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Pseudo-peptides Based on Methyl Cysteine or Methionine Inspired from Mets Motifs Found in the Copper Transporter Ctr1

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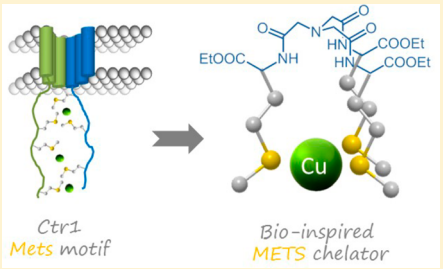
Supporting Information

ABSTRACT: Most proteins involved in Cu homeostasis bind to intracellular Cu(I) in stable Cu(S-Cys)$_x$ environments, thanks to well-conserved cysteine-rich sequences. Similarly, the Cu(I) transport protein Ctr1, responsible for copper acquisition, binds Cu(I) in Cu(S-Met)$_3$ environments in conserved methionine-rich MXMXXM sequences, referred as Mets motifs. Pseudo-peptides based on a nitrilotriacetic acid scaffold and functionalized with three amino acids bearing thioether side chains, either methyl cysteine in **T**^{1} or methionine in **T**^{2}, were synthesized as mimics of the Mets sequences found in Ctr1. These two ligands were obtained with good overall yields from commercial amino acids and demonstrate efficient chelating ability for Cu(I). Only one species, the mononuclear [Cu**T**^{1,2}]$^+$ complex, was evidenced by electrospray ionization−mass spectroscopy (ESI-MS) and the circular dichroism signature obtained for the most constrained Cu**T**^{1} complex having the shortest side chains showed reorganization of the pseudo-peptide scaffold upon Cu(I) complexation. Considering that thioether functions are neutral sulfur donors, the stability constants measured by competition with ferrozine are quite large: log $K \approx 10.2-10.3$. The Cu**T**^{1,2} complexes are significantly more stable that those formed with linear peptides, mimicking isolated Mets motifs MXMXXM of the Cu transport protein Ctr1 (log $K \approx 5-6$). This may be attributed to the preorganized pseudo-peptide scaffold, which arranges the three neutral sulfur donors toward the metal center. Such moderate affinity Cu(I) chelators are interesting for applications in chelation therapy, for instance, to induce minimum disturbance of Cu homeostasis in Wilson's disease treatments.



INTRODUCTION

The molecular mechanisms of copper (Cu) trafficking are now quite well elucidated, especially in the hepatocytes. Copper in the +2 oxidation state (Cu(II)) in the oxidizing extracellular environment is first reduced to Cu(I) via a reductase inserted in the membrane.[1] Then, a decisive step occurs: Cu(I) entry in the hepatocytes by crossing the plasma membrane, taking advantage of a favorable concentration gradient via the Cu(I)-specific transporter Ctr1.[2] The Cu(I) transport proteins (Ctr1) are integral membrane proteins that are widely conserved from yeast to humans.[3,4] Genetic studies have confirmed that Ctr1 is essential for copper acquisition, as yeast mutants lacking Ctr1 show clear signs of Cu deficiency[5,6] and mice lacking mCtr1 die *in utero*.[7] Such studies have clearly established the essential role of Ctr1 in Cu acquisition, which is of great importance for mammalian development.

Ctr1 contains three transmembrane regions, with the amino terminus in the extracellular space and the carboxyl terminus facing the cytosol.[3,6] The protein is made of a homotrimer,[4−6,8] the center of which allows Cu(I) transport as evidenced by the tryptophan scanning analysis performed by De Feo et al.[9] Conserved cysteine and histidine residues located at the cytosolic terminus have been shown to bind copper.[2,10] The extracellular domain of yeast Ctr1 (yCtr1), which is responsible for Cu entrance in the cells, is mainly made of methionine-rich domains (Mets motifs) arranged in both MetXXMet and MetXMet motifs containing 3−5 methionine residues per Mets motif. The yeast Ctr1 (yCtr1) has 8 Mets motifs, with a total of 30 methionine residues in the 140-residues extracellular domain, whereas the human Ctr1 (hCtr1) has 2 Mets motifs in a 66-residue extracellular region. The Mets motifs have been assumed to be involved in extracellular Cu acquisition ever since Ctr1 was identified as a copper transporter.[5,6,11] Other proteins involved in copper transport use methionines for Cu(I) binding such as Pco,[12] Cop,[13] and CusF,[14] which are three proteins found in copper-resistant bacteria. In all of the Cu(I) transport proteins, the Mets motifs seem to be suitable for both copper acquisition and copper internalization, since these peptide sequences are able to stabilize reduced Cu(I), which is more kinetically labile than Cu(II), in the extracellular oxidizing environment.

