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New strategies towards advanced CT contrast agents. Development of neutral and monoanionic sulfur-bridged W(V) dimeric complexes†

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Multinuclear tungsten complexes are intriguing candidates for new contrast media that can provide substantial improvements in CT imaging diagnostics. Herein, we present a ligand strategy, based on amino acids, and mono- and disubstituted EDTA derivatives, that enables the development of stable complexes with high tungsten content and reasonably low osmolality. Accordingly, a series of neutral and monoanionic di-μ-sulfido W(V) dimers have been synthesized *via* a convenient procedure utilizing microwave heating in combination with ion-pair HPLC reaction monitoring. The compounds were characterized in detail by various techniques, including ESI-HRMS, NMR spectroscopy, HPLC, elemental analysis, and X-ray crystallography. The aqueous stability of the complexes under physiologically relevant conditions, and during heat sterilization was also examined as an initial assessment of their potential applicability as radiocontrast agents. Monoanionic complexes featuring monosubstituted EDTA derivatives have demonstrated high stability, while producing a lower number of ions in solution (resulting in lower osmolality) in comparison to their bis-anionic EDTA counterparts. Nevertheless, they exhibited insufficient water solubility for application as intravascular contrast agents. However, our study showed that aqueous solubility of this type of complexes can be tuned by small modifications in the ligand structure.

Introduction

X-ray computed tomography (CT) is a well-established imaging technique that is routinely used for a wide range of clinical problems, such as uncovering and evaluation of different organ abnormalities and neoplastic formations.[1,2] Contrast media are utilized in about 40% of the CT scans undertaken to increase the visibility of soft tissues and fluids, heighten the differences between adjacent tissues and improve the images obtained.[3,4] Contrast-enhanced CT is an indispensable tool in the diagnosis and monitoring of severe pathologies, such as neoplastic diseases and pulmonary embolism (also a frequently observed complication in patients with COVID-19 pneumonia).[5–8] However, current clinically approved iodinated radiocontrast agents possess several features that could be improved on, such as low CT imaging efficacy, lack of specificity, and high dose requirements.[2,3,9–11] Most of these limitations derive from the suboptimal attenuating characteristics of iodine atoms. Utilizing elements with higher atomic numbers (X-ray absorption and absorption edge K value increase with Z) can significantly enhance the X-ray attenuation capabilities and overall performance of radiocontrast agents. Accordingly, contrast media, based on elements heavier than iodine, would provide substantial improvements in image contrast and quality, along with reduced toxicity and radiation burden for the patient (especially for patients with obesity).[4,12,13]

Advances in CT imaging instrumentation have further stimulated the search for new contrast agents with improved attenuation profiles.[4] Consequently, various design strategies, employing electron-dense heavy elements have been explored.[12,14,15] Nanoparticle formulations, containing metallic Au, Ta, Bi or W, respectively their salts or oxides, have demonstrated several advantages (*e.g.*, superior X-ray attenuation and long circulation times) towards conventional contrast agents in various studies.[14,16–19] However, difficulties with size and shape control, poor elimination profiles, safety concerns, and high market costs (in the case of Au) are

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amongst the obstacles impeding their successful clinical development so far. Heavy metal chelate complexes have also been investigated as candidates for CT contrast media. Promising results have been reported for lanthanide complexes with polyaminocarboxylate ligands (like the Gd compounds used in MRI) as well as for some Hf and W multinuclear cluster compounds (see below).[12,20–22]

Several studies revealed the potential of W(V) and W(IV) cluster complexes stabilized by chelating ligands as radiocontrast agents.[12,20,21,23] These compounds contain two or more tungsten atoms and thus provide a high degree of X-ray attenuation per molecule, in resemblance with the triiodobenzene moiety in iodinated contrast media. Prominent examples comprise W(V) dinuclear EDTA complexes,[24–27] and trinuclear cuboidal W_3S_4 clusters[20,28] (see Fig. 1). They have shown encouraging results in *in vitro* and *in vivo* X-ray imaging studies providing high contrast enhancement with noticeable improvements in image quality along with suitably low acute toxicities in mice.[12,20] The main disadvantages of the reported tungsten multinuclear complexes come from their ionic character (*i.e.*, bis-, tris- or poly-anionic), which results in high osmolality and dose-limiting side effects, hindering their further clinical development.

The abovementioned limitations, however, can be overcome by a thoughtful design of multidentate chelating ligands that would reduce the magnitude of the overall electrical charge of the complex formed. Accordingly, we aimed to explore the applicability of EDTA derivatives, where one or two of the carboxylates are exchanged to neutral coordinating moieties, as potential ligands to develop neutral and monoanionic W(V) dinuclear complexes with superior X-ray imaging efficacy and desirable level of biological tolerance.

$Na_2[W_2O_4(EDTA)]$ is usually synthesized from the respective W(V) oxalato complex,[24,29] a precursor, that suffers from several drawbacks, such as unsatisfactory reproducibility of its preparation and purity, poorly defined stoichiometry, and limited stability towards air, particularly in solution.[30–32] The preparation of the S,S-bridged analog, $Na_2[W_2O_2S_2(EDTA)]$, utilizes $Na_2[W_2O_2S_2(Cys)_2]$, where H_2Cys is L-cysteine, as a precursor and comprises a straightforward procedure.[27] Furthermore, both $Na_2[W_2O_2S_2(EDTA)]$ and the cysteinato precursor showed to be resistant towards air oxidation in aqueous solutions. Accordingly, di-μ-sulfido W(V) dimers can provide a suitable platform for the development of novel tungsten-based radiocontrast agent candidates.

In this study, we report on the synthesis of a series of new neutral and monoanionic W(V) complexes featuring the $W_2O_2(\mu\text{-}S)_2$ core and amino acids or EDTA derivatives as ligands. Thus, a convenient synthetic procedure utilizing microwave heating in combination with reverse-phase ion-pair high-performance liquid chromatography (RPIP-HPLC) reaction monitoring, was developed for compound preparation. The complexes were characterized in detail by electrospray ionization high-resolution mass spectrometry (ESI-HRMS), NMR spectroscopy, HPLC, elemental analysis, and in two cases by X-ray crystallography. Furthermore, their stability under physiological conditions (pH, temperature, and the presence of competing ligands) and during heat sterilization was also examined as an initial assessment of their potential applicability as radiocontrast agents.

Results and discussion

Di-μ-sulfido W(V) dinuclear complexes with amino acid ligands. Synthesis and characterization

Dianionic di-μ-sulfido cysteinato dimeric complexes $M_2[W_2O_2S_2(Cys)_2]$ (M = K **1a**; Na **1b**; NH_4 **1c**) were synthesized following the method of Yamasaki and Shibahara[27] with $(NH_4)_2[WS_4]$ as starting material (Scheme 1) in yields of 42–45%. Subsequently, the procedure was adapted using other amino acids (*i.e.*, methionine, serine, asparagine, histidine, 2,3-diaminopropionic acid, and 1,2-ethanediylbiscysteine[33]) as potential ligands to afford neutral analogs of **1a-c**. Non-ionic complexes of the type $[W_2O_2S_2(AA)_2]$ could be successfully obtained only with histidine and 2,3-diaminopropionic acid. Nevertheless, preparations of $[W_2O_2S_2(His)_2]$ (**2**) and $[W_2O_2S_2(DAP)_2]$ (**3**) suffered from poor yields (<10%) and difficulties with purification. Thus, alternative approaches utilizing $Na_2[W_2O_2S_2(Cys)_2]$ (**1b**) and $Na_2[W_2O_2S_2(EDTA)]$ (**9b**) as

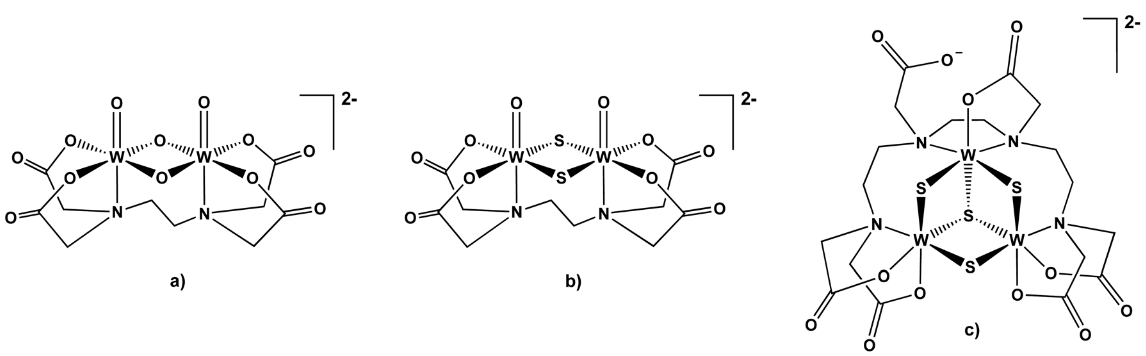
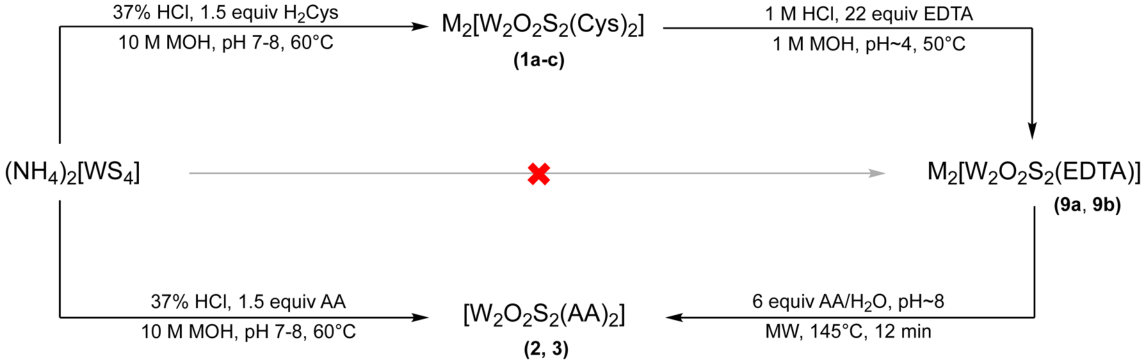


Fig. 1 Chemical structures of $[W_2O_4(EDTA)]^{2-}$ (a), $[W_2O_2S_2(EDTA)]^{2-}$ (b) and $[W_3S_4(TTHA)]^{2-}$ (c). Suitable counterions include Na^+, NH_4^+, NMG^+, *etc.* H_4EDTA: ethylenediaminetetraacetic acid, H_6TTHA: triethylenetetranitrilohexaacetic acid, NMG: *N*-methyl-D-glucamine.



