

Supporting Information for:

## **Active uptake of the hydrophilic copper complex Cu(II)-TETA in primary cultures of neonatal rat cardiomyocytes**

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## Supplementary materials and methods

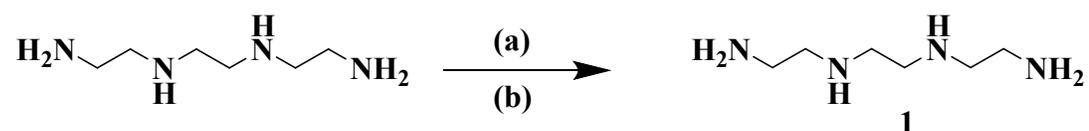
### Materials and methods

#### General

All solvents and chemicals were purchased from Sigma-Aldrich (USA) and Kelong (CN) and used as received. The  $^1\text{H}$  and  $^{13}\text{C}$  NMR spectroscopic measurements were conducted in deuterated solvents at the Analysis & Testing Center Sichuan University (Chengdu, P, R, China), using a Bruker AV II NMR spectrometer at -600 MHz and 150 MHz, respectively.

GC-MS analysis was performed with the Shimadzu GCMS-QP2010 Plus of the Department of Analysis & Testing Center Sichuan University (Chengdu, PR China). High-resolution Electrospray Ionization (ESI) time-of-flight (TOF) mass spectrometry analysis was performed using the Bruker Maxis Impact at the Department of Regenerative Medicine Research Center, West China Medical School / West China Hospital, Sichuan University. Electrochemistry experiments were carried out using a CHI 660D electrochemical workstation from the Department of Regenerative Medicine Research Center, West China Medical School / West China Hospital, Sichuan University. X-ray crystallography was done using a Bruker Technologies Xcalibur E using a  $\mu$ -focus Cu  $K\alpha$  radiation source ( $\lambda=1.5406\text{\AA}$ ) with collimating mirror monochromators. UV-Vis analysis was performed using the Bio-Tek SynergyMx of the Department of Regenerative Medicine Research Center, West China Medical School / West China Hospital, Sichuan University. Fourier transformed infrared spectrometer (FT-IR) analysis was performed using the Thermo Fisher Nicolet 6700 at the Department of Analysis & Testing Center Sichuan University (Chengdu, P, R, China).

#### Synthesis

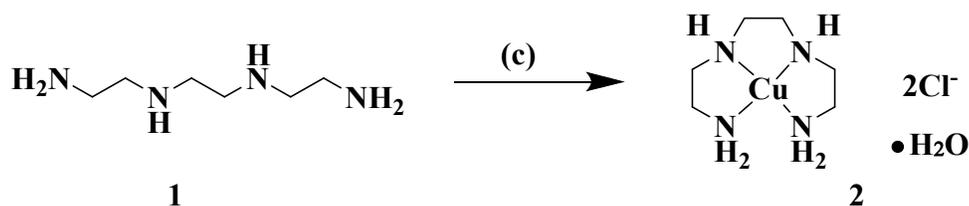


**Scheme S1. Purification of TETA.** Conditions: (a) TETA, dichloromethane, di-*tert*-butyl dicarbonate, petroleum ether,  $\text{H}_2\text{O}$ , room temperature, 2 hours (50%); (b) Dichloromethane,  $\text{Na}_2\text{CO}_3$ , trifluoroacetic acid.

#### Compound 1

Step (a): TETA (14.6 g, 100 mmol) was dissolved in 200 mL dichloromethane, and di-*tert*-butyl dicarbonate (10.0 g 500

