Exploring the copper binding ability of Mets7 hCtr-1 protein domain and His7 derivative: an insight in Michael addition catalysis

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Abstract. Mets7 is a methionine-rich motif present in hCtr-1 transporter that is involved in copper cellular trafficking. Its ability to bind Cu(I) was recently exploited to develop metallopeptide catalysts for Henry condensation. Here, the catalytic activity of **Mets7-Cu(I)** complex in Michael addition reactions has been evaluated. Furthermore, **His7** peptide, in which Met residues have been substituted with His ones, was also prepared. This substitution allowed **His7** to coordinate Cu(II), with the obtainment of a stable turn conformation as evicted by CD experiments. **His7-Cu(II)** proved also to be a better catalyst than **Mets7-Cu(I)** in the addition reaction. In particular, when the substrate was the (*E*)-1-phenyl-3-(pyridin-2-yl)prop-2-en-1-one, a conversion of 71% and a significative 58 % of e.e. was observed.

Keywords
Metallopeptide, Hybrid catalyst, Copper, Michael reaction, chalcone

Introduction

Metal coordination to proteins is often a necessary requirement to provide functional structures as well as enzymatic catalytic activities.[1] Commonly, amino acids bind metal ions through the formation of stable five-membered chelates, but the presence of different side chains, comprising more than one set of donor atoms, greatly increases coordination geometries variability and thermodynamic stability[2-4] Enzymes active sites possess unique architectures determined by the nature and the position of the amino acid residues involved in metal ion coordination.[5] This intricate three-dimensional network of interactions creates an asymmetric environment around the metal centre allowing an efficient substrate recognition and orientation. It is thus not surprising that chemists have been exploring the advantageous properties of peptides as ligands for metal ions for asymmetric catalysis.[6-9] Indeed, despite the achievements obtained using transition metal catalysts based on chiral diphosphines and *N*-chelating ligands, many transformations are still hampered by several issues concerning reactivity and selectivity.[10-16] Conversely, enzymes often outperform transition metal catalysts with higher levels of efficiency and of chemo-, regio- and stereoselectivity,[17-20] giving thus an inspiration for the development of enzyme-like transition metal hybrid catalysts.[19-23] Combining the rational design of the peptide ligand and the choice of an appropriate metal, it is possible to merge the high reactivity of transition metals with the structural and functional groups versatility of peptides, achieving levels of reactivity otherwise unrealisable. A common approach in this regard takes advantage of natural peptide sequences known for their ability to coordinate to metal ions.[24] Indeed peptides can be used as templates for inserting non-natural amino acids or synthetic scaffolds stabilizing a specific secondary structure,[25-27] and/or allowing the coordination to a specific transition metal.[28] In this context, Roelfes' research group modified bovine pancreatic polypeptide (bPP), a 36-mer member of pancreatic hormones,[29] by truncating it to a 31-mer derivative and introducing non-proteinogenic amino acids in order to develop a functional ligand for Cu^{2+} catalysis. These hybrid systems, applied to the asymmetric Diels-Alder and Michael addition reactions on α,β-unsaturated carbonyl compounds afforded the desired products in moderate-to-good enantioselective levels. Recently, starting from ATCUN motif,[30, 31] a Cu(II)- and Ni(II)-binding sequence found at the *N*-terminus of many naturally occurring proteins, ultrashort peptides were developed as iridium binding catalytic systems for asymmetric transfer hydrogenation of substituted ketones in water.[32, 33] The same scaffolds were successfully employed in the preparation of cobalt-based hybrid electrocatalysts, for the selective six-electron reduction of nitrite and hydroxylamine intermediates to ammonium in aqueous buffer.[34, 35]

In our research group we recently reported the catalytic ability of the complex between **Mets7** and Cu(I) in Henry condensation reaction.[28] **Mets7** is a methionine-rich motif naturally present in hCtr1copper transporters, devoted to the trafficking of cellular copper and recognised for being involved in inducing resistance to therapeutically approved platinum drugs by their sequestration.[36] In our previous work, we developed a series of hybrid catalysts obtaining significative e.e. and yield in the reaction between substituted benzaldehydes with nitromethane.

