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Electronic Supporting Information

Versatile Quantitative Biopsy: Approach for Cost-effective Detection of Hydrogen Peroxide in Tissue Specimen

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Materials and Instruments

Absorption spectra were recorded using a Hitachi U−4100 UV−vis spectrophotometer (Kyoto, Japan) in 1 cm path length quartz cuvettes. ^{1}H and ^{13}C NMR spectra were recorded on an Invoa−400 (Invoa 400) spectrometer and referenced to solvent signals. The number-average molecular weight (M_n) and molecular distribution (polydispersity index, PDI = M_w/M_n) of polymers were determined at room temperature (RT) using a Waters GPC liquid chromatograph (Waters, USA) equipped with four TSK HXL series of polystyrene divinylbenzene gel columns (300 7.8 mm). Calibration was established with polystyrene standards from Polymer Laboratories. THF was used as solvent with a flow rate of 1 mL min^{-1}. Scanning electron microscopy (SEM) images were obtained on an S-4800 scanning electron microscope (Hitachi) with a working voltage of 5 kV. A drop of the micelle aqueous solution (0.05 mg mL^{-1}) was deposited onto a silicon slice and allowed to dry at RT before measurement. The mean size of the micelles was determined by dynamic light scattering (DLS) using a Malvern Nano S instrument (Malvern, UK).

The detailed synthesis and structural characterizations of (1)-(6)

Synthesis of L-Tyrosine-N-carboxyanhydride (Tyr-NCA) (1)

In a 100 mL two-neckround-bottom flask, L-tyrosine (1.00 g, 5.52 mmol) was dissolved in anhydrous THF (20 mL), and under a N_2 atmosphere, triphosgene (0.65 g, 2.21 mmol) was added and reacted for 5.5 h under reflux (70 ^{o}C), then cooled to 0 ^{o}C in an ice bath, the solvent was evaporated under reduced pressure. The crude product was recrystallized three times from THF/hexane to obtain the product Tyr-NCA. Yield: 1.09 g, 95.61%. ^{1}H NMR (400 MHz, DMSO): δ = 9.33 (s, 1H, OH), 9.03 (s, 1H, NH), 6.98-6.96 (d, *J*=8.0 Hz, 2H, ArH),

6.70-6.68 (d, *J*=8.0 Hz, 2H, ArH), 4.71-4.69 (t, *J*=4.0 Hz, 1H, OCOCH), 2.92-2.90 (d, *J*=8.0 Hz, 2H, $ArCH_2$). ^{13}C NMR (100 MHz, DMSO): δ = 171.34, 156.92, 152.13, 131.14, 125.01, 115.65, 58.95, 35.92.

Synthesis of PEG-Tyr (2)

Under N_2 atmosphere, Tyr-NCA (0.21 g, 1.01mmol) was added to a dry reaction flask, then anhydrous N,N-dimethylformamide was added, and after stirring for 10 min, PEG-NH_2 (0.50 g, 0.1 mmol) was added, stirred at 0 ℃ for 2 d, the reaction was stopped, precipitated with anhydrous ether and dried in vacuum to obtain the product PEG-Tyr. Yield: 0.55 g, 82.96%. ^1H NMR (400 MHz, DMSO): δ = 9.09 (s, OH), 7.94 (s, CHNH), 6.96-6.94(d, ArH), 6.60-6.58 (d, ArH), 4.42 (s, C(O)CHNH), 3.51 (s, CH_2CH_2O), 3.24 (s, CH_3O), 2.82 -2.50 (d, $ArCH_2$).

Synthesis of PEG-*b*-PPRLT (3)

In a 50 mL round bottom flask, PEG-Tyr (0.40g, 0.06 mmol), K_2CO_3 (0.25 g, 1.80 mmol) and anhydrous DMF (4 mL) were added, and 3-bromopropyne (0.21 g, 1.80 mmol) was dropped at 0 ℃, stirred at room temperature for 24 h, dialyzed with deionized water for 3 days (MWCO 3500), freeze-dried to obtain the product PEG-*b*-PPRLT. Yield: 0.36 g, 85.71%. ^1H NMR (400 MHz, DMSO): δ =8.12 (s, CHNH), 7.14 (s, ArH), 6.89-6.82 (d, ArH), 4.72-4.68 (d, CCCHO), 4.52 (s, C(O)CHNH), 3.53 (s, CH_2CH_2O), 3.26 (s, CH_3O), 2.92 -2.70 (d, $ArCH_2$).

Synthesis of 2,5-dioxopyrrolidin-1-yl 4-(4,4,5,5-tetramethyl-1,3,2-dioxaborolan-2-yl)benzyl carbonate (4)

In a 50 mL round-bottom flask, N,N'-disuccinimidyl carbonate (0.80 g, 3.14 mmol) was dissolved in dry acetonitrile (3.5 mL), then 4-(hydroxymethyl)phenylboronic acid pinacol

ester (0.50 g, 2.14 mmol) and N,N-diisopropylethylamine (0.81 g, 6.28 mmol) were added, and reacted at 0 ^{o}C under N_2 protection for 30 min, then for 2 h at room temperature. The solvent was evaporated under reduced pressure, and the residues were dissolved in 50 mL of DCM and extracted three times with saturated salt water (3 × 10 mL). The organic phase was dried with anhydrous sodium sulfate, filtered, and concentrated to yield crude product, which was purified by column chromatography (eluent: dichloromethane/ethyl acetate=10/1, v/v) to obtain a white solid product 4. Yield: 0.75 g, 93.75%. ^{1}H NMR (400 MHz, DMSO): δ = 7.74-7.72 (d, 2H, ArH), 7.47-7.45 (d, 2H, ArH), 5.43 (s, 2H, $ArCH_2O$), 2.81 (s, 4H, $COCH_2CH_2CO$), 1.30 (s, 12H, $(CH_3)_2CC(CH_3)_2$). ^{13}C NMR (100 MHz, DMSO): δ = 170.33, 151.69, 137.48, 135.16, 129.13, 128.38, 84.29, 72.49, 25.83, 25.14.

Synthesis of 4-(4,4,5,5-tetramethyl-1,3,2-dioxaborolan-2-yl)benzyl (2-bromoethyl)carbamate (5)

The above product 4 (0.71 g, 1.89 mmol) was dissolved in THF (10 mL), and then added to the aqueous solution (1.5 mL) of bromoethylamine hydrobromide (0.57g, 2.78 mmol) and N,N-diisopropylethylamine (0.36g, 2.79 mmol), stirred at room temperature for 5 h. The solvent was evaporated under reduced pressure, the residue was acidified with 5% citric acid (10 mL), then extracted with ethyl acetate (3 × 20 mL), and the organic phase was washed with brine (3 × 10 mL), dried over anhydrous sodium sulfate, filtered, and concentrated to yield crude product, which was purified by column chromatography (eluent: ethyl acetate/petroleum ether = 1/5, v/v) to obtain a colorless oily liquid product 5. Yield: 0.69 g, 95.07%. ^{1}H NMR (400 MHz, $CDCl_3$): δ = 7.81-7.80 (d, 2H, ArH), 7.36-7.34 (d, 2H, ArH), 5.24 (s, 1H, NH), 5.13 (s, 2H, $ArCH_2O$), 3.61-3.60 (d, 2H, $NHCH_2$), 3.48-3.46 (d, 2H, CH_2Br),

1.34 (s, 12H, $(CH_3)_2CC(CH_3)_2$). ^{13}C NMR (100 MHz, $CDCl_3$): δ = 156.12, 139.26, 135.02, 127.16, 83.88, 66.85, 42.78, 32.45, 24.86.

