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Novel Crown-Ether- Methylenediphosphonotetrathioate Hybrids as Zn(II) Chelators

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Hybrids of methylenediphosphonotetrathioate and crown-ether (MDPT-CE) were synthesized forming 7-,8-,9-,10- and 13-membered rings. Both 7- and 13-memebered ring-containing compounds were found to be highly stable to air-oxidation for at least four weeks. These hybrids bind Zn(II) by both MDPT and CE moieties, forming a 2:1 L:Zn(II) complex. Interestingly, 13-membered ring MDPT-CE showing a high affinity to Zn(II) (K_a 3±0.5*10^6 mol^{-2}*L^2), does not bind Li(I) or Na(I). 13-Membered MDPT-CE hybrid is a promising water-soluble, air-stable, high-affinity Zn(II)-chelator, exhibiting selectivity to Zn(II) vs. Mg(II), Na(I), and Li(I).

Introduction

The fraction of the world's population affected by diseases involving abnormal Zn(II) homeostasis including diabetes, prostate and breast cancers, arthritis, and Alzheimer's disease is already large and growing. Therefore, Zn(II) chelators are required as potential therapeutic agents for the treatment of various health disorders involving excess of Zn(II) ions or unwanted activity of Zn(II)-enzymes.[1]

For instance, nucleoside 5'-phosphorothioate analogues may be used for the treatment of bone diseases by inhibition of the overexpressed nucleotide pyrophosphatase, NPP1.[2] Another potential application of nucleoside 5'-phosphorothioate analogues may be for the treatment of type 2 diabetes, where insulin receptor was found to be damaged due to overexpression of NPP1.[3]

Additional Zn(II) chelators include carbonic anhydrase inhibitors which are currently clinically used for the treatment of glaucoma and epilepsy;[4,5] and Clioquinol, a lipophilic Zn(II)/Cu(II) chelator which attenuates Aβ deposition in a mouse model for Alzheimer's disease suggesting therapeutic utility in the treatment of Alzheimer's disease.[6,7] Likewise, 8-hydroxyquinoline-2-carboxaldehyde isonicotinoyl hydrazone is able to disrupt zinc and copper interactions with the Aβ-peptide by efficiently competing for Zn(II) and Cu(II) binding.[8]

Recently, we have developed a novel scaffold of Zn(II)-chelator. This scaffold is based on methylene diphosphonate (MDP), **1**,[9] to provide metabolic and chemical stability, and four sulfur atoms replacing disphosphonate oxygen atoms to confer a 'soft-base' character to MDP and increase selectivity towards Zn(II)-ions. Specifically, MDP binds Ca(II) ions (logK 5.97)[10,11] and hence is used for treating bone diseases.[12] However, MDP also binds Mg(II),[10] Cu(II),[13] and other ions,[14-16] thus making MDP and its derivatives non-selective chelators in a physiological environment.

Therefore, we recently developed methylenediphosphono-tetrathioate (MDPT),[17] **2**, its O,O'-diester-MDPT derivatives,[18] **3**, and nucleoside-5'-MDPT analogues **4** and **5** (Fig. 1), as selective Zn(II) chelators. All these derivatives were synthesized from methylene-bis(1,3,2-dithiaphospholane-2-sulfide), **6**.[19] MDPT scaffold is highly selective to border-line /soft metal-ions vs. alkali earth metal-ions. This metal-ion selectivity is significant in the Ca(II) and Mg(II) rich physiological medium.[20-22]

Specifically, MDPT, **2**, exhibited high affinity to Zn(II) (logK 10.84), and was 10^7-times more selective to Zn(II) vs. Ca(II)-ions, however, its relative sensitivity to air-oxidation limited its use.[17]

O,O'-diester-MDPT derivatives, **3**, selectively chelate soft/ borderline metal-ions (Zn(II), Ni(II), Cu(I), and Fe(II)). However, M(II)-complexes of **3** are water insoluble and dissolve only in organic solvents such as DMSO and EtOH.[18]

Nucleoside-5'-MDPTs, **4-5**, also prefer Zn(II) over Mg(II).[19] Both **4a** and **5a** were highly stable under basic conditions (pD 11). Under acidic conditions (pD 1.5) **5a** was more stable than **4a**. Furthermore, unlike nucleotide **5a**, dinucleotide **4a** was stable to air-oxidation.[19]

Hence, here we aimed to develop water soluble, high-affinity and selective MDPT-based Zn(II) chelators, with improved chemical stability, as compared to MDPT. For these purposes we designed novel hybrids of crown-ether and methylenediphosphonotetra-thioate, MDPT-CE (Fig. 2). We hypothesized that fusing CE to MDPT will improve water solubility of M(II)-complexes of MDPT-CE vs. that of O,O'-diester-MDPT, **3**, and enhance Zn(II)-coordination by MDPT-CE vs. **3**, due to additional interactions with CE-oxygen atoms. Finally, we hypothesized that unlike MDPT, MDPT-CE ring will resist air-oxidation, to avoid formation of a fused disulfide ring.

Specifically, we report on the synthesis of MDPT-CE analogues, **7-11**, evaluation of their selectivity to Zn(II) vs. Mg(II), and the mode of Zn(II) binding. In addition, we report the association constants of MDPT-CE-Zn(II) complexes, the chemical stability of **7**-**11**, under

acidic or oxidizing conditions, and the selectivity of the most promising chelator, **11**, to Zn(II) vs. Na(I) and Li(I).

