Supporting Information

Gold(I) compounds with lansoprazole-type ligands: synthesis, characterization and anticancer properties *in vitro*

M. Serratice,[a, †] B. Bertrand,[b,c, †] E. F. J. Janssen,[b] E. Hemelt,[b] A. Zucca,[a] F. Cocco,[a] M. A. Cinellu[a, *] and A. Casini[b, *]

[a] Department of Chemistry and Pharmacy, University of Sassari, Via Vienna 2, 07100 Sassari, Italy

[b] Department of Pharmacokinetics, Toxicology and Targeting, Research Institute of Pharmacy, University of Groningen, Antonius Deusinglaan 1, Groningen 9713 AV (The Netherlands)

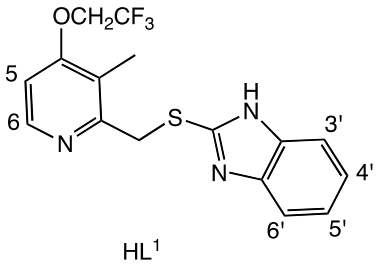
[c] Institut de Chimie Moléculaire de l'Université de Bourgogne, UMR 6302 CNRS Université de Bourgogne, Dijon, France.

[†] These two authors contributed equally to the work

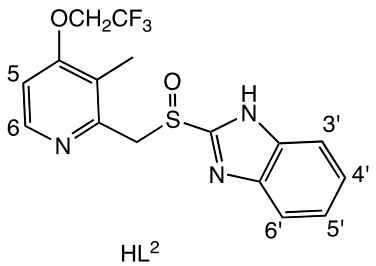
Experimental section

General procedures

All starting materials were used as received from commercial sources; 2-((3-methyl-4-(2,2,2-trifluoroethoxy)pyridin-2-yl)methylthio)-1*H*-benzo[*d*]imidazole (HL^1) and 2-((3-methyl-4-(2,2,2-trifluoroethoxy)pyridin-2-yl)methylsulfinyl)-1*H*-benzo[*d*]imidazole (HL^2) were synthesized according to a literature method.[1] The precursors $(PPh_3)AuCl$, [2] $(TPA)AuCl$,[3] $(THT)AuCl$[4] and $Na[Au(sac)_2]$[5] were prepared according to literature procedures. Elemental analyses were performed with a Perkin-Elmer elemental analyzer 240B by Mr. Antonello Canu (Dipartimento di Chimica e Farmacia, Università degli Studi di Sassari, Italy). Infrared spectra were recorded with a FT-IR Jasco 480P using nujol mulls. ^1H and $^{31}P\{^1H\}$ NMR spectra were recorded with a Bruker Avance III 400 and with a Varian VXR 300 spectrometers at 298 K. Chemical shifts are given in ppm relative to internal TMS or to the solvent residual peak(s) for ^1H and external 85% H_3PO_4 for $^{31}P\{^1H\}$; *J* values are given in Hz.



HL^1



HL^2

Spectroscopic data of HL^1. Selected IR bands (ν/cm^{-1}, nujol mull): 3556 (NH), 1660 (C-C, C-N ring stretch.), 1577, 1444, 1410, 1290, 1256 (asym. C-O-C), 1167, 1109, 976, 746. ^1H NMR ($CDCl_3$): δ 12.6 (s, 1H, NH), 8.41 (d, $J_{H\text{-}H}$ = 6.0 Hz, 1H, H^6), 7.54 (m, $J_{H\text{-}H}$ = 9.3, 6.0, 3.3 Hz, 2H, $H^{3'}$, $H^{6'}$), 7.19 (m, $J_{H\text{-}H}$ = 9.3, 6.0, 3.3 Hz, 2H, $H^{4'}$, $H^{5'}$), 6.72 (d, $J_{H\text{-}H}$ = 6.0 Hz, 1H, H^5), 4.43 (q, $J_{H\text{-}F}$ = 8.0 Hz, 2H, CH_2CF_3), 4.42 (s, 2H, CH_2S), 2.32 (s, 3H, CH_3); (CD_2Cl_2): δ 12.8 (s, 1H, NH), 8.46 (d, $J_{H\text{-}H}$ = 5.7 Hz, 1H, H^6), 7.55 (m, $J_{H\text{-}H}$ = 9.3, 6.0, 3.3 Hz, 2H, $H^{3'}$, $H^{6'}$), 7.20 (m, $J_{H\text{-}H}$ = 9.3, 6.0, 3.3 Hz, 2H, $H^{4'}$, $H^{5'}$), 6.79 (d, $J_{H\text{-}H}$ = 5.7 Hz, 1H, H^5), 4.49 (q, $J_{H\text{-}F}$ = 7.8 Hz, 2H, CH_2CF_3), 4.46 (s, 2H, CH_2S), 2.34 (s, 3H, CH_3).

Spectroscopic data of HL^2. Selected IR bands (ν/cm^{-1}, nujol mull): 3416 (NH), 1585 (C-C, C-N ring str.), 1267 (asym. C-O-C), 1164, 1113, 1039 (S=O), 973, 746. ^1H NMR ($CDCl_3$): δ 11.4 (br, 1H, NH), 8.35 (d, $J_{H\text{-}H}$ = 5.6 Hz, 1H, H^6), 7.81 (m, 1H, $H^{6'}$), 7.50 (m, 1H, $H^{3'}$), 7.34 (m, $J_{H\text{-}H}$ = 9.6, 6.4, 3.2 Hz, 2H, $H^{4'}$, $H^{5'}$), 6.68 (d, $J_{H\text{-}H}$ = 5.6 Hz, 1H, H^5), 4.77 (AB quartet, J_{AB} = 13.6 Hz, 2H,

CH_2SO), 4.38 (q, $J_{H\text{-}F}$ = 8.0 Hz, 2H, CH_2CF$_3$), 2.23 (s, 3H, CH$_3$); (DMSO-d_6): δ 8.29 (d, $J_{H\text{-}H}$ = 5.6 Hz, 1H, H^6), 7.62 (m, AA' part of an AA'BB', $J_{H\text{-}H}$ = 9.3, 6.1, 3.2 Hz, 2H, H$^{3'}$, H$^{6'}$), 7.25 (m, BB' part, $J_{H\text{-}H}$ = 9.3, 6.1, 3.2 Hz, 2H, H$^{4'}$, H$^{5'}$), 7.09 (d, $J_{H\text{-}H}$ = 5.6 Hz, 1H, H^5), 4.90 (q, $J_{H\text{-}F}$ = 8.6 Hz, 2H, CH_2CF$_3$), 4.77 (AB quartet, J_{AB} = 13.7 Hz, 2H, CH_2SO), 2.18 (s, 3H, CH$_3$).

Synthesis

Mononuclear complexes

[Au(HL^1)Cl] (1a) A solution of HL^1 (177.0 mg, 0.5 mmol) in CH_2Cl_2 (20 mL) was added to a solution of (THT)AuCl (160.3 mg, 0.5 mmol) in the same solvent (20 mL). The resulting mixture was stirred for 24 h at room temperature in the dark. Afterward the solution was filtered through Celite and concentrated to a small volume. Addition of diethyl ether afforded a white solid that was filtered off and vacuum-dried. Yield 75.4%. Mp 170 °C. Anal. Calcd. for $C_{16}H_{14}AuClF_3N_3OS$ (585.78): C, 32.81; H, 2.41; N, 7.17. Found: C, 32.80; H, 2.31; N, 7.25. Selected IR bands (ν/cm^{-1}, nujol mull): 1587, 1508, 1265, 1176, 1113, 744, 341 (Au-Cl). ^{1}H NMR (CD_2Cl_2): δ 8.49 (d, $J_{H\text{-}H}$ = 5.6 Hz, 1H, H^6), 7.71 (m, $J_{H\text{-}H}$ = 9.6, 6.6, 3.3 Hz, 1H, H$^{6'}$), 7.63 (m, $J_{H\text{-}H}$ = 9.3, 6.0, 3.3 Hz, 1H, H$^{3'}$), 7.38 (m, $J_{H\text{-}H}$ = 9.3, 6.6, 3.6 Hz, 2H, H$^{4'}$, H$^{5'}$), 6.86 (d, $J_{H\text{-}H}$ = 6.0 Hz, 1H, H^5), 4.54 (s, 2H, CH_2-S), 4.53 (q, $J_{H\text{-}F}$ = 8.0 Hz, 2H, CH_2CF$_3$), 2.37 (s, 3H, CH$_3$).

[Au(HL^1)(PPh$_3$)](BF$_4$) (2a-BF$_4$) To a solution of (PPh$_3$)AuCl (274.4 mg, 0.5 mmol) in dichloromethane (20 mL) was added a solution of $AgBF_4$ (107.0 mg, 0.55 mmol) in the same solvent (10 mL); the resulting suspension was stirred in the dark until AgCl precipitation was completed; then the filtered solution was added to a solution of HL^1 (177.0 mg, 0.5 mmol) in the same solvent (10 mL) and the reaction mixture was stirred for 3 h at room temperature in the dark. Afterward the solution was filtered through Celite and concentrated to a small volume, addition of diethyl ether afforded a white solid that was filterd off and vacuum-dried to give the analytical sample. Yield 40 %. Mp 155 °C. Anal. Calcd for $C_{34}H_{29}AuBF_7N_3OPS$ (899.42): C, 45.40; H, 3.25; N, 4.67. Found: C, 45.18; H, 3.14; N, 4.69. Selected IR bands (ν/cm^{-1}, nujol mull): 1581, 1438, 1291, 1262, 1166, 1103 (PPh$_3$), 1057 (BF_4^-), 970, 745, 693. ^{1}H NMR ($CDCl_3$): δ 12.6 (br, 1H, NH), 8.09 (d, br, $J_{H\text{-}H}$ = 5.2 Hz, 1H, H^6), 7.71 (m, br, 2H, H$^{3'}$, H$^{6'}$), 7.64-7.52 (m, 15H, PPh$_3$), 7.28 (m, br, 2H, H$^{4'}$, H$^{5'}$), 6.65 (d, $J_{H\text{-}H}$ = 6.0 Hz, 1H, H^5), 4.64 (s, 2H, CH_2-S), 4.32 (q, $J_{H\text{-}F}$ = 8.0 Hz, 2H, CH_2CF$_3$), 2.00 (s, 3H, CH$_3$). ^{31}P NMR ($CDCl_3$): δ 31.6 ppm (s, PPh$_3$).

