Design and synthesis of thiazoline–thiazole hybrid macrocycles possessing strong binding affinity to Pb^{2+} and Cd^{2+}†

Fu She Han,‡[a] Hidetoshi Tokuyama§[b] and Tohru Fukuyama*[c]

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Thiazoline–thiazole hybrid macrocycles were synthesized *via* head-to-tail cyclooligomerization. The macrocycle consisting of eight heterocyclic subunits displays high binding affinity to the heavy metal toxins Pb^{2+} and Cd^{2+}.

Development of macromolecular architectures possessing defined structural motifs is a focus of intense research in the area of molecular or ion recognition,[1] supramolecular chemistry,[2] and drug design.[3] A fundamental step to realize structure-based interactions should be an appropriate design and synthesis of structural modules, which can be assembled to build tailored molecular structures. In this context, nitrogen containing heterocycles have been widely used as building blocks since they may take part in a variety of non-covalent interactions, such as dipole–dipole interactions, hydrogen-bonding, and chelation. With this background, numerous molecular architectures comprising pyridine and pyrrole rings have so far been synthesized and have proven to possess significant properties.[4]

As a new entry into the subunits of novel artificial macromolecules, we have focused our particular interest on thiazoline and thiazole heterocycles. These heterocycles are found in numerous natural products[5] and exhibit interesting properties, including biological activities. Furthermore, they have been demonstrated to interact with small molecules or metal cations by hydrogen-bonding and coordination, thereby rendering attractive building blocks to the macromolecules for construction of the structural motifs possessing molecular or ion recognition sites. Indeed, our previous studies have demonstrated that cyclic macromolecular architectures fabricated from chiral thiazolines exhibit interesting binding affinities towards small molecules (Fig. 1, A).[6] However, the conformational flexibility of oligothiazolines made it difficult to construct thiazoline-based macromolecules having defined recognition sites. In this study, we designed a thiazoline–thiazole hybrid-type macrocycle (Fig. 1, B) with decreased conformational flexibility by the incorporation of a planar thiazole ring. Herein, we describe the synthesis of thiazoline–thiazole hybrid macrocycles and their significant properties as good receptors for heavy metal toxins.[7]

The key structural module **4** for the synthesis of thiazoline–thiazole hybrid macrocycles was prepared from thiazolidinone **1**.[6] Esterification of **1** and subsequent treatment with NH_3 in MeOH afforded amide **2**. Amide **2** was then converted to thiazole **3** through the modified Hantzsch reaction.[8] Thus, treatment of amide **2** with Lawesson's reagent gave thioamide regioselectively, which was followed by S-alkylation with ethyl bromopyruvate and dehydration with TFAA to yield the desired thiazole **3**. Finally, the key unit **4** was obtained by ring-opening of the thiazolidinone ring after activation with a Boc group and hydrolysis of the ester. In order to form a macrocyclic structure, we employed the cyclooligomerization protocol which was developed in our synthetic studies on macrocyclic oligothiazolines.[6] Thus, the key module **4** bearing thiol and carboxylic acid functionalities was oligomerized in a head-to-tail manner to produce the cyclic tetramer **5** and pentamer **6** in excellent total yield (68%, ratio of **5** : **6** = *ca.* 1.9 : 1) under the optimized conditions (BOP–Cl (3.0 eq.), Et_3N (6.0 eq.), CH_2Cl_2 (4 mM), r.t., 20 h). Finally, a one-pot, multiple thiazoline formation was carried out by sequential treatment of the mixture of **5** and **6** with TFA and heating in benzene, leading to **7** and **8** in good yield (68% for **7**, 64% for **8**). The two macrocyclic compounds **7** and **8** were easily separated by the conventional chromatographic method.

The structure of macrocycles **7** and **8** were determined by standard spectroscopic techniques including ^{1}H- and ^{13}C-NMR, and high resolution mass spectrometry. The observations of only a single set of signals both in their ^{1}H- (Fig. 2) and ^{13}C-NMR spectra suggested that macrocycles **7** and **8** possess a C_4 and C_5 symmetry, respectively. 1D NOESY experiments of **7** revealed a strong nuclear Overhauser effect between the methyl and methylene signals at 3.28 ppm (H_a); however, no effect was observed between methyl and methylene signals at 4.57 ppm (H_b),

