Electronic Supporting Information

A therapeutically viable photo-activated manganese-based CO-releasing molecule (photo-CO-RM)

Jonathan S. Ward, Jason M. Lynam, James W. B. Moir, David E. Sanin, Adrian P. Mountford, and Ian J. S. Fairlamb

Contents

S1 - General experimental details

All reactions were carried out under a nitrogen atmosphere unless otherwise stated. Chemical reagents were purchased from Sigma Aldrich, Alfa Aesar or Frontier Scientific and used as received. All dry solvents were obtained from a Pure Solv MD-7 solvent machine and were stored in ampoules under nitrogen until required. Ethers from this machine were deoxygenated by sonication with nitrogen bubbling for 30 minutes.

All TLC analysis was carried out using Merck 5554 silica plates and spots were visualised using UV light at 254 and 365 nm. Column chromatography was carried out using silica gel 60 purchased from Sigma Aldrich.

Solution ^{1}H, and ^{13}C NMR analysis was carried out on Jeol ESC400 or ESX400 spectrometers. These were operating at 400 MHz (^{1}H), 100 MHz (^{13}C) frequencies for the appropriate experiments. All chemical shifts in ^{1}H NMR spectra are reported in ppm (δ) and are referenced to the residual NMR solvent ($CHCl_3$: 7.26 ppm DMSO: 2.54 ppm). The spectra were processed in Mnova v.6 software. All

chemical shifts in reported ^{13}C NMR spectra are reported in ppm (δ) and are referenced to the NMR solvent. ($CHCl_3$: 77.36 ppm DMSO: 40.45 ppm). For the ^{13}C NMR of the tetracarbonyl manganese(I) complexes, the metal carbonyl peaks were not observed due to long relaxation time in this solvent. IR spectroscopy confirms the presence of these metal carbonyls.

Mass spectrometry was carried out using a Bruker microTOF instrument. All data were acquired in positive ion mode using ESI or LIFDI ionisation. High resolution spectrometry data is reported with less than 5 ppm error unless otherwise stated. All LIFDI data reported is within 120 ppm error. Degradation experiments were carried out using a capillary exit voltage of 46 V with a skimmer voltage of 45 V. This reduces the amount of fragmentation from the molecular ion so that new peaks can be spotted more easily. This also stops the detector being overloaded with other ions.

Melting points of all complexes and ligands were obtained on a Perkin Elmer DSC 7 machine. Experiments were run using a ramp rate of 10 °C min^{-1} to above the required melting temperature. The melting point was taken as the onset of the observed endothermic peak. The maximum error in the melting point data obtained is ± 2 °C.

IR spectroscopy was carried out on a Thermo-Nicolet Avatar-370 FT-IR spectrometer. Spectra were taken in either solid state (KBr disc), or in solution with THF or methanol as solvents.

UV-Visible spectroscopy for the myoglobin assay and molar absorption co-efficient determination was carried out on a Jasco V-560 spectrometer. A baseline in the required solvent was carried out prior to starting an assay. Photo-initiated carbon monoxide release was carried out using either a 365 nm 6 W TLC lamp or a 5 W 400 nm LED directly above the solution drawing 2.4 W.

S2 - UV irradiation system

A new method of carrying out photochemical reactions and assays has been developed at the chemistry department at the University of York. An LED has been mounted on a special plastic cap that fits over the top of a PMMA cuvette (shown in Figure 1).

Figure 1 – An LED mounted on a special plastic cap fitted in to a PMMA cuvette.

This LED is connected to the control unit shown in Figure 2 and the functions are shown in Table 1.

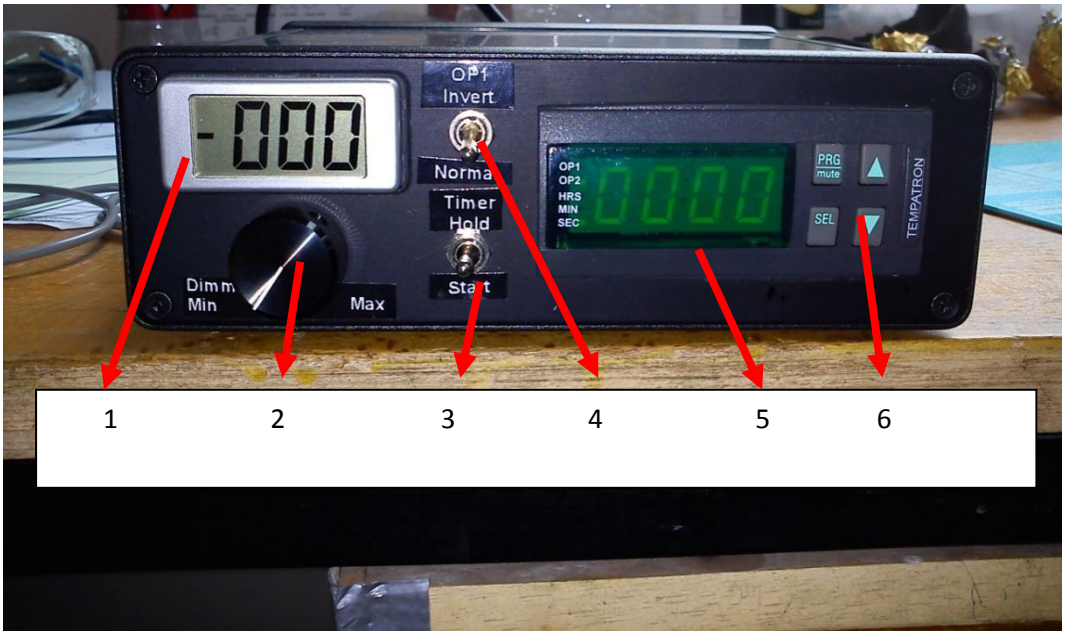


Figure 2 - Control unit for the irradiation system

Number	Name/Brief function
1	Current Monitor – Measures power that the LED is drawing in milliamps
2	Dimmer Switch – Turn to increase or decrease power
3	Start/Hold button, Up = hold (time will not count), Down = Start/Reset counter
4	Mode inverter- Can be used to change off/on modes
5	Timer display : Left two digits = Minutes, Right two digits = Seconds
6	Timer controls – can be used to change how long the LEDs come on and off for

Table 1 – Functions of different switches on the irradiation system

This irradiation system allows light intensity to be varied. It also allows for automated on and off cycles to be programmed into the system making it possible to automate experiments. This system ensures that consistent irradiation of a well defined wavelength is produced for the desired experiments.

S3 - Experimental procedure for the myoglobin assay

Lyophilised equine heart myoglobin (11 mg, purchased from Sigma Aldrich), was dissolved in 10 ml of 0.01 M PBS buffer solution (pH 7.4) to give an absorbance of approx.0.7 at 550 nm. This mass of myoglobin will give a solution with an approximate myoglobin concentration of 50 μM depending on the quality of the myoglobin. Sonication for five minutes will usually give full myoglobin dissolution. 1 ml of myoglobin solution was added to a PMMA 1 ml UV cuvette and approx. 1 mg of sodium dithionite was added. The solution was mixed with a micropipette twice. The UV spectra of the deoxy-Mb solution was then obtained. CO gas was bubbled through the 1 ml of solution for 15 seconds. The Mb-CO UV spectrum was then obtained.

A new 1 ml cuvette containing 1 ml of myoglobin stock solution was then prepared and incubated at 37 °C ready for the assay. 1 mg of CO-RM was dissolved in 100 µl of DMSO. Using the CO-RM stock solution, 100 µl of a second stock solution was prepared. The second stock solution is 200 times the concentration required in actual assay (*e.g* 8 mM for a 40 µM assay). Sodium dithionite (approx. 1 mg) was added to the new 1 ml myoglobin cuvette and the solution was mixed twice.

Immediately after this, 5 µl of the secondary CO-RM stock solution was added to the new deoxy-Mb solution giving final CO-RM concentrations of 10, 20, 40 and 60 µM as required. The solution was mixed again twice and the solution was layered with light mineral oil (500 µl) to prevent CO escape. The assay was then started immediately. UV spectra were acquired every five minutes and irradiation was used when required. The sample was irradiated from above at either 365 or 400 nm for two minutes every five minute period. The assay was not carried out for longer than 2.5 hours due to myoglobin degradation.

S4 - Standard data treatment for the myoglobin assay

Deoxy-Mb contains an absorption maximum at 560 nm. As CO is released from the administered CO-RM, the absorption maximum at 560 nm decreases and two new peaks at 540 nm and 578 nm arise in the spectra. These new peaks come from Mb-CO. The total concentration of myoglobin in the stock solution prepared is calculated from the maximum absorption peak at 540 nm in the saturated Mb-CO solution. Equation 1 shows how this is calculated.

$$\text{Mb-CO}_{\max} = (\text{OD}_{540}/\,\varepsilon) \times 1000$$

Equation 1: Calculating the total myoglobin concentration for an assay stock solution.ε = excition co-efficient of Mb-CO= 15.4 mM cm^{-1}, OD_{540} = Absorbance of saturated Mb-CO solution at 540nm.

The calculation of Mb-CO concentrations throughout the assay are also calculated from the OD_{540}. A second coefficient (ε_2) is required for these experiments as there are two species absorbing in solution, deoxy-Mb and Mb-CO. This coefficient takes in to account the change in absorbance at 540nm (ΔOD_{540}). This calculation also requires a second wavelength. This is used as a reference point to increase the accuracy of the calculations. The UV-Visible spectra of deoxy-Mb and Mb-CO share four isosbestic points (OD_{iso}) at 510, 550, 570, and 585 nm. The isosbestic point at 510 nm was used as the reference point to calculate the coefficient ε_2. This was done using equation 2.

$$\varepsilon_2 = \frac{\Delta\text{OD}_{540} - \Delta\text{OD}_{\text{iso510}} \times 1000}{\text{Mb}{-}\text{CO}_{\max}}$$

Equation 2: Calculating the second Mb-CO extinction coefficient(ε_2).ΔOD_{iso510} = Change in absorbance at the 510 nm isosbestic point, ΔOD_{540} = change in absorbance at 540 nm. Mb-CO_{max} =maximum concentration of myoglobin. ε_2 = new extinction coefficient.

