

Supporting Information

In situ Bioorthogonal-Modulation of m⁶A RNA Methylation in Macrophages for Efficient Eradication of Intracellular Bacteria

Mengyu Sun, [a,b] Jinsong Ren*, [a,b] and Xiaogang Qu*, [a,b]

[a] State Key Laboratory of Rare Earth Resources Utilization and Laboratory of Chemical Biology, Changchun Institute of Applied Chemistry, Chinese Academy of Sciences, Changchun 130022, China

[b] School of Applied Chemistry and Engineering, University of Science and Technology of China, Hefei 230026, China

Experimental Section

Materials: 5,10,15,20-tetrakis(4-aminophenyl) porphyrin (TAPP) was purchased from Energy Chemical (Anhui, China). 2,5-dihydroxyterephthalaldehyde (Dha) was obtained from Shanghai Kaiwei Chemical Technology Co., LTD (Shanghai, China). Iron(III) chloride hexahydrate was purchased from Sigma-Aldrich. Methyl piperidine-3-carboxylate hydrochloride (MPCH), rhodamine 110 (RH 110) were purchased from Energy Chemical (Anhui, China). Sodium ascorbate was purchased from Shanghai Macklin Biochemical Co., Ltd. (Shanghai, China). H₂O₂ was obtained from Beijing Chemicals (Beijing, China). 1-ethyl-3-(3-dimethylaminopropyl) carbodiimide (EDC), and N-hydroxysuccinimide (NHS) were acquired from Aladdin Reagent (Shanghai, China). 3,3',5,5'-Tetramethylbenzidine (TMB), N-acetyl cysteine (NAC) was obtained from Adamas-beta. Phosphate buffer solution (PBS) was obtained from MultiSciences. Cell counting kit-8 (CCK-8) solution and qPCR-related reagents were acquired from Beyotime Biotechnology Ltd. Cell culture dishes were bought from Guangzhou Jet Bio-Filtration Co., Ltd, 20 mm glass-bottom dishes and centrifuge tubes were obtained from NEST Biotechnology Co., Ltd (Wuxi, China). Recombinant murine interleukin-4 (IL-4) and gene sequences were obtained from Sangon Biotechnology Inc. (Shanghai, China). ECL was purchased from Shangdong Sparkjade Biotechnology Co., Ltd. All other reagents were analytical reagent grade and used as received. Ultrapure water (18.2 MΩ; Millipore Co., USA) was used in all experiments and to prepare all buffers.

Measurements and Characterizations: TEM measurements were carried out on a FEI TECNAI G2 F20 at 200 kV. The energy dispersive X-ray spectroscopy (EDS) was acquired on the Hitachi S-4800 FS-SEM at the working voltage of 20 kV and working current of 15 μA without platinum coating. The crystalline structures of the asprepared samples were evaluated by X-ray diffraction (XRD) analysis on a D8 Focus diffractometer (Bruker) using Cu Kα radiation ($\lambda = 0.15405$ nm). Inductively coupled plasma mass spectrometry (ICP-MS) measurements were performed on a Thermo Scientific X series II inductively coupled plasma mass spectrometer. UV-vis absorbance measurement was carried out on a JASCO V-550 UV-vis spectrophotometer (JASCO International Co., LTD., Tokyo, Japan) with a Peltier temperature control accessory. Fluorescence measurements were carried out on a JASCO FP-6500 spectrofluorometer. The flow cytometry data was obtained by BD LSRFortessa Cell Analyzer. All the photos were taken with a Canon camera.

Preparation of Fe-TAPP: TAPP (100 mg) and anhydrous ferric chloride (250 mg) were added to anhydrous DMF (15 mL) under a nitrogen atmosphere. After being stirred at 150 °C for 4 h, cold water was added and then filtered. The precipitate was dissolved in CH₂Cl₂ and washed with pure water. The organic layer was dried with Na₂SO₄ and the solvent was evaporated in vacuo to give Fe-TAPP.

Preparation of COFe: The COFe nanoparticles were prepared according to the literature by mixing Dha (19.9 mg, 0.12 mmol) and Tph (40.5 mg, 0.06 mmol) in dichlorobenzene/butyl alcohol/6 M acetic acid (5/5/1, v/v/v, 3.3 mL). After sonication for 10 min, the mixture was degassed in a Pyrex tube (20 mL) through freeze-pump-thaw cycles for three times and then

sealed off. The tube was heated 3 days at 120 °C. After that, the product was collected and washed with anhydrous THF, acetone. Then dried under vacuum overnight to obtain COFe.

Preparation of CID and CIDM: In brief, COFe (1 mg mL⁻¹) in DMF/water (9/1, v/v) was sonicated in an ultrasonic bath for 5 h at the power of 300 W. The dispersion was then sonicated with a sonic probe for 5 h at the power of 1200 W, 30%. The sonic probe worked every 3 s with the interval of 4 s. Next, the dispersion was again sonicated with ultrasonic bath for 5 h. After that, the resulting solution was centrifuged at 12,000 rpm for 15 min. Finally, the supernatant containing COF nanodots was collected for future use. Then, the dispersion containing COF nanodots was mixed with DSPE-PEG-mannose (1 mg mL⁻¹) under sonication in an ultrasonic bath for 30 min. After that, DMF in the mixture was removed by vacuum rotary evaporation and the resulted sample was dissolved in water and dialyzed against water to remove unreacted DSPE-PEG-mannose. In addition, for removing the micelles formed by DSPE-PEG-mannose, the sample was further filtered by 100 nm membrane.

Preparation of FITC-CIDM: CIDM (10 mg) was dissolved in MES buffer (10 mL, pH 6.0, 20 × 10⁻³ M) and stirred to obtain dispersed solution, then the FITC dissolved in MES buffer (10 mL, pH 6.0, 20 × 10⁻³ M) was added and stirred for 24 h. The product was centrifuged at 12,000 rpm for 10 min and washed three times. The FITC-CID and FITC-COFe were prepared in the same method.

The Catalytic Reduction of Azide Group Mediated by catalysts: The reaction activity of CIDM was evaluated by the cleavage of pro-rhodamine 110 (pro-RH 110, 3). In detail, CIDM (50 µg mL⁻¹), 10 µM pro-RH 110 (20 mM in DMSO) and 5 mM sodium ascorbate (50 mM in PBS) were dissolved in H₂O at 37 °C, respectively. After 24 h, 200 µL of reaction medium was taken and mixed with 400 µL of water. Then the catalyst was removed by centrifugation and the supernatant was detected for fluorescence. Measured the fluorescence intensity of rhodamine 110 (RH 110) (Ex = 488 nm, Em = 530 nm) by fluorescence spectrometer. The three reaction conditions (H₂O, PBS and DMEM) were tested by the same method.

