Electronic Supporting Information (ESI)

A novel aminotriazole-based NHC complex for the design of gold(I) anti-cancer agents: synthesis and biological evaluation

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Materials and methods

General

All reagents and the solvents were used as received from Sigma, Aldrich, or Acros Organics. 1-tert-butyl-4-(tert-butylamino)-4*H*-1,2,4-triazol-1-ium perchlorate (**1**) was synthesized as described earlier.[1] ^{1}H and ^{13}C NMR spectra were recorded on a Bruker DRX-400 AS NMR System. HRESI(+) mass spectra were recorded on a Bruker micrOTOF instrument using dichloromethane as a solvent. The purity of the target compound (>95%) was confirmed by elemental analysis. IR spectra of powder samples were registered on a Nicolet Thermo Avatar 330 spectrometer using Smart Diffuse Reflection accessory in the range of 4000–400 cm^{-1}. The TG and DSC curves were obtained with a NETZSCH STA 429 thermoanalyzer in a dynamic nitrogen atmosphere (heating rate of 10 $K \cdot min^{-1}$, aluminum oxide, mass 5–6 mg and temperature range from room temperature up to 600 °C).

TrxR/GR inhibition assay

To determine the inhibition of TrxR an established microplate reader based assay was performed. For this purpose commercially available rat liver TrxR was used and diluted with distilled water to achieve concentrations of 0.4 U/mL (TrxR). The compounds were freshly dissolved as stock solutions in DMF. To each 25 *µ*L aliquots of the enzyme solution each 25 *µ*L of potassium phosphate buffer pH 7.0 containing the compounds in graded concentrations or vehicle (DMF) without compounds (control probe) were added and the resulting solutions (final concentration of DMF: max. 0.5% V/V) were incubated with moderate shaking for 75 min at 37 °C in a 96 well plate. To each well 225 µL of reaction mixture (1000 µL reaction mixture consisted of 500 µL potassium phosphate buffer pH 7.0, 80 µL 100 mM EDTA solution pH 7.5, 20 µL BSA solution 0.05%, 100 µL of 20 mM NADPH solution and 300 µL of distilled water) were added and the reaction was started by addition of 25 µL of an 20 mM ethanolic dithio-bis-2-nitrobenzoic acid (DTNB) solution. After proper mixing, the formation of 5-thio-2-nitrobenzoic acid (5-TNB) was monitored with a microplate reader (Perkin Elmer Victor X4) at 405 nm in 35 s intervals for 350 s. For each tested compound the non interference with the assay components was confirmed by a negative

control experiment using an enzyme free solution. The IC_{50} values were calculated as the concentration of compound decreasing the enzymatic activity of the untreated control by 50% and are given as the means and error of 3 independent experiments.

Cell culture and Cytotoxicity Assay in MDA-MB-231 and HT-29 Cells

The antiproliferative effects in MDA-MB-231 and HT-29 cells after 72 h (HT-29) or 96 h (MDA-MB-231) exposure to the gold complexes were evaluated using the crystal violet assay. In short: HT-29 human colon carcinoma cells and MDA-MB-231 breast cancer cells were maintained in cell culture medium (minimum essential eagle medium supplemented with 2.2 g $NaHCO_3$, 110 mg L^{-1} sodium pyruvate and 50 mg L^{-1} gentamicin sulfate adjusted to pH 7.4) containing 10% (V/V) fetal calf serum at 37 °C/5% CO_2 and passaged once a week according to standard procedures. For the experiments, the compounds were prepared freshly as stock solutions in DMF and diluted with the cell culture medium to the final assay concentrations (0.1% V/V DMF). The assay procedure is described in more detail in our recent paper.[2] The IC_{50} value was described as that concentration reducing proliferation of untreated control cells by 50%.

