

Supplementary Information

A {Cd₄Cl₂O₁₄} cluster functionalized sandwich-type tungsto-arsenate as conformation modulator for misfolding A β peptide†

Jiai Hua,^{*a†} Xueman Wei,^{d‡} Xiang Ma,^{*a} Jinzhe Jiao,^b Binghui Chai,^b Chenbin Wu,^a Changli Zhang,^{*c} and Yulan Niu^{*b}

a. Chemistry and Chemical Engineering Department, Taiyuan Institute of Technology, Taiyuan, 030008, P. R. China. E-mail: maxiang@tit.edu.cn; jjaihua10660@163.com; Fax: +86 351 3569476.

b. Laboratory of Biochemistry and Pharmacy, Taiyuan Institute of Technology, Taiyuan, 030008, P. R. China. E-mail: Niuy@tit.edu.cn

c. School of environmental science, Nanjing Xiaozhuang University, Nanjing, 2111171, P. R. China. E-mail: zhangcl@njxzc.edu.cn.

d. Department of Geriatrics, First affiliated Hospital of Naval Medical University, Shanghai, 200081, P. R. China. E-mail: wxmshchyy@163.com.

1. Supplementary Tables and Figures

Table S1 Crystallographic data and structural refinements for CdAW

Empirical formula	C ₁₈ H ₈₈ N ₁₂ As ₂ Cd ₄ Cl ₂ W ₁₈ O ₇₆
Formula weight	5668.64
Crystal system	Triclinic
Space group	<i>P</i> -1
<i>a</i> / Å	12.5169(15)
<i>b</i> / Å	12.5282(16)
<i>c</i> / Å	15.952(3)
α / deg	99.623(3)
β / deg	98.647(3)
γ / deg	112.828(2)
<i>V</i> / Å ³	2208.5(6)
<i>Z</i>	1
<i>D_c</i> / g cm ⁻³	4.262
μ / mm ⁻¹	25.189
<i>T</i> / K	296(2)
Limiting indices	-14 ≤ <i>h</i> ≤ 14 -14 ≤ <i>k</i> ≤ 14 -12 ≤ <i>l</i> ≤ 18
Measured reflections	11000
Independent reflections	7654

R_{int}	0.0939
Data / restraints / parameters	7654 / 200 / 585
GOF on F^2	1.004
Final R indices [$I > 2\sigma(I)$]	$R_1 = 0.0792$, $wR_2 = 0.1755$
R indices (all data)	$R_1 = 0.0942$ $wR_2 = 0.1874$
Completeness	98.5 %

Table S2 Selected bond length (Å) for CdAW

W(1)-O(1)	1.753	W(2)-O(2)	1.756
W(1)-O(10)	1.771	W(2)-O(11)	1.769
W(1)-O(12)	1.925	W(2)-O(13)	1.942
W(1)-O(30)	1.961	W(2)-O(12)	1.947
W(1)-O(25)	2.017	W(2)-O(14)	2.000
W(1)-O(31)	2.322	W(2)-O(31)	2.361
W(3)-O(3)	1.718	W(4)-O(4)	1.715
W(3)-O(25)	1.859	W(4)-O(20)	1.761
W(3)-O(14)	1.884	W(4)-O(13)	1.891
W(3)-O(19)	1.934	W(4)-O(17)	1.993
W(3)-O(15)	1.942	W(4)-O(18)	1.998
W(3)-O(31)	2.479	W(4)-O(32)	2.349
W(5)-O(5)	1.720	W(6)-O(6)	1.729
W(5)-O(18)	1.883	W(6)-O(22)	1.782
W(5)-O(21)	1.885	W(6)-O(17)	1.874
W(5)-O(15)	1.891	W(6)-O(28)	1.986
W(5)-O(16)	1.956	W(6)-O(21)	1.998
W(8)-O(8)	1.722	W(6)-O(32)	2.363
W(8)-O(26)	1.807	W(7)-O(7)	1.710
W(8)-O(29)	1.879	W(7)-O(27)	1.840
W(8)-O(28)	1.898	W(7)-O(19)	1.898
W(8)-O(23)	1.976	W(7)-O(16)	1.907
W(8)-O(33)	2.330	W(7)-O(23)	1.912
W(9)-O(9)	1.710	Cd(1)-O(11)	2.223
W(9)-O(24)	1.778	Cd(1)-O(10)	2.244
W(9)-O(30)	1.889	Cd(1)-O(26)#1	2.293
W(9)-O(29)	1.971	Cd(1)-O(22)#1	2.321
W(9)-O(27)	2.061	Cd(1)-Cl(1)	2.511
W(9)-O(33)	2.376	Cd(1)-O(34)	2.512
Cd(2)-O(24)	2.165	Cd(2)-O(34)	2.341
Cd(2)-O(20)#1	2.177	As(1)-O(34)	1.663
Cd(2)-O(22)#1	2.253	As(1)-O(32)	1.680
Cd(2)-O(26)	2.280	As(1)-O(33)	1.689
Cd(2)-O(34)#1	2.330	As(1)-O(31)	1.708

