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Vanadium Complexes with Mixed *O,S* Anionic Ligands Derived from Maltol: Synthesis, Characterization, and Biological Studies

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Four mixed *O,S* binding bidentate ligand precursors derived from maltol (3-hydroxy-2-methyl-4-pyrone) have been chelated to vanadium to yield new bis(ligand)oxovanadium(IV) and tris(ligand)vanadium(III) complexes. The four ligand precursors include two pyranthiones, 3-hydroxy-2-methyl-4-pyranthione, commonly known as thiomaltol (Htma), and 2-ethyl-3-hydroxy-4-pyranthione, commonly known as ethylthiomaltol (Hetma), as well as two pyridinethiones, 3-hydroxy-2-methyl-4(H)-pyridinethione (Hmppt) and 3-hydroxy-1,2-dimethyl-4-pyridinethione (Hdppt). Vanadium complex formation was confirmed by elemental analysis, mass spectrometry, and IR and EPR (where possible) spectroscopies. The X-ray structure of oxobis(thiomaltolato)vanadium(IV),$VO(tma)_2$, was also determined; both cis and trans isomers were isolated in the same asymmetric unit. In both isomers, the two thiomaltolato ligands are arranged around the base of the square pyramid with the V═O linkage perpendicular; the vanadium atom is slightly displaced from the basal plane [V(1) = 0.656(3) Å, V(2) = 0.664(2) Å]. All of the new complexes were screened for insulin-enhancing effectiveness in streptozotocin-induced diabetes in rats, and $VO(tma)_2$ was profiled metabolically for urinary vanadium and ligand clearance by GFAAS and ESIMS, respectively. The new vanadium complexes did not lower blood glucose levels acutely, possibly because of rapid dissociation and excretion.

Introduction

The coordination chemistry of vanadium with sulfur-containing ligands is an emerging field of interest with relevance to several disparate biological systems.[1−4] The presence of vanadium−sulfur bonding in the active site of certain nitrogenase enzymes has been well established,[5] and vanadium−sulfur coordination also appears to be pivotal to the well-known tyrosine phosphatase inhibition through binding to cysteine at the putative active site.[6] A third area of interest, vanadium complexes with ligands that supply both sulfur and oxygen donors for candidate therapeutic agents,[7] is one that dovetailed nicely with our previous explorations in the more general area of vanadium-containing insulin mimics.[8]

The insulin-enhancing properties of vanadium complexes have been thoroughly investigated, both in vitro and in vivo.[7−10] The absolute or relative lack of insulin, a hallmark of all types of diabetes mellitus, can be partially overcome

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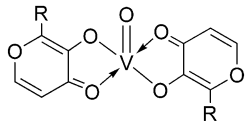
by oral administration of either vanadium salts (e.g., $VOSO_4$ or sodium vanadate) or organically chelated V(III),[11] V(IV),[12] or V(V)[13] complexes. Numerous studies in experimental animals have shown that tolerable levels of vanadium-containing compounds can normalize plasma glucose, lipids, and the thyroid hormone and at least partially counteract the increased oxidative stress that accompanies diabetes mellitus and contributes to secondary complications. Nonetheless, an obviously limited "window of optimal utility"[14,15] has spurred continuing efforts to further tailor the ligands used for vanadium complexation such that greater potency and efficacy could be achieved.

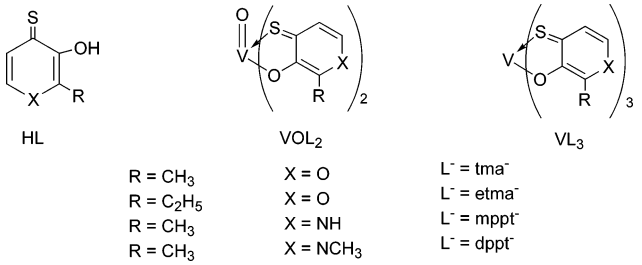
In previous studies in our laboratories, both vanadium(III) and oxovanadium(IV) complexes with, primarily, oxygen donor ligands have been shown effective, with one compound, bis(ethylmaltolato)oxovanadium(IV), having completed phase I clinical trials.[16]



$R = CH_3$ BMOV
$R = C_2H_5$ BEOV

Inorganic vanadium compounds, although they are effective, have poor gastrointestinal absorption and require relatively high doses for therapeutic efficacy.[14] As shown previously by our group,[12,16] the vanadyl complexes with maltol and ethylmaltol (approved food additives), namely, bis(maltolato)oxovanadium(IV) (BMOV) and bis(ethylmaltolato)oxovanadium(IV) (BEOV), are several times more potent than vanadyl sulfate.

Among the many different complexes of vanadium that have been synthesized, characterized, and biologically tested by us[8,9] and by others,[10,17] there have been vanadium complexes with mixed *O,S* donors and mixed *O,N* donors, but so far none have proven to be substantially more potent than BMOV or BEOV in vivo. In this work, we revisit the maltol motif with small but significant modifications. The aim of this study was to test the insulin-enhancing properties of several new vanadium complexes with mixed *O,S* donor ligands. The syntheses and characterization of four mixed *O,S* donor ligands and their corresponding vanadyl(IV) and vanadium(III) complexes are described here. The ketonic oxygen in maltol and ethylmaltol was replaced by sulfur, yielding thiomaltol (Htma) and ethylthiomaltol (Hetma), respectively. These compounds should bind to vanadium through the thiocarbonyl sulfur and deprotonated hydroxyl oxygen atoms, thus comprising mixed *O,S* donor ligands. Two other ligands with mixed *O,S* donor atoms with the N−R group substituted for the ring oxygen were also synthesized: 3-hydroxy-2-methyl-4-pyridinethione (Hmppt) and 3-hydroxy-1,2-dimethyl-4-pyridinethione (Hdppt). The corresponding oxygen analogues of these compounds (i.e., C═O instead of C═S) are ligands that have been previously synthesized and tested as vanadyl complexes for insulin-like properties.[10,16] The substitution of N−R in the ring provides versatility in terms of varying the R group on the nitrogen and, hence, changing chemical properties such as lipophilicity, solubility, and stability of the vanadium complex.



HL: $R = CH_3$; $R = C_2H_5$; $R = CH_3$; $R = CH_3$

VOL_2: X = O; X = O; X = NH; $X = NCH_3$

VL_3: $L^- = tma^-$; $L^- = etma^-$; $L^- = mppt^-$; $L^- = dppt^-$

The syntheses and characterizations of four mixed *O,S* donor ligands and their corresponding vanadyl(IV) and vanadium(III) complexes are described herein. Furthermore, the results of the biological tests of the vanadium complexes as insulin-enhancing agents as compared to BMOV are presented.

Experimental Section

Materials and Methods. The preparation and determination of the acidity constants of the ligand precursors have been fully described in an accompanying article.[18] All solvents were reagent-grade and were obtained from Fisher Scientific. All other chemicals [$VOSO_4 \cdot H_2O$, VCl_3, NEt_3, P_2S_5, NaCl, 1 M NaOH, 40% aqueous $MeNH_2$, NH_4OH, HCl, and DTT (dithiothreitol)]were obtained from Sigma-Aldrich or Fisher Scientific and were used as received without further purification. Potassium biphthalate (KHP) was obtained from Anachemia, and silica gel 230−400 mesh was obtained from Silicycle. Water was deionized (Barnstead D8902 and D8904 Cartridges) and distilled (Hytrex II GX50-9-7/8 and GX100-9-7/8 Cartridge filters) before use. Atomic absorption standard (AAS) vanadyl solution for potentiometric and spectrophotometric titrations was obtained from Sigma-Aldrich. Instrument-quality (all metals <1 ppb) concentrated nitric acid was from Seastar Chemicals Inc., Sidney, BC, Canada.