Synthesis of 4-(4,4,5,5-tetramethyl-1,3,2-dioxaborolan-2-yl)benzyl(2-azidoethyl)carbamate (BPmoc-N_3) (6)

The above product 5 (1.69 mmol, 0.65 g) and NaN_3 (3.38 mmol, 0.22 g) were dissolve in DMF (10 mL), stirred at 80 ℃ for 24 h, then stopped the reaction, added CH_2Cl_2 (20 mL), and extracted with saturated sodium chloride solution (3 × 10 mL), the organic phase was collected to obtain the crude product, which was purified by column chromatography (eluent: ethyl acetate/petroleum ether = 1/5, v/v) to obtain the white solid product 6. Yield: 0.55 g, 94.08%. ^1H NMR (400 MHz, $CDCl_3$): δ = 7.81-7.79 (d,2H, ArH), 7.35-7.34 (d, 2H, ArH), 5.12 (s, 3H, $ArCH_2O$+NH), 3.43-3.42 (d, 2H, CH_2N_3), 3.36-3.35 (d, 2H, $NHCH_2$), 1.34 (s, 12H, $(CH_3)_2CC(CH_3)_2$). ^{13}C NMR (100 MHz, $CDCl_3$): δ = 156.24, 139.31, 135.01, 127.14, 83.87, 66.80, 51.13, 40.48, 24.86.

Preparation of Micelles and Amylose Loaded Micelles

Micelles of PEG-*b*-PPRLT and PEG-*b*-(PPRLT-*g*-BPmoc) were prepared by a dialysis method. The amphiphilic block copolymer (25.0 mg) was dissolved in DMF (1.5 mL), and then DI water (10 mL) was slowly added with vigorous stirring. After vigorous stirring for another 2 h at RT, the resulting solution was got and submitted to dialyze against DI water that was renewed regularly for every 4 hour to remove DMF (MWCO 1 kDa). The final micelle concentration was adjusted by adding DI water to 0.5 mg mL^{-1}.

Measurement of the Critical Micellar Concentration (CMC)

The CMC of PEG-*b*-PPRLT and PEG-*b*-(PPRLT-*g*-BPmoc) amphiphiles were determined

by a dye solubilization method. Nile Red (NR) in THF (0.1 mg mL^{-1}, 30 μL) was added to a vial using a pipettor. After THF was evaporated, micellar solution (0.1 to 5×10^{-4} mg mL^{-1}, 2 mL) was added and stirred for 12 h. The fluorescence intensity of the resulting solutions was measured under the excitation wavelength of NR (550 nm).

Calculation of Loading Content (ALC)

The trapped content of amylose is directly related to the sensitivity of the system. Therefore the trapping capacity of PEG-*b*-(PPRLT-*g*-BPmoc) micelle with concentrations of 2.0 mg mL^{-1} was studied. The different concentrations of amylose (0, 0.05, 0.10, 0.15, 0.20, 0.25, 0.30, 0.35, 0.40, 0.45, 0.50, 1.0 and 2.0 mg mL^{-1}) were added to the micelle solution. After vigorous stirring under RT for 6 h, the same concentration of KI/I_2 was added. UV-vis spectrophotometer was used to monitor the amylose leakage. It was confirmed that once the amylose was trapped completely, the absorbance of amylose-KI/I_2 complex at 574 nm was not changed obviously. The ALC was calculated according to the following equation:

$$ALC\ (\%) = W_{loaded}/(W_{polymer} + W_{loaded}) \times 100\%$$

The W_{loaded} and $W_{polymer}$ are the weight of the loaded amylose and PEG-*b*-(PPRLT-*g*-BPmoc) micelles, respectively.

Cell culture and tumor xenografts model construction

MCF-7 cells were obtained from the biomedical engineering center of Hunan University (Changsha, China). Those cell was cultured using high-glucose Dulbecco's modified Eagle's medium (DMEM, GIBCO) with 1% penicillin-streptomycin (10,000 U mL^{-1}, 10,000 μg mL^{-1}, Invitrogen) and 10% fatal bovine serum (GIBCO) in an atmosphere of 5% CO_2 and 95% air at 37 ℃.

Eight-week-old BALB/c nude mice (male) purchased from SJA Co., Ltd. (Changsha, China) were used as the previous procedure of cervical tumor tissue slices preparation. All animal operations were in accord with institutional animal use and care regulations, according to protocol No. SYXK (Xiang) 2008-0001, approved by Laboratory Animal Center of Hunan. Preparation oftumor samples follow the previous procedure: a total of 2×10^6 MCF-7 cells diluted in 100 μL of serum-free DMEM medium were injected subcutaneously into the right flank of 6 to 8-week-old BALB/c nude mice to inoculate tumors. After 6-30 days, the mice were sacrificed, and the tumor samples were obtained.

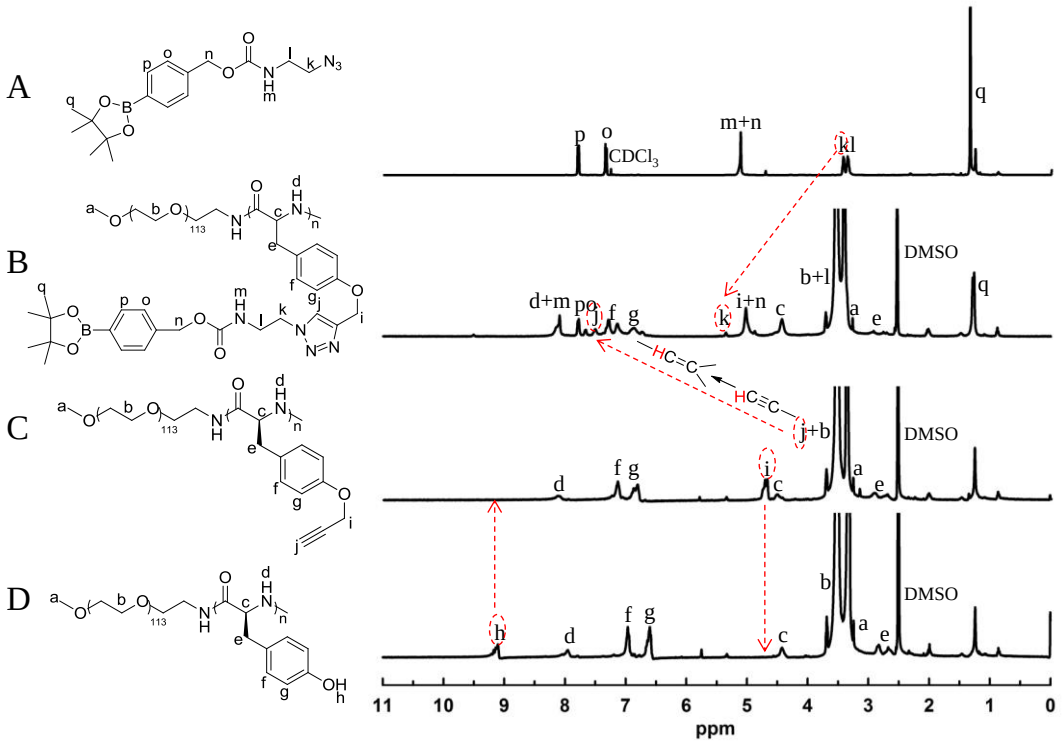


Figure S1. ^{1}H NMR spectra of (A) BPmoc-N_3, (B) PEG-*b*-(PPRLT-*g*-BPmoc), (C) PEG-*b*-PPRLT and (D) PEG-Tyr.