Scheme 1 Synthesis of di-μ-sulfido W(v) dinuclear complexes with amino acid ligands and EDTA. AA = His (**2**) or DAP (**3**), $M^+ = K^+$ (**1a** and **9a**), Na^+ (**1b** and **9b**) or NH_4^+ (**1c**); MOH: KOH, NaOH or NH_4OH, respectively; H_2Cys: Cysteine, His: Histidinate, DAP: 2,3-diaminopropionate.

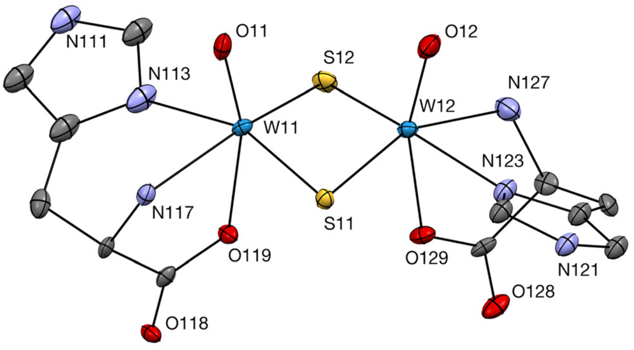


Fig. 2 ORTEP view of $[W_2O_2S_2(His)_2]$ (**2**) with atom labeling scheme. The thermal ellipsoids have been drawn at 50% probability level. Hydrogen atoms as well as solvent molecules are omitted for clarity.

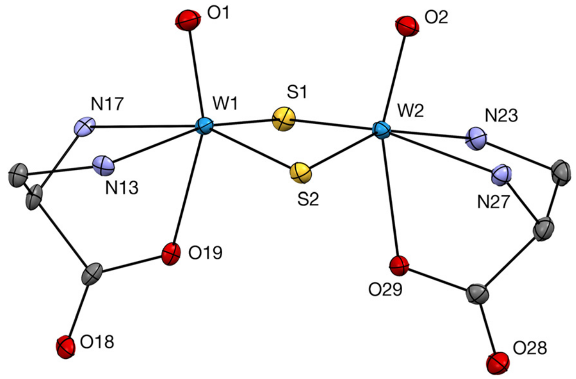


Fig. 3 ORTEP view of $[W_2O_2S_2(DAP)_2]$ (**3**) with atom labeling scheme. The thermal ellipsoids are drawn at the 50% probability level. Hydrogen atoms as well as solvent molecules, $[WS_4]^{2-}$ anions, and K^+ cations are omitted for clarity.

starting materials were explored (Fig. S1 and S2†). Accordingly, complexes **2** and **3** could be obtained in higher yields (82% and 37%, respectively) and satisfactory purity from **9b** *via* microwave-assisted ligand-exchange reaction at pH 8 (Scheme 1). Their identities were unambiguously confirmed by ESI-HRMS and X-ray crystallography (see below).

The molecular structures of $[W_2O_2S_2(His)_2]$ (**2**) and $[W_2O_2S_2(DAP)_2]$ (**3**), determined by single-crystal X-ray diffraction analysis, are shown in Fig. 2 and 3, respectively. Selected bond lengths and angles are listed in Table 1. Crystal data, data collection parameters, and structure refinement details are given in the ESI.† Compound **2** crystallized in the orthorhombic space group $P2_12_12_1$, with an asymmetric unit consisting of six $[W_2O_2S_2(His)_2]$ molecules and 17 water molecules connected by inter-molecular hydrogen bonds. Complex **3** crystallized in the trigonal space group $R\bar{3}$. Since the crystals were obtained from the filtrate of the reaction mixture (after storage at 4 °C for 3 days), the determined structure consisted of three molecules of $[W_2O_2S_2(DAP)_2]$, four $[WS_4]^{2-}$ anions, eight K^+ cations, and 14 water molecules (see Fig. S4†). Both complexes **2** and **3** contain the $W_2O_2(\mu\text{-}S)_2$ core with two histidinato or diaminopropionato, respectively, ligands coordinated to the W atoms in a tridentate mode. The central W_2S_2 rings are non-planar with S–W–S–W torsion angles, that are well comparable with those observed in the structurally similar cysteinato dimer[27] (Table 1). The W atoms have distorted octahedral environments with the O atoms of the carboxylate groups in *trans* positions to the W═O oxygen atoms, and the amino groups (respectively the amino groups and the N atoms of the imidazole rings in the case of **2**) *trans* to the bridging S atoms. The W–OCO bonds in **1b**, **2**, and **3** (2.137–2.296 Å) are longer than the W–OCO bonds in $[W_2O_2S_2(EDTA)]^{2-}$ (2.083–2.124 Å)[27] and the average value of 2.1 Å found for W–OCOMe bond lengths in literature.[34] The observed bond lengthening is a consequence of the *trans* influence of the W═O moiety.

Table 1 Selected bond lengths (Å) and angles (°) for complexes **1b**,[27] **2** and **3**

	1b	**2**	**3**
W═O	1.711–1.723	1.692–1.722	1.723–1.724
W–S	2.320–2.364	2.313–2.348	2.323–2.330
W–O (W–OCO)	2.220–2.296	2.137–2.203	2.182–2.194
O═W–O	162.34–164.20	156.52–161.91	156.97–159.01
S–W–S	103.38–104.12	102.15–104.29	104.04–104.19
W–S–W	74.73–74.90	74.10–75.27	73.97–74.12
S–W–S–W	12.86–13.15	12.08–18.56	14.67–14.71

Non-ionic compounds **2** and **3** have a high tungsten content (48% and 55%, respectively) and thus could provide a high degree of X-ray attenuation per molecule. However, their poor solubility in most common solvents (*e.g.*, water, MeOH, EtOH, DMSO) impeded detailed characterization in solution, subsequent analytical studies, and potential applications as intravenous radiocontrast agents.

Reactivity of $M_2[W_2O_2S_2(Cys)_2]$ towards EDTA derivatives. Reaction monitoring and optimization.

Tungsten(v) complexes of the type $M_2[W_2O_2S_2(EDTA)]$, where $M^+ = K^+$ or Na^+, could not be synthesized directly from $(NH_4)_2[WS_4]$ following the method for preparation of cysteinato complexes **1a–c**. Their synthesis, however, can be accomplished through aquation of $M_2[W_2O_2S_2(Cys)_2]$ in 1 M HCl and subsequent coordination of EDTA (taken in excess) after increasing the pH of the reaction mixture to 4–5 (Scheme 1).[27] To develop neutral and monoanionic analogs of $Na_2[W_2O_2S_2(EDTA)]$, we have designed a series of bis- and monosubstituted EDTA derivatives as potential ligands (see Fig. 4). Accordingly, one or two carboxylates from EDTA have been exchanged by neutral moieties with high coordination ability to the W(v) core (*e.g.*, NH_2, imidazole, pyridine). However, di-μ-sulfido W(v) dimers featuring these ligands could not be obtained following the procedure for the preparation of $Na_2[W_2O_2S_2(EDTA)]$, and thus alternative synthetic approaches needed to be developed.

The reaction between aqueous solutions of the cysteinato complex **1a** and ligands L1–8 (3 equiv., pH ~ 1) led in all cases to orange-yellow precipitates, which are poorly soluble in water and common organic solvents. ESI-MS measurements revealed, that the crude products obtained contain the desired W(v) complexes. Several other species, including free ligand and different adducts with cysteine (*e.g.*, $[W_2O_2S_2(L)(CysH)]^-$) were also detected (Fig. S6 and S7†). A pure product could be isolated only in the case of L1 after multiple purification steps. The compound was characterized as $[W_2O_2S_2(L1)]$ (**4**) by HRMS, NMR spectroscopy (Fig. S18 and S19†), and elemental analysis. Intriguingly, ESI-MS spectra of the crude products obtained with L5–8 showed the presence of both $[W_2O_2S_2(L)]^-$ and the double-charged dimer $[W_2O_2S_2(L)]_2^{2-}$ in a ratio dependent on the pH of the sample solution (Fig. S8†). RPIP-HPLC (*vide infra*) and ^{1}H NMR experiments have indeed confirmed that $[W_2O_2S_2(L)]^-$ and $[W_2O_2S_2(L)]_2^{2-}$ are different species.

