Supporting Information for:

Synthesis and Anticancer Properties of Water-Soluble Zinc Ionophores

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Materials and Methods

Materials. Motexafin gadolinium (MGd) was used as a 2 mM (2.3 mg/mL) formulation in 5% aqueous mannitol. Zinc acetate (99.99%, $ZnOAc_2$) was purchased from Aldrich Chemical Co. and prepared as a 2 mM formulation in 5% aqueous mannitol. PCI-5002 and PCI-5003, synthesized as described below, were formulated in 5% mannitol at 2 to 8 mM concentrations for both in vitro and in vivo experiments. PCI-5001 and the zinc salt of 1-hydroxypyridine-2-thione (ZnHPT, Sigma Chemical) were formulated in DMSO for in vitro experiments. The zinc-free ligands were freely soluble in aqueous media. The lowest energy extinction coefficient of ZnHPT ($\varepsilon 280 = 22{,}082\ M^{-1}\ cm^{-1}$; $\varepsilon 332 = 6{,}187\ M^{-1}\ cm^{-1}$; DMSO) was used to quantify solutions of ZnHPT analogues, which were in some cases isolated as oils. The extinction coefficients of PCI-5001 ($\varepsilon 284 = 24{,}416\ M^{-1}\ cm^{-1}$; $\varepsilon 336 = 6{,}228\ M^{-1}\ cm^{-1}$; DMSO) were nearly identical to those of ZnHPT. ACS grade water, purchased from Aldrich Chemical Co., was used to prepare (10X) secondary stocks of MGd and zinc.

Cells and Cell Culture Reagents. A549 lung cancer and PC3 prostate cancer lines were obtained from the American Type Culture Collection. Unless otherwise indicated, all cell

culture reagents were purchased from Invitrogen. Cells were cultured in RPMI 1640 medium supplemented with 20 mM HEPES, 2 mM L-glutamine, 10% heat inactivated fetal bovine serum (Hyclone) and antibiotics (200 U/mL penicillin and 200 μg/mL streptomycin).

Cellular Proliferation. The proliferation of exponential phase cultures of A549 and PC3 cells was assessed by tetrazolium salt reduction (1). In brief, A549 (2000 cells/well) or PC3 (4000 cells/well) cells were seeded on 96-well microtiter plates and allowed to adhere overnight. Stock solutions of ZnHPT or analogues in medium were serially diluted in a ratio of 1:4. Stock solutions of MGd or $ZnOAc_2$ or 5% mannitol in ACS grade water were added and plates were incubated at 37 ^{o}C under a 5% CO_2/95% air atmosphere. After 72 hours, medium was replaced with fresh medium (150 μL/well) supplemented with the tetrazolium dye, 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide (MTT, Sigma Chemical, 0.5 mg/mL). Plates were incubated at 37 ^{o}C and viable cells measured as described (1). Cellular reduction within plateau phase cultures was measured as above after 24 hours of treatment.

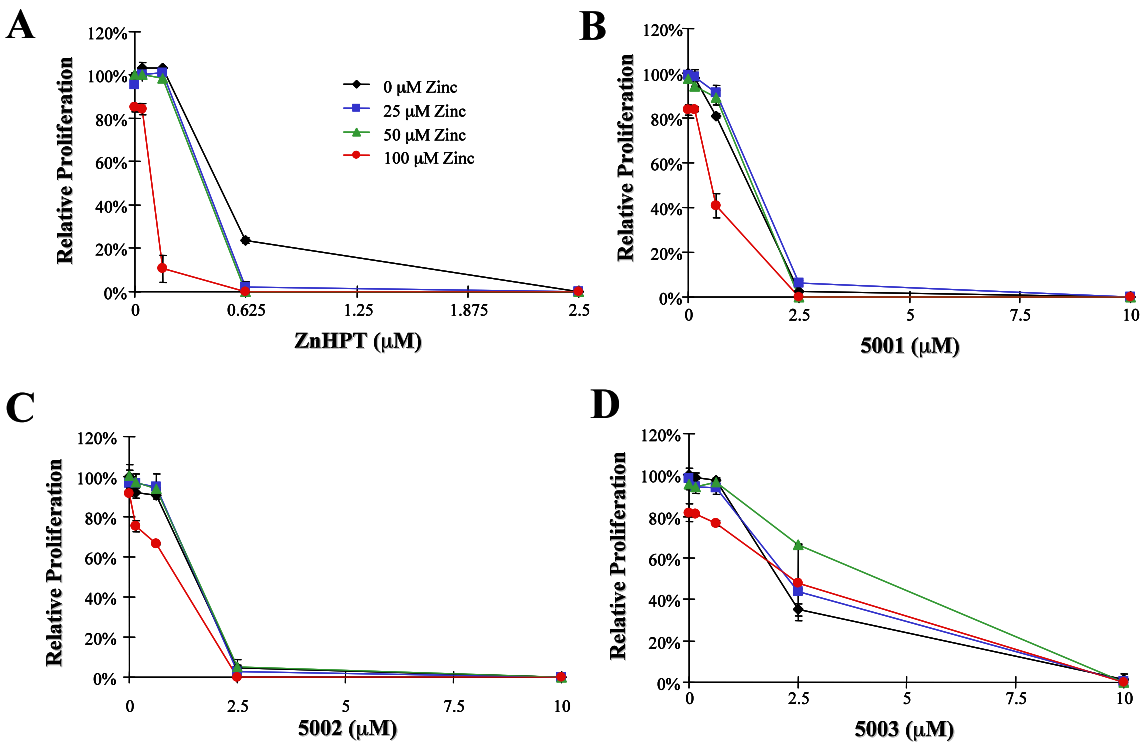
Lipoate Reduction. Thioredoxin reductase activity was assessed by measuring the rate of lipoate reduction (2). In brief, A549 or PC3 cells (10,000 cells/well) were seeded on 96-well plates and allowed to adhere overnight and grow two additional days until confluent. Cells were treated with ZnHPT, ZnHPT analogues, zinc, or 5% mannitol as described above for 2 or 4 hours. Medium was removed, cells were washed with Hanks balanced salt solution (HBSS), and a solution of 5 mM lipoic acid and 1 mM 5,5'-dithiobis(2-nitrobenzoic acid) in HBSS (100 μL/well) was added. Plates were incubated at ambient temperature in the dark. At chosen time intervals, plate absorbances were measured at 405-650 nm. Rates were normalized to those of wells containing neither exogenous zinc nor ZnHPT analogues to allow plate-to-plate comparison.

Intracellular Free Zinc and Cell Viability. The concentration of intracellular free zinc was assessed using the ion-specific fluorescent probe, FluoZin-3-AM™ (FluoZin-3, Molecular Probes, Inc.) (3). Plateau phase cultures were treated with control 5% mannitol vehicle or $ZnOAc_2$ in the presence or absence of ZnHPT or ZnHPT analogues for 4 hours. Following treatment, cells were isolated by centrifugation. Cell pellets were washed and re-suspended in a solution of 0.5% BSA in PBS. An aliquot of 10^6 cells (200 μL) was removed, centrifuged, and treated with FluoZin-3 reaction buffer as described (3). An aliquot of the cell suspension was supplemented with 2 μg/mL propidium iodide (Sigma Biochemical), incubated for 5 minutes,

