Supplementary information –Chemical Communications

Investigation of a new "$N_2S_2O_2$" chelating agent with high Po(IV) affinity

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1) General chemical procedures and analytical data

Materials NMR spectra were recorded at room temperature with a Bruker Avance 300 Ultra Shield or eBruker Avance III 400 spectrometer and chemical shifts are reported in parts per million relative to tetramethylsilane or a residual solvent peak peak ($CHCl_3$: 1H: δ=7.26, 13C: δ=77.2; DMSO-d6: 1H: δ=2.54, 13C: δ=40.4). Peak multiplicity is reported as: singlet (s), doublet (d), triplet (t), quartet (q), multiplet (m), and broad (br). High resolution mass spectra HRMS where obtained by Electrospray Ionisation (ESI) on a Micromass-Waters Q-TOF Ultima Global or with a Bruker Autoflex III SmartBeam spectrometer (MALDI). Low-resolution mass spectra (MS) were recorded with a Thermo electron DSQ spectrometer. All reagents were purchased from Acros Organics or Aldrich and were used without further purification. Column chromatography was conducted on silica gel Kieselgel SI60 (40-63 μm) from Merck. Reactions requiring anhydrous conditions were performed under argon. Dichloromethane was distilled from calcium hydride under nitrogen prior to use. Microwave experiments were conducted in sealed vials in commercial microwave reactors especially designed for synthetic chemistry. (MultiSYNTH, Milestone). The instrument features a special shaking system that ensures high homogeneity of the reaction mixtures.

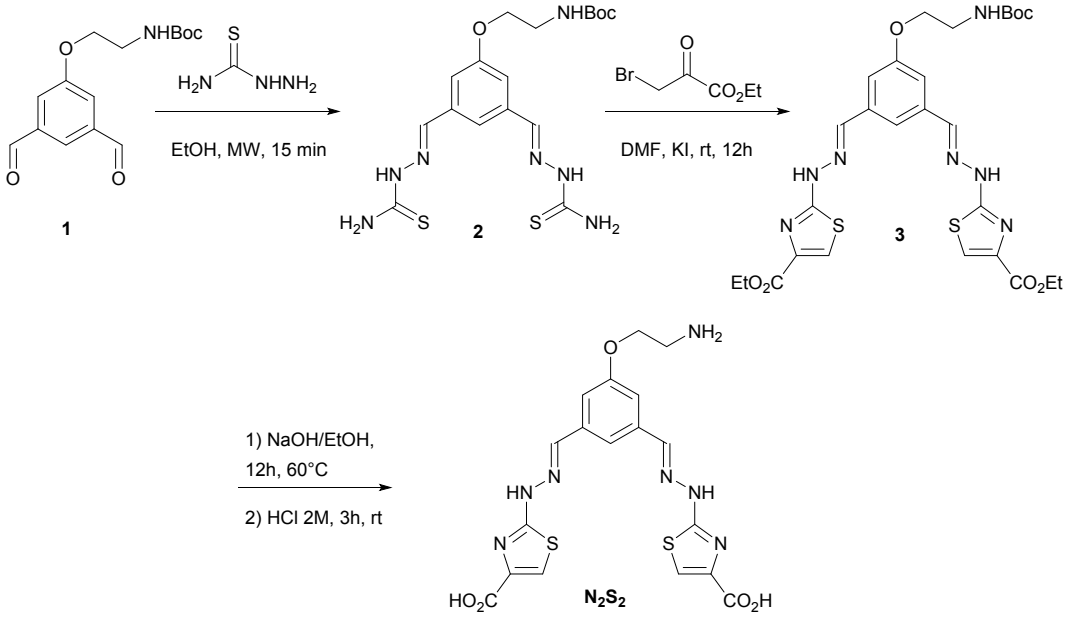

Figure 1. Synthesis of N_2S_2 ligand.