Here, we investigated the catalytic ability of **Mets7-Cu(I)** complex in asymmetric Michael addition of nitromethane to a series of homo- and heterocycle chalcones. This reaction was selected as it affords valuable intermediates in the synthetic pathway for $GABA_B$ receptor agonists.[37] Furthermore, we developed a new chiral ligand called **His7**, in which Met residues have been replaced with His. This replacement allowed Cu(II) binding[38, 39], with the obtainment of a stable turn conformation as evicted by CD experiments. In Michael addition,[40] **His7-Cu(II)** proved to be a better catalyst than **Mets7-Cu(I)**. In particular, when the substrate was the (*E*)-1-phenyl-3-(pyridin-2-yl)prop-2-en-1-one, a conversion of 71% and a significant 58 % of e.e. was obtained.

Materials and Methods

General. All reactions were carried out in oven-dried glassware and dry solvents under nitrogen atmosphere. Fmoc Rinkamide resin, Fmoc-protected (L)-amino acids, HBTU, HOBt and DIEA were purchased from Iris Biotech Gmbh (Germany). Solvents, piperidine and other reagents were purchased from Sigma-Aldrich (Germany). **His7** and **Mets7** were synthesized using a CEM Liberty Blue peptide synthesizer and purified using RP-HPLC with a Jasco BS-997-01 instrument and a DENALI C-18 column from GRACE VYDAC (10 µm, 250 x 22 mm). MS analyses were performed with a Thermo Finnigan (MA, USA) LCQ Advantage system MS spectrometer with an electrospray ionization source and an 'Ion Trap' mass analyser. The MS spectra were obtained by direct infusion of a sample solution in MeOH under ionization, ESI positive. Circular Dichroism (CD) spectra were recorded on a Jasco-810 spectropolarimeter. Catalytic reactions were monitored by HPLC analysis with Merck-Hitachi L-7100 equipped with Detector UV6000LP and chiral column (AD, OJ-H Chiralcel, Lux Cellulose-4, Lux Cellulose-2 or Lux Amylose-2). ^{1}H NMR and ^{13}C NMR spectra were recorded with Varian Oxford 300 MHz spectrometer at 300 and 75 MHz respectively. Chemical shifts are given as δ values in ppm relative to residual solvent peaks as the internal reference. ^{13}C NMR spectra are ^{1}H-decoupled and the determination of the multiplicities was achieved by the APT pulse sequence.

General procedure for the synthesis of peptide

Peptide **His7** and **Mets7** were synthetized on Rink-amide resin (250 mg, 0.69 loading) using Microwave assisted solid phase peptide synthesis.[41] A five-fold molar excess of Fmoc-protected amino acids (0.2 M in DMF), and Oxima/DIC (5 : 5 eq) as activators were used. Coupling reactions were performed for 5 min at 40 W with a maximum temperature of 75 °C. His coupling was performed for 2 min at rt at 0W followed by 4 min at 35W with a maximum temperature of 50 °C. Deprotection was performed twice using 20% piperidine in DMF (5 and 10 min each). Acetylation was performed manually on resin using Ac_2O (10 eq.) and DIPEA (10 eq.), two couplings of 1 h each. The final cleavage cocktail was made by trifluoroacetic acid (TFA, 8 mL), triisopropylsilane (TIS, 400 µl), thioanisole (400 µl), water (200 µl) and phenol (0.6 g) for 4 h. After the cleavage, the peptides were precipitated in cold diethyl ether and centrifuged three times (3 min, 6000 rpm, 4°C). **Mets7** was purified by RP-HPLC using a gradient elution of 5–70% solvent B (solvent A: water/acetonitrile/trifluoroacetic acid 95:5:0.1; solvent B: water/acetonitrile/trifluoroacetic acid 5:95 :0.1) over 20 min at a flow rate of 20 mL/min. **His7** was purified by fractioned precipitation from tBuMe.ether/hexane 1:1 mixture. The peptides were analyzed by analytical RP-HPLC, showing a purity higher than 90%, and, then freeze-dried and stored at 0 °C.

Peptide	***Sequence***	***Calcd MW***	***Found MW***	***rt[a] (min)***	***yield***
Mets7	*M T G M K G M S*	*882.36*	*883.61*	*17.5*	*50 mg*

His7	*H T G H K G H S*	*900.94*	*901.72*	*11.8*	*80 mg*

[a] gradient elution of 2–60% solvent B (solvent A: water + 0.1%TFA; solvent B: acetonitrile/+ 0.1% TFA) over 20 min at a flowrate of 0.8 ml/min.

