Electronic Supplementary Material (ESI) for Dalton Transactions.

Supplementary Material

Chemistry and Radiochemistry of Arsenic, Rhenium and Rhodium Isotopes Relevant to Radiopharmaceutical Applications: Development of High Specific Activity Radionuclides for Imaging and Treatment

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Supplementary Material for Arsenic Section:

Materials, Physical Measurements and Results

2-ethyl-2-(hydroxymethyl)propane-1,3-diol, ethanethiol, and triethylamine (TEA) were obtained from Sigma-Aldrich (St. Louis, MO), and arsenic trioxide (***CAUTION!*** Arsenic is highly toxic and should be handled with care) was obtained from Thermo Fisher Scientific (Guilford, CT). All solvents used were reagent grade, and all reagents were used without further purification. ^{1}H- and ^{13}C-NMR spectra were obtained in $CDCl_3$ with 1% TMS (Cambridge Isotope Laboratories, MA) on a Bruker DRX 500 MHz Spectrometer. Electrospray Ionization Mass Spectra (ESI-MS) were collected on a Thermo Finnigan TSQ7000 triple-quadrupole instrument with an API2 source. Infrared (IR) spectra were obtained as KBr pellets on a Thermo Nicolet Nexus 670 FT-IR instrument.

4-ethyl-2,6,7-trioxa-1-arsabicyclo[2.2.2]octane

1,1,1-Tris(hydroxymethyl)propane (1.3 g, 9.7 mmol) and arsenic trioxide (1.0 g, 5.1 mmol) were heated to 125 °C in toluene (25 mL) in a 2-necked round bottom flask equipped with a short vigreux distillation head. After stirring overnight, water had distilled off from the reaction. The reaction mixture was filtered, taken to dryness by vacuum distillation, and recrystallized from hexanes to obtain the pure product as a white solid. X-ray quality crystals were obtained by slow evaporation from hexanes. Yield: 1.5 g, 70%. ^{1}H NMR ($CDCl_3$ d_1; 500 MHz) δ ppm: 0.790 (t, 3H, $CH_2C\mathbf{H_3}$), 1.094 (q, 2H, $C\mathbf{H_2}CH_3$), 4.075 (s, 6H, $OC\mathbf{H_2}$). ^{13}C NMR ($CDCl_3$ d_1; 500 MHz) δ ppm: 7.15 ($CH_2\mathbf{C}H_3$), 25.1 ($\mathbf{C}H_2CH_3$), 35.62 ($\mathbf{C}CH_2$), 72.36 ($\mathbf{C}H_2O$). ESI/APCI MS (*m/z*): 221.06 (220.98 calcd for $C_6H_{11}O_4As$ $[M+O-H]^-$). **Figures S1**-**S4** show the ^{1}H and ^{13}C NMR spectra of the ligand and its arsenic complex.

Thiol challenge study

4-ethyl-2,6,7-trioxa-1-arsabicyclo[2.2.2]octane (20 mg, 0.097 mmol) was dissolved in 1 mL of deuterated chloroform in a capped scintillation vial (solution A, 97 mM). 1-Ethanethiol (17.8 µL, 15.3 mg, 0.24 mmol) and TEA (34 µL, 24.7 mg, 0.24 mmol) were dissolved in 947.2 µL of deuterated chloroform in a capped scintillation vial (solution B, 240 mM). Three NMR samples were prepared as follows: 1) 500 µL each of solution A and deuterated chloroform; 2) 500 µL each of solution B and deuterated chloroform; and 3) 500 µL each of solution A and solution B. All solutions were monitored by ^{1}H NMR at 0 h, 4 h, 24 h, and 72 h. **Figures S5** and **S6** show the time t = 0 ^{1}H-NMR spectra.

X-ray determination of 4-ethyl-2,6,7-trioxa-1-arsabicyclo[2.2.2]octane

The crystal structure data for the $As(O_3R)$ bicyclic complex was obtained at -100 °C on a Bruker APEX II CCD Area Detector system using the ω scan technique with Mo Kα radiation from a graphite monochromator. The crystal size was approximately 0.50 x 0.25 x 0.15 mm. Intensities were corrected for Lorentz and polarization effects. The structure was solved by direct methods with full-matrix least-squares refinement, using the SHELX package.[S1] All non-hydrogen atoms were refined with anisotropic thermal parameters. The hydrogen atoms were placed at calculated positions and included in the refinement using a riding model, with fixed isotropic U. Other crystal refinement data are reported in **Table S1**. Final difference maps contained no features of chemical significance.

A search of the Cambridge Structural Database[S2,S3] revealed no crystal structures of 4-ethyl-2,6,7-trioxa-1-arsabicyclo[2.2.2]octane. Crystal data, data collection and structure refinement details are summarized in **Table S1**. The C-bound H atoms were included in calculated positions and treated as riding: C-H = 0.95 – 0.99 Å with Uiso(H) = 1.2 Ueq(C). The final least-squares cycle for the crystal structure of 4-ethyl-2,6,7-trioxa-1-arsabicyclo[2.2.2]octane was calculated with R = 0.0722 and R_w = 0.2069. The bond angles and distances are summarized in **Table S2**. The As-O bond lengths for 4-ethyl-2,6,7-trioxa-1-arsabicyclo[2.2.2]octane range from 1.7777(13) to 1.7858(14), with an average of 1.7827 (Å) (**Table S2**). These bond lengths are in good agreement with the As(III)-O bond distance of 1.77 ± 0.03 (Å) reported in the Cambridge Structural Database.[S2-S4] The crystal structure shows no sign of an As=O double bond, however existence of an $O=As(OR)_3$ species has been reported but no crystal structure was obtained.[S5] In the crystal, molecules pack as dimers about inversion centers, with a close intermolecular contact of 2.868(2) Å between O3 and As1, which is accompanied by a contact distance of 2.823(3) Å between O3 and the symmetry generated O3. Such close interactions are common in the packing of similar metal-O systems. These dimer pairs are then stacked into columns along the **a** direction, as shown in **Figure S7**.

References

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S6. *APEX 3, SAINT*. Bruker AXS Inc., Madison, Wisconsin, USA.

