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Cytotoxic gold(I)-bearing dendrimers from alkyne precursors†

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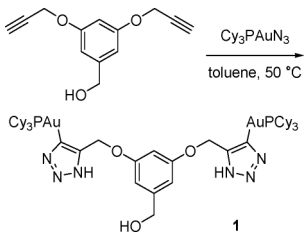
A series of four dendrimers end-functionalized with gold(I) has been prepared from alkyne-terminated precursors and (tricyclohexylphosphine)gold(I) azide. Isolated yields range from 84–89%, based on gold. The first-generation dendrimer is cytotoxic toward 3T3 mouse fibroblast cells. Apoptosis ensues within 6 h of treatment with gold(I).

Gold is prominent in restorative and prosthetic dentistry.[1] Its history in rheumatoid arthritis treatment reaches across decades.[2] In recent years, the focus of medicinal gold(I) chemistry has shifted toward cancer therapy in response to observations that gold(I) anti-arthritics possess anti-inflammatory and immunosuppressive activity.[3,4] The orally bioavailable prodrug auranofin is toxic to P388 leukemia and B16 melanoma cell lines.[5] Results from Berners-Price, Filipovska, and co-workers find bis(diphosphine) gold(I) cations to be membrane-permeable, and indeed, to trigger apoptosis in human breast-cancer cell lines.[6–8] The mechanism of this biological activity is drawing scrutiny. One hypothesis having experimental support is that gold(I) inhibits the mitochondrial form of the enzyme thioredoxin reductase. The single SeCys residue of this enzyme, which lies near the protein's *C*-terminus, is thought to bind gold in a favorable soft-soft match.[9] The inhibited (gold-bound) reductase then fails to reduce thioredoxin, and thereby triggers mitochondrial membrane permeability. Apoptosis ensues. Malignant tissues up-regulate thioredoxin reductase, and this enzyme is emerging as a chemotherapeutic target. The anticancer propensities of gold(I) compounds continue to attract clinical attention, and the medicinal prospects of gold chemistry are far from exhausted.

Dendrimers are branched polymers having precisely controllable architectures and uniform composition. Their use as targetable drug carriers now represents a mature field of study.[10] Dendrimers exploit the enhanced permeability and retention of tumors, where the blood vessels of cancerous tissue trap macromolecules. Polymeric drugs, or polymer-immobilized prodrugs, linger inside tumors and acquire a longer residence time. Selectivity results for malignant over healthy tissues.

We have reported that the [3 + 2] cycloaddition of (phosphine)gold(I) azides with terminal alkynes affords triazolato complexes in good to excellent yields.[11,12] In the product complexes, triazolate ligands bind gold through *carbon*. The resulting organometallics are stable to air, moisture, and laboratory lighting.[13–15] We find this method of metalating alkyne precursors simpler than classical deprotonation/metalation of alkynes.[16]

The reaction of Cy_3PAuN_3 (Cy = cyclohexyl) with the ethynyl-terminated dendrimers of Hawker and co-workers[17] yields dendrimers bearing *C*-bound (phosphine)gold(I) triazolates. A representative example appears in Scheme 1. Four generations of dendrimer have been metalated this way, and the number of bound gold atoms is 2^n, where n is the cardinal number corresponding to the dendrimer's generation (*e.g.*, the fourth-generation dendrimer has $2^4 = 16$ gold(I) centers). Isolated yields based on Cy_3PAuN_3 are as follows: 84%, **1**; 89%, **2**; 89%, **3**; 95%, **4**. The dendrimers are soluble in THF, dimethyl sulfoxide (DMSO), and DMSO/water mixtures. Solubility decreases with each higher generation. The fourth-generation dendrimer, with 16 gold atoms, is insoluble in common solvents, and only the first three generations are considered here. Fig. 1 depicts line-drawings of the first three dendrimer generations. End-to-end diameters, estimated from molecular mechanics minimization, range from 2 nm for the first-generation dendrimer to 4 nm for the fourth. These sizes fall below the ≤ 5.5-nm limit encountered for kidney clearance of nanocrystals.[18] Thus the new dendrimers are potentially compatible with renal elimination.



Scheme 1 Synthesis of first-generation dendrimer **1**.

Organometallic products were characterized by multinuclear NMR and infrared spectroscopies. Mass spectra and elemental analysis indicate that all terminal alkynyl groups in the organic precursors are transformed into (triazolato)gold(I) moieties. Vibrational signatures corresponding to terminal alkynes or azide complexes are absent in the products' infrared spectra. The organogold dendrimers are colorless, and the

