Ctr-1 Mets7 motif inspiring new peptide ligands for Cu(I)-catalyzed asymmetric Henry reaction under green conditions

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Taking inspiration from Ctr-1 Mets7 Cu(I) binding motif, effective hybrid catalysts have been developed for asymmetric Henry condensation under green conditions. The introduction of an unnatural dipeptide mimic allowed to increase the catalytic performance. Metal transporters' binding domains could be exploited as strategy for designing hybrid catalysts for homogeneous catalysis.

Hybrid catalyst attracted the attention of several research groups in the last years.[1-3] The development of a hybrid catalyst involves the synergic combination of a biomacromolecular scaffold such as a protein, and an active catalytic moiety such as a transition metal complex.[4] The so obtained artificial metalloenzymes have indeed shown high selectivity in the synthesis of enantiomerically enriched compounds in aqueous media.[5, 6] These successful stories have been possible thanks to the intimate knowledge of both the protein scaffold that hosts the metal entity and of the interaction(s) between the metal and its chelating ligands (the first sphere of coordination), as well as with the protein environment (the second sphere of coordination). Nevertheless, the use of modified protein scaffolds requires studies of site-direct mutagenesis and molecular biology techniques.[7] The use of small peptides capable of binding transition metals could be thus a much more convenient approach thanks to the easiness and modularity of their synthesis. On the other hand, reproducing the functional groups' spatial arrangement of a catalytic site with small peptides is still a challenge.[8-10] Metal transporters are proteins[11, 12] devoted to the trafficking of ions across the membranes and contain peptide domains able to bind metal ions.[13-18] A powerful approach to design hybrid metal-peptide catalysts could thus take advantage of the modulation of their binding activity. The native amino acid sequence of the binding domains could be optimized by inserting appropriate functional amino acids.[19-22] In this way it could be possible to increase the ability to form the complex with the metal. Furthermore, the introduction of unnatural amino acids and of molecular scaffolds could stabilize specific conformations of the peptide, creating the molecular architecture of a catalytic site.[23-25]

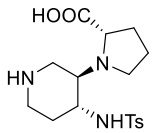
It has been established that copper binding sites in proteins are normally dominated by histidine, cysteine and methionine residues.[26, 27] The main differences among them are that methionine does not form cross linking or bridge two metal ions and does not contain a protonable side chain. The thioether indeed is a functional group that does not have a pH dependency, and is less prone to oxidation. Human Ctr-1 (hCtr-1) is a glycosylated membrane protein of 190 amino acids, belonging to the highly conserved family of copper transporters.[8] This domain is particularly important in defining the pore's dimensions and controlling Cu(I) trafficking across the membrane bilayer. It stands out for the presence of methionine (Met)-rich regions arranged as MXXM or MXM motifs containing three to five methionine residues. Starting from the sequence of the Met-rich motif present in the N-terminal domain of yeast yCtr1, Arnesano and co-workers designed a 8-mer sequence (MTGMKGMS), called Mets7, particularly able in binding Cu(I) and Pt(II) drug complexes.[28, 29] Circular dichroism studies indicated that Mets7 tends to adopt a random-coil structure in aqueous solution, while, upon coordination with Cu(I), it undergoes a large conformational change obtaining two consecutive β-turns.

The aim of our work has been the preliminary evaluation of Mets7, and a small library of its derivatives, as chiral ligands of Cu(I) to be used in homogenous catalysis. We hypothesized that Mets7 could favor Cu(I) coordination environments avoiding the redox cycling of copper between Cu(I) and Cu(II).[30, 31] This feature is particularly profitable in view of using this system as catalyst preserving the metal centre. Furthermore, the stable conformation of the peptide upon Cu(I) complexation could influence enantioselectivity in asymmetric reactions. To the best of our knowledge Mets7, as well as to other metal binding domains from metal transporters, has been never used as ligands for asymmetric homogenous catalysis.

