Electronic Supplementary Material (ESI) for Journal of Materials Chemistry B.

Supporting information

In cellulo Synthesis of Dendrimeric Sensors For Fluorescence-on Imaging of Bacterial Phagocytosis

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CONTENTS

Scheme S1 Synthetic route of ^{6Az}ProRed-TPP. S3

Scheme S2 Synthetic route of ^{4Az}Red and ^{6Az}Red.....S3

Fig. S1 In vitro SPAAC of ^{4Az}Red (A), ^{6Az}Red (B) or ^{6Az}ProRed-TPP (C) with ^{DBCO}Blue-TPP. S4

Fig. S2 Probe targeting for bacteria.....S5

Fig. S3 Tagging of stressed bacteria with the adduct of ^{2Az}Red and ^{DBCO}Blue-TPP.....S5

Fig. S4 Tagging of stressed bacteria with the adduct of ^{4Az}Red and ^{DBCO}Blue-TPP.....S6

Fig. S5 Tagging of stressed bacteria with the adduct of ^{6Az}Red and ^{DBCO}Blue-TPP.....S6

Fig. S6 Efficiently SPAAC of ^{2Az}Red (A), ^{4Az}Red (B) or ^{6Az}Red (C) and ^{DBCO}Blue-TPP in *S.aureus*. S7

Fig. S7 Tagging of stressed bacteria with the adduct of ^{0Az}Red and ^{DBCO}Blue-TPP.....S8

Fig. S8 Colocalization of Dendritic sensors with FITC-D-Lys-labeled bacteria.....S8

Fig. S9 Optical properties of the adduct of ^{6Az}ProRed-TPP and ^{DBCO}Blue-TPP.....S9

Fig. S10 Tagging of stressed Bacteria with dendritic pH sensors (pH 7.0). S9

Fig. S11 Tagging S.aureus with Den-pH.....S9

Fig. S12 Colocalization of Dendritic pH sensors with FITC-D-Lys labelled bacteria. S10

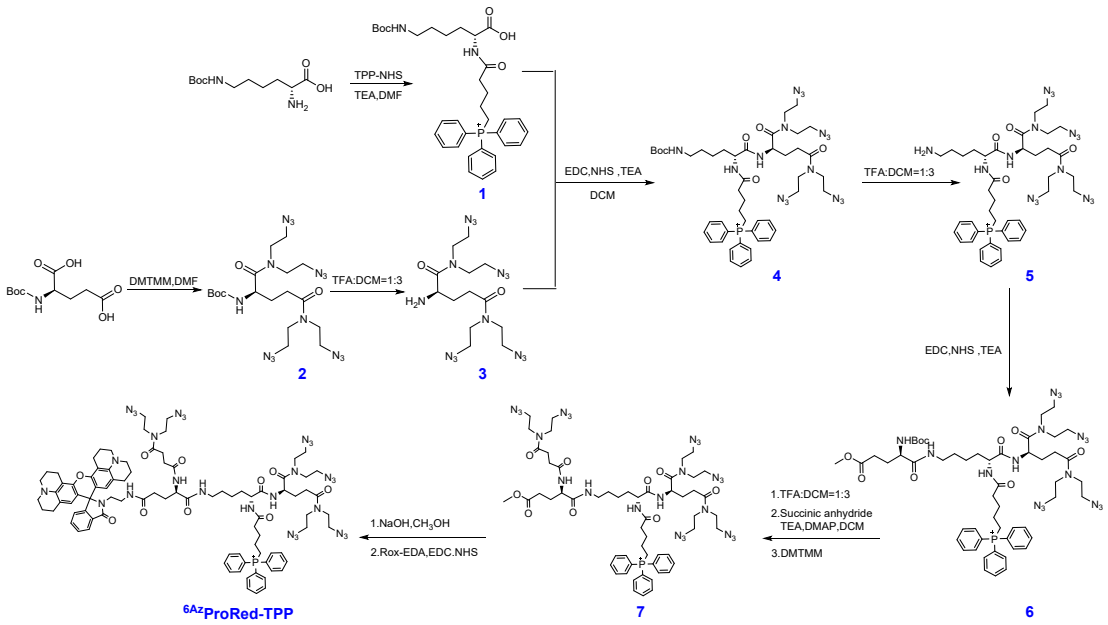
Fig. S13 The Organelle localization of ^{6Az}ProRed-TPP leaked from engulfed bacteria. S10

Fig. S14 The Organelle localization of ^{DBCO}Blue-TPP. S10

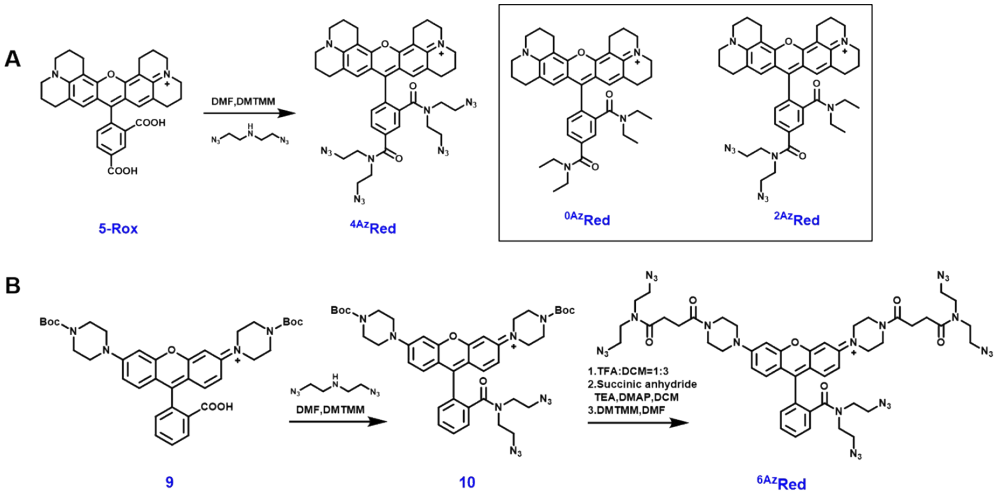
Fig. S15 Inability of Den-pH to tag *S.aureus*.....S10

NMR spectra. S11

Mass spectra S20



Scheme S1 Synthetic route of ^{6Az}ProRed-TPP.



Scheme S2 Synthetic route of ^{4Az}Red and ^{6Az}Red.

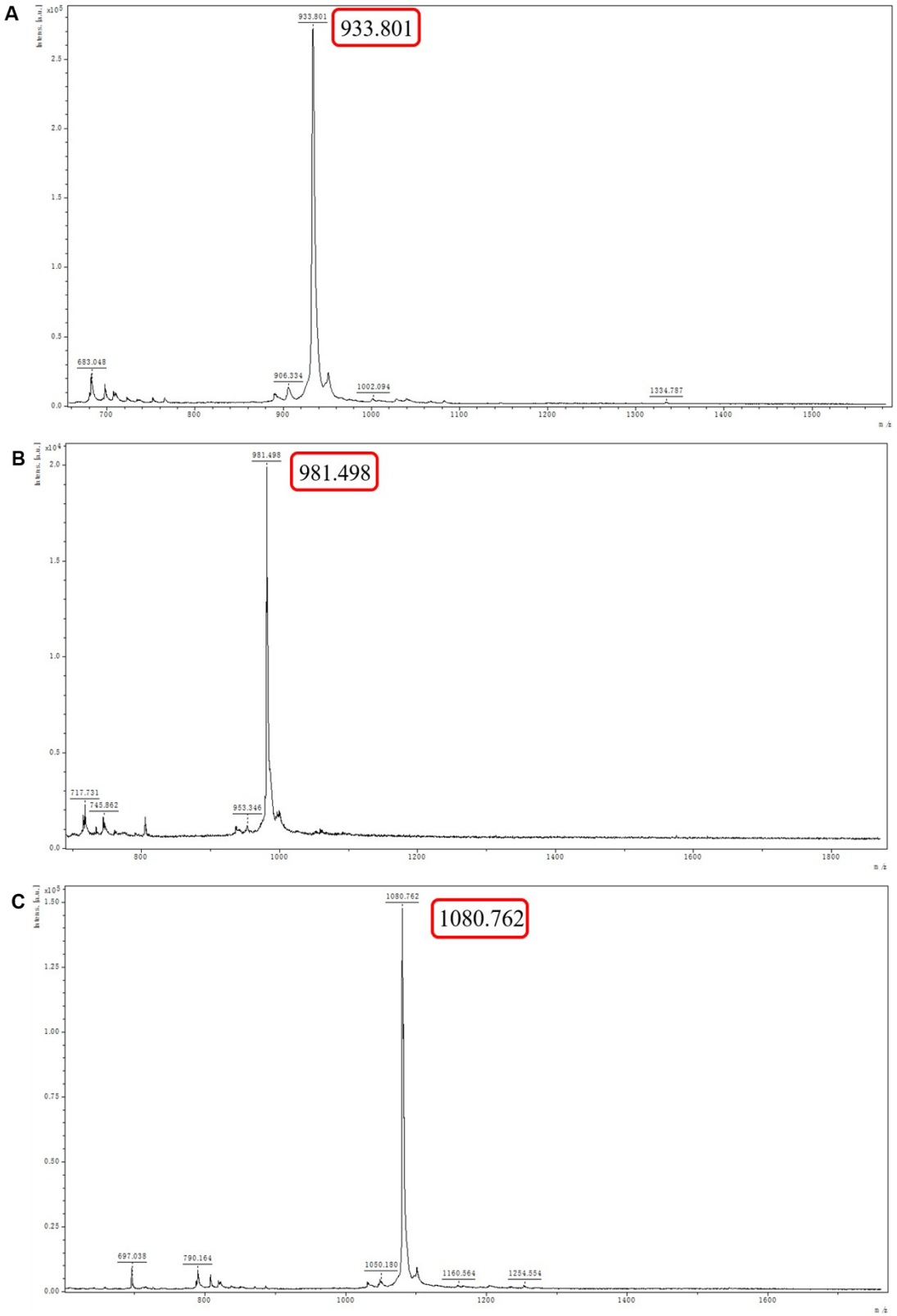


Fig. S1 In vitro SPAAC of ^{4Az}Red (A), ^{6Az}Red (B) or ^{6Az}ProRed-TPP (C) with ^{DBCO}Blue-TPP.

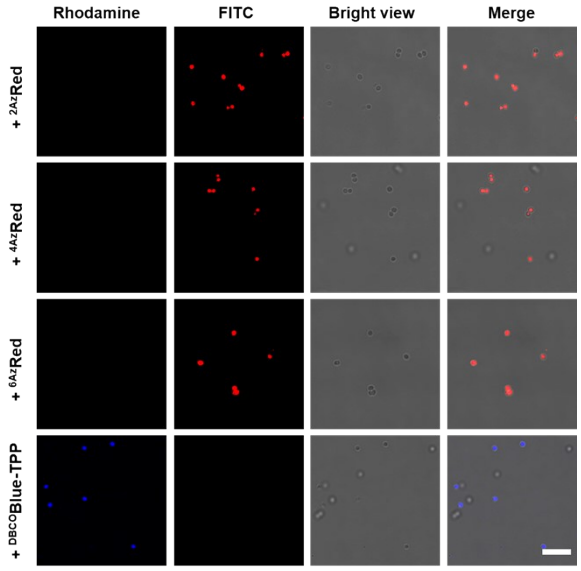


Fig. S2 Probe targeting for bacteria. *S. aureus* were incubated at 37°C for 1 h in LB medium supplemented with ^{2Az}Red (50 μM), ^{4Az}Red (50 μM), ^{6Az}Red (200 μM) and ^{DBCO}Blue-TPP (50 μM) respectively. The *S. aureus* were washed with PBS, and then visualized by confocal microscopy.