***Tert*-butyl 2-(3,5-bis(-(2-carbamothioylhydrazono)methyl)phenoxy)ethylcarbamate 2.** Thiosemicarbazide (2.19 mmol, 2 eq) and *tert*-butyl 2-(3,5-diformylphenoxy)ethylcarbamate[1] (1.10 mmol, 1 eq) were suspended in 4 mL of ethanol in a 10 mL-microwave reactor. The reactor

was placed in the microwave oven and heated from room temperature to 120 °C (optic fiber) in 5 min by monomode microwave irradiation (power: 150 W, stirring: 50%, ventilation: 1/3), maintained at 120 °C for 20 min (power: 50 W, stirring: 50%, ventilation: 1/3) and left to cool down to room temperature for 5 min (power: 0 W, stirring: 50%, ventilation: 3/3). The mixture was filtered and washed with cold ethanol. Off white powder, yield: 92%. Melting point 237°C. **^{1}H NMR (300 MHz, [D6]DMSO):** 1.37 (s, 9H, C(CH_3)$_3$), 3.28 (m, 2H, CH_2), 4.05 (m, 2H, CH_2), 7.01 (bs, 1H, N*H*Boc), 7.42 (s, 2H, *Har*), 7.68 (s, 1H, *Har*), 8.01 (s, 2H, C*H*N), 8.15 (bs, 2H, NH_2), 8.25 (bs, 2H, NH_2), 11.51 (bs, 2H, N*H*). **^{13}C NMR (75 MHz, [D6]DMSO):** 28.2, 39.8, 66.8, 77.8, 113.9, 119.8, 136.0, 141.6, 155.7, 159.0, 178.1. **MS (MALDI) m/z (%):** 462 (M+Na$^+$).

Diethyl 2,2'-(5-(2-((*tert*-butoxycarbonyl)amino)ethoxy)-1,3-phenylene)bis(methanylylidene))bis(hydrazin-1-yl-2-ylidene))bisthiazole-4-carboxylate 3.

Tert-butyl 2-(3,5-bis((2-carbamothioylhydrazono)methyl)phenoxy)ethylcarbamate 2 (0.36 mmol, 1 eq) was dissolved in 8 mL of anhydrous DMF. After total dissolution, ethyl bromopyruvate (1.1 mmol, 3 eq) was added, followed by potassium iodide (0.5 mmol). The reaction was stirred for 12 h at room temperature under an inert system and was concentrated under reduced pressure. The residue was suspended in 100 mL of dichloromethane and 100 mL of saturated NH_4Cl solution. The organic layer and the solid formed between the two phases were washed with 5 mL of methanol and then the solid compound was filtered on a micropore plate. The solid was a pale orange color, yield 63%. **^{1}H NMR (300 MHz, [D6]DMSO):** 1.28 (t, 6H, CH_3, J = 7.1 Hz), 1.38 (s, 9H, C(CH_3)$_3$), 3.3 (m, 2H, CH_2), 4.03 (m, 2H, CH_2), 4.24 (q, 4H, CH_2, J = 7.1 Hz), 7.06 (bs, 1H, N*H*Boc), 7.22 (s, 2H, *Har*), 7.54 (s, 1H, *Har*), 7.79 (s, 2H, *CHN*), 7.98 (s, 2H, *Har*), 12.4 (bs, 2H, N*H*). **^{13}C NMR (75MHz, [D6]DMSO):** 14.1, 28.2, 45.7, 60.3, 66.6, 77.8, 112.8, 117, 119.1 , 136.1, 141.1, 142,8, 155.6, 159.0, 160.9, 168.0. **MS (MALDI) m/z (%):** 654 (M+Na$^+$).

2,2'-(5-(2-aminoethoxy)-1,3 phenylene)bis(methanylylidene))bis(hydrazin-1-yl-2-ylidene))bis(thiazole-4-carboxylic acid) N_2S_2.

