Electronic Supplementary Material (ESI) for Materials Advances.

Supporting Information

Photofabrication of fluorescent nanospheres from *de novo* designed peptides, and their enzyme-responsive dissociation in living cells

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Characterization. Transmission electron microscope (TEM) images were conducted by JEM-2100F microscope at accelerating voltage of 160 kV. High-resolution electrospray ionization mass spectrometries (HR-ESI MS) were performed on a ThermoScientific Q Exactive UHMR Hybrid Quadrupole-Orbitrap Mass Spectrometer. ^{1}H nuclear magnetic resonance (NMR) spectra were recorded on a Bruker Ascend 600 MHz spectrometer. Fourier transform infrared (FT-IR) spectra were recorded on a Thermo Scientific Nicolet iS5 system. UV-visible (UV-vis) absorption spectra were recorded on a Shimadzu UV-2600 spectrophotometer. Fluorescence measurements were run on a Hitachi F-2500 fluorescence spectrophotometer. Circular dichroism (CD) spectra were measured on a Jasco J-810 spectrometer using 1-mm quartz cuvette. Dynamic light scattering (DLS) and zeta potential (ζ) were determined by a Malvern Nano-ZS90 Zetasizer.

Table S1. Loading content (%) and encapsulation efficiency (%) of PNS for fluorescent dyes.

Sample[a]	Loading content (%)	Encapsulation efficiency (%)
RhB@YXD-PNS	17.1	73.8
DPA@YX-PNS	12.8	70.8
FL@YXR-PNS	14.0	72.6

[a] The feeding ratio of dye to peptide in weight was 0.2:1. The loading content and encapsulation efficiency were calculated by the following formulas: loading content = (weight of loaded dye/weight of dye-loaded PNS) × 100%, encapsulation efficiency = (weight of loaded dye/weight of feeding dye) × 100%.

Table S2. Encapsulation efficiency (%) of PNS for different fluorescent dyes.

Dye \ Peptide	YXD	YX	YXR
RhB	73.8	70.9	68.2
DPA	64.2	70.8	60.5
FL	38.9	62.7	72.6

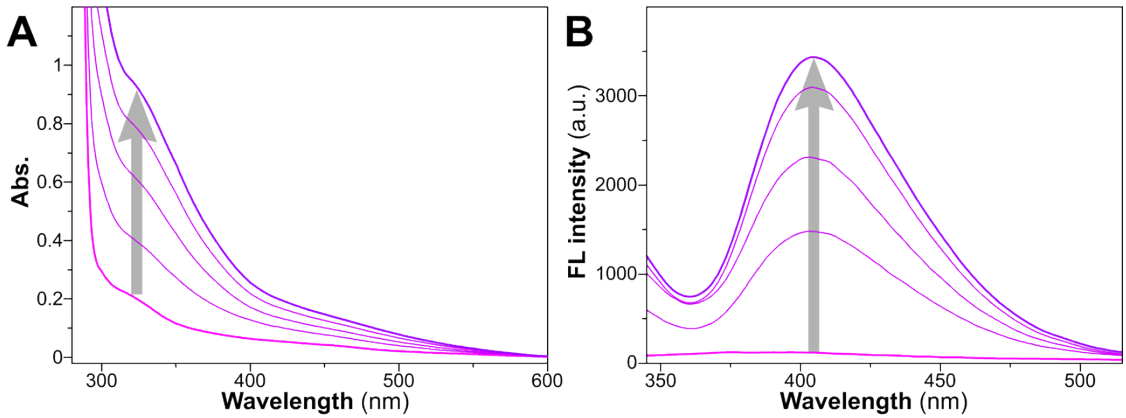


Fig. S1 Time-dependent changes of UV-vis absorption and fluorescence emission spectra for YX peptide upon irradiation of 0, 2, 4, 6, 10 min.

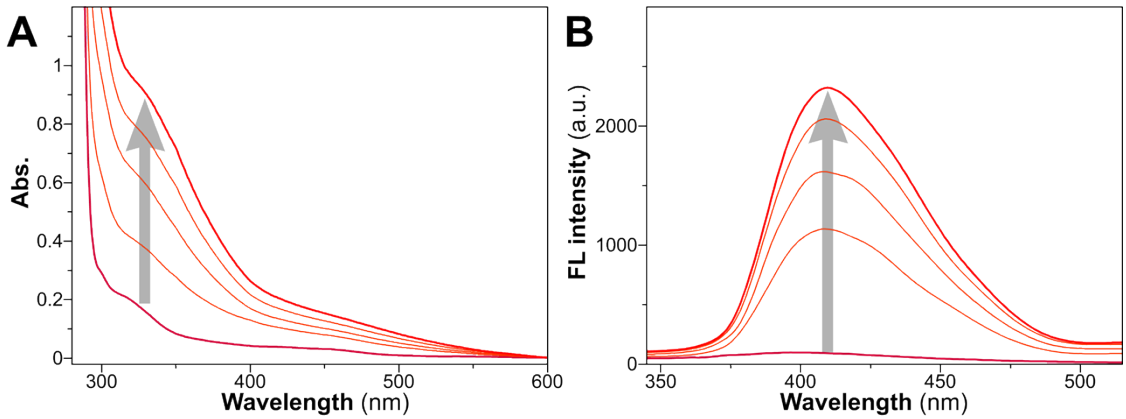


Fig. S2 Time-dependent changes of UV-vis absorption and fluorescence emission spectra for YXR peptide upon irradiation of 0, 2, 4, 6, 10 min.

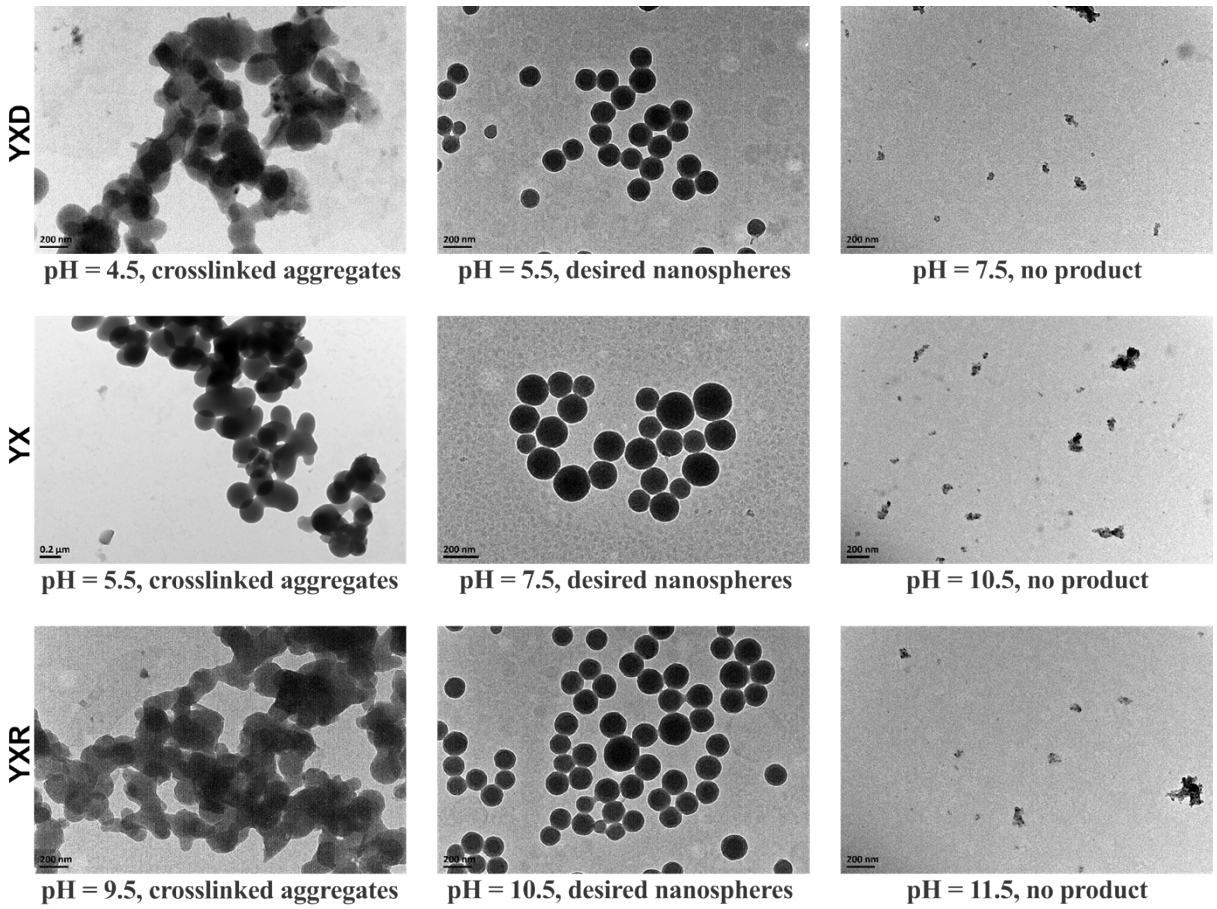


Fig. S3 TEM images of the photocrosslinking products of MMP-responsive peptides at different pH conditions.

