Electronic Supplementary Material (ESI) for New Journal of Chemistry.

A biocompatible Mn-decorated metal organic cage with sustainable CO releasing

Xiaomei Ning,[a,b] Peilin Yin,[b] Lixia Zhang,[b] Feng Gao,*[a] Youfu Wang*[b] and Jinghui Yang*[c]

a. School of Resources, Environment and Materials, Guangxi University, Nanning 530004, China. E-mail: gaofeng@gxu.edu.cn

b. School of Chemistry and Chemical Engineering, Frontiers Science Center for Transformative Molecules, Shanghai Jiao Tong University, Shanghai 200240, China. E-mail: wyfown@sjtu.edu.cn

c. Department of Organ Transplantation, Shanghai Changzheng Hospital, Naval Medical University, Shanghai, 200003, China. E-mail: yjh@smmu.edu.cn

Table of Contents

Experimental Section

Materials

2,2-bipyridine-5,5-dicarboxylic acid, zirconocene dichloride, horse myoglobin, and bromopentacarbonylmanganese(I) were commercially available and used as received.

Characterizations

NMR: MOC and MOC-Mn are structurally confirmed by AVANCE III HD 500 NMR spectrometer (Bruker 500) (Germany).

ESI: High-resolution mass spectra (HRMS) were recorded on a Bruker impact II UPLC-QTOF instruments (Germany).

FTIR: Fourier transform infrared (FTIR) spectra were recorded on a Bruker Tensor 27 FTIR using ATR measurements for solids as neat samples.

XPS: The bonding situation within MOC-Mn was evaluated by Thermo Field X-ray photoelectron spectrometer (America).

ICP: The loading amount of $Mn(CO)_5Br$ within MOC-Mn was evaluated using the Thermo inductively coupled plasma emission spectrometer (America).

SEM: The structure and element distribution of MOC and MOC-Mn were confirmed by Nova NanoSEM 450 field emission scanning electron microscope.

Preparation of MOC

The preparation of MOC is following literature with some modifications.[1] 2,2-bipyridine-5,5-dicarboxylic acid (0.50 mmol, 122 mg), zirconocene dichloride (0.15 mmol, 44 mg) was mixed in DMSO (10 ml) and water (1 ml) in a 20 ml vial, sonicated for 10 min, and then placed in an oven at 65 °C for 8 hours to obtain off-white crystals. After cooling to room temperature, the crystals were centrifuged (12000 rpm, 10 min), then washed with ethanol and centrifuged for three times to recover the resulting MOC crystals.

Preparation of MOC-Mn

$Mn(CO)_5Br$ (0.083 mmol, 23 mg) and MOC (0.014 mmol, 50 mg) were added to a mixture of THF and toluene (1:1, 10 ml), stirred at room temperature for 12 h, then heated at 60 °C for 3 h. After cooling to room temperature, the mixture was centrifuged (12,000 rpm, 10 min) and washed with THF to obtain orange microcrystals. Finally, the recovered microcrystals were dried under vacuum at 50 °C for 12 h to obtain dry orange powder, and the resulting sample is store under argon gas.[2] (All the above processes are protected from light)

MbCO test

First, horse myoglobin solution (80 μM, 890 μl) and sodium dithionite solution (0.2 M, 100 μl) in PBS were mixed to reduce the Mb to deoxy-Mb, which was incubated at 37 °C for 10 min, and then degassed with argon for 10 min. Second, MOC-Mn solution in DMSO (15 μM, 10 μl) was added to above solution and was degassed by bubbling with argon for 10 min. Finally, the mixed solution was placed vertically under a UV lamp (5 W, 365 nm) with a distance of 10 cm, and then irradiated continuously for 10 min, and then the spectra of MbCO were collected with UV-vis spectrophotometer, and the process was repeated until there was no longer a difference in the concentration of MbCO.[3] The conversion of MbCO is evaluated by calculating the absorbance at 540 nm as follows: Ɛ is extinction coefficient of MbCO = 15.4 $mM^{-1}cm^{-1}$ and OD_{540} is the absorbance of MbCO solution at 540 nm.[4]

$MbCO=(OD_{540}/Ɛ)\times 1000$

Animals and ethics statement

Female C57BL/6 mice (8-9 weeks old, weight 20-22 g) were purchased from Cyagen Biosciences (Suzhou, China). All animal experiments were performed in accordance with the guidelines of the National Institute of Health for the Care

and Use of Laboratory Animals and approved by the Scientific Investigation Board of Shanghai Jiaotong University.