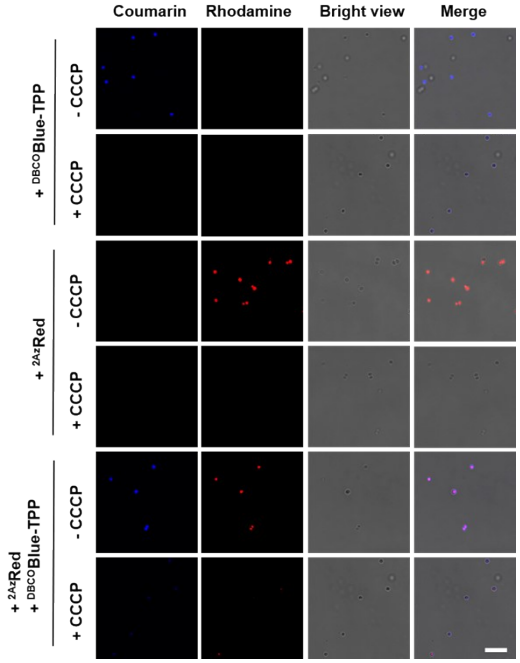


Fig. S3 Tagging of stressed bacteria with the adduct of ^{2Az}Red and ^{DBCO}Blue-TPP. *S. aureus* were incubated at 37°C for 1 h in LB medium supplemented with ^{2Az}Red (50 μM) or no addition, and then cultured in LB medium spiked with or without ^{DBCO}Blue-TPP (100 μM) for 1 h. *S. aureus* were washed with PBS, and then visualized by confocal microscopy. *S. aureus* were washed with PBS, and then maintained in LB medium containing CCCP (300 μM) for 1 h, and then visualized by confocal microscopy.

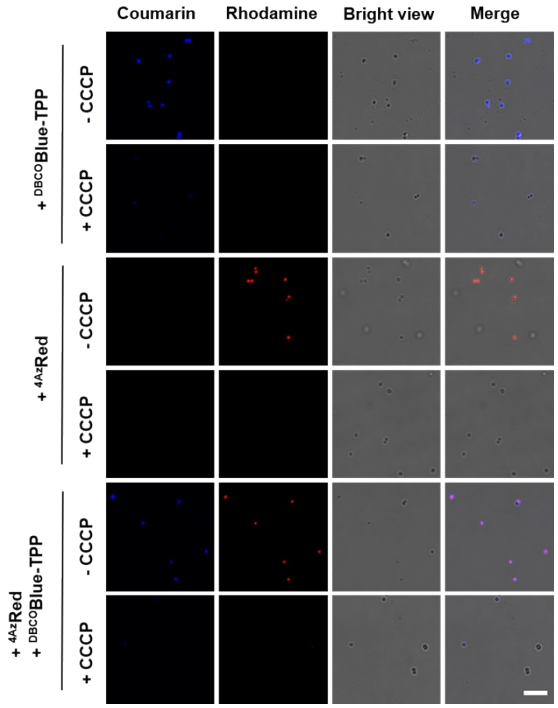


Fig. S4 Tagging of stressed bacteria with the adduct of ^{4Az}Red and ^{DBCO}Blue-TPP. *S. aureus* were incubated at 37°C for 1 h in LB medium supplemented with ^{4Az}Red (50 μM) or no addition, and then cultured in LB medium spiked with or without ^{DBCO}Blue-TPP (200 μM) for 1 h. *S. aureus* were washed with PBS, and then visualized by confocal microscopy. *S. aureus* were washed with PBS, and then maintained in LB medium containing CCCP (300 μM) for 1 h, and then visualized by confocal microscopy.

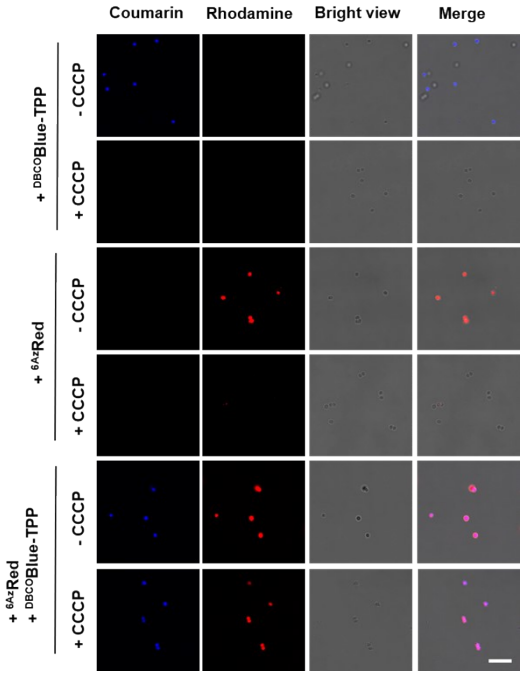


Fig. S5 Tagging of stressed bacteria with the adduct of ^{6Az}Red and ^{DBCO}Blue-TPP. *S. aureus* were incubated at 37°C for 1 h in LB medium supplemented with ^{4Az}Red (200 μM) or no addition, and then cultured in LB medium spiked with or without ^{DBCO}Blue-TPP (300 μM) for 1 h. *S. aureus* were washed with PBS, and then visualized by confocal microscopy. *S. aureus* were washed with PBS, and then maintained in LB medium containing CCCP (300 μM) for 1 h, and then visualized by confocal microscopy

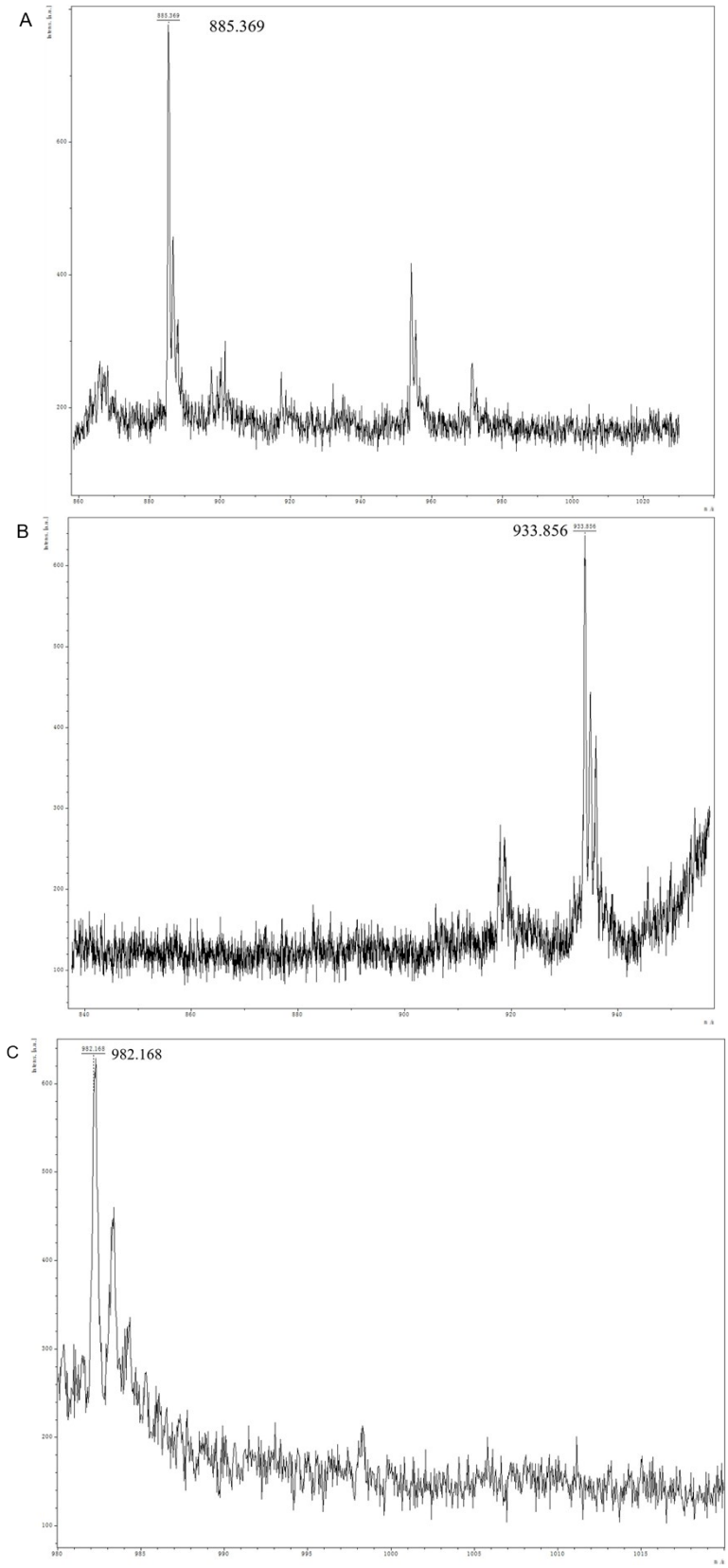


Fig. S6 SPAAC of ^{2Az}Red (A), ^{4Az}Red (B) or ^{6Az}Red (C) and ^{DBCO}Blue-TPP in *S.aureus*.

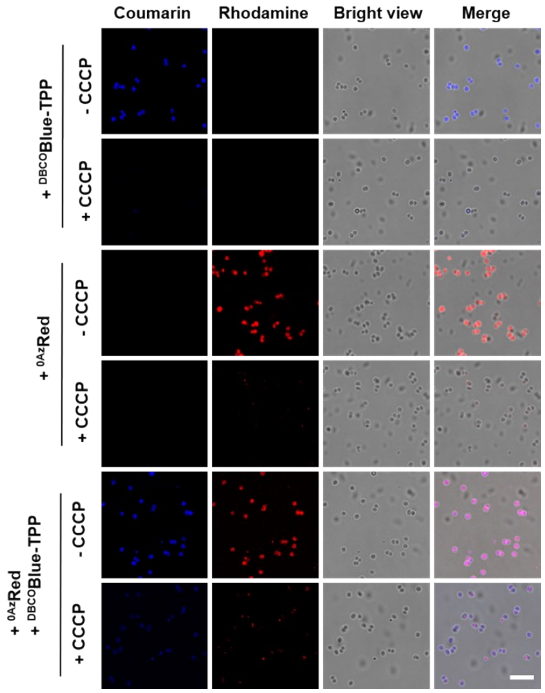


Fig. S7 Tagging of stressed bacteria with the adduct of ^{0Az}Red and ^{DBCO}Blue-TPP. *S. aureus* were incubated at 37°C for 1 h in LB medium supplemented with ^{0Az}Red (50 μM) or no addition, and then cultured in LB medium spiked with or without ^{DBCO}Blue-TPP (50 μM) for 1 h. *S. aureus* were washed with PBS, and then visualized by confocal microscopy. *S. aureus* were washed with PBS, and then maintained in LB medium containing CCCP (300 μM) for 1 h, and then visualized by confocal microscopy.