mmol) was then added in small portions. The solution became milky and was stirred at room temperature for a further 2 hours. Product was collected as white powder by filtration, washed with water (3×100 mL) and petroleum ether (3×100 mL). Step (b): The white powder was dissolved in 200 mL dichloromethane, and excess trifluoroacetic acid was then added in small portions until adjusting to pH=2. Excess saturated Na<sub>2</sub>CO<sub>3</sub> aqueous solution was then added in small portions until adjusting to pH=9~10. The inorganic salt was removed by filtration and the mother liquor was concentrated after evaporation of solvent. The desired complex was washed by ethanol (3×100 mL) and obtained as a pale yellow oily liquid after evaporation of solvent (7.3 g, 50% yield). <sup>1</sup>H NMR (600 MHz, d<sub>2</sub>-H<sub>2</sub>O, 25 °C): δ 3.53 (s, 4H), 3.48 (t, 4H), 3.38 (t, 4H). <sup>13</sup>C NMR (150 MHz, d<sub>2</sub>-H<sub>2</sub>O, 25 °C): δ 44.72, 43.40, 35.43. HR ESI-MS (ESI<sup>+</sup>, MeOH): *m/z* calculated for [C<sub>6</sub>H<sub>19</sub>N<sub>4</sub>]<sup>+</sup> 147.1610, found 147.1569.



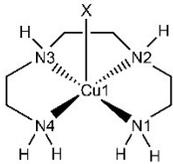
**Scheme S2. Synthesis of Cu(II)-TETA.** Conditions: TETA, CuCl<sub>2</sub>•2H<sub>2</sub>O, ethanol, n-hexane, chloroform, room temperature, 3 hours (81%).

### Compound 2

Cu(II)-TETA was synthesized via chelating reaction with ligands TETA (14.6 g, 100 mmol) and CuCl<sub>2</sub>•2H<sub>2</sub>O (17.0 g, 100 mmol) in 300 mL ethanol. The hydrophilic reaction products were purified by flash column chromatography using a gradient of 20% to 50% methanol in chloroform to yield the final complex as the monohydrate, light blue solid (24.3 g, 81% yield). Single crystals of Cu(II)-TETA were grown in ethanol/n-hexane system. HR ESI-MS (ESI<sup>+</sup>, MeOH): *m/z* calculated for [C<sub>6</sub>H<sub>18</sub>N<sub>4</sub>CuCl]<sup>+</sup> 244.0516, found 244.0486.

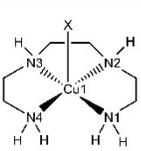
## Supplementary Tables

Supplementary Table S1. Comparison of selected bond lengths (Å) of [Cu(TETA)Cl] and related structures.<sup>a</sup>

	[Cu(TETA)Cl]Cl	[Cu(TETA)I]I	[Cu(TETA)(SCN)] [SCN]	[Cu(TETA)(NCS)] [ClO <sub>4</sub> ] <sup>b</sup>	[Cu(TETA)] [PF <sub>6</sub> ] <sub>2</sub>
CCDC#	1535585	1317289	1263305	1269536	1227418
Cu1-N1	2.026(4)	2.045(7)	2.008(7)	1.98(2)/2.05(3)	1.993(8)
Cu1-N2	2.035(4)	2.040(5)	2.015(6)	2.08(2)/2.04(2)	1.971(10)
Cu1-N3	2.026(4)	2.025(6)	2.030(5)	1.98(2)/1.98(2)	1.993(8) <sup>e</sup>
Cu1-N4	2.035(4)	2.019(6)	2.013(7)	2.10(2)/2.07(2)	1.971(10) <sup>e</sup>
Cu1-X <sup>c</sup>	2.4741(12)	2.7728(19)	2.607(2)	2.12(3)/2.19(3)	2.59(3) <sup>f</sup>
Cu1 ··· N <sub>4</sub> plane <sup>d</sup>	0.4178(5)	0.4387(13)	0.370	0.427/0.392	-0.096 <sup>g</sup>

a) Atom positions are numbered per [Cu(TETA)Cl]Cl (see inset), which may differ from original report for [Cu(TETA)(SCN)][SCN] and [Cu(TETA)(NCS)][ClO<sub>4</sub>]. b) Asymmetric unit contains two unique Cu complexes. c) X = axial donor d) Distance from Cu1 to square base plane defined by N1, N2, N3, and N4. e) N3 and N4 are symmetry generated (x, ½ -y, -z) from N1 and N2, respectively. f) distance represents a weak interaction, value is 90% of the sum of the van der Waal distances. g) Cu1 lies below the N<sub>4</sub> plane with respect to the axial position X.