Instrumentation. Infrared spectra were recorded as KBr disks in the range of 4000−500 cm^{-1} on a Galaxy Series 5000 FTIR spectrometer and were standardized to polystyrene. Mass spectra were obtained with a Kratos MS 50 (electron-impact ionization mass spectrometry, EIMS), a Kratos Concept II H32Q (Cs^+ liquid secondary ion mass spectrometry, LSIMS), or a Micromass LCT or Bruker Esquire ion trap (electrospray ionization, ESIMS) spectrometer. Room-temperature (293 K) magnetic susceptibilities

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were measured on a Johnson Matthey magnetic susceptibility balance; diamagnetic corrections were based on Pascal's constants. Mr. Peter Borda or Mr. Minaz Lakha in the Department of Chemistry, University of British Columbia, performed the elemental analyses of C, H, N, and S. Room-temperature and frozen-solution X-band EPR spectra were recorded on a Bruker ECS-106 X-band spectrometer. Temperature (125 or 298 K) was maintained by liquid nitrogen flowing through a cryostat in conjunction with a Eurotherm B-VT-2000 variable-temperature controller. The microwave frequency and magnetic field were calibrated with an EIP 625A microwave-frequency counter and a Varian E500 gaussmeter, respectively. Computer simulations of the isotropic EPR spectra were performed using Bruker's WINEPR/SIMFONIA package. The solution (298 K) spectrum of $VO(dppt)_2$ (in MeOH) was recorded in a 20-μL quartz capillary. Quartz tubes (4 mm i.d.) were used to record the solution and frozen-solution spectra of $VO(tma)_2$ and bis(ethylthiomaltolato)oxovanadium(IV), $VO(etma)_2$, in DMF and bis(2-methyl-3-oxy-4-pyridinethionato)oxovanadium(IV), $VO(mppt)_2$, and bis(1,2-dimethyl-3-oxy-4-pyridinethionato)oxovanadium(IV), $VO(dppt)_2$, in MeOH/DMF (1:1). A Varian model SpectrAA 300 graphite furnace atomic absorption spectrometer (GFAAS) with Zeeman background correction equipped with an autosampler was used for the determination of vanadium concentrations in urine samples of treated diabetic rats.

Preparation of the Complexes. All complexation reactions and the filtering of the vanadium(III) products were carried out under Ar. Yields are presented for the analytically pure compounds and were calculated on the basis of the respective vanadium starting material for the vanadium complexes.

(i) Oxobis(thiomaltolato)vanadium(IV), $VO(tma)_2$. $VOSO_4 \cdot 3H_2O$ (5.55 g, 0.026 mol) was added to a solution of Htma (7.27 g, 0.051 mol) in 100 mL of degassed water (78 °C). As the pH of the solution was raised slowly (over 10 min) with 1 M NaOH to ~5, a solid precipitated from the solution. The resulting mixture along with the precipitate was refluxed for 3 h, cooled to room temperature, and vacuum filtered to yield a dark olive-green compound. The precipitate was washed with 5 mL of cold water and dried overnight in vacuo to yield 7.6 g, 84% based on V. Anal. Calcd (found) for $C_{12}H_{10}O_5S_2V$: C, 41.27 (40.99); H, 2.89 (2.85); S, 18.36 (18.20). MS *m*/*z* (+LSIMS): 350 ($[HVOL_2]^+$), 333 ($[VL_2]^+$). IR (cm^{-1}, ± 4 cm^{-1}): 974 ($\nu_{V=O}$). Magnetic moment: $\mu_{eff} = 1.65$ μ_B.

(ii) Bis(ethylthiomaltolato)oxovanadium(IV), $VO(etma)_2$. $VO(etma)_2$ was synthesized according to the same method as employed for $VO(tma)_2$, replacing Htma with Hetma. A brown precipitate was collected by vacuum filtration, washed with 5 mL of cold water, and dried overnight in vacuo to yield 74% based on V. Anal. Calcd (found) for $C_{14}H_{14}O_5S_2V$: C, 44.56 (44.16); H, 3.74 (3.71); S, 16.99 (16.75). MS *m*/*z* (+LSIMS): 378 ($[HVOL_2]^+$), 361 ($[VL_2]^+$). IR (cm^{-1}, ± 4 cm^{-1}): 972 ($\nu_{V=O}$). Magnetic moment: $\mu_{eff} = 1.71$ μ_B.

(iii) Bis(2-methyl-3-oxy-4-pyridinethionato)oxovanadium(IV) Trihydrate, $VO(mppt)_2 \cdot 3H_2O$. $VO(mppt)_2$ was synthesized according to the same method as employed for $VO(tma)_2$, replacing Htma with Hmppt. A grayish-brown precipitate was collected by vacuum filtration, washed with 5 mL of cold water, and dried overnight in vacuo to yield 78% based on V. Anal. Calcd (found) for $C_{12}H_{12}N_2O_3S_2V \cdot 3H_2O$: C, 35.91 (35.99); H, 4.52 (4.57); N, 6.98 (6.91); S, 15.96 (15.82). MS *m*/*z* (+LSIMS): 348 ($[HVOL_2]^+$), 331 ($[VL_2]^+$). IR (cm^{-1}, ± 4 cm^{-1}): 968 ($\nu_{V=O}$). Magnetic moment: $\mu_{eff} = 1.62$ μ_B.

(iv) Bis(1,2-dimethyl-3-oxy-4-pyridinethionato)oxovanadium-(IV), $VO(dppt)_2$. Method A. $VO(dppt)_2$ was synthesized according to the same method as employed for $VO(tma)_2$, replacing Htma with Hdppt. A pine-green precipitate was collected by vacuum filtration, washed with 5 mL of cold water, and dried overnight in vacuo to yield 42% based on V.

Method B. $VOSO_4 \cdot 3H_2O$ (4.30 g, 0.020 mol) was added to a solution of Htma (5.62 g, 0.040 mol) dissolved in 250 mL of hot water (78 °C). The $N-CH_3$ pyridinethione was prepared in situ by slowly (over ~2 h) adding 40% methylamine (17 mL, 0.20 mol). Lowering the pH of the solution from ~11 to ~9 by addition of concentrated H_2SO_4, dropwise, resulted in a solid precipitating from the solution. The reaction mixture along with the precipitate was refluxed overnight, cooled to room temperature, and vacuum filtered to obtain a pine-green compound. The precipitate was washed twice with 10 mL of cold water and dried overnight in vacuo. Analysis verified the product to be identical to $VO(dppt)_2$ synthesized by method A, yield 5.48 g, 73% based on V. Anal. Calcd (found) for $C_{14}H_{16}N_2O_3S_2V$: C, 44.80 (44.87); H, 4.30 (4.42); N, 7.46 (7.51); S, 17.08 (17.22). MS *m*/*z* (+LSIMS): 376 ($[HVOL_2]^+$), 359 ($[VL_2]^+$). IR (cm^{-1}, ± 4 cm^{-1}): 962 ($\nu_{V=O}$). Magnetic moment: $\mu_{eff} = 1.66$ μ_B.

(v) Tris(thiomaltolato)vanadium(III), $V(tma)_3$. VCl_3 (1.42 g, 0.009 mol) was added to a solution of Htma (3.84 g, 0.027 mol) in 300 mL of degassed hot water and 50 mL of methanol at 80 °C. The black reaction mixture was refluxed for 3 h and then cooled to room temperature. The black, flaky precipitate formed was collected by vacuum filtration, washed twice with 5 mL of cold water, and dried overnight in vacuo to yield 2.77 g, 65% based on V. Anal. Calcd (found) for $C_{18}H_{15}O_6S_3V$: C, 45.57 (45.67); H, 3.19 (3.11); S, 20.27 (20.21). MS *m*/*z* (EIMS): 474 ($[VL_3]^+$), 333 ($[VL_2]^+$). IR (cm^{-1}, ± 4 cm^{-1}): 1580, 1500 (ring vibrations), 673 (ν_{V-O}). Magnetic moment: $\mu_{eff} = 2.53$ μ_B.

