A colorimetric ligand for mercuric ion

by

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Supporting Information

This section provides complete procedures, experimental details and characterization data. A detailed synopsis of the synthesis of each material, the method used for determining the dissociation constants, evidence used for characterization of the complexation of chemosensor **1** with mercuric ion, and procedure used for each assay is provided within.

Chemical Synthesis.

General Procedures: Unless otherwise stated, all reactions were performed under an inert atmosphere with dry reagents and solvents and flame-dried glassware. Analytical thin layer chromatography (TLC) was performed using 0.25 mm silica gel coated Kieselgel 60 F_{254} plates. Visualization of the chromatogram was by UV absorbance, ninhydrine and *p*-anisaldehyde. Liquid chromatography was performed using compressed air (flash chromatography) with the indicated solvent system and silica gel 60 (230-400 mesh). Preparative TLC was performed using Merck 1 mm coated silica gel Kieselgel 60 F_{254} plates. ^{1}H NMR spectra were recorded on a Bruker AMX-400 spectrometer. Chemical shifts are reported in parts per million (ppm) on the δ scale from an internal standard. ^{13}C NMR (proton decoupled) were recorded on a Bruker AMX-400 spectrometer at 100 MHz. ^{31}P NMR (proton decoupled) spectra were recorded on a Bruker AMX-400 spectrometer at 101 MHz. High-resolution mass spectra (HRMS) were recorded on a VG ZAB-VSE mass spectrometer. Analytical HPLC was performed on a Hitachi 655A series HPLC using a Vydac 201TP54 reversed phase column.

Synthesis of **3**: Iodine (3.0 g, 11.8 mmol) was added over 45 min to *N*-2-hydroxyethyl-*N*-methylaniline (1.88 g, 12.5 mmol) and $NaHCO_3$ (1.57 g, 18.7 mmol) in 48 ml of CH_2Cl_2 and 36 ml of water at 0 °C. After the addition was complete, the mixture was warmed to rt and kept there for 30 min. The mixture was then diluted with 300 ml of CH_2Cl_2 and 40 ml of water, the organic phase was collected and consecutively washed with 50 ml of water, 50 ml of satd. sodium thiosulfate, 50 ml of water (2x), 50 ml of brine, and dried with Na_2SO_4. The crude product was dissolved in EtOAc, filtered through 80 g of SiO_2, concentrated and recrystallized from 10 : 1 n-hexane / THF to yield 2.46 g (92 %) of **3**. ^{1}H NMR (400 MHz, $CDCl_3$): δ = 7.44 (dt, *J* = 9.0, 1.5 Hz, 2H), 6.53 (dt, *J* = 9.0, 1.5 Hz, 2H), 3.77 (sbr, 2H), 3.43 (t, J = 6.0 Hz, 2H), 2.92 (s, 3H); ^{13}C NMR (100 MHz, $CDCl_3$): δ = 149.3, 137.6, 114.9, 77.9, 59.9, 55.0, 38.7; HR-MS: calcd. for $C_9H_{12}ONI$ [M^+]: 276.9964, found: 276.9973.

Synthesis of **4**: A mixture of iodide **3** (1.55 g, 6.0 mmol), $Cl_2Pd(PPh_3)_2$ (42.4 mg, 0.064 mmol) and CuI (10.8 mg, 0.064 mmol) in 2 ml of THF and 8 ml of triethylamine was degassed by rapid bubbling of dry argon. After 15 min of degassing, 2-propyn-1-ol (0.93 ml, 16.0 mmol) was added to the vigorously stirred mixture under argon. The crude solution was filtered through 200 g of silica gel with 5 % methanol in ethyl acetate and concentrated. Pure material was obtained through flash chromatography (SiO_2, 25% ethyl acetate / hexanes), yielding 1.14 g (93 %) of **4**. ^{1}H NMR (400 MHz, CD_3OD): δ = 7.22 (d, J = 9.0 Hz, 2H), 6.64 (d, J = 9.0 Hz, 2H), 4.36 (s, 2H), 3.68 (t, J = 6.0 Hz, 2H), 3.44 (t, J = 6.0 Hz, 2H), 2.96 (s, 3H); ^{13}C NMR (100 MHz, CD_3OD): δ = 150.6, 133.7, 112.6, 110.7, 86.7, 86.1, 60.1, 55.3, 51.4, 39.2; HR-MS: calcd. for $C_{12}H_{17}O_2N$ [M^++2H]: 207.1259, found: 207.1251.