Table S3 Bond valence and Σs of W, As, and Cd in CdAW

Bond	Valence	Bond	Valence	Bond	Valence	Atom	Σs
W(1)-O(1)	1.558	W(1)-O(12)	0.978	W(1)-O(25)	0.763		
W(1)-O(10)	1.484	W(1)-O(30)	0.888	W(1)-O(31)	0.335	W(1)	6.006
W(2)-O(2)	1.545	W(2)-O(13)	0.935	W(2)-O(14)	0.799		
W(2)-O(11)	1.492	W(2)-O(12)	0.922	W(2)-O(31)	0.301	W(2)	5.994
W(3)-O(3)	1.712	W(3)-O(14)	1.093	W(3)-O(15)	0.935		
W(3)-O(25)	1.170	W(3)-O(19)	0.955	W(3)-O(31)	0.219	W(3)	6.084
W(4)-O(4)	1.726	W(4)-O(13)	1.073	W(4)-O(18)	0.803		
W(4)-O(20)	1.524	W(4)-O(17)	0.814	W(4)-O(32)	0.311	W(4)	6.252
W(5)-O(5)	1.703	W(5)-O(21)	1.090	W(5)-O(32)	0.164		
W(5)-O(18)	1.096	W(5)-O(15)	1.073	W(5)-O(16)	0.900	W(5)	6.026
W(6)-O(6)	1.662	W(6)-O(17)	1.123	W(6)-O(21)	0.803		
W(6)-O(22)	1.440	W(6)-O(28)	0.830	W(6)-O(32)	0.300	W(6)	6.159
W(7)-O(7)	1.750	W(7)-O(19)	1.053	W(7)-O(33)	0.180		
W(7)-O(27)	1.231	W(7)-O(16)	1.027	W(7)-O(23)	1.014	W(7)	6.255
W(8)-O(8)	1.694	W(8)-O(29)	1.108	W(8)-O(23)	0.853		
W(8)-O(26)	1.346	W(8)-O(28)	1.053	W(8)-O(33)	0.328	W(8)	6.381
W(9)-O(9)	1.750	W(9)-O(30)	1.079	W(9)-O(27)	0.678		
W(9)-O(24)	1.456	W(9)-O(29)	0.864	W(9)-O(33)	0.289	W(9)	6.115
Cd(1)-O(11)	0.422	Cd(1)-O(26) #1	0.349	Cd(1)-Cl(1)	0.446		
Cd(1)-O(10)	0.399	Cd(1)-O(22) #1	0.324	Cd(1)-O(34)	0.193	Cd(1)	2.134
Cd(2)-O(24)	0.494	Cd(2)-O(22) #1	0.389	Cd(2)-O(34)#1	0.316		
Cd(2)-O(20) #1	0.478	Cd(2)-O(26)	0.362	Cd(2)-O(34)	0.307	Cd(2)	2.347
As(1)-O(34)	1.325	As(1)-O(32)	1.265	As(1)-O(33)	1.235		
As(1)-O(31)	1.173					As(1)	4.997

