Electronic Supplementary Material (ESI) for Dalton Transactions.

Dinuclear gold(I) complexes with N-phosphanyl, N-heterocyclic carbene ligands: synthetic strategies, luminescence properties and anticancer activity

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Supporting Information

General remarks

All manipulations of air and moisture sensitive compounds were carried out using standard Schlenk techniques or in a glove box under an atmosphere of argon or dinitrogen. The reagents were purchased from Aldrich as high-purity products and generally used as received; all solvents were purified and dried by standard methods. Complexes **1a**,[1] **3** and **4**,[2] the azolium precursors[1,2] and the dicationic silver complex[2] employed as reagent in the preparation of **4** and **5** were prepared according to previously reported procedures. NMR spectra were recorded at 25°C on a Bruker Avance spectrometer working at 300 MHz (300.1 MHz for ^{1}H, 75.5 MHz for ^{13}C and 121.5 MHz for ^{31}P); chemical shifts (δ) are reported in units of ppm relative to the residual solvent signals and to external 85% H_3PO_4 (for ^{31}P). ESI-MS analyses were performed using a LCQ-Duo (Thermo-Finnigan) operating in positive ion mode. Instrumental parameters: capillary voltage 10 V, spray voltage 4.5 kV; capillary temperature 200 °C; mass scan range from 150 to 2000 amu; N_2 was used as sheath gas; the He pressure inside the trap was kept constant. The pressure directly read by an ion gauge (in the absence of the N_2 stream) was 1.33×10^{-5} Torr. Sample solutions were prepared by dissolving the compounds in acetonitrile. Sample solutions were directly infused into the ESI source by a syringe pump at 8μL/min flow rate. Elemental analyses were carried out by the microanalytical laboratory of Chemical Sciences Department (University of Padova) with a Fisons EA 1108 CHNS-O apparatus.

*Synthesis of complexes **1b***

The parent azolium salt (167.8 mg, 0.4 mmol), [AuCl(SMe$_2$)] (117.3 mg, 0.4 mmol), K_2CO_3 (60 mg, 0.4 mmol) and KCl (29.2 mg, 0.4 mmol) were placed in a 50 mL two-necked round-bottomed flask equipped with a magnetic stirring bar under inert atmosphere. Dry acetonitrile (5 mL) was subsequently added and the reaction mixture was maintained at room temperature whilst stirring for 18 hours, then filtered, and the solid residue was washed twice with dry acetonitrile (2.5 mL). The solution was evaporated to dryness under reduced pressure and the resulting solid was washed twice with diethylether (10 mL) and dried under reduced pressure. The solid was finally recrystallized by slow diffusion of diethylether into an acetonitrile solution of the crude isolated solid. Yield 43%. Anal. Calcd for $C_{32}H_{58}Au_2F_6N_4O_6P_2S_2$: C, 31.28; H, 4.76; N, 4.56; S, 5.22%. Found: C, 31.32; H, 4.77; N, 4.49, S, 5.53%. ^{1}H NMR (CD$_3$CN) δ 0.94 (t, $^3J_{HH}$ = 7.2 Hz, 3H, CH$_3$), 1.29-1.44 (m, 2H, CH$_2$), 1.50 (d, $^3J_{PH}$ = 17.7 Hz, 18H, CH$_3$ of ^{t}Bu), 1.87-1.98 (m, 2H, CH$_2$), 4.35 (t, $^3J_{HH}$ = 7.2 Hz, 2H, N-CH$_2$), 7.73 (s, 2H, CH). ^{13}C NMR (CD$_3$CN) δ 13.8 (s, CH$_3$), 20.5 (s, CH$_2$), 29.0 (d, $^2J_{PC}$ = 7.9 Hz, CH$_3$ of ^{t}Bu), 33.7 (s, CH$_2$), 40.1 (d, $^1J_{PC}$=16 Hz, C of ^{t}Bu), 53.5 (s, CH$_2$), 126.1 (s, CH Im), 126.5 (s, CH Im), carbene carbon not detected. ^{31}P NMR (CD$_3$CN) δ 130.68 (s). ESI-MS (positive ions): *m/z* 1079.1 $[Au_2L_2OTf]^+$

*Synthesis of complex **2***

Complex **2** was previously prepared upon transmetallation from the corresponding silver complex.[1] In this work, a more direct route similar to that described above for complex **1b** was successfully employed. The parent azolium salt (93.4 mg, 0.2 mmol), [AuCl(SMe$_2$)] (93.4 mg, 0.2 mmol), K_2CO_3 (30 mg, 0.2 mmol) and KCl (14.6 mg, 0.2 mmol) were placed in a 50 mL two-necked round-bottomed flask equipped with a magnetic stirring bar under inert atmosphere. Dry acetonitrile (2.5 mL) was subsequently added and the reaction mixture was maintained at room temperature whilst stirring for 18 hours, then filtered, and the solid residue was washed twice with dry acetonitrile (2.5 mL). The solution was evaporated to dryness under reduced pressure and the resulting solid was washed twice with diethylether (5 mL) and dried under reduced pressure. The solid was finally recrystallized by slow diffusion of diethylether into an acetonitrile solution of the crude isolated solid. Yield 76%. Analytical data match those of the sample from the previously reported synthesis.[1]