Using the new extinction coefficient, the concentration of myoglobin in an unknown sample can be calculated. This is done using equation 3.

$$\text{Mb-CO} = \frac{(\Delta\text{OD}_{540} - \Delta\text{OD}_{\text{iso510}})}{\varepsilon_2} \times 1000$$

Equation 3 – Calculating the unknown Mb-CO concentration in a sample. ΔOD_{540} = change in absorbance at 540 nm, ΔOD_{iso510} = Change in absorbance at the 510 nm isosbestic point, ε_2 = new absorption coefficient.

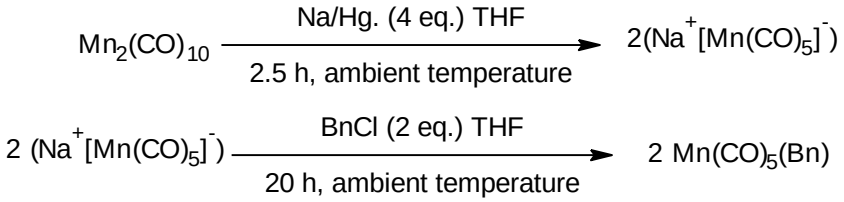
$t_{1/2}$ is reported as the time taken for the Mb-CO concentration to equal half that of the initial CO-RM concentration. t_0 is taken as the time when irradiation is started. This is because the complexes tested did not release CO until irradiation was initiated. The values are reported consistently regardless of how many CO molecules are released per CO-RM. The kinetics of these compounds are affected by CO-RM concentration, irradiation intensity and the rate of each CO release from the same metal centre will vary.

S4.1 - The correction of myoglobin assay data using non-linear regression.

The data treatment for myoglobin assay data detailed in S4 is only sufficient for CO-RM compounds that do not absorb in the 500-600 nm region of the UV-vis spectrum, and also for compounds which precipitate to any extent causing turbidity. The turbidity can be accounted for by the correction at 510 nm, however it does not account for the difference in light scattering across the 500-600 nm region. A procedure by Fairlamb and co-workers was developed to account for this turbidity/CO-RM absorbance.[1] This procedure can also be used to account for other minor differences between the reference spectra and the spectra measured during the assay.

S5 - Synthesis of ligands and corresponding complexes with characterisation and NMR spectra

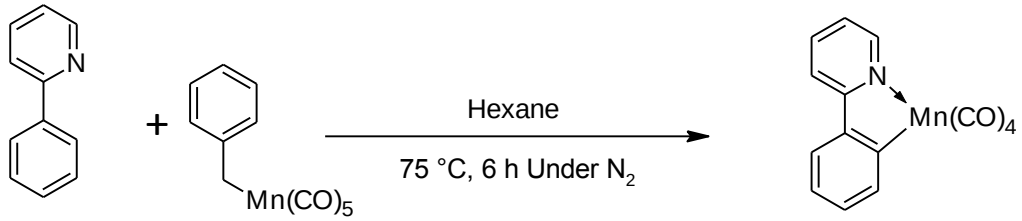
Synthesis of benzyl pentacarbonyl manganese(I). [2]



To an oven-dried Schlenk tube equipped with a magnetic stirrer under nitrogen was added mercury (3 ml). Sodium metal (4 eq., 1.07 mmol, 246 mg) was added in small pieces with rapid stirring to allow dissolution. In a separate Schlenk tube under nitrogen was added $Mn_2(CO)_{10}$ (1 eq., 2.68 mmol, 1.04 g), followed by anhydrous, deoxygenated THF (40 ml). The THF solution was then transferred by cannula on to the sodium amalgam and stirred for 3 hours. In a separate Schlenk tube equipped with a magnetic stirrer under nitrogen was added benzyl chloride (2 eq., 5.36 mmol, 617 µl, 678 mg). This was then incubated in ice cold water and was put under vacuum for 1 minute to remove oxygen. At room temperature, the THF solution of $NaMn(CO)_5$ was transferred *via* cannula into the benzyl chloride. The mixture was stirred at room temperature (20 °C) for 20 h. The solution was then filtered through a bed of Celite™ and washed with diethyl ether (5 × 20 ml). The crude mixture was loaded on to silica and this was added on to a pad of silica (5 cm). The pad was washed with petroleum ether (3 × 40 ml). The solvent was removed to yield product containing residual benzyl chloride. Benzyl chloride was removed under high vacuum (< 1 mbar). A pale yellow crystalline product was obtained. (1.179g, 76% yield).

M.P. (DSC): 40 °C; ^{1}H NMR (400 MHz, $CDCl_3$) δ: 7.18 (m, 4H), 6.97 (m, 1H), 2.41 (s, 2H); IR (THF): 2107, 2047, 2009, 1987 cm^{-1}; ESI-MS *m/z:* 286.9748 $[M+H]^+$ (calc. for $MnO_5C_{12}H_8$: 286.9747).

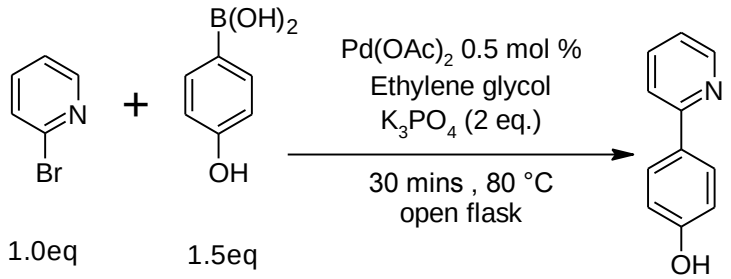
General procedure 1 - Synthesis of tetracarbonyl (2-phenylpyridine-κ^{2}N,C^8) manganese(I).[3, 4]



To an oven dried Schlenk tube equipped with a magnetic stirrer under nitrogen was added 2-phenylpyridine (1 eq., 1 mmol, 143 µl, 155 mg) and $Mn(CO)_5(Bn)$ (1 eq., 1 mmol, 286 mg) followed by dry deoxygenated hexane (16 ml). The mixture was refluxed with stirring for 6 h, and was allowed to cool to room temperature. The solution was filtered through a pipette packed with cotton wool and removal of solvent under reduced pressure yielded a yellow crystalline solid (284 mg, 88% yield).

M.P. (DSC): 114 °C; ^{1}H NMR(400 MHz, $CDCl_3$) δ: 8.72 (d, *J* = 5.6 Hz, 1H), 7.97 (d, *J* = 7.5 Hz, 1H), 7.87 (d, *J* = 8.1 Hz, 1H), 7.81-7.74 (m, 2H), 7.28 (td, *J* = 7.5 Hz, 1.0 Hz 1H), 7.17 (td *J* = 7.5, 1.5 Hz, 1H), 7.11 (ddd, *J* = 7.5Hz, 5.7 Hz, 1.5 Hz 1H); ^{13}C NMR (100 MHz, $CDCl_3$) δ: 174.9, 166.4, 153.9, 146.2, 141.7, 137.9, 130.3 124.2, 124.0, 122.4, 119.3; Elemental Analysis (CHN): C: 56.65% H: 2.98% N: 4.06% (calc.: C: 56.10% H: 2.51% N: 4.36%; LIFDI-MS: *m/z* = 321.0202 $[M]^+$ (calc. for $MnC_{15}H_8NO_4$ = 320.9834); IR (THF): 2071, 1986, 1972, 1928, 1600, 1576, 1477 cm^{-1}.

Synthesis of 2(4-hydroxy-phenyl)pyridine[5].

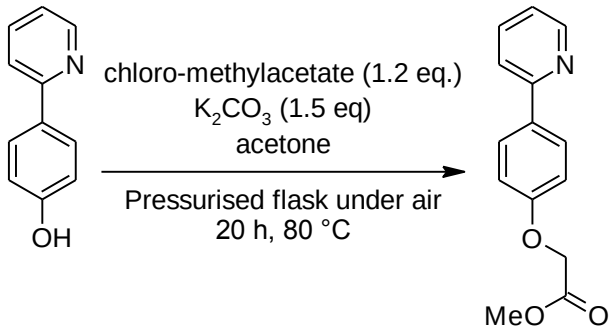


To a 250 ml round bottomed flask equipped with a magnetic stirrer was added $Pd(OAc)_2$ (0.005 eq., 50 µmol, 11.2 mg), 4-hydroxybenzeneboronic acid (1.5 eq., 15 mmol, 505 mg), potassium tri-phosphate (2 eq., 20 mmol, 4.24 g), 2-bromopyridine (1 eq., 10 mmol, 950 µl/1.58 g), and ethylene glycol (60 ml). The reaction was heated to 80 °C for 30 minutes and was allowed to cool to room temperature. Water (75 ml) and saturated brine (75 ml) was added and the aqueous layer was extracted with dichloromethane (4 × 100 ml). The organic layer was dried with $MgSO_4$ and filtered. Solvent was removed under reduced pressure to yield crude product. The crude product was purified by silica gel column chromatography (40:60 PET ether/ethyl acetate followed by 50:50 PET ether/ethyl acetate). The solvent was removed to isolate the title compound as a white solid (1.37 g, 80% yield).

