	8	**1b**	-2.65	-10.79	2.54	13.19	6.43	1.56	-2.04

Table 10S. Minimum inhibitory concentration (MIC, µg/mL) on all tested microorganisms, in a range of 0.25 - 32 µg/mL of the drug (complexes **2**-**4**) concentration; CC_{50} (cytotoxicity, µg/mL) with HEK-293 cells; HC_{10} (haemolytic activity, µg/mL) with RBC cells.

	Entry	Compound	G +ve	G -ve				Yeast		Human	
			S. aureus (Sa)	*E. coli* (Ec)	*K. pneumoniae* (Kp)	*P. aeruginosa* (Pa)	*A. baumannii* (Ab)	*C. albicans* (Ca)	*C. neoformans var. grubii* (Cn)	Embryonic kidney cells HEK-293 (Hk)	Red blood cells RBC (Hm)
complexes	1	**2a**	2	>32	>32	>32	>32	16	1	0.9567	>32
	2	**2b**	≤0.25	>32	>32	>32	32	≤0.25	≤0.25	1.066	>32
	3	**3b**	>32	>32	>32	>32	>32	32	>32	>32	>32
	4	**4a**	16	32	>32	>32	>32	4	16	>32	>32
	5	**4b**	16	≤0.25	>32	>32	>32	4	≤0.25	>32	≤0.25

7. Anticancer studies

Cell viability assays were performed using MDA-MB-231 human breast adenocarcinoma cells. The cells were routinely cultured in high glucose Dulbecco's Modified Eagle Medium(DMEM) phenol red-free medium supplemented with L-glutamine (1%), Fetal Bovine Serum (FBS, 10%) and sodium pyruvate (1 mM). The cells were subcultured (1:4 – 1:12) every 3-5 days, when they reached near confluence in 75 cm^2 Nunc Easy flasks. For chemosensitivity studies, stock solutions of bis(pyridyl)allenes **1a**-**b** and complexes **2**-**4** (20 mM) in DMSO (molecular biology grade) were prepared and further diluted with complete culture medium to obtain a range of desired concentrations. MDA-MB-231 cells were washed with PBS-B buffer, trypsinated and counted using a Neubauer haemocytometer. The cell suspension of concentration of 2 x 10^4 cell/mL was prepared and used to seed a number of 96-well round bottom plates. Black bottom plates were used for CellTiter-Blue® assay and transparent plates were used for the MTT assay. Each well was filled with 100 µL of cell suspension (2000 cells/well). The plates were incubated for 24 h at 37 °C at 5% CO_2. After that time, 100 µL of solution of the drug in complete culture medium was added to each experimental well giving a total of 200 µL per well. Control wells were treated with 100 µL of DMSO solution in complete culture media alone. Additionally, controls not containing cells were prepared (DMSO solution in complete culture medium, 200 µL per well). Drug solutions were added so that the final DMSO concentration did not exceed 0.5% (v/v) and the same DMSO concentration was used across all the wells. The plates were incubated for 24 h at 37 °C at 5% CO_2. **For CellTiter-Blue (CTB)** assay, after the corresponding incubation, 20 µL of CTB reagent was added to each well and the plate was shaken for 10 s. The plate was returned to the incubator (37 °C, 5% CO_2) for 4 h. The fluorescence emission intensity of the wells was then measured (excitation – 561 nm, emission – 594 nm). Background fluorescence was corrected by subtracting the average value of the fluorescence emission intensity of cell-free wells (media controls). **For MTT** assay, after the corresponding incubation, 20 µL of a yellow MTT reagent (5 mg/mL in PBS-B buffer) was added to each well. The plate was returned to the incubator (37 °C, 5% CO_2) for 3.5 h. The solution was then removed from each well with a pipette. 150 µL of DMSO (molecular biology grade) was added to each well and the plate was shaken resulting in colourless to bright pink solutions. The absorbance intensity of each well was measured at 560 nm. Background absorbance was corrected by subtracting the average value of the absorbance intensity of cell-free wells (media controls).

