SUPPLEMENTARY INFORMATION

Cytotoxic Gold(I)-Bearing Dendrimers from Alkyne Precursors

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List of Compounds

$[(PCy_3)Au(triazolate)]_2$-[G-1]-OH	**1**
$[(PCy_3)Au(triazolate)]_4$-[G-2]-OH	**2**
$[(PCy_3)Au(triazolate)]_8$-[G-3]-OH	**3**
$[(PCy_3)Au(triazolate)]_{16}$-[G-4]-OH	**4**

G = Generation; acet = –C≡CH

Structures of dendrimeric, terminal alkyne precursors appear in Chart S1.

List of Procedures

Fluorescence-activated cell sorting analysis of 3T3 mouse fibroblast cells with varying concentrations of $[(PCy_3)Au(triazolate)]_2$-[G-1]-OH after 6 hours

Fluorescence-activated cell sorting analysis of 3T3 mouse fibroblast cells with varying concentrations of $[(PCy_3)Au(triazolate)]_2$-[G-1]-OH after 20 hours

Fluorescence-activated cell sorting analysis of 3T3 mouse fibroblast cells with a (+)-camptothecin solution

Experimental

(Tricyclohexylphosphine)gold(I) azide was prepared as previously.[1] Other commercial solvents and reagents were used as received. Microanalyses (C, H, and N) were performed by Robertson Microlit Laboratories. ^{1}H and ^{31}P{^{1}H} nuclear magnetic resonance spectra were recorded on a Varian AS-400 spectrometer operating at 399.7 and 161.8 MHz respectively. Chemical shifts were determined relative to the solvent residual peaks and an external concentrated phosphoric acid standard, respectively.

Synthesis of di-gold complex 1. In 4 mL of toluene was stirred $(Acet)_2$-[G-1]-OH (Supporting Information) (0.0090 g, 0.041 mmol) and $(PCy_3)AuN_3$ (0.065 g, 0.11 mmol). The solution was degassed under argon for 30 minutes and allowed to stir for 72 hours at 50°C. The solution produced a white precipitate after 72 hours. The resultant suspension was allowed to cool to room temperature and the precipitate was allowed to settle. The toluene was pipetted off. The white precipitate was washed with toluene (3 × 20 mL) and then with *n*-pentane (3 × 20 mL). The precipitate was collected and dried under vacuum. Yield: 44 mg (88%). ^{1}H NMR (DMSO-d_6): δ 14.01 (s br, 2H, N***H***), 6.51-6.93 (m, 3H, *o,p*-Ar), 4.97 (s, 4H, OCH_2-triazole), 4.38 (s, 2H, CH_2OH), 0.98-2.21 (m, 66H, cyclohexyl) ppm. ^{31}P{^{1}H} NMR (DMSO-d_6): δ 59.52 ppm. IR (KBr): 3211 ($\nu_{s\ NH}$, m, br), 2927 ($\nu_{as\ -CH_2-}$, s), 2850 ($\nu_{s\ -CH_2-}$, s), 1594 ($\nu_{triazole}$, s), 1447 ($\nu_{triazole}$, s), 1346 ($\nu_{triazole}$, m) cm^{-1}. Electrospray mass spectrum: Calcd for $C_{49}H_{78}N_6O_3Au_2P_2$: 1255.06. Found: 1255.3.

[1] Partyka, D. V.; Robilotto, T. J.; Zeller, M.; Hunter, A. D.; Gray, T. G. *Proc. Natl. Acad. Sci., U.S.A.* **2008**, *105*, 14293–14297.

Anal. Calcd for $C_{49}H_{78}N_6O_3Au_2P_2$: C, 46.89; H, 6.26; N, 6.70. Found: C, 46.68; H, 5.98; N, 6.44.