M.P. (DSC): 164 °C; ^{1}H NMR (400 MHz, $CDCl_3$) δ: 8.62 (ddd, *J* = 5.0, 1.8, 1.0 Hz, 1H), 7.79-7.71 (m, 3H), 7.65(d, *J* = 8.0 Hz, 1H), 7.21 (dd, *J* = 7.4, 4.9 Hz, 1H), 6.80 (d, *J* = 8.5 Hz, 2H); ^{13}C NMR(100 MHz, $CDCl_3$) δ: 158.2, 157.9, 149.2, 137.7, 130.8, 128.9, 121.9, 121.1, 116.2; Elemental Analysis (CHN) C: 76.85% H: 5.31% N: 8.04% (calc.: C: 76.17% H: 5.30% N:8.18%); ESI-MS *m/z* = 172.0755 $[M+H]^+$ (calc. for $C_{11}H_{10}NO$= 172.0762); IR (KBr disc): 3367-2120, 1603, 1560, 1523,

1470, 1425, 1381, 1273, 1245, 1183, 1153, 1097, 998, 966, 839, 778, 744, 714, 646, 623, 580, 552, 492, 473 cm^{-1}.

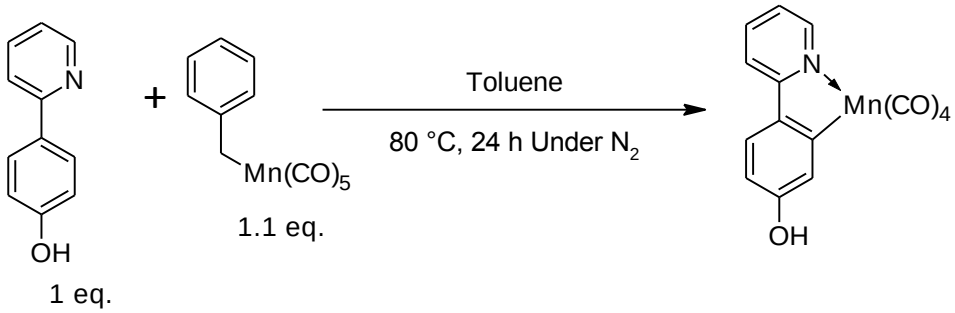
Synthesis of methyl [4-(pyridin-2-yl)phenoxy]acetate (1).[6]



To a thick walled round bottomed flask equipped with a magnetic stirrer was added 2-(4-hydroxy-phenyl)pyridine (1 eq., 5.84 mmol, 1.00 g), potassium carbonate (1.5 eq., 8.76 mmol, 1.21 g) and chloromethylacetate (1.2 eq., 7.01 mmol, 760 mg/615 µl). Acetone (50 ml) was added and the flask was sealed with a screw lid. The mixture was the heated to 80 °C for 20 hours. The reaction mixture was allowed to cool to room temperature and deionised water was added (60 ml). The aqueous layer was extracted with dichloromethane (3 × 60 ml) and the organic layer was dried with $MgSO_4$ and filtered. Removal of solvent under reduced pressure yielded pure product (1.30 g, 92% yield).

M.P.(DSC): 95 °C ; ^{1}H NMR (400 MHz, $CDCl_3$) δ: 8.64(ddd, *J* = 4.8, 1.7 Hz, 0.9 Hz, 1H), 7.94(d, *J* = 8.9 Hz, 2H), 7.71(d, *J* = 7.7, 1.7 Hz, 1H), 7.65(dt, *J* = 8.0, 1.1 Hz, 1H), 7.17(ddd, *J* = 7.2, 4.8, 1.2 Hz, 1H), 6.99(d, *J* = 9.0 Hz, 2H), 4.69(s, 2H), 3.81(s, 3H);^{13}C NMR(100 MHz, $CDCl_3$) δ: 169.6, 158.9, 157.2, 149.9, 137.1, 133.5, 128.6, 122.0, 120.2, 115.1, 65.7, 52.7; ESI-MS *m/z* = 244.0971 $[M+H]^+$ (calc. for $C_{14}H_{14}NO_3$ = 244.0974); IR (Pressed KBr disc): 1757, 1601, 1587, 1561, 1512, 1467, 1435, 1396, 1314, 1307, 1275, 1212, 1177, 1157, 1114, 1080, 1029, 1005, 982, 913, 840, 825, 781, 743, 715, 702, 639, 603, 551, 490 cm^{-1}.

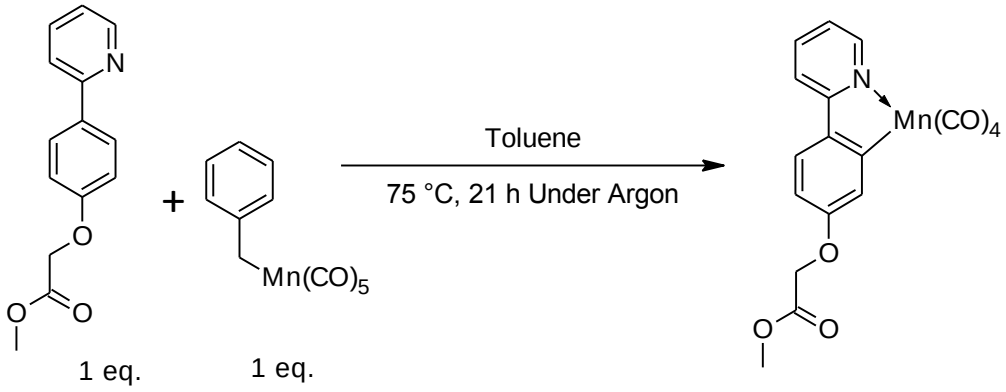
Synthesis of tetracarbonyl (2-(4-hydroxy-phenyl)κ,C^8-pyridine-κ,N) manganese(I) (6)



Using the details from general procedure 1, $Mn(CO)_5(Bn)$ (1.1 eq., 2.57 mmol, 735 mg), 2-(4-hydroxy-phenyl)pyridine (1 eq., 2.336 mmol, 400 mg) and toluene (30 ml) were used to prepare the title complex **2c**. At the end of the reaction, the solvent was removed under reduced pressure. The crude mixture was loaded on to silica using dichloromethane. The compound was purified by silica gel column chromatography (30:70 diethyl ether/PET ether). Removal of solvent under reduced pressure yielded product as an off white solid (250 mg, 32% yield).

M.P. (DSC): 127 °C (crystalline change) 171 °C (melt); ^{1}H NMR (400 MHz, $CDCl_3$) δ: 8.64 (d, *J* = 5.6 Hz, 1H), 7.74 (d, *J* = 4.2 Hz, 2H), 7.69 (d, *J* = 8.4 Hz, 1H), 7.42 (d, *J* = 2.2 Hz, 1H), 7.03 (apr. q *J* = 5.4, 2.0 Hz, 1H), 6.66 (d, *J* = 8.2 Hz, 1H) , 4.98 (s, 1H); ^{13}C NMR (100 MHz $CDCl_3$) δ: 178.4 ,166.3, 157.2, 154.0, 139.6, 138.0, 127.5, 125.8, 121.6, 118.9, 111.9; Elemental Analysis (CHN) C: 53.38% H: 2.46% N: 3.99% (calc.: C: 53.28 % H: 2.68 % N: 4.14 %); ESI-MS *m/z* = 337.9871 $[M+H]^+$ (calc. for $MnC_{15}H_9NO_5$= 337.9856); IR (Solution: THF): 3264, 2072, 1988, 1971, 1929, 1604, 1580, 1556, 1481, 1467, 1432, 1268, 1221, 1162 cm^{-1}.

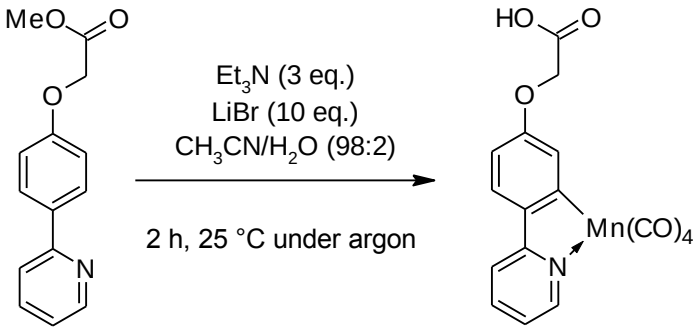
Synthesis of tetracarbonyl (methyl [4-(pyridin-2-yl-κ,N)phen-κ,C^2-oxy]acetate) manganese(I) (2)



Using the details from general procedure 1, $Mn(CO)_5$(Bn) (1 eq., 1.82 mmol, 521 mg), methyl [4-(pyridin-2-yl)phenoxy]acetate (1 eq., 1.82 mmol, 472 mg) and toluene (29 ml) were used to prepare the title complex. After heating, the reaction mixture was allowed to cool to room temperature. The solvent was removed under reduced pressure and crude solid was loaded on to silica using dichloromethane. The crude mixture was purified using silica gel column chromatography (80:20 PET ether/ethyl acetate). Removal of solvent under reduced pressure gave pure product (600 mg, 81% yield).