Synthesis of complex ***5***

A solution of [AuCl(SMe$_2$)] (155.8 mg, 0.53 mmol) in acetonitrile (15 mL) was added to a stirred solution of the silver complex precursor (161 mg, 0.133 mmol) in acetonitrile (5 mL). The reaction mixture was stirred overnight at room temperature. A black precipitate was filtered off and the clear filtrate was concentrated under vacuum to ca. 2 mL; addition of diethyl ether (15 mL) afforded the precipitation of an off-white solid, which was collected by filtration, washed with diethylether (3 mL) and dried in vacuum. The product was finally recrystallized by slow diffusion of diethylether into an acetonitrile solution of the crude isolated solid. Yield 57%. Anal. Calcd for $C_{24}H_{41}Au_2ClF_3N_2O_3PS_2$: C, 29.20; H, 4.19; N, 2.84; S, 6.50%. Found: C, 29.53; H, 4.26; N, 2.84; S, 6.36%. ^{1}H NMR (CD$_3$CN) δ 1.61 (d, J_{PH} = 16.8 Hz, 18H, CH$_3$ of ^{t}Bu), 2.22 (s, 6H, CH$_3$), 2.27 (bm, 2H, CH$_2$), 2.31 (s, 3H, CH$_3$), 2.32 (s, 6H, SCH$_3$), 3.55 (bt, 2H, NCH$_2$), 3.93 (bt, 2H, NCH$_2$), 7.08 (s, 2H, CH Ar). ^{13}C NMR (CD$_3$CN) δ 16.9 (s, CH$_3$), 20.2 (s, CH$_3$), 20.3 (bs, CH$_2$), 22.6 (s, SCH$_3$), 29.6 (d, $^2J_{PC}$=8.3 Hz, CH$_3$ of ^{t}Bu), 41.0 (d, $^1J_{PC}$=18 Hz, C of ^{t}Bu), 46.2 (s, CH$_2$), 47.7 (s, CH$_2$), 130.0 (CH Ar), 134.8 (C Ar), 139.7 (C Ar), 141.8 (C Ar), carbine carbon not detected. ^{31}P NMR (CD$_3$CN) δ 140.4 (s). ESI-MS (positive ions): *m/z* 792.3 [Au$_2$LCl(H$_2$O)]$^+$, 815.7 [Au$_2$LCl(CH$_3$CN)]$^+$. Crystals were obtained by slow diffusion of ether into an acetonitrile solution of the complex.

Synthesis of complex ***6***

The parent azolium salt (220 mg, 0.23 mmol), [AuCl(SMe$_2$)] (135.0 mg, 0.46 mmol), K$_2$CO$_3$ (31.7 mg, 0.23 mmol) and KCl (68 mg, 0.92 mmol) were placed in a 50 mL two-necked round-bottomed flask equipped with a magnetic stirring bar under inert atmosphere. Dry acetonitrile (5 mL) was subsequently added and the reaction mixture was maintained at room temperature whilst stirring for 18 hours, then filtered, and the solid residue was washed thrice with dry acetonitrile (7.5 mL). The solution was evaporated to dryness under reduced pressure and the resulting solid was washed twice with diethylether (10 mL) and dried under reduced pressure. The solid was finally recrystallized by slow diffusion of diethylether into a chloroform solution of the crude isolated solid. Yield 43%. Anal. Calcd for $C_{33}H_{45}Au_2ClF_3N_4O_3PS$: C, 36.19; H, 4.14; N, 5.12; S, 2.93%. Found: C, 36.51; H, 4.25; N, 5.00, S, 2.70%. ^{1}H NMR (CD$_3$CN) δ 1.57

(d, $^3J_{PH}$ = 17.6 Hz, 18H, CH_3 of ^{t}Bu), 2.09 (s, 6H, CH_3), 2.15 (s, 6H, CH_3), 2.34 (s, 3H, CH_3), 2.37 (s, 3H, CH_3), 7.03 (s, 1H, Im-CH), 7.08 (s, 2H, Ar-CH), 7.15 (s, 2H, Ar-CH), 7.25 (s, 1H, Im-CH), 7.70 (d, J_{HH} = 2.1 Hz, 1H, Im-CH), 7.93 (d, J_{HH} = 2.1 Hz, 1H, Im-CH), 8.11 (s, 1H, N-CH-N). ^{13}C NMR (CD_3CN) δ 16.7 (s CH_3), 17.1 (s, CH_3), 20.2 (s, CH_3), 20.4 (s, CH_3), 28.4 (d, J_{PC} = 7.7 Hz, CH_3 ^{t}Bu), 39.5 (d, J_{PC} = 18.2 Hz, C ^{t}Bu), 122.1, 124.9, 126.9 (s, C H Ar), 129.2, 129.3, 129.4, 134.4, 135.1, 135.2, 140.4, 140.8, 141.7. ^{31}P NMR (CD_3CN) δ 126.94 (s). ESI-MS (positive ions): *m/z* 945.0 $[Au_2LCl(MesIm)]^+$.

*Synthesis of gold complexes 7 and **8***

A solution of $[AuCl(SMe_2)]$ (72.0 mg, 0.244 mmol) in acetonitrile (10 mL) was added to a stirred solution of the silver complex precursor (68.4 mg, 0.061 mmol) in acetonitrile (10 mL). The reaction mixture was stirred overnight at room temperature. A black precipitate was filtered off and the clear filtrate was concentrated under vacuum to ca. 2 mL; addition of diethyl ether (25 mL) afforded the precipitation of an off-white solid, which was collected by filtration, washed with diethylether (3 mL) and dried in vacuum. NMR characterization of the product provided evidence of a mixture of complexes **7** and **8** in 77:23 ratio, as apparent from the signal of the t-butyl protons in the ^{1}H NMR (300 MHz, CD_3CN) at δ 1.58 and 1.24 (d, $^3J_{PH}$ = 17 Hz), and from the phosphorus signal ^{31}P NMR (121 MHz, CD_3CN) δ 140.14 and 138.54 (s).