[(PCy_3)Au(triazolate)]$_4$-[G-2]-OH (2). In 6 mL of tetrahydrofuran was stirred $(Acet)_4$-[G-2]-OH (0.020 g, 0.038 mmol) and $(PCy_3)AuN_3$ (0.11 g, 0.20 mmol). The solution was degassed under argon for 30 minutes and allowed to stir for 96 hours at 50 °C. The solution was allowed to cool to room, was filtered, and vacuum-evaporated to ~0.5 mL of solution remaining. Toluene (20 mL) was added and a white solid precipitated. The white precipitate was washed with toluene (3 × 20 mL), and n-pentane (3 × 20 mL). The white precipitate was collected and vacuum dried. Yield: 88 mg (89%). ^{1}H NMR (DMSO-d_6): δ 14.02 (s br, N**H**, 4H), 6.41-6.63 (m, *o,p*-Ar, 9H), 4.99 (s, OC**H**$_2$-triazole, 8H), 4.91 (s, Ar-OC**H**$_2$-Ar, 4H), 4.41 (s, C**H**$_2$OH, 2H), 0.98-2.17 (m, cyclohexyl, 132H) ppm. ^{31}P NMR (DMSO-d_6): δ 59.49 ppm. IR (KBr): 3145, 2925, 2850, 1594, 1446, 1344 cm^{-1}. MALDI mass spectrum: 2614.17 (M^+). Anal. Calcd for $C_{105}H_{160}N_{12}O_7Au_4P_4$: C, 48.24; H, 6.17; N, 6.43. Found: C, 48.27; H, 5.89; N, 6.13.

[(PCy_3)Au(triazolate)]$_8$-[G-3]-OH (3). In 6 mL of tetrahydrofuran was stirred $(Acet)_8$-[G-3]-OH (0.021 g, 0.018 mmol) and $(PCy_3)AuN_3$ (0.12 g, 0.23 mmol). The solution was degassed under argon for 30 minutes and allowed to stir for 120 hours at 50 °C. The solution was allowed to cool to room, was filtered, and vacuum-evaporated to ~0.5 mL of solution remaining. Toluene (30 mL) was added and a white solid precipitated. The white precipitate was washed toluene (3 × 30 mL), and three times with n-pentane (30 mL). The white precipitate was then collected and vacuum dried. Yield: 85 mg (89%). ^{1}H NMR (DMSO-d_6): δ 14.02 (s br, N**H**, 8H), 6.43-6.63 (m, *o,p*-Ar, 21H) , 4.80-5.06 (m, OC**H**$_2$-triazole, Ar-OC**H**$_2$-Ar, 28H), 4.4 (s, C**H**$_2$OH, 2H), 0.97-2.19 (m, cyclohexyl, 264H) ppm. ^{31}P NMR (DMSO-d_6): δ 59.47 ppm. IR (KBr): 3146, 2926, 2850, 1594, 1446, 1343 cm^{-1}. MALDI mass spectrum: 5329.37 $(M - 3)^+$. Anal. Calcd for $C_{217}H_{324}N_{24}O_{15}Au_8P_8$: C, 48.88; H, 6.12; N, 6.30. Found: C, 48.62; H, 6.00; N, 6.53.

[(PCy_3)Au(triazolate)]$_{16}$-[G-4]-OH (4). In 6 mL of tetrahydrofuran was stirred $(Acet)_{16}$-[G-4]-OH (0.084 g, 0.034 mmol) and $(PCy_3)AuN_3$ (0.057 g, 0.11 mmol). The solution was degassed under argon for 30 minutes and allowed to stir for 120 hours at 50 °C. A white precipitate formed upon completion. The suspension was allowed to cool to room and vacuum-evaporated to ~0.5 mL of solution remaining. The white precipitate was washed toluene (3 × 30 mL), and three times with n-pentane (30 mL). The white precipitate was then collected and vacuum dried. Yield: 33 mg (90%). The material was insoluble in all solvents tested and analytical data were not obtained. IR (KBr): 3141, 2925, 2849, 1594, 1446, 1342 cm^{-1}.

Fluorescence-activated cell sorting analysis of 3T3 mouse fibroblast cells with varying concentrations of [(PCy_3)Au(triazolate)]$_2$-[G-1]-OH after 6 hours.

NIH/3T3 mouse fibroblast cells (ATCC.org) were maintained in medium consisting of Dulbecco's Modified Eagle's Medium, 10% fetal bovine serum, and 1% penicillin/streptomycin solution, called D8 medium, and incubated in a humidified incubator at 37 °C and 5% CO_2. All solution and medium materials were obtained from

Fisher-Thermo Scientific. The number of apoptotic cells was quantified using an annexin-V apoptosis staining kit with 7-Amino-actinomycin D (7-AAD) vital stain (AnnexinV- PE Apoptosis Detection Kit; BD Biosciences Pharmingen, San Diego, CA). Cytometric analysis was performed on a FACSCalibur (Becton-Dickinson, San Jose, CA). The excitation wavelength was 488 nm, and emission was collected on channel FL2 (564-601 nm) and 7-AAD on FL3 (670-nm long pass filter). Microscopy images were obtained from a Nikon Eclipse TE300 microscope using a Q-Imaging Retiga-SRV Fast 1394 camera and Image-Pro 6.2 software.

