

Supplementary Information for

Clickable, Acid Labile Immunosuppressive Prodrugs for In Vivo Targeting

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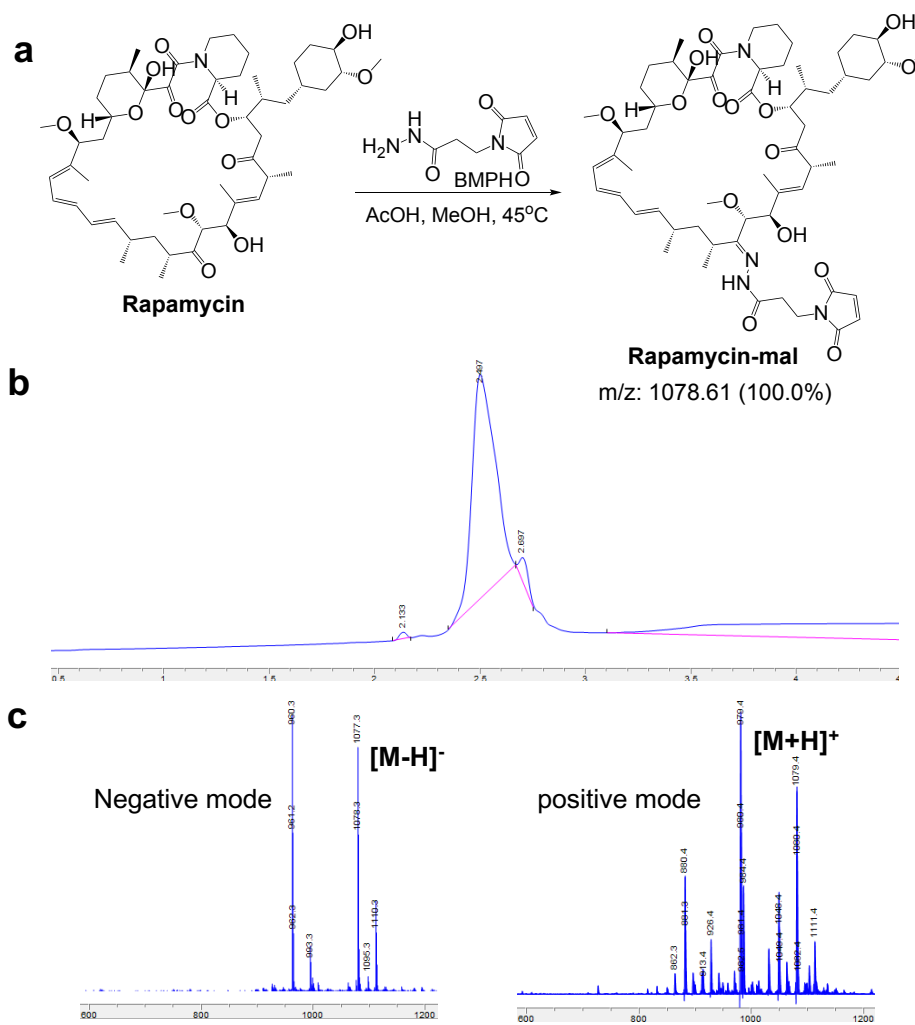


Figure S1. Synthesis and characterizations of rapamycin-mal. (a) Synthetic route of rapamycin-mal. (b) HPLC profile of rapamycin-mal, detected at 254 nm. (c) Mass spectra of rapamycin-mal in negative and positive mode, respectively.