Sample preparation for cellular uptake studies

The cellular uptake was measured according to a previously described procedure.[3] In short: cells were grown until at least 70% confluency in 75 cm^2 cell culture flasks. Stock solutions of the gold complexes in DMF were freshly prepared and diluted with cell culture medium to the desired concentration (final DMF concentration: 0.1% V/V, final gold complex concentration: 2 and 10 µM). The cell culture medium of the cell culture flasks was replaced with 4 mL of the cell culture medium solutions containing the compounds and the flasks were incubated at 37 °C / 5% CO_2 for 1, 4 or 8 h. The cell pellets were isolated by trypsinisation and centrifugation (room temperature, 3500 g, 5 min), resuspended in double distilled water, lysed by sonication and appropriately diluted using double distilled water. An aliquot was removed for the purpose of protein quantification by the Bradford method. The determination of the gold content of the samples was performed by high resolution continuum source atomic absorption spectroscopy (see below). Results were calculated from the data of 3 independent experiments and are given as nmol gold per mg cellular protein.

Atomic absorption spectroscopy (AAS)

Gold contents were measured with a graphite furnace high resolution continuum source atomic absorption spectrometer (contrAA®700, Analytik Jena AG) at 242.795 nm according to a recently described method with minor modifications.[4] In short: to 200 µL aliquots of the diluted lysates 20 µL triton X-100 (1%) and 20 µL ascorbic acid (1%) were added and the

probes were measured as described below. The gold content of the lysates was accessed by an extrapolation method using freshly prepared matrix-matched solutions of test compounds for calibration. Probes were injected at a volume of 25 μL into graphite wall tubes. The mean absorbance of triplicate injections was used throughout the study. Drying, atomization and tube cleaning steps were performed as outlined in more detail in the literature.[3]

X-ray Study

For single crystal X-ray diffraction experiment, a crystal of **3** was fixed on a micro mount and placed on a Agilent Technologies SuperNova diffractometer and measured at a temperature of 100K using monochromated CuKα radiation. The unit cell parameters of **3** (Table S1) were refined by least square techniques using 26372 reflections in the 2θ range of 7.11–134.96. The structure have been solved by the direct methods and refined R_1 = 0.08 (wR_2 = 0.204) for 4127 unique reflections with $|F_o| \geq 4\sigma_F$ by means of the SHELX program[5] incorporated in the *OLEX2* program package.[6] The crystallographic data and some parameters of refinement are placed in Table S1. The carbon-bound H atoms were placed in calculated positions and were included in the refinement in the 'riding' model approximation, with U_{iso}(H) set to 1.5U_{eq}(C) and C–H 0.96 Å for CH_3 groups. High values of the refinement parameters and rather low bonds precision in the structural model are due to the low quality of the crystals. Empirical absorption correction was applied in CrysAlisPro[7] program complex using spherical harmonics, implemented in SCALE3 ABSPACK scaling algorithm. Supplementary crystallographic data for this paper have been deposited at Cambridge Crystallographic Data Centre (CCDC 1053436) and can be obtained free of charge *via* www.ccdc.cam.ac.uk/data_request/cif.

References

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5 G. M. Sheldrick, *Acta Cryst.,* 2008, **A64**, 112.

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7 CrysAlisPro, Agilent Technologies, Version 1.171.36.20 (release 27-06-2012).

