

Supplementary Materials for

ROS-responsive chitosan-SS31 prodrug for AKI therapy via rapid distribution in the kidney and long-term retention in the renal tubule

Di Liu, Gaofeng Shu, Feiyang Jin, Jing Qi, Xiaoling Xu, Yan Du, Hui Yu, Jun Wang, Mingchen Sun, Yuchan You, Minxia Zhu, Meixuan Chen, Luwen Zhu, Qiying Shen, Xiaoying Ying, Xuefang Lou*, Saiping Jiang*, Yongzhong Du*

*Corresponding author. Email: duyongzhong@zju.edu.cn (Y.D.); j5145@zju.edu.cn (S.J.); louxf@zucc.edu.cn (X.L.)

Published 9 October 2020, *Sci. Adv.* **6**, eabb7422 (2020)
DOI: 10.1126/sciadv.abb7422

This PDF file includes:

Figs. S1 to S10

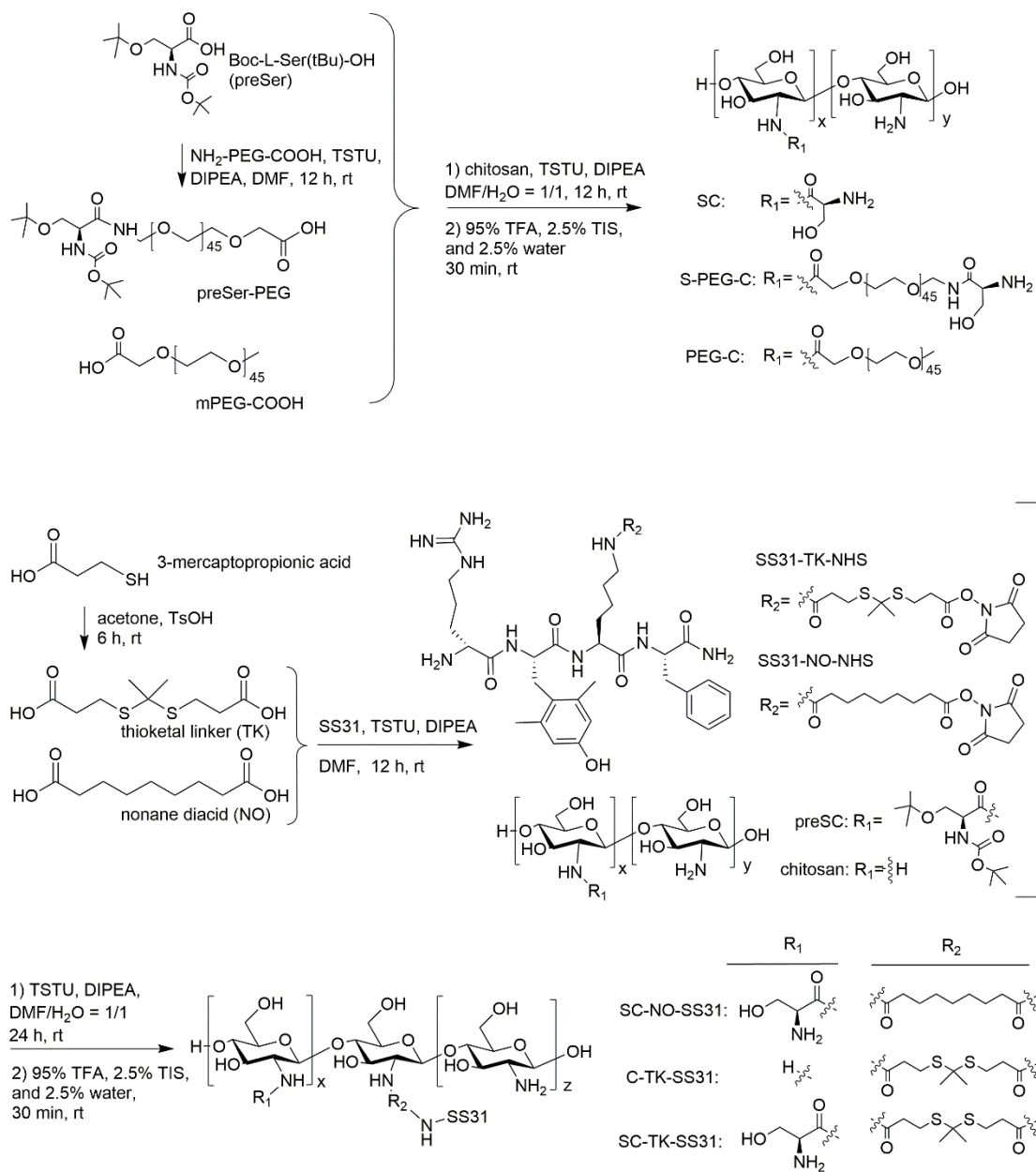
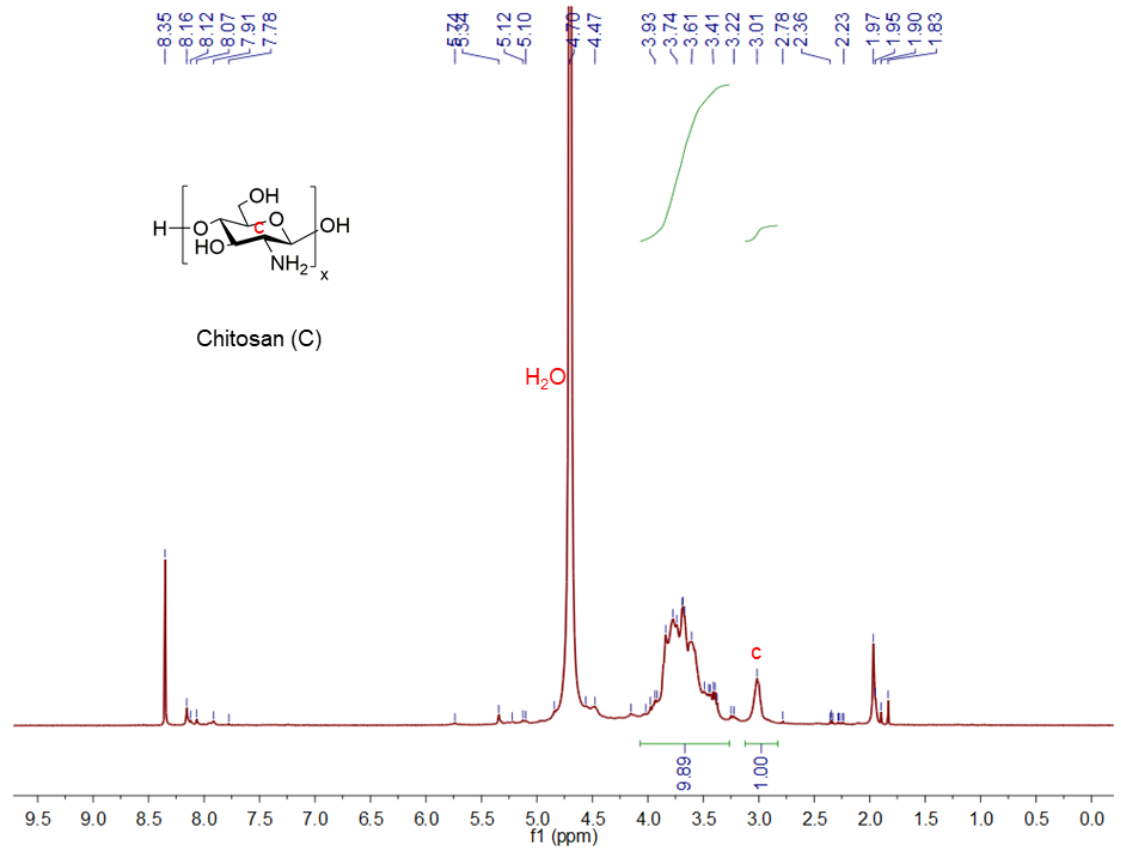
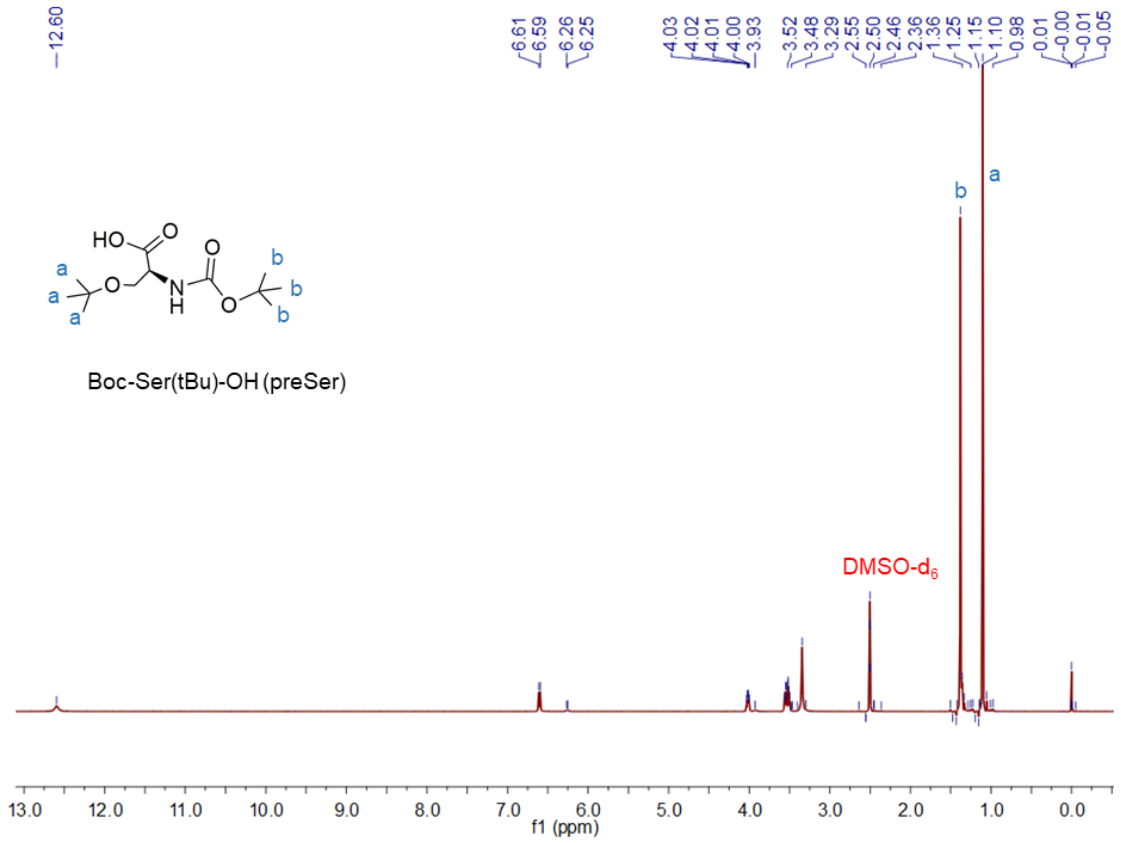
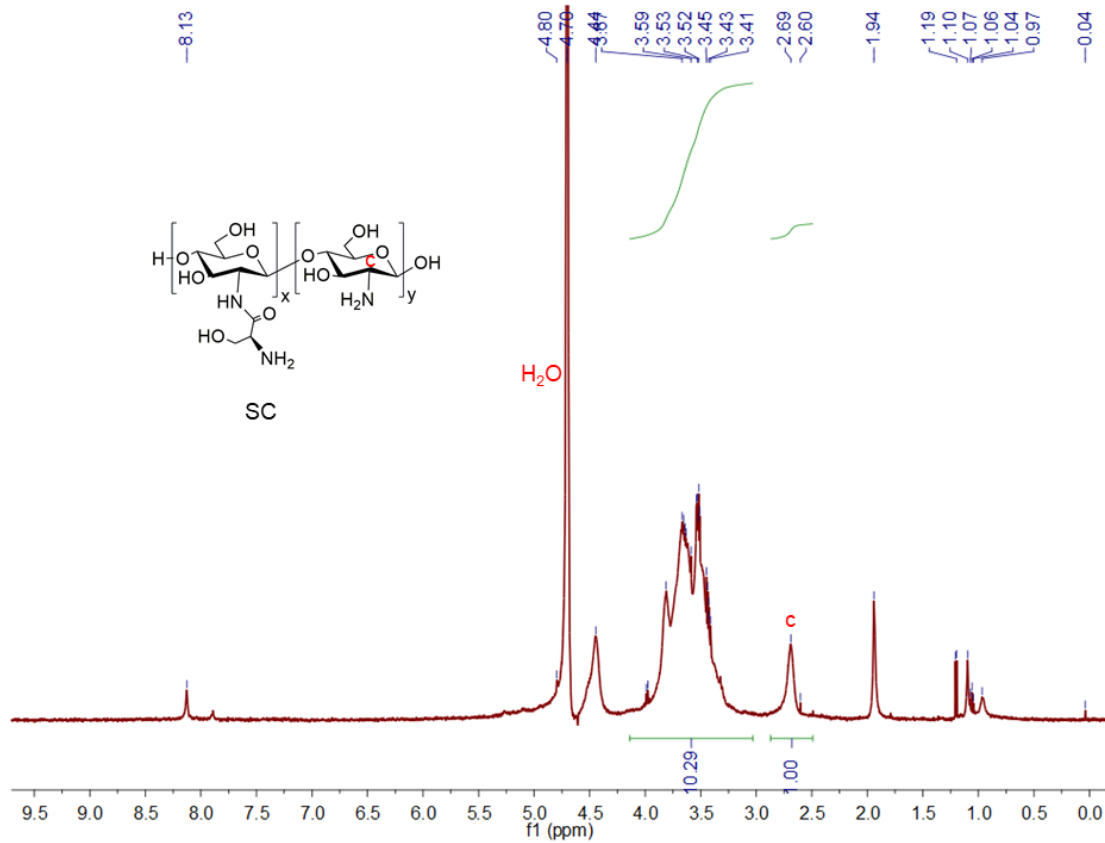
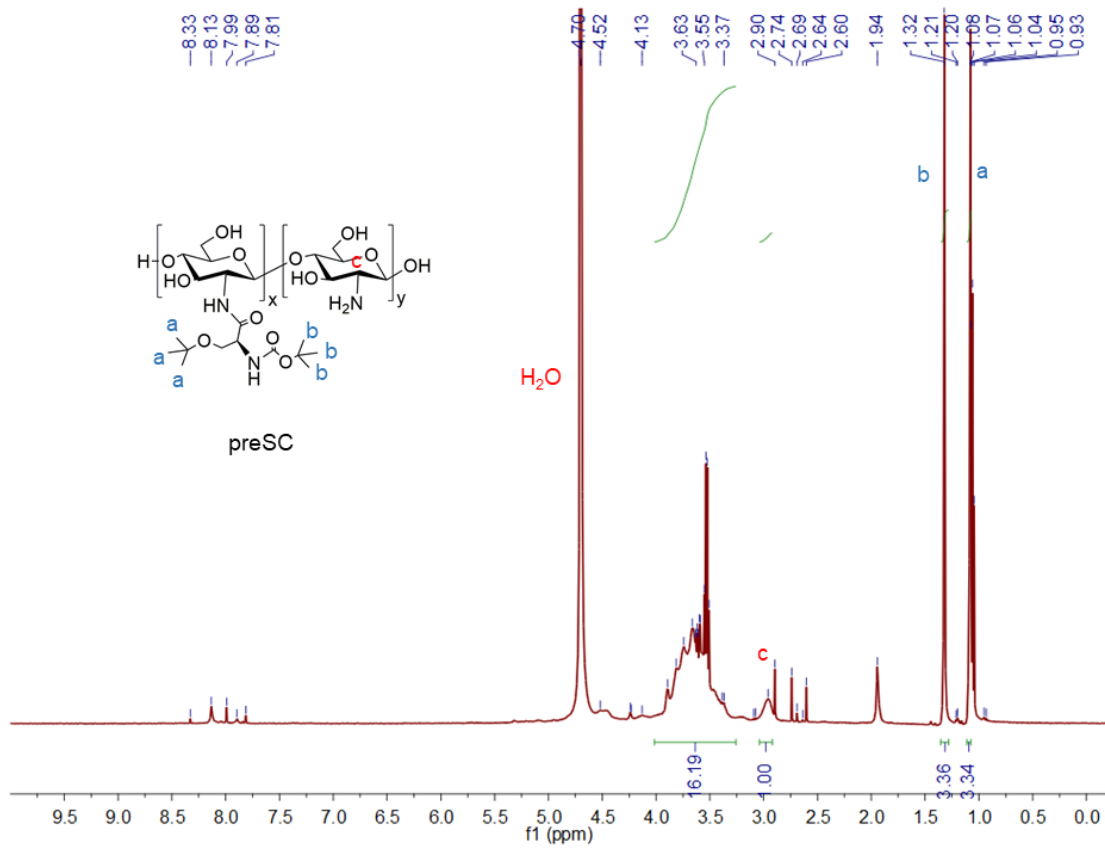
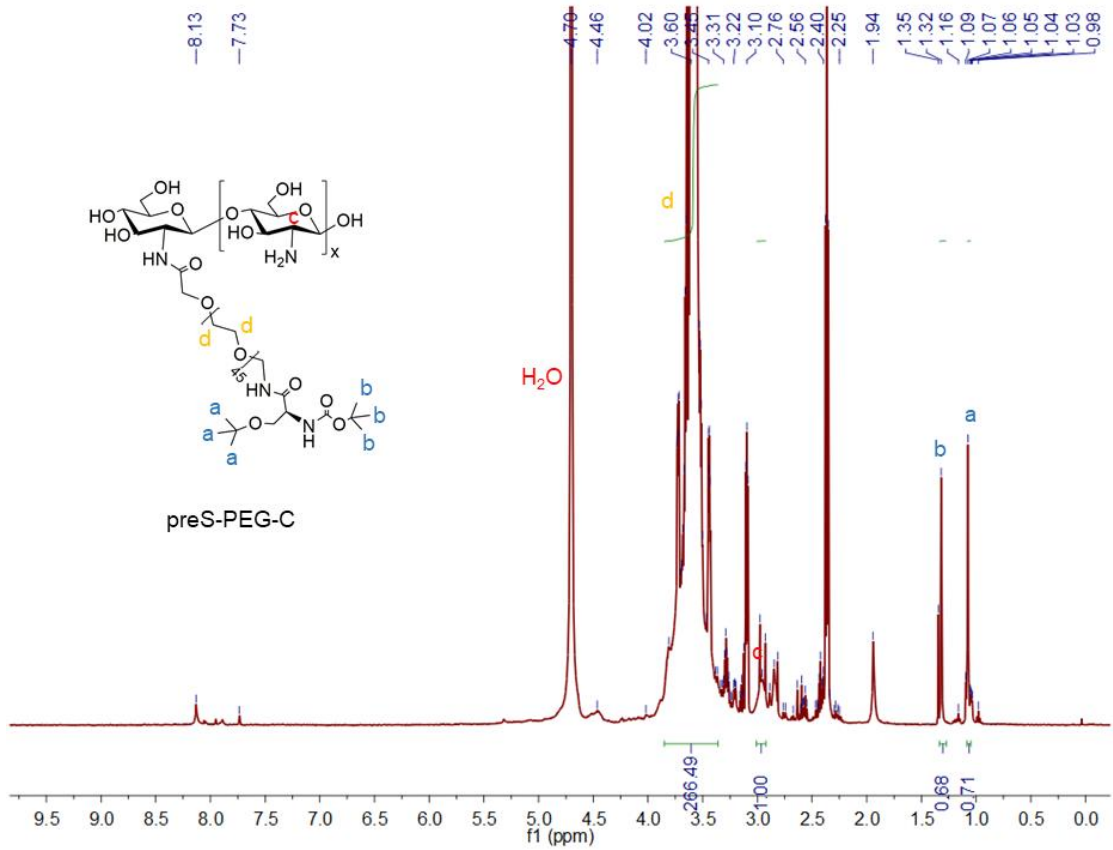
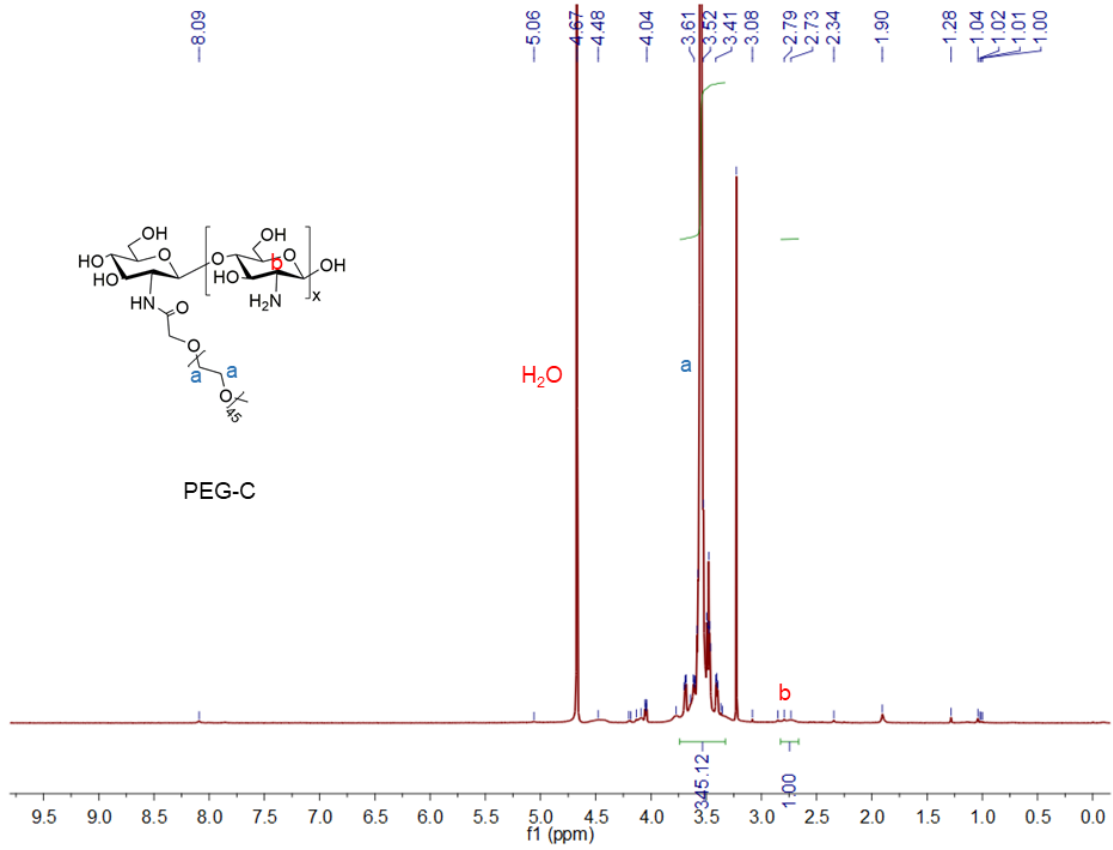
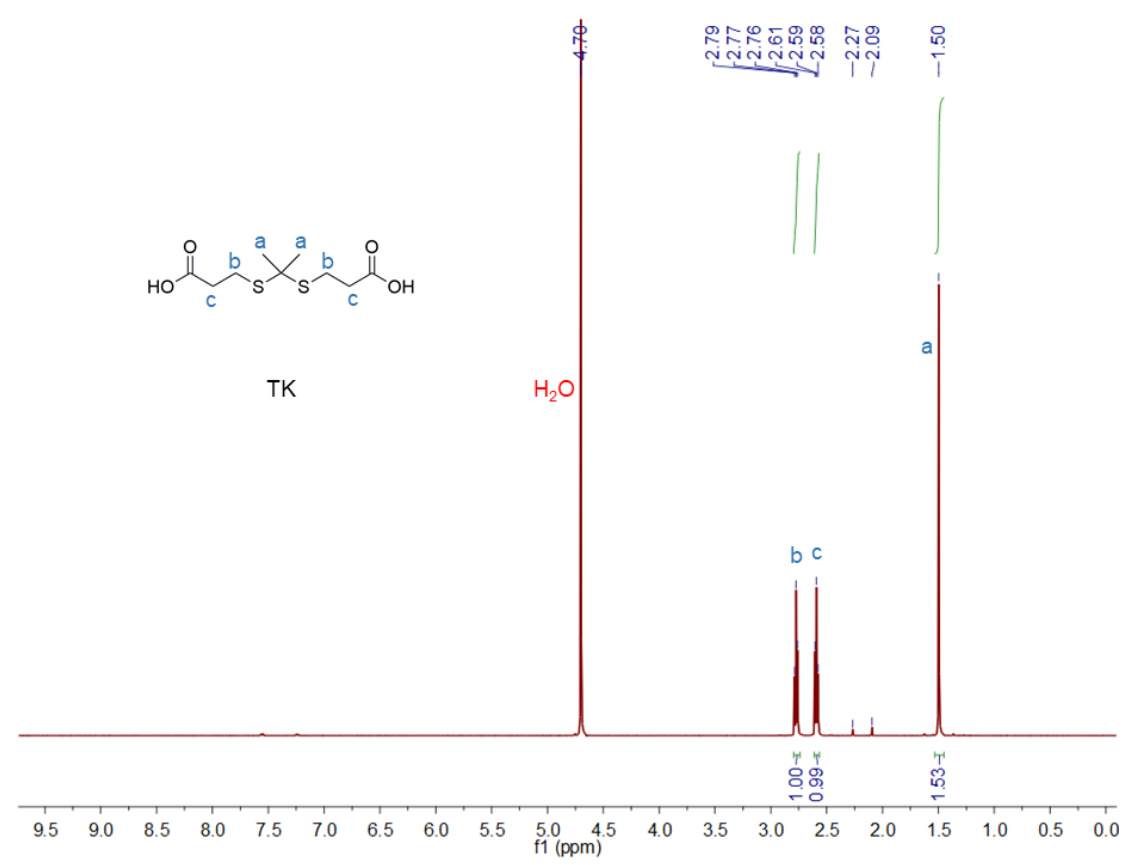
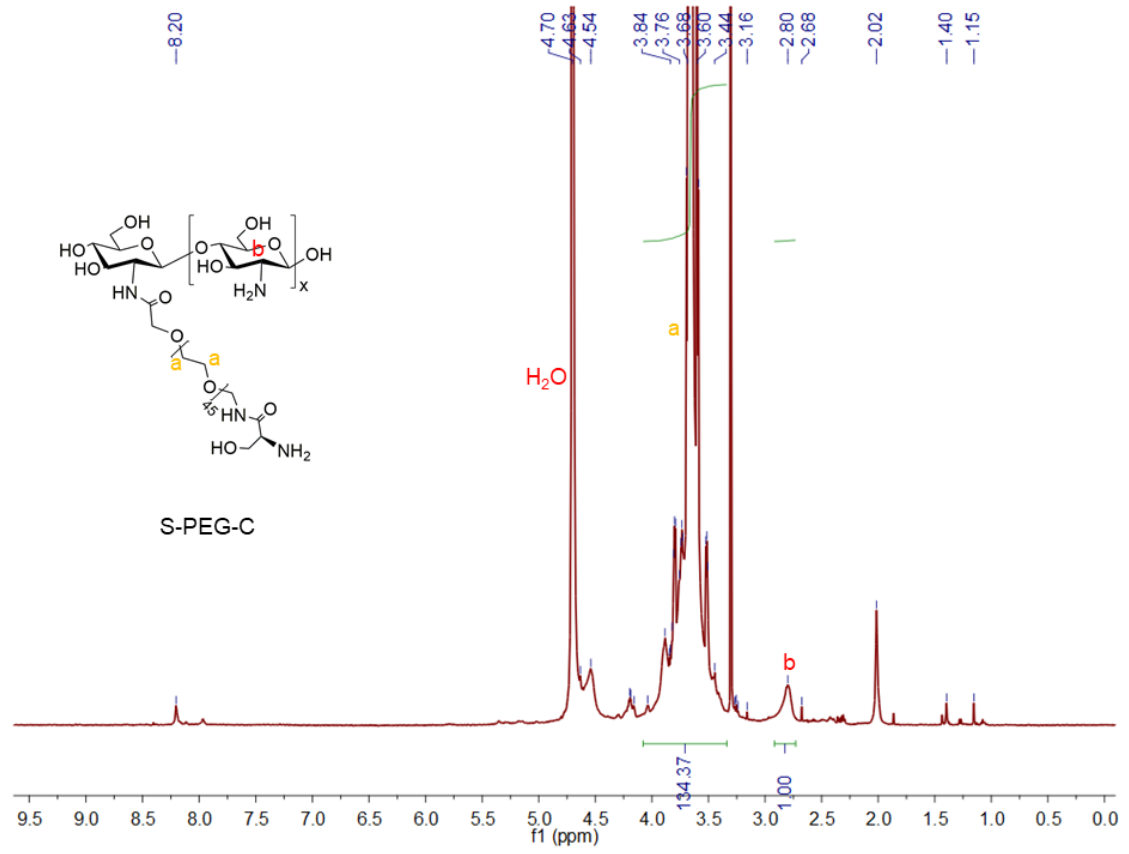