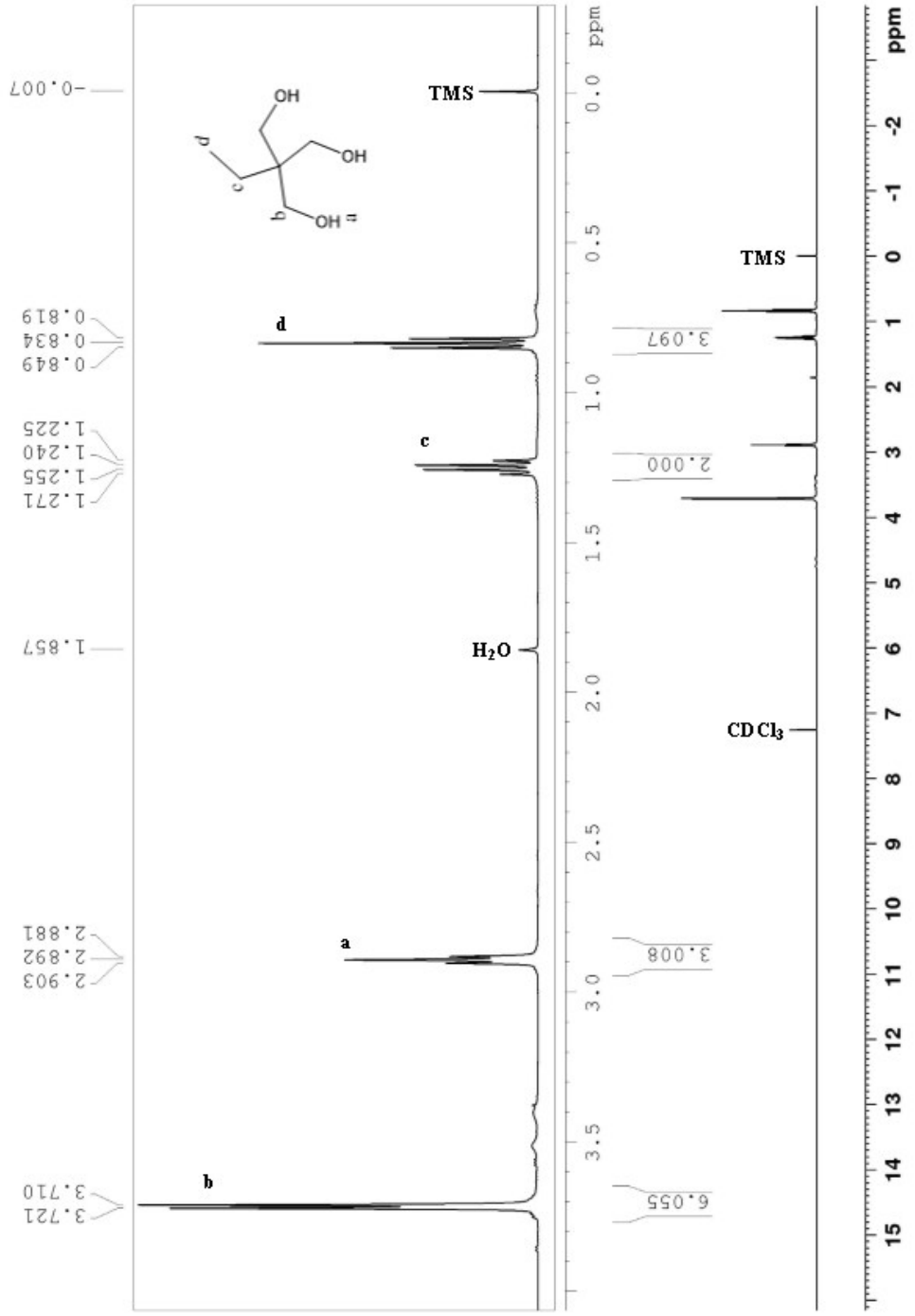

Figure S1. ^{1}H-NMR spectrum of 2-ethyl-2-(hydroxymethyl)propane-1,3-diol ($CDCl_3$ d_1; 500 MHz).