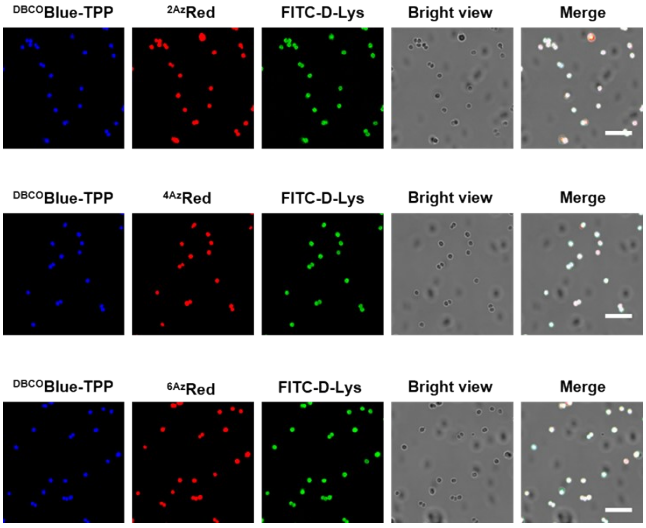


Fig. S8 Colocalization of Dendritic sensors with FITC-D-Lys-labeled bacteria. *S. aureus* prelabeled with FITC-D-Lys were incubated at 37 °C for 1 h in LB medium supplemented with ^{2Az}Red (50 μM), ^{4Az}Red (50 μM) and ^{6Az}Red (200 μM) respectively, and then cultured in LB medium spiked with ^{DBCO}Blue-TPP (100 μM, 200 μM, 300 μM) for 1 h. The cells were collected, washed with PBS, and then visualized by confocal microscopy

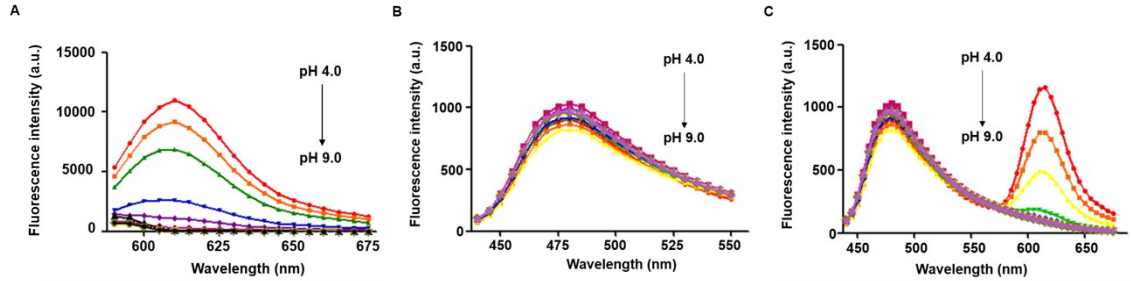


Fig. S9 Optical properties of the adduct of ^{6Az}ProRed-TPP and ^{DBCO}Blue-TPP. (A) Acidity-reporting red fluorescence of ^{6Az}ProRed-TPP. (B) Unaltered blue fluorescence of coumarin in the adduct of ^{6Az}ProRed-TPP and ^{DBCO}Blue-TPP at different pH. (C) pH titration curves of the adducts were plotted using fluorescence emission of coumarin (I_{475} nm) and Rox(I_{610} nm) over pH. The adducts were added to PBS buffer (10 mM) of varied pH (4.0–9.0) to a final concentration of 10 μM, and the solutions were analyzed for fluorescence emission using λex = 430 nm for adducts from ^{6Az}ProRed-TPP/^{DBCO}Blue-TPP.

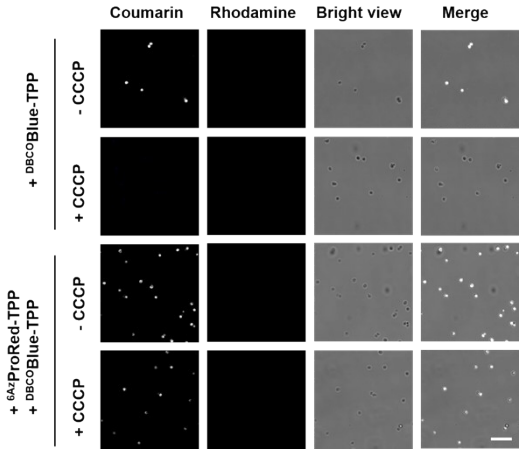


Fig. S10 Tagging of stressed Bacteria with dendritic pH sensors (pH 7.0). *S. aureus* were incubated at 37 °C for 1 h in LB medium supplemented with ^{6Az}ProRed-TPP (200 μM), and then cultured in LB medium spiked with or without ^{DBCO}Blue-TPP (600 μM) for 1 h. *S. aureus* were washed with PBS, and then maintained in buffer of pH 7.0 or buffer of pH 7.0 containing CCCP (300 μM) for 1h, and then visualized by confocal microscopy.

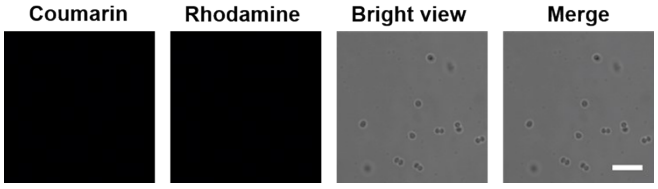


Fig. S11 Inability of Den-pH synthesized in vitro to tag *S.aureus*. *S. aureus* were incubated at 37 °C for 1 h in LB medium supplemented with Den-pH (200 μM) for 1h, and then visualized by confocal microscopy.

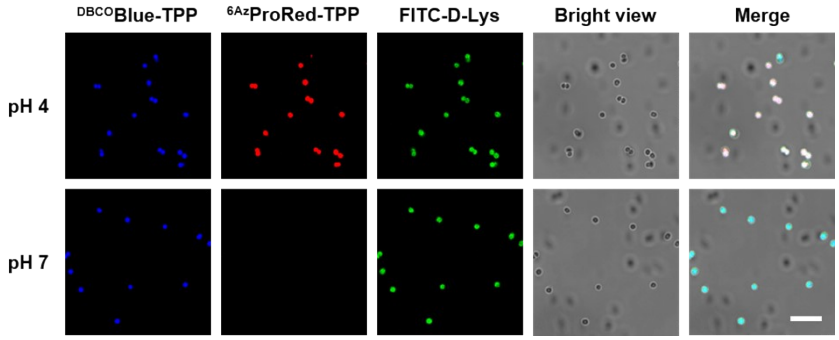


Fig. S12 Colocalization of Dendritic pH sensors with FITC-D-Lys labelled bacteria. S. aureus prelabeled with FITC-D-Lys were incubated at 37 °C for 1 h in LB medium supplemented with ^{6Az}ProRed-TPP (200 μM), and then cultured in LB medium spiked with ^{DBCO}Blue-TPP (300 μM) for 1 h. The cells were collected, washed with PBS, and then maintained in buffer of pH 4.0 or buffer of pH 7.0. The cells were analyzed by confocal fluorescence microscopy.

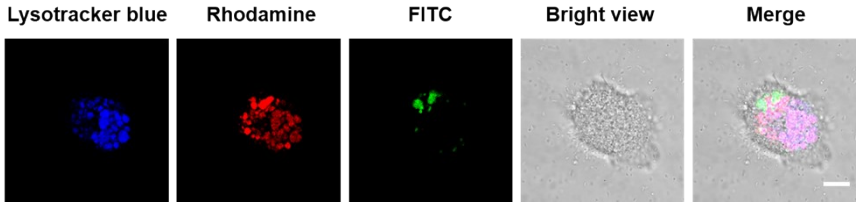


Fig. S13 The Organelle localization of ^{6Az}ProRed-TPP leaked from engulfed bacteria. *S. aureus* prelabeled with FITC-D-Lys were incubated with ^{6Az}ProRed-TPP (200 μM) and ^{DBCO}Blue-TPP (600 μM) for 1 h, respectively. The bacteria were co-cultured with BMDMs for 1 h. The BMDM were further treated with lysotracker blue (2 μM) for 30 min, and then visualized by confocal fluorescence microscopy.

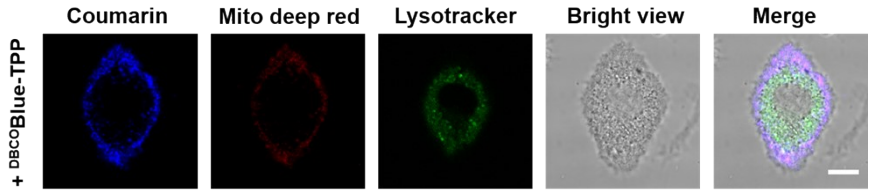


Fig. S14 Organelle localization of ^{DBCO}Blue-TPP. BMDMs were incubated with ^{DBCO}Blue-TPP (5 μM) for 1 h. BMDMs were further treated with lysotracker green (2 μM) for 1 h, and then with mitotracker deep red (1 μM) for 10 min. The cells visualized by confocal fluorescence microscopy.

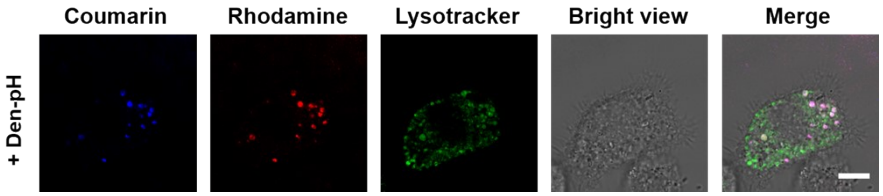


Fig. S15 The Organelle localization of Den-pH. BMDMs were incubated with Den-pH (5 μM) for 1 h. BMDMs were further treated with lysotracker green (2 μM) for 1 h, and then visualized by confocal fluorescence microscopy.

NMR spectra.

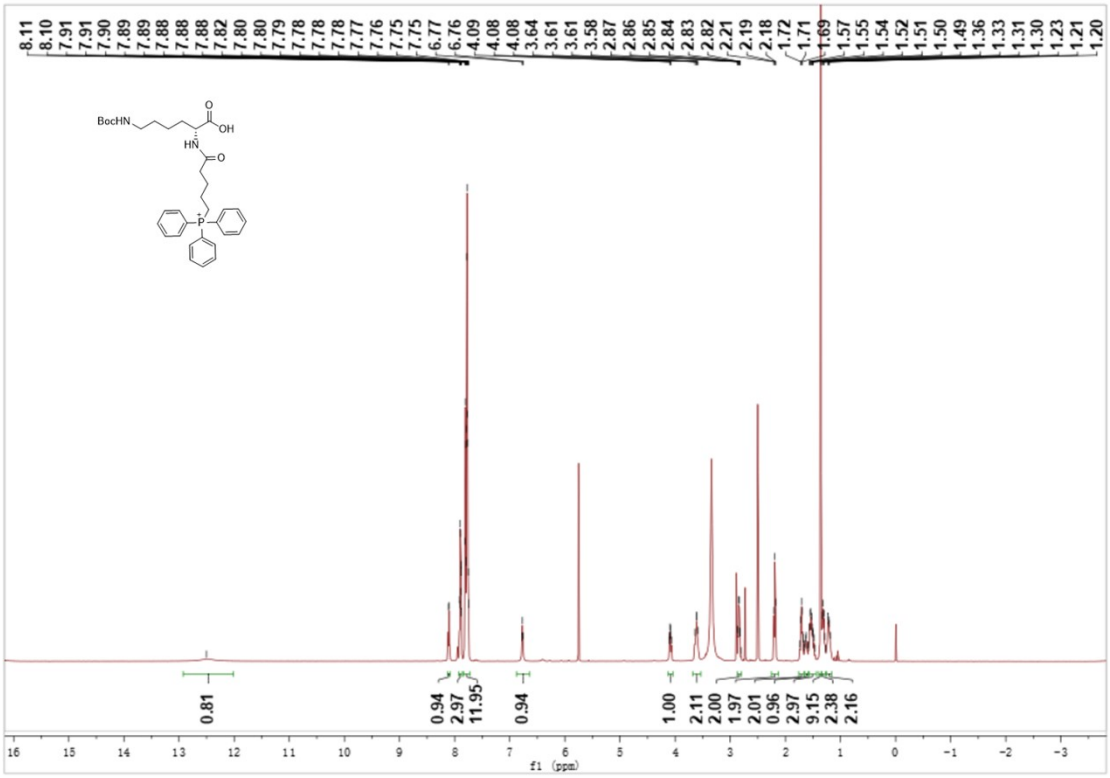


Fig. S11 ^{1}H NMR spectrum of compound **1** ($CDCl_3$).