[Au(HL^2)(PPh$_3$)](BF$_4$) (2b-BF$_4$) Same procedure as for **2a**-BF$_4$. Yield 65%. Mp 130 °C. Anal. Calcd for $C_{34}H_{29}AuBF_7N_3O_2PS$ (915.42): C, 44.61; H, 3.19; N, 4.59. Found: C, 44.38; H, 3.19; N,

4.67. Selected IR bands (ν/cm^{-1}, nujol mull): 3419, 1632, 1579, 1437, 1261, 1165, 1102 (PPh_3), 1060 (BF_4^-), 748, 712, 693. ^{1}H NMR ($CDCl_3$): δ 8.33 (br, 1H, H^6), 7.74 (br, 2H, $H^{3'}$, $H^{6'}$), 7.53 (br, 15H, PPh_3), 7.36 (m, $J_{H\text{-}H}$ = 9.1, 6.0, 3.0 Hz, 2H, $H^{4'}$, H^5), 6.67 (d, $J_{H\text{-}H}$ 5.6 Hz, 1H, H^5), 4.76 (AB, J_{AB} = 13.6 Hz, 2H, CH_2SO), 4.36 (br, 2H, OCH_2CF$_3$), 2.18 (br, 3H, CH_3). ^{31}P NMR ($CDCl_3$): δ 31.2 ppm (s, PPh_3).

Na[Au(L^1)(sac)] (Na-3a) A solution of HL^1 (177.0 mg, 0.5 mmol) in MeCN (20 mL) was added to a solution of Na[Au(sac)$_2$] (292.0 mg, 0.5 mmol) in the same solvent (20 mL). The resulting solution was stirred for 24 h at room temperature in the dark. Afterward the solvent was removed under vacuum, the residue taken up with $CHCl_3$ and the filtered solution concentrated to a small volume. Addition of diethyl ether afforded a white solid that was filtered off and vacuum-dried to give the analytical sample. Yield 45%. Mp 220 °C. Anal. Calcd for $C_{23}H_{17}AuF_3N_4NaO_4S_2$ (754.49): C, 36.61; H, 2.27; N, 7.43. Found: C, 36.45, H, 2.37, N, 7.37. Selected IR bands (ν/cm^{-1}, nujol mull): 1689 (C=O), 1581, 1290 (asym SO_2), 1261, 1171 (sym SO_2), 1111, 972, 746. ^{1}H NMR (CD_2Cl_2): δ 8.51 (d, $J_{H\text{-}H}$ = 5.2 Hz, 1H, H^6), 7.94 (d, $J_{H\text{-}H}$ = 6.4 Hz, 1H, $H^{3''}$ sac), 7.87 (d, $J_{H\text{-}H}$ = 6.8 Hz, 1H, $H^{6''}$ sac), 7.76 (m, 3H, $H^{3'}$ + $H^{4''}$, $H^{5''}$ sac), 7.67 (m. br 1H, $H^{6'}$), 7.40 (m, CD part, $J_{H\text{-}H}$ = 9.0, 6.0, 2.7 Hz, 2H, $H^{4'}$, $H^{5'}$), 6.88 (d, $J_{H\text{-}H}$ = 5.6 Hz, 1H, H^5), 4.57 (s, 2H, CH_2-S), 4.53 (q, $J_{H\text{-}F}$ = 7.8 Hz, 2H, CH_2CF_3), 2.36 (s, 3H, CH_3).

[Au(L^1)(PPh$_3$)] (4a) To a solution of (PPh_3)AuCl (69.3 mg, 0.14 mmol) in acetone (20 mL) was added a solution of $AgBF_4$ (27.3 mg, 0.14 mmol) in the same solvent (10 mL); the resulting mixture was stirred in the dark until AgCl precipitation was completed. Then, the filtered solution was added to a solution of HL^1 (50.0 mg, 0.14 mmol) and KOH (7.9 mg, 0.14 mmol) in MeCN (5 mL) and H_2O (20 mL) and stirred for 24 h at room temperature in the dark. Afterwards, the solvent was removed under vacuum and the residue taken up with dichloromethane; the filtered solution was concentrated to a small volume and diethyl ether added. The precipitate obtained was filtered, washed with diethyl ether, and vacuum-dried to give the analytical sample as a white solid. Yield 40 %. Mp 155 °C. Anal. Calcd for $C_{34}H_{28}AuF_3N_3OPS$ (811.61): C, 50.32; H, 3.48; N 5.18. Found: C, 50.25; H, 3.31; N, 5.06. Selected IR bands (ν/cm^{-1}, nujol mull): 1656, 1294, 1263, 1153, 1113 (PPh_3), 972, 750. ^{1}H NMR ($CDCl_3$): δ 8.21 (d, $J_{H\text{-}H}$ = 5.6 Hz, 1H, H^6), 7.62-7.48 (m, 17H, $H^{3'}$+$H^{6'}$ + H-PPh_3), 7.08 (m, 2H, $H^{4'}$, $H^{5'}$), 6.59 (d, $J_{H\text{-}H}$ = 5.6 Hz, 1H, H^5), 4.84 (s, 2H, CH_2-S), 4.34 (q, $J_{H\text{-}F}$ = 8.0 Hz, 2H, CH_2CF$_3$), 2.29 (s, 3H, CH_3). ^{31}P NMR ($CDCl_3$): δ 32.2 ppm (s, PPh_3).

[Au(L[1])(TPA)] (5a) An aqueous solution of KOH (28.05 mg, 0.5 mmol) (20 mL) was added to a solution of HL[1] (177.0 mg, 0.5 mmol) in MeCN (3 mL); the resulting solution was added to an aqueous suspension of [(TPA)AuCl] (195.0 mg, 0.5 mmol) (20 mL) and the resulting mixture stirred for 24 h at room temperature in the dark. Afterward the white solid was collected by filtration under vacuum and washed with H_2O, EtOH, Et_2O. Recrystallization from acetone/Et_2O gave the analytical sample. Yield 55%. Mp 130 °C. Anal. Calcd for $C_{22}H_{25}AuF_3N_6OPS$ (706.47): C, 37.40; H, 3.57; N, 11.90. Found: C, 37.21; H, 3.48; N, 11.75. Selected IR bands (ν/cm^{-1}, nujol mull): 1579, 1392, 1284, 1167, 1109, 1012, 972, 742. ^{1}H NMR ($CDCl_3$): δ 8.36 (d, $J_{H\text{-}H}$ = 6.0 Hz, 1H, H^6), 7.57 (m, br, 2H, H$^{3'}$, H$^{6'}$), 7.07 (m, br, 2H, H$^{4'}$, H$^{5'}$), 6.64 (d, $J_{H\text{-}H}$ = 5.2 Hz, 1H, H^5), 4.85 (s, 2H, CH_2S), 4.50 (q, AB, J_{AB} = 13.6 Hz, 6H, NCH_2N), 4.39 (q, $J_{H\text{-}F}$ = 8.0 Hz, 2H, CH_2CF$_3$), 4.22 (s, 6H, NCH_2P), 2.35 (s, 3H, CH_3). ^{31}P NMR ($CDCl_3$): δ -58.8 ppm (TPA).

[Au(L[2])(TPA)] (5b) Same procedure as for **5a**. Recrystallization from acetone/Et_2O gave the analytical sample. Yield 68%. Mp 120 °C. Anal. Calcd for $C_{22}H_{25}AuF_3N_6O_2PS$ (722.47): C, 36.57; H, 3.49; N, 11.63. Found C, 36.39; H, 3.43; N, 11.48. Selected IR bands (ν/cm^{-1}, nujol mull): 1680, 1583, 1405, 1284, 1265, 1163, 1106, 1038, 1016, 973, 748. ^{1}H NMR ($CDCl_3$): δ 8.36 (d, $J_{H\text{-}H}$ = 5.6 Hz, 1H, H^6), 7.74 (br, 2H, H$^{3'}$,H$^{6'}$), 7.23 (m, $J_{H\text{-}H}$ = 9.2, 6.0, 3.2 Hz, 2H, H$^{4'}$, H$^{5'}$), 6.68 (d, $J_{H\text{-}H}$ = 5.6 Hz, 1H, H^5), 4.70 (q, AB, J_{AB} = 13.5 Hz, 2H, CH_2SO), 4.57 (q, AB, J_{AB} = 13.5 Hz, 6H, NCH_2N), 4.41 (q, $J_{H\text{-}F}$ = 8.0 Hz, 2H, OCH_2CF$_3$), 4.36 (s, 6H, N-CH_2-P), 2.28 (s, 3H, CH_3). ^{1}H NMR (acetone-d_6): δ 8.34 (d, $J_{H\text{-}H}$ = 5.4 Hz, 1H, H^6), 7.62 (m, AA' part of an AA'BB', $J_{H\text{-}H}$ = 9.0, 6.0, 3.3 Hz, 2H, H$^{3'}$, H$^{6'}$), 7.12 (m, BB' part, $J_{H\text{-}H}$ = 9.0, 5.7, 2.7 Hz, 2H, H$^{4'}$, H$^{5'}$), 7.07 (d, $J_{H\text{-}H}$ = 5.4 Hz, 1H, H^5), 4.83 (q, $J_{H\text{-}F}$ = 8.4 Hz, 2H, OCH_2CF$_3$), 4.71 (AB, J_{AB} = 12.9 Hz, 6H, N-CH_2-N), 4.57 (s, 2H, CH_2SO), 4.52 (s, 6H, N-CH_2-P), 2.27 (s, 3H, CH_3). ^{31}P NMR ($CDCl_3$): δ -58.6 ppm (s, TPA).

