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Supporting Information

Post-functionalization of platinum-NHC complexes by oxime ligation for ligand targeted therapy.

by

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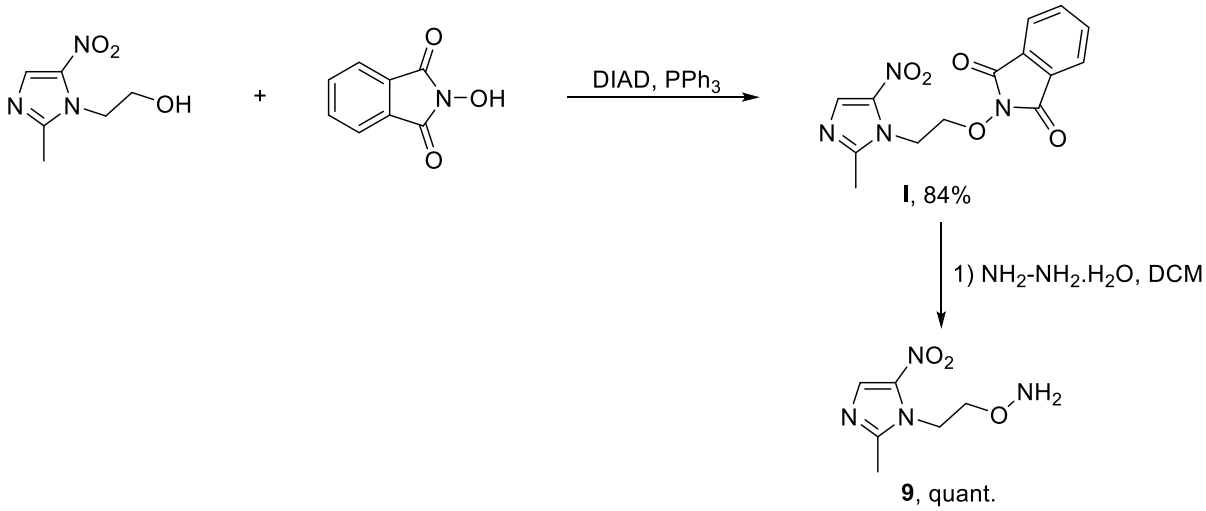
General Considerations

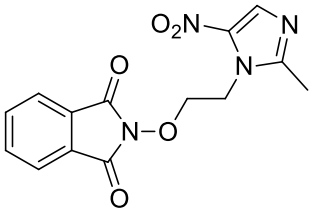
All manipulations were performed under inert atmosphere (argon) by using standard vacuum line and Schlenk tube techniques. Glassware was dried at 120 °C in an oven for at least three hours. Toluene, CH_2Cl_2 and THF were dried using an SPS system and water content was quantified by Karl Fischer titration. Ethanol and acetonitrile were deoxygenated by bubbling argon for 30 min and stored over activated 4Å molecular sieves. Cyclohexylamine, pyridine and NEt_3 were distilled over CaH_2 and stored under argon. NaI and K_2CO_3 were dried over two days under reduced pressure at 200 °C. All other reagents were commercially available and used as received. NMR spectra were recorded on Bruker AV300 spectrometer. Chemical shifts are reported in ppm (δ) compared to TMS (^1H and ^{13}C) using the residual peak of deuterated solvent as internal standard. MS spectra were performed by the mass spectrometry service of the "Institut de Chimie de Strasbourg" on microTOF, Bruker Daltonics. Elemental analyses were performed by the 'service d'analyse élémentaire' of the Strasbourg chemistry department.

Synthetic pathways and procedures

Ligand **9** was synthetized using the following synthetic pathway:



Synthesis of **I**

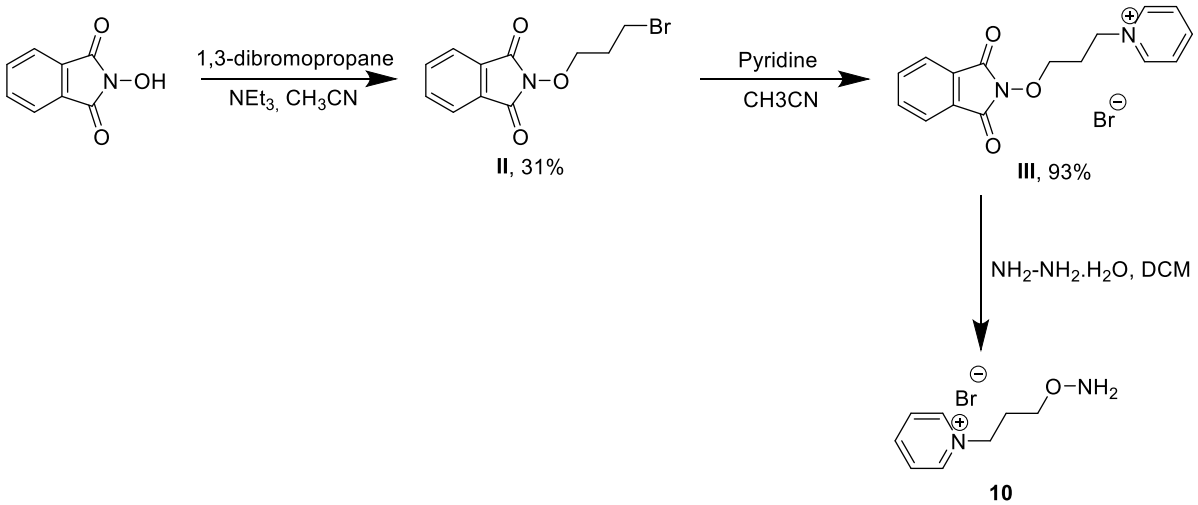


To a stirred solution of metronidazole (1.027 g, 6 mmol), N-hydroxyphtalimide (1.468 g, 9 mmol) and PPh_3 (2.361 g, 9 mmol) in dry THF (50 mL) at 0°C, a solution of DIAD (3.780 mL, 19.2 mmol) was added dropwise. When the addition was done, the solvent was evaporated and the oily residue was triturated with Et_2O. The precipitate was filtered off to give **I** as a white powder (1.587 g, 84%).

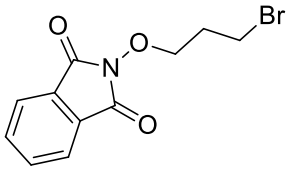
^{1}H-NMR ($CDCl_3$, 300 MHz, 20 °C): δ 2.61 (s, 3H), 4.57 (t, J = 4.8, 2H), 4.73 (t, J = 4.8 Hz, 2H), 7.74-7.79 (m, 2H), 7.80-7.83 (m, 2H), 8.04 (s, 1H). **^{13}C-NMR** ($CDCl_3$, 75 MHz, 20 °C): δ 14.6, 45.1, 77.5, 123.7, 128.5, 133.6, 134.8, 138.3, 151.9, 162.9. MS (positive ESI) [M+Na]: calculated for $C_{14}H_{12}N_4O_5Na$ 339.07, found 339.07.

9 was synthesized by mixing **I** (200 mg, 0.63 mmol) with hydrazine monohydrate (154 μL, 3.16 mmol, 5 eq) in chloroform at 0°C. After stirring 1h, the solid formed was removed by filtration on a celite plug. The volatiles were removed under vacuum and **9** was used without further purification and characterization.

Ligand **10** was synthetized using the following synthetic pathway:



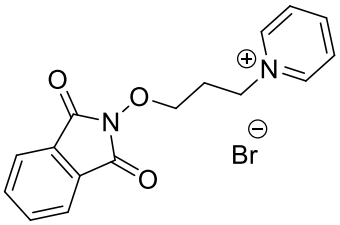
Synthesis of **II**



N-hydroxyphtalimide (4.895 g, 30 mmol), 1,3-dibromopropane (6.090 mL, 60 mmol) and NEt_3 (8.363 mL, 60 mmol) were added to CH_3CN (100 mL) and heated to reflux overnight. After evaporation of the volatiles, the crude mixture was purified on SiO_2 column chromatography (cyclohexane/AcOEt 98/2 to 8/2) to afford **II** as a white powder (2.616 g, 31%).

^{1}H-NMR ($CDCl_3$, 300 MHz, 20 °C): δ 2.30 (quint, J = 6.0 Hz, 2H), 3.70 (t, J = 6.4 Hz, 2H), 4.36 (t, J = 5.8 Hz, 2H), 7.73-7.78 (m, 2H), 7.81-7.85 (m, 2H). **^{13}C-NMR** ($CDCl_3$, 75 MHz, 20 °C): δ 29.3, 31.4, 76.1, 123.6, 128.8, 134.6, 163.6.

Synthesis of **III**

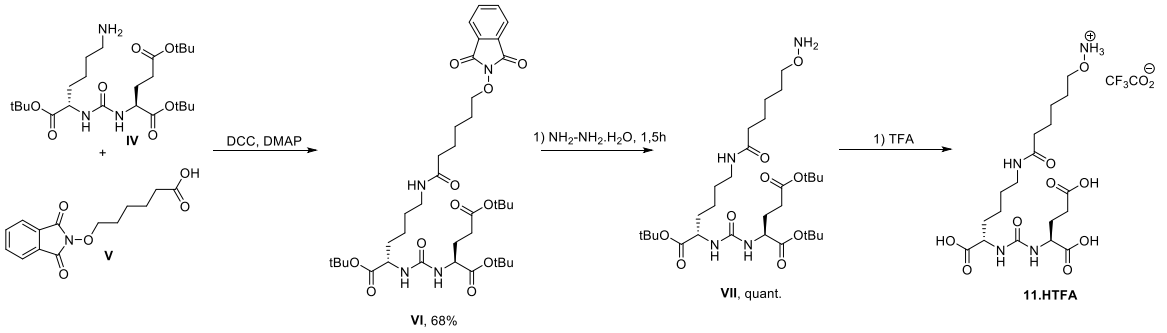


II (1 g, 3.52 mmol) and pyridine (1.708 mL, 21.1 mmol) were added to CH_3CN and heated to reflux overnight. After cooling down to room temperature, Et_2O was added to mixture giving a white precipitate which was filtered on frit to afford **III** (1.193 g, 93%) as a white powder.

^{1}H-NMR (Methanol-d4, 300 MHz, 20 °C): δ 2.42 (quint, J = 6.2 Hz, 2H), 4.25 (t, J = 5.7 Hz, 2H), 4.89 (t, J = 7.0 Hz, 2H), 7.87 (s, 4H), 8.21 (t, J = 7.0 Hz, 2H), 8.65 (t, J = 7.5 Hz, 1H), 9.20 (d, J = 5.7 Hz, 2H). **^{13}C-NMR** ($CDCl_3$, 75 MHz, 20 °C): δ 29.4, 58.0, 74.3, 123.3, 128.1, 128.5, 134.9, 145.1, 145.7. MS (positive ESI) [M+]: calculated for $C_{16}H_{15}N_2O_3$ 283.11, found 283.11.

10 was synthesized by mixing **III** (30 mg, 0.08 mmol) with hydrazine monohydrate (20 μL, 0.41 mmol, 5 eq) in chloroform at 0°C. After stirring 1h, the solid formed was removed by filtration on a celite plug. The volatiles were removed under vacuum and **10** was used without further purification and characterization.

Ligand **11** was synthetized using the following synthetic pathway:



Synthesis of **VI**

V (47 mg, 0.17 mmol) was slowly added to a solution of DCC (35 mg, 0.17 mmol) and DMAP (2 mg, 0.017 mmol) in DCM (10 mL) at 0°C. TRhe solution was stirred at room temperature during 1 hour. Then, a solution of urea **IV** (54 mg, 0.11 mmol) in DCM (10 mL) was added dropwise to the mixture. The solution was stirred overnight at room temperature. The solution was filtered on celite plug and the volatiles were removed. The crude mixture was purified using SiO_2 column chromatography (cyclohexane/EtOAc, 5/5 to 0/1). **VI** was obtained as a white powder (58 mg, 68%).

^{1}H-NMR ($CDCl_3$, 300 MHz, 20 °C): δ 1.39 (s, 9H), 1.41 (s, 9H), 1.44 (s, 9H), 1.50-1.60 (m, 6H), 1.67-1.89 (m, 6H), 1.99-2.10 (m, 2H), 2.21-2.33 (m, 2H), 3.08-3.16 (m, 1H), 3.26-3.37 (m, 1H), 4.19 (t, J = 6.4 Hz, 2H), 4.27-4.30 (m, 2H), 5.46-5.48 (m, 2H), 6.27-6.41 (m, 1H), 7.71-7.75 (m, 2H), 7.80-7.84 (m, 2H). **^{13}C-NMR** ($CDCl_3$, 75 MHz, 20 °C): δ 22.4, 25.2, 25.3, 27.8, 28.0, 28.8, 31.6, 32.4, 36.3, 38.9, 53.1, 53.2, 78.3, 80.4, 81.5, 82.0, 123.5, 128.9, 134.4, 157.2, 163.7, 172.2, 172.4, 172.7, 173.2.

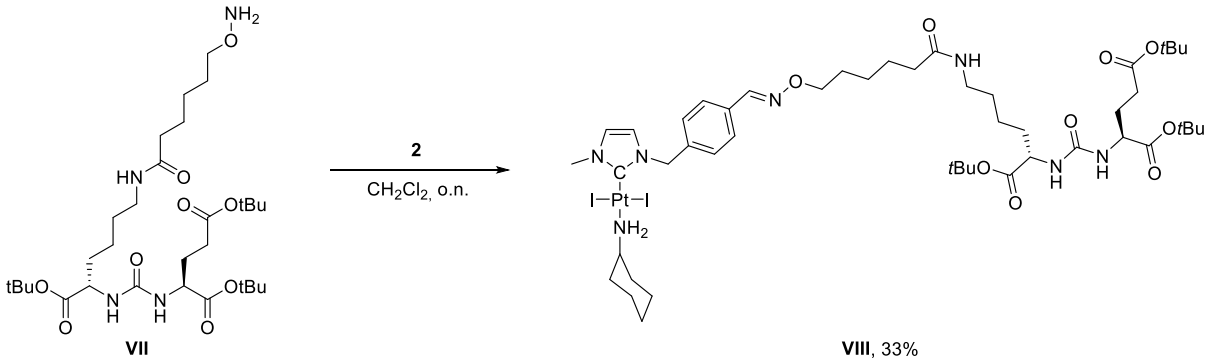
Synthesis of **VII**

VI (60 mg, 0.08 mmol) was dissolved in CHCl3 (10 mL) and NH_2-NH_2.H_2O (20 µl, 0.4 mmol) was added dropwise. The mixture was stirred at room temperature during 1h30. The solids were filtered on a celite plug. After removal of the volatiles, **VII** was obtained as a white powder (50 mg, quant.).

