Transition State Structure of RNA Depurination by Saporin L3

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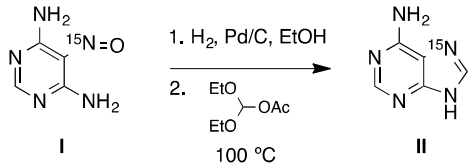
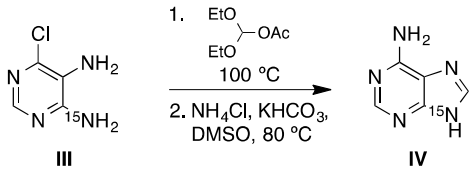
SUPPORTING INFORMATION

Synthesis of [7–^{15}N]– and [9–^{15}N]adenines.



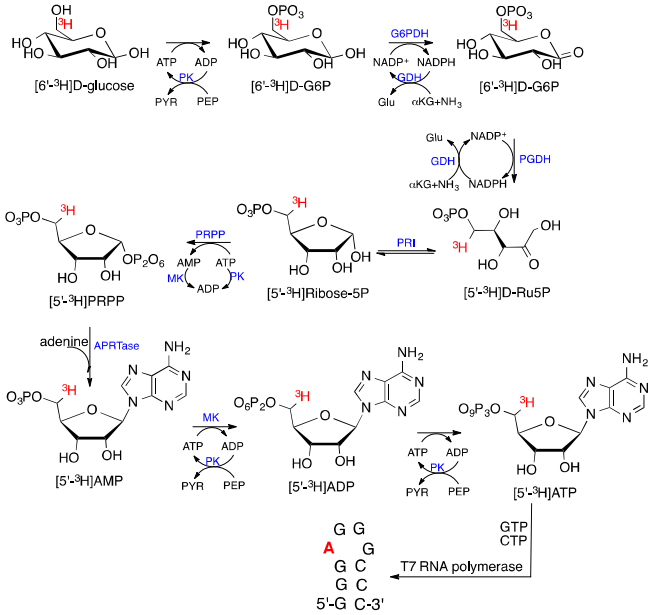
Supplementary Figure S1. Synthesis of [7–^{15}N]adenine (**II**).

[7–^{15}N]Adenine (II). [7–^{15}N]adenine (II) was synthesized from 4,6–diamino–5–[^{15}N]nitrosopyrimidine (I) using methods adapted from Sethi *et al*.[52] In a flame–dried, Ar–flushed flask equipped with magnetic stir bar and septum, nitrosopyrimidine (I) (20.0 mg, 0.144 mmol) was suspended in absolute EtOH (16.0 mL). A spatula tip of Pd on carbon (10 wt. %) was added to the suspension and the flask was purged with H_2 for 20 minutes. The flask was fitted with a balloon of H_2 and the suspension was stirred overnight at room temperature. The next day, the suspension was filtered through a pad of celite (EtOH) and the filtrate was concentrated to dryness by rotary evaporation. The crude product was suspended in diethoxymethyl acetate (2.5 mL) and stirred at 100 ºC overnight in a sealed, thick–walled flask. The next morning, the reaction was cooled to room temperature and concentrated by rotary evaporation. The crude product was purified via HPLC on a Phenomenex Luna® 5 µm C_{18} column (250 x 10.0 mm, 100 Å) using an isocratic solvent system of 4% MeCN in H_2O. Fractions containing [7–^{15}N]adenine were combined, frozen, and the solvent was removed via lyophilization to provide (**II**) as a white solid. Analytical data were in agreement with literature precedent.



Supplementary Figure S2. Synthesis of [9–^{15}N]adenine (IV).

[9–^{15}N]Adenine (IV). [9–^{15}N]Adenine (IV) was synthesized from [4–^{15}N]diamino–6–chloropyrimidine (III) using methods adapted from Sethi *et al*.[52] and Jones *et al*.[53] In a flame–dried, Ar–flushed thick–walled flask, chloropyrimidine (III) (10.0 mg, 0.0687 mmol) was dissolved in diethoxymethyl acetate (1.5 mL). The flask was sealed and the mixture was stirred at 100 ºC overnight. The next day, the reaction was cooled to room temperature and concentrated to dryness by rotary evaporation. The crude product was dissolved in dry DMSO (2.0 mL) in a flame–dried, Ar–flushed 4.0 mL vial equipped with magnetic stir bar and septum. $KHCO_3$ (41.2 mg, 0.412 mmol) and NH_4Cl (14.6 mg, 0.274 mmol) were added to the solution, the vial was sealed with a Teflon cap, and the reaction was stirred at 80 ºC for three days. The reaction was then cooled to room temperature, diluted with H_2O, and filtered through a pad of celite (H_2O). The crude product was purified via HPLC on a Phenomenex Luna® 5 µm C_{18} column (250 x 10.0 mm, 100 Å) using an isocratic solvent system of 4% MeCN in H_2O. Fractions containing [9–^{15}N]adenine were combined, frozen, and solvent was removed via lyophilization to afford (IV) as a white solid. Analytical data were in agreement with literature precedent.



Supplementary Figure S3. Synthesis of isotopically labeled stem–loop RNAs.