M.P. (DSC): 144 °C; ^{1}H NMR (400 MHz, $CDCl_3$) δ: 8.65 (d, *J* = 5.6 Hz, 1H), 7.75 (d, *J* = 3.6 Hz, 2H), 7.90 (d, *J* = 8.6 Hz, 1H), 7.52 (d, *J* = 2.5 Hz, 1H), 7.04 (m, 1H), 6.71 (dd, *J* = 8.5, 2.5 Hz, 1H), 4.75 (s, 2H), 3.84 (s, 3H); ^{13}C NMR (100 MHz, $CDCl_3$) δ: 175.1, 169.5, 165.9, 158.8, 153.8, 140.2, 137.8, 126.1, 125.2, 121.6, 118.9, 110.8, 65.3, 52.4; Elemental Analysis (CHN) C: 52.80% H: 2.99% N: 3.20% (calc.: C: 53.10% H: 2.48% N: 3.44%); ESI-MS *m/z* = 410.0061, $[M+H]^+$ (calc. for $MnC_{18}H_{13}NO_7$ = 410.0067); IR (THF): 2073, 1990, 1973, 1931, 1767, 1743, 1604, 1581, 1554, 1467, 1428 cm^{-1}.

Synthesis of tetracarbonyl (methyl [4-(pyridin-2-yl-κ,N)phen-κ,C^2-oxy]acetic acid) manganese(I) (3).[7]



To a round bottomed flask equipped with a magnetic stirrer was added (methyl [4-(pyridin-2-yl-κ,N)phen-κ,C_2-oxy]acetate)Manganese(I) (1 eq., 0.733 mmol, 300 mg), and lithium bromide (10 eq., 7.33 mmol, 636 mg). The flask was purged with argon using a balloon. Triethylamine (3 eq., 2.19 mmol, 223 mg/307 µl), deionised water (140 µl) and acetonitrile (7 ml) were then added. The acetonitrile was degassed by sonication with argon bubbling from a balloon for 15 minutes prior to addition. The reaction was stirred at 25 °C for 2 h. Deionised water (15 ml) was added to the reaction and the mixture was acidified with 2M HCl to pH 2-3. The aqueous layer was extracted with dichloromethane (3 × 30 ml). The organic extract was washed with water (20 ml) and was dried with $MgSO_4$ and filtered. The solvent was removed under reduced pressure to yield pure product as a yellow solid (246 mg, 84% yield).

M.P. (DSC): 153 °C (broad); ^{1}H NMR (400 MHz, d_6-DMSO) δ: 13.15 (broad s, 1H) 8.72 (d, *J* = 5.6 Hz, 1H), 8.15 (d, *J* = 8.0 Hz, 1H), 8.00 (t, *J* = 8.0 Hz, 2H), 7.35 (d, *J* = 2.5 Hz, 1H), 7.31 (ddd, *J* = 7.0, 5.7, 1.2 Hz, 1H) 6.75 (d, *J* = 7.9 Hz, 1H), 4.78 (s, 2H); ^{13}C NMR (100 MHz, d_6-DMSO) δ: 221.0, 215.4, 214.4, 175.8, 171.1, 165.7, 159.8, 155.1, 140.6, 139.8, 126.9, 126.6, 123.6, 120.3, 111.1, 65.3; Elemental Analysis (CHN) C: 51.65% H: 2.71% N: 3.49% (calc.: C: 51.67% H: 2.56% N: 3.54%); ESI-MS *m/z* = 395.9916, $[M+H]^+$ (calc. for $MnC_{17}H_{11}NO_7$ = 395.9911); IR (THF): 2071, 1989, 1972, 1931, 1766, 1731, 1604, 1581, 1554, 1469, 1428, 1314, 1284 cm^{-1}.

S6 – ^{1}H and ^{13}C NMR spectra of synthesised compounds

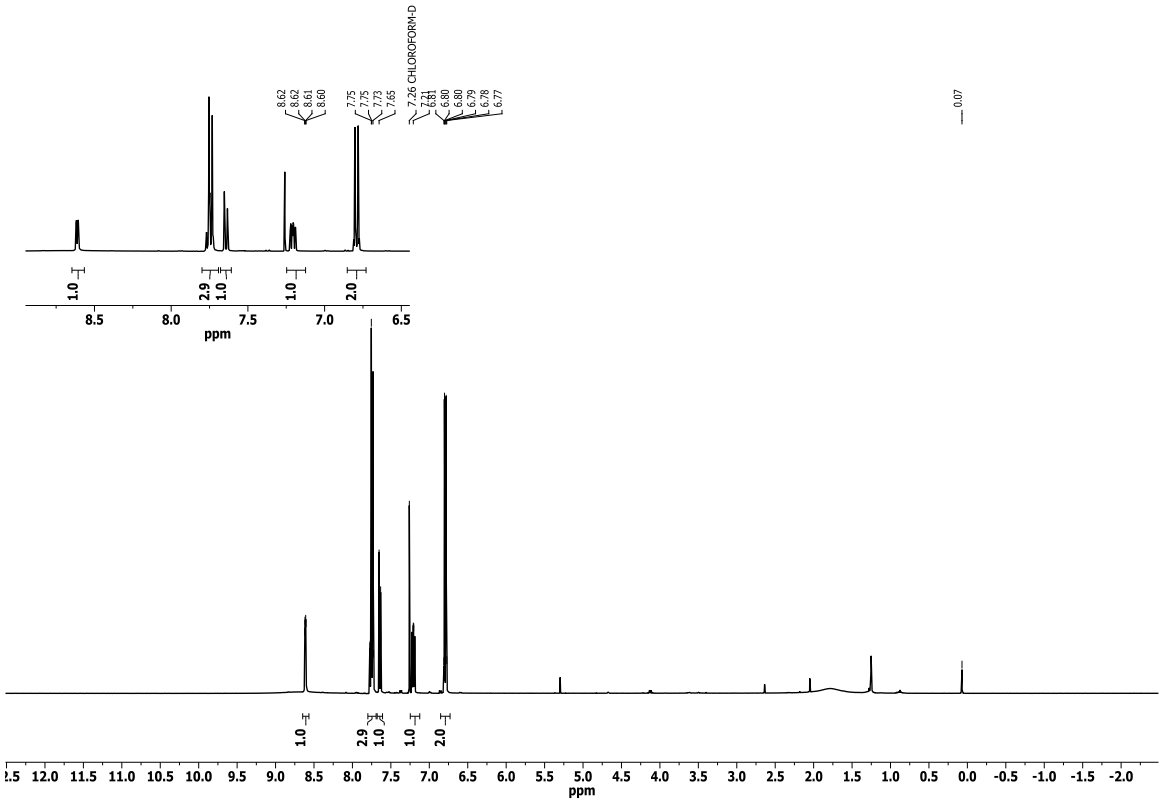


Figure 3 - ^{1}H NMR spectrum in $CDCl_3$ of 2(4-hydroxy-phenyl)pyridine.

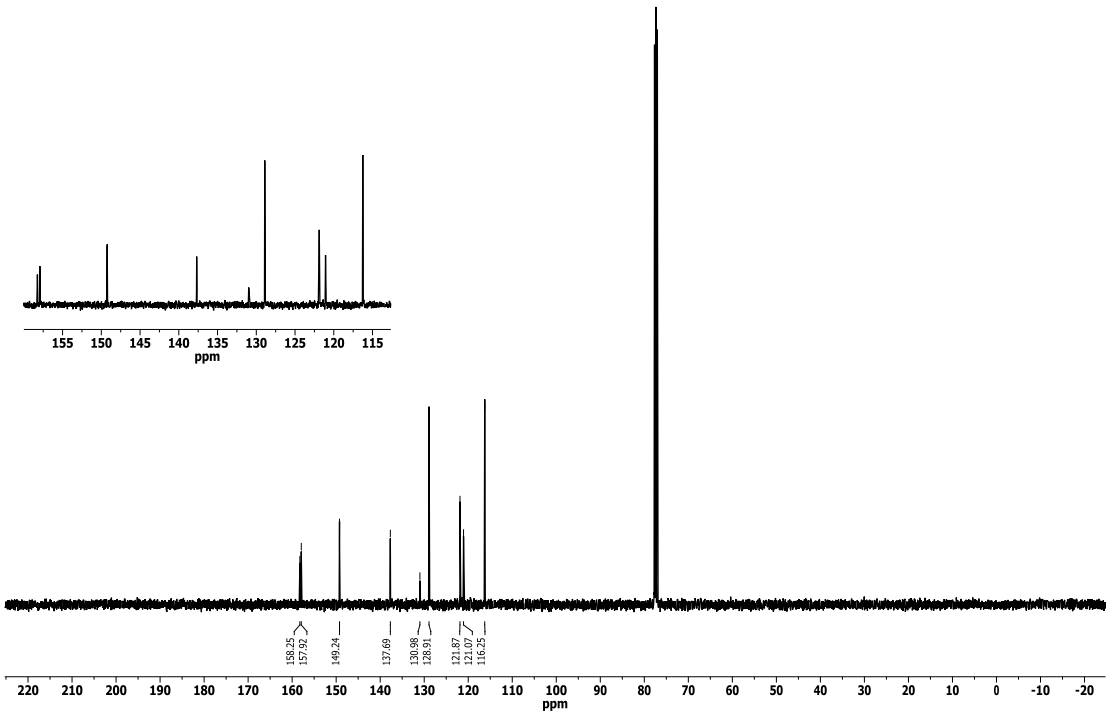


Figure 4 - ^{13}C NMR spectrum in $CDCl_3$ of 2(4-hydroxy-phenyl)pyridine.

