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Dimeric W_3SO_3 Cluster Complexes: Synthesis, Characterization, and Potential Applications as X-ray Contrast Agents

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Our continued research on the use of heavy metal cluster complexes as a new class of X-ray contrast agents in medical diagnostic imaging is described. A series of 2:3 cluster−ligand complexes, $[(W^{IV}_3SO_3)_2L_3]^{4-}$ (L = linear polyaminopolycarboxylate ligands), were isolated from the reaction of aqua ion $[W^{IV}_3SO_3(H_2O)_9]^{4-}$ (prepared in large quantities through an improved literature process) with respective ligands in refluxing DMF. The salts of $[(W^{IV}_3SO_3)_2L_3]^{4-}$ complex anions were fully characterized using routine techniques such as elemental analysis, MS, HPLC, UV−vis, IR, and NMR. The solid structures of two complex anions, $[(W^{IV}_3SO_3)_2(PDTA)_3]^{4-}$ and $[(W^{IV}_3SO_3)_2(HO{-}PDTA)_3]^{4-}$, were determined by X-ray crystallography. They are the first examples wherein two $W^{IV}_3SO_3$ clusters are complexed and linked by three ligands that contain two terminal iminodiacetate (bis-IDA) groups. Complexation of the unstable aqua ion $[W^{IV}_3SO_3(H_2O)_9]^{4-}$ with ligands has imparted desired biological compatibility to the tungsten metal cluster. These complexes are stable and highly soluble in H_2O. The potential utility of such tungsten cluster complexes as X-ray contrast agents was evaluated in both in vitro and in vivo animal studies. In addition, the syntheses of several new linear polyaminopolycarboxylate ligands used in this study are reported.

Introduction

Following the discovery of X-ray radiation by W. C. Roentgen more than a century ago,[1] X-ray imaging procedures have became a group of widely accepted noninvasive, versatile diagnostic techniques to study internal anatomy and disease states in patients.[2] To be clinically useful in resolving lesions from normal tissue, X-ray contrast agents are frequently administrated to patients during such procedures.[3−5] Examples of currently approved X-ray contrast media include $BaSO_4$, an insoluble agent used exclusively for gastrointestinal tract imaging; and water-soluble, nonionic iodinated agents such as Iohexol and Iodixanol, which are based on the 1,3,5-triiodobenzene platform and used in many intravascular applications such as angiography and CT procedures.[6]

The search for a new generation of water-soluble X-ray contrast agents has led us to explore the use of heavy metal ($Z > 53$) cluster complexes, based on their superior X-ray attenuation characteristics when compared to iodine-based agents.[6−9] The cuboidal, trinuclear tungsten cluster aqua ion $[W^{IV}_3S_4(H_2O)_9]^{4+}$ was selected as an entry point for this application based on its synthetic accessibility, chemical stability, and presumed ability to strongly bind polyaminopolycarboxylate based ligands (Figure 1, with their usual abbreviations[10]).[11,12] The chloride salt of the aqua ion, $[W^{IV}_3S_4(H_2O)_9]Cl_4$ (**1**), was prepared in large quantities through an improved method.[8] The reaction of **1** with TTHA in refluxing DMF produced a complicated mixture from which the 1:1 cluster−ligand complex, $Na_2[W^{IV}_3S_4(TTHA)]$ (**2**), was isolated and characterized.[9] The reaction of **1** with EGTA under similar conditions produced $Na_4[(W^{IV}_3S_4)_2(EGTA)_3]$ (**3**) in slightly better yields and whose 2:3 (cluster−ligand) complex anion structure was determined by X-ray crystallography.[8] Complexation of the unstable aqua ion **1** with these two polyaminopolycarboxylate ligands imparted improved biological properties to the metal cluster. The tungsten cluster complexes **2** and **3** are water soluble and stable under a wide range of pH conditions, exhibited low acute toxicity in mice, and demonstrated sufficient efficacy in both in vitro and in vivo X-ray contrast experiments.[6−9] Two potential benefits derived from this class of X-ray contrast media include (1) a level of X-ray

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(10) Abbreviations: CT, computed tomography; TTHA, triethylenetetraamine hexaacetate; EGTA, ethyleneglycoldiamine tetraacetate; EDTA, ethylenediamine tetraacetate; PDTA, propylenediamine tetraacetate; HO-PDTA, 2-hydroxy-1,3-propylenediamine tetraacetate; NMG, *N*-methyl-D-glucamine.

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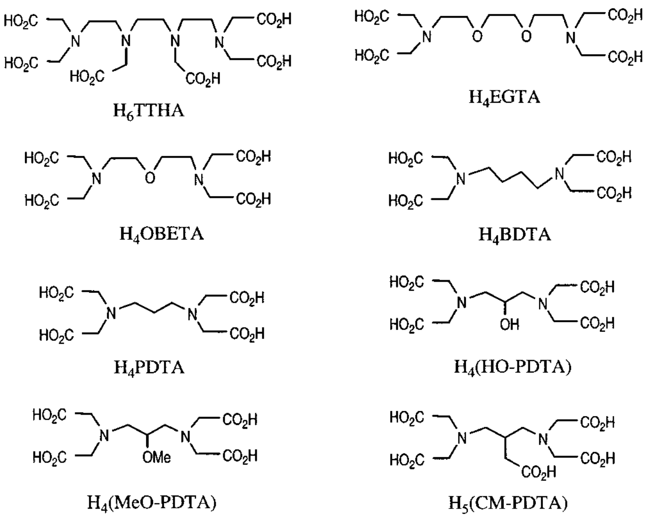

Figure 1. Schematic representations of linear polyaminopolycarboxylate ligands.

contrast enhancement that is unattainable from the current agents at comparable doses; (2) reduced radiation exposure to patients by using a higher energy X-ray beam to take the advantage of the higher K-edge for tungsten.