Examples of NMR spectra

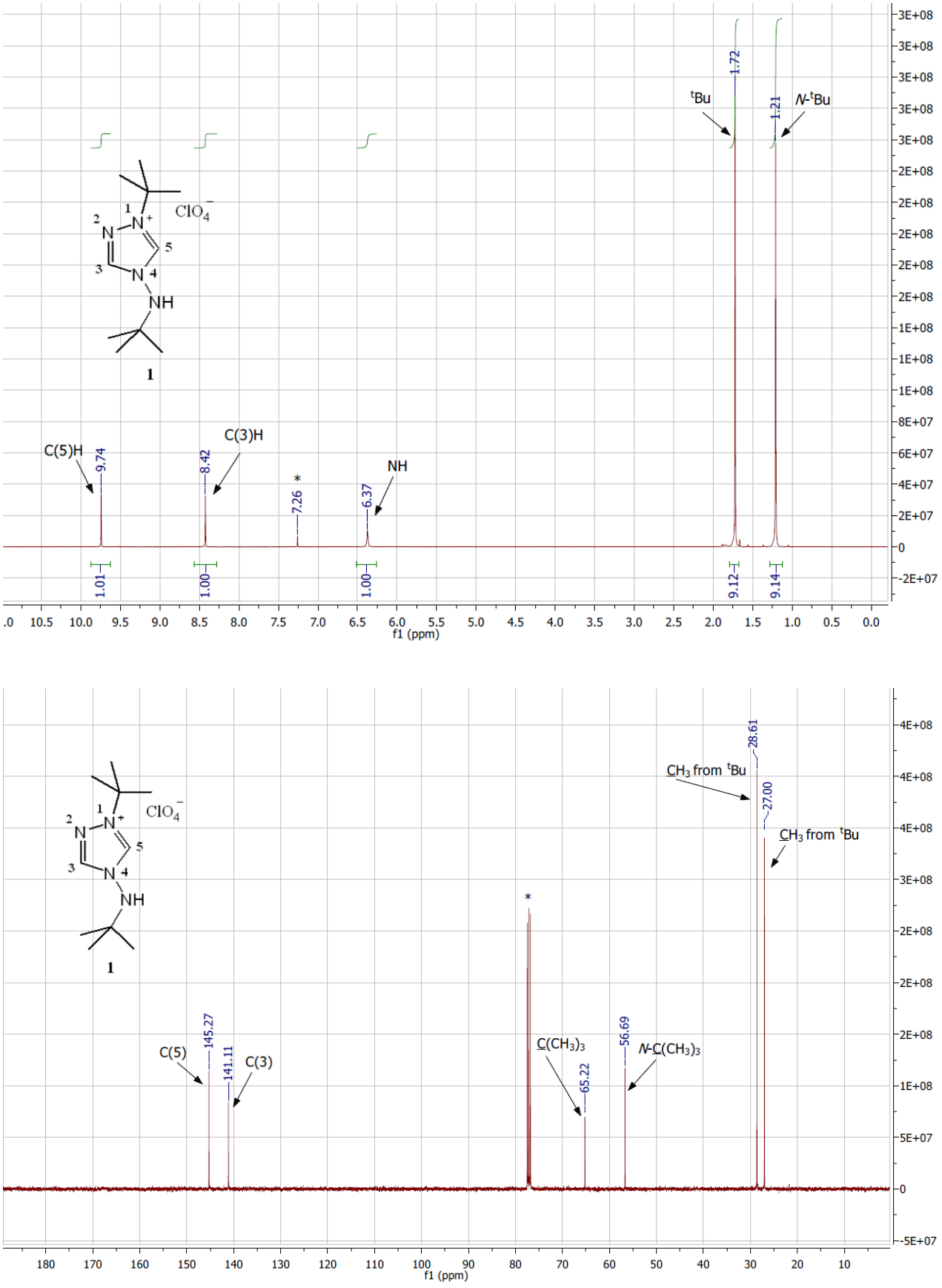


Figure S1. ^{1}H and ^{13}C NMR spectra of **1**.