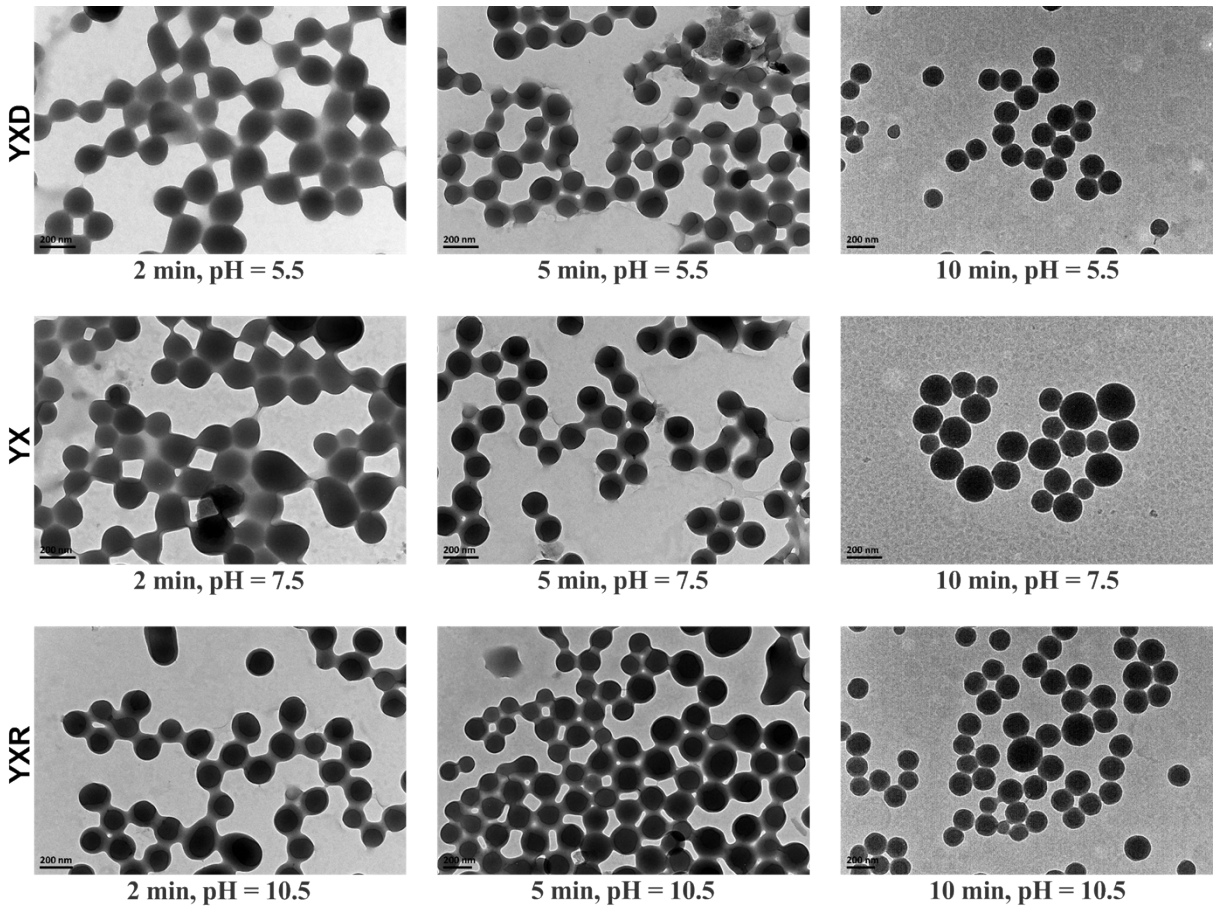


Fig. S4 TEM image for MMP-responsive peptides after treated with light irradiation (405-nm LEDs, 112 mW cm^{-2}) for 2, 6, and 10 min, respectively.

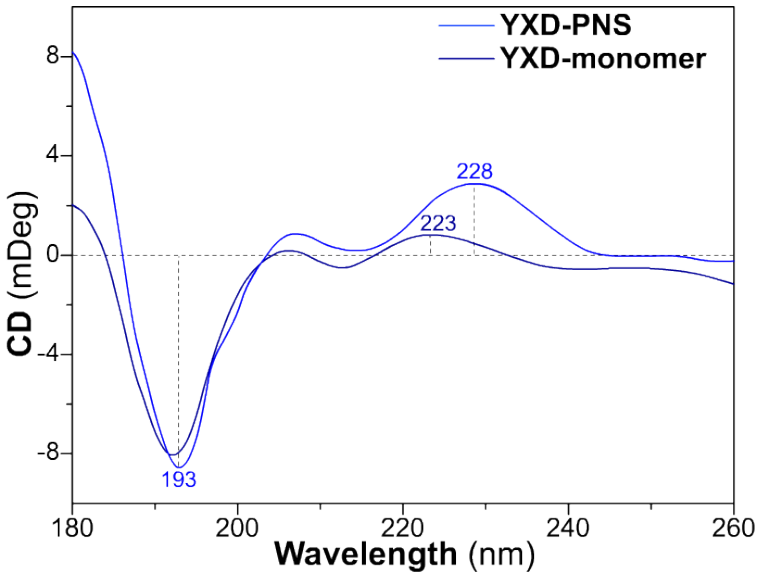


Fig. S5 CD spectra of YXD peptide and YXD-PNS in PBS.

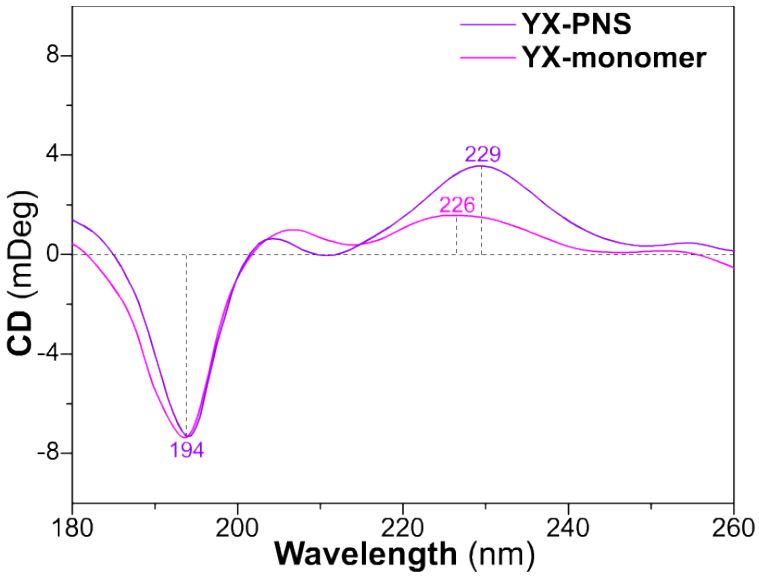


Fig. S6 CD spectra of YX peptide and YX-PNS in PBS.

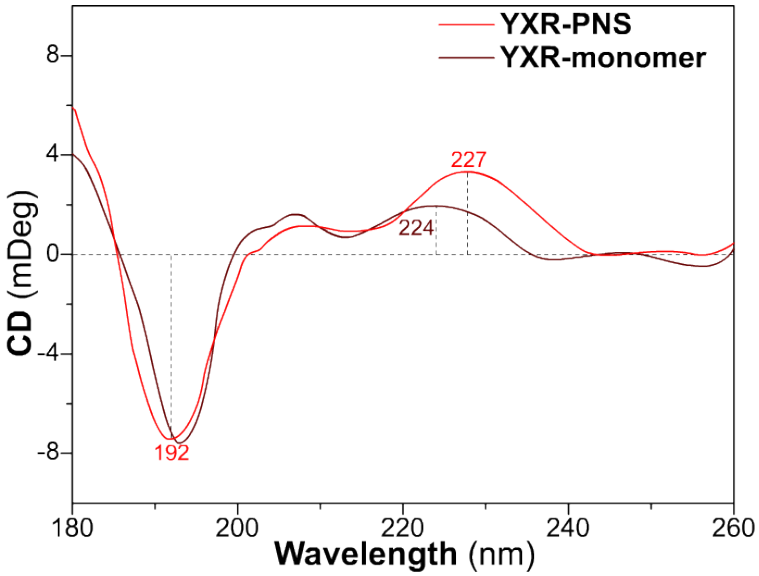


Fig. S7 CD spectra of YXR peptide and YXR-PNS in PBS.

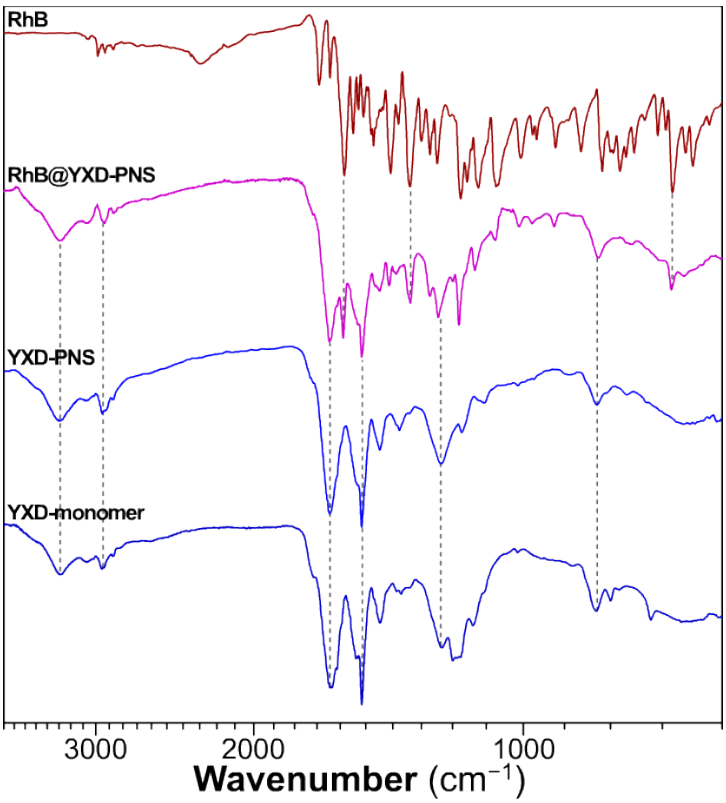


Fig. S8 FT-IR spectra of RhB, RhB@YXD-PNS, YXD-PNS, YXD peptide.