Recently, our research effort in this area was extended to another symmetric aqua ion, $[W^{IV}{}_3SO_3(H_2O)_9]^{4+}$, in the $[W^{IV}{}_3S_xO_{4-x}]^{4+}$ ($x = 0-4$) series.[13-21] In this report, we describe an improved, large-scale synthesis of its chloride salt, $[W^{IV}{}_3SO_3(H_2O)_9]Cl_4$ (**4**), the synthesis of some uncommon linear polyaminopolycarboxylate ligands with two terminal iminodiacetate groups (bis-IDA), and the preparation of a series of 2:3 cluster−ligand complexes with the $W^{IV}{}_3SO_3$ core. These dimeric trinuclear tungsten cluster complexes were fully characterized using routine analytical techniques and the solid state structures of two representatives were determined by X-ray crystallography. The results of limited biological studies will also be reported.

Experimental Section

Materials and Synthesis. All chemicals were used as received. The water content in purified products was determined by a TGA method described earlier.[8] The purity of the cluster complexes was routinely determined by HPLC using the following conditions: ion-pairing reverse phase, C18 column; mobile phase: aqueous MeOH containing 10 mM tetrabutylammonium bromide (TBAB) gradient, monitored at 240 nm.

Preparation of Aqua Ion. $[W_3SO_3(H_2O)_9]Cl_4$ (4). This compound was prepared by a modified literature procedure.[14] To a flask containing $W(CO)_6$ (90 g) and Na_2S (36 g) under a blanket of N_2 was added acetic anhydride (3 L). The flow of N_2 was stopped and the mixture stirred with a mechanical stirrer. The mixture was heated at reflux for 72 h, and the resulting black solution was cooled to room temperature and filtered. The solid collected (dark brown powder and clear needles, which can be stored in the freezer for later workup) was taken up in 12 N HCl (100 mL) and stirred briefly. The mixture was allowed to stand for 2.5 h, diluted with H_2O (1 L) and then filtered. The filtrate was concentrated under vacuum in a water bath (below 50 °C) to a volume of 200 mL. Half of this solution (100 mL) was loaded onto a Sephadex (G-25, 500 g) column and eluted with 2 N HCl, yielding three distinctly colored fractions: a pale orange band, followed by a ruby red/brown band, and finally a purple band. Following a similar purification of the other half, the ruby red/brown fractions were combined and concentrated to dryness in vacuo to give 30.2 g (37.9%) of **4** as a red solid. In addition, from the pale orange fractions, 3 g of orange solid was obtained, which contains 50% of **4**; and from the purple fractions, 7 g of purple solid was obtained, which contains 75% of **4**. The later two solids can be treated again with Sephadex columns to increase the yield of **4**. UV−vis: λ_{max}, nm (ϵ, $M^{-1}\,cm^{-1}$): 460 (360). Anal. Calcd for $[W_3SO_3(H_2O)_9]Cl_4$ ($Cl_4H_{18}O_{12}SW_3$): S, 3.43; W, 58.95. Found: S, 3.63; W, 59.40.

Preparation of Ligands. Oxybis(ethylamine)tetraacetic acid (H_4OBETA, 5). To a solution of chloroacetic acid (13.3 g, 141 mmol) in 15 mL of 30% NaOH was added 2,2′-oxybis(ethylamine) dihydrochloride (5.0 g, 28.2 mmol) with stirring. Then additional 30% NaOH was added to the reaction mixture to maintain its pH between 10 and 11.9. After 2.5 h at room temperature, the reaction mixture was heated at 40 °C for 3 h, then at 90 °C for 15 h until no more NaOH was need to maintain the pH. ^1H NMR analysis indicated the reaction to be complete at this stage. The product was then loaded onto an AG1X8 (acetate form) ion exchange resin column and eluted with 4 N HOAc and then 5 N HOAc. The fractions containing desired product were combined and treated with aqueous NaOH to adjust the pH to basic, and the solution was subjected to a second anion chromatography procedure. Rotary evaporation of the eluate afforded 6 g (63%) of **5** as a white foam. ^1H NMR (D_2O, δ, ppm): 3.42 (t, 4H); 3.67 (t, 4H); 3.89 (s, 8H). ^{13}C NMR (D_2O, δ, ppm): 55.3, 57.4, 65.0, 170.0. Anal. Calcd for $H_4OBETA\cdot 0.1H_2O$ ($C_{12}H_{20.2}N_2O_{9.1}$): C, 42.63; H, 6.02; N, 8.29. Found: C, 42.54; H, 5.94; N, 8.34.

2-Methoxy-1,3-propylenediamine Tetraacetic Acid (H_4MeO-PDTA, 6). The amino groups of 2-hydroxy-1,3-diaminopropane were protected in quantitative yield using $(BOC)_2O$. This product, in anhydrous tetrahydrofuran, was sequentially treated with NaH (1.1 equiv) for 1 h and then MeI (2 equiv) at 0 °C. The mixture was allowed to warm to ambient temperature overnight. The product was isolated in 43% yield following a silica gel chromatography procedure, eluting with 10−20% ethyl acetate in hexane. The protecting groups were removed by stirring overnight at room temperature with 6 N HCl, and the intermediate diamine was alkylated with bromoacetic acid at pH 9−10 overnight. The crude ligand was purified by chromatography on AG1X8 resin (OH-form), eluting with 10−15 N acetic acid. Compound **6** was obtained as a white foam in 90% yield after evaporation to dryness of the eluate. MS (FAB, $[M + H]^+$): calcd 337, found 337. ^1H NMR (D_2O, δ, ppm): 3.7 (s, 9H); 3.3 (m, 4H); 3.1 (s, 3H).