Dinuclear Complexes

[Au$_2$(L[1])(PPh$_3$)$_2$](BF$_4$) (7a-BF$_4$) A solution of HL[1] (98.9 mg, 0.28 mmol) and NaH (6.72 mg, 0.28 mmol) in THF (20 mL) was stirred for 10 minutes. Then the solvent was removed under vacuum and a dichloromethane solution of [(PPh$_3$)Au](BF$_4$) (0.56 mmol in 20 mL) added to the residue. The resulting mixture was stirred for 5 h at room temperature, in the darkness. Afterward the suspension was filtered through Celite and concentrated to a small volume. Addition of diethyl ether afforded a white solid of **7a**-BF$_4$. Yield: 74%. Mp 190 °C. Anal. Calcd for $C_{52}H_{43}Au_2BF_7N_3OP_2S$ (1357.66): C, 46.00; H, 3.19; N, 3.10. Found: C, 46.02; H, 3.23; N, 2.97.

Selected IR bands (ν/cm^{-1}, nujol mull): 1679, 1581, 1261, 1165, 1105 (PPh_3), 1059 (BF_4^-), 970, 837, 746, 692. ^{1}H NMR ($CDCl_3$): δ 8.03 (d, $J_{H\text{-}H}$ = 5.2 Hz, 1H, H^6), 7.75 (m, 2H, AA' part of an AA'BB', $J_{H\text{-}H}$ = 8.8, 5.6, 3.2 Hz, H$^{3'}$, H$^{6'}$), 7.63-7.55 (m, 30H, PPh_3), 7.34 (m, BB' part, $J_{H\text{-}H}$ = 8.8, 5.6, 2.8 Hz, 2H, H$^{4'}$, H$^{5'}$), 6.67 (d, $J_{H\text{-}H}$ = 5.6 Hz, 1H, H^5), 4.59 (s, 2H, CH_2S), 4.30 (q, $J_{H\text{-}F}$ = 8.0 Hz, 2H, CH_2CF$_3$), 1.92 (s, 3H, CH_3). ^{31}P NMR (CD_2Cl_2): δ 31.2 and 33.1 ppm.

[Au$_2$(L^2)(PPh$_3$)$_2$](BF$_4$) (7b-BF$_4$) Same procedure as for **7a-BF$_4$**. Yield 57%. Mp 135 °C. Anal. Calcd for $C_{52}H_{43}Au_2BF_7N_3O_2P_2S$ (1373.66): C, 45.47; H, 3.16; N, 3.06. Found: C, 45.33; H, 2.92; N, 2.88. Selected IR bands (ν/cm^{-1}, nujol mull): 1682, 1579, 1261, 1167, 1103 (PPh_3), 1057 (BF_4^-), 970, 810, 748, 694. ^{1}H NMR ($CDCl_3$): δ 7.95 (d, br, $J_{H\text{-}H}$ = 4.8 Hz, 1H, H^6), 7.81 (m, AA' part of an AA'BB', $J_{H\text{-}H}$ = 8.8, 5.6, 2.8 Hz, 2H, H$^{3'}$, H$^{6'}$), 7.59 (m, br, 30H, PPh_3), 7.39 (m, br, BB' part, $J_{H\text{-}H}$ = 9.2, 5.2, 3.2 Hz, 2H, H$^{4'}$, H$^{5'}$), 6.78 (d, $J_{H\text{-}H}$ = 5.6 Hz, 1H, H^5), 4.78 (q, AB, $J_{H\text{-}H}$ = 13.2 Hz, 2H, CH_2SO), 4.27 (qd, $J_{H\text{-}F}$ = 8.0, 3.2 Hz, 2H, CH_2CF$_3$), 1.93 (s, 3H, CH_3). ^{31}P NMR ($CDCl_3$): δ 31.0 and 33.2 ppm.

Cell viability assay

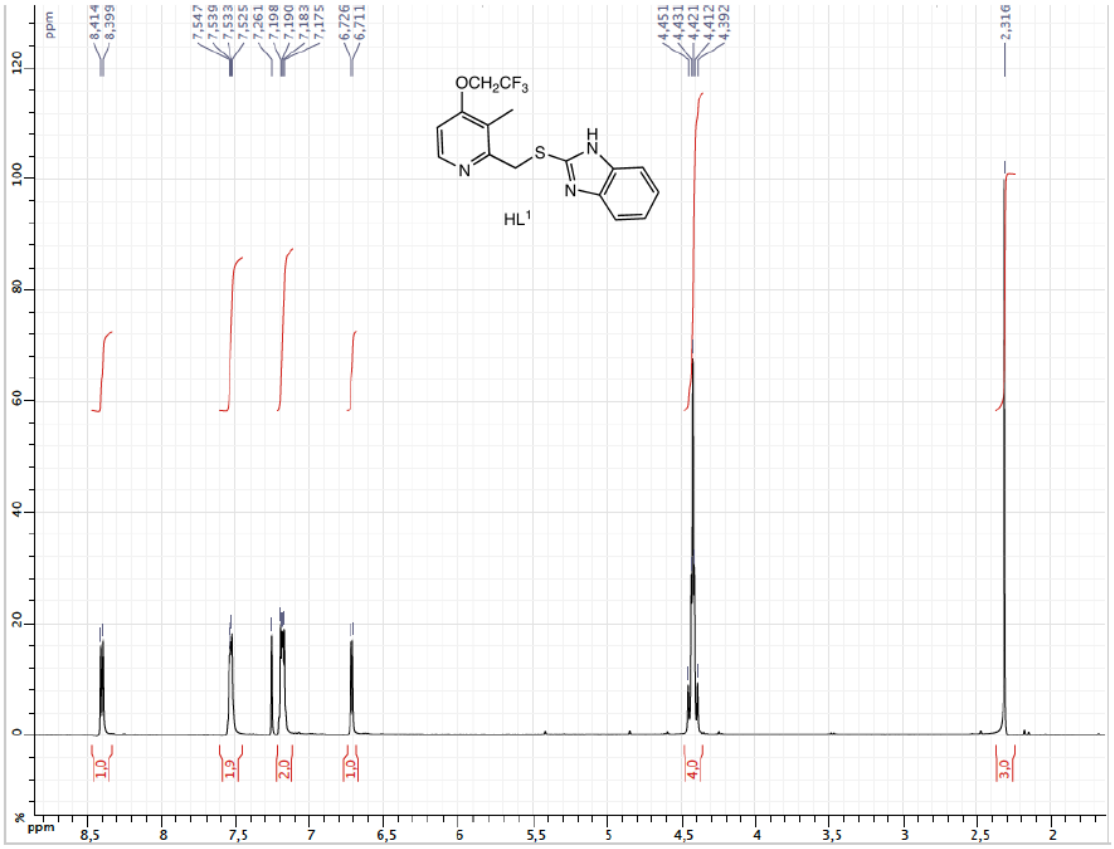
The human ovarian cancer A2780 and A2780cisR cells, (obtained from the European Centre of Cell Cultures ECACC, Salisbury, UK) were cultured in RPMI medium containing GlutaMax-I supplemented with 10% FBS and 1% penicillin/streptomycin (all from Invitrogen), at 37°C in a humidified atmosphere of 95% of air and 5% CO_2 (Heraeus, Germany). Non-tumoral human embryonic kidney cells HEK-293T were cultivated in DMEM medium with GlutaMax-I, 10% FBS and 1% penicillin/streptomycin, incubated in the same conditions as other cell lines. For evaluation of growth inhibition, cells were seeded in 96-well plates (Costar, Integra Biosciences, Cambridge, MA) at a concentration of 10000 cells/well and grown for 24 h in complete medium. Solutions of the compounds were prepared by diluting a freshly prepared stock solution (10^{-2} M in DMSO) of the corresponding compound in aqueous media (RPMI or DMEM depending on the cell lines). The percentage of DMSO in the culture medium never exceeded 0.2%: at this concentration DMSO has no effect on the cell viability. Cisplatin (Sigma-Aldrich) stock solutions were prepared at 1 mM conc. in MilliQ water. Afterwards, the intermediate dilutions of the compounds were added to the wells (200 µL) to obtain a final concentration ranging from 0 to 200 µM, and the cells were incubated for 72 h. Following 72 h drug exposure, 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide (MTT) was added to the cells at a final concentration of 0.50 mg.ml^{-1} incubated for 3-4 h, then the culture medium was removed and the violet formazan dissolved in DMSO. The optical density of each well (96-well plates) was quantified in quadruplicate at 540 nm

using a multi-well plate reader and the percentage of surviving cells was calculated from the ratio of absorbance between treated and untreated cells. The IC_{50} value was calculated as the concentration reducing the proliferation of the cells by 50% and is presented as a mean (± SE) of at least three independent experiments.