^{1}H-NMR ($CDCl_3$, 300 MHz, 20 °C): δ 1.41 (s, 9H), 1.42 (s, 9H), 1.44 (s, 9H), 1.50-1.60 (m, 6H), 1.47-1.65 (m, 6H), 1.68-2.10 (m, 4H), 2.27-2.33 (m, 2H), 3.06-3.12 (m, 1H), 3.25-3.36 (m, 1H), 3.65 (t, J = 6.5 Hz, 2H), 4.22-4.32 (m, 2H), 5.40 (bs, 2H), 5.57 (d, J = 8.0 Hz, 2H), 5.65 (d, J = 8.3 Hz, 2H), 6.74 (t, J = 8.0 Hz, 1H). **^{13}C-NMR** ($CDCl_3$, 75 MHz, 20 °C): δ 22.5, 25.6, 25.6, 28.0, 28.0, 28.8, 31.6, 32.4, 36.3, 38.8, 53.1, 53.3, 76.0, 80.5, 81.5, 82.1, 157.3, 172.2, 172.4, 173.0, 173.3.

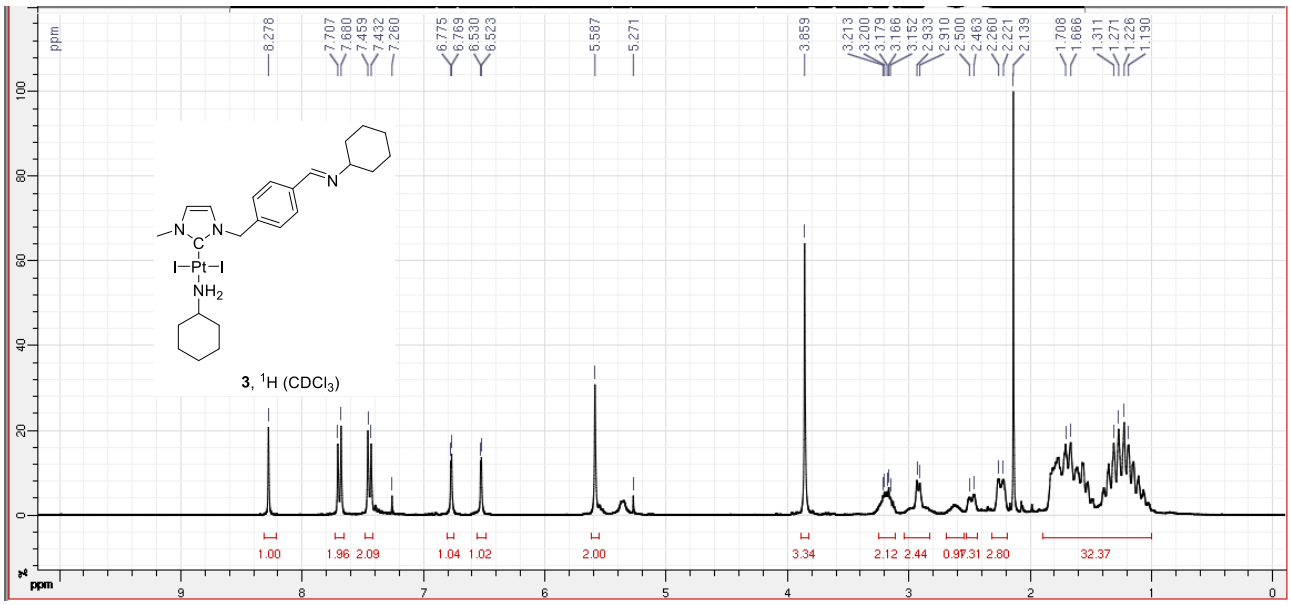
11.HTFA was synthesized by mixing **VII** (30 mg, 0.08 mmol) with TFA (20 µL, 0.41 mmol, 5 eq) in DCM at 0°C. After stirring 10 minutes, the mixture was quenched with water and the volatiles were removed under vaccuo. **11.HTFA** was used without any further characterization.

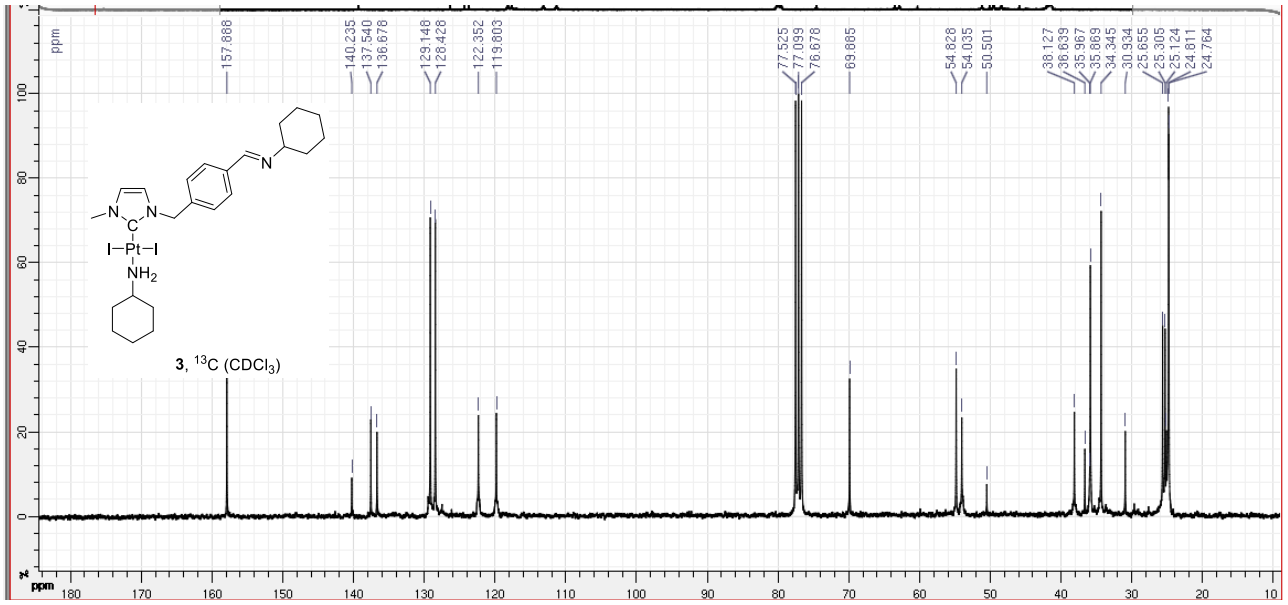
*t*Bu-protected PSMA complex (**VIII**) could be synthesized using the following synthetic pathway:

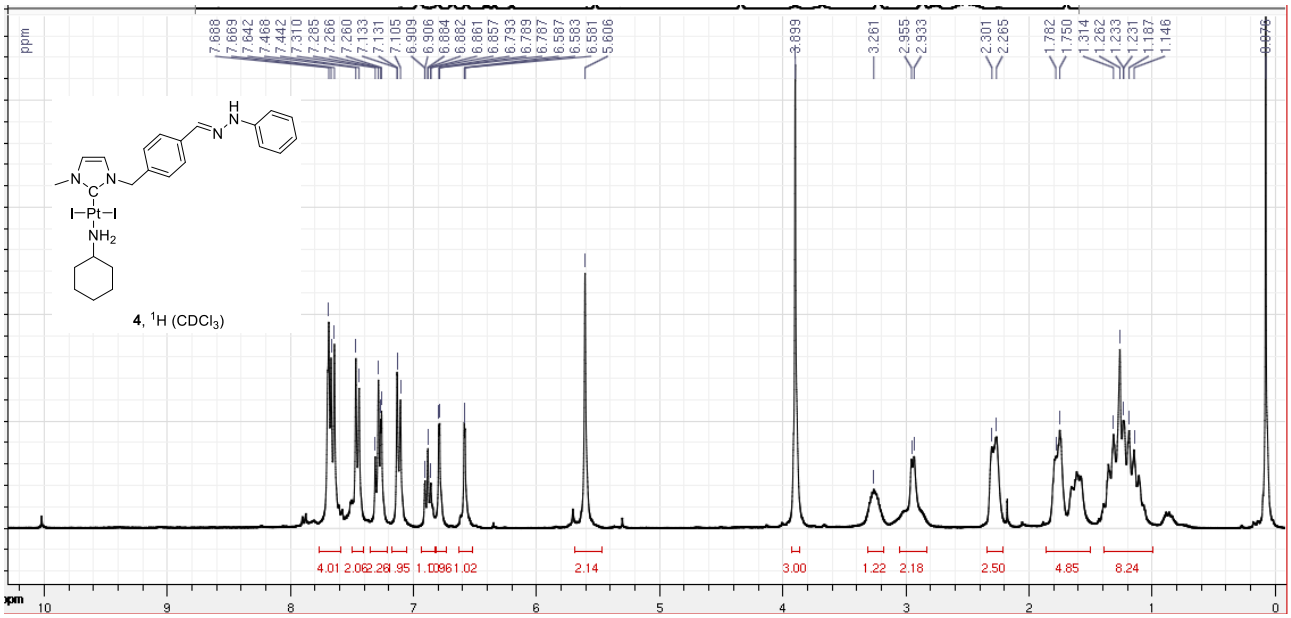


Complex VIII. Complex **2** (76 mg, 0.1 mmol) and **VII** (53 mg, 0.09 mmol) in anhydrous dichloromethane was stirred overnight at room temperature. The volatiles removed under reduced pressure and the crude product was purified by chromatography (SiO_2, CH_2Cl_2/cyclohexane) to afford **VIII** as a yellow solid (38 mg, 33%). ^{1}H-NMR ($CDCl_3$, 300 MHz, 20 °C): δ 1.07-1.39 (m, 18H), 1.43 (s, 9H), 1.45 (s, 9H), 1.46 (s, 9H), 1.56-1.95 (m, 15H), 1.98-2.15 (m, 2H), 2.15-2.40 (m, 7H), 2.80-3.05 (m, 2H), 3.09-3.36 (m, 4H), 3.90 (s, 3H), 4.16 (t, J = 6.7 Hz, 2H), 4.22-4.37 (m, 2H), 5.30 (t, J = 7.3 Hz, 2H), 5.61 (s, 2H), 6.00-6.09 (m, 1H), 6.57 (d, J = 2.1 Hz, 1H), 6.80 (d, J = 2.1 Hz, 1H), 7.45 (d, J = 8.0 Hz, 2H), 7.57 (d, J = 8.0 Hz, 2H), 8.06 (s, 1H). ^{13}C-NMR ($CDCl_3$, 75 MHz, 20 °C): δ 14.0, 22.4, 24.8, 25.2, 25.6, 25.6, 28.0, 28.0, 28.1, 28.8, 31.6, 32.5, 35.9, 36.5, 38.1, 38.9, 53.1, 53.2, 53.9, 54.8, 74.2, 80.6, 81.7, 82.1, 119.8, 122.4, 127.3, 128.9, 129.2, 131.2, 132.4, 136.9, 140.2, 147.7, 157.0, 172.3, 172.4, 173.2. MS (positive ESI) [M+Na]: calculated for $C_{48}H_{79}I_2N_7O_9PtNa$ 1369.36, found 1369.36.