As a next step, the pH- and temperature-dependent reactivity of $[W_2O_2S_2(Cys)_2]^{2-}$ towards the ligands depicted in Fig. 4 was investigated in order to find appropriate reaction conditions that allow preparation of the respective complexes with sufficient purity. Accordingly, the cysteinato precursor (**1a** or **1b**) was incubated with the corresponding ligands (3–4 equiv.) in buffered solutions (pH values ranging from 2 to 7) for 24 h at 50–80 °C and at rt. To enable reaction monitoring RPIP-HPLC methodology, utilizing 0.1% tetrabutylammonium chloride (Bu_4NCl) as ion-pairing reagent, has been developed. Thus, sufficient retention of the analyzed ionic species and separation based on both the charge and lipophilicity of the analytes could be achieved. All examined ligands showed limited to no reactivity at pH > 6 even after 20 h of heating at 80 °C. These findings corroborate the higher affinity to the $W_2O_2(\mu\text{-}S)_2$ core for cysteine in comparison to EDTA at pH > 5. Rapid formation of insoluble adducts with L1–4 at pH < 5 and with L5–8 at pH < 3 precluded HPLC reaction monitoring at these conditions. In the case of EDTA and HEDTA (*N*-(2-hydroxyethyl)ethylenediaminetriacetic acid), no precipitation was observed, but the low solubility of the former in its protonated form obstructed reaction monitoring at pH < 3. Transformation of the cysteinato precursor into a new complex *via* formation of intermediate multiple-charged adducts (with high retention times) could be clearly observed for the reactions with L5–8 and EDTA at pH 3–4 (Fig. S9†). The changes in the reaction mixtures, evident from the HPLC experiments, as well as the formation of the desired products, were further confirmed by ESI-MS measurements. The effect of pH, time of heating, and the structure of the ligand on the reaction kinetics is illustrated in Fig. 5.

Reaction conversion above 80% was achieved for EDTA and L6–8 at pH 3 after heating at 75 °C for 20 h; in the case of L5 less than 30% conversion was observed under the same conditions (Fig. 5). Microwave heating was utilized to further improve the overall reaction behavior. Indeed, microwave heating at 140–150 °C resulted in complete conversion in dramatically shortened reaction time (2–5 min) while a lower amount of ligand was needed (Fig. 6). Thus, performing the reactions at pH 3–3.5 and microwave heating (140–150° C for

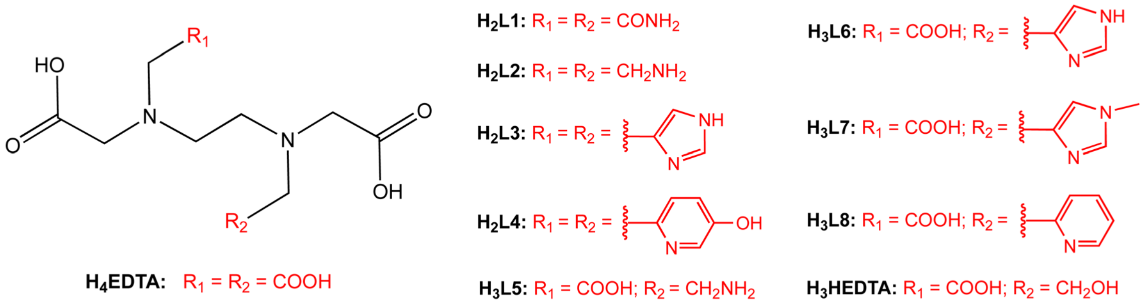


Fig. 4 EDTA and analogues explored as potential ligands for the development of new dinuclear W(v) complexes.

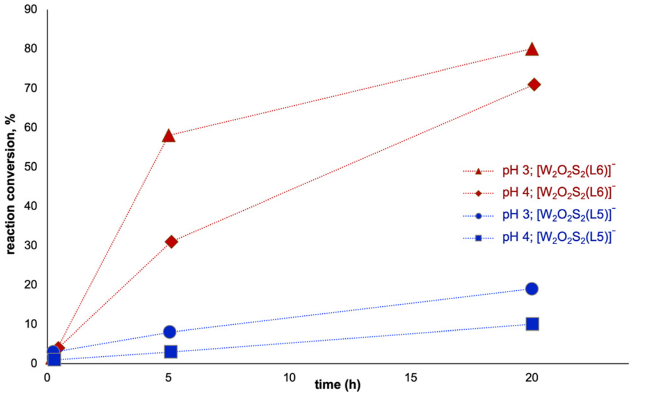


Fig. 5 Reactivity of **1b** towards L5 (blue) and L6 (red) at pH = 3 and pH = 4 as followed by RPIP-HPLC; the graph shows the amounts of final complexes formed (in %) after 0.2, 5 and 20 h of incubation at 75 °C, as calculated from the chromatograms recorded at 230 nm.

2–5 min) proved to be the method of choice for the synthesis of the desired complexes with L5–8.

Synthesis and characterization of monoanionic di-μ-sulfido W(v) dinuclear complexes with EDTA derivatives

Monoanionic di-μ-sulfido W(v) complexes with L5–8 were synthesized from **1a-c** by microwave-assisted ligand-exchange reaction at pH 3–3.5 and temperatures of 140–150 °C (Scheme 2) in accordance with the findings described above. The preparations did not require air-free conditions. However, purging with N_2 prior microwave heating was carried out as a precaution to minimize the possibility of formation of insoluble cystine (*via* oxidation of the released cysteine), which could co-precipitate with the desired product. The compounds were obtained as yellow-orange powders in yields above 80%, except for M[$W_2O_2S_2$(L5)] (**5a**, **5b**), where yields were lower (~45%), in part due to impurities presented in L5, which resulted in side product formation. Analogous complexes could not be synthesized with HEDTA. Regardless of the reaction conditions (pH, reaction time and temperature, conventional or microwave heating) used, incomplete reaction conversion with formation of several species (identified by HPLC and ESI-MS) was observed. Attempts to separate the species and isolate the desired Na[$W_2O_2S_2$(HEDTA)] complex by RP-flash chromatography were unsuccessful.

$$\underset{\textbf{(1a-c)}}{M_2[W_2O_2S_2(Cys)_2]} \xrightarrow[\text{MW, 140-150° C, 2-6 min}]{\text{2-3 equiv L/H}_2\text{O, pH 3-3.5}} \underset{\textbf{(5-8a-c)}}{M[W_2O_2S_2(L)]}$$

5a, **5b**: L = L5
6a-c: L = L6
7a, **7b**: L = L7
8a, **8b**: L = L8

Scheme 2 Synthesis of monoanionic di-μ-sulfido W(v) dinuclear complexes with EDTA derivatives. M$^+$ = K$^+$ (**5–8a**), Na$^+$ (**5–8b**) or NH$_4^+$ (**6c**).

The identity of all synthesized W(v) complexes was unambiguously confirmed by ESI-HRMS measurements in the negative ion mode; in all cases the detected *m*/*z* values and isotopic distribution patterns were in good agreement with calculated data (Fig. S10–S13†). Purity of the compounds was further verified by elemental analysis (C, H, N, and S) and RPIP-HPLC (for retention times and HPLC purity, see Table S5 and Fig. S14†). The ^{1}H NMR spectra of monoanionic W(v) complexes showed relatively complicated spectral patterns due to the double sets of signals observed for the respective coordinated ligand. This is particularly distinct in the spectra of compounds **6a** and **7a** as displayed in Fig. 7. We assume the presence of two isomers in solution as observed with other W(v) dimers featuring EDTA ligands.[26] The relative ratio of the isomers was found to depend on the solvent used, being nearly 1 : 1 in DMSO-d$_6$ and 1 : 2 in D_2O (Fig. S15†). Subsequent measurements at various temperatures in DMSO-d$_6$ (25 to 65 °C), as well as in D_2O (10 to 60 °C) did not show any meaningful change in the ratio of the signal sets and overall of the observed spectral pattern (Fig. S26 and S27†), implying that the isomers are not in dynamic equilibrium with each other. These findings are in agreement with the very slow rates of conformational interconversion in solution observed for other S,S-bridged W(v) complexes.[25,35] Double sets of signals were also detected in the ^{13}C NMR spectra of the compounds (Fig. S21, S23 and S24†).

Solubility and stability studies

Non-ionic complexes **2–4** exhibited very limited solubility in water (<0.5 mg ml^{-1}) and most organic solvents (except for **4**, which showed moderate solubility in DMSO). All monoanionic W(v) dimers are well soluble in DMSO (>60 mg ml^{-1}), insoluble

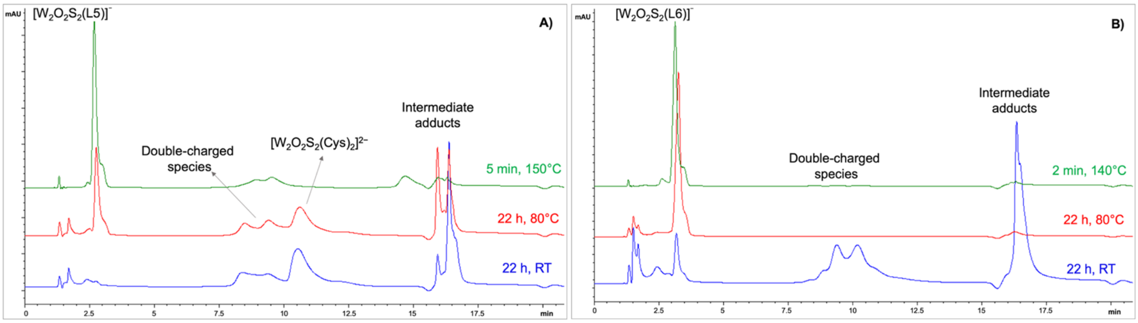


Fig. 6 RP-HPLC chromatograms of the reaction mixture of **1a** and L5 (A), **1a** and L6 (B) at pH 3.5 after 22 h at rt (blue), 22 h at 80 °C (red) or microwave heating (green). Mobile phase consisted of 0.1% Bu$_4$NCl/MeOH (65/35 at 0–14 min, 40/60 at 14.5–18.5 min); overlay of chromatograms recorded at 230 nm is shown.