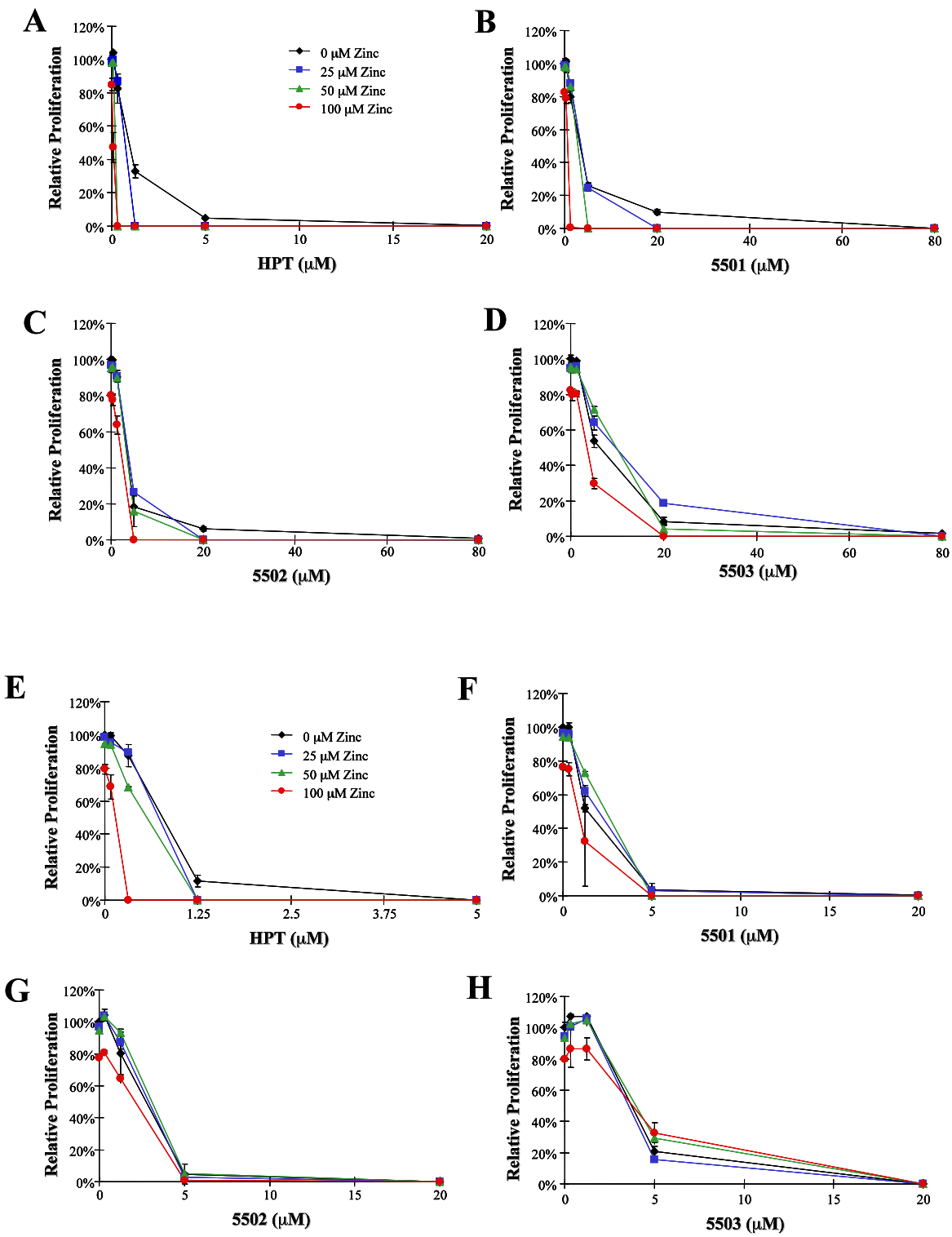
and subjected to two-parameter flow cytometric analysis as described previously (4). Cell viability and apoptosis in like-treated plateau phase cultures was measured using the ANNEXIN-V PI Kit (BD Biosciences, San Jose, CA) after 24 hours of treatment as described (5).

Measurement of reactive oxygen species (ROS). Reactive oxygen species were measured in live cells as intracellular peroxides by monitoring the oxidation of 2',7'-dichlorofluorescin-diacetate (DCFA) to 2',7'-dichlorofluorescein (DCF) (Molecular Probes). Cells (1 x 10^6 per mL) were incubated in a solution of 1 μg per mL DCFA in 0.5% BSA in HBSS for 15 minutes at 37 ^oC. Two mL additional 0.5% BSA in HBSS was added, cells were isolated by centrifugation, and the pellet re-suspended in a solution of 50 μg/mL 7-aminoactinomycin D (7-AAD) in 0.5% BSA in HBSS. Cell suspensions were incubated at ambient temperature for 2 to 3 minutes, and stored on ice until analysis. The fluorescent intensity in live (i.e., 7-AAD impermeable) cells was analyzed by flow cytometry.

Mouse Xenograft Models. Animal care was in accordance with NIH and institutional guidelines. For the A549 xenograft model, 1.25 x 10^6 A549 cells were injected subcutaneously/intramuscularly into the right hind flank of 6 week old CD-1 nude mice that had been irradiated with 4 Gy of total body irradiation from a ^{137}Cs radiation source one day prior to tumor implantation (6). For the PC3 xenograft model, 1.5 x 10^6 cells were used. When the average size of tumors reached approximately 100 mm^3, mice were randomized by tumor size to treatment groups, typically containing 6-8 mice per group. Mice were treated intravenously with 2-4 doses of zinc ionophore, typically 100 μmol/kg, on consecutive days. Vehicle control treated animals received 5% mannitol on the same schedule. Tumor and body weight measurements were performed three times per week. Tumor volume was calculated using the equation V (mm^3) = a x $b^2/2$, where a is the largest diameter and b is the smallest diameter. No significant body weight loss was observed. To perform gene expression profiling, mice were treated intravenously with one dose (100 μmol/kg) PCI-5002, PCI-5003, or control vehicle (4 mice per group) when the average A549 tumor size reached 500 mm^3. After four hours, tumors were harvested and snap frozen immediately on dry ice. Tumor tissue was dissected, homogenized in Trizol (Invitrogen, Omni International, Model TH-115), and total RNA was isolated and subjected to analysis using Human Genome U133 Plus 2.0 Arrays as described above.