Cell survival was calculated as a percentage of non-treated cells. The half maximal inhibitory concentration (IC_{50}) values were determined from the plots of % cell survival *vs.* concentration.

The CTB assay was performed in technical triplicate; the MTT assay was performed in technical and experimental triplicate.

Table 11S. IC_{50} values [μM] (± SD for MTT) for MDA-MB-231 cell line (24 h incubation) of complexes **2**-**4** and bis(pyridyl)allenes **1**-**b** using CellTiter-Blue® and MTT assays.

	Entry	Compound	IC_{50} [μM] CTB	IC_{50} [μM] MTT
complexes	1	**2a**	0.29	3.32 ± 1.22
	2	**2b**	>100	-
	3	**3a**	N/A[a]	-
	4	**3b**	>100	-
	5	**4a**	5.87	2.50 ± 1.63
	6	**4b**	66.12	-
ligands	7	**1a**	>100	-
	8	**1b**	>100	-

[a] Lack of clear viability profile.

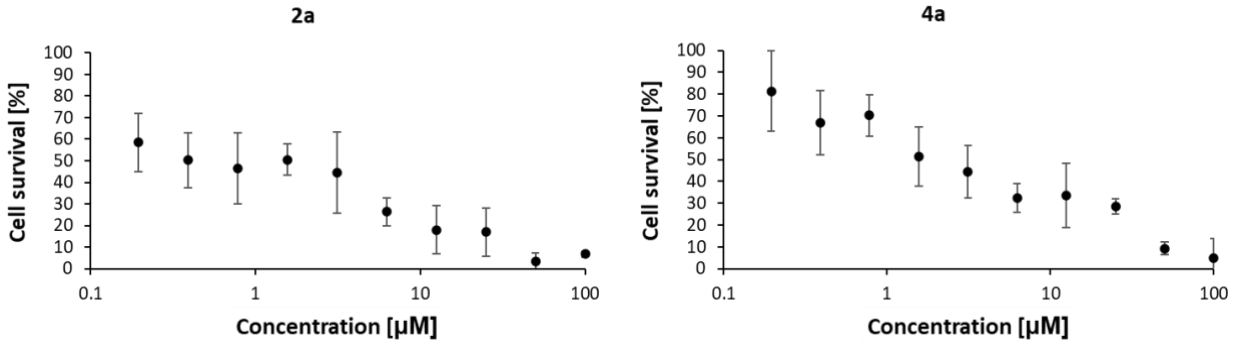

Figure 7S. MTT assay - viability of MDA-MB-231 cells treated with **2a** (left) and **4a** (right) after 24 h of incubation.

8. DNA studies

8.1. General experimental

Oligonucleotides were purchased from Eurogentec and were HPLC purified. Solid DNA samples were initially dissolved as a stock solution in purified water (1 mM); further dilutions were carried out in the respective sodium cacodylate buffer. Samples were thermally annealed in a heat block at 95 °C for 5 minutes and cooled slowly to room temperature overnight. Stock solution of the ligands were made at 10 mM in DMSO and was stored at −20 °C, subsequent dilutions were made in the appropriate buffer.

Name	Sequence 5′-3′
DS	GGC-ATA-GTGCGT-GGG-CGT-TAG-C was annealed with its complementary sequence GCT-AAC-GCC-CACGCA-CTA-TGC-C
hTeloC	TAA-CCC-TAA-CCC-TAA-CCC-TAA-CCC
DAP	CCC-CCG-CCC-CCG-CCC-CCG-CCC-CCG-CCC-CC
hif-1-α	CGC-GCT-CCC-GCC-CCC-TCT-CCC-CTC-CCC-GCG-C
hTeloG	GGG-TTA-GGG-TTA-GGG-TTA-GGG