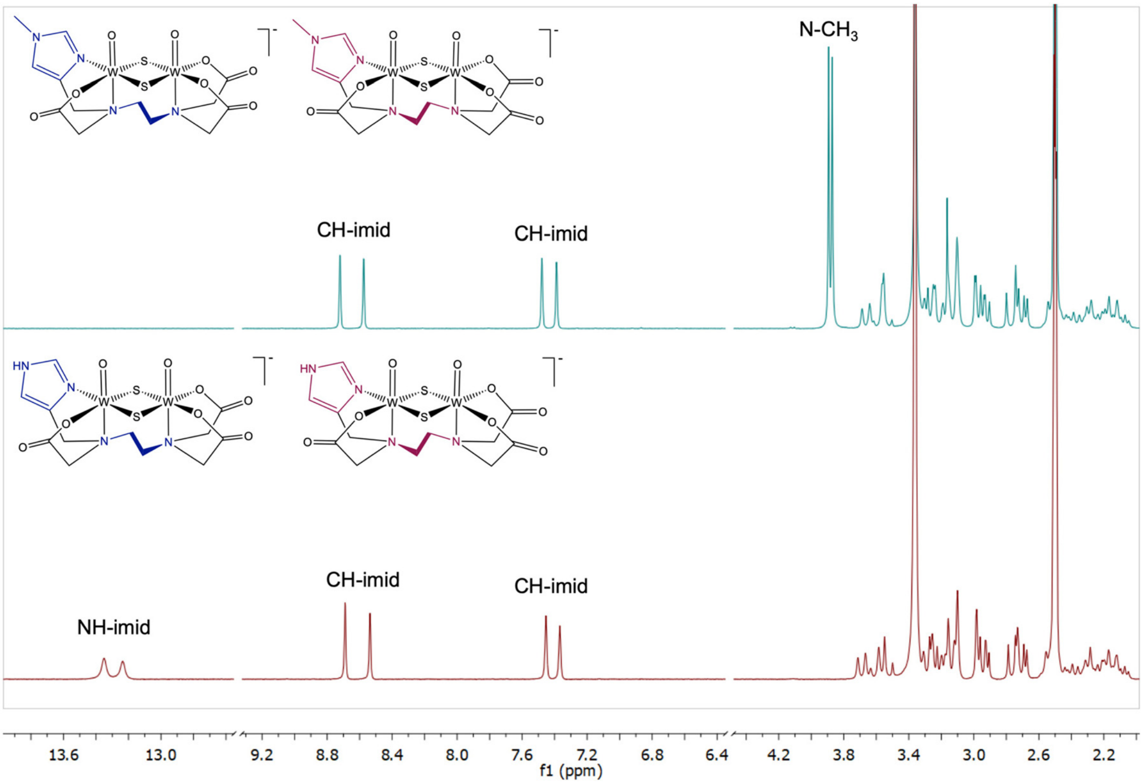


Fig. 7 ^{1}H NMR spectra of **6a** (bottom) and **7b** (top) in DMSO-d$_6$ at rt; chemical formulae of the two possible isomeric forms of the compounds are also shown.

in MeOH and Et$_2$O, and sparingly soluble in water upon heating. Aqueous solutions with concentrations of 5–15 mg ml^{-1} could be prepared at 50 °C with most of the complexes, while no signs of precipitation were observed after cooling the solutions to rt. The counterion had only minor influence on the solubility of the monoanionic dimers, whereas the most water-soluble agents were the one featuring ligands L7 and L8 (Table S5†). Nevertheless, none of the investigated compounds exhibited sufficient water solubility for use as an intravascular contrast agent.

Sulfido-bridged dinuclear complexes showed to be stable in DMSO and aqueous solutions over days of incubation at rt, as evidenced by ^{1}H NMR spectroscopy and RPIP-HPLC measurements. High stability under physiological conditions and during heat sterilization is crucial for metal complexes to be candidates for contrast media, as any release of free metal ions carries the risk of considerable side effects. Thus, stability of the most water-soluble compounds (*i.e.*, **1b**, **7a**, **8b** and **9b**) at physiological pH was examined over 20 h of incubation at 25 °C and during autoclaving (121 °C, 45 min) by means of UV-Vis spectroscopy and RPIP-HPLC. No signs of decomposition could be observed for any of the investigated complexes in the experimental settings used; integrity of the compounds after heat sterilization was >99% as determined by RPIP-HPLC, indicating their high hydrothermal stability at physiological pH.

The abundance of cysteine (and derived species) in the human body,[36] and its affinity towards the $W_2O_2(\mu\text{-}S)_2$ core at pH > 5 (*vide supra*) prompted us to investigate the potential interactions between di-μ-sulfido dimers and cysteine at physiologically-relevant conditions (pH 7.4, 37 °C). Thus, selected complexes were incubated with excess of L-cysteine (3 equiv.) at phosphate buffer (20 mM, pH 7.4) for 24 h at 37 °C, and RPIP-HPLC was used to monitor possible ligand-exchange reactions. Indeed, all tested compounds react with cysteine with formation of $[W_2O_2S_2(Cys)_2]^{2-}$. Nevertheless, the rate of the reaction is rather slow; under the deliberately harsh experimental setting, still nearly 70% of the parent complex survive after 24 h of incubation (Fig. 8). The reaction is even slower at lower pH (*e.g.*, in unbuffered aqueous solutions) and at lower cysteine/complex ratio. However, the results indicate that interaction with cysteine is a possibility which needs to be taken into account when compounds of this type are profiled for *in vivo* use.

Molar conductivity

Molar conductivity of the aqueous solutions of selected di-μ-sulfido complexes, as well as of NaCl and $Na_2[WO_4]$ have been determined and listed in Table 2. Double-charged W(V) complexes showed similar conductivity values, however lower than expected for 2 : 1 type of electrolytes (*e.g.*, $Na_2[WO_4]$). Analogously, monoanionic S,S-bridged dimers showed comparable conductivity values, which were lower than those measured for NaCl and expected for 1 : 1 type of electrolytes. A possible explanation for the lower conductivity of single- and

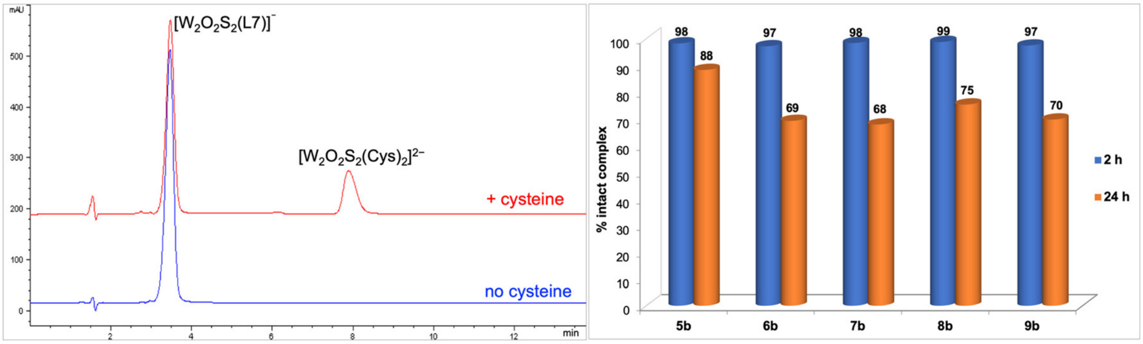


Fig. 8 Reactivity of di-μ-sulfido W(v) complexes towards cysteine at pH 7.4. Left: RPIP-HPLC chromatograms of pure **7b** and in a mixture with cysteine (3 equiv.) after 24 h of incubation at 37 °C. Right: amounts of remaining intact complex after 2 and 24 h of incubation with cysteine for **5–9b**, as calculated from the chromatograms recorded at 230 nm.

Table 2 Molar conductivity of compounds under investigation (1 mM) in Milli-Q water at 25 °C

Compound	Formula	Λ_m (S cm^2 mol^{-1})
NaCl	NaCl	131
Na_2WO_4	$Na_2[WO_4]$	204
6a	$K[W_2O_2S_2(L6)]$	88
7b	$Na[W_2O_2S_2(L7)]$	71
8b	$Na[W_2O_2S_2(L8)]$	68
1b	$Na_2[W_2O_2S_2(Cys)_2]$	165
9b	$Na_2[W_2O_2S_2(EDTA)]$	169

double-charged W(v) dimeric complexes can be their incomplete dissociation and/or ion pair formation in aqueous solution. Hence, lower than expected osmolality and fewer adverse side effects by potential intravascular administration might be expected for this class of compounds.

Conclusions

An efficient methodology, utilizing microwave heating and IP-RP-HPLC monitoring, for the preparation and examination of S,S-bridged W(v) dimers has been developed. Subsequently, a series of new neutral and monoanionic complexes featuring the $W_2O_2(\mu\text{-}S)_2$ core and amino acids or EDTA derivatives as ligands have been synthesized and characterized by various techniques. All compounds have a high tungsten content (between 44 and 55%, respectively) and thus can provide a high degree of X-ray attenuation per molecule, superior to clinically used iodinated contrast agents, especially at higher tube voltages.[12,14,37] Monoanionic complexes have demonstrated high stability under physiologically relevant conditions as well as during heat sterilization while producing lower number of ions in solution (reflecting in lower osmolality) in comparison to their bis-anionic EDTA counterparts. Although the here investigated compounds displayed insufficient water solubility for application as intravascular contrast media, the study revealed the potential of monoanionic W(v) complexes as viable leads for the development of metal-based contrast agents. Their solubility can be further tuned by minor modifications of the ligand structure and/or a change of the W-bridging atoms. For instance, *N*-methylation of imidazole or its exchange with pyridine in a monosubstituted EDTA ligand resulted in several times increase of the solubility for the respective complexes. Furthermore, a suitable formulation (*e.g.*, using advanced drug delivery systems) could allow complexes that provide a high amount of tungsten per molecule, but have sub-optimal solubility and/or osmolality to be utilized as X-ray imaging agents. Promising approaches to enhance solubility, stability, blood circulation time, and tissue targeting efficiency of contrast agent candidates include incorporation into biological transport vehicles and nanoparticles (*e.g.*, liposomes, lipoproteins, polymeric nanoparticles, nanosuspensions, nanocapsules, *etc.*).[2,12,14,18,38]