2-Carboxymethyl-1,3-propylenediamine Tetraacetic Acid (H_5CM-PDTA, 7). To a solution of malononitrile (30 g, 0.45 mol) in anhydrous tetrahydrofuran (3 L) was added NaH (14.4 g, 60%, 0.36 mol), and the mixture was stirred for 15 min. Benzyl bromoacetate (57 mL, 0.36 mol) in tetrahydrofuran (500 mL) was added slowly dropwise at 0 °C and the mixture stirred overnight. The solution was filtered and the filtrate evaporated to give an orange gum. This material was treated with ethanol to yield a white solid (12.5 g) whose ^1H NMR spectrum indicated the product to be a mixture of 93% monoalkylated malononitrile and 7% of the dialkylated product. A second treatment with ethanol increased the purity of monoalkylated malononitrile to 97%. The monoalkylated compound in HOAc was reduced overnight in a Parr hydrogenator using Perlman's catalyst to yield 2-carboxymethyl-1,3-propanediamine as a pale yellow gum. MS (FAB, $[M + H]^+$): calcd 133, found 133. This product was alkylated using the method described to prepare **6** (5 equiv of *tert*-butylbromoactetate was used since the carboxyl group is also alkylated) to yield the ester form of the ligand. The product was purified by silica gel chromatography, eluting with 2−3% MeOH in CH_2Cl_2 to give a colorless, viscous oil. The oil was hydrolyzed to the free ligand by stirring overnight in 6 N HCl. Further purification with anionic chromatography on AG1X8 resin, eluting with

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6 N HCl, afforded the title compound **7** as a colorless foam after evaporation of solvent. MS (FAB, $[M + H]^+$): calcd 365, found 365. ^{1}H NMR (D_2O, δ, ppm): 3.8, 3.7, 3.0, 2.2. ^{13}C NMR (D_2O, δ, ppm): 184, 181, 63, 61.5, 43, 34.

1,4-Butylenediamine Tetraacetic Acid (H_4BDTA, 8). A solution of 6.0 g of 1,4-diaminobutane, 56.4 g (4.25 equiv) of *tert*-butylbromoacetate and 77 g of trisodium phosphate in 200 mL of acetonitrile was heated to reflux overnight with mechanical stirring. TLC analysis of the reaction mixture on the next day showed one major spot with several minor impurities. The mixture was filtered, and the salt cake was washed with CH_3CN and discarded. The filtrate was evaporated to dryness to give a thick yellow oil, which was then diluted with methanol and treated with 6 N HCl (200 mL). After stirring for 1 h, the solution was evaporated to dryness and again treated with 6 N HCl for 1 h. The solution was evaporated to dryness, and the acid was completely removed with repeated addition of water and rotary evaporation to give 34 g of solid; ^{1}H NMR indicated approximately 98% deprotection. A portion of the crude *tert*-butyl ester was chromatographed on silica gel, eluting with 3−4% MeOH in CH_2Cl_2 to afford a pure sample of the ester. FAB MS (FAB, $[M + H]^+$): calcd 545, found 545. This ester was hydrolyzed with triflic acid to yield the free carboxylic acid ligand. The final product **8** was purified by dissolution in 2 N NH_4OH followed by precipitation with 12 N HCl. MS (FAB, $[M + H]^+$): calcd 321, found 321. ^{1}H NMR (D_2O, δ, ppm): 2.8 (s, 8H); 2.2 (s, 4H); 1.1 (s, 4H). ^{13}C NMR (D_2O, δ, ppm): 181, 60, 56, 25. Anal. Calcd for $H_4BDTA\cdot 1.29HCl\cdot 1.10H_2O$ ($C_{12}H_{23.49}N_2O_{9.1}Cl_{1.29}$): C, 37.23; H, 6.12; N, 7.34. Found: C, 37.22; H, 6.06; N, 7.17.

Preparation of Cluster Complexes. $Na_4[(W_3SO_3)_2(EGTA)_3]$ (9). To a solution of 5.0 g of $[W_3SO_3(H_2O)_9]Cl_4$ in 700 mL of DMF was added 3.0 g of H_4EGTA solid. The mixture was refluxed for 3 h, whereupon a red solid separated. The solid was collected by filtration and dissolved in H_2O. The solution was passed through an AG50X8 cation exchange column (Na^+ form), and the eluate was evaporated to dryness under vacuum. The resulting red solid was dissolved with a minimal amount of water, and the solution was loaded on a Sephadex G-25 column. Elution with water yielded three bands. The last band was collected, dried under vacuum, and again passed through a Sephadex G-25 column, as described above. The third band was collected and concentrated under vacuum to give 1.0 g (14.0%) of red solid product **9** as a 10 H_2O hydrate. UV−vis: λ_{max}, nm (ϵ, M^{-1} cm^{-1}): 475 (1250). ^{1}H NMR (D_2O, δ, ppm): 4.26 (d, 12H), 4.07 (d, 12H), 3.55 (t, 12H), 3.44 (t, 12H), 3.35 (s, 12H). ^{13}C NMR (D_2O, δ, ppm): 181.0, 70.3, 66.8, 64.1. ^{183}W NMR (D_2O, δ, ppm in reference to Na_2WO_4): 1206.5. MS (FAB, $[M + Na]^+$): calcd 2507, found 2507. Anal. Calcd for $Na_4[(W_3SO_3)_2(EGTA)_3]\cdot 10H_2O$ ($C_{42}H_{80}N_6O_{46}Na_4S_2W_6$): C, 18.93; H, 3.03; N, 3.15; Na, 3.45; S, 2.41; W, 41.1. Found: C, 18.74; H, 2.88; N, 3.08; Na, 3.44; S, 2.43; W, 39.8.

$Na_4[(W_3SO_3)_2(OBETA)_3]$ (10). To a solution of 3.8 g of $[W_3SO_3(H_2O)_9]Cl_4$ in 400 mL of DMF was added 1.83 g of H_4OBETA solid. After refluxing for 3 h, a red-orange solid separated. The solid was collected, and 2-propanol was added to the filtrate, causing the precipitation of additional solid. Two batches of solid were combined and treated by cation exchange and size exclusion chromatographic techniques as described in the preparation of **9**. The pure product **10**, 1.8 g (35.0%), was obtained as a red solid (a 10 H_2O hydrate). UV−vis: λ_{max}, 475 nm. ^{1}H NMR (D_2O, δ, ppm) 4.28 (d, 12H), 4.17 (d, 12H), 3.55 (t, 12H), 3.39 (t, 12H). ^{13}C NMR (D_2O, δ, ppm) 182.0, 67.9, 65.3. ^{183}W NMR (D_2O, δ, ppm) 1203.5. MS (FAB, $[M + Na]^+$): calcd 2374.8, found 2374. Anal. Calcd for $Na_4[W_3SO_3)_2(OBETA)_3]\cdot 10H_2O$ ($C_{36}H_{68}N_6O_{43}Na_4S_2W_6$): C, 17.08; H, 2.71; N, 3.32; Na, 3.63; S, 2.53; W, 43.56. Found: C, 16.98; H, 2.49; N, 3.34; Na, 3.18; S, 2.70; W, 43.77.