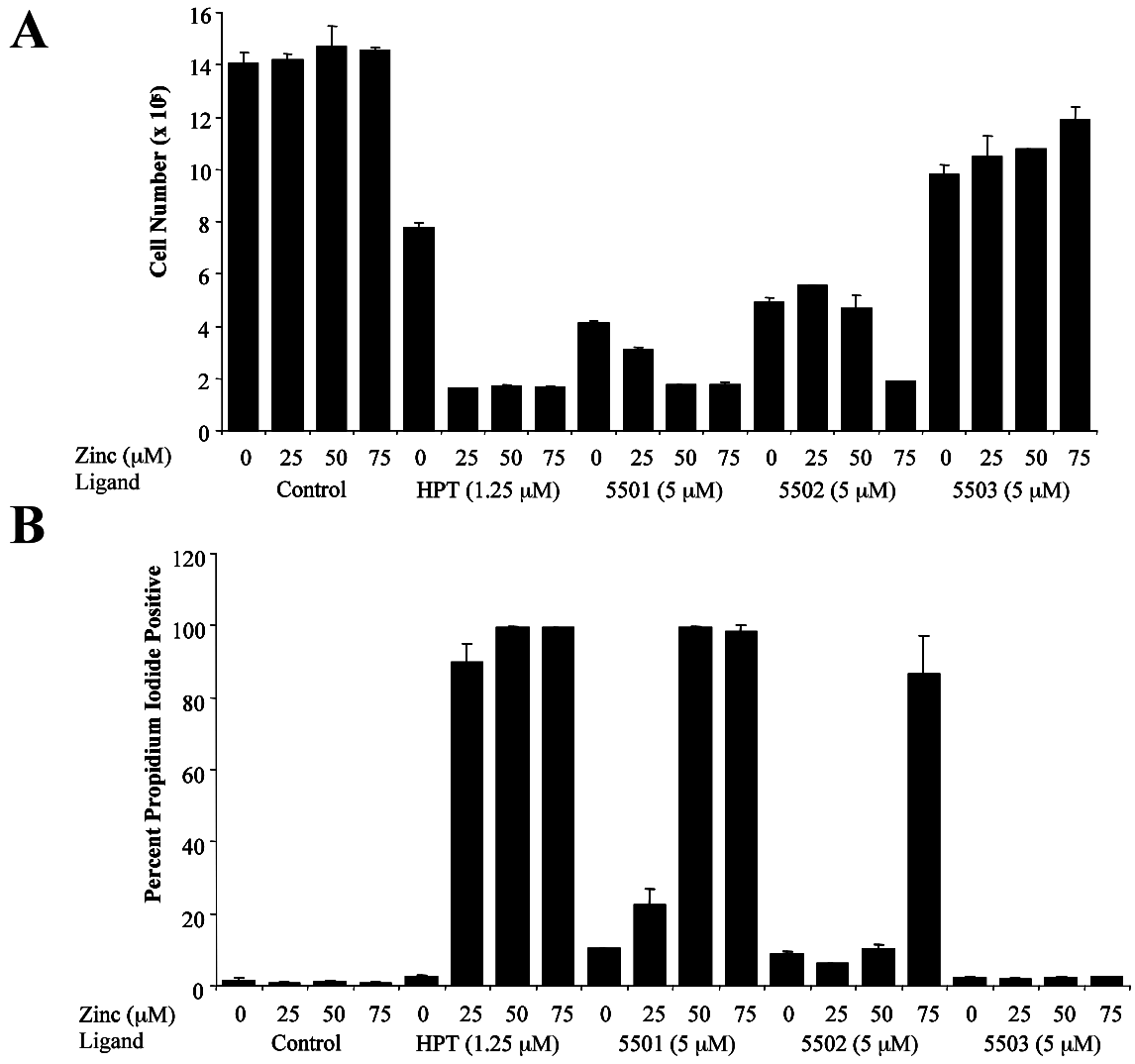


Supplemental Figure 1. Zinc ionophores inhibit proliferation of PC3 cells. Exponential phase cultures of PC3 cells were treated with zinc ionophores (A to D) at the indicated concentrations in the presence or absence of zinc acetate [0 µM (black line), 25 µM (blue line), 50 µM (green line), and 100 µM (red line)] for 72 hours in duplicate experiments. Error bars indication one standard deviation.

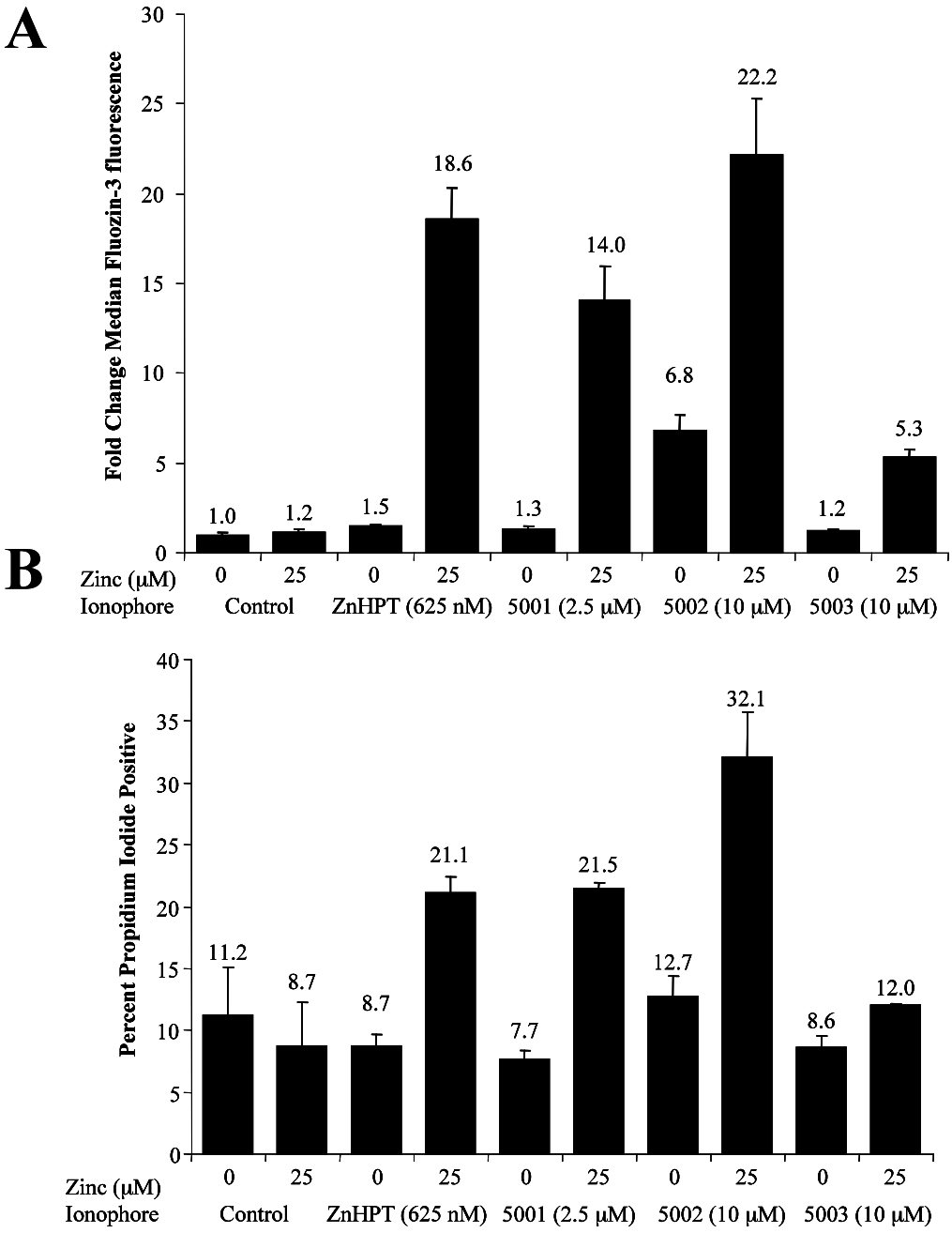


Supplemental Figure 2. Treatment with zinc ligands inhibits A549 and PC3 cell proliferation. Exponential phase A549 cultures (A to D) or PC3 cultures (E to H) were treated with zinc ligands at the indicated concentrations in the presence or absence of zinc acetate [0 µM

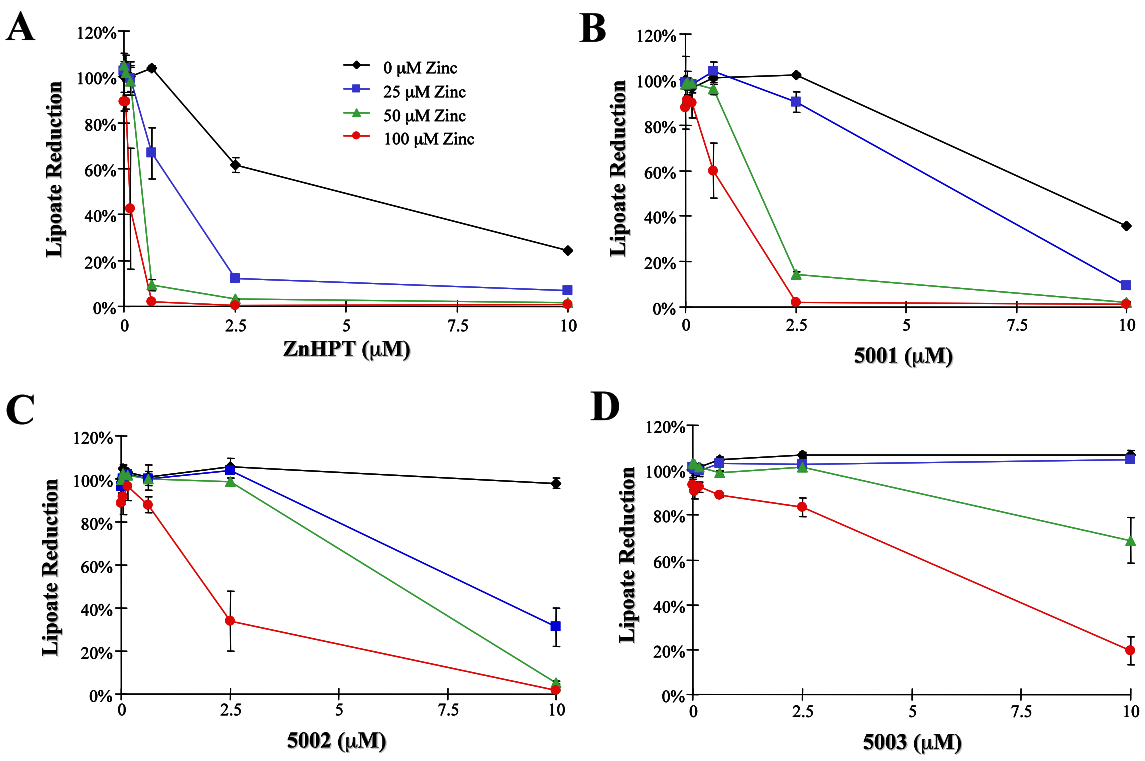
(black line), 25 μM (blue line), 50 μM (green line), and 100 μM (red line)] for 72 hours in duplicate experiments. Error bars indication one standard deviation.