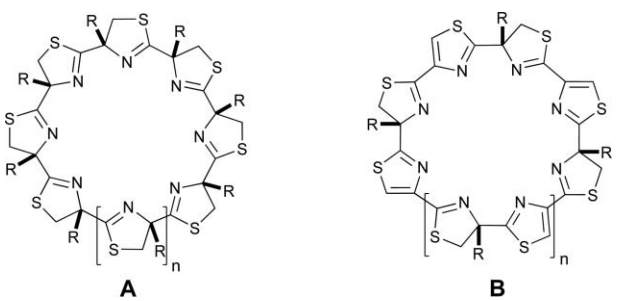
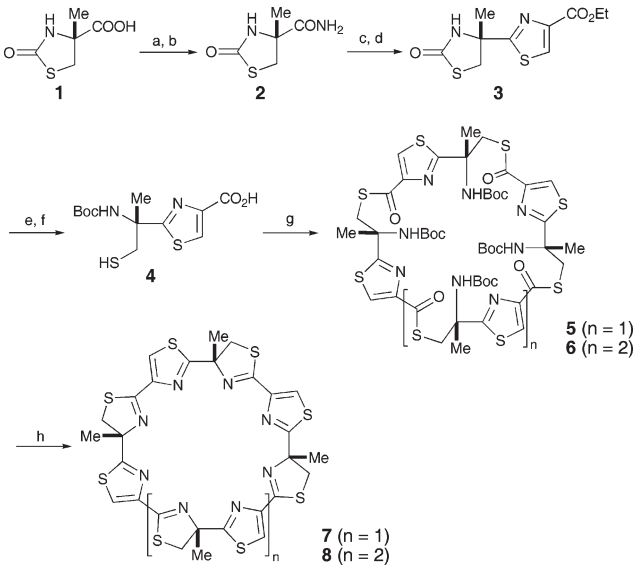


Fig. 1 Structures of cyclic macromolecules constructed from thiazoline and thiazole heterocycles.

[a]*PRESTO, The Japan Science and Technology Agency (JST), 7-3-1 Hongo, Bunkyo-ku, Tokyo, 113-0033, Japan*
[b]*Graduate School of Pharmaceutical Sciences, PRESTO, The Japan Science and Technology Agency, University of Tokyo, 7-3-1 Hongo, Bunkyo-ku, Tokyo, 113-0033, Japan*
[c]*Graduate School of Pharmaceutical Sciences, University of Tokyo, 7-3-1 Hongo, Bunkyo-ku, Tokyo, 113-0033, Japan. E-mail: fukuyama@mol.f.u-tokyo.ac.jp; Fax: +81-3-5802-8694*
† Electronic supplementary information (ESI) available: Structural characterization of macrocycles **7** and **8**, UV-vis and ^{1}H-NMR titrations. See DOI: 10.1039/b707904d
‡ Current address: National Institute for Materials Science, 1-1 Namiki, Tsukuba, Ibaraki 305-0044, Japan
§ Current Address: Graduate School of Pharmaceutical Sciences, Tohoku Univesrity, Aramaki, Aoba-ku,Sendai 980-8975, Japan



Scheme 1 *Reagents and conditions*: (a) CH_2N_2 (3.0 eq.), THF, 0 °C, 0.5 h, quant.; (b) NH_3, MeOH, r.t., overnight, quant.; (c) Lawesson's reagent, THF, 60 °C, 1.5 h, 88%; (d) Ethyl bromopyruvate (3.0 eq.), K_2CO_3 (5.0 eq.), THF, 0 °C to r.t., 2 h; then TFAA (1.1 eq.), pyridine (2.0 eq.), 0 °C, 84%; (e) Boc_2O (1.2 eq.), Et_3N (2.0 eq.), DMAP (cat.), r.t., 93%; (f) LiOH (3.0 eq.), THF–H_2O, r.t., 84%; (g) BOP–Cl (3.0 eq.), Et_3N (6.0 eq.), CH_2Cl_2 (4 mM), r.t., 20 h, 68% (molar ratio of **5** : **6** = *ca.* 1.9 : 1); (h) TFA, r.t., 20 min, evaporated; then benzene, reflux, 4 h, 68% for **7**; 64% for **8**.

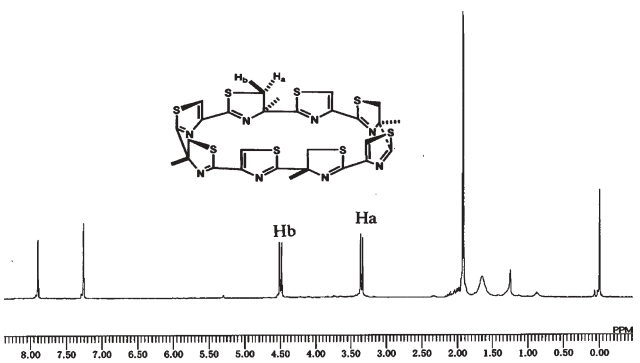


Fig. 2 ^{1}H-NMR spectrum of macrocycle **7** in $CDCl_3$.

suggesting H_a points outward from and H_b points inward towards the cavity of the macrocycle, respectively. UV-vis analysis showed that **7** and **8** have a characteristic absorption at *ca.* 255 nm in a mixed solvent of MeOH and H_2O (v/v = 95 : 5).