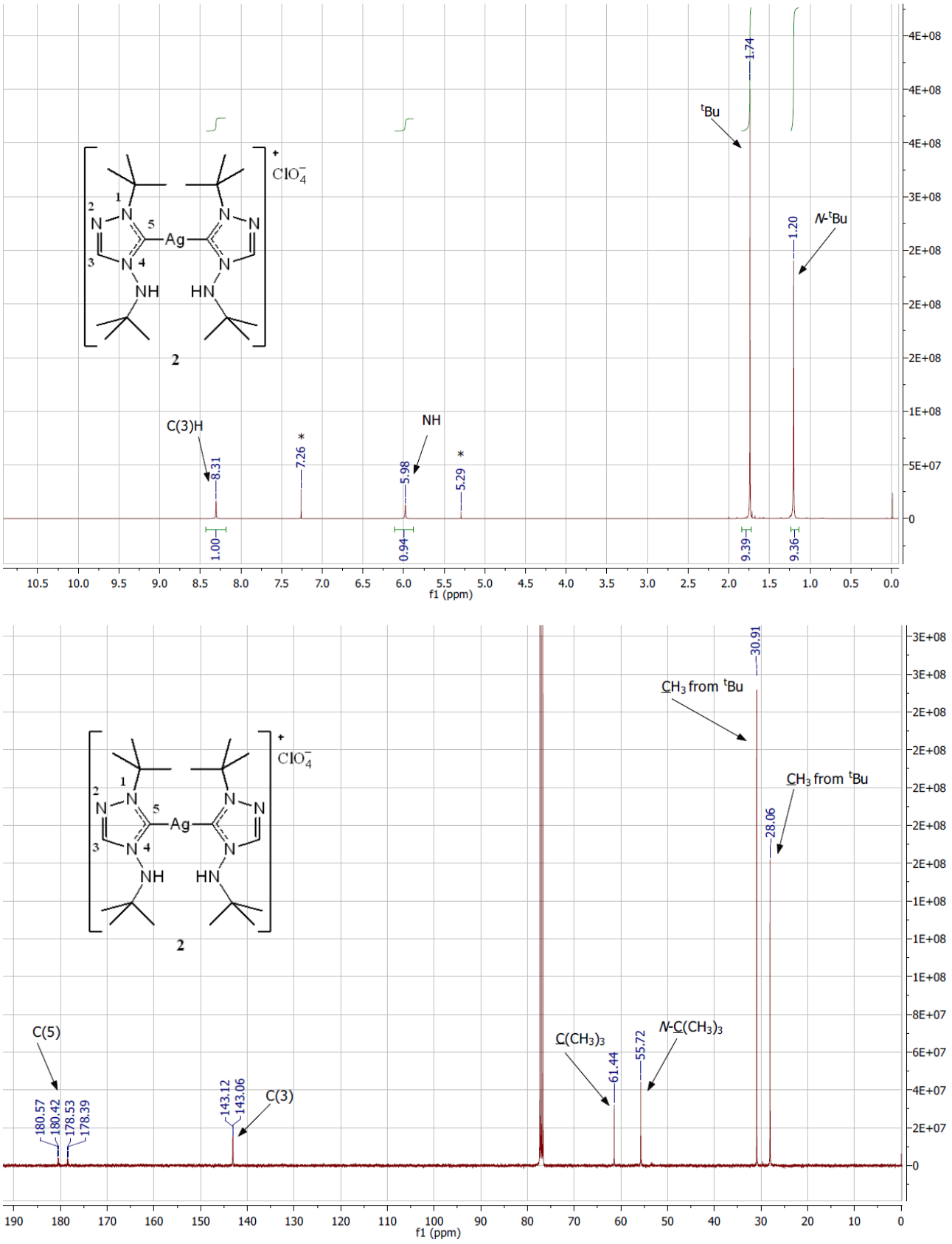
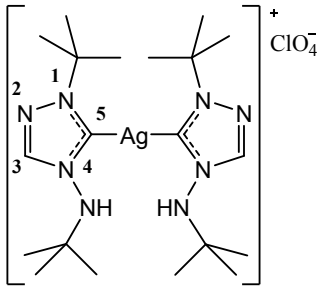


Figure S2. ^{1}H and ^{13}C NMR spectra of **2**.



2

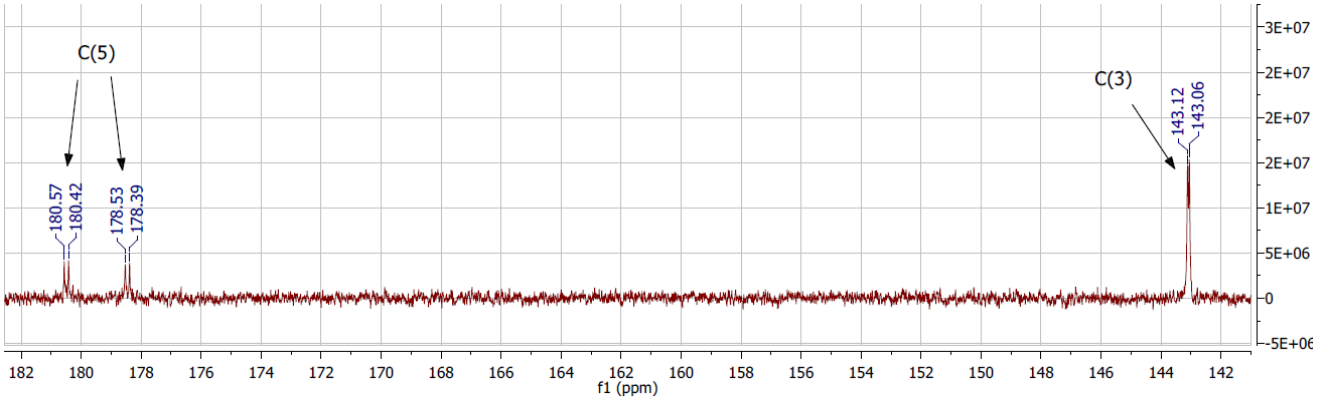


Figure S3. Inset showing splitting of C(3) and C(5) resonances due to ^{13}C-$^{107/109}Ag$ spin coupling in the ^{13}C NMR spectrum of **2**.