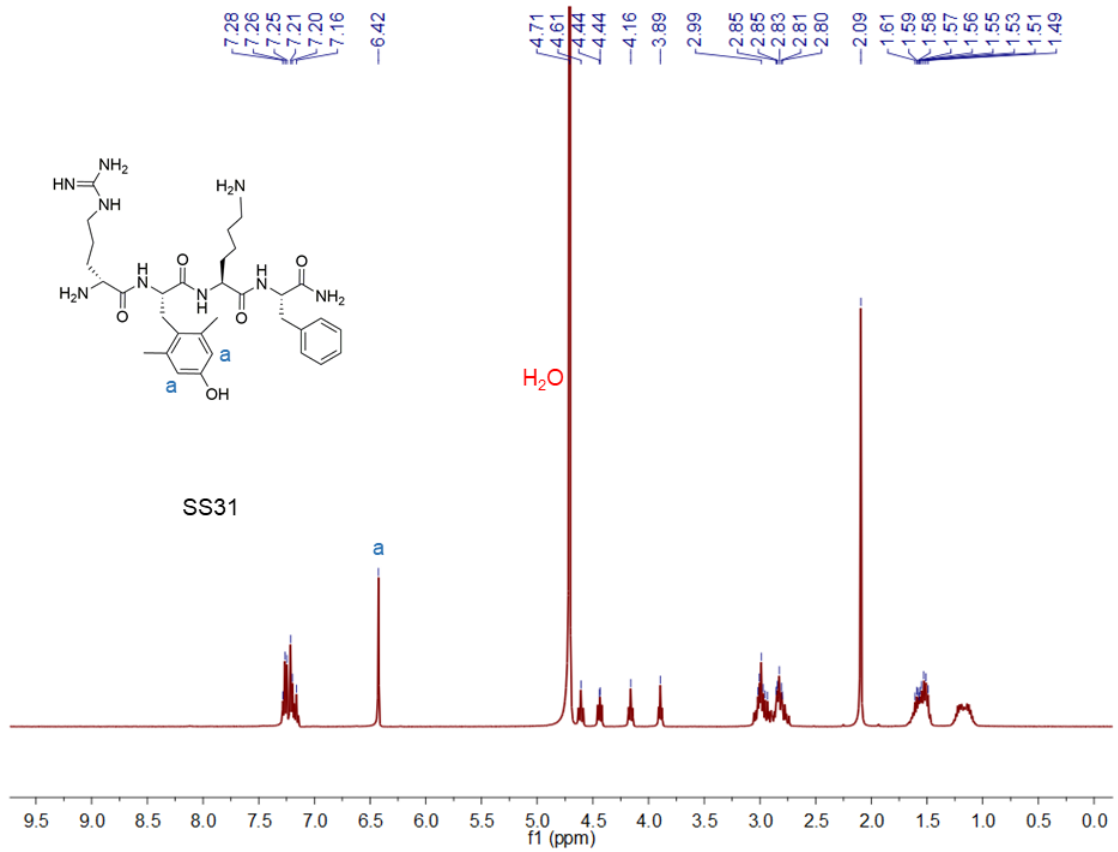
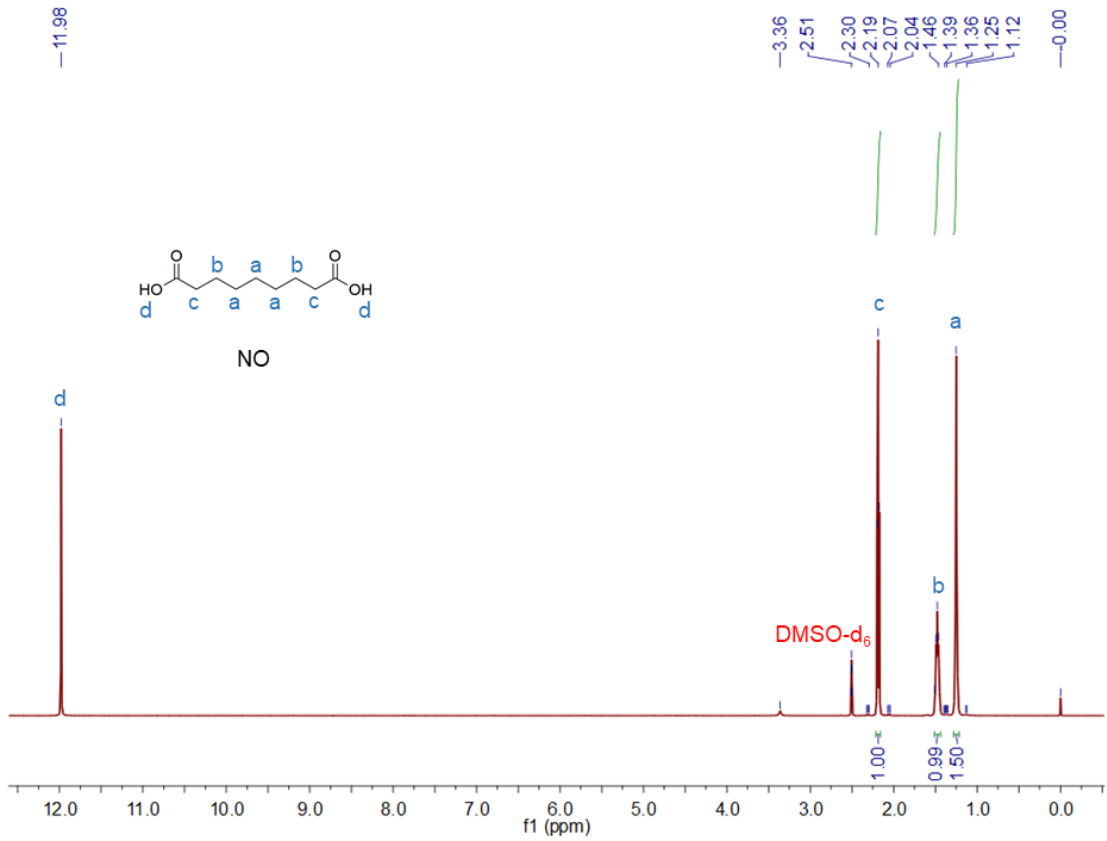
Fig. S1. Syntheses of SC, S-PEG-C, PEG-C, SC-TK-SS31, C-TK-SS31 and SC-NO-SS31.