*X-ray crystal structure determination of compounds **5**, **6**, **8***

The crystallographic data for both compounds were collected on a APEX II Bruker CCD diffractometer. Unit-cell geometrical parameters and the data collection (20 s/frame scan time for a sphere of diffraction data) were obtained by using the APEX 3 program package,[3] SAIN[3] and SADABS[4] were used to process the raw frame data to obtain the data file of the reflections. The structures were solved using SHELXT[5] (the Intrinsic Phasing method in the APEX 3 program). The refinement of the structures was performed using the SHELXTL-2018/3 program[6] in the WinGX suite v.2020.1.[7] The hydrogen atoms were incorporated in the refinement in specified geometry and refined “riding” on the related carbon atoms. For compound **6** the calculated molar mass, density and absorption coefficient include one acetonitrile solvent molecule per cell which does not appear in the final files because of the refinements carried out with data subjected to

SQUEEZE. Crystallographic data were deposited with the Cambridge Crystallographic Data Centre as supplementary publication (CCDC 2096907-2096909). Copies of the data can be obtained free of charge on application to the CCDC, 12 Union Road, Cambridge CB2 1EZ, U.K. (fax, (+44) 1223 336033; e-mail, deposit@ccdc.cam.ac.uk).

Luminescence measurements

Emission and excitation spectra were obtained with an Edinburgh FLS980 spectrometer equipped with a peltier-cooled Hamamatsu R928 photomultiplier tube (185-850 nm). An Edinburgh Xe900 450 W Xenon arc lamp was used as exciting light source. Corrected spectra were obtained *via* a calibration curve supplied with the instrument. Emission lifetimes in the ps-μs range were determined with the single photon counting technique by means of the same Edinburgh FLS980 spectrometer using a laser diode as excitation source (1 MHz, λ_{exc} = 407 nm) and a Hamamatsu MCP R3809U-50 (time resolution 20 ps) as detector. The luminescence lifetimes in the microsecond-millisecond scales were measured by using the same system equipped by pulsed microsencond flash lamp with variable repetition rate and elaborated with standard software fitting procedures. For solid samples, Φ_{em} have been calculated by corrected emission spectra obtained from an apparatus consisting of a barium sulphate coated integrating sphere (4 or 6 inches), a He-Cd laser (λ_{exc}: 325 nm, 5mW) or a 450W Xe lamp (λ_{exc} = tunable by a monochromator supplied with the instrument) as light sources, and a R928 photomultiplier tube, following the procedure described by De Mello et al.[8]

Experimental uncertainties are estimated to be ± 8% for lifetime determinations, ± 20% for emission quantum yields, ± 2 nm and ± 5 nm for excitation and emission peaks, respectively.

Cell viability assays.

10^3 cells/well HCT116 and 8 x 10^3 cells/well MRC5 were seeded in 96-well plates. One day later, cells were treated using serial dilutions of drugs and viability were evaluated after 96 hours with CellTiter-Glo assay (Promega, Madison, WI, USA) with a Tecan M1000 instrument. IC50 values were calculated from a dose-response curve. Averages and standard deviations were obtained from triplicates.

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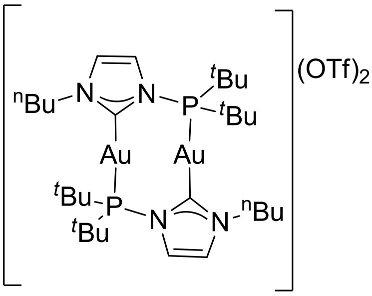
Complex 1b



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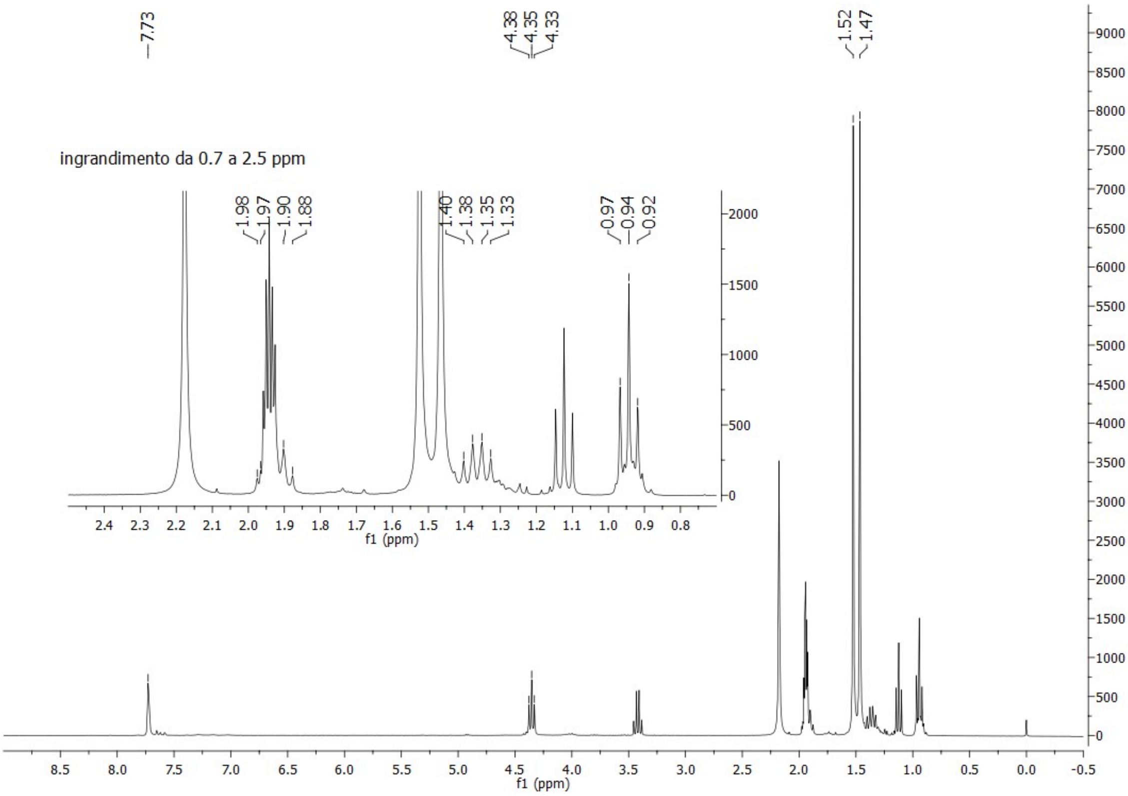

