Supporting Information

Decahexanuclear Zinc(II) Coordination Container Featuring a Flexible Tetracarboxylate Ligand: A Self-Assembly Supermolecule for Highly Efficient Drug Delivery of Anti-Inflammatory Agents

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Supplementary Methods

General methods

^{1}H NMR spectra were recorded on a Bruker 400 MHz instrument with trimethylsilane (TMS) as internal standard. 2D DOSY NMR and NOESY experiments were performed on JNM-ECZ400S/L1 spectrometer at 20°C. UV-Vis absorption spectra were measured on a Perkin-Elmer Lambda 35 UV-Vis spectrophotometer. The excitation and emission spectra were collected using an Edinburgh FLS-920 fluorescence spectrometer. Powder X-ray diffraction results were obtained from RIGAKU Miniflex with Cu *Kα* radiation of λ = 1.5405 Å operated at 30 kV at the scan rate of 2 degree/min.

X-ray crystallography

Crystallographic data of crystal **ZnPMTC** were collected on Hybrid Pixel Array detector equipped with Ga-Kα radiation (λ = 1.3405 Å) at about 105 K. The structure was solved and refined using the Bruker SHELXTL Software Package, a computer program for automatic solution of crystal structures, and refined by the full-matrix least-squares method with ShelXle Version 4.8.6, a Qt graphical user interface for the SHELXL.[S1-S2] The electron count due to disordered solvent molecules in the void space of the crystals was calculated using the program SQUEEZE in PLATON software package[S3] and refined further using the data generated. Crystallographic data and structure refinements are summarized in Table S1. CCDC 2070333 contain the supplementary crystallographic data for this paper. These data can be obtained free of charge from the Cambridge Crystallographic Data Centre via www.ccdc.cam.ac.uk/data_request/cif.

Drugs encapsulation experiments

The host-guest interaction of **ZnPMTC** with drug molecules of diclofenac sodium (DCF),

naproxen (NPX), or aspirin (AP) was probed using the NMR titration technique. Stock solutions of **ZnPMTC** and drugs (DCF, NPX, or Aspirin) were prepared in DMSO-d_6 at a concentration of ~4×10^{-3} mol/L and ~0.03 mol/L, respectively. 0.40 mL of the **ZnPMTC** stock solution was then placed in an NMR tube, to which 2 to 200 μL of the drug solution was added in successive portions. After each addition, the tube was capped and inverted to ensure that the components were fully mixed to reach equilibration. The ^{1}H, 2D-DOSY, and 2D-NOESY NMR measurements were collected at room temperature.

Drugs release experiments

The sample of NPX@**ZnPMTC**, DCF@**ZnPMTC**, or Aspirin@**ZnPMTC** (~2–4 mg) was suspended in 15.0 mL of PBS buffer solution (pH = 7.4, 6.0, or 5.0) with stirring. The UV−vis spectra of the solutions were monitored at different time intervals to determine the release percentage of NPX, DCF or Aspirin. The amount of RAPA or FA released was determined from the UV−vis absorbance at specific wavelength (295 nm for RAPA and 350 for FA).

Cell viability assay

RAW 264.7 macrophages, purchased from Stem Cell Bank, Chinese Academy of Sciences (Shanghai, China), were cultured in complete α-MEM (α-MEM supplemented with 10% FBS, 100 U/mL penicillin/streptomycin). To assess the effect of **ZnPMTC** on cell viability, the cells were treated with increased concentration of **ZnPMTC** (0, 2.5, 5, 10, 20, 40 μg/ml) for 24 h. After treatment, the cells were incubated for Calcein-AM and PI solution (Invitrogen, CA, USA) for 15 min. Living cells were detected in green fluorescence, while dead cells were detected in red fluorescence, respectively.

To measure the cell viability in a quantitative approach, RAW 264.7 macrophages were seeded into 96-well plates at a density of 8×10^3 cells/well. After adhering to the wall, the cells were incubated with increased concentrations of **ZnPMTC** (0, 2.5, 5, 10, 20, 40 μg/ml) for 48 and 96 h, respectively. After treatment, 10 μL CCK-8 solution (Dojindo Molecular Technologies, Kumamoto, Japan) was added into each well. After incubating for 2 h, the absorbance was detected at a wavelength of 450 nm (630 nm as the reference wavelength) by using a microplate reader. The cell viability was indicated as percent cell viability, with the viability of the control cells set at 100%.

Hemolysis test

To further evaluate the hemocompatibility of ZnPMTC, fresh blood was harvested from healthy C57BL/6 mice, collected in anticoagulated tubes. The red blood cells (RBCs) were collected after being centrifuged and washed for several times. The RBCs were diluted with PBS for 5% (v/v) erythrocyte suspension. Then a suspension of RBCs was added into the tubes with various concentrations of ZnPMTC. Double distilled water and PBS were used as positive and negative controls, respectively. The mixture was incubated for 2 h at 37 °C and centrifuged at 3,000 rpm for 5 min. The absorbance of the supernatant was determined at 540 nm by a microplate reader. The hemolytic rate was calculated as follows: Hemolytic rate (%) = (ODsample-ODnegative)/(ODpositive-ODnegative) × 100%.

Cell cycle analysis

For cell cycle analysis, cells were exposed to various concentrations of ZnPMTC for 24 h, then collected and fixed in cold 70 % ethanol at 4 °C for 30 min. After centrifugation at 1000 rpm for 5 min, the cells were suspended and incubated with 50 μg/mL PI (Beyotime, Shanghai, China) for 30

min. Then the cell cycle analysis was performed by a FACScan flow cytometer (BD, CA, USA) for at least 20,000 cells per sample, then analyzed by FlowJo software.

Cell Uptake test

To investigate the endocytosis of ZnPMTC, RAW 264.7 cells were seeded and incubated with ZnPMTC or RB@ZnPMTC for 6 h. Then the cells were observed under a confocal fluorescence microscope (Leica TCS-SP5, DM6000-CFS). To explore the intracellular transportation of ZnPMTC, RAW 264.7 cells were incubated with RB@ZnPMTC for 2, 12 and 24 h, respectively. Then the cells were treated with LysoTracker Green (Yeasen, China) for 1 h. After washing with PBS for 3 times, Hoechst 33342 was used for nuclear staining.

Quantitative PCR analysis

RAW 264.7 macrophages were seeded in 6-well plates at a density of 4×10^5 cells/well. Pretreated with various treatment groups (ZnPMTC, NPX, NPX@ ZnPMTC) for 24 h, cells were stimulated with LPS plus IFN-γ for another 24 h, with various treatment groups during the inflammatory induction. According to the manufacturer's instructions, total RNA was extracted by Axygen RNA Miniprep Kit (Axygen, Union City, CA, USA). Reverse transcription was performed by the Prime Script RT reagent Kit (Takara Biotechnology, Otsu, Shiga, Japan) to obtain cDNA from the RNA template. Subsequently, a real-time PCR assay was performed on an ABI 7500 Sequencing Detection System (Applied Biosystems, Foster City, CA) using the SYBR® Premix Ex Taq™ II (Takara Biotechnology, Otsu, Shiga, Japan). Briefly, 5 μL of TB Green, 3 μL of ddH_2O, 1 μL of cDNA, 0.4 μL of each primer and 0.2 μL ROX Dye2 were mixed to make up a total volume of 10 μL for each PCR. Cycling conditions were 40 cycles of 95 °C for 5 s and 60 °C for 30 s. The specificity of

amplification was verified by performing reverse transcription PCR and analyzing the melting curves. The comparative $2^{-\Delta\Delta CT}$ method was used to calculate the relative expression levels of each gene. GAPDH was included as housekeeping gene, and all reactions were run in triplicate. The sequences of the primers are listed as follows: mouse GAPDH: forward, 5′-ACCCAGAAGACTGTGGATGG-3′ and reverse, 5′-CACATTGGGGGTAGGAACAC-3’; mouse TNF-α: forward, 5′-GCCTCTTCTCATTCCTGCTTGTGG-3′ and reverse, 5′- GTGGTTTGTGAGTGTGAGGGTCTG-3’; mouse IL-1β: forward, 5′- TCGCAGCAGCACATCAACAAGAG-3′ and reverse, 5′-AGGTCCACGGGAAAGACACAGG-3’; mouse IL-6: forward, 5′- TGCCTTCTTGGGACTGAT -3′ and reverse, 5′-CTGGCTTTGTCTTTCTTGTT-3’; mouse iNOS: forward, 5′-ACTCAGCCAAGCCCTCACCTAC-3′ and reverse, 5′- TCCAATCTCTGCCTATCCGTCTCG-3’.

Immunofluorescence for polarized macrophages

Pretreated with various treatment groups for 24 h, RAW 264.7 macrophages were stimulated with LPS plus IFN-γ for M1 polarization for another 24 h. The polarized macrophages were fixed in 4% paraformaldehyde for 20 minutes, washed three times with PBS. After being blocked in 5% goat serum for 60 min, the cells were incubated with primary antibodies against iNOS (Abcam, dilution 1:100) overnight. The fluorescent second antibodies were incubated for 1 h. Further, the nucleus was stained with 4,6-diamidino-2-phenylindole (DAPI) before imaging.

Table S1. Crystallographic Data for Coordination container ZnPMTC.

Empirical formula	$C_{256}H_{248}N_8O_{84}S_{16}Zn_{16}$
Formula weight	6339.49
Temperature (K)	105(8)
Wavelength	1.34050
Crystal system	Monoclinic
space group	P2(1)/c
a (Å)	26.4005(7)
b (Å)	17.3776(8)
c (Å)	47.1016(8)
α (°)	90
b (°)	96.7505(19)
γ (°)	90
V (Å^3)	21459.3(12)
Z	2
D(calcd) (g cm^{-3})	0.981
μ (Mo $K_α$) (mm^{-1})	1.413
F(000)	6496
θ range (°)	2.326 - 48.800
Limiting indices	$-29 \le h \le 29$; $-19 \le k \le 18$; $-52 \le l \le 52$
Reflections collected / unique	109084 / 30780 [R_{int} = 0.0337]
Data / restraints / parameters	30780 / 1268 / 1927
GOF	1.059
R_1 ($I>2\sigma(I)$)	0.0931
wR_2 ($I>2\sigma(I)$)	0.2318
R_1 (all data)	0.1349
wR_2 (all data)	0.2590
Δρ / e Å^{-3}	0.700, -0.442

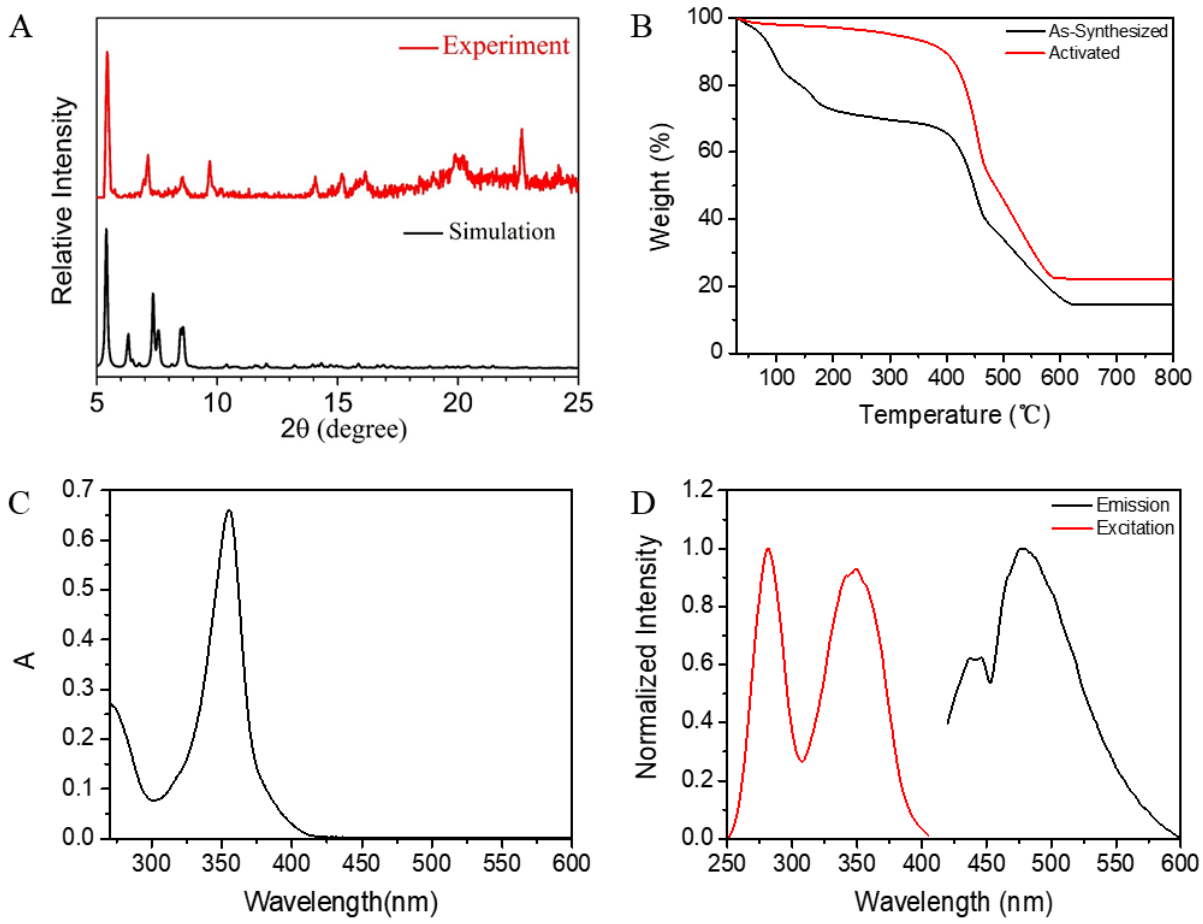

Figure S1. (A) Powder X-ray diffraction patterns, (B) TGA, (C) UV-vis absorption spectrum, and (D) excitation (red) and emission (black) spectra of **ZnPMTC**.