A mixture of compound **3** (112 mg, 0.17 mmol), ethanol (12 mL) and 2 M aqueous solution of NaOH (12 mL) was stirred for one night at 60 °C. The solution was evaporated to dryness, and the residue was dissolved in HCl solution (2 M, 12 mL). The mixture was shaken for 3 h at room temperature. The solvent was evaporated under reduced pressure and the residue was dissolved in cooled acidic water. The dark orange precipitated compound was filtered on a micropore plate. Orange solid, yield: 83%. **^{1}H NMR (300 MHz, [D6]DMSO):** 3.28 (m, 2H, CH_2), 4.29 (m, 2H, CH_2), 7.27 (s, 1H, *Har*), 7.72 (s, 2H, *Har*), 8.06 (s, 1H, *CHN*), 8.34 (bs, 4H, *Har* and NH_2). **^{13}C NMR (75MHz, [D6]DMSO):** 39.2, 65.4, 120.2, 121.3, 124.3, 136.5, 143.1, 143.5, 158.6, 161.9, 168.6. **MS (MALDI) m/z (%):** 498 (M+Na$^+$).

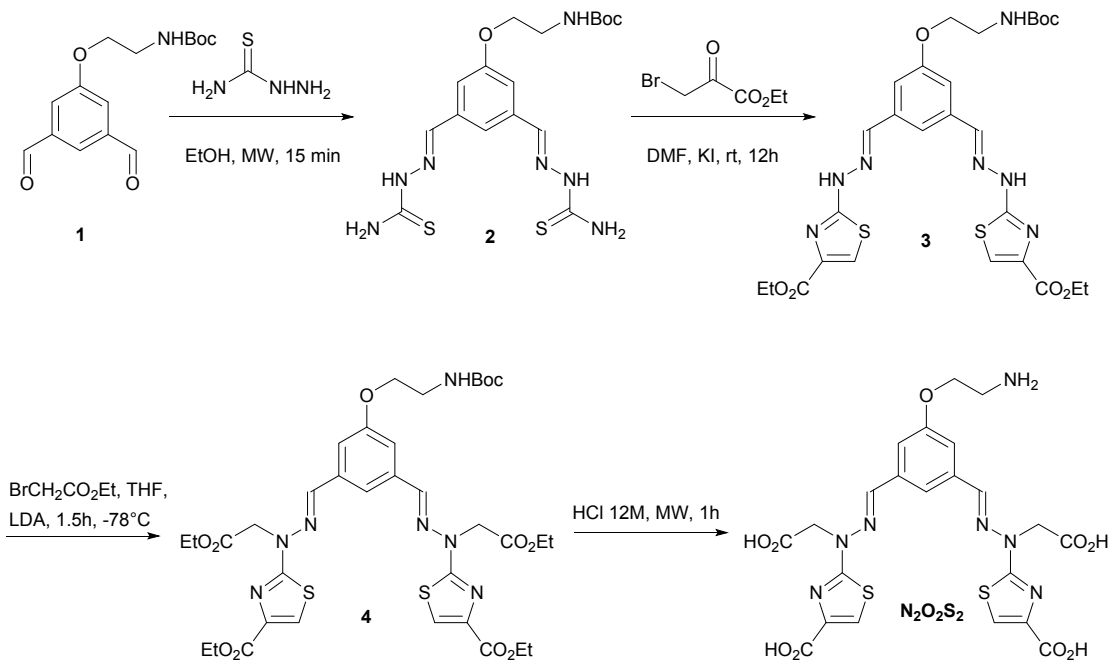

Figure 2. Synthesis of $N_2S_2O_2$ ligand.