Synthesis of **5**: Activated MnO_2 (1.57 g, 18.0 mmol) was added to **4** (821 mg, 4.0 mol) in 45 ml of CH_2Cl_2 at rt. After 6 h, the reaction was concentrated to 10 ml and directly purified by flash chromatography (SiO_2, 25% ethyl acetate / hexanes), yielding 729 mg (89 %) of **5**. ^{1}H NMR (400 MHz, $CDCl_3$): δ = 9.31 (s, 1H), 7.44 (d, J = 9.0 Hz, 2H), 6.67 (d, J = 9.0 Hz, 2H), 3.81 (dt, J = 6.0 Hz, 2H), 3.55 (t, J = 6.0 Hz, 2H), 3.05 (s, 3H); ^{13}C NMR (100 MHz, $CDCl_3$): δ = 176.6, 151.5, 135.5, 111.7, 105.0, 100.2, 90.1, 60.0, 54.2, 38.9 ; HR-MS: calcd. for $C_{12}H_{14}O_2N$ [MH^+]: 204.1025, found: 204.1022.

Synthesis of **7**: Sodium bis-(trimethylsilyl)amide (10.0 ml, 1.0 M in THF, 10.0 mmol) was added to phosphonate **6** (1.30 g, 4.49 mmol) in 10 ml of DMF at 0 °C. After 30 min, the solution was warmed to rt, kept there for 30 min, and then recooled to - 20 °C. Aldehyde **5** (772 mg, 3.8 mmol) in 20 ml of THF was added *via* canula. After 8 h at rt, 30 ml of water was added, the pH was adjusted to 7 with 5 % aqueous HCl and then further diluted with 50 ml brine. Crude product was obtained by extraction with 150 ml of 10 % THF in CH_2Cl_2 (3x), dried with Na_2SO_4, and concentrated. Flash chromatography (SiO_2, 25% $CHCl_3$ / hexanes) and recrystallization from n-hexane / THF (10 : 1) yielded 950 mg (74 %) of **7**. ^1H NMR (400 MHz, $CDCl_3$): $\delta = 7.90$ (d, $J = 9.0$ Hz, 1H), 7.19 (d, $J = 9.0$ Hz, 2H), 6.96 (s, 1H), 6.89 (d, $J = 9.0$ Hz, 1H), 6.71 (d, $J = 16.0$ Hz, 1H), 6.53 (d, $J = 9.0$ Hz, 2H), 6.44 (d, $J = 16.0$ Hz, 1H), 3.60 (t, $J = 6.0$ Hz, 2H), 3.35 (t, $J = 6.0$ Hz, 2H), 2.89 (s, 3H); ^{13}C NMR (100 MHz, $CDCl_3$): $\delta = 155.2$, 149.7, 146.0, 136.0, 133.1, 125.5, 117.6, 116.2, 115.0, 111.7, 109.2, 97.6, 87.0, 59.5, 54.4, 38.7; HR-MS: calcd. for $C_{19}H_{19}O_4N_2$ [MH^+]: 339.1345, found: 339.1339.

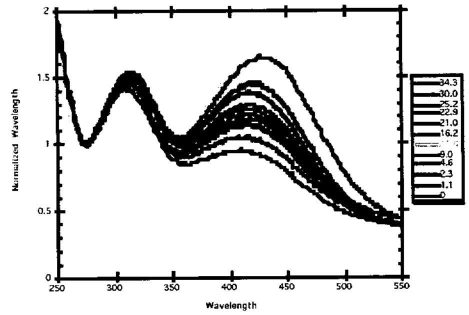
Synthesis of **1**: 2-Alkoxy-2-thio-1,3,2-dithiaphospholane **8** (42.5 mg, 0.139 mmol) was mixed with **7** (47.0 mg, 0.139 mmol) and DBU (21 mg, 0.139 mmol) in 8 ml of acetonitrile. Synthesis of **8** is described in O. Brümmer, *et. al., Lett. Pep. Sci.* **1999**, *in press*. After 4 h, the acetonitrile was removed and the crude product was subjected to flash chromatography (SiO_2, 12% MeOH in CH_2Cl_2), yielding 25 mg (24%) of **1** along with 24.2 mg (55%) of recovered **7**. ^1H NMR (400 MHz, $CDCl_3 + CD_3OD$): $\delta = 8.03$ (d, $J = 9.0$ Hz, 1H), 7.41 (d, $J = 9.0$ Hz, 2H), 7.29 (d, $J = 9.0$ Hz, 2H), 7.21 (dd, $J = 9.0$, 2.0 Hz, 2H), 7.15 (s, 1H), 7.13 (dd, $J = 9.0$, 2.0 Hz, 1H), 6.88 (d, $J = 16.0$ Hz, 1H), 6.75 (d, $J = 9.0$ Hz, 2H), 6.69 (d, $J = 16.0$ Hz, 1H), 4.23 (dt, $J = 10$, 6 Hz, 2H), 3.71 (t, $J = 6.0$ Hz, 2H, DBU), 3.58-3.55 (m, 2H, DBU), 3.51 (t, $J = 6$ Hz, 2H, DBU), 3.31 (t, $J = 6.0$ Hz, 2H), 3.05 (s, 3H), 2.66-2.62 (m, 2H, DBU), 2.11 (s, 3H), 2.00 (tt, $J = 6.0$ Hz, 2H, DBU), 1.79-1.66 (m, 6H, DBU); ^{13}C NMR (100 MHz, $CDCl_3 + CD_3OD$): $\delta = 171.2$, 156.2, 150.7 (DBU), 147.0, 137.1, 135.3, 134.2, 134.0, 126.5, 122.9, 121.7, 118.4, 117.5, 115.9, 112.7, 110.3, 98.4, 87.8, 63.8, 55.3 (DBU), 53.1, 49.5 (DBU), 39.3 (DBU), 39.2, 33.7 (DBU),