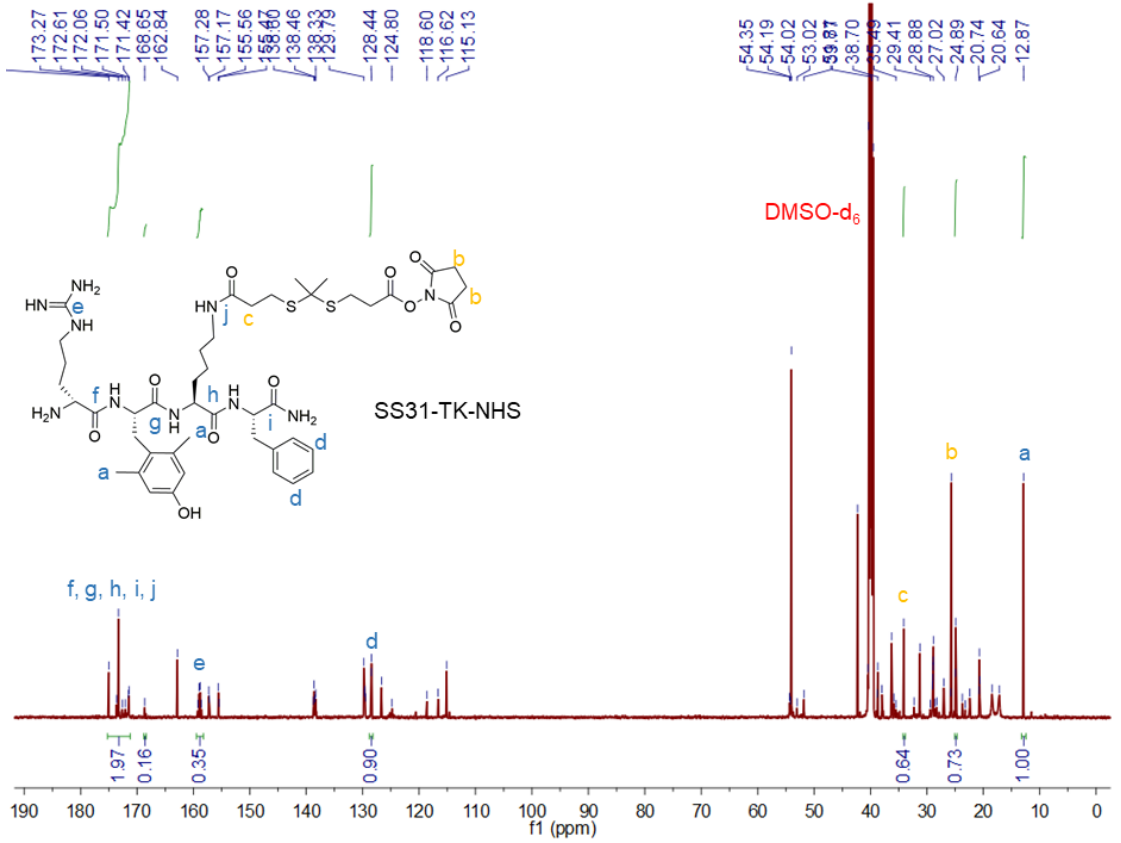
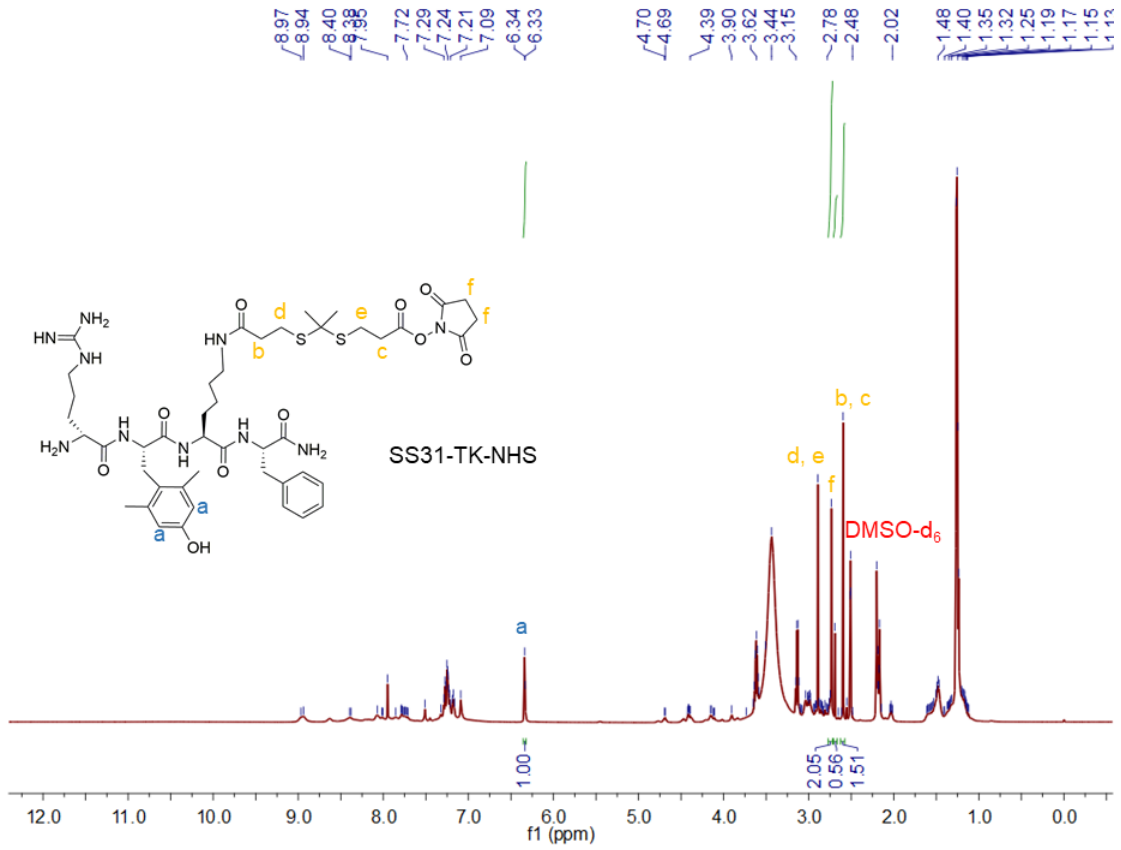


