Gold Luminescent Probes

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Luminescence Studies of the Intracellular Distribution of a Dinuclear Gold(I) N-Heterocyclic Carbene Complex**

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Gold compounds are of significant importance in medicine, primarily for the treatment of rheumatoid arthritis.[1] We have

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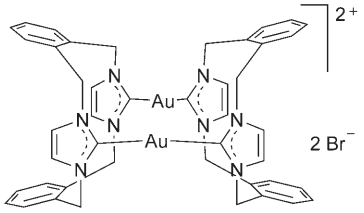
Supporting information for this article is available on the WWW under http://www.angewandte.org or from the author.

been interested in the development of Au^I complexes of phosphine[2] and more recently N-heterocyclic carbene (NHC) ligands as potential antitumor agents that selectively target the mitochondria of cancer cells.[3]

Recently, we reported the synthesis and structures of a family of fascinating dinuclear Au^I–carbene complexes, for example, **1** and **2**.[4] These complexes have demonstrated significant antimitochondrial activity[3] and, interestingly, the cyclophane ligand framework allows fine control over the intramolecular distance between the gold atoms. Compound **1** supports a short Au···Au interaction of 3.0485(3) Å and is luminescent, whereas compound **2**, with a significantly longer Au···Au distance (3.7917(4) Å), is not.[4] The luminescence associated with attractive Au···Au (aurophilic) interactions has been of great interest, both from experimental[5] and theoretical[6] points of view, and complexes possessing such properties offer potential as luminescent display devices and sensors.[7]



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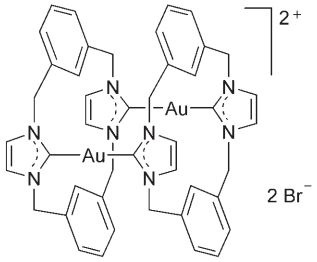
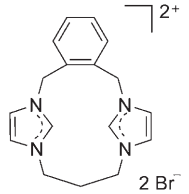


2

Our interest in targeting specific cellular organelles (mitochondria) led us to consider the possibility of exploiting the native luminescence of **1** to determine its intracellular distribution by using fluorescence microscopy. However, the luminescence profile for **1** (λ_{ex} 260 nm, λ_{em} 400 nm; ex = excitation, em = emission)[4] is unsuitable for such studies, as the high energy excitation would lead to interference from other molecules in the cell.

Previous studies have shown that the excitation and emission energies associated with short Au···Au interactions are, in some cases, red shifted as a result of a contraction of the Au···Au distance.[8,9] Herein, we report studies on a new dinuclear Au^I–NHC complex in which the ligand is designed to support a shorter Au···Au distance than that of **1**.

The imidazolium salt, NHC-ligand precursor **3**, was synthesized from an equimolar ratio of 1,3-bis(1-imidazolyl)-propane[10] and α,α′-dibromo-*o*-xylene in refluxing acetonitrile under high dilution conditions. The dinuclear Au^I complex **4** was synthesized from an equimolar ratio of **3** and $(Me_2S)AuCl$ in hot *N*,*N*-dimethylformamide (DMF; ≈90 °C), with sodium acetate acting as a mild base.[4] The imidazolium salt **3** is unsymmetrical about the imidazolium units, resulting in *cis* and *trans* isomers of the dinuclear Au^I complex **4**.



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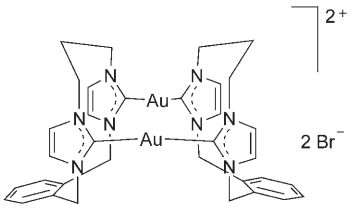
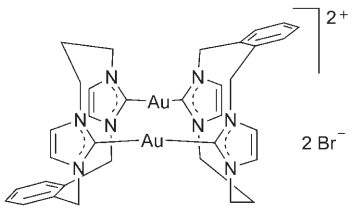