8.2. FID assay

FID experiments were performed on a BMG CLARIOstar plate reader using Corning 96-Well Solid Black Flat Bottom plates. A 10 mM stock solution of TO was prepared in DMSO and dilution to 2 μM in the appropriate buffer. 90 μL of the 2 μM TO solution were added to each well and the fluorescence emission at 450 nm was measured with excitation at 430 nm; this was normalised to 0% representing background fluorescence. 1 μL of 90 μM DNA was then added, shaken using 114 double orbital shaking at 700 rpm in the plate reader for 15 s, and allowed to equilibrate for 15 minutes. After equilibration, fluorescence emission was measured once again and normalised to 100% representing maximum fluorescence enhancement from the TO probe binding the DNA secondary structure. 0.9 μL aliquots of ligand were titrated into each well (in triplicate) and measured as before. Fluorescence measurements after ligand addition were normalised between the 0 and 100% levels determined per the respective well. Percentage TO displacement was calculated as the difference between the normalised 100% fluorescence level and the normalised fluorescence measured after each ligand addition.

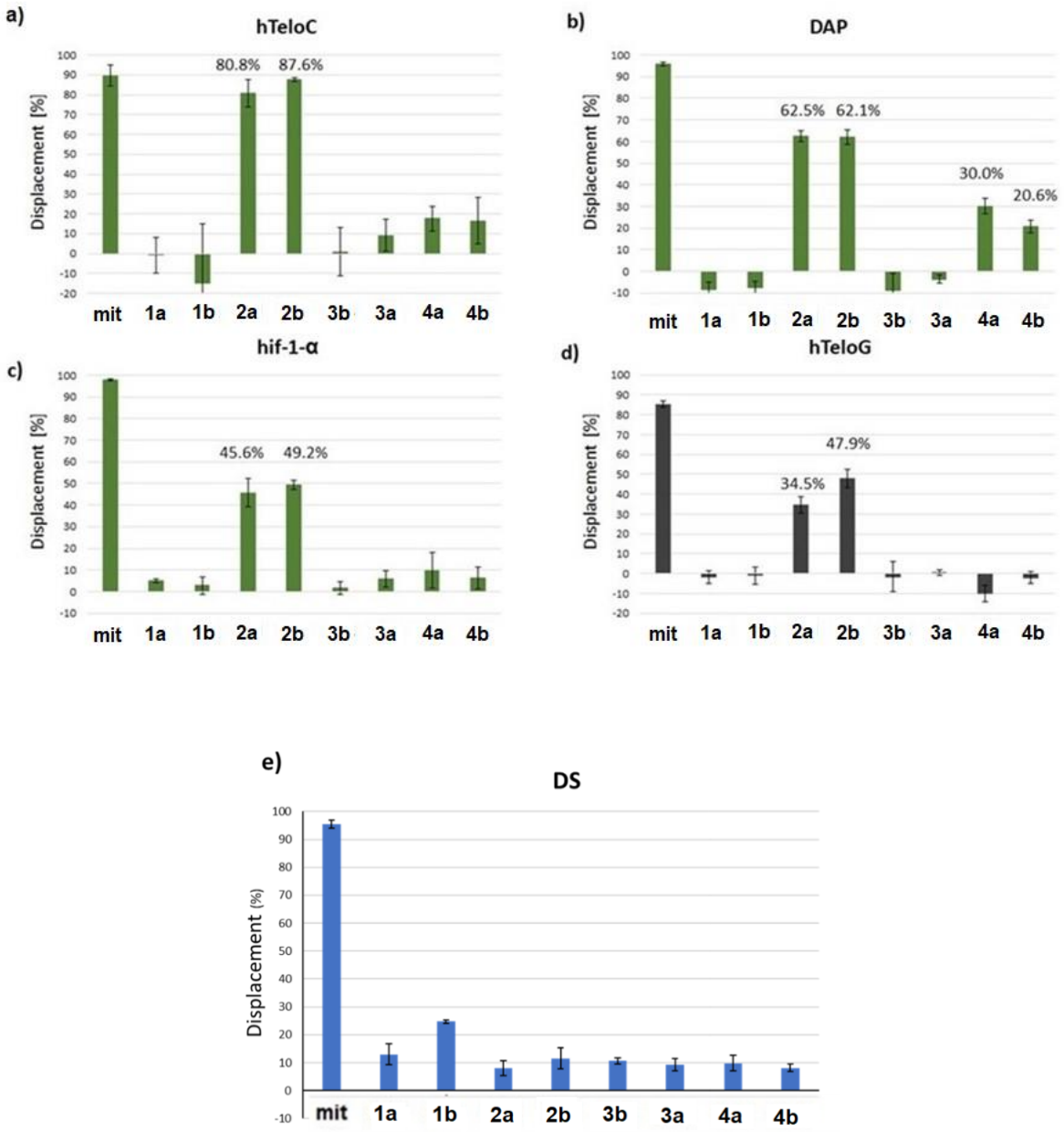

Figure 8S. TO displacement assay for allene ligands **1a**-**b** and allene-containing complexes at 2.5 μM in 10 mM sodium cacodylate, 100 mM KCl: a) 0.5 μM hTeloC, pH 5.5; b) 0.5 μM DAP, pH 6.8; c) 0.5 μM hif-1-α, pH 6.8; d) 0.5 μM hTeloG, pH 7.0; e) 0.5 μM DS, pH 7.0.