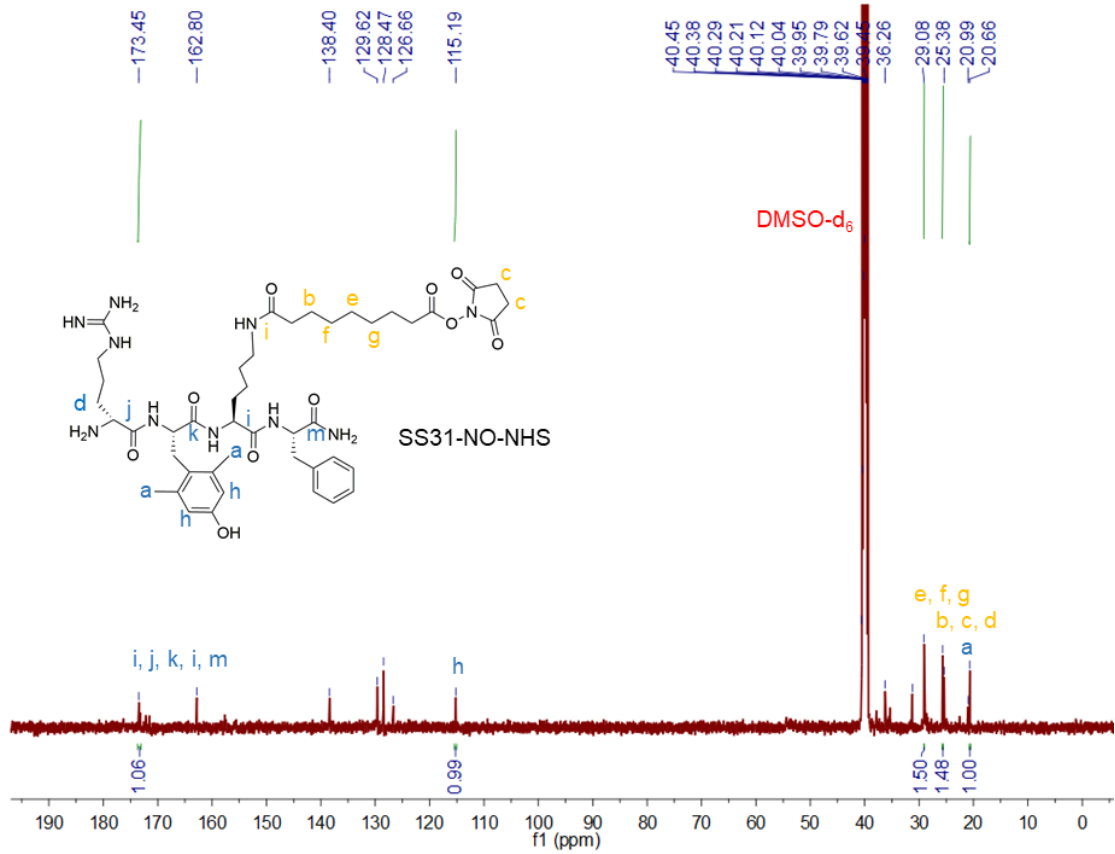
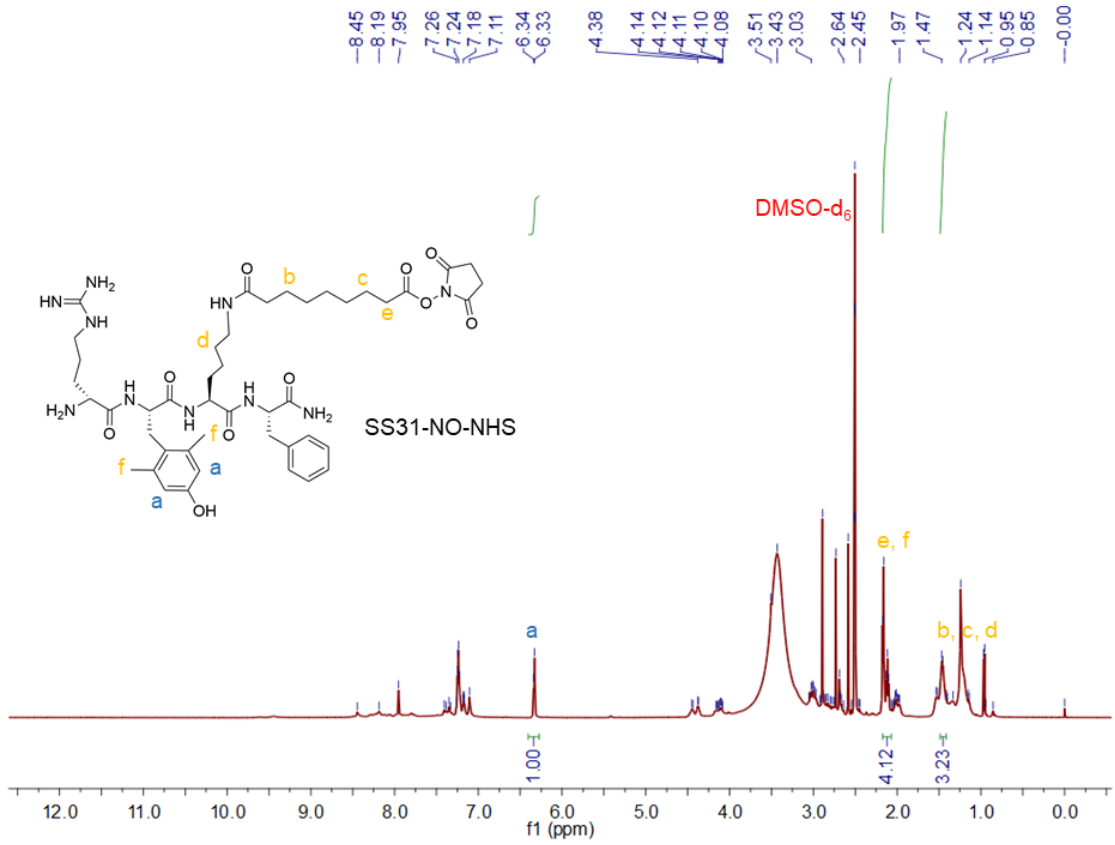