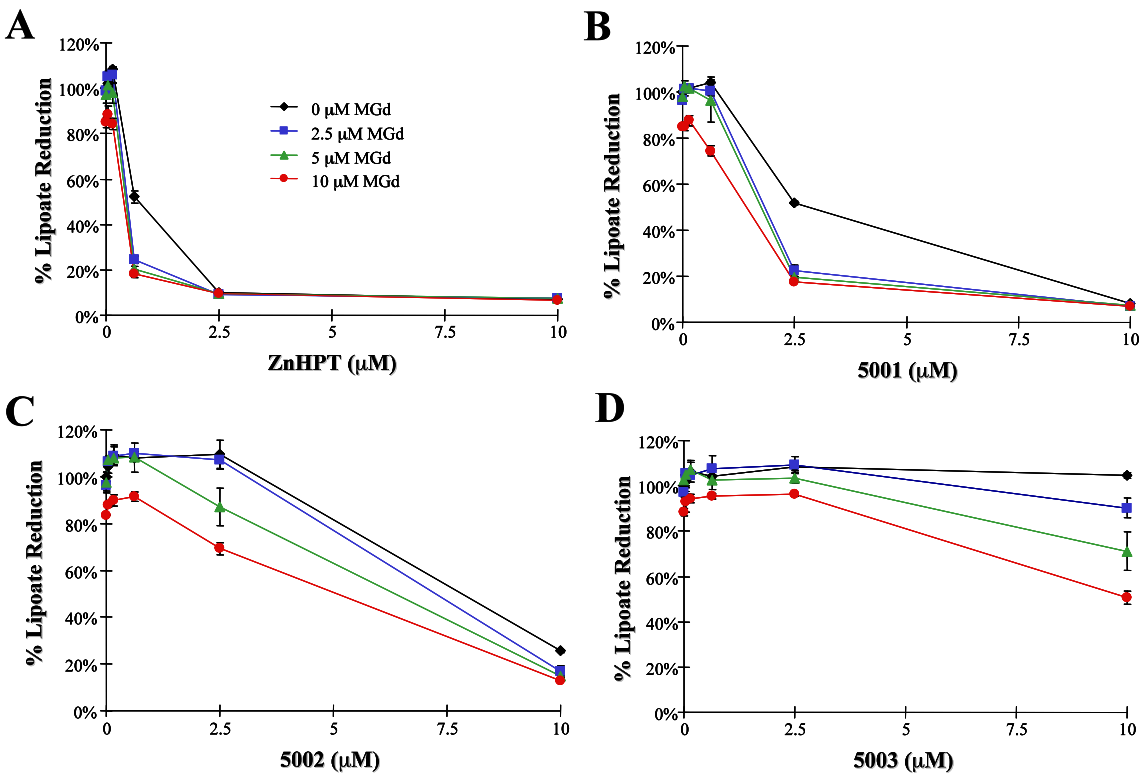
Supplemental Figure 3. Treatment with zinc ligands inhibits A549 cell proliferation and causes cell death in response to zinc. Exponential phase A549 cultures were treated with zinc ligands (1.25 or 5 μM) in the presence or absence of zinc acetate (Zinc, 0-75 μM) for 72 hours in duplicate experiments. Error bars indication one standard deviation. A. Cell density by Coulter counting after 72 hours of treatment. B. Percentage of propidium iodide stained cells after 72 hours.



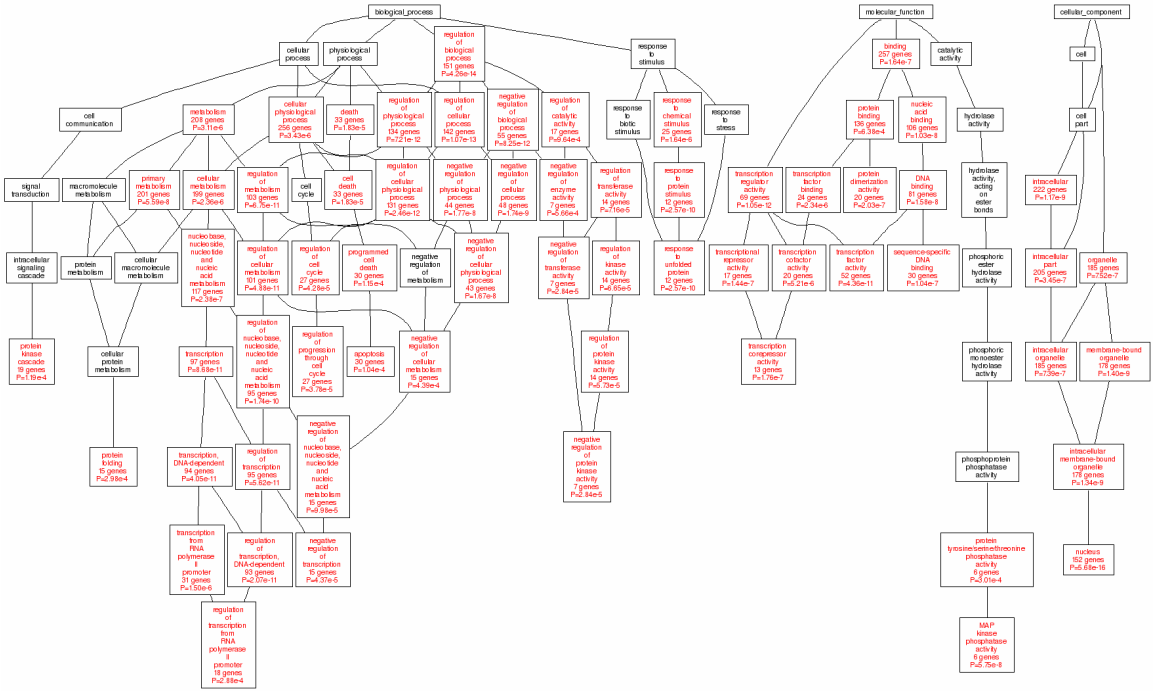
Supplemental Figure 4. Zinc ionophore treatment alters levels of intracellular free zinc and leads to cell death of PC3 cells in response to zinc. Plateau phase PC3 cultures were treated with zinc ionophores (0, 0.625, 2.5 or 10 μM) in the presence or absence of zinc acetate (Zinc, 25 μM) for 2 hours in duplicate experiments. Error bars indication one standard deviation. A. Fold increase of FluoZin-3 fluorescence in live-gated cells after 2 hours. B. Percentage of propidium iodide stained cells after 2 hours.