The catalytic efficiency of CIDM was evaluated by the cleavage of pro-rhodamine 110 (pro-RH 110, 3) with different times. In detail, CIDM (50 µg mL⁻¹), 10 µM pro-RH 110 (20 mM in DMSO) and 5 mM sodium ascorbate (50 mM in PBS) were dissolved in H₂O at 37 °C, respectively. At different time periods, 200 µL of reaction medium was taken and mixed with 400 µL of water. Then the catalyst was removed by centrifugation and the supernatant was detected for fluorescence. Measured the fluorescence intensity of rhodamine 110 (RH 110) (Ex= 488 nm, Em = 530 nm) by fluorescence spectrometer. The catalytic efficiency of CIDM compared with bulk COFe and CID (with the same amount of Fe) was using the same method.

OXD-like Activity of CIDM. The OXD-like activity of CIDM was studied through the catalytic oxidation of the TMB by spectrophotometrically monitoring the absorbance changes at 652 nm. Typically, chemicals were added into 480 µL of PB buffer solution (10 mM, pH 3.0) in the order 10 µL of CIDM (500 µg mL⁻¹) and 10 µL of TMB (50 mM) under 37 °C, unless otherwise stated. The concentration, pH, and temperature-dependent OXD-like activity were investigated by the same method.

Cell Culture: The 4T1, RAW264.7 and 3T3 cells were supplied by American Type Culture Collection (ATCC). The cells were cultured in Dulbecco's modified eagle medium (DMEM) supplemented with 10% fetal bovine serum and 1% Penicillin-Streptomycin at 37 °C in a humidified atmosphere of 5% CO₂.

Cellular Uptake Behaviors of CIDM: RAW264.7 cells were randomly planted in 24-well plates and allowed to adhere for 24 h. FITC-CID (50 µg mL⁻¹), FITC-COFe (50 µg mL⁻¹) and FITC-CIDM (50 µg mL⁻¹) were added and incubated with the cells for 4 h, respectively. And then, the cells were washed by PBS for 3 times before fluorescence analysis.

In vitro Cytotoxicity Measurement of CIDM: RAW264.7 were seeded in 96-well plates with a density of 5 × 10³ cells per well and cultured overnight. CIDM with different concentrations was added to the wells. After being cultured for 48 h, the medium was removed, followed by washing with PBS. 100 µL of 10% MTT solution was added to the above each well, and then the plate was incubated for 4 h at 37 °C. A microplate reader was used to measure the absorbance at 490 nm.

CIDM Mediated Cleavage Reaction Inside Living Cells: RAW264.7 cells were plated in 24-well plates and incubated with CIDM (50 µg mL⁻¹) for 12 h. After washing twice with PBS, the cells were incubated with a medium containing 20 µM pro-RH 110 (20 mM in DMSO) and 5 mM sodium ascorbate for about 12 h. Finally, the cells were washed twice with PBS, and the production of fluorescent RH 110 in the RAW264.7 was monitored by flow cytometry and fluorescence microscopy.

Determination of ROS Generation in vitro: For ROS detection, RAW264.7 cells were randomly seeded in 24-well plates in DMEM for 24 h before further manipulation. Afterward, different formulation was added and incubated for 24 h. The treated cells were washed with PBS twice and incubated with 10 µM of DCFH-DA for 30 min. After removing the unloaded probe with PBS, the fluorescence intensity of cells was monitored by confocal laser scanning microscope (CLSM).

MS Study of MPCH Synthesis in Living Cells: RAW264.7 cells were plated in 24-well plates and incubated with CIDM (50 µg mL⁻¹) for 12 h. After washing twice with PBS, the cells were incubated with a medium containing pro-MPCH (100 nM, 10 µM in DMSO) + sodium ascorbate (5 mM) for about 24 h. Finally, the cells were washed twice with PBS. Then the cells were resuspended in water and lysed by sonication. At the last, the supernatant was harvested by centrifugation for 8 min at 12,000 rpm and detected by MS.

Dot blot assay: The total RNA was extracted with RNAeasy™ Animal RNA Isolation Kit according to the manufacturer's instruction. Then the RNA samples were diluted in nuclease-free water, denatured at 95 °C for 3 minutes and placed on ice. Then 1 µL of the RNA samples was dotted onto a positively charged nylon membrane (Beyotime, FFN10). The RNA samples were cross-linked onto the membrane via UV irradiation at 254 nm (30 minutes). After UV crosslinking, the membrane was blocked with 5% nonfat dry milk for 1 hour at room temperature and incubated

with rabbit-anti-m⁶A antibody (1: 1000) at 4 °C overnight. Finally, the membrane was washed with 1 × PBST buffer 3×, then incubated with the HRP-conjugated goat anti-rabbit IgG (1:1000) for 2 h at room temperature. The luminescence signal was performed using the enhanced chemiluminescence solution reaction. The image results were analysis by ImageJ.

The re-education of macrophages in vitro: The fluorescence intensity of macrophages was detected by flow cytometry. RAW264.7 macrophages were seeded into 6-well plates and incubated with 25 ng mL⁻¹ IL-4 for 12 h. Then old media were removed and replaced by fresh media containing CIDM (50 µg mL⁻¹) and incubated for 12 h. After washing twice with PBS, the macrophages were incubated with a medium containing 100 nM pro-MPCH (10 µM in DMSO) and sodium ascorbate (5 mM) for 24 h. After that, the treated macrophages were washed and stained with anti-FITC-CD86 and anti-PE-CD206 respectively.

Quantitative Real-time Polymerase Chain Reaction (qRT-PCR): After incubating with different groups, total RNA was isolated from macrophages with RNAeasy™ Animal RNA Isolation Kit. Then 1 µg of total RNA was reverse-transcribed into complementary DNAs with the BeyoRT II First Strand cDNA Synthesis Kit. After that, qPCR was performed with primers described previously on the Toptical 96 Real-Time PCR System using the BeyoFast™ SYBR Green qPCR Mix. Primers for amplification are shown in Table S2. STAT1 qPCR Primer Pair (QM05278S) was obtained by Beyotime Biotechnology Ltd.

Global m⁶A level: Total RNA was extracted from treated lung tissues and cells as described above. The global m⁶A level was measured by an m⁶A RNA Methylation Quantification Colorimetric Kit (Epigentek, Farmingdale, P-9005) according to the manufacturer's manual. Briefly, total RNA solution (200 ng, 2 µL) standard samples and negative control were added to the strip wells and incubated for 1.5 h at 37°C. Then, 50 µL of diluted capture antibody solutions, diluted detection antibody solutions, and diluted enhancer solutions were added to each well step by step, and the samples were covered and incubated at room temperature. Subsequently, 100 µL of detection solution and 100 µL of stop solution were added into each well step by step at room temperature. The optical density was measured using a microplate reader at 450 nm within 2–15 min.