Table 12S. Summary of the FID results for allene ligands and allene-containing complexes. 0.5 µM DNA and 2.5 µM of compound in 10 mM sodium cacodylate, 100 mM KCl at pH 5.5 (hTeloC), 6.8 (hif-1-α, DAP) and pH 7.0 (hTeloG, DS).

		Displacement [%]				
Entry	**Compound**	**DS**	**hTeloC**	**hif-1-α**	**DAP**	**hTeloG**
1	**1a**	9.8	-0.9	4.9	-8.6	-1.7
2	**1b**	8.1	-14.8	2.8	-7.4	-1.1
3	**2a**	13.0	80.8	45.6	62.5	34.5
4	**2b**	24.7	87.6	49.2	62.1	47.9
5	**3b**	8.0	0.8	1.6	-8.9	-1.6
6	**3a**	11.5	9.2	5.9	-3.6	0.7
7	**4a**	10.6	17.6	9.8	30.0	-10.0
8	**4b**	9.3	16.5	6.2	20.6	-2.1

8.3. CD-melting experiments

Circular dichroism was performed using a Jasco J-810 spectropolarimeter using a 1 mm path length quartz cuvette. Scans were performed with a scanning speed of 200 nm/min, response time of 1 s, 0.5 nm pitch and 2 nm bandwidth. The spectra are an accumulation of 3 or 4 scans and are zero corrected at 320 nm. Melting experiments were performed using the same measurement parameters while heating the sample at a rate of 1 °C/min within the desired temperature range and measuring a complete spectrum at 5 °C intervals. The temperature at which 50% of the thermal denaturation had taken place (T_m) was calculated by plotting normalised ellipticity at 288 nm against temperature. These data were fitted with sigmoidal curves to give the T_m values. Examples of CD-melting curves for **2a** are shown in the Figure 9S - Figure 12S.

