Supplementary Material

The Identity of the Nucleophile Substitution may Influence Metal Interactions with the Cleavage Site of the Minimal Hammerhead Ribozyme

Edith M. Osborne, W. Luke Ward, Max Z. Ruehle, and Victoria J. DeRose

Department of Chemistry, University of Oregon, Eugene, OR 97403-1253

Table of Contents

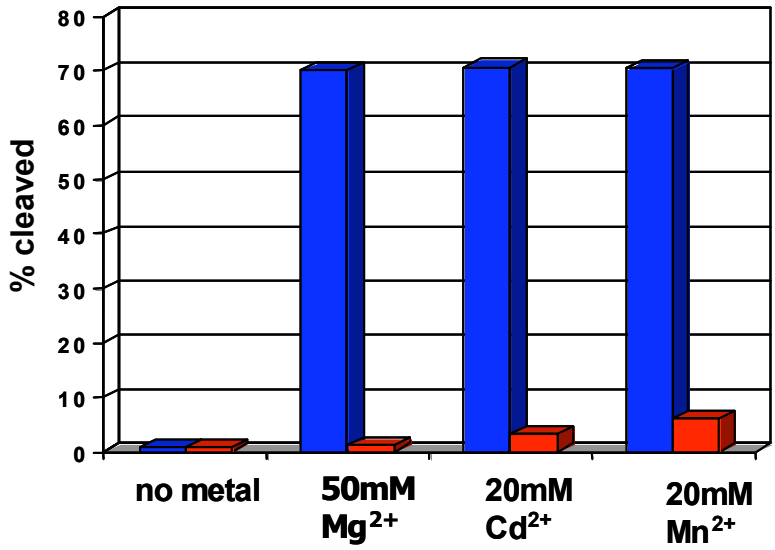


Figure S1. Activity of the mHHRz with a nucleophile C17 (cleavage site) 2'-NH_2 substitution in comparison with WT. Data are plotted as % cleaved substrate following incubation for discrete time periods. WT HHRz (in blue) were incubated for 1 hr and C17 2'-NH_2-substituted HHRz (in red) were incubated for 36 hours. Reaction conditions: 5 mM Hepes pH 8.5, 100 mM NaCl, 24 ^{0}C, and indicated concentrations of divalent cations (as Cl^- salts).