In vitro antimicrobial assay. RAW264.7 cells (1 × 10⁵) were seeded on coverslips in 24-well plate and incubated for 20 h at 37°C with 5% CO₂. RAW264.7 macrophages were seeded into 6-well plates and incubated with 25 ng mL⁻¹ IL-4 for 12 h. Then old media were removed and replaced by fresh media containing CIDM (50 µg mL⁻¹) and incubated for 12 h. After washing twice with PBS, the macrophages were incubated with a medium containing 100 nM pro-MPCH (10 µM in DMSO) + sodium ascorbate (5 mM) for 24 h. Afterward, the medium of each well was replaced with 500 µL fresh complete medium containing MRSA solution at a multiplicity of infection (MOI) of 20. After 1 h infection, the medium was removed and the samples were washed with PBS for 3 times to remove the bacteria outside of macrophages. Then, antibiotic gentamycin (50 µg mL⁻¹) was added into infected macrophages with an incubation time of 60 min, to eliminate MRSA adhered to the surface of macrophage cells. Then cell nucleus was labeled by DAPI (2 µg/mL) for 15 min. Samples were then washed three times with PBS for intracellular bacteria imaging by using CLSM.

For flow cytometry sample preparation, MRSA was incubated with 5 µg/mL FITC for 2 h (FITC-MRSA) to tag MRSA with fluorescent. RAW264.7 were infected with FITC-MRSA (MOI = 10) after different treatments for 1 h. Intracellular MRSA was collected by lysing cells with HBSS containing 0.1% BSA and 0.1% Triton X100 for flow cytometry.

For measure the intracellular bacteria ablation effect, after various treatment, macrophages were infected with MRSA at a ratio of 20 bacteria per macrophage. After 1 h infection, the medium was removed and washed with fresh culture medium to wash away extracellular MRSA. Fresh culture medium containing 50 µg mL⁻¹ gentamycin was then added and incubated for 1 h to inhibit the growth of extracellular bacteria on the surface of RAW 264.7 cells. After 10 h, macrophages were lysed with PBS solution supplemented with 1 % Triton-X100, and serial dilutions of the lysate were made in PBS. The number of the surviving intracellular bacteria was determined by plating on LB agar plates.

For the growth-inhibition assay in liquid medium, five groups of as-prepared bacterial suspensions treated with (A) PBS, (B) pro-MPCH, (C) CIDM, (D) MPCH and (E) pro-MPCH + CIDM were incubated separately with at 37 °C under 180 rpm. After 5 h incubation, the absorbance at 600 nm was recorded.

For the inhibitory studies, after pretreatment of RAW264.7 cells, 1 mM of the ROS inhibitor NAC was incubated for 4 h. The treated cells were washed with PBS twice and incubated with 10 µM of DCFH-DA for 30 min. Then cells were washed with PBS twice, resuspended in 500 µL PBS for flow cytometry analysis. For measure the intracellular bacteria ablation effect, after 1 mM NAC was incubated for 4 h, macrophages were infected with MRSA at a ratio of 20 bacteria per macrophage. The number of the surviving intracellular bacteria was determined by plating on LB agar plates .

Statistical Analysis: All data were expressed in this article as mean result ± standard deviation (s.d.). All figures shown in this article were obtained from three independent experiments with similar results unless specific mention. The statistical analysis was performed by using Origin 8.0 software. Statistical evaluation was performed using unpaired Student's two-sided t test analysis. Asterisks indicate significant differences (*P < 0.05, **P < 0.01, ***P < 0.001, ****p < 0.0001).

Table S1. Antibody list.

Antibody	company	Catalog	Application
anti-CD206 (PE - conjugated)	BioLegend	104713	FC
anti-CD86 (FITC- conjugated)	BioLegend	159203	FC
anti-m⁶A (Rabbit Polyclonal)	Beyotime	AF7407	DB
HRP-conjugated mouse anti-rabbit immunoglobulin G (IgG)	Sangon Biotech	D110065	WB
anti-STAT1 (Rabbit Polyclonal)	Sangon Biotech	D120084	WB

**Anti-beta-Actin
(Rabbit Polyclona)**

Bioss

bs-0061R

WB

Table S2. Gene primers used for qRT-PCR.

Gene	Forward primer	Reverse primer
TNF-a	CCTGTAGCCACGTCGTAGC	AGCAATGACTCCAAAGTAGACC
Arginase I	GAACACGGCAGTGGCTTTAAC	TGCTTAGCTCTGTCTGCTTTGC
IL-10	CAGGGATCTTAGCTAACGGAAA	GCTCAGTGAATAAATAGAATGGGAA C
iNOS	CCCTTCAATGGTTGGTACATGG	ACATTGATCTCCGTGACAGCC
GAPDH	CGGAGTCAACGGATTTGGTCGT	TCTCAGCCTTGACGGTGCCA

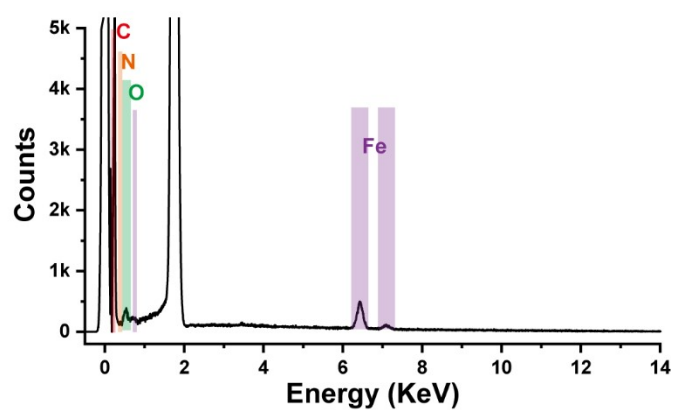


Figure S1. EDS of COFe nanoparticles.

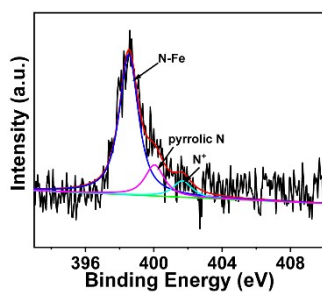


Figure S2. The XPS N 1s analysis of CID.

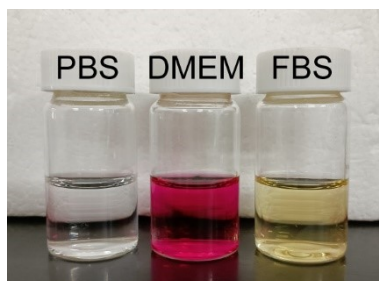


Figure S3. Photograph of CIDM nanocatalysts dissolved in different solutions.

