Supporting Information

Two Novel Chiral Tetranucleate Copper-based Complexes: Syntheses, Crystal Structures, Inhibiting Angiogenesis and the Growth of Human Breast Cancer in Vitro and Vivo

Xiao-Xiao Hou,[† a] Ya-Ping Ren,[† a] Zhao-Hui Luo,[a] Bing-Li Jiang,[a] Tian-Tian Lu, [a] Fu-Ping Huang,[b] and Xiu-Ying Qin [*a,b]

Instruments and materials

Materials. All materials and solvents were purchased commercially and used without further purification unless specifically noted. Ultrapure Milli-Q water was used in all experiments. O-vanillin and 3,5-Di-tert-butylsalicylaldehyde reagents were purchased from SAAN Chemical Technology Co., Ltd. *L*-Methioninol was purchased from Sequoia precision Chemical Co., Ltd. Potassium hydroxide(A.R.), Anhydrous methanol and ethanol(A.R.) and Copper nitrate trihydrate (A.R.) were purchased from Xilong Science Co., Ltd. MTT, penicillin/streptomycin, and dimethylsulfoxide was purchased from Sigma-Aldrich, USA. Dulbecco's modified eagle medium (DMEM, Gibco), Fetal bovine serum (FBS, GEMINI), pancreatic enzyme(Gibco), cell culture plates(Corning) were used. Annexin V/PI apoptosis kit was purchased from BD Bioscience. The JC-1 mitochondrial membrane potential detection kit was from Beyetime (Shanghai, China). HUVECs and MDA-MB-231 cell lines were purchased from Shanghai oulu biological technology Co, ltd. Cell culture: HUVECs were cultured in DMEM medium supplemented with FBS(10%), penicillin(100 μg/mL), and streptomycin (100 μg/mL); MDA-MB-231 cells were cultured in DMEM medium supplemented with FBS(15%), penicillin (100 μg/mL), and streptomycin (100 μg/mL). They were incubated at 37℃ in a humidified incubator with 5 % CO_2 and 95 % air, and the medium was changed thrice weekly. Matrigel was purchased from Corning.

Instruments. IR spectras were taken on a IRAffinity-1 FT-IR spectrometer with KBr pallets in the range of 4000～400 cm^{-1}. The crystal structures were determined by a four-circle CCD diffractometer (SuperNova, Single source at offset, Eos). Mass spectra were recorded on a Liquid Chromatography Mass Spectrometry (Exactive, Thermo Fisher Scientific) with DMSO as solvent and CH_3CH_2OH diluent. C, H, and N elemental analyses were performed using a PerkinElmer 2400 II elemental analyzer. The ICP –MS (Inductively coupled plasma mass spectrometry) data were recorded using a FLexar-NexION300X inductively coupled plasma OES spectrometer. Apoptosis assays and mitochondrial membrane potential detection were determined by BD FACSCanto. The animal experiments were approved by the Institutional Animal Care and Use Committee of Guilin

Medical University. Cells were cultured in a CO_2 incubator (170S, Galaxy, New Brunswick). Cells were observed with a inverted microscope (OLYMPUS CKX35, Japan).

Supporting Tables

Table S1. Crystal data and structure refinement parameters for TNCu-A and TNCu-B.