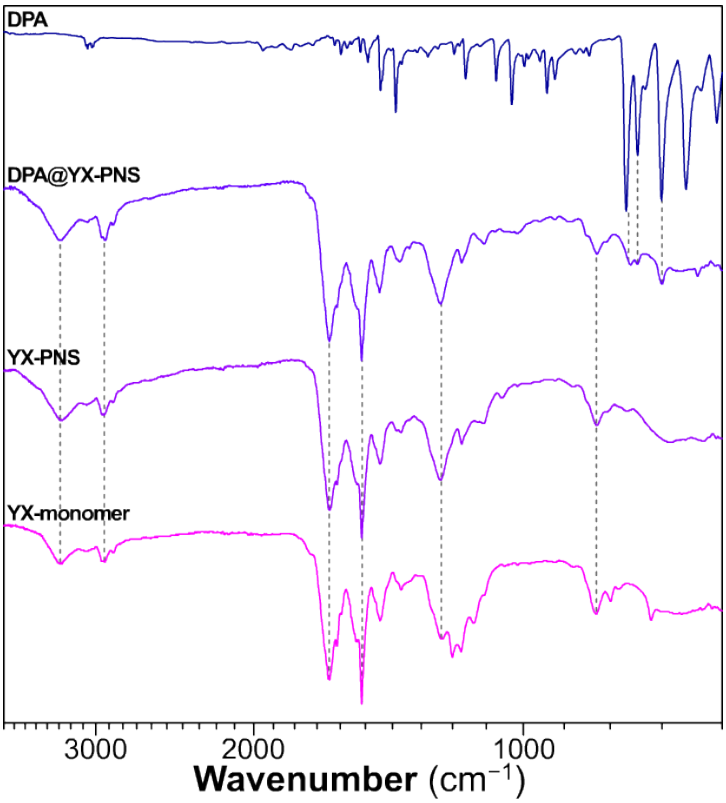


Fig. S9 FT-IR spectra of DPA, DPA@YX-PNS, YX-PNS, YX peptide.

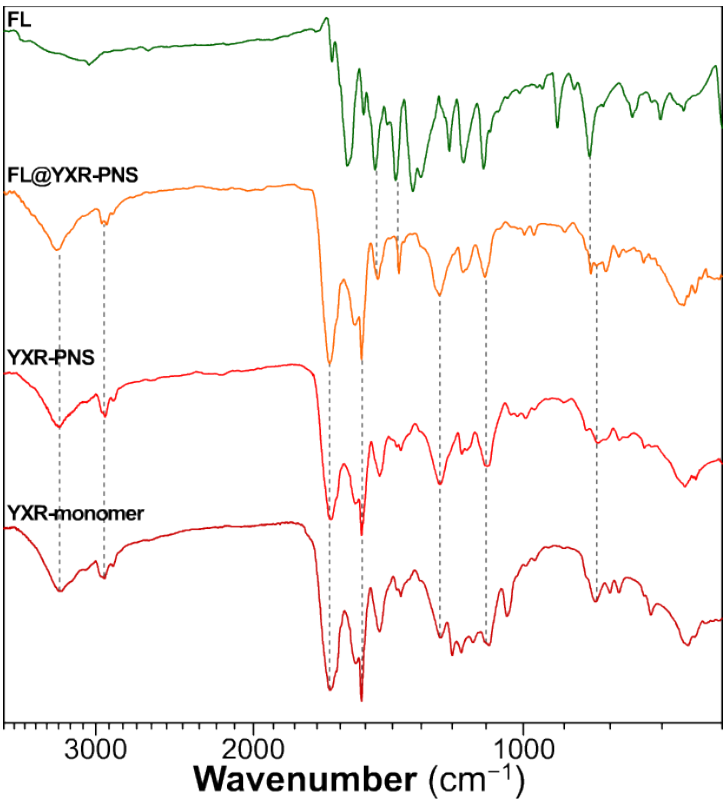


Fig. S10 FT-IR spectra of FL, FL@YXR-PNS, YXR-PNS, YXR peptide.

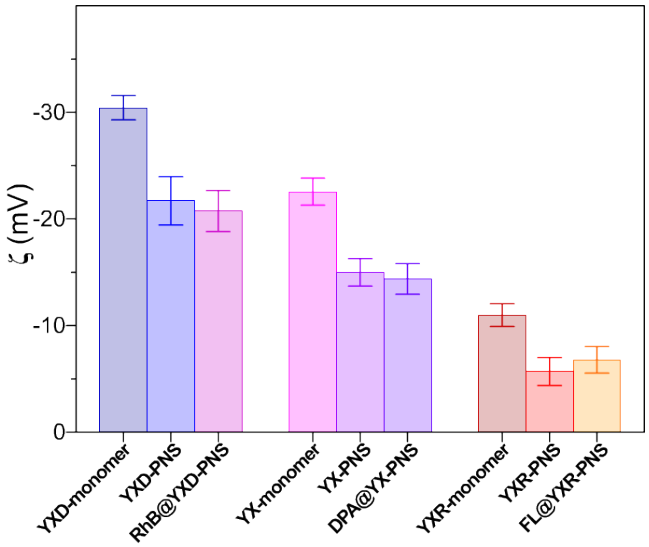


Fig. S11 Zeta potentials of YXD peptide, YXD-PNS, RhB@YXD-PNS, YX peptide, YX-PNS, DPA@YX-PNS, YXR peptide, YXR-PNS, FL@YXR-PNS.

Fig. S12 Photographs of RhB@YXD-PNS, DPA@YX-PNS, and FL@YXR-PNS solution of water under daylight (right) and a 365 nm UV lamp (left).

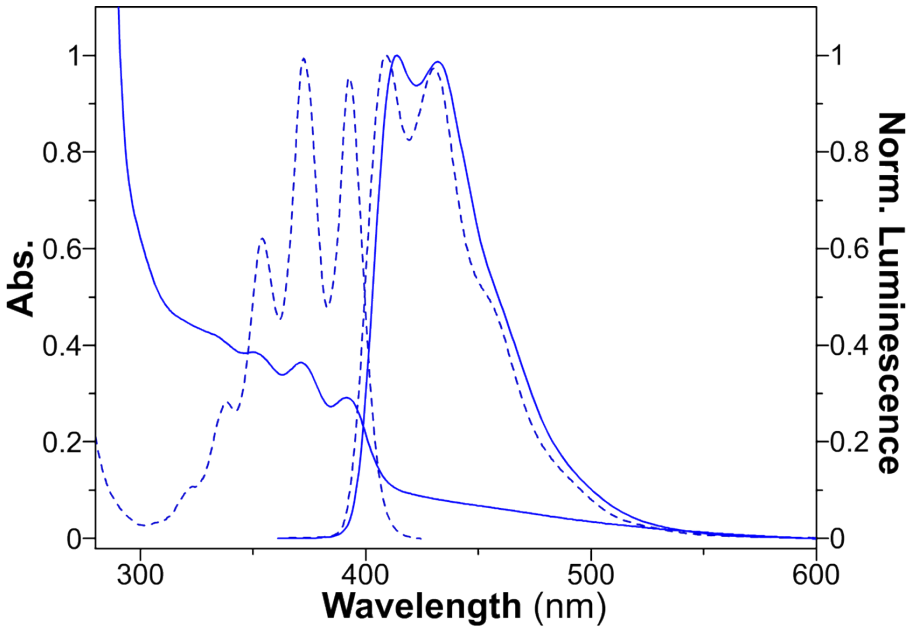


Fig. S13 UV-vis absorption and normalized fluorescence spectra of free DPA (dotted line) and DPA@YX-PNS (solid line).

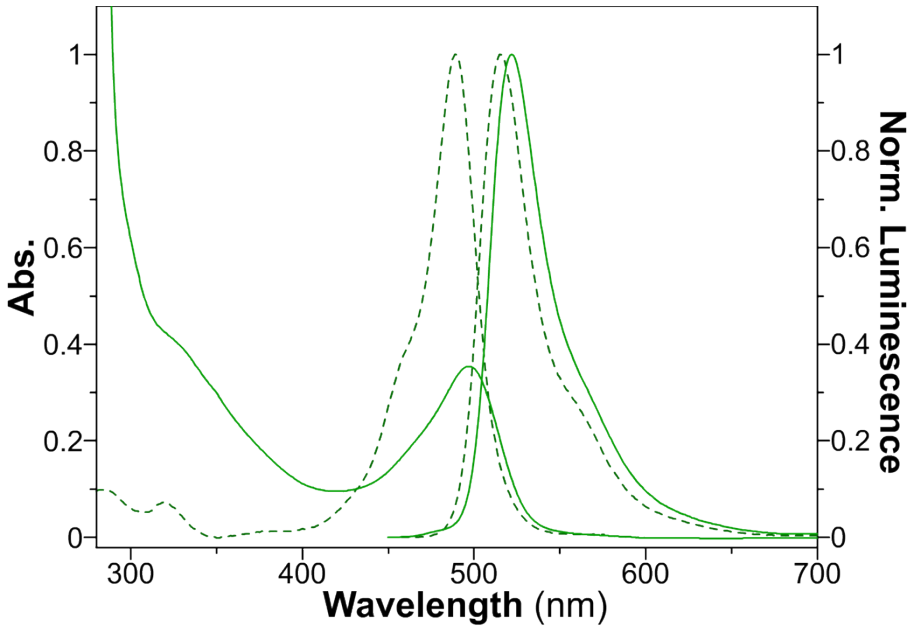


Fig. S14 UV-vis absorption and normalized fluorescence spectra of free FL (dotted line) and FL@YXR-PNS (solid line).