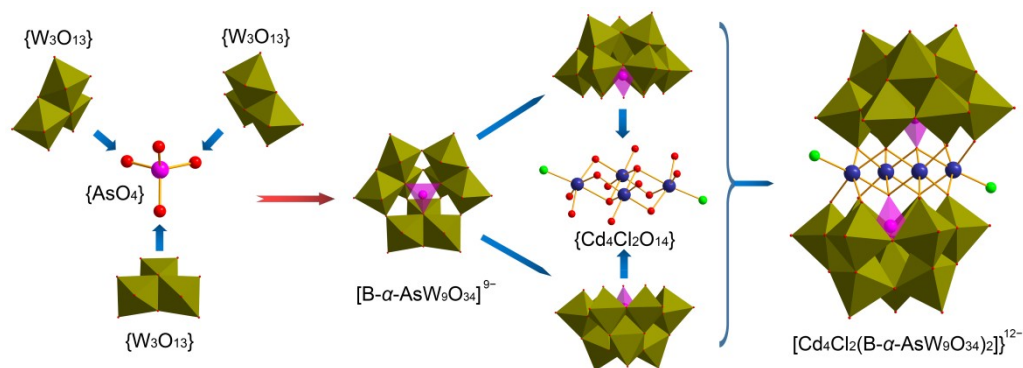


Fig. S1 The anatomical view of the $[\text{Cd}_4\text{Cl}_2(\text{B-}\alpha\text{-AsW}_9\text{O}_{34})_2]^{12-}$ in CdAW.

The IR spectrum of CdAW shows similar asymmetric vibrations to other $[\text{B-}\alpha\text{-AsW}_9\text{O}_{34}]^{9-}$ containing species.¹ As shown in Fig. S2, three characteristic bands assigned to the $\nu(\text{W-O}_t)$, $\nu(\text{W-O}_{\mu 2})$, and $\nu(\text{W-O}_{\mu 3})$ appear at 940, 783, 733–625 cm^{-1} , respectively.¹ The band at 874 cm^{-1} is attributed to $\nu(\text{As-O}_{\mu 4})$.¹ In addition, characteristic bands at 3410 cm^{-1} are ascribed to stretching vibrations of N–H which confirms the presence of en. Vibration at 1614 cm^{-1} can be assigned to –OH stretching.¹ The IR spectrum is in good agreement with the result of X-ray diffraction structural analysis.

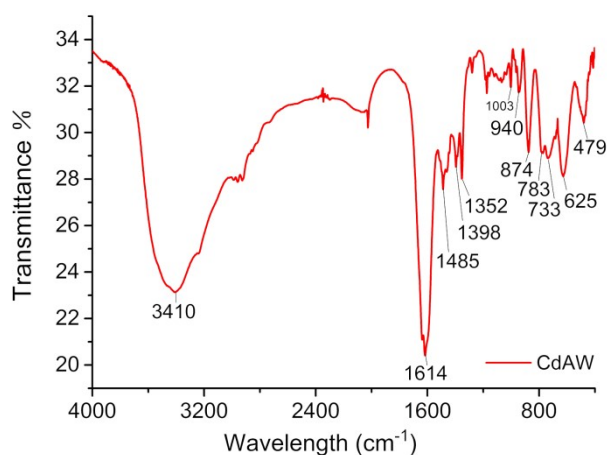


Fig. S2 IR spectrum for CdAW.