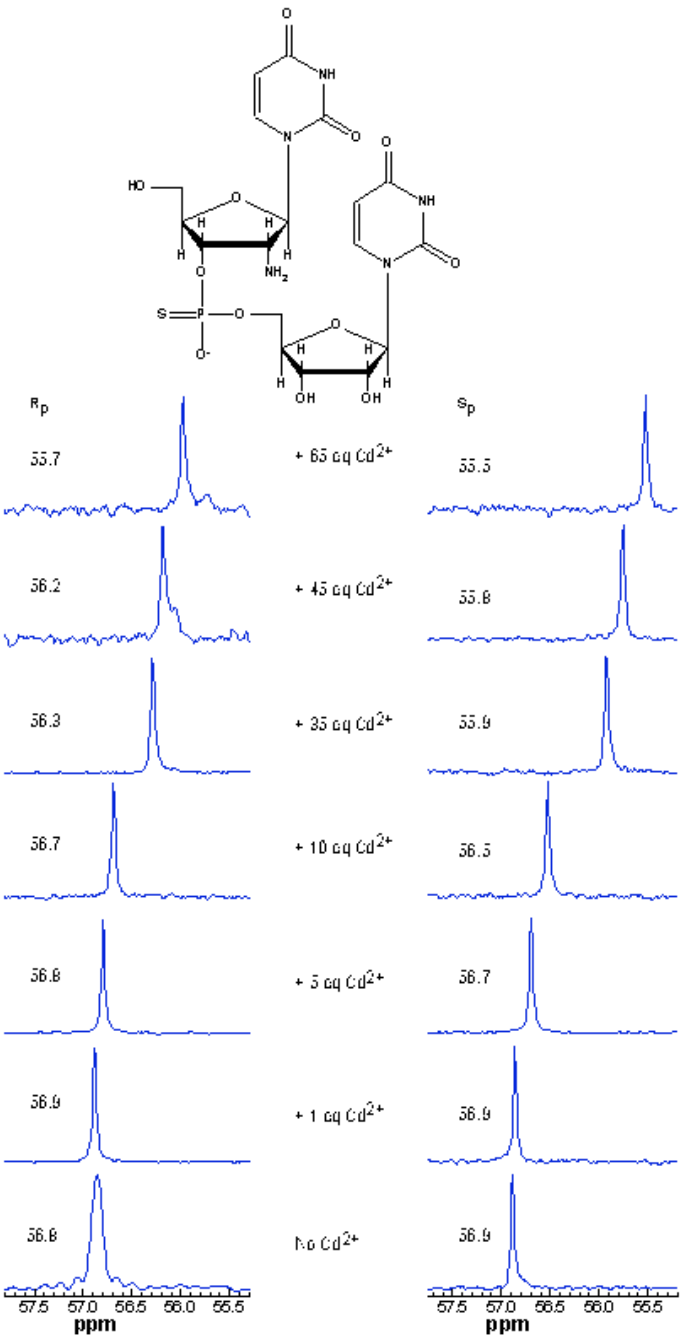


Figure S2. Cd^{2+}-dependent $^{am}U_{PS}U$ $\{^1H\}$ ^{31}P NMR spectra. Data are shown for the separated R_p (left) and S_p (right) diastereomers. Titration conditions are 15 ^0C, 5 mM Hepes pH 8.0, 100 mM NaCl.

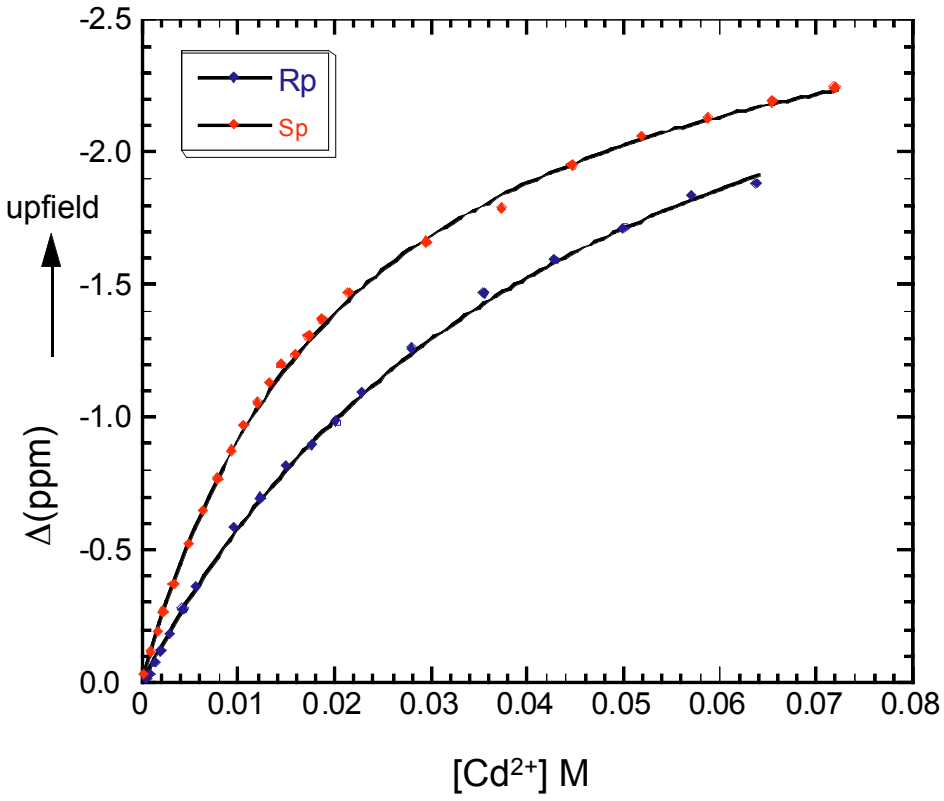


Figure S3. Fit of Cd^{2+}-dependent $^{