Mets motifs have been extensively studied by Franz et al.,[15−18] who have focused on the thioether-only binding sites found in these motifs, without any histidine residue in the sequence.[15,16] These methionine-only binding sites have been found to bind Cu(I) with three methionine units in Cu(S-Met)$_3$ environments. The corresponding binding constant values have been measured by both an ascorbic assay at pH 4.5 and mass spectrometry (MS). Binding constant values (log K)

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between 5 and 6 have been determined. These values are quite low and indicate that the complexes formed with Mets motifs are significantly less stable, with respect to Cu(I) species formed with cysteine-rich proteins such as Cu chaperones or metallothioneins (log $K \approx 16-19$).[2,19−23] This is in accordance with the function of the Mets Motifs dedicated to the transport of Cu(I) from the extracellular medium to the cytosol.

The understanding of Cu homeostasis is of great interest for inorganic medicinal chemists, since proteins involved in Cu transport or sequestration are sources of inspiration for the design of metal chelators. Our laboratory has been involved for years in the design of bioinspired intracellular Cu(I) chelators to treat copper overload in the orphan Wilson's disease.[24] In particular, pseudo-peptides built up on a nitrilotriacetic acid (NTA) template and extended with three cysteines or D-penicillamines have been designed and extensively described in previous publications.[24−28] It has been demonstrated that these tripodal architectures tightly bind Cu(I) in well-defined CuS_3 cores either in mononuclear Cu(I) complexes or in Cu_4S_6 or Cu_6S_9 metal−thiolate clusters. The affinities range from log $K \approx 16$ to log $K \approx 19$, and they are therefore competitive with those of metallothioneins and copper chaperones found in cells. Hence, tripodal architectures derived from NTA are particularly efficient in binding Cu(I) and are therefore promising chelators for the detoxification of Cu in Wilson's disease.

Therefore, these efficient pseudo-peptide scaffolds have been exploited to design Cu(I) chelators inspired from Mets motifs found in Ctr1. The tripodal architectures $\mathbf{T^1}$ and $\mathbf{T^2}$ based on the NTA template and extended by three amino acids bearing thioether side chains (see Figure 1), either methyl cysteine ethyl ester ($\mathbf{T^1}$) or methionine ethyl ester ($\mathbf{T^2}$) have been synthesized. These new pseudo-peptides are expected to complex Cu(I) in solution with properties close to Mets motifs found in Cu transporters.

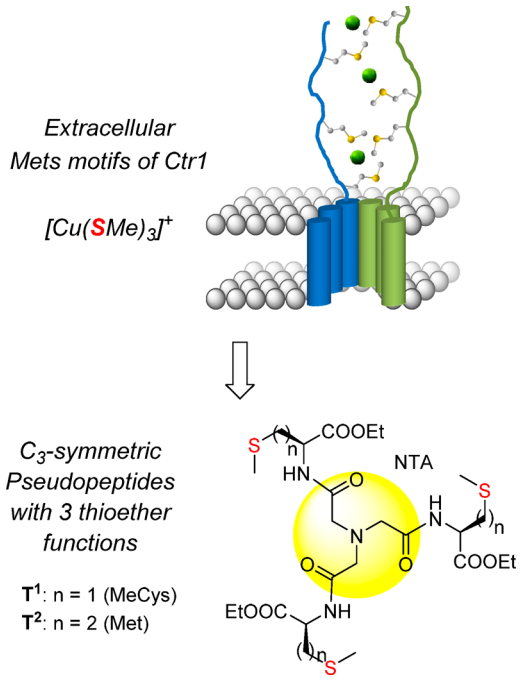

Figure 1. Structures of the thioether-based scaffolds $\mathbf{T^1}$ and $\mathbf{T^2}$ inspired from the Mets motifs found in the extracellular domain of Ctr1.