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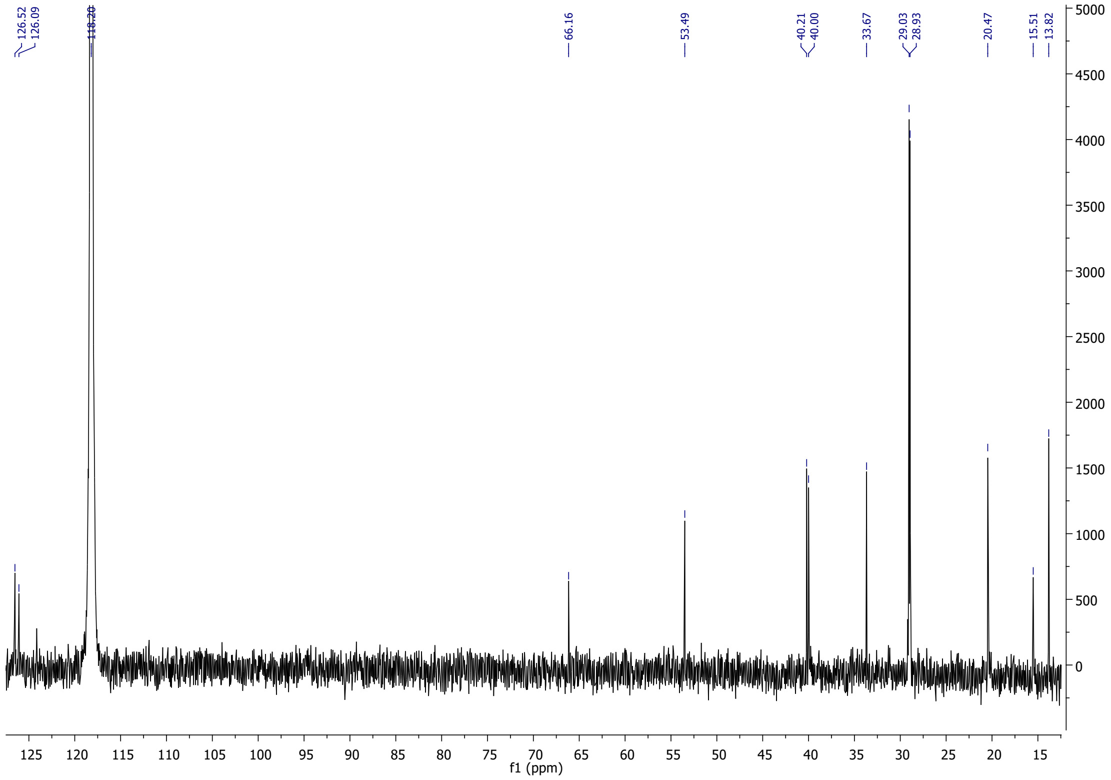

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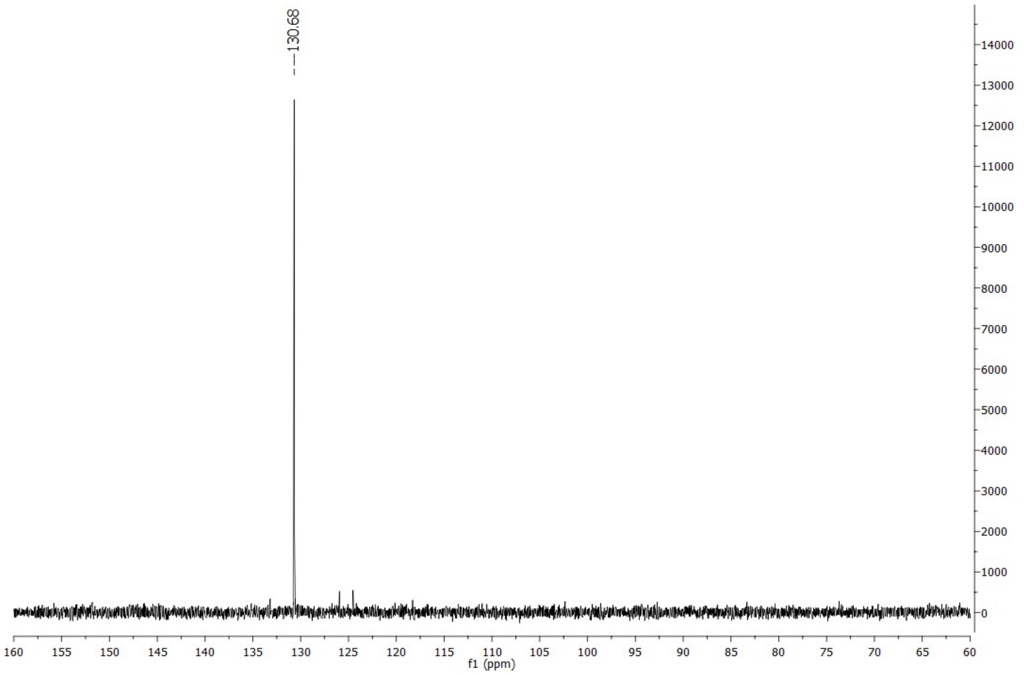

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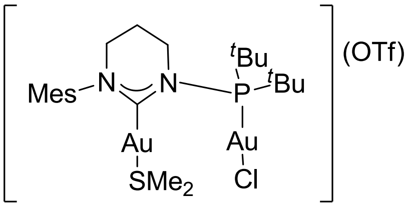