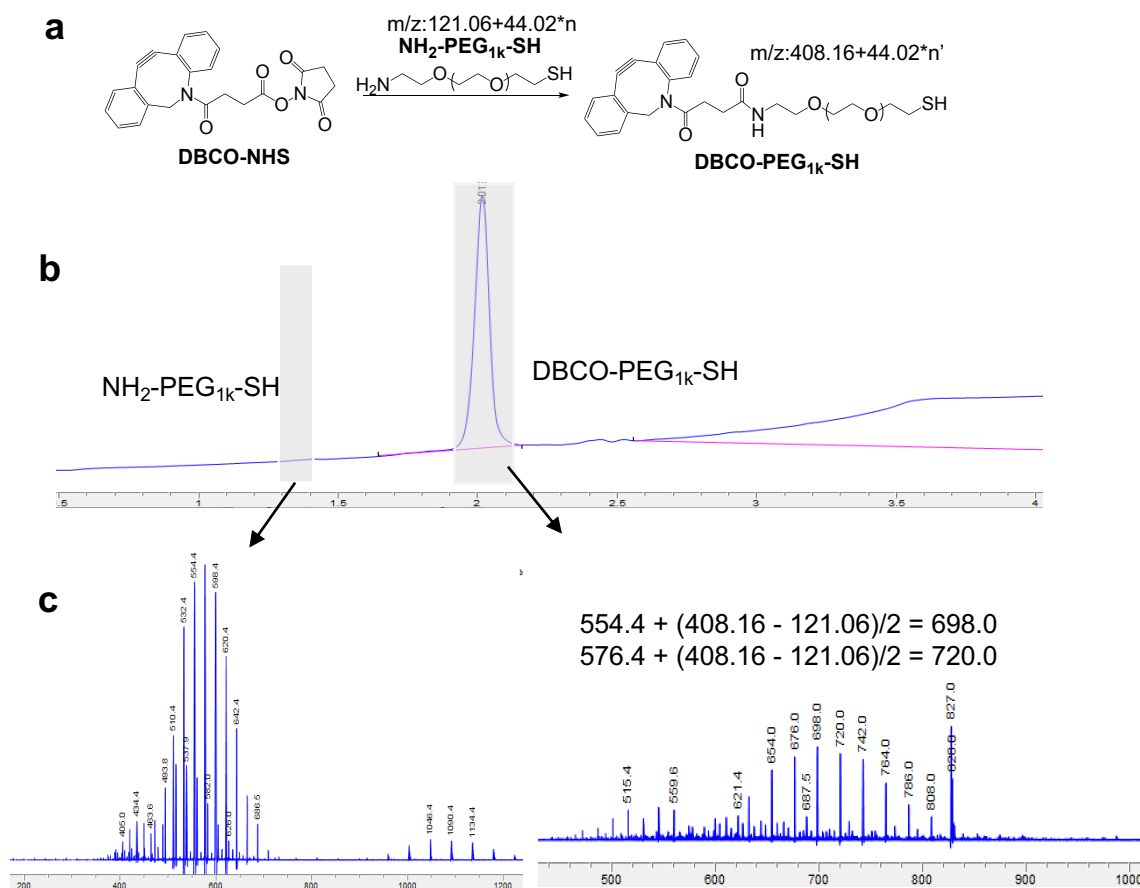


Figure S2. Synthesis and characterizations of DBCO-PEG_{1k}-SH. (a) Synthetic route of DBCO-PEG_{1k}-SH. (b) HPLC profile of the mixture of DBCO-NHS and NH₂-PEG_{1k}-SH after 24-h reaction. (c) Mass spectra of NH₂-PEG_{1k}-SH and DBCO-PEG_{1k}-SH, respectively. Peak shift confirmed the successful conjugation.