In this article, we report the design and synthesis of the two new pseudo-peptides, $\mathbf{T^1}$ and $\mathbf{T^2}$, which bear three neutral soft sulfur donors, as well as their Cu(I) complexation properties. Interestingly these tripodal thioether chelating agents demonstrate lower affinity for Cu(I) than the corresponding thiolate compounds derived from cysteine and D-penicillamine but significantly higher affinities than linear peptides, mimicking isolated Mets motifs found in the Ctr1 extracellular domain.

EXPERIMENTAL SECTION

General Procedure for Sample Preparation. The procedures used for the syntheses of the chelators $\mathbf{T^{1,2}}$ are described in the Supporting Information. Since Cu(I) is sensitive to air oxidation, samples used for physicochemical studies were all prepared in a glovebox under argon atmosphere and sealed if used outside the glovebox. All the solutions used for the sample preparation were deoxygenated and stored in the glovebox. The chelators $\mathbf{T^{1,2}}$ were weighted and introduced in the glovebox via a sealed flask under an argon atmosphere. All the solutions were prepared in the glovebox. The samples were dispersed in mixtures containing different proportions of Millipore water buffered or not. At least 10% of acetonitrile was added to each sample to prevent Cu(I) disproportionation in solution.[29] The Cu(I) solutions were prepared by dissolving the appropriate amount of $Cu(CH_3CN)_4$ PF_6 in acetonitrile. The final concentration was determined by adding an excess of sodium bathocuproine disulfonate (BCS) and measuring the absorbance of $Cu(BCS)_2^{3-}$ (λ^{max} = 483 nm, ε = 13 300 M^{-1} cm^{-1}).[2]

Electrospray Ionization−Mass Spectroscopy (ESI-MS) Titrations with Cu(I). Mass spectra were acquired on a LXQ-linear ion trap (THERMO Scientific, San Jose, USA) equipped with an electrospray source. Electrospray full scan spectra in the range m/z = 50−2000 amu or 2000−3000 amu were obtained by infusion through a fused silica tubing at 2−10 μL/min. The solutions were analyzed both in positive and negative modes. The LXQ calibration (m/z = 50−2000) was achieved according to the standard calibration procedure from the manufacturer (mixture of caffeine, MRFA and Ultramark 1621). An ES-Tuning Mix solution (Agilent) was used to calibrate the spectrometer between 2000 amu and 3000 amu. The temperature of the heated capillary for the LXQ was set in a range of 200−250 °C, the ion-spray voltage was in the range of 3−6 kV and the injection time was 5−200 ms. Samples (∼100 μM) were prepared in MeCN/ammonium acetate buffer (10 mM, pH 7) or MeCN/water (1/9, v/v) and aliquots of the metal solution were added.

Circular Dichroism (CD) Titrations with Cu(I). The circular dichroism (CD) spectra were acquired with an Applied Photo-Physics Chirascan spectrometer outside the glovebox. A 2-mL ligand solution (∼10−35 μM) in Millipore water/CH_3CN (9/1, v/v) was transferred in a sealed UV cell (1 cm path) and the metal solution was prepared in a sealed flask. Both samples were then stirred under argon atmosphere outside the glovebox. Aliquots corresponding to 0.25 equiv of the metal solution were successively added. The solution was stirred during 10 min before each measurement to ensure the complete formation of the metallic species.