Experimental section

Materials and methods

All reagents and solvents were obtained from commercial suppliers and used without further purification. High purity water used in the synthetic procedures and HPLC experiments was obtained from a Milli-Q water purification system (Millipore GmbH, Vienna, Austria). Microwave-assisted experiments were carried out in a Biotage® Initiator + (Biotage AB, Uppsala, Sweden) single-mode microwave instrument producing controlled irradiation at 2.45 GHz in Pyrex microwave reaction vials. The vials were equipped with magnetic stirring bars and magnetic stirring at a rate of 720 rpm was used throughout the experiments. Reaction times refer to hold times at the temperatures indicated, not to total irradiation times. The temperature was measured with an IR sensor on the outside of the reaction vessel. HPLC analyses were performed on Agilent 1200 series, equipped with Agilent Zorbax Eclipse XDB-C18 (4.6 × 150 mm, 5 μm) column at 25 °C, a flow rate of 1.0 ml min^{-1} and UV-Vis detection. Mobile phase consisted of 0.1% Bu_4NCl in water/MeOH mixtures; stepwise gradient elution (*e.g.*, 35% MeOH for 14 min, followed by 60% MeOH for 4 min) was used. High resolution mass spectra (HRMS) were obtained on an Agilent Technologies 6230 TOF LC/MS instrument equipped with an ESI ion source (negative ionization mode); acetonitrile/water (40 : 60) mixture with 0.1% 5 M $HCOONH_4$ was used as a solvent. NMR spectra were recorded on a Bruker Avance III 300 MHz spectrometer in D_2O or DMSO-d_6 at ambient temperature; in certain cases, various temperature measurements were also performed. Chemical shifts δ were referenced with the residual solvent peaks of the respective NMR solvents used, and are given in ppm. J values are given in Hz. The multiplicity of peaks is denoted as singlet (s), broad signal (br), doublet (d), doublet of doublets (dd), triplet (t), or multiplet (m). NMR and HRMS data were processed using MestreNova. Elemental analyses (C, H, N, S) were performed at the Department of Inorganic Chemistry at the University of Technology in Graz using a Heraeus Vario Elementar automatic analyzer, and are within ±0.4% of the calculated values, confirming ≥95% purity of the respective compounds.

Synthesis

Synthesis and characterization of ligands L1–8 is given in the ESI.† The other ligands used were purchased from commercial vendors.

Tungsten(V) cysteinato precursors (**1a-c**) were synthesized according to a slightly modified literature procedure.[27] Thus, 37% HCl was added dropwise to an aqueous solution of $(NH_4)_2[WS_4]$ to obtain a dark black-brown slurry. Subsequent addition of L-cysteine and increasing the pH to 7–8 by using of 10 M KOH, 10 M NaOH or 35% NH_4OH, respectively, resulted in a dark red-brown solution, which turned into green-yellow after heating for 30 minutes at 60 °C. Crystallization of the crude products was achieved after addition of KCl (resp. NaCl or NH_4Cl) and storage at 4 °C for 48 h. The desired products were obtained as orange-yellow crystalline powders after recrystallization(s) from hot water.

Dipotassium (di-μ-sulfido)bis[(L-cysteinato)-oxotungstate(V)] pentahydrate, $K_2[W_2O_2S_2(Cys)_2]\cdot 5H_2O$ (1a)

$(NH_4)_2[WS_4]$ (2.50 g, 0.0072 mol) in water (25 ml), 37% HCl (5 ml), L-cysteine (1.50 g, 0.01201 mol, 1.6 equiv.), 10 M KOH (6.2 ml), KCl (2.50 g). Yield (1.34 g, 43%), orange powder. Elemental analysis, found: C, 8.31; H, 2.30; N, 3.17; S, 14.65. Calc. for $C_6H_{10}N_2O_6S_4W_2K_2\cdot 5H_2O$: C, 8.28; H, 2.32; N, 3.22; S, 14.74%. ESI-HRMS (−) found (calculated): m/z $[M + HCOO^-]^-$, 824.7680 (824.7696); $[M - K^+]^-$, 740.8068 (740.8083); $[M - 2K^+ + H^+]^-$, 702.8508 (702.8519). ^1H NMR (300 MHz, D_2O): δ 4.86 (dd, J = 3.6, 1.7 Hz, 2H, C**H**NH_2), 3.58 (dd, J = 12.5, 3.6 Hz, 2H, C**H**$_2$S), 3.00 (dd, J = 12.5, 1.8 Hz, 2H, C**H**$_2$S) ppm. ^{13}C NMR (75 MHz, D_2O): δ 179.0 (**C**OO), 63.4 (**C**HNH_2), 27.8 (**C**H$_2$S) ppm.

Disodium (di-μ-sulfido)bis[(L-cysteinato)-oxotungstate(V)] tetrahydrate, $Na_2[W_2O_2S_2(Cys)_2]\cdot 4H_2O$ (1b)

$(NH_4)_2[WS_4]$ (2.50 g, 0.0072 mol) in water (25 ml), 37% HCl (5 ml), L-cysteine (1.50 g, 0.0120 mol, 1.6 equiv.), 10 M NaOH (6.4 ml), NaCl (2.50 g). Yield (1.26 g, 43%), orange-yellow powder. Elemental analysis, found: C, 8.66; H, 2.31; N, 3.25; S 15.39. Calc. for $C_6H_{10}N_2O_6S_4W_2Na_2\cdot 4H_2O$: C, 8.79; H, 2.21; N, 3.42; S, 15.64%. ESI-HRMS (m/z, negative mode), found (calculated): $[M + HCOO^-]^-$, 792.8212 (792.8218); $[M - Na^+]^-$, 724.8333 (724.8343); $[M - 2Na^+ + H^+]^-$, 702.8516 (702.8519). ^1H NMR (300 MHz, D_2O): δ 4.84 (dd, J = 3.6, 1.7 Hz, 2H, C**H**NH_2), 3.56 (dd, J = 12.5, 3.6 Hz, 2H, C**H**$_2$S), 2.97 (dd, J = 12.5, 1.8 Hz, 2H, C**H**$_2$S) ppm. ^{13}C NMR (75 MHz, D_2O): δ 179.0 (**C**OO), 63.4 (**C**HNH_2), 27.8 (**C**H$_2$S) ppm.

Diammonium (di-μ-sulfido)bis[(L-cysteinato)-oxotungstate(V)] trihydrate, $(NH_4)_2[W_2O_2S_2(Cys)_2]\cdot 3H_2O$ (1c)

$(NH_4)_2[WS_4]$ (2.50 g, 0.0072 mol) in water (26 ml), 37% HCl (5 ml), L-cysteine hydrochloride (1.55 g, 0.0090 mol, 1.3 equiv.), 35% NH_3 (5.2 ml), NH_4Cl (2.50 g). Yield (1.20 g, 42%), orange-yellow powder. Elemental analysis, found: C, 8.90; H, 3.06; N, 6.80; S, 16.30. Calc. for $C_6H_{10}N_2O_6S_4W_2(NH_4)_2\cdot 3H_2O$: C, 9.10; H, 3.05; N, 7.07; S, 16.19%. ESI-HRMS (−) found (calculated): m/z $[M - 2NH_4^+ + H^+]^-$, 702.8542 (702.8519). ^1H NMR (300 MHz, DMSO-d_6): 7.26 (br d, J = 10.1 Hz, 2H, N**H**$_2$), 6.74 (s, 8H, NH_4), 5.45 (br d, J = 10.5 Hz, 2H, N**H**$_2$), 4.24 (br s, 4H, 2H, C**H**NH_2), 3.22 (dd, J = 11.4, 3.5 Hz, 2H, C**H**$_2$S), 2.52 (under solvent peak, C**H**$_2$S) ppm. ^{13}C NMR (75 MHz, D_2O): δ 179.1 (**C**OO), 63.4 (**C**HNH_2), 27.9 (**C**H$_2$S) ppm.

Neutral complexes of the type $[W_2O_2S_2(AA)_2]$, AA = His or DAP

Method A: $[W_2O_2S_2(His)_2]$ (**2**) and $[W_2O_2S_2(DAP)_2]$ (**3**) could be obtained employing the procedure for synthesis of **1a-c** with minor modifications, however in poor yields (<10%) after multiple recrystallizations from boiling water.

Method B (general procedure): An aqueous solution of $Na_2[W_2O_2S_2(EDTA)]$ (**9b**, 1 equiv.) was added dropwise to an aqueous solution of the respective amino acid (L-histidine or DL-2,3-diaminopropionic acid, 6 equiv.) in a 20 ml microwave reaction vial with stirring. The pH of the yellow-orange solution obtained was adjusted to ~7.5–8 (indicator control) by addition of 1 M NaOH. The microwave vial was then tightly sealed with a Teflon septum and an aluminum crimp, placed in the microwave instrument, and heated at 145 °C for 12 min. Thereafter, the vial was removed from the instrument and cooled to 4 °C for 16 h to promote product precipitation. The fine yellow precipitate was collected by vacuum filtration *via* a sintered glass filter (P5), washed with minimal amounts of cold water and MeOH, and dried *in vacuo*. The volume of the filtrate was reduced (by rotary evaporation) to approx. 4–5 ml, placed in a 5 ml microwave vial, and subjected to microwave heating at 145 °C for 5 min. A second fraction of the product was collected after cooling to 4 °C for 5 h. In the case of complex **2**, a third pure fraction could be obtained from the filtrate after another cycle of volume reduction, microwave heating and storage at 4 °C. Reaction monitoring was carried out by RPIP-HPLC (see Fig. S2†).