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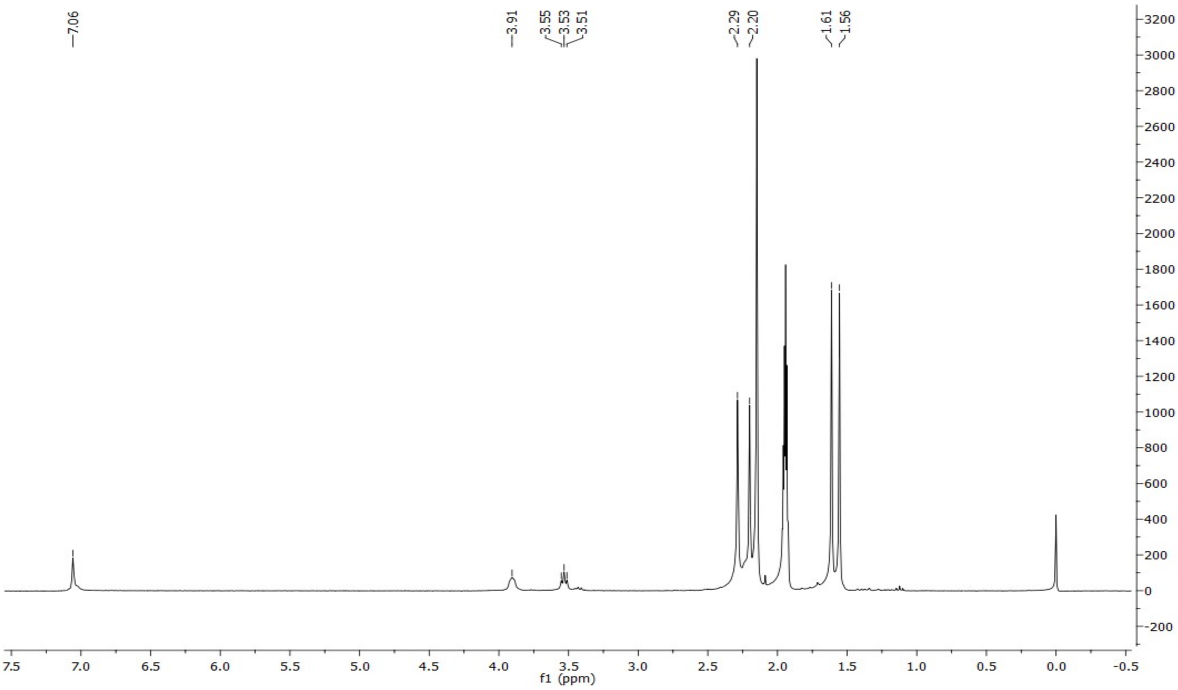

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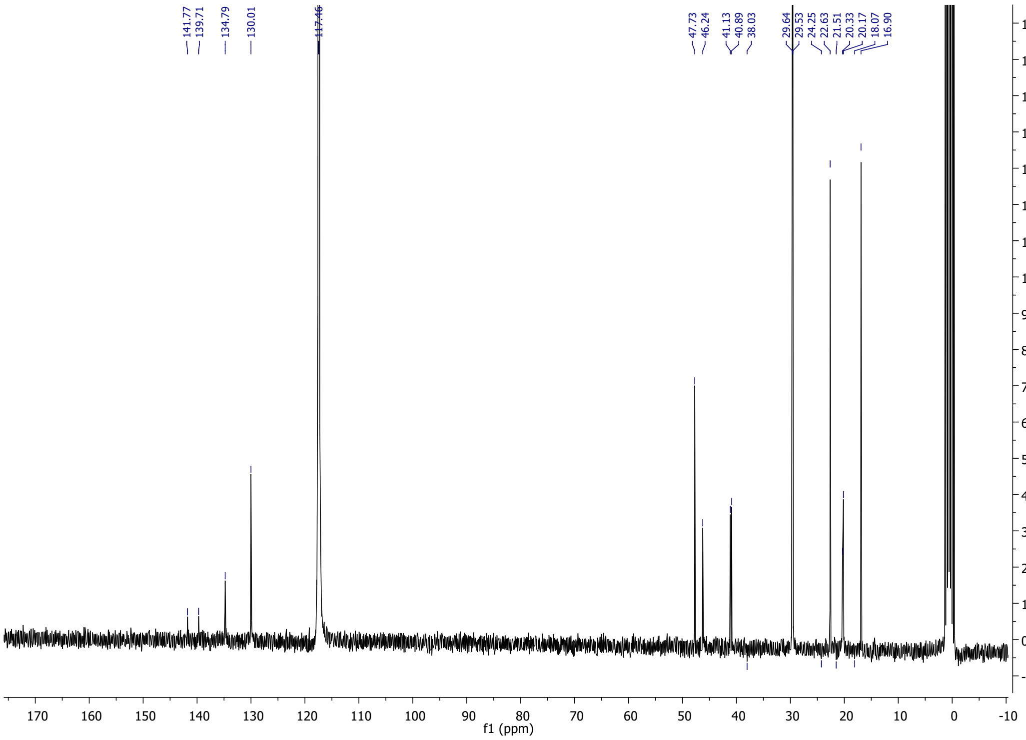