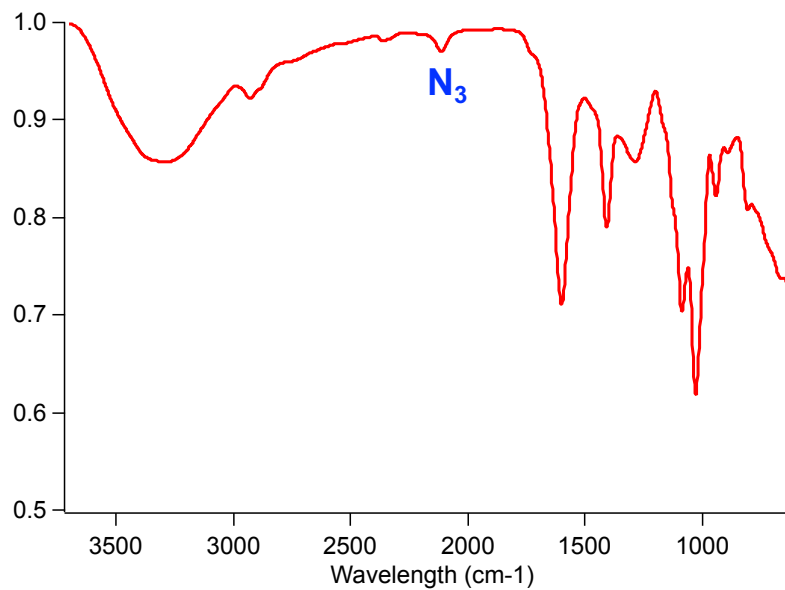


Figure S3. FTIR spectrum of alginate-N₃. The peak at 2106 cm⁻¹ confirms the existence of azido groups.