(Di-μ-sulfido)bis[(L-histidinato)-oxotungsten(V)] hydrate, $[W_2O_2S_2(His)_2]\cdot H_2O$ (2)

9b (100 mg, 0.123 mmol) in water (6 ml), L-histidine (114 mg, 0.735 mmol, 6 equiv.) in water (5 ml), 1 M NaOH (50 μl). Yield

(80 mg, 82%), yellow powder. Elemental analysis, found: C, 18.09; H, 2.28; N, 10.57; S, 8.15. Calc. for $C_{12}H_{16}N_6O_6S_2W_2{\cdot}H_2O$: C, 18.24; H, 2.30; N, 10.63; S, 8.12%. ESI-HRMS (−) found (calculated): *m*/*z* $[M - H^+]^-$, 770.9496 (770.9519). Crystals suitable for X-ray data collection were obtained in a closed HPLC vial after slow cooling down to rt of a pre-saturated at 90 °C aqueous solution of **2**.

(Di-μ-sulfido)bis[(2,3-diaminopropionato)-oxotungsten(v)] hydrate, $[W_2O_2S_2(DAP)_2]{\cdot}1.5H_2O$ (3)

9b (100 mg, 0.123 mmol) in water (6 ml), DL-2,3-diaminopropionic acid hydrochloride (110 mg, 0.782 mmol, 6 equiv.) in water (5 ml), 1 M NaOH (0.6 ml). Yield (32 mg, 37%), yellow powder. Elemental analysis, found: C, 10.45; H, 2.50; N, 7.97; S, 9.15. Calc. for $C_6H_{14}N_4O_6S_2W_2{\cdot}1.5\ H_2O$: C, 10.34; H, 2.46; N, 8.04; S, 9.20. ESI-HRMS (−) found (calculated): *m*/*z* $[M - H^+]^-$, 668.9278 (668.9301).

Crystals suitable for X-ray data collection were obtained after storage the filtrate of the reaction mixture (synthetic method A, employing $(NH_4)_2[WS_4]$ as a precursor) at 4 °C for 3 days.

$[W_2O_2S_2(L1)]{\cdot}2H_2O$ (4)

$H_2L1{\cdot}HCl$ (300 mg, 0.918 mmol, 3 equiv.) was dissolved in water (12 ml) with gentle warming and addition of few drops of 10 M KOH (until pH 6 was reached). The solution was added to **1a** (250 mg, 0.287 mmol, 1 equiv.), dissolved in water (10 ml), and the reaction mixture was stirred for 10 min at rt. Subsequently, concentrated HCl (1.5 ml) was added dropwise causing an immediate precipitation. The resulted orange suspension was stirred at 60 °C for 15 min and another 1 h at rt. The fine orange precipitate was collected by vacuum filtration *via* sintered glass filter (P5), washed with water and acetone and dried in vacuum. The crude product was suspended in water while stirring for 30 min at rt; the fine orange suspension acquired was centrifuged to deposit an orange solid, which was dried in vacuum. The purification procedure was executed twice. Yield (172 mg, 77%), orange powder. Elemental analysis, found: C, 15.07; H, 2.43; N, 6.91; S, 7.92. Calc. for $C_{10}H_{16}N_4O_8S_2W_2{\cdot}2H_2O$: C, 15.24; H, 2.56; N, 7.11; S, 8.14%. ESI-HRMS (−) found (calculated): *m*/*z* $[M - H^+]^-$, 750.9318 (750.9350). ^1H NMR (300 MHz, DMSO-d_6): δ 9.94–9.56 (m, 4H, CON**H₂**), 3.65 (m, 2H, C**H₂**CON), 3.50 (m, 1H), 3.25 (m, under solvent peak), 2.93 (m, 2H), 2.33 (m, 4H) ppm.

General procedure for the synthesis of monoanionic complexes of the type $M[W_2O_2S_2(L)]$, L = L5–8, $M^+ = K^+$, Na^+ or NH_4^+

An aqueous solution of the tungsten cysteinato precursor (**1a**-**c**, 1 equiv.) was added dropwise to an aqueous solution of the respective ligand (L5–8, 2–3 equiv.) in a 20 ml microwave reaction vial with stirring. The pH of the yellow suspension formed was adjusted to ~3–3.5 by addition of 1 M solution of the respective base (KOH, NaOH or NH_3), to give a clear orange-yellow solution. After flushing the reaction mixture with N_2 for 10 min the microwave vial was tightly closed under a flow of N_2, placed in the microwave instrument, and heated at 140–150 °C for 2–6 min. Subsequently, the reaction vial was removed from the instrument, cooled to rt, and then placed at 4 °C for 5–20 h to complete product precipitation. The yellow-orange fine precipitate was collected by vacuum filtration with a sintered glass filter (P5), washed with minimal amounts of cold water and MeOH, and dried in vacuum. In some cases, additional amounts of pure product could be obtained after reducing the volume of the filtrate to approx. 1/2 to 1/3 of the original and cooling at 4 °C. Reaction conversion was monitored by RPIP-HPLC before and after microwave heating.

$K[W_2O_2S_2(L5)]{\cdot}2H_2O$ (5a)

1a (200 mg, 0.230 mmol) in water (7 ml), $H_3L5{\cdot}HCl$ (205 mg, 0.652 mmol, 2.8 equiv.) in water (6 ml), 1 M KOH (1.1 ml), 150 °C for 6 minutes. The pure product was obtained after recrystallization from hot water. Yield (76 mg, 41%), yellow-orange powder. Elemental analysis, found: C, 14.53; H, 2.33; N, 5.12; S, 7.49. Calc. for $C_{10}H_{16}N_3O_8S_2W_2K{\cdot}2H_2O$: C, 14.76; H, 2.48, N, 5.17; S, 7.88%. ESI-HRMS (−) found (calculated): *m*/*z* $[M - K^+]^-$, 737.9375 (737.9403). ^1H NMR (300 MHz, DMSO-d_6): δ 6.92 (br, 1H, NH₂), 6.15 (br, 1H, NH₂), 3.29–2.99 (m, overlapped, 6H, **CH₂**COO and **CH₂**NH₂), 2.98–2.86 (m, 1H), 2.76–2.61 (m, 2H, C**H₂**COO), 2.44–2.22 (m, overlapped, 3H), 2.14–2.06 (m, 1H, NC**H₂**CH₂N), 1.99–1.89 (m, 1H, NC**H₂**CH₂N) ppm. ^{13}C NMR (75 MHz, D_2O): δ 175.2 (**C**OO), 173.9 (**C**OO), 173.7 (**C**OO), 67.6 (**C**H₂NH₂), 63.7 (**C**H₂COO), 62.9 (**C**H₂COO), 60.7 (**C**H₂COO), 55.2, 54.0, 39.7 (under solvent peak) ppm.

$Na[W_2O_2S_2(L5)]{\cdot}2H_2O$ (5b)

1b (195 mg, 0.238 mmol) in water (5 ml), $H_3L5{\cdot}HCl$ (212 mg, 0.681 mmol, 2.8 equiv.) in water (7 ml), 1 M NaOH (1.2 ml), 150 °C for 6 minutes. Yield (88 mg, 46%), yellow-orange powder. Elemental analysis, found: C, 15.04; H, 2.59; N, 5.25; S, 7.70. Calc. for $C_{10}H_{16}N_3O_8S_2W_2Na{\cdot}2H_2O$: C, 15.07; H, 2.53; N, 5.27; S, 8.04%. ESI-HRMS (−) found (calculated): *m*/*z* $[M + HCOO^-]^-$, 805.9279 (805.9277); $[M - Na^+]^-$, 737.9386 (737.9403). ^1H NMR (300 MHz, DMSO-d_6): δ 6.93 (br, 1H, **NH₂**), 6.15 (br, 1H, **NH₂**), 3.29–3.00 (m, overlapped, 6H, **CH₂**COO and **CH₂**NH₂), 2.98–2.86 (m, 1H), 2.77–2.61 (m, 2H, **CH₂**COO), 2.44–2.22 (m, overlapped, 3H), 2.14–2.06 (m, 1H, N**CH₂CH₂**N), 1.99–1.88 (m, 1H, NC**H₂CH₂**N) ppm. ^{13}C NMR (75 MHz, D_2O): δ 175.2 (**C**OO), 173.9 (**C**OO), 173.7 (**C**OO), 67.6 (**C**H₂NH₂), 63.7 (**C**H₂COO), 62.9 (**C**H₂COO), 60.7 (**C**H₂COO), 55.2, 54.0, 39.7 (under solvent peak) ppm.

$K[W_2O_2S_2(L6)]{\cdot}H_2O$ (6a)

1a (200 mg, 0.230 mmol) in water (7 ml), $H_3L6{\cdot}3HCl$ (165 mg, 0.389 mmol, 1.7 equiv.) in water (5 ml), 1 M KOH (0.95 ml), 140 °C for 2.5 minutes. Yield (158 mg, 83%), yellow-orange powder. Elemental analysis, found: C, 17.49; H, 2.02; N, 6.74; S, 7.47. Calc. for $C_{12}H_{15}N_4O_8S_2W_2K{\cdot}H_2O$: C, 17.32; H, 2.06; N, 6.73; S, 7.71%. ESI-HRMS (−) found (calculated): *m*/*z* $[M - H^+]^-$, 812.8901 (812.8914); $[M - K^+]^-$, 774.9341 (774.9355). ^1H NMR (300 MHz, DMSO-d_6): δ 13.35 and 13.24 (s + s, 1H, imid-

N**H**), 8.69 and 8.53 (s + s, 1H, imid-C**H**), 7.45 and 7.37 (s + s, 1H, imid-C**H**), 3.75–3.49 (m, 2H), 3.28–3.05 (m, 4H), 3.02–2.88 (m, 2H), 2.82–2.65 (m, 2H), 2.47–2.00 (m, 3H) ppm. ^{13}C NMR (75 MHz, DMSO-d$_6$): δ 173.8–173.5 (**C**OO), 138.7 and 138.0 (imid-**C**H), 134.7 and 134.6 (imid-**C**=N), 115.0 and 114.3 (imid-**C**H), 67.8, 67.7, 66.5, 62.8, 61.8, 60.8, 60.2, 57.6, 55.5, 55.4, 54.9, 54.8 ppm.