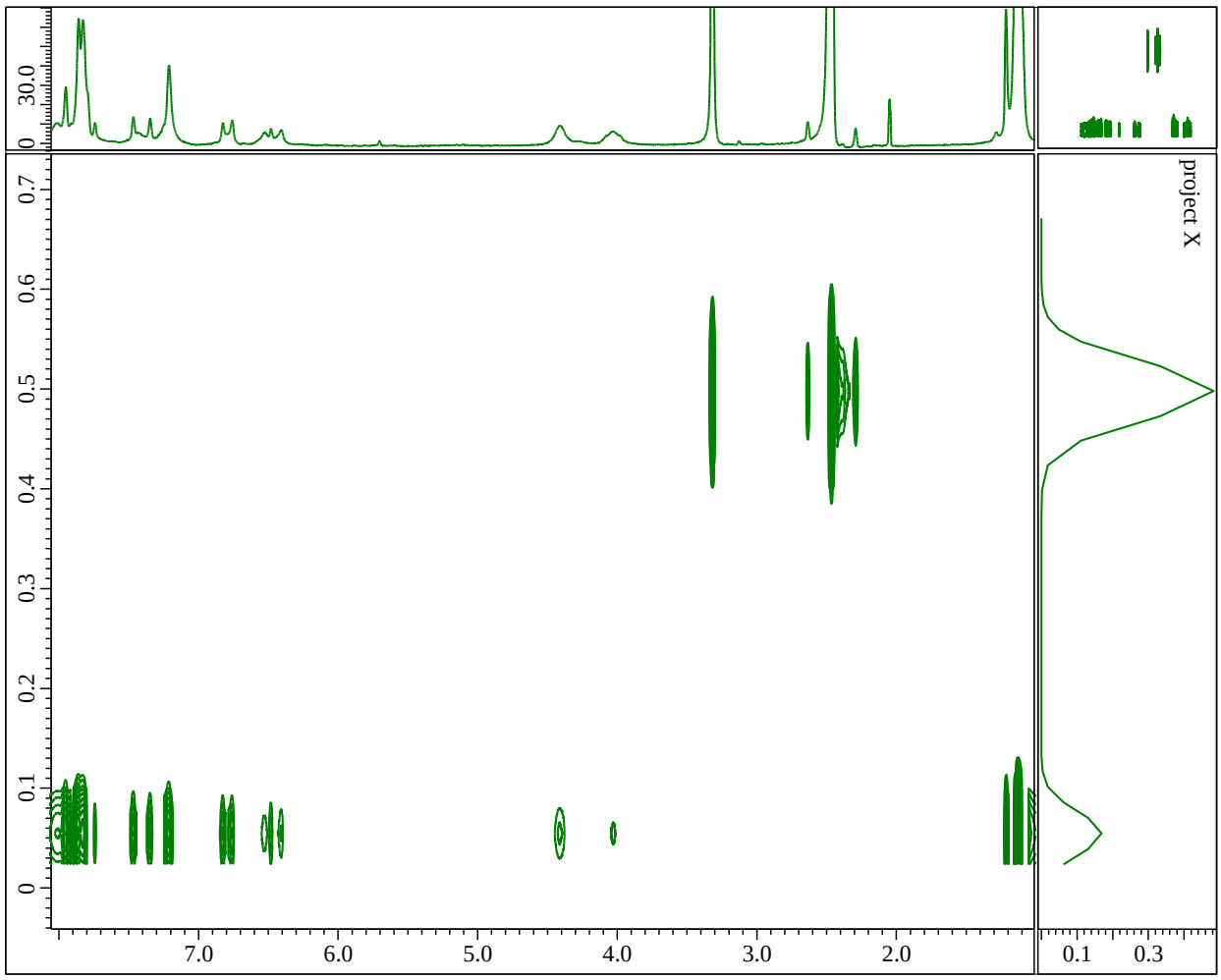

Figure S2. DOSY NMR (400 MHz, DMSO-d_6, 25°C**)** spectrum of **ZnPMTC**, indicating a single species of ZnPMTC with diffusion co-efficient value of 8.12×10-11 m^2/s.

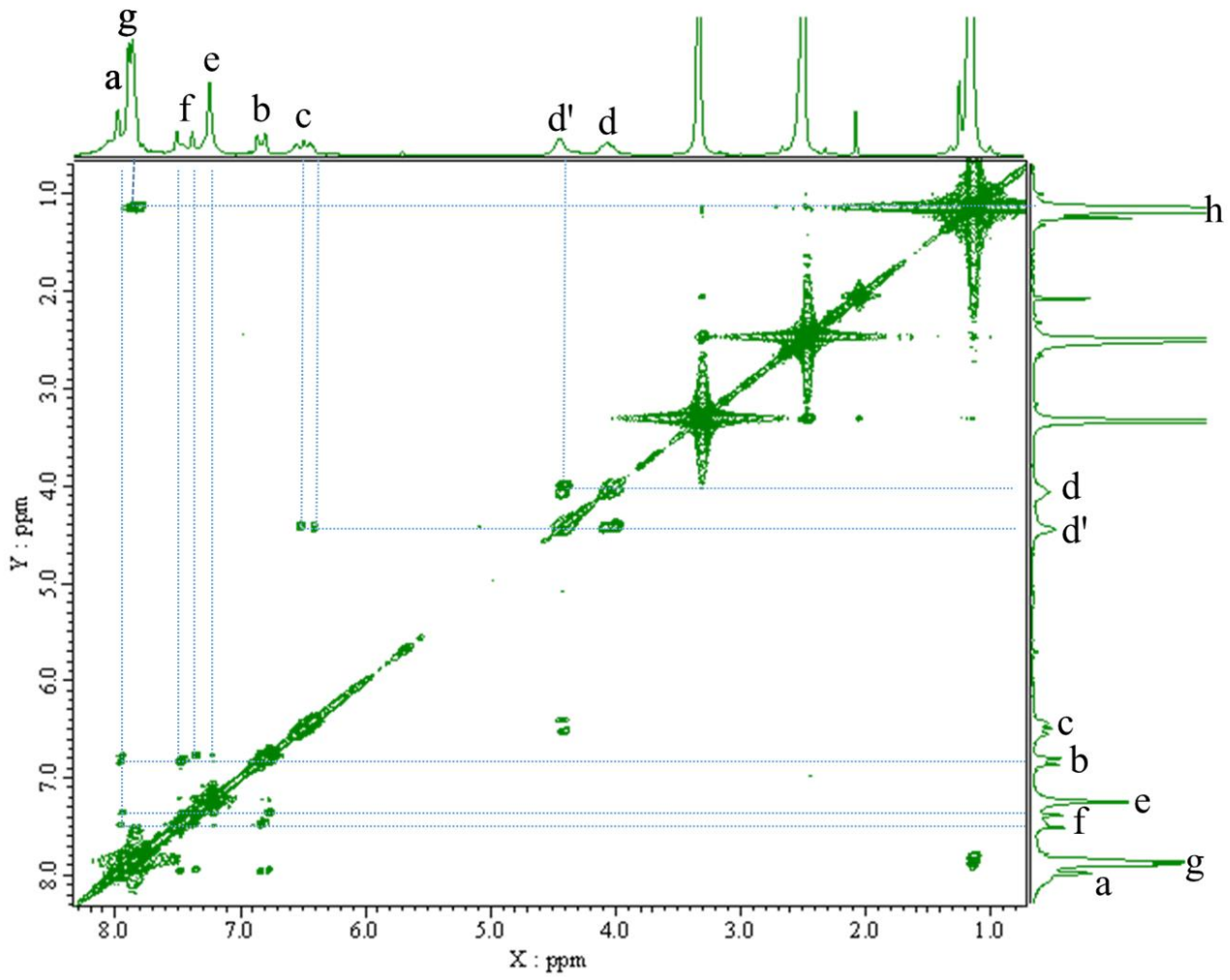

Figure S3. COSY NMR (400 MHz, DMSO-d_6, 25°C**)** spectrum of **ZnPMTC**

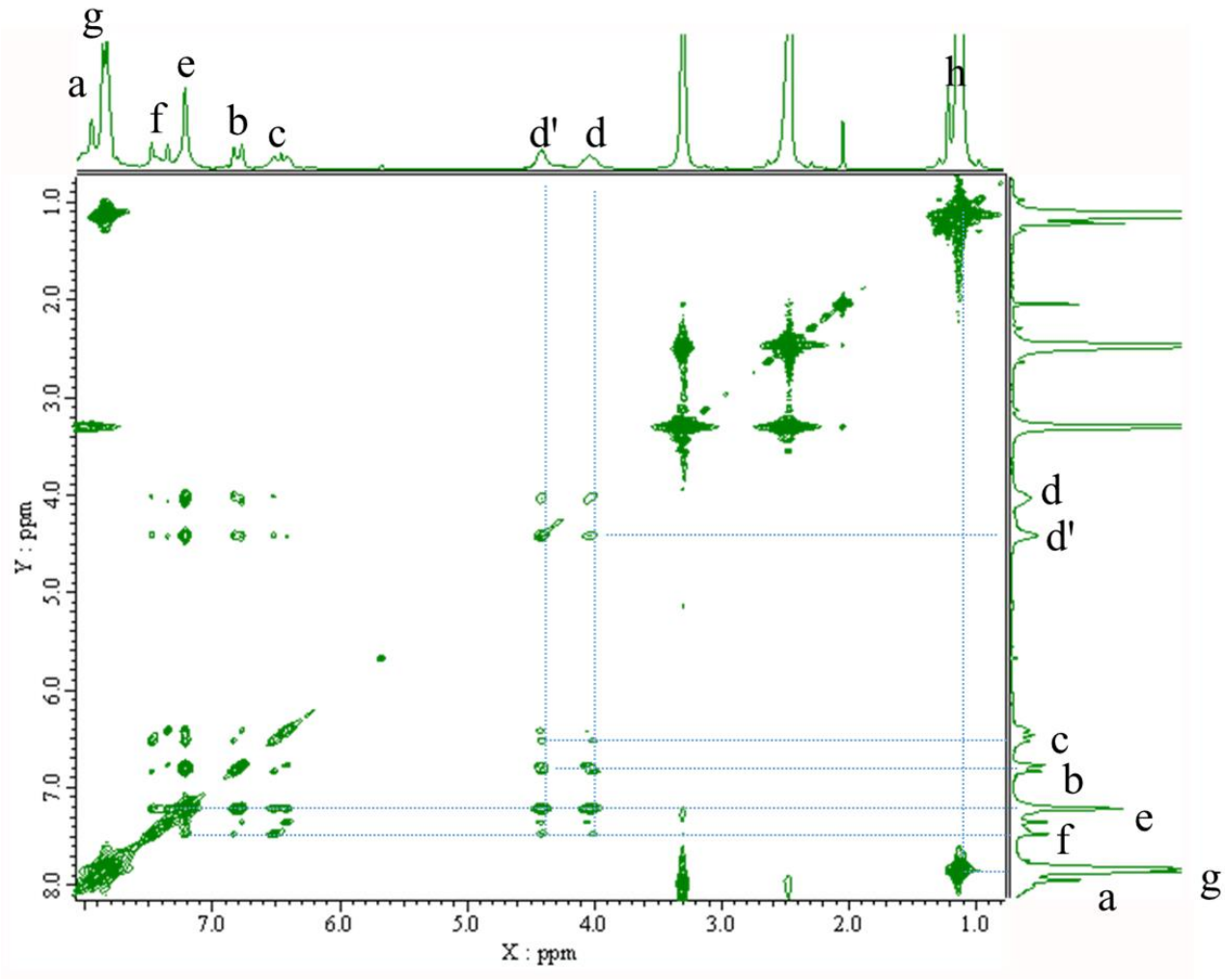

Figure S4. NOEY NMR (400 MHz, DMSO-d_6, 25°C**)** spectrum of **ZnPMTC**

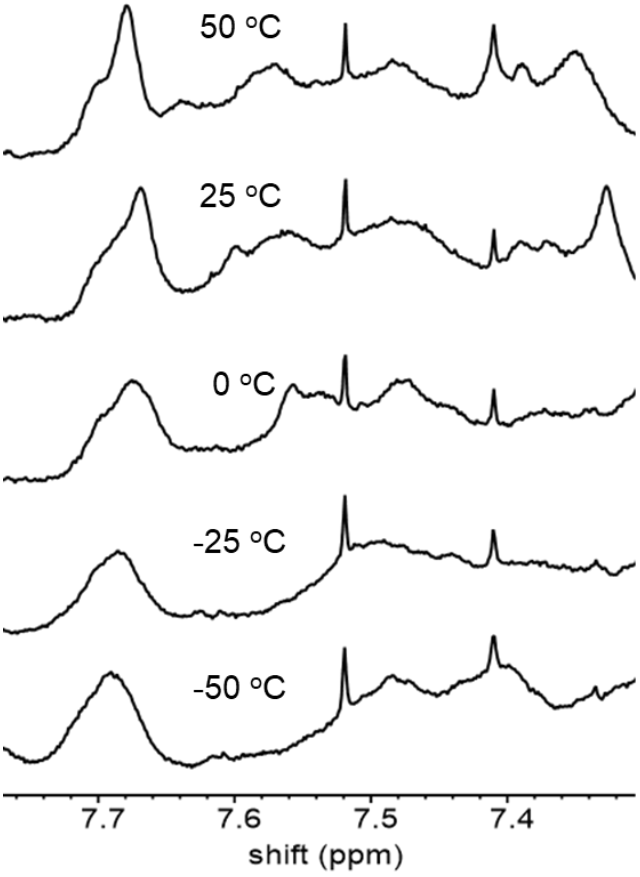

Figure S5. Variable temperature ^{1}H NMR spectra of **ZnPMTC** in $CDCl_3$ solution.