Having established an efficient protocol to synthesize the thiazoline–thiazole hybrid macromolecules, we next studied the properties of the novel macrocycles possessing a well-defined cavity. Interestingly, preliminary binding studies with metal ions revealed that this class of molecules have a high binding affinity particularly to heavy metal ions. Thus, **7** and **8** were mixed with a variety of metal ions (1 : 1 or 1 : 2 stoichiometic mixture of L : M^{n+}, where L = **7** or **8**, M^{n+} = Na^+, K^+, Cs^+, Mg^{2+}, Ba^{2+}, Mn^{2+}, Co^{2+}, Ni^{2+}, Cu^{2+}, Zn^{2+}, Ag^+, Pd^{2+}, Cd^{2+}, Hg^{2+} and Pb^{2+}), and their UV-vis spectra were determined. Among 15 metal ions examined, the mixtures of **7**–Pb^{2+} and **7**–Cd^{2+} displayed a large increase in molar absorptivity (ε_{max}), accompanied by a small increase in λ_{max}. However, mixtures of **7** with other metal ions showed zero or only slight changes as compared to the free sprectrum of **7**. These observations suggested that macrocycle **7** coordinates strongly with Pb^{2+} and Cd^{2+} ions among various metal ions studied. On the other hand, similar experiments showed that **8** exhibits very weak or no binding ability to all metal ions, probably due to the excessively large size of **8**.

The binding property of **7** was further investigated by using the standard ^{1}H-NMR titrations in $CDCl_3$ and CD_3OD (v/v = 1 : 1). Thus, a stock solution (25 mM containing 2 mM of **7**) of M^{n+}, where M^{n+} = Mg^{2+}, Ni^{2+}, Zn^{2+}, Mn^{2+}, Hg^{2+}, Cd^{2+} and Pb^{2+}, was added gradually in an aliquot manner to a solution of **7** (2 mM in v/v = 1 : 1 $CDCl_3$ and CD_3OD), and the ^{1}H-NMR spectrum was determined after standing for 30 min at room temperature (a time dependent study showed no further chemical shift change upon standing overnight).

As illustrated in Fig. 3 (a), chemical shift and shape of the thiazole proton peak of **7** at 8.05 ppm remained unchanged even in the presence of a large excess (up to eight equivalents) of Mg^{2+}, Ni^{2+}, and Zn^{2+} ions, although Mn^{2+} and Hg^{2+} ions caused a significant broadening of the peaks. In sharp contrast, addition of Pb^{2+} led to a gradual down-field shift of the peak, which reached a plateau at 8.23 ppm when more than two equivalents of Pb^{2+} was added. We also observed the significant down-field shift of the peak when Pb^{2+} (two equivalents) was added to the mixture of **7** and Zn^{2+} (two equivalents), suggesting that receptor **7** binds exclusively to Pb^{2+}. On the other hand, addition of Cd^{2+} produced a new signal at 8.22 ppm. The intensity of the new peak, which would be assigned to the complex of **7**/Cd^{2+}, increased gradually with the incremental addition of Cd^{2+} (until more than two equivalents was added) as the signal, due to free **7**, weakened synchronously (Fig. 3, b).

The composition of the complex was determined on the basis of the NMR titration (*vide supra*) and continuous variation method[9] using UV-vis spectroscopies. A combination of these results suggested that a 1 : 2 complex was formed between **7** and Pb^{2+} and Cd^{2+} as well. The evaluated stepwise stability constants for the 1 : 2 complexes of [**7**]·[Pb^{2+}]$_2$, and [**7**]·[Cd^{2+}]$_2$ are K_1 = 6.86 × 10^4 and K_2 = 3.01 × 10^3, and K_1 = 4.73 × 10^3 and K_2 = 1.95 × 10^3, respectively.[10]

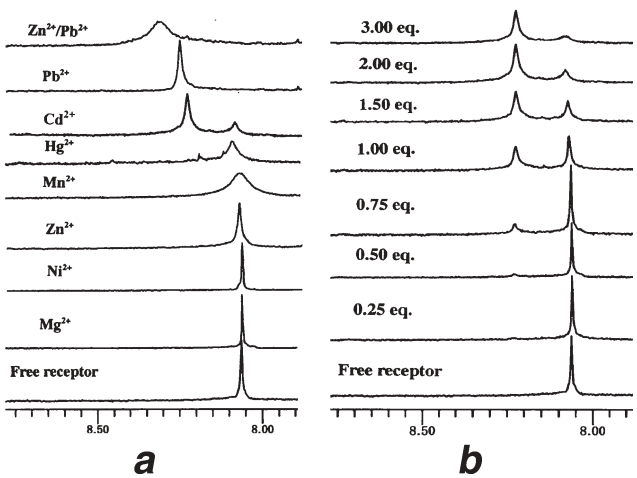


Fig. 3 The thiazole proton region of overlaid ^{1}H-NMR spectra of 1 : 2 mixtures of **7** with various metal ions (a); and the mixtures of **7** with Cd^{2+} at various molar ratio (b). All titrations were duplicated.