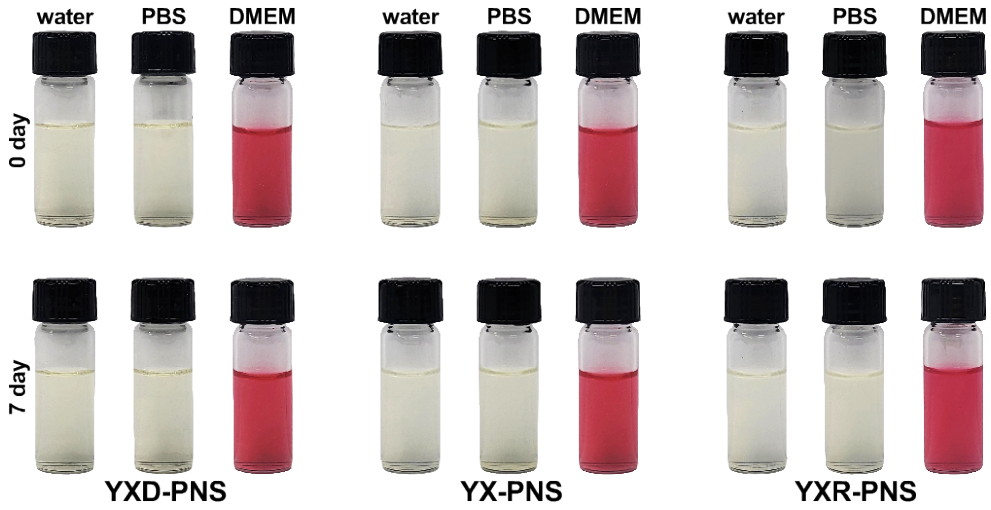


Fig. S15 Photographs of YXD-PNS, YX-PNS, and YXR-PNS solution of water, PBS, and DMEM after placing at room temperature for 0 and 7 days.

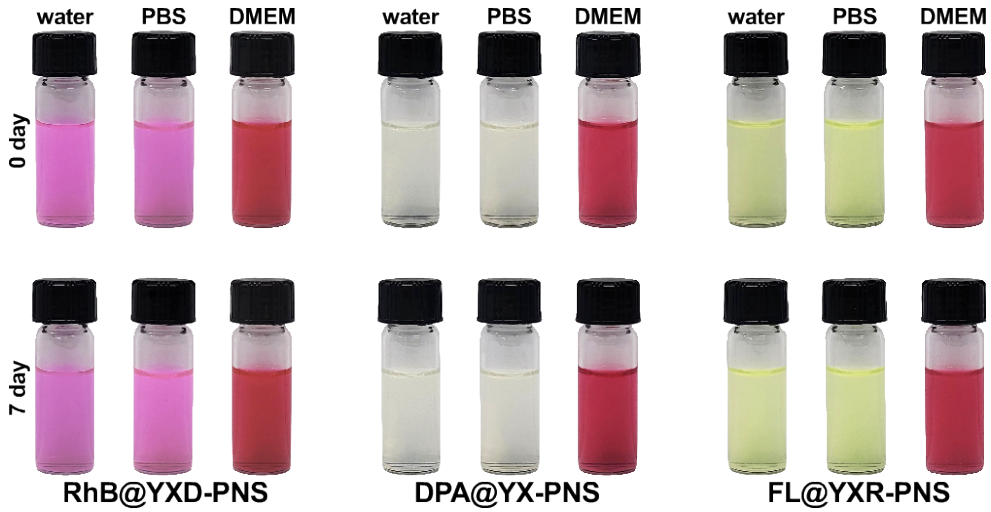


Fig. S16 Photographs of RhB@YXD-PNS, DPA@YX-PNS, and FL@YXR-PNS solution of water, PBS, and DMEM after placing at room temperature for 0 and 7 days.

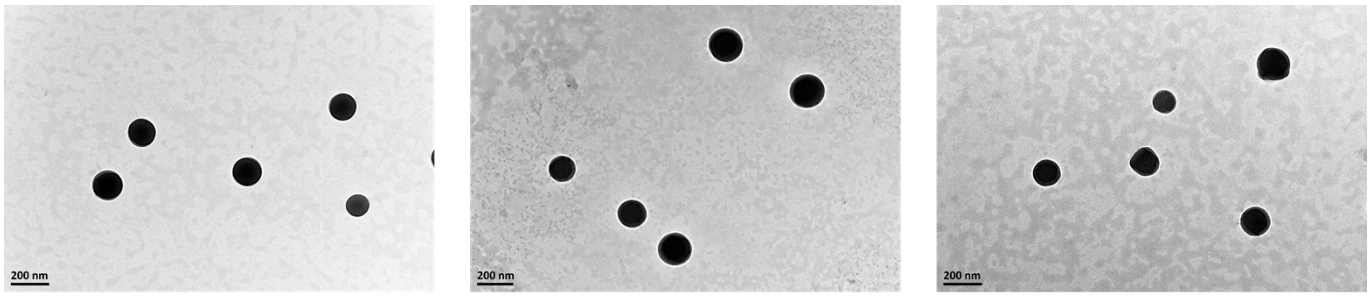


Fig. S17 TEM images of YYXALGLPXYY PNS incubated with MMP at 37 °C for 12, 24, and 48 h, respectively.

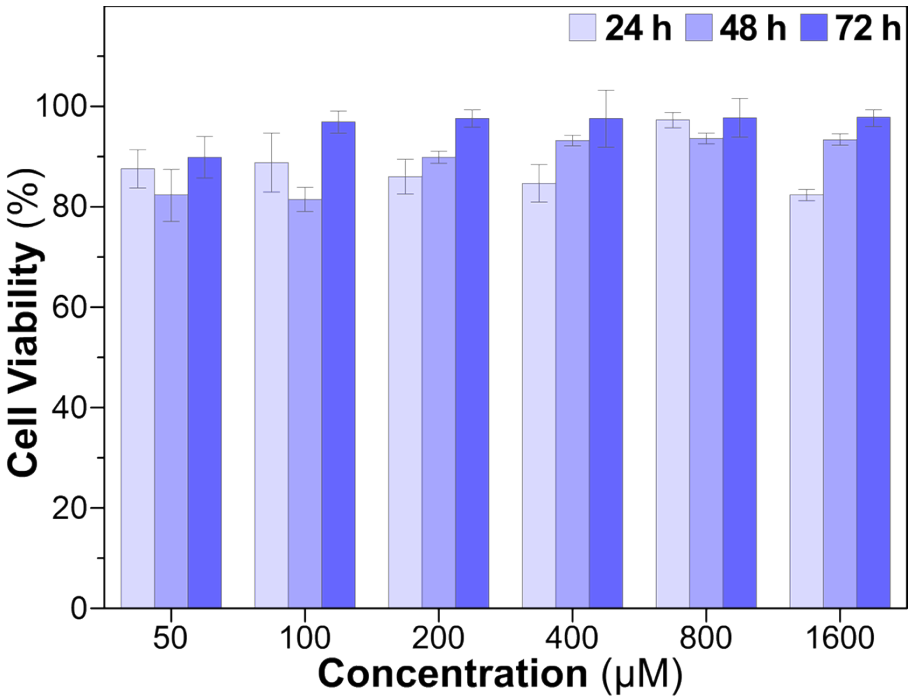


Fig. S18 Cytotoxicity of RhB@YXD-PNS at various peptide concentrations in A549 cells after 24, 48, and 72 h incubation (n = 3).

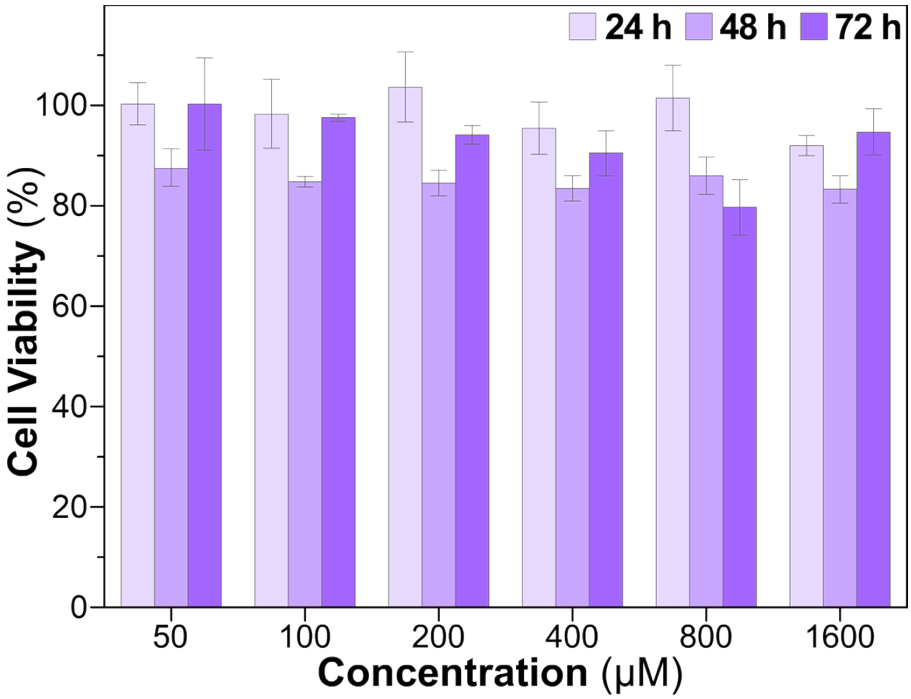


Fig. S19 Cytotoxicity of DPA@YX-PNS at various peptide concentrations in A549 cells after 24, 48, and 72 h incubation (n = 3).