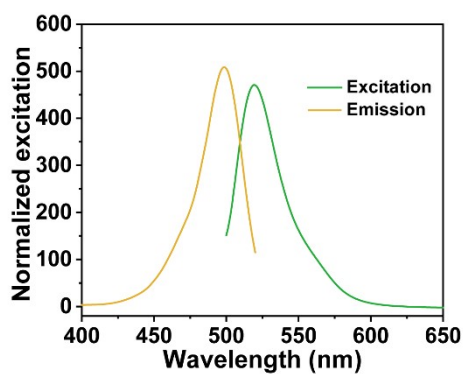


Figure S4. Normalized excitation (pink line) and emission (blue line) of RH 110 (4) in H₂O. The cleavage of pro-RH 110 (3) to afford 4 ($\lambda_{\text{ex}} = 488 \text{ nm}$ and $\lambda_{\text{em}} = 530 \text{ nm}$).

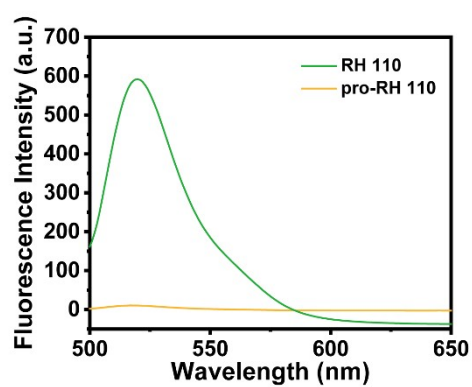


Figure S5. Fluorescence spectrum of pro-RH 110 (green) and pro-RH 110 (orange).



Figure S6. The fluorescence photographs of the reactions in H₂O with or without CIDM catalysts.

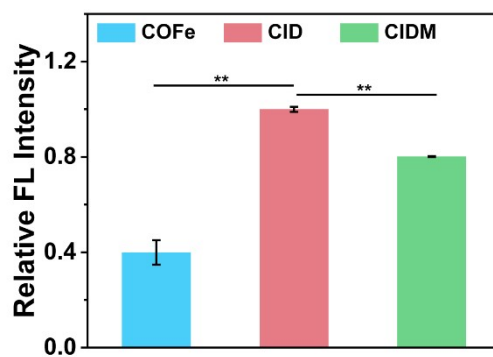


Figure S7. The relative fluorescence intensity of the control experiments with bulk COFe, CID and CIDM.

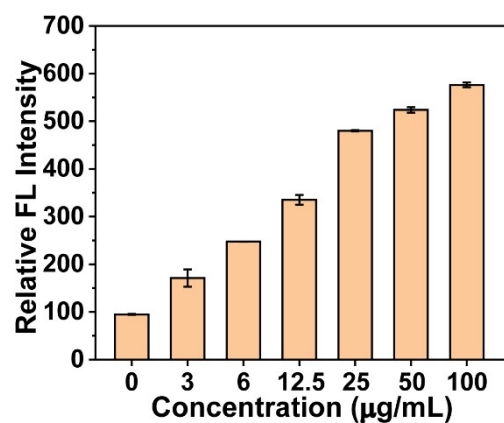


Figure S8. The different concentrations of CIDM on catalytic activity. Each fluorescence intensity of CIDM with different concentrations was normalized by control (0 µg mL⁻¹).

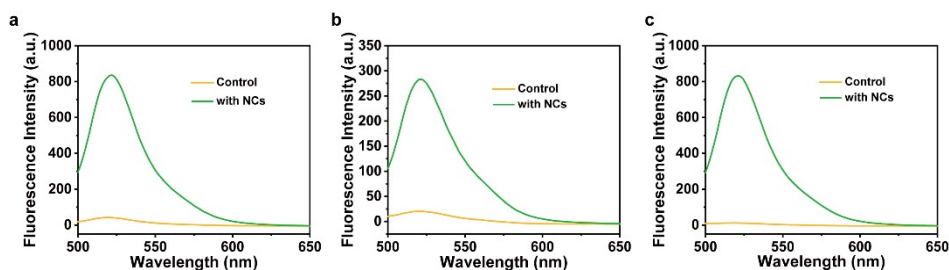


Figure S9. The fluorescence spectra of CIDM NCs in different conditions. The reactions occurred in DMEM; PBS; H₂O.

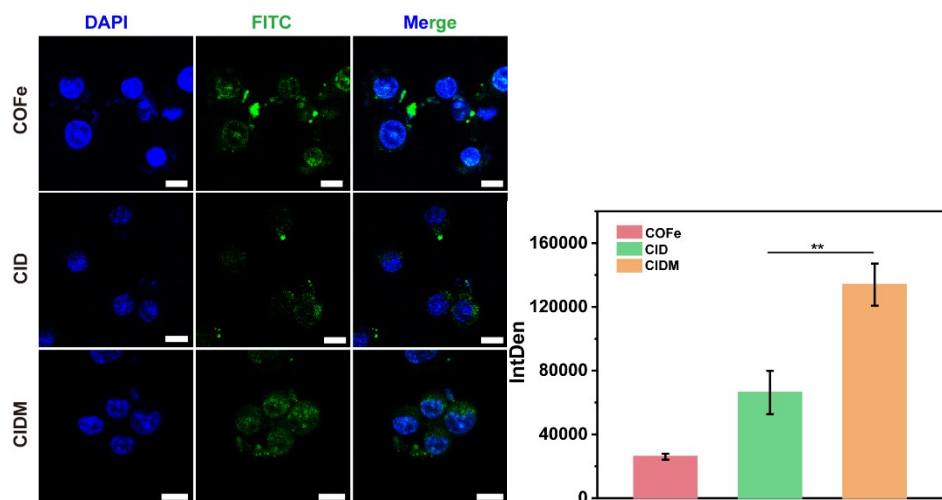


Figure S10. Fluorescence micrograph analysis and fluorescence quantitative analysis of the cellular uptakes of FITC-COFe, FITC-CID and FITC-CIDM of RAW264.7 cells. Scale bar = 10 µm. Data are shown as mean ± SD, n = 3.

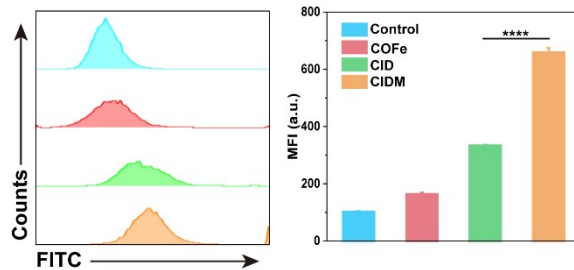


Figure S11. Flow cytometry analysis of cellular uptakes against FITC-COFe, FITC-CID and FITC-CIDM of RAW264.7 cells.