As the model reaction to test our hypothesis, we selected the Henry condensation.[32-35] This reaction is an important synthetic tool to form a C–C bond, widely used for the obtainment of β-hydroxy nitro derivatives. Henry condensation is normally base-catalyzed, leading to side reactions such as the Cannizzaro aldehyde disproportionation or water elimination of the product. Due to the low enantioselectivity evinced under aqueous or green-solvent conditions, it's clear the necessity of exploring new types of environmentally friendly base-free catalysts.[36, 37]

Table 1. Amino acid sequence modifications starting from Mets7 sequence and molecular structure of unnatural scaffold **X**

Peptide	Sequence
Mets7	M T G M K G M S
A	K G K P G M S
B	K G M P G M S
B1	K T G M P G M S
C	M G K P G M S
S1	M T G M (X) M S



X

Starting from Mets7 sequence, we modified the number and the position of methionine residues (Table 1). Met position could vary the coordination geometry of Cu(I) complex, from linear to trigonal planar or tetrahedral. We expected that the so obtained peptides could assume different ordered or unordered secondary structures upon coordination with Cu(I),

thus affecting the ability to induce chirality in the corresponding hybrid catalysts. Furthermore, the synthesis of peptide mimetic **S1** containing the unnatural scaffold **X** was also planned with the idea of stabilizing the turn conformation. Indeed, compound **X** is a easily accessible delta amino acid able to stabilize β-structures when inserted in model peptides.[38, 39]

To support this hypothesis, we performed REMD calculations to compare the conformational behavior of metal-free Mets7 and S1.[40] Simulations were conducted as suggested by a recent article where several methods were compared for their ability to predict the native conformations of peptides (see ESI for details).[41] The room temperature trajectories of Mets7 and S1 were analyzed by clustering, H-bonds and other geometrical analyses. Data obtained from the cluster analysis are summarized in Table 2, while representative conformations of clusters #1 and #2 of peptide S1 are shown in Figure 1.

As expected, the replacement of KG with scaffold **X** reduces the conformational freedom of peptide **S1**, if compared to Mets7. Indeed, only two principal conformations are predicted by cluster analysis for the former peptide (clusters #1 and #2, with a percentage population (pop%) of 49 and 42%, respectively), principally differing in the orientation of the N-terminal segment (residues M1-M4) due to the rotation of the amidic bond linking M4 to scaffold **X**.

Table 2. Results[a] from the Cluster Analysis of the 75-100 ns Section of the 302.8 K REMD Trajectory for Mets7 and S1.

	Mets7		S1	
#	pop%	ψ,ϕ avg[b]	pop%	ψ,ϕ avg[b]
1	29.8	13.6±55.5, -98.8±29.3	49.0	67.1±157.7, -54.8±62.0
2	27.5	103.4±65.1, -91.3±80.7	42.0	119.2±121.0, -55.0±64.9
3	19.0	38.0±117.8, -88.9±23.2	8.0	43.0±154.8, -60.1±57.2
4	15.6	12.5±66.5, -40.3±84.2	0.9	-10.0±171.2, -67.5±67.4
5	8.1	35.7±62.6, -35.6±83.3	0.1	74.0±159.5, -16.7±97.5

[a]The full set of dihedrals is reported in Table TS1, ESI while representative geometries are depicted in Figure FS1, ESI. [b]Average ψ and ϕ dihedrals (deg.) and corresponding standard deviations are obtained for each cluster representative structure by averaging the corresponding values for all residues, without the capping Ac and NH_2 groups.

Moreover, the ψ and ϕ dihedrals of representative geometries of both clusters #1 and #2 of **S1** are compatible with a turn-like conformation, even if H-bond analysis did not show any of the hydrogen bonds typical of a β-turn (Table TS2, ESI). Conversely, four similarly populated clusters are obtained from the analysis of the Mets7 trajectory, suggesting a conformational freedom which is compatible with an intrinsically disordered peptide. Finally, the distances between the three methionine sulfur atoms of Mets7 and **S1** (d1, d2 and d3, Figure 1) were found to be generally larger for the former (d1 = 9.5±3.0 Å, d2 = 12.7±4.3 Å, d3 = 10.9±3.1 Å) than for the latter peptide (d1 = 9.8±1.6 Å, d2 = 9.3±3.2 Å, d3 = 7.7±2.0 Å) in the respective REMD trajectories, suggesting that complexation of copper should be favored for **S1**.

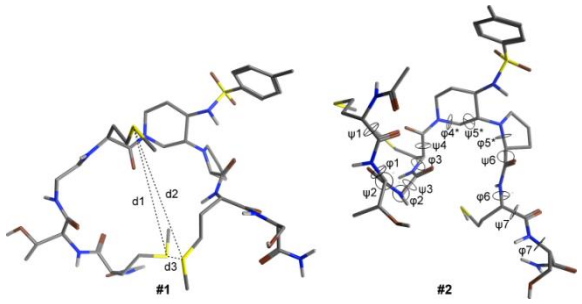


Figure 1. Representative conformations of clusters #1 and #2 as obtained from cluster analysis of the 302.8 K REMD trajectory of peptide **S1**. Non-standard dihedrals of the dipeptide mimetic X are marked with an asterisk.