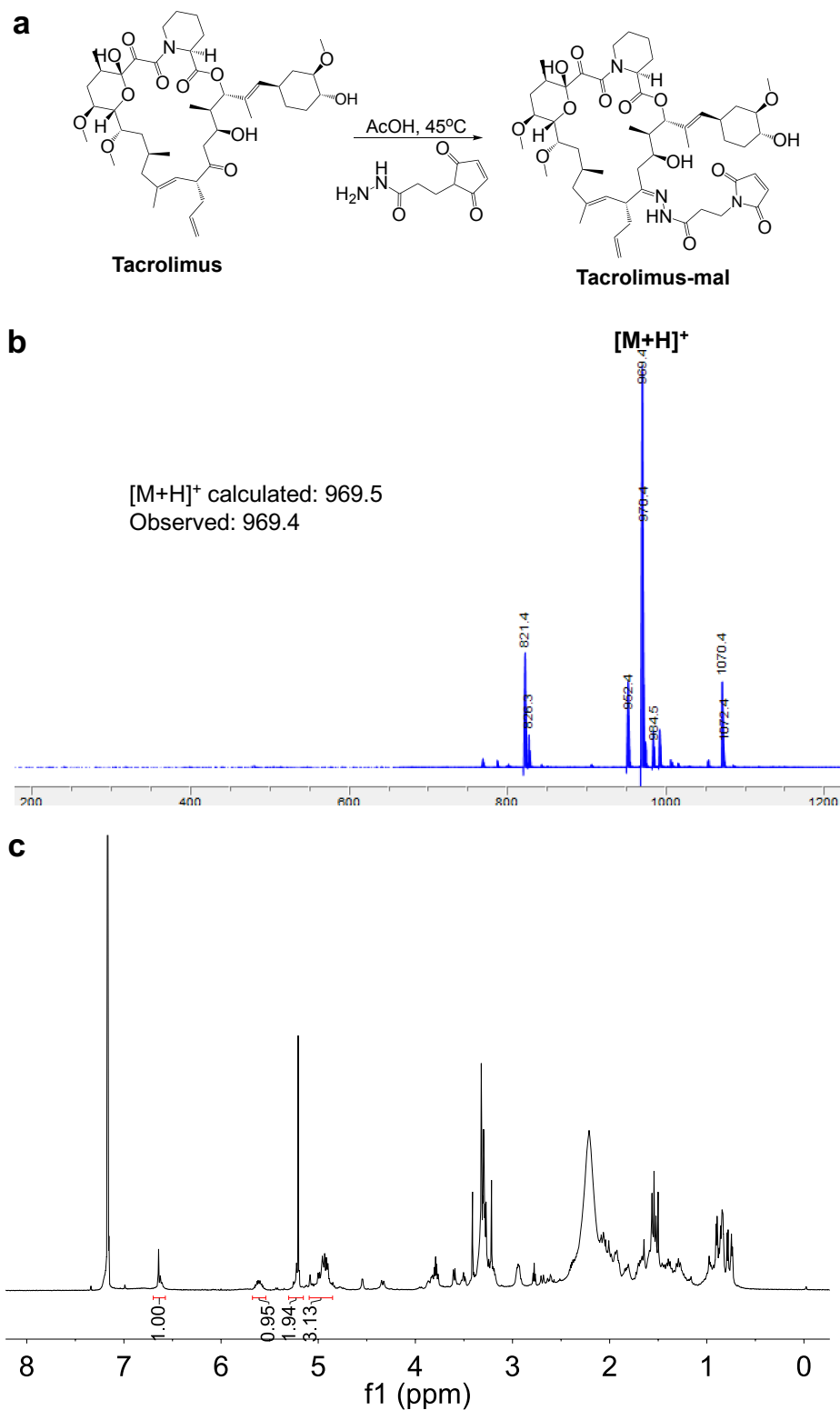


Figure S4. Synthesis and characterizations of tacrolimus-mal. (a) Synthetic route of tacrolimus-mal. (b) Mass spectrum of tacrolimus-mal in positive mode. (c) ^1H NMR spectrum of tacrolimus-mal in CDCl_3 .