Into a six-well culture plate was added 1.0 mL of D8 medium containing ~100,000 3T3 cells. This mixture was allowed to stand for one hour for the cells to attach to the plate. To individual wells was added 100 µL of a $[(PCy_3)Au(triazolate)]_2$-[G-1]-OH dendrimer solution at 110, 55, 11, and 1.0 µM in 20% DMSO, giving final concentrations of solutions of 10, 5.0, 1.0, and 0.1 in 1.8% DMSO respectively. The cell culture plate was then incubated at 37 °C and 5% CO_2 concentration for 6 hours. The cell plate medium was collected into a 3 mL test tube and then the well was washed with 1 mL of 1x PBS (phosphate-buffered saline) buffer solution and that was collected and added to the 3 mL test tube. 100 µL of 0.25% trypsin solution was added to the plate well. The well was then incubated at 37 °C and 5% CO_2 concentration for 5 minutes. To the well was added 1 mL of D8 medium, stirred and then collected into the 3 mL test tube. The 3 mL test tube was centrifuged at 1060 rpm to obtain a cell pellet. The medium was then removed via vacuum, and the cell pellet was washed with 1 mL of 1x PBS buffer solution. The 3 mL test tube was then centrifuged to obtain a cell pellet and this washing process was repeated. The cells were re-suspended in 200 µL of 1x annexin V binding buffer and 100 µL of this solution was transferred to a 3 mL culture tube. Annexin V-PE (5.0 µL) and 5.0 µL of 7-amino-actinomycin stain were added and the suspension and was gently mixed and then incubated at room temperature in the dark for 15 minutes. A 500 µL-aliquot of 1x annexin V binding buffer was added and the culture tube and gentle mixing and fluorescence-activated cell analysis was performed.

Fluorescent-activated cell sorting analysis of 3T3 mouse fibroblast cells with varying concentrations of $[(PCy_3)Au(triazolate)]_2$-[G-1]-OH after 20 hours. Into a 6-well culture plate was added 1.0 mL D8 medium containing ~100,000 3T3 cells, which were allowed to stand for 1 hour for the cells to attach to the plate. To individual wells was added 100 µL of a $[(PCy_3)Au(triazolate)]_2$-[G-1]-OH dendrimer solution at 110, 55, 11, and 1.0 µM in 20% DMSO, giving final concentrations of solutions of 10, 5.0, 1.0, and 0.1 in 1.8% DMSO respectively. The cell culture plate was then incubated at 37°C and 5% CO_2 concentration for 20 hours. The cell plate medium was collected into a 3 mL test tube and then the well was washed with 1 mL of 1x PBS buffer solution and that was collected and added to the 3 mL test tube. 100 µL of 0.25% trypsin solution was added to the plate well. The well was then incubated at 37°C and 5% CO_2 concentration for 5 minutes. To the well was added 1 mL of D8 medium, which was stirred and then collected into a 3 mL test tube. The 3 mL test tube was centrifuged at 1060 rpm to obtain a cell pellet. The medium was then removed via vacuum, and the cell pellet was washed with 1 mL of 1x PBS buffer solution. The 3 mL test tube was then centrifuged to obtain a cell pellet and this washing process was repeated. The cells were resuspended in 200 µL

of 1x annexin V binding buffer and 100 µL of this solution was transferred to a 3 mL culture tube. 5.0 µL of annexin V-PE and 5.0 µl of 7-amino-actinomycin stain was added and the suspension and was gently mixed and incubated at room temperature in the dark for 15 minutes. 500 µL of 1x annexin V binding buffer was added and the culture tube and was gently mixing and fluorescent-activated cell sorting analysis was performed.