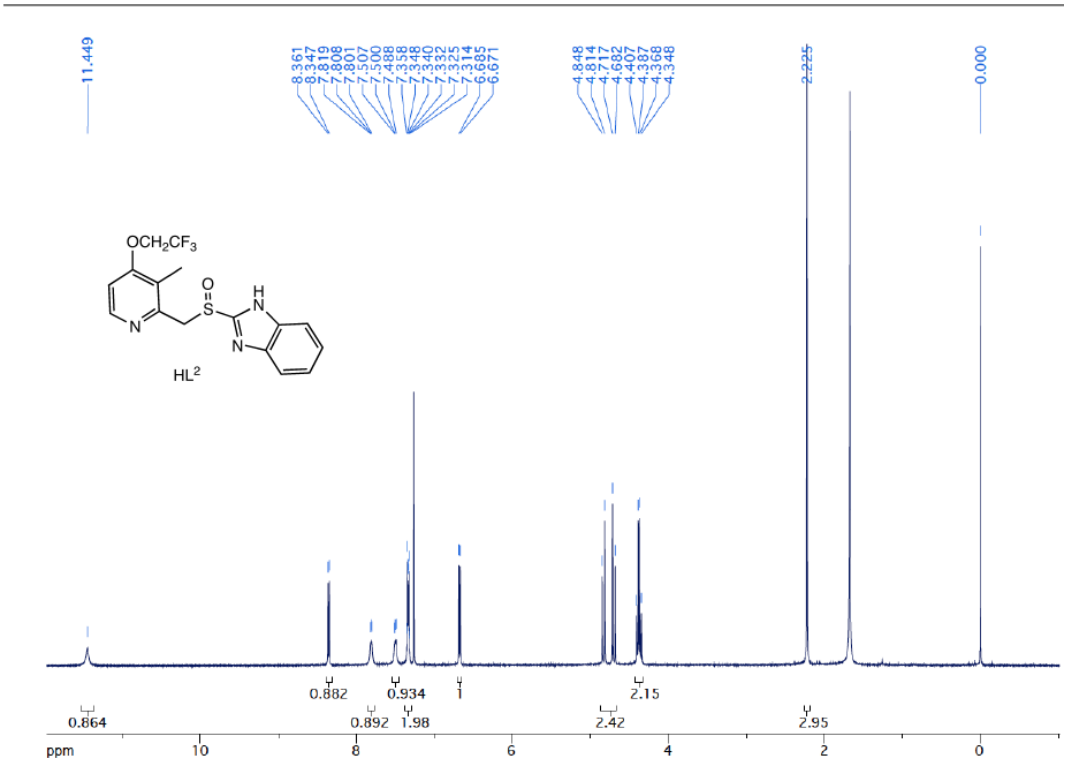
Measurement of intracellular ATP content

Cellular ATP levels as an indirect parameter of the activity of V-H^+-ATPase were determined using a bioluminescence-based assay (Roche diagnostic, Mannheim, Germany). Thus, $1x10^4$ cells per well were allowed to attach in white-bottom 96-well plates overnight. Then cells were treated with compounds at different concentrations (2 μM and 5 μM) for 3 h at 37°C. The medium was then removed, and the cells were lysed as previously reported (Schneider V. et al, JBIC 2013, 18, 165). ATP content was quantified using the above mentioned detection kit and measuring the luminescence signal in a black 96-well plate Lucy1 luminometer (Anthos, Durham, NC, USA) using a standard ATP calibration curve. The ATP content was related to protein content determined by the Lowry protein assay (Bio Rad, Veenendaal, The Netherlands). Results are expressed as a mean (± SE) of at least three independent experiments.