Hemolysis Test

Appropriate amount of heparin anticoagulant was added to fresh mouse blood (1 ml) in a centrifuge tube and stirred for 10 min. PBS (8 ml) was added, shake well, and then centrifuged (1500 rpm, 15 min) to remove the supernatant. The precipitated erythrocytes were washed with PBS for 2-3 times in the same way until no red color in the supernatant. The washed erythrocytes in PBS (100 μl) was added to MOC-Mn solution (1 ml, different concentrations), incubated at 37 °C for 1 hour and centrifuged (3000 rpm, 5 min).

MTT Studies

The HeLa cells were inoculated in 96-well plates and incubated at 37 °C in an incubator for 24 h. Then different concentrations of MOC and MOC-Mn solutions were added to HeLa cells and incubated for another 24 h. Then the HeLa cells was treated with or without light irradiation for 10 minutes. After that, MTT agent is add to each well and incubated for 4 h. Finally, DMSO (100 μl) is added to each well, and the absorbance at 570 nm is measured by UV-vis spectrophotometer and cell viability was calculated compared with the untreated blank control group.[5]

Cell uptake assay

For in-vitro cellular uptake assays, HeLa cells were inoculated into 10 mm tissue plates at the density of 10000 cells/mL for 24 h. MOC-Mn (125 μg/ mL, 10 μL) was added to each plate and cultured for 2 h, 4 h, 8 h or 12 h, respectively.

For in-vitro cellular retention assays, HeLa cells were inoculated into 10 mm tissue plates at the density of 10000 cells/mL for 24 h. MOC-Mn (125 μg/ mL, 10 μL) was added to each plate and cultured for 12h. Then, the medium was

extracted and fresh medium was added. Subsequently, the cells were collected at 4 h (12+4 h) and 8 h (12+8 h) respectively.

We used ICP-MS to quantify the cellular uptake of MOC-Mn. Then the cells were trysinized with trysin, and collected by centrifugation, washed with PBS buffer for 3 times. For traditional ICP-MS analysis, the samples were digested in a solution containing 0.25 mL HNO_3 (68%) and 0.75 mL HCl (38%) for 4 h at 110 °C. After cooling, the samples were diluted with HCl (2%) to 10 mL. Mn and Zr contents were then detected by ICP-MS.

In vivo Biocompatibility

MOC and MOC-Mn were dissolved in DMSO (50 ul) and then diluted with PBS (2 ml) to a concentration of 800 ug/ml, and then 200 μl of each solution of PBS, MOC, and MOC-Mn were injected through the tail vein into C57/B6 mice. The PBS group was used as a blank control group.

The blood, heart, liver, spleen, lung and kidney of each mouse were acquired after 24 hours of administration. The blood was used for injury biomarker detection, and the organs were used for H&E staining experiments.

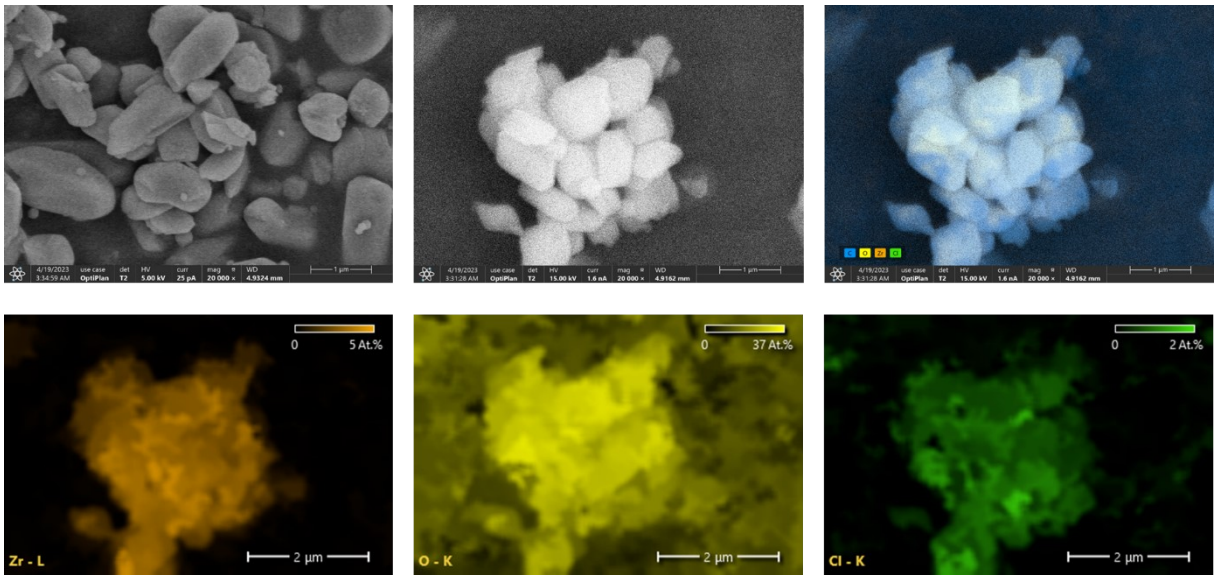

Fig. S1 The SEM and EDS mapping images of MOC.