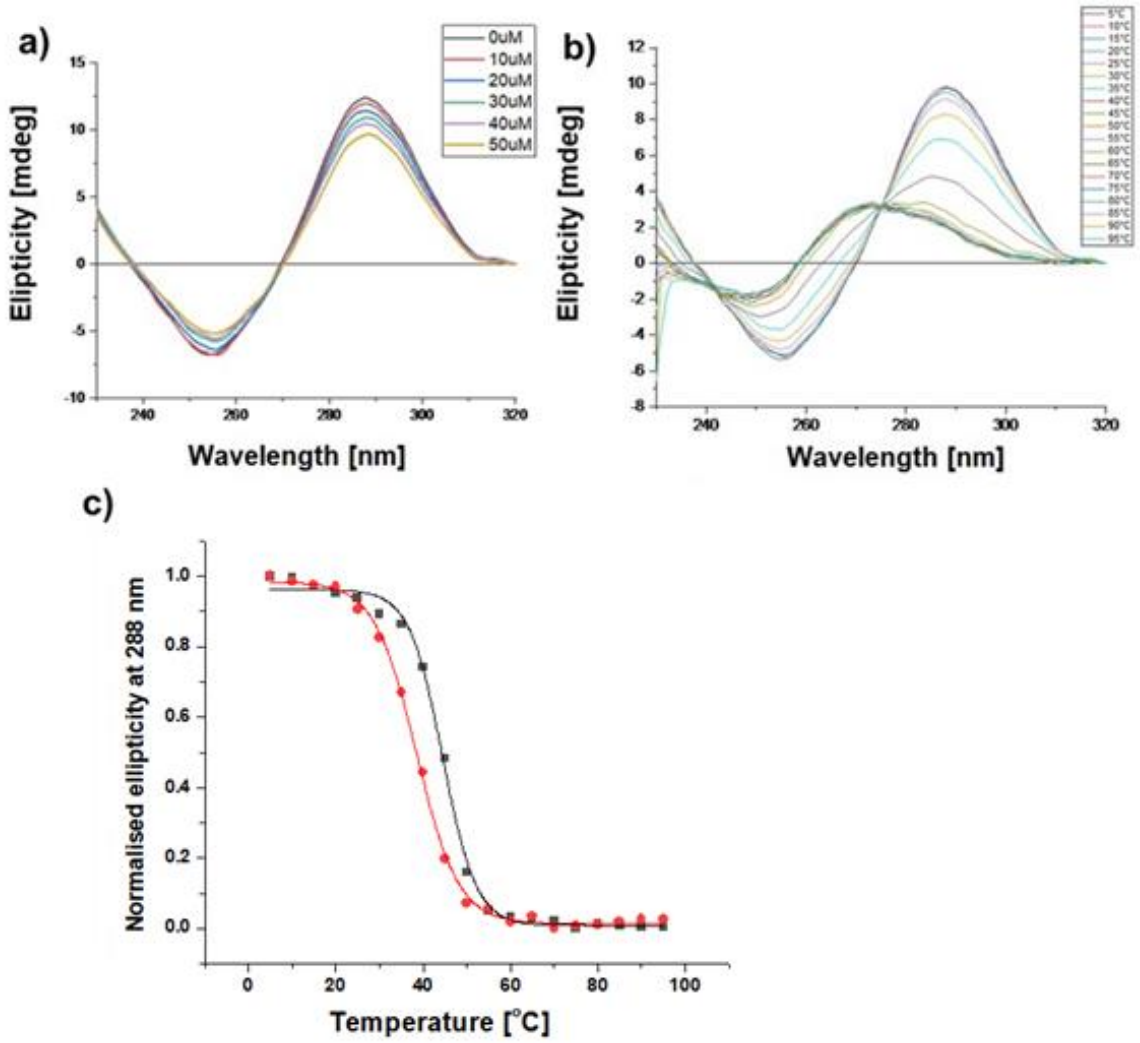

Figure 9S. CD experiments of 10 μM hTeloC in 10 mM sodium cacodylate, 100 mM KCl, pH 5.5 with compound **2a**: a) titration with 0-50 μM (0-5 eq) of **2a**; b) CD-melt with 50 μM of **2a**; c) melting curve derived from the normalised ellipticity at 288 nm in the absence (black) and presence (red) or compound **2a**.

