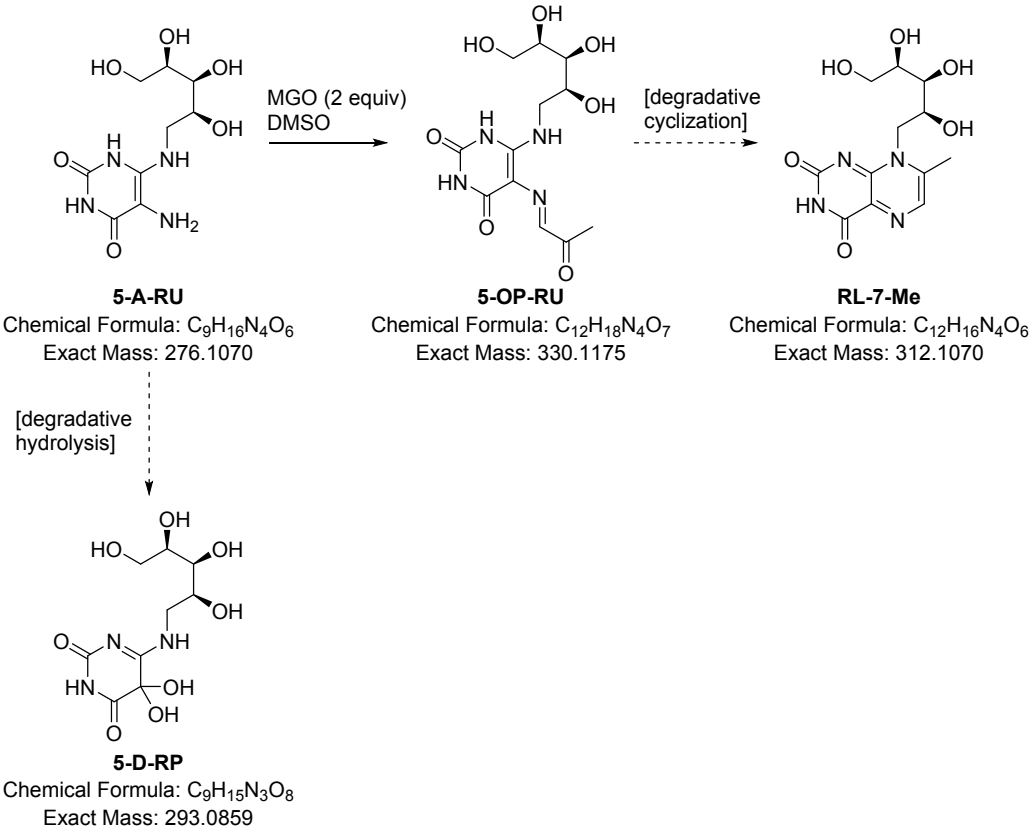