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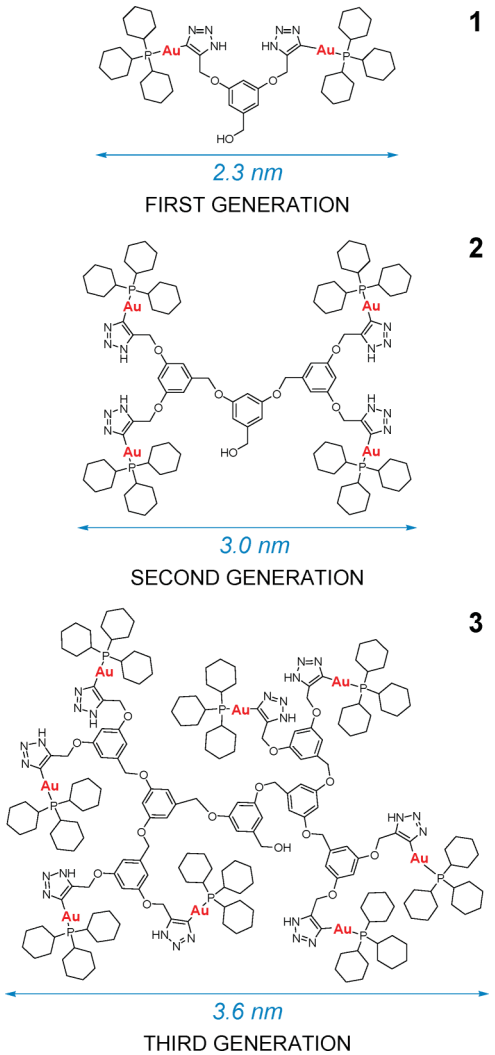

Fig. 1 (Triazolato)gold(I) dendrimers. End-to-end dimensions are estimated from molecular mechanics minimizations. A fourth-generation dendrimer (not shown) has been prepared; its diameter is estimated to be 4.0 nm.

absorption spectra are featureless in the near-UV and visible regions.

Initial experiments sought to determine cytotoxicity of a representative dendrimer. Solutions of **1** at concentrations of 230 μM, 100 μM, and 50 μM were prepared in 12-well plates, as was a DMSO control with no drug. To each well, which contained 10^5 cells of 3T3 (immortalized mouse fibroblast) in 1 mL of D8 medium, was added 100 μL of a stock solution of **1**. Cells were incubated at 37 °C and 5% CO_2 for 6 h. Experiments were conducted in duplicate. Phase-contrast microscopy images of selected wells appear in Fig. 2. For all concentrations within 4.55–20.9 μM **1**, the cytotoxicity is estimated to exceed 95%. Fig. 2b shows that cells with no drug in D8 medium containing 4.5% DMSO remained attached to the dish and viable, as indicated by their elongated shape. Cells that are dead or dying are spherical, and detach readily with mechanical agitation. The amount of DMSO used in this study was optimized to maintain viability for the duration of the experiments. Control experiments with the

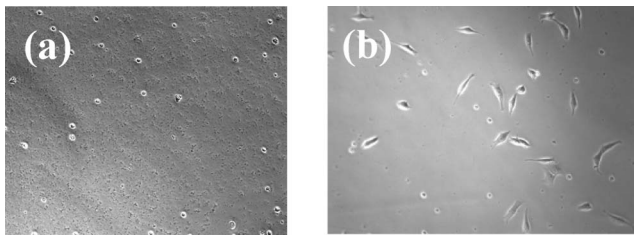

Fig. 2 Phase-contrast microscopy images of 3T3 fibroblast cells. (a) 4.55 μM **1**, following addition to medium in cell-culture well. (b) Without **1**. All samples are in D8 medium with 4.5% v:v DMSO after 6 h of treatment.

unmetallated alkyne dendrimer indicated toxicity similar to that of the cell-culture medium.

Apoptosis was assayed using annexin-V and the vital stain 7-aminoactinomycin D (7-AAD). Cells alive but undergoing apoptosis are defined as being positive for annexin-V and negative for 7-AAD (red bars in Fig. 3). Cells that were positive for both annexin-V and 7-AAD are dead (black, Fig. 3); they can have died from apoptosis or cytotoxic necrosis. Cells that are undergoing apoptosis or dead are plotted to demonstrate the change in cell state over the course of the study as compared to controls. Incubation of 3T3 cells with micromolar solutions **1** results in cell death that increases with increasing concentrations of **1**. These were seeded at 10^5 cells per well in a 12-well polystyrene tissue culture dish with 1 mL D8 medium per well, and were allowed to attach over a period of several hours. Experimental details appear in the Supporting Information.†

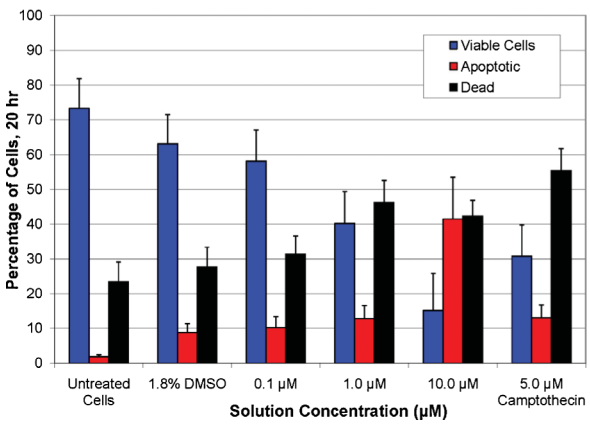

Fig. 3 Fluorescence-activated cell analysis of 3T3 mouse fibroblast cells with solutions of **1** at various concentrations, after 20 h. The blue column indicates cells that are still viable (living) in solution. The red column indicates cells that are currently undergoing apoptosis or have died through apoptosis. The black column shows cells that have died through an unknown (apoptosis *vs.* necrosis) process. Concentrations are of **1** unless otherwise indicated.