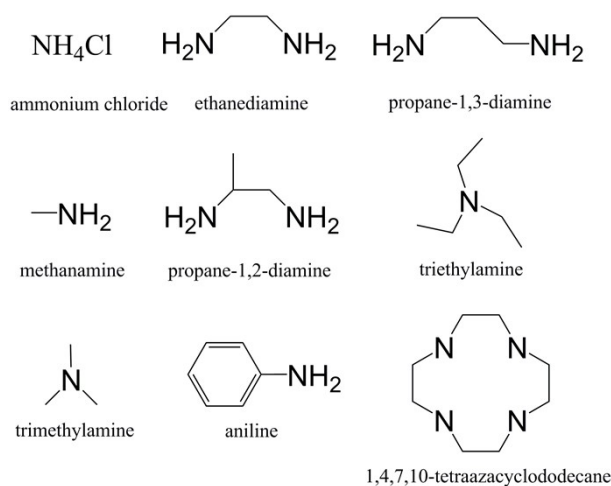


Fig. S3 The structures of a series of amines used in parallel experiments.

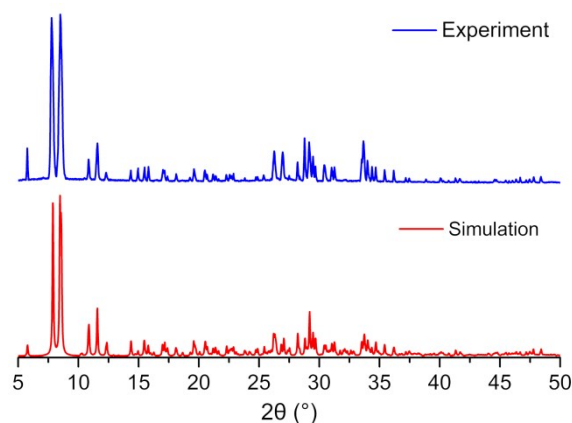


Fig. S4 Comparison of the simulated and experimental XRPD patterns of CdAW.

As shown in Fig. S5, the thermogravimetric (TG) curve of CdAW displays two stages of weight loss, giving a total loss of 19.06% in the temperature range of 25–600 °C. The first stage from 40 to 130 °C is attributed to the loss of eight lattice water molecules, and the observed weight loss 2.90% is consistent with the calculated value 2.86%. The second stage with the weight loss of 16.16% occurs between 190 and 566 °C, which may be assigned to the removal of six 1,2-dap molecules, one As_2O_3 and four structural water molecule (calcd. 16.10%).²

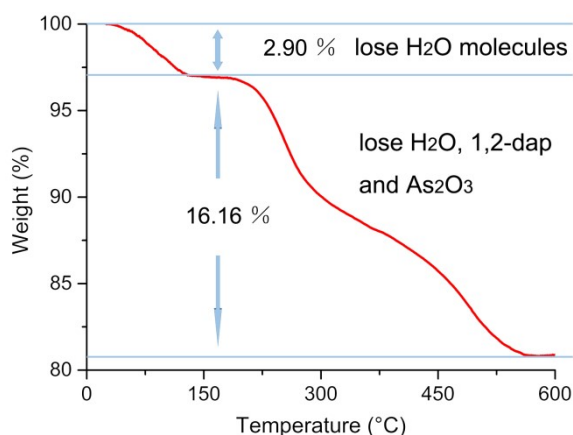


Fig. S5 TGA curve of CdAW.

As shown in Fig. S6, the UV spectrum displays two absorption peaks in aqueous solution, one locating at 193.8 nm and the other with a wide shoulder absorption at 257.5 nm in the region of 190~600 nm. These two peaks may be assigned to $\text{O}_t \rightarrow \text{W}$ and $\text{O}_\mu \rightarrow \text{W}$ charge transfer transitions, respectively.³

The influences of pH value on the stability of CdAW have also been investigated by UV-visible spectra. As shown in the inset of Fig. S6, the UV-visible absorption peaks of CdAW showed no significant change between pH 4.50 and 8.00 in intensity. Beyond the above pH range, absorption peaks at both 193.8 nm gradually change in intensity, which may indicate that the skeleton of CdAW began to collapse.