Diethyl 2,2'-((5-(2-((tert-butoxycarbonyl)amino)ethoxy)-1,3-phenylene)bis(methanylylidene))bis(1-(2-ethoxy-2-oxoethyl)hydrazin-1-yl-2-ylidene))bis(thiazole-4-carboxylate) 4

Compound **3** (0.2 mmol, 1 eq) was dissolved in freshly distillated THF (20 mL), temperature is lowered to -78°C, and LDA (0.6 mmol, 3 eq) is added. The mixture is stirred 45 min, ethyle bromoacetate is added (0.6 mmol, 3 eq), and the mixture is stirred at 0°C for 45 min, quenched by water and concentrated under vacuum. The crude product was then purified by column chromatography over silicagel (EP/AcOEt) Yield: 65%. **^{1}H NMR (300 MHz, $CDCl_3$)**: 1.28 (t, 6H, *CH*$_3$, *J* = 7.2 Hz), 1.39 (t, 6H, *CH*$_3$, *J* = 7.2 Hz), 1.46 (s, 9H, C(C*H*$_3$)$_3$), 3.58 (m, 2H, *CH*$_2$), 4.12 (t, 2H, *CH*$_2$, *J* = 4.8 Hz), 4.25 (q, 4H, *CH*$_2$, *J* = 7.2 Hz), 4.37 (q, 4H, *CH*$_2$, *J* = 7.2 Hz), 5.11 (s, 4H, *CH*$_2$), 7.24 (d, 2H, *Har, J* = 1.1 Hz), 7.42 (s, 2H, C*HN*), 7.48 (s, 1H, *Har*), 7.66 (s, 2H, *Har*). **^{13}C NMR (75MHz, $CDCl_3$):** 14.3, 14.5, 28.6, 40.1, 47.0, 61.3, 62.1, 67.7, 113.6, 119.5, 121.1, 136.2, 136.3, 143.6, 159.4, 161.7, 166.9, 169.9. **MS (MALDI) m/z (%):** 882 ($M+Na^+$).

2,2'-(((5-(2-aminoethoxy)-1,3-phenylene)bis(methanylylidene))bis(1-(carboxymethyl)hydrazin-1-yl-2-ylidene))bis(thiazole-4-carboxylic acid) $N_2S_2O_2$

Compound **4** (62 mg, 0.14 mmol) was suspended in 4 mL of HCl 12M in a 10 mL-microwave reactor. The reactor was placed in the microwave oven and heated from room temperature to 100

°C (optic fiber) in 5 min by monomode microwave irradiation (power: 150 W, stirring: 50%, ventilation: 1/3), maintained at 10 °C for 1 h (power: 50 W, stirring: 50%, ventilation: 1/3) and left to cool down to room temperature for 5 min (power: 0 W, stirring: 50%, ventilation: 3/3). The mixture was concentrated under vacuum, then co-evaporated with water to remove HCl. Brown solid, yield: 89%.**^{1}H NMR (400 MHz, [D6]DMSO):** 3.30 (m, 2H, CH_2), 4.28 (m, 2H, CH_2), 5.05 (s, 4H, CH_2), 7.34 (s, 2H, *Har*), 7.75 (s, 1H, *Har*), 7.92 (s, 2H, *CHN*), 7.98 (s, 2H, *Har*), 8.08 (m, 2H, NH_2). **^{13}C NMR (100MHz, [D6]DMSO):** 39.1, 46.42, 65.5, 120.2, 121.3, 124.5, 136.4, 143.2, 143.9, 158.9, 162.0, 168.1, 168.7. **MS (MALDI) m/z (%):** 614 ($M+Na^+$)

2) Experimental details for the determination of the reaction constant of the Po(IV)/L system