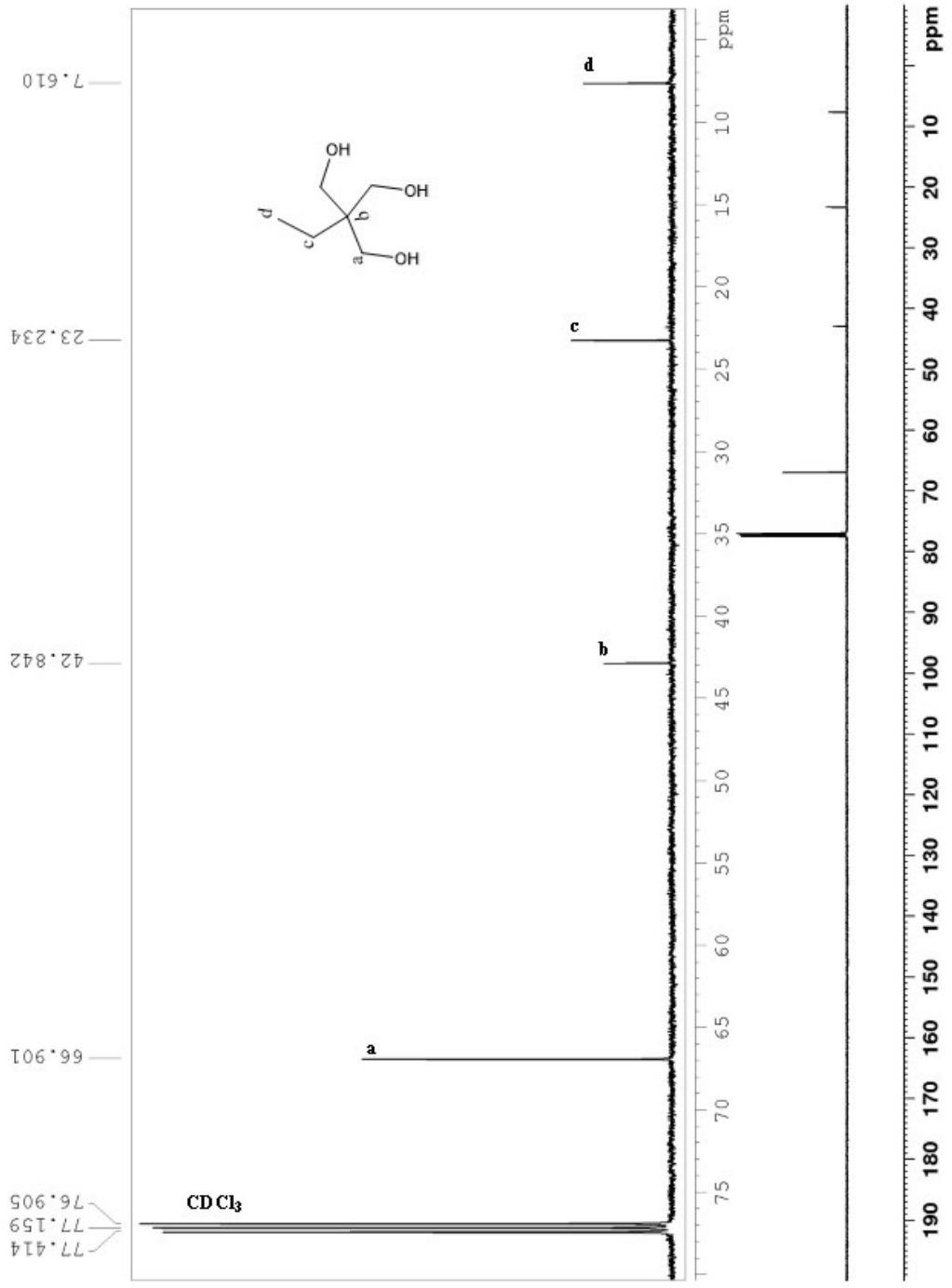

Figure S2. ^{13}C-NMR spectrum of 2-ethyl-2-(hydroxymethyl)propane-1,3-diol ($CDCl_3$ d_1; 500 MHz).

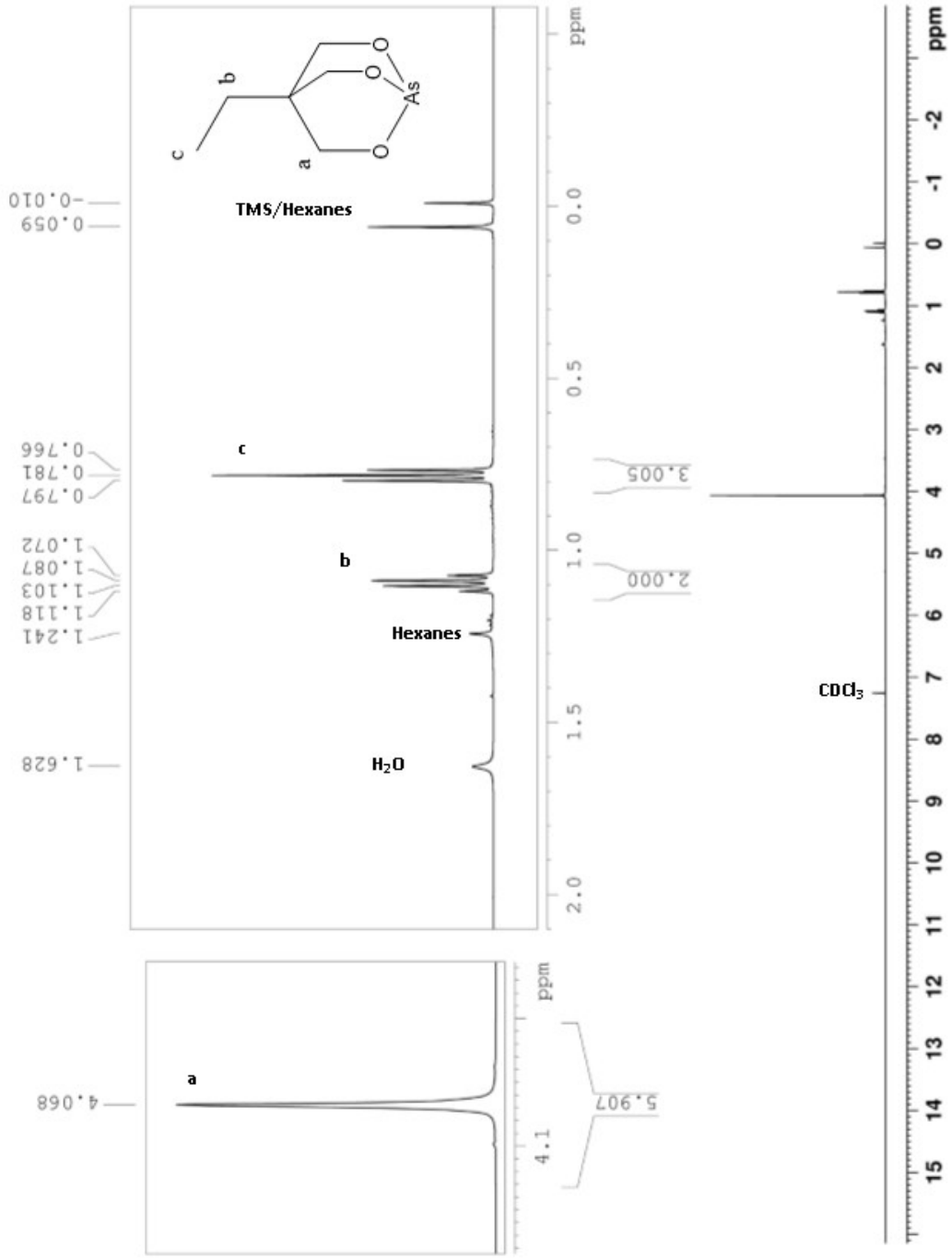

Figure S3. ^{1}H-NMR spectrum of 4-ethyl-2,6,7-trioxa-1-arsabicyclo[2.2.2]octane ($CDCl_3$ d_1; 500 MHz).