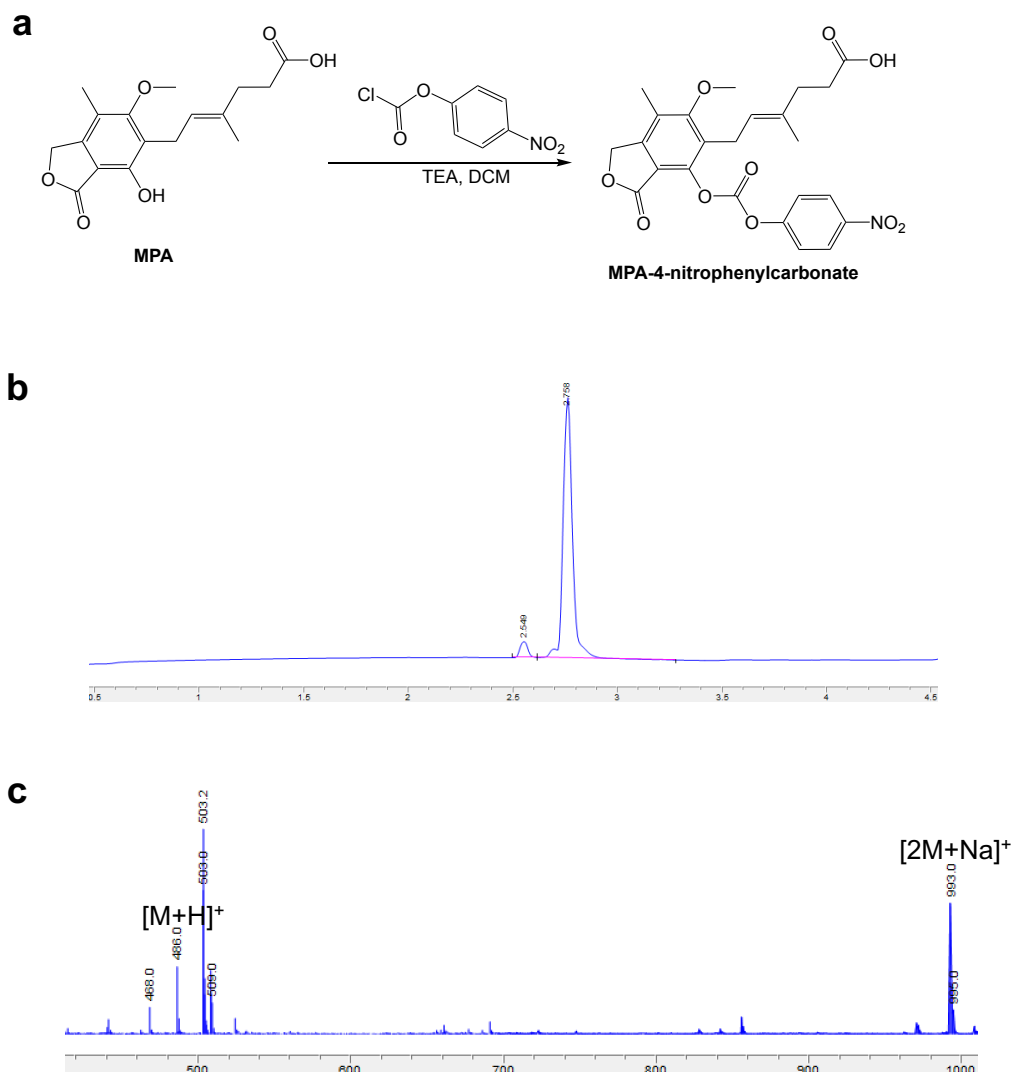


Figure S5. Synthesis and characterizations of MPA-4-nitrophenylcarbonate. (a) Synthetic route of MPA-4-nitrophenylcarbonate. (b) HPLC profile of MPA-4-nitrophenylcarbonate. (c) Mass spectrum of MPA-4-nitrophenylcarbonate in positive mode.

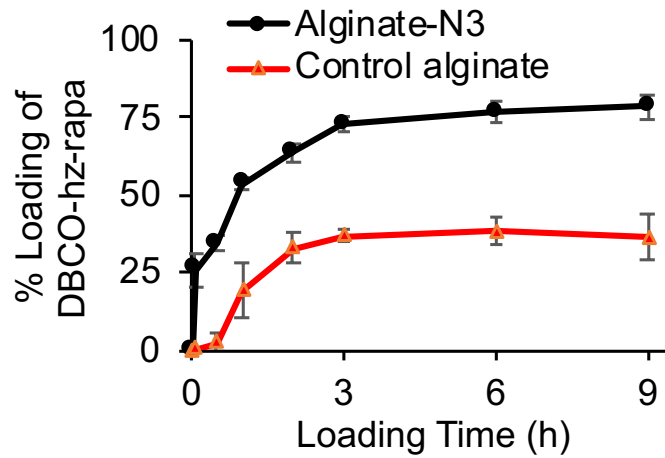


Figure S6. Loading kinetics of DBCO-hz-ropa into alginate-N₃ and unmodified alginate gels, respectively. Data are presented as mean \pm SD (n=4).

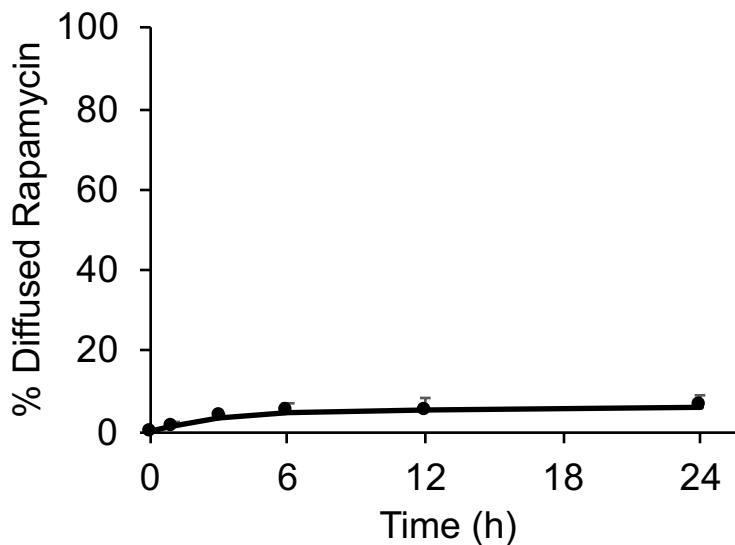


Figure S7. Loading kinetics of rapamycin into alginate-N₃ gels via diffusion. Rapamycin was first dissolved in dimethyl sulfoxide before being added into the medium. Data are presented as mean \pm SD (n=3).