Fig. 3 indicates apoptosis brought about by **1** after 20 h, with notable activity at 10.0 μM **1**. Apoptosis and total cell death increases with concentration from 0.1–10.0 μM **1**. Little apoptosis or cell death is seen in 3T3 cells treated with **1** over 6 h (Figure S1, Supporting Information†), with one exception, namely apoptosis at 10 μM concentration, indicating a rapid mechanism of action. The LD_{50} was determined to be 90 ng mL^{-1}.

Dendrimers bearing terminal alkyne functionalities have been made into gold-bearing scaffolds. Cycloaddition reactions of

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(tricyclohexylphosphine)gold(I) azide with pendant –C≡CH moieties attach gold through irreversible triazolate formation. Four generations of gold(I) dendrimer have been prepared. First-generation di-gold complex **1** is cytotoxic toward rapidly-dividing mammalian cells. Compound **1** induces apoptosis at 1–10 μM concentrations over a period of hours. Cellular evaluation of higher-generation dendrimers and synthetic studies of gold-bearing macromolecules are ongoing.

Notes and references

1 J. Rothat, in *Gold: Progress in Chemistry, Biotechnology, and Technology*, ed. H. Schmidbaur, Wiley, New York, 1999, p. 173.
2 C. F. Shaw, III, in *Gold: Progress in Chemistry, Biotechnology, and Technology*, ed. H. Schmidbaur, Wiley, New York, 1999, p. 259.
3 S. Y. Ho and E. R. T. Tiekink, in *Metallotherapeutic Drugs & Metal-based Diagnostic Agents: The Use of Metals in Medicine*, ed. M. Gielen and E. R. T. Tiekink, Wiley, New York, 2005, p. 507.
4 K. P. Bhabak, B. J. Bhuyan and G. Mugesh, *Dalton Trans.*, 2011, **40**, 2099–2111.
5 C. K. Mirabelli, R. K. Johnson, D. T. Hill, L. F. Faucette, G. R. Girard, G. Y. Kuo, C. M. Sung and S. T. Crooke, *J. Med. Chem.*, 1986, **29**, 218.
6 P. J. Barnard and S. J. Berners-Price, *Coord. Chem. Rev.*, 2007, **251**, 1889.
7 J. L. Hickey, R. A. Ruhayel, P. J. Barnard, M. V. Baker, S. J. Berners-Price and A. Filipskova, *J. Am. Chem. Soc.*, 2008, **130**, 12570.
8 O. Rackham, S. J. Nichols, P. J. Leedman, S. J. Berners-Price and A. Filipovska, *Biochem. Pharmacol.*, 2007, **74**, 992.
9 M. P. Rigobello, A. Folda, M. C. Baldoin, G. Maria, G. Scutari and A. Bindoli, *Free Radical Res.*, 2005, **39**, 687.
10 E. R. Gillies and J. M. J. Fréchet, *Drug Discovery Today*, 2005, **10**, 35.
11 D. V. Partyka, J. B. Updegraff, III, M. Zeller, A. D. Hunter and T. G. Gray, *Organometallics*, 2007, **26**, 183.
12 D. V. Partyka, L. Gao, T. S. Teets, J. B. Updegraff, III, N. Deligonul and T. G. Gray, *Organometallics*, 2009, **28**, 6171.
13 D. V. Partyka, M. Zeller, A. D. Hunter and T. G. Gray, *Angew. Chem., Int. Ed.*, 2006, **45**, 8188.
14 D. V. Partyka, J. B. Updegraff, III, M. Zeller, A. D. Hunter and T. G. Gray, *Organometallics*, 2009, **28**, 1666.
15 L. Gao, M. A. Peay, D. V. Partyka, J. B. Updegraff, III, T. S. Teets, A. J. Esswein, M. Zeller, A. D. Hunter and T. G. Gray, *Organometallics*, 2009, **28**, 5669.
16 L. Gao, D. V. Partyka, J. B. Updegraff, III, N. Deligonul and T. G. Gray, *Eur. J. Inorg. Chem.*, 2009, 2711.
17 M. Malkoch, K. Schleicher, E. Drockenmuller, C. J. Hawker, T. P. Russel, P. Wu and V. V. Fokin, *Macromolecules*, 2005, **38**, 3663.
18 H. S. Choi, W. Liu, P. Misra, E. Tanaka, J. P. Zimmer, B. I. Ipe, M. G. Bawendi and J. V. Frangioni, *Nat. Biotechnol.*, 2007, **25**, 1165.