Affinity for Cu(I). The apparent affinity constants at pH 7.4 of the Cu(I) complexes were measured by ultraviolet−visible (UV-vis) titrations in the presence of ferrozine (Fz) as a competitor.[30,31] The spectra were recorded with a Varian Cary50 spectrophotometer equipped with optical fibers

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connected to an external cell holder in the glovebox. A solution of the Cu(I) complex with Fz, $Cu(Fz)_2^{3-}$ (ca. 80 μM), was prepared in 100 mM Hepes buffer/MeCN (9/1, v/v), pH 7.4 in the UV cell (1 cm path). The mixture was stirred for 10 min to ensure the formation of the complex. Aliquots of the pseudo-peptide solution (~2 mM in 100 mM Hepes buffer, pH 7.4) were then added to $Cu(Fz)_2^{3-}$ previously formed in the UV cell. The spectra were then recorded and show the decrease of the orange $Cu(Fz)_2^{3-}$ complex, which absorbs at 470 nm with a molar extinction coefficient value ε = 4320 M^{-1} cm^{-1}. The stability of the absorbance at 470 nm was controlled before the addition of any other aliquots of competitor. The stability constants were then determined using the binding constant of the $Cu(Fz)_2^{3-}$ complex (log β_{12} = 15.1)[31] and the equations reported in the Boxed Text S1 in the Supporting Information. Constants given in Table 1 are averaged values of significant data points, i.e., points showing Cu displacements in the range of 30%−70%.

Table 1. Conditional Stability Constants of the Cu(I) Complexes with the Pseudo-peptides $T^{1,2}$, Compared to Ctr1 Sequences Reported in the Literature

ligand	coordinating amino acids	log K^a	method[b]	reference
T^1	M	10.3(1)	Fz	this work
T^2	M	10.1(1)	Fz	this work
yMets1	M	5.0	AAA	ref 16
hMets1	M	5.0	AAA/ESI-MS	ref 16
hMets2	M	5.3	AAA/ESI-MS	ref 16
PcoC	M	5.6	AAA/ESI-MS	ref 16
Ctr1-14	M/H	12.8	BCA	ref 17

[a]Errors in the last figures are reported in parentheses. [b]Legend: Fz = ferrozine; AAA = ascorbic oxidation essay, 400 μM ascorbic acid (H_2Asc), pH 4.5; ESI-MS = 5 mM, H_2Asc, pH 3.5 or 10 mM, NH_4OAc, 5 mM H_2Asc, pH 7; BCA = Hepes buffer, pH 7.4, 0.5% MeCN (where y denotes yeast, h denotes human, and M denotes methionine or a methionine derivative); and H = histidine.

RESULTS

Syntheses of the Pseudo-peptides T^1 and T^2. The synthetic strategies used to elaborate the ligands T^1 and T^2 are depicted in Scheme 1. These syntheses are based on functional transformations from either Boc-Cys(Me)−OH or Boc-Met-OH and are widely inspired from our previous work on D-penicillamine-based tripods.[28] The first step consists in an esterification reaction with cesium carbonate (Cs_2CO_3) and ethyl bromide used as reagents in either pure acetonitrile (MeCN) or in an acetonitrile/dimethylformamide mixture (MeCN/DMF), depending on the solubility of the starting materials. This reaction leads to **1a** or **1b** (86%), which are Boc-deprotected to provide **2a** and **2b** quantitatively. The latters were involved in coupling reactions with the activated ester $NTA(NHS)_3$ using diisopropylethylamine as a base to set the pH at 7−8, leading to the pure products T^1 (67%, 58% global yield) and T^2 (63%, 54% global yield).