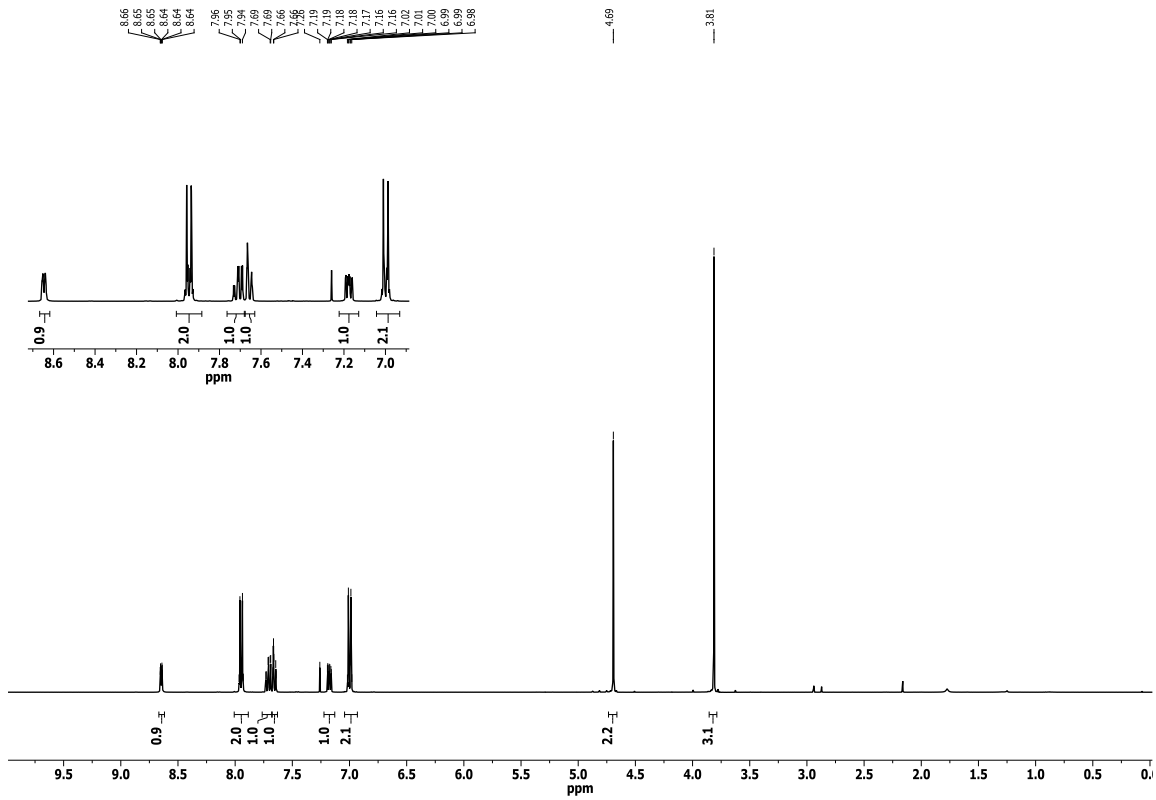


*Figure 5 - ^{1}H NMR spectrum in $CDCl_3$ of **1***

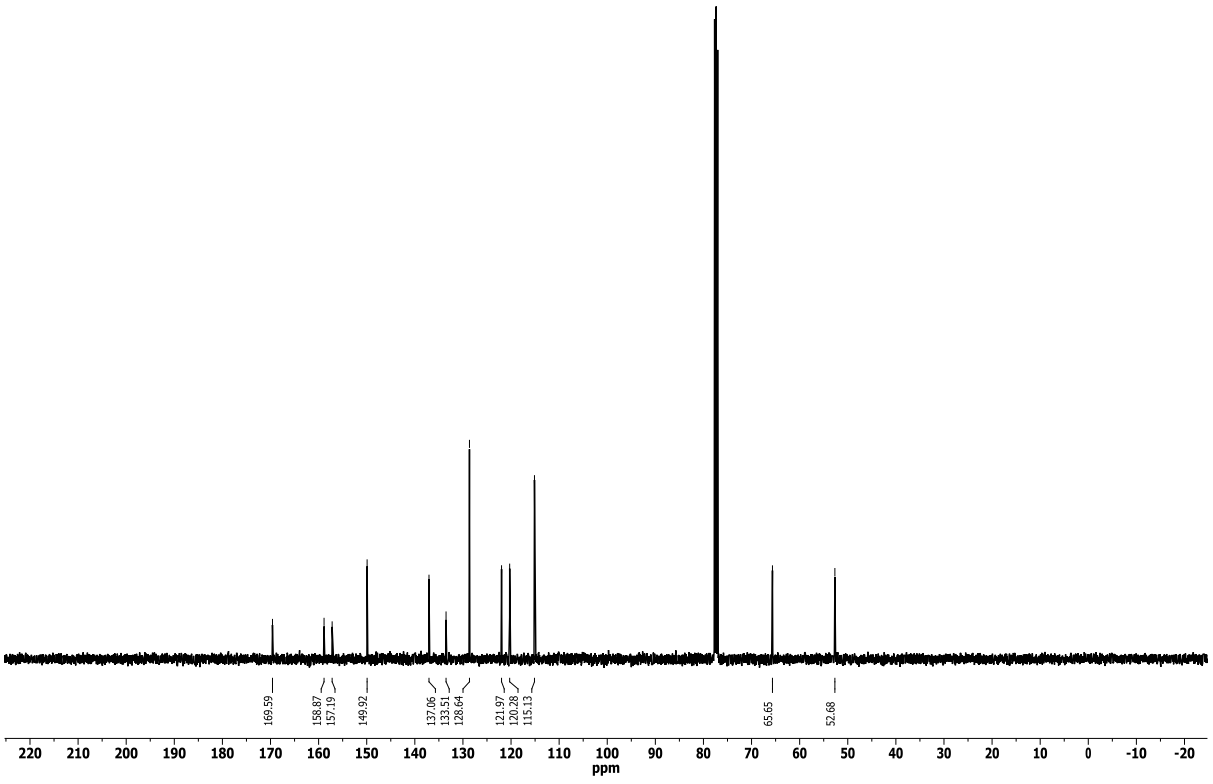


*Figure 6 – ^{13}C NMR spectrum in $CDCl_3$ of **1***

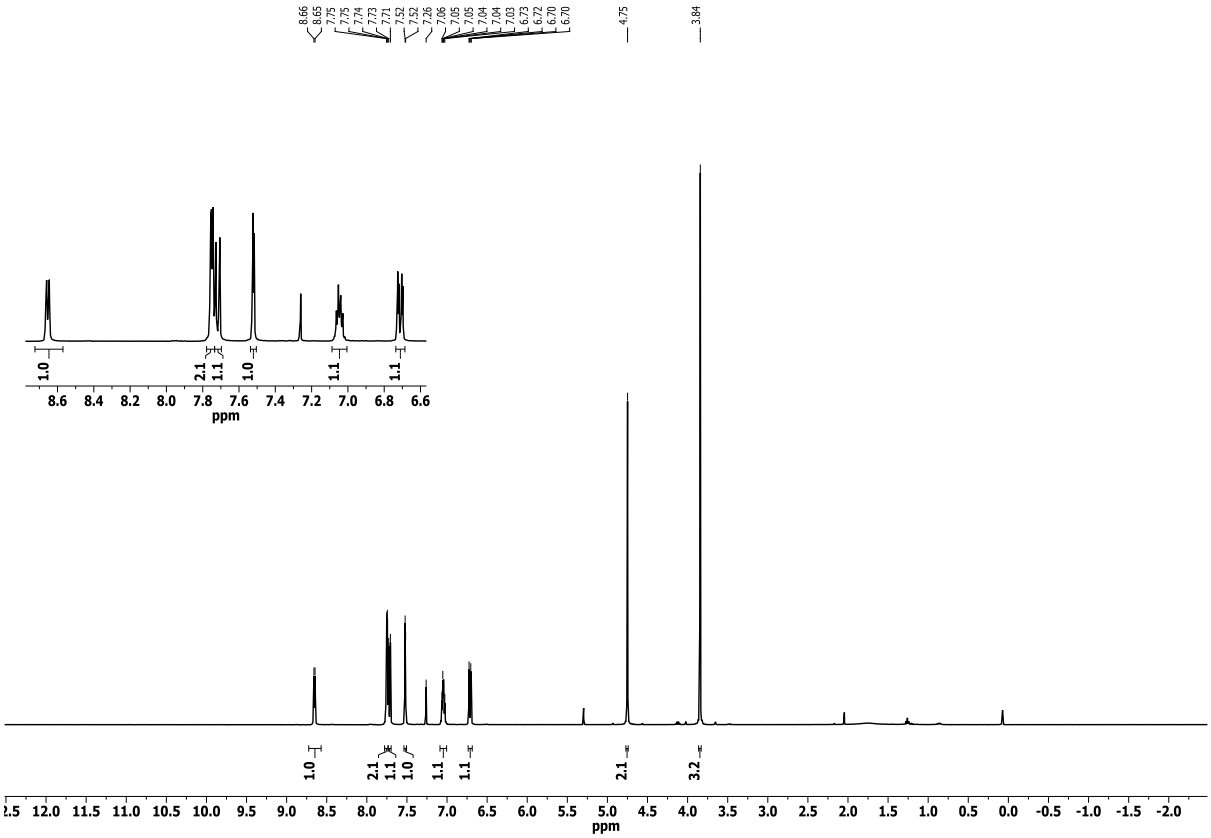


*Figure 7 – ^{1}H NMR spectrum in $CDCl_3$ of complex **2***

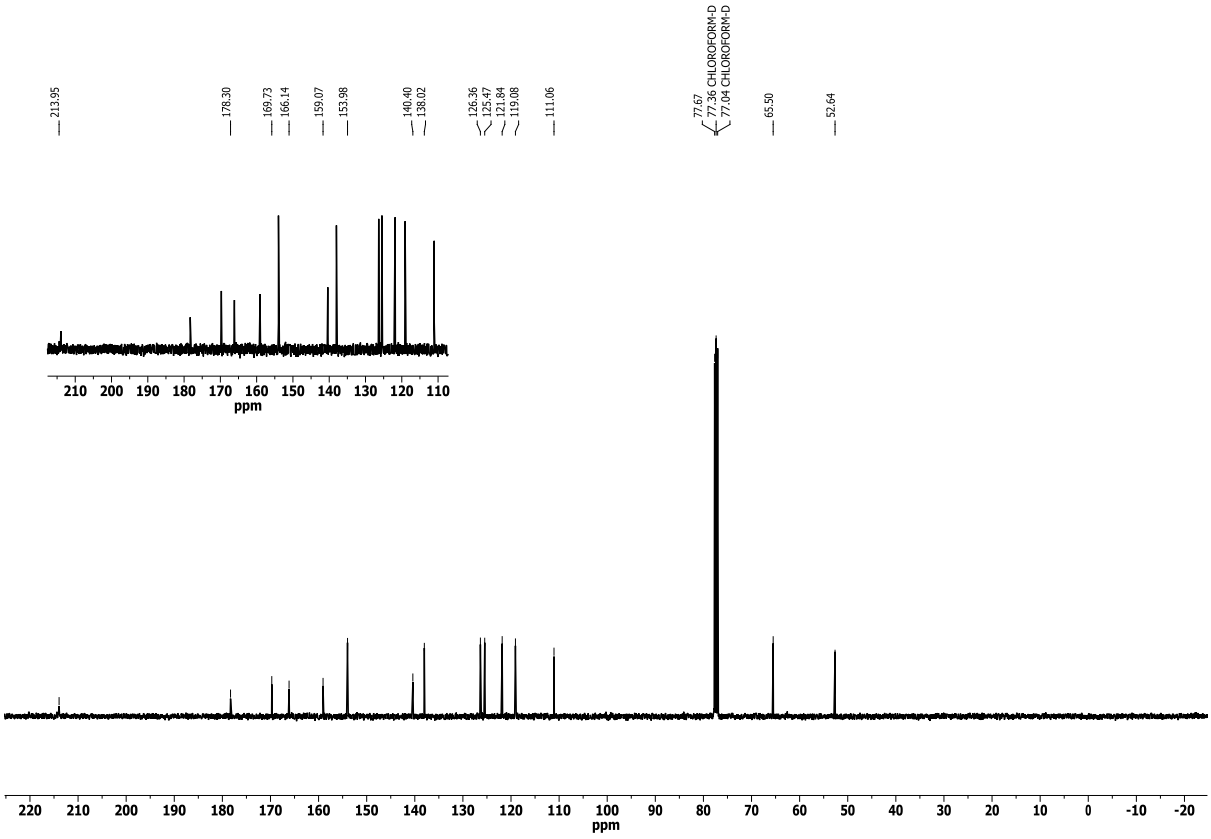


*Figure 8 - ^{13}C NMR spectrum in $CDCl_3$ of complex **2.***

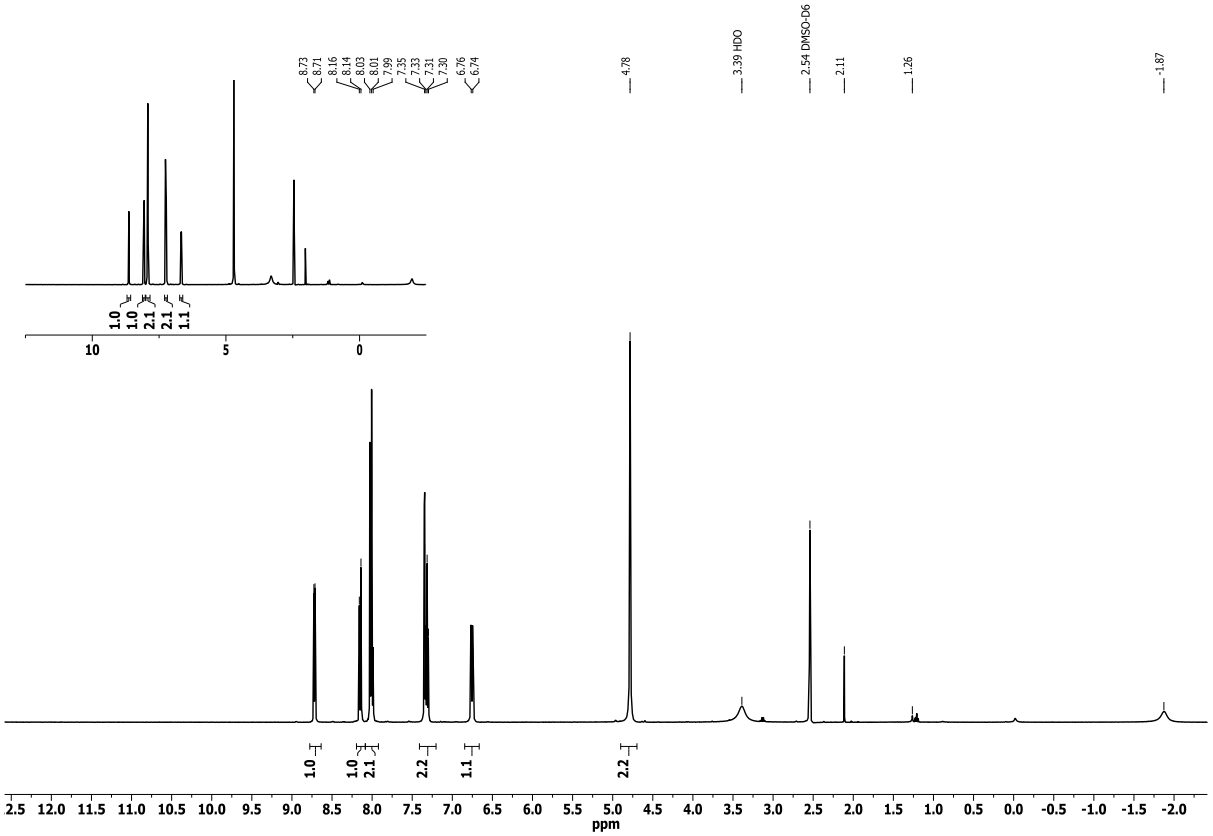


*Figure 9 – ^{1}H NMR spectrum in $CDCl_3$ of complex **3***

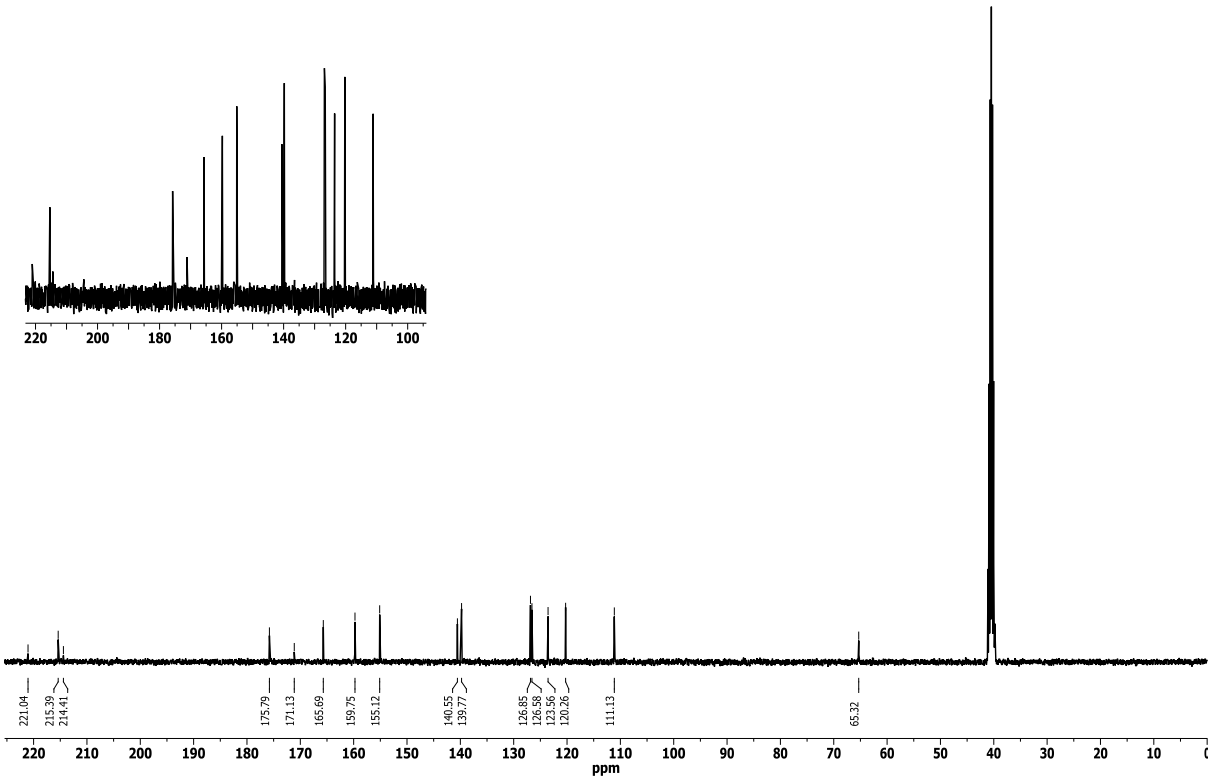


*Figure 10 – ^{13}C NMR spectrum in $CDCl_3$ of complex **3**.*

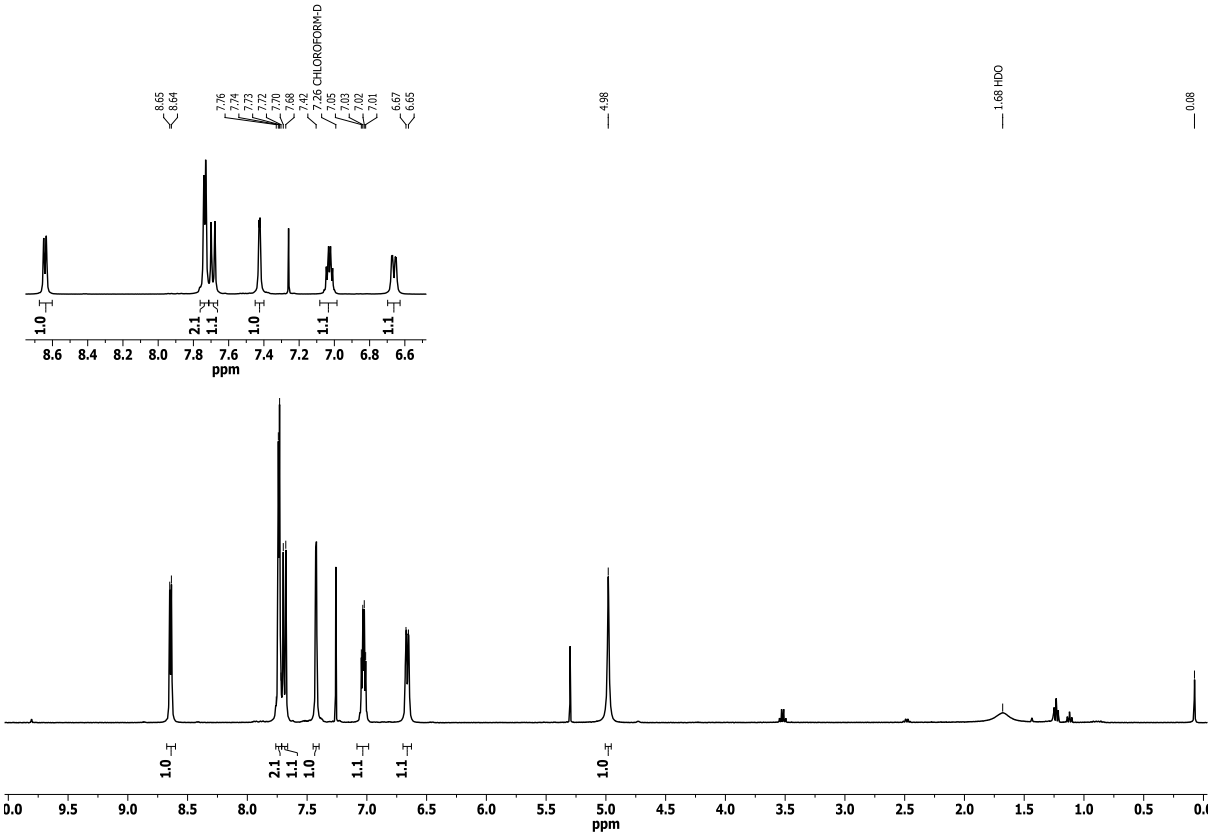


Figure 11 – ^{1}H NMR spectrum in $CDCl_3$ of complex ***6***

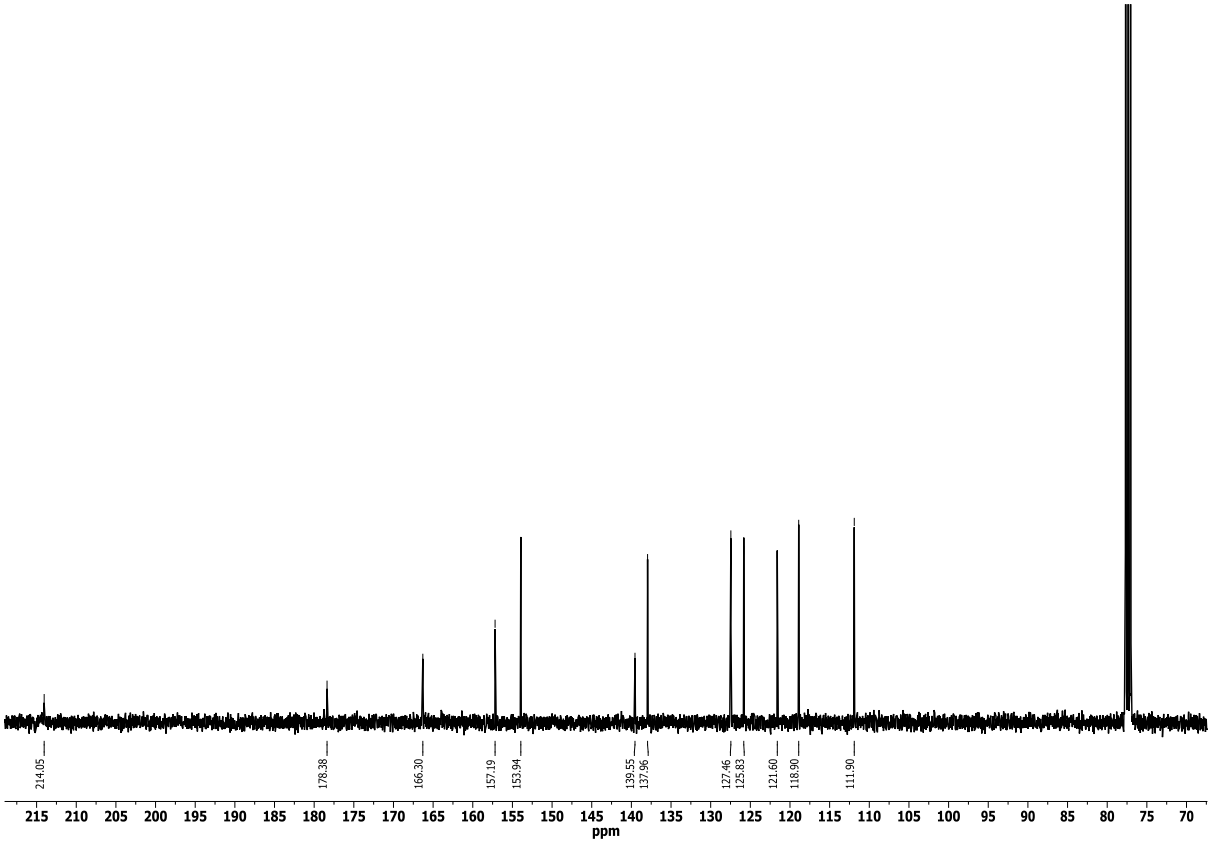


Figure 12 – ^{13}C NMR spectrum in $CDCl_3$ of complex ***6.***

S7 - Biological testing on RAW 264.7 cells - Experimental details

RAW 264.7 cell culture

RAW 264.7 cells (a murine macrophage cell line) were obtained from cryopreserved stocks and cultured in complete DMEM® (Gibco) containing 10% heat inactivated foetal calf serum (FCS), 2mM L-glutamine, 100 units