Parameters	**TNCu-A**	**TNCu-B**
Empirical formula	$C_{52}H_{68}Cu_4N_4O_{12}S_4$	$C_{80}H_{124}Cu_4N_4O_8S_4$
Formula moiety	$2(C_{26}H_{34}Cu_2N_2O_6S_2)$	$C_{80}H_{124}Cu_4N_4O_8S_4$
Formula weight	1323.50	1652.22
Temperature (K)	293(2)	296(2)
Wavelength (Å)	0.71073	0.71073
Crystal system	monoclinic, I_2	orthorhombic, $P2_12_12$
a (Å)	23.0626(7)	26.102(5)
b (Å)	13.2138(3)	11.779(2)
c (Å)	19.8116(8)	14.489(3)
α (°)	90	90
β (°)	105.730(4)	90
γ (°)	90	90
V (Å ^{3})	5811.4(3)	4454.7(16)
Z, D_{Calcd} (Mg.m $^{-3}$)	4, 1.513	2, 1.232
Abs. coefficient (mm $^{-1}$)	1.649	1.086
F (000)	2736	1752
Limiting indices	$-31 \le h \le 31$ $-18 \le k \le 16$ $-27 \le l \le 25$	$-32 \le h \le 32$ $-14 \le k \le 12$ $-17 \le l \le 17$
Θ measurement and refinement (°)	3.8550~ 26.0870; θ_{max} = 29.166, θ_{min} = 3.366	2.228~24.033; θ_{max} = 26.386, θ_{min} = 1.405
Reflections collected	21873	33587
Independent reflections	12555(Rint = 0.0263)	9077(Rint = 0.0324)
Observed data	8062($I > 2\sigma(I)$)	7546 ($I > 2\sigma(I)$)
Refinement method	Full-matrix least-squares on F2	Full-matrix least-squares on F2
Nref / Npar / Nres	12555/726/92	9077/497/86
Flack parameter *	0.010(10)	0.005(5)
Final R_1, R_2, *Goodness* [$I > 2\sigma(I)$]	R_1= 0.0522, R_2 = 0.1064, *Goodness* = 1.027 =1/[$\sigma^2(F_o^2)$ + (0.0410P)^2 + 12.9435P] Where P=(F_o^2 + 2F_c^2)/3	R_1 = 0.0466, R_2=0.1233, *Goodness* = 1.058 = 1/[$\sigma^2(F_o^2)$ + (0.0878P)^2+ 0.7380P] where P = (F_o^2 + 2F_c^2)/3
Final R_1 , R_2 , S (all data)	R_1 = 0.0974, R_2 = 0.1323, S = 1.030	R_1 = 0.0599, R_2 = 0.1370, S =1.087
$(\Delta/\sigma)_{max}$	0.001	0.001

* For TNCu-A and TNCu-B: Parsons, Flack and Wagner, Acta Cryst. B69 (2013) 249-259.

Table S2. Selected bond distances (Å) and angles (°) for complexes TNCu-A.

Bond	Dist. (Å)	Bond	Dist. (Å)	Bond	Dist. (Å)
Cu(1)—O(2)	1.908 (9)	Cu(2)—O(3)	1.958 (8)	Cu(4)—O(9)	1.966 (7)
Cu(1)—O(3)	1.949 (8)	Cu(2)—O(5)	1.897 (6)	Cu(4)—O(11)	1.887 (8)
Cu(1)—O(6)^{i}	1.960 (8)	Cu(2)—O(6)	1.961 (7)	Cu(4)—O(12)	1.951 (7)
Cu(1)—N(1)	1.925 (9)	Cu(2)—N(2)	1.930 (10)	Cu(4)—N(4)	1.956(9)
Cu(3)—O(9)	1.945 (6)	Cu(3)—O(12)^{ii}	1.937(8)	S(3)—C(38)	1.86 (3)
Cu(3)—O(8)	1.879(6)	Cu(3)—N(3)	1.923 (9)	S(3A)—C(38A)	1.897 (16)
S(1)—C(12)	1.821 (17)	S(2)—C(26)	1.721 (7)	S(4)—C(51)	1.779(15)
S(1)—C(13)	1.785 (19)	S(2A)—C(26A)	1.927 (15)	S(4A)—C(51A)	1.81(3)
Angle	**(°)**	**Angle**	**(°)**	**Angle**	**(°)**
N(1)—Cu(1)—O(3)	83.6 (4)	O(5)—Cu(2)—O(3)	95.2(3)	N(3)—Cu(3)—O(12)^{ii}	166.9(3)
O(3)—Cu(1)—O(6)^{i}	86.8(4)	O(5)—Cu(2)—N(2)	93.4 (4)	O(8)—Cu(3)—O(9)	178.4(4)
O(2)—Cu(1)—N(1)	93.6(4)	O(3)—Cu(2)—O(6)	86.9(3)	N(3)—Cu(3)—O(9)	83.8 (3)
O(2)—Cu(1)—O(6)^{i}	95.7(4)	N(2)—Cu(2)—O(6)	84.7 (4)	O(8)—Cu(3)—N(3)	94.9 (3)
O(2)—Cu(1)—O(3)	177.0 (4)	O(5)—Cu(2)—O(6)	177.8(4)	O(8)—Cu(3)—O(12)^{ii}	93.7 (3)
N(1)—Cu(1)—O(6)^{i}	167.2 (4)	N(2)—Cu(2)—O(3)	165.0(5)	O(12)^{ii}—Cu(3)—O(9)	87.8(3)
O(11)—Cu(4)—O(9)	95.8 (4)	O(12)—Cu(4)—N(4)	83.7 (4)	Cu(3)—O(9)—Cu(4)	112.6(4)
O(11)—Cu(4)—N(4)	93.1(4)	O(11)—Cu(4)—O(12)	176.7 (4)	Cu(3)^{ii}—O(12)—Cu(4)	107.6(4)
O(12)—Cu(4)—O(9)	87.2(3)	N(4)—Cu(4)—O(9)	166.4 (4)	C(39)—S(3)—C(38)	123.0(16)
Cu(1)^{i}—O(6)—Cu(2)	106.7(4)	Cu(1)—O(3)—Cu(2)	111.8(4)	C(52)—S(4)—C(51)	98.4(12)
C(13)—S(1)—C(12)	102.6(10)	C(26)—S(2)—C(25)	104.8(10)	C(52A)—S(4A)—C(51A)	122(4)