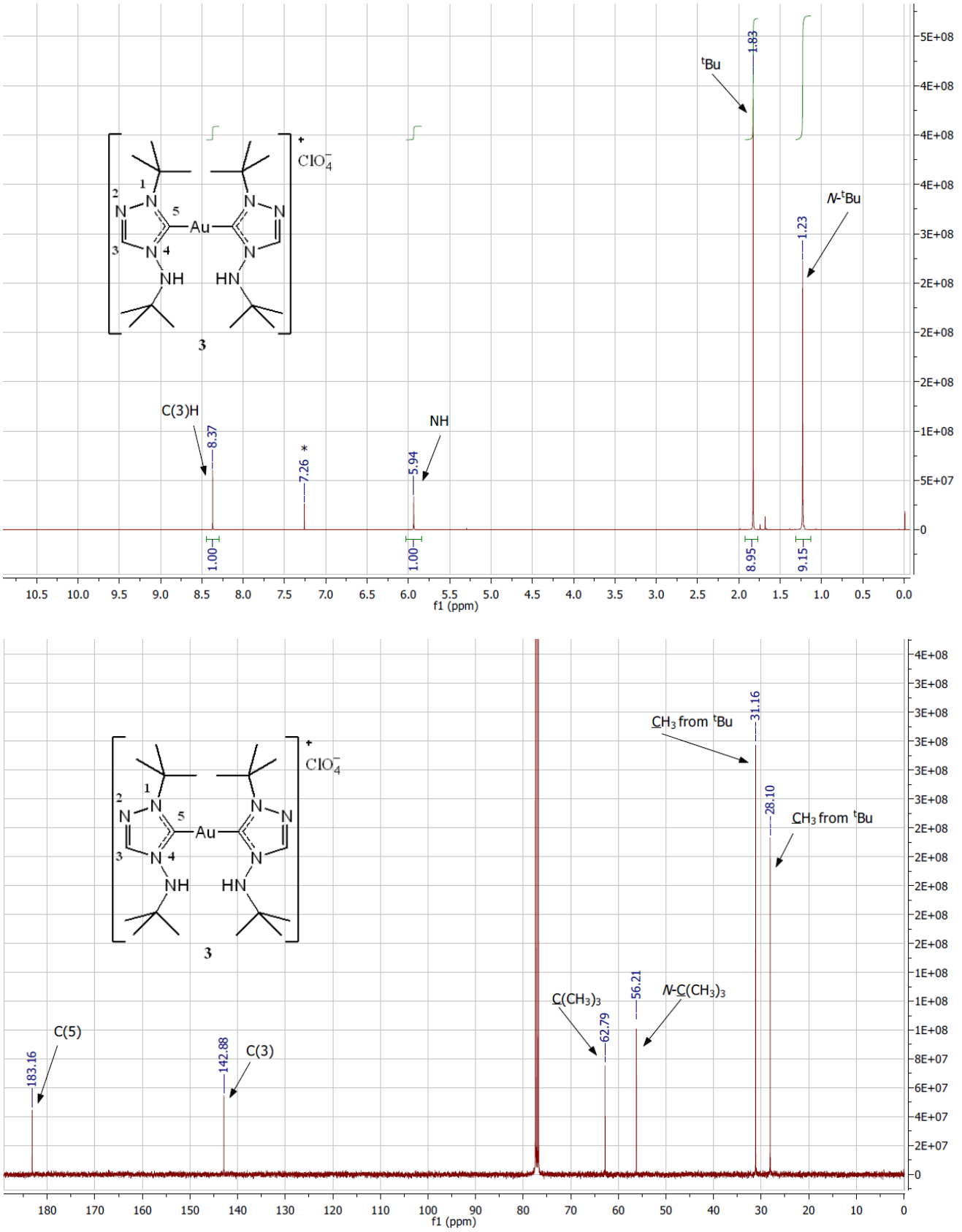


Figure S4. ^{1}H and ^{13}C NMR spectra of **3**.