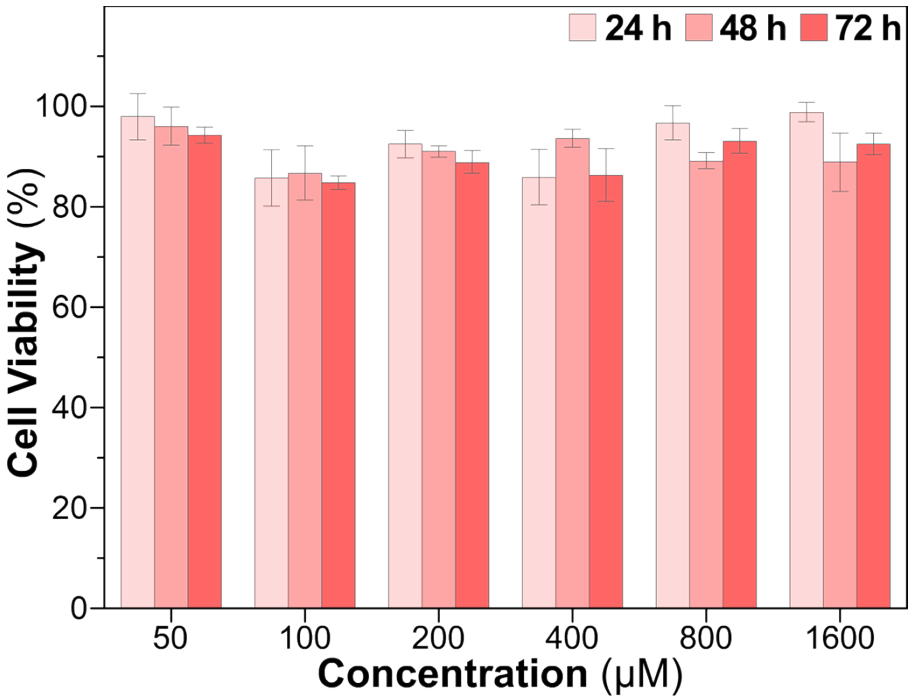


Fig. S20 Cytotoxicity of FL@YXR-PNS at various peptide concentrations in A549 cells after 24, 48, and 72 h incubation (n = 3).

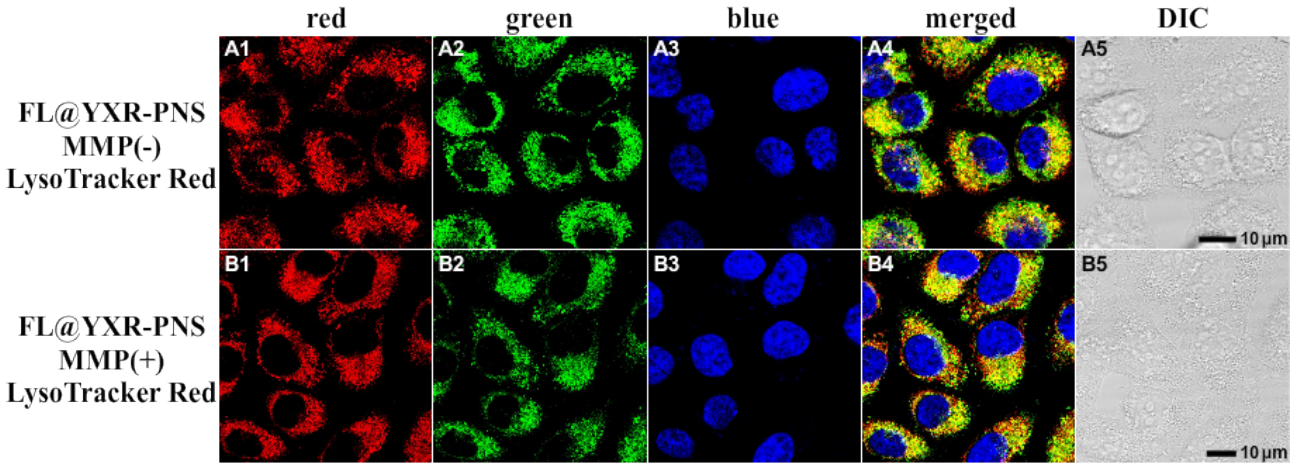


Fig. S21 (A) Colocalization of FL@YXR-PNS with LysoTracker Green in A549 cells. (B) CLSM images for A549 cells incubated with FL@YXR-PNS upon stimuli of MMP. Cell were counterstained with Hoechst 33342 (blue) for nuclei.

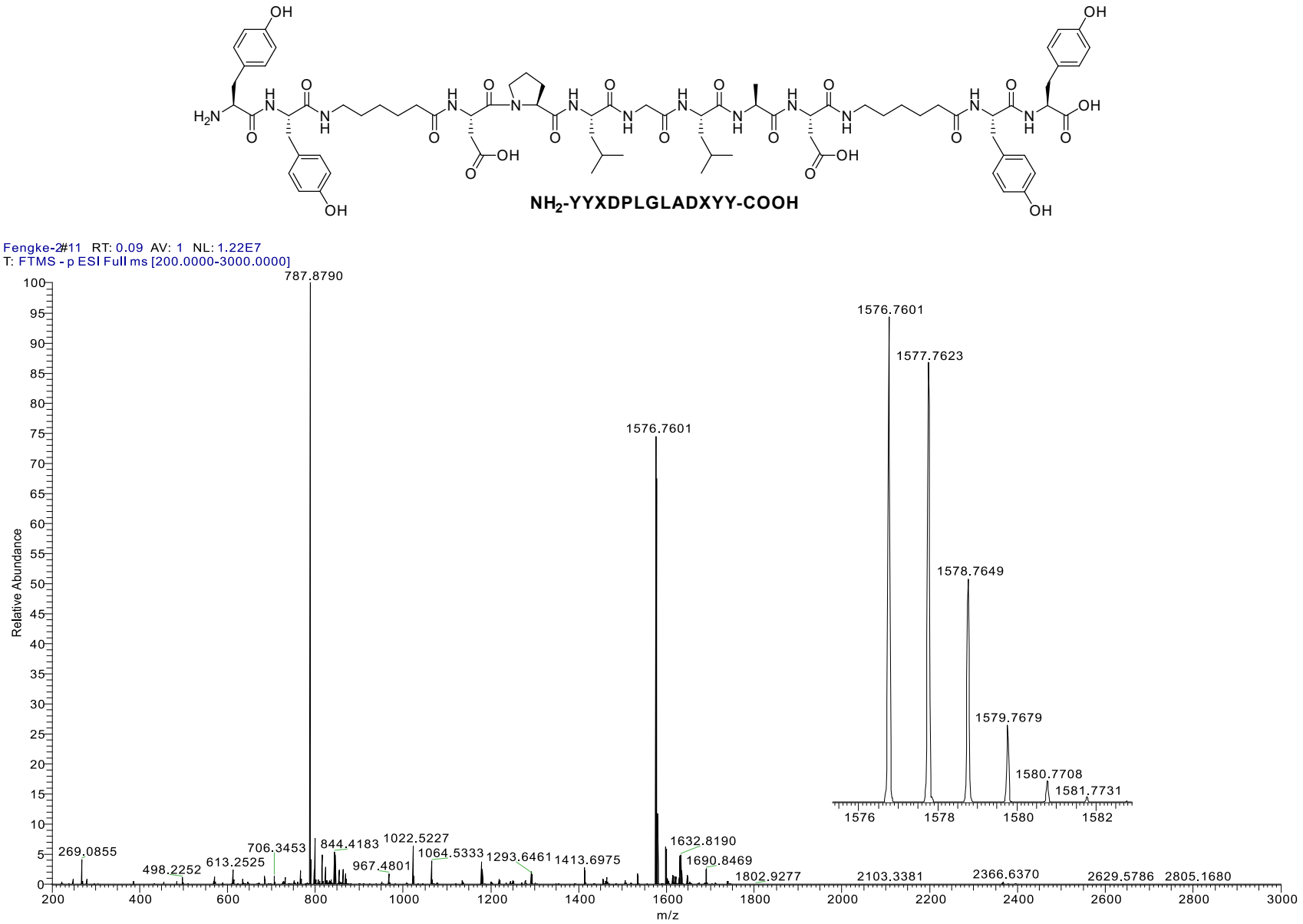
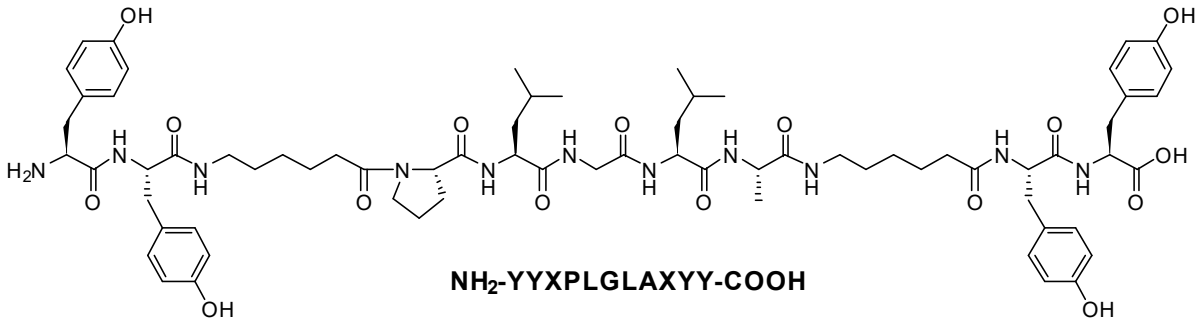


Fig. S22 HR-ESI MS spectrum for YXD peptide.