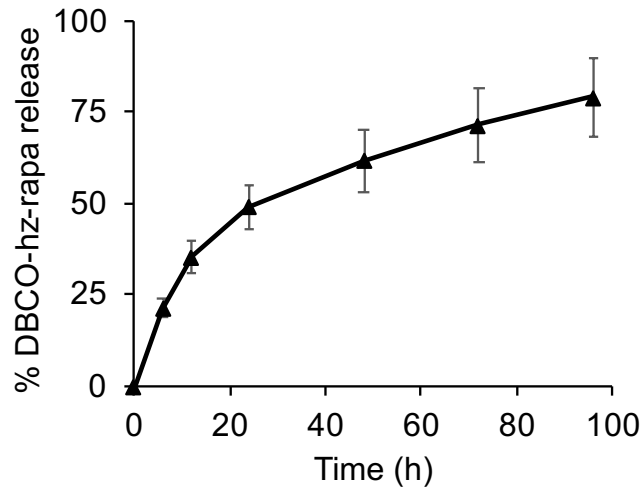


Figure S8. Release profile of DBCO-hz-rapa from unmodified alginate gels at pH 7.4 (n=4). Gels with diffusively loaded DBCO-hz-rapa were placed in fresh medium and monitored for drug release. Data are presented as mean \pm SD.

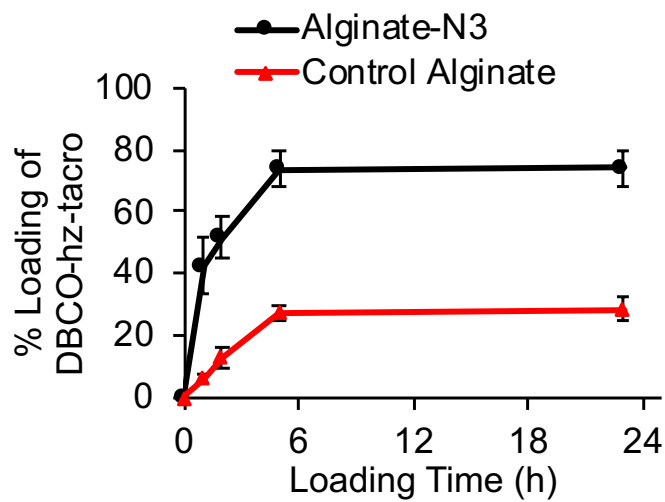


Figure S9. Loading kinetics of DBCO-hz-tacro into alginate-N₃ and unmodified alginate gels, respectively. Data are presented as mean \pm SD (n=4).

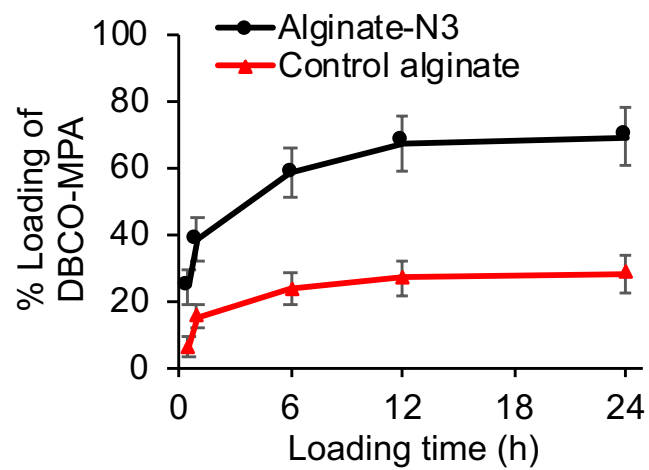


Figure S10. Loading kinetics of DBCO-MPA into alginate-N₃ and unmodified alginate gels, respectively. Data are presented as mean \pm SD (n=4).