$Na_4[(W_3SO_3)_2(BDTA)_3]$ (11). To a solution of 4.0 g of $[W_3SO_3(H_2O)_9]Cl_4$ in 400 mL of DMF was added 2.54 g of H_4BDTA solid. The mixture was refluxed for 4 h, and a red solid separated. This reaction mixture was treated with a workup procedure similar to that described for **10**. The desired product **11**, 1.1 g (20.4%), was obtained as a red solid (a 12 H_2O hydrate). UV−vis: λ_{max}, 475 nm. ^{1}H NMR (D_2O, δ, ppm): 4.17 (d, 12H), 3.92 (d, 12H), 3.15 (b, 12H), 1.28 (b, 12H).^{183}W NMR (D_2O, δ, ppm): 1209. MS (FAB, $[M + Na]^+$): calcd 2326.8, found 2327. Anal. Calcd for $Na_4[(W_3SO_3)_2(BDTA)_3]\cdot 12H_2O$ ($C_{36}H_{72}N_6O_{42}Na_4S_2W_6$): C, 17.16; H, 2.88; N, 3.33; Na, 3.65; S, 2.54; W, 43.77. Found: C, 16.93; H, 2.96; N, 3.15; Na, 3.50; S, 2.51; W, 44.29.

$Na_4[(W_3SO_3)_2(PDTA)_3]$ (12). To a solution of 2.0 g of $[W_3SO_3(H_2O)_9]Cl_4$ in 160 mL of DMF was added 0.91 g of H_4PDTA solid. The mixture was refluxed for 4 h, and a red solid separated. This reaction mixture was treated with a workup procedure similar to that described for **10**. The pure product **12**, 1.2 g (45.6%), was obtained as a red solid (an 11 H_2O hydrate). UV−vis: λ_{max}, 475 nm. ^{1}H NMR (D_2O, δ, ppm): 4.30 (d, 12H), 4.09 (12, 4H), 2.93 (m, 12H), 2.07(m, 6H). ^{13}C NMR (D_2O, δ, ppm): 182.6, 66.8, 63.9, 19.2. ^{183}W NMR (D_2O, δ, ppm): 1189. MS (FAB, $[M + H]^+$): calcd 2262.8, found 2262. Anal. Calcd for $Na_4[(W_3SO_3)_2(PDTA)_3]\cdot 11H_2O$ ($C_{33}H_{64}N_6O_{41}Na_4S_2W_6$): C, 16.11; H, 2.61; N, 3.42; Na, 3.74; S, 2.61; W, 44.84. Found: C, 16.65; H, 2.30; N, 3.46; Na, 3.81; S, 2.69; W, 44.46.

$Na_4[(W_3SO_3)_2(HO\text{-}PDTA)_3]$ (13). To a solution of 2.0 g of $[W_3SO_3(H_2O)_9]Cl_4$ in 100 mL of DMF was added 1.10 g of HO-PDTA acid as a solid. The mixture was refluxed for 3 h, at which point a small amount of red solid separated. The DMF solvent was removed under vacuum, leaving a red residue. The residue was dissolved in H_2O, and 1 N NaOH was added to adjust the pH of the solution from pH 2 to pH 9. The solution was then evaporated to dryness and the residue was dissolved in water and filtered through a 0.22 μm filter. The pH of the solution was then adjusted to pH 2 by the addition of 1 N HCl. A solution of 1.7 g of $[(Ph_3P)_2N]Cl$ (PPNCl) in warm water was added, and a fine red solid separated. This solid was collected by filtration and purified by multiple crystallization from MeOH/H_2O. The recrystallization procedure consisted of diluting the MeOH solution of $(PPN)_4[(W_3SO_3)_2(HO\text{-}PDTA)_3]$ with distilled water and allowing the crystals to grow slowly. The crystals were collected and washed with hot distilled water. The Na^+ salt of the complex was obtained through a cation exchange procedure using AG50X8 resin (Na^+ form). The pure product **13**, 0.76 g (29.4%), was obtained as a red solid (a 6 H_2O hydrate). UV−vis: λ_{max}, 475 nm. ^{1}H NMR (D_2O, δ, ppm): 4.7 (m, 3H), 4.2−4.6 (m, 24H), 3.33 (m, 6H), 2.74 (m, 6H).^{183}W NMR (D_2O, δ, ppm): 1207.7, 1201.3, 1196.2, 1190.7. MS (FAB, $[M + H]^+$): calcd 2310.8, found 2310. Anal. Calcd for $Na_4[(W_3SO_3)_2(HO\text{-}PDTA)_3]\cdot 6H_2O$ ($C_{33}H_{54}N_6O_{39}Na_4S_2W_6$): C, 16.39; H, 2.25; N, 3.48; Na, 3.80; S, 2.65; W, 45.62. Found: C, 16.02; H, 2.66; N, 3.40; Na, 3.54; S, 2.68; W, 46.05.