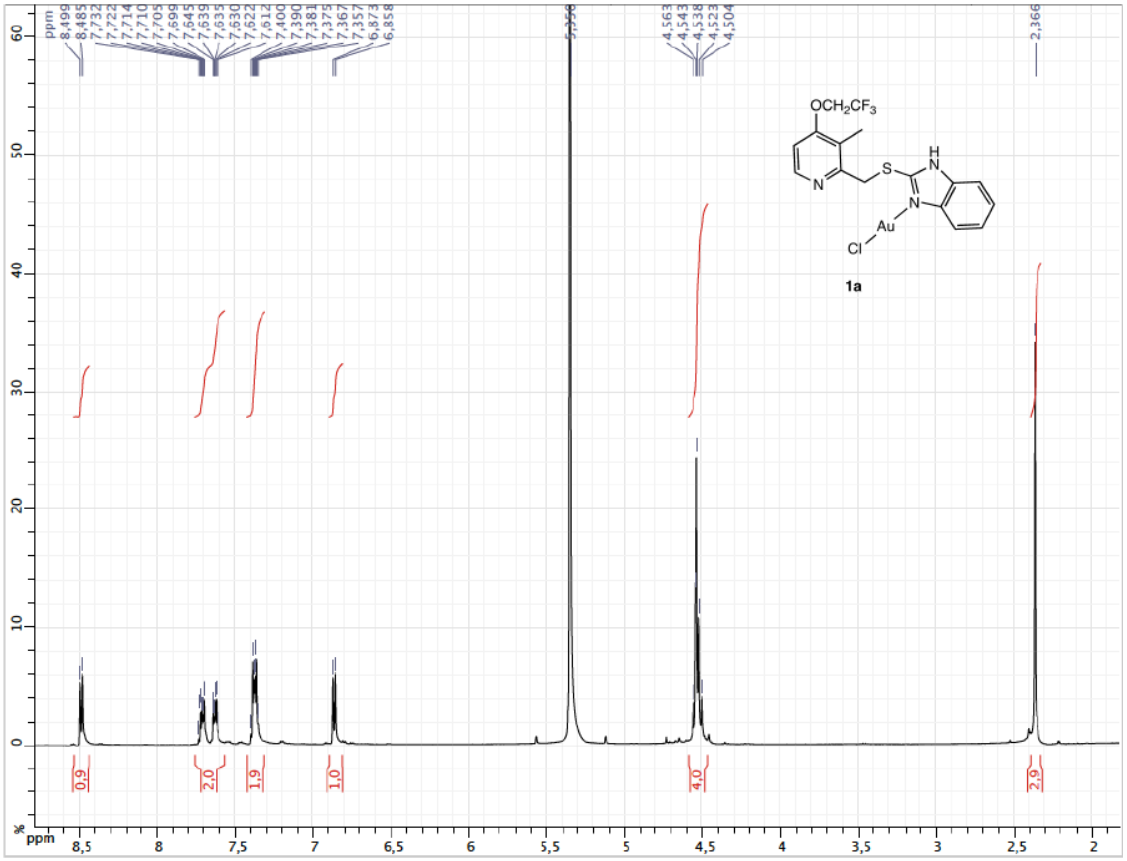
NMR spectra



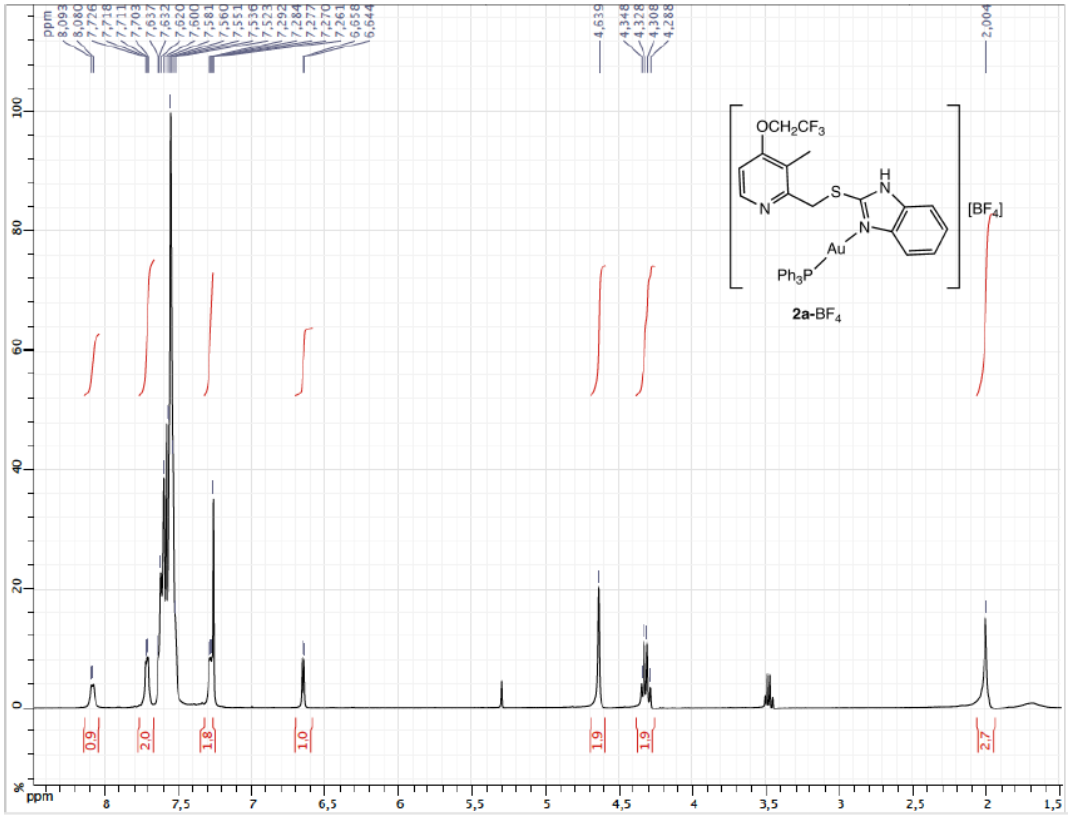
^{1}H NMR ($CDCl_3$) HL^1



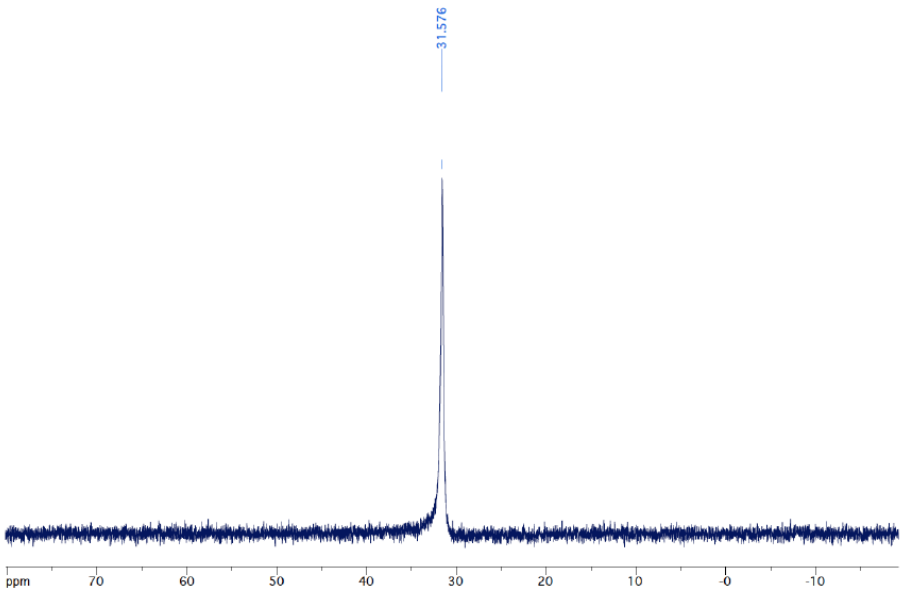
^{1}H NMR ($CDCl_3$) HL^2



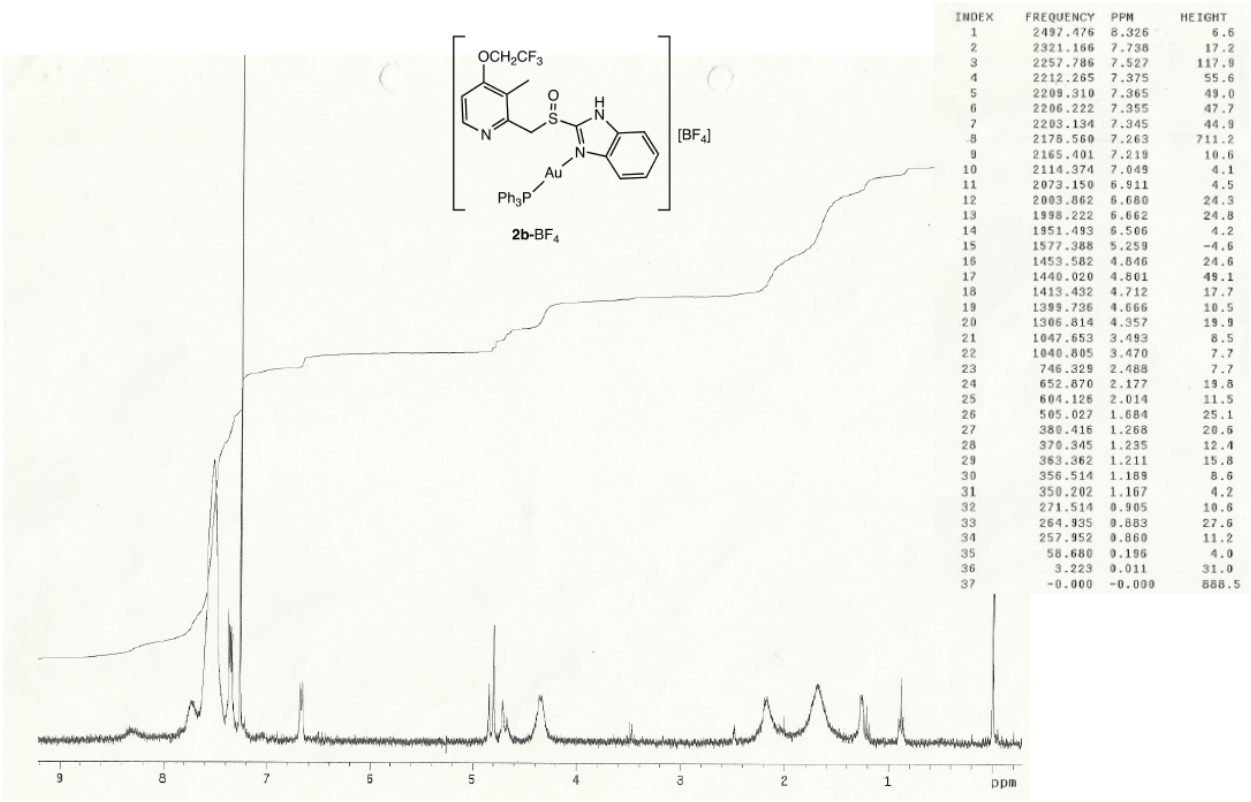
^{1}H NMR (CD_2Cl_2) **1a**



^{1}H NMR ($CDCl_3$) **2a**-BF_4

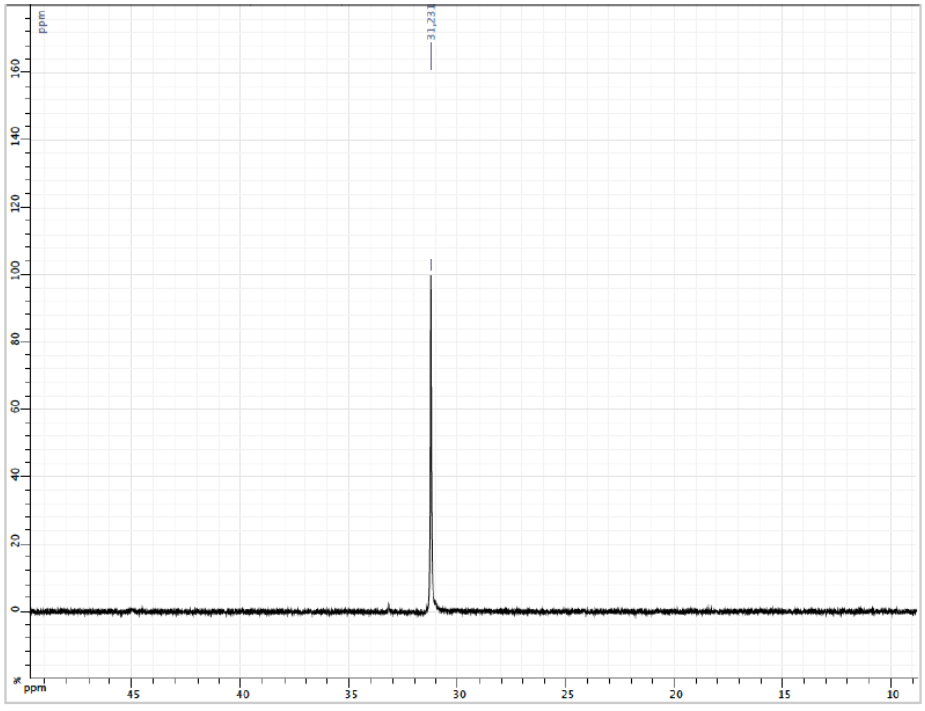


^{31}P NMR ($CDCl_3$) **2a**-BF_4

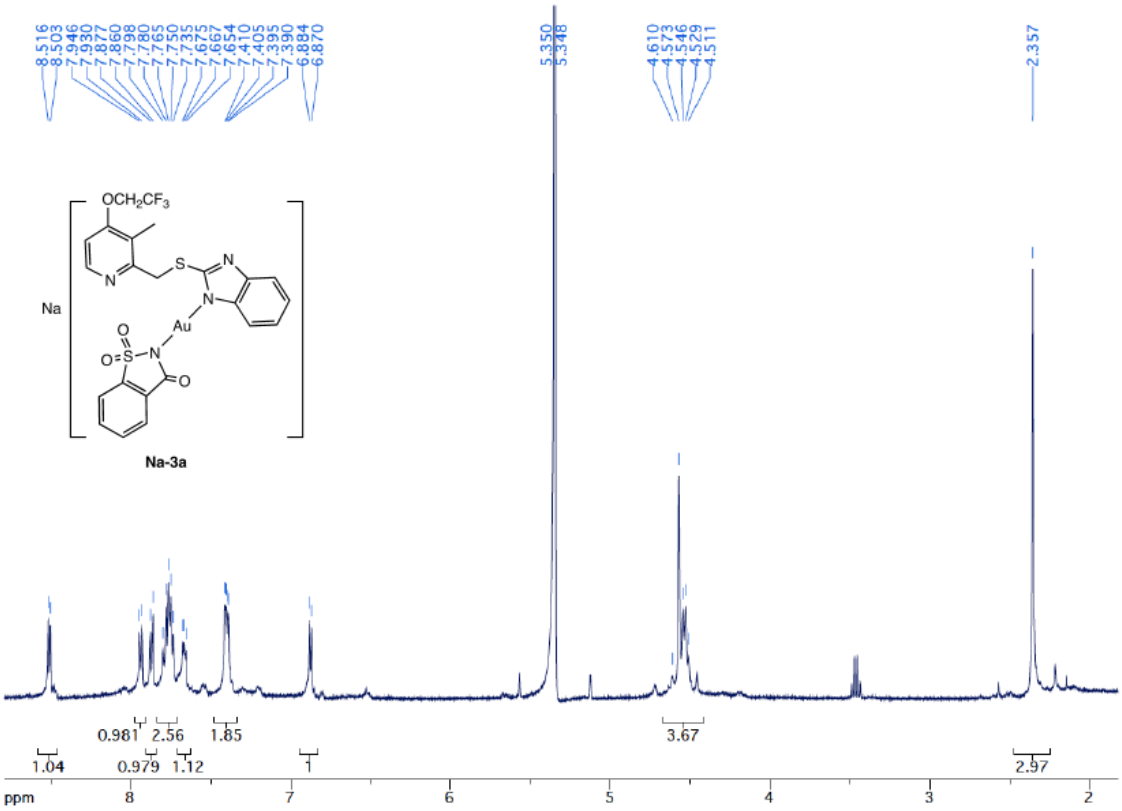


INDEX	FREQUENCY	PPM	HEIGHT
1	2497.476	8.326	6.6
2	2321.166	7.738	17.2
3	2257.786	7.527	117.9
4	2212.265	7.375	55.6
5	2209.310	7.365	49.0
6	2206.222	7.355	47.7
7	2203.134	7.345	44.9
8	2178.560	7.263	711.2
9	2165.401	7.219	10.6
10	2114.374	7.049	4.1
11	2073.150	6.911	4.5
12	2003.862	6.680	24.3
13	1998.222	6.662	24.8
14	1951.493	6.506	4.2
15	1577.388	5.259	-4.6
16	1453.582	4.846	24.6
17	1440.020	4.801	49.1
18	1413.432	4.712	17.7
19	1399.736	4.666	10.5
20	1306.814	4.357	19.9
21	1047.653	3.493	8.5
22	1040.805	3.470	7.7
23	746.328	2.488	7.7
24	652.870	2.177	19.8
25	604.126	2.014	11.5
26	505.027	1.684	25.1
27	380.416	1.268	20.6
28	370.345	1.235	12.4
29	363.362	1.211	15.8
30	356.514	1.189	8.6
31	350.202	1.167	4.2
32	271.514	0.905	10.6
33	264.935	0.883	27.6
34	257.952	0.860	11.2
35	58.680	0.196	4.0
36	3.223	0.011	31.0
37	-0.000	-0.000	888.5