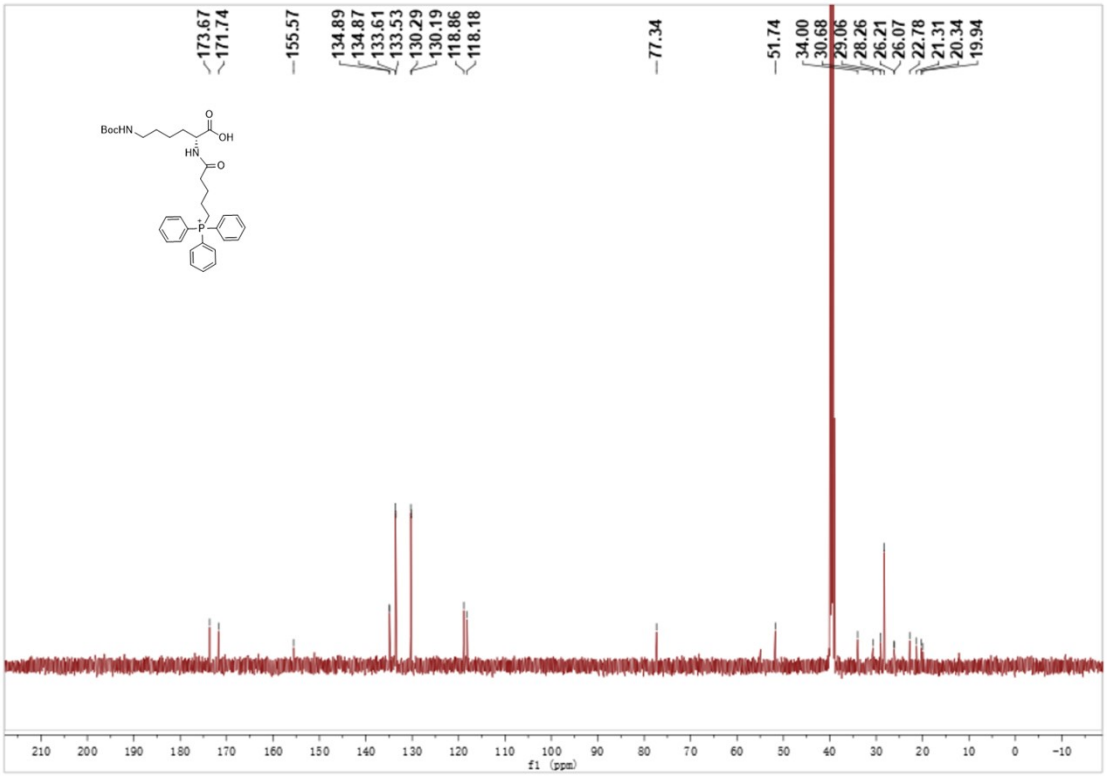


Fig. S12 ^{13}C NMR spectrum of compound **1** ($CDCl_3$).

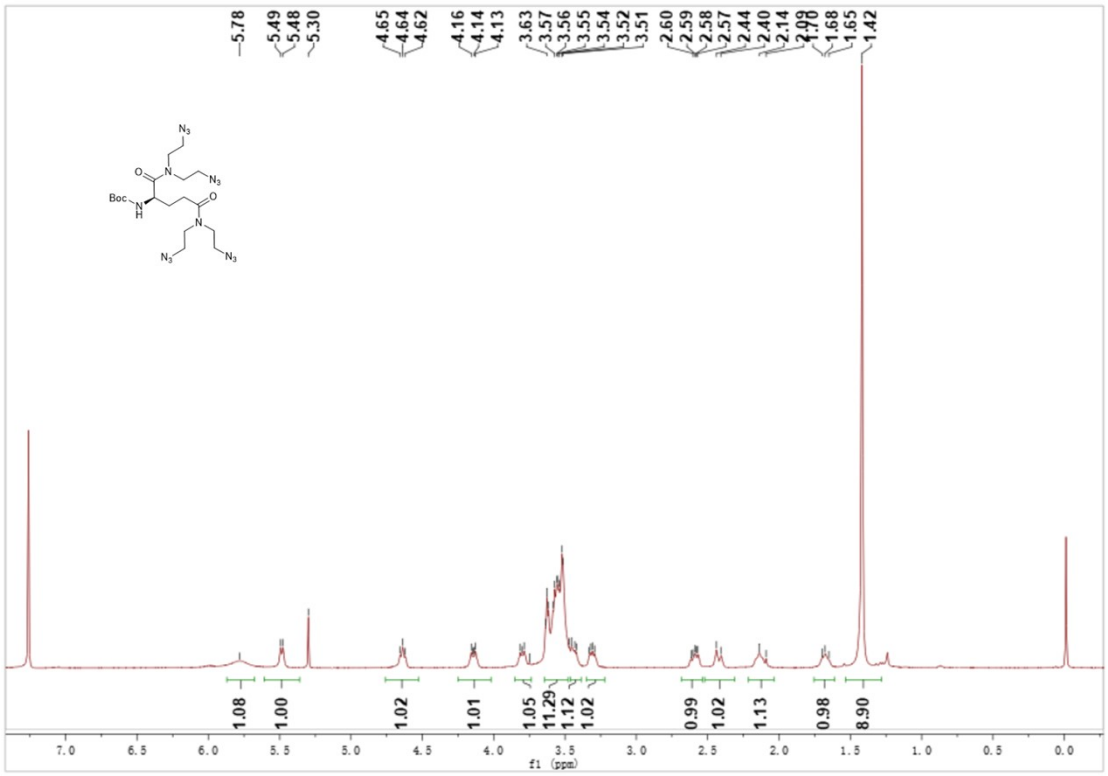


Fig. S13 ^{1}H NMR spectrum of compound **2** ($CDCl_3$).

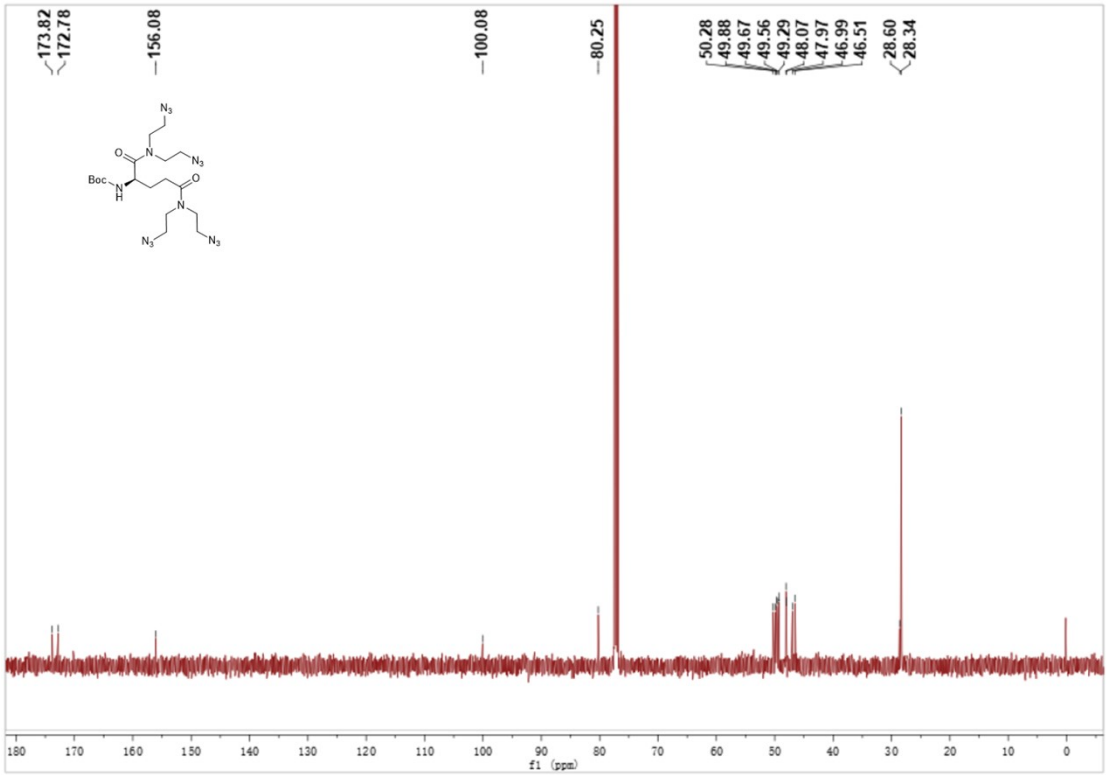


Fig. S14 ^{13}C NMR spectrum of compound **2** ($CDCl_3$).