(vi) Tris(ethylthiomaltolato)vanadium(III), $V(etma)_3$. VCl_3 (0.165 g, 1.1 mmol) was added to a solution of Hetma (0.51 g, 3.3 mmol) in 10 mL of degassed water and 3 mL of MeOH at 83 °C with Ar sparging. Triethylamine, $N(Et)_3$ (0.45 mL, 3.2 mmol), was added, and the solution was refluxed for 3 h and then cooled to room temperature. A black crystalline, air-sensitive precipitate [decomposes to $VO(etma)_2$ in 5−6 days] was filtered under Ar, washed with 5 mL of cold, degassed water, and dried overnight in vacuo to yield 0.43 g, 79% based on V. Anal. Calcd (found) for $C_{21}H_{21}O_6S_3V$: C, 48.83 (48.50); H, 4.10 (4.06). MS *m*/*z* (ESI-MS): 516 ($[VL_3]^+$), 361 ($[VL_2]^+$). IR (cm^{-1}, ± 4 cm^{-1}): 1576, 1499 (ring vibrations), 673 (ν_{V-O}). Magnetic moment: $\mu_{eff} = 2.52$ μ_B.

Spectrophotometric Titrations. The stepwise equilibrium constants of the vanadyl complexes were determined by performing spectrophotometric and potentiometric titrations. For the spectrophotometric titrations, 30.00 mL of an aqueous solution containing 0.16 M NaCl, 16.2 mM $[VO^{2+}]_{stock}$ [prepared by diluting a V(IV) AAS solution], and ~0.9 mM ligand precursors was titrated with ~0.1 M NaOH. The analytical solution was kept under constant Ar flow in a titration vessel maintained at 25 ± 0.1 °C using a Julabo UC circulating bath equipped with a Metrohm 6.0123.100 pH glass electrode, a Metrohm 6.0726.100 silver chloride reference electrode, a model 665 Metrohm Dosimat autoburet, and a Metrohm 713 pH meter. An aliquot from the solution was transferred into a cuvette (path length = 1 cm), and the UV absorption was measured. After measuring the absorbance, we carefully returned the aliquot to the original solution, added titrant in 0.1-mL increments with constant stirring, and recorded the next measurement after the pH stabilized (±0.1 mV) . Absorbance data were corrected for dilution factors and were fitted by nonlinear regression analysis using an iterative procedure in the SigmaPlot 2001 program.

Potentiometric Measurements. The general procedure for the potentiometric titrations has been described in detail in an accompanying article.[18] All solutions were prepared with freshly boiled deionized, distilled water that was purged with Ar during boiling and cooling to ensure removal of CO_2. The ionic strength (I) of the titration solutions was kept constant with 0.16 M NaCl. The value of pK_w used at $I = 0.16$ M NaCl and $T = 25$ °C was 13.76.[19] The titrations were controlled by a locally written Qbasic program. The acidity constants of the ligand precursors were calculated using the data within the range of $\sim 2.5 \leq pH \leq 10.5$ on an IBM-compatible computer containing a Pentium II processor using a curve-fit procedure (a Newton−Gauss nonlinear least-squares program). The final values of the acidity constants for all of the compounds[18] were obtained from the average of at least 10 independent titrations. Potentiometric measurements of the ligand precursors (HL) were conducted in the presence and absence of the vanadyl ion. For the metal titrations, the ligand-to-metal ratio was at least 3:1 to prevent hydrolysis. Stability constants of the vanadyl complexes were determined under the same conditions as employed for the free ligand and were calculated using the data within the range of $\sim 4.5 \leq pH \leq 10.0$ on an IBM-compatible computer containing a Pentium II processor using a curve-fit procedure (a Newton−Gauss nonlinear least-squares program). The first equilibrium constant K_{110} was determined spectrophotometrically and was maintained as a constant in potentiometric calculations of K_{120} and K_{12-1}. The hydrolysis model of a vanadyl system was also included in the model.[20,21] The final values of the equilibrium constants of all of the complexes were obtained from the average of eight independent titrations.

X-ray Crystallographic Analysis of $VO(tma)_2$. Crystals were mounted on a glass fiber, and measurements were made on a Bruker X8 diffractometer with graphite monochromated Mo Kα radiation. Data were collected and integrated using the Bruker SAINT software package.[22] All data were processed and corrected for Lorentz and polarization effects and absorption using the multiscan technique (SADABS).[23] The structure was solved by direct methods.[24] The material crystallizes with both the cis and trans isomers in the asymmetric unit. Complete details of the X-ray crystallographic analysis of $VO(tma)_2$ can be found in the Supporting Information.

Biological Studies. Male Wistar rats weighing 190−220 g were cared for in accordance with the principles and guidelines of the Canadian Council on Animal Care. The animals (two per cage) were housed on a 12-h light/dark schedule. The cages were maintained at 21 ± 1 °C, and the humidity was maintained at 54 ± 2%. The animals were allowed ad libitum access to food (Purina rat chow no. 5001) and tap water. Several experimental comparisons were run sequentially to facilitate the care of the large number of animals. $VO(tma)_2$, $V(tma)_3$, $VO(dppt)_2$, and $V(etma)_3$ were administered by oral gavage; $VO(etma)_2$ and $VO(mppt)_2$ were administered by ip (intraperitoneal) injections. In each case, after a 5-day acclimatization period, animals were randomly assigned to one of the following groups: control (C, nondiabetic), control plus BMOV (CB), diabetic (D), diabetic treated with BMOV (DB), or diabetic treated with a new thio-vanadium complex (DT); in the last three screening assays [for $V(etma)_3$, $VO(mppt)_2$, and $VO(etma)_2$], animals were assigned to D, DB, and DT groups only. The number of rats in each group for each study is given in the insets of the individual graphs in the Supporting Information.

For animals in the D, DB, and DT groups, diabetes was induced by tail vein injection of 60 mg/kg of streptozotocin (STZ, Sigma, St. Louis, MO) in 0.9% NaCl under light halothane anaesthesia. Diabetic status was confirmed 3 days after the STZ injection by a glucometer (Ames Glucometer II, Miles Inc., Elkhart, Ind., and Glucostix, Miles Canada Inc., Etobicoke, ON, Canada); blood glucose levels greater than 13 mM were considered diabetic. For the duration of the experiments (72 h), the plasma glucose levels were monitored by using the Beckman Glucose Analyzer 2. Plasma glucose levels less than 9 mM were considered normoglycemic.

Suspensions of the vanadium compounds to be tested were prepared in a uniform volume (5 mL) of 1% carboxymethylcellulose (CMC, 0.12 M) prepared immediately before administration. The established ED_{50} dose for BMOV or BEOV was used for each screening assay:[8,16] 0.6 mmol kg^{-1} po (per os) and 0.1 mmol kg^{-1} ip in a total volume of 5 mL kg^{-1}. The control and diabetic groups received the equivalent volume of 1% CMC alone.

The study was commenced 7 days after the STZ injection. Plasma glucose levels were determined by collecting 100 μL of whole blood prior to the start of treatment (time 0) and at set intervals up to 72 h following compound administration. Results were analyzed by GLM ANOVA or two-way ANOVA followed by the Newman−Keuls test ($p < 0.05$ accepted as significantly different) using the Number Cruncher Statistical System (NCSS) and are presented as means plus/minus the standard error of the mean (SEM) in the graphs (see Supporting Information).

Determination of Vanadium and Vanadyl Complex Urinary Excretion. Three STZ-diabetic male Wistar rats were administered $VO(tma)_2$, 0.1 mmoL kg^{-1} (1 mg V kg^{-1}), ip. Immediately following injection, the animals were placed in metabolic cages equipped with urinary collection containers. These collection containers were removed at the end of 2, 4, 8, 12, and 24 h; the total volume of each urine sample was recorded; and the samples were analyzed by positive ion mode electrospray ionization mass spectrometry (+ESIMS) to identify the presence of $VO(tma)_2$ or its metabolites in the urine. The urine samples were also evaluated for the total vanadium excreted by each animal up to 24 h after injection. All glassware used in sample preparation was rinsed with 20% HNO_3. Instrument-quality concentrated nitric acid (Seastar Chemicals Inc., Sidney, BC, Canada; distilled in quartz, all metals <1 ppb) and distilled, deionized water were used to prepare the solutions and serial dilutions. The vanadium stock solution (100 ppb) was prepared by adding 10 μL of commercially available standard vanadyl atomic absorption solution, 1020 μg mL^{-1} as purchased from Sigma-Aldrich, to 990 μL of 5% HNO_3 solution. The urine samples were wet digested overnight with 50% HNO_3 and then diluted as required for vanadium concentration measurement by GFAAS.