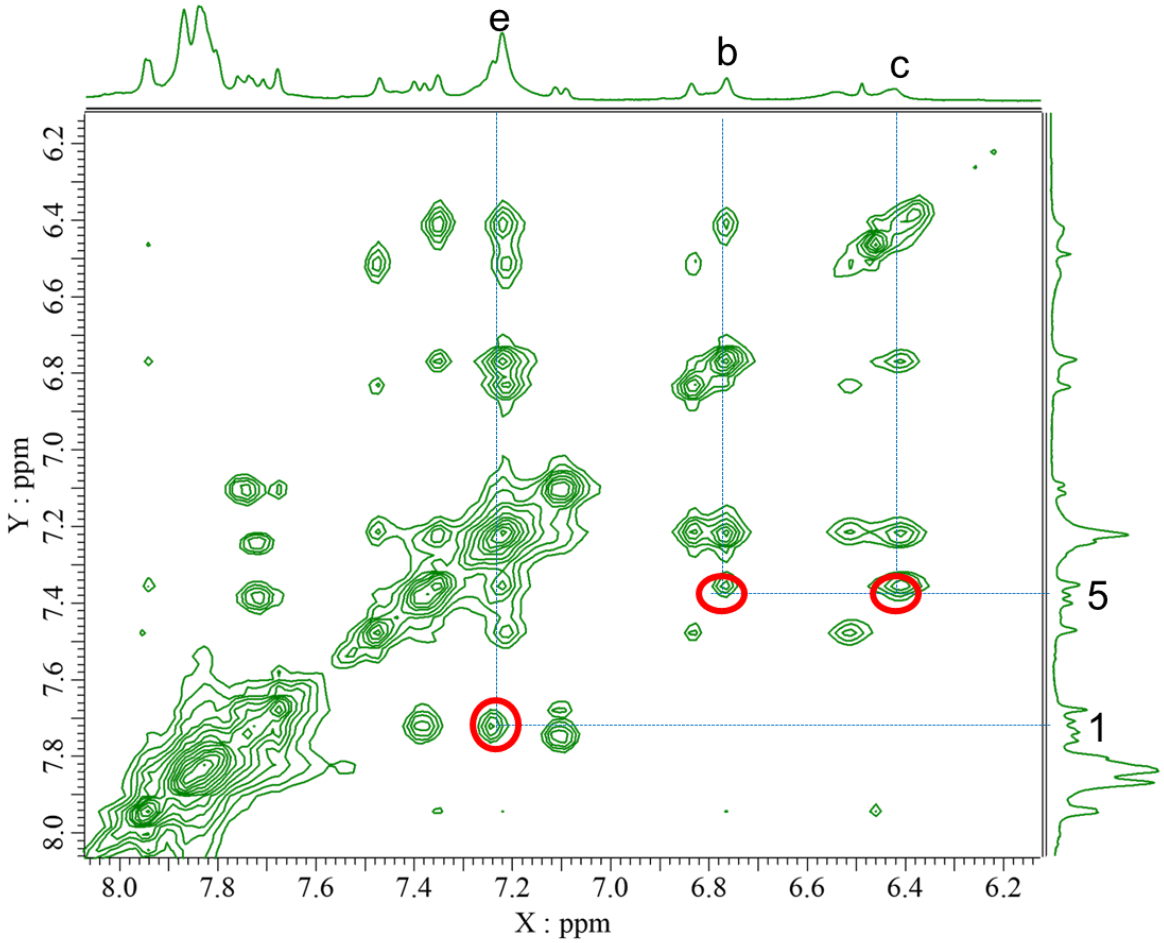

Figure S6. The ^{1}H-^{1}H NOESY spectrum of NPX@**ZnPMTC** at the ratio of **ZnPMTC** : NPX = 1 : 2 in DMSO-d_6. The red circles highlight the *endo*-encapsulation of two equiv. of NPX.

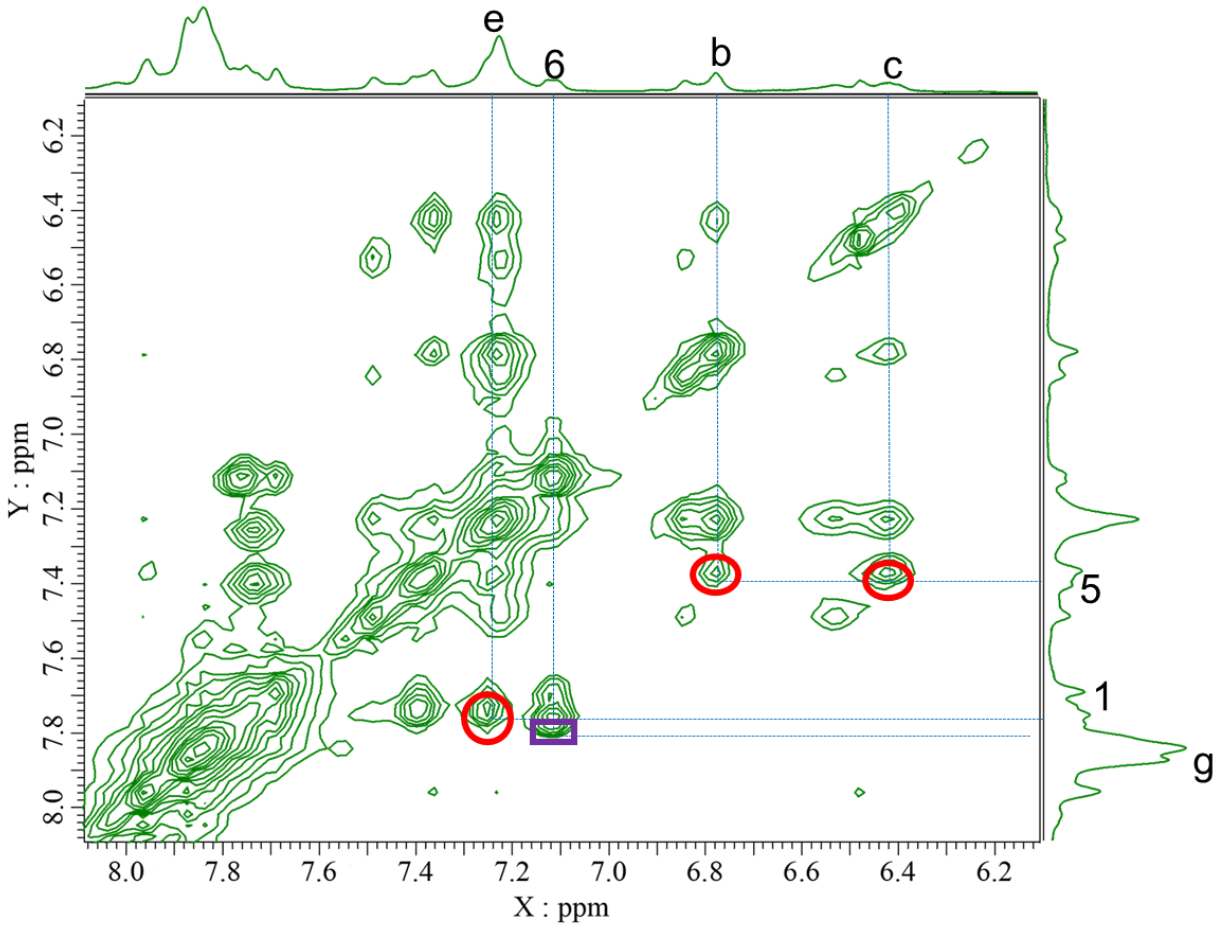

Figure S7. The ^{1}H-^{1}H NOESY spectrum of NPX@**ZnPMTC** at the ratio of **ZnPMTC** : NPX = 1 : 3 in DMSO-d_6. The red circles highlight the *endo*-encapsulation of two equiv. of NPX, and the purple square indicate the *exo*-encapsulation of one equiv. of NPX.

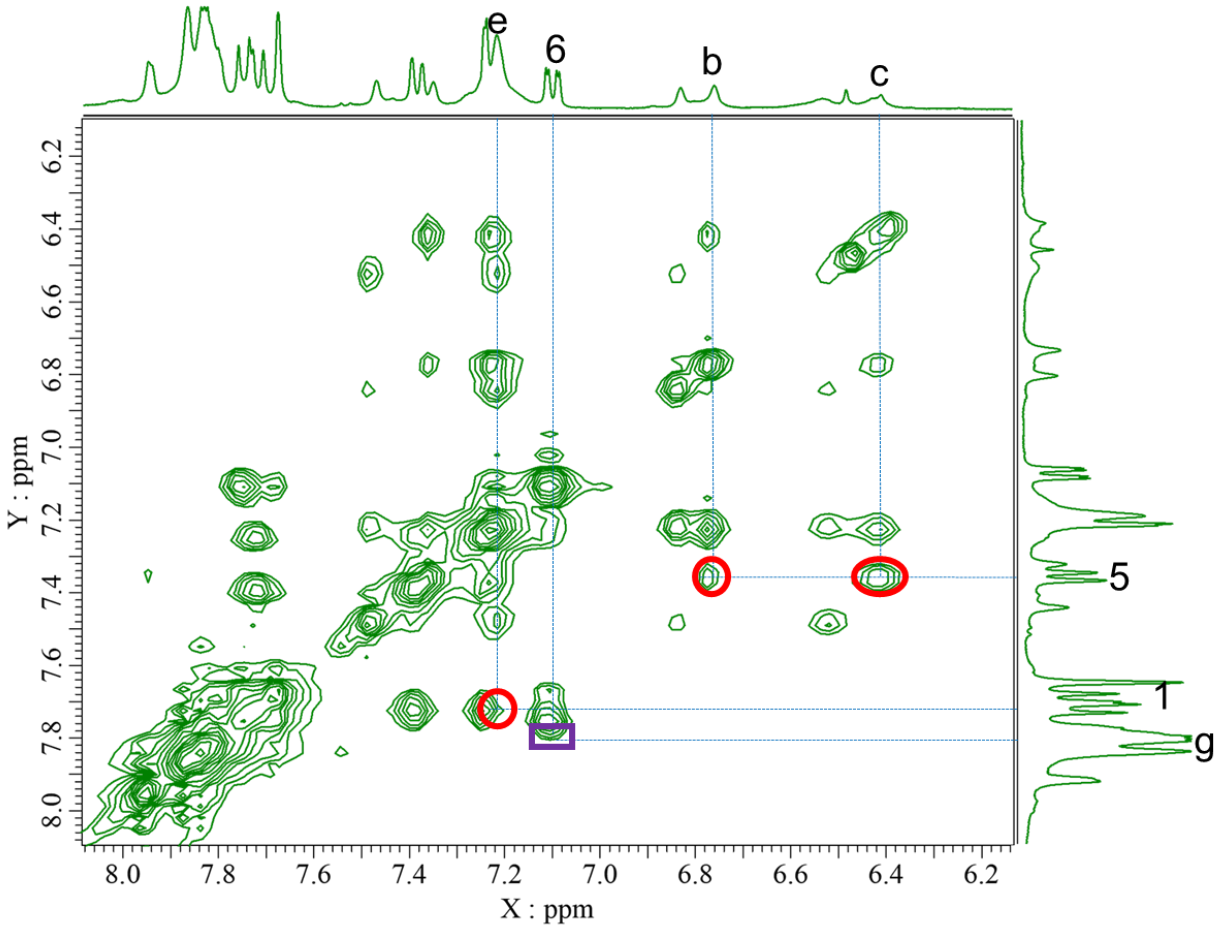

Figure S8. The ^{1}H-^{1}H NOESY spectrum of NPX@**ZnPMTC** at the ratio of **ZnPMTC** : NPX = 1 : 6 in DMSO-d_6. The red circles highlight the *endo*-encapsulation of two equiv. of NPX, and the purple square indicate the *exo*-encapsulation of four equiv. of NPX.