Spectroscopic Characterization of the Cu(I) Complexes. As expected, no characteristic signal of the binding of the two ligands $T^{1,2}$ to Cu(I) is detected in the UV spectra acquired in the range of 200−800 nm. The coordination of the neutral thioether groups to Cu(I) does not give rise to LMCT bands, as in the case of the negatively charged thiolate donors.[25,28] Therefore, the complexation properties of the chelators $T^{1,2}$ have been investigated by circular dichroism (CD). Aliquots of Cu(I) in acetonitrile have been added to a solution containing the ligand from 0 to 2 equiv per ligand. CD signals shifts or appearance of novel signals are expected due to conformational changes of the ligand backbones caused by Cu(I) binding. The titration recorded for the methyl cysteine derivative T^1 is represented in Figure 2 and shows relevant changes evidencing Cu(I) binding. An optimal concentration of T^1 ≈ 20 μM allows to extract relevant signals from the noise without saturation and to detect the small evolution of the signals when the Cu(I) complex forms. Curves depicted in Figure 2 show that Cu(I) binding induces conformational changes of the ligand backbone, which are evidenced by both

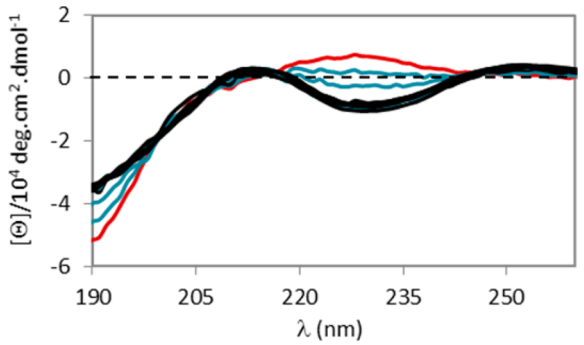
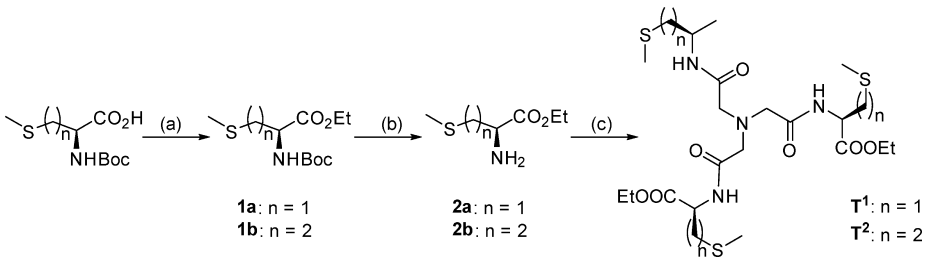


Figure 2. Circular dichroism (CD) titrations of T^1 (19 μM) in MeCN/water (1/9, v/v) with Cu(I) (0−2 equiv): (red trace) free T^1; (blue trace) 0.25−0.75 equiv of Cu(I); and (black trace) 1−2 equiv of Cu(I).

Scheme 1. Syntheses of the Thioether-Based Scaffolds $T^{1,2}$ [a]



[a]Experimental conditions: (a) Cs_2CO_3, Et-Br in MeCN (**1a**) or DMF/MeCN (**1b**), RT, 24 h (**1a**, 86% ; **1b**, 86%) ; (b) TES, DCM/TFA, RT, 1.5 h (quantitative reactions); (c) 1 equiv of $NTA(NHS)_3$ for 3 equiv of **2**, DIEA, MeCN, pH 7−8, RT, 24 h (T^1: 67%, 58% global yield ; T^2: 63%, 54% global yield).

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the negative band centered at 230 nm, the ellipcity of which decreases with the additions of Cu(I), and the negative band detected below 190 nm, the ellipcity of which increases with the additions of Cu(I). Above 1 equiv of Cu(I) added, all the curves are superimposed, which clearly ensures the formation of Cu$\mathbf{T}^1$ species in solution. These results are also in good agreement with the observations made by Franz et al.,[15] who demonstrated that Cu(I) binding makes the high-energy negative band (195 nm) of some Mets motifs sequences (hMet2, Pco) shift to less-negative ellipcity values. These shifts have also been attributed to conformational changes of the peptide backbones caused by Cu(I) binding. More precisely, an evolution from random coil states to β-type turn conformations with the addition of Cu(I) has been found responsible for the changes observed on the spectra.