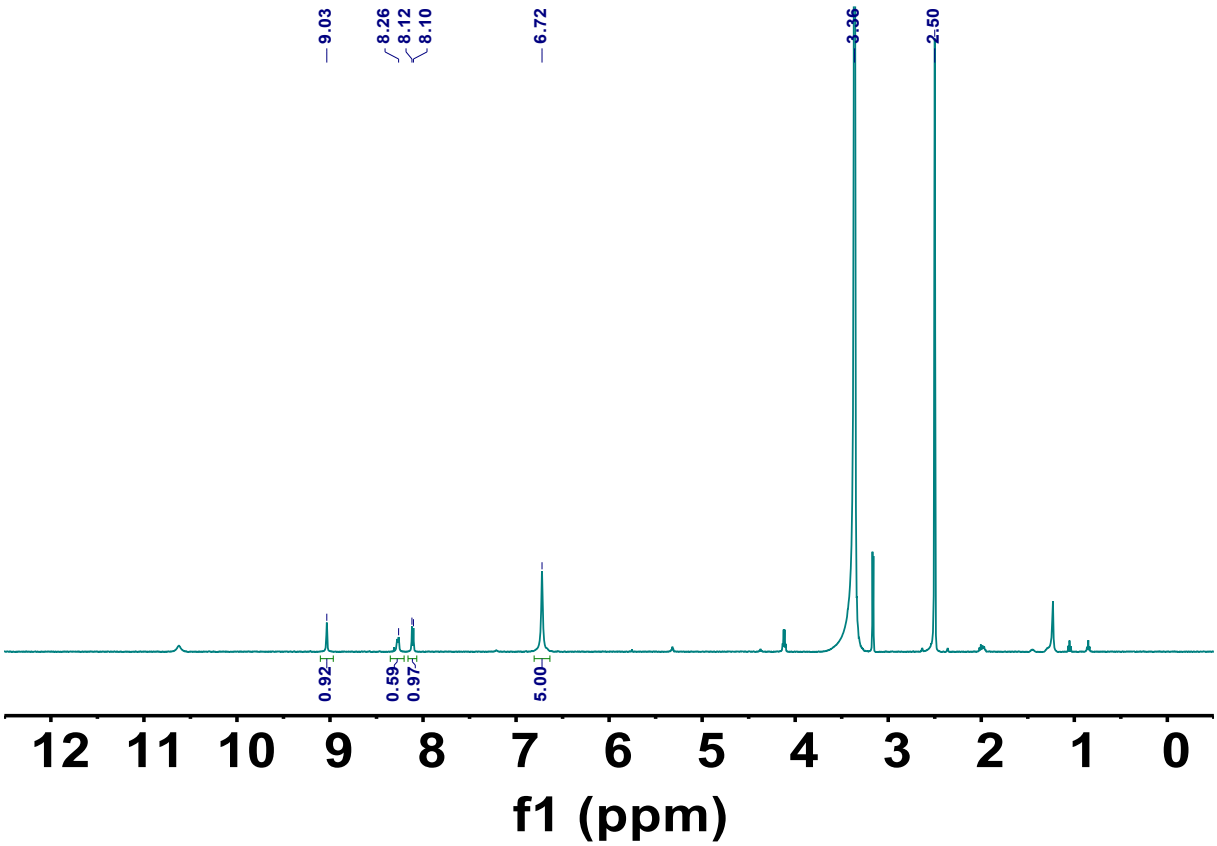

Fig. S2 The ^{1}H-NMR spectrum of MOC in DMSO-d_6.