cis-**4**



trans-**4**

C*is*- and *trans*-**4** cocrystallized as their bromide salts in a 1:1 ratio. Single crystals suitable for X-ray diffraction studies were grown from a methanol solution of both isomers. In the solid state, in space group *C*2/*c*,[11] the cation of *cis*-**4** is disposed about a twofold axis (Au···Au′ 2.9290(4); Au···C 2.035(6), 2.033(6) Å; C–Au–C 177.4(3)°), whereas that of *trans*-**4** is disposed about a crystallographic inversion center (Au···Au′ 2.9582(4); Au···C 2.036(6), 2.029(6) Å; C–Au–C 175.5(3)°), (Figure 1). Deviations of the Au atoms from the C_3N_2 carbene planes are $\delta(Au) =$ 0.19(1), 0.21(1) (*trans*); 0.07(1), 0.15(1) Å (*cis*). The *cis* cation of **4** is twisted about the Au···Au line (τ(C–Au···Au–C) 12.6(3)°), which is seemingly a consequence of inter- rather than intramolecular interactions.

The isomeric forms of **4** were separated by fractional crystallization from water, with *trans*-**4** being the less soluble isomer. After recrystallization from ethanol, *cis*-**4** was obtained as a colorless microcrystalline powder and *trans*-**4** as colorless needles. Upon exposure to an ambient atmosphere, crystals of *trans*-**4** rapidly developed a pale green color.[12] The ^1H and ^{13}C NMR signals for *cis*- and *trans*-**4** (298 K, CD_3OD) are sharp. The number of signals detected is the same for each isomer as would be expected for relatively rigid complexes with either a mirror plane (*cis*-**4**) or an inversion centre and a twofold axis (*trans*-**4**) through the Au centers. Consistent with the proposed structures, the ^1H NMR signals for the benzylic protons for *cis*- and *trans*-**4** give rise to sharp AX patterns, and the protons in the propylene chain give rise to signals showing splitting patterns with the expected geminal and vicinal couplings.

Aqueous solutions of each isomer display identical electronic absorption (see the Supporting Information) and emission properties and *cis*-**4** was selected for cellular localization studies based on its higher aqueous solubility. In aqueous solution, two emission bands are seen with relative intensities that depend on the excitation wavelength used. With excitation at 313 nm, an emission centered at 396 nm is obtained, whereas excitation at 355 nm yields a band centered at 496 nm, and each of the emission bands show distinct excitation spectra (Figure 2). Very similar spectral properties have been reported previously for a system involving short Au···Au interactions.[9] The emission quantum yield (Φ) is also dependent on the excitation wavelength and values of 0.29 (λ_{ex} 313 nm) and 0.98 (λ_{ex} 350 nm) were determined by using quinine sulfate in H_2SO_4 (0.1M) as a reference. Importantly, the lower energy excitation and emission bands are of suitable wavelengths for cellular distribution studies. A further