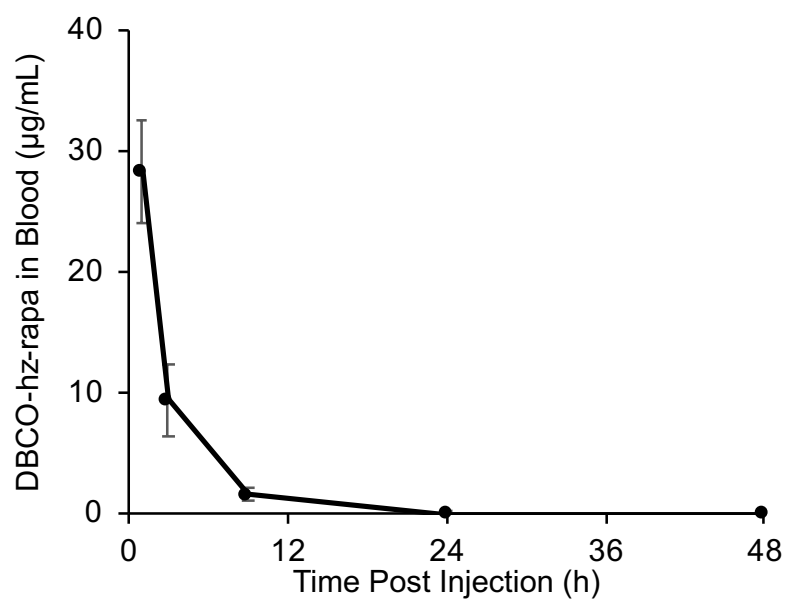


Figure S11. Pharmacokinetics of DBCO-hz-rapa. DBCO-hz-rapa (50 mg/kg) was tail vein injected to Balb/c mice. Blood (10 µL) was collected from orbital sinus at selected time points (1, 3, 9, 24, and 48 h), lysed, and diluted with water/methanol (1/2, v/v, 90 µL) prior to HPLC analyses.

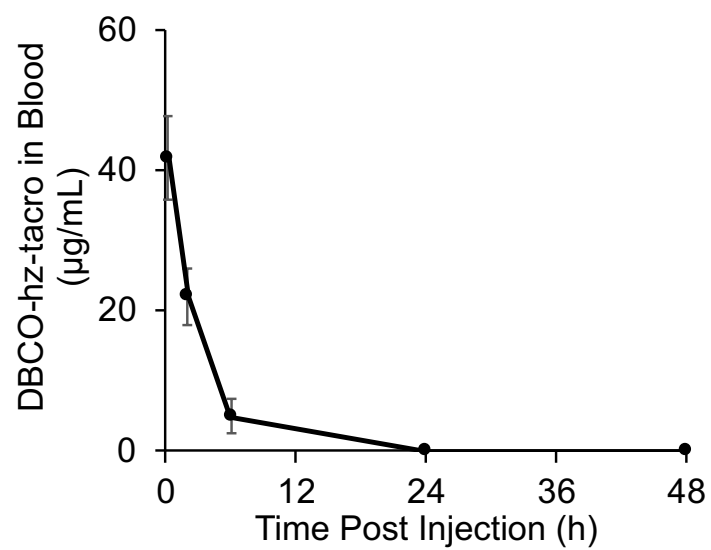


Figure S12. Pharmacokinetics of DBCO-hz-tacro. DBCO-hz-tacro (50 mg/kg) was tail vein injected to Balb/c mice. Blood (10 µL) was collected from orbital sinus at selected time points (0.2, 2, 6, 24, and 48 h), lysed, and diluted with water/methanol (1/2, v/v, 90 µL) prior to HPLC analyses.