Materials

All solutions were prepared using Milli-Q water and all experiments were conducted at room temperature (22±3˚C). All the chemicals used were of analytical reagent grade. Polonium-210 radionuclide was produced and purified according to the protocol developed by A. Younes *et al.*[1] To prevent potential unsuitable complexation, the polonium solution was evaporated to dryness and recovered in the appropriate medium twice which is 0.1M $NaClO_4$ and HEPES 10^{-3}M (pH=7.4). In this aqueous medium, speciation of polonium is difficult to investigate from a chemist's point of view and is not very well defined. [2-5] It was reported that the oxidation states of polonium are -2, +2, +3, +4 and +6. [6,7] The tetravalent state is the most stable in many kinds of solutions for a wide range of pH values. Hydrolysis of polonium (IV) ions at different pH has also been investigated using solvent-extraction procedures by Sugunama *et al*.[8,9] They conclude that polonium ion is easily hydrolyzed in aqueous solutions. The dependence of the chemical behavior of polonium as a function of hydrogen-ion concentration clearly show the importance of the hydrolysis processes. Hataye *et al.* demonstrate that there are two hydrolysis species existing in perchlorate medium: $Po(OH)_3^+$ and $Po(OH)_4$. Due to a lack of thermodynamic data associated to the hydrolysis equilibrium, we consider that the species present in our condition is at the oxidation +4: Po(IV).[10,11]

Competition method

Since polonium has no stable nuclides, radiochemical studies were performed at trace or ultra-trace concentrations of Po(IV), typically less than 10^{-12} M. As a result, most of the chemical analytical tools usually available, such as NMR and mass spectrometry, could not be applied to evaluate polonium chemistry. The reaction constants between Po(IV) and the organic ligands (L) were determined by the competition method proposed by Champion *et al.*[12] The method consists in studying the distribution of Po-210 between an aqueous phase and an organic one as a function of the initial inorganic ligand concentrations present in the aqueous solution. A given species is characterized by a given distribution coefficient (D) and a change in D arising from a perturbation of the system indicates a change in speciation. A quantitative analysis of the data based on the law

of mass action will give the number of ligand ([ligand] perturbation) exchanged in the chemical process. The systems (5 mL of organic phase: 10^{-5} M dithizone in chloroform and 5 mL of aqueous phase, 0.1M $NaClO_4$ and HEPES 10^{-3}M , were brought into contact in super-polyethylene tubes) were first equilibrated before Po-210 (20 Bq) addition. After the addition of Po-210, two hours of shaking were done to achieve distribution equilibrium of Po-210 between the two phases. This time proved to be sufficient to achieve distribution equilibrium between the phases for all the studied systems. Then the two phases were separated, and an aliquot of both the aqueous and organic phases was withdrawn to derive the distribution coefficient D:

$$D = \frac{A_{org} \times V_{aq}}{A_{aq} \times V_{org}} \qquad \text{(S1)}$$

where V_{org} and V_{aq} represent given phase volumes, and A_{org} and A_{aq} are the polonium activities in the organic and aqueous phases at equilibrium, respectively. Uncertainties associated with D values were calculated according to the following equation:

$$\sigma_D = D \times \sqrt{\frac{\sigma_{A_{org}}^2}{A_{org}} + \frac{\sigma_{A_{aq}}^2}{A_{aq}} + \frac{\sigma_{V_{org}}^2}{V_{org}} + \frac{\sigma_{V_{aq}}^2}{V_{aq}}} \qquad \text{(S2)}$$

Po-210 activity in both phases (organic / aqueous) were determined by liquid scintillation using a Packard 2550 TR Liquid Scintillation analyzer. The samples were prepared by mixing an aliquot of 2 mL of the solution to be measured and 3 mL of ultimate gold AB scintillation cocktail. The measuring time was fixed at 1 h. Quenching arising from the organic solvent was taken into account according to the following relation:

$$A = A_m (5.10^{-12} \times TSIE^5 - 10^{-8} \times TSIE^4 + 10^{-5} \times TSIE^3 - 4.310^{-3} \times TSIE^2 + 0.9587 \times TSIE + 10.988) \qquad \text{(S3)}$$

where A_m is the activity measured by liquid scintillation and TSIE is an independent parameter from polonium analysis defined by the apparatus to determine the quenching parameter.

Experiments were twice repeated with the same experimental conditions and the mean values are given together with uncertainties corresponding to a 95% confidence interval.

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