The same experiments run with the methionine derivative $\mathbf{T}^2$ did not show any significant evolution of the CD spectra, regardless of the stoichiometry and the concentrations used (data not shown). The lack of sensitivity of the CD signature to Cu(I) binding may be related to the longer, and therefore more-flexible, arms of the tripodal ligand $\mathbf{T}^2$, in comparison to $\mathbf{T}^1$. Indeed, the CD evolution seen for Cu(I) binding by $\mathbf{T}^1$ is rather small. Since $\mathbf{T}^2$ is expected to be more flexible due to the presence of one extra methylene group in each of the three thioether arms, the effect of Cu(I) binding on the chirality of the molecule is likely to escape from the detection by CD spectroscopy.

Mass Spectrometry Signatures of the Cu(I) Complexes. All the mass spectra have been performed on samples prepared in MeCN/water (1/9, v/v) mixture with a chelator concentration [**T**] ≈ 100 μM. Aliquots of Cu(I) in acetonitrile have been added from 0 to 4 equiv per ligand **T**. The samples were carefully maintained under argon until the injection in the mass spectrometer to avoid any Cu(I) oxidation into Cu(II). Some of the mass spectra acquired in the positive mode for $\mathbf{T}^2$ are shown in Figure 3.

In the presence of equimolar Cu(I), the free form $\mathbf{T}^2$, detected as the sodium adduct $[\mathbf{T}^2+Na]^+$ at 691.4 and the Cu(I) complex Cu$\mathbf{T}^2$ detected as $[\mathbf{T}^2+Cu]^+$ at 731.3 are both observed, the latter being significantly more intense. This equilibrium is displaced toward the Cu$\mathbf{T}^2$ complex with the addition of Cu(I), and above 2 equiv, the only signal of the Cu$\mathbf{T}^2$ complex is found to remain on the spectrum. The examination of the Cu complex isotopic pattern confirms that Cu is in the +1 oxidation state (Cu(I)). Very similar data are observed for the Cu(I) complex of $\mathbf{T}^1$, which is detected as $[\mathbf{T}^1+Cu]^+$ at 689.3 in Figure S1 in the Supporting Information and corroborate the CD experiment results with the formation of a 1:1 Cu(I) complex.

Hence, electrospray ionization−mass spectrometry (ESI-MS) clearly indicates that the two tripodal pseudopeptides $\mathbf{T}^1$ and $\mathbf{T}^2$ form monometallic Cu**T** complexes of significant stabilities in water. This was confirmed by measuring the conditional stability constants of these two Cu(I)−thioether species.

Affinity for Cu(I). The affinities of the chelators $\mathbf{T}^{1,2}$ for Cu(I) have been determined by competition experiments using ferrozine (Fz) as a competitor.[30,31] Fz is known to form a 1:2 stoichiometry complex $Cu(Fz)_2^{3-}$ that absorbs at 470 nm ($\varepsilon_{Cu(Fz)_2}^{470\ nm}$ = 4320 M^{-1} cm^{-1}). The conditional stability constant at neutral pH of the complex $Cu(Fz)_2^{3-}$, log $K_{Cu(Fz)2}^{pH=7}$ = 15.1 has been recently determined and is rather small compared to those reported for other Cu(I) competitors