Synthesis of Cu(I) complex

In a Schlenk tube containing 8 mg of **Mets7** or **His7** in 2 mL of MeOH, 4.6 mg of solid $Cu(OAc)_2$ (1 eq.) was added. 8.1 mg of ascorbic acid (2 eq.) was introduced for *in situ* reduction of Cu(II) to Cu(I). The solution was stirred overnight at room temperature and a formation of precipitate was observed. The precipitate was filtered and dried in vacuum. The solid was washed three times with Et_2O and analyzed by ESI-MS. **Mets7-Cu(I)**: pale yellow powder. 4.5 mg, yield 49 %. ESI-MS for $[C_{34}H_{62}N_{10}O_{11}S_3Cu]^+$: calcd 945.31 found 945.3 **His7-Cu(I)**: pale brown powder. 5.2 mg, yield 57 %. ESI-MS: calculated for $[C_{37}H_{56}N_{16}O_{11}Cu]^+$: 962.36; found 963.38 $[M+H]^+$

Synthesis of Cu(II) complex

In a Schlenk tube containing 8 mg of **His7** in 2 mL of MeOH, 4.6 mg of solid $Cu(OAc)_2$ (1 eq.) was added. The solution was stirred overnight at room temperature and a formation of precipitate was observed. The precipitate was filtered and dried in vacuum. The solid was washed three times with Et_2O and analysed by ESI-MS. **His7-Cu(II)**: blue-green powder. 7.3 mg, yield 80 %. ESI-MS: calculated for $[C_{37}H_{56}N_{16}O_{11}Cu]^{2+}$: 962.36; found 963.44 $[M+H]^+$

Circular dichroism

Solutions of peptides **Mets7, His7** and complexes **Mets7-Cu(I), His7-Cu(I), His7-Cu(II)** were prepared in H_2O (50 µM, 1.5 mL). Spectra were obtained from 195 to 250 nm with a 0.1 nm step and 1 s collection time per step, measuring three averages. The spectrum of the solvent was subtracted to eliminate interference from cell, solvent and optical equipment. The CD spectra were plotted as mean residue ellipticity θ (degree x cm2 x dmol-1) versus wave length λ (nm). Noise-reduction was obtained using a Fourier-transform filter program from Jasco.

General procedure for asymmetric Michael reaction. A mixture of powdered, flame-dried CsF (2 eq.), peptide (0.022 eq.), $Cu(OAc)_2$ (0.02 eq.), and chalcone (1 eq.) in toluene (0.15 ml) was treated with nitromethane (10 eq.). The mixture was stirred at rt for 18 h and then diluted with 400 µl of Et_2O and 400 µl of H_2O. The organic phase was separated, dried over anhydrous Na_2SO_4 and evaporated.
In the case of **Mets7-Cu(I)** and **His7-Cu(I)** the reaction was performed applying the same procedure by using the pre-formed complex as described above.
The conversion and the enantiomeric excess were determined by HPLC analysis (see Table T2 in SI).

Results and Discussion

Mets7 (MTGMKGMS) is a 8-mer peptide derived from hCtr1 transport protein able to chelate Cu(I).[42, 43] In 2007, Arnesano and co-workers demonstrated that the sulphur atoms of **Mets7** are able to chelate both Cu(I) and its Pt(II) bioisoster. This coordination allows the peptide conformational change from an unordered to two consecutive β-turns structure. This structuring process is responsible of the copper trafficking inside the cell and of cis-platinum transport through the membranes.[42] In our previous work,[28] we demonstrated the catalytic ability of **Mets7-Cu(I)** complex in Henry condensation reaction. In particular, the presence of a stable β-turn conformation was found crucial for enhancing both the reaction rate and the enantioselection. Here **Mets7-Cu(I)** catalyst was investigated in asymmetric Michael addition of nitromethane to a series of homo- and heterocycle chalcones. Furthermore, we developed **His7** peptide (HTGHKGHS), in which the Met residues have been replaced by His ones. The presence of the imidazole ring of His could indeed allow the binding of both Cu(I) and Cu(II), without interfering with the peptide structural rearrangement due the metal complexation. Furthermore, the HSAB principles establish border-line Cu(II) ion preference for the imidazole histidyl *N*-donor as the best match allowing thus a substantial change in the geometry of the related complex

achieving a square-planar rather than tetrahedral complex. The obtainment of metal complexes with Cu(II) could give the possibility to extend the scope of reactions thanks to different coordination geometries and to improve stability towards oxidation.