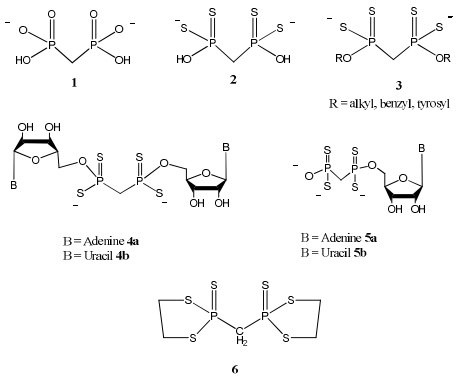


Fig. 1. Methylene diphosphonate (MDP), **1**, methylenediphosphonotetrathioate (MDPT), **2**, O,O'-diester-MDPT derivatives, **3**, dinucleoside- and nucleoside-5'-MDPT, **4** -**5** and starting material, **6**.

Results and Discussion

Synthesis

We synthesized MDPT-CEs from a 7-membered ring (**7**) up to a 13-membered ring (**11**). Compound **9**, contains a phenyl ring, as additional chelation site with metal-ions (π-cation interactions). Specifically, **7**-**11** were obtained in a one-step reaction, upon addition of the appropriate diol to **6**,[18] DBU, and molecular sieves 4Å in dry chloroform. The reaction progress was monitored by ^{31}P-NMR. The appearance of a new singlet at 102-105 ppm and the disappearance of starting material singlet at 92 ppm, indicated the completion of the reaction. The crude residue of the mentioned reactions was separated on a silica-gel column. Next, $DBUH^+$ counter ions were exchanged to Na(I) ions on Dowex 50wx8-20 Na^+-form. Finally, separations were performed on a reverse phase column eluting the products with TEAA/ACN. Compounds **7**, **9**, and **11** were obtained in 43, 10, and 15% yield, respectively, however, during the synthesis of **8** and **10**, acyclic by-products **12** and **13** were isolated as well (Table 1). By-product **14** was obtained in varying amounts in all syntheses, as indicated by typical doublets at 70 and 60 ppm in ^{31}P-NMR spectrum. The smallest MDPT-CE ring (7-membered ring), **7**, was obtained at the highest yield (43%). Larger rings were obtained at lower yields (5-16%). The low yield of **8** and **10** was related to formation of acyclic by-products **12** and **13**, respectively. The formation of **14**, due to hydrolysis and oxidation of **6**, also limited MDPT-CEs' yields. The amount of **14** was moisture-dependent. For instance, reaction of **6** with non-distilled ethylene glycol resulted in a low yield of **7**, but ca. 50% **14**, despite the presence of molecular sieves in the reaction mixture. The formation of **12** and **13** during the synthesis of **8** and **10**, and the lack of acyclic by-products during the synthesis of **7** and **11**, could be related to the size of the formed MDPT-CE ring, as interpreted below.

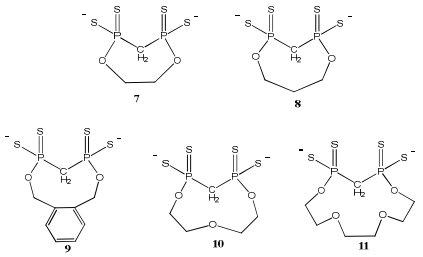


Fig. 2. MDPT-CE products **7**-**11**

Illuminati *et al.* reported that the rate of cyclization of ω-bromoalkanoates to form 8-membered ring lactone was 100-times slower than that of the 7-membred ring.[23,24] This supports our findings that the yield of an 8-memered ring, **8**, is lower (16%), than that of a 7-membered ring, **7** (43%). Likewise, the relative stability of a 7-membred ring, explains why acyclic by-product **12** was formed (30% yield) in the synthesis of **8**, but not in the synthesis of **7**. Illuminati *et al.* also reported a 10-fold increase in the cyclization rate of ω-bromoalkanoates to form 13- vs. 10-membered ring.[24] This is also consistent with the finding that during the synthesis of **11** (13-membered ring), no acyclic by-product was formed, while in the synthesis of a 10-membered-ring, **10** (5%), acyclic by-product **13** was formed (6%). The formation of the 9-memberd ring, **9**, was not accompanied by an acyclic by-product, although the rate of formation of 8- and 9-membered rings was reported to be similar,[24] possibly due to the phenyl moiety which introduces rigidity to the ring, thus facilitating the formation of **9** (15% yield).

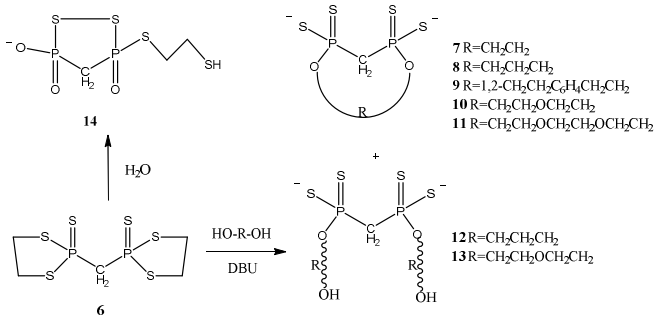
Stability assays

The stability of representative MDPT-CE analogues, containing the smallest and the largest ring sizes (7 and 13 membered rings, respectively), **7** and **11**, was explored under acidic conditions, and their susceptibility to oxidation was studied by bubbling air into their D_2O solutions. These experiments were monitored by ^{31}P-NMR.