Na[W$_2$O$_2$S$_2$(L6)]·H$_2$O (6b)

1b (200 mg, 0.244 mmol) in water (7 ml), H$_3$L6·3HCl (182 mg, 0.429 mmol, 1.8 equiv.) in water (7 ml), 1 M NaOH (1.0 ml), 140 °C for 2.5 minutes. Yield (152 mg, 76%), yellow-orange powder. Elemental analysis, found: C, 17.45; H, 2.24; N, 6.95; S, 7.91. Calc. for C$_{12}$H$_{15}$N$_4$O$_8$S$_2$W$_2$Na·H$_2$O: C, 17.66; H, 2.10; N, 6.87; S, 7.86%. ESI-HRMS (−) found (calculated): *m*/*z* [M − H$^+$]$^-$, 796.9150 (796.9174); [M − Na$^+$]$^-$, 774.9340 (774.9355). ^{1}H NMR (300 MHz, DMSO-d$_6$): δ 13.35 and 13.23 (s + s, 1H, imid-N**H**), 8.69 and 8.54 (s + s, 1H, imid-C**H**), 7.45 and 7.37 (s + s, 1H, imid-C**H**), 3.75–3.49 (m, 2H), 3.28–3.05 (m, 4H), 3.02–2.88 (m, 2H), 2.82–2.65 (m, 2H), 2.47–2.00 (m, 3H). ^{13}C NMR (75 MHz, DMSO-d$_6$): δ 173.8–173.5 (**C**OO), 138.7 and 138.0 (imid-**C**H), 134.7 and 134.6 (imid-**C**=N), 115.0 and 114.3 (imid-**C**H), 67.8, 67.7, 66.5, 62.7, 61.8, 60.8, 60.2, 57.5, 55.5, 55.3, 54.9, 54.8 ppm.

(NH$_4$)[W$_2$O$_2$S$_2$(L6)]·H$_2$O (6c)

1c (198 mg, 0.250 mmol) in water (6 ml), H$_3$L6·3HCl (185 mg, 0.438 mmol, 1.8 equiv.) in water (5 ml), 5% NH$_4$OH (0.55 ml), 140 °C for 2.5 minutes. Yield (165 mg, 82%), yellow-orange powder. Elemental analysis, found: C, 17.82; H, 2.61; N, 8.55; S, 7.77. Calc. for C$_{12}$H$_{15}$N$_4$O$_8$S$_2$W$_2$(NH$_4$)·H$_2$O: C, 17.77; H, 2.61; N, 8.63; S, 7.91%. ESI-HRMS (−) found (calculated): *m*/*z* [M − NH$_4$$^+$]$^-$, 774.9342 (774.9355). ^{1}H NMR (DMSO-d$_6$, 300 MHz): δ 13.35 and 13.24 (s + s, 1H, imid-N**H**), 8.69 and 8.54 (s + s, 1H, imid-C**H**), 7.45 and 7.37 (s + s, 1H, imid-C**H**), 7.09 (t, $^1J_{\mathrm{N,H}}$ = 51.3 Hz, 4H, N**H**$_4$), 3.75–3.49 (m, 2H), 3.28–3.05 (m, 4H), 3.03–2.89 (m, 2H), 2.82–2.65 (m, 2H), 2.45–2.02 (m, 3H) ppm. ^{13}C NMR (75 MHz, DMSO-d$_6$): δ 173.8–173.5 (**C**OO), 138.7 and 138.0 (imid-**C**H), 134.7 and 134.6 (imid-**C**=N), 115.0 and 114.3 (imid-**C**H), 67.8, 67.7, 66.5, 62.8, 61.8, 60.8, 60.2, 57.5, 55.5, 55.4, 54.9, 54.8 ppm.

K[W$_2$O$_2$S$_2$(L7)]·H$_2$O (7a)

1a (200 mg, 0.230 mmol) in water (7 ml), H$_3$L7·3HCl (200 mg, 0.460 mmol, 2 equiv.) in water (6 ml), 1 M KOH (1.15 ml), 140 °C for 2.5 minutes. Yield (160 mg, 82%), yellow-orange powder. Elemental analysis, found: C, 18.51; H, 2.17; N, 6.64; S, 7.44. Calc. for C$_{13}$H$_{17}$N$_4$O$_8$S$_2$W$_2$K·H$_2$O: C, 18.45; H, 2.26; N, 6.62; S, 7.58%. ESI-HRMS (−) found (calc.): *m*/*z* [M + HCOO$^-$]$^-$, 872.9122 (872.9125); [M − K$^+$]$^-$, 788.9505 (788.9513). ^{1}H NMR (300 MHz, DMSO-d$_6$): δ 8.73 and 8.58 (s + s, 1H, imid-C**H**), 7.48 and 7.39 (s + s, 1H, imid-C**H**), 3.89 and 3.87 (s + s, 3H, imid-N–C**H**$_3$), 3.71–3.49 (m, 2H), 3.30–3.06 (m, 4H), 3.02–2.88 (m, 1.5H), 2.82–2.65 (m, 1.5H), 2.45–2.02 (m, 3H) ppm. ^{13}C NMR (75 MHz, DMSO-d$_6$): δ 173.8–173.5 (**C**OO), 140.2 and 139.5 (imid-**C**H), 135.1 (imid-**C**=N), 119.1 and 118.3 (imid-**C**H), 67.8, 67.6, 66.5, 62.7, 61.8, 60.8, 60.3, 57.6, 55.6, 55.4, 55.0, 54.8; 34.6 and 34.5 (imid-N–**C**H$_3$) ppm.

Na[W$_2$O$_2$S$_2$(L7)]·2H$_2$O (7b)

1b (205 mg, 0.249 mmol) in water (5 ml), H$_3$L7·3HCl (211 mg, 0.482 mmol, 2 equiv.) in water (5 ml), 1 M NaOH (1.2 ml), 140 °C for 2.5 minutes. Yield (182 mg, 86%), yellow-orange powder. Elemental analysis, found: C, 18.25; H, 2.53; N, 6.57; S, 7.42. Calc. for C$_{13}$H$_{17}$N$_4$O$_8$S$_2$W$_2$Na·2H$_2$O: C, 18.40; H, 2.50; N, 6.61; S, 7.56%. ESI-HRMS (−) found (calculated): *m*/*z* [M + HCOO$^-$]$^-$, 856.9378 (856.9386); [M − Na$^+$]$^-$, 788.9503 (788.9513). ^{1}H NMR (300 MHz, DMSO-d$_6$): δ 8.72 and 8.58 (s + s, 1H, imid-C**H**), 7.48 and 7.39 (s + s, 1H, imid-C**H**), 3.89 and 3.87 (s + s, 3H, imid-N–C**H**$_3$), 3.71–3.48 (m, 2H), 3.30–3.05 (m, 4H), 3.02–2.88 (m, 1.5H), 2.82–2.64 (m, 1.5H), 2.45–2.02 (m, 3H) ppm. ^{13}C NMR (75 MHz, DMSO-d$_6$): δ 173.8–173.5 (**C**OO), 140.2 and 139.5 (imid-**C**H), 135.1 (imid-**C**=N), 119.1 and 118.3 (imid-**C**H), 67.8, 67.6, 66.5, 62.7, 61.8, 60.8, 60.3, 57.6, 55.6, 55.4, 55.0, 54.8; 34.6 and 34.5 (imid-N–**C**H$_3$) ppm.

K[W$_2$O$_2$S$_2$(L8)] (8a)

1a (208 mg, 0.239 mmol) in water (7 ml), H$_3$L8·3HCl (200 mg, 0.461 mmol, 2 equiv.) in water (7 ml), 1 M KOH (1.25 ml), 140 °C for 3 minutes. Yield (172 mg, 87%), yellow-orange powder. Elemental analysis, found: C, 20.40; H, 1.86; N, 5.13; S, 7.74. Calc. for C$_{14}$H$_{16}$N$_3$O$_8$S$_2$W$_2$K: C, 20.38; H, 1.95; N, 5.09; S, 7.77%. ESI-HRMS (−) found (calculated): *m*/*z* [M + HCOO$^-$]$^-$, 869.9014 (869.9016); [M − K$^+$]$^-$, 785.9395 (785.9404). ^{1}H NMR (300 MHz, DMSO-d$_6$): δ 9.36 (br d, *J* = 5.7 Hz, 0.5H, py-**H**) and 9.28 (br d, *J* = 4.8 Hz, 0.5H, py-**H**), 8.28 (m, 1H, py-**H**), 7.86–7.73 (m, 2H, py-**H**), 4.20 (br d, *J* = 15.5 Hz, 0.5 H, C**H**$_2$-py), 4.07 (br s, 1H, C**H**$_2$-py), 3.78 (d, *J* = 15.5 Hz, 0.5 H, C**H**$_2$-py), 3.30–3.09 (m, 4H, C**H**$_2$COO), 3.05–2.93 (m, 1H, C**H**$_2$COO), 2.82–2.70 (m, 1H, C**H**$_2$COO), 2.58 (m overlapped with solvent peak, 1H, NC**H**$_2$C**H**$_2$N), 2.43–2.08 (m overlapped, 3H, NC**H**$_2$C**H**$_2$N) ppm. ^{13}C NMR (75 MHz, DMSO-d$_6$): δ 173.6–173.2 (**C**OO), 156.6, 152.5, 152.3, 141.6, 141.5, 126.1, 125.6, 124.7 and 123.8 (py); 68.1, 67.7, 67.5, 66.8, 64.0, 62.8, 62.0, 61.6, 58.5, 56.0, 55.2, 54.5, 48.6 ppm.