Both **His7** and **Mets7** peptides were synthetized by Fmoc solid phase peptide synthesis, using Rink-amide resin (0.69 mmol/g) following standard procedures.[41, 44] The complexes were prepared by mixing equimolar quantities of **Mets7** or **His7** with $Cu(OAc)_2$ in MeOH and adding 2 equivalent of ascorbic acid for inducing the Cu(II) reduction to Cu(I). The formation of the corresponding Cu(I)-complexes was evinced from ESI-MS spectra. For **His7**, using the same copper precursor, the Cu(II) complex was synthetized and, also in this case, its formation was proved by ESI-MS. The coordination of **His7** to copper metal center was then confirmed by the bathochromic shift of the metal-to-ligand charge transfer transition (MLCT) band[45] in the UV spectra at 407 nm, more intense for **His7-Cu(II)** complex than in the case of the **His7-Cu(I)** one (See SI).
The conformational behaviour of peptides and their complexes was investigated in water by circular dichroism (CD) experiments (Figure 1). CD is indeed a powerful tool to detect conformational changes also in short peptides. [41, 46] As expected, the CD spectra of **His7** and **Mets7** alone were characterized by the presence of a negative band at around 195-198 nm, indicating the absence of a preferred conformation. CD spectra of Cu(I) complexes suggested for both peptides a conformational rearrangement. For **Mets7-Cu(I)**, the metal coordination between the three sulfur atoms present on the three Met, led to an important structural change in the peptide, as previously reported.[28] In the CD spectrum, indeed, the blue shift of the band at 195 nm and the positive band at around 208 nm indicated the presence of a turn or a bended conformation. In the case of **His7-Cu(I)** a change of peptide conformation was also suggested by the presence of a negative peak at 195 nm and two shoulders at 208 and 235 nm. Anyway, the conformational induction upon complexation was more evident in the case of **His7-Cu(II)** complex, with the obtainment of a β-turn-like spectrum characterized by a strong negative value at 190 nm, a positive Cotton effect at 205 nm and a negative minimum at 225 nm. From these findings we can thus confirm that the His/Met substitution does not interfere with the peptide rearrangement upon complexation.

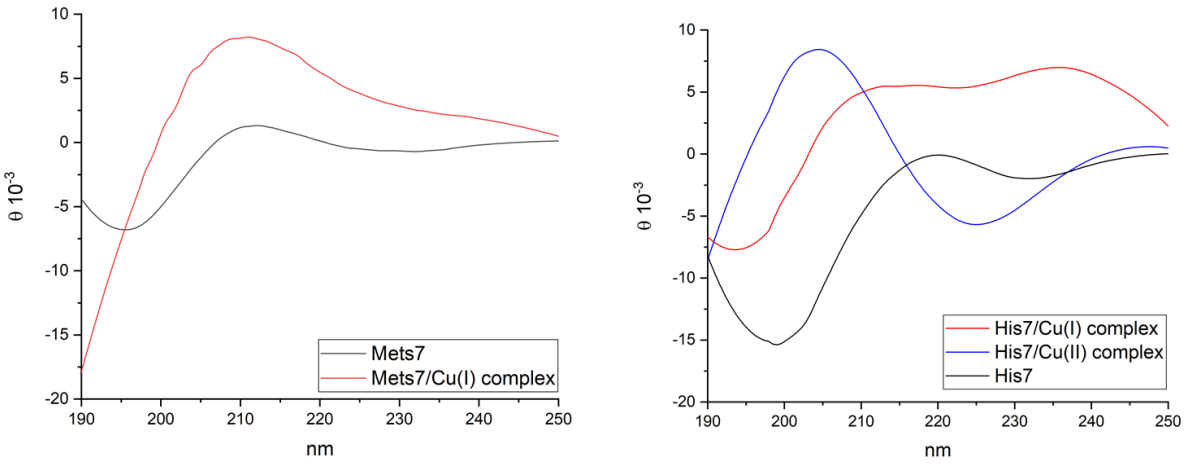
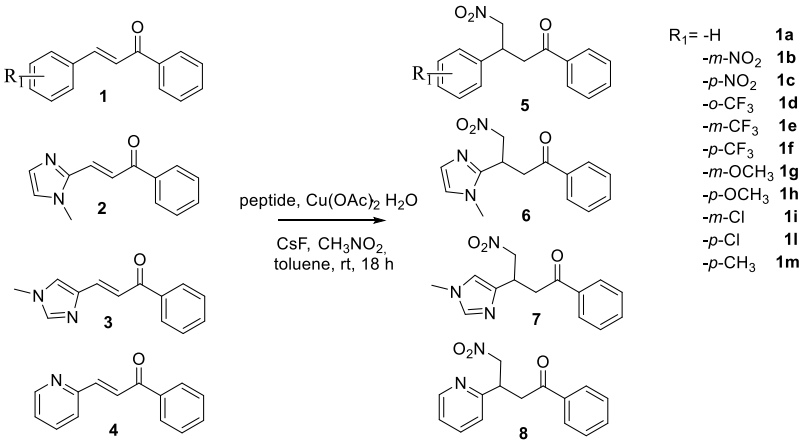