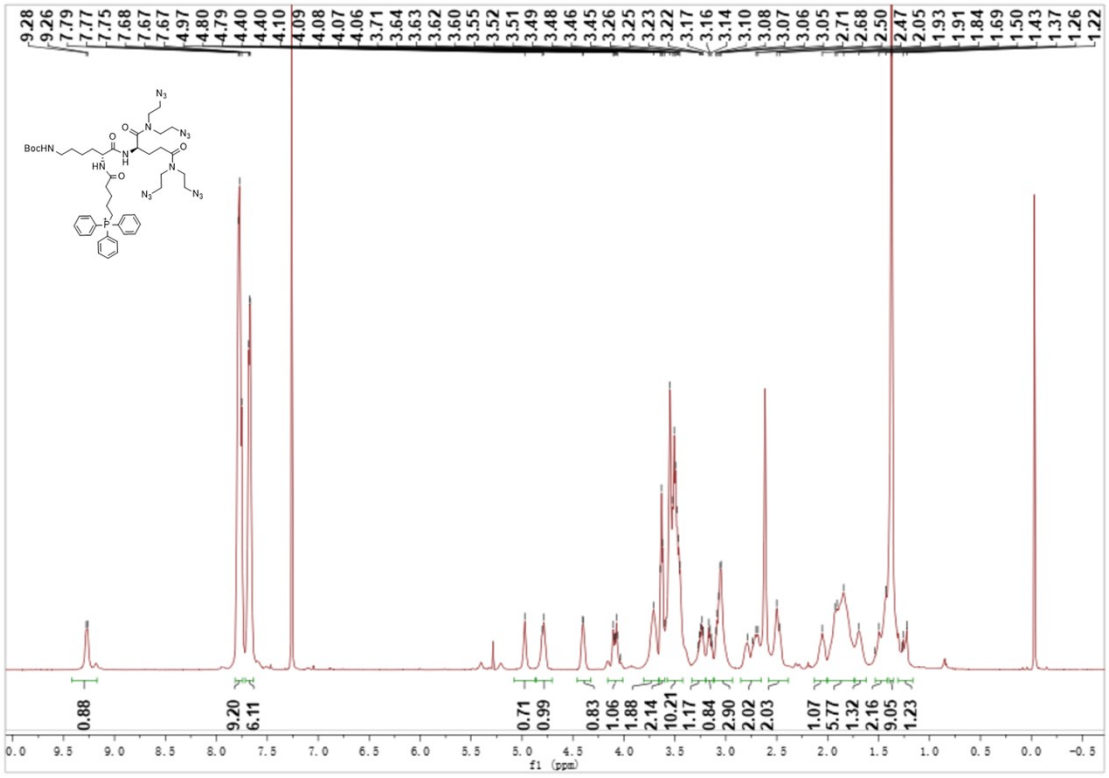


Fig. S15 ^{1}H NMR spectrum of compound **4** ($CDCl_3$).

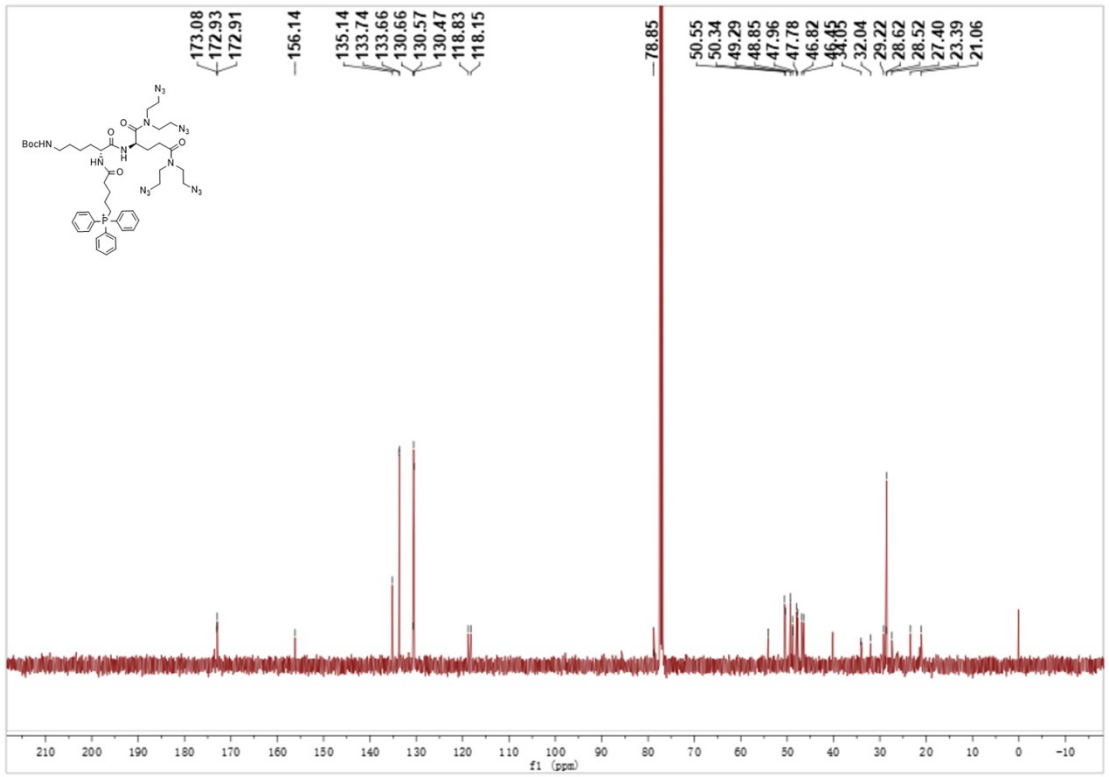


Fig. S16 ^{13}C NMR spectrum of compound **4** ($CDCl_3$).

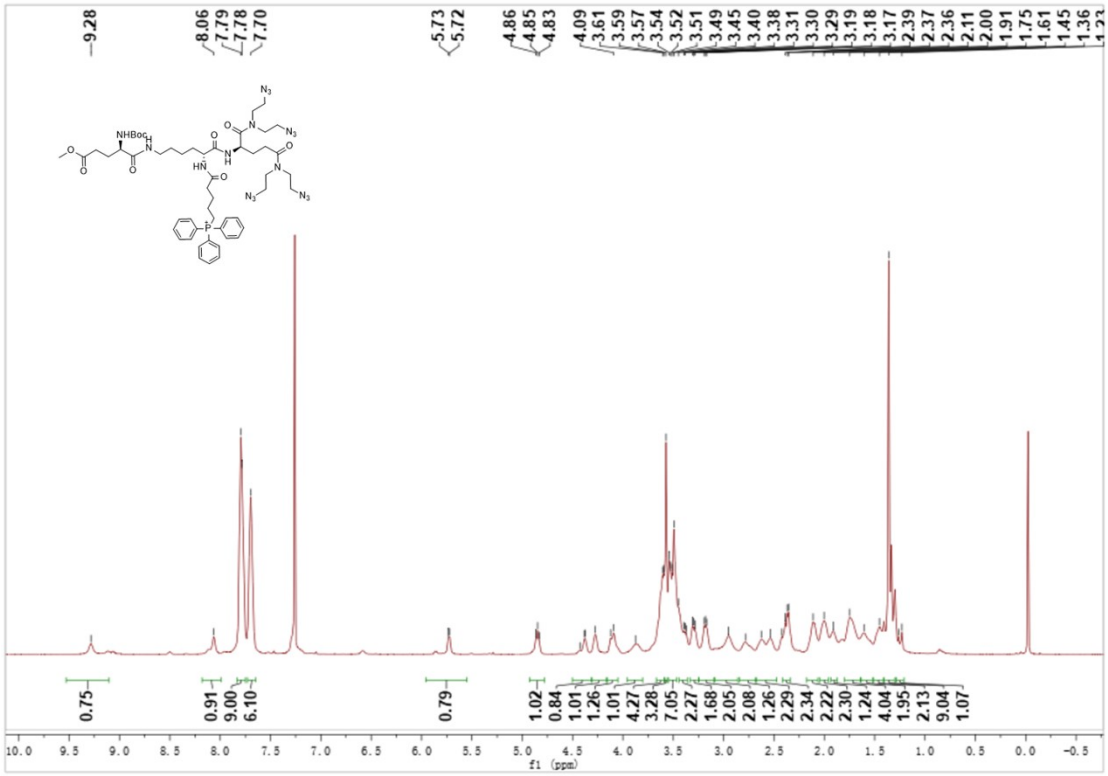


Fig. S17 ^{1}H NMR spectrum of compound **6** ($CDCl_3$).

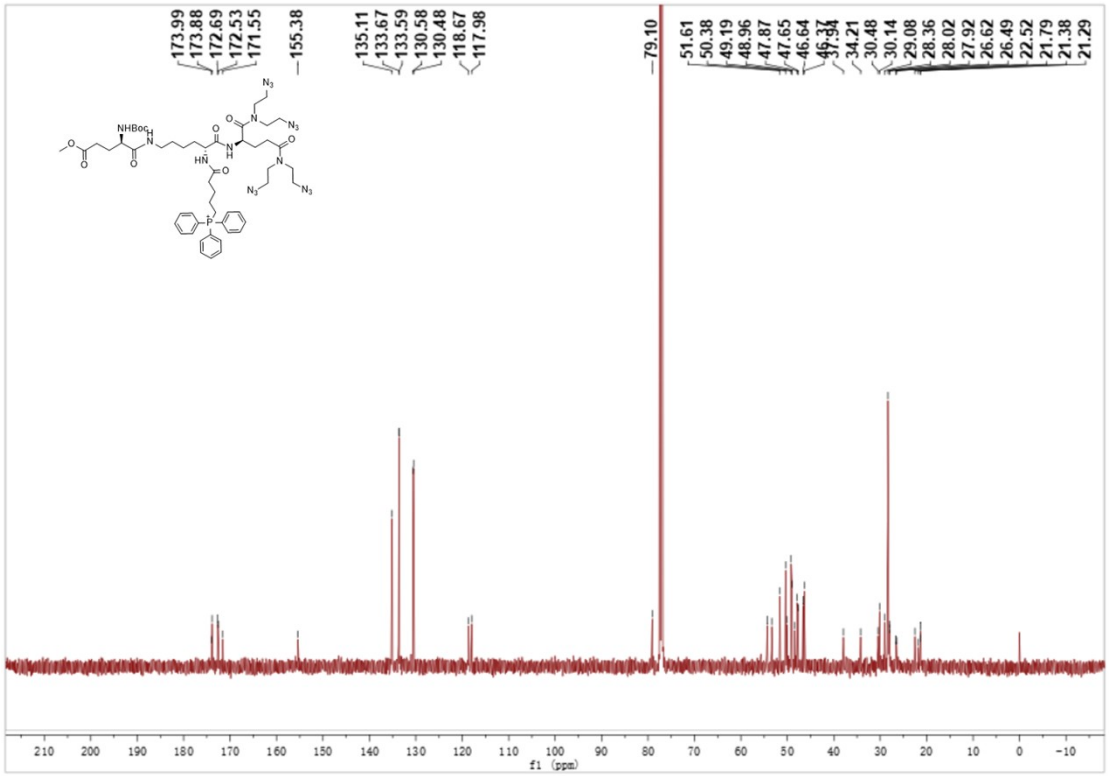


Fig. S18 ^{13}C NMR spectrum of compound **6** ($CDCl_3$).