NMR spectra



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6.861
6.857
6.793
6.789
6.787
6.587
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2.26
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4.85
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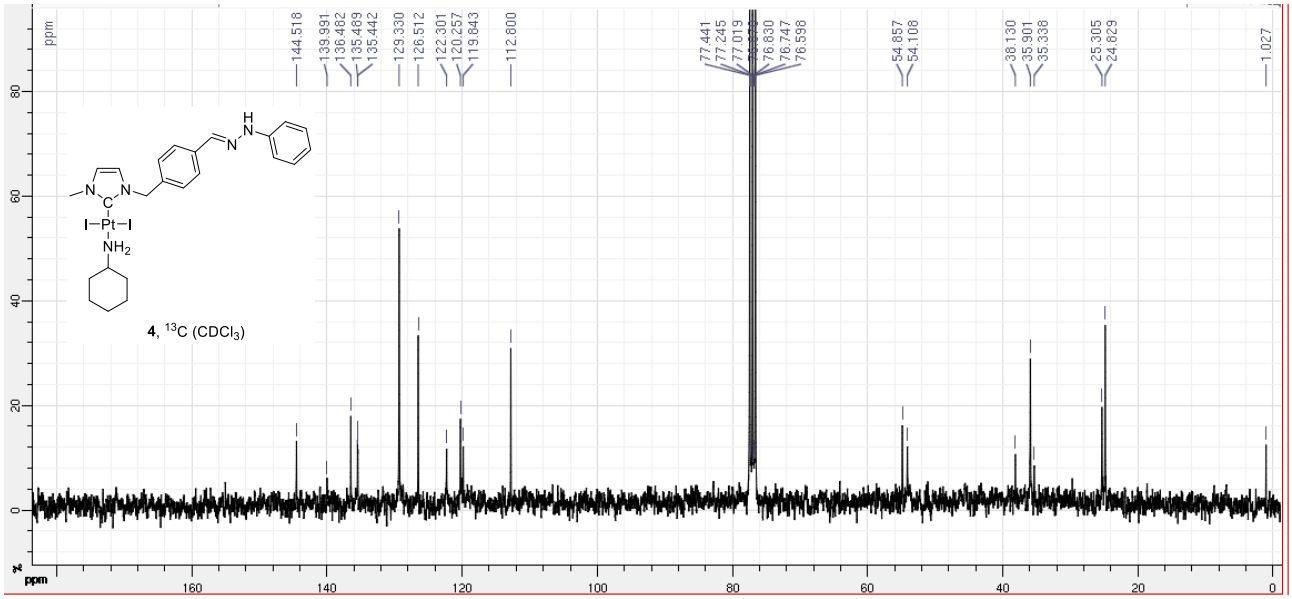
4, ^{1}H ($CDCl_3$)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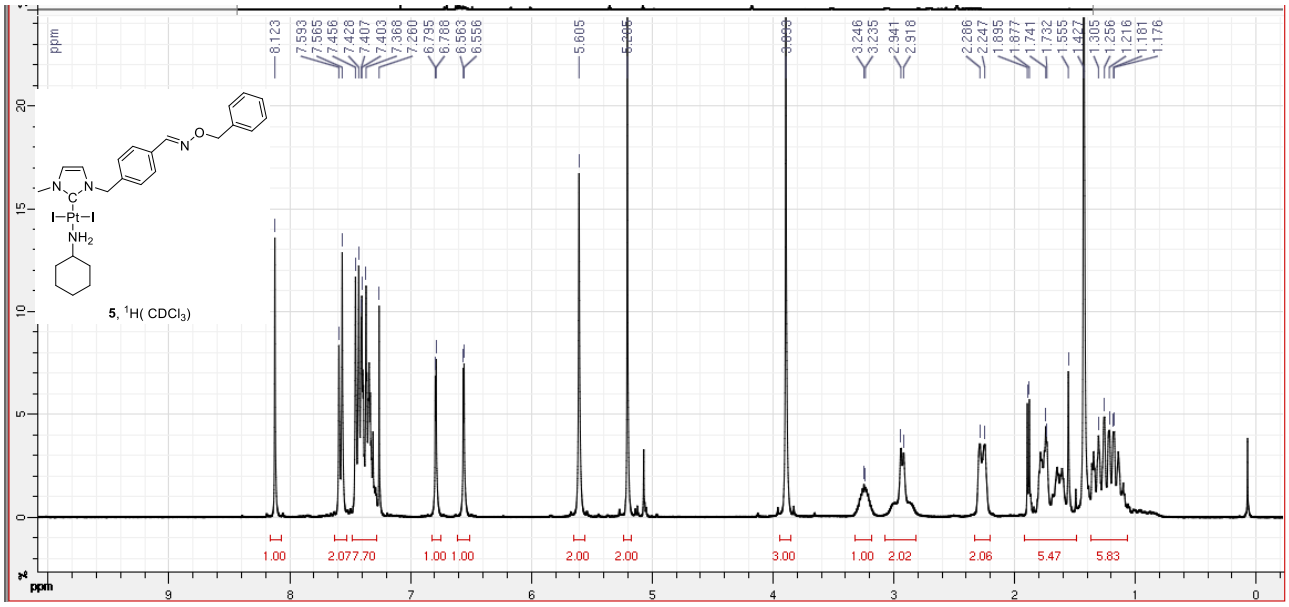
4, ^{13}C ($CDCl_3$)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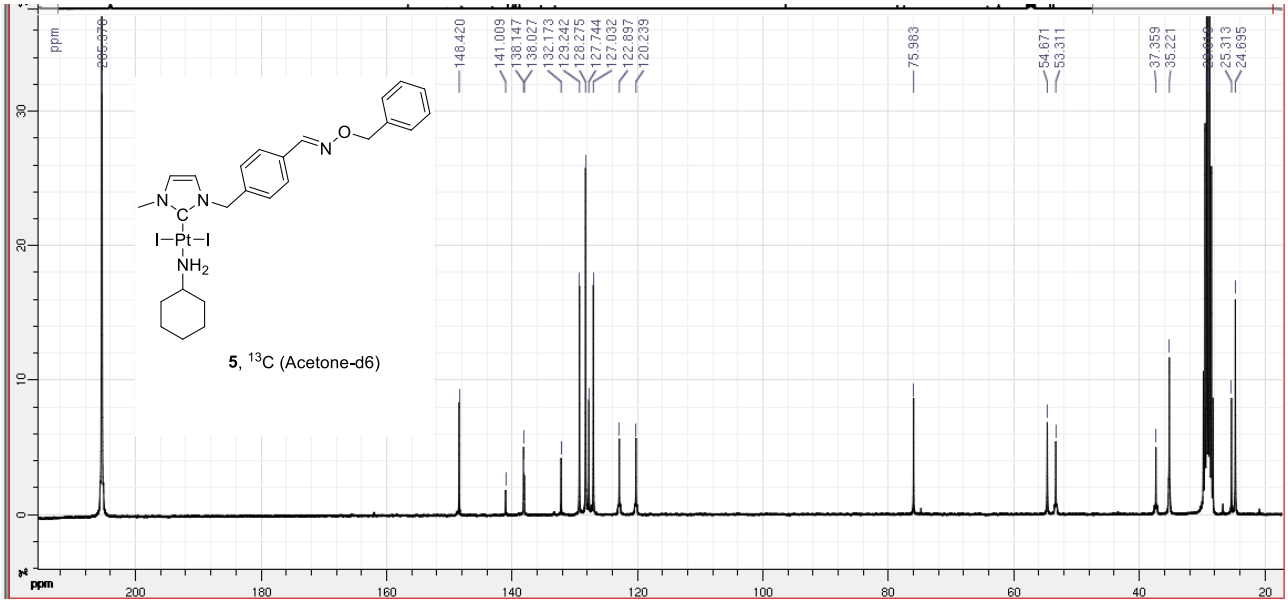
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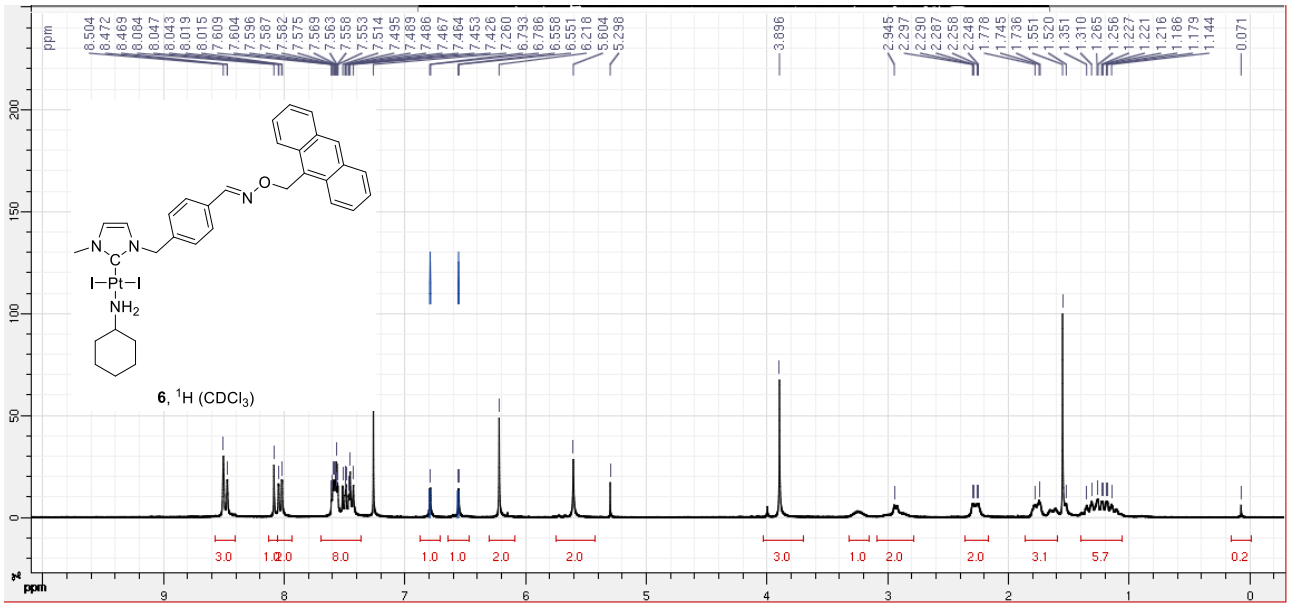
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6, ^{1}H (CDCl$_3$)
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5.7
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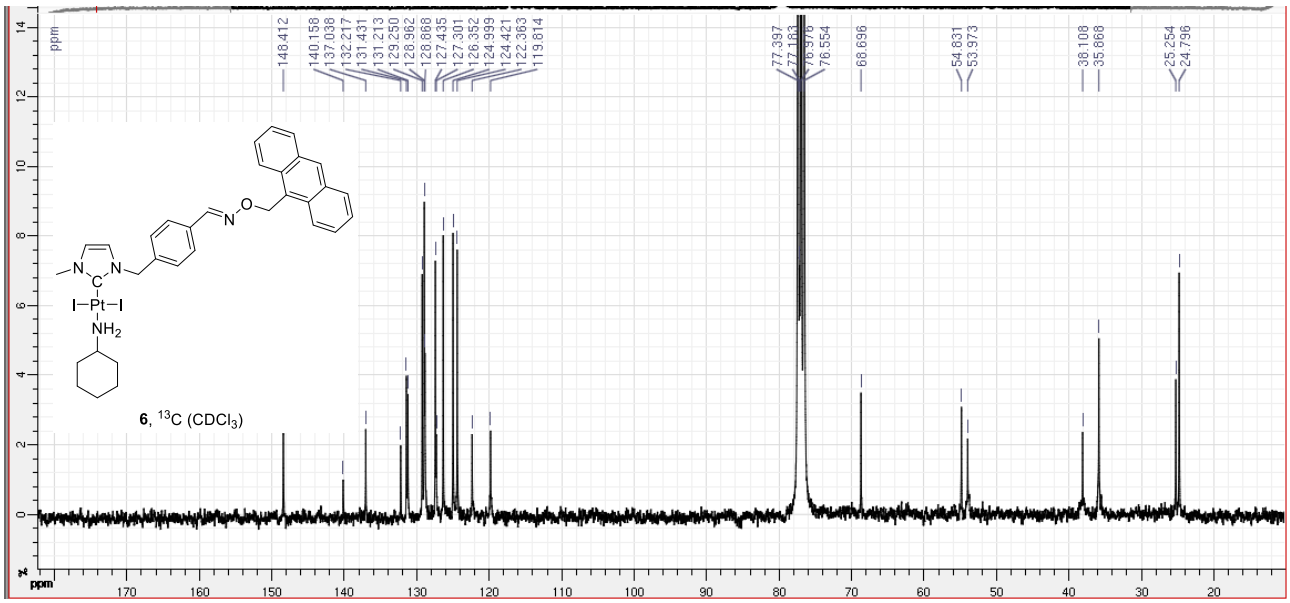
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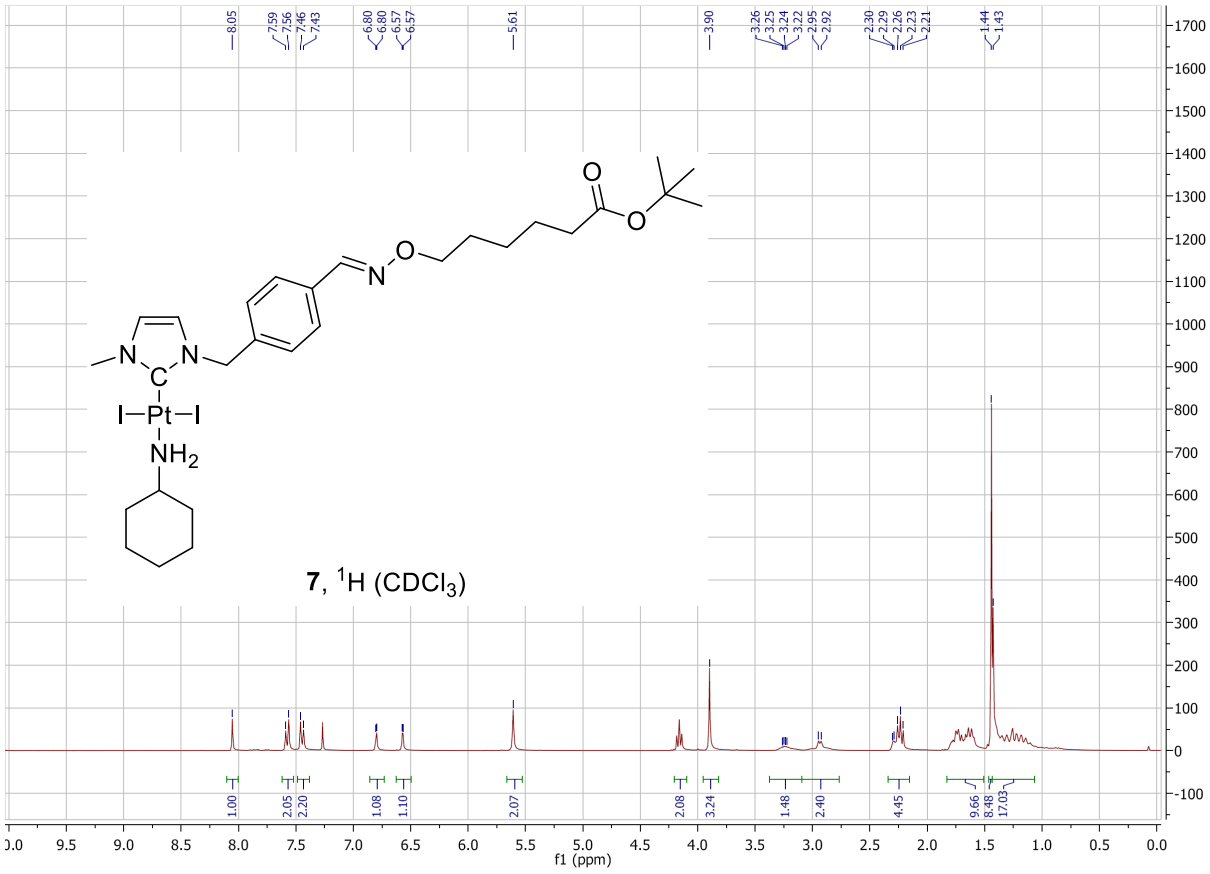
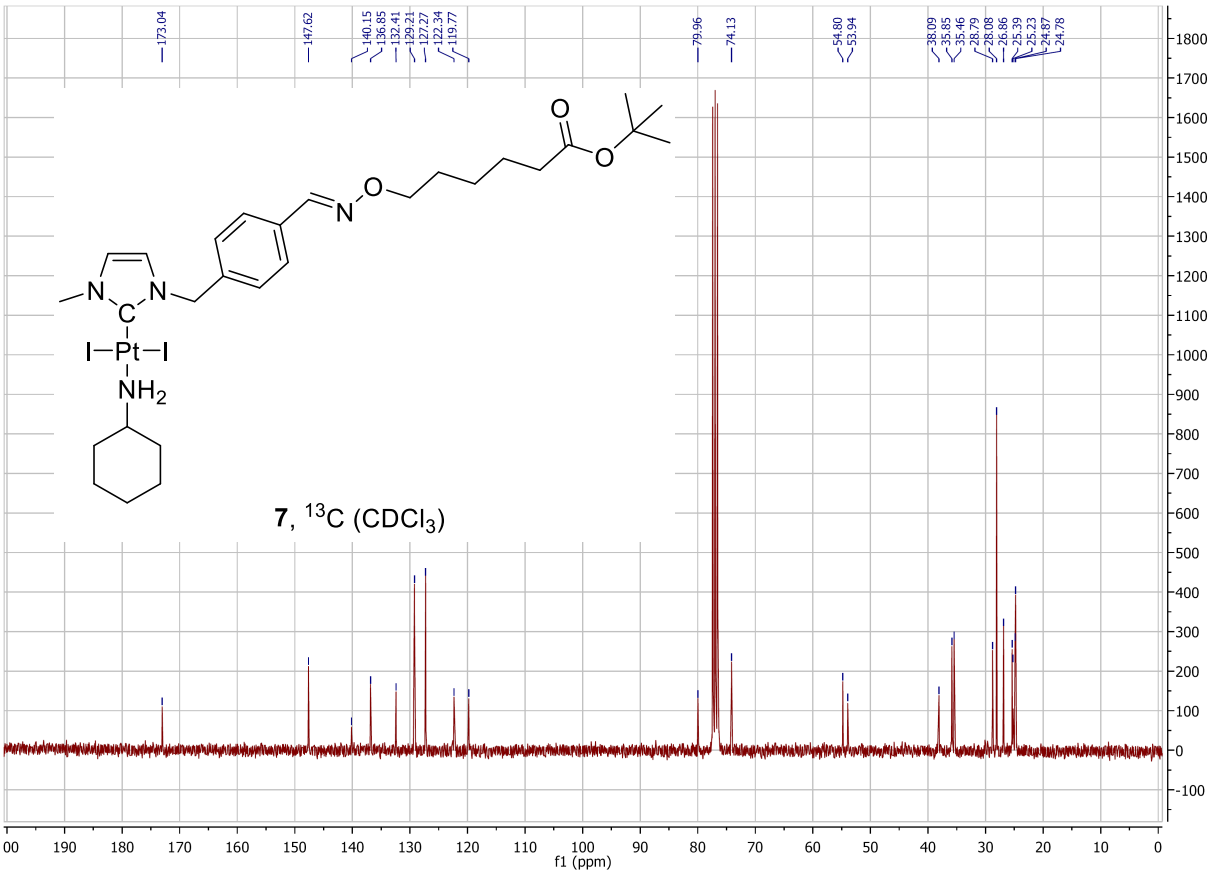
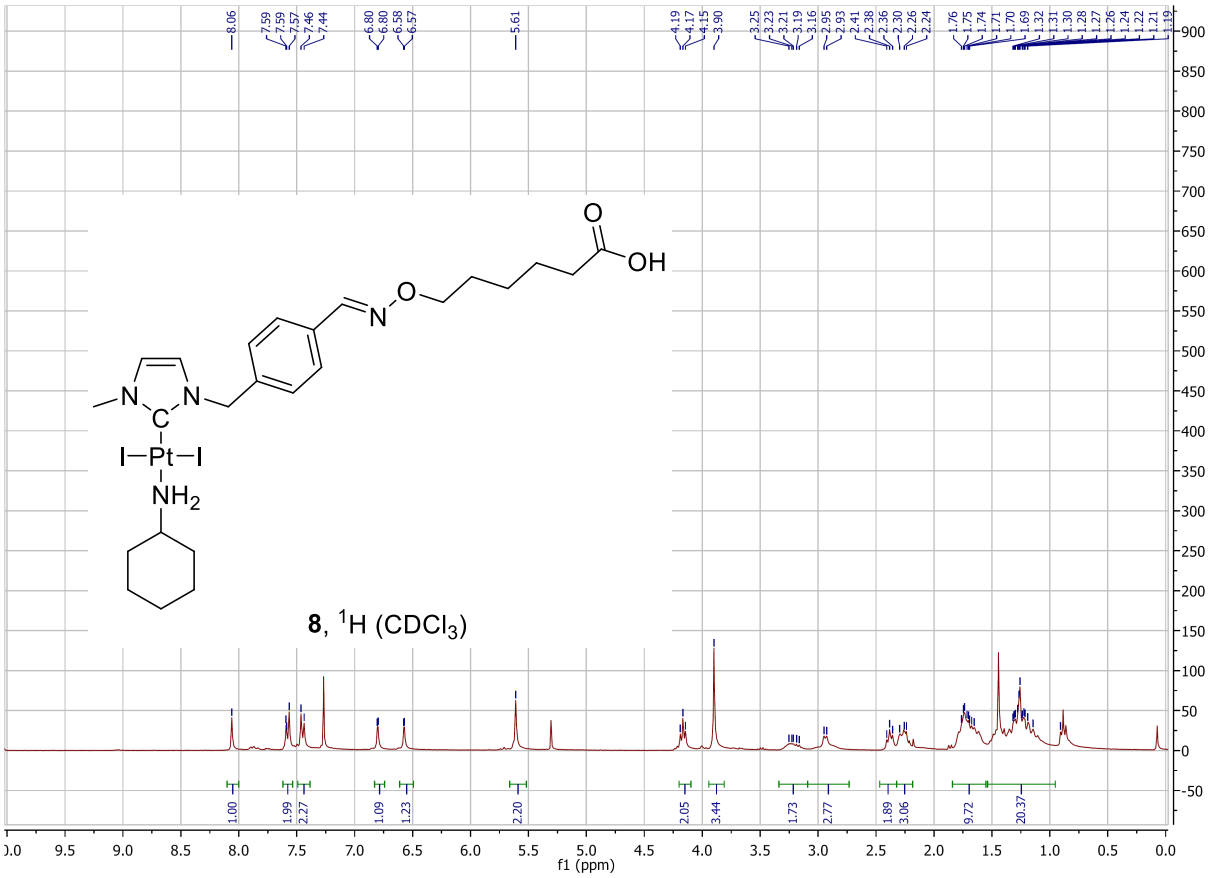
6, ^{13}C (CDCl$_3$)