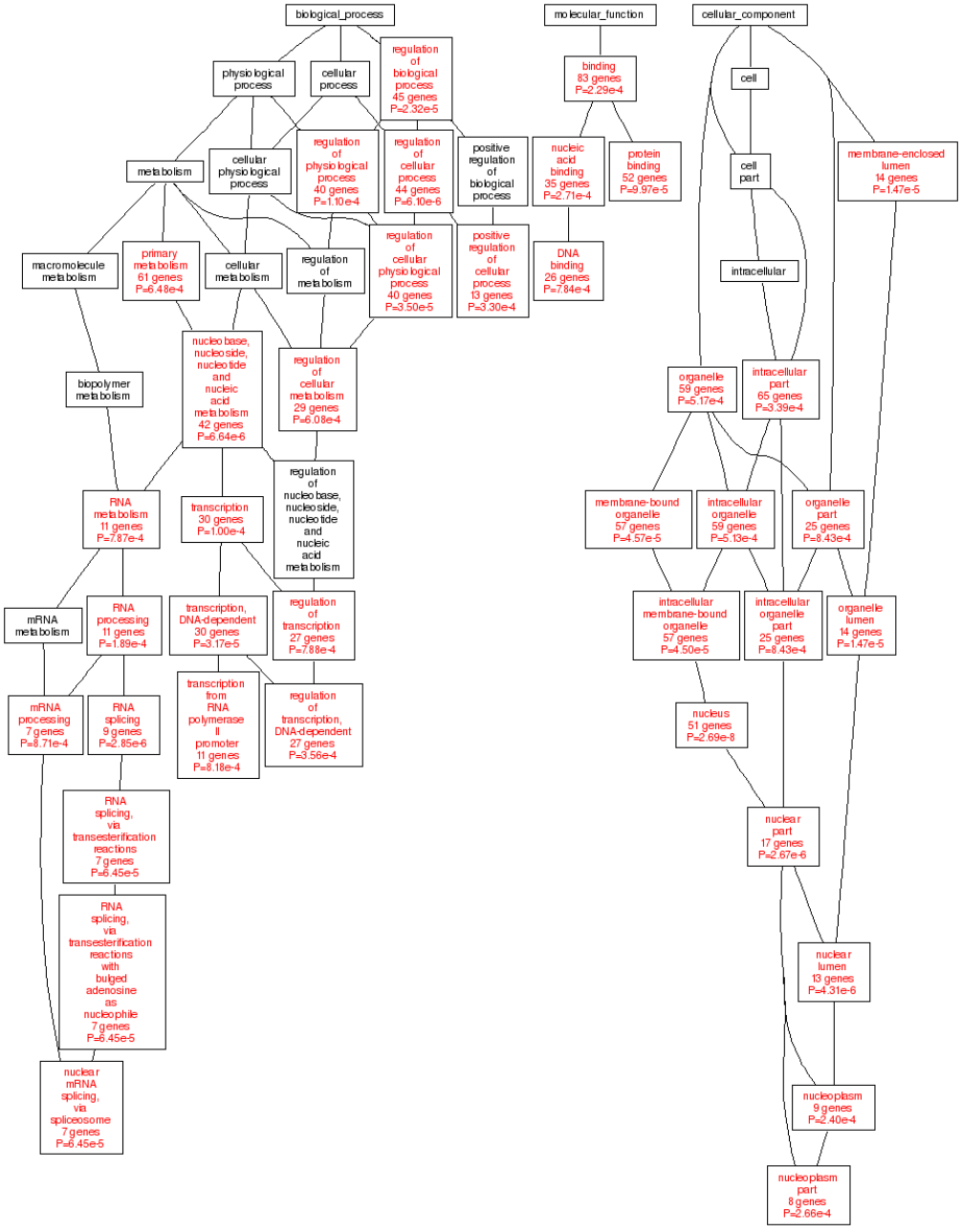
Supplemental Figure 5. Inhibition of lipoate reduction in A549 cells. Plateau phase A549 cultures were treated for 2 hours with zinc ionophore (0-10 µM) and zinc acetate [0 µM (black line), 25 µM (blue line), 50 µM (green line), and 100 µM (red line)], medium exchanged, and relative lipoate reduction measured after 20 minutes as described in Materials and Methods. Error bars indicate one standard deviation.



Supplemental Figure 6. Inhibition of lipoate reduction in A549 cells. Plateau phase A549 cultures were treated for 2 hours with zinc ionophore (0-10 µM) and motexafin gadolinium [0 µM (black line), 2.5 µM (blue line), 5 µM (green line), and 10 µM (red line)], medium exchanged, and relative lipoate reduction measured after 40 minutes as described in Materials and Methods. Error bars indicate one standard deviation.



Supplemental Figure 7. Gene Ontology (GO) analysis of the 608 transcripts showing up-regulation in A549 cultures treated with 10 µM PCI-5002 in the presence of 25 µM exogenous zinc. Analyses were conducted using WebGestalt software, as described in the methods. In the provided Directed Acyclic Graph (DAG), the functional categories showing significant enrichment ($P < 0.001$ based on a hypergeometric test) in this dataset are colored in red.



Supplemental Figure 8. Gene Ontology (GO) analysis of the 309 transcripts showing down-regulation in A549 cultures treated with 10 µM PCI-5002 in the presence of 25 µM exogenous zinc. Analyses were conducted using WebGestalt software, as described in the methods. In the provided Directed Acyclic Graph (DAG), the functional categories showing significant enrichment ($P < 0.001$) in this dataset are colored in red.

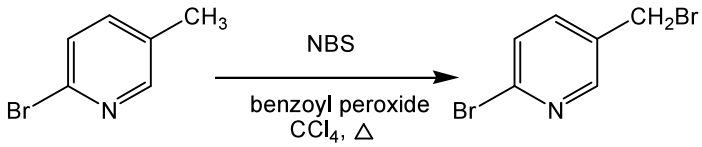
Supplemental Figure 9. Hierarchical clustering analysis of gene expression data from A549 cell cultures. The dendrograms were generated based on average linkage hierarchical clustering of expression data from 538 transcripts whose coefficient of variation was greater than 0.10 across all in vitro groups. Data from untreated (mannitol), zinc treated (25 uM zinc acetate), PCI-5002 treated (10 μM), and combination treated (zinc (25 μM) and PCI-2002 (10 μM)) A459 cultures are provided in triplicate.

Supplemental Figure 10. Hierarchical clustering analysis of gene expression data from A549 xenografts. The dendrograms were generated based on average linkage hierarchical clustering of expression data from 100 transcripts whose coefficient of variation was greater than 0.10 across all groups. Data from control (untreated), PCI-5002 treated (100 μmol / kg), and PCI-5003 treated (100 μmol / kg) xenografts are provided in quadruplicate. The names of samples treated with PCI-5003 are coded in blue for clarity.

Synthesis of PCI-5001, PCI-5002, and PCI-5003

General. All starting materials were purchased and used without further purification unless otherwise noted. Proton and ^{13}C NMR spectra were recorded using a 250 MHz Varian, 300 MHz GE Tacmag spectrometer, 400 MHz Varian MERCURY or 500 MHz Varian INOVA. UV/Vis spectra were taken on Beckman DU-640B or Agilent 8453 Spectrophotometer. Column chromatography was run using ICN-Silitech 32-63 D60 Å silica gel or Sorbent Technologies standard activity 50-200 μm neutral alumina. Sep-Pak reverse-phase tC18 cartridge columns were purchased from Waters.

Bromination of 2-Bromo-5-methyl-pyridine



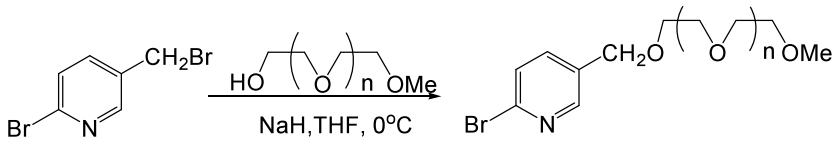
1 2

A solution of 2-bromo-5-methyl-pyridine (5g, 29.06mmol), N-bromosuccinimide (5.34g, 30mmol), benzoyl peroxide (60mg, 0.248mmol) in 40mL of dry CCl_4 was heated at reflux under N_2 for 8h. Large amount of solid was seen to float on the surface.

The hot mixture was filtered and the solid on the funnel was washed successively with two 10mL portions of hot CCl_4. The solvent was removed using a rotary evaporator.