Supplementary Table S2. Comparison of selected bond angles (°) of [Cu(TETA)Cl] and related structures.<sup>a</sup>

	[Cu(TETA)Cl]Cl	[Cu(TETA)I]I	[Cu(TETA)(SCN)] [SCN]	[Cu(TETA)(NCS)] [ClO <sub>4</sub> ] <sup>b</sup>	[Cu(TETA)] [PF <sub>6</sub> ] <sub>2</sub>
CCDC#	1535585	1317289	1263305	1269536	1227418
N1-Cu1-N2	84.60(16)	85.0(3)	84.7(3)	85(1)/83(1)	86.2(4)
N1-Cu1-N3	163.99(15)	150.5(3)	154.0(3)	165(1)/147(1)	169.0(9) <sup>d</sup>
N1-Cu1-N4	97.79(15)	96.1(3)	98.9(3)	95(1)/97(1)	103.0(3) <sup>d</sup>
N2-Cu1-N3	84.54(15)	85.0(3)	84.3(2)	83(1)/88(1)	84.2(4) <sup>d</sup>

N2-Cu1-N4	146.08(15)	157.8(2)	161.0(3)	144(1)/167(1)	169.0(9) <sup>d</sup>
N3-Cu1-N4	84.82(15)	85.4(2)	84.6(3)	89(1)/85(1)	86.2(4) <sup>d</sup>
X-Cu1-N1 <sup>c</sup>	99.60(11)	102.8(2)	101.1(2)	102(1)/105(1)	
X-Cu1-N2 <sup>c</sup>	106.25(11)	96.58(16)	100.1(2)	99(1)/96(1)	
X-Cu1-N3 <sup>c</sup>	94.72(10)	105.26(16)	104.0(2)	89(1)/107(1)	
X-Cu1-N4 <sup>c</sup>	106.67(11)	104.78(17)	97.5(2)	116(1)/97(1)	

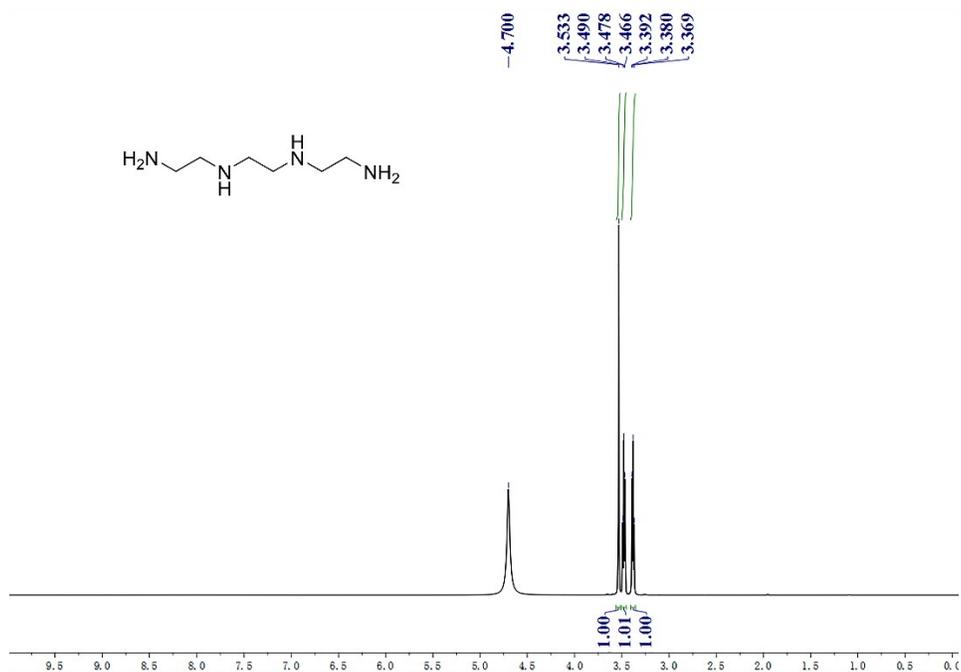
a) Atom positions are numbered as per [Cu(TETA)Cl]Cl (see inset), which may differ from original report for [Cu(TETA)(SCN)][SCN] and [Cu(TETA)(NCS)][ClO<sub>4</sub>]. b) Asymmetric unit contains two unique Cu complexes. c) X = axial donor d) N3 and N4 are symmetry generated ( $x, \frac{1}{2} -y, -z$ ) from N1 and N2, respectively.