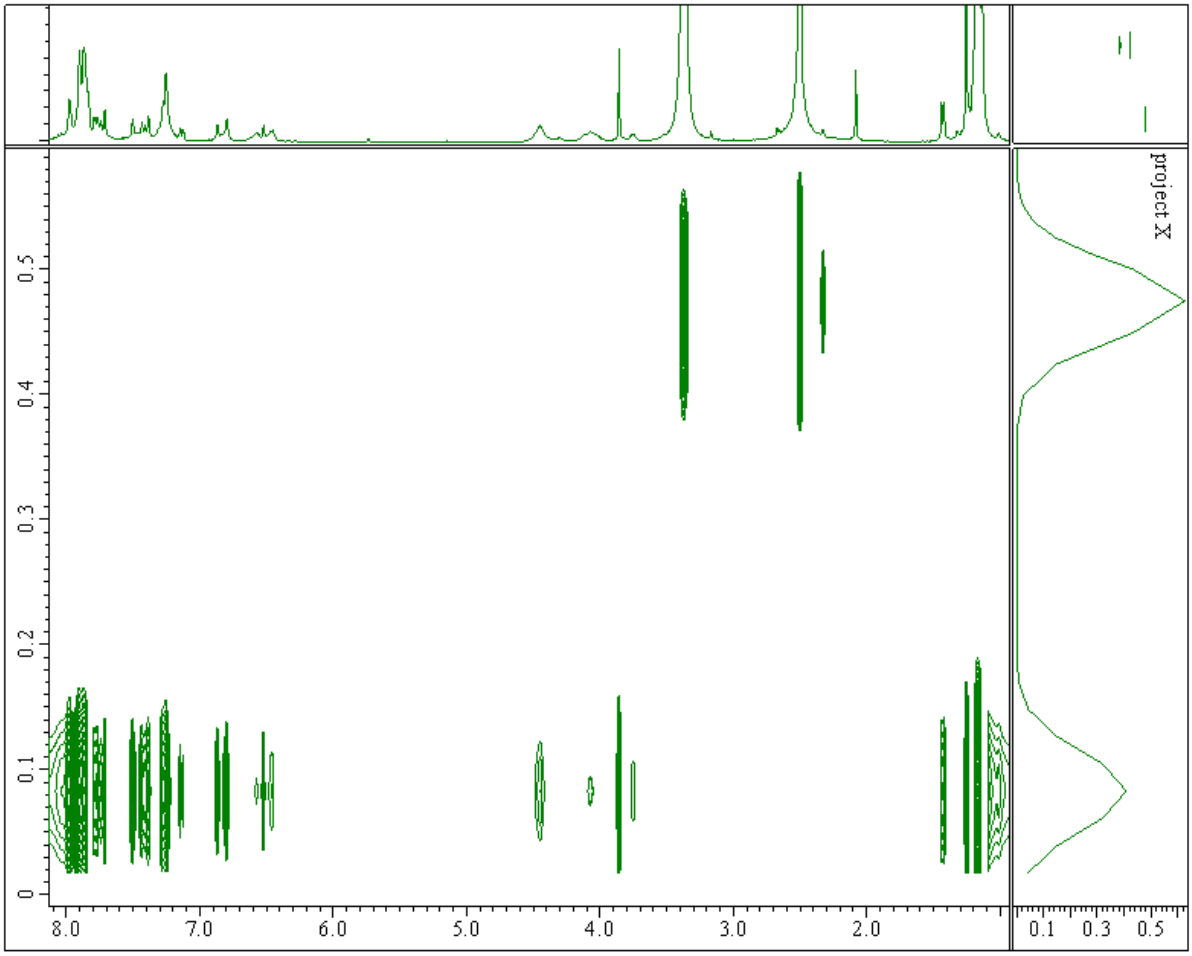

Figure S9. DOSY NMR (400 MHz, DMSO-d_6, 20°C**)** spectrum of NPX@**ZnPMTC** at the ratio of **ZnPMTC** : NPX = 1 : 2. Signals diffuse with observed diffusion coefficient value of 7.70×10^{-11} m^2/s.

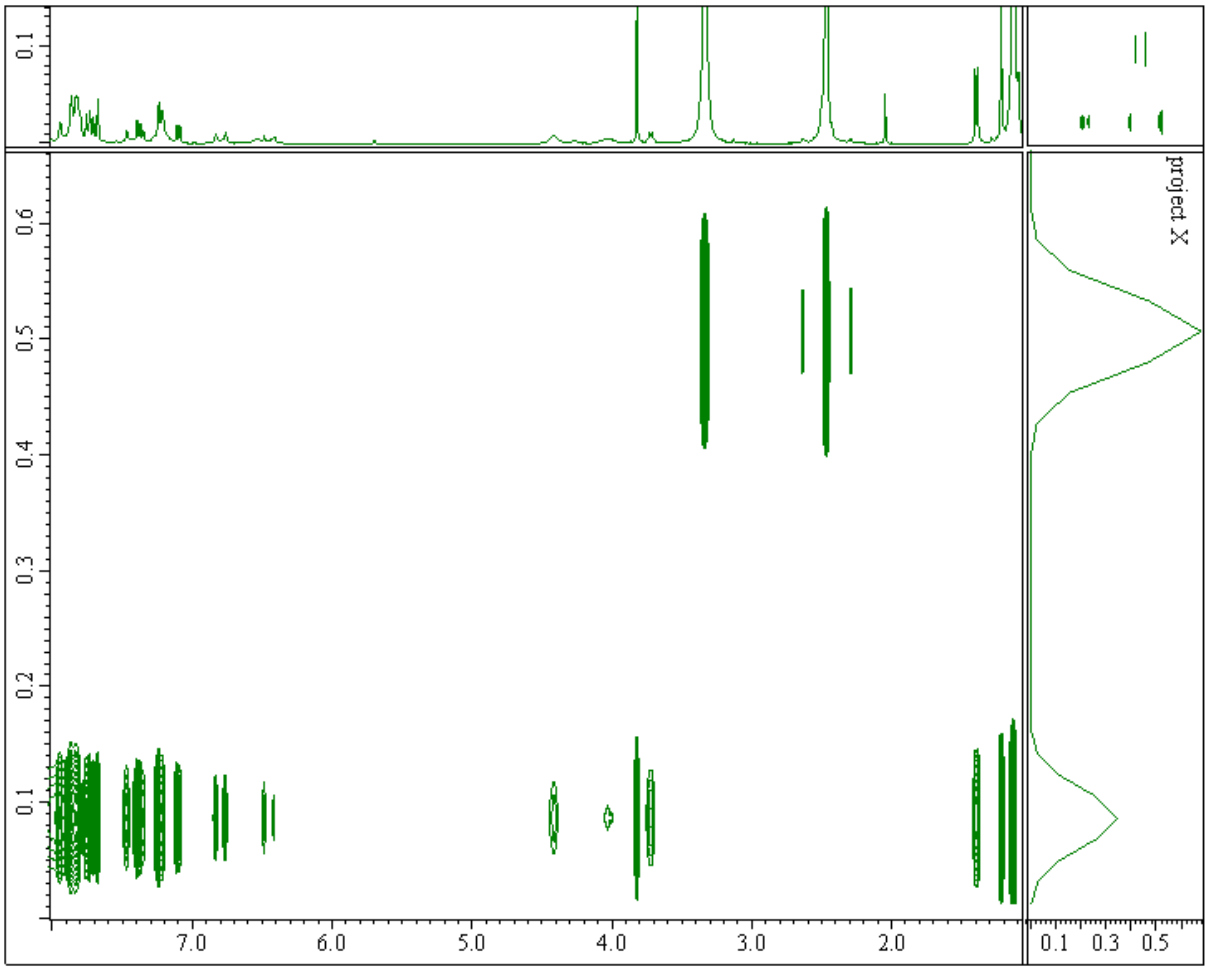

Figure S10. DOSY NMR (400 MHz, DMSO-d_6, 20°C**)** spectrum of NPX@**ZnPMTC** at the ratio of **ZnPMTC** : NPX = 1 : 6. Signals diffuse with observed diffusion coefficient value of 7.12×10^{-11} m^2/s.

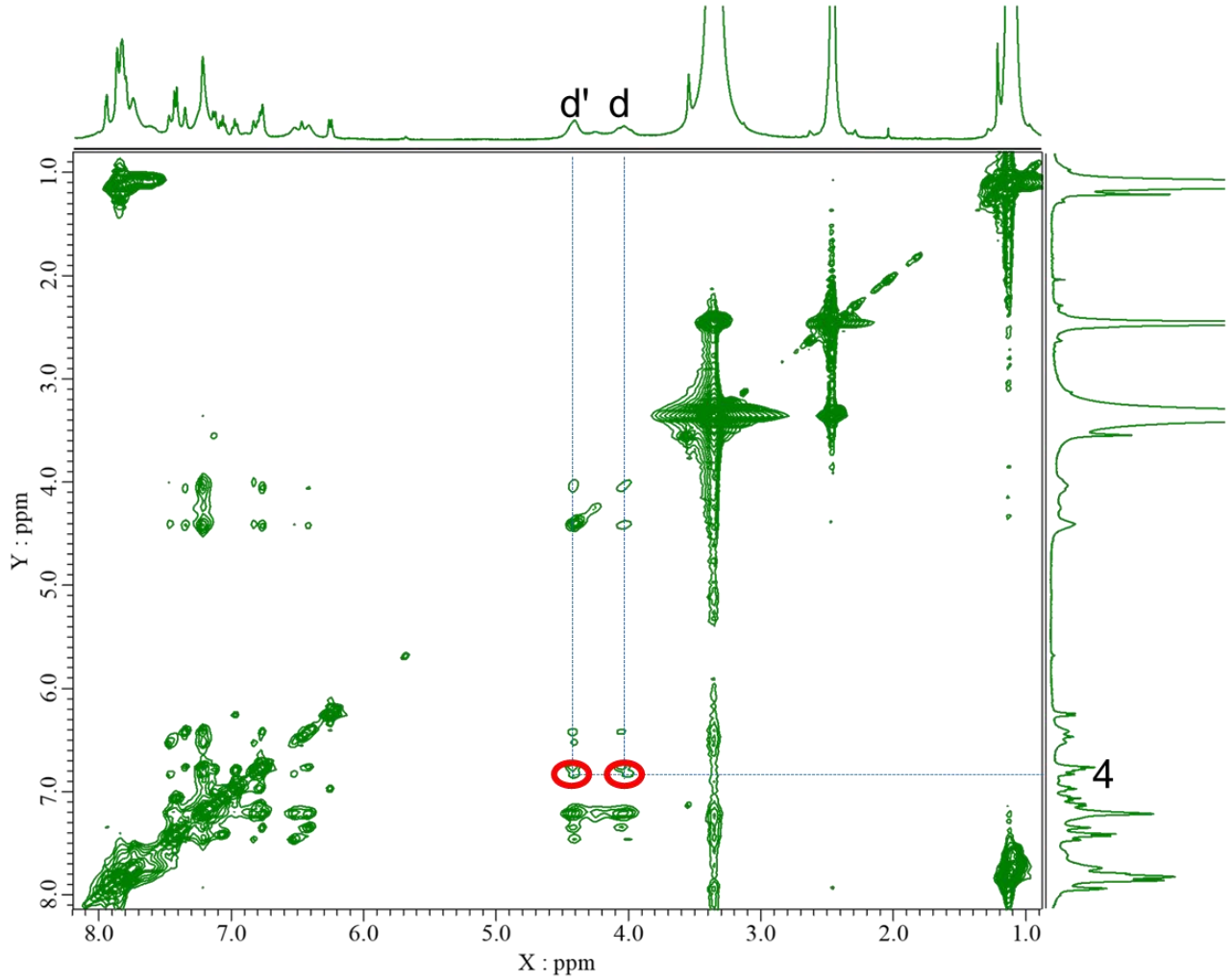

Figure S11. The ^{1}H-^{1}H NOESY spectrum of DCF@**ZnPMTC** at the ratio of **ZnPMTC** : DCF = 1 : 3 in DMSO-d_6. The red circles highlight the *endo*-encapsulation of three equiv. of DCF.