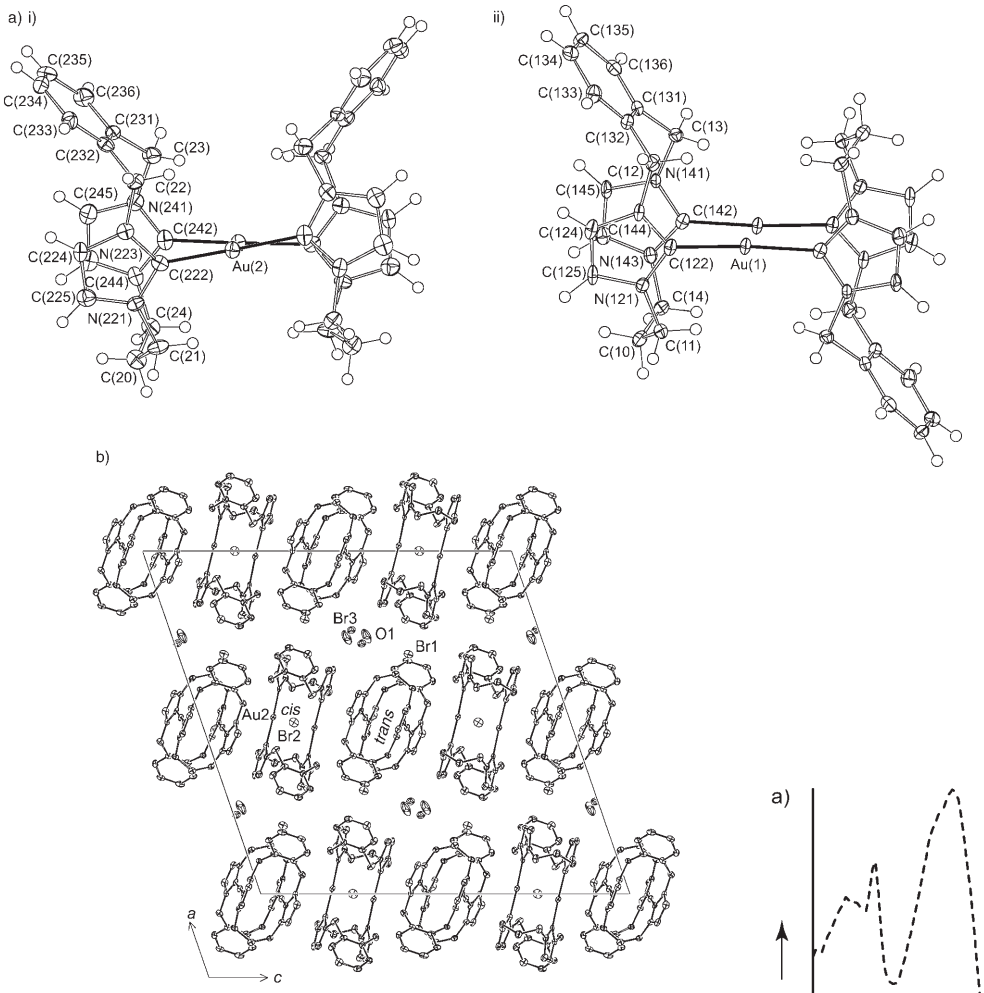


Figure 1. a) The i) *cis*- and ii) *trans*-cations of **4**·H_2O. b) Unit cell contents, projected down *b*. Amplitude displacement ellipsoids are depicted at 50% probability; hydrogen atoms, where shown, have arbitrary radii of 0.1 Å.

consideration in assessing the feasibility of such studies is whether the Au^I complex is likely to retain its dinuclear (luminescent) structure under biological conditions as it might be expected that the structure would be degraded by facile ligand exchange reactions with biological thiols. Remarkably, ^1H NMR spectroscopy experiments showed no evidence of reaction between *cis*-**4** and glutathione over a period of 28 h (see the Supporting Information).

The uptake and distribution of *cis*-**4** were evaluated in RAW264.7 cells (a mouse macrophage cancer cell line)[13] by using fluorescence confocal microscopy. Figure 3 shows typical brightfield and luminescence images of RAW264.7 cells treated for 15 h with *cis*-**4**. Preserved cell morphology confirmed viability after treatment (Figure 3a). Cellular uptake of *cis*-**4** yielded a clear punctate pattern of luminescence, indicating localization into cellular organelles (Figure 3b). Cell nuclei (identifiable on brightfield images) carried little or no luminescence, indicating that *cis*-**4** was not concentrated in the nucleus. No cellular luminescence was detected in the absence of added gold complex.

Colocalization studies of *cis*-**4** with a lysosomal dye, lysotracker red, and a mitochondrial dye, mitotracker green, provided evidence for localization of *cis*-**4** within lysosomes rather than mitochondria. Luminescence images of RAW264.7 cells treated with *cis*-**4** and lysotracker red (Figure 4b and c) show clear regional colocalization. We observed that the punctate distribution of *cis*-**4** luminescence became less pronounced in cells exposed to lysotracker red, suggesting physicochemical interaction between the two compounds in lysosomes. Mitochondrial mitotracker green luminescence did not colocalize with *cis*-**4** (see the Supporting Information).