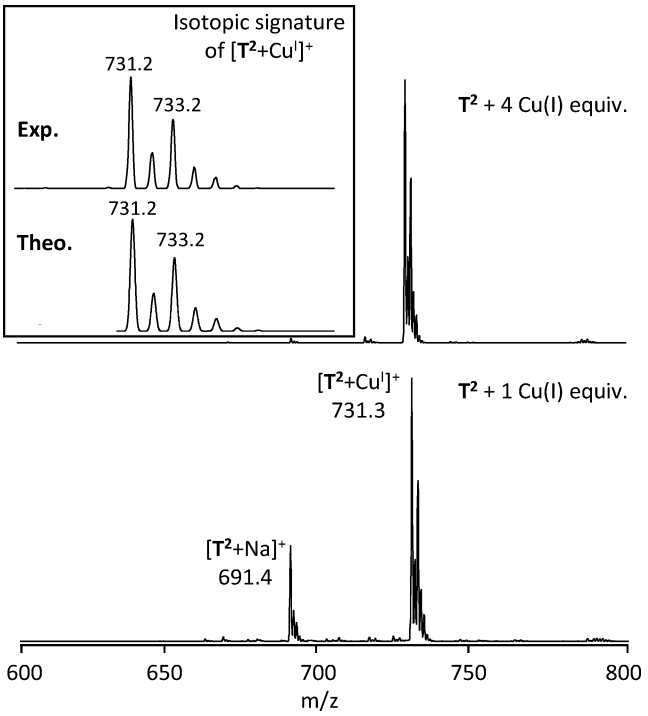

Figure 3. (+) ESI-MS spectra of $\mathbf{T}^2$ (100 μM) with equimolar Cu(I) (down) and 4 equiv (top) in water/MeCN (9/1, v/v). Inset shows isotopic patterns (experimental (Exp.) and calculated (Theo.)).

such as BCA (log β_2 = 17.3)[32] and BCS (log β_2 = 19.8).[2] Thus, competition experiments performed using Fz allow for the measurement of quite low affinity constants, with pCu^+ in the range of 9.8−13.8, with enough accuracy.[31] Recently, Wedd et al. emphasized that Fz may be sensitive to reducing agents while BCA and BCS are not. Therefore, the competitive experiments were performed without any reducing agent and in the glovebox to avoid any trace of oxygen, which may lead to Cu(II).

The complex $Cu(Fz)_2^{3-}$ was mixed with aliquots of the two ligands $\mathbf{T}^1$ and $\mathbf{T}^2$ at pH 7.4 to induce Cu displacements of 30%−70%. The affinity constants (K) were obtained, considering the formation of a single Cu(I) complex, as evidenced by mass spectrometry and circular dichroism, according to eq 1.

$$Cu(I) + \mathbf{T}^{1,2} = Cu\mathbf{T}^{1,2}\ K \qquad (1)$$

The equations used for the determination of the constants are reported in Boxed Text S1 in the Supporting Information.

As seen in Table 1, affinity constants values of log K = 10.3(1) and 10.1(1) have been determined for Cu(I) binding by the two pseudo-peptides, $\mathbf{T}^1$ and $\mathbf{T}^2$, respectively. These two compounds bind Cu(I) with greater affinities than linear peptides mimicking isolated Mets motifs found in yeast or human Ctr1 (log K = 5−6).[15] Although different methods have been used to measure these binding constants, striking differences are observed between the tripodal chelators $\mathbf{T}^{1,2}$ and peptides containing Mets-only sequences. The larger affinity for Cu(I) demonstrated by the pseudo-peptides $\mathbf{T}^1$ and $\mathbf{T}^2$ may be attributed to the rigid NTA chemical scaffold, which provides more preorganized binding sites and contributes to reinforce the Cu(I) binding, with respect to the more-flexible Mets Motifs. As expected, the affinities of $\mathbf{T}^{1,2}$ for Cu(I) are lower than those measured for peptide sequences that include

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both methionine and histidine residues (log $K \approx 13$), since histidine is known to coordinate more efficiently Cu(I) than methionine at physiological pH.[17,18]

DISCUSSION

The pseudo-peptides $\mathbf{T^1}$ and $\mathbf{T^2}$ are inspired from $Cu(S\text{-}Met)_3$ coordination spheres of the methionine-only binding sites found in the Cu(I) transporter Ctr1 and many bacteria copper-resistant proteins.[12−16] These ligands are based on a tripodal nitrilotriacetic acid (NTA) scaffold functionalized with three converging thioether functions, either from three methionine esters in $\mathbf{T^2}$ or from three more constrained methyl cysteine esters in $\mathbf{T^1}$. The synthesis of these two neutral soft sulfur donors were performed using synthetic strategies established in previous work on cysteine and D-penicillamine derivatives.[25,26,28] $\mathbf{T^1}$ and $\mathbf{T^2}$ were obtained from commercial amino acids with 58% and 54% global yields, respectively.