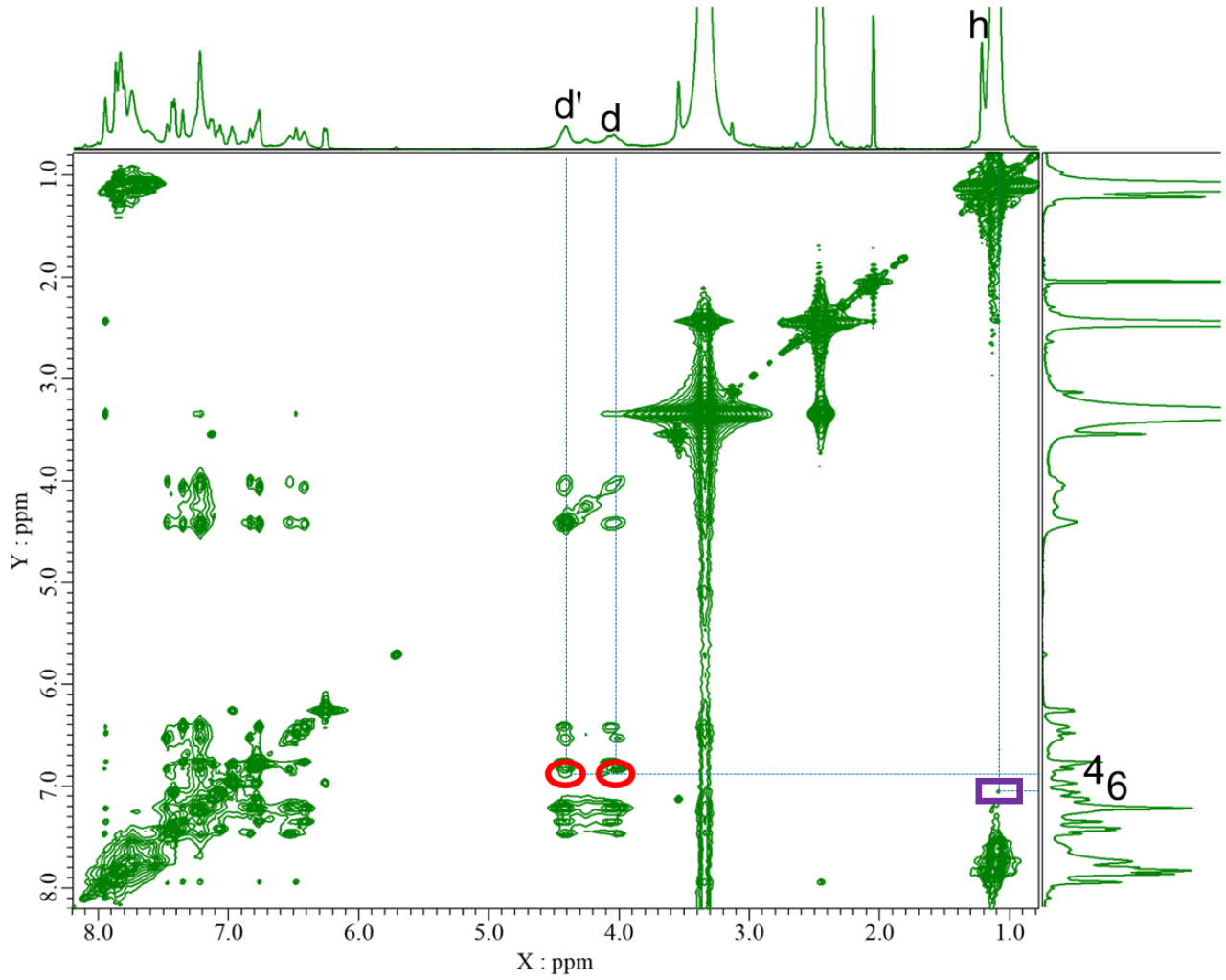

Figure S12. The ^{1}H-^{1}H NOESY spectrum of DCF@**ZnPMTC** at the ratio of **ZnPMTC** : DCF = 1 : 4 in DMSO-d_6. The red circles highlight the *endo*-encapsulation of three equiv. of DCF, and the purple square indicate the *exo*-encapsulation of one equiv. of DCF.

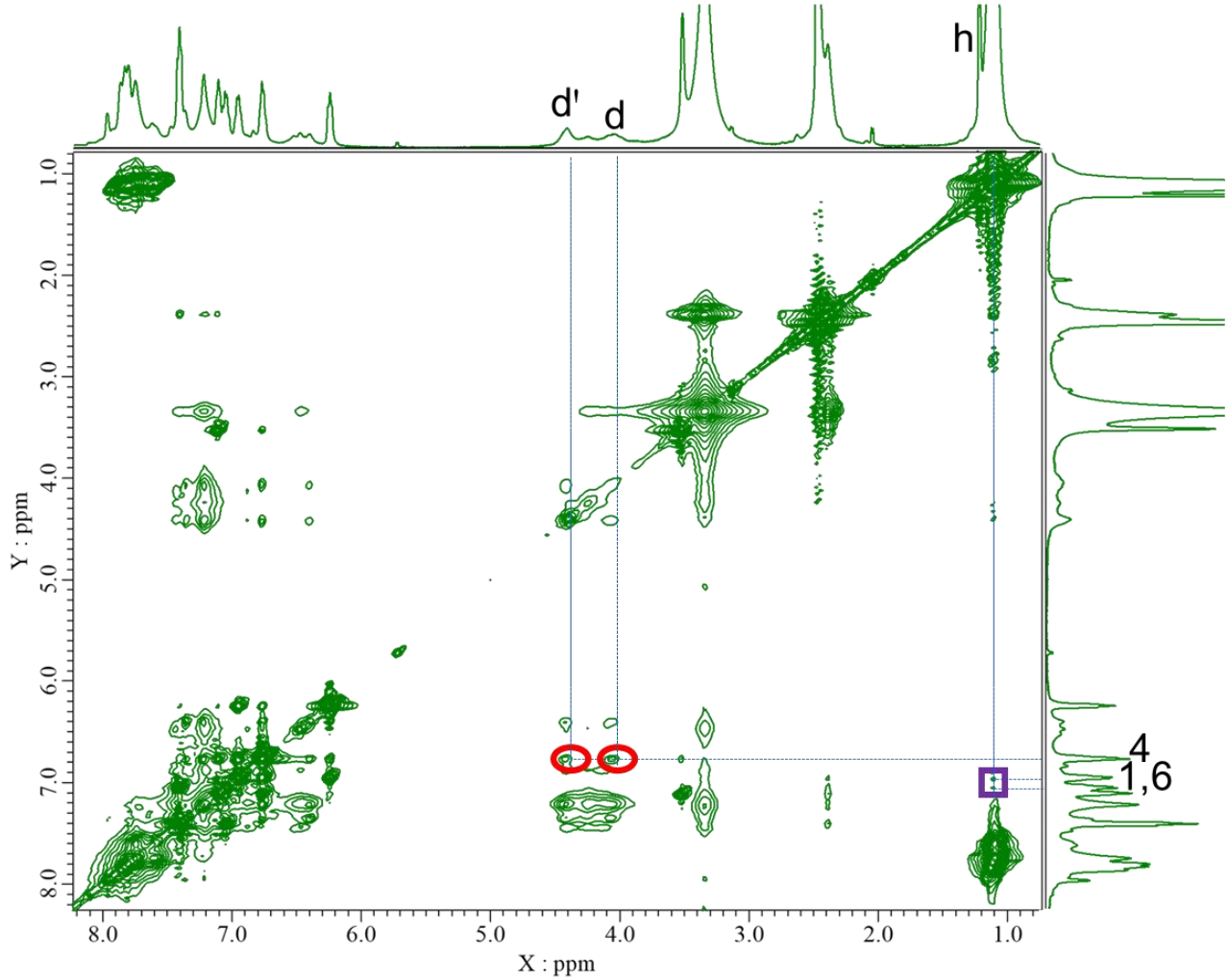

Figure S13. The ^{1}H-^{1}H NOESY spectrum of DCF@**ZnPMTC** at the ratio of **ZnPMTC** : DCF = 1 : 7 in DMSO-d_6. The red circles highlight the *endo*-encapsulation of three equiv. of DCF, and the purple square indicate the *exo*-encapsulation of four equiv. of DCF.

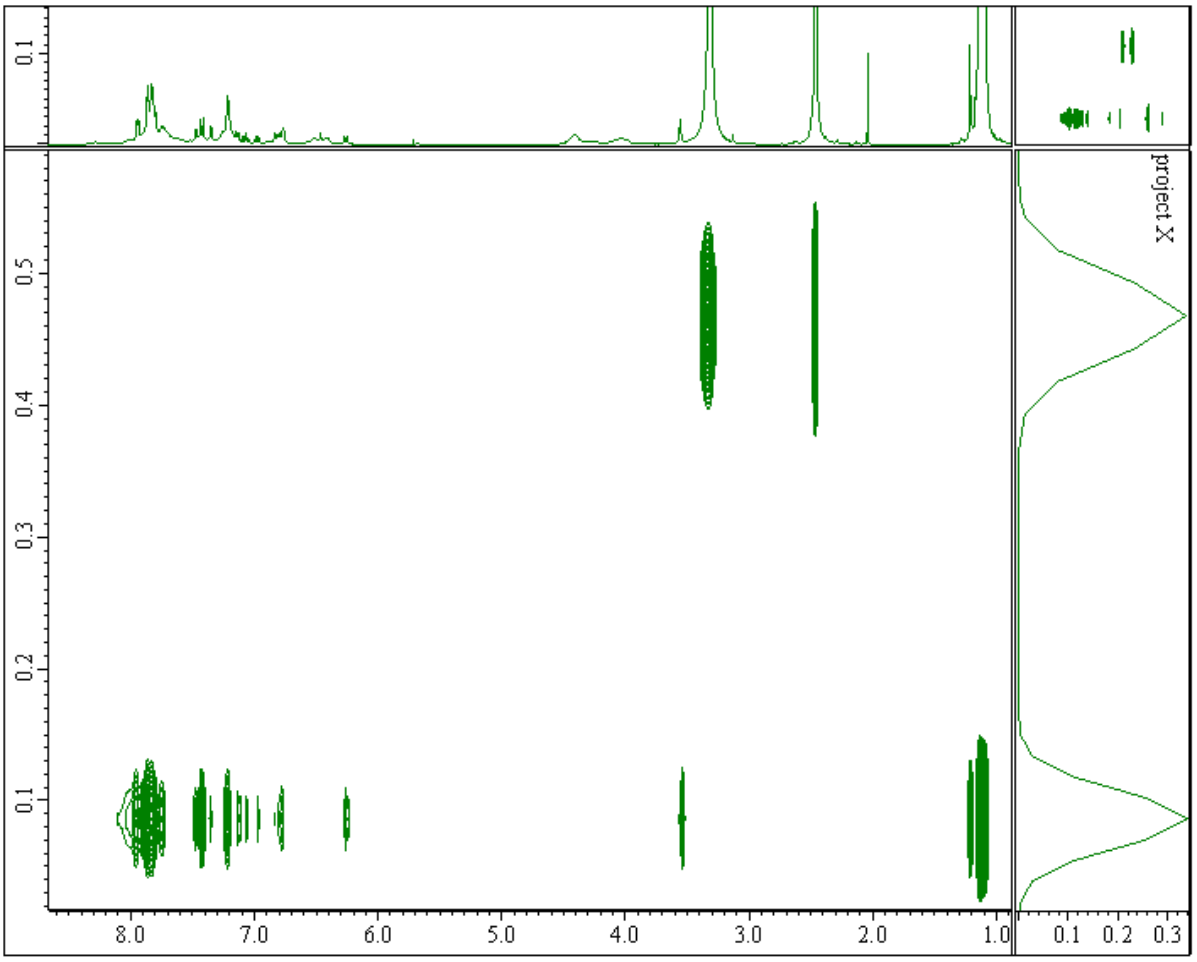

Figure S14. DOSY NMR (400 MHz, DMSO-d_6, 20°C**)** spectrum of DCF@**ZnPMTC** at the ratio of **ZnPMTC** : DCF = 1 : 3. Signals diffuse with observed diffusion coefficient value of 7.56×10^{-11} m^2/s.

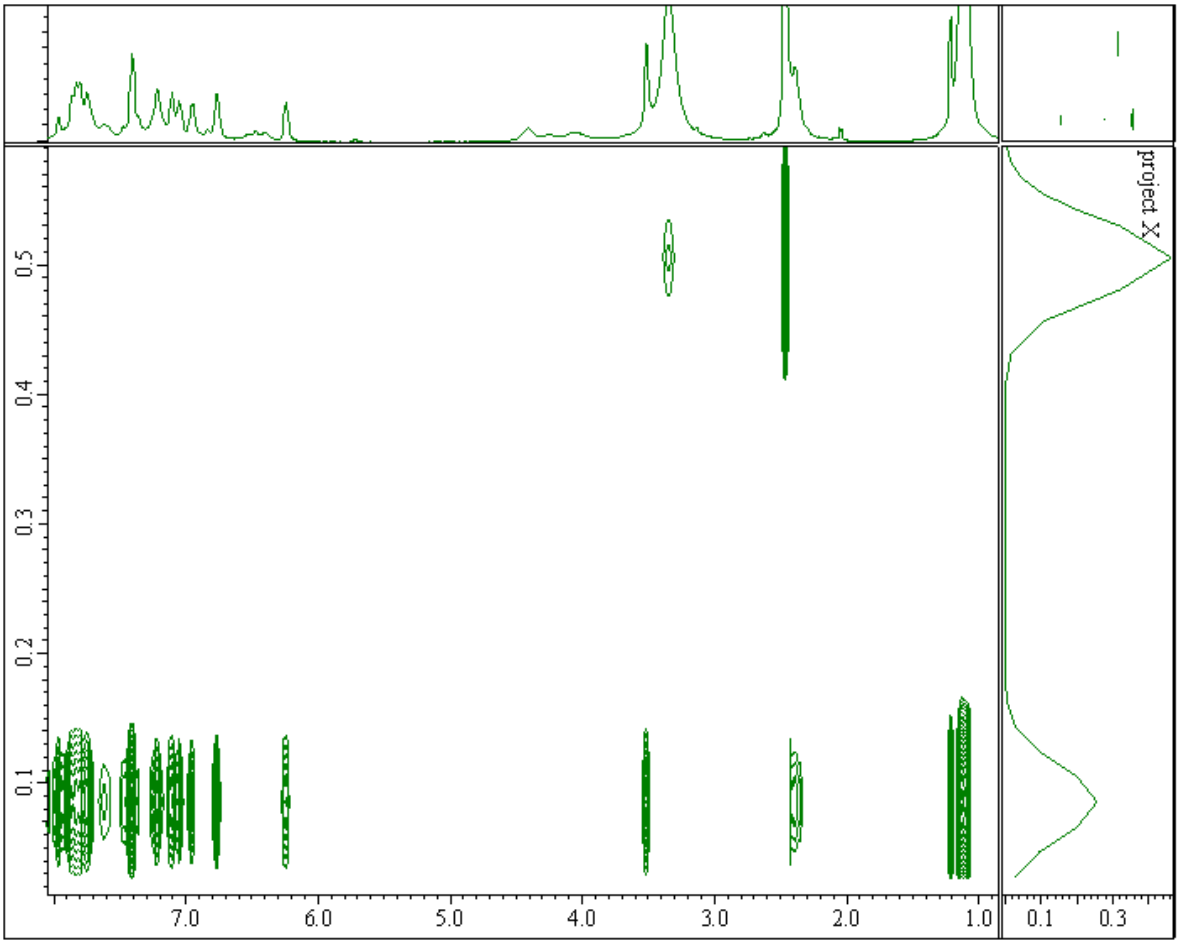

Figure S15. DOSY NMR (400 MHz, DMSO-d_6, 20°C**)** spectrum of DCF@**ZnPMTC** at the ratio of **ZnPMTC** : DCF = 1 : 7. Signals diffuse with observed diffusion coefficient value of 7.11×10^{-11} m^2/s.