Symmetry code: (i) −x+1, y, −z+1; (ii) −x+1, y, −z.

Table S3. Selected bond distances (Å) and angles (°) for complexes TNCu-B.

Bond	Dist. (Å)	Bond	Dist. (Å)	Bond	Dist. (Å)
Cu(1)—O(1)	1.949 (4)	Cu(2)—O(3)	1.952(4)	S(2)—C(25A)	1.81(2)
Cu(1)—O(3)^{i}	1.945(3)	Cu(2)—O(4)	1.891(4)	S(2A)—C(25)	1.992(17)
Cu(1)—O(2)	1.900(4)	Cu(2)—N(2)	1.934(4)	C(24)—S(2)	1.905(16)
Cu(1)—N(1)	1.925(4)	Cu(2)—O(1)	1.951(4)	C(24A)—S(2A)	1.817(17)
S(1)—C(2)	1.760(18)	C(1)—S(1A)	1.716(19)		
S(1)—C(1A)	1.75(6)	C(2A)—S(1A)	1.98(4)		
Angle	**(°)**	**Angle**	**(°)**	**Angle**	**(°)**
O(2)—Cu(1)—O(3)^{i}	95.81(16)	N(1)—Cu(1)—O(3)^{i}	168.34(18)	O(4)—Cu(2)—O(3)	175.66(16)
O(2)—Cu(1)—N(1)	93.53(17)	Cu(1)^{i}—O(3)—Cu(2)	108.05(17)	O(4)—Cu(2)—N(2)	93.24(17)
O(3)^{i}—Cu(1)—O(1)	87.27(16)	Cu(1) —O(1)—Cu(2)	116.06(19)	O(4)—Cu(2)—O(1)	95.75(17)

N(1)—Cu(1)—O(1)	83.51(18)	N(2)—Cu(2)—O(1)	165.93(19)	N(2)—Cu(2)—O(3)	82.85(16)
O(2)—Cu(1)—O(1)	176.79(16)	C(1)—S(1A)—C(2A)	97.5(16)	O(1)—Cu(2)—O(3)	88.45(16)

Symmetry code: (i) –x+1, −y+1, z.

Table S4. Hydrogen bond lengths (Å) and angles (°) for complexes TNCu-A and TNCu-B.