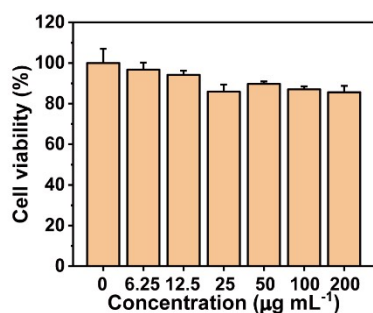


Figure S12. Cell viability of RAW264.7 cells treated with CIDM at different concentrations for 48 h.

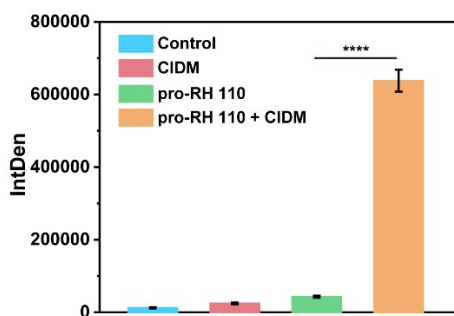


Figure S13. Quantitative analysis of RAW264.7 cells treated with different formulations (Figure 3c). Data are shown as mean \pm SD, n = 3.

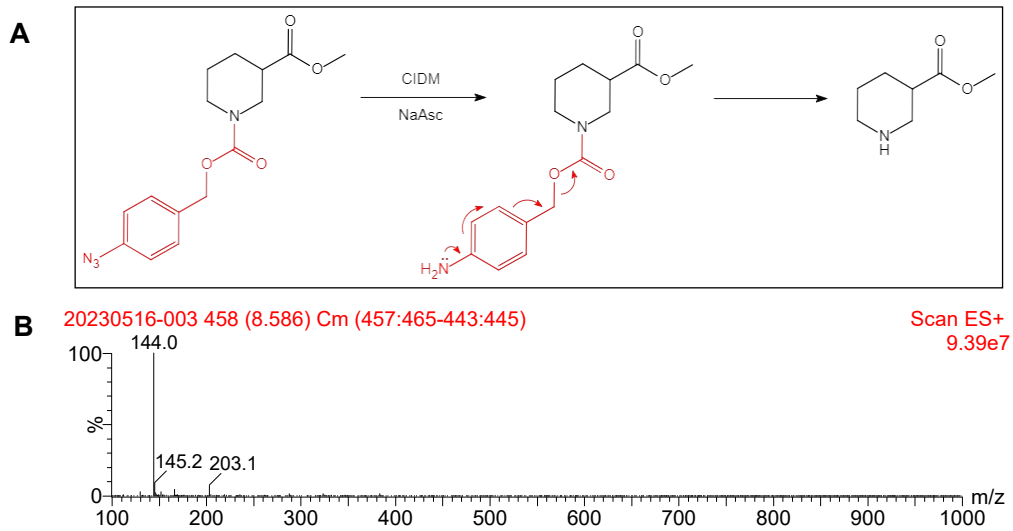


Figure S14. (A) Feasible mechanism for the pro-MPCH activation in the presence of catalyst and reducing agent. (B) The MS analysis from the cell lysate confirming the presence of cleavage product MPCH.

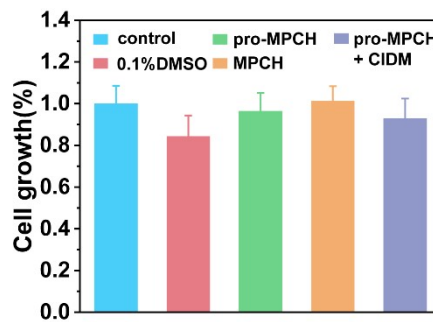


Figure S15. The viability of RAW264.7 cells treated with different formulations.

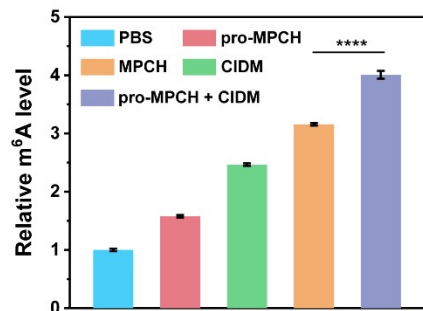


Figure S16. The relative global m⁶A levels after various treatments.

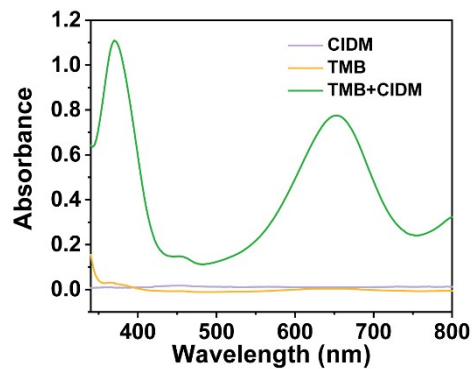


Figure S17. UV/vis absorption spectra of different solutions.

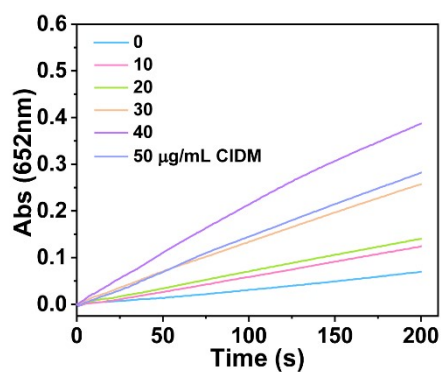


Figure S18. The OXD-like activity of CIDM is concentration-dependent.

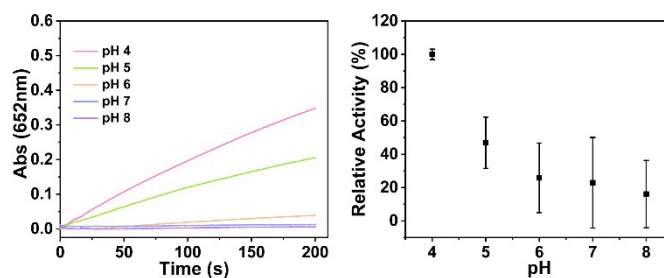


Figure S19. The OXD-like activity of CIDM is pH-dependent.

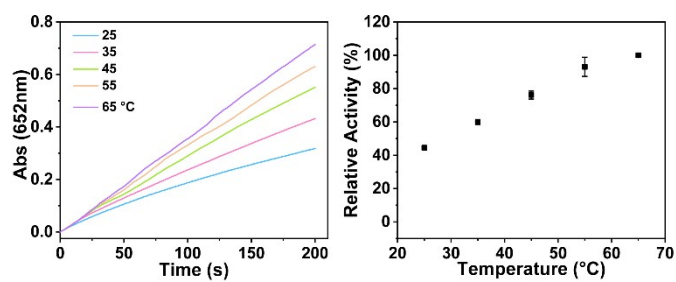


Figure S20. The OXD-like activity of CIDM is temperature-dependent.