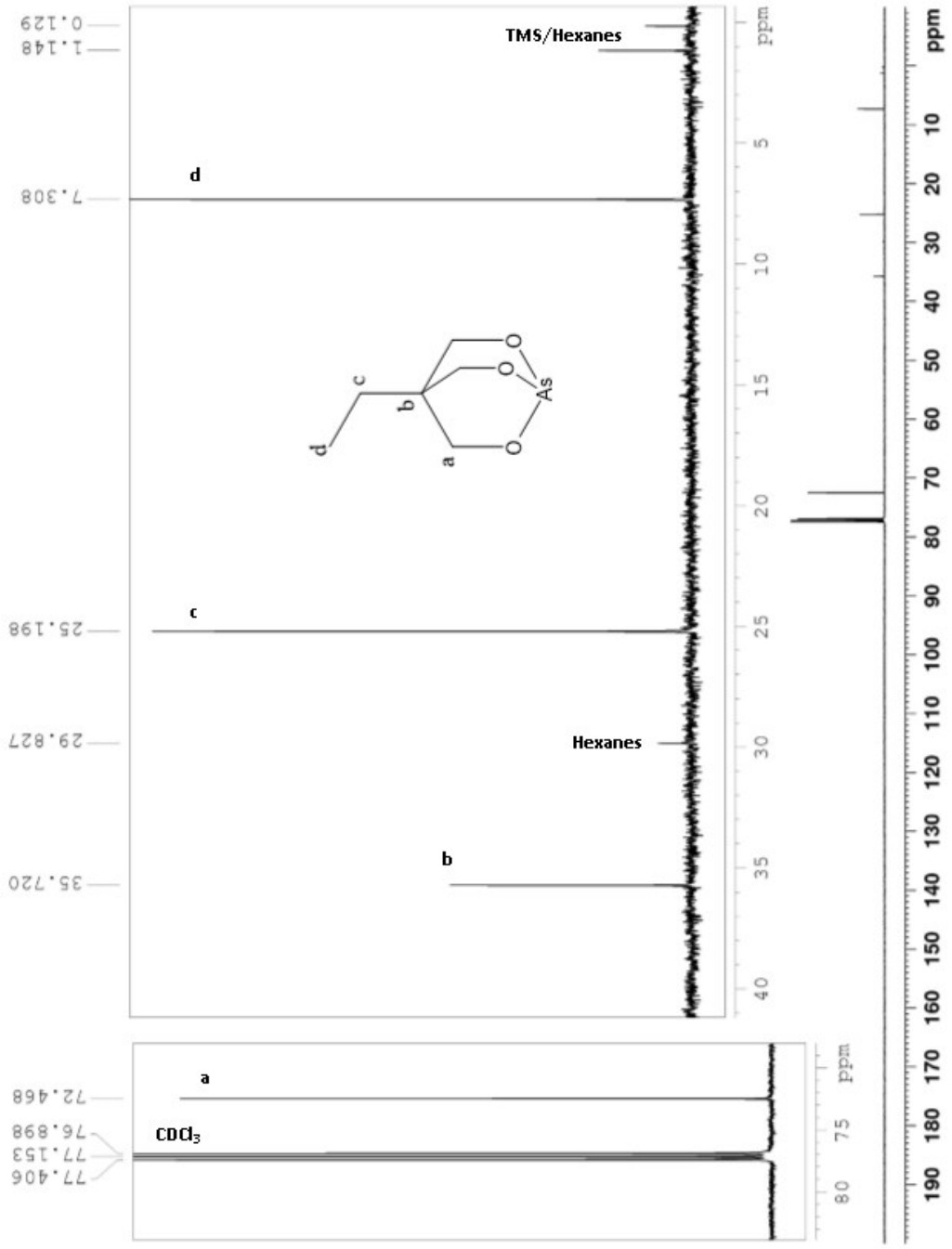

Figure S4. ^{13}C-NMR spectrum of 4-ethyl-2,6,7-trioxa-1-arsabicyclo[2.2.2]octane ($CDCl_3$ d_1; 500 MHz)

Table S1. X-ray crystal data, data collection parameters, and refinement parameters for 4-ethyl-2,6,7-trioxa-1-arsabicyclo[2.2.2]octane.

Empirical formula	$C_6H_{11}AsO_3$
CCDC #	1456953
Formula weight	206.07
Temperature	100(2) K
Wavelength	0.71073 Å
Crystal system	Monoclinic
Space group	P 21/n
Unit cell dimensions	a = 6.0059(10) Å α= 90°. b = 11.602(2) Å β= 92.272(2)°. c = 10.6270(18) Å γ = 90°.
Volume	739.9(2) $Å^3$
Z	4
Density (calculated)	1.850 mg/m^3
Absorption coefficient	4.537 mm^{-1}
F (000)	416
Crystal size	0.500 x 0.250 x 0.150 mm^3
Theta range for data collection	2.600 to 27.538°
Index ranges	$-7 \leq h \leq 7$, $-14 \leq k \leq 14$, $-13 \leq l \leq 13$
Reflections collected	8432
Independent reflections	1691 [R(int) = 0.0230]
Completeness to theta = 25.242°	99.9 %
Absorption correction	Semi-empirical from equivalents
Max. and min. transmission	0.55 and 0.40
Refinement method	Full-matrix least-squares on F^2
Data / restraints / parameters	1691 / 0 / 92
Goodness-of-fit on F^2	1.079
Final R indices [I>2sigma(I)]	R1 = 0.0211, wR2 = 0.0546
R indices (all data)	R1 = 0.0234, wR2 = 0.0555
Extinction coefficient	n/a
Largest diff. peak and hole	0.677 and -0.542 e. $Å^{-3}$

Computer programs: *APEX 3*, *SAINT*[S6], SHELXS2014[S1].

Table S2. Selected bond distances (Å) and angles (°) for 4-ethyl-2,6,7-trioxa-1-arsabicyclo[2.2.2]octane.

Selected distance (Å)		Selected bond angles (°)	
As(1)-O(1)	1.7777(13)	O(1)-As(1)-O(2)	96.23(6)
As(1)-O(2)	1.7847(14)	O(1)-As(1)-O(3)	97.51(6)
As(1)-O(3)	1.7858(14)	O(2)-As(1)-O(3)	96.03(6)
O(1)-C(1)	1.450(2)	C(1)-O(1)-As(1)	114.93(10)
O(2)-C(2)	1.451(2)	C(2)-O(2)-As(1)	114.84(11)
O(3)-C(3)	1.446(2)	C(3)-O(3)-As(1)	115.51(11)
C(1)-C(4)	1.535(2)	O(1)-C(1)-C(4)	112.22(14)
C(2)-C(4)	1.537(2)	O(2)-C(2)-C(4)	112.63(15)
C(3)-C(4)	1.533(2)	O(3)-C(3)-C(4)	112.19(14)
C(4)-C(5)	1.536(2)	C(3)-C(4)-C(1)	109.02(14)
C(5)-C(6)	1.532(3)	C(3)-C(4)-C(5)	110.93(15)
		C(1)-C(4)-C(5)	110.87(15)
		C(3)-C(4)-C(2)	108.43(15)
		C(1)-C(4)-C(2)	109.12(15)
		C(5)-C(4)-C(2)	108.42(15)
		C(6)-C(5)-C(4)	115.51(15)