Results and Discussion

Four neutral oxovanadium(IV) complexes, $VO(tma)_2$, $VO(etma)_2$, $VO(mppt)_2$, and $VO(dppt)_2$, analogous to their non-thio-containing pyrone and pyridinone analogues, have been synthesized and characterized both physicochemically and biologically. In each case, the ligand precursor had an *O,S* binding moiety and was derived from one of two ligand classes: 3-hydroxy-4-pyranthiones or 3-hydroxy-4-pyridine-

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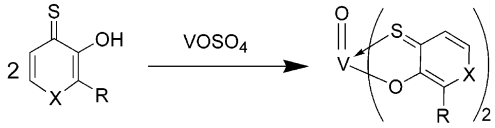
Scheme 1. Syntheses of the Oxovanadium(IV) Complexes (1:2 VO^{2+}/HL) in H_2O, ~80 °C, pH ~5



R = CH_3	X = O	$VO(tma)_2$
R = C_2H_5	X = O	$VO(etma)_2$
R = CH_3	X = NH	$VO(mppt)_2$
R = CH_3	X = NCH_3	$VO(dppt)_2$

thiones. The synthesis and complete characterization, including acidity constants and X-ray crystal structures, of the ligand precursors have been reported in an accompanying article.[18]

Unlike their oxygen counterparts, all of the thio-vanadyl complexes synthesized in this work were found to be stable to oxidation both in the solid state and in solution in various solvents (i.e., water, alcohols, CH_3CN, CH_2Cl_2, toluene, and THF). Of the four complexes, $VO(dppt)_2$ was found to have the lowest solubility.

Synthesis of the vanadyl complexes was accomplished readily by adding vanadyl sulfate to aqueous solutions of the corresponding ligand precursor in a 1:2 ratio, adjusting the pH to ~5 (with NaOH), and refluxing the resulting solution for 3 h (Scheme 1). The reactions were carried out at high temperatures (~80 °C) because of the low solubilities of the ligand precursors in water. The product that precipitated from each reaction mixture was collected by vacuum filtration, washed with cold water, and dried overnight in vacuo. $VO(tma)_2$, $VO(etma)_2$, $VO(mppt)_2$, and $VO(dppt)_2$ were obtained in high yields (42−84%) as olive-green, dark brownish-green, brown, and pine-green solids, respectively. Interestingly, for $VO(dppt)_2$, sometimes a light-gray hydrated form of the complex was isolated [$VO(dppt)_2 \cdot H_2O$].

An additional one-pot method was also used to synthesize $VO(dppt)_2$ (Scheme 2). The original method was similar to that of the other VOL_2 complexes, reacting Hdppt with $VOSO_4$ (Scheme 1), and while this work was ongoing, there was a report of the synthesis of $VO(dppt)_2$ from $VOSO_4$ and Hdppt.[25] The one-pot method (vide infra) is more efficient (Scheme 2); it was adapted from the previously reported syntheses by our group[26,27] wherein various metal ions (VO^{2+}, Al^{3+}, Ga^{3+}, and In^{3+}) were complexed with Hdpp (3-hydroxy-1,2-dimethyl-4-pyridinone), the oxygen analogue of Hdppt. In this method, $VOSO_4$ is added to a solution of Htma, the precursor of Hdppt. The metal−pyranthione complex is formed in situ, and addition of $MeNH_2$ to this solution converts the bound pyranthione to the N−CH_3 pyridinethione. The conversion of a pyranthione to a pyridinethione involves nucleophilic attack by the primary amine at C-2 followed by the elimination of water and ring closure. The nucleophilic attack is hindered by increased electron density at C-2 because of the CH_3 substituent, an electron-donating group, and the anionic hydroxyl oxygen. Metal complexation to the pyranthione anion tma^- has an electron-withdrawing effect on the ring. The electropositive metal ion pulls electron density away from oxygen and the ring, aiding the nucleophilic attack by the amine on the ring. This one-pot synthesis avoids the extra steps involved in isolating Hdppt and yields $VO(dppt)_2$ directly from Htma. The procedure was easier and more efficient than the metathesis method and gave a significantly higher overall yield (73% vs 42%).

New V(III) complexes with pyranthiones, $V(tma)_3$ and $V(etma)_3$, were synthesized in good yields (65−79%) from VCl_3 and the respective ligand precursors (Scheme 3). Because of unavoidable partial hydrolysis/methanolysis of VCl_3, the complexation reaction did not yield the products in 100% yield. For $V(tma)_3$, the black solid precipitated almost immediately upon addition of VCl_3 to the solution, but the reaction mixture was refluxed for 3 h and cooled before filtering to maximize the yield. The synthesis of $V(etma)_3$ was similar, apart from the addition of a base (NEt_3) to precipitate the pure complex. Synthesis of the vanadium(III) complexes of the pyridinethiones, $V(mppt)_3$ and $V(dppt)_3$, was also attempted in a fashion analogous to that for $V(etma)_3$ (Scheme 3). It was found that, despite various conditions employed (i.e., temperatures, solvents, and bases), the solid product that precipitated was the oxidized vanadyl complex (VOL_2). The vanadium(III) complex remained dissolved in the solvent. Attempts were made to isolate the pure complex by extracting the filtrant with CH_2Cl_2. Black solids containing $V(mppt)_3$ or $V(dppt)_3$ could be isolated, but in poor yields (11−21%) with some inseparable impurities and suspect elemental analyses. Any further attempts to recrystallize the complexes resulted in oxidation of the oxovanadium(IV) complexes. Similarly to their oxygen counterparts, the thio-V(III) complexes were also found to be air-sensitive to varying extents. $V(tma)_3$ was found to be the most stable (months), whereas $V(etma)_3$ oxidized in 2−4 days and $V(mppt)_3$ and $V(dppt)_3$ decomposed within 24 h.

Previously, vanadium(III) complexes of the corresponding pyrones and pyridinones have been prepared by in situ reduction of the vanadyl complexes with sodium dithionite.[11,28] For the thio-vanadyl complexes, in situ reduction of vanadyl to vanadium(III) could not be achieved despite several attempts with different reducing agents and solvents. Because the vanadyl complexes could be neither oxidized nor reduced easily under various conditions, it appears that these sulfur-substituted complexes are very stable under ambient conditions.

All of the complexes were characterized by elemental analysis, electrospray ionization (ESI) or liquid secondary ion (LSI) mass spectrometry, magnetic moments, and IR and EPR [for V(IV)] spectroscopies. Elemental analyses of all of the complexes correlated well with the calculated values. For $VO(mppt)_2$, the complex was always isolated as a trihydrate, indicating the increased hydrogen-bonding ability of the N−H protons in the ring. For the N−CH_3 pyridine-

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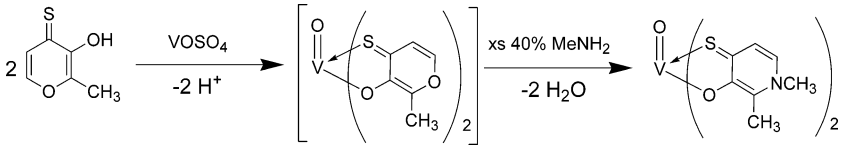
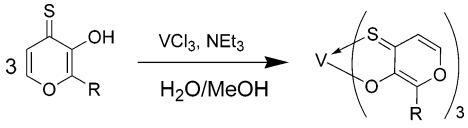
Scheme 2. One-Pot Synthesis of $VO(dppt)_2$ from $VOSO_4$, Htma, and 40% $MeNH_2$



Scheme 3. Syntheses of Tris(ligand)vanadium(III) Complexes



R = CH_3 V(tma)$_3$
R = C_2H_5 V(etma)$_3$

thione, the oxovanadium complex was isolated as a pine-green solid for $VO(dppt)_2$ and as a light-gray complex for the monohydrate $VO(dppt)_2 \cdot H_2O$. The monohydrate could not be converted to the anhydrate even after extensive heating.