Table S1. Molecular Characteristics of Amphiphiles PEG-*b*-PPRLT and PEG-*b*-(PPRLT-*g*-BPmoc)

Entry	M_w/M_n [a]	$M_{n,GPC}$ [a]	$M_{n,NMR}$ [b]	CMC[c] (mg mL^{-1})
PEG-*b*-PPRLT	1.0221	7175	7035	0.0105
PEG-*b*-(PPRLT)-g-BPmoc	1.0613	11124	10495	0.0112

[a] Both molecular weight ($M_{n,GPC}$) and the polydispersity (M_w/M_n) of the amphiphiles were determined by GPC.
[b] $M_{n,NMR}$ was determined by ^{1}H NMR.
[c] CMC: the critical micellar concentration of the amphiphiles was determined by fluorescence spectroscopy (Figure S3).

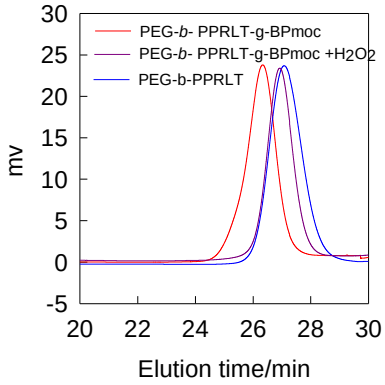


Figure S2. GPC traces of PEG-*b*-PPRLT (Blue line), PEG-*b*-(PPRLT-*g*-BPmoc) (Red line) and PEG-*b*-(PPRLT-*g*-BPmoc)+H_2O_2 (Purple line).

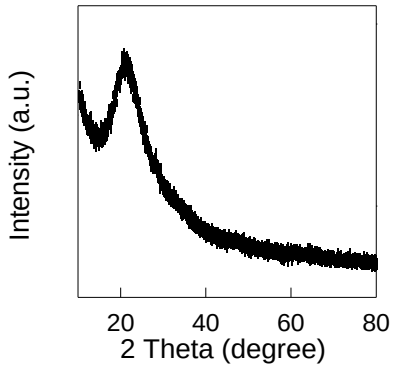


Figure S3. XRD pattern of PEG-*b*-(PPRLT-*g*-BPmoc)

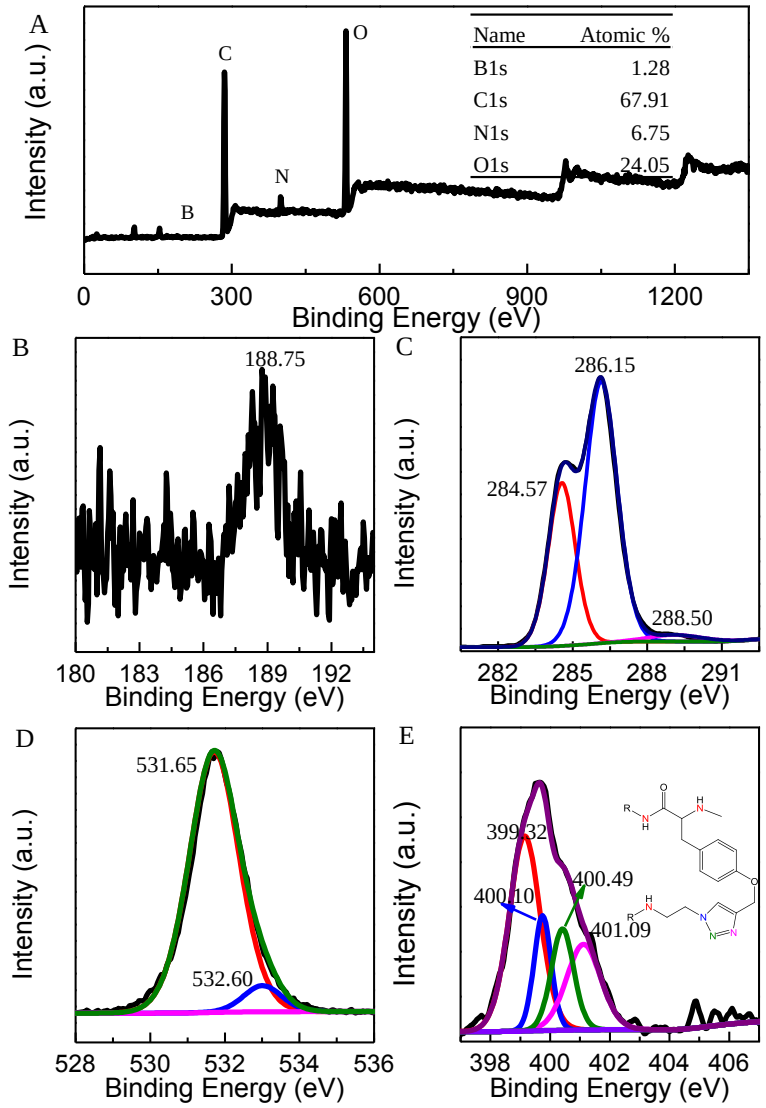


Figure S4. (A) XPS survey spectroscopy of PEG-*b*-(PPRLT-*g*-BPmoc). HRXPS spectra of : (B) B 1s, (C) C 1s, (D) O 1s and (E) N 1s.

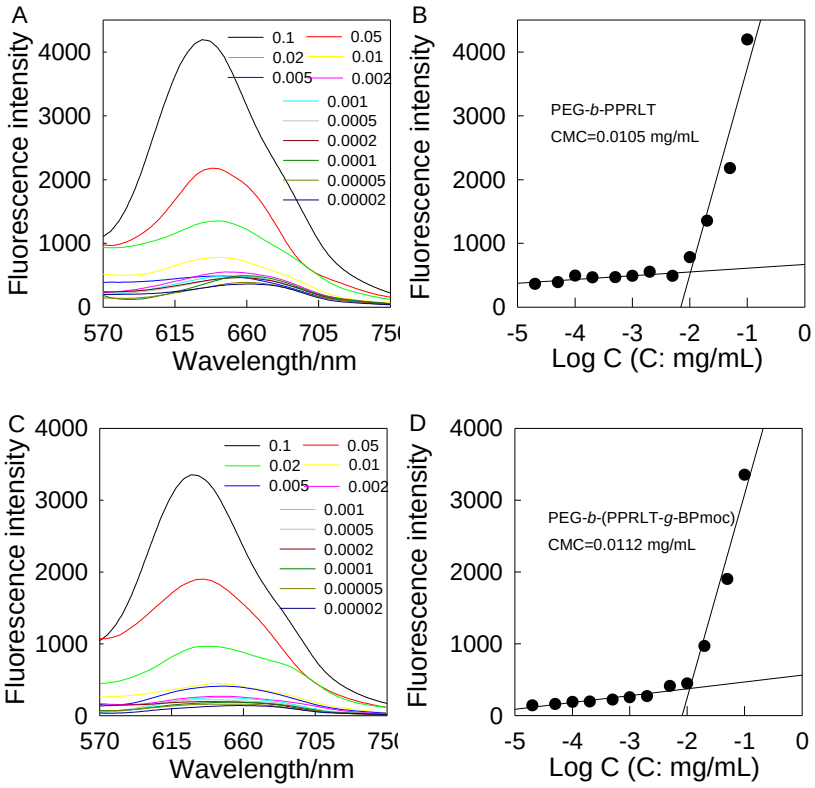


Figure S5. Fluorescence emission spectra of Nile Red in (A) PEG-*b*-PPRLT micelles and (C) PEG-*b*-(PPRLT-*g*-BPmoc) micelles of varying concentrations and the relevant emission intensity at 630 nm versus the log of concentration for (B) PEG-*b*-PPRLT micelles and (D) PEG-*b*-(PPRLT-*g*-BPmoc) micelles.