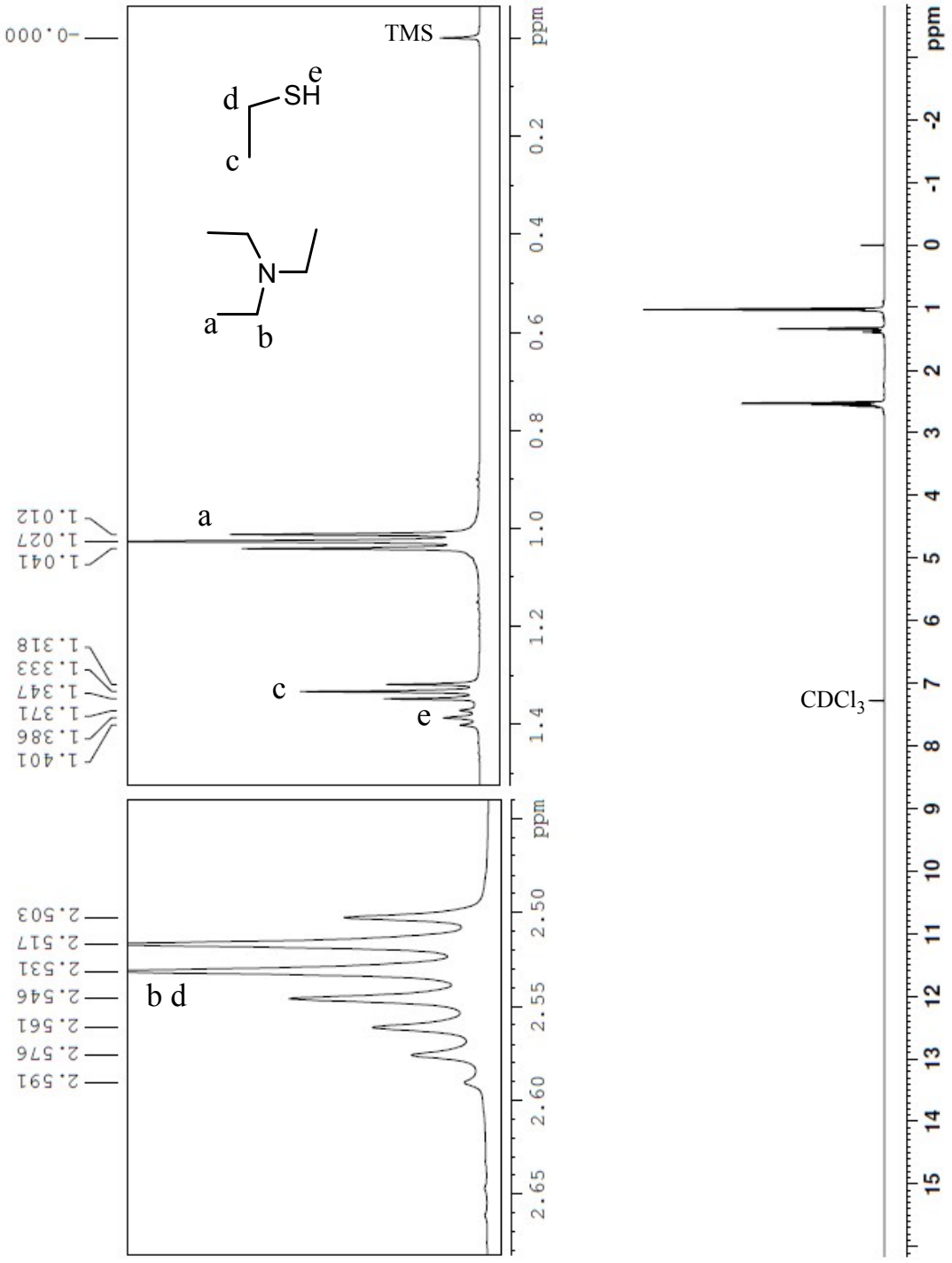

Figure S5. ^{1}H-NMR spectrum of a mixture of 1-ethanethiol and triethylamine (stock solution B, 0.24 mmol, 1:1 ratio, affording thiolates); this is the reference spectrum for the t = 0 h time point ($CDCl_3$ d_1; 500 MHz).