All the peptides were prepared by solid phase synthesis using the Fmoc strategy (see ESI).[42] Cu(I) complexes were prepared by mixing equimolar amounts of peptide with copper(II) acetate in methanol. Two equimolar amounts of ascorbic acid were added to reduce Cu(II) to Cu(I). This methodology was applied taking into consideration that Cu(I) is sensitive to air oxidation until stabilized upon coordination. The ESI-MS spectra (Figure FS8-13, ESI) showed the formation of complexes for all the screened peptide ligands. Furthermore, only the mononuclear $[Cu(pep)]^+$ complex was evinced. In order to exclude any unspecific binding event, an Ala-scan sequence ATGAKGAS, in which the three methionines were replaced by alanine, was also prepared. The ESI-MS analysis showed that no interaction occurred (Figure FS14, ESI), indicating that the binding under the same analysis conditions is solely due to the presence of the coordinating sulphur of methionine.[29]

The conformational behavior of compounds **A**, **B**, **B1**, **C** and **S1** and their complexes was investigated in water. As expected, the CD spectra of **A**, **B**, **B1**, **C** alone were characterized by the presence of a negative band at 195 nm, indicating the absence of a preferred conformation (Figure FS15, ESI). NMR experiments on peptide mimic **S1** indicated that it is present in solution as a mixture of *cis/trans* rotamers of the tertiary amide on the piperidine ring, confirming the molecular modelling hypothesis. Furthermore, a turn conformation of the unnatural scaffold **X** was also observed (see ESI for details).

CD spectra of Cu(I) complexes confirmed in all cases the binding with the metal (Figure 2), as evinced by the increase of the intensity of the negative band below 195 nm.[31] Furthermore, only for Mets7 and **S1** complexes, a blue shift of this band was observed, indicating the presence of a stable beta-turn conformation upon complexation. These data indicated that the MXXMXXM binding motif is necessary to obtain an ordered secondary structure in the presence of Cu(I). On the contrary, the bidentate motif (MXXM in peptides **B** and **B1**, and MXXXXM in peptide **C**) is not sufficient for stabilizing the molecular architecture of the complex.

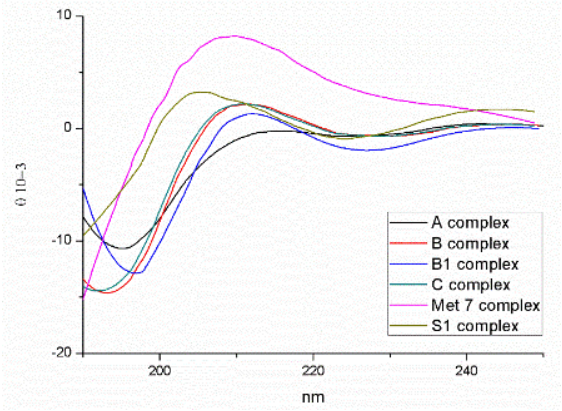
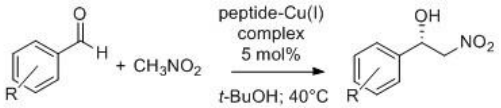


Figure 2. CD spectra of Cu(I) peptide complex

The catalytic performance of peptide-Cu(I)-complexes was thus investigated in the asymmetric nitro-aldol Henry condensation under green conditions. [43]

Benzaldehyde, 4-NO_2- and 4-Cl-benzaldehyde were selected as model substrates in a preliminary screening using nitromethane both as co-substrate and co-solvent. Firstly, different solvents (water, 2-propanol, methanol and *t*-BuOH) and different temperature values (10, 25 and 40 °C) were evaluated (see table TS3 in SI). In particular, we decided to screen solvents that, based on recent solvent selection rules,[44, 45] are considered preferable for working in green conditions. The reactions were thus carried out using *t*-BuOH for avoiding the competitive spontaneous condensation reaction observed to take place with the other screened solvents. Furthermore, *t*-BuOH was able to completely dissolve the peptide complexes, allowing to use them in catalytic amount. Moreover, no base was added and the temperature was maintained at 40 °C to increase the reaction rate without harming the enantioselectivity.