29.8 (DBU), 27.4 (DBU), 24.8 (DBU), 23.7 (DBU), 20.3; ^{31}P NMR (100 MHz, $CDCl_3$ + CD_3OD): δ = 113.9; ESI-MS for $C_{27}H_{25}O_6N_3S_2P$ [M-DBUH$^+$]: 582.734.

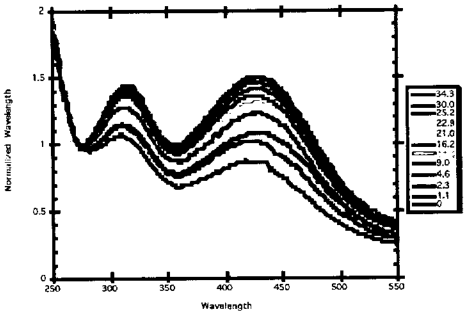
Isolation and characterization of the 2:1 complex of **1** *to* **Hg^{2+}.** 200 µL of $Hg(NO_3)_2$ (10 mM in D_2O) was added to solution of 2.0 mg of **1** (0.0027 mmol) in D_2O (200 µL) and CD_3CN (200 µL), resulting in the immediate formation of a red precipitate. The clear D_2O/CD_3CN phase was removed and the precipitate was washed three times with D_2O (400 µL each time). After drying *in vacuo* the precipitate was dissolved in d_6-DMSO for further characterization. ^{1}H NMR (400 MHz, d_6-DMSO): δ = 7.89 (d, *J* = 9 Hz, 2 × 1H),7.53 (d, *J* = 9 Hz, 2 × 2H), 7.29 (d, *J* = 8 Hz, 2 × 2H), 7.22-7.19 (m, 2 × 2H), 7.13 (d, *J* = 9 Hz, 2 × 2H), 6.97 (d, *J* = 16 Hz, 2 × 1H), 6.79-6.71 (m, 2 × 3H), 4.29-4.22 (m, 2 × 2H), 3.75-3.69 (m, 2 × 2H), 2.97 (s, 2 × 3H), 2.02 (s, 2 × 3H); ^{31}P NMR (101 MHz, d_6-DMSO): δ = 99.70; ESI-MS for $C_{54}H_{50}O_{12}N_6P_2S_4HgH$ [M+H]$^+$: 1367; the theoretical isotope pattern for $C_{54}H_{50}O_{12}N_6P_2S_4HgH$ is identical with the observed one: 1363:1364:1365:1366:1367:1368:1369:1370 = 1:2:3:3:4:3:2:1; HPLC-trace: 8.68 min for **1**, 112.54 min for the **1**-Hg precipitate when eluted with 45% acetonitrile / water containing 0.1% TFA.