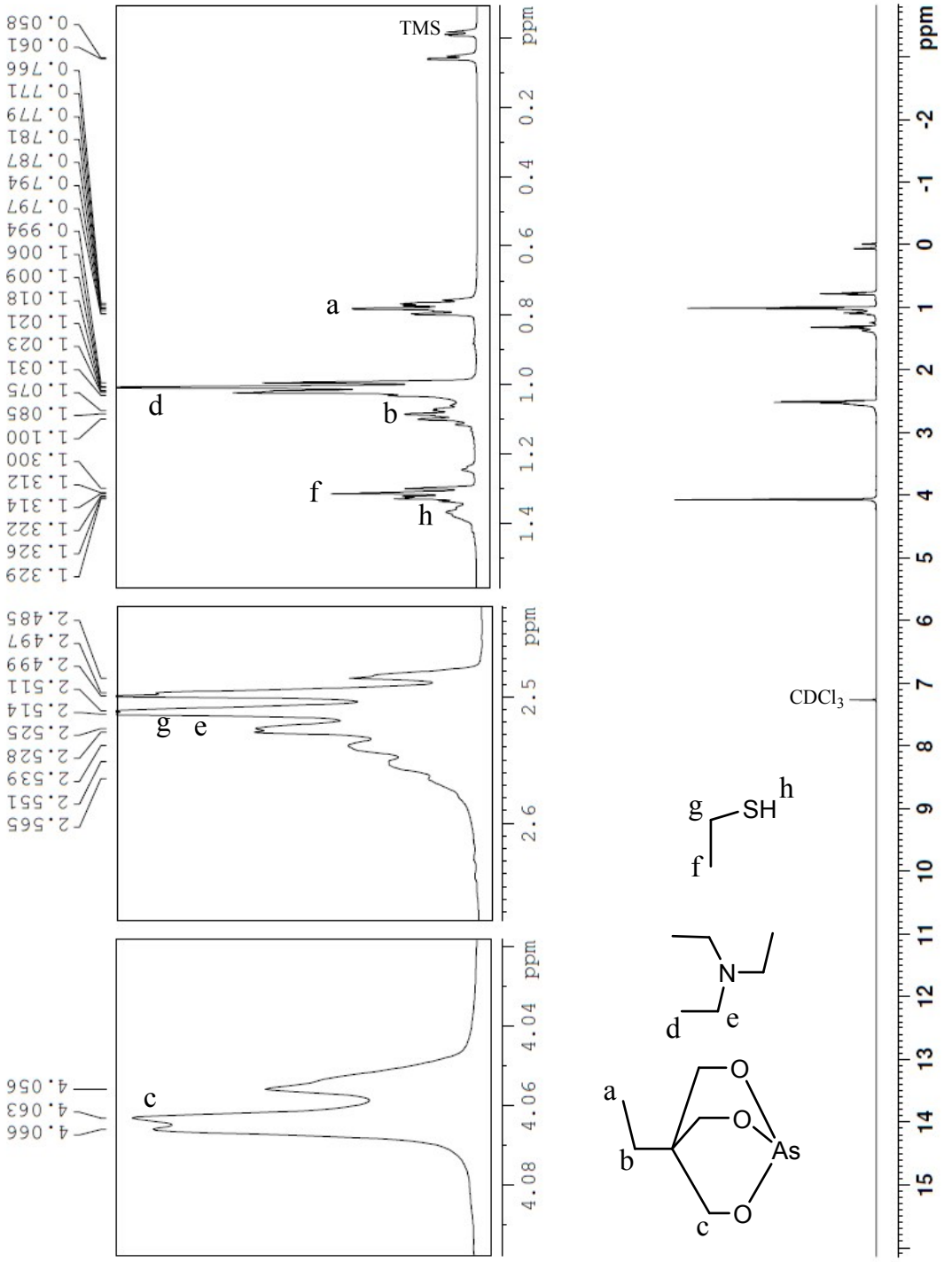

Figure S6. ^{1}H-NMR spectrum of a mixture of 1-ethanethiol, triethylamine and 4-ethyl-2,6,7-trioxa-1-arsabicyclo[2.2.2]octane (thiol challenge study; free thiol: As complex 2.5: 1); this is the t = 0 h time point spectrum.

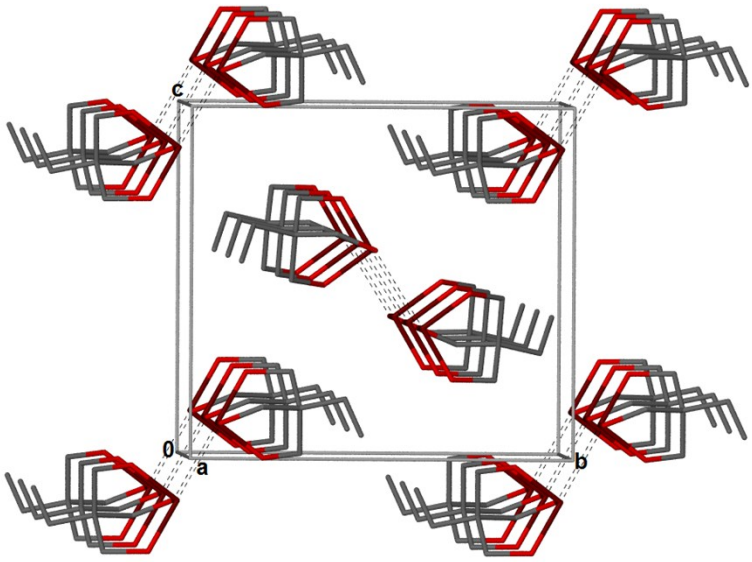

Figure S7. The packing diagram of 4-ethyl-2,6,7-trioxa-1-arsabicyclo[2.2.2]octane.

Supplementary Material for Rhodium Section [S7]:

Materials, Physical Measurements and Results

Rink Amide resin, Fmoc amino acids and coupling reagents were purchased from EMD Biosciences (San Diego, CA, USA). ^{125}I-Tyr^4-BBN was purchased from Perkin Elmer (Waltham, MA, USA). All other reagents were purchased from either Fisher Scientific (Pittsburg, PA, USA) or Sigma Aldrich (St. Louis, MO, USA). Triethylamine was dried over calcium sulfate overnight. All other reagents were used without further purification.

Dimethyl-3,7,11,15-tetrathiaheptadecane-1,17-dioic acid

Dimethyl-3,7,11,15-tetrathiaheptadecane-1,17-dioic acid (333-S_4-$(CH_2COOH)_2$) was synthesized as previously published and analyzed by NMR using either a Bruker DRX 500 MHz or a DRX 300 MHz widebore spectrometer (Billerica, MA, USA).[S8,S9] Analytical HPLC was performed using a Shimadzu SCL-10A (Koyoto, Japan) system with a binary gradient system [solvent A = DI water with 0.1% trifluoroacetic acid (TFA), B = Acetonitrile with 0.1% TFA] and an in line SPD-10A UV absorbance detector (280 nm, 220 nm).

Synthesis of coupled 333-S_4-BBN(7-14)NH_2

Fmoc-8Aoc-BBN(7-14) was prepared using automated solid phase Fmoc chemistry with an Omega AAPPTEC 396 peptide synthesizer on a Rink Amide resin support. The resin bound amino acids were deprotected with a 0.1 M solution of hydroxybenzoltriazole (HOBT) and 20% Piperdine. Then the carboxyl groups were activated with 0.5 M of O-benzotriazole-N,N,N',N'-tetramethyl-uronium-hexafluorophosphate (HBTU) in dimethylformamide (DMF), and a mixture of 34.8 mL (0.2 mol) diisopropylethylamine (DIEA) in 65.2 mL (0.7 mol) N-methyl-2-pyrrolidone (NMP). The activated end of the growing resin-bound peptide was flushed with an excess of Fmoc-amino acid resulting in the formation of an amide bond. Manual coupling was used to conjugate the tetrathioether chelate by adding 60 µmol of Fmoc-8Aoc-BBN(7-14)NH_2 on the resin support to a stirring solution containing DIEA (50 µL, 300 µmol), NMP (100 µL, 130 µmol), 3,3,3-S4-$(COOH)_2$ (65 mg, 17 µmol), HBTU (45.5 mg, 120 µmol), and HOBT (27 mg, 200 µmol). The solution was stirred at 60 ^oC for 30 min, cooled and then filtered. S4-8Aoc-BBN(7-14)NH_2 was cleaved from the resin support in a solution of 5% water, 5% triisopropyl silane (TIS), and 5% phenol in TFA, filtered and precipitated in 12 mL of cold *t*-butyl ether. The desired product was HPLC purified using a Prep Nova-Pak HR C18 Waters column (6 µm, 7.8 x 300 mm, 60 Å) and a binary solvent gradient (80% A, 20% B shifted to 50% A, 50% B over 60 min). The purified product was then analyzed via LC-MS using a Kromasil C18 HPLC column (5 µm, 150 x 4.6 mm, 100 Å) and a Finnigan TSQ7000 mass spectrometer with the same solvent gradient. Thiol containing scavengers must be omitted from the cleavage solution as they may react with the chelate thioethers. LC-MS analysis **(Figure S8**) confirmed formation of the 94.66% pure 333-S_4-BBN(7-14)NH_2 with a retention time of 42.26 min and m/z of 1436.2 Da (calculated = 1435.68 Da).