ml^{-1} penicillin and 0.1 mg ml^{-1} streptomycin at 37 °C and 5% CO_2. When cells reached confluency, they were washed with 1x PBS pH 7.2, and then recovered using a plastic cell scraper before being re-suspended in fresh media, at a 1:5 ratio for routine passage of the cell line. For experimentation, cell number was determined using a haemocytometer and plated at 5 × 10^5 or 5× 10^4 cells per well in 24 or 96 well plates, respectively in a final volume of 1 ml or 200 µl respectively. The cells were incubated at 37 °C overnight and were 80-90% confluent the next day.

Addition of CO-RMs

Medium from overnight cell cultures was replaced with fresh complete DMEM containing the various CO-RMs at different concentrations, without altering the final concentration of DMSO (in which all compounds were diluted prior to the experiment). Cells were incubated with the various CO-RMs overnight.

Alamar blue assay in a 24 well plate.

After incubation with the specified CO-RM, the media from each well was removed and replaced with 1 ml of a 10% Alamar blue (Sigma Aldrich)/90% complete DMEM. Cells were then incubated for 4 hours at 37 °C, after which 200 µl of the culture supernatants were transferred into flat bottomed 96 well plates and absorbance determined using a plate reader at 570 nm with a background subtraction at 630 nm. Results were expressed as a percentage scaled between the difference in absorbance of a 1% Triton-X-114 control (dead cells) and a live control with no compound.

LDH assay in a 96 well plate

After incubation with the specified CO-RM, cell cultures were centrifuged at 800 *g* for 5 minutes to pellet cell debris. 50 µl aliquots of supernatant were then removed from each well and added to a flat bottomed 96 well plate alongside 100 µl of LDH reaction mixture (Sigma Aldrich). Absorbance was measured at various time intervals at 490 nm (subtraction at 630 nm) until the absorbance of the 1% Triton control reached at least 1.6, which will allow detection of low toxicity, without saturating the reaction. Results were expressed as a percentage scaled between the difference in absorbance of a 1% Triton-X-114 control (dead cells) and no compound control. Blank media was also used in this assay and the absorbance from this was subtracted from all wells.