Supplementary Table S3. RT-PCR primer sequences for rat cardiomyocytes.

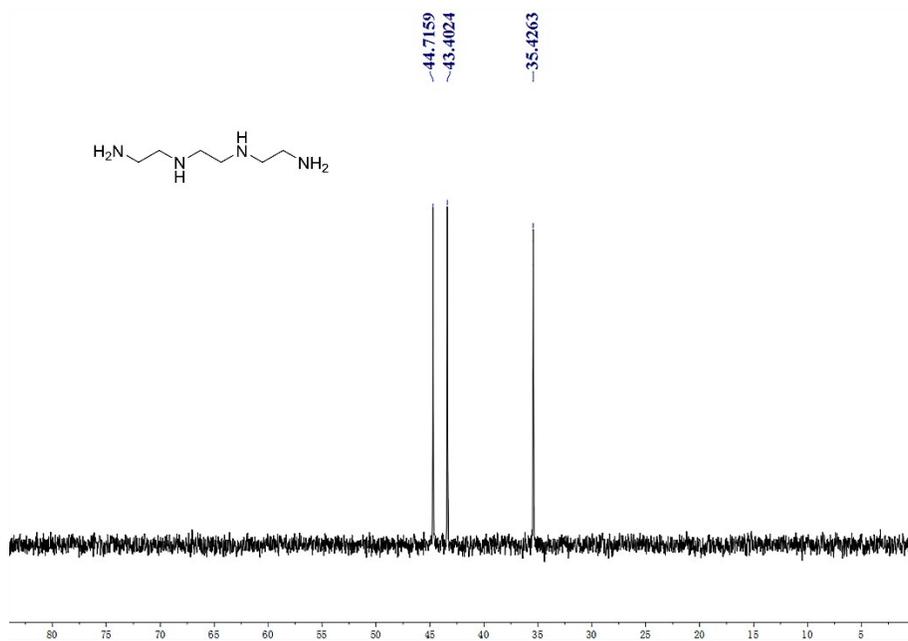
<b>Primer</b>	<b>Forward sequence</b>	<b>Reverse sequence</b>
CTR1	5'-CCCACGAGATGATGATGCCT-3'	5'-AGCCATTTCTCCAGGTGTGTT-3'
$\beta$ -actin	5'-TGGCTCCTAGCACCATGAAG-3'	5'-AAACGCAGCTCAGTAACAGT-3'

RT-PCR, reverse transcriptase polymerase chain reaction; CTR1, copper transporter 1