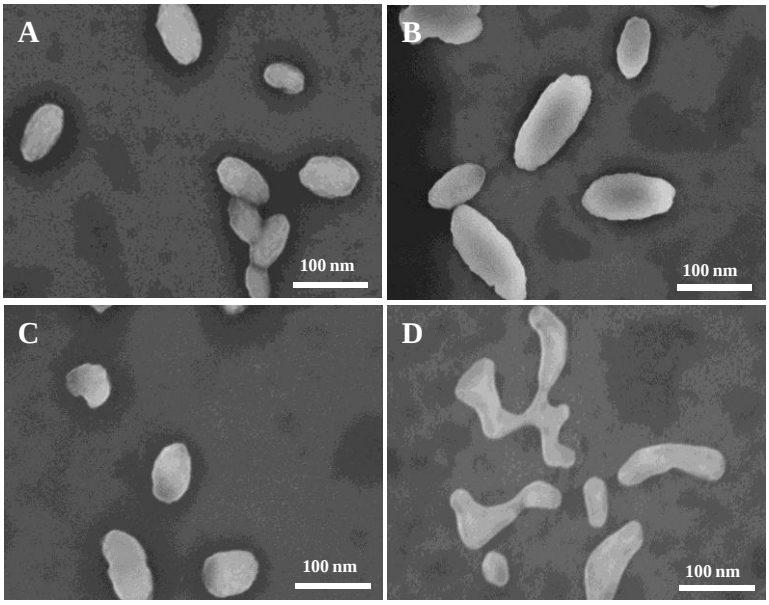


Figure S6. SEM photographs of the micelles: (A) PEG-*b*-PPRLT, (B) PEG-*b*-(PPRLT-*g*-BPmoc), (C) PEG-*b*-PPRLT treated by H_2O_2 and (D) PEG-*b*-(PPRLT-*g*-BPmoc) treated by H_2O_2.

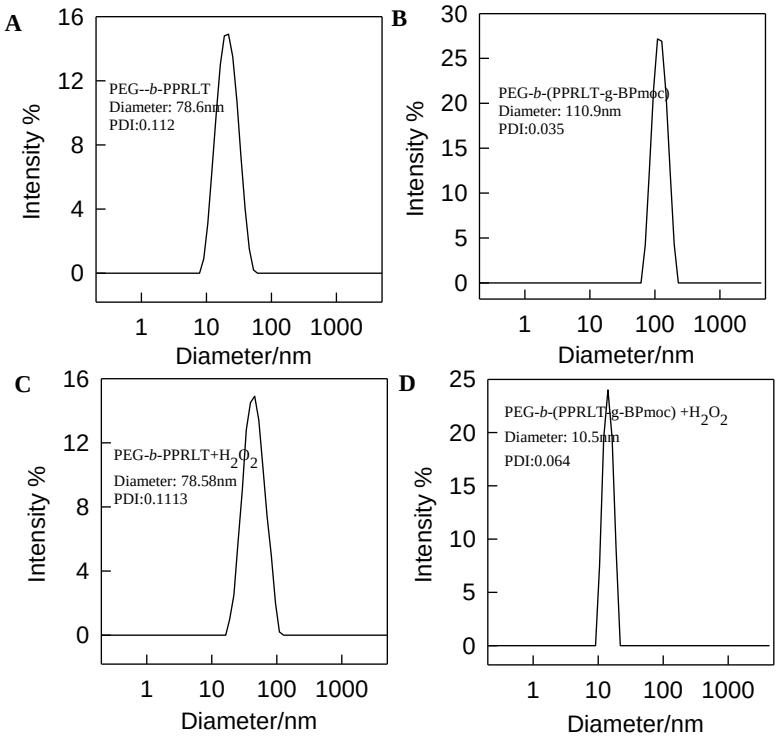


Figure S7. Mean size distributions of the micelles determined by DLS: (A) PEG-*b*-PPRLT, (B) PEG-*b*-(PPRLT-*g*-BPmoc), (C) PEG-*b*-PPRLT after 60 min treated by H_2O_2 and (D) PEG-*b*-(PPRLT-*g*-BPmoc) treated by H_2O_2 (1 μM).

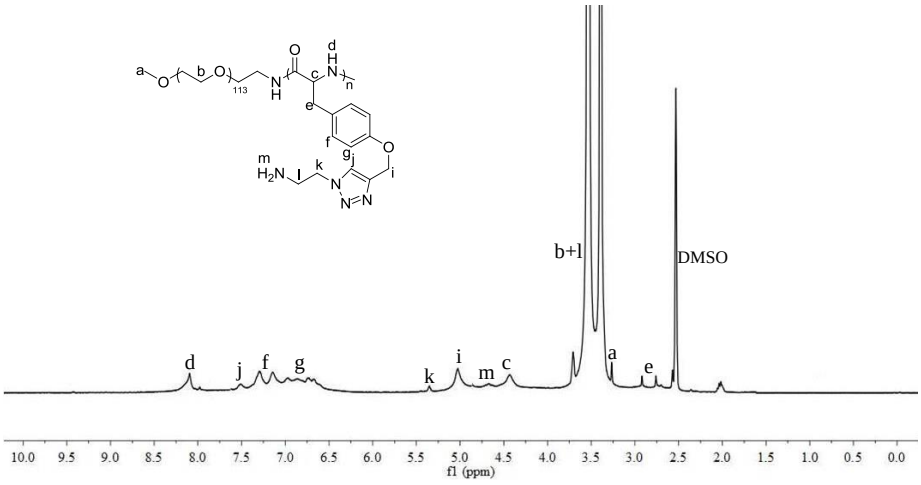


Figure S8. ^{1}H NMR of PEG-*b*-(PPRLT-*g*-BPmoc) micelles (0.5 mg mL^{-1}) treated with H_2O_2 (1 μM). (The product was allowed to dialyze in aqueous solution and lyophilize for ^{1}H NMR detection.)