Our hypothesis was that incorporation of MDPT unit into a ring would inhibit its air-oxidation, since oxidation may result in a strained 5-membered disulphide-ring fused to CE ring. This notion is supported by the resistance of hindered **4a** to air-oxidation.[19]

Both **7** and **11** were found to be highly stable to air-oxidation for at least four weeks. Compound **7** was found to be stable under pD 1.5 for at least four weeks, while **11** decomposed with $t_{1/2}$ 42 h. Using ^{31}P-NMR and MS analyses we elucidated the species formed during the decomposition of **11** at pD 1.5 (Fig. 3).[19] Compound **11** was ca. 5-times more stable than dinucleotide-MDPT, **4a**, at pD 1.5 ($t_{1/2}$ 9 h). The higher stability of **7** vs. **11** to acid-catalysed hydrolysis may be correlated with the higher rate of formation of 7- vs. 13-membered ring.[23]

Table 1. Yields and ^{31}P-NMR data of derivatives **7-11** and by-products **12-13**



Compound	R	% Yield[a]	^{31}P-NMR[b] ppm
7	CH_2CH_2	43	105.3
8	$CH_2CH_2CH_2$	16	102.5
9	1,2-$CH_2CH_2C_6H_4CH_2CH_2$	15	103.4
10	$CH_2CH_2OCH_2CH_2$	5	104.1
11	$CH_2CH_2OCH_2CH_2OCH_2CH_2$	10	104.7
12	$CH_2CH_2CH_2$	30	103.6
13	$CH_2CH_2OCH_2CH_2$	6	102.7

[a] Yields of products after both LC and HPLC separations. [b] ^{31}P-NMR (at 162 MHz) data of products **7-13** disodium salts in D_2O.

Nucleoside **5a**, underwent spontaneous air-oxidation in water ($t_{1/2}$ 14 h).[19] Likewise, MDPT underwent 33% oxidation after 27 h.[17] However, dinucleotide, **4a**, resisted air-oxidation for at least 3 d.

Air-oxidation of **7** and **11** results in a strained system in which a 5-membered disulfide ring is fused to a 7/13-membered ring. The resistance of **7** and **11** to formation of a strained system is reminiscent of the stability of dinucleotide **4a**. In both cases the formation of a sterically hindered system is avoided.

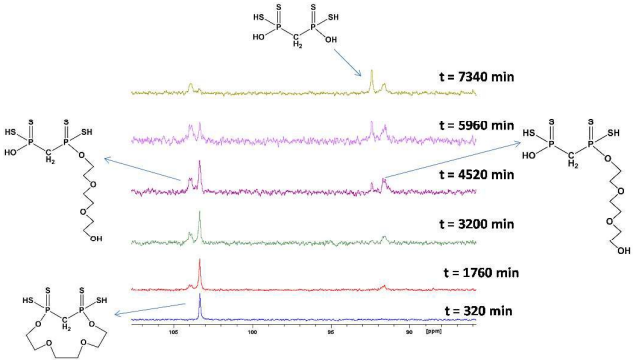


Fig. 3. Time-dependent ^{31}P-NMR spectra of **11** at pD 1.5, measured at 160 MHz, 300 K.

Characterization of representative compounds 7, 9 and 11 as Zn(II) chelators

High Zn(II) selectivity vs. alkali earth metal-ions, which are abundant in a physiological medium, is a pre-requisite for zinc chelators used for pharmacological purposes. We explored Zn(II) vs. Mg(II)-coordination by **7**, **9**, and **11**. These representative analogues were chosen due to their ease of preparation (easier separation and no formation of acyclic by-products, unlike compounds **8** and **10**), and since each represents another type of MDPT-CE (aliphatic, aromatic and tri-ether rings, respectively). Specifically, D_2O solutions of **7**, **9** and **11** were titrated by 0.1-5 eq Zn(II)/Mg(II) and monitored by ^{1}H- and ^{31}P-NMR. Titrations with Zn(II), resulted in ~1 log unit decrease of the original pD (7.3), while no change of pD was observed for Mg(II) titrations. To discern whether shifts of signals in ^{31}P-NMR spectra during Zn(II)-titration are due to Zn(II)-coordination or pD changes, we measured ^{31}P-NMR spectra of **7** as a function of pD (Fig. S1, ESI†). No change of ^{31}P-NMR shift of signals of **7** was observed upon pD increase from 2 to 11.

Addition of 0.1 eq Zn(II) to **11** caused line-broadening and an upfield shift of the dithiophosphonate signal in ^{31}P-NMR spectra (Fig. 4). Line-broadening was a result of dynamic equilibrium (from the NMR line-widths and using the Eyring equation, a free energy of activation *ca*. 14.0 kcal*mol^{-1} was estimated) between the free ligand, e.g. **11**, and the Zn(II)-ligand complex.[25] After addition of 0.5 eq Zn(II) a singlet at 100.7 ppm was observed. The shift of the phosphorus signal (from 103.4 to 100.7 ppm) and line-sharpening indicated the formation of Zn(II) complex at a 2:1 ligand:metal stoichiometric ratio. Addition of up to 5 eq of Zn(II) resulted in no change in ^{31}P-NMR spectrum.