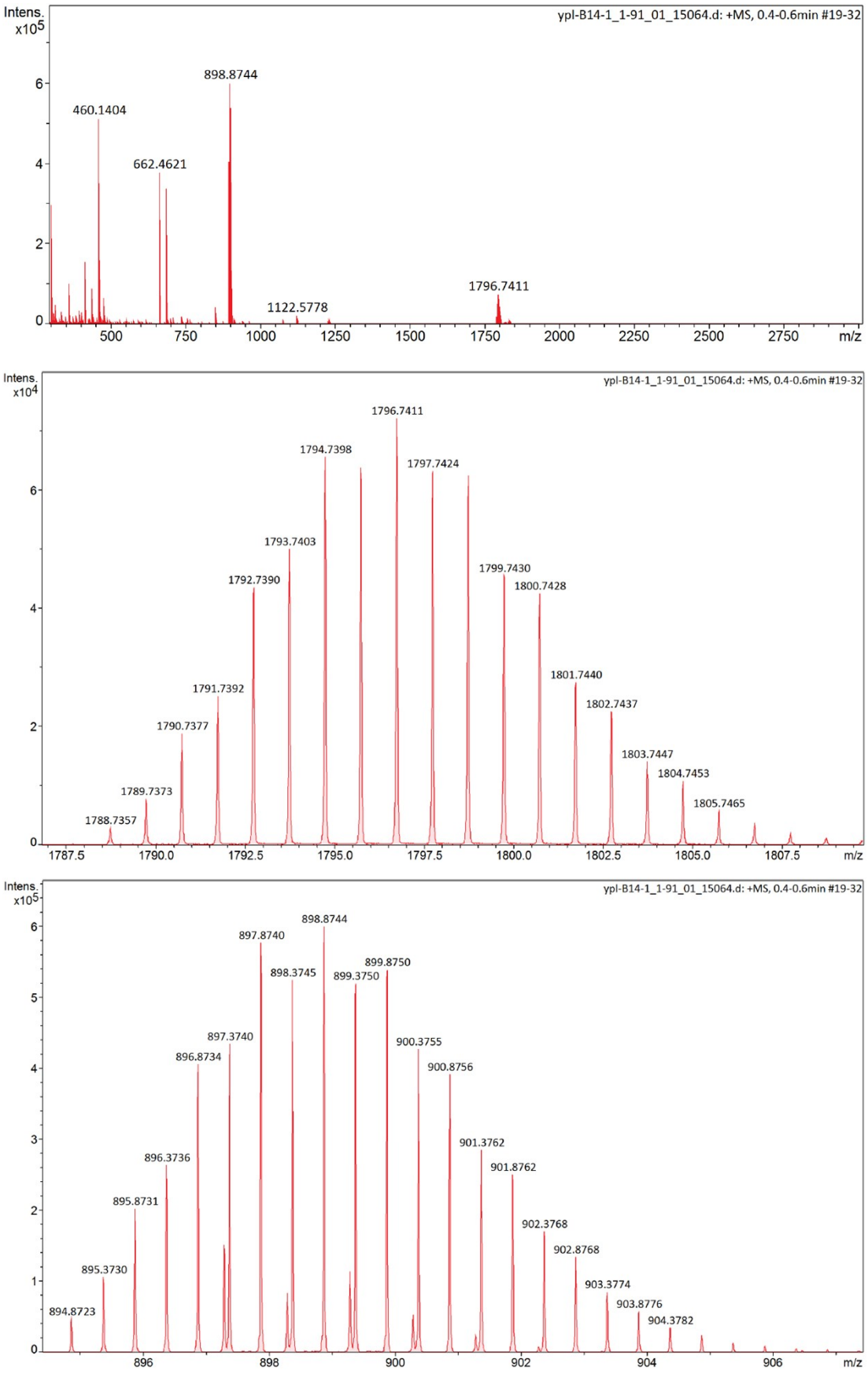

Fig. S3 The ESI mass spectrum of MOC.