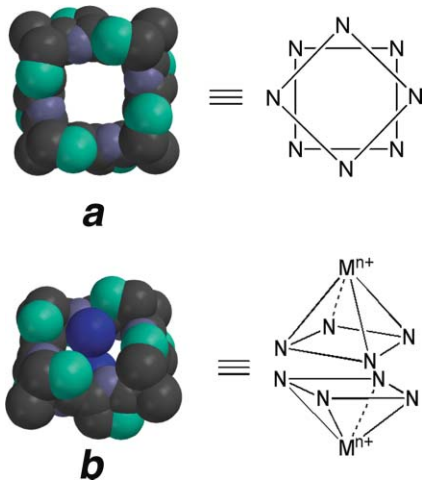


Fig. 4 (a) HF/3-21G optimized lowest-energy conformer of macrocycle **7**; (b) proposed model structure of **7**·2Pb^{2+}. Atom colors: nitrogen: gray; sulfur: green; carbon: black; lead: blue.

The structures of the complexes of [**7**]·[Pb^{2+}]$_2$ and [**7**]·[Cd^{2+}]$_2$ were deduced from the behavior of methylene protons in the thiazoline ring. Namely, addition of Pb^{2+} or Cd^{2+} to **7** gives rise to a down-field shift of the outward proton H$_a$ but an up-field shift of the inward proton H$_b$. This could be attributed to a higher shielding effect of the metal ions on the inward proton H$_b$ than on the outward H$_a$, suggesting that Pb^{2+} or Cd^{2+} is buried within the cavity of the macrocycle and forms an inclusion complex. The importance of the cavity structure for strong binding to metal ions such as Pb^{2+} or Cd^{2+} was also supported by comparing the binding affinities of the cyclic compound **7** with those of its linear analogues. As a result, the linear compounds revealed not only weak binding ability but also poor selectivity for various metal ions.[11]

Taking into account of all these experimental results, we propose a model structure for the [host]·[guest]$_2$ complexes. A preliminary lowest-energy conformer search using HF/3-21G revealed that macrocycle **7** has C_4 symmetry (Fig. 4, a), which is in good agreement with its ^{1}H- and ^{13}C-NMR spectroscopic properties. These data also suggest that the macrocyclic molecule possesses a well-defined cavity with an estimated pore size of d = 6.07 Å. All of the eight nitrogen atoms settle in the inner side of the cavity, such that the four nitrogens in the thiazole rings are located at the front side and the remaining four are in the thiazoline rings occupying the back side, thereby assuming an anti-prism geometry. Consequently, the well-defined cavity as well as the nature of the uniquely arranged nitrogen atoms of **7** allows this macrocycle to coordinate selectively with two metal ions as governed by structure complementation of the complex between the macrocycle and metal ions. In the case of Pb^{2+}, the four nitrogen atoms in the thiazole ring coordinate with one Pb^{2+} ion (Fig. 4, b), while the other four nitrogen atoms in the thiazoline ring bind to another Pb^{2+} ion.

In summary, we have developed an efficient synthesis of novel thiazoline-thiazole hybrid-type macrocycles **7** and **8**. Metal coordination studies revealed that macrocycle **7** exhibits strong binding affinity to Pb^{2+} and Cd^{2+} ions. In addition, the preliminary competitive binding experiment showed that this type of macrocycle has a potential as highly selective receptor of heavy metal ions. This subject continues to be a topic of great concern given the problem of environmental pollutants. Further studies on the utility of this novel class of macrocycles, including chiral discrimination through H-bonding, π–π interaction, and the (*R*) and (*S*) chirality of the thiazoline moiety,[6] are currently underway in our laboratories.

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11 Structures of the reference compounds **9** and **10**. NMR titration showed that, among the five metal ions, Hg^{2+}, Cd^{2+}, Pb^{2+}, Zn^{2+}, and Mg^{2+}, Hg^{2+} showed a maximum chemical shift change with $\Delta\delta = \delta_{\mathbf{9}\cdot Hg^{2+}} - \delta_{\mathbf{9}} < 0.02$ ppm for dimer **9** and $\Delta\delta = \delta_{\mathbf{10}\cdot Hg^{2+}} - \delta_{\mathbf{10}} < 0.07$ ppm for tetramer **10**, respectively, in the presence of 4.0 eq. of Hg^{2+}. A careful inspection of the NMR spectra revealed a binding order of Hg^{2+} ⩾ Cd^{2+} ≈ Pb^{2+} ⩾ Zn^{2+} ≈ Mg^{2+} for the five metal ions.