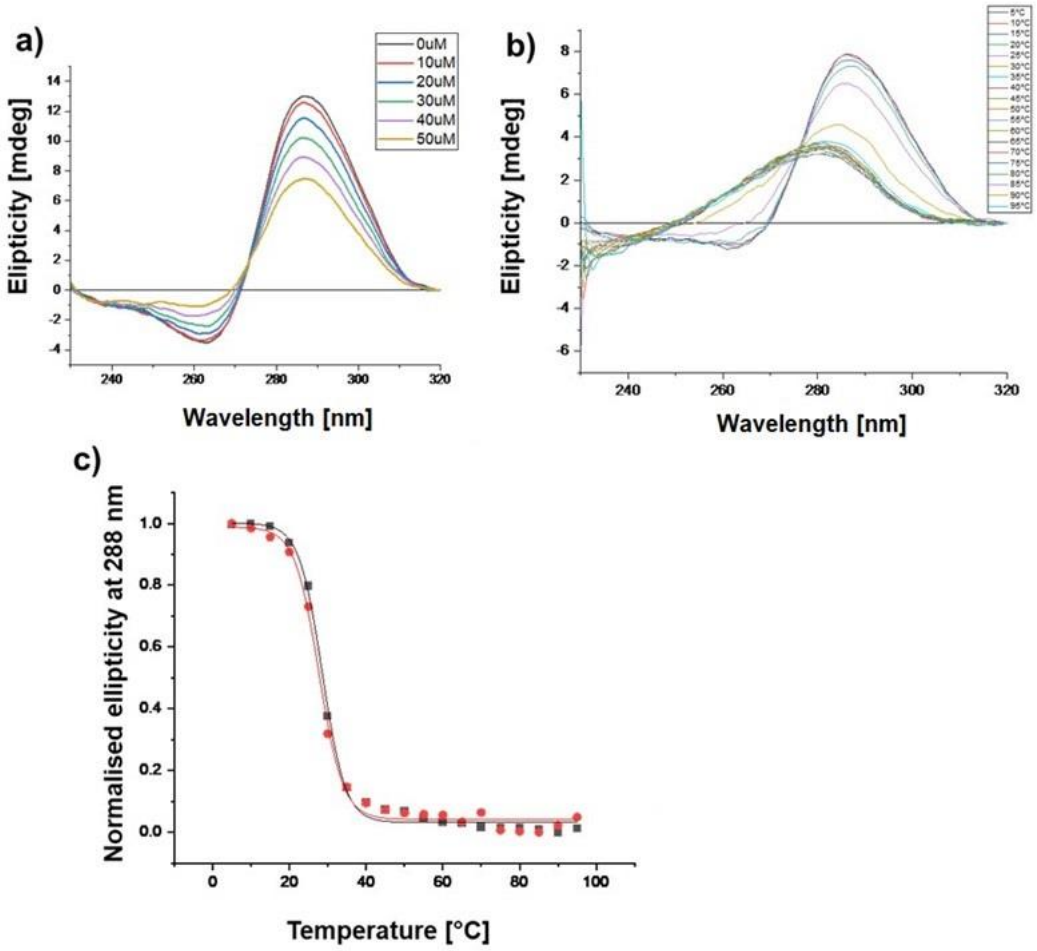

Figure 10S. CD experiments of 10 μM DAP in 10 mM sodium cacodylate, 100 mM KCl, pH 6.8 with compound **2a**: a) titration with 0-50 μM (0-5 eq) of **2a**; b) CD-melt with 50 μM of **2a**; c) melting curve derived from the normalised ellipticity at 288 nm in the absence (black) and presence (red) or compound **2a**.