Purification on silica gel, using a gradient of hexane/ ethyl acetate as the eluent yielded 3.4g product (46%). The characterization data are identical to those reported in the literature. CI-MS: m/z 252 $[M+H]^+$.

PEGylation of 2-Bromo-5-bromomethylpyridine



2

3a: n = 0
3b: n = 1
3c: n = 2

The PEGylation reactions with different length polyethylene glycols were carried out following the same procedure. An example is given below: To a stirred suspension of NaH (60% suspension in mineral oil, 640mg, 16mmol) in 100mL of anhydrous THF at 0°C was added diethyleneglycol methyl ether (0.95mL, 8mmol). The solution was allowed to stir at 0°C for 30min under N_2. Then 2-Bromo-5-bromomethylpyridine (1.004g, 4mmol) in 5ml of THF was added dropwise to the solution. The resulting mixture was stirred at 0°C for another one hour. The mixture was concentrated under reduced pressure and 20 mL of CH_2Cl_2 was added to dissolve the product. The inorganic salts were removed by vacuum filtration, and the filtrate was

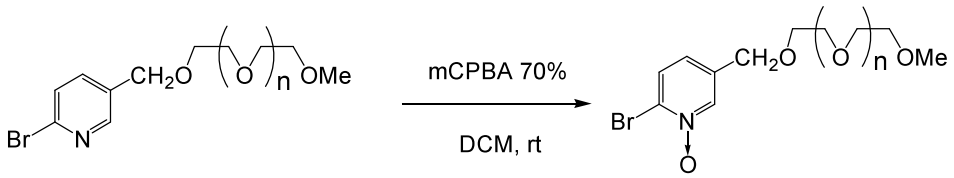
concentrated on a rotary evaporator. Column chromatography on silica gel using CH_2Cl_2 as the eluent yielded 1.1g PEGylation product **3b** (90%).

2-Bromo-5-((2-methoxyethoxy)methyl)pyridine (3a) Yield: 85%. ESI-MS: *m/z* 246 $[M+H]^+$; CI-HRMS $[M+H]^+$ calcd. for $C_9H_{13}BrNO_2$: 246.0130 found: 246.0131. ^1H NMR (300 Hz, $CDCl_3$): δ 8.27 (s, 1H), 7.52 (d, J = 8.2 Hz, 1H), 7.41 (d, J = 8.2 Hz, 1H), 4.49 (s, 2H), 3.60-3.58 (m, 2H), 3.53-3.51 (m, 2H), 3.33 (s, 3H) ppm. ^{13}C NMR (300 Hz, $CDCl_3$): δ 149.1, 141.0, 137.9, 133.0, 127.7, 71.7, 69.8, 69.7, 58.9 ppm.

2-Bromo-5-((2-(2-methoxyethoxy)ethoxy)methyl)pyridine (**3b)** Yield: 90%. ESI-MS: *m/z* 290 $[M+H]^+$; CI-HRMS $[M+H]^+$ calcd. for $C_{11}H_{17}$ $BrNO_3$: 290.0392 found: 290.0389. ^1H NMR (300 Hz, $CDCl_3$): δ 8.28 (s, 1H), 7.53 (d, J = 8.2 Hz, 1H), 7.41 (d, J = 8.2 Hz, 1H), 4.50 (s, 2H), 3.64-3.63 (m, 4H), 3.61-3.59 (m, 2H), 3.51-3.49 (m, 2H), 3.33 (s, 3H) ppm. ^{13}C NMR (300 Hz, $CDCl_3$): δ 149.1, 141.0, 137.9, 133.0, 127.7, 71.7, 70.4(2), 70.4(1), 69.8, 69.7, 58.9 ppm.

2-Bromo-5-((2-(2-(2-methoxyethoxy)ethoxy)ethoxy)methyl)pyridine (3c) Yield: 88%. ESI-MS: *m/z* 334 $[M+H]^+$; CI-HRMS $[M+H]^+$ calcd. for $C_{13}H_{21}BrNO_4$: 334.0654 found: 334.0656. ^1H NMR (300 Hz, $CDCl_3$): δ 8.28 (s, 1H), 7.53 (d, J = 8.2 Hz, 1H), 7.42 (d, J = 8.2 Hz, 1H), 4.50 (s, 2H), 3.64-3.59 (m, 10H), 3.52-3.49 (m, 2H), 3.33 (s, 3H) ppm. ^{13}C NMR (300 Hz, $CDCl_3$): δ 149.3, 141.2, 138.0, 133.2, 127.8, 71.9, 70.6, 70.5(4), 70.4(6), 69.9, 69.8 and 59.0 ppm.

Oxidation of 2-Bromo-5-CH_2OPEG-pyridine



3a: n = 0
3b: n = 1
3c: n = 2

4a: n = 0
4b: n = 1
4c: n = 2

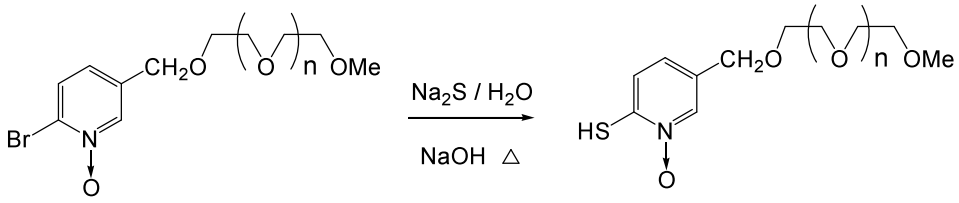
The oxidation reactions of **3a-c** were carried out following the same procedure. An example is given below: To a solution of 2-Bromo-5-CH_2PEG(2)-pyridine (**3b**, 1.1g, 3.79mmol) in 15mL of CH_2Cl_2 was added 3-chloroperoxybenzoic acid (1.7g, 7.58mmol). After being stirred at room temperature overnight, the reaction mixture was concentrated under reduced pressure. De-ionized water (DI H_2O, 10 mL) was added to dissolve the product. The insoluble solid was removed by vacuum filtration and the solid on the funnel was washed with DI H_2O (5mL x 2). The combined filtrate and washings were loaded on a tC-18 Sep-pak column and purified to provide 950mg (82%) of **4b**.

2-Bromo-5-((2-methoxyethoxy)methyl)pyridine-N-oxide (4a, PCI-5501) Yield: 76%. ESI-MS: *m/z* 262 $[M+H]^+$. ^{1}H NMR (300 Hz, $CDCl_3$): δ 8.35 (s, 1H), 7.56 (d, J = 8.2 Hz, 1H), 7.04 (d, J = 8.2 Hz, 1H), 4.46 (s, 2H), 3.62-3.60 (m, 2H), 3.54-3.51 (m, 2H), 3.33 (s, 3H) ppm. ^{13}C NMR (300 Hz, $CDCl_3$): δ 139.1, 136.6, 131.1, 130.0, 124.6, 71.5, 69.9, 68.8, 58.8 ppm.

2-Bromo-5-((2-(2-methoxyethoxy)ethoxy)methyl)pyridine-N-oxide (4b, PCI-5502) Yield: 78%. ESI-MS: *m/z* 306 $[M+H]^+$; CI-HRMS $[M+H]^+$ calcd. for $C_{11}H_{17}BrNO_4$: 306.0341 found: 306.0328. ^{1}H NMR (300 Hz, $CDCl_3$): δ 8.29 (s, 1H), 7.51 (d, J = 8.8 Hz, 1H), 6.99 (d, J = 8.8 Hz, 1H), 4.41 (s, 2H), 3.57-3.56 (m, 4H), 3.54-3.52 (m, 2H), 3.45-3.43 (m, 2H), 3.26 (s, 3H) ppm. ^{13}C NMR (300 Hz, $CDCl_3$): δ 138.8, 136.7, 131.2, 130.0, 124.8, 71.6, 70.3, 70.2(7), 70.0, 68.8, 58.8 ppm.