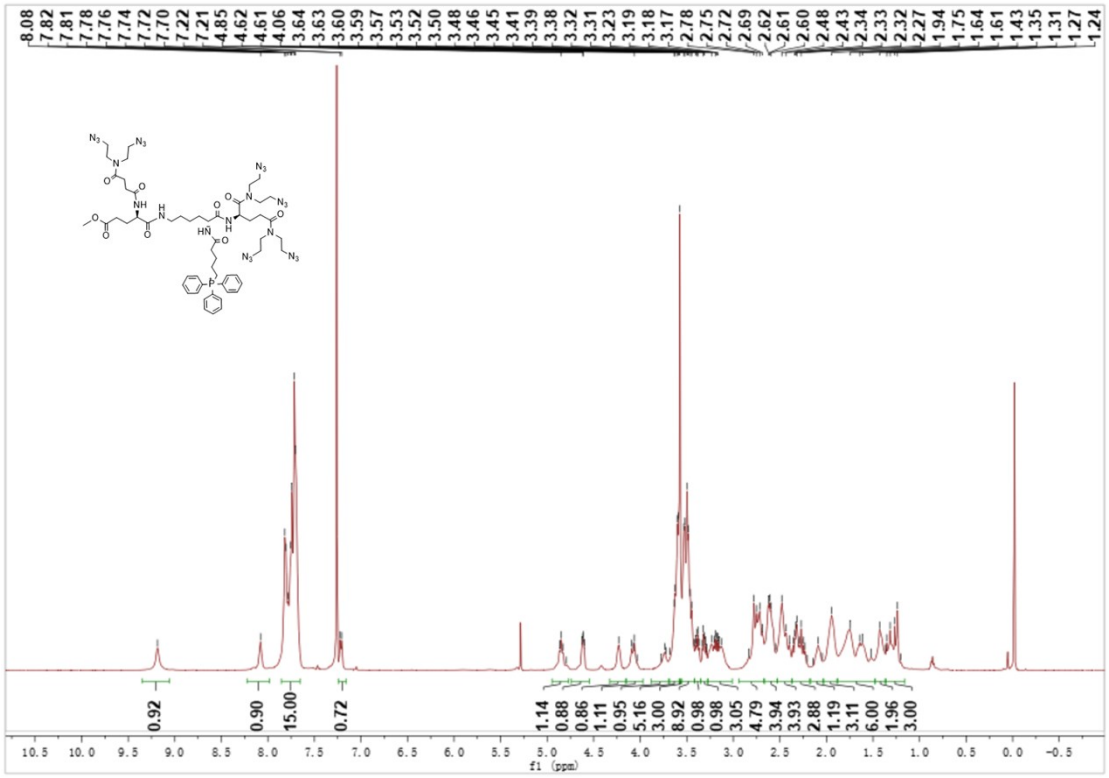


Fig. S19 ^{1}H NMR spectrum of compound **7** ($CDCl_3$).

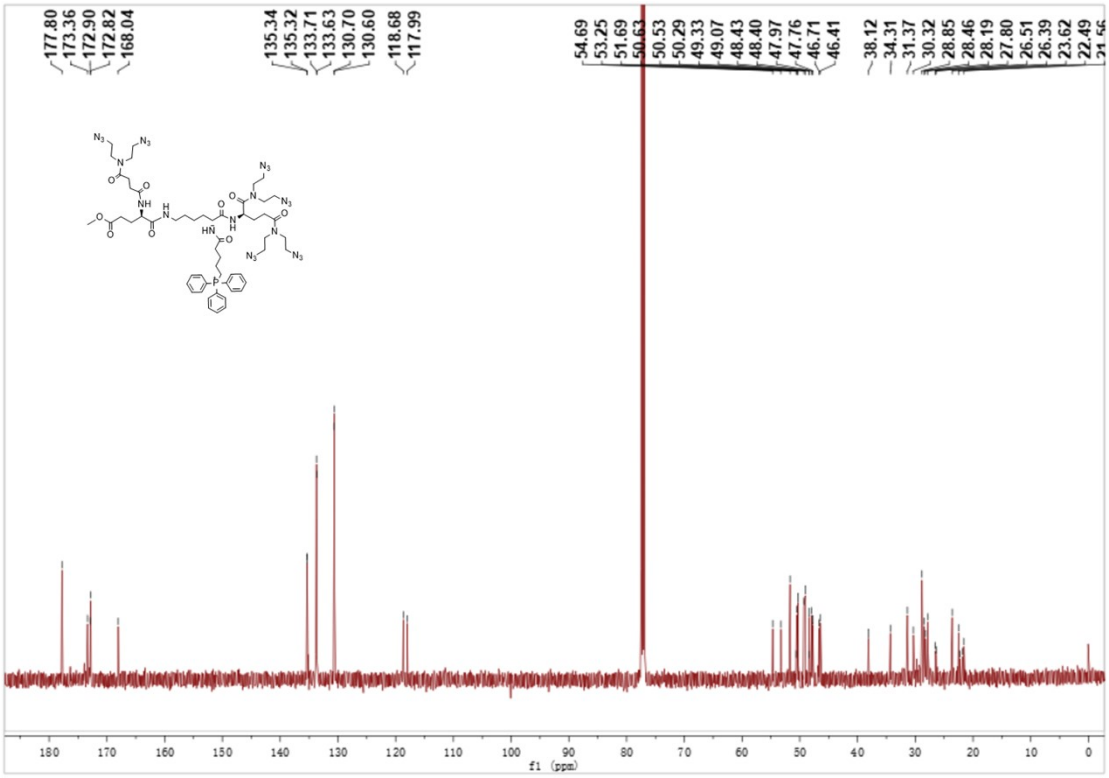


Fig. S20 ^{13}C NMR spectrum of compound **7** ($CDCl_3$).

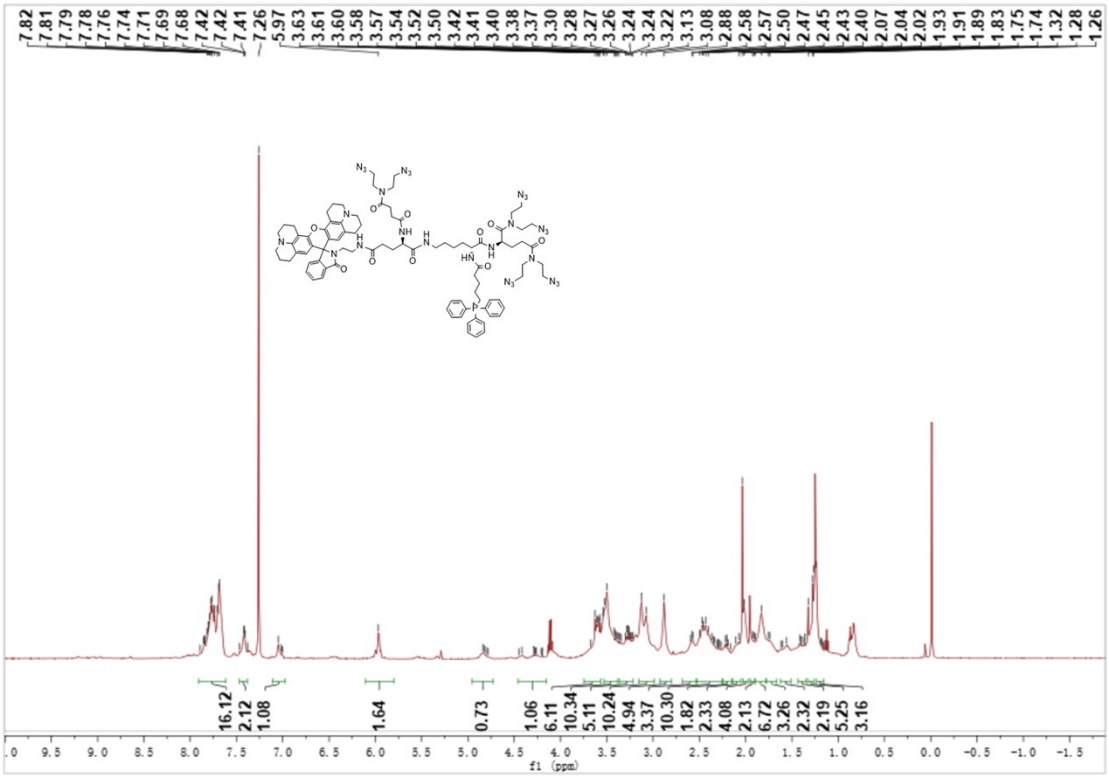


Fig. S21 ^{1}H NMR spectrum of **^{6Az}ProRed-TPP** ($CDCl_3$).

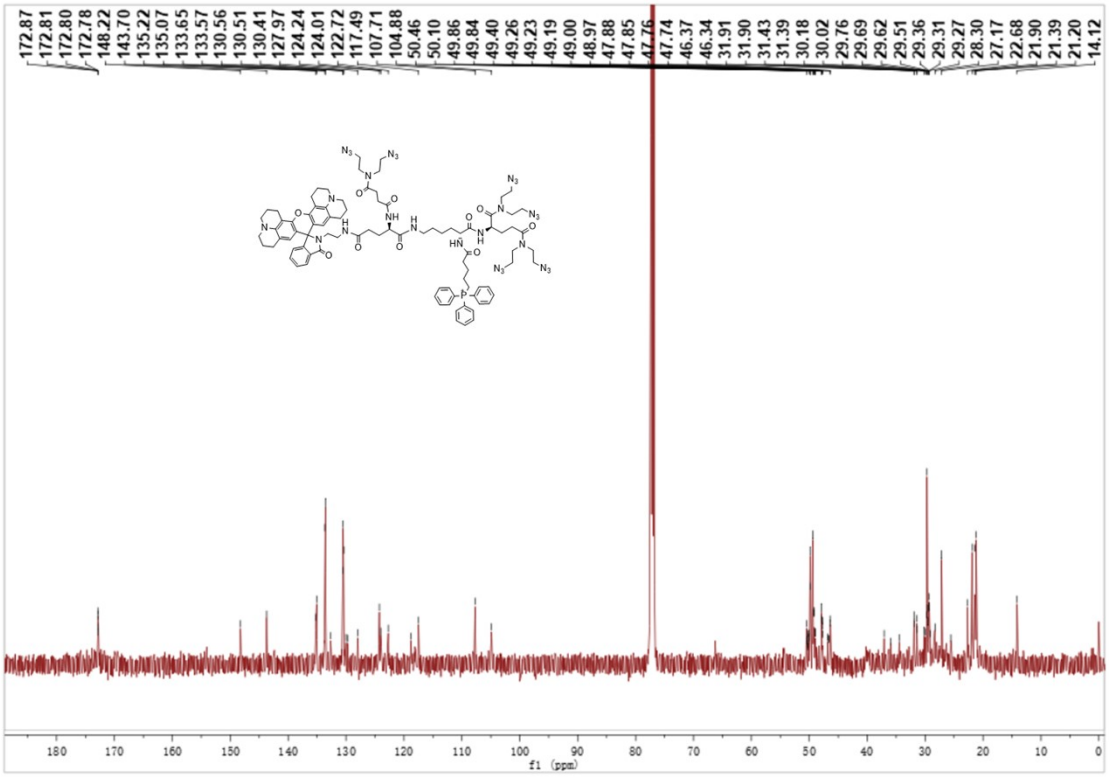


Fig. S22 ^{13}C NMR spectrum of **^{6Az}ProRed-TPP** ($CDCl_3$).