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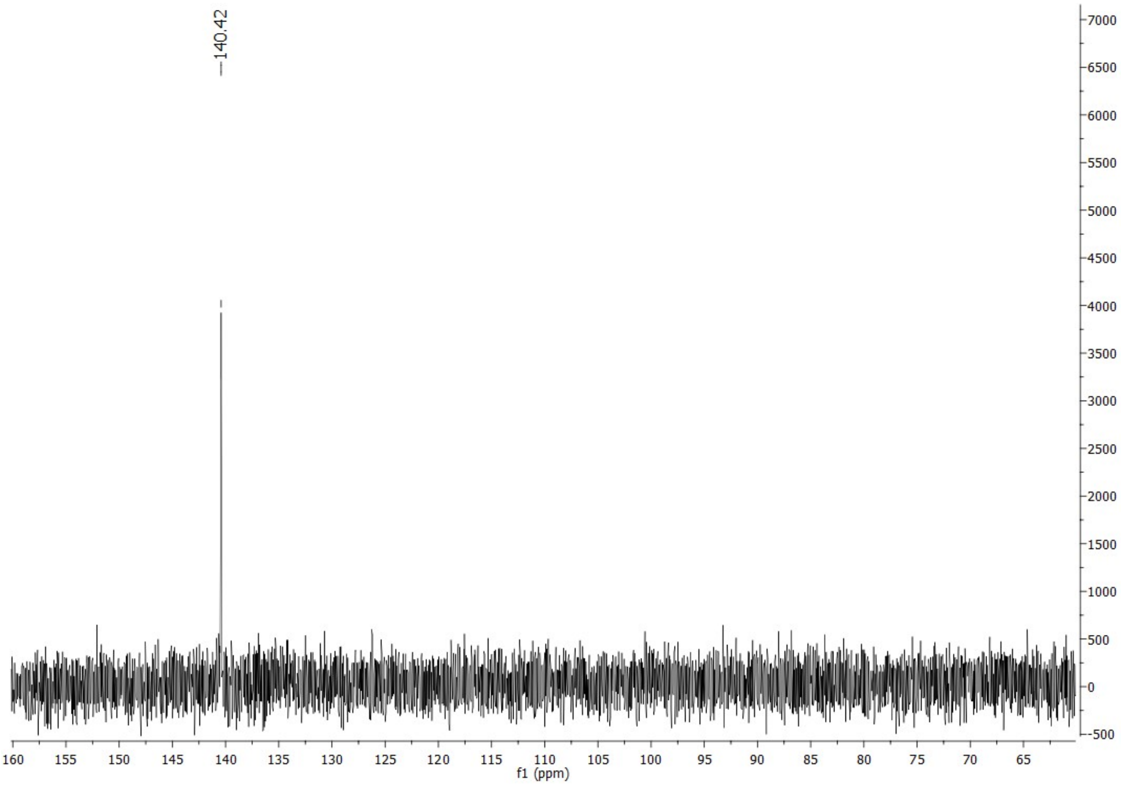

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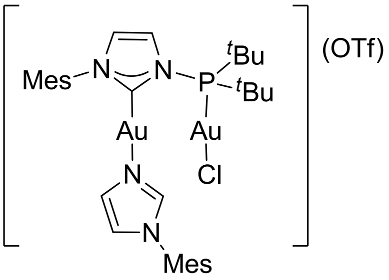

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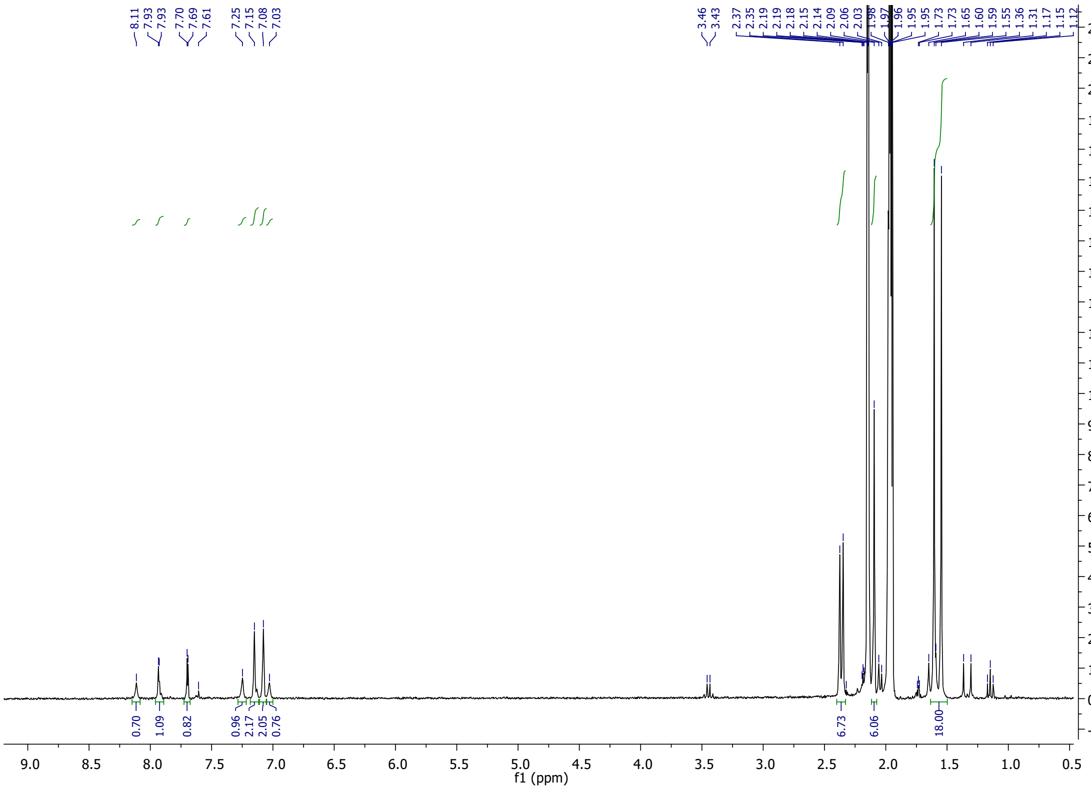