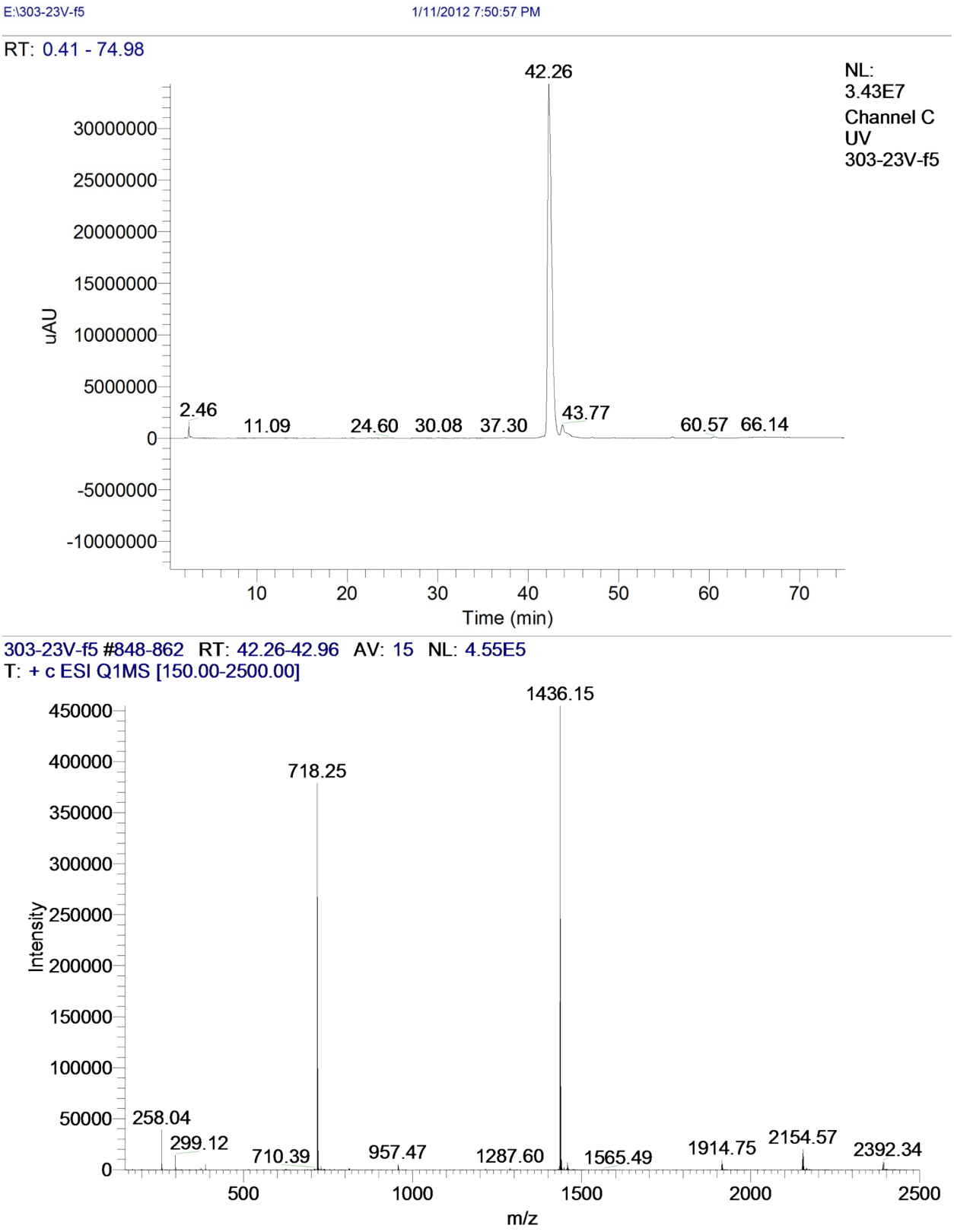

Figure S8. LC-MS of 3,3,3-S_4-BBN(7-14)NH_2.

[RhCl(333-S4-BBN(7-14)NH_2]$^+$ TFA^- complex

333-S4-BBN(7-14)NH_2 (0.5 mg, 0.31 µmol) was dissolved in 5 mL of 4% ethanol/acetonitrile solution. The solution was brought to reflux at 90 ^oC and 40 µL of $RhCl_3 \cdot 3H_2O$ (0.9 mg, 0.34 mmol) in acetonitrile was added dropwise. The mixture was refluxed at 90 ^oC for 1 h, cooled and then lyophilized. The resulting pale yellow solid was analyzed by LC-MS using a Kromasil C18 HPLC column (5 µm, 150 x 4.6 mm, 100 Å) with a Finnigan TSQ7000 mass spectrometer using a solvent gradient of 90% A , 10% B shifted to 50% A, 50% B over a period of 30 min. The product was also analyzed by MALDI TOF MS with an AB Sciex4700 mass spectrometer.

The metal complex was formed by adding $RhCl_3 \cdot 3H_2O$ to a refluxing solution of 333-S_4-BB(7-14)NH_2 in an ethanolic solution. LC-MS analysis (**Figure S9**) supports formation of [RhCl(333-S_4-BBN(7-14)NH_2]$^+$ based on the proposed structure (**Figure S10b**), which shows the Rh(III) coordinated to four sulfur atoms, one chloride and a pendant carboxylate as evidenced by a m/z of 1571.8 Da (calculated = 1571.02 Da) for the [RhCl(333-S_4-BBN(7-14)NH_2]$^+$ and a m/z of 786.3 Da (calculated = 786.51 Da) for the protonated [RhCl(333-S_4-BBN(7-14)NH_2]$^{2+}$ species with a retention time of 20.1 min. This configuration was unexpected based on previous studies with 333-S_4-$(CH_2COOH)_2$ in which the Rh(III) was coordinated to two chlorides in addition to the four sulfur atoms but not with either of the two pendant carboxylic acid groups (**Figure S10a**).[S8,S9]