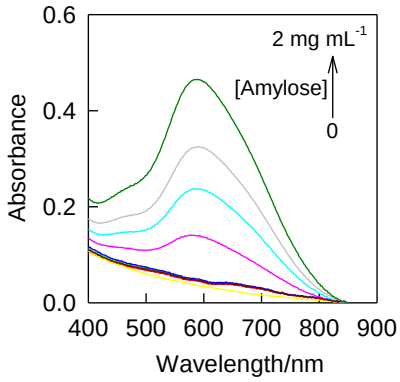


Figure S9. Trapping capacity of PEG-*b*-(PPRLT-*g*-BPmoc)@Amylose micelle (2.0 mg mL^{-1}) to different concentrations of amylose (0, 0.05, 0.10, 0.15, 0.20, 0.25, 0.30, 0.35, 0.40, 0.45, 0.50, 1.0 and 2.0 mg mL^{-1}) were researched. All of the experiments were performed with the same concentration of KI/I_2 (1 mM).

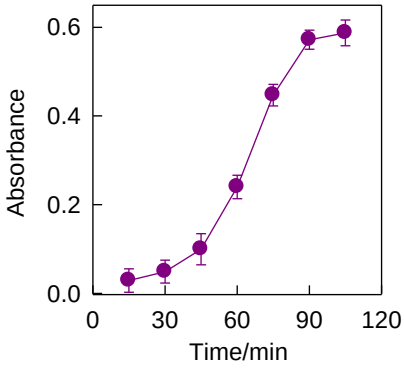


Figure S10. Absorption spectra changed with the time history of the PEG-*b*-(PPRLT-*g*-BPmoc)@Amylose micelle (0.5 mg mL^{-1}) treated with the H_2O_2 (1 μM). Soon afterwards, the solution was treated by KI/I_2 at the time of 0, 15, 30, 45, 60, 75, 90 and 105 min, respectively. Absorbance intensity was recorded at 574 nm.

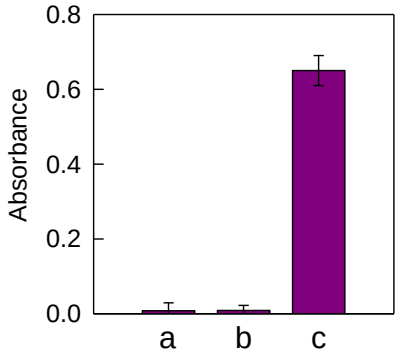


Figure S11. Absorption spectra changes of PEG-*b*-(PPRLT-*g*-BPmoc)@Amylose micelle (0.5mg mL^{-1}) under different conditions: free PEG-*b*-(PPRLT-*g*-BPmoc)@Amylose micelle (a), treated by H_2O_2 (1μM) for 90 min (b), and upon addition of KI/I_2 (c).

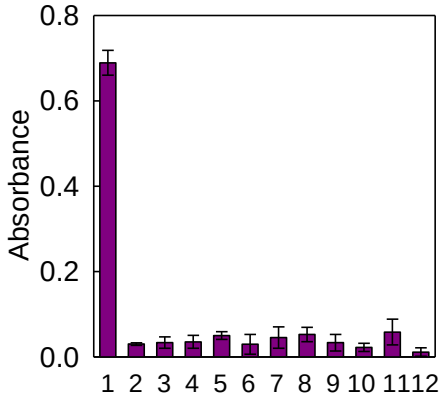


Figure S12. The selectivity of PEG-*b*-(PPRLT-*g*-BPmoc)@Amylose micelle (0.5 mg mL^{-1}) was toward various ROS. 1-12 are H_2O_2, CH_3CO_3H, $^1O_2$, O^tBu, OH, $O_2^{-\cdot}$, HClO, Fe^{3+}, NO^{2-}, NO^{3-}, tert-butyl hedroperoxide (TBHP) and NO, respectively. The absorbance of PEG-*b*-(PPRLT-*g*-BPmoc)@Amylose micelle in PBS (pH 7.4, 20 mM) solution and treated by KI/I_2 90 min later after addition of ROS (1 μM), respectively.