Fluorescence-activated cell sorting analysis of 3T3 mouse fibroblast cells with a (+)-camptothecin solution. An initial solution of 1.0 mM (+)-camptothecin in DMSO was prepared. To a six-well cell culture plate was added 1 mL of D8 medium containing ~100,000 3T3 cells. The plate allowed to stand for 1 hour for the cells to attach. To the well was added 5 µL of the 1.0 mM (+)-camptothecin solution giving a final concentration of 5 µM. The cell culture plate was incubated at 37 °C and 5% CO_2 concentration for 6 hours. The cell plate medium was collected into a 3 mL test tube and the well was washed with 1 mL of 1x PBS buffer solution. The resulting mixture was collected and added to the 3 mL test tube. A 0.25% (100 µL) trypsin solution was added to the plate well. The well was then incubated at 37 °C and 5% CO_2 concentration for 5 minutes. To the well was added 1 mL of DMEM medium. The contents of the well were stirred and collected into the 3 mL test tube. The 3 mL test tube was centrifuged at 1060 rpm to obtain a cell pellet. The medium was then removed via vacuum, and the cell pellet was washed with 1 mL of 1x PBS buffer solution. The 3 mL test tube was then centrifuged to obtain a cell pellet and this washing process was repeated. The cells were re-suspended in 200 µL of 1x annexin V binding buffer and 100 µL of this solution was transferred to a 3 mL culture tube. Annexin V-PE (5.0 µL) and 5.0 µL of 7-amino-actinomycin stain were added and the suspension and was gently mixed and then incubated at room temperature in the dark for 15 minutes. Another 500 µL-aliquot of 1x annexin V binding buffer was added and the culture tube and gentle mixing and fluorescence-activated cell analysis was performed.