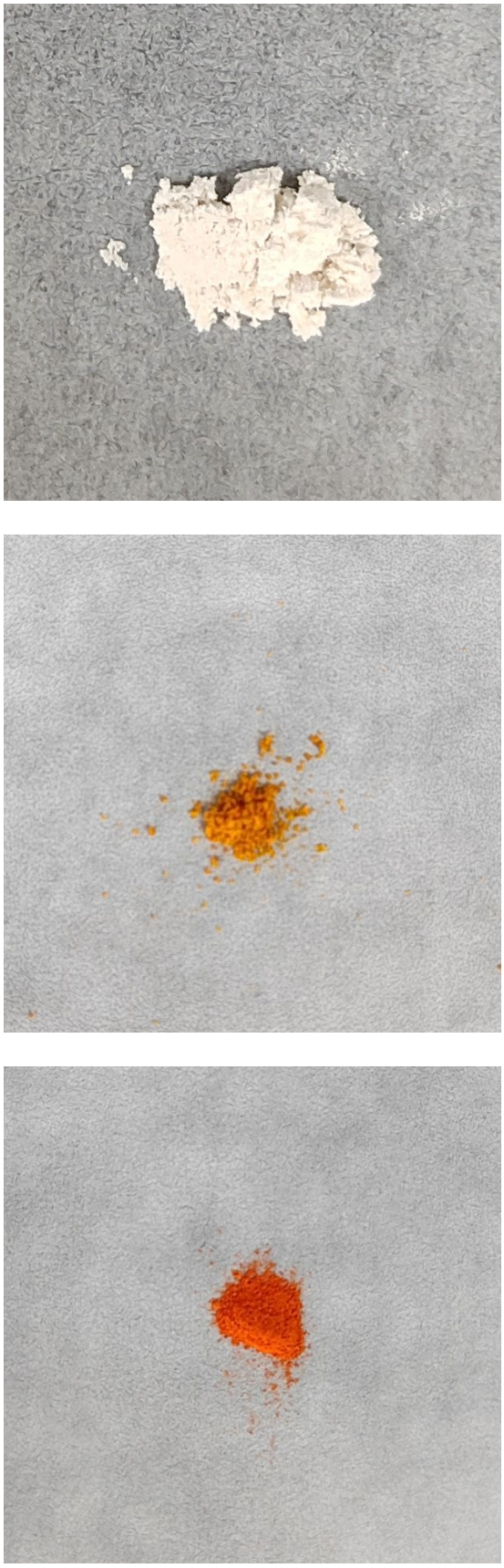

Fig. S4 The photographs of MOC, $Mn(CO)_5Br$ and MOC-Mn.

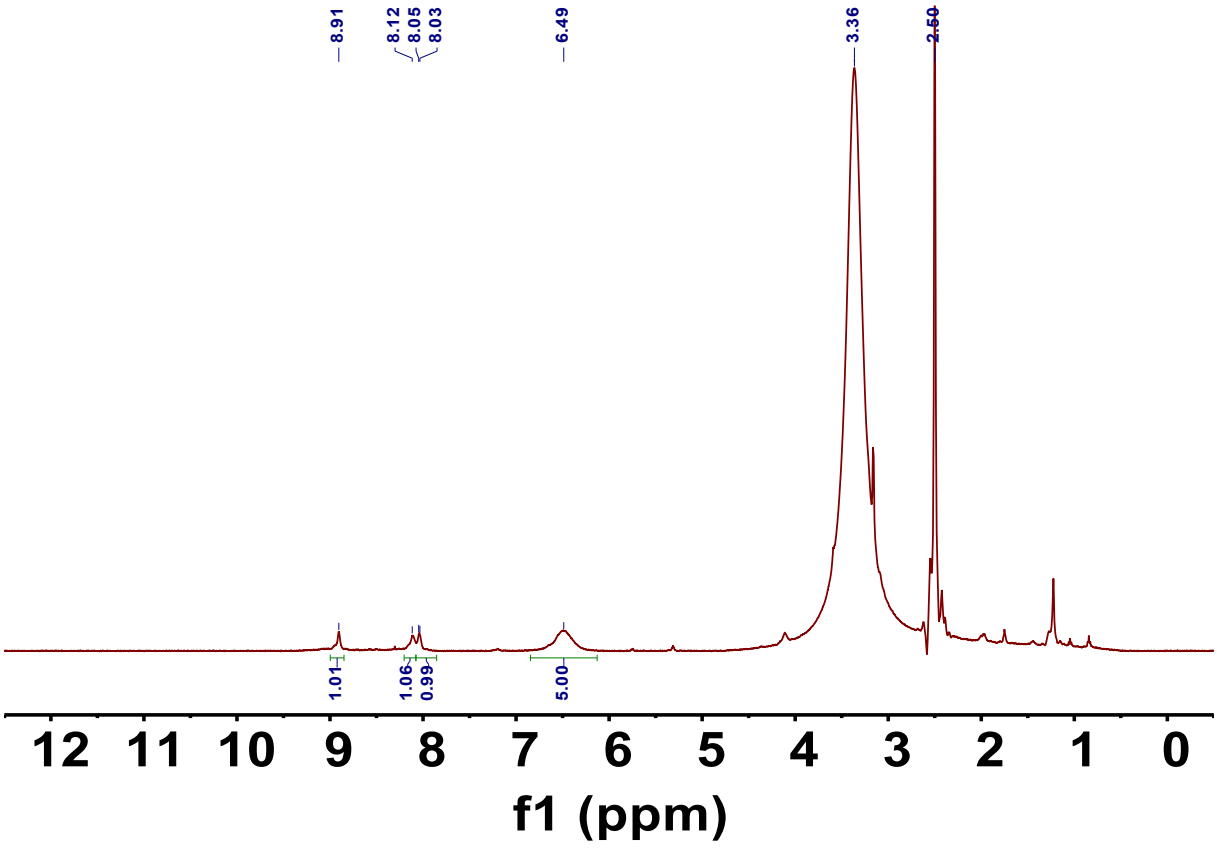

Fig. S5 The ^{1}H-NMR spectrum of MOC-Mn in DMSO-d_6.