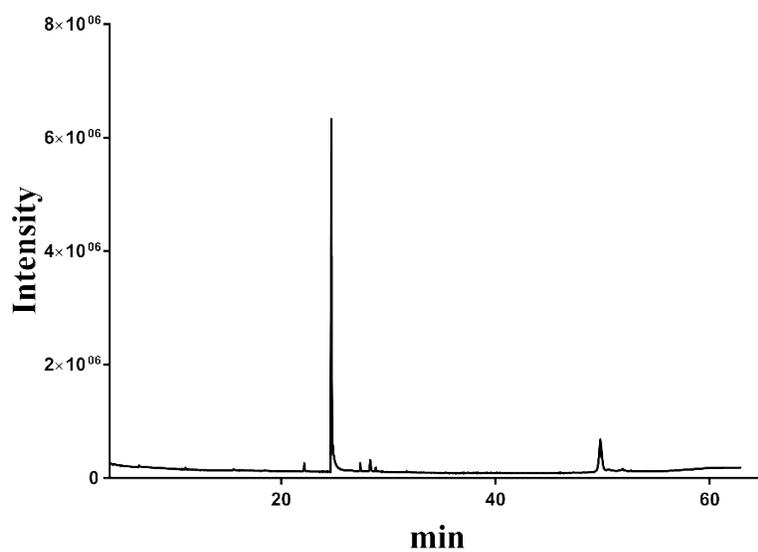
## Supplementary Figures



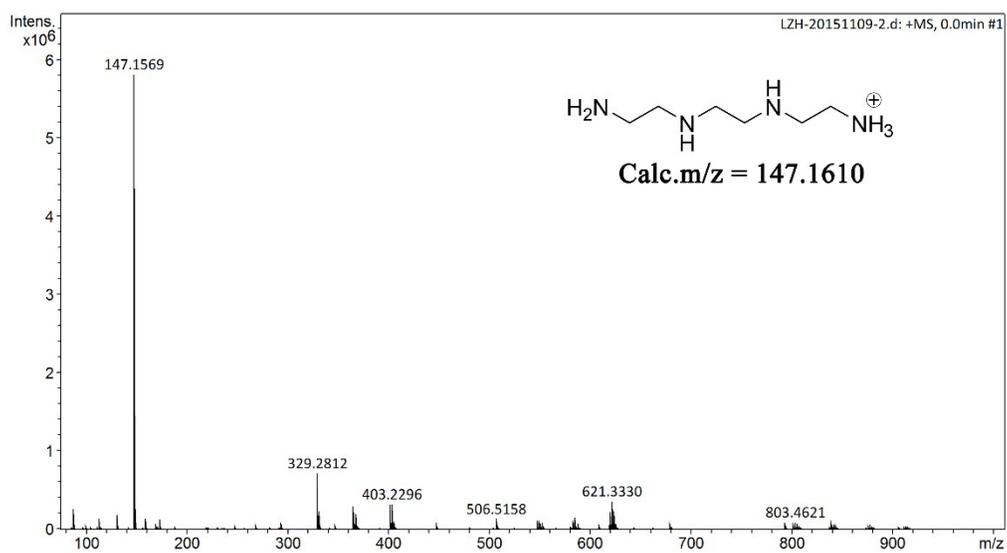
Supplementary Figure S1.  $^1\text{H}$  NMR of compound 1 (TETA) in  $\text{D}_2\text{O}$ .



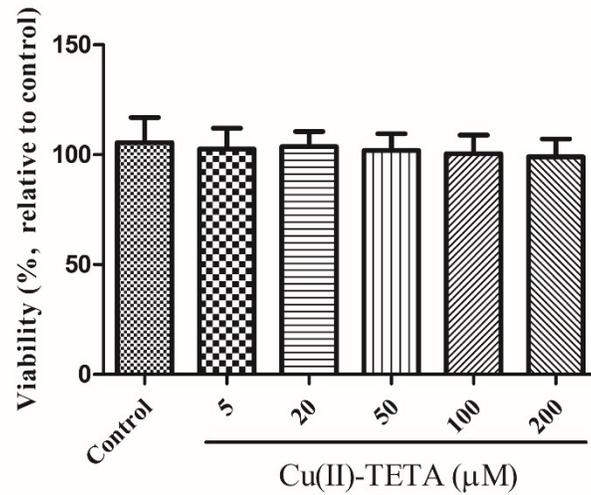
Supplementary Figure S2.  $^{13}\text{C}$  NMR of compound 1 (TETA) in  $\text{D}_2\text{O}$ .



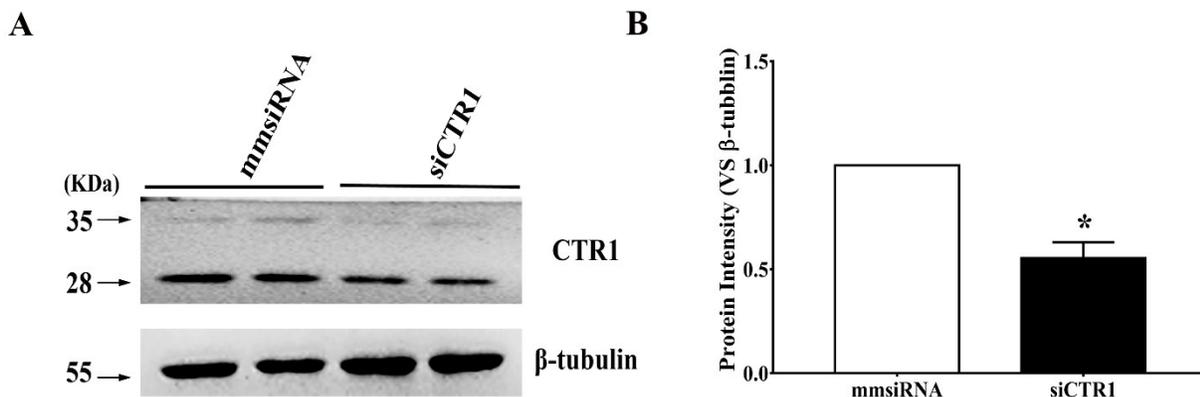
Supplementary Figure S3. GC-MS analysis of compound 1(TETA).