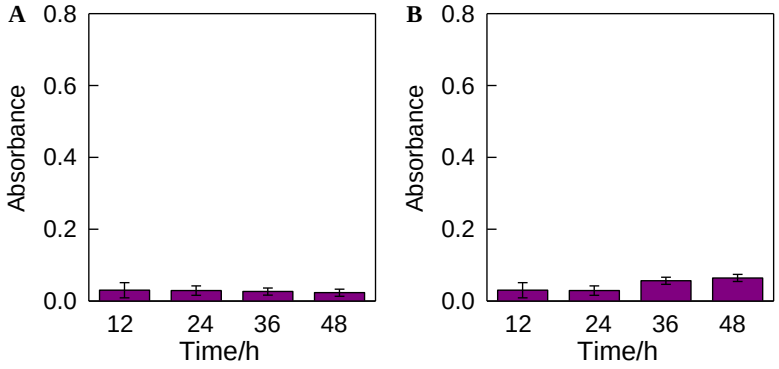
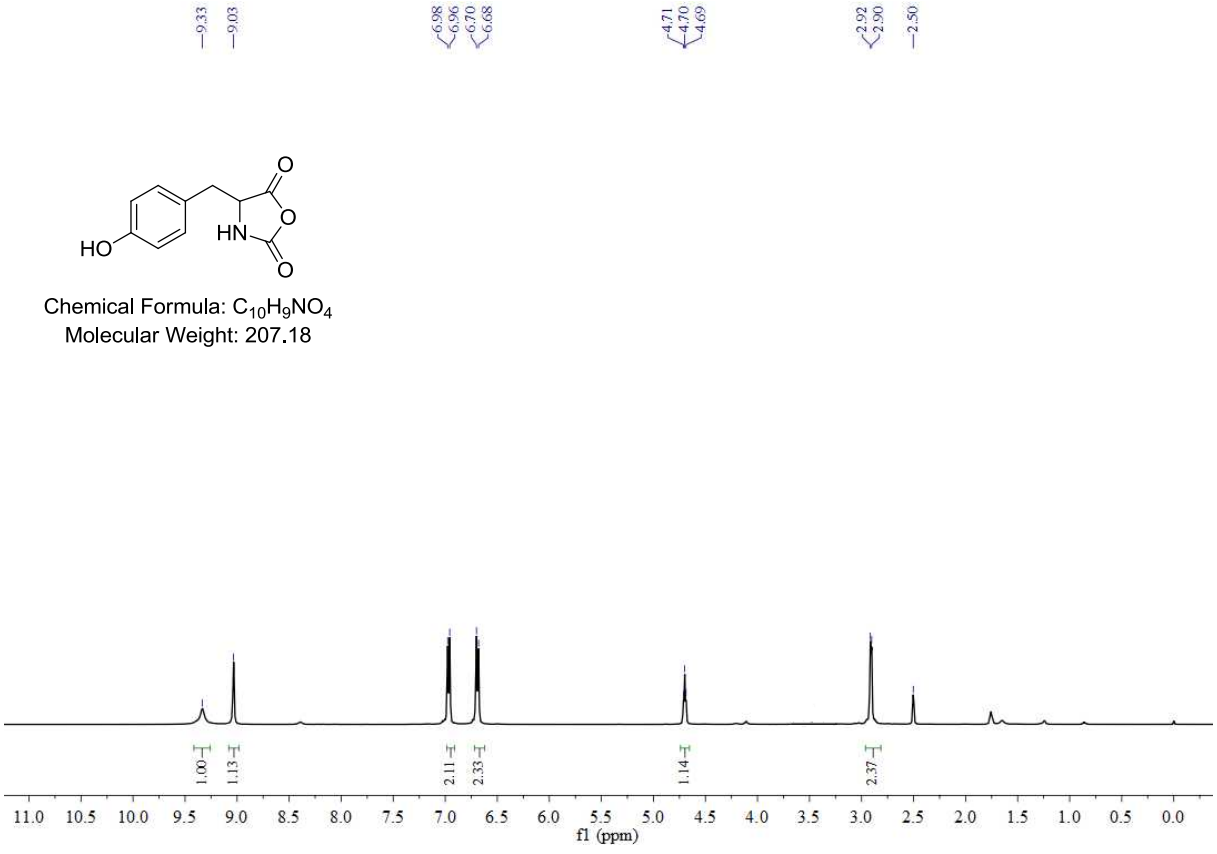
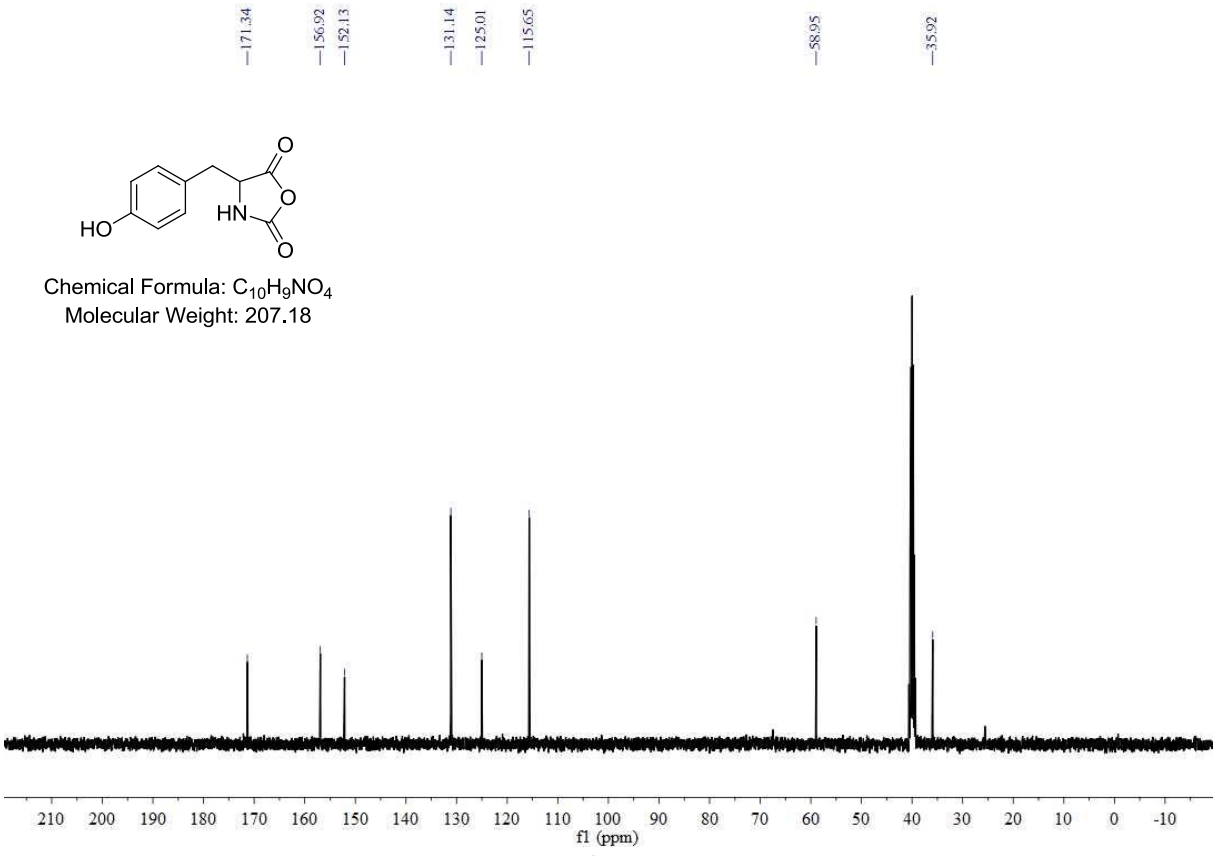
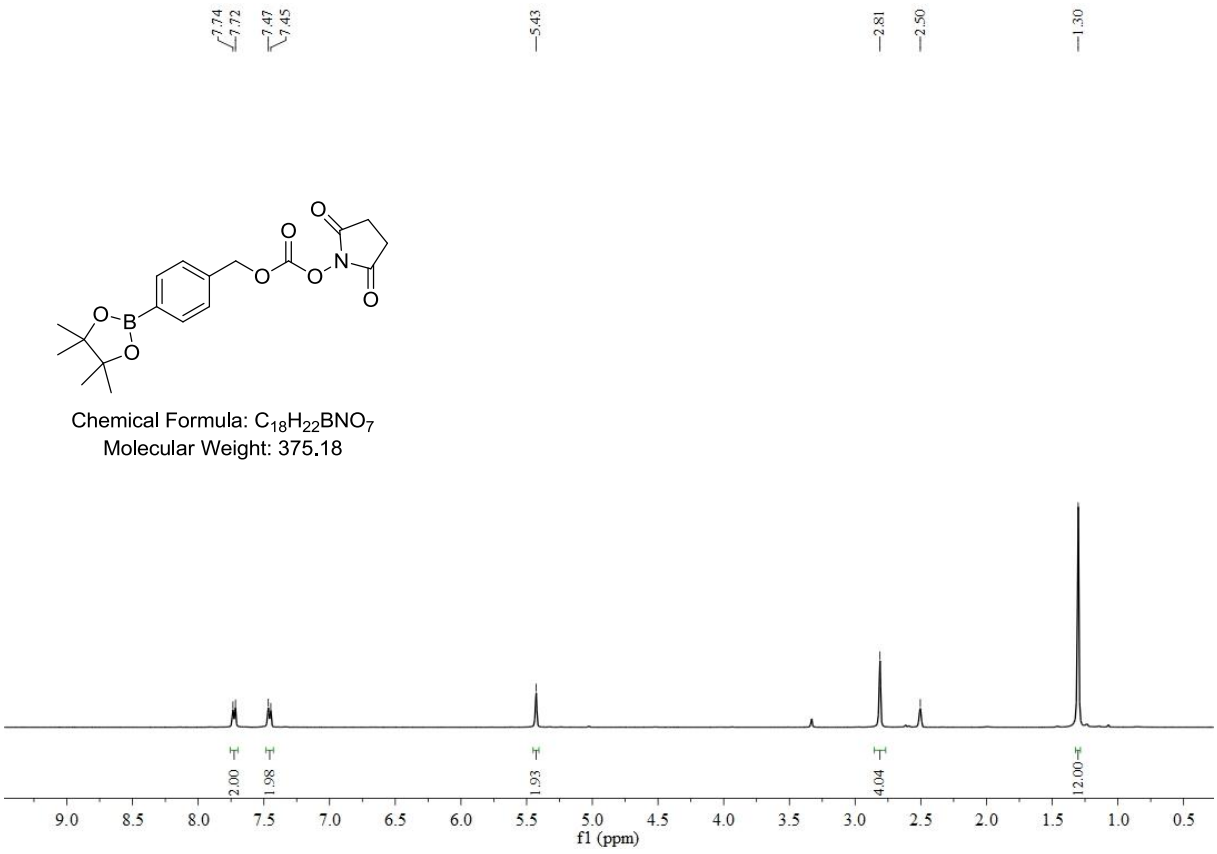


Figure S13. (A) Effect of human cell lysate-contained biological fluids on PEG-*b*-(PPRLT-*g*-BPmoc)@Amylose at different times (12, 24, 36 and 48 h). (B) Effect of bovine serum albumin on PEG-*b*-(PPRLT-*g*-BPmoc)@Amylose at different times (12, 24, 36 and 48 h). All the needed PEG-*b*-(PPRLT-*g*-BPmoc)@Amylose concentration was 0.5 mg mL^{-1} and the KI/I_2 was 1 mM.