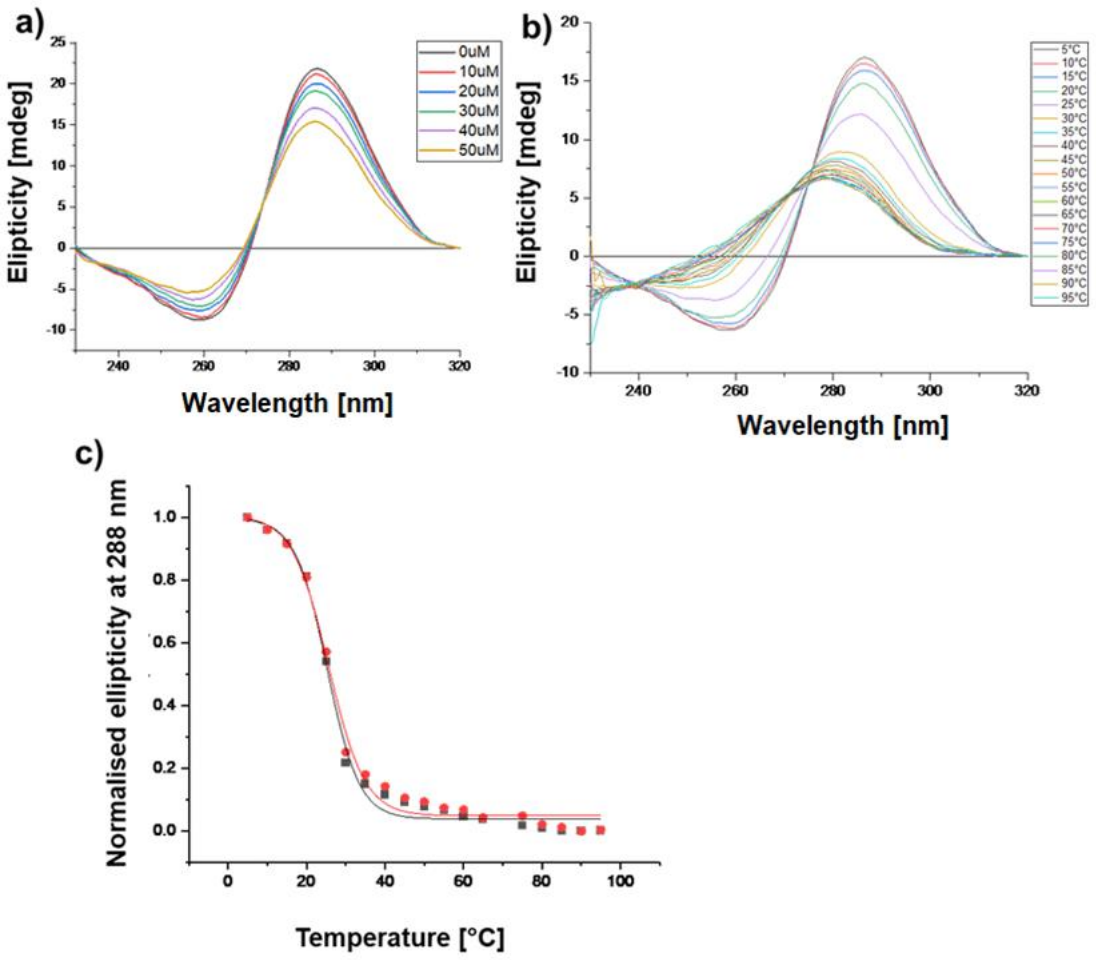

Figure 11S. CD experiments of 10 μM hif-1-α in 10 mM sodium cacodylate, 100 mM KCl, pH 6.8 with compound **2a**: a) titration with 0-50 μM (0-5 eq) of **2a**; b) CD-melt with 50 μM of **2a**; c) melting curve derived from the normalised ellipticity at 288 nm in the absence (black) and presence (red) or compound **2a**.