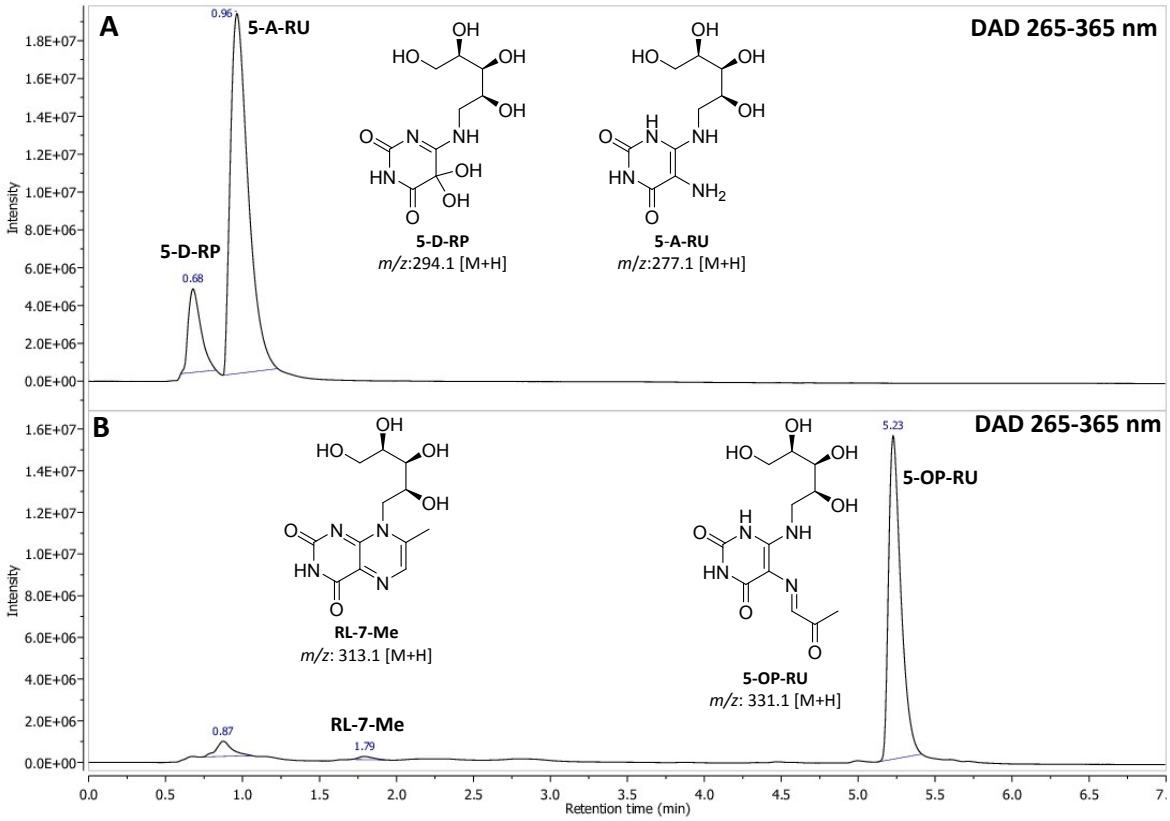
Spectroscopic Analysis of 5-OP-RU