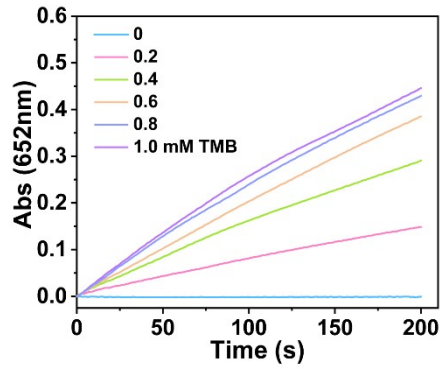


Figure S21. The OXD-like activity of CIDM is TMB concentration dependent.

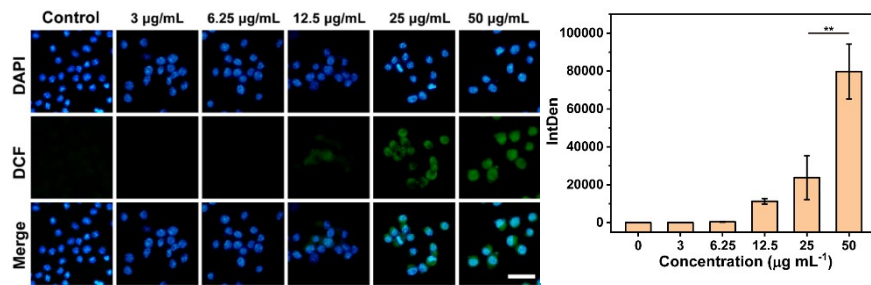


Figure S22. Detection and quantitative analysis of ROS in RAW264.7 cells after different concentrations. Scale bar = 20 µm. Data are shown as mean ± SD, n = 3.

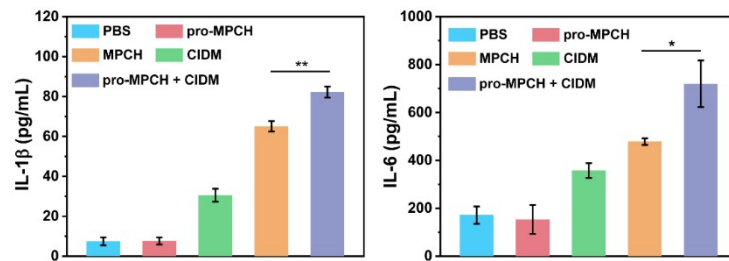


Figure S23. Production of IL-1 β and IL-6 in the supernatant of RAW264.7 cells after different treatments. Data are shown as mean ± SD, n = 3.

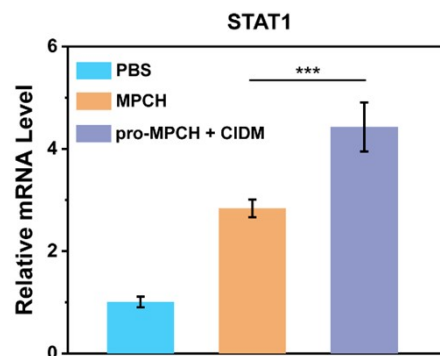


Figure S24. RNA expression levels of STAT1 were validated by using qPCR. Data are shown as mean ± SD, n = 4.

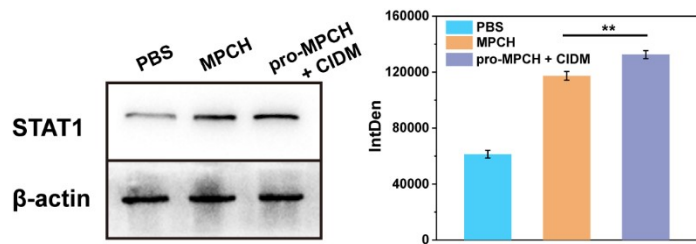


Figure S25. Western blot analysis and quantification of STAT1 expression. Data are shown as mean \pm SD, n = 3.

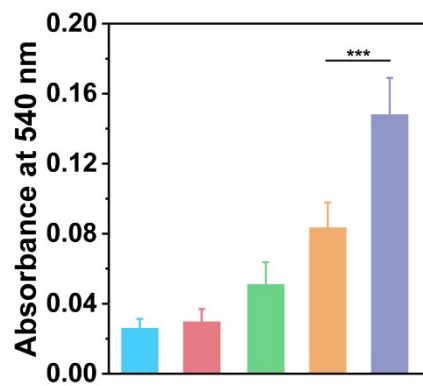


Figure S26. Effect of different treatments on the phagocytosis of macrophages. The absorbance of neutral red at a wavelength of 540 nm represented phagocytosis by macrophages. The group A to E was PBS, pro-MPCH, CIDM, MPCH and CIDM + pro-MPCH, respectively.

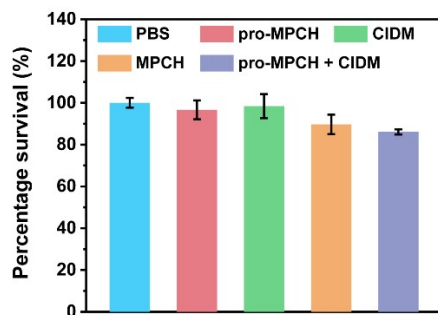


Figure S27. Viability analysis of MRSA. Data were presented as mean \pm s.d. (n = 3).

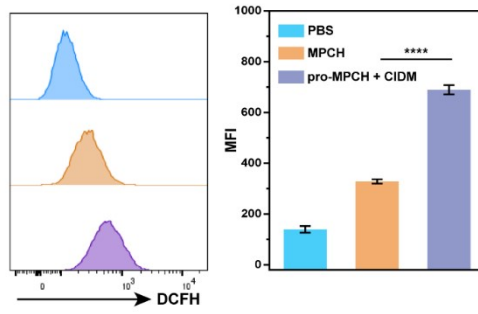


Figure S28. Flow cytometry analysis and quantitative analysis of ROS generation. Data were presented as mean \pm s.d. (n = 3).