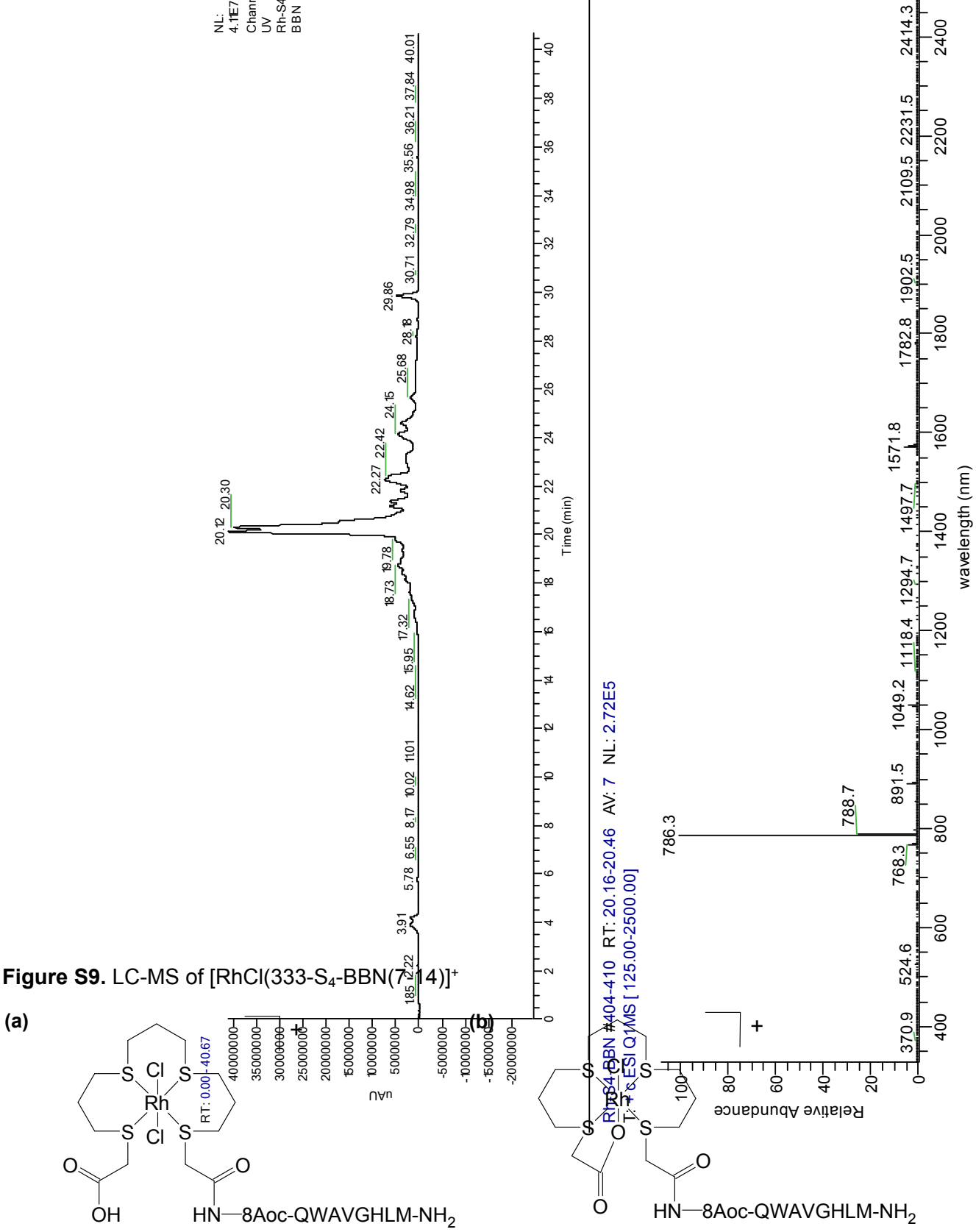

Figure S9. LC-MS of $[RhCl(333\text{-}S_4\text{-}BBN(7\text{-}14)]^+$

Figure S10. Expected structure of $[RhCl_2(333\text{-}S_4\text{-}BBN(7\text{-}14)NH_2)]^+$ (**a**)[S4] and proposed structure of $[RhCl(333\text{-}S_4\text{-}BBN(7\text{-}14)NH_2)]^+$ (**b**).

In vitro analysis of $[RhCl\text{-}S4\text{-}8Aoc\text{-}BBN(7\text{-}14)NH_2]^+$ TFA^-

The affinity of [RhCl(333-S_4-BBN(7-14)NH_2)]$^+$ for the GRP receptor was evaluated using a competitive binding assay compared to ^{125}I-Tyr^4-BBN with GRP receptor positive PC-3 human prostate cancer cells. In a micro-well plate approximately 3 x 10^5 PC-3 cells were suspended in Roswell Park Memorial Institute (RPMI) medium at pH 7.4 with 4.8 mg/mL of 4-(2-hydroxyethyl)-1-piperazineethanesulfonic acid(HEPES), and 2 mg/mL of bovine serum albumin (BSA). The mixture was incubated at 37 ^{o}C for 1 h with 30,000 cpm ^{125}I-Tyr^4-BBN and increasing concentrations of [RhCl(333-S_4-BBN(7-14)NH_2)]$^+$ from 3.3x10^{-13} to 3.3x10^{-6} M. The cells were washed four times with media to release any non-specifically bound peptide and then counted on a Multi-Wiper (Laboratory Tecnologies, Maple Park, IL, USA) multiwell NaI gamma scintillation detector. The average concentration, derived from three experiments, of [RhCl(333-S_4-BBN(7-14)NH_2)]$^+$ needed to inhibit ^{125}I-Tyr^4-BBN binding by 50% (IC_{50}) was determined to be 2.2 ± 0.3 nM. The IC_{50} curve was obtained by plotting the percent of ^{125}I-Tyr-BBN bound to the cells as a function of the concentration of [RhCl(333-S_4-BBN(7-14)NH_2)]$^+$ added using GraphFit software version 4 (Erithacus Software Limited, Middlesex, UK).

Synthesis of ^{105}Rh(333-S_4-BBN(7-14)NH_2

Ethanolic solutions of 333-S_4-BBN(7-14)NH_2 were added to 0.5 - 1 mCi of ^{105}Rh-chloride in dilute HCl (pH 3-4). The reaction solution was heated at 80 ^{o}C for 1 h and analyzed by HPLC. Labeling conditions were varied from 2.5% - 57% ethanol and 5.8 x 10^{-5} M - 1.16 x 10^{-3} M 333-S_4-BBN(7-14)NH_2.

Repeated attempts to synthesize ^{105}RhCl(333-S_4-BBN(7-14)NH_2) under various radiolabeling conditions failed to result in a single species of the radiolabeled complex with significant yield (>10%). All conditions resulted in low labeling yields (< 5 - 10%) as measured by analytical HPLC. Additional heating (85 ^{o}C, 2 h) resulted in formation of many radiolabeled species observed by HPLC.

References

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