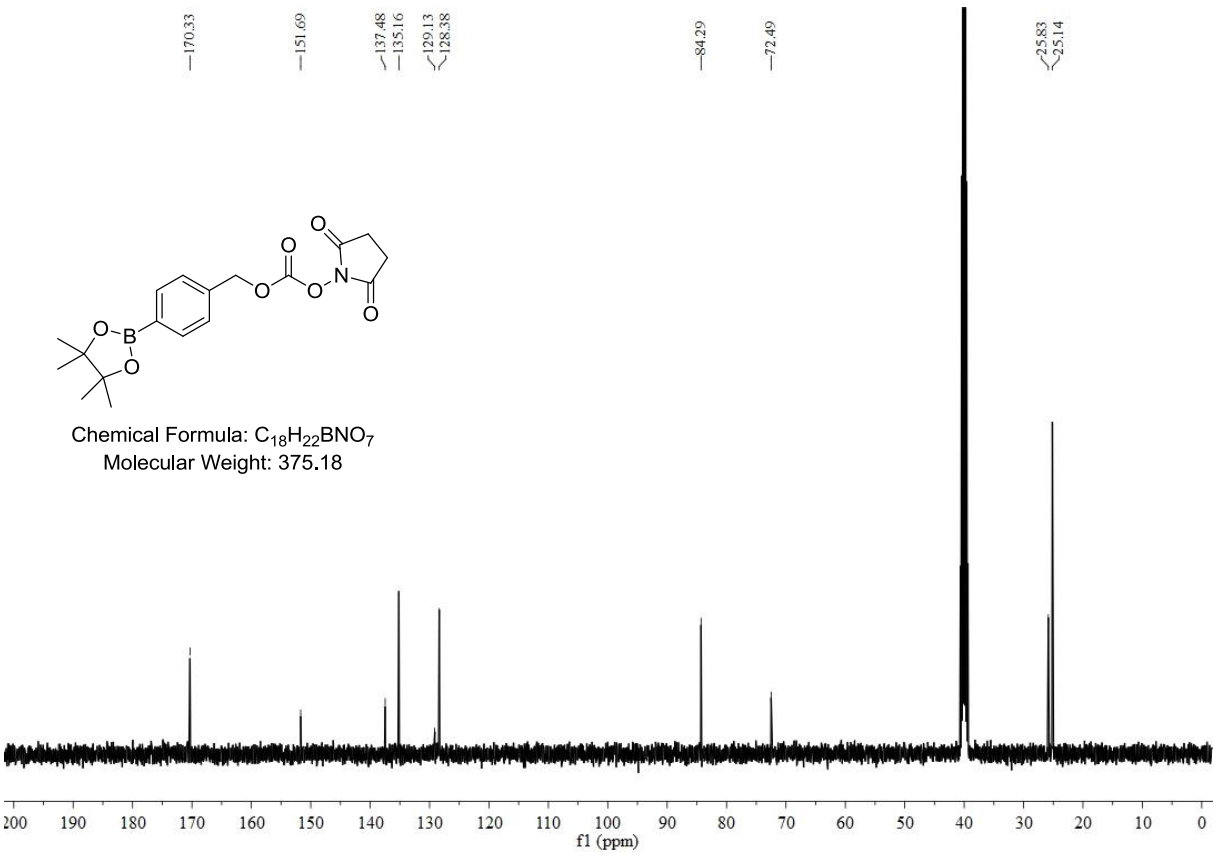
^{1}H NMR and ^{13}C NMR







Chemical Formula: $C_{18}H_{22}BNO_7$
Molecular Weight: 375.18



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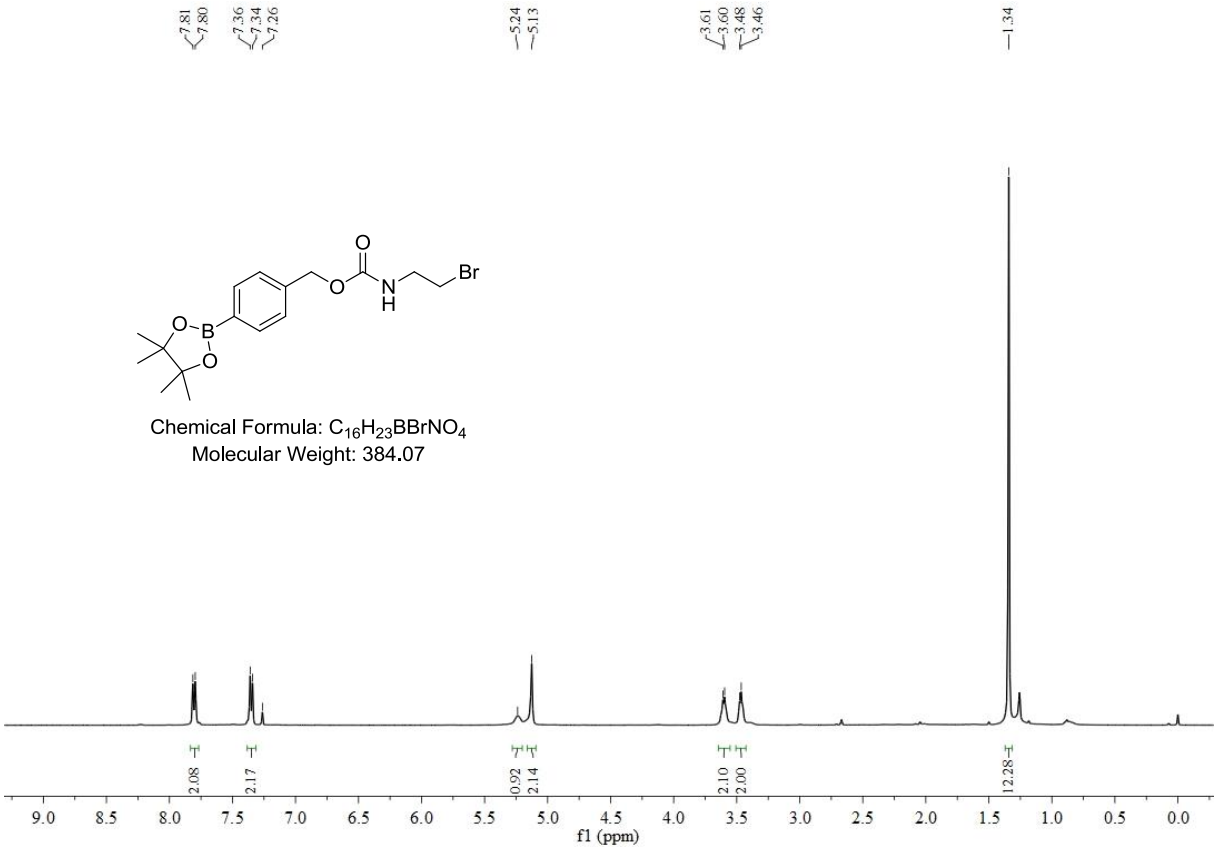