Positive ion detection mode mass spectrometry was used to analyze all of the complexes, and diagnostic mass spectra were obtained with the expected isotopic patterns. The most intense peak for the vanadyl complexes corresponded to $[VL_2]^+$; other major peaks in the spectra were the protonated parent-ion peak, $[HVOL_2]^+$, and its fragment, $[VOL]^+$. A characteristic small peak corresponding to $[VL_3]^+$ was also usually observed for vanadyl complexes. The mass spectra of the vanadium(III) complexes were dominated by the parent-ion peak, $[VL_3]^+$, and the characteristic $[VL_2]^+$ peak. The absence of the $[HVOL_2]^+$ peak from the spectra of V(III) complexes confirms that the complex has not been oxidized to the V(IV) species, even in the instrument. A small peak corresponding to the $[V_2L_5]^+$ ion, characteristic of tris-(bidentate ligand) trivalent metal ion complexes, was also observed for some of the complexes.[11]

Diagnostic shifts in the ligand stretches of the IR spectra of both the oxovanadium(IV) and vanadium(III) complexes were observed. In the oxovanadium(IV) and vanadium(III) complexes, the pyranthione vibrations undergo a significant bathochromic shift of $\sim$40$-$50 cm^{-1}, whereas the pyridinethione peaks shift by only $\sim$10$-$23 cm^{-1} to lower energy. This bathochromic shift was also seen for the oxygen analogues.[11,26] The coordination of the ligand to vanadium was confirmed by the disappearance of the strong $\nu_{C=S}$ band and the appearance of a new peak around $\sim$673$-$681 cm^{-1} for V$-$O stretches. The most characteristic difference between the IR spectra of the vanadyl(IV) and vanadium-(III) complexes is the vanadyl stretch $\nu_{V=O}$ at 985 $\pm$50 cm^{-1} in the vanadyl(IV) complexes. The absence of a $\nu_{V=O}$ stretch in the IR spectra of the vanadium(III) complexes confirmed the lack of oxidation [of V(III) to VO^{2+}]. For these thio-vanadyl complexes, the $\nu_{V=O}$ stretch was observed between 962 and 974 cm^{-1}, which is consistent with other similar vanadyl complexes containing an O_2S_2 coordination sphere ($\sim$951$-$965 cm^{-1}).[29,30] Compared to the oxygen analogues, these complexes had lower $\nu_{V=O}$ stretching frequencies, as expected because of the replacement of oxygen with the less-electronegative and heavier sulfur atom.

The experimental magnetic moments of the four vanadyl complexes were found to be in the range of 1.62$-$1.71 μ_B at room temperature, which is consistent with the value predicted from the spin-only formula (1.73 μ_B)[31] of a vanadium(IV) system. These values are slightly lower than those of their corresponding oxygen analogues ($\sim$1.72$-$1.77 μ_B)[12,16,26] but are consistent with the presence of a single unpaired electron and are within the range of the values of other similar $VO(O_2S_2)$ complexes ($\sim$1.69 μ_B).[30,32] The spin-only magnetic moment that is expected for vanadium(III) complexes is 2.83 μ_B.[31] The room-temperature magnetic moments of the pyranthione-V(III) complexes were found to be 2.52$-$2.53 μ_B. These values are typical of octahedral vanadium(III) complexes[33,34] with two unpaired electrons and are in the same range as the corresponding oxygen analogues (2.5$-$2.7 μ_B). Because of the small amounts of products obtained, the magnetic moments of the pyridinethione$-$vanadium(III) complexes could not be obtained.

Room-temperature EPR spectra of the vanadyl complexes show the expected eight-line pattern characteristic of a d^1 V(IV) system. The isotropic EPR spectrum (298 K) of VO-(tma)$_2$ was obtained in toluene (g_{iso} = 1.974, A_{iso} = 85.0 $\times$ 10^{-4} cm^{-1}), and the parameters are remarkably similar to the values reported previously for VO(tma)$_2$ in $CHCl_3$ (g_{iso} = 1.976, A_{iso} = 83.0 $\times$ 10^{-4} cm^{-1}).[35] The frozen-solution X-band EPR spectra of all four thio-vanadyl complexes were recorded. Figure 1 shows the anisotropic EPR spectrum (125 K) of VO(mppt)$_2$ in MeOH/DMF (1:1). DMF was used because of the limited solubility of the complex in MeOH alone. The g and A_z values of all four compounds (Table 1) are within the ranges typical for an O_2S_2 coordination sphere around a vanadyl ion.[36,37] As expected, the g values of the thio compounds are slightly higher than those of their oxygen counterparts ($g_{iso} \approx$ 1.96$-$1.98), but the A values are lower [$A_{iso} \approx$ (90$-$96) $\times$ 10^{-4} cm^{-1}].[16,38] Evidence of cis/trans isomers was seen in the anisotropic spectra, but it was unnecessary to include both isomers to obtain an acceptable simulation fit.

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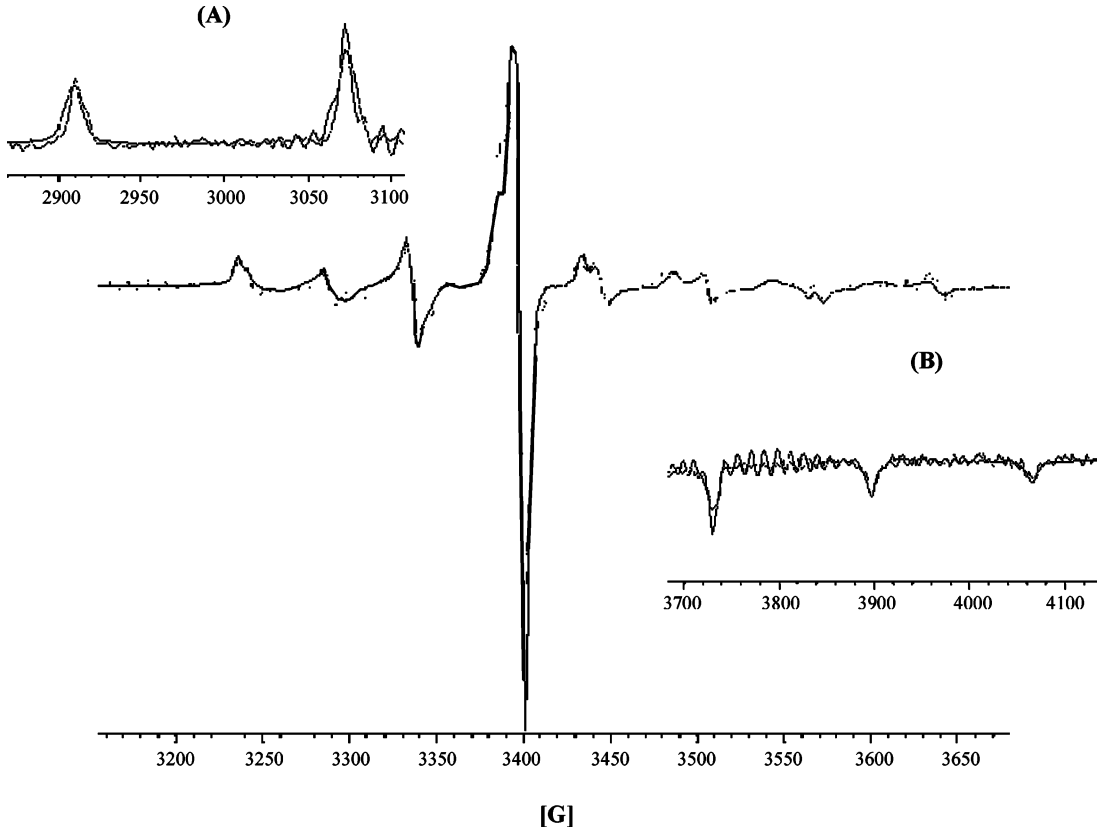


Figure 1. Frozen-solution EPR spectrum of $VO(mppt)_2$ in MeOH/DMF, experimental (dark gray) and simulated (light gray). (A and B) Expansions of the vanadium hyperfine resonances associated with the principal z axis in the spectrum ($T = 125$ K, $\nu = 9.560$ GHz).