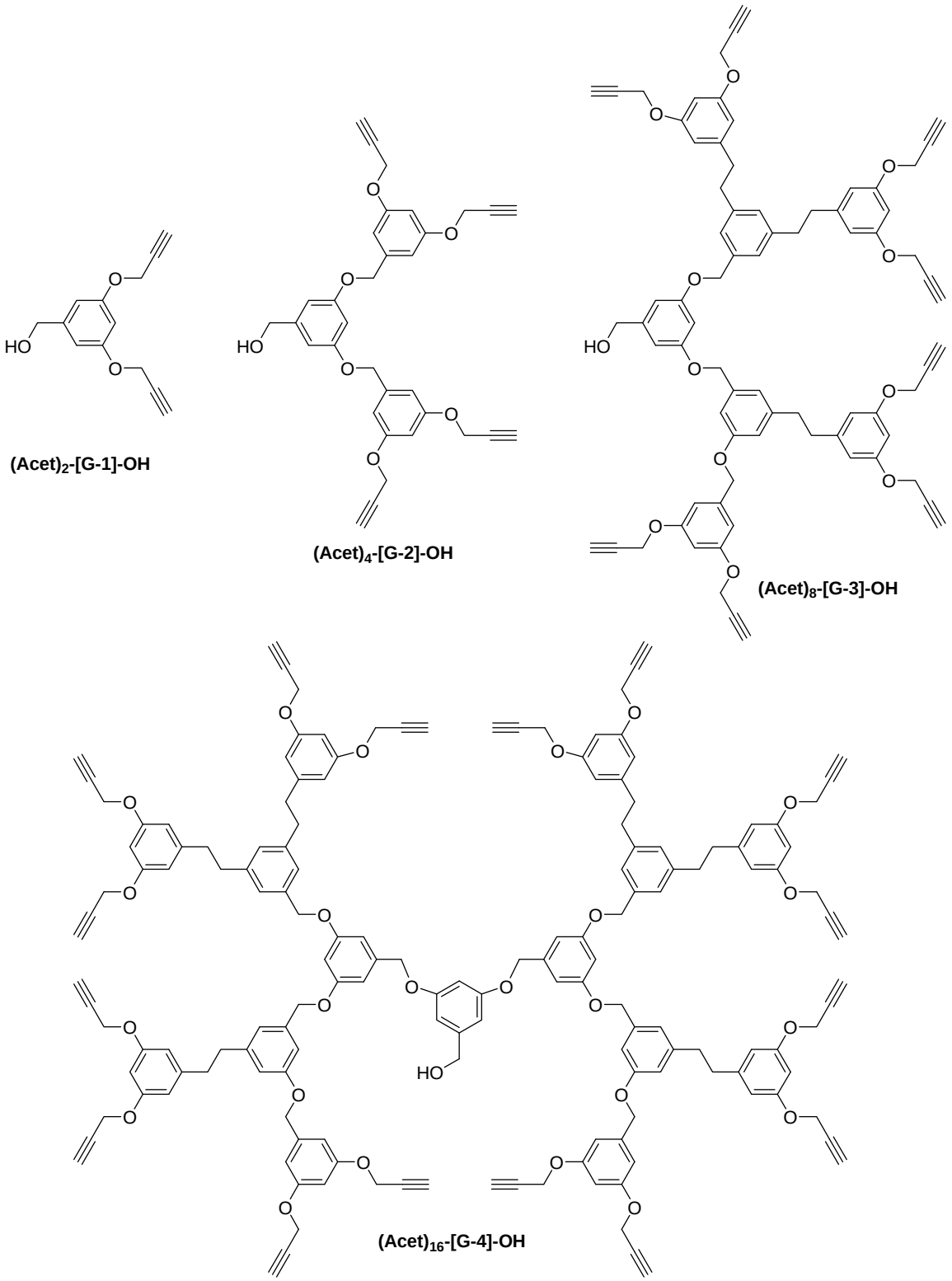

Chart S1. Terminal alkyne-bearing precursors and their abbreviations.