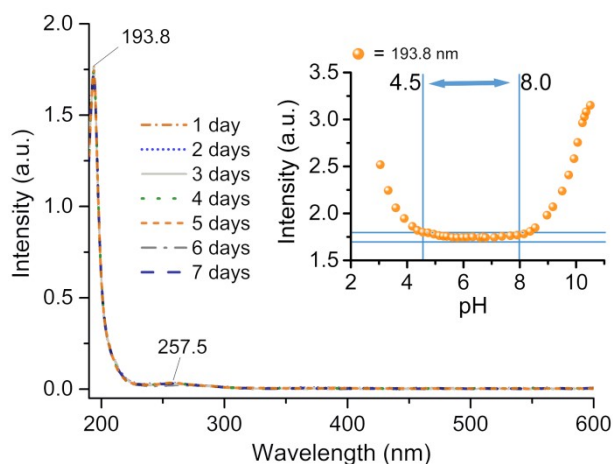


Fig. S6 UV-Vis spectra of CdAW in deionized water for 1–7 days; and pH stability of CdAW (inset a and b) .

2. Experimental Section

2.1 Materials and methods

In this study, reagents used are all of analytical grade, and purchased from commercial suppliers and used as received unless otherwise stated. Human A β 40/42 was purchased from Macklin agent Ltd. (China), which were verified by HPLC and electrospray ionization mass spectrometry (ESI-MS). 2',7'-dichlorofluorescein diacetate (DCFH-DA), 3-(4,5-dimethyl-2-thiazolyl)-2,5-diphenyl-2-H-tetrazolium bromide (MTT), nerve growth factor 7S (NGF-7S) and tris(hydromethyl)aminomethane (Tris) were purchased from Sigma-Aldrich Inc. (USA). Cadmium chloride (CdCl₂), sodium tungstate (Na₂WO₄·2H₂O), sodium arsenite (NaAsO₂), zinc acetate (Zn(OAc)₂), copper(II) chloride (CuCl₂) and 1,2-diaminopropane (dap) were purchased from J & K. Pheochromocytoma cells (PC12 cells) were purchased from American Type Culture Collection (ATCC). Stock solutions of A β 40, Zn(OAc)₂ and CuCl₂ were prepared according to the reported procedures;⁴ and stock solution of CdAW was prepared by dissolving the compound in DMSO to give a final concentration of 2 mM. All the solutions were prepared with Milli-Q water and filtered through a 0.22 μ m filter (Millipore).

A single crystal of CdAW was mounted on a Bruker Apex-2 diffractometer with a CCD detector using graphite monochromatized MoK α radiation ($\lambda = 0.71073$ Å) at 296 K. Data integration was performed using SAINT.⁵ Routine Lorentz and polarization corrections were applied. Multiscan absorption corrections were performed using SADABS.⁶ The structure was solved by direct methods and refined by full-matrix least-squares on F^2 . The remaining atoms were found from successive full-matrix least-squares refinements on F^2 and Fourier syntheses. All calculations were performed using the SHELXL-97 program package.⁷ No hydrogen atoms associated with the water molecules were located from the difference Fourier map. Positions of the hydrogen atoms attached to the carbon and nitrogen atoms were geometrically placed. All hydrogen atoms were refined isotropically as a riding mode using the default SHELXTL parameters.

DCF fluorescence were conducted on a Thermo Scientific Varioskan Flash microplate reader. The morphological analysis were examined on a JEOL JEM-2100 LaB6 (HR) transmission

electron microscope. The CD spectrum of the sample solution was measured on a JASCO J-810 automatic recording spectropolarimeter (Tokyo, Japan). Elemental analysis was performed on a PQEXCe II ICP-MS. IR/UV spectra were recorded on a NICOLET iS10 and UV-3600 spectrometer respectively. The TG was tested on a STA449F3 TG-DSC from 25 to 600 °C.