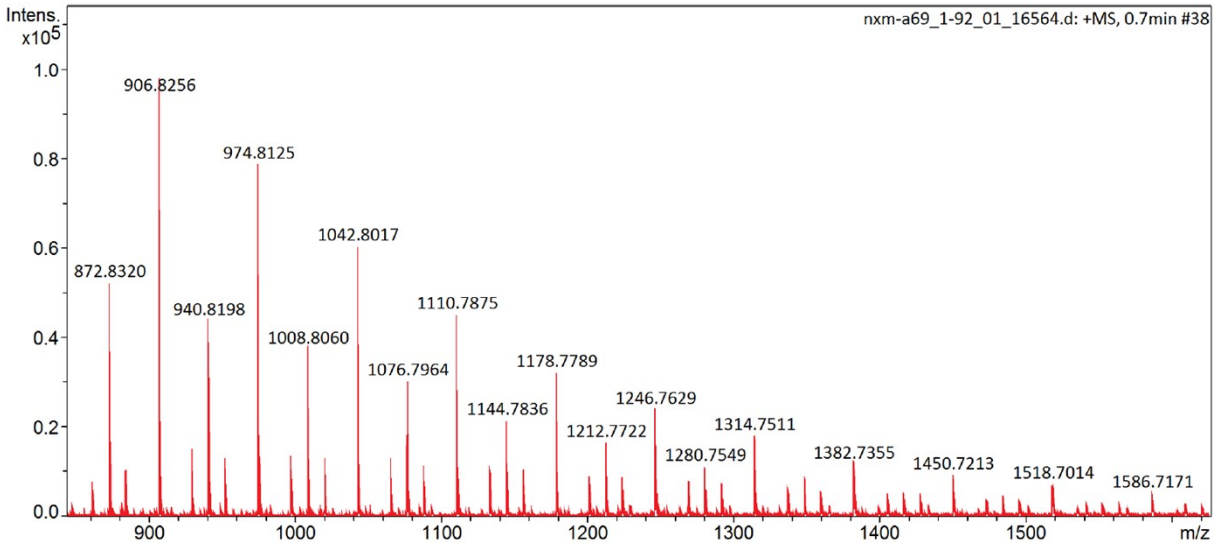

Fig. S6 The ESI mass spectrum of MOC-Mn.

Table S1. The molecular weights of possible MOC-Mn fragments.

Loading Number of $Mn(CO)_5Br$	Number of lost CO	Calculated m/z	Observed m/z
2	0	1008.35	1008.8060
3	3	1042.07	1042.8017
4	1	1110.79	1110.7875
4	6	1075.79	1076.7964
5	4	1144.51	1144.7836
6	2	1213.24	1212.7722
6	7	1178.24	1178.7789