Similar to **11**, the stoichiometry of **9**-Zn(II) and **7**-Zn(II) complexes is 2:1 (Figs. S15-S16, ESI†). Zn(II) addition to **11** and **7**, resulted in maximum Δδ of 3 to 1 ppm of MDPT signal in ^{31}P-NMR spectra, respectively. These Δδ values may imply on inner- and outer-sphere coordination for **11**-Zn(II) and **7**-Zn(II) complexes, respectively.[26]

Unlike Zn(II) binding to **7**, **9** and **11**, no shift of the signals was observed upon Mg(II)-titration (Figs. S8-S13, ESI†), indicating clear preference of MDPT-CE analogues to Zn(II).

The mode of coordination of Zn(II) by MDPT-CEs was also studied by UV. Analogue **9**, in Tris buffer (pH 7.4), was titrated with $ZnCl_2$ in H_2O and the absorbance was measured (Fig. S17, ESI†). The absorbance of $ZnCl_2$ in buffer solution appeared at 200 nm, and an increasing shoulder at ~220 nm indicated zinc-thiolate ligation.[27]

Zn(II)-complexes of **7** and **11** were also characterized by FT-IR (Figs. 5 and S18, ESI†). The P=S stretching band[28,29] of **7** and **11** appeared at 633 cm^{-1} (Fig. 5A) and 625 cm^{-1} (Fig. S18A), respectively. The P=S stretching bands of **7**-Zn(II) 2:1 (Fig. 5B) and 1:1 (Fig. 5C) complexes shifted to 616 cm^{-1}, and that of **11**-Zn(II) 2:1 to 604 cm^{-1}, (Fig. S18B), thus indicating the involvement of MDPT sulfur atoms in Zn(II)-binding.

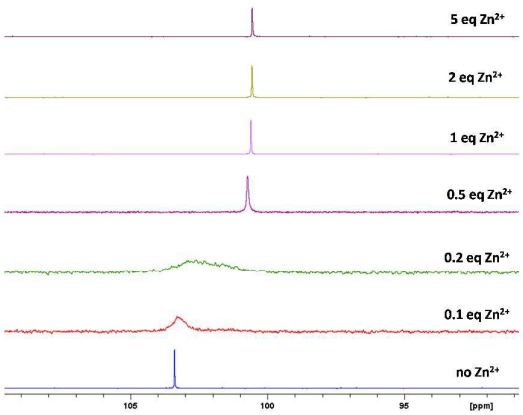


Fig. 4. Titration of 6 mM **11** in D_2O with 0.2 M $ZnCl_2$ solution monitored by ^{31}P-NMR spectra measured at 243 MHz, 300K.

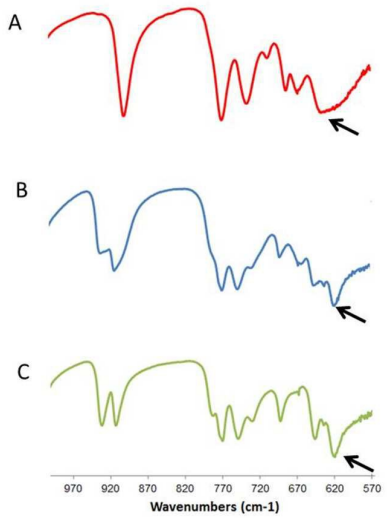


Fig. 5. FT-IR spectra 1900-500 cm^{-1} region, of A) compound **7**, B) **7**-Zn(II) (2:1) and C) **7**-Zn(II) (1:1).

Determination of association constants of Zn(II) complexes of representative analogues 7, 9, and 11

To quantify the affinity of representative compounds to Zn(II), association constants were extracted from ^{31}P-NMR monitored Zn(II)-titrations data. NMR studies and data analysis by the Yoe-Jones method[30] (Figs. 4, S15 and S16, ESI†) indicated a ML_2 stoichiometry. A 2:1 ligand:metal stoichiometry provided an equilibrium equation as follows:

1) $2L + M \rightleftarrows L_2M$

2) $K_a = [L_2M]/[L]^2[M]$

3) $[L_2M] = X$; $[L] = L_0-2X$; $[M] = M_0-X$

4) $K_a = X/(L_0-2X)^2(M_0-X)$

5) $4K_aX^3 - 4K_a(L_0+ M_0)X^2 + (4K_aL_0M_0 + K_aL_0^2+1)X - K_aL_0^2M_0 = 0$

6) $\delta_P = 2*D*X/L_0$; $D = \Delta\delta_{max}$

L = compound **7**, **9** or **11**. M = Zn(II). L_0 and M_0 = concentration of the ligand and Zn(II), respectively, at a given point.

From equation 5 we generated a calculated curve for the ^{31}P-NMR chemical shifts by assuming starting values for K_a and $\Delta\delta_P$. We then manually fitted these two parameters so there would be an optimal fit of the calculated curve to the experimentally measured chemical shifts (Figs. 4, S3 and S6, ESI†). We were thus able to determine the association constants of **7**-, **9**-, **11**- Zn(II) complexes (Table 2). As **11** showed the highest affinity to Zn(II), the kinetics of the complex formation was the slowest, as manifested in line broadening of the signals in ^{31}P-NMR (Fig. 4). The chemical shifts of the broadened signals were set at the centre of gravity of the peak (the middle of the integration curve); not surprisingly, for this compound the fitting for intermediate amounts of Zn(II), namely 0.1 and 0.2 eq of Zn(II), was the poorest. The NMR data are summarized in Fig. 6, in which the vertical axis corresponds to a normalized $\Delta\delta$, i.e., $\Delta\delta = [\delta_{P(\text{observed at a given [Zn(II)]})} - \delta_{P(\text{ligand})}]/[\delta_{P(\text{complex})} - \delta_{P(\text{ligand})}]$. The points on the graphs indicate experimentally measured chemical shifts; the continuous curves passing through the calculated chemical shifts for each ligand were obtained using the values of the association constant, K_a, given in Table 2.