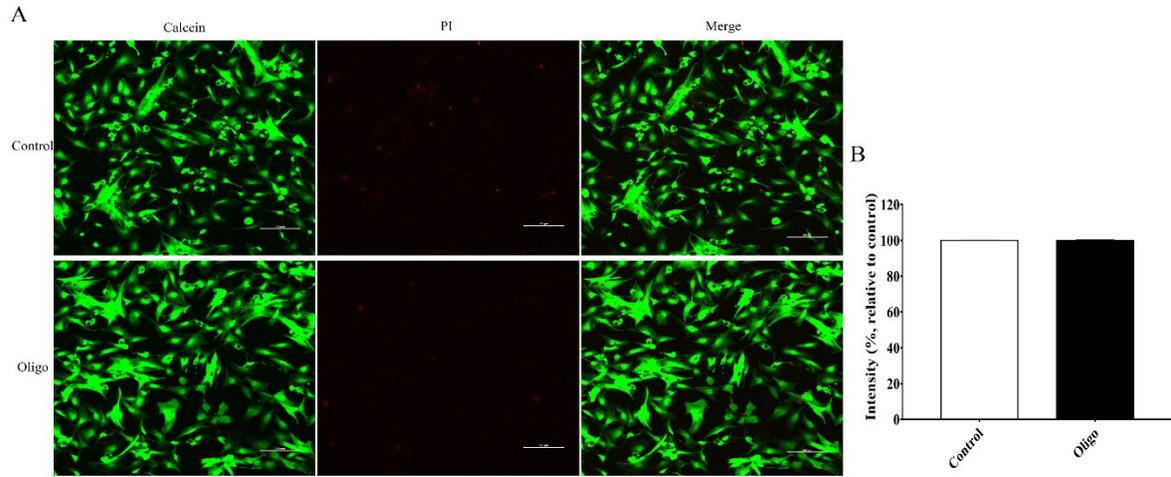
Supplementary Figure S4. High resolution +ESI-MS of compound 1 (TETA).



Supplementary Figure S5. The effect of Cu(II)-TETA on the viability of primary cardiomyocytes in cultures. The cell viability measures the cytotoxic effect of Cu(II)-TETA treatment for 6 h. The data expressed in ‘percentage’ refer to 100% as the control, other treatment groups relative to the control group. All of the data were collected from three independent experiments and expressed as mean  $\pm$ S.E.M.



Supplementary Figure S6. The detection of the efficacy of gene silencing of CTR1 by western blotting. (A) Western blotting analysis of changes in CTR1 protein levels in response to siRNA targeting CTR1. (B) Semi-quantitative analysis of CTR1 protein level detected by western blotting. All of the data were collected from five independent experiments and expressed as mean  $\pm$ S.E.M. \*significantly different from untreated controls ( $p < 0.05$ ).



Supplementary Figure S7. The effect of oligomycin on the viability of primary cardiomyocytes in cultures. (A) Primary cardiomyocytes were exposed to oligomycin in medium with a final concentration of 2  $\mu\text{g}/\text{mL}$  for 48 h. Living cells after treatment were detected as described in cell viability method using fluorescence microscope. All above pictures of rulers are 100  $\mu\text{m}$ . (B) Fluorescence intensity analysis of cardiomyocytes exposed to oligomycin by IPP. All of the data were collected from five independent experiments and expressed as mean  $\pm$ S.E.M.