Table 1. Spin Hamiltonian Parameters of the Vanadyl Complexes (DMF, $T = 125$ K, $g \pm 0.001$, $A \pm 0.1 \times 10^{-4}$ cm^{-1}

complex	g_x	g_y	g_z	g_{avg}	A_x	A_y	A_z	A_{av}	ν (GHz)
$VO(tma)_2$	1.982	1.982	1.954	1.973	−52.0	−51.5	−153.0	−85.5	9.568
$VO(etma)_2$	1.983	1.983	1.950	1.972	−50.0	−52.0	−150.2	−84.1	9.565
$VO(mppt)_2$[a]	1.985	1.981	1.958	1.975	−42.0	−52.0	−151.0	−81.7	9.560
$VO(dppt)_2$[a]	1.986	1.979	1.957	1.974	−45.2	−51.3	−151.0	−82.5	9.567

[a] MeOH/DMF (1:1), $g_{av} = (g_x + g_y + g_z)/3$, $A_{av} = (A_x + A_y + A_z)/3$.

The solution equilibria of vanadyl (VO^{2+}) with each of the four thio ligands were obtained by potentiometric and spectrophotometric titrations. The acidity constants of the ligands have been described in an accompanying article.[18] The stepwise stability constants of the 1:1 and 1:2 metal-to-ligand species (K_{110} and K_{120}) and the overall stability constant (β_{120}) were determined as defined in eqs 1−5.

$$HL \overset{K_a}{\rightleftharpoons} H^+ + L^- \quad (1)$$

$$VO^{2+} + L^- \overset{K_{110}}{\rightleftharpoons} [(VO)L]^+ \quad (2)$$

$$[(VO)L]^+ + L^- \overset{K_{120}}{\rightleftharpoons} (VO)L_2 \quad (3)$$

$$VO^{2+} + 2L^- \overset{\beta_{120}}{\rightleftharpoons} (VO)L_2 \quad (4)$$

$$(VO)L_2 + OH^- \overset{K_{12-1}}{\rightleftharpoons} [(VO)L_2(OH)]^- \quad (5)$$

The 1:1 vanadyl/pyranthione (Htma and Hetma) species form significantly even at pH less than 2; therefore, variable-pH spectrophotometry was used to determine the 1:1 stability constant (log K_{110}) accurately. High metal-to-ligand ratios (M/L ≈ 12:1) were used, and the absorbance changes were monitored between 290 and 310 nm as the pH was varied from ∼2.4 to 3.8 (at pH > 4, metal hydrolysis can occur). The high metal-to-ligand ratios were used to ascertain that the absorbance changes are due to only the $[(VO)L]^+$ species (Figure 2).

As the ligand is deprotonated and chelated to the vanadyl ion, the absorbance at ∼355 nm decreases, and the absorbance at ∼300 nm increases. The experimental spectrophotometric data were fitted by nonlinear regression analysis. By using mass balance equations, the pH of the solution, and the original analyte concentrations and constants (i.e., K_a and K_h), a theoretical data set could be generated. The resultant mass balance equation in terms of K_{110} and $[(VO)L]^+$ is shown in eq 6 (charges have been omitted for clarity).

$$K_{110} = \frac{K_a K_h + \left(\frac{K_h}{[H]}\right) + K_a[H] + 1}{\frac{[VO]_t L_t}{[(VO)L]} - [VO]_t - L_t + [(VO)L]} \quad (6)$$

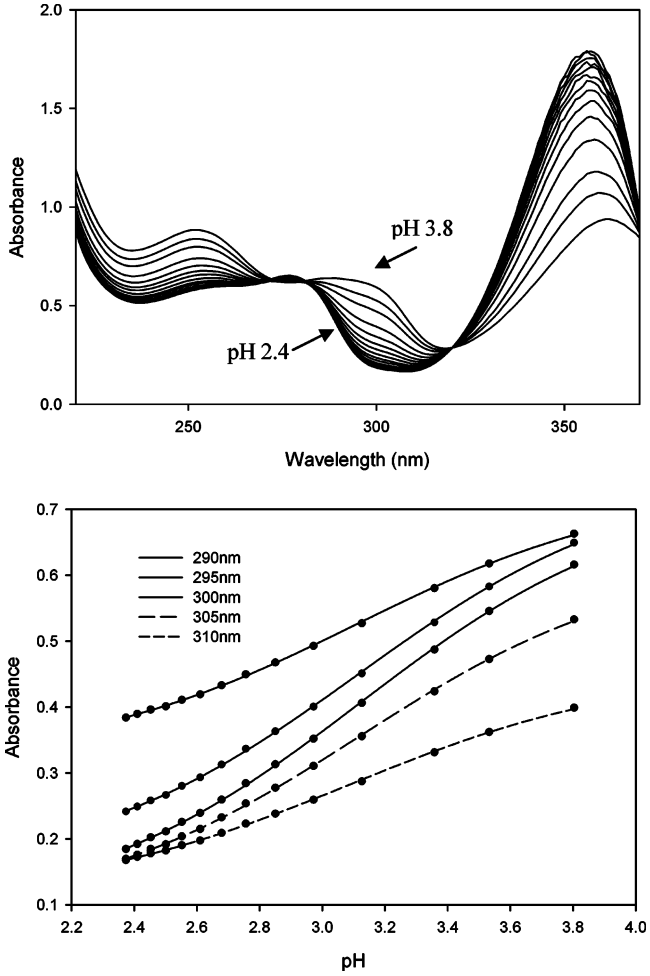


Figure 2. (Top) Variable-pH UV spectrophotometric titration of VO^{2+}/Htma ($[VO^{2+}] = 1.08$ mM, [Htma] = 0.09 mM) at 25 °C and $I = 0.16$ M NaCl. (Bottom) Experimental (data points) and calculated (lines) absorbance values vs pH for the determination of log K_{110} at different wavelengths.

K_a is the acidity constant of the HL ligand, $[VO]_t$ is the total concentration of vanadyl, L_t is the total concentration of the ligand, [H] is the acid concentration derived from the pH of the solution, [(VO)L] is the concentration of the 1:1 vanadyl/pyranthione species at a given pH, and $K_h = 2.14 \times 10^{-6}$ is the first hydrolysis constant of VO^{2+} (eq 7).

$$K_h = \frac{[(VO)OH][H]}{[VO]} \tag{7}$$

Rewriting eq 6, the equation can be solved for [(VO)L] in terms of K_{110} using the quadratic formula (eq 8).

$$[(VO)L] = \frac{-B \pm \sqrt{B^2 - 4(K_{110})(K_{110}L_tM_t)}}{2K_{110}} \tag{8}$$

where

$$B = K_{110}L_t + K_{110}M_t + K_aK_h + \left(\frac{K_h}{[H]}\right) + K_a[H] + 1$$

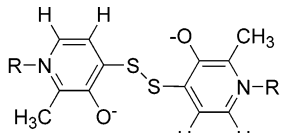
The concentration of (VO)L can be calculated from the Beer−Lambert law: $[(VO)L]^+ = abs/\epsilon$. Therefore, iterating K_{110} and ϵ as a function of pH results in the least-squares fitting of the theoretical absorbance values to the experimental data. Five wavelengths with 14 data points in the range of $2.4 \leq pH \leq 3.8$ were fitted with calculated curves, and the averages of at least three separate trials were taken to obtain the final log K_{110} values of $VO(tma)^+$ and $VO(etma)^+$. Figure 2 (bottom) shows the experimental data (symbols) and calculated fit (solid lines) for one of the trials of the vanadyl thiomaltolato system.

Table 2. Stability Constants of the Species Formed between V(IV) (as VO^{2+}) and the Various (Pro)Ligands HL[a]

	Htma	Hetma	Hmppt	Hdppt
pK_a[b]	8.12(1)	8.20(1)	8.92(1)	9.44(1)
log K_{110}	8.04(2)[c]	8.07(2)[c]	9.2(1)	7.95(2)
log K_{120}	5.86(2)	6.53(1)	6.9(1)	5.58(8)
log β_{120}	13.9(2)	14.6(1)	16.1(1)	13.53(8)
log K_{12-1}	6.39(4)	5.9(1)	5.53(5)	5.03(4)

[a] Potentiometric titrations, $I = 0.16$ M NaCl, 25 °C. Values in parentheses refer to the error (3σ) in the last digit. [b] Reference 18. [c] Spectrophotometric titrations.