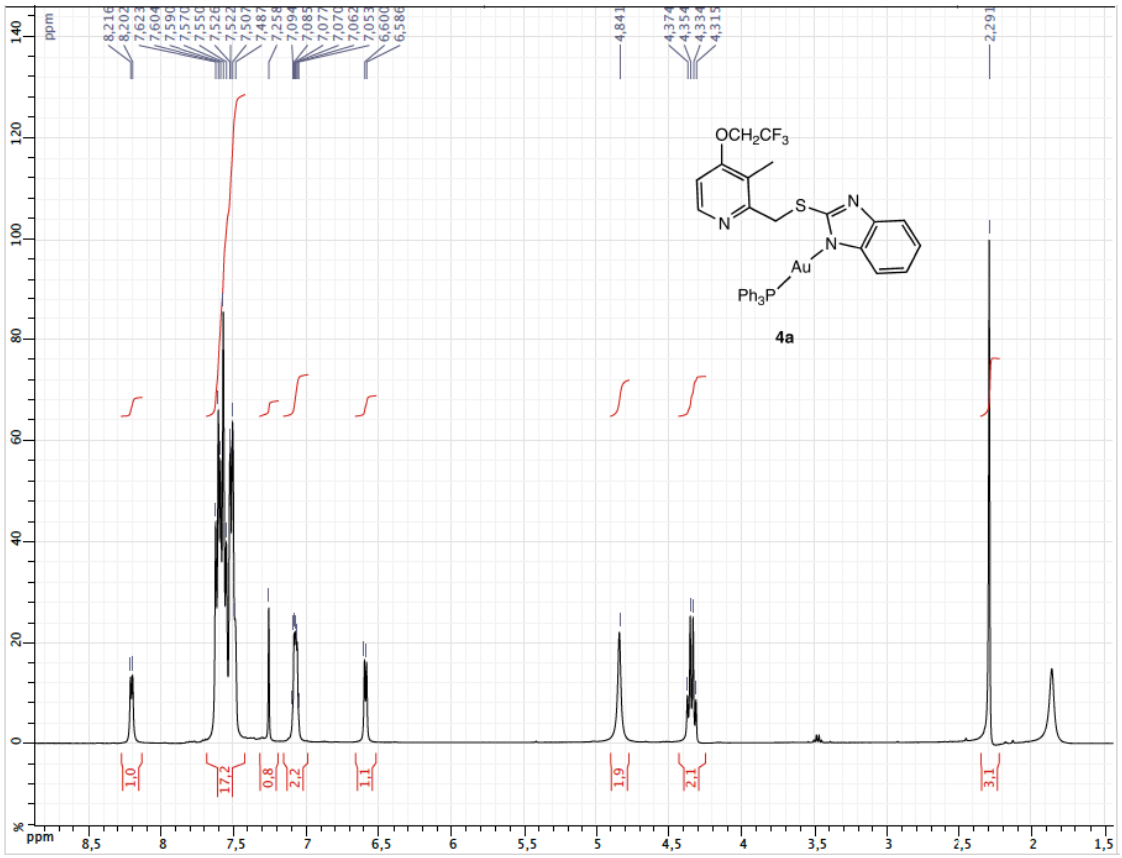
^{1}H NMR ($CDCl_3$) **2b**-BF_4



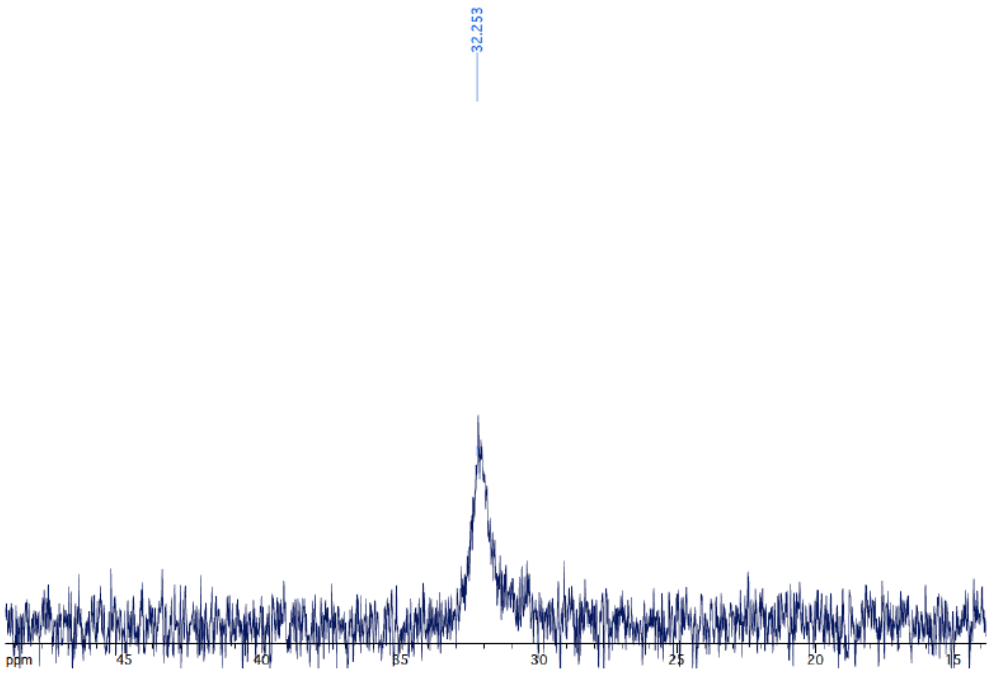
^{31}P NMR($CDCl_3$) **2b**-BF_4



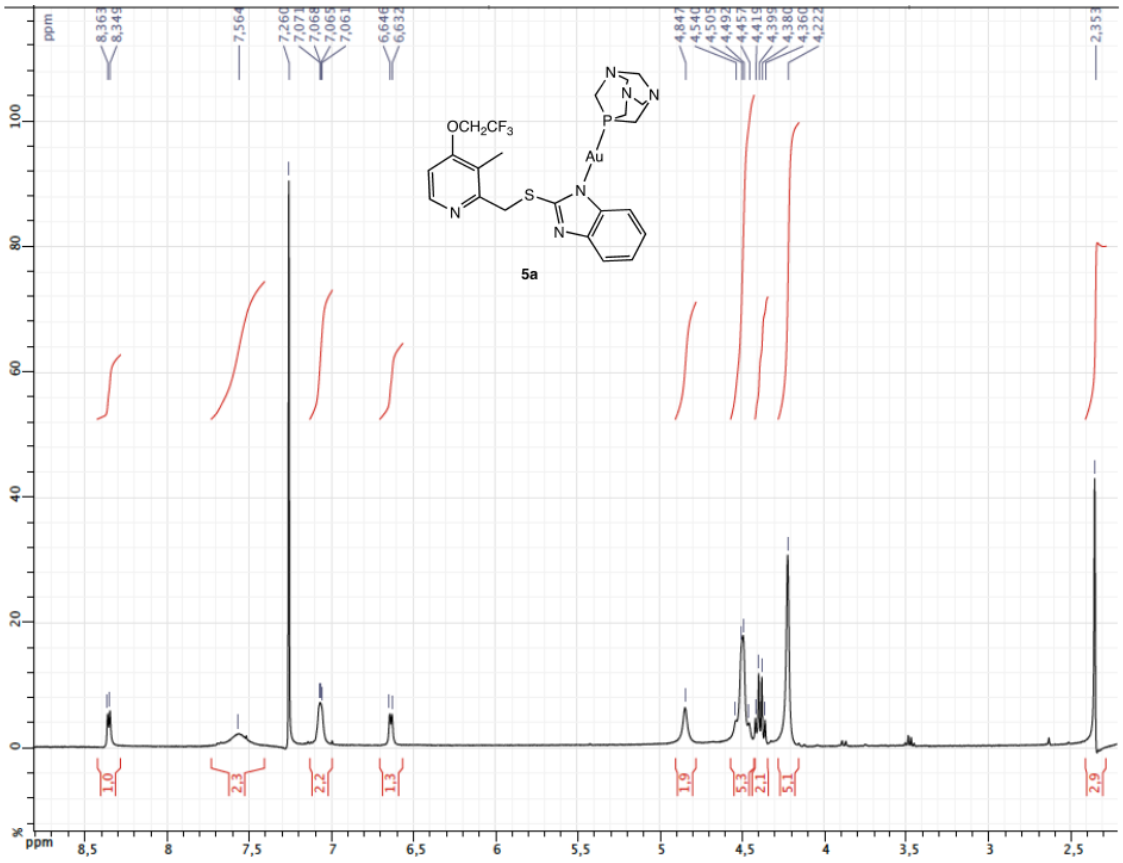
^{1}H NMR (CD_2Cl_2) Na-**3a**



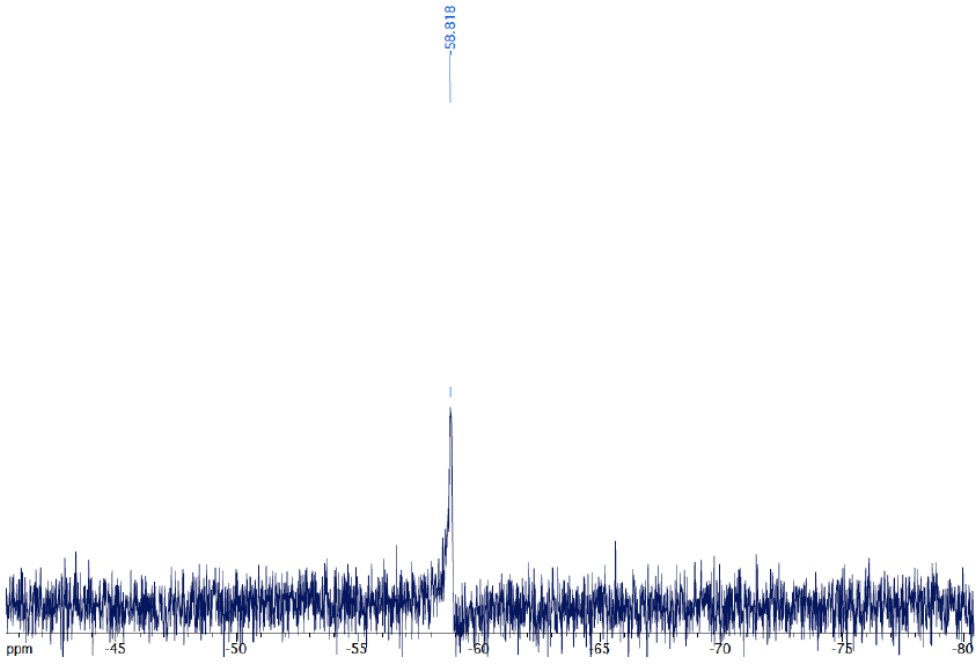
^{1}H NMR ($CDCl_3$) **4a**



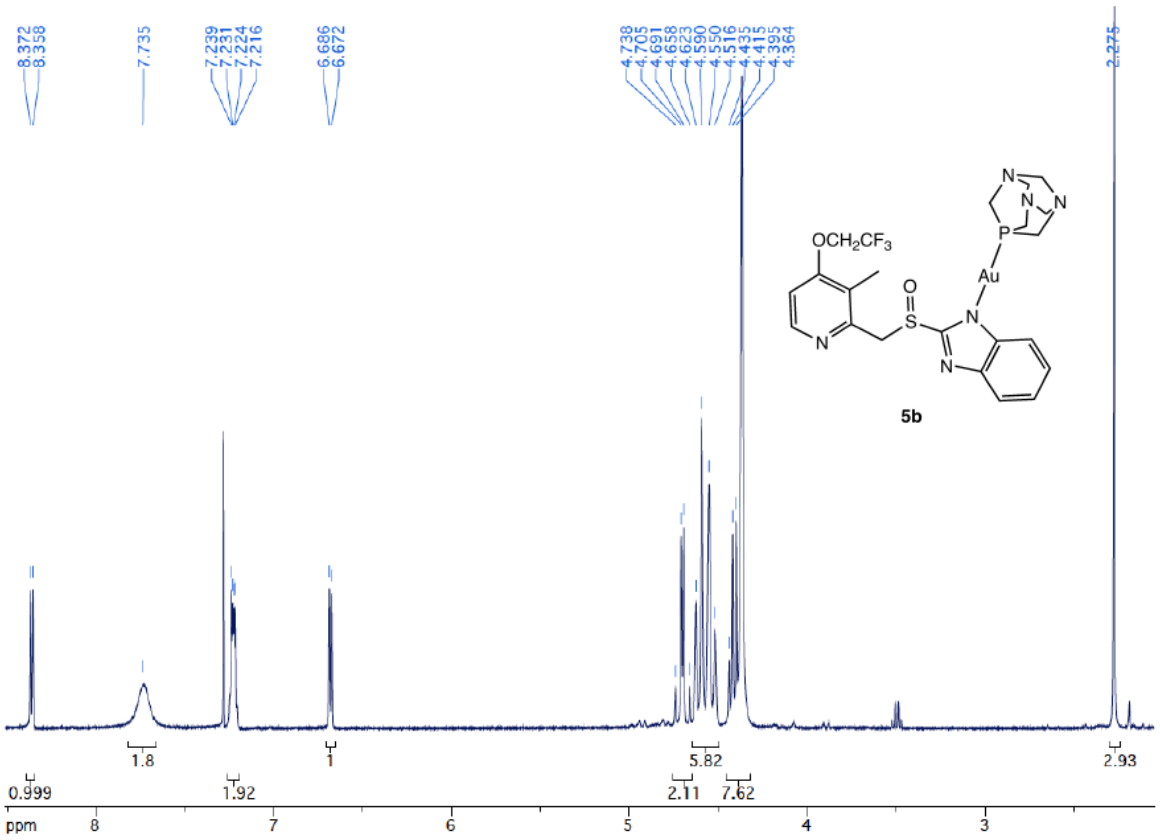
^{31}P NMR ($CDCl_3$) **4a**



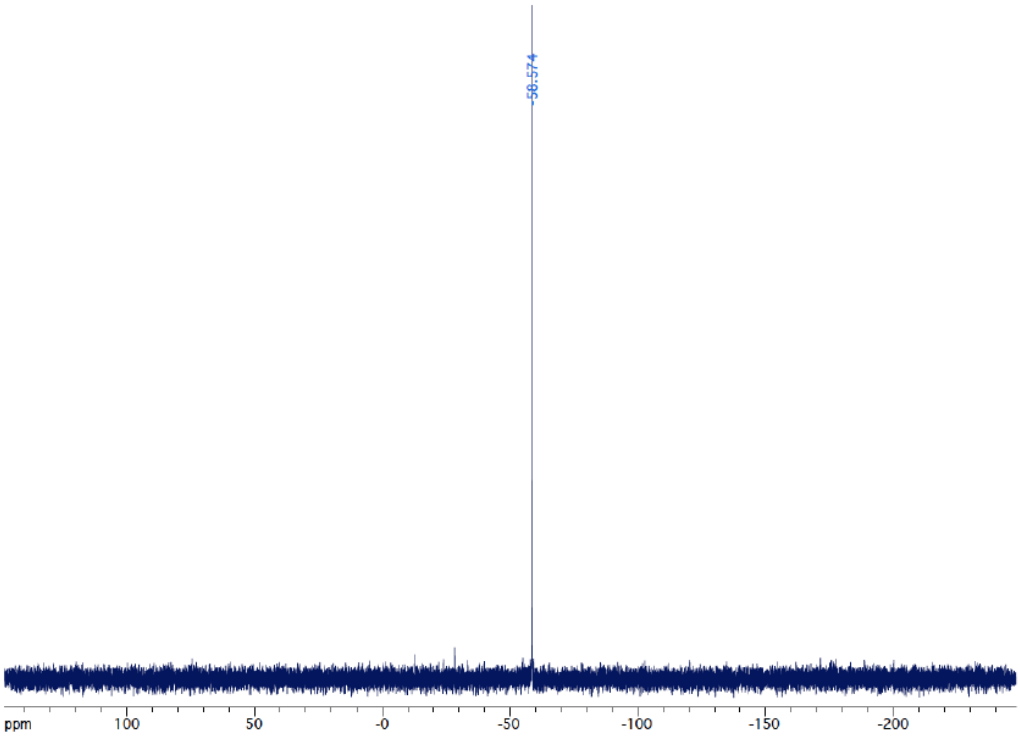
^{1}H NMR ($CDCl_3$) **5a**



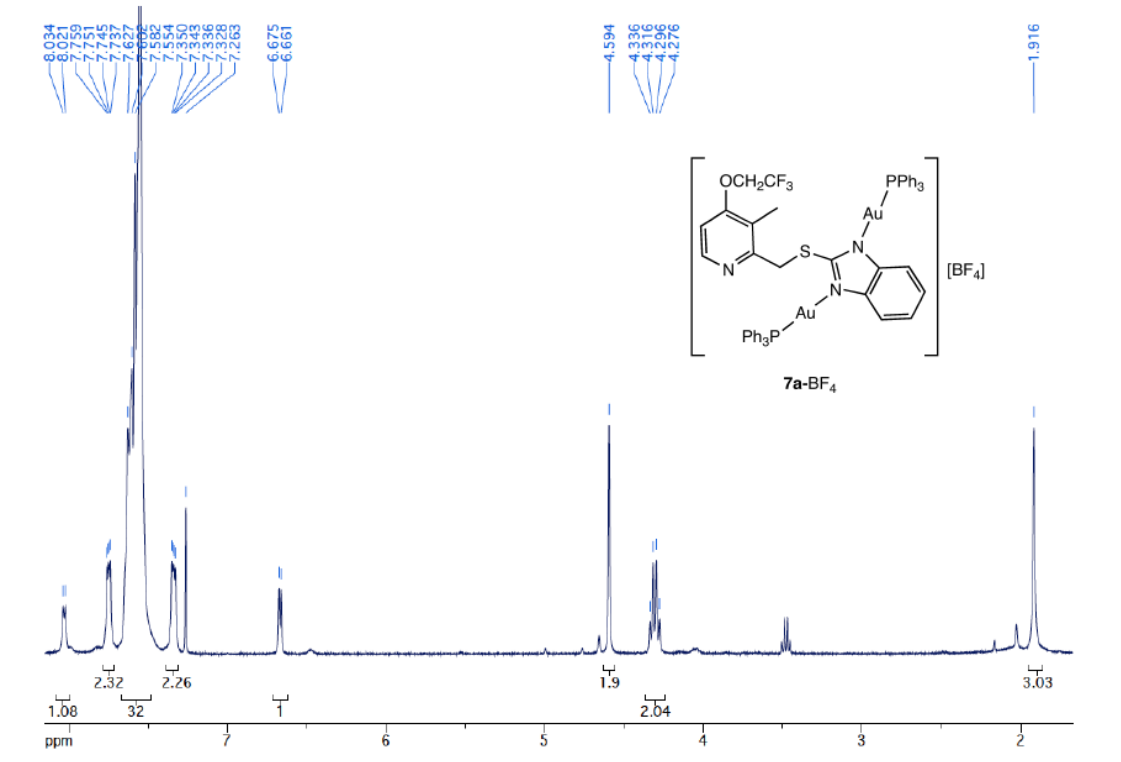