7, ^{1}H ($CDCl_3$)
f1 (ppm)


7, ^{13}C ($CDCl_3$)
f1 (ppm)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3.90
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3.23
3.21
3.19
3.16
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2.93
2.41
2.38
2.36
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2.24
1.76
1.75
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1.19
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N
O
N
C
N
I—Pt—I
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1.00
1.99
2.27
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3.06
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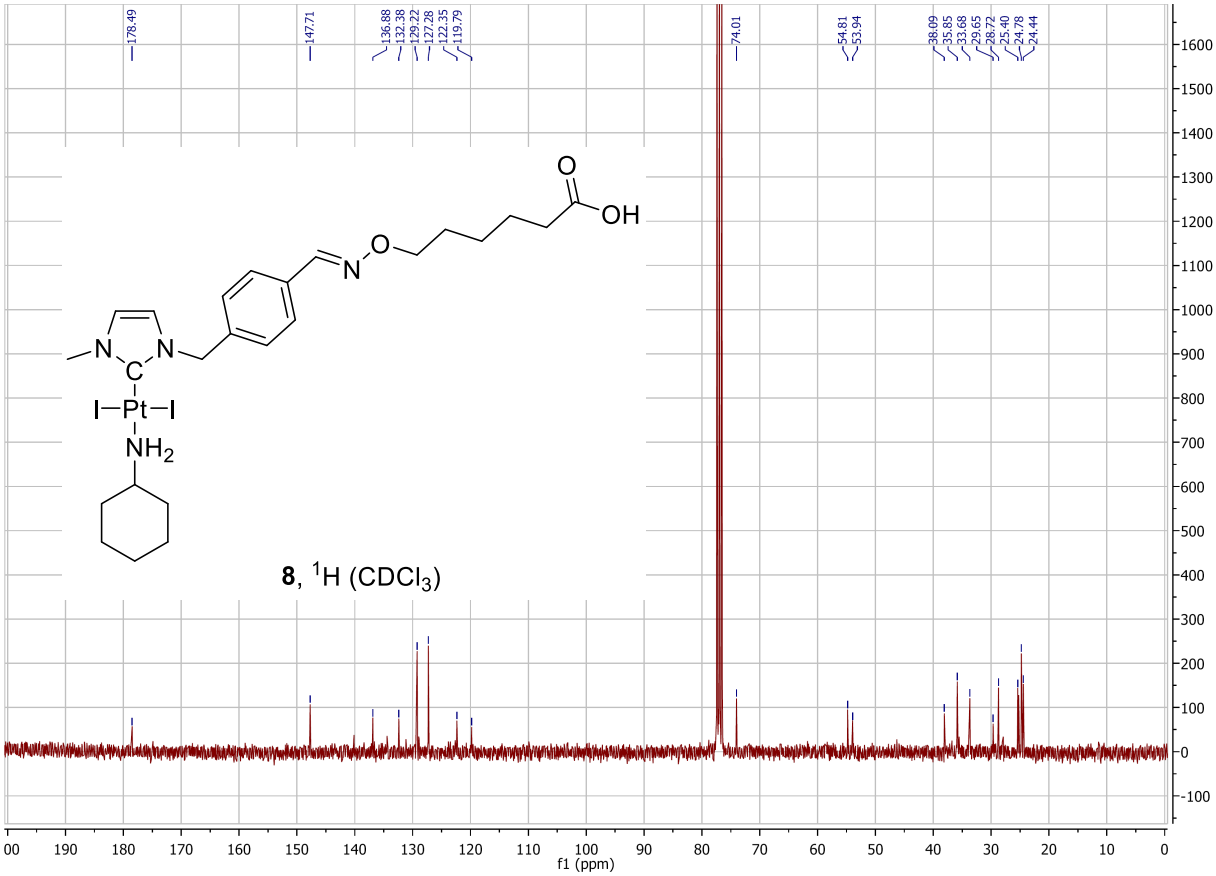
20.37
f1 (ppm)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29.65
28.72
25.40
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N
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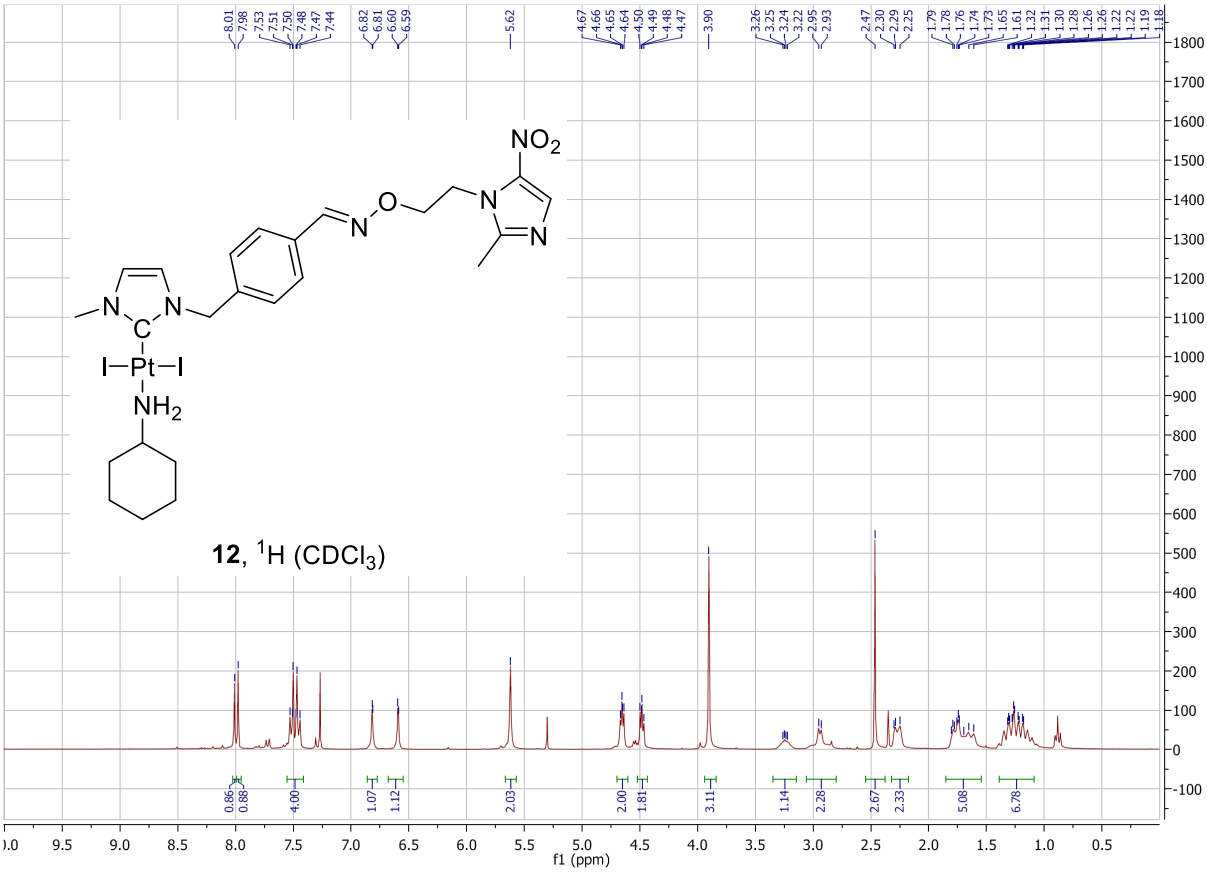
N
C
N
I—Pt—I
NH2
8, 1H (CDCl3)
f1 (ppm)