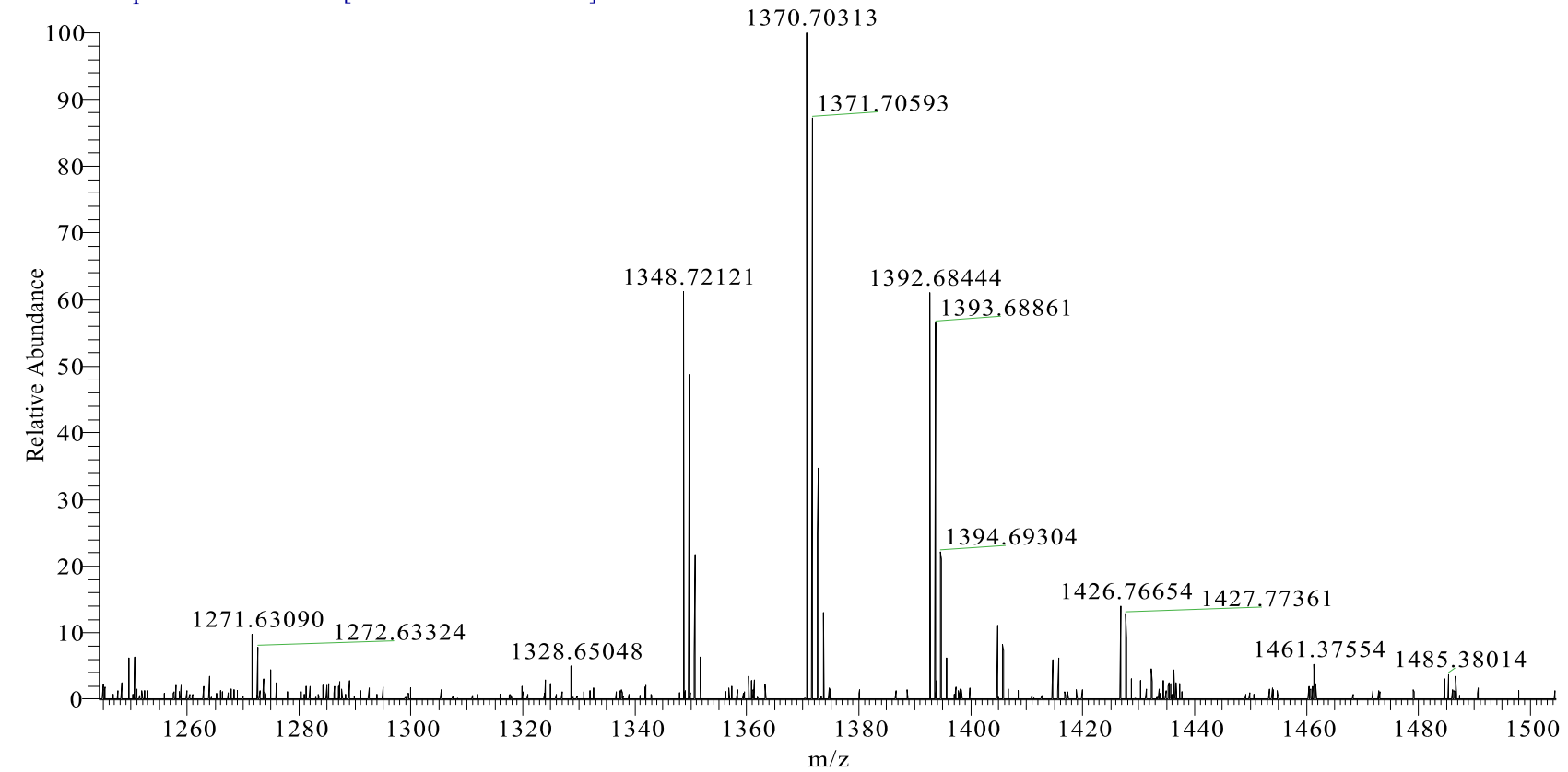


Fig. S23 HR-ESI MS spectrum for YX peptide.

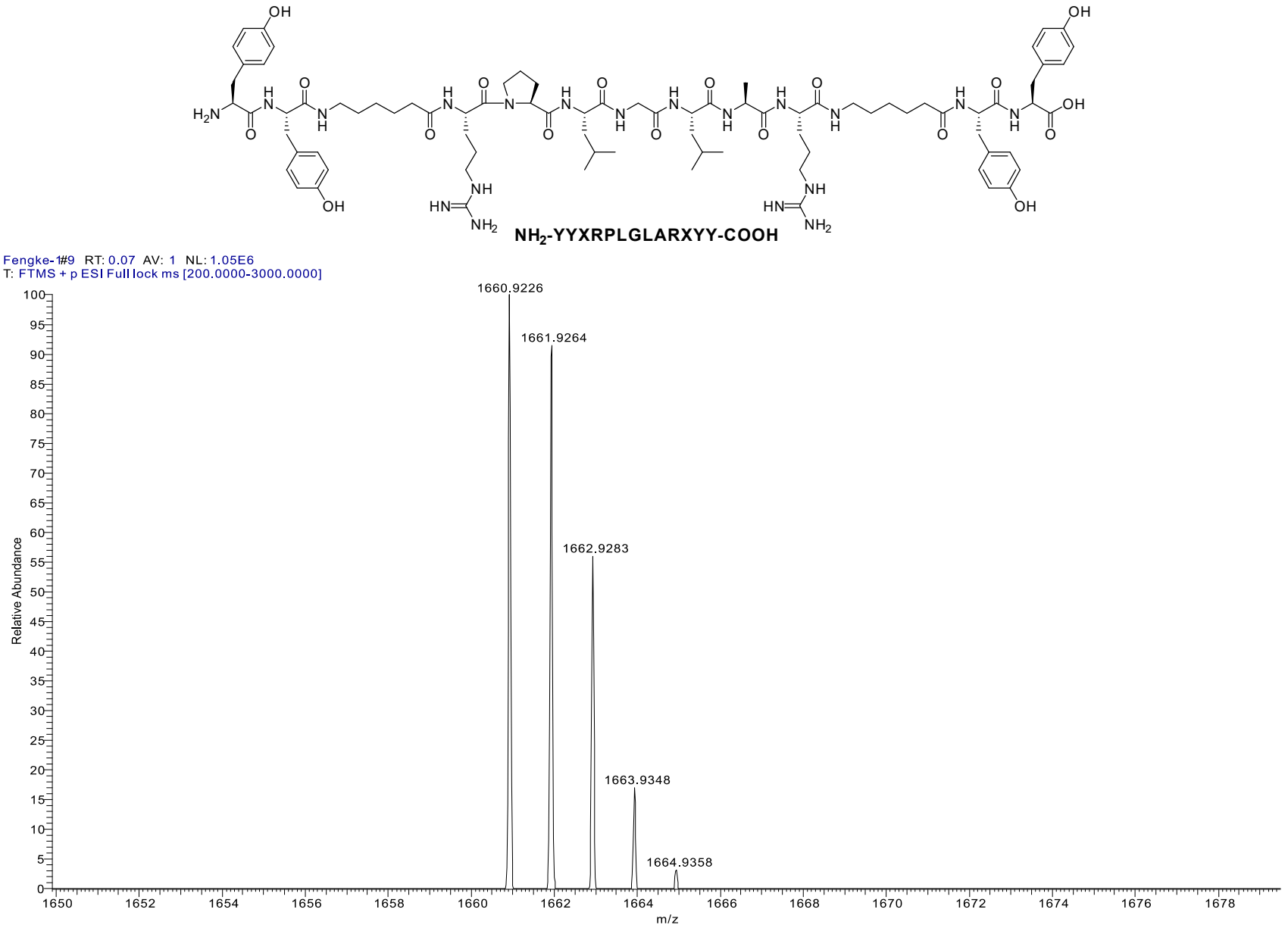
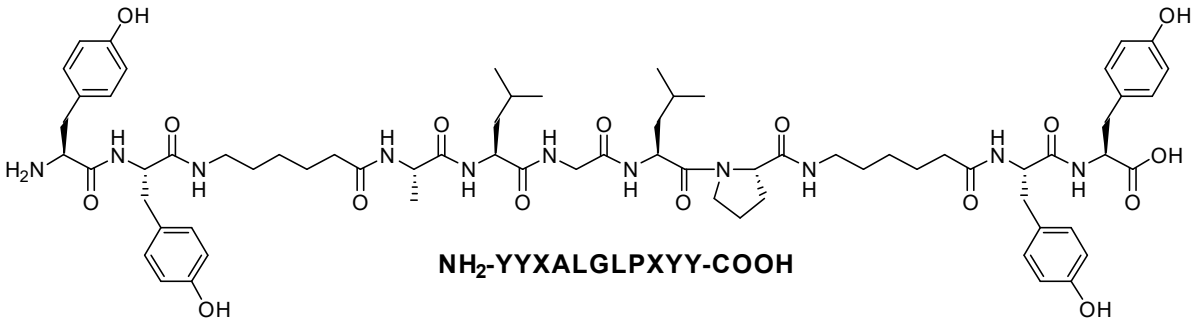


Fig. S24 HR-ESI MS spectrum for YXR peptide.