2.2 Synthesis of CdAW

Two solutions were prepared separately. Solution A: Na₂WO₄·2H₂O (3.299 g, 10.00 mmol) and NaAsO₂ (0.430 g, 3.00 mmol) were dissolved in water (100 mL) under stirring. Solution B: CdCl₂ (0.916 g, 5.00 mmol) and dap (0.06 mL, 0.850 mmol) were added to water (100 mL) under stirring. The resulting mixture of B is added to solution A. The mixture was stirred for 10 min at room temperature and then the pH value was adjusted to 5.50 by adding 6 mol·L⁻¹ HCl dropwise. The solution was sealed in a 25 mL Teflon-lined autoclave kept at 160 °C for 3 days and then cooled to room temperature. Transparent rhomboic crystals were collected. Yield: 0.61 g. ca. 22% (based on Na₂WO₄·2H₂O).

2.3 Bond valence sums (Σs)

The bond valence sums (Σs) of oxygen atoms in CdAW were further calculated according to the reported methods⁸. Briefly, the oxidation states of the O atoms in SCW were calculated on the following formula:

$$V_i = \sum_j s_{ij} = \sum_j \exp\left(\frac{r_0' - r_{ij}}{B}\right)$$

where r_{ij} represents the observed values of bond distances listed in Table S2, and r_0' the theoretical value of bond distance between two atoms; B was set to 0.37.⁹ The theoretical values of W–O, As–O, Cd–Cl and Cd–O come from literatures, in which the r_0' (W⁶⁺–O) is 1.917 Å, r_0' (As⁵⁺–O) is 1.767 Å, r_0' (Cd²⁺–Cl) is 2.212 Å, and r_0' (Cd²⁺–O) is 1.904 Å.^{6, 10}

2.4 Stability of CdAW

The stability of CdAW was studied by using UV-Vis spectroscopy according to the reported method.¹¹

2.5 Morphological analysis

Aβ40 (20 μM) in Tris buffer solution (20 mM Tris-HCl/150 mM NaCl, 990 μL) was incubated alone or with Zn(OAc)₂ / CuCl₂(4 μL, 10 mM) in the absence or presence of CdAW (with the final concentration of 20 μM) or DMSO at 37 °C for 24 h. A drop of solution (10 μL) was spotted on the 300-mesh carbon-coated copper grids at room temperature. After 2 min, the excess solution was removed. The grids were stained with uranyl acetate (10 μL, 1%, w/v) for 2 min; Then, they were washed with Milli-Q water (10 μL). The samples were examined on a JEOL JEM-2100 LaB6 (HR) transmission electron microscope.

2.6 Conformational modulation

Aβ40 (20 μM) was dissolved in the Tris buffer solution (20 mM Tris-HCl/150 mM NaCl) and incubated without or with Zn(OAc)₂ or CuCl₂ (40 μM) in the absence or presence of CdAW (with the final concentration of 20 μM) at 37 °C for 24 h, respectively. The CD spectrum of the sample solution was measured on a JASCO J-810 automatic recording spectropolarimeter (Tokyo, Japan) in the range of 190–260 nm. The data acquired in the absence of protein was subtracted from the spectrum. In the control tests, DMSO gave negative results.

2.7 ThT assay

Aβ40 (20 μM) in buffer solution (20 mM Tris-HCl/150 mM NaCl, 993.6 μL) was incubated at

37 °C for 24 h. ThT (2 μ L, 5 mM) was added to each solution simultaneously in the dark and incubated at 37 °C for 0.5 h. CdAW (4 μ L, 5 mM) or DMSO (4 μ L, final concentration 0.40 %) was added into the system with stirring. Each sample was transferred to a well of a flat-bottomed 96-well black plate (Corning Costar Corp). The fluorescence spectra ($\lambda_{\text{ex}} = 415$ nm) were measured by a Varioskan Flash microplate reader (Thermo Scientific) every 15 min from 0 to 120 min.

2.8 $^1\text{H-NMR}$ spectra

The samples of $^1\text{H-NMR}$ spectra were prepared by dissolving CdAW (200 μM) in a mixture containing A β 40 (200 μM), 10% D_2O , 85% H_2O and 5% DMSO- d_6 and incubating at 37 °C for 24 h and then were centrifuged to get the soluble samples. The $^1\text{H-NMR}$ spectra were recorded on a Bruker DRX-600 spectrometer.