NO2
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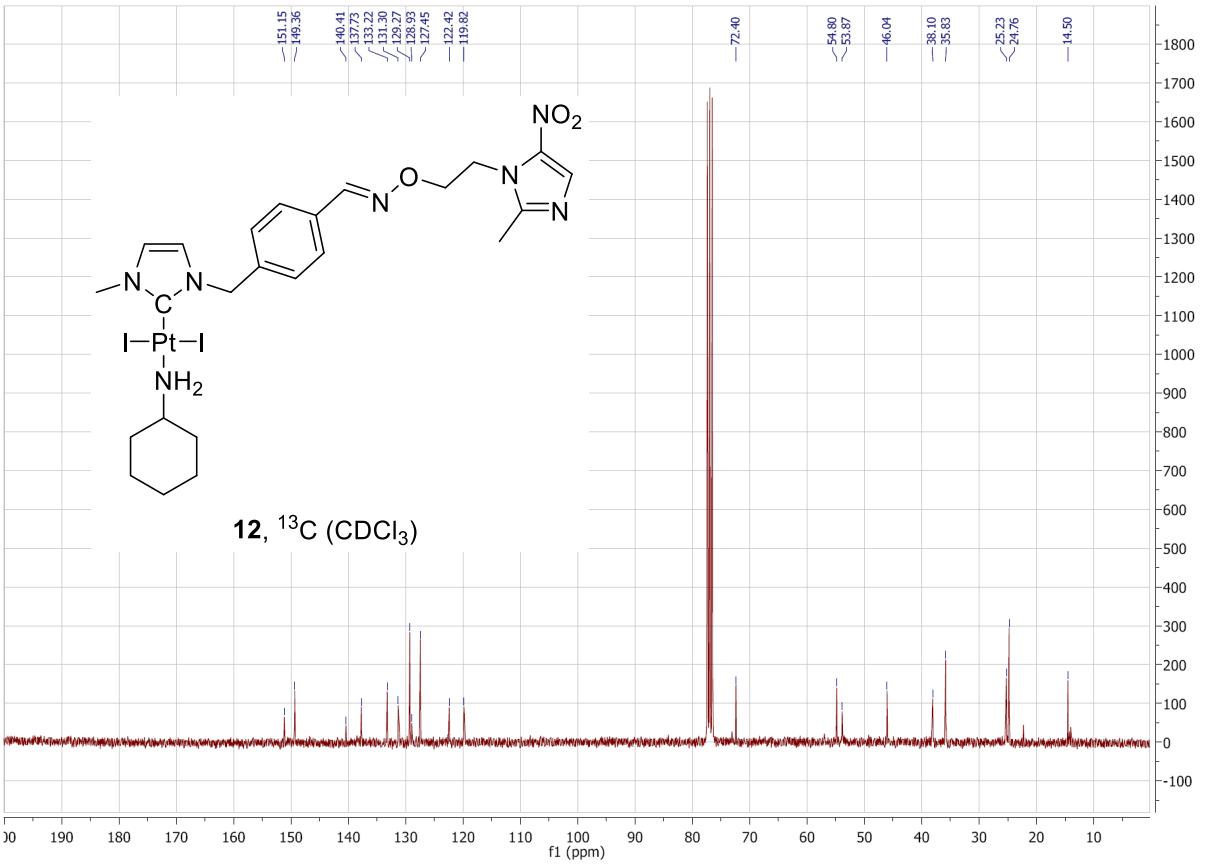
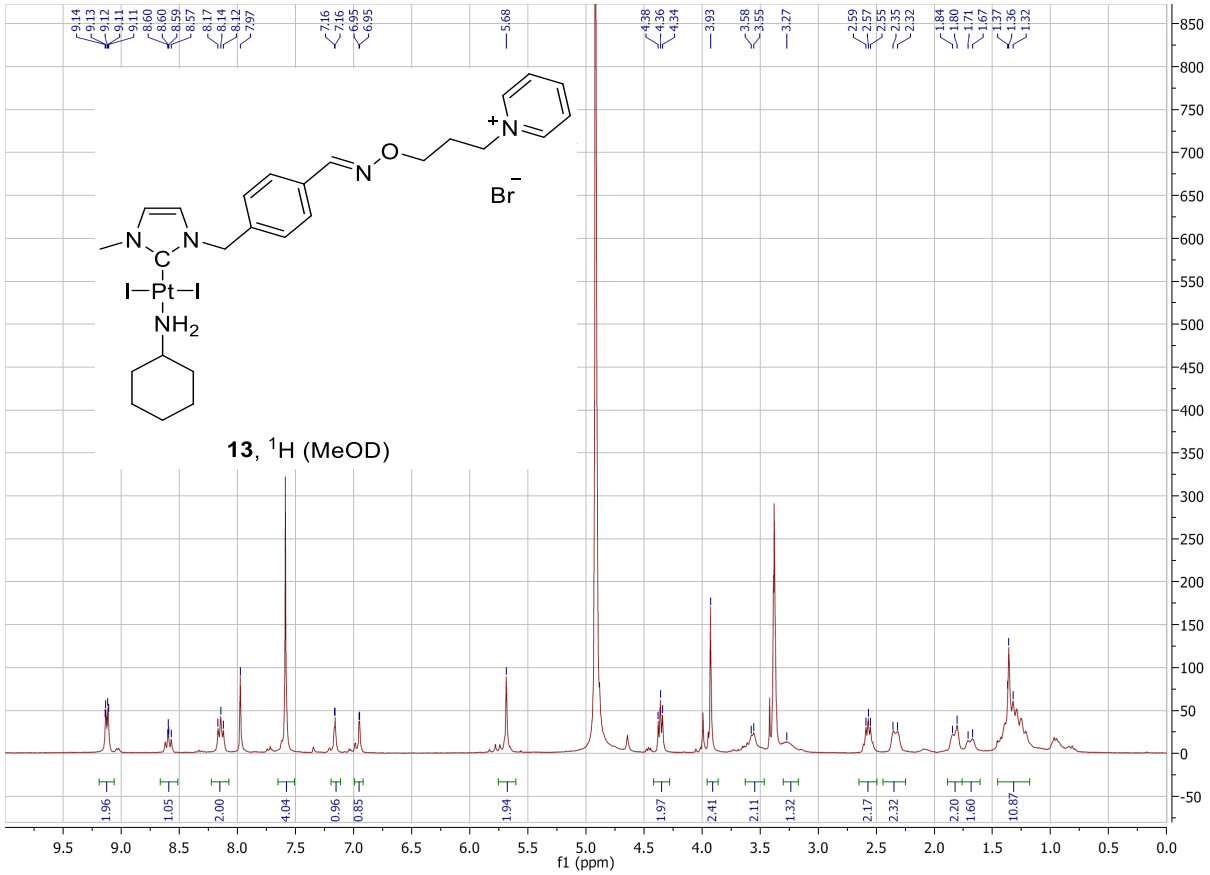
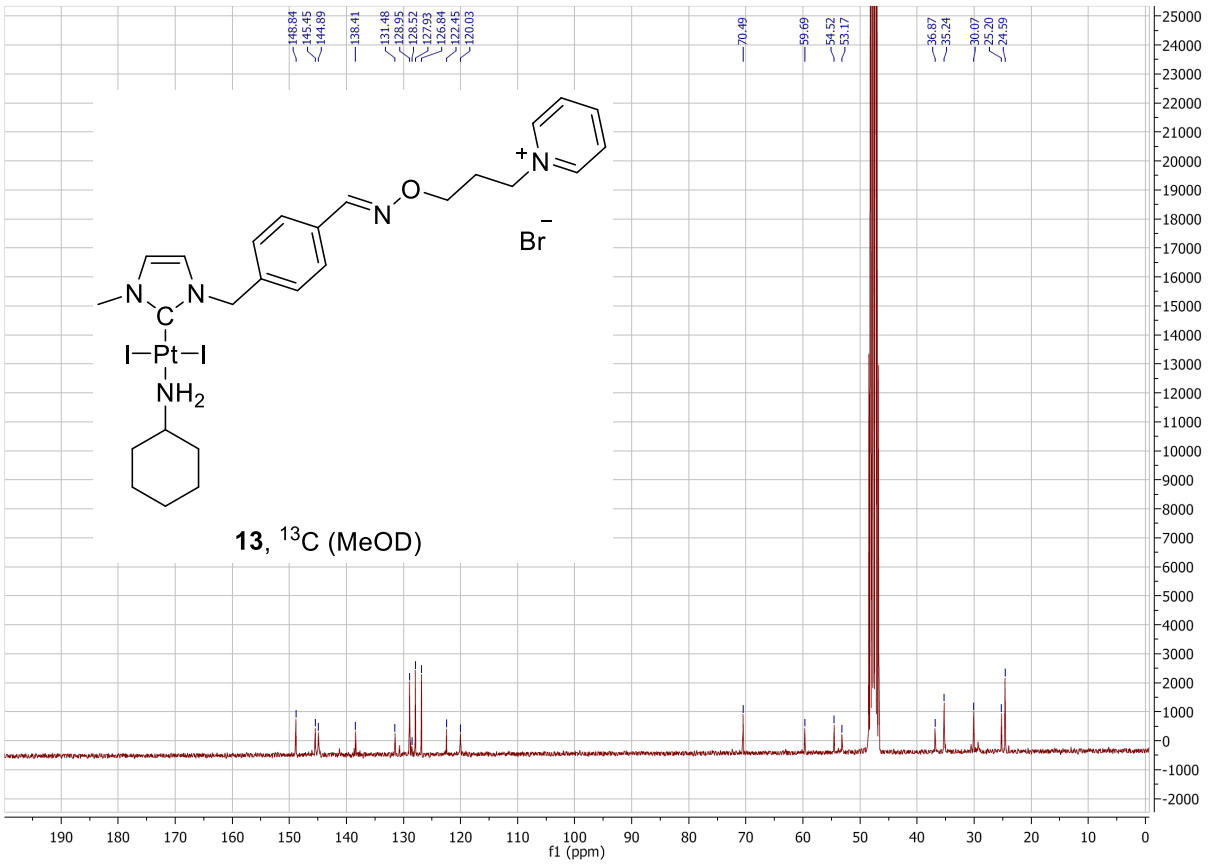
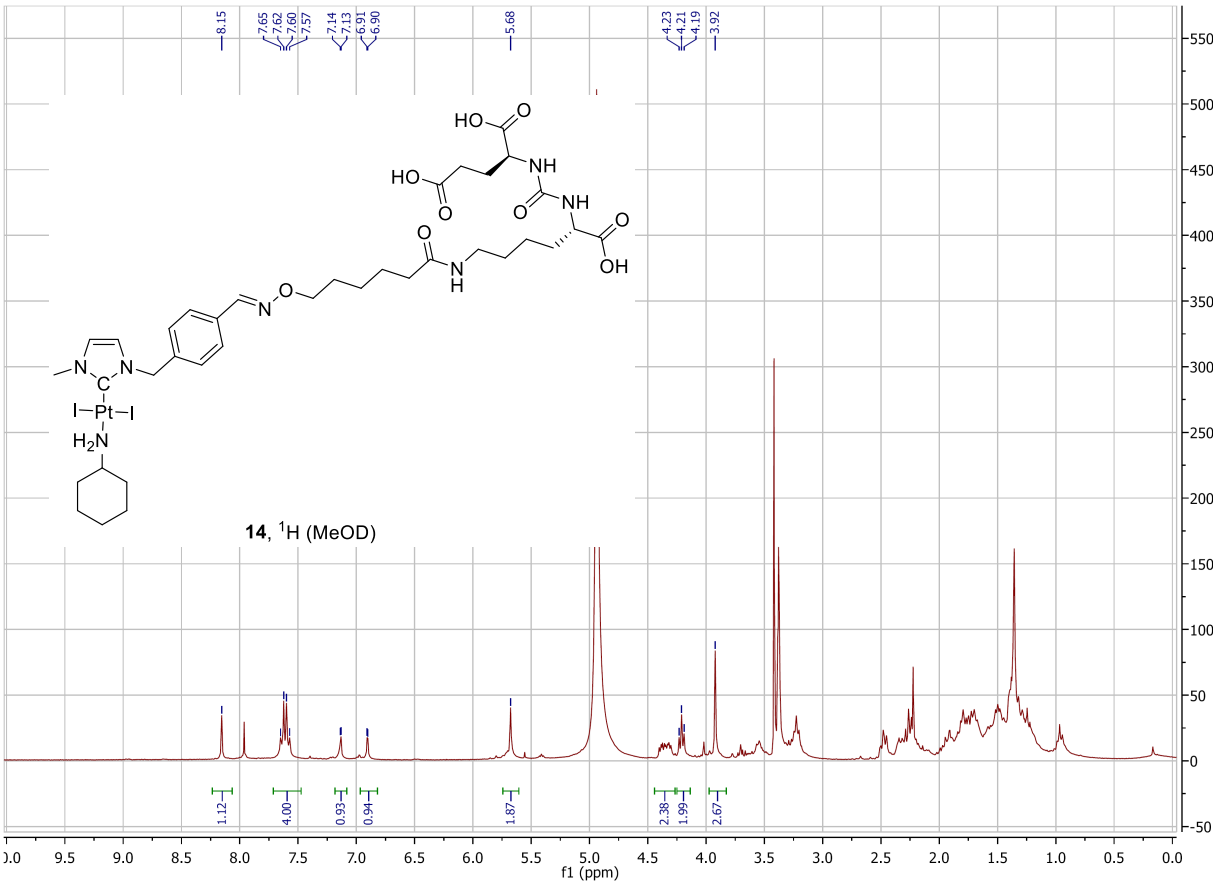
O
N
N
N
C
I–Pt–I
NH2
12, 1H (CDCl3)
f1 (ppm)

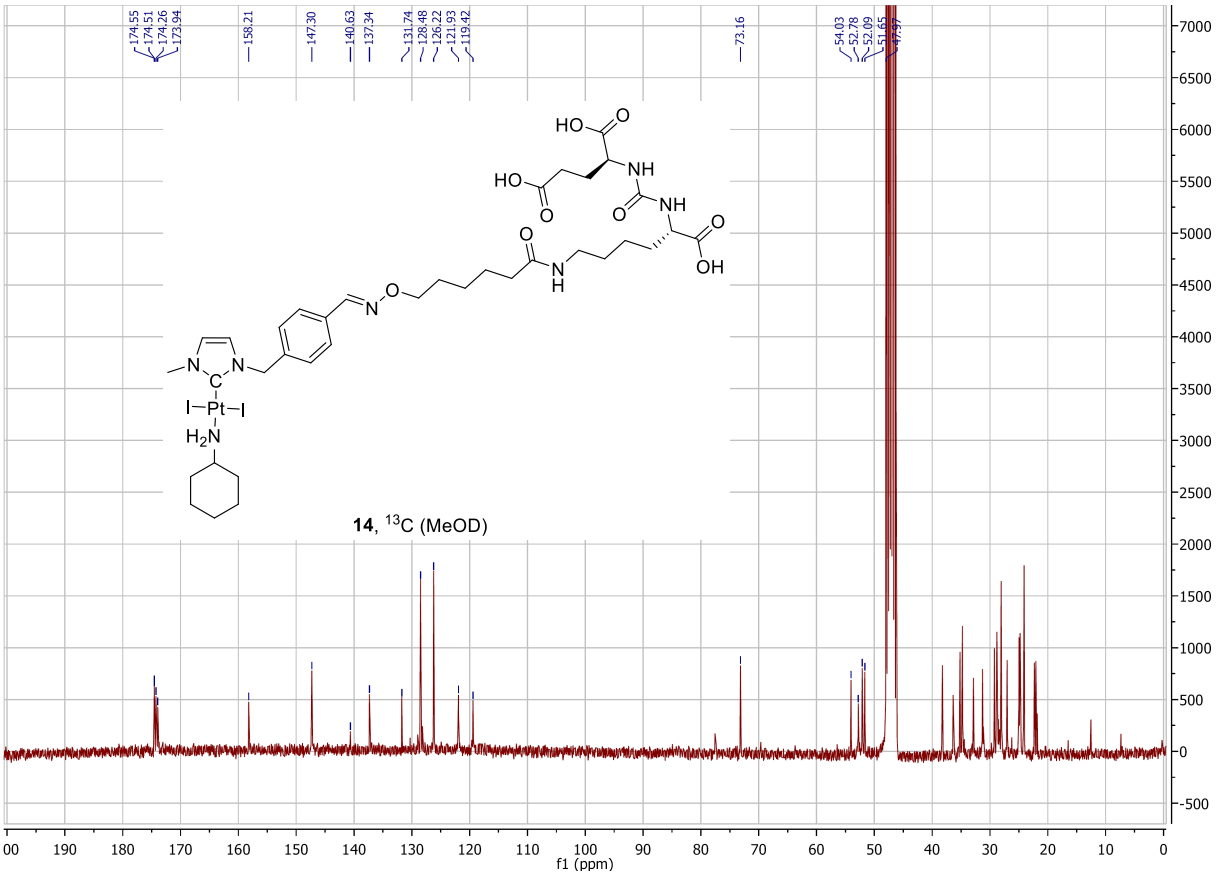

NO2
N
O
N
N
N
C
I–Pt–I
NH2
12, 13C (CDCl3)
f1 (ppm)


13, 1H (MeOD)
f1 (ppm)