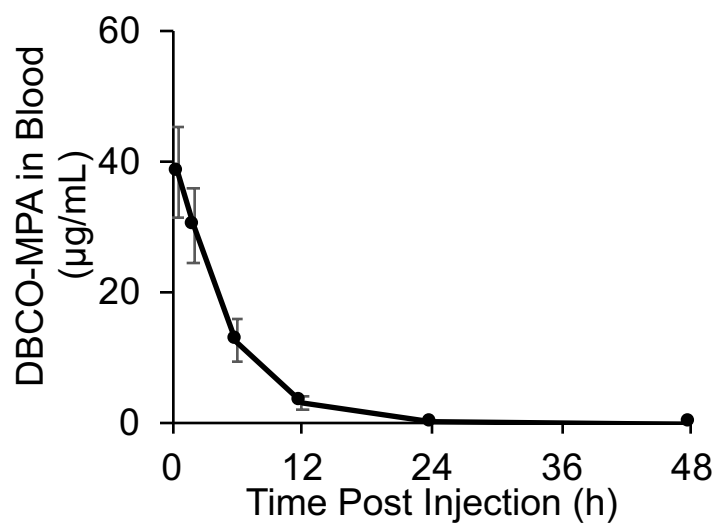


Figure S13. Pharmacokinetics of DBCO-MPA. DBCO-MPA (50 mg/kg) was tail vein injected to Balb/c mice. Blood (10 µL) was collected from orbital sinus at selected time points (0.5, 2, 6, 12, 24, and 48 h), lysed, and diluted with water/methanol (1/2, v/v, 90 µL) prior to HPLC analyses.

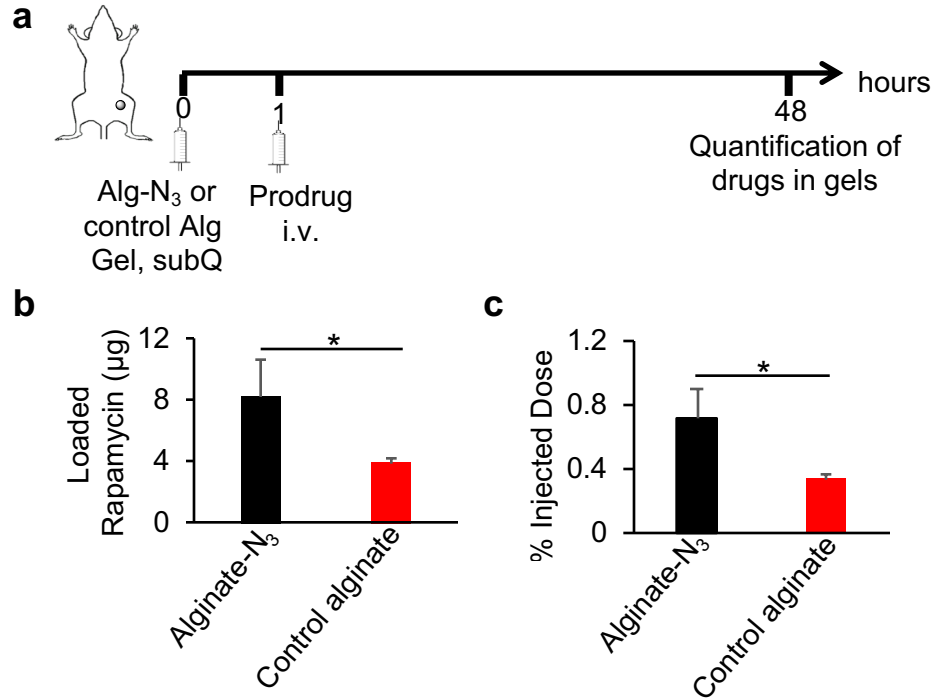


Figure S14. In vivo reloading of DBCO-hz-rapa to alginate-N₃ gels. (a) Schematic illustration of in vivo reloading study. Alginate-N₃ or control alginate gels (100 μL) were subcutaneously injected into the right flank of Balb/c mice. After 1 h, DBCO-hz-rapa (150 mg/kg) was i.v. injected via tail vein. At 48 h, gels were dissociated in the presence of EDTA and treated with formic acid prior to HPLC analyses. (b) Amount of rapamycin recovered from alginate-N₃ or control alginate gels at 48 h (n=3). (c) Amount of loaded rapamycin presented as the percentage of injected dose (n=3). Data were presented as mean ± SD and analyzed by two-tailed t-test (0.01 < *P ≤ 0.05; **P ≤ 0.01; ***P ≤ 0.001).