$Na_4[(W_3SO_3)_2(MeO\text{-}PDTA)_3]$ (14). To a solution of 2.0 g of $[W_3SO_3(H_2O)_9]Cl_4$ in 30 mL of DMF at 150 °C was added dropwise (30 min) a solution of 1.08 g of MeO-PDTA acid in 30 mL of DMF. The mixture was refluxed for 3 h followed by the precipitation of a red solid. This reaction mixture was treated with the workup procedure described for **10**. The pure product **14**, 1.4 g (52.9%), was obtained as a red solid (a 7 H_2O hydrate). UV−vis: λ_{max}, 475 nm. ^{1}H NMR (D_2O, δ, ppm): 4.2−4.6 (m, 27H), 3.71 (s, 9H), 3.33 (m, 12H), 2.8 (m, 12H). ^{183}W NMR (D_2O, δ, ppm): 1212.4, 1205.8, 1201.7, 1195.4. MS (FAB, $[M + H]^+$): calcd 2352.8, found 2349. Anal. Calcd for $Na_4[(W_3SO_3)_2(MeO\text{-}PDTA)_3]\cdot 7H_2O$ ($C_{36}H_{62}N_6O_{40}Na_4S_2W_6$): C, 17.45; H, 2.52; N, 3.39; Na, 3.71; S, 2.59; W, 44.51. Found: C, 16.77; H, 2.91; N, 3.17; Na, 3.89; S, 2.35; W, 45.13.

$Na_7[(W_3SO_3)_2(CM\text{-}PDTA)_3]$ (15). This compound was prepared in a procedure similar to that described for **13** using 2.0 g of $[W_3SO_3(H_2O)_9]Cl_4$, 100 mL of DMF, and 1.21 g of CM-PDTA acid solid; however, a red solid did not precipitate during the reaction. The product was purified by multiple recrystallizations of the PPN^+ salt in a procedure similar to that described for **13**. The pure Na^+ salt **15**, 0.50 g (18.3%), was obtained as a red solid through a cation exchange procedure. It was a 7 H_2O hydrate. UV−vis: λ_{max}, 475 nm. ^{1}H NMR (D_2O, δ, ppm): 4.1−4.6 (m, 8H), 3.7 (m, 2H), 2.5−3.0 (m, 7H). ^{183}W NMR (D_2O, δ, ppm): 1235.7, 1228.1, 1216.7, 1212.6. MS (FAB, $[M + H]^+$): calcd 2502.7, found 2504. Anal. Calcd for $Na_7[(W_3SO_3)_2(CM\text{-}PDTA)_3]\cdot 7H_2O$ ($C_{39}H_{62}N_6O_{43}Na_7S_2W_6$): C,17.82; H, 2.26; N, 3.20; Na, 6.12; S, 2.44; W, 41.97. Found: C, 18.22; H, 2.79; N, 3.20; Na, 6.09; S, 2.62; W, 41.70.

X-ray Crystallographic Analysis. $(PPh_4)_4[(W_3SO_3)_2(PDTA)_3]\cdot 39H_2O$ (16). Complex **16** was obtained by cation metastasis using PPh_4Cl and **12** in water. The red precipitate was collected and dissolved in MeOH. Water was added to the point of cloudiness, and the solution

Table 1. Crystallographic Data for $(PPh_4)_4[(W_3SO_3)_2(PDTA)_3]\cdot39H_2O$ (**16**) and $(PPh_4)_4[(W_3SO_3)_2(HO\text{-}PDTA)_3]\cdot27.5H_2O$ (**17**)

	16	**17**
formula	$C_{129}H_{200}N_6O_{69}P_4S_2W_6$	$C_{129}H_{177}N_6O_{60.5}P_4S_2W_6$
fw	4230.1	4070.96
temp (°C)	−129	−130
λ (Å)	0.71073	0.71069
space group	$P2/n$	$P\bar{1}$
a (Å)	15.389(5)	19.968(1)
b (Å)	13.496(4)	20.197(1)
c (Å)	39.511(6)	22.873(1)
α (deg)		113.352(1)
β (deg)	93.670(19)	99.748(1)
γ (deg)		108.289(1)
V (Å^3)	8189(6)	7570.7(8)
Z	2	2
D_{calcd} (g/cm^3)	1.72	1.78
R; R_w	3.68%; 4.12%	4.6%; 5.9%
GOF	1.59	1.99

was slowly evaporated to afford the red crystalline plates. A fragment, 0.40 × 0.40 × 0.25 mm, was cut from one of the crystals and was mounted on a glass fiber using Paratone N hydrocarbon oil. An Enraf-Nonius CAD-4 diffractometer, equipped with a N_2 flow low-temperature device, was used to collect 11237 raw intensity data. A summary of selected crystallographic data for **16** is given in Table 1. The raw data were converted to structure factors by applying corrections for scan speed, background, Lorentz, and polarization effects. Patterson method was used to solve the structure, and its refinement was done using standard least-squares and Fourier techniques. Several atoms (C12, C14, O7, and eight oxygen atoms of the solvate water molecules) are severely disordered and thus were modeled with 50% occupancy at two positions and were refined isotropically. All other atoms were refined anisotropically. Hydrogen atoms were included only to the full occupancy atoms and were not refined. The final residuals were obtained using 7374 accepted data points ($F^2 > 3\sigma(F^2)$) against 963 variables.

$(PPh_4)_4[(W_3SO_3)_2(HO\text{-}PDTA)_3]\cdot27.5H_2O$ (17). Red layered blocky crystals of complex **17** were obtained through a procedure similar to that described for **16**. A fragment, 0.11 × 0.17 × 0.35 mm, cut from one of the crystals was mounted on a glass fiber using Paratone N hydrocarbon oil. A Siemens SMART system was used to collect 28666 raw intensity data. Its crystallographic data is also listed in Table 1. The raw data were integrated and corrected for Lorentz and polarization effects using the program SAINT. Absorption correction was applied to the data set using the program XPREP. The structure was solved by and expanded using Fourier techniques. All full occupancy non-hydrogen atoms were refined anisotropically, while partial occupancy atoms were refined isotropically. Hydrogen atoms were included only to the full occupancy atoms and were not refined. The final residuals were obtained using 15857 accepted data points ($I > 3\sigma(I)$) against 1867 variables.

Results and Discussion

Synthesis. The preparations of the new polyaminopolycarboxylate ligands are straightforward, involving routine functional group protections and the alkylation of the primary amine with bromoacetic acid or esters. Pure ligand products (acid form) were generally obtained through anion exchange chromatography.