S7.1 - LDH assay data on RAW 264.7 macrophages with complexes 2, 3 and 6

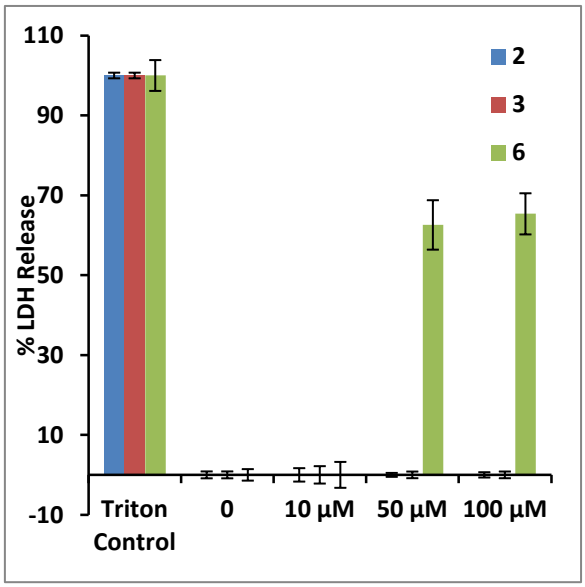


*Figure 13 - LDH assay data on RAW 264.7 macrophages with complexes **2**, **3** and **6**. Values are scaled between a 100% control (live cells in media) and a 0% control (1% Triton X-114 added).*

S8 - Degradation study of complex 3 in 50:50 CH_3CN/H_2O by ESI-MS

Complex **3** was dissolved in 5 ml 50:50 CH_3CN/H_2O at a concentration of 0.5 mg ml^{-1}. The solution was passed through a 0.45 µl syringe and a 100 µl aliquot was taken and diluted by 10. The solution was analysed using a capillary exit voltage of 46 V with a skimmer voltage of 45 V. 4 µl injections were used for all measurements.

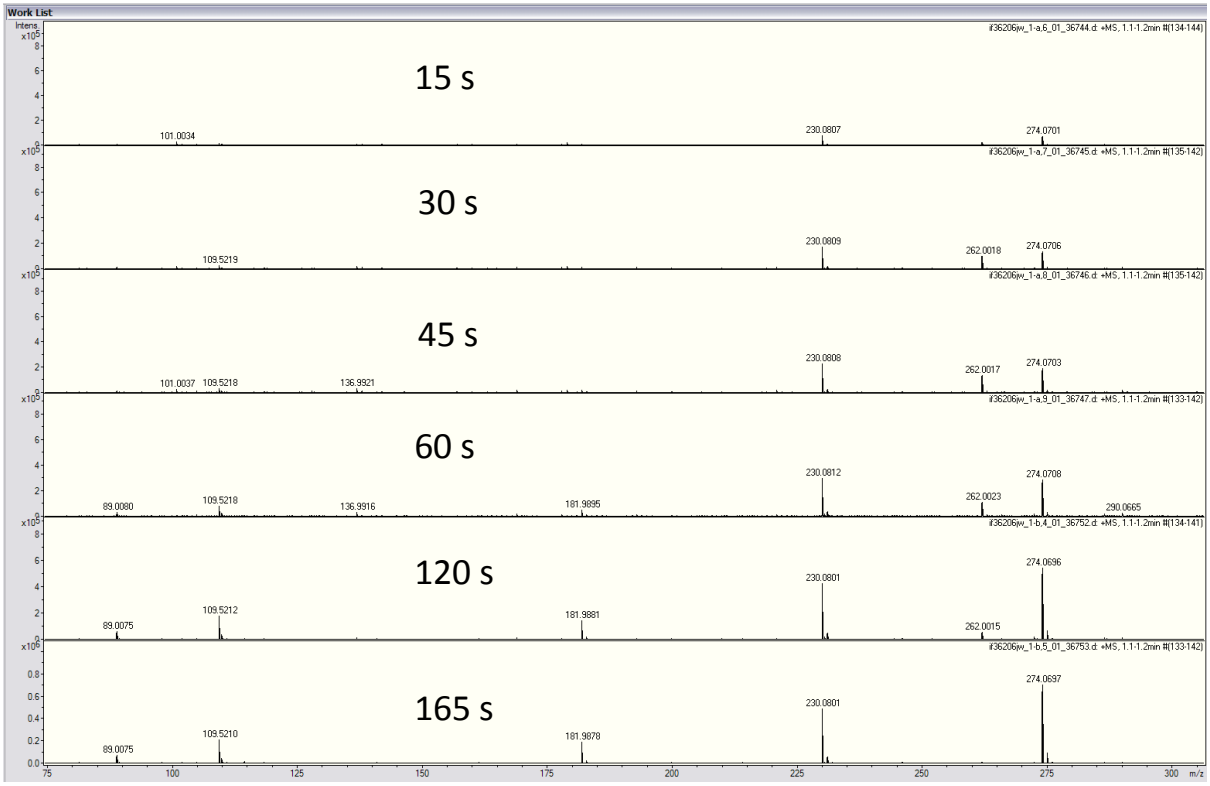


*Figure 14 - The photo-degradation of complex **3** from 75-300 m/z by ESI-MS analysis. Total irradiation time indicated above.*

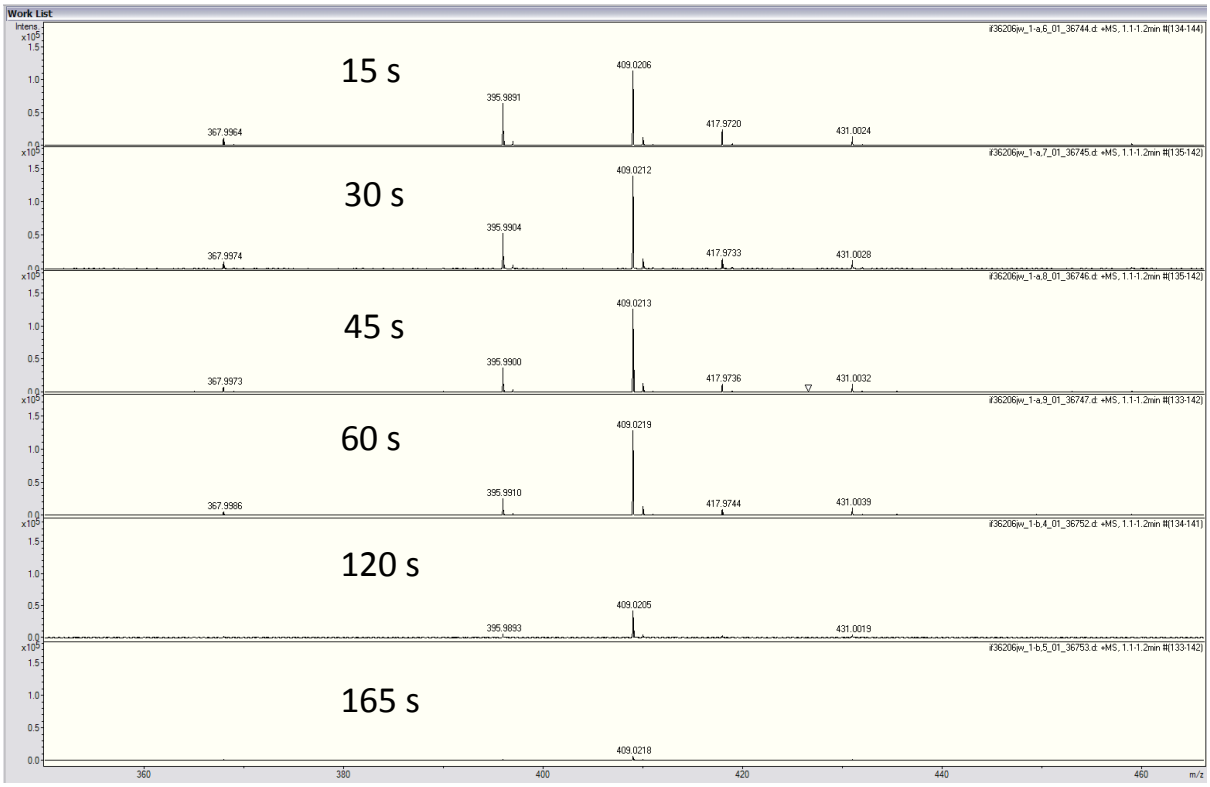


*Figure 15 - The photo-degradation of complex **3** from 300-460 m/z by ESI-MS analysis. Total irradiation time indicated above.*

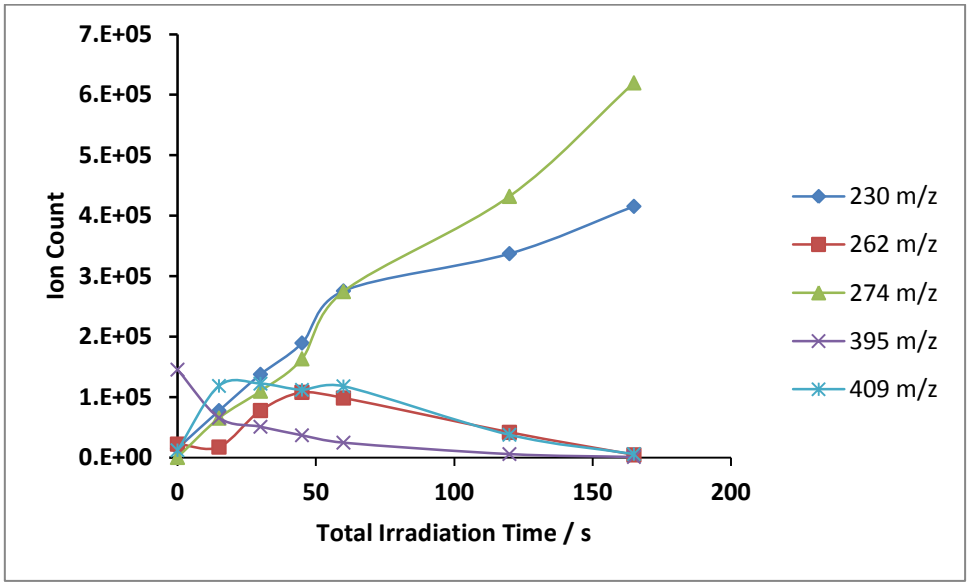


*Figure 16 - The ion count for key species over time during the photo-degradation of complex **3**. Irradiation carried out with an LED (400 nm, 2.4 W).*

S9 – References

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