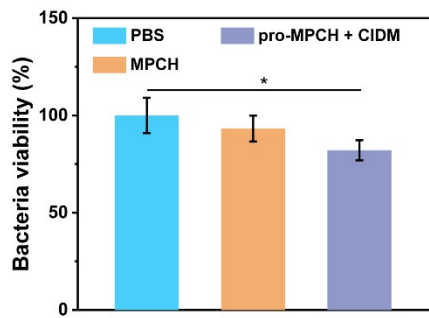
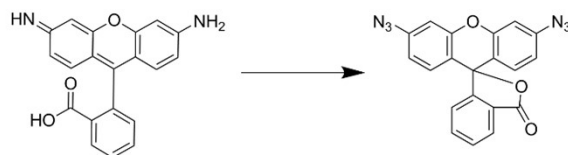
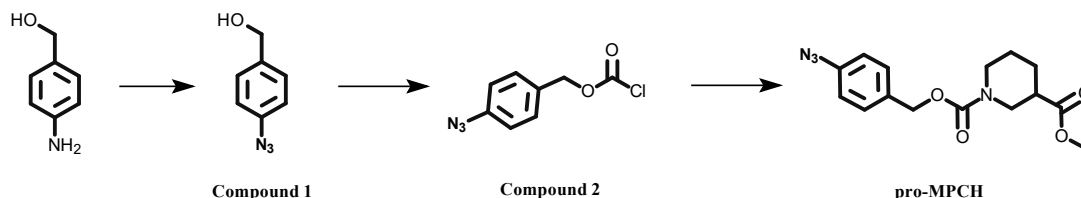


Figure S29. Viability analysis of MRSA after being treated with NAC. Data were presented as mean \pm s.d. (n = 3).

Synthesis of chemical substrate molecules in this study



Synthesis of pro-RH 110: Rhodamine 110 hydrochloride (50 mg, 0.136 mmol) was dissolved in water (4 mL) and HCl (12 N, 1 mL) under nitrogen atmosphere. Sodium nitrite (38 mg, 0.551 mmol) was added and the reaction mixture was stirred at room temperature. After 2 hours, NaN₃ (53 mg, 0.815 mmol) was added slowly to the above reaction mixture and the color of the solution changed from pink to yellow. The reaction was further stirred at room temperature for 2.5 hours and monitored by TLC. The product was extracted into DCM (3 × 20 mL), washed with brine, dried over Na₂SO₄, and concentrated in vacuo. The crude material was purified by flash column chromatography (ethyl acetate: petroleum ether 1:3). The resulting product was isolated as a pale yellow solid.



Compound 1: To a solution of NaNO₂ (200 mg, 3 mmol) in water (5 mL), round bottom flask cooled at 0 °C containing 98% H₂SO₄ in water (4.8 mL) was added dropwise a solution of 4-aminobenzyl alcohol (246 mg, 2 mmol) dissolved in HCl 6N (2 mL). To the mixture, a solution of NaN₃ (1.8 g, 28.9 mmol) in water (5 mL) was dropped slowly. The reaction was stirred at room temperature overnight and monitored by TLC. The reaction mixture was then extracted with DCM and water. Organic layers were combined, dried over anhydrous Na₂SO₄, concentrated and purified by flash column chromatography (ethyl acetate: petroleum ether 1:5). The resulting product was isolated as a yellow oil.

Compound 2: Na₂CO₃ (2.5 g, 23 mmol) and triphosgene (994 mg, 3.4 mmol) were vacuo dried in a round-bottom flask. The flask was cooled in an ice bath and THF (15 mL) was added. After stirring under nitrogen in the dark for 1 h at 0 °C, a solution of compound 1 (1.0 g, 6.7 mmol) in dry THF (15 mL) was added dropwise and the stirring was continued for additional 6 h at room temperature. After completion of the reaction, the solvent was removed under reduced pressure and was used without further purification for the next step.

pro-MPCH: To a solution of compound 2 (100 mg, 0.14 mmol) and pyridine (0.1 mL, 1.2 mmol) in dry CH₂Cl₂ (10 mL), a solution of methyl piperidine-3-carboxylate hydrochloride (29 mg, 0.14 mmol) in CH₂Cl₂ was added dropwise and the reaction mixture was allowed to stand for 6 h at room temperature under nitrogen atmosphere. After that, the mixture was washed with HCl (1M, 20 mL), saturated NaCl (20 mL), dried over anhydrous Na₂SO₄ and concentrated. The residue was

purified via pre-TLC (petroleum ether: ethyl acetate=2:1) to give the desired product as brownish yellow oil.

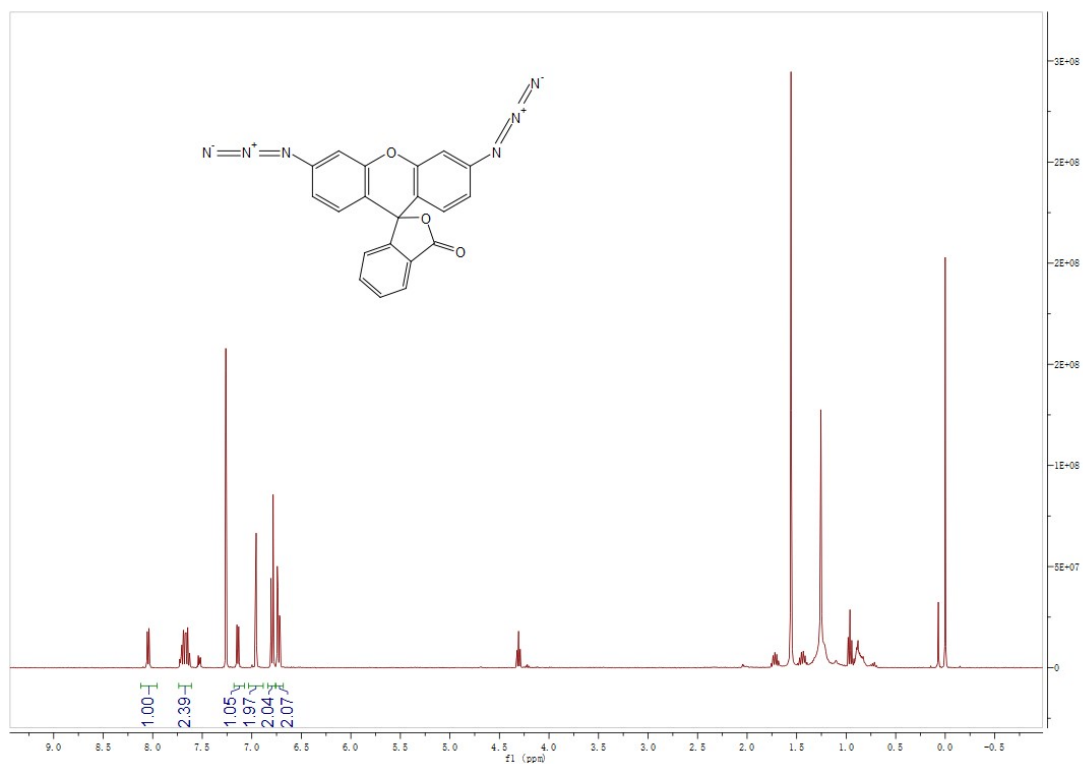


Figure S30. HNMR spectra of pro-RH 110. ^1H NMR (400 MHz, CDCl_3) δ 8.05 (d, $J = 7.0$ Hz, 1H), 7.74 – 7.61 (m, 2H), 7.14 (d, $J = 7.3$ Hz, 1H), 6.96 (d, $J = 2.2$ Hz, 2H), 6.80 (d, $J = 8.5$ Hz, 2H), 6.73 (dd, $J = 8.5, 2.2$ Hz, 2H).