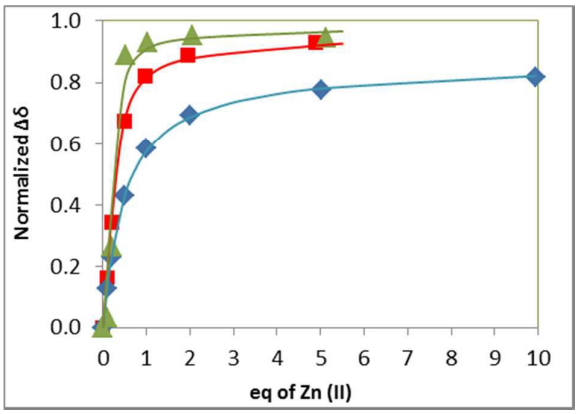


Fig. 6. Experimental Δδ values and calculated curves (see text) *vs.* number of equivalents of Zn(II) for compounds **7** (◆), **9** (■) and **11** (▲).

Association constants obtained from Zn(II)-titrations (Table 2) imply that Zn(II) interacts not only with the MDPT moiety but also with the CE-moiety.

Table 2. Calculated association constants of Zn(II) complexes of **7**, **9,** and **11**.

Zn(II) complexes	K_a, Calculated association constant $[mol^{-2}*L^2]$
7-Zn(II) complex	$0.05\pm0.003*10^6$
9-Zn(II) complex	$0.5\pm0.03*10^6$
11-Zn(II) complex	$3\pm0.5*10^6$

X-ray crystal structure of 11-Zn(II) complex

Compound **11** shows the highest affinity towards Zn(II) (K_a $3\pm0.5*10^6$ $mol^{-2}*L^{-2}$) and also contains a crown ether moiety of a size suitable for Na(I)/Li(I) chelation. Hence, we next characterized **11**-Zn(II) complex X-ray crystal structure (Fig. 7). Zn(II) complex of **11** was recrystallized from a mixture of water, ethanol, and methanol (1:1:1 v/v/v) and slow evaporation of the solvents to obtain yellowish crystals. X-ray data demonstrate a 2:1 ligand:metal complex, supporting the ^{31}P-NMR data for Zn(II)-titration of **11** (Fig. 4). The X-ray crystal structure also reveals an inner-sphere coordination of Zn(II) by sulfur atoms, as implied by NMR and IR data (Figs. 4 and S18). The hypothesis that Zn(II) interacts also with CE oxygen atoms is supported by solution ^{13}C-NMR spectra measured before and after Zn(II)-addition to **11** in D_2O (Fig. 8) confirmed the interaction of the CE-moiety with Zn(II). The CE carbon signals shifted by 0.5-0.8 ppm upon addition of 10 eq of Zn(II) vs. internal standard (MeOH). Carbon atoms adjacent to O5 and O6 shifted by ~0.8 ppm, while more distant carbon atoms in the CE moiety, shifted by ~0.5 ppm, thus indicating differential coordination of Zn(II) with CE oxygen atoms. Analogue **9** has a 10-fold higher affinity to Zn(II) than that of analogue **7**, possibly due to interaction of Zn(II) with the aromatic system[31,32] in addition to that with CE oxygen atoms.

X-ray data reveals that **11** interacts with Na(I) ions, in addition to Zn(II), by the oxygen atoms of the CE ring. In this way it forms a long, endless polymer chain linked together by the Na(I). Et_3NH^+ ions and water molecules are present between these chains, making layers (Fig. 7B). Na(I) ions serve as counter-ions of these polymeric chains. Indeed, ^{13}C-NMR spectra measured before and after addition of 5 eq Na(I)-ions to Et_3NH^+ salt of **11** (obtained after HPLC separation of **11** using triethyl ammonium acetate buffer as eluent) (Fig. S19, ESI†) showed no change of the CE carbon signals vs. internal standard (MeOH), indicating no binding interaction of CE with Na(I) in solution, unlike with Zn(II). Likwise, no change in ^{13}C-NMR was observed upon Li(I) addition to **11** (Fig. S20, ESI†).

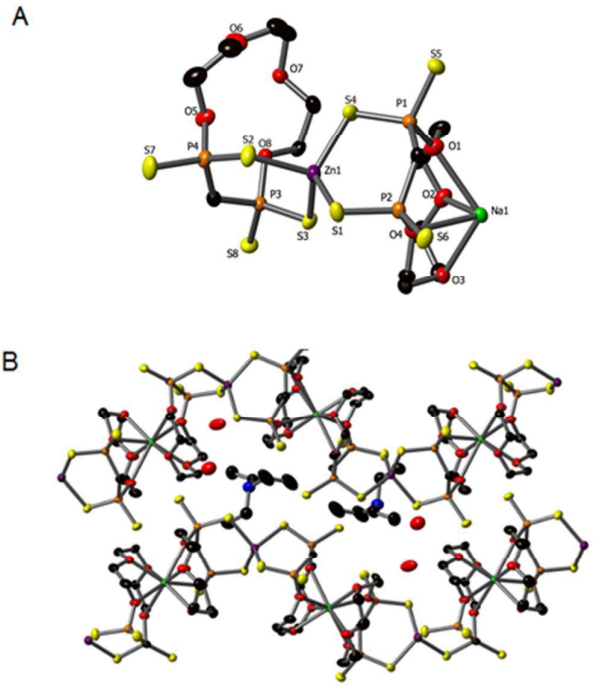


Fig. 7. X-ray structure of single crystal of **11**-Zn(II) complex (Et_3NH^+ ions, water molecules and hydrogen atoms were omitted for clarity) (A) and an endless polymer chain linked together by the Na(I)-ions and water molecules of **11**-Zn(II) complexes (B).