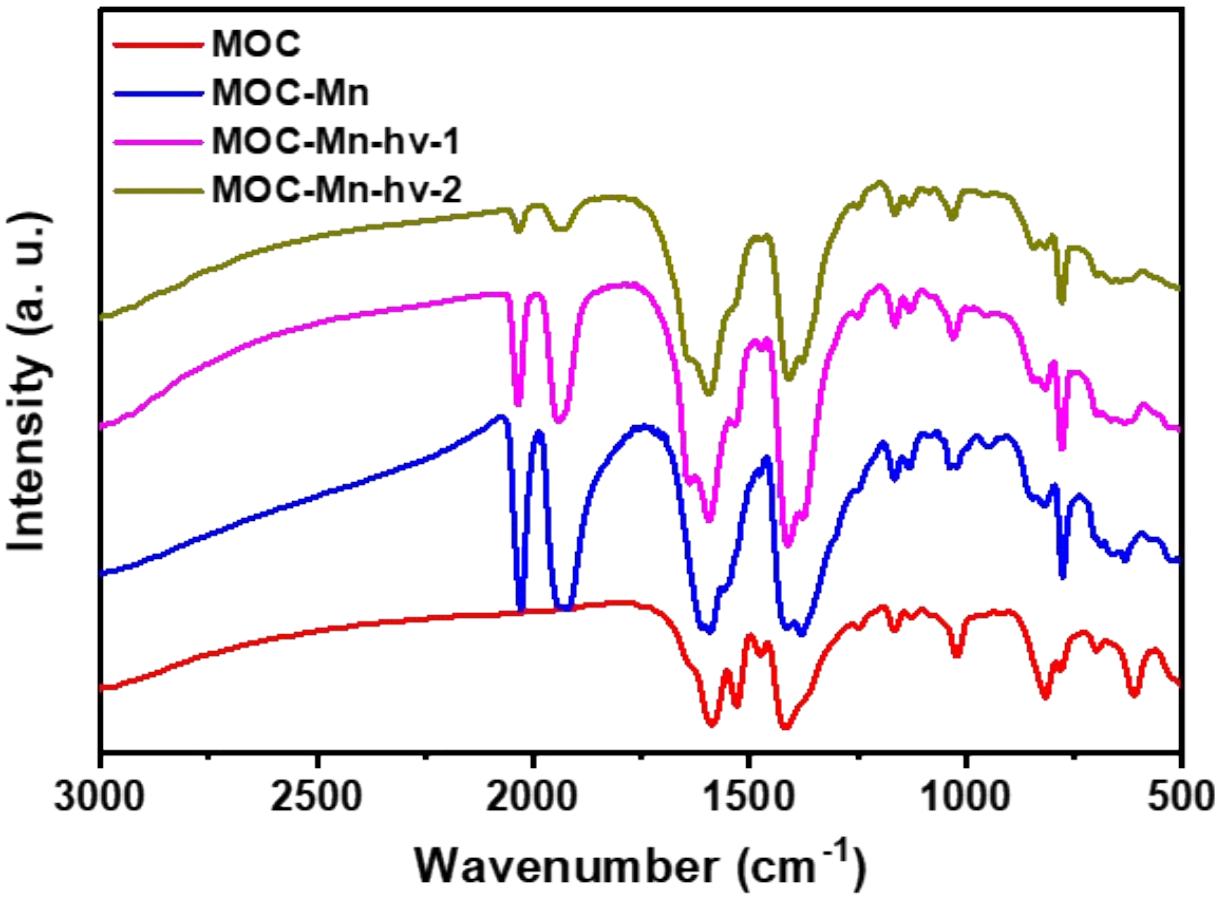

Fig. S7 The FTIR spectrum of MOC, MOC-Mn, light-trigged MOC-Mn.

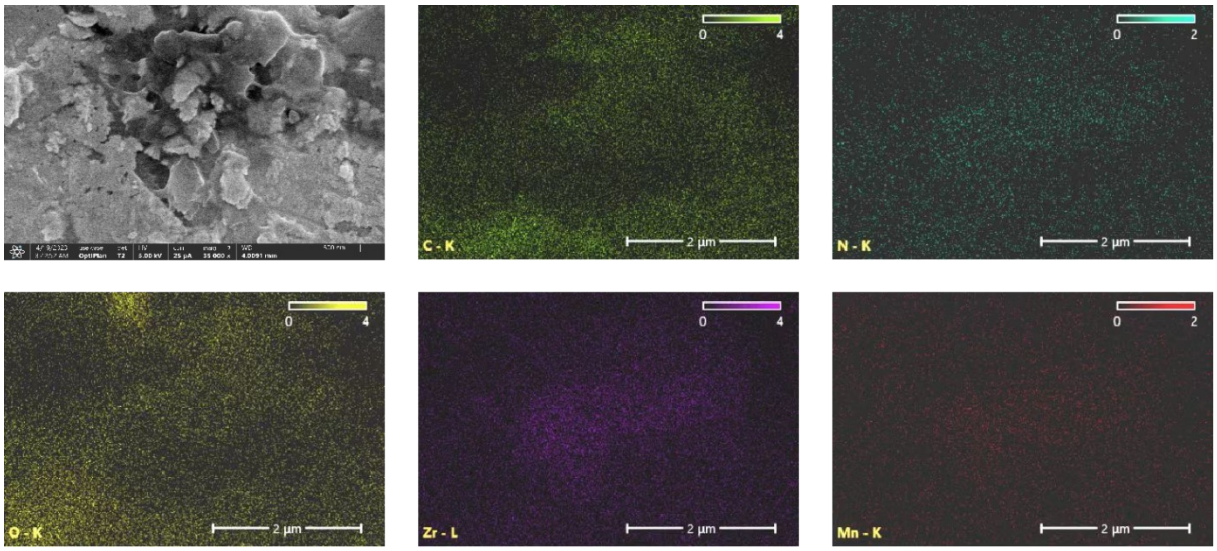

Fig. S8 The SEM and EDS mapping images of MOC-Mn.