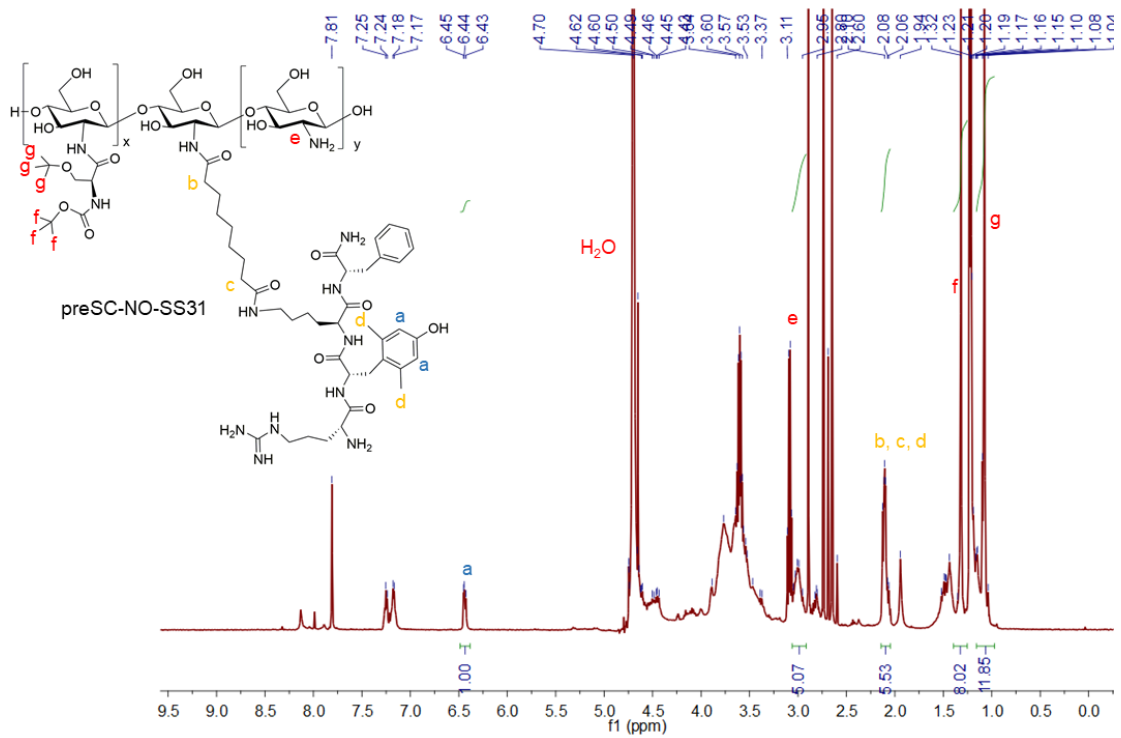
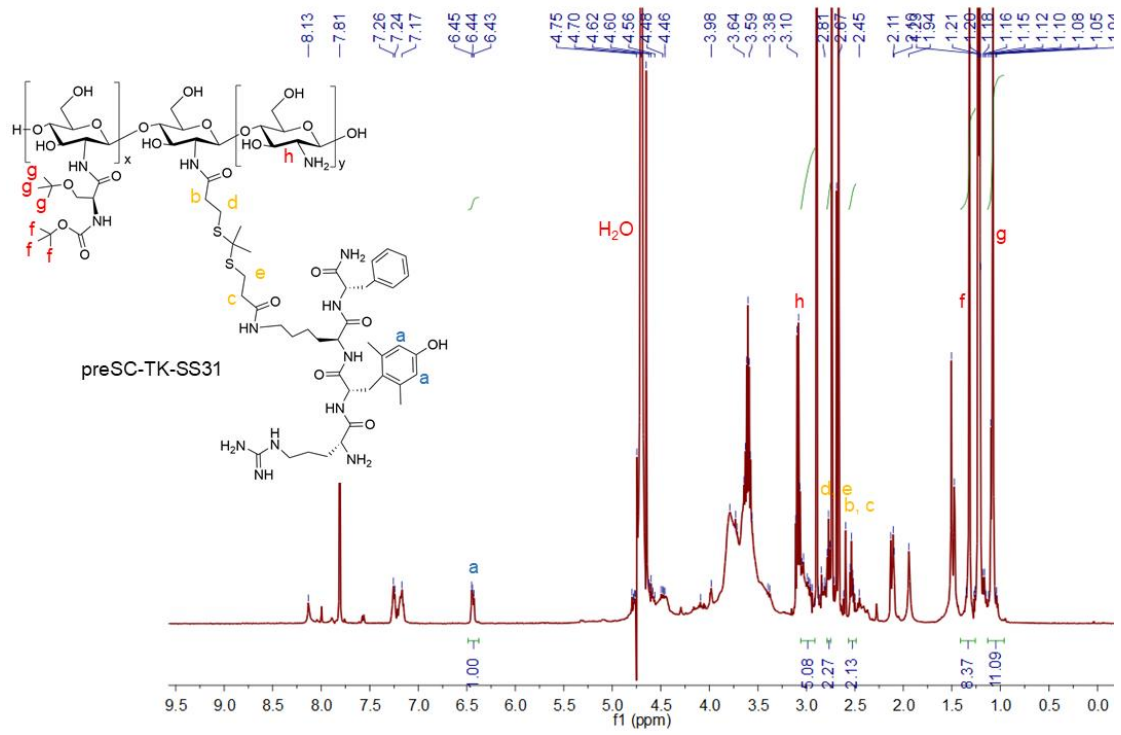


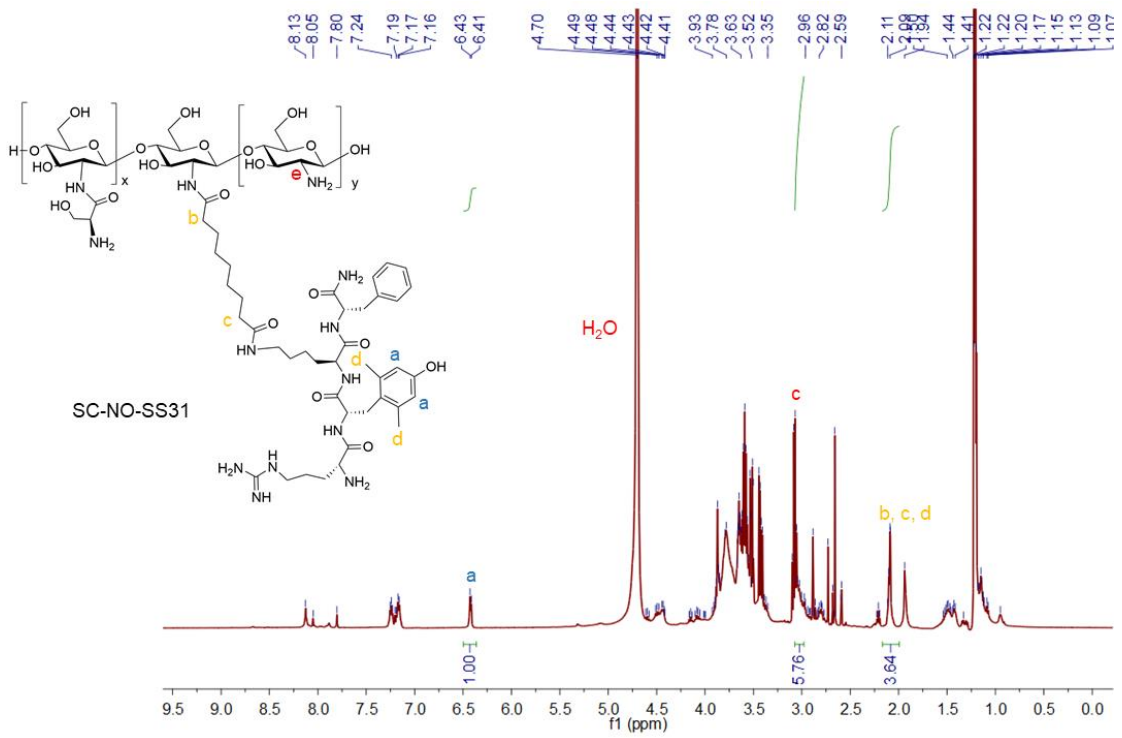
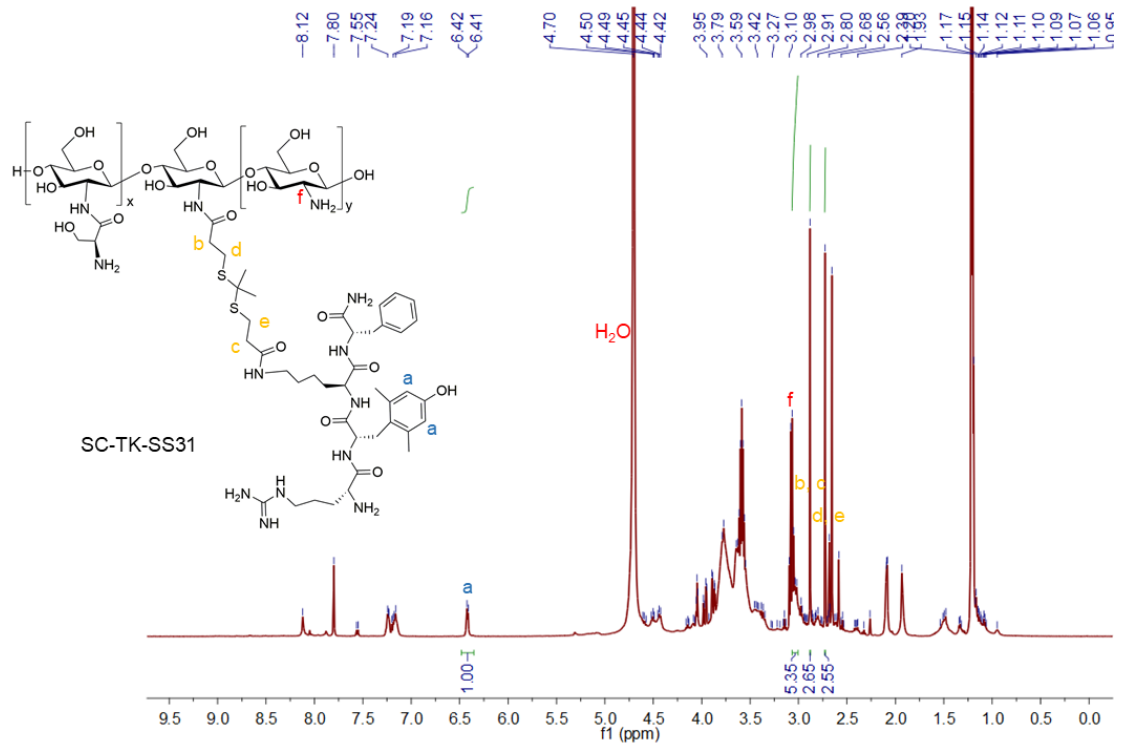