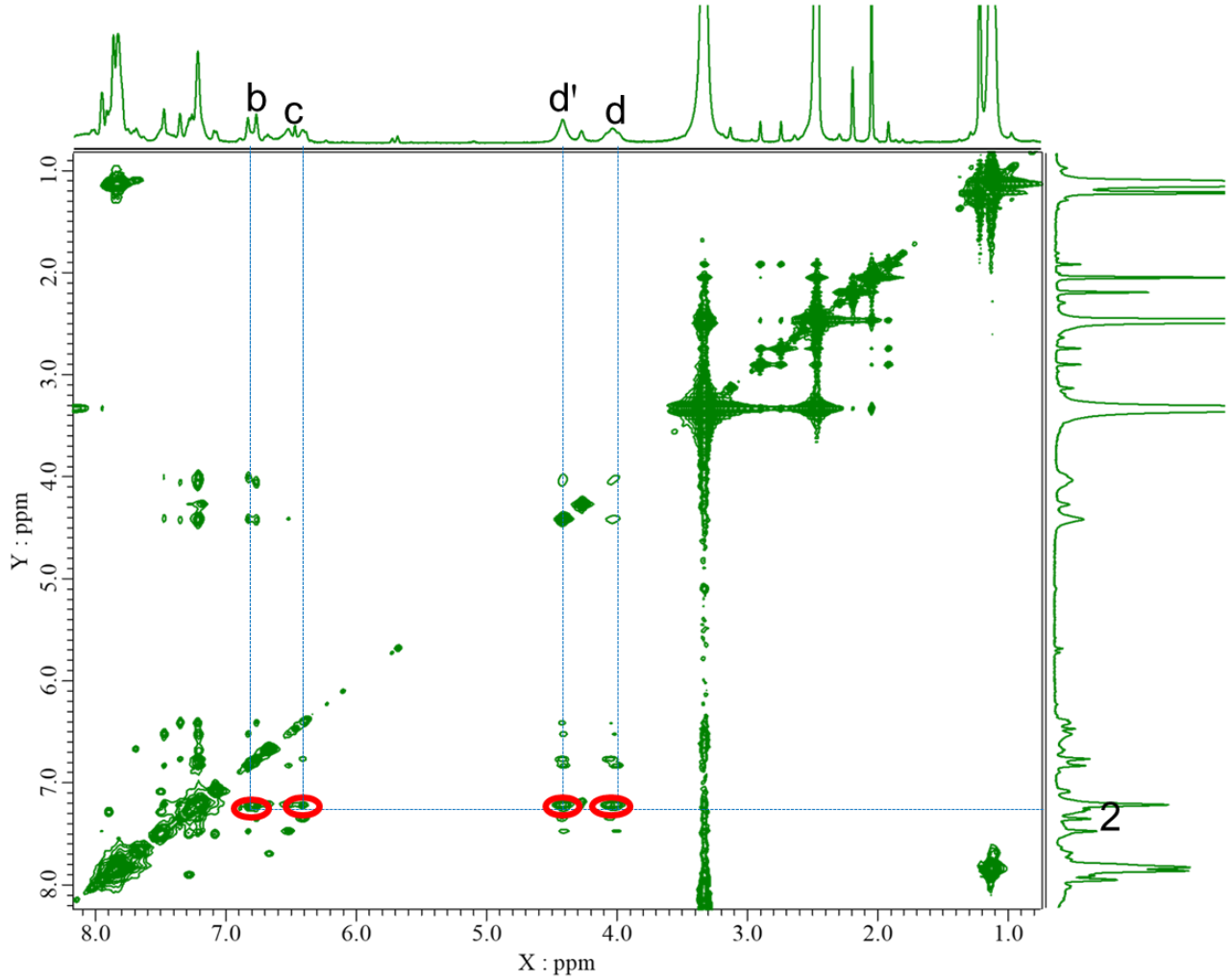

Figure S16 The ^{1}H-^{1}H NOESY spectrum of AP@**ZnPMTC** at the ratio of **ZnPMTC** : AP = 1 : 4 in DMSO-d_6. The red circles highlight the *endo*-encapsulation of four equiv. of AP.

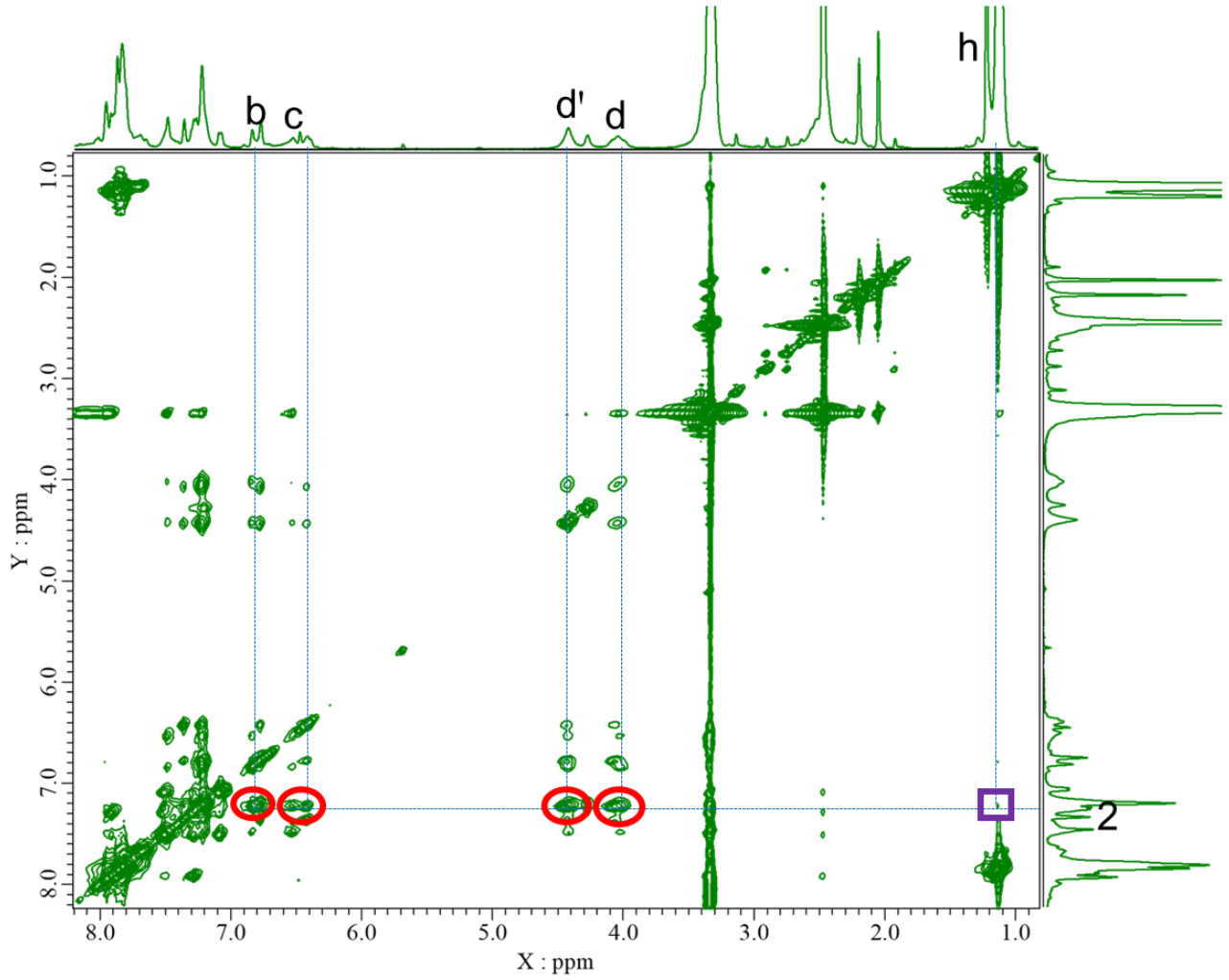

Figure S17. The ^{1}H-^{1}H NOESY spectrum of AP@**ZnPMTC** at the ratio of **ZnPMTC** : AP = 1 : 5 in DMSO-d_6. The red circles highlight the *endo*-encapsulation of four equiv. of AP, and the purple square indicate the *exo*-encapsulation of one equiv. of AP.

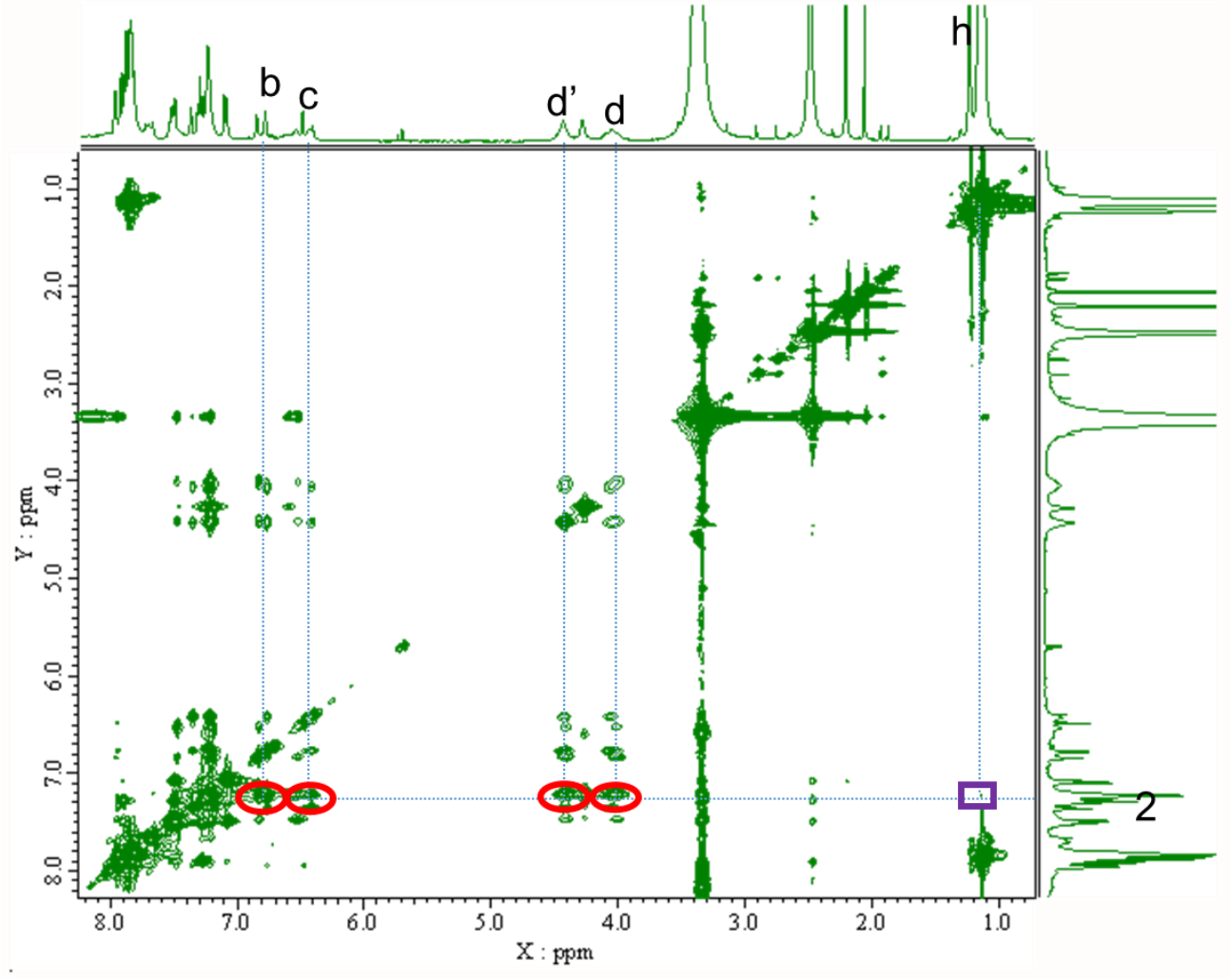

Figure S18. The ^{1}H-^{1}H NOESY spectrum of AP@**ZnPMTC** at the ratio of **ZnPMTC** : AP = 1 : 8 in DMSO-d_6. The red circles highlight the *endo*-encapsulation of four equiv. of AP, and the purple square indicate the *exo*-encapsulation of four equiv. of AP.