Intracellular localization to lysosomes has been noted for several other metal complexes. For example the antiarthritic drug, gold thiomalate (myocrisin), is readily taken up by macrophages and the gold is almost

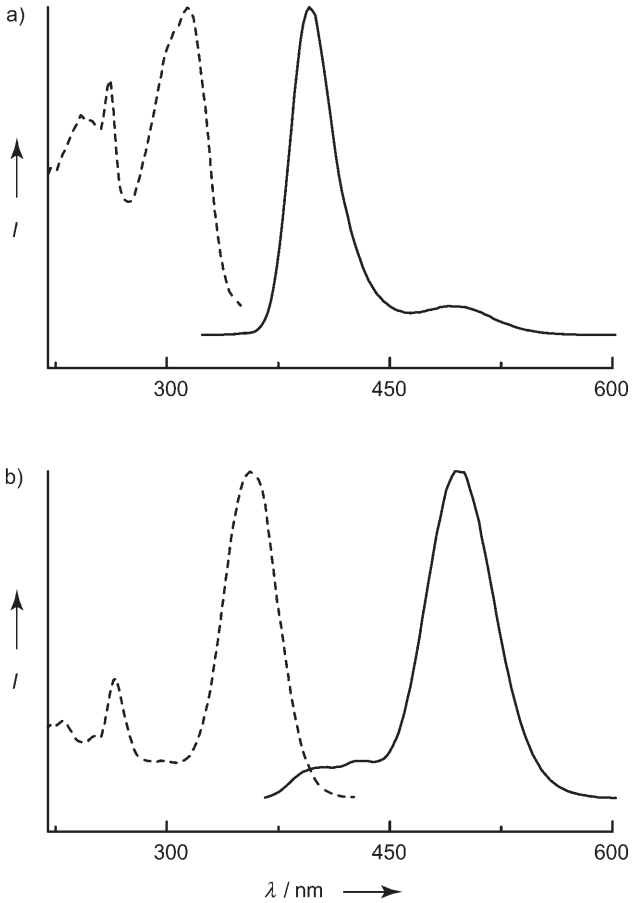


Figure 2. Normalized electronic emission (solid line) and excitation spectra (dashed line) for an aqueous solution of *cis*-**4** (5×10^{-5} M) at 25 °C, a) λ_{ex} 313 nm, λ_{em} 396 nm ($\Phi=0.29$) and b) λ_{ex} 355 nm, λ_{em} 496 nm ($\Phi=0.98$).