Table S1. Crystallographic data for **3**.

Formula	$C_{20}H_{36}N_8AuClO_4$
Formula weight	684.98
Temperature K	100(2)
Crystal system	orthorhombic
Space group	$Pna2_1$
a (Å)	11.5632(4)
b (Å)	12.4279(4)
c (Å)	19.2238(9)
α (°)	90
β (°)	90
γ (°)	90
V (Å^3)	2762.58(18)
Z	4
D_{calc} (g/cm^3)	1.647
μ (mm^{-1})	11.230
F(000)	1360.0
Crystal size/mm^3	0.25 × 0.17 × 0.12
Radiation	CuKα (λ = 1.54184)
Angle range (°)	7.112 to 134.964
Index ranges	-13 ≤ h ≤ 13, -14 ≤ k ≤ 14, -23 ≤ l ≤ 22
Total reflections	26372
Unique reflections	4486 [R_{int} = 0.1207, R_{sigma} = 0.0549]
Data/restraints/parameters	4486/1/146
Goodness-of-fit on F^2	1.084
Final R indexes [I>=2σ (I)]	R_1 = 0.0804, wR_2 = 0.2039
Final R indexes [all data]	R_1 = 0.0855, wR_2 = 0.2075
Largest diff. peak/hole / e Å^{-3}	6.18/-2.20

$R_1 = \Sigma||F_o| - |F_c||/\Sigma|F_o|$; $wR_2 = \{\Sigma[w(F_o^2 - F_c^2)^2]/\Sigma[w(F_o^2)^2]\}^{1/2}$;
$w = 1/[\sigma^2(F_o^2)+(aP)^2 + bP]$, where $P = (F_o^2 + 2F_c^2)/3$; $s = \{\Sigma[w(F_o^2 - F_c^2)]/(n - p)\}^{1/2}$ where *n* is the number of reflections and *p* is the number of refinement parameters.

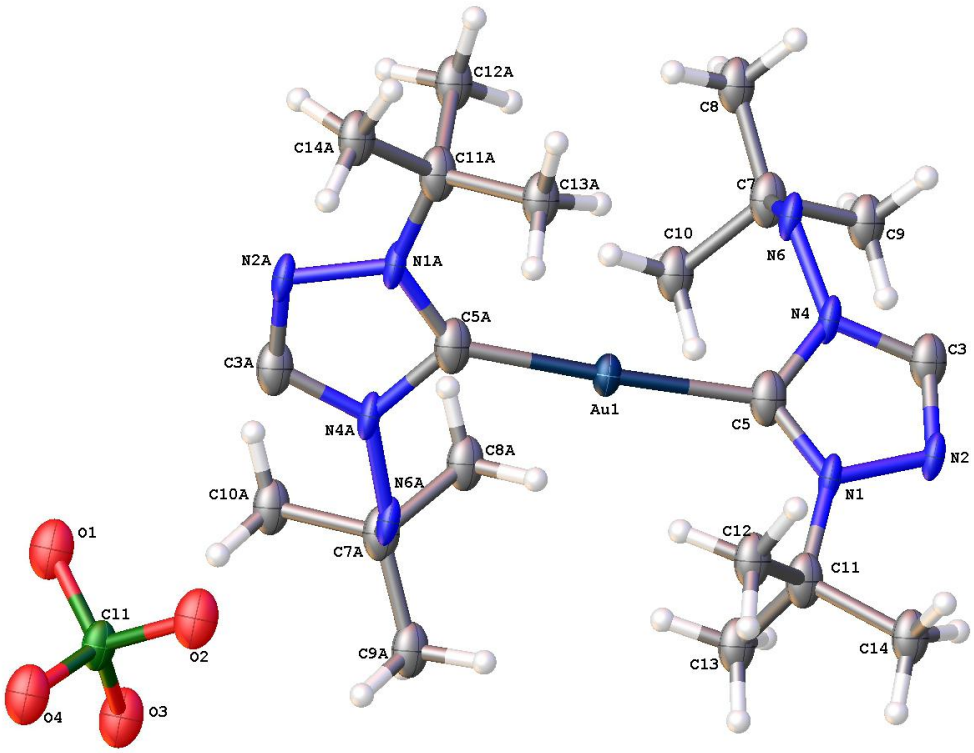


Figure S5. Molecular structure of **3** with atom numbering scheme.