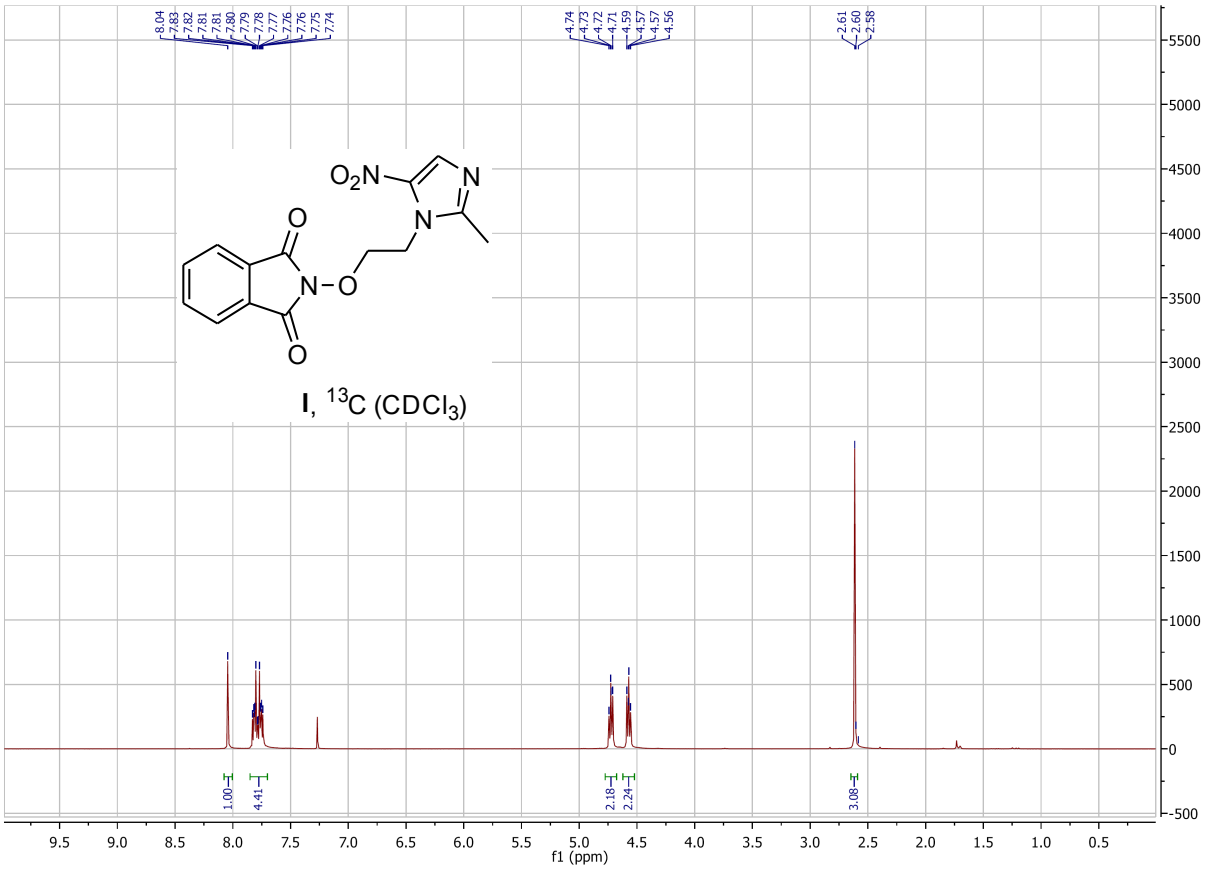

13, 13C (MeOD)
f1 (ppm)

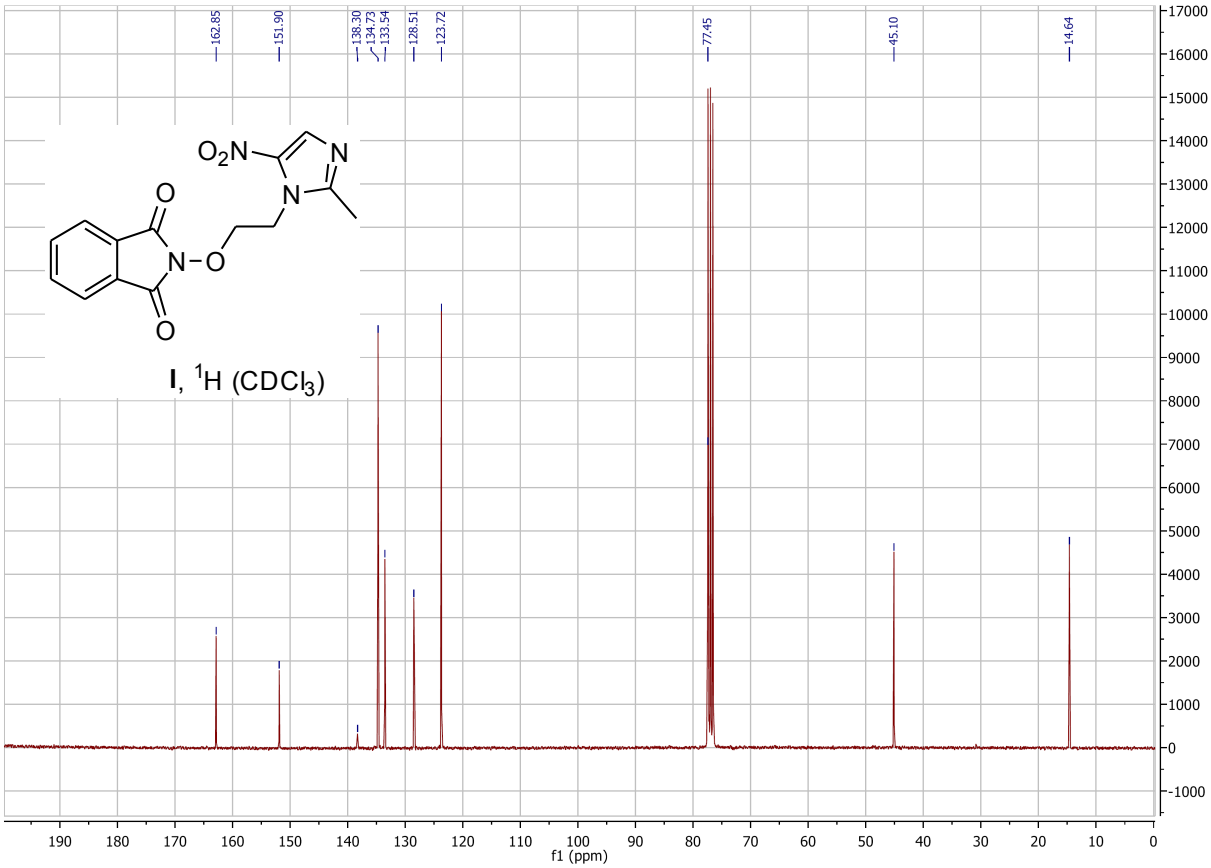


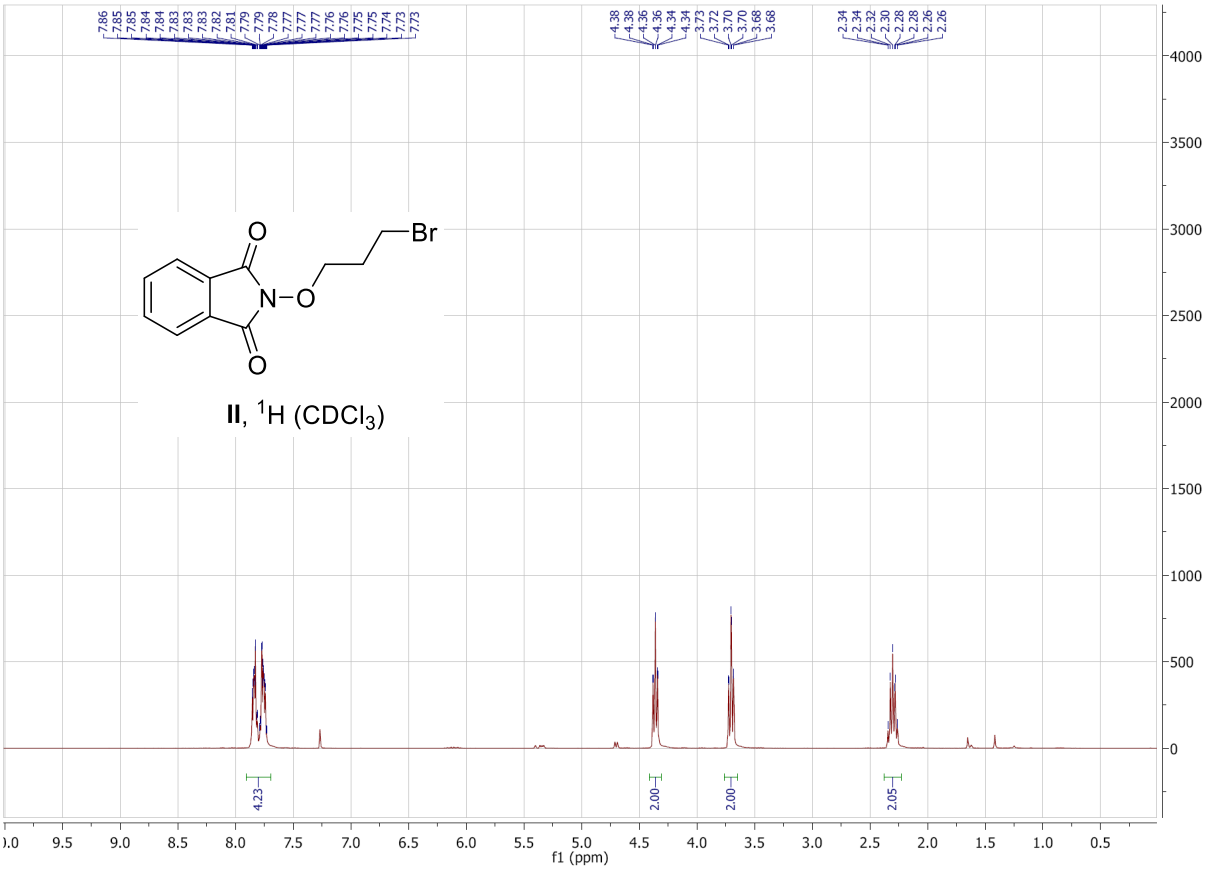
14, ^{1}H (MeOD)



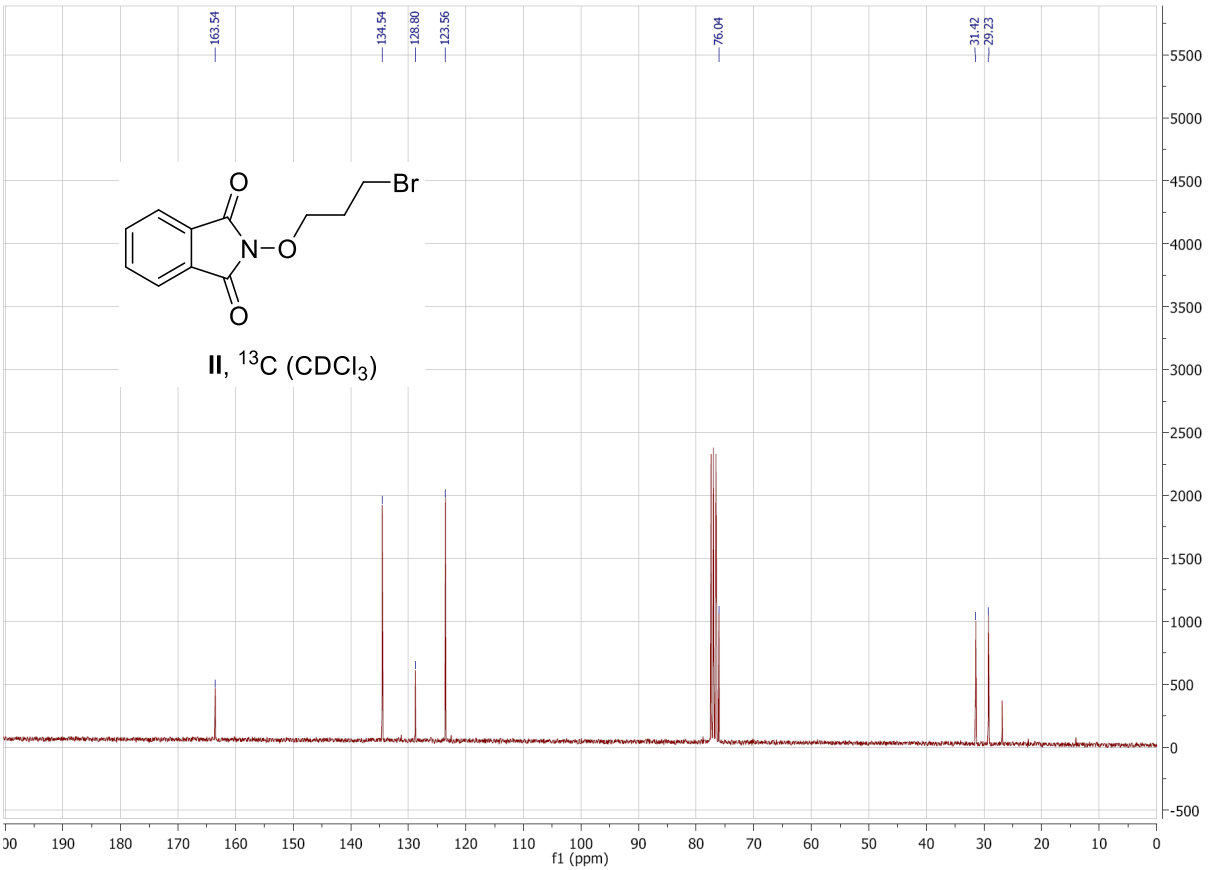
14, ^{13}C (MeOD)


8.04
7.83
7.82
7.81
7.81
7.80
7.79
7.78
7.77
7.76
7.76
7.75
7.74
4.74
4.73
4.72
4.71
4.59
4.57
4.57
4.56
2.61
2.60
2.58
O2N
N
N
O
N–O
O
I, 13C (CDCl3)
1.00
4.41
2.18
2.24
3.08
f1 (ppm)