TNCu-A				
D—H•••A	*D*—H	H•••A	*D*•••A	<*D*HA
C(8)—H(8B)•••S(2A) ^{iii}	0.96	2.80	3.74(3)	166.4
C(8)—H(8C)•••O(2)	0.96	2.51	3.06(2)	116.6
C(10)—H(10)•••S(1)	0.98	2.84	3.356(12)	113.4
C(24A)—H(24D)•••N(2) ^{i}	0.97	2.26	3.19(4)	161.1
C(11)—H(11B)•••O(10)	0.97	2.39	3.221(13)	143.7
C(36)—H(37)•••S(3)	0.98	2.83	3.32(3)	112.1
C(37)—H(37A)•••S(4) ^{v}	0.97	2.79	3.729(11)	162.3
C(47)—H(47A)•••O(11)	0.96	2.47	2.969(17)	111.9
C(51)—H(51A)•••O(1) ^{vi}	0.97	2.42	3.39(2)	173.3
C(12)—H(12B)•••S(2) ^{iv}	0.97	2.73	3.396(18)	126.4
C(48)—H(48A)•••O(8)) ^{ii}	0.97	2.47	2.987(15)	112.8
TNCu-B				
D—H•••A	*D*—H	H•••A	*D*•••A	<*D*HA
C(21)—H (21A)•••O(2)^{i}	0.97	2.60	3.129(7)	114.3
C(5)—H (5BD)•••O(4)	0.97	2.62	3.214(8)	120.1
C(24A)—H (24C)•••S(2A)^{i}	0.97	2.25	3.101(19)	145.2

Symmetry codes:

For complex TNCu-A：(i) −x+1, y, −z+1; (ii) −x+1, y, −z;; (iii) x+1/2, y+1/2, z+1/2; (iv) −x+1, y+1, −z+1; (v) −x+1, y+1, −z; . (vi) x, y, z−1.

For complex TNCu-B：(i) -x+1, -y+1, z.

Supplementary Figures

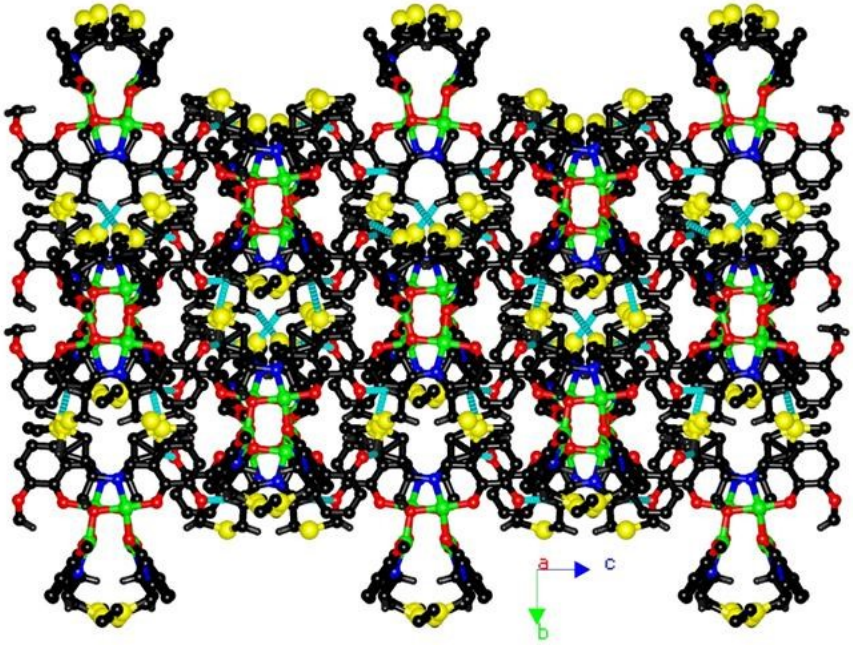

Figure S1 The two-dimensional network structure of TNCu-A in the *bc* plane. The dotted lines in light blue indicate hydrogen bondings. Most of the hydrogen atoms bonded to carbon atoms were omitted for clarity.