2.9 Inhibition of ROS generation

DCF stock solution (1 mM) and horseradish peroxidase (HRP) stock solution (4 μM) were prepared with a Tris buffer (20 mM Tris-HCl/150 mM NaCl, pH 7.4) as described in the reported procedures.¹ Samples containing A β 40 (20 μM) and CuCl_2 (40 μM) were incubated without or with CdAW (20 μM , with final DMSO content: 1.5 μL) at 37 °C. Then, ascorbate solution (10 μM) was added to each sample and incubated at 37 °C for 10 min. The samples (200 μL) were injected to the wells of a flat-bottomed 96-well black plate. HRP (0.04 μM) and DCFH (100 μM) were then injected to each solution and incubated in the dark at 37 °C. Fluorescence intensity ($\lambda_{\text{ex}} = 485$ nm) from 505 to 650 nm was measured by a Varioskan Flash microplate reader (Thermo Scientific).

2.10 Inhibition of neurotoxicity

The PC12 cells used for the neurotoxicity, and synaptic dysfunction analysis were prepared as described in the previous literature.¹² The effects of CdAW on the inhibition neurotoxicity were evaluated by using the MTT assay. PC12 cells were incubated with A β 40 (20 μM) alone or with Zn^{2+} - or Cu^{2+} (40 μM)-induced A β 40 complexes in absence or presence of CdAW (20 μM) for 24 h. The results are obtained from three independent experiments and presented as the mean \pm standard deviation of the independent experiments. The results were compared using a twoway ANOVA (* $p \leq 0.05$, ** $p \leq 0.01$, *** $p \leq 0.001$).

2.11 Cell morphological analysis

The PC12 cells used for this morphological analysis were prepared as above. After incubation for 24 h, the morphological pictures of those cells were captured by a microscope.

¹ J. Y. Niu, X. Ma, J. W. Zhao, P. T. Ma, C. Zhang and J. P. Wang, *CrystEngComm* 2011, **13**, 4834–4837.

² C. Y. Sun, Y. G. Li and E. B. Wang, *Inorg. Chem.*, 2007, **46**, 1563–1574.

³ X. Ma, P. T. Ma, D. D. Zhang, J. A. Hua, C. Zhang, T. F. Huang, J. P. Wang, J. Y. Niu, *Dalton Trans.* 2013, **42**, 874–878.

⁴ X. H. Wang, X. Y. Wang, C. L. Zhang, Y. Jiao and Z. J. Guo, *Chem. Sci.*, 2012, **3**, 1304–1312.

⁵ *Saint*. Bruker, AXS Inc., Madison WI **2007**.

⁶ N. E. Brese and M. O’Keeffe, *Acta Crystallogr. B Struct. Sci.*, 1991, **47**, 192–197.

⁷ G. M. Sheldrick, *SHELXL-97, Programs for Crystal Structure Refinements*. University of Göttingen, Germany **1997**.

⁸ S. T. Zheng, J. Zhang, J. M. Juan, D. Q. Yuan and G. Y. Yang, *Angew. Chem. Int. Ed. Engl.*, 2009, **48**, 7176–7179.

⁹ I. D. Brown and D. Altermatt, *Acta Crystallogr. B Struct. Sci.* 1985, **41**, 244–247.

¹⁰ G. P. Shields, P. R. Raithby, F. H. Allen and W. D. S. Motherwell, *Acta Crystallogr. B Struct. Sci.*, 2000, **56**, 455–465.

¹¹ N. Gao, H. J. Sun, K. Dong, J. S. Ren, T. C. Duan, C. Xu and X. G. Qu, *Nature Commun.*, 2014, **5**,

3422–3431.

¹² T. Yang, X. H. Wang, C. L. Zhang, X. Ma, K. Wang, Y. Q. Wang, J. Luo, L. Yang, C. Yao and X. Y. Wang, *Chem. Commun.*, 2016, **52**, 2245–2248.