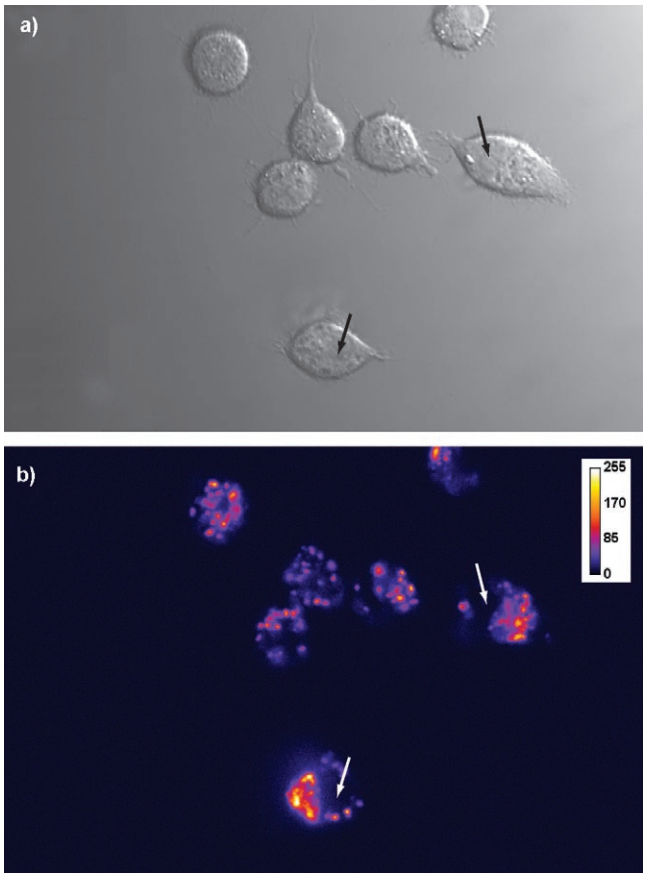


Figure 3. Cellular distribution of *cis*-**4** (200 μM) in RAW264.7 cells after incubation for 15 h at 37 °C; a) brightfield image and b) luminescence image (λ_{ex} 351 nm). The black (a) and white (b) arrows mark the position of the nucleus.

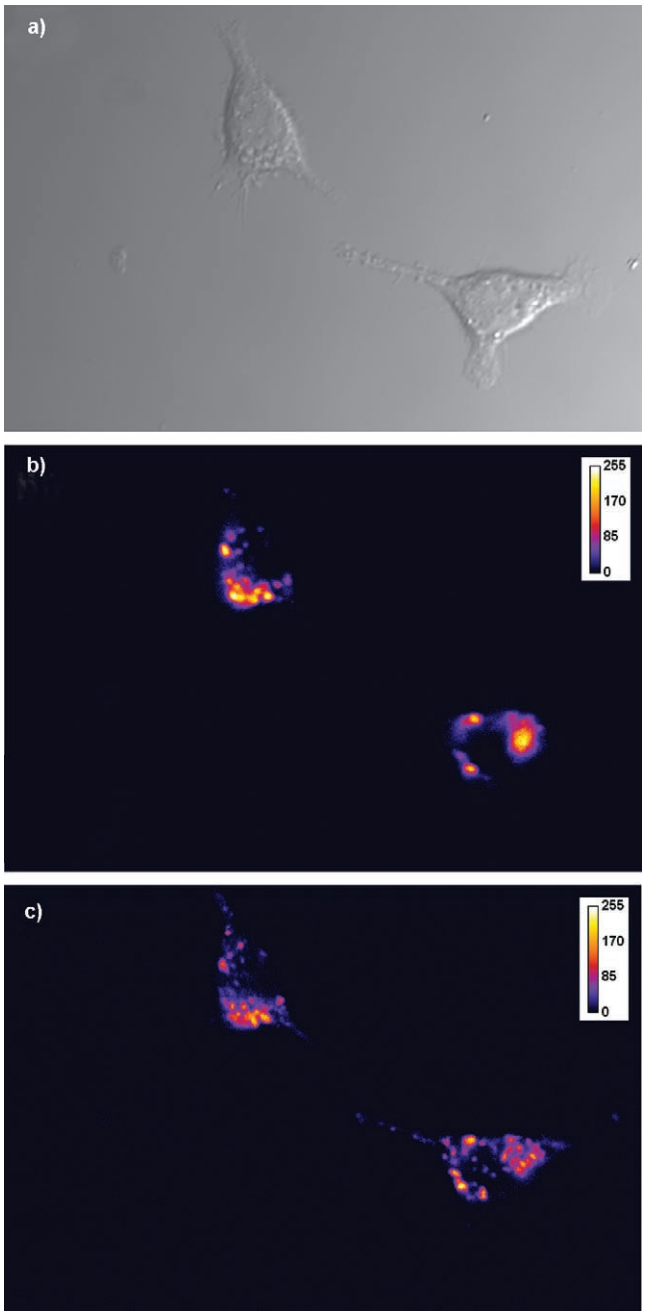


Figure 4. Cellular distribution of *cis*-**4** and lysotracker red in RAW264.7 cells after incubation with *cis*-**4** (15 h, 200 μM) and lysotracker red (20 min, 50 nM) at 37 °C; a) brightfield image and b) luminescence image (λ_{ex} 351 nm) showing *cis*-**4** distribution and c) luminescence image (λ_{ex} 543 nm) showing lysotracker red distribution.