Figure 1. CD spectra (50 μM in water) of: left) **Mets7** and **Mets7/Cu(I)** complex; right) **His7**, **His7/Cu(I)** and **His7/Cu(II)** complexes.

The catalytic performance of peptide-copper-complexes was then investigated in the asymmetric Michael condensation of different substituted chalcones[47] with nitromethane. This reaction represents an important synthetic tool to form new C–C bonds, and it is commonly used for the obtainment of valuable synthetic intermediates. Three different benzoyl group-activated olefins such as the 2- or 4-imidazolyl enones (**2** and **3** respectively **)** and the 2-pyridinyl enone **4** were synthesized and evaluated as substrates for the synthesis of the corresponding chiral products using the obtained **Mets7** and **His7** complexes.[48] A preliminary screening (See SI) in which different reaction conditions were tested, changing the solvent, substrate/catalyst ratio, basic additive and temperature, was first performed. As a result, toluene was confirmed as the solvent of choice and CsF as the best performing base. A substrate/catalyst ratio of 50/1 was used for determining

conversion in 18 h and the reaction was conducted at room temperature to increase the reaction rate without harming the enantioselectivity.

Table 1. Enantioselective Michael addition.



Entry	Catalyst	Substrate	Conversion % [a]	e.e.% [a]
1	**His7-Cu(II)**	**1a**	85	-
2	**His7-Cu(II)**	**1b**	99	32 (1 ent)
3	**His7-Cu(II)**	**1c**	99	-
4	**His7-Cu(II)**	**1d**	99	-
5	**His7-Cu(II)**	**1e**	99	-
6	**His7-Cu(II)**	**1f**	99	5 (2 ent)
7	**His7-Cu(II)**	**1g**	99	-
8	**His7-Cu(II)**	**1h**	99	-
9	**His7-Cu(II)**	**1i**	99	8 (2 ent)
10	**His7-Cu(II)**	**1l**	99	10 (1 ent)
11	**His7-Cu(II)**	**1m**	99	-
12	**His7-Cu(II)**	**2**	14	7 (1 ent)
13	**His7-Cu(II)**	**3**	15	11 (2 ent)
14	**His7-Cu(II)**	**4**	71	58 (2 ent)
15	**Mets7-Cu(I)**	**1a**	99	-
16	**Mets7-Cu(I)**	**3**	39	-
17	**Mets7-Cu(I)**	**4**	83	13 (2 ent)
18	**His7-Cu(I)**	**1a**	35	-
19	**His7-Cu(I)**	**3**	25	5
20	**His7-Cu(I)**	**4**	43	-

¶ Reaction were conducted with peptide (0.022 eq.), $Cu(OAc)_2$ (0.02 eq.), CsF (2 eq.) and CH_3NO_2 (10 eq.) in toluene, final substrate concentration of 350 mM at room temperature for 18 h. [a]Conversion and enantiomeric excess were determined by HPLC (see Table T2 in SI).