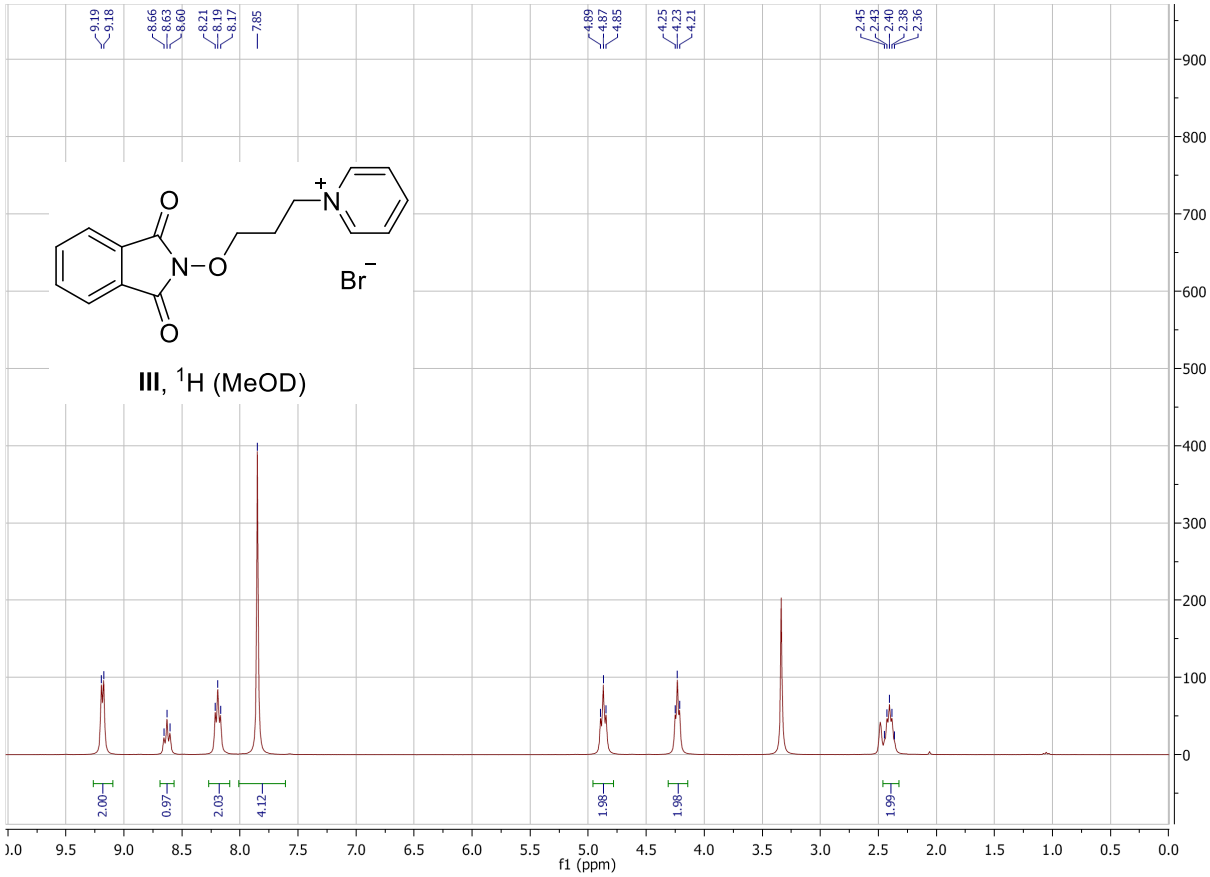

162.85
151.90
138.30
134.73
133.54
128.51
123.72
77.45
45.10
14.64
O2N
N
N
O
N–O
O
I, 1H (CDCl3)
f1 (ppm)

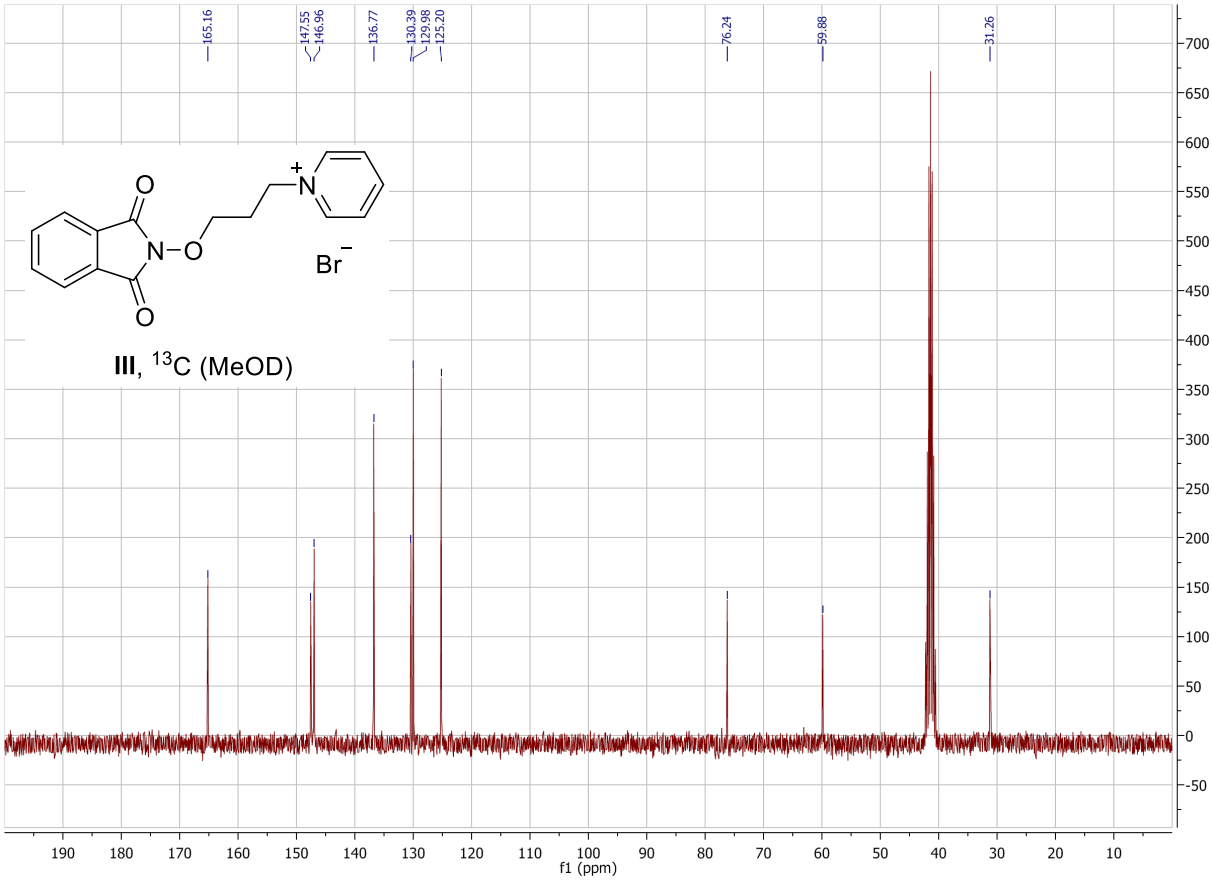


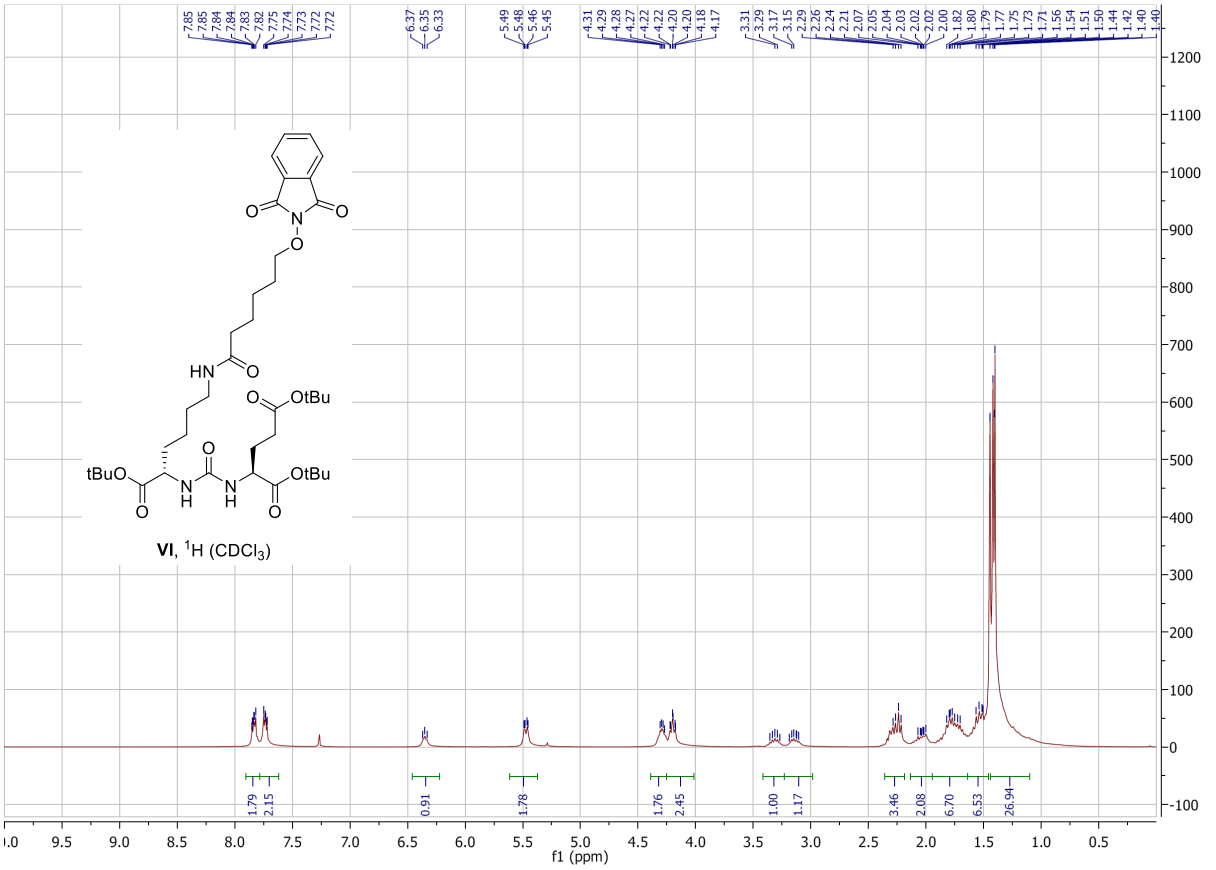
II, ^{1}H ($CDCl_3$)



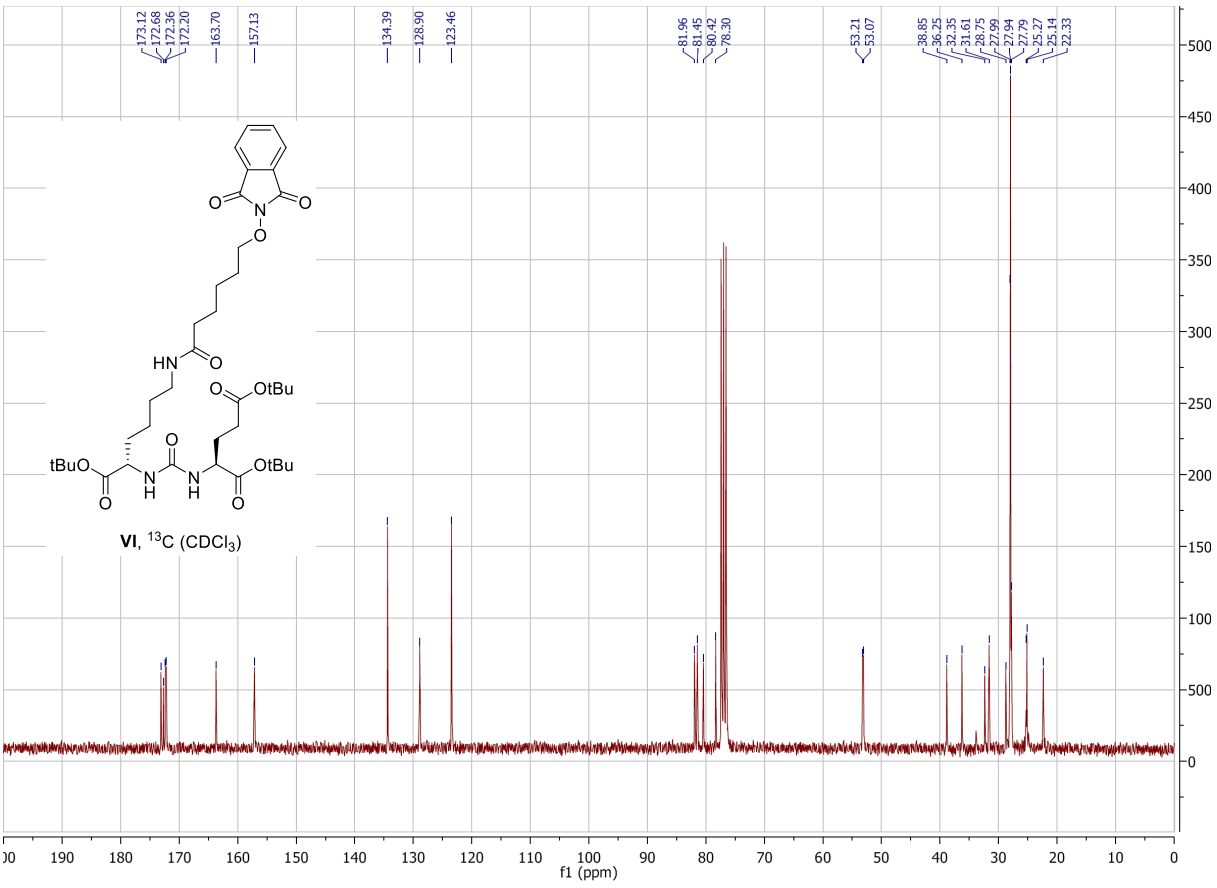
II, ^{13}C ($CDCl_3$)


9.19
9.18
8.66
8.63
8.60
8.21
8.19
8.17
7.85
4.89
4.87
4.85
4.25
4.23
4.21
2.45
2.43
2.40
2.38
2.36
O
N–O
O
+
N
Br
–
III, 1H (MeOD)
2.00
0.97
2.03
4.12
1.98
1.98
1.99
f1 (ppm)