The W_3SO_3 core is prepared from $W(CO)_6$ through an oxidation process whose mechanism is not clearly understood.[14−16] The presence of a small amount of water (<6% w/w $H_2O:Na_2S$) in commercially supplied anhydrous Na_2S starting material is necessary for a high-yield preparation; however, excess water (>1 equiv of $H_2O:Na_2S$) is detrimental to the preparation. A single size exclusion chromatographic procedure afforded the W_3SO_3 core in high purity. Additional size exclusion procedures on the mixture fractions improved the overall yield of W_3SO_3 cores. The red solid obtained by rotary evaporation under vacuum can be stored in a freezer for a long period (more than 2 years) with no noticeable degradation.

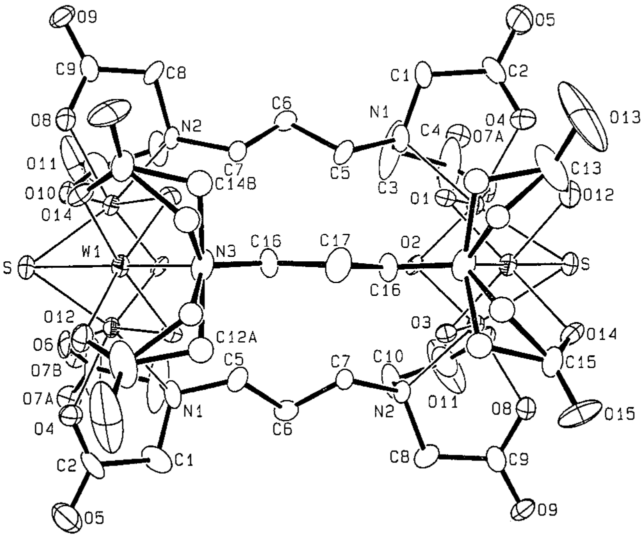

Figure 2. ORTEP representation for the crystal structure of $[(W_3SO_3)_2(PDTA)_3]^{4-}$, showing 50% thermal ellipsoids and partial atom-labeling scheme.

Similar to the preparation of tungsten cluster complexes **2** and **3**, reactions of the aqua ion **4** and the linear bis-IDA ligands in refluxing DMF produced crude solid mixtures that either separated spontaneously or were precipitated by the addition of 2-propanol to the cold reaction solution. Subsequent workup using ion exchange, size exclusion chromatography, and multistep crystallization techniques afforded the pure products, **9**−**15**, as red solids in reasonable yields. The reactivity of aqua ion **4** is similar to that of **1**, and the yields of their EGTA complexes are comparable. Within the series of $[(W^{IV}{}_3SO_3)_2L_3]$ cluster complexes, the reaction yields increase (up to about 60% in the cases of **12** and **14**) when the distance between two terminal IDA groups is shortened. The presence of reactive functional groups, such as −OH or $-CO_2H$, generally has a deleterious effect on yields. The reaction between **4** and H_4EDTA is an exception: even though mass spectroscopic studies have found mass peaks that match the stoichiometry of the 2:3 cluster complex in the crude product mixture, no pure $[(W^{IV}{}_3SO_3)_2(EDTA)_3]^{4-}$ complex was isolated after numerous attempts. This may be caused by the propensity for the EDTA ligand to preferentially chelate two tungsten atoms in one cluster in a fashion similar to that found in the $[W^V{}_2Q_4EDTA]^{2-}$ (Q = O, S) complexes.[22−25] In fact, mass peaks corresponding to the 1:2 cluster−ligand complex (possibly $[W^{IV}{}_3SO_3(EDTA)_2]^{4-}$) was always detected in the MS studies of the crude product mixtures.

Description of Structures. Prior examples of structurally characterized complexes having the trinuclear W_3SO_3 core are limited to two cases: $[W^{IV}{}_3SO_3(NCS)_9]^{5-}$ and $[W^{IV}{}_3SO_3(Hnta)_3]^{2-}$.[14,15] In the present X-ray crystallographic analyses, the structures of **16** (Figure 2) and **17** (Figure 3) are the first known examples of two W_3SO_3 clusters both complexed and linked by three polyaminopolycarboxylate ligands to form 2:3

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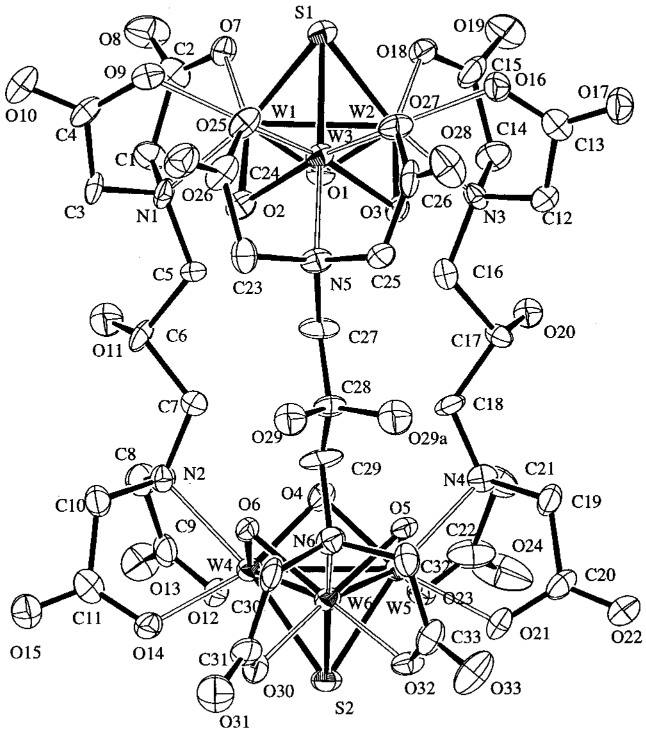