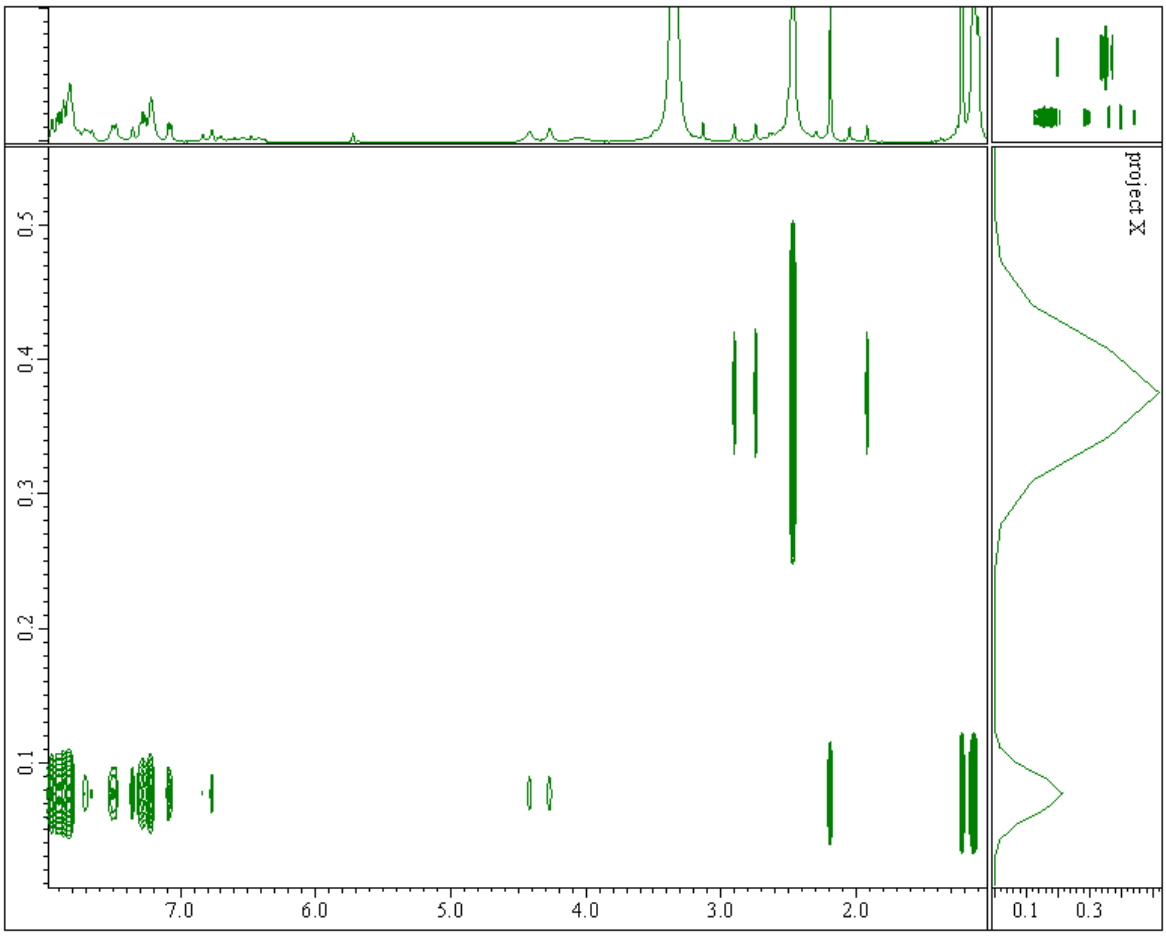

Figure S19. DOSY NMR (400 MHz, DMSO-d_6, 20°C**)** spectrum of AP@**ZnPMTC** at the ratio of **ZnPMTC** : AP = 1 : 4. Signals diffuse with observed diffusion coefficient value of 7.41×10^{-11} m^2/s.

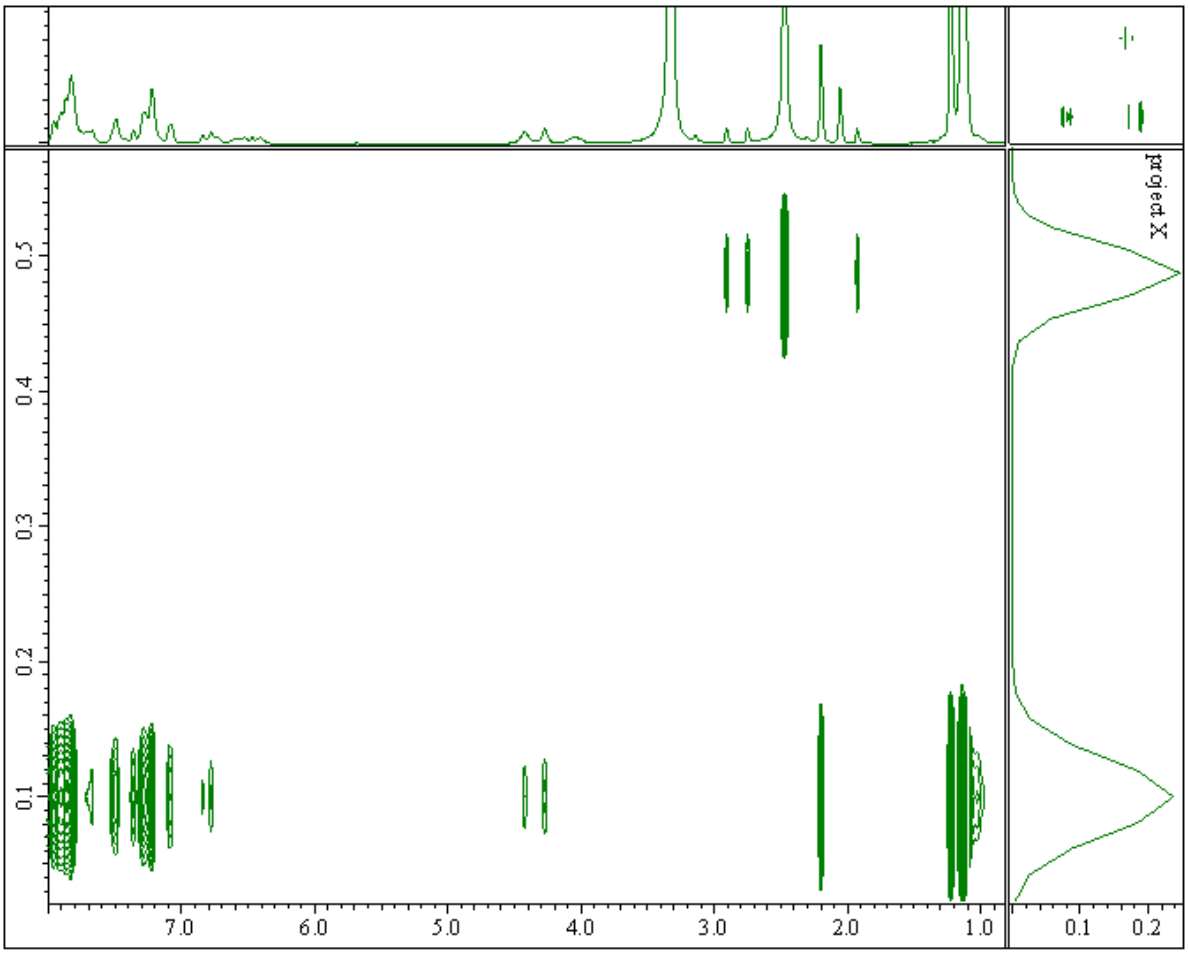

Figure S20. DOSY NMR (400 MHz, DMSO-d_6, 20°C**)** spectrum of AP@**ZnPMTC** at the ratio of **ZnPMTC** : AP = 1 : 8. Signals diffuse with observed diffusion coefficient value of 7.13×10^{-11} m^2/s.

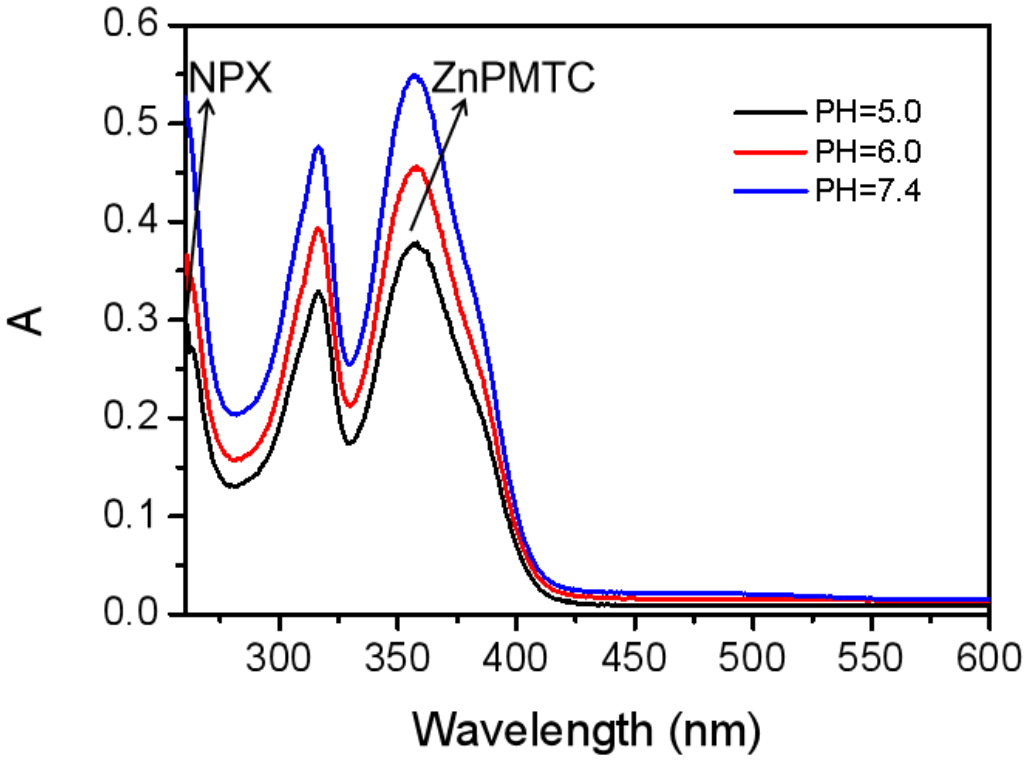

Figure S21. UV-Vis absorption spectra of NPX@**ZnPMTC** samples after 7 days of drugs release process.

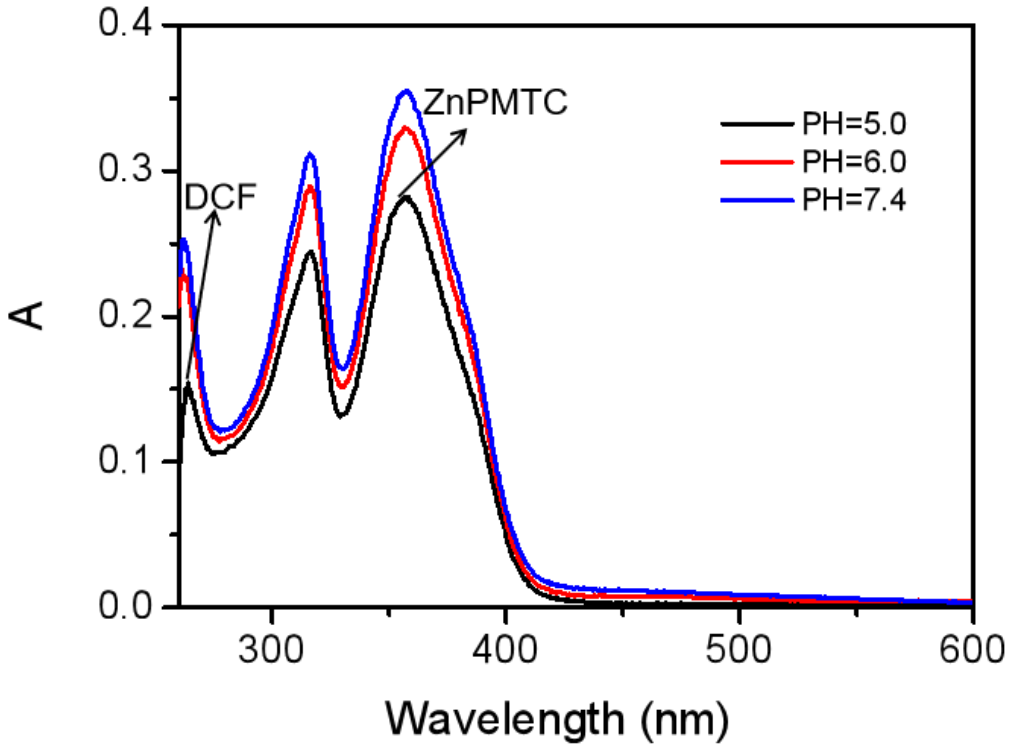

Figure S22. UV-Vis absorption spectra of DCF@**ZnPMTC** samples after 7 days of drugs release process.