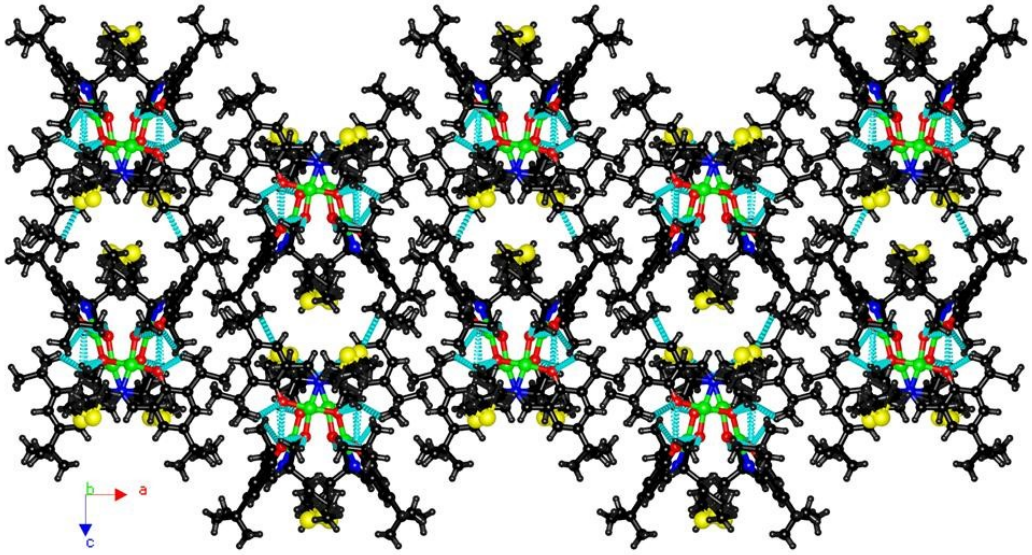

Figure S2 The two-dimensional network structure of TNCu-B in the *ac* plane. The dotted lines in light blue indicate hydrogen bondings.