Figure 3. ORTEP representation for the crystal structure of $[(W_3SO_3)_2(HO\text{-}PDTA)_3]^{4-}$, showing 50% thermal ellipsoids and atom-labeling scheme.

cluster–ligand complexes. The only other examples of 2:3 cluster–ligand complexes with a M_3Q_4 (Q = O, S) core are $[(Mo^{IV}{}_3O_4)_2(EDTA)_3]^{4-}$ and $[(W^{IV}{}_3S_4)_2(EGTA)_3]^{4-}$.[8,26] The structure of **16** contains a crystallographically imposed C_2 symmetry in the center of the molecule which relates the two complexed W_3SO_3 units. Since the spatial arrangement of the W_3SO_3 core and the coordination of the tungsten atoms by IDA groups are highly symmetrical, one can easily imagine a C_3 axis existing through the two μ_3-S atoms. The local coordination of tungsten atoms by IDA groups is similar to that of $[(W_3SO_3)(Hnta)_3]^{2-}$; selected bond distances and angles (or their ranges) for complexes **16** and **17** are listed in Tables 2 and 3, respectively.

Close inspection of the structure of **17** revealed an interesting phenomenon. The oxygen atoms on the propylene backbone are modeled with occupancy disorder. Atom O29 was refined in two positions with equal occupancy (plotted), while O11 and O20 were modeled in two positions with 80:20 occupancy (only the dominant ones are plotted). There are two possible isomers for this complex with respect to the spatial arrangement of the –OH groups: the cis ("pedal wheel") configuration where all three –OH groups point to one direction and the trans configuration where one –OH points to the other direction. Statistically, the ratio between cis and trans isomers is 1:3. Therefore, cocrystallization of the cis and trans isomers is the cause of the disorder in crystallography.

NMR studies also confirm this hypothesis. Figure 4 contains the ^{183}W NMR spectra for complexes **12**, **13**, and **15**. In complex **12**, all three tungsten atoms are equivalent; therefore only one peak is present. In complex **13**, since three tungsten atoms in the cis isomer are equivalent, three tungsten atoms in the trans isomer are not equivalent, and the ratio between the cis and trans isomers is 1:3, therefore four peaks with similar intensity are observed. As for complex **15**, since the bulky carboxymethyl group has modified the complexation kinetics of the cis and trans isomers and influenced their thermodynamic properties, the ratio between cis and trans isomers is not 1:3. In this particular case, the trans isomer is the favored reaction product; therefore there are three peaks with higher intensity.

Table 2. Selected Bond Lengths (Å) and Angles (deg) for **16**

Bond Lengths

W1–W2	2.620 (1)	W2–W3	2.774(2)
W1–W3	2.611(1)	W1–S	2.389 (3)
W2–S	2.379(3)	W3–S	2.387(2)
W1–O1	1.919(6)	W1–O3	1.937(6)
W2–O1	1.934(7)	W2–O2	1.922(6)
W3–O2	1.931(6)	W3–O3	1.892(6)
W1–N3	2.247(8)	W2–N1	2.259(8)
W3–N2	2.243(7)		
W1–O12	2.075(6)	W1–O14	2.094(6)
W2–O4	2.082(6)	W2–O6	2.074(7)
W3–O8	2.080(6)	W3–O10	2.113(7)

Angles

W–S–W	66.15(7)–66.29(6)
S–W–$O_{carboxy}$	92.1(2)–94.0(2)
S–W–N	168.3(2)–169.2(2)
O(1,2,3)–W–N	83.8(3)–86.1(3)
$O_{carboxy}$–W–$O_{carboxy}$	81.2(3)–83.0(3)
S–W–O(1,2,3)	101.8(2)–103.6(2)
W–O(1,2,3)–W	84.9(2)–86.0(2)
O (1,2,3)–W–O(1,2,3)	92.8(3)–93.7(3)
N–W–$O_{carboxy}$	77.2(3)–79.1(3)
O (1,2,3)–W–$O(_{carboxy})_{trans}$	161.4(3)–163.4(3)
O (1,2,3)-W–$O(_{carboxy})_{cis}$	87.0(2)–93.6(3)

Table 3. The Ranges of Bond Lengths (Å) and Angles (deg) for **17**

Bond Lengths

W–W	2.6082(6)–2.6318(6)	W–S	2.375(3)–2.401(3)
W–O_{bridge}	1.901(7)–1.948(7)	W–$O_{carboxy}$	2.063(7)–2.121(7)
W–N	2.225(8)–2.269(8)		
C28–O29	1.34(2)	C28–O29a	1.43(2)
C6–O11	1.43(1)	C6–O11a	1.48(3)
C17–O20	1.43(1)	C17–O20a	1.27(4)

Angles

W–S–W	65.99(7)–66.87(7)
S–W–$O_{carboxy}$	93.5(2)–95.5(2)
S–W–N	168.5(2)–171.2(2)
O_{bridge}–W–N	81.9(3)–86.5(3)
$O_{carboxy}$–W–$O_{carboxy}$	80.0(3)–81.2(3)
O_{bridge}–W–$O(_{carboxy})_{cis}$	87.6(2)–92.3(3)
S–W–O_{bridge}	102.0(2)–103.0(2)
W–O_{bridge}–W	84.9(2)–86.1(3)
O_{bridge}–W–O_{bridge}	93.0(3)–94.4(2)
N–W–$O_{carboxy}$	76.7(3)–78.9(3)
O_{bridge}–W–$O(_{carboxy})_{trans}$	159.4(3)–162.3(3)
O29–C28–O29a	86(1)
O20–C17–O20a	75(1)
O11–C6–O11a	102(1)

Biological Studies. Similar to the aqua ion **1**, which decomposes quickly in strong acidic and anaerobic conditions, aqua ion **4** exhibited an even stronger propensity for decomposition. However, complexes **9**–**15** are remarkably stable in aqueous solutions under a wide range of pH conditions. No degradation (monitored by UV–vis and NMR techniques) was observed with compounds **9** and **10** in aqueous solutions (pH 7) for a long period of time (>60 days, open to air and at room temperature).