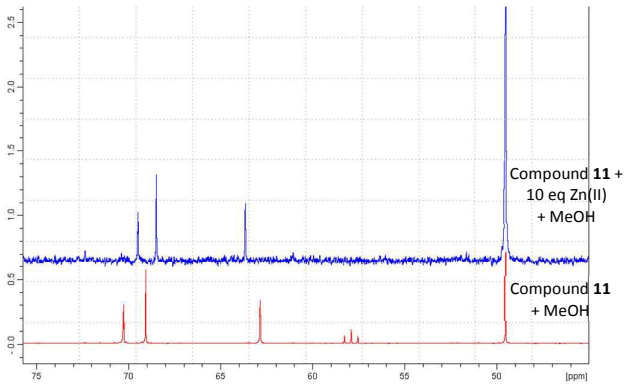


Fig. 8. ^{13}C-NMR of compound **11** before and after Zn(II)-addition in D_2O, with MeOH as internal standard (the absence of the MDPT methylene protons upon Zn(II) addition is due to their exchange with deuterium).

Conclusions

We synthesized novel hybrids of crown-ether and methylenediphosphonotetrathioate, **7**-**11**, in a one-step synthesis from **6**. Compounds **7** and 11 were found to be highly stable to air oxidation. Compound **7** was highly stable also at pD 1.5, while **11** decomposed with $t_{1/2}$ 42 h. Compound

11 is a high-affinity Zn(II)-chelator (K_a= 3±0.5*10^6 $mol^{-2}*L^2$) while **7** and **9** (K_a= 0.05±0.003*10^6 and 0.5±0.03*10^6 $mol^{-2}*L^2$, respectively), are weaker Zn(II)-chelators. Furthermore, **7-11** bind preferentially Zn(II) over Mg(II). The stoichiometry of **11**-Zn(II) complex is 2:1 ligand: metal. MDPT sulfur atoms coordinate Zn(II) via an inner-sphere mode. Association constants of Zn(II)-complexes of **7**, **9** and **11** in aqueous solution implied the involvement of CE-oxygen atoms in Zn(II)-coordination in addition to that with MDPT. This hypothesis was supported by NMR data for **11** in the presence of Zn(II)/ Na(I)/ Li(I) ions vs. free **11**. In summary, MDPT-CE hybrid **11**, is a water-soluble, air-stable, high-affinity Zn(II)-chelator, exhibiting selectivity to Zn(II) vs. Mg(II), Na(I), and Li(I).

Experimental Section

General

Reactions were performed in oven dried flasks under N_2 atmosphere. 1,8-Diazabicyclo[5.4.0]undec-7-ene (DBU), ethylene glycol, diethylene glycol, triethylene glycol and propylene glycol were distilled under reduced pressure before use. $CHCl_3$ was distilled over P_4O_{10}. Purification was performed using flash chromatography on silica-gel and C_{18} reverse phase supports. ^{1}H-NMR spectra were obtained using a 400 or 600 MHz spectrometer. ^{13}C-NMR spectra were obtained at 100 or 150 MHz. ^{31}P-NMR spectra were obtained at 162, or 243 MHz. HRMS and MS spectra were measured on a MALDI-TOF or ESI-TOF instruments. Final purification of the products was achieved on an HPLC system, using a semipreparative reverse-phase column. IR spectra were recorded on FT-IR spectrometer using ZnSe crystal for powder samples pressed.

Typical procedure for preparation of cyclic tetrathio-bisphosphonate analogues 7-11

To a two-necked round bottom flask containing activated molecular sieves, compound **6**[18] (0.46 mmol) and dry chloroform (9.6 mL) was added. To this suspension, DBU (0.92 mmol) and the appropriate diol (0.46 mmol) were added. The reaction mixture was heated under reflux and nitrogen atmosphere for 45 minutes. Progress of the reaction was monitored by ^{31}P-NMR in $CDCl_3$. After cooling to room temperature, the mixture was filtered, molecular sieves were washed with chloroform and the solvent was evaporated. The crude residue was separated on silica-gel column upon $CHCl_3$:MeOH elution. To increase water solubility the product containing fraction was dissolved in a water:THF 6:4 mixture and passed through Dowex 50wx8-20 Na^+-form. THF was evaporated and the remaining aqueous solution was freeze-dried. Final purification was carried out by HPLC, using a semipreparative reverse-phase column, applying TEAA/ACN (the gradient for each material described below). The solvent was freeze-dried several times until a constant weight was obtained. Triethyl ammonium ions in the product were replaced by sodium by passing the products' solution through Dowex 50wx8-20 Na^+-form and freeze-drying again to obtain the desired products.