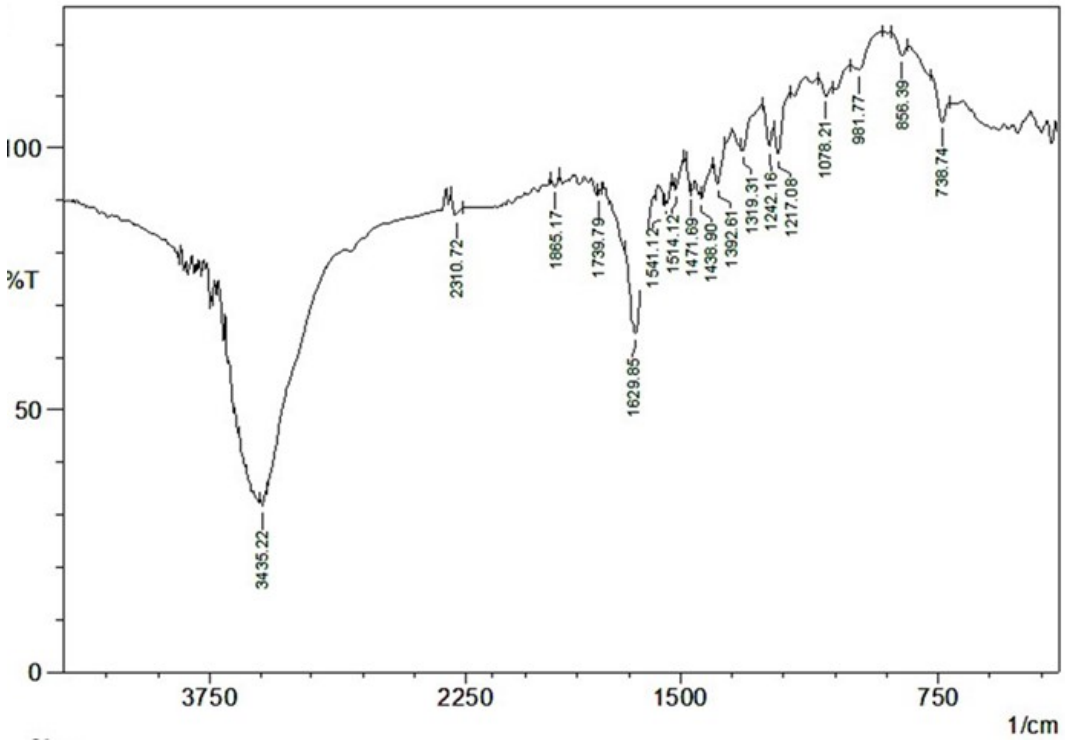

Figure S3 FT-IR of $\{[Cu_4(C_{13}H_{17}O_3NS)_4]\}_2$ (abbreviated as TNCu-A), and $H_2(C_{13}H_{17}O_3NS)$ = $C_6H_3(OH)(OCH_3)CH{=}NCH(CH_2OH)(CH_2CH_2SCH_3)$.

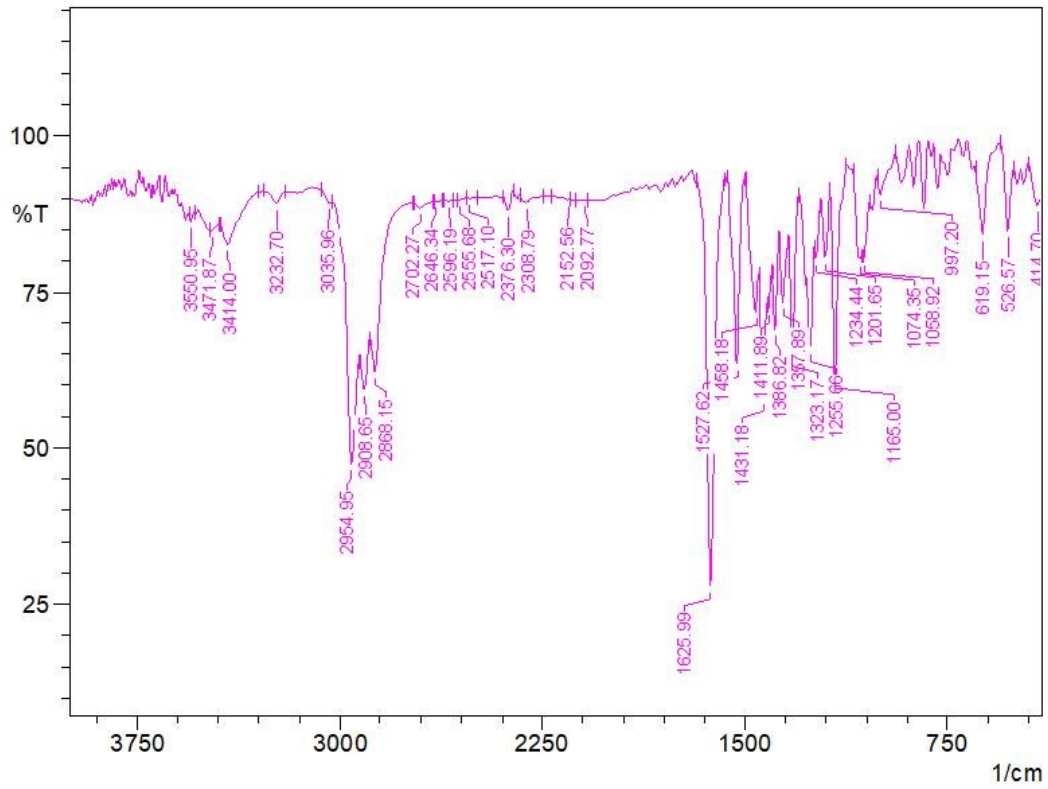

Figure S4 FT-IR of $[Cu_4(C_{20}H_{31}O_2NS)_4]$, (abbreviated as TNCu-B, and $H_2(C_{20}H_{31}O_2NS)$ = $C_6H_2(C(CH_3)_2(OH)CH{=}NCH(CH_2OH)(CH_2CH_2SCH_3)$.