For the pyranthiones, log K_{110} values were fixed for working up the potentiometric titration data to obtain K_{120} and K_{12-1}. For the pyridinethiones, Hmppt and Hdppt, both log K_{110} and log K_{120} were obtained from potentiometric titrations. For the VO^{2+}/Hmppt system, most of $[(VO)L]^+$ did form at pH less than 2, but spectrophotometric titrations could not be done with this system because of problems with ligand dimerization. The pyridinethiones have a tendency to tautomerize to the thiol form; in the presence of water and molecular oxygen, two adjacent molecules in their thiol forms can oxidatively dimerize to form a disulfide bond. Because there is a higher concentration of the ligand precursor in spectrophotometric titrations (as compared to potentiometric titrations), the UV−vis spectrum indicated dimerization before the ligand precursor could be deprotonated and complexed to oxovanadium(IV). Therefore, the values given here for the VO^{2+}/Hmppt system are the best possible estimates from potentiometric titrations.



R = H, 4,4'-dithiobis(2-methylpyridinium-3-oxide), Hmppt-dimer
R = CH_3, 4,4'-dithiobis(1,2-dimethylpyridinium-3-oxide), Hdppt-dimer

The titrations were carried out at $3 < pH < 11$ (25 °C). Vanadyl hydrolysis was included in the model using constants from the literature.[20] As seen in the oxygen analogues,[12,16] a hydroxo complex, $[(VO)L_2(OH)]^-$ [incorporating a deprotonated water molecule coordinated to $(VO)L_2$], was also formed at higher pH in addition to the $[(VO)L]^+$ and $(VO)L_2$ species. The stepwise formation constants, log K_{110}, log K_{120}, and log K_{12-1}, as well as the overall stability constants, log β_{120}, of all four thio-vanadyl systems are listed in Table 2.

Figure 3 shows the speciation diagrams of the VO^{2+}/Hetma and VO^{2+}/Hdppt systems in the $2 < pH < 10$ range. These diagrams were calculated for a VO^{2+}/L ratio of 1:3 and were

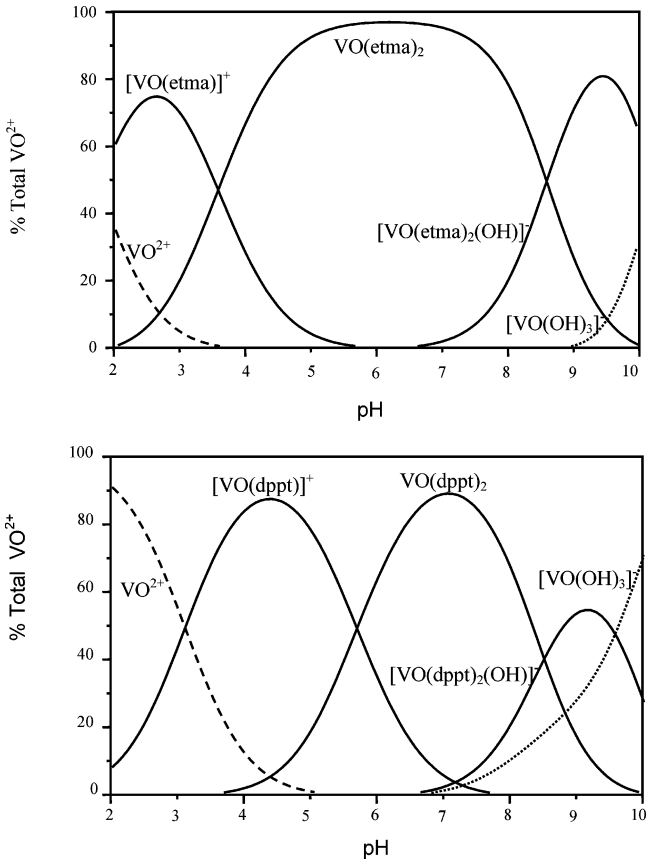


Figure 3. Speciation diagrams of VO^{2+}/etma (top) and VO^{2+}/dppt (bottom) as a function of percent VO^{2+} versus pH (I = 0.16 M NaCl, T = 25 °C, ligand/metal ratio ≥ 3:1).

Table 3. Comparison of the Overall Stability Constants of the Thio-Vanadyl Complexes and Their Oxygen Analogues

	log β_{120}	ref
$VO(tma)_2$	13.9(2)	this work
$VO(ma)_2$	16.31(3)	12
$VO(etma)_2$	14.6(1)	this work
$VO(ema)_2$	16.41(3)	16
$VO(mppt)_2$	16.1(1)	this work
$VO(dppt)_2$	13.53(8)	this work
$VO(dpp)_2$	22.25(8)	38

based on the stability constants listed in Table 2. The diagrams clearly indicate that the dominant species at physiological pH ≈ 7 are $(VO)L_2$ and $[(VO)L_2(OH)]^-$. For the biological activity of a complex, it is desired that the bis complex species stays intact at high pH, a result seen in the speciation diagrams. The anionic hydroxo complex could, however, hinder the cellular absorption of the complex.

Compared to those of their oxygen analogues, the overall stability constants of the thio-vanadyl complexes are lower (Table 3). This was expected because vanadium is a hard acid and sulfur is a soft donor. Although the mixed *O,S* bidentate ligands are strong chelators (based on log β_{120} values), they are weaker than the corresponding *O,O* bidentate ligands. Because free vanadyl might be responsible for the insulin-enhancing effect, a slightly weaker coordinated ligand might release the metal ion at the appropriate site more easily than the more strongly chelating ligand, hence

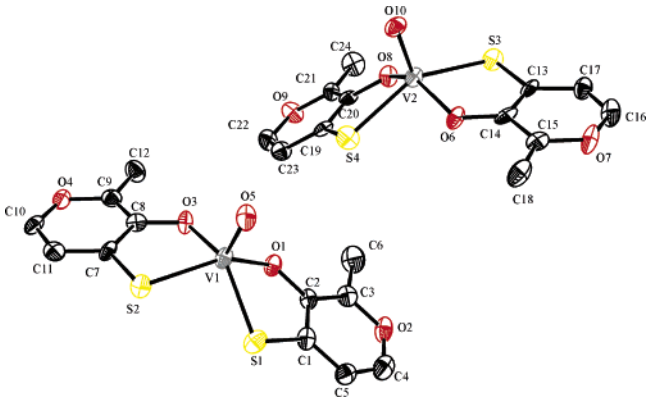


Figure 4. ORTEP diagrams of *cis*- and *trans*-$VO(tma)_2$ (50% thermal ellipsoids).

Table 4. Selected Bond Lengths (Å) in $VO(tma)_2$

cis-$VO(tma)_2$		*trans*-$VO(tma)_2$	
V(1)−O(5)	1.614(5)	V(2)−O(10)	1.610(5)
V(1)−O(1)	1.958(5)	V(2)−O(6)	1.949(5)
V(1)−O(3)	1.936(5)	V(2)−O(8)	1.954(5)
V(1)−S(1)	2.409(3)	V(2)−S(3)	2.432(3)
V(1)−S(2)	2.403(3)	V(2)−S(4)	2.429(3)
C(1)−S(1)	1.698(8)	C(13)−S(3)	1.686(8)
C(7)−S(2)	1.712(8)	C(19)−S(4)	1.715(7)
C(2)−O(1)	1.331(9)	C(14)−O(6)	1.328(9)
C(8)−O(3)	1.315(9)	C(20)−O(8)	1.325(9)
C(1)−C(2)	1.403(10)	C(13)−C(14)	1.429(11)
C(7)−C(8)	1.419(11)	C(19)−C(20)	1.410(10)