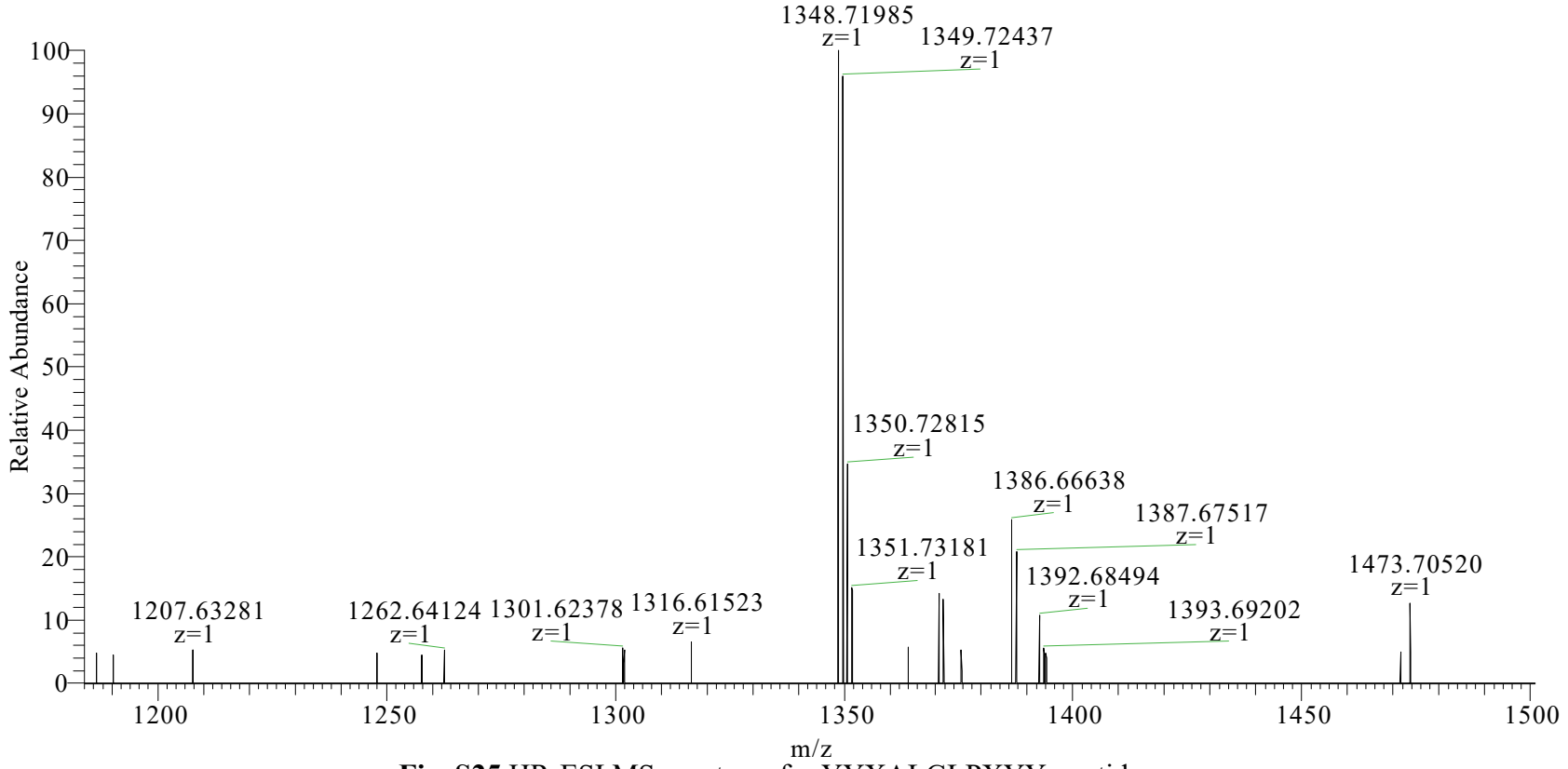


Fig. S25 HR-ESI MS spectrum for YYXALGLPXYY peptide.

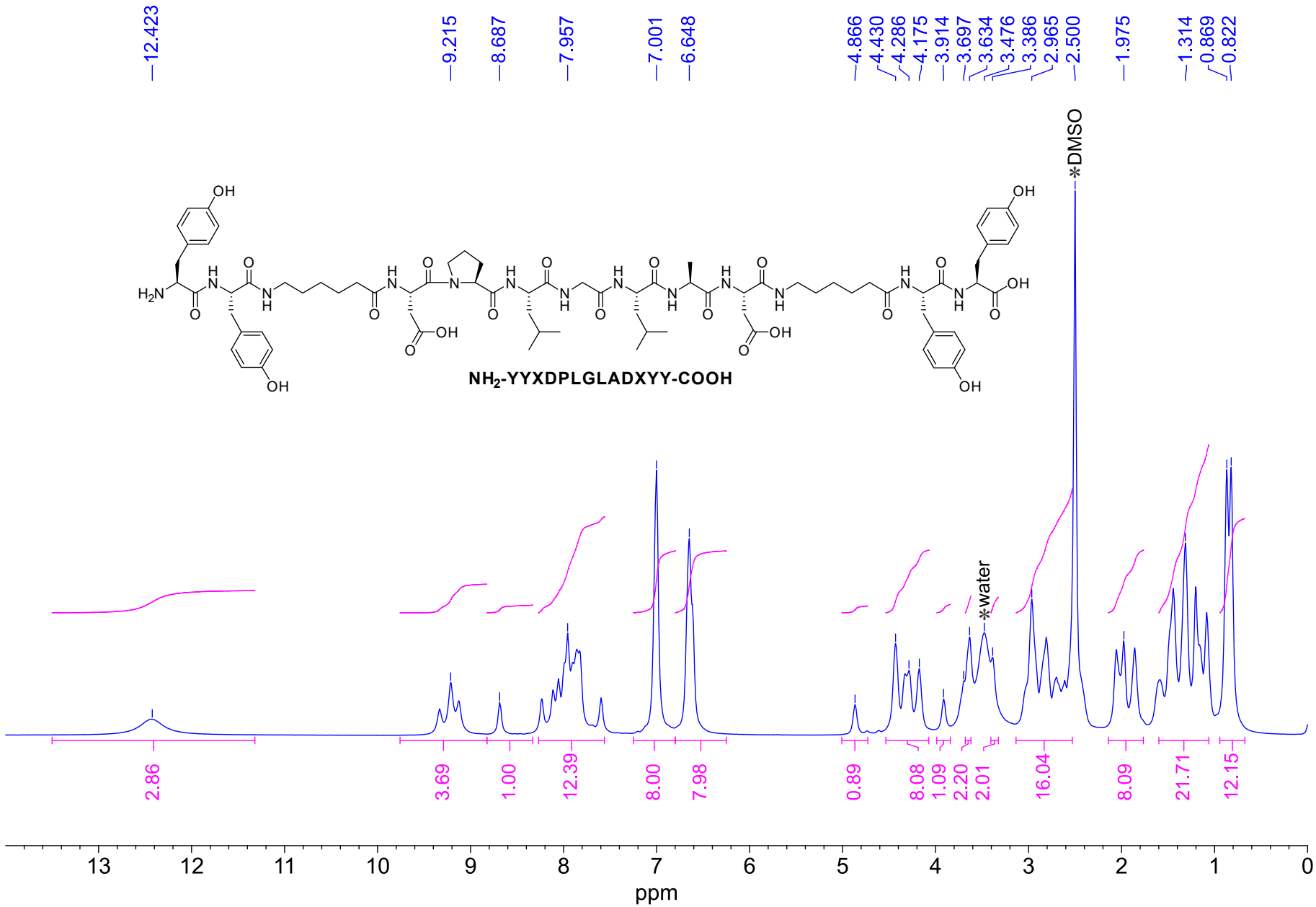


Fig. S26 ^{1}H NMR spectrum for YXD peptide in DMSO-d_6.