An acceptable level of biological tolerance was achieved for the W_3SO_3 cluster through complexation with bis-IDA ligands. The acute toxicity of these cluster complexes in mice was assessed following tail vein bolus injections of the test substances in ~pH 7 aqueous solutions. Due to limited sample sizes, only maximum tolerated doses (MTD) were determined, which

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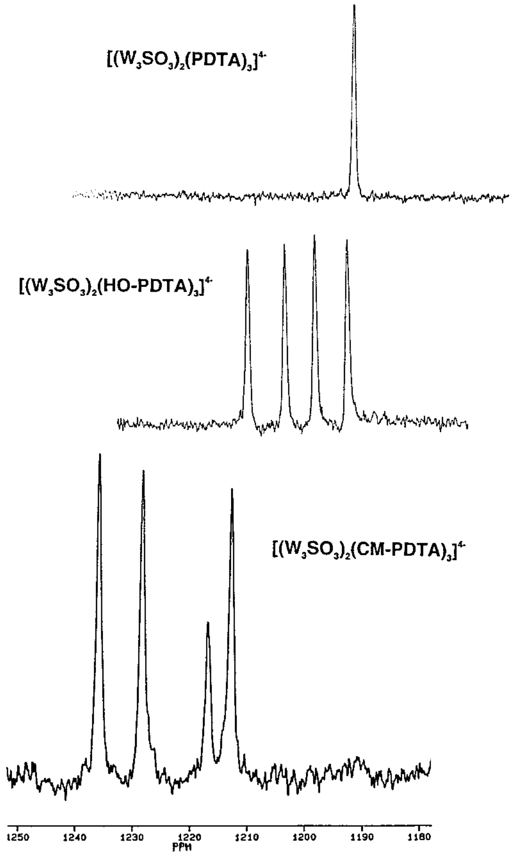

Figure 4. ^{183}W NMR spectra of $[(W_3SO_3)_2(PDTA)_3]^{4-}$, $[(W_3SO_3)_2(HO\text{-}PDTA)_3]^{4-}$, and $[(W_3SO_3)_2(CM\text{-}PDTA)_3]^{7-}$.

Table 4. Acute Murine Toxicity of W_3SO_3 Clusters Complexes

complexes	MTD (mmol/kg)	test concn (mM)
$Na_4[(W_3SO_3)_2(EGTA)_3]$	7.8	160
$(NMG)_4[(W_3SO_3)_2(EGTA)_3]$	4.5	150
$Na_4[(W_3SO_3)_2(OBETA)_3]$	4.4	107
$Na_4[(W_3SO_3)_2(BDTA)_3]$	2.6	120
$Na_4[(W_3SO_3)_2(PDTA)_3]$	6.5	79
$Na_4[(W_3SO_3)_2(HO\text{-}PDTA)_3]$	2.2	153
$Na_4[(W_3SO_3)_2(MeO\text{-}PDTA)_3]$	5.2	140

often approximate the LD_{50}. Table 4 lists the MTDs and the testing concentration for selected W_3SO_3 cluster complexes. The NMG salt of $[(W_3SO_3)_2(EGTA)_3]^{4-}$ was obtained through the neutralization of $H_4[(W_3SO_3)_2(EGTA)_3]$, which in turn was obtained by passing a solution of complex **9** through an AG50X8 cation exchange column in H^+ form. Overall, the toxicity and solubility of these W_3SO_3 complexes are similar to those of the W_3S_4 complexes,[6−9] though the W_3SO_3 complexes have a slightly higher solubility and are somewhat less toxic. For a theoretical imaging dose of 1 mmol/kg, all W_3SO_3 complexes would display safety margins that are approximately 5-fold. While it is well-known that acute toxicity can vary significantly in a compound with multiple isomers, difficulty in separating cis and trans components in complexes (i.e., **17**) containing both isomers precluded individual acute toxicity determinations. Although it is interesting to speculate that an individual cis or trans isomer from a pair might display dramatically decreased toxicity compared to the mixture, the salient feature of this work is the demonstration of acceptable biological tolerance of these novel prototype cluster complexes, sufficient to conduct in vivo studies. A comparative safety assessment of the individual isomers would be required for any agent of sufficient interest for advanced evaluation.

In vitro and in vivo (rat) comparative studies performed on commercial CT scanners using complex **9** have demonstrated that its X-ray attenuation capability is twice that of **2**, and four times that of iohexol on a molar basis. Complexes **2**, **3**, and **9** demonstrated two-compartment extracellular pharmacokinetic profiles in rats, similar to the profile observed for the commercial agent iohexol.[6−9,27]

Concluding Remarks

In summary, the aqua ion **4** can be reproducibly prepared in large quantities and stored as a solid with negligible degradation. Pure $W_3SO_3{-}L$ complexes with 2:3 stoichiometry can be obtained through a simple reaction in refluxing DMF followed by size exclusion chromatography. In comparison with the synthetic pathway for $W_3S_4{-}L$ complexes, the route to W_3SO_3 complexes is simpler with higher preparative yields for both the aqua ion and its complexes. The starting material for the W_3SO_3 aqua ion, $W(CO)_6$, is also readily available commercially. Complexation of the W_3SO_3 cluster with bis-IDA ligands imparts a necessary degree of water solubility and chemical stability required for biological testing. Furthermore, tungsten cluster complexes **9**−**15** have demonstrated low acute toxicity in mice and, for complex **9**, an acceptable pharmacokinetic profile in rats. The superior X-ray contrast efficacy of complex **9** was confirmed by in vitro and in vivo animal X-ray imaging studies. Currently, the research and development of such tungsten cluster complexes as next generation X-ray contrast agents is under further exploration.

Acknowledgment. We thank Dr. F. Hollander of University of California Berkeley for experimental assistance (X-ray crystal structure determination).

Supporting Information Available: Listings of crystallographic data, positional and thermal parameters, and bond lengths and angles for complex **16** and **17**. This material is available free of charge via the Internet at http://pubs.acs.org.

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