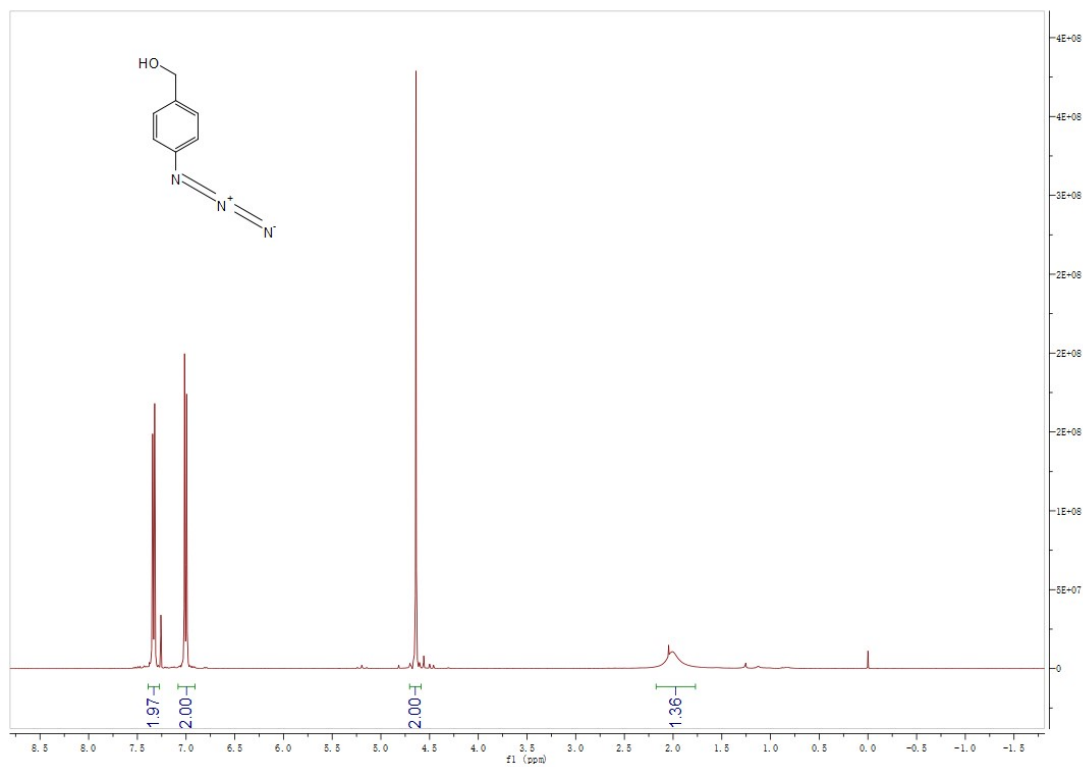


Figure S31. HNMR spectra of compound 1. ^1H NMR (400 MHz, CDCl_3) δ 7.33 (d, $J = 8.5$ Hz, 1H), 7.06 – 6.96 (m, 2H), 4.64 (s, 1H), 2.14 – 1.87 (m, 1H).

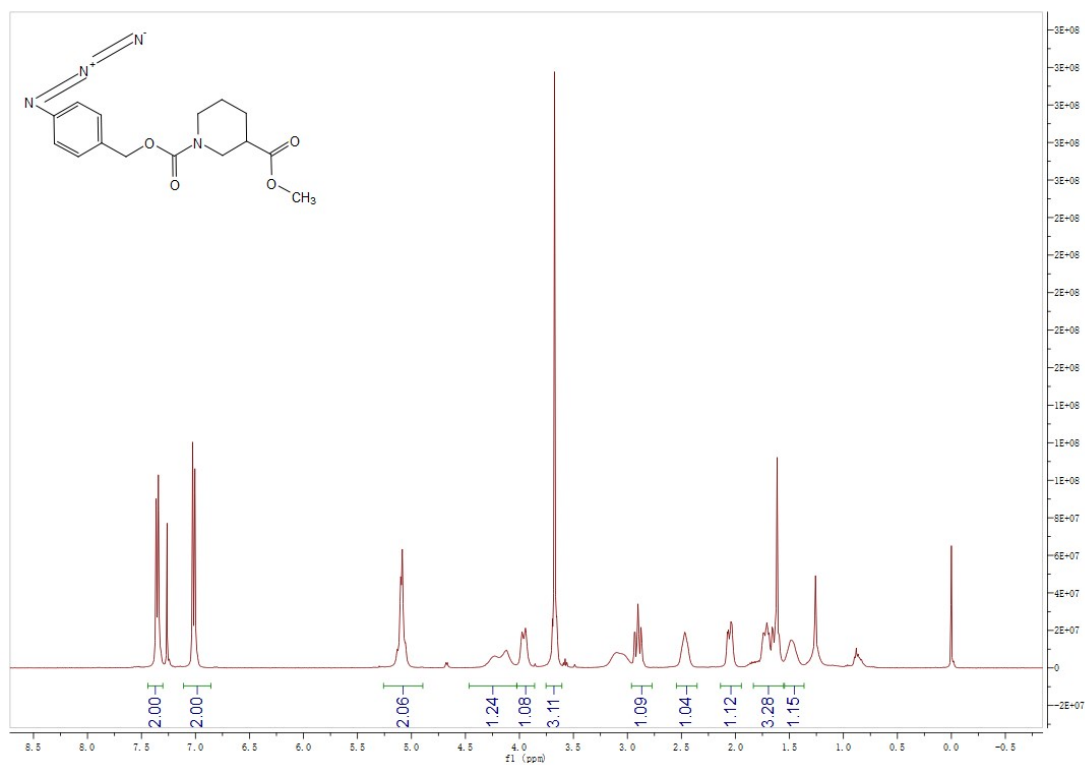


Figure S32. HNMR spectra of pro-MPCH. ^1H NMR (400 MHz, CDCl_3) δ 7.35 (d, $J = 8.2$ Hz, 1H), 7.02 (d, $J = 8.4$ Hz, 2H), 5.26 – 4.89 (m, 1H), 4.17 (d, $J = 42.7$ Hz, 1H), 3.96 (d, $J = 12.9$ Hz, 1H), 3.75 – 3.61 (m, 2H), 2.96 – 2.77 (m, 1H), 2.47 (s, 1H), 2.14 – 1.95 (m, 1H), 1.83 – 1.55 (m, 2H), 1.48 (s, 1H).