His7-Cu(II) revealed to be the best catalyst, both in terms of conversion and enantioselection, except in the case of substrates **2** and **3** carrying the imidazole moiety (Table 1, entries 12, 13, 16 and 19). In particular, significative e.e. were obtained for compounds **1b**, the (*E*)-3-(3-nitrophenyl)-1-phenylprop-2-en-1-one (Table 1, entry 2,), and **4**, the (*E*)-1-phenyl-3-(pyridin-2-yl)prop-2-en-1-one (Table 1, entry 14). In the case of substrate **4** a modest 13 % e.e. was also obtained when **Mets7-Cu(I)** was used as catalyst, probably due to an additional coordination in the transition state of the pyridinic *N* to the metal.[49]

The obtained data agreed with the proposed reaction mechanism depicted in Figure 2. First, nitromethane displaces acetate in the His7-Cu(II) complex, with the obtainment of complex **A**. Then a nucleophilic addition

to the chalcone occurred with the formation of O-enolate. The pivotal role of the peptide turn conformation around the copper centre makes the catalytic moiety assessable only by one of the two pro-chiral faces of the substrate dictating the enantioselectivity of the product. The elimination of the product regenerates the pre-catalyst, able to react with another nitromethane.

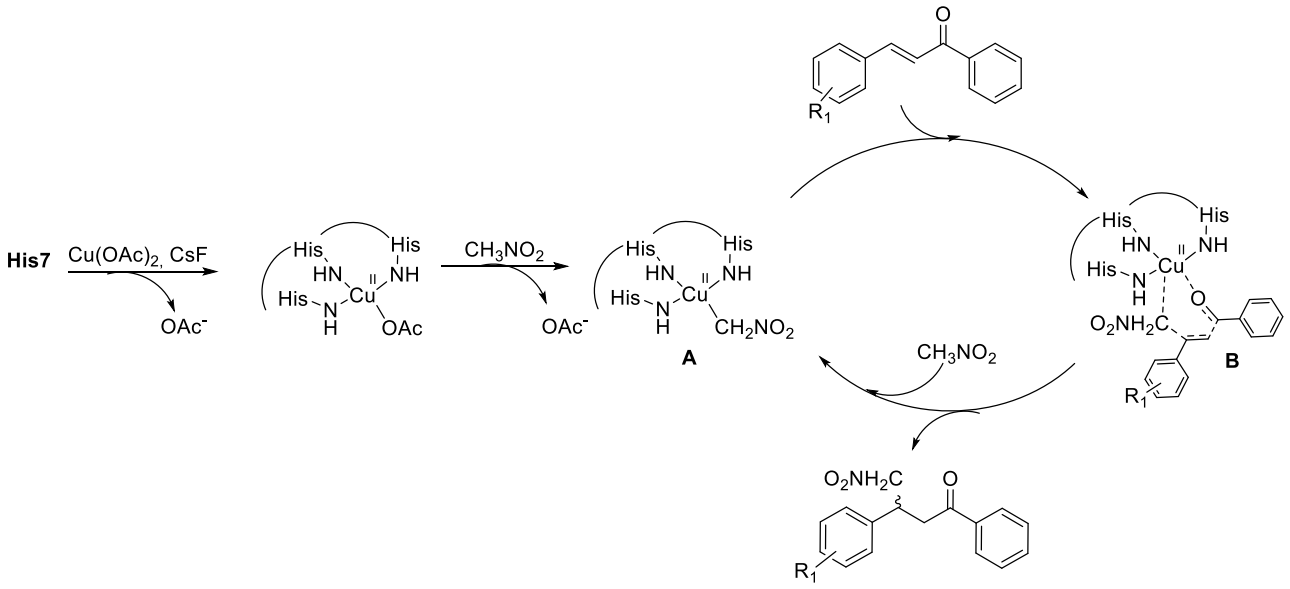


Figure 2. Proposed catalytic cycle for asymmetric Michael addiction with **His7-Cu(II)**

Furthermore, both **His7-Cu(II)** and **Mets7-Cu(I)** complexes, led to the same enantiomers, suggesting a similar coordination mode of the two peptides around the metal centre as highlighted by CD data. On the other hand, the drastic decrease of the enantioselectivity for **Mets7-Cu(I)** and **His7-Cu(I)** complexes could be ascribed to a thermodynamically more stable conformation of **His7** when coordinated to Cu(II).

Conclusion

In this work, we investigated the catalytic ability of peptides derived from hCtr1 copper transporter domain in asymmetric Michael addition of nitromethane to chalcones. The introduction of His residues favoured the formation of a stable Cu(II) complex characterized by a β-turn conformation. In particular, the reaction proceeded with good enantioselectivity when compound **4** ((*E*)-1-phenyl-3-(pyridin-2-yl)prop-2-en-1-one) was used as the substrate, reaching a valuable 58 % e.e..

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