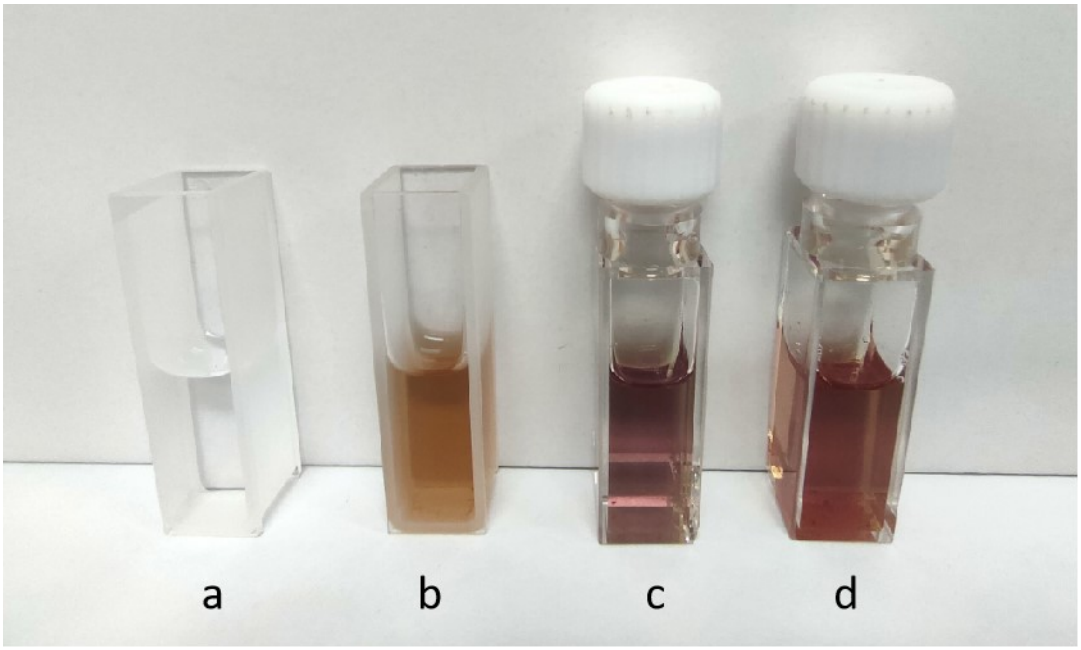

Fig. S9 The photographs of solutions during the MbCO assay process. a) PBS, b) Mb in PBS, c) Mb and $Na_2S_2O_4$ in PBS, d) Mb, $Na_2S_2O_4$ and MOC-Mn in PBS after 365 nm irradiation.

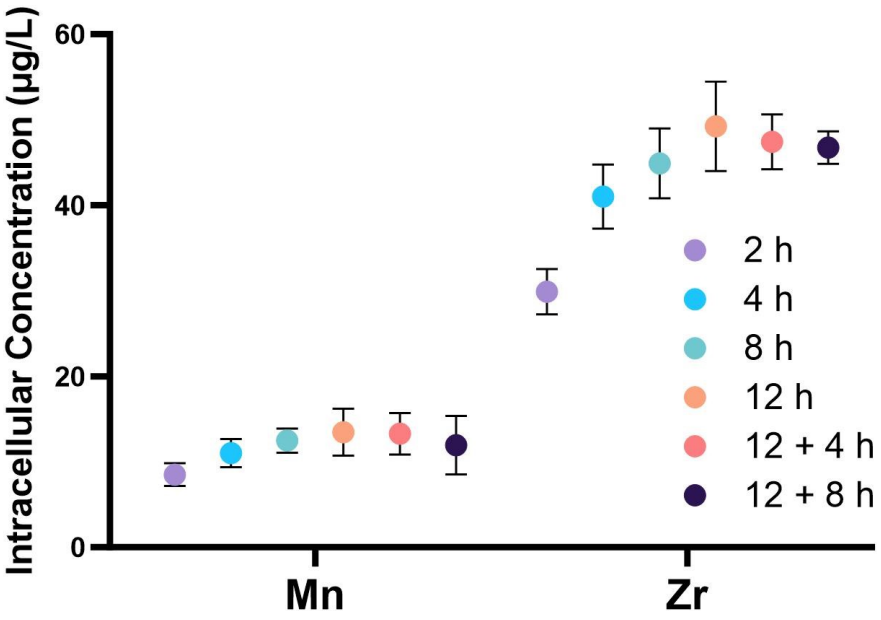

Fig. S10 ICP-MS results of Mn and Zr during uptake of MOC-Mn into HeLa cells.

Notes and references

1. X. J. Qi, M. Sun, B. S. Hou, X. Yu, G. G. Shan, C. Y. Sun, A. Yousaf, X. L. Wang and Z. M. Su, *Nanotechnology*, 2021, **32**, 075602.

2. S. Diring, A. Carné-Sánchez, J. Zhang, S. Ikemura, C. Kim, H. Inaba, S. Kitagawa and S. Furukawa, *Chem. Sci.*, 2017, **8**, 2381-2386.

3. C. Nagel, S. McLean, R. K. Poole, H. Braunschweig, T. Kramer and U. Schatzschneider, *Dalton Trans.*, 2014, **43**, 9986-9997.

4. A. J. Atkin, J. M. Lynam, B. E. Moulton, P. Sawle, R. Motterlini, N. M. Boyle, M. T. Pryce and I. J. Fairlamb, *Dalton Trans.*, 2011, **40**, 5755-5761.

5. O. S. Qureshi, A. Zeb, M. Akram, M. S. Kim, J. H. Kang, H. S. Kim, A. Majid, I. Han, S. Y. Chang, O. N. Bae and J. K. Kim, *Eur. J. Pharm. Biopharm.*, 2016, **108**, 187-195.