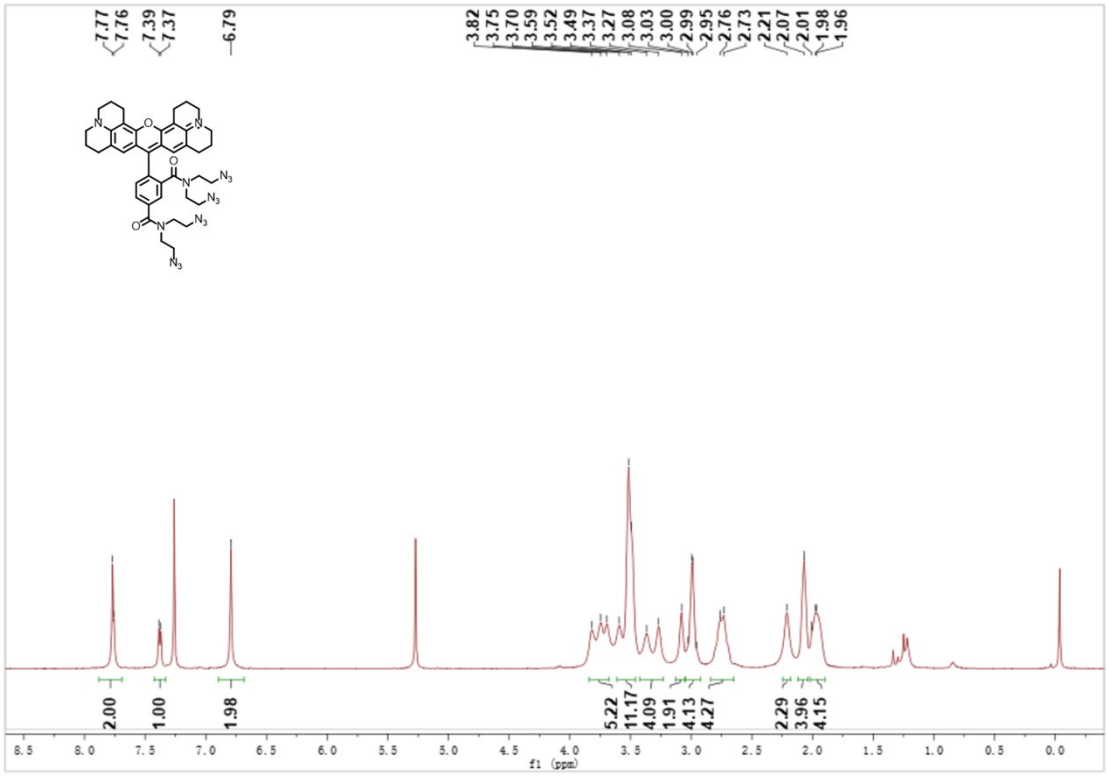


Fig. S23 ^{1}H NMR spectrum of **^{4Az}Red** ($CDCl_3$).

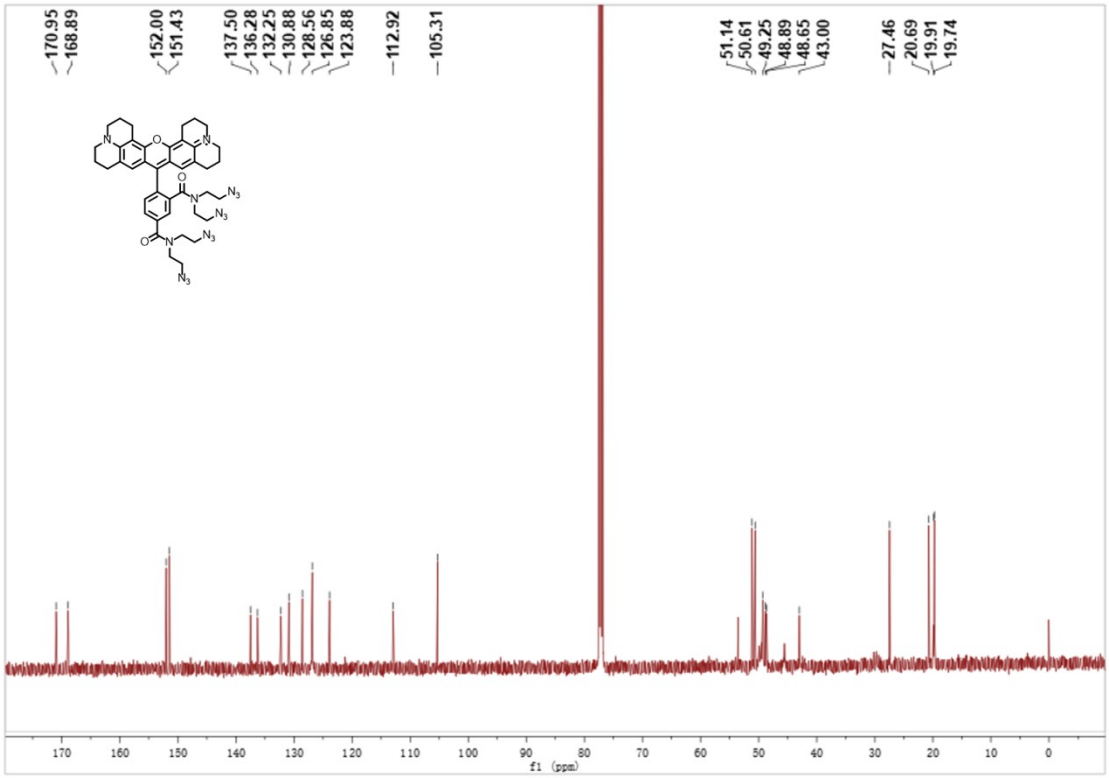


Fig. S24 ^{13}C NMR spectrum of **^{4Az}Red** ($CDCl_3$).

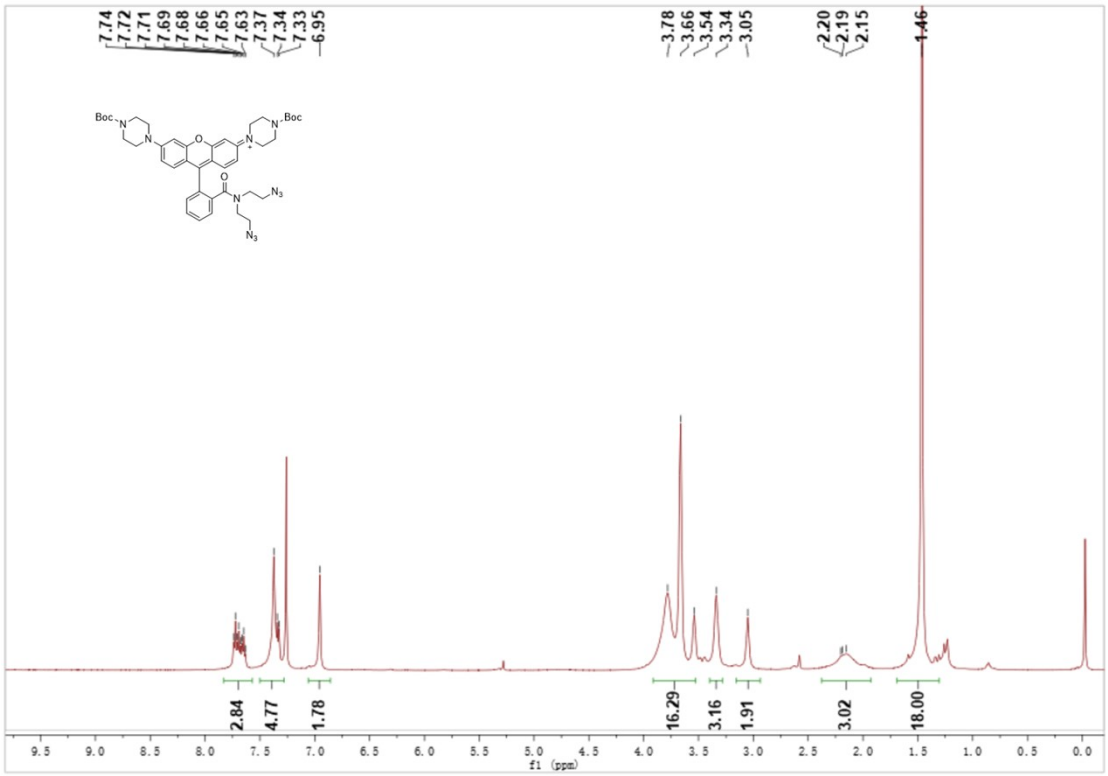


Fig. S25 ^{1}H NMR spectrum of compound **9** ($CDCl_3$).

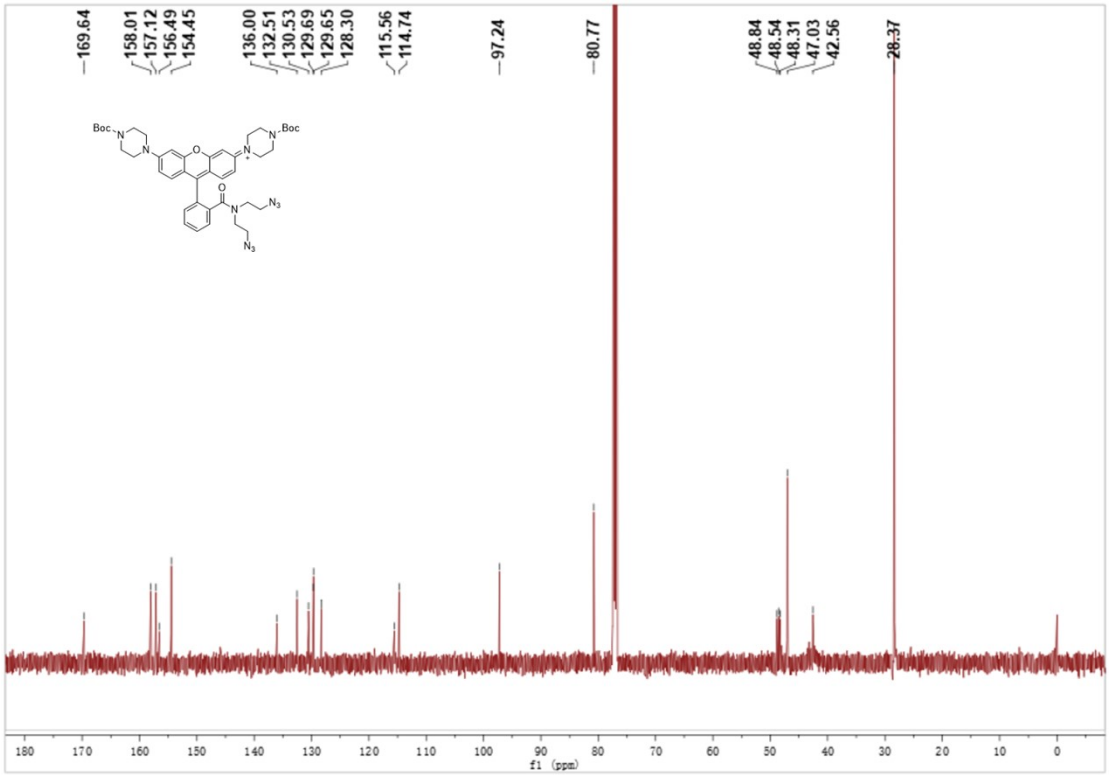


Fig. S26 ^{13}C NMR spectrum of compound **9** ($CDCl_3$).