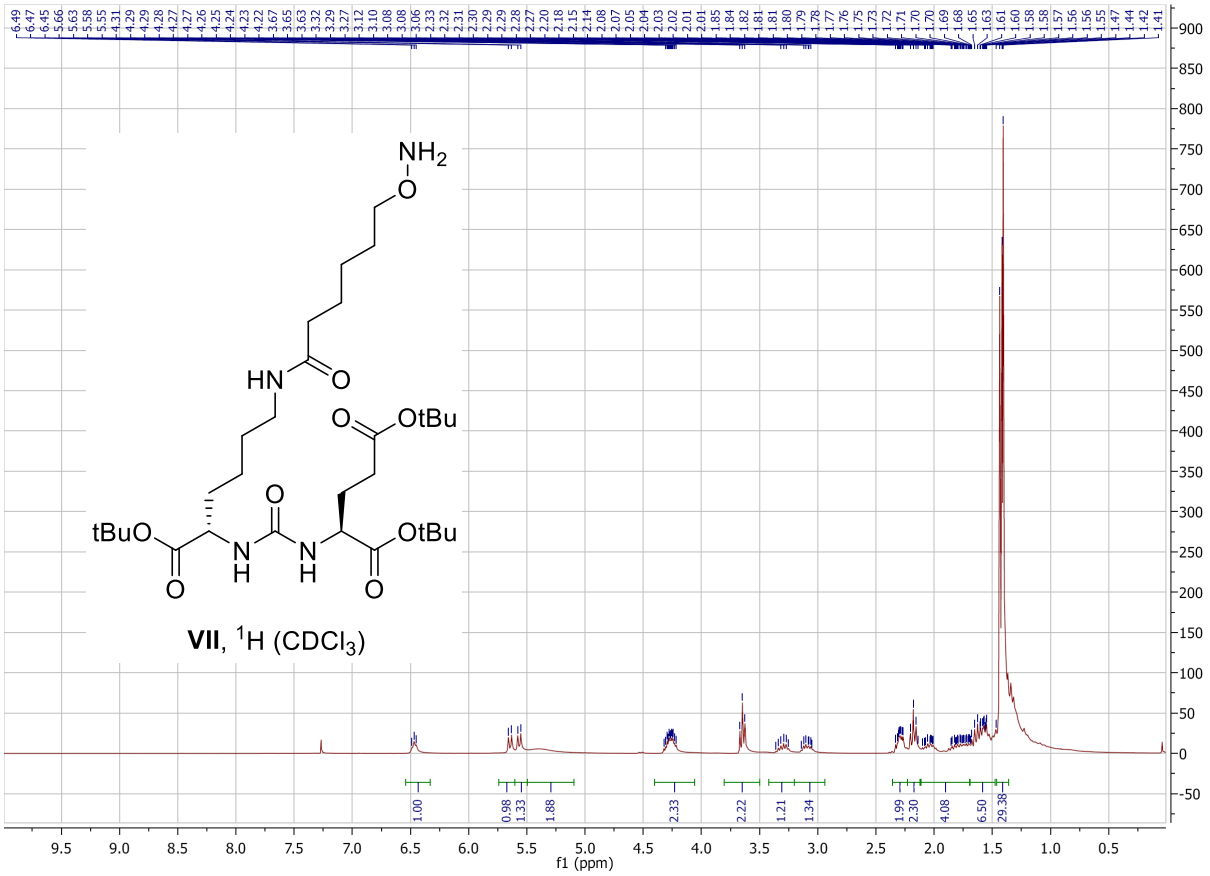

165.16
147.55
146.96
136.77
130.39
129.98
125.20
76.24
59.88
31.26
O
N–O
O
+
N
Br
–
III, 13C (MeOD)
f1 (ppm)

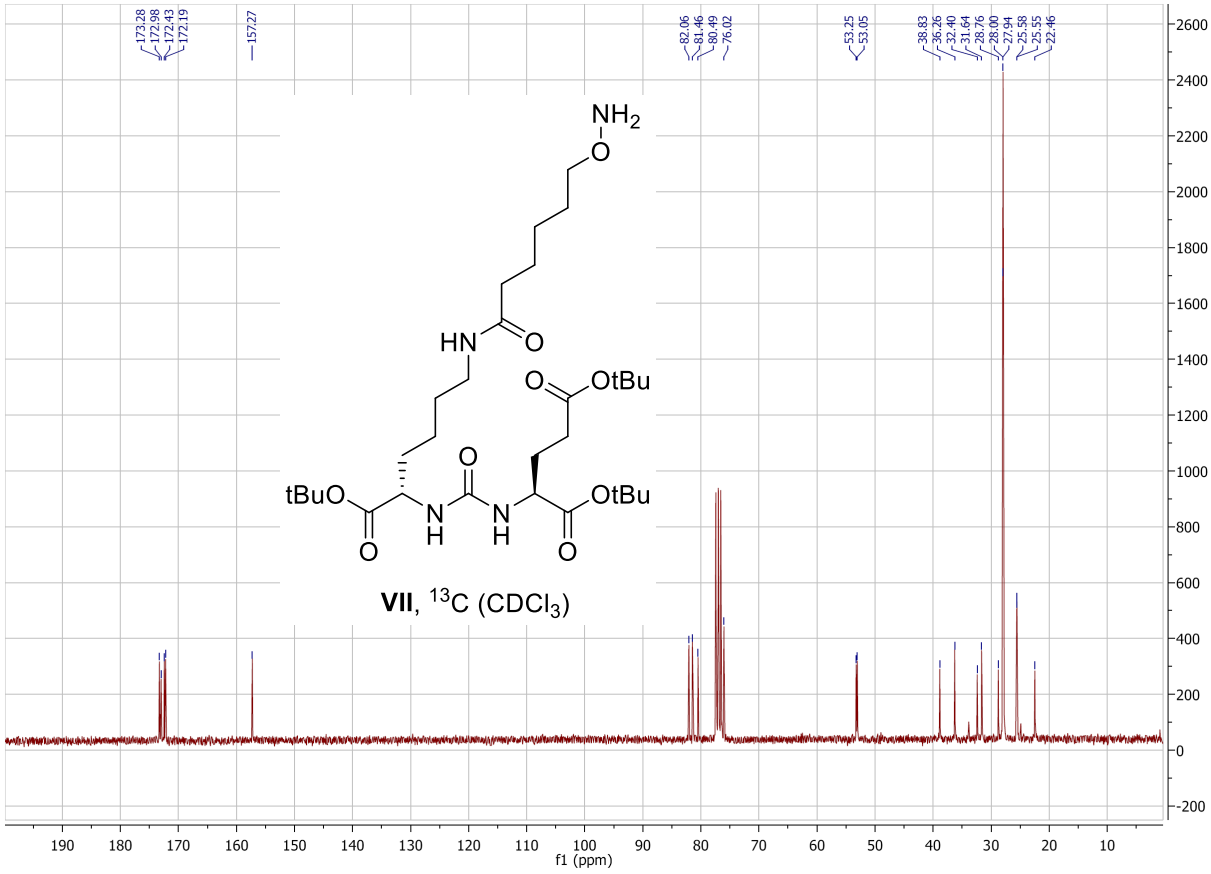


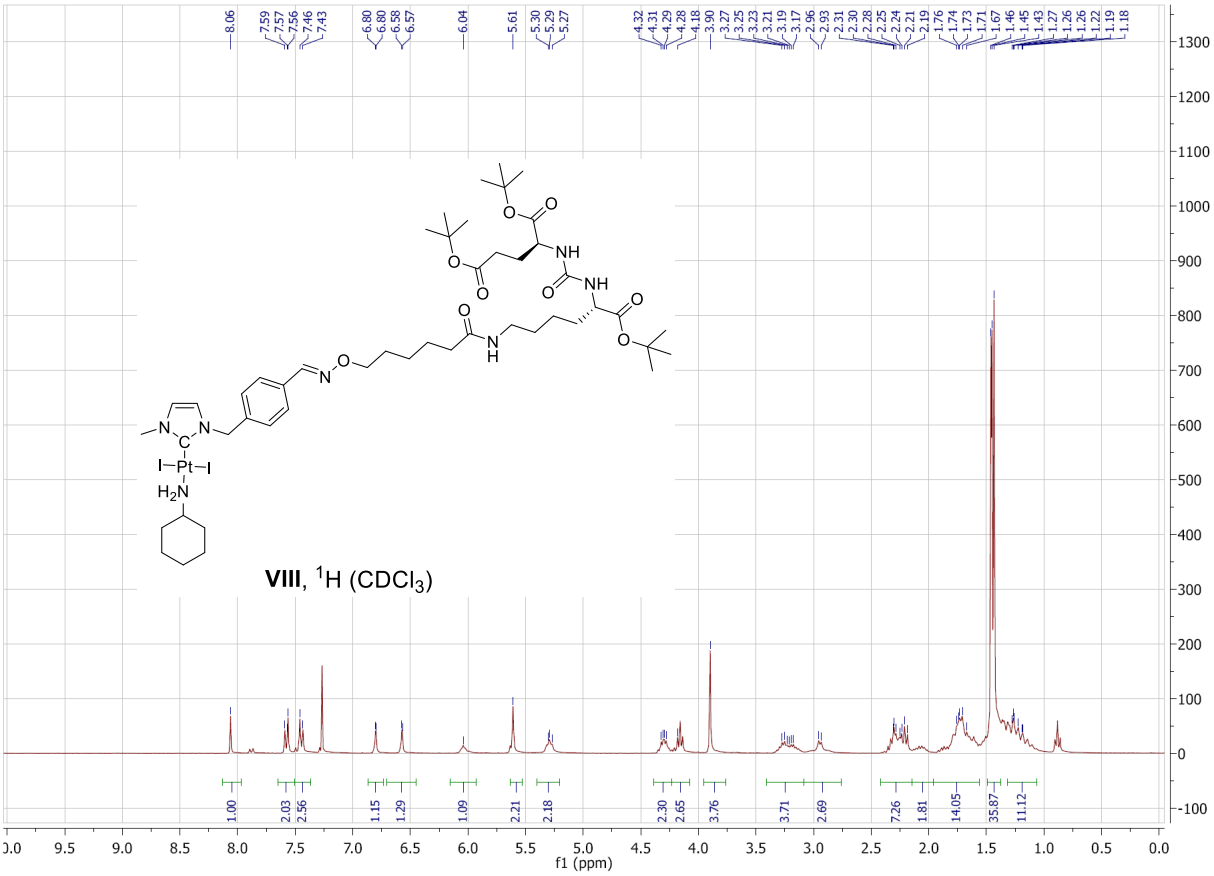
VI, ^{1}H ($CDCl_3$)

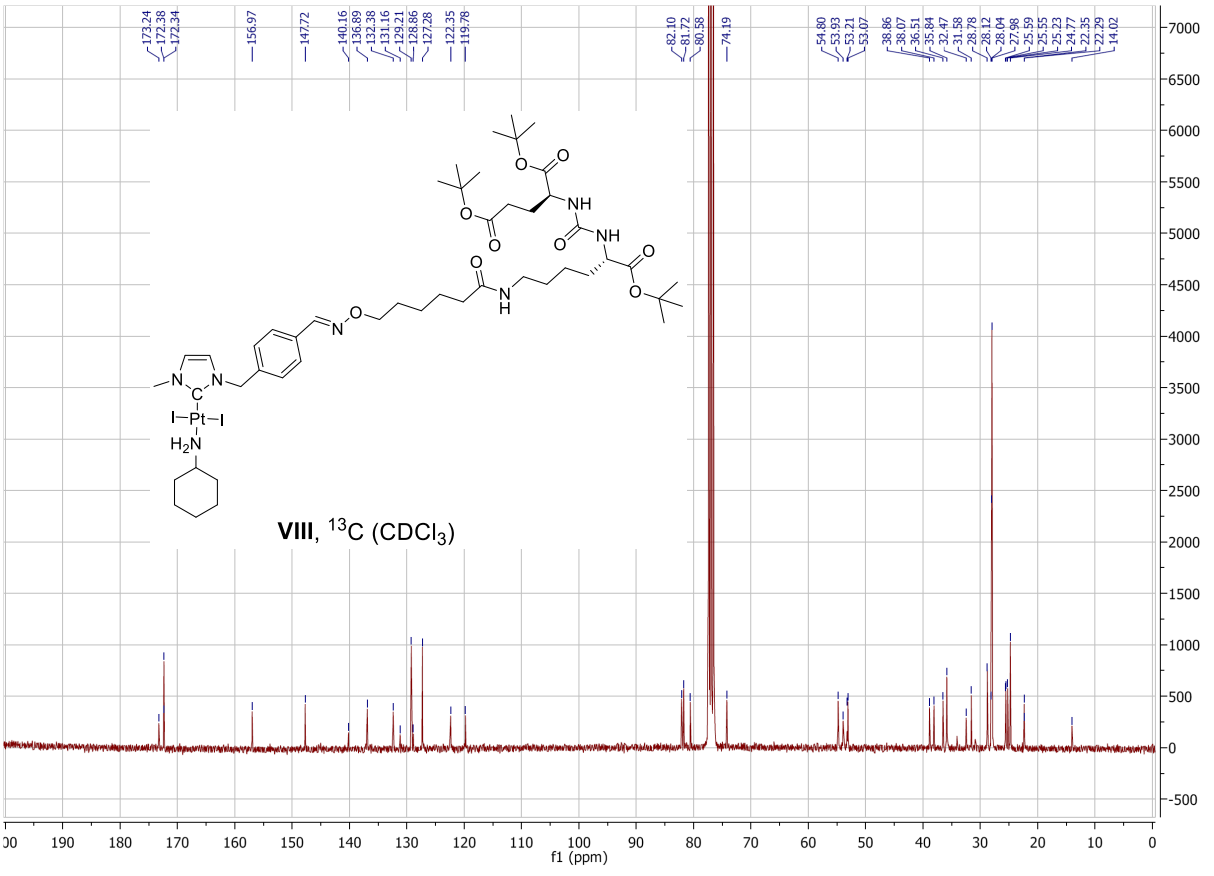


VI, ^{13}C ($CDCl_3$)


NH2
O
HN
O
O
OtBu
tBuO
O
N
H
N
H
O
OtBu
O
O
VII, 1H (CDCl3)
f1 (ppm)


NH2
O
HN
O
O
OtBu
tBuO
O
N
H
N
H
O
OtBu
O
O
VII, 13C (CDCl3)
f1 (ppm)


VIII, ^{1}H ($CDCl_3$)
f1 (ppm)


VIII, ^{13}C ($CDCl_3$)
f1 (ppm)

Inhibition of cell proliferation

Samples were prepared by dissolution of the compounds in DMSO (except for cisplatin that was dissolved in water) at stock concentrations of 10 mM. MCF7, HCT116, PC3 and SK-OV3 cell lines were maintained as monolayers in RPMI 1640 medium supplemented with 10% fetal calf serum, in the presence of penicilline, streptomycine and fungizone in 75cm2 flask under 5%CO2, while MRC5 and EPC were grown in complete D-MEM medium. Cells were plated in 96-well tissue culture plates in 200µl complete medium at a density of 1000-2500 cells per well and treated 24h later with 2µl of compounds using a Biomek 3000 automation workstation (Beckman-Coulter). Controls received the same volume of the appropriate vehicle (DMSO or water, 1% final volume). After 72h exposure, MTS reagent (CellTiter 96® Aqueous One, Promega) was added and incubated for 3h at 37°C: the absorbance was monitored at 490nm and results expressed as the inhibition of cell proliferation calculated as the ratio [(1-(OD490 treated/OD490 control))×100] in triplicate experiments after subtraction of the blank without cells. Positive controls (cells incubated with a reference drug at its IC50 concentration) were routinely added to check the responsiveness of cells. For IC50 determination [50% inhibition of cell proliferation], cells were incubated for 72 h following the same protocol with compound concentrations ranged 5nM to 100µM in separate duplicate experiments. At these concentrations, no interference with Pt complexes was noticed at 490 nm.