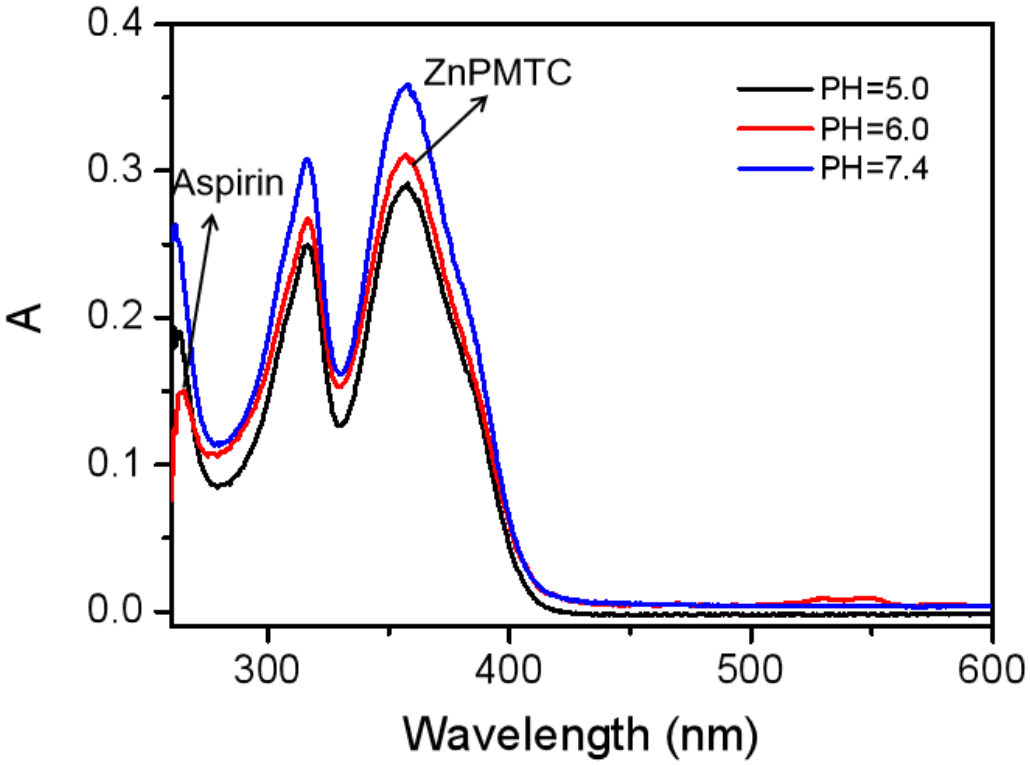

Figure S23. UV-Vis absorption spectra of AP@**ZnPMTC** samples after 7 days of drugs release process.

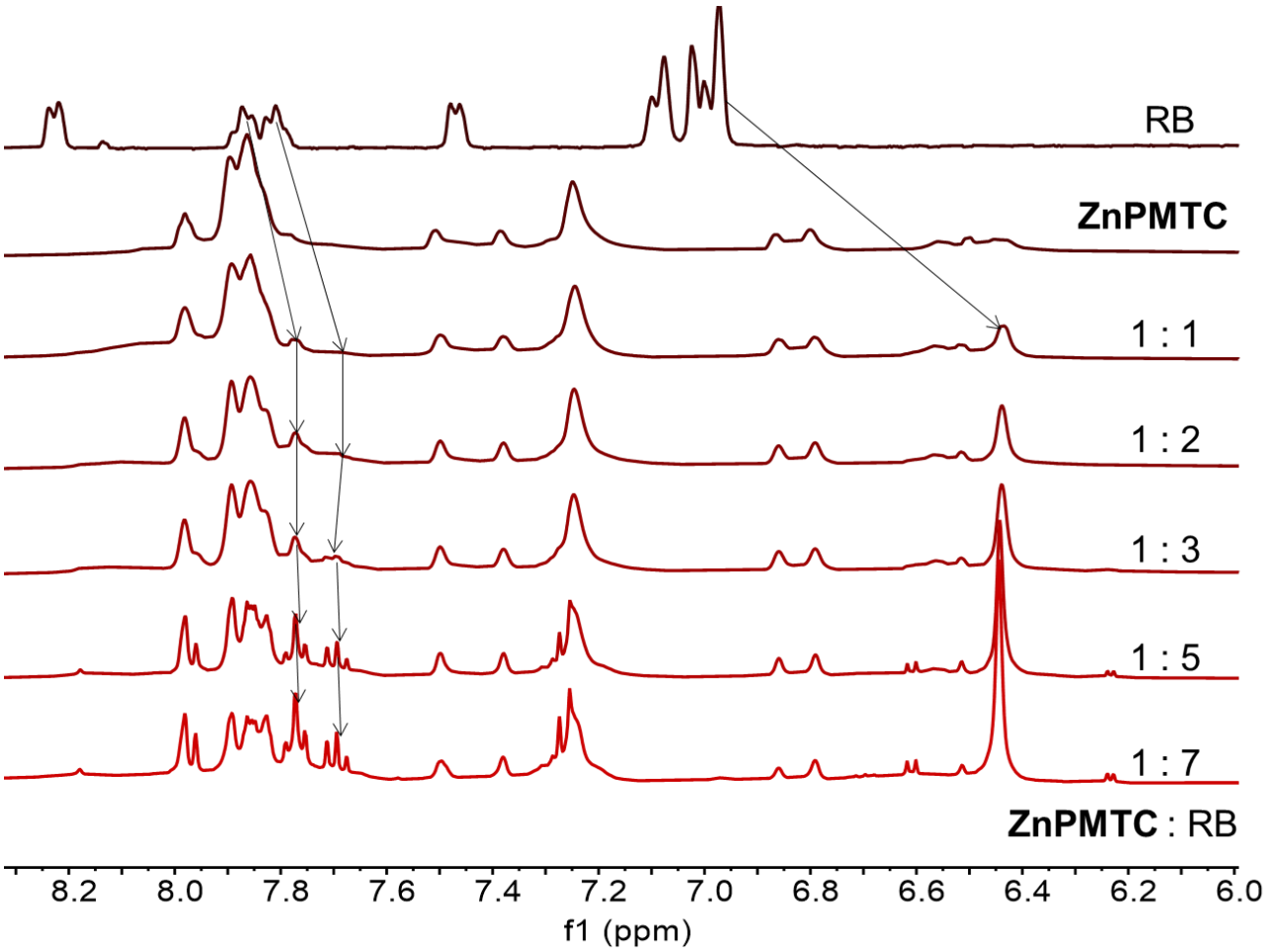

Figure S24. ^{1}H NMR spectral changes (400 MHz, 5×10^{-4} M in DMSO-d_6) of RB upon addition to **ZnPMTC**.

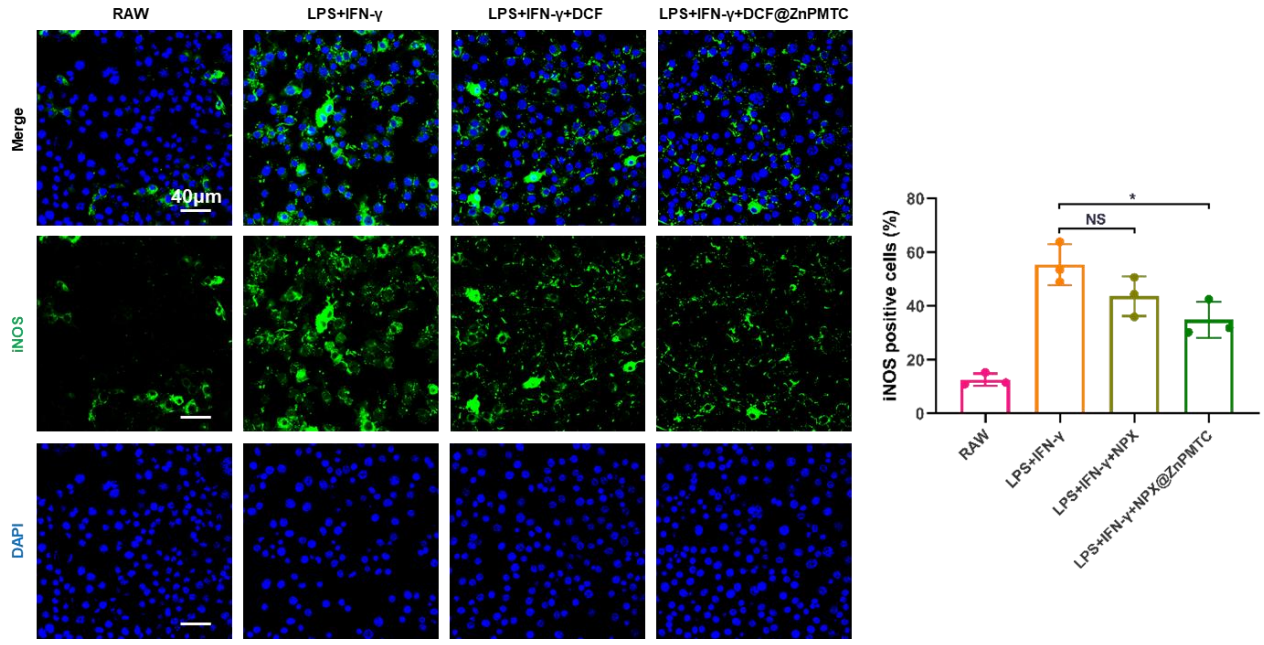

Figure S25. Confocal images of RAW 264.7 treated with DCF@**ZnPMTC** in the presence of LPS plus IFN-γ for 24 h. iNOS was used as the markers for M1 phenotype.

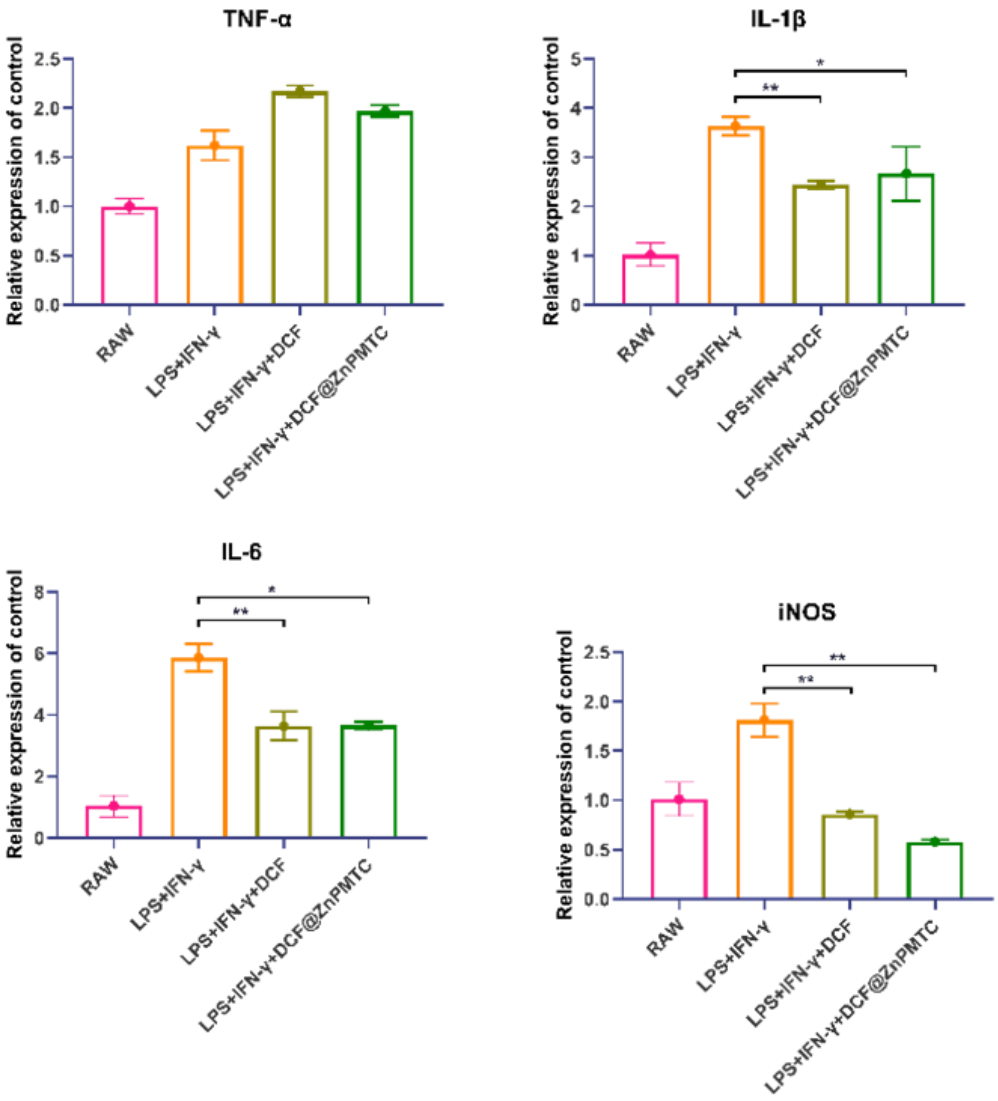

Figure S26. Expression of M1-related genes analyzed by RT-qPCR in RAW 264.7 macrophages.

References

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(S2) Hubschle, C. B.; Sheldrick, G. M.; Dittrich, B. ShelXle: a Qt Graphical User Interface for SHELXL. *J. Appl. Crystallogr.* **2011,** *44*, 1281-1284.

(S3) Spek, A. PLATON SQUEEZE: a Tool for the Calculation of the Disordered Solvent Contribution to the Calculated Structure Factors. *Acta Crystallogr. C* **2015,** *71*, 9-18.