The data presented in this paper show the formation of mononuclear $Cu^{(I)}\mathbf{T^{1,2}}$ complexes. The affinity for Cu(I) was measured by using ferrozine (Fz) as a competitor. Indeed, Fz is a Cu(I) chelating agent with a moderate affinity and therefore allows determination of the affinity constants for Cu(I) inferior to 10^{13}. The two pseudo-peptides $\mathbf{T^1}$ and $\mathbf{T^2}$ demonstrate interesting affinity for Cu(I) with log $K \approx 10.1-10.3$. The constants are very similar, demonstrating that the modification of the chemical architecture due to the substitution of methyl cysteine arms in $\mathbf{T^1}$ with methionines in $\mathbf{T^2}$ does not affect the Cu(I) chelation properties. Interestingly, these values are significantly larger than those reported for linear peptides mimicking isolated methionine-only binding sites found in Ctr1 sequences (log $K \approx 5-6$).[15,16] This may be assigned to the preorganization of the tripodal ligands $\mathbf{T^1}$ and $\mathbf{T^2}$ that present three converging sulfur donors to coordinate the Cu(I) ion. Therefore, these pseudo-peptides are likely to compete with the Mets motifs for Cu(I) *in vivo*.

The two neutral sulfur donors $\mathbf{T^1}$ and $\mathbf{T^2}$ may also be compared to the previously reported pseudo-peptides $\mathbf{L^1}$ (derived from cysteine[25,26]) and $\mathbf{L^4}$ (derived from D-penicillamine[28]), which present three negatively charged sulfur thiolate donors and mimic $[CuS_3]^{2-}$ coordination sites found in metallothioneins. $\mathbf{T^1}$ and $\mathbf{T^2}$ have the same chemical architecture than the thiolate derivatives $\mathbf{L^1}$ and $\mathbf{L^4}$ but present three neutral sulfur donors of the soft thioether groups to chelate Cu(I) in $[CuS_3]^+$ environments. So, as expected, they show significantly lower affinity than the cysteine or D-penicillamine derivatives, which chelate Cu(I) with extremely large affinities, log $K = 17-19$.

Ligands based on thioethers as neutral sulfur donors could contribute to the development of novel agents for chelation therapy, which is a field that is under constant evolution.[33] Indeed, in contrast to thiol derivatives, they are not sensitive to extracellular oxidizing environments. Therefore, they are interesting candidates for extracellular Cu(I) chelation, for instance when Cu(I) is formed before cellular incorporation via Ctr1. Such extracellular chelators are currently proposed to promote cisplatin entrance in cisplatin-resistant cancer cells. Indeed, cisplatin is known to interfere in Cu homeostasis and interacts with Cu transporting proteins such as Atox1 or Ctr1.[34,35] In particular, its cellular uptake is proposed to be mediated by the copper transporter Ctr1; therefore, extracellular Cu(I) competes with cisplatin for binding to Ctr1.[35] Some Cu chelators such as tetrathiomolybdate (TTM) have already been used to deplete Cu and to promote cisplatin entrance in cancer cells.[36] Considering the affinity of $\mathbf{T^1}$ and $\mathbf{T^2}$ for Cu(I), they may be appropriate candidates to compete for Cu(I) with Ctr1 and to enhance cisplatin incorporation and efficacy. However, since Pt(II) is known to bind to sulfur donors and in particular to methionine of Mets motifs,[37] the interaction of $\mathbf{T^1}$ and $\mathbf{T^2}$ with cisplatin should also be taken into account.

The moderate affinity of the two methionine- or methyl cysteine-based pseudo-peptides may also be of interest to induce minimum disturbance of Cu homeostasis in chelation therapy treatments such as in Wilson's disease.

ASSOCIATED CONTENT

Supporting Information

Syntheses and characterizations of chelators $\mathbf{T^{1,2}}$, supplementary MS figures, equations for the calculation of the stability constants. This material is available free of charge via the Internet at http://pubs.acs.org.

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Notes

The authors declare no competing financial interest.

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