2-Bromo-5-((2-(2-(2-methoxyethoxy)ethoxy)ethoxy)methyl)pyridine-N-oxide (4c, PCI-5503) Yield: 70%. ESI-MS: *m/z* 350 $[M+H]^+$. ^1H NMR (300 Hz, $CDCl_3$): δ 8.33 (s, 1H), 7.55 (d, *J* = 8.8 Hz, 1H), 7.04 (d, *J* = 8.8 Hz, 1H), 4.45(s, 2H), 3.62-3.56 (m, 10H), 3.49-3.40 (m, 2H), 3.30 (s, 3H) ppm. ^{13}C NMR (300 Hz, $CDCl_3$): δ 139.8, 136.8, 131.2, 130.2, 124.7, 71.8, 70.4(4) broad, 70.4, 70.1, 68.9, 58.9 ppm.

Sodium sulfide reaction with 2-Bromo-5-CH_2PEG-pyridine-N-Oxide



4a: n = 0
4b: n = 1
4c: n = 2

5a: n = 0
5b: n = 1
5c: n = 2

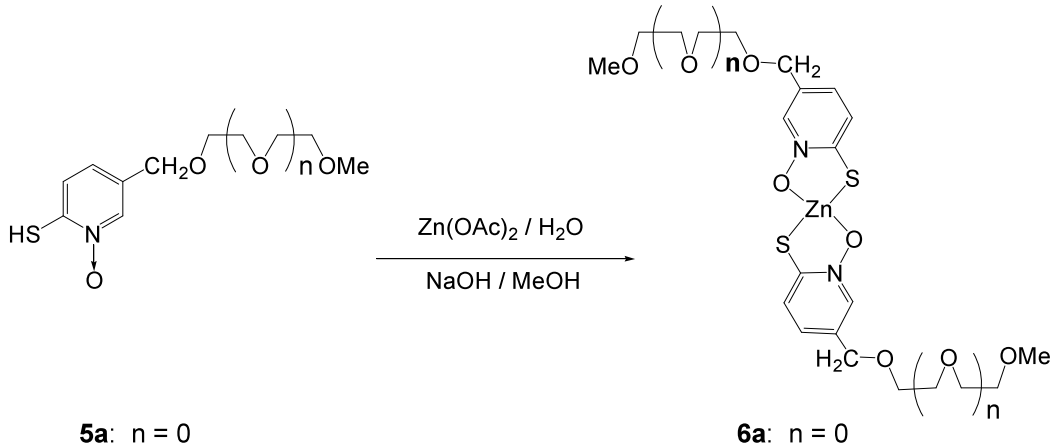
The reactions of **4a-c** with sodium sulfide were carried out following the same procedure. An example is given below: A 50mL three-neck round-bottom flask equipped with a reflux condenser was charged with **4b** (950mg, 3.1mmol), 0.1N NaOH solution (0.3 mL) and 5mL of DI H_2O. The resulting solution was heated to 80°C. Na_2S (484 mg, 6.2 mmol) in 2mL of DI H_2O was added dropwise via a syringe over a period of 1h. The reaction mixture was allowed to stir for an additional 1 h. After cooling to room temperature, acetic acid was added to adjust pH to 5. The mixture was concentrated under vacuum to give **5b** as yellow oil, which was used in the next reaction without further purification. Small amounts of analytical samples were prepared by silica gel column chromatography followed by Sep-Pak treatment.

2-Mercapto-5-((2-methoxyethoxy)methyl)pyridine-N-oxide (5a). ESI-MS: *m/z* 216 $[M+H]^+$. ^1H NMR (300 Hz, $CDCl_3$): δ 8.10 (s, 1H), 7.60 (d, *J* = 8.4 Hz, 1H), 7.21 (d, *J* = 8.4 Hz, 1H), 4.44 (s, 2H), 3.65-3.62 (m, 2H), 3.57-3.53 (m, 2H), 3.36 (s, 3H) ppm. ^{13}C NMR (300 Hz, $CDCl_3$): δ 165.6, 132.1, 131.5, 129.9, 125.7, 71.7, 69.9, 68.9, 60.0 ppm.

2-Mercapto -5-((2-(2-methoxyethoxy)ethoxy)methyl)pyridine-N-oxide (5b) ESI-MS: *m/z* 260 $[M+H]^+$; ^1H NMR (300 Hz, $CDCl_3$): δ 8.12 (s, 1H), 7.58 (d, *J* = 8.4 Hz, 1H), 7.19 (d, *J* = 8.4 Hz, 1H), 4.53 (s, 2H), 3.66-3.65 (m, 4H), 3.62-3.60 (m, 2H), 3.54-3.51 (m, 2H), 3.35 (s, 3H) ppm. ^{13}C NMR (300 Hz, $CDCl_3$): δ 165.7, 132.1, 131.5, 130.0, 125.9, 71.8, 70.5, 69.9, 68.9, 59.0 ppm.

2-Mercapto-5-((2-(2-(2-methoxyethoxy)ethoxy)ethoxy)methyl)pyridine-N-oxide (5c) ESI-MS: *m/z* 304 $[M+H]^+$. ^1H NMR (300 Hz, $CDCl_3$): δ 8.12 (s, 1H), 7.57 (d, *J* = 8.4 Hz, 1H), 7.18 (d, *J* = 8.4 Hz, 1H), 4.43(s, 2H), 3.63-3.59 (m, 10H), 3.50-3.48 (m, 2H), 3.31 (s, 3H) ppm. ^{13}C NMR (300 Hz, $CDCl_3$): δ 165.7, 132.1, 131.4, 130.0, 125.9, 71.8, 70.6, 70.5, 70.4, 68.9 and 59.0 ppm.

Preparation of zinc complex of 2-mercapto-5-CH_2PEG-pyridine-N-Oxide---General Procedure



5a: n = 0
5b: n = 1
5c: n = 2

6a: n = 0
6b: n = 1
6c: n = 2

To a solution of crude **5b** from the previous step, 5mL of MeOH and 1mL of DI H_2O, 1N NaOH solution was added to adjust pH to 8 at room temperature. Then 1 equivalent of $Zn(OAc)_2$ was added and the resulting mixture was heated to 70^oC and stirred under N_2 atmosphere for 1h. The reaction mixture was concentrated under reduced pressure. The residue was purified by Sep-pak chromatography to give **6b** (630mg, yield for 2 steps combined: 70%) as a light brown solid.

2-Mercapto-5-((2-methoxyethoxy)methyl)pyridine-N-oxide, Zn complex (6a, PCI-5001) Yield: 65% (for 2 steps). ESI-MS: *m/z* 493 $[M+H]^+$. CI-HRMS $[M+H]^+$ calcd. for $C_{18}H_{25}N_2O_6S_2Zn$: 493.0446 found: 493.0440. ^1H NMR (300 Hz, $CDCl_3$): δ 8.26 (d, J = 2.0 Hz, 2H), 7.62 (d, *J* = 8.4 Hz, 2H), 7.18 (dd, *J* = 8.4, *J* = 2.0 Hz, 2H), 4.43 (s, 4H), 3.57-3.55 (m, 4H), 3.51-3.49 (m, 4H), 3.32 (s, 6H) ppm. ^{13}C NMR (300 Hz, $CDCl_3$): δ 158.1, 136.0, 129.6, 129.5, 129.2, 71.7, 69.7, 69.1 and 59.0 ppm.

2-Mercapto -5-((2-(2-methoxyethoxy)ethoxy)methyl)pyridine-N-oxide, Zn complex (6b, PCI-5002) Yield: 70% (for 2 steps). ESI-MS: *m/z* 581 $[M+H]^+$; FAB-HRMS $[M+H]^+$ calcd. for $C_{22}H_{33}N_2O_8S_2Zn$: 581.0967; found: 581.0958. ^1H NMR (300 Hz, $CDCl_3$): δ 8.29 (d, J = 2.0 Hz, 2H), 7.65 (d, *J* = 8.4 Hz, 2H), 7.20(dd, *J* = 8.4, *J* = 2.0 Hz, 2H), 4.46 (s, 4H), 3.63-3.60 (m, 12H), 3.53-3.51 (m, 4H), 3.35 (s, 6H) ppm. ^{13}C NMR (300 Hz, $CDCl_3$): δ 158.2, 136.1, 129.8, 129.6, 129.3, 71.9, 70.6, 70.5, 69.9, 69.2 and 59.0 ppm.