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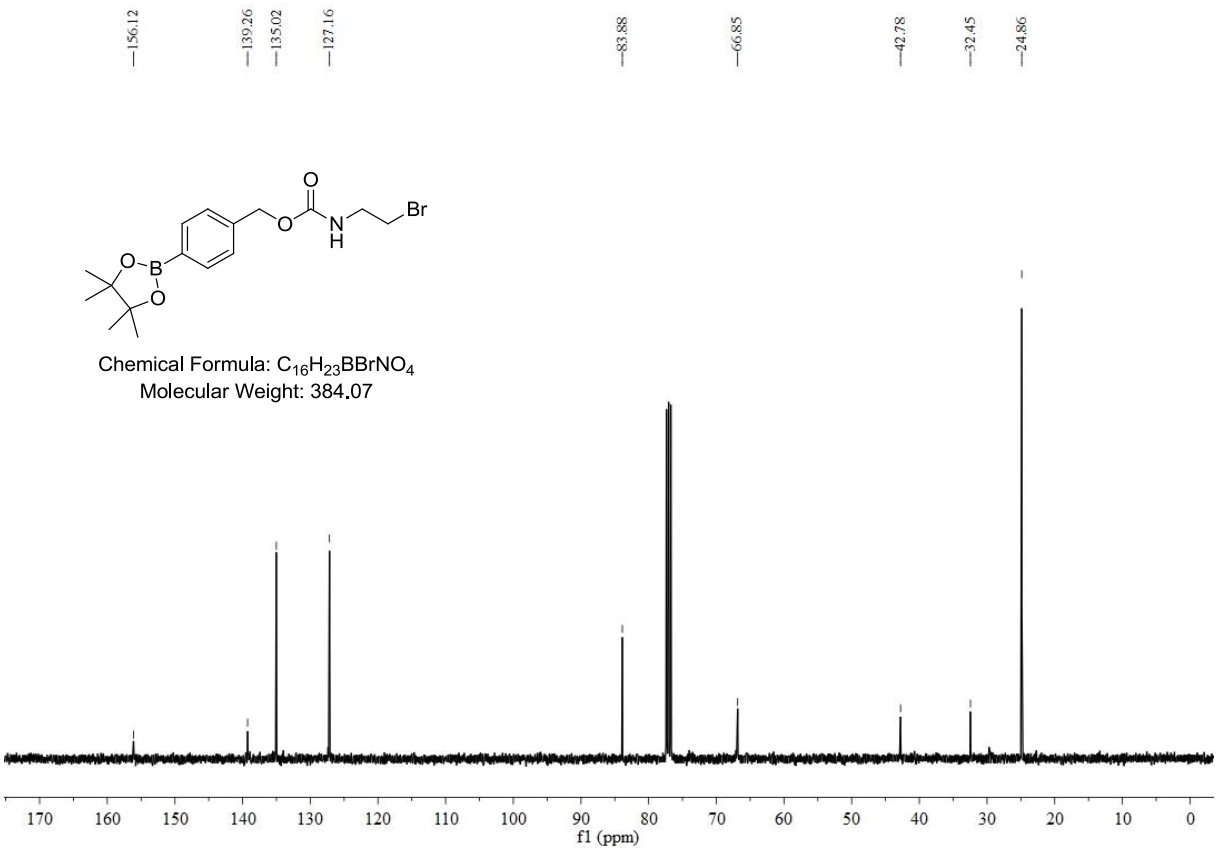
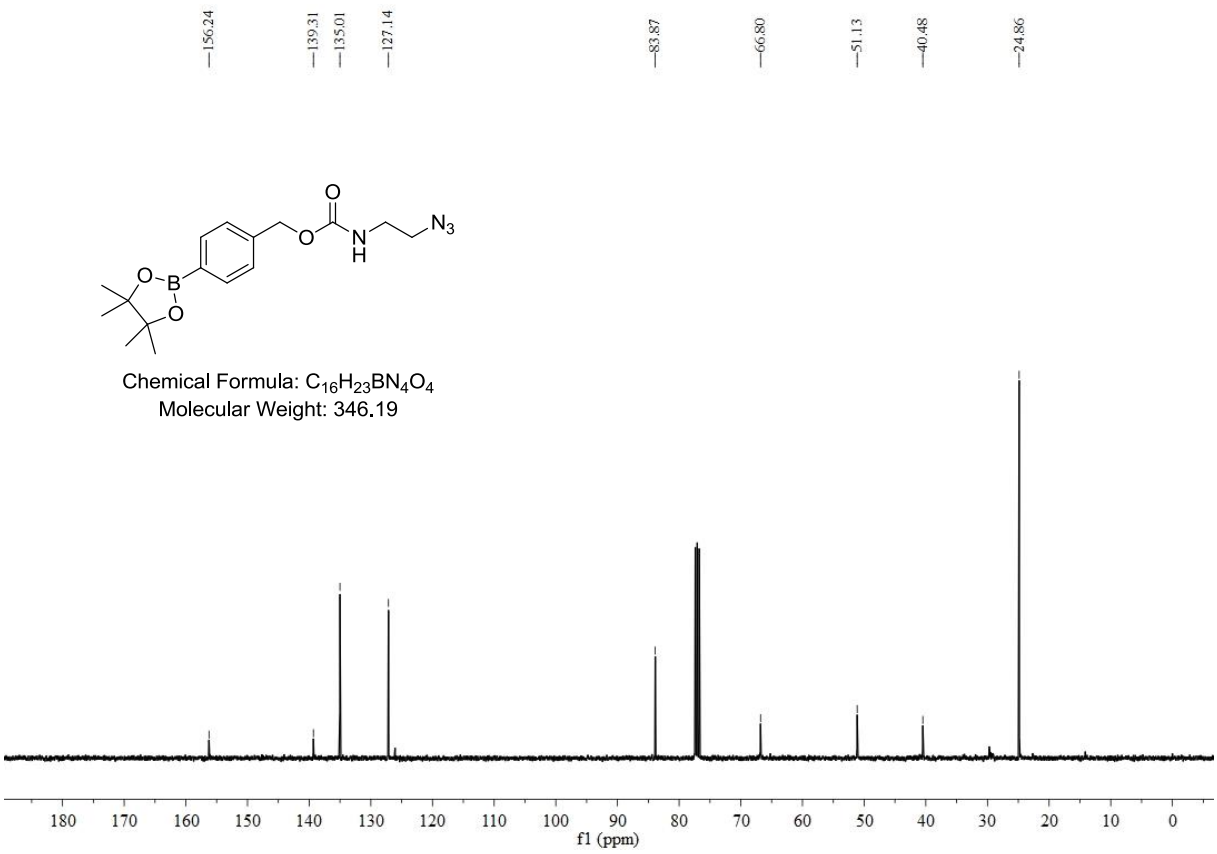


Chemical Formula: $C_{16}H_{23}BBrNO_4$
Molecular Weight: 384.07



Chemical Formula: $C_{16}H_{23}BN_4O_4$
Molecular Weight: 346.19