exclusively deposited in lysosomes.[14] Fluorescence microscopy studies of both zinc–bis(thiosemicarbazone) complexes[15] and a dinuclear platinum complex labeled with a fluorescent tag[16] have shown sequestration of the complexes in lysosomes. These latter studies have demonstrated also that intracellular distribution is strongly dependent on the cell type. RAW264.7 cells were selected for the present study because their adherent properties make them ideal for investigation by confocal microscopy. Notably, *cis*-**4** exhibits only modest cytotoxicity in this cell line (IC_{50} 52 μM, 48 h incubation, see the Supporting Information). Preliminary studies indicate greater potency in other cell lines, such as the human promyelocytic leukaemia cell line, HL 60[17] (IC_{50} 10 μM, 48 h incubation, see the Supporting Information). Further studies are in progress to investigate the intracellular distribution of *cis*-**4** in other cell lines to determine whether a correlation exists between cytotoxicity and localization to mitochondria.

In summary, we have synthesized a dinuclear gold(I) complex in which the bidentate N-heterocyclic carbene ligand has been specifically designed to support Au···Au interactions of a predetermined length. Shortening the Au···Au distance allows the luminescence profile of the resultant dinuclear complex to be red shifted and thereby "tuned" to the desired range. By exploiting the inherent luminescent properties of antimitochondrial dinuclear Au^I complexes, there is potential to investigate their cellular processing in different cancer cell lines by using confocal fluorescence microscopy. This approach is particularly advantageous as it allows cellular imaging studies to be conducted without the need to attach a fluorescent tag. Apart from the synthetic challenges involved in such an approach, the attached emissive group will most likely change the intracellular distribution of the Au^I complex

by altering the fundamental properties (e.g. charge, lipophilicity) that determine the potential localization to mitochondria.

Experimental Section

Full details of the synthesis and spectroscopic characterization of **3** and **4**, the determination of the emission quantum yields for **4**, and the experimental procedures used in the cytotoxicity and confocal microscopy studies are given in the Supporting Information.

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[11] (*cis*-**4**, *trans*-**4**. 4Br. 2"H_2O")$_{0.5} \equiv C_{34}H_{38}Au_2Br_2N_8O$, $M = 1128.5$. Monoclinic $C2/c$, $a = 24.939(1)$, $b = 11.3040(5)$, $c = 25.394(1)$ Å, $\beta = 109.042(2)°$, $V = 6767$ Å^3. ρ_{calcd} $(Z = 8) = 2.215$ g cm^{-3}. $\mu = 3.9$ mm^{-1}; specimen: $0.11 \times 0.03 \times 0.02$ mm^3; "T"$_{min/max} = 0.78$. $2\theta_{max} = 37°$ (monochromatic synchrotron radiation, $\lambda = 0.48595$ Å); $N_t = 66750$, $N = 7859$ ($R_{int} = 0.048$), $N_o = 6933$; $R = 0.036$, $R_w = 0.072$ ($w = (\sigma^2(F^2) + 32\ F^2)^{-1}$); T ca. 103 K. No hydrogens located in association with "O(1)"; Br(3) is modeled as disordered over a pair of well-separated, inversion related sites (3.291(2) Å). CCDC-295083 contains the supplementary crystallographic data for this paper. These data can be obtained free of charge from The Cambridge Crystallographic Data Centre via www.ccdc.cam.ac.uk/data_request/cif.

[12] The color of **4** in solution is solvent dependent, with colorless solutions formed in organic solvents (methanol, ethanol, dimethyl sulfoxide (DMSO), and DMF) and pale green solutions formed in water. It is possible that the green color of solid samples of *trans*-**4** results from the initially colorless crystals absorbing atmospheric water. The fascinating solvent dependence of the emissive behavior of **4** is currently under further investigation, and the results of these studies will be reported in due course.

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