2-Mercapto-5-((2-(2-(2-methoxyethoxy)ethoxy)ethoxy)methyl)pyridine-N-oxide, Zn complex (6c, PCI-5003) Yield: 61% (for 2 steps). CI-MS: *m/z* 669 $[M+H]^+$. FAB-HRMS $[M+H]^+$ calcd. for $C_{26}H_{41}N_2O_{10}S_2Zn$: 669.1494; found: 669.1488. ^1H NMR (300 Hz, $CDCl_3$): δ 8.29 (d, J = 2.0 Hz, 2H), 7.66 (d, *J* = 8.4 Hz, 2H), 7.21 (dd, *J* = 8.4, 2.0 Hz, 2H), 4.46(s, 4H), 3.65-3.59 (m, 20H), 3.53-3.50 (m, 4H), 3.34 (s, 6H) ppm. ^{13}C NMR (300 Hz, $CDCl_3$): *δ* 158.2, 136.1, 129.8, 129.7, 129.3, 71.8, 71.9, 70.7, 70.6, 70.5, 69.9, 69.2 ppm.

Supplemental Data Table 1. Bond lengths [Å] and angles [°] for PCI-5001.

Zn1-O1	2.0516(11)	C5-H5	0.896(19)
Zn1-O4#1	2.1065(11)	C6-H6A	0.99(2)
Zn1-O4	2.1656(10)	C6-H6B	0.96(2)
Zn1-S1	2.3106(4)	C7-C8	1.491(3)
Zn1-S2	2.3203(4)	C7-H7A	0.98(2)
S1-C1	1.7220(16)	C7-H7B	0.98(2)
S2-C10	1.7202(16)	C8-H8A	0.99(2)
O1-N1	1.3441(16)	C8-H8B	0.98(2)
O2-C6	1.409(2)	C9-H9A	0.95(2)
O2-C7	1.428(2)	C9-H9B	1.01(3)
O3-C8	1.414(2)	C9-H9C	0.97(2)
O3-C9	1.418(2)	C10-C11	1.410(2)
O4-N2	1.3567(15)	C11-C12	1.376(2)
O4-Zn1#1	2.1065(11)	C11-H11	0.943(18)
O5-C15	1.418(2)	C12-C13	1.398(2)
O5-C16	1.419(2)	C12-H12	0.942(19)
O6-C18	1.416(2)	C13-C14	1.373(2)
O6-C17	1.418(2)	C13-C15	1.507(2)
N1-C5	1.361(2)	C14-H14	0.927(18)
N1-C1	1.368(2)	C15-H15A	1.01(2)
N2-C14	1.351(2)	C15-H15B	1.01(2)
N2-C10	1.3668(19)	C16-C17	1.498(3)
C1-C2	1.406(2)	C16-H16A	0.97(2)
C2-C3	1.365(2)	C16-H16B	0.98(2)
C2-H2	0.969(19)	C17-H17A	0.99(2)
C3-C4	1.399(2)	C17-H17B	0.98(2)
C3-H3	0.94(2)	C18-H18A	0.97(3)
C4-C5	1.374(2)	C18-H18B	0.94(2)
C4-C6	1.502(2)	C18-H18C	1.01(2)
O1-Zn1-O4#1	97.49(4)	O4#1-Zn1-O4	79.64(4)
O1-Zn1-O4	176.76(4)	O1-Zn1-S1	85.70(3)

O4#1-Zn1-S1	106.37(3)	C4-C5-H5	123.5(12)
O4-Zn1-S1	93.64(3)	O2-C6-C4	111.54(13)
O1-Zn1-S2	99.89(3)	O2-C6-H6A	109.2(12)
O4#1-Zn1-S2	109.63(3)	C4-C6-H6A	109.1(12)
O4-Zn1-S2	82.56(3)	O2-C6-H6B	112.3(12)
S1-Zn1-S2	142.391(17)	C4-C6-H6B	106.2(12)
C1-S1-Zn1	95.94(5)	H6A-C6-H6B	108.5(16)
C10-S2-Zn1	98.52(5)	O2-C7-C8	110.44(15)
N1-O1-Zn1	115.88(9)	O2-C7-H7A	105.1(12)
C6-O2-C7	110.08(13)	C8-C7-H7A	111.9(13)
C8-O3-C9	112.48(14)	O2-C7-H7B	109.2(13)
N2-O4-Zn1#1	114.93(8)	C8-C7-H7B	110.0(13)
N2-O4-Zn1	115.44(8)	H7A-C7-H7B	110.0(18)
Zn1#1-O4-Zn1	100.36(4)	O3-C8-C7	109.53(15)
C15-O5-C16	113.76(13)	O3-C8-H8A	109.3(12)
C18-O6-C17	112.26(16)	C7-C8-H8A	112.3(12)
O1-N1-C5	116.56(13)	O3-C8-H8B	110.0(13)
O1-N1-C1	121.01(12)	C7-C8-H8B	109.5(13)
C5-N1-C1	122.43(13)	H8A-C8-H8B	106.1(17)
C14-N2-O4	116.46(12)	O3-C9-H9A	107.7(14)
C14-N2-C10	123.39(13)	O3-C9-H9B	109.6(14)
O4-N2-C10	120.15(12)	H9A-C9-H9B	107.6(19)
N1-C1-C2	116.34(14)	O3-C9-H9C	110.4(14)
N1-C1-S1	121.32(11)	H9A-C9-H9C	108.4(19)
C2-C1-S1	122.33(12)	H9B-C9-H9C	112.9(19)
C3-C2-C1	121.77(16)	N2-C10-C11	115.64(14)
C3-C2-H2	120.7(11)	N2-C10-S2	121.51(11)
C1-C2-H2	117.5(11)	C11-C10-S2	122.84(12)
C2-C3-C4	120.41(15)	C12-C11-C10	121.82(15)
C2-C3-H3	119.6(12)	C12-C11-H11	120.7(11)
C4-C3-H3	120.0(12)	C10-C11-H11	117.5(11)
C5-C4-C3	117.49(15)	C11-C12-C13	120.09(14)
C5-C4-C6	122.87(15)	C11-C12-H12	119.2(11)
C3-C4-C6	119.64(14)	C13-C12-H12	120.7(11)
N1-C5-C4	121.54(15)	C14-C13-C12	117.59(15)
N1-C5-H5	114.9(12)	C14-C13-C15	118.63(14)

C12-C13-C15	123.76(14)
N2-C14-C13	121.45(15)
N2-C14-H14	116.7(11)
C13-C14-H14	121.9(11)
O5-C15-C13	107.89(13)
O5-C15-H15A	112.3(12)
C13-C15-H15A	110.1(12)
O5-C15-H15B	108.6(11)
C13-C15-H15B	109.3(11)
H15A-C15-H15B	108.7(15)
O5-C16-C17	108.33(15)
O5-C16-H16A	110.1(12)
C17-C16-H16A	109.9(12)
O5-C16-H16B	110.4(12)
C17-C16-H16B	109.8(12)
H16A-C16-H16B	108.2(17)
O6-C17-C16	109.86(15)
O6-C17-H17A	109.3(14)
C16-C17-H17A	110.1(13)
O6-C17-H17B	108.5(12)
C16-C17-H17B	110.4(12)
H17A-C17-H17B	108.6(18)
O6-C18-H18A	108.4(15)
O6-C18-H18B	111.5(15)
H18A-C18-H18B	109(2)
O6-C18-H18C	108.5(14)
H18A-C18-H18C	113(2)
H18B-C18-H18C	107(2)

Symmetry transformations used to generate equivalent atoms:

#1 -x+1,-y+1,-z+1

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