Table 5. Selected Bond Angles (deg) in $VO(tma)_2$

cis-$VO(tma)_2$		*trans*-$VO(tma)_2$	
S(2)−V(1)−O(5)	106.8(2)	S(4)−V(2)−O(10)	105.9(2)
S(2)−V(1)−O(3)	83.58(17)	S(4)−V(2)−O(8)	82.15(17)
S(2)−V(1)−O(1)	144.48(17)	S(4)−V(2)−O(6)	87.88(18)
S(1)−V(1)−O(5)	106.3(2)	S(3)−V(2)−O(10)	107.4(2)
S(1)−V(1)−O(3)	144.43(18)	S(3)−V(2)−O(8)	85.90(17)
S(1)−V(1)−O(1)	82.61(17)	S(3)−V(2)−O(6)	82.78(18)
S(2)−V(1)−S(1)	86.73(9)	S(4)−V(2)−S(3)	146.70(10)
O(5)−V(1)−O(3)	109.3(3)	O(10)−V(2)−O(6)	109.9(2)
O(5)−V(1)−O(1)	108.8(3)	O(10)−V(2)−O(8)	107.9(2)
O(3)−V(1)−O(1)	85.7(2)	O(6)−V(2)−O(8)	142.2(2)
V(1)−S(1)−C(1)	95.7(3)	V(2)−S(3)−C(13)	96.4(3)
V(1)−S(2)−C(7)	95.8(3)	V(2)−S(4)−C(19)	96.7(3)
V(1)−O(1)−C(2)	119.7(5)	V(2)−O(6)−C(14)	121.3(5)
V(1)−O(3)−C(8)	122.1(5)	V(2)−O(8)−C(20)	122.0(5)

increasing drug potency. This can be proven only after a detailed study of the mechanism of action of these complexes in vivo.

An X-ray crystal structure of $VO(tma)_2$ was also obtained. Green needle-shaped crystals of $VO(tma)_2$ suitable for X-ray diffraction were grown by mixing warm solutions of $VO(acac)_2$ in MeOH and Htma in THF (VO^{2+}/Htma in a 1:2 ratio) followed by slow evaporation of the resulting solution. The material crystallizes with both the cis and trans (1:1) isomers in the asymmetric unit (Figure 4). To our knowledge, this is the first example of a trans isomer of a square-pyramidal vanadyl complex with an O_2S_2 coordination sphere. All of the other similar complexes have been found to form only the cis geometry in the solid state.[29]

Selected bond lengths and angles of both isomers are listed in Tables 4 and 5. In both isomers, the two thiomaltolato ligands are arranged around the base of the square pyramid with the V═O linkage perpendicular. The vanadium atom

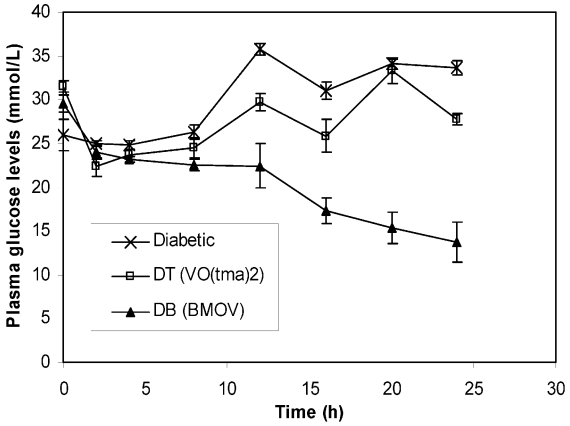


Figure 5. Plasma glucose levels in two treated groups of STZ rats compared to a diabetic group. (For all other DT groups, please see the Supporting Information.)

is slightly displaced from the basal plane [V(1) = 0.656(3) Å, V(2) = 0.664(2) Å]. The V=O bond distance is the same in both isomers (cis, trans 1.61 Å), and the value for the cis isomer of $VO(tma)_2$ matches the V=O distances that were reported for other *cis*-$VO(O_2S_2)$ structures {e.g., V=O is 1.691(2) Å for $[VO(mmp)_2]^{2-}$, where $mmpH_2$ = 2-mercapto-4-methylphenol}.[29] Compared to the structure of its oxygen analogue, $VO(ma)_2$, the V=O bond length in $VO(tma)_2$ is longer [1.597(7) Å) in $VO(ma)_2$], as suggested by the IR data (vide supra).

Biological Studies

None of the treated rats showed any overt signs of toxicity (noted principally as gastrointestinal distress in other studies), nor were there significant changes in body weight over the course of the 72-h screening, either by oral gavage or ip. The only significant glucose lowering observed was in BMOV-treated rats; all of the others showed little or no effect of treatment up to the 24-h time point (Figure 5). No further changes were noted for the duration of the screening assay. As compared to the diabetic rats and diabetic rats treated with BMOV, these thio-vanadium complexes did not produce significant glucose lowering ($p < 0.05$) under these conditions (Figures S1−S3).

During the acute screening trials, however, it was noted that, for $VO(tma)_2$, the rats excreted unusually bright yellow urine 4 h postadministration. Because the ligand precursor, thiomaltol, is a bright-yellow compound, it was predicted that exceedingly fast decomposition of the complex and faster excretion of vanadium (as compared to that from BMOV) in the urine might be the reason that no significant glucose lowering was observed for the vanadium complexes with thio-substituted ligands. To test the hypothesis that thio substitution promoted rapid excretion of both vanadium and the ligand precursor, we measured urinary vanadium excretion (GFAAS) over a 72-h period in three treated rats and also analyzed urine samples by mass spectrometry (ESIMS). Electrospray mass spectrometry was used to determine the molecular weight of the major components of the urine samples. The major peak in all of the spectra corresponded to thiomaltol (m/z = 143, $[M + 1]^+$) with the expected isotope pattern. No peaks corresponding to $VO(tma)_2$ or $VO(tma)^+$ were observed, suggesting that the complex was not excreted intact but was absorbed and rapidly decomposed before excretion.

Figure S4 shows the plot of amount of V (in milligrams) per sample and percent administered dose (%AD) versus time (in hours) for rat 2 as an example with a clear pattern of excretion (total %AD excreted by the other two rats was <5% each at 24 h). For rat 2, the amount of vanadium excreted (~30%) with respect to the dose administered was comparable to that reported in other studies in which vanadium excretion was estimated.[39−41] Sabbioni and Marafante studied the metabolic patterns of vanadium in rats for 21 days.[41] Measuring the excretion of vanadium in urine (and feces), they found that ~32% of the iv (intravenous) administered dose of vanadium was excreted in 2−3 days. Heinemann and co-workers studied the pharmacokinetics of vanadium in five healthy volunteers and found that 52% of the (iv) administered dose of vanadium was recovered in the urine after 12 days.[42] The data for these studies suggests that the peak excretion occurs only after ~24 h. Low vanadium recovery precluded any definitive pharmacokinetic profiling; however, the appearance of ligand-precursor peaks, with no apparent intact complex, suggests that rapid dissociation of the complex following injection had occurred.

While the present work was in progress, Sakurai and coworkers[25] reported positive glucose-lowering results of an in vivo trial of $VO(dppt)_2$ administered ip (10 mg V d^{-1}) to STZ-diabetic rats for 21 d, suggesting that, given a long enough treatment period, this compound might yield therapeutically relevant relief of diabetic symptomatology.

Conclusions

Four vanadium(IV) and vanadium(III) complexes were synthesized with each of four mixed *O,S* donor ligands, two pyranthiones and two pyridinethiones. Although the synthesis of the vanadyl complexes was uncomplicated, the vanadium(III) complexes were air-sensitive, and purification of the product was difficult in some cases. At physiological pH, the dominant forms of all four vanadyl complexes are $(VO)L_2$ and $(VO)L_2(OH)$, as expected (and desired). A crystal structure determination of $VO(tma)_2$ elucidated both the cis and trans isomers in the same asymmetric unit, which was not heretofore observed for vanadyl complexes with mixed *O,S* ligation. No significant glucose lowering by thio-vanadyl or V(III) complexes was apparent. Hence, for this model, sulfur substitution did not increase the efficacy of the vanadium complexes as insulin-enhancing agents as compared to BMOV.

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Supporting Information Available: Crystallographic data for $VO(tma)_2$, bond lengths of *cis*- and *trans*-$VO(tma)_2$, bond angles of $VO(tma)_2$, and plasma glucose levels of the DT groups. This material is available free of charge via the Internet at http://pubs.acs.org.

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