Na[W$_2$O$_2$S$_2$(L8)]·2H$_2$O (8b)

1b (204 mg, 0.249 mmol) in water (6 ml), H$_3$L8·3HCl (217 mg, 0.499 mmol, 2 equiv.) in water (5 ml), 1 M NaOH (1.2 ml), 140 °C for 3 minutes. Yield (175 mg, 84%), yellow-orange powder. Elemental analysis, found: C, 19.99; H, 2.16; N, 5.03; S, 7.28. Calc. for C$_{14}$H$_{16}$N$_3$O$_8$S$_2$W$_2$Na·2H$_2$O: C, 19.90; H, 2.38; N, 4.97; S, 7.59%. ESI-HRMS (−) found (calculated): *m*/*z* [M + HCOO$^-$]$^-$, 853.9288 (853.9277); [M − Na$^+$]$^-$, 785.9402 (785.9404). ^{1}H NMR (300 MHz, DMSO-d$_6$): δ 9.35 (br d, *J* = 5.6 Hz, 0.5H, py-**H**) and 9.28 (br d, *J* = 4.1 Hz, 0.5H, py-**H**), 8.27 (m, 1H, py-**H**), 7.85–7.74 (m, 2H, py-**H**), 4.19 (br d, *J* = 15.6 Hz, 0.5 H, C**H**$_2$-py), 4.07 (br s, 1H, C**H**$_2$-py), 3.78 (br d, *J* = 15.6 Hz, 0.5 H, C**H**$_2$-py), 3.32–3.01 (m, 4H, C**H**$_2$COO), 3.02–2.94 (m, 1H, C**H**$_2$COO), 2.82–2.70 (m, 1H, C**H**$_2$COO), 2.57 (m overlapped with solvent peak, 1H, NC**H**$_2$C**H**$_2$N), 2.44–2.10 (m overlapped, 3H, NC**H**$_2$C**H**$_2$N) ppm. ^{13}C NMR (75 MHz, DMSO-d$_6$): δ

173.7–173.2 (**C**OO), 156.6, 152.5, 152.3, 141.6, 141.5, 126.1, 125.6, 124.8 and 123.8 (py); 68.1, 67.9, 67.5, 66.8, 64.0, 62.8, 62.0, 61.6, 58.5, 56.0, 55.2, 54.5, 48.6 ppm.

Synthesis of $M_2[W_2O_2S_2(EDTA)]$, $M^+ = K^+$ or Na^+

The procedure for microwave-assisted synthesis of monoanionic sulfido-bridged complexes was adopted to afford the corresponding EDTA complexes. An aqueous solution of the tungsten cysteinato precursor (**1a-b**, 1 equiv.) was added dropwise to an aqueous solution of Na_2H_2EDTA (or H_4EDTA, partially neutralized with KOH) in a 20 ml microwave reaction vial with stirring. The pH of the reaction mixture was adjusted to ~3–3.5 by addition of 1 M HCl. After flushing with N_2 for 10 min, the microwave vial was tightly closed under a flow of N_2, placed in the microwave instrument, and heated at 140 °C for 2 min. Thereafter, the vial was removed from the instrument and cooled down to rt. The orange solution obtained was concentrated (by rotary evaporation) to *ca.* 1/3 of the original volume and placed at 4 °C for 16 h to crystallize the desired product. The latter was collected by filtration, washed with minimal amounts of cold water and MeOH, and dried *in vacuo*. Reaction monitoring was carried out by IP-RP-HPLC before and after microwave heating.

Dipotassium (μ-ethylenediaminetetraacetato)-(di-μ-sulfido)bis[(oxotungstate(V)] dihydrate, $K_2[W_2O_2S_2(EDTA)]\cdot 2H_2O$ (9a)

1a (201 mg, 0.231 mmol) in water (7 ml), H_4EDTA (135 mg, 0.462 mmol, 2 equiv.) in warm water (7 ml) with addition of 1 M KOH (0.9 ml), 1 M HCl (0.35 ml), 140 °C for 2 minutes. Yield (175 mg, 89%), orange crystalline powder. Elemental analysis, found: C, 13.82; H, 1.82; N, 3.31; S, 7.04. Calc. for $C_{10}H_{12}N_2O_{10}S_2W_2K_2\cdot 2H_2O$: C, 13.87; H, 1.86; N, 3.24; S, 7.40%. ESI-HRMS (−) found (calculated): *m*/*z* $[M + HCOO^-]^-$, 874.8192 (874.8203); $[M - K^+]^-$, 790.8582 (790.8594); $[M - 2K^+ + H^+]^-$, 752.9016 (752.9031); $[M - 2K^+]^{2-}$, 375.9483 (375.9434). ^1H NMR (300 MHz, D_2O): δ 3.64 (br d, *J* = 16.9 Hz, 4H, C**H₂**COO), 3.42 (d, ^2J = 17.1 Hz, 2H, C**H₂**COO), 3.16 (d, ^2J = 16.2 Hz, 2H, C**H₂**COO), 2.56 (s+d, $^3J_{W,H}$ = 30.5 Hz, 4H, N–C**H₂**C**H₂**N) ppm. ^{13}C NMR (75 MHz, D_2O): δ 178.2 (**C**OO), 67.2 (**C**H₂COO), 62.6 (**C**H₂COO), 56.2 (N–**C**H₂**C**H₂N) ppm.

Disodium (μ-ethylenediaminetetraacetato)-(di-μ-sulfido)bis[(oxotungstate(V)] hydrate, $Na_2[W_2O_2S_2(EDTA)]\cdot H_2O$ (9b)

1b (200 mg, 0.244 mmol) in water (7 ml), $Na_2H_2EDTA\cdot 2H_2O$ (182 mg, 0.488 mmol, 2 equiv.) in warm water (7 ml), 1 M HCl (0.44 ml), 140 °C for 2 minutes. Yield (134 mg, 68%), orange powder. Elemental analysis, found: C, 14.68; H, 1.80; N, 3.73; S, 8.02. Calc. for $C_{10}H_{12}N_2O_{10}S_2W_2Na_2\cdot H_2O$: C, 14.72; H, 1.73; N, 3.43; S, 7.86%. ESI-HRMS (−) found (calculated): *m*/*z* $[M + HCOO^-]^-$, 842.8763 (842.8724); $[M - Na^+]^-$, 774.8864 (774.8855); $[M - 2Na^+ + H^+]^-$, 752.9056 (752.9031); $[M - 2Na^+]^{2-}$, 375.9479 (375.9434). ^1H NMR (300 MHz, D_2O): δ 3.60 (br d, ^2J = 17.0 Hz, 4H, C**H₂**COO), 3.38 (d, ^2J = 17.2 Hz, 2H, C**H₂**COO), 3.11 (d, ^2J = 16.3 Hz, 2H, C**H₂**COO), 2.51 (br s+d, $^3J_{W,H}$ = 30.7 Hz, 4H, N–C**H₂**C**H₂**N) ppm. ^{13}C NMR (75 MHz, D_2O): δ 178.2 (**C**OO), 67.2 (**C**H₂COO), 62.6 (**C**H₂COO), 56.2 (N–**C**H₂**C**H₂N) ppm.

Crystallographic structure determination

Single-crystal X-ray diffraction measurements were performed on a Bruker APEX-II CCD diffractometer using monochromatized Mo-K_α radiation at 100 K. Molecular structures were solved by direct methods (SHELXS-97)[39] and refined by full-matrix least-squares techniques against F^2 (SHELXL-2014/6).[40] Further details, including crystal data, data collection parameters, and structure refinement are given in the SI.

Stability studies

Stability of selected complexes (*i.e.*, **1b**, **7a**, **8b** and **9b**) over 20 h of incubation in phosphate buffer (20 mM, pH 7.4) at 25 °C and under ambient atmosphere was examined by means of UV-Vis spectroscopy scanning kinetic measurements at the range λ = 300–800 nm. The measurements were performed on a Varian Cary® 50 spectrophotometer equipped with VWR 1140S recirculating thermostat. The solvent absorption was subtracted as the background. In addition, the same complexes were investigated for their stability during heat sterilization. Compounds were dissolved in 20 mM phosphate buffer (pH 7.4) to yield final concentrations of 2 mM. Solutions were filtered through a nylon membrane (0.45 μm) filter and filled in 2 ml microwave vials. After purging with N_2 for 5 min, the vials were tightly closed under a flow of N_2, placed in the microwave instrument, and heated at 121 °C for 45 min. All solutions were clear and without signs of precipitation after the heat treatment. A small aliquot (150 μl) was taken before and after heating treatment and subjected to RPIP-HPLC analysis after dilution to 1 ml with Milli-Q water. Absolute stability was calculated from the HPLC chromatograms as the ratio of the peak area of the compound after and before the heat treatment.

Incubation with L-cysteine

Selected di-μ-sulfido W(V) complexes (1.5 mM) were incubated with L-cysteine (3 equiv.) in 20 mM phosphate buffer (pH 7.4) for 24 h at 37 °C. Reactivity and new adducts formation was followed by means of RPIP-HPLC. Amounts of the remaining intact complex after 2 and 24 h of incubation with cysteine were calculated from the HPLC chromatograms as the ratio of the peak area of the compound after the respective incubation time (2 or 24 h) and at time point 0 (*i.e.*, promptly after mixing the solutions).

Molar conductivity measurements

Solutions containing 1 mM of the complexes under investigation, were prepared in Milli-Q water. 1 mM solutions of NaCl and $Na_2[WO_4]$ were examined for comparative purposes. Measurements were performed with WTW Portable Conductivity Meter ProfiLine Cond 3110 at 25 ± 1 °C.

Conflicts of interest

There are no conflicts to declare.

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