1,5,2,4-Dioxadiphosphepane-2,4-bis(thiolate) 2,4-disulfide disodium salt, 7, was obtained in 43% yield from **6** (0.21 mmol) and ethylene glycol (0.21 mmol) following the typical procedure mentioned above. HPLC separation was carried out using isocratic elution with TEAA/ACN 98:2. ^{1}H NMR (600 MHz, D_2O): δ 4.37-4.34 (m, 4H), 3.81 (t, J = 12.0 Hz, 2H) ppm. ^{13}C NMR (150 MHz, D_2O): δ 67.2 (t, J = 4.4 Hz), 64.4 (t, J = 50.7 Hz) ppm. ^{31}P NMR (162 MHz, D_2O): δ 105.3 ppm. HRMS (MALDI) m/z: calcd for $C_3H_7O_2P_2S_4^-$ $[M-H]^-$ 264.8810; found, 264.8852. **7-Zn(II) complex.** ^{31}P NMR (162 MHz, D_2O): δ 104.2 ppm. IR (ZnSe): ν 1611, 1431, 1346, 1279, 1236, 1146, 1106, 1060, 1049, 1015, 920, 914, 767, 745, 726, 689, 653, 635, 626, 616 cm^{-1}. MS (ESI) m/z: calcd for $C_6H_{15}O_4P_4S_8Zn^+$ $[M+3H]^+$ 596.98; found, 597.18.

1,5,2,4-Dioxadiphosphocane-2,4-bis(thiolate) 2,4-disulfide-disodium salt, **8,** was obtained in 16% yield from **6** (0.3 mmol) and 1,3-propanediol (0.3 mmol) following the typical procedure mentioned above. HPLC separation was carried out using isocratic elution with TEAA/ACN 98:2. ^{1}H NMR (700 MHz, D_2O): δ 4.18-4.16 (m, 4H), 3.58 (t, J =11.7 Hz, 2H), 1.97 (quin, J = 5.7 Hz, 2H) ppm. ^{13}C NMR (175 MHz, D_2O): δ 64.3 (t, J = 4.3 Hz), 57.2 (t, J = 50.9 Hz), 28.3 ppm. ^{31}P NMR (162 MHz, D_2O): δ 104.7 ppm. HRMS (MALDI) calcd for $C_4H_9O_2P_2S_4^-$ $[M-H]^-$, 278.8955; found, 278.8924.

1H-Benzo[1,5,2,4]dioxadiphosphonine-3,5(4H,7H)-bis(thiolate) 3,5-disulfide disodium salt, **9,** was obtained in 15% yield from **6** (0.3 mmol) and 1,2-benzenedimethanol (0.3 mmol) following the typical procedure mentioned above. HPLC separation was carried out using isocratic elution with TEAA/ACN 84:16. ^{1}H NMR (600 MHz, D_2O): δ 7.43(s, 4H), 5.12 (t, J = 3.0 Hz, 4H), 3.65 (t, J = 11.4 Hz, 2H) ppm. ^{13}C NMR (150 MHz, D_2O): δ 136.3, 131.1, 129.8, 69.0, 57.5 (t, J = 53.6 Hz) ppm. ^{31}P NMR (162 MHz, D_2O): δ 104.1 ppm. HRMS (MALDI) calcd for $C_9H_{11}O_2P_2S_4^-$ $[M-H]^-$, 340.9112; found, 340.9103. **9-Zn(II) complex.** ^{31}P NMR (162 MHz, D_2O): δ 102.1 ppm. IR (ZnSe): ν 1622, 1441, 1385, 1381, 1278, 1260, 1212, 1170, 1141, 1046, 981, 945, 937, 898, 857, 832, 749, 711, 674, 657 cm^{-1}. MS (ESI) m/z: calcd for $C_{18}H_{23}O_4P_4S_8Zn^+$ $[M+3H]^+$ 749.17; found, 749.03.

1,5,8,2,4-Trioxadiphosphecane-2,4-bis(thiolate) 2,4-disulfide disodium salt, 10, was obtained in 5% yield from **6** (0.92 mmol) and diethylene glycol (0.92 mmol) following the typical procedure mentioned above. HPLC separation was carried out using isocratic elution with TEAA/ACN 96:4. ^{1}H NMR (600 MHz, D_2O): δ 4.03-3.99 (m, 4H), 3.76 (t, J = 4.0 Hz, 4H), 3.50 (t, J = 12.0 Hz, 2H) ppm. ^{13}C NMR (150 MHz, D_2O): δ 70.8, 65.2, 58.6 (t, J = 60.8 Hz) ppm. ^{31}P NMR (162 MHz, D_2O): δ 102.5 ppm. HRMS (MALDI) calcd for $C_5H_{11}O_3P_2S_4^-$ $[M-H]^-$, 308.9061; found, 308.9023.

1,5,8,11-Tetraoxa-2,4-diphosphacyclotridecane-2,4-bis(thiolate) 2,4-disulfide disodium salt, **11**, was obtained in 10% yield from **6** (0.46 mmol) and triethylene glycol (0.46 mmol) following the typical procedure mentioned above. HPLC separation was carried out using isocratic elution with TEAA/ACN 94:6. ^{1}H NMR (600 MHz, D_2O): δ 4.07-4.03 (m, 4H), 3.75-3.73 (m, 4H), 3.66 (s, 4H), 3.47 (t, J = 13.2 Hz, 2H) ppm. ^{13}C NMR (150 MHz, D_2O): δ 70.3, 69.1, 62.9, 57.9 (t, J = 63.9 Hz) ppm. ^{31}P NMR (162 MHz, D_2O): δ 103.4 ppm. HRMS (MALDI) calcd for $C_7H_{15}O_4P_2S_4^-$ $[M-H]^-$, 352.9323; found, 352.9341.