^{1}H NMR spectra were recorded on a Bruker 500 MHz spectrometer and referenced to tetramethylsilane at 0 ppm (internal standard). High resolution electrospray ionization (ESI) mass spectra were undertaken on a Waters Q-TOF Premier™ Tandem Mass spectrometer fitted with a Waters 2795 HPLC. HPLC-MS analysis was performed on a Phenomenex Synergi™ Fusion-RP C18 column (2.5 M, 3x50 mm), heated to 40 °C, with 10 mM NH_4OAc/water used as eluent A and MeOH as eluant B (flow = 0.5 mL/min). The following gradient method was used: T0 (min) = 100:0 A:B, T2 = 100:0 A:B, T7 = 50:50 A:B, T8 = 100:0 A:B, T10 = 100:0 A:B. Peaks were detected by UV absorbance, primarily at 254 nm, and identified with the inline MS detector.

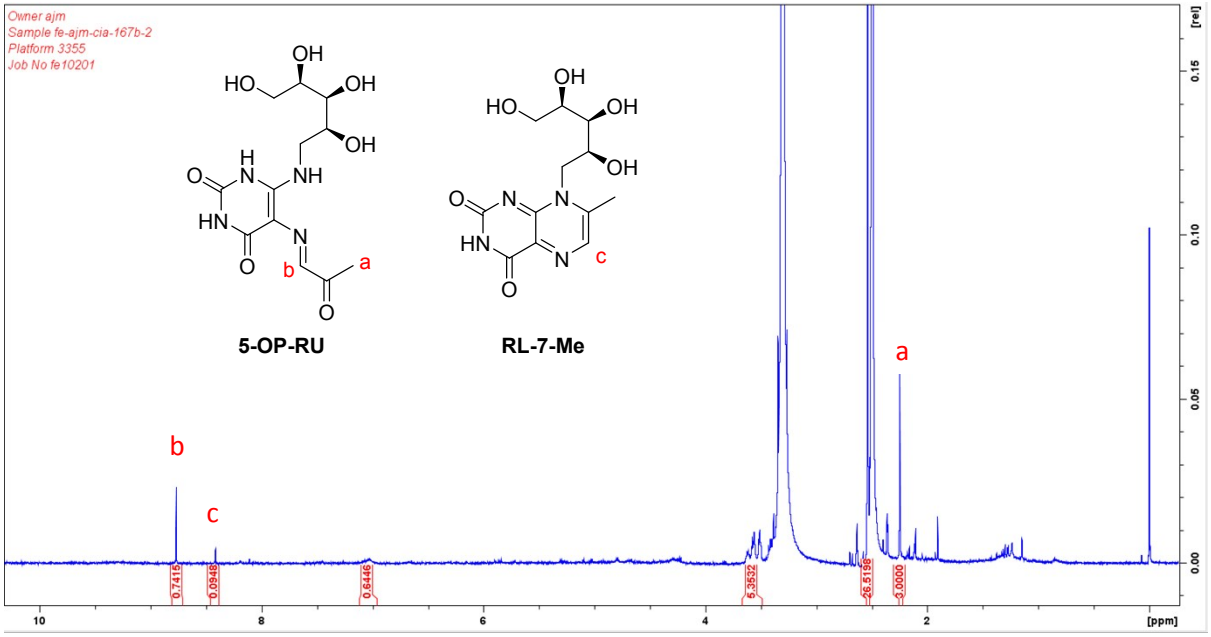


SI Scheme 1: Synthesis of 5-OP-RU and its degradative by-products.
5-A-RU, 5-amino-6-D-ribitylaminouracil; **5-D-RP**, 5,5-dihydroxy-6-(D-ribitylamino)pyrimidine-2,4(3*H*,5*H*)-dione; **MGO**, methylglyoxal; **5-OP-RU**, 5-(2-oxopropylideneamino)-6-D-ribitylaminouracil; **RL-7-Me**, 7-methyl-8-D-ribityllumazine.



SI Fig. 1 Analytical HPLC spectra for the reaction between 5-A-RU and MGO.
(**A**) A freshly prepared solution of 5-A-RU (5 mg/mL, DMSO) was diluted into deoxygenated water (*c* = 0.5 mg/mL) and analysed by HPLC-MS (DAD 265-365 nm). Exposure to air during sample preparation leads to the appearance of a degradative compound (eluting at 0.58 min) which corresponds to the putative hydrate 5-D-RP (m/z 294.10).[1] (**B**) Crude reaction between 5-A-RU (5 mg/mL DMSO) and MGO (2 equiv). After a 30 min incubation period, an aliquot was diluted into deoxygenated water (*c* = 0.5 mg/mL) and analysed by HPLC-MS (DAD 265-365 nm).

^{1}H NMR spectrum of 5-OP-RU (500 MHz, d_6-DMSO)



SI Fig. 2 ^{1}H NMR spectrum of 5-OP-RU.
^{1}H NMR spectrum of 5-OP-RU obtained in this work which matched that previously reported.[2] The presence of some RL-7-Me is evidenced by the lumazine methine signal (c).

References

1. J. Lange, R. J. Anderson, A. J. Marshall, S. T. S. Chan, T. S. Bilbrough, O. Gasser, C. Gonzalez-Lopez, M. Salio, V. Cerundolo, I. F. Hermans and G. F. Painter, The Chemical Synthesis, Stability, and Activity of MAIT Cell Prodrug Agonists That Access MR1 in Recycling Endosomes, *ACS chemical biology*, 2020, **15**, 437-445.
2. J. Y. W. Mak, W. Xu, R. C. Reid, A. J. Corbett, B. S. Meehan, H. Wang, Z. Chen, J. Rossjohn, J. McCluskey, L. Liu and D. P. Fairlie, Stabilizing short-lived Schiff base derivatives of 5-aminouracils that activate mucosal-associated invariant T cells, *Nature communications*, 2017, **8**, 14599.