Table 3. Henry reaction catalyzed by peptide ligand-Cu(I) complexes



Entry	Peptide	Substrate	C (%)[a]	e.e. (%)[b]	TON
1	Mets7	R = H	54	48 (*R*)	12
2	Mets7	R = 4-NO_2	90	46 (*S*)	20
3	Mets7	R = 4-Cl	65	rac	14
4	A	R = H	-	-	-
5	A	R = 4-NO_2	-	-	-
6	A	R = 4-Cl	10	rac	2
7	B	R = H	10	22 (*S*)	2
8	B	R = 4-NO_2	25	7 (*R*)	6
9	B	R = 4-Cl	15	9 (*R*)	3
10	B1	R = H	10	5 (*R*)	2
11	B1	R = 4-NO_2	20	rac	4
12	B1	R = 4-Cl	10	rac	2
13	C	R = H	20	24 (*S*)	4
14	C	R = 4-NO_2	50	rac	11
15	C	R = 4-Cl	20	18 (*R*)	4
16	S1	R = H	50	75 (*R*)	11
17	S1	R = 4-NO_2	62	40 (*S*)	14
18	S1	R = 4-Cl-	54	rac	12

All reactions were carried out for 12 h using 5 mol % complex in *t*-BuOH:CH_3NO_2=50:50 (2 mL) [a]Conversion (C) was obtained by ^{1}H-NMR analysis. [b]Data were compared by taking the average of three independent experiments. Enantiomeric excess was determined using HPLC equipped with chiral OD-H column. Eluent: hexane/2-propanol=90/10 flow=1.0 mL/min, λ = 215 nm or hexane/2-propanol=80/20 flow=0.5 mL/min, λ = 254 nm. $[sub]_f$ = 10 mM, $[cat]_f$ = 0.45 mM.

As reported in Table 3, only the catalytic complex with the monocoordinating peptide **A**, resulted inactive or less active as catalyst (entries 4-6). In all the other cases, the reaction was found effective especially using Mets7 and **S1** complexes (entries 1, 2, 16, 17). In the case of Mets7 complex the yield was up to 90 % within 12 h (entry 2). For this reason this was the reaction time set for the evaluation of catalyst performance (TON).

Regarding the enantiomeric excess, using 4-Cl-benzaldehyde as the substrate, only a racemic mixture was obtained (entries 3, 6, 9, 12 and 18). As an exception, a 18 % e.e. in favor of *R* configuration was obtained when peptide **C** was employed. In the case of benzaldehyde and 4-NO_2-benzaldehyde, different enantioselectivities were observed depending on the catalyst geometry. Indeed, low enantioselectivity was obtained by using **B**, **B1** and **C** complexes that would lead to a bidentate complex and in which the peptides do not assume a stable β-turn conformation (entries 7-15). This behavior might depend on the higher molecular mobility of these complexes making them capable to accommodate different labile geometries. As evinced by using peptide **B** or **C**-copper(I) complexes the condensation product was obtained in *S* configuration for benzaldehyde while the other enantiomer was achieved for both 4-NO_2 and 4-Cl-benzaldehyde.

This inversion of product configuration might be due to the MXXM (peptide **B**) or MXXXXM (peptide **C**) motifs causing a different chirality arrangement around the metal (entries 7-9 and 13-15).On the other hand, Mets7 and **S1** beta-turn complexes, gave the *R* isomer as the main enantiomer with good e.e. in the condensation reaction of benzaldehyde (entries 1 and 16) whereas when starting from 4-NO_2-benzaldehyde the reaction led to the *S* enantiomer (entries 2 and 17).

From these results, it is evident that the presence of a stable beta-turn conformation is crucial for enhancing both the reaction rate and the enantioselection. This ordered conformation could influence the coordination arrangement ($Cu(S\text{-}Met)_3$) playing thus an important role on the stabilization of the reduction potential of the metal centre. This feature is indeed critical for avoiding redox cycling between copper(I) to copper(II). In this way, the catalytically active form of the complex results much more stabilized, and thus functional to carry out the condensation reaction. Indeed, Mets7 and **S1** peptides probably act as catalytic pocket creating a second coordination sphere arrangement. The chemical fine-tuning of the catalytic moiety results adjusted in a localization of the substrate inside the mimicking "funnel-shape cavity" of the peptide influencing the enantioselectivity and consequently the absolute configuration of the products

In conclusion, we designed and prepared modified peptide sequences inspired to Ctr1 Mets7 copper binding motif. These compounds were able to bind copper(I) and they were preliminarily tested as hybrid catalysts in asymmetric Henry condensation under green reaction conditions. The introduction of the unnatural scaffold **X**, able to stabilize the geometry and the conformation of the complex, allowed to obtain good e.e. and yield when benzaldehyde and 4-NO_2-benzaldehyde were used as the substrates. We can thus conclude that the use of modified metal binding protein domains from metal transporters can be a valuable strategy for designing hybrid catalysts for homogeneous asymmetric reactions.

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