11-Zn(II) complex. ^{31}P NMR (162 MHz, D_2O): δ 100.7 ppm. IR (ZnSe): v 1607, 1443, 1346, 1345, 1286, 1243, 1237, 1161, 1114, 1072, 1040, 1035, 934, 839, 794, 737, 721, 686, 646, 630, 604 cm^{-1}. MS (ESI) m/z: calcd for $C_{14}H_{31}O_8P_4S_8Zn^+$ $[M+3H]^+$ 773.18; found, 772.85.

Crystallization of 11-Zn(II) complex

Triethylammonium salt of compound **11** (10.3 mg, 0.018 mmol) was dissolved in 1 mL of HPLC-grade water (Carlo Erba Reagents). Then, zinc acetate (1.7 mg, 0.0093 mmol) dissolved in 0.5 mL of HPLC-grade water was added and the solution was freeze-dried to obtain a white powder of Zn(II) complex of **11**. Next, the powder was recrystallized from a mixture of water, ethanol and methanol (1:1:1 v/v/v) and slow evaporation of the solvents to obtain yellowish crystals.

O,O'-Bis(3-hydroxypropyl) methylenediphosphonodithioate disodium salt, **12,** was obtained in 30% yield as a by-product in the synthesis of compound **8**. ^{1}H NMR (600 MHz, D_2O): δ 4.12-4.07 (m, 4H), 3.76 (t, J = 6.3 Hz, 4H), 3.46 (t, J = 13.6 Hz, 2H), 1.91 (quin, J = 6.2 Hz, 4H) ppm. ^{13}C NMR (150 MHz, D_2O): δ 62.1, 59.5, 57.7 (t, J = 64.5 Hz), 32.9 ppm. ^{31}P NMR (162 MHz, D_2O): δ 102.7 ppm. HRMS (TOF ES^-) Calcd for $C_7H_{16}O_4NaP_2S_4^-$ $[M-2H+Na]^-$, 376.9304; found, 376.9308.

O,O'-Bis(2-(2-hydroxyethoxy)ethyl) methylenediphosphonodithioatedisodium salt, **13,** was obtained in 6% yield as a by-product in the synthesis of compound **10**. ^{1}H NMR (400 MHz, D_2O): δ 4.15-4.08 (m, 4H), 3.77-3.72 (m, 4H), 3.69-3.65 (m, 4H), 3.65-3.61 (m, 4H), 3.41 (t, J = 14.10 Hz, 2H) ppm. ^{13}C NMR (150 MHz, D_2O): δ 72.4, 70.6, 63.7, 61.1, 57.3 (t, J = 64.7 Hz) ppm. ^{31}P NMR (162 MHz, D_2O): δ 103.6 ppm. HRMS (MALDI) calcd for $C_9H_{21}O_6P_2S_4^-$ $[M-H]^-$, 414.9691; found, 414.9724.

5-((2-Mercaptoethyl)thio)-1,2,3,5-dithiadiphospholan-3-olate 3,5-dioxide triethylammonium salt, 14, was obtained as a by-product in the synthesis of compounds **7-11**. ^{1}H NMR (600 MHz, D_2O): δ 3.79-3.74 (m, 1H), 3.68-3.62 (m, 1H), 3.46-3.28 (m, 3H), 3.14-3.03 (m, 1H) ppm. ^{13}C NMR (150 MHz, D_2O): δ 69.1 (dd, J = 58.2 Hz, 37.2 Hz), 38.6 (d, J = 3.9 Hz), 35.0 (d, J = 3.2 Hz) ppm. ^{31}P NMR (162 MHz, D_2O): δ 71.7 (d, J = 23.8 Hz, 1P), 63.3 (d, J = 23.8 Hz, 1P) ppm. HRMS (TOF ES^-) Calcd for $C_3H_7O_3P_2S_4^-$ $[M-H]^-$, 280.8753; found, 280.8762.

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Notes and references

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† Electronic Supplementary Information (ESI) available: [^{1}H-, and ^{31}P-NMR monitored titrations of compounds **7**, **9** and **11** with Zn(II), Mg(II), Na(I), and Li(I), spectral data (^{1}H-, ^{13}C-, and ^{31}P-NMR) of compounds **7-13**, X-ray data for **11**-Zn(II) complex (CCDC 1039223), calculated and experimental Δδ of compounds **7**, **9** and **11**]. See DOI: 10.1039/x0xx00000x

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Novel Crown-Ether- Methylenediphosphonotetrathioate Hybrids as Selective Zn(II) Chelators

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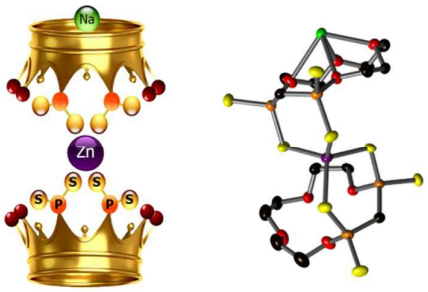

A 13-membered methylene-diphosphonotetrathioate-crown ether hybrid is a water-soluble, air-stable, high-affinity Zn(II)-chelator, exhibiting selectivity to Zn(II) vs. Mg(II), Na(I), and Li(I).