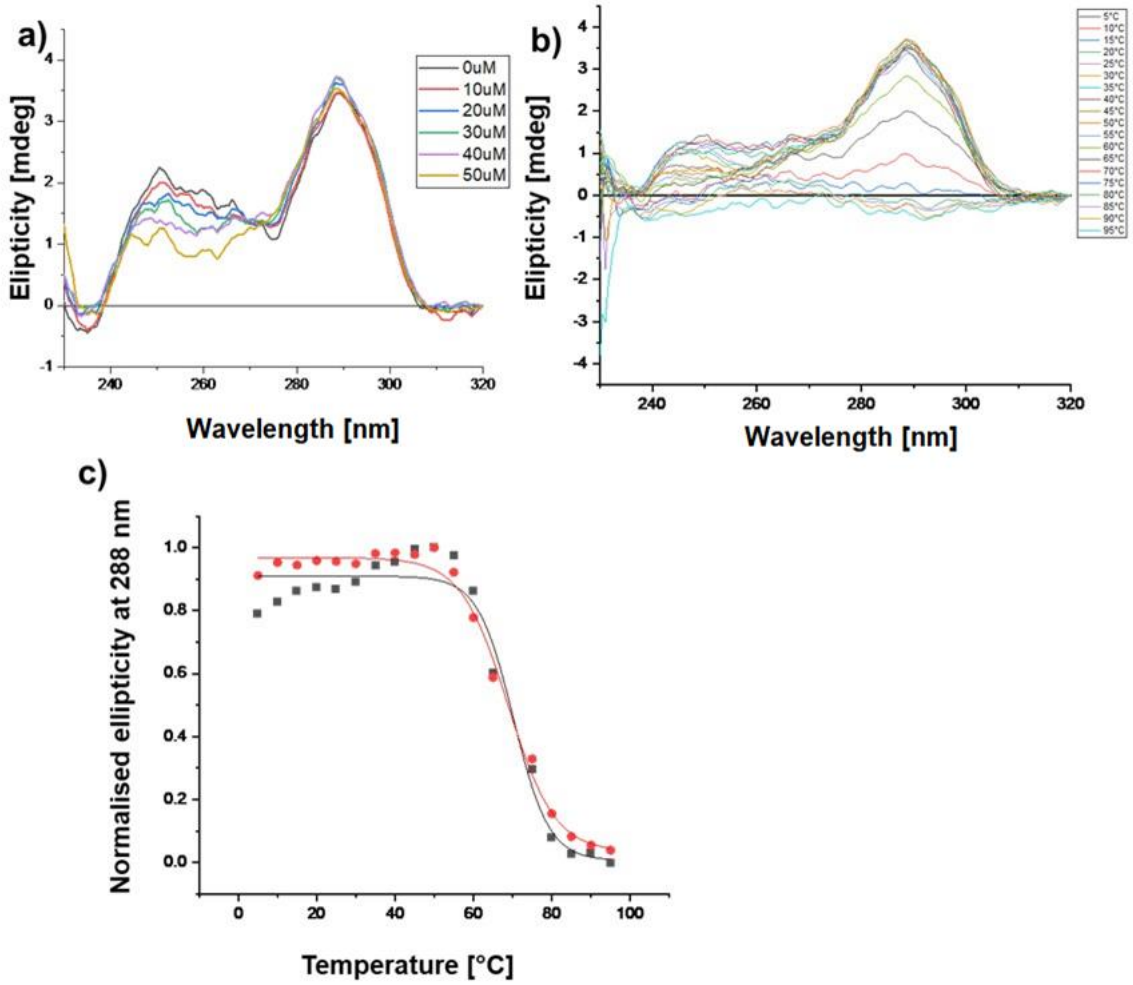

Figure 12S. CD experiments of 10 μM hTeloG in 10 mM sodium cacodylate, 100 mM KCl, pH 7.0 with compound **2a**: a) titration with 0-50 μM (0-5 eq) of **2a**; b) CD-melt with 50 μM of **2a**; c) melting curve derived from the normalised ellipticity at 288 nm in the absence (black) and presence (red) or compound **2a**.

Table 13S. Change in melting temperature (ΔT_m) of hTeloC, DAP, hif-1-α and hTeloG measured by CD-melting experiments for 2a-b. Errors represent standard deviations from at least duplicate experiments.

		ΔT_m / [°C]			
Entry	**Compound**	**hTeloC**	**DAP**	**hif-1-α**	**hTeloG**
1	**2a**	-8.1 ± 0.8°C	-1 ± 0.1°C	0.1 ± 0.3°C	-0.3 ± 0.8°C
2	**2b**	-2.1 ± 0.1°C	-0.7 ± 0.6°C	0.3 ± 0.9°C	-0.7 ± 0.6°C

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