Determination of dissociation constants (***K***$_d$). A series of 11 metals was screened using a plate reader (Molecular Devices, Spectra MAX 250) on 300 µl 96-well quartz plate. Twelve wells, displayed horizontally along the plate, were loaded with a gradient of metal ions ranging from 0 to 360 µM in 35 mM HEPES (pH 7.0, 140 µL) and a 10.5 µM stock solution of either chromophor in acetonitrile (60µL) and the changes in absorption were examined. Four repetitions of each run were simultaneously collected and averaged in order to minimize error. Therefore, an individual run allowed each metal to be simultaneously screened against both **1** and **7**. Comparision of the titration curves (for some examples see below) reveals that the addition of Cd^{2+}, Eu^{3+}, Gd^{3+}, Hg^{2+}, In^{3+}, La^{3+}, Pb^{2+}, or Tb^{3+} results in a reduction of absorption about $\lambda_{max} = 415$ nm and minor reduction at $\lambda_{max} = 310$ nm. The intensities of these two maxima were only modestly altered upon addition of non-thiophylic metals, such as K^+, Zn^{2+}, or Co^{2+}. These weak binding metals salts required up to 150 equivalent of metal to alter the absorption of **1**. This alteration was independent of metal and likely originated from adjustment of the ionic strength of the media. Assuming a steady-state, affinity of each interaction, expressed in terms of K_d, was determined by plotting the absorption at 415 nm vs. the inverse of the metal concentration.

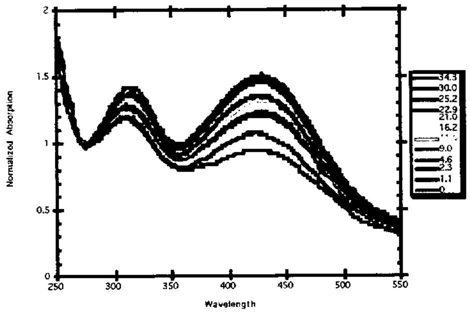
$Cd(NO_3)_2$



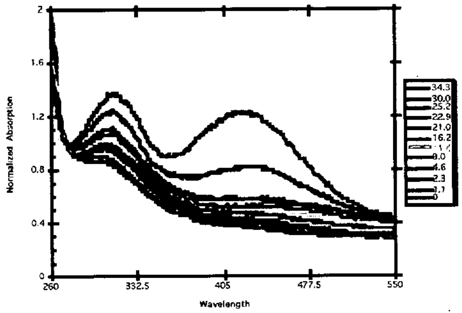
$Eu(NO_3)_3$



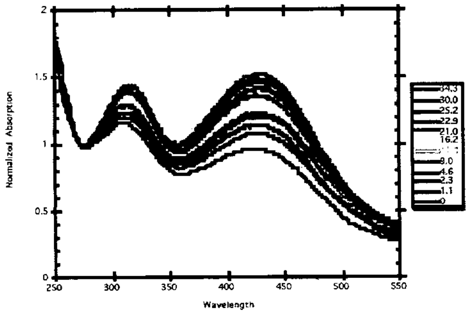
$La(NO_3)_3$



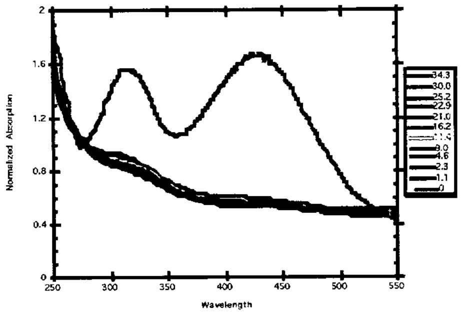
$Pb(NO_3)_2$



$Tb(NO_3)_3$



$Hg(NO_3)_2$



Assay of metal binding as illustrated in Figures 1 and 2. A 96-welled plate was loaded with 60 µl of a 35 µM solution of **1** in acetonitrile and 130 µl of 50 mM HEPES pH 7.0 per well. Ten µl of a 200 µM stock of each metal was added to a distinct well. Immediately following mixing, the mother liquor was transferred by pipette to an opaque Teflon plate (Berghof, America). A colorless solution indicated the presence of strong binding. Verification of this result can be enhanced by the addition of 60 µL of 10 µM bromophenol blue in ethanol (Fig 3). Bromophenol blue was chosen based on its ability to differentiate its color upon mixing. Yellow solutions mixed with one equivalent of bromophenol blue per **1** gave violet, and clear solutions remained blue. Addition of less than 0.2 equivalents of metal per **1** left the unreacted solutions yellow and the bleached solutions blue. The test was further verified by dissolving the precipitate in the original plate with 80 µl of acetone. Appearance of yellow occurred in wells, which contained high affinity metals. The effective concentration of each metal, depicted in Fig. 4, was examined after incubating a plate loaded with 60 µl of a designated concentration of each metal, 60 µl of a 35 µM acetonitrile solution of **1** and 80 µl of 50 mM HEPES pH 7.0 for 30 min at 23 °C.