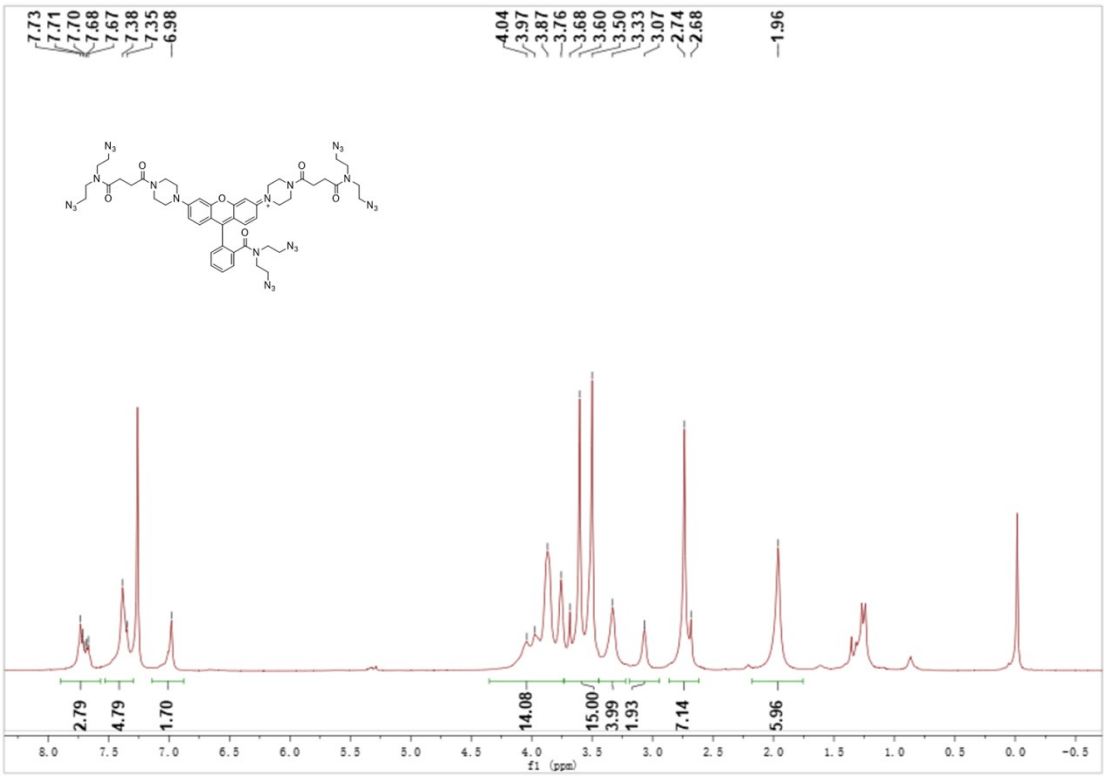


Fig. S27 ^{1}H NMR spectrum of **^{6Az}Red** ($CDCl_3$).

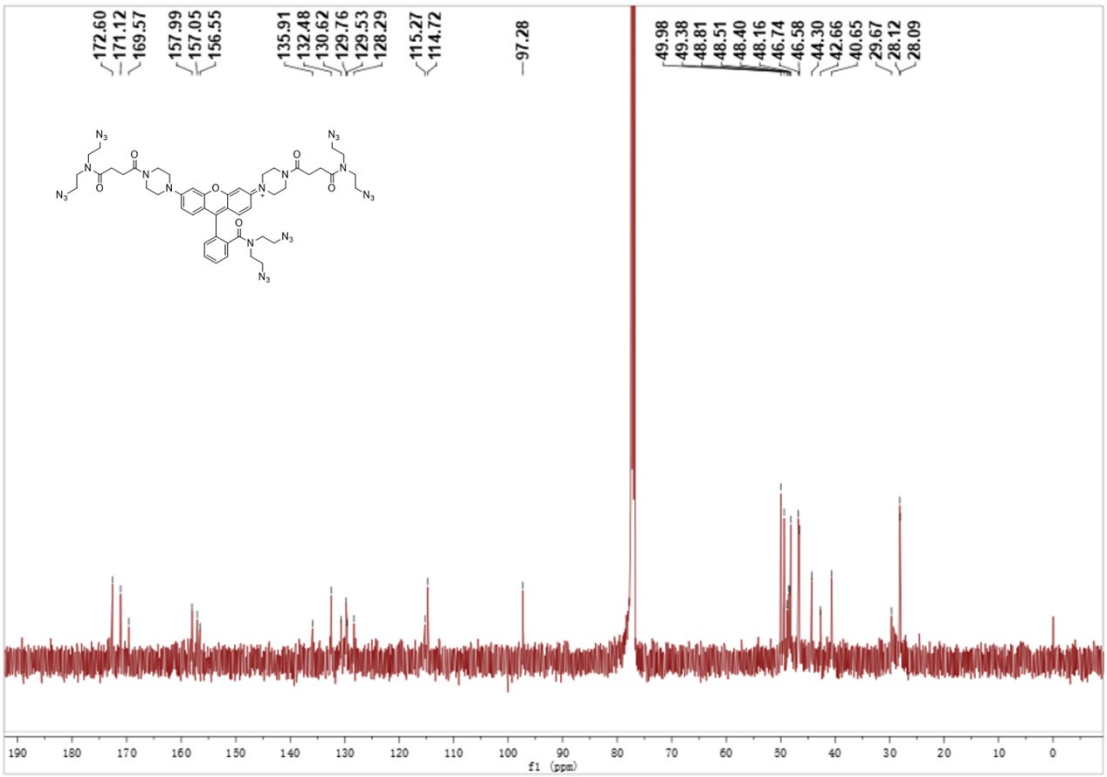


Fig. S28 ^{13}C NMR spectrum of **^{6Az}Red** ($CDCl_3$).

Mass spectra

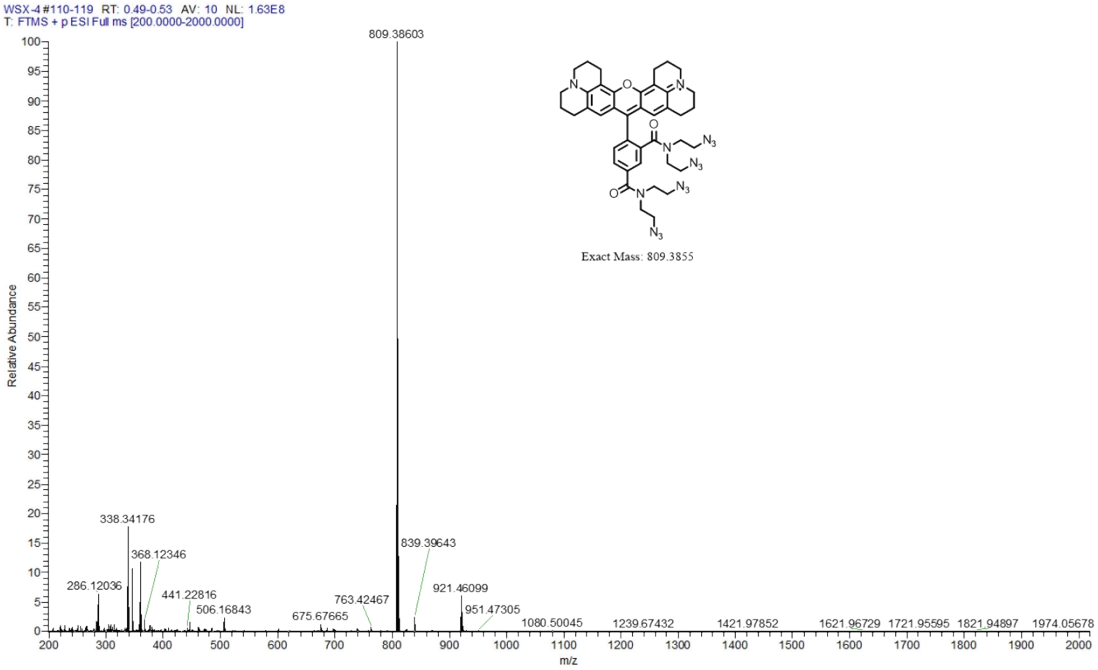


Fig. S29 HRMS of **^{4Az}Red**.

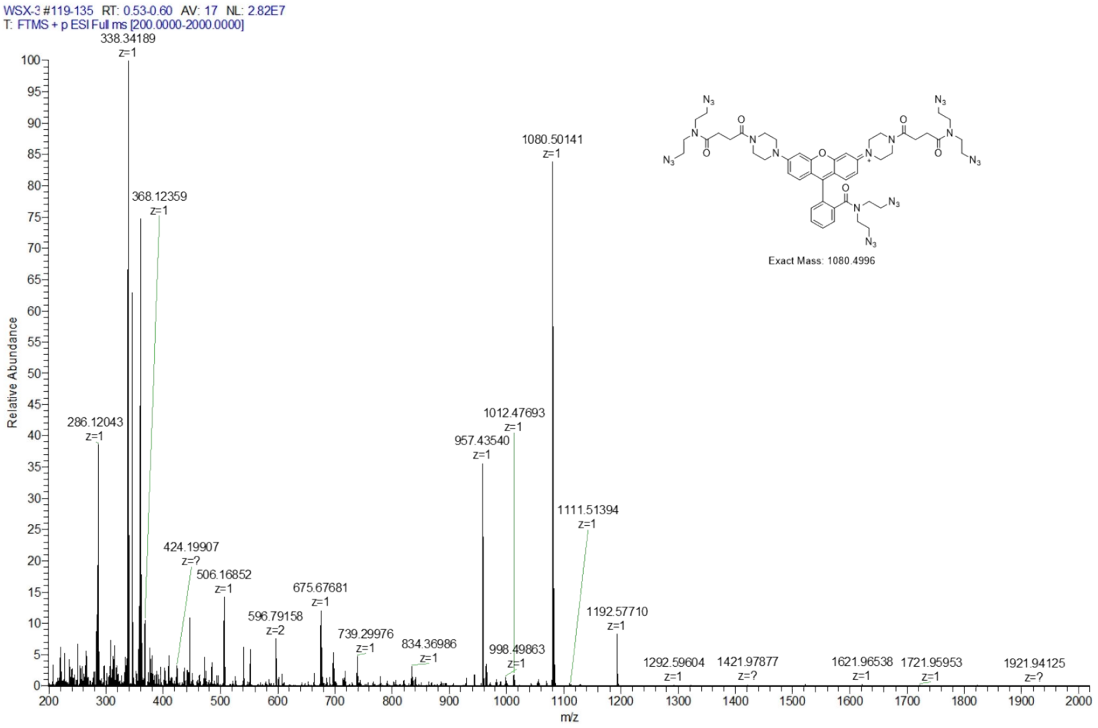


Fig. S30 HRMS of **^{4Az}Red**.

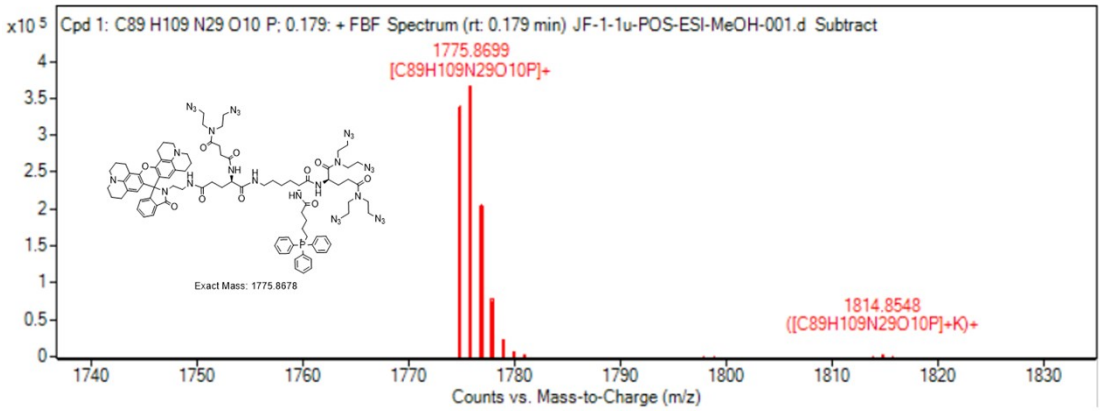


Fig. S31 HRMS of ^{6Az}**ProRed-TPP**.