^{31}P NMR ($CDCl_3$) **5a**



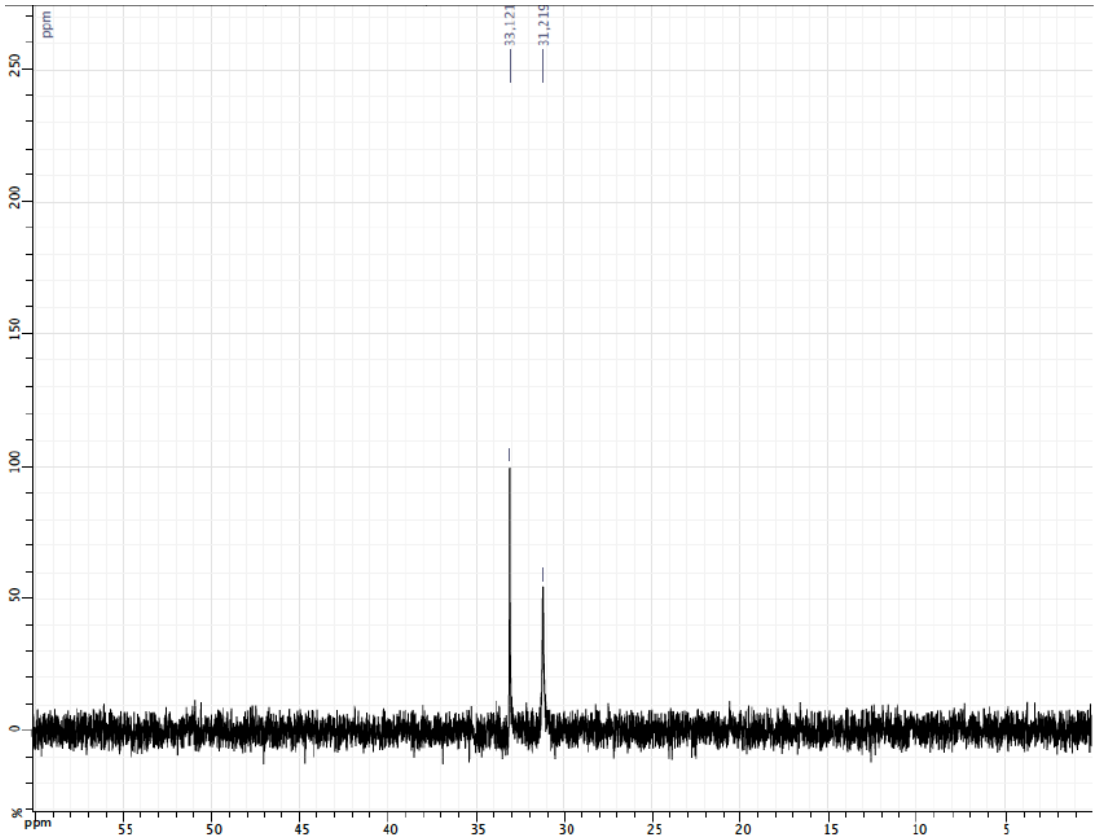
^{1}H NMR ($CDCl_3$) **5b**



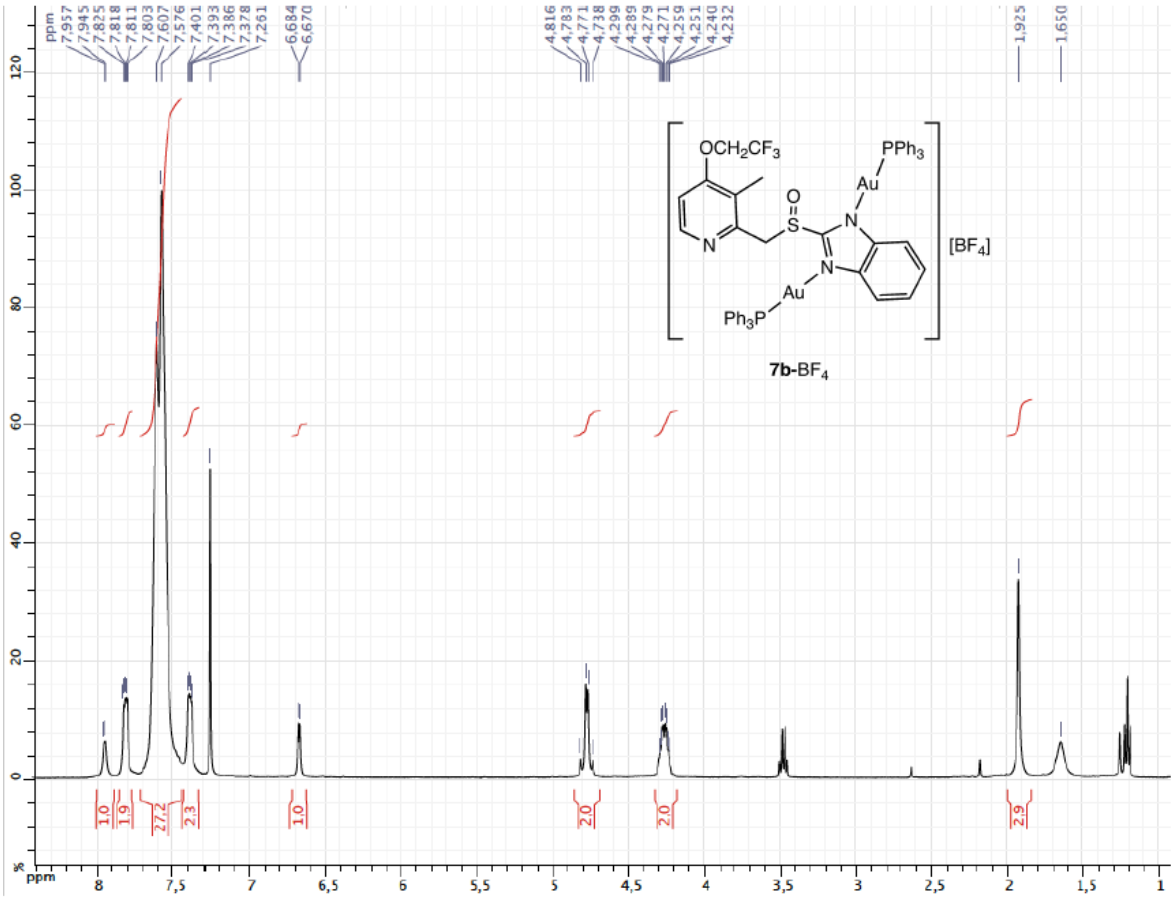
^{31}P NMR ($CDCl_3$) **5b**



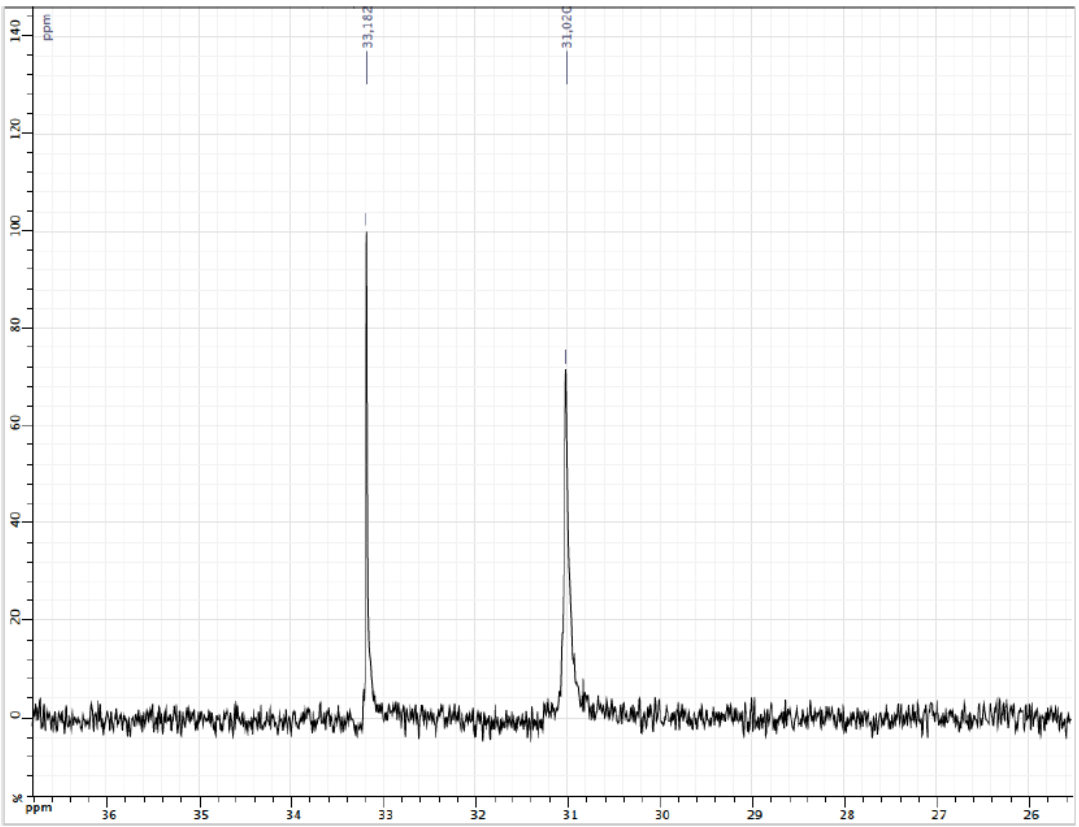
^{1}H NMR ($CDCl_3$) **7a**-BF_4



^{31}P NMR ($CDCl_3$) **7a**-BF_4



^{1}H NMR ($CDCl_3$) **7b**-BF_4



^{31}P NMR ($CDCl_3$) **7b**-BF_4

[1] S. Gangula, C. R. Elati, A. Neredla, S. R. Baddam, U. K. Neelam, R. Bandichhor and A. Dongamanti, *Org. Process Res. Dev.*, **2010**, *14*, 229-233.

[2] C.A. McAuliffe, R.V. Parish and P.D. Randall, *J. Chem. Soc., Dalton Trans.* **1979**, 1730−1735.

[3] Z. Assefa, B. G. McBurnett, R. J. Staples, J. P. Fackler, B. Assmann, K. Angermaier, H. Schmidbaur, *Inorg. Chem.* **1995**, *34*, 75-83.

[4] R. Uson, A. Laguna and M. Laguna, *Inorg. Synth*. **1989**, *26*, 85.

[5] M. A. Cinellu, L. Maiore, A. Schier, H. Schmidbaur, D. Rossi. *Z. Naturforsch. B*, **2008**, *63b*, 1027-1034.