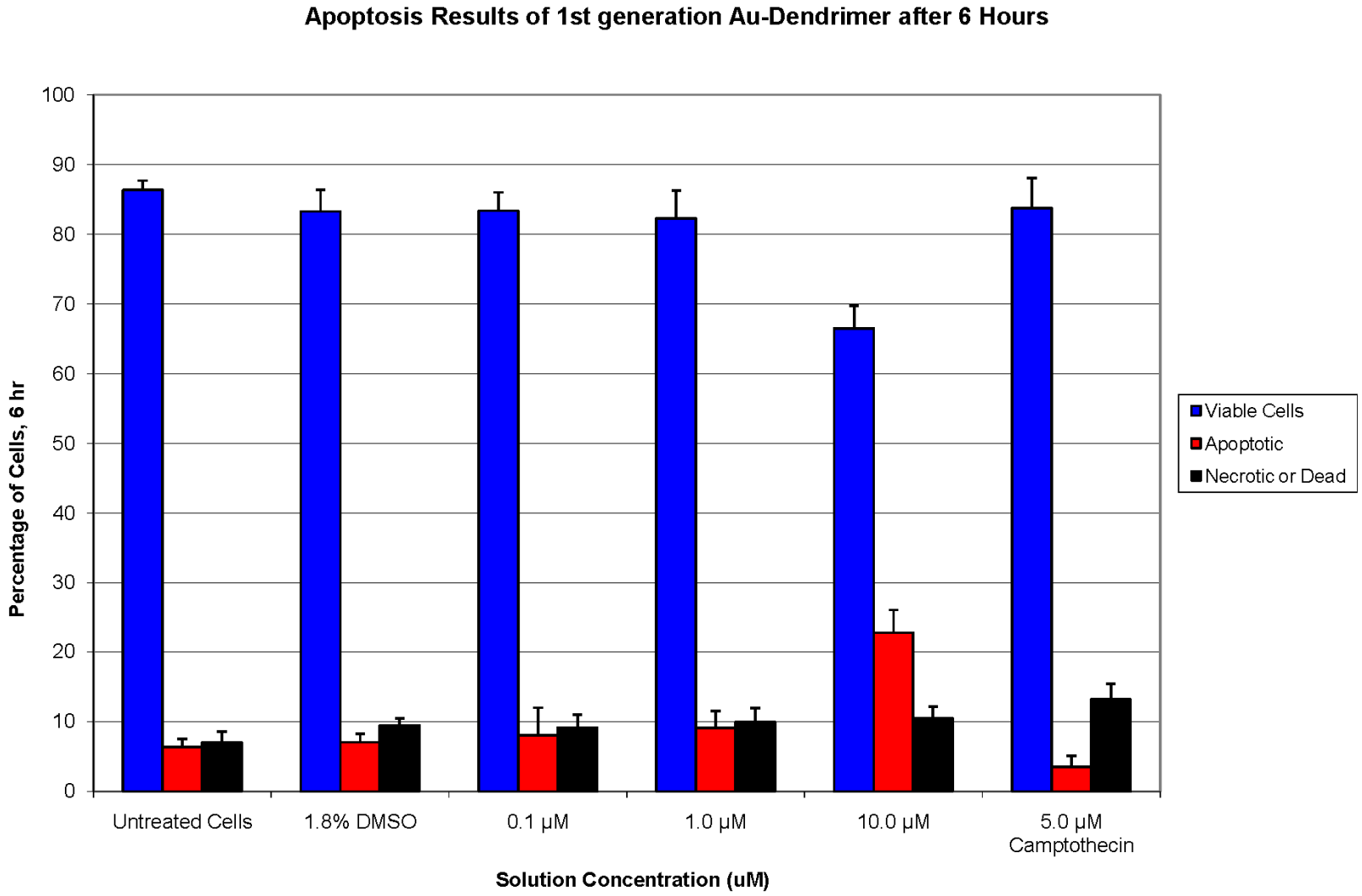

Figure S1. Fluorescence-activated cell analysis of 3T3 mouse fibroblast cells with solutions of **1** at various concentrations, after 6 h. Concentrations are of **1** unless otherwise indicated. The blue column indicates cells that are still viable (living) in solution. The red column indicates cells 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. The vertical scale is identical to that in Figure 3 of the main text.

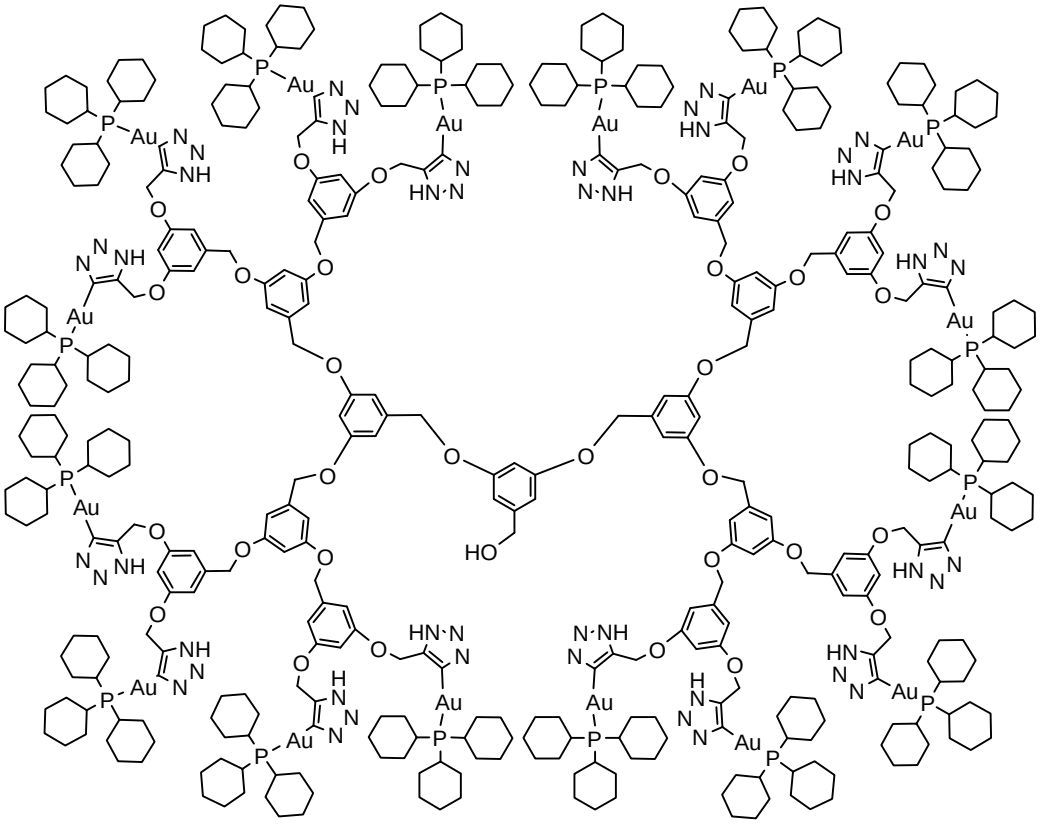

Figure S2. Line drawing of fourth-generation dendrimer **4**.