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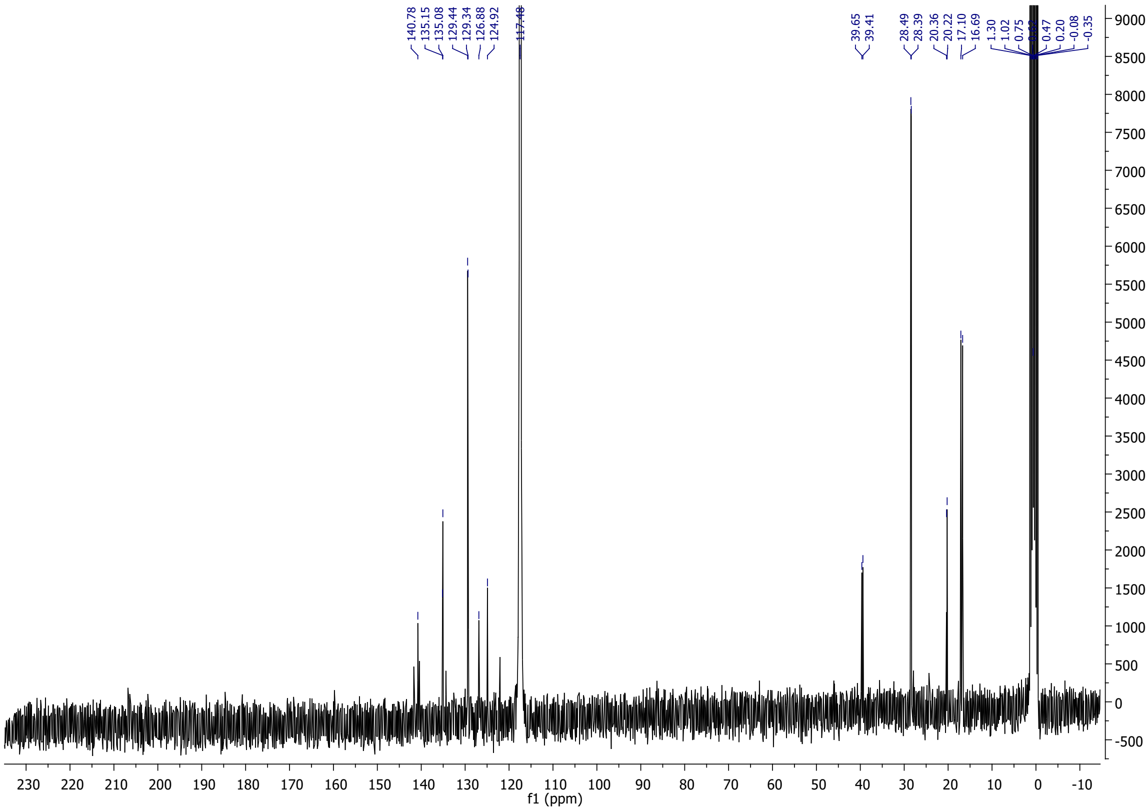

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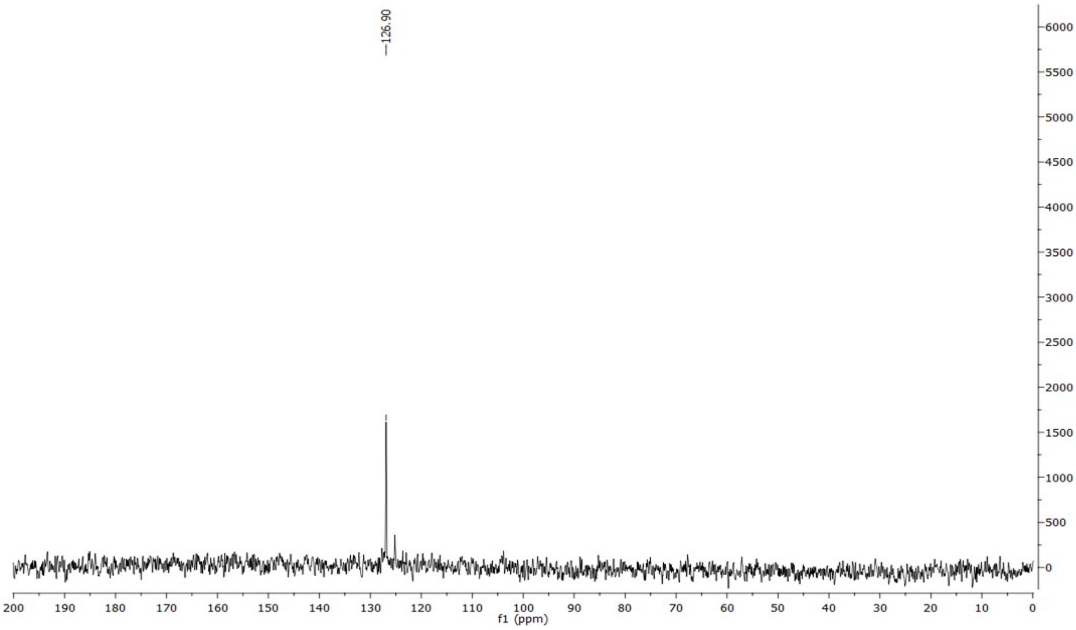
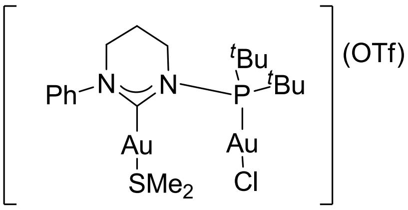


Complex 7+8



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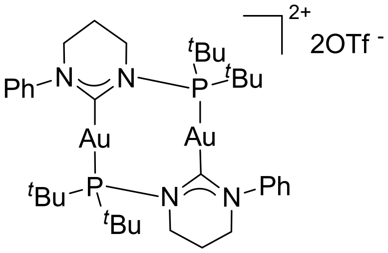