Table S2. Bond lengths for **3**.

Atom	Atom	Length/Å	Atom	Atom	Length/Å
Au1	C5	2.07(2)	Cl1	O4	1.40(3)
Au1	C5A	1.98(2)	Cl1	O2	1.43(2)
C5	N4	1.38(5)	Cl1	O1	1.43(3)
C5	N1	1.28(4)	Cl1	O3	1.46(2)
C5A	N4A	1.45(6)	N6A	C7A	1.49(3)
C5A	N1A	1.34(6)	C11A	C13A	1.51(4)
N6	N4	1.42(3)	C11A	C14A	1.50(4)
N6	C7	1.49(3)	C11A	C12A	1.51(4)
N4A	C3A	1.34(4)	C11	C13	1.51(4)
N4A	N6A	1.43(3)	C11	C14	1.53(4)
N1A	N2A	1.40(3)	C11	C12	1.50(4)
N1A	C11A	1.46(4)	C7A	C10A	1.52(4)
N4	C3	1.34(3)	C7A	C8A	1.51(4)
N1	N2	1.40(3)	C7A	C9A	1.52(4)
N1	C11	1.47(4)	C7	C10	1.54(4)
N2	C3	1.29(4)	C7	C8	1.49(4)
C3A	N2A	1.31(4)	C7	C9	1.54(4)

Table S3. Bond angles for **3**.

Atom	Atom	Atom	Angle/˚	Atom	Atom	Atom	Angle/˚

C5A	Au1	C5	176.5(19)	O1	Cl1	O3	109.2(13)
N4	C5	Au1	121(2)	N2	C3	N4	111(2)
N1	C5	Au1	131(3)	N4A	N6A	C7A	116(2)
N1	C5	N4	107(2)	N1A	C11A	C13A	113(3)
N4A	C5A	Au1	122(3)	N1A	C11A	C14A	109(2)
N1A	C5A	Au1	136(4)	N1A	C11A	C12A	108(2)
N1A	C5A	N4A	101.1(19)	C13A	C11A	C12A	111(2)
N4	N6	C7	116(2)	C14A	C11A	C13A	108(2)
C3A	N4A	C5A	108(3)	C14A	C11A	C12A	107(3)
C3A	N4A	N6A	128(2)	N1	C11	C13	108(2)
N6A	N4A	C5A	124(2)	N1	C11	C14	108(2)
C5A	N1A	N2A	115(3)	N1	C11	C12	110(2)
C5A	N1A	C11A	125(3)	C13	C11	C14	111(3)
N2A	N1A	C11A	119(2)	C12	C11	C13	114(2)
C5	N4	N6	124(2)	C12	C11	C14	106(2)
C3	N4	C5	106(2)	N6A	C7A	C10A	114(2)
C3	N4	N6	130(2)	N6A	C7A	C8A	109(2)
C5	N1	N2	110(2)	N6A	C7A	C9A	103(2)
C5	N1	C11	129(2)	C10A	C7A	C9A	112(3)
N2	N1	C11	121(2)	C8A	C7A	C10A	110(2)
C3	N2	N1	105(2)	C8A	C7A	C9A	109(2)
N2A	C3A	N4A	114(3)	N6	C7	C10	109(2)
C3A	N2A	N1A	103(2)	N6	C7	C8	104(2)
O4	Cl1	O2	110.2(13)	N6	C7	C9	113(2)
O4	Cl1	O1	109.2(17)	C8	C7	C10	111(2)
O4	Cl1	O3	109.5(15)	C8	C7	C9	110(2)
O2	Cl1	O1	108.7(14)	C9	C7	C10	109(2)
O2	Cl1	O3	110.0(12)				