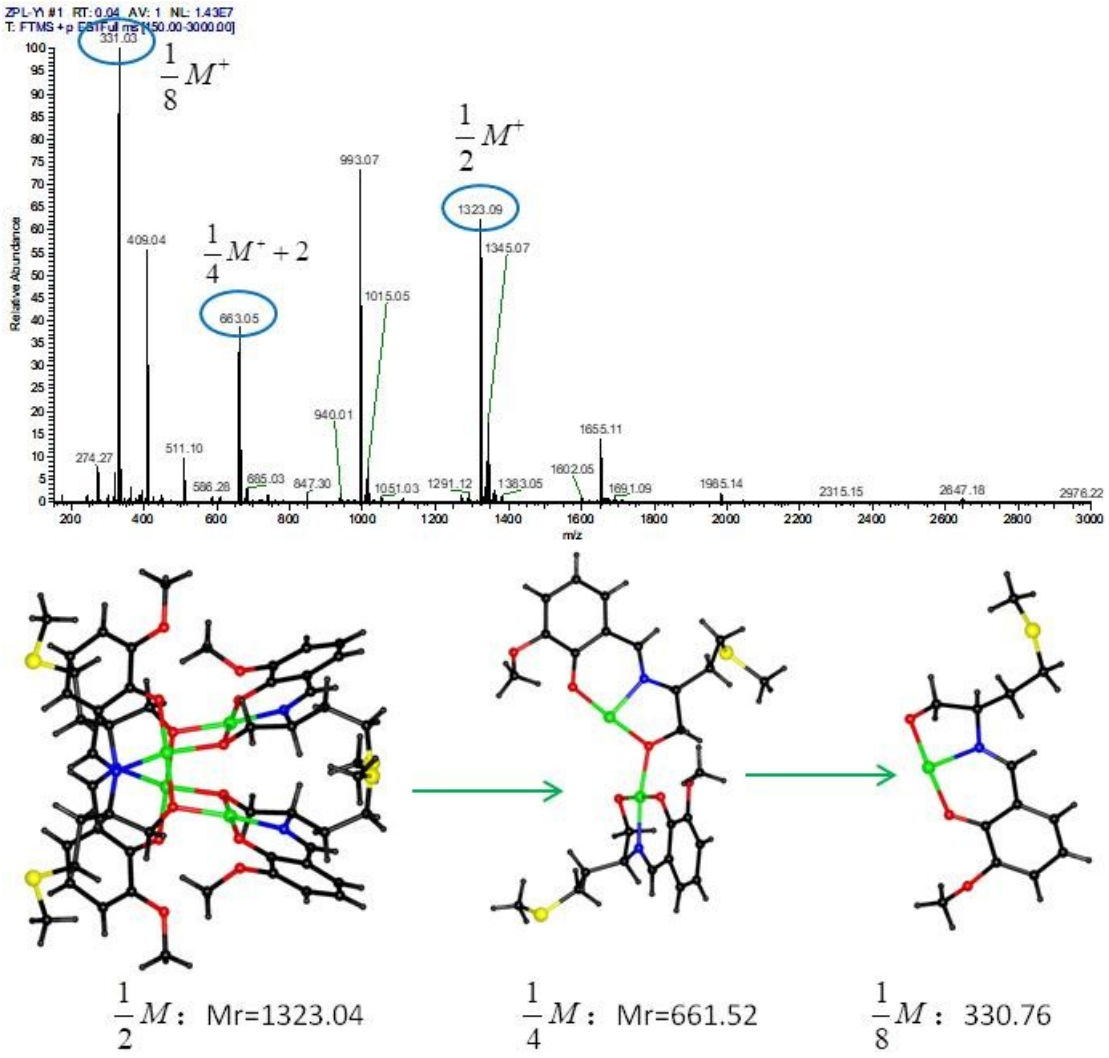

Figure S5. Liquid chromatography mass spectrometry of positive ion of $\{[Cu_4(C_{13}H_{17}O_3NS)_4]\}_2$ (abbreviated as TNCu-A).