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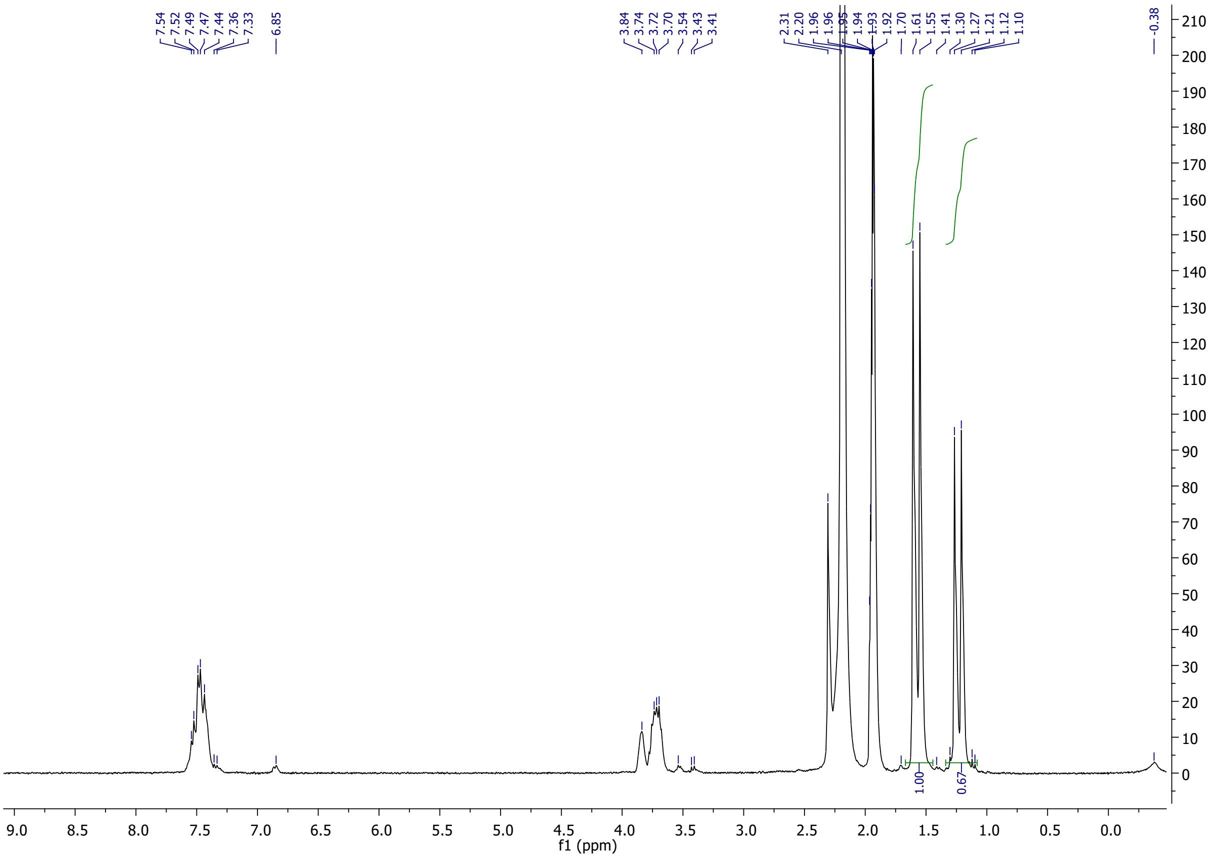

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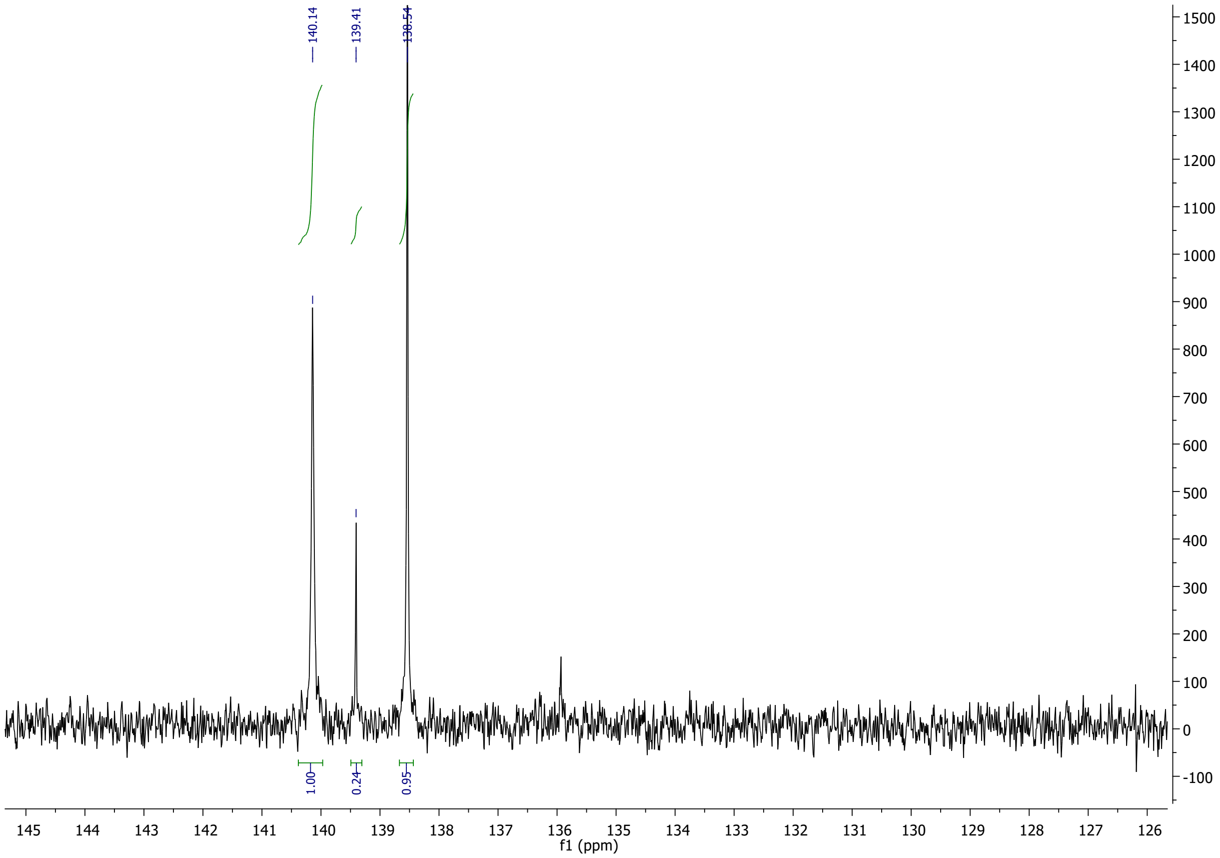

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