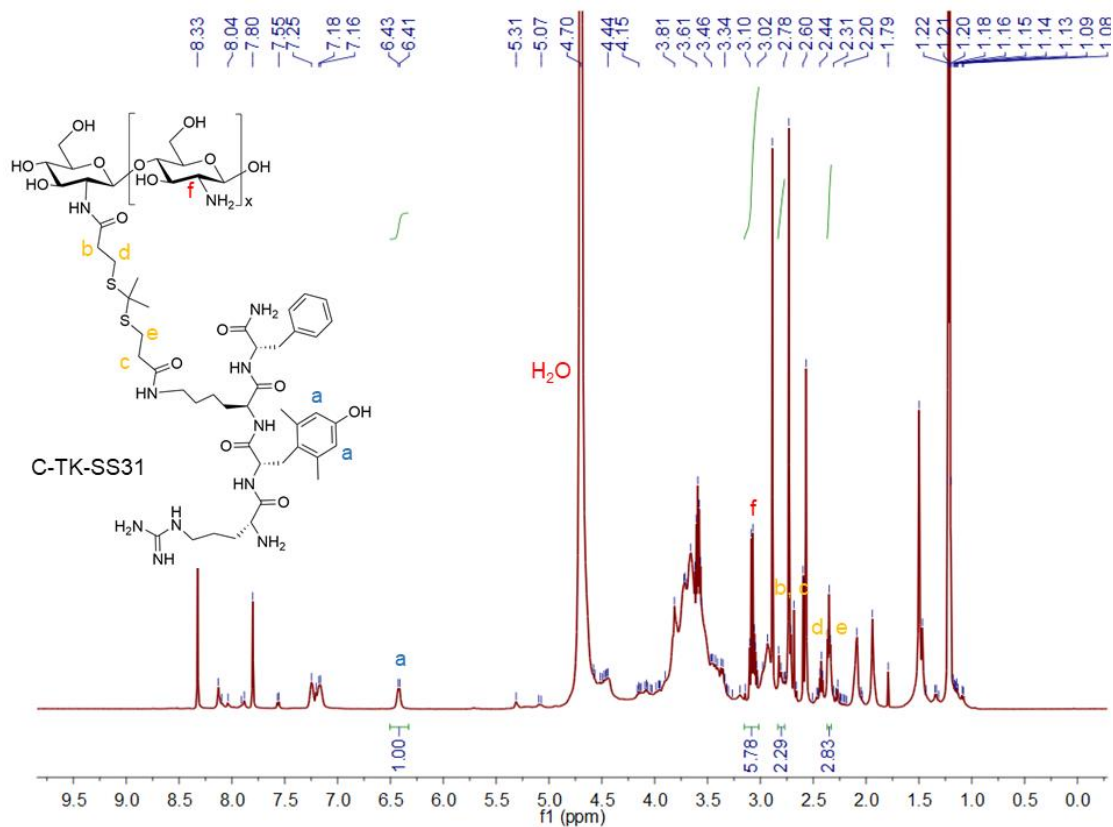


Fig. S2. ¹H-NMR spectra of reagents, intermediates and products in Fig. S1.

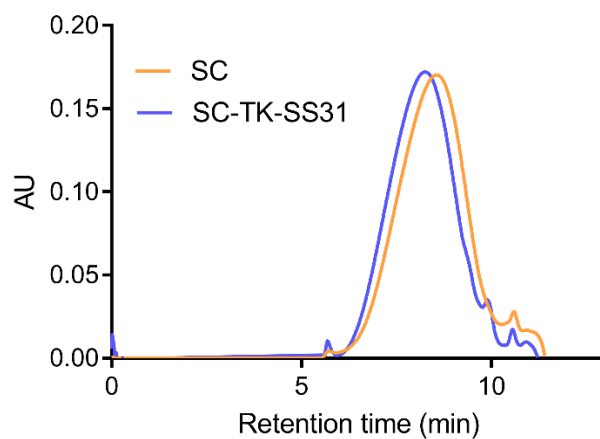


Fig. S3. The standard GPC traces of SC and SC-TK-SS31.

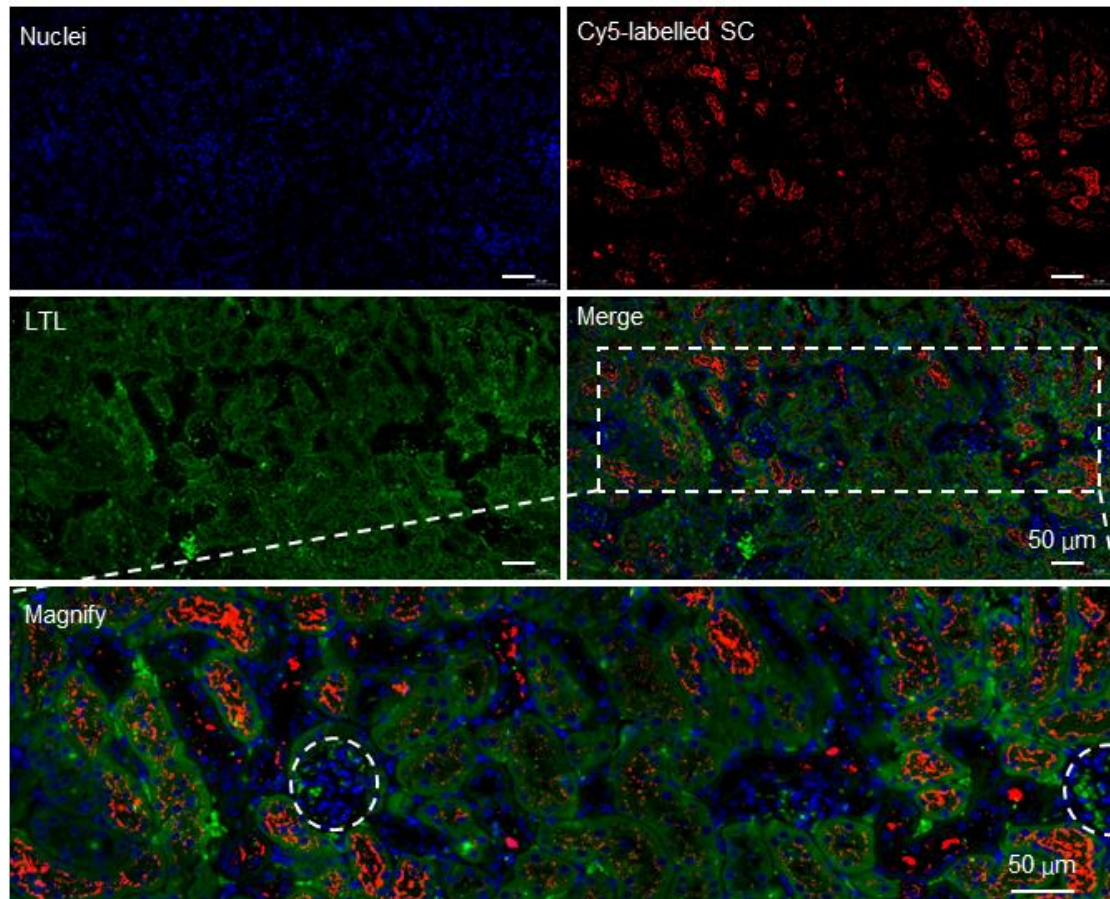


Fig. S4. SC located in renal tubules. Representative confocal images of AKI kidney sections after intravenous injection of Cy5-SC (red signal) for 4 h. Blue indicates DAPI staining. Immunostaining for *Lotus-tetragonolobus Lectin* (LTL), the proximal tubule marker, is shown in green. White dashed circles denote glomeruli. Scale bar = 50 μm.

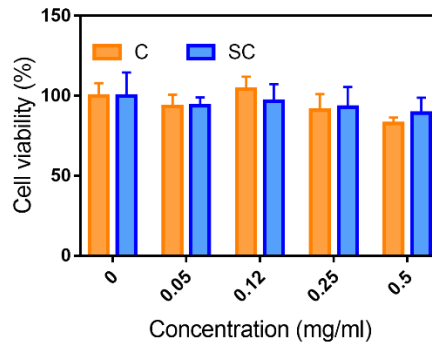


Fig. S5. *In vitro* biocompatibility of SC. C indicates chitosan. The data are the mean \pm s.d. n = 6.