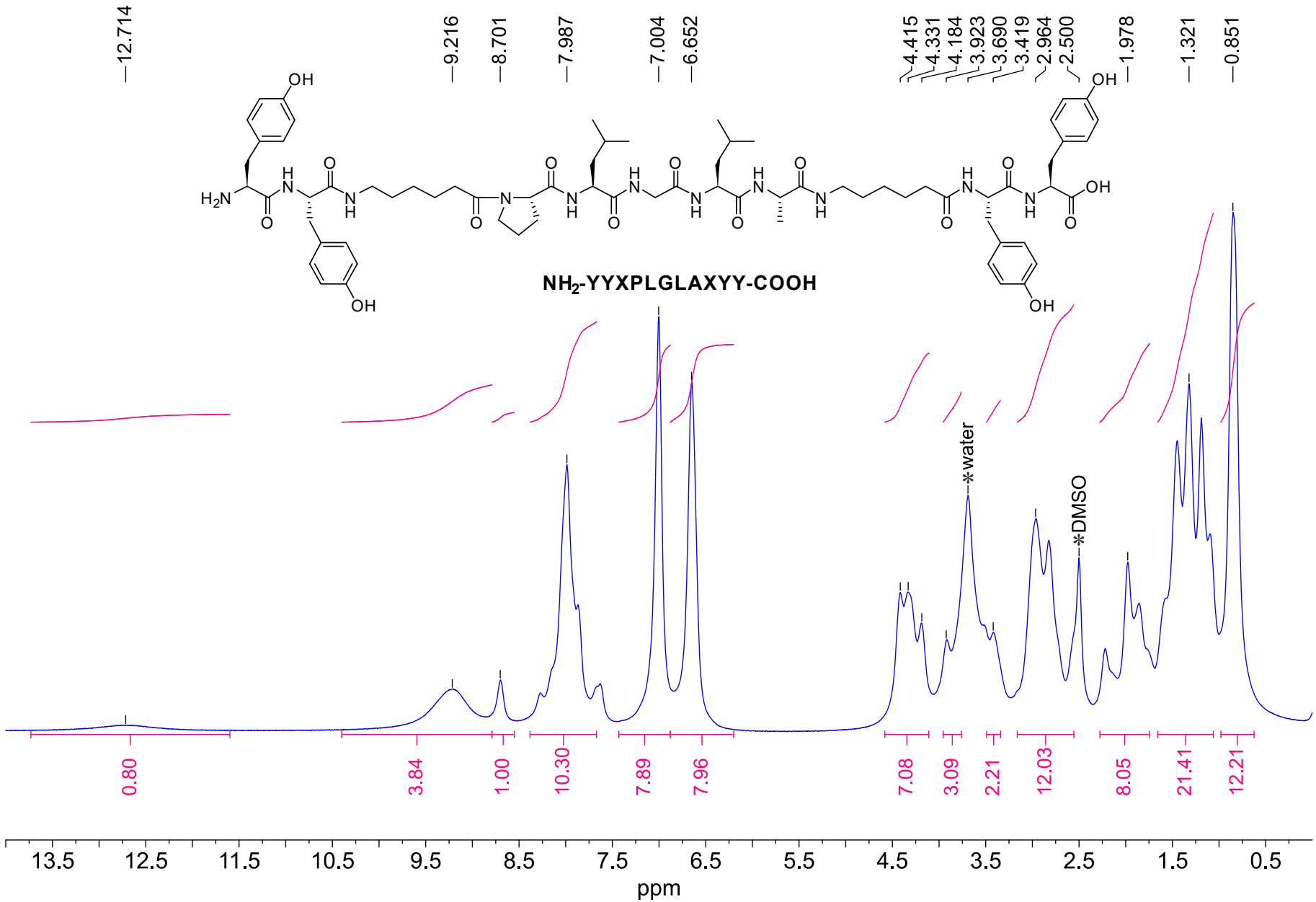


Fig. S27 ^{1}H NMR spectrum for YX peptide in DMSO-d_6.

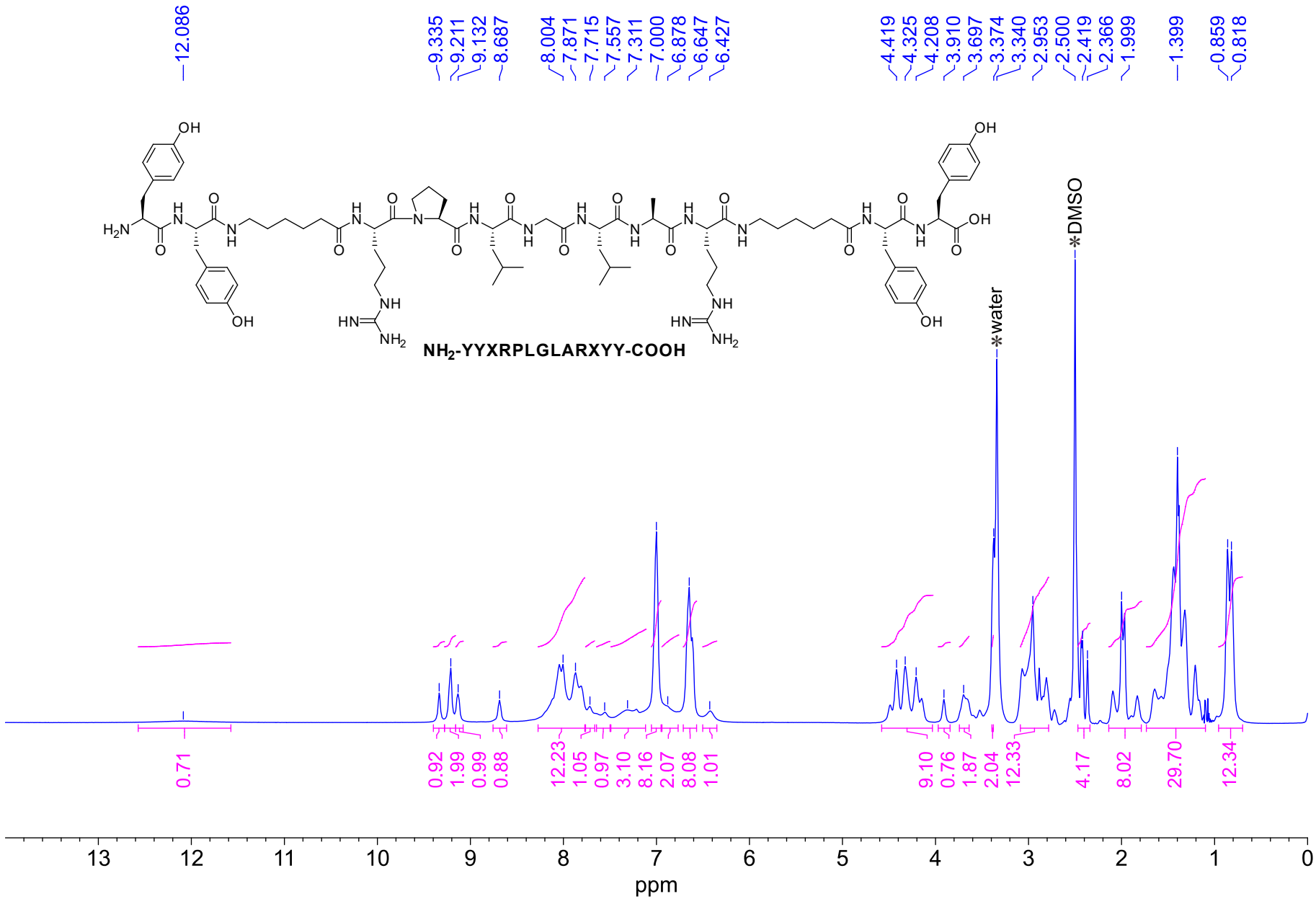


Fig. S28 ^{1}H NMR spectrum for YXR peptide in DMSO-d_6.

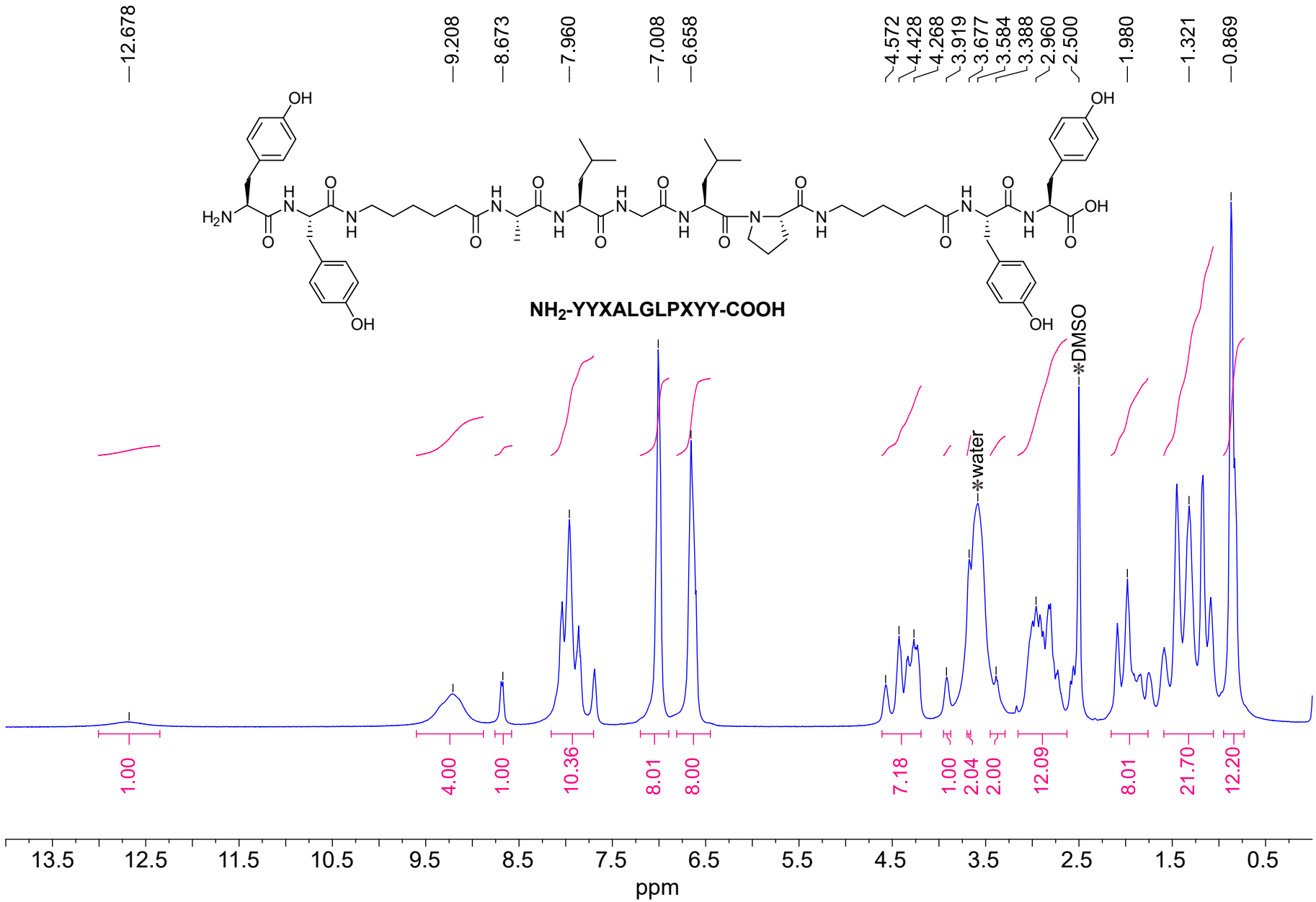


Fig. S29 ^{1}H NMR spectrum for YYXALGLPXYY peptide in DMSO-d_6.