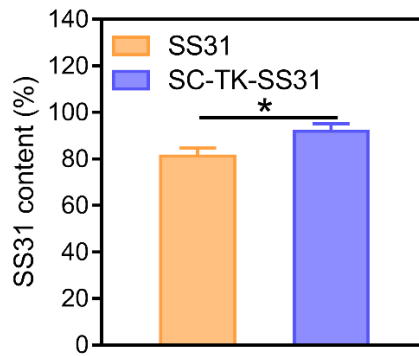


Fig. S6. Plasma stability of SS31 and SC-TK-SS31. The remaining SS31 content in plasmas after incubating with SS31 or SC-TK-SS31 at a concentration of 10 μ g/ml SS31 in a 37 °C incubator for 24 h. The data are the mean \pm s.d. n = 3, * p < 0.05 between groups as indicated.

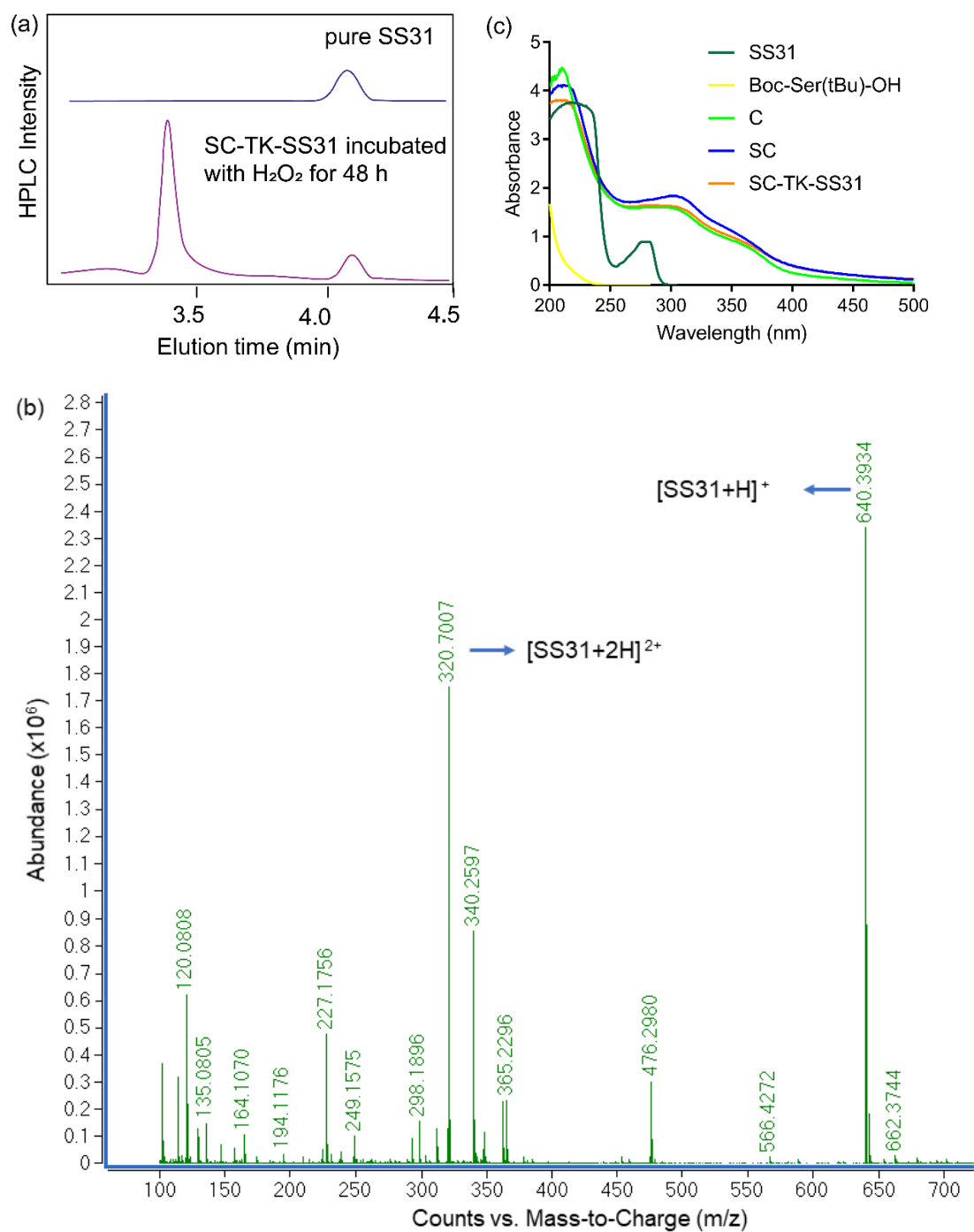


Fig. S7. Pure SS31 release of SC-TK-SS31 triggered by H_2O_2 . (a) HPLC trace of pure SS31 (elution time, 4.1 min) and SC-TK-SS31 incubated with H_2O_2 (10 mM) for 48 h. (b) LC-MS spectrum of the component of SC-TK-SS31 with an elution time of 4.1 min after 48 h treatment in the presence of 10 mM H_2O_2 . Molecular weight of SS31 is 639.3875 g/mol. (c) UV-Vis absorbance spectra of different contents of SC-TK-SS31.

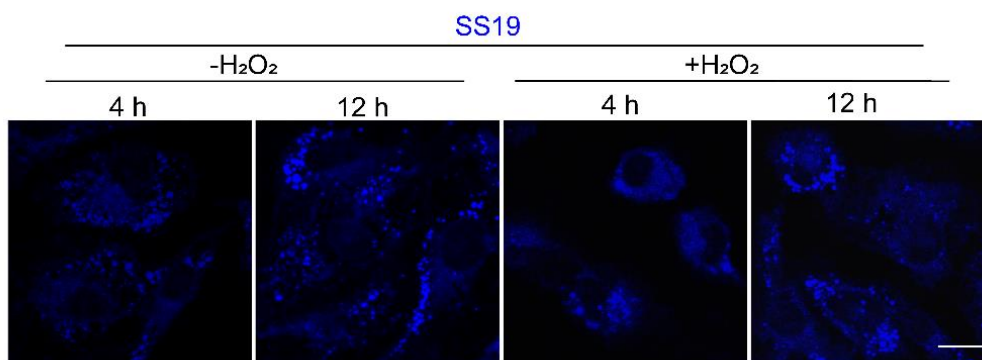


Fig. S8. Cellular uptake of pure SS31. Representative confocal images of normal or H₂O₂-stimulated HK-2 cells after incubated with SS19 for 4 h or 12 h at a concentration of 20 µg/mL. Blue indicates SS19, representing SS31. Scale bar = 20 µm.

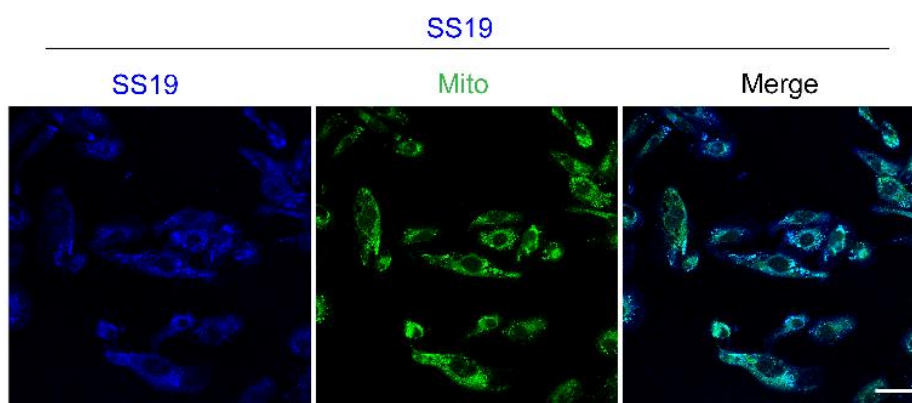


Fig. S9. Mitochondria targeting ability of pure SS31. Representative confocal images of H₂O₂-stimulated HK-2 cells after incubated with SS19 for 12 h. Blue indicates SS19, representing SS31. MitoTracker-stained mitochondria are shown in green. Scale bar = 40 µm.

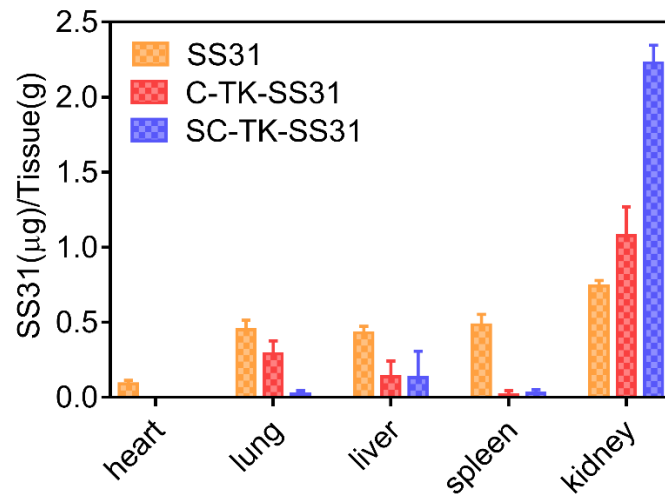


Fig. S10. SS31 concentrations in the main organs. SS31 concentrations in heart, lung, liver, spleen, and kidney of AKI mice at 4 h post injection of formulations (pure SS31, C-TK-SS31 and SC-TK-SS31) at a dose of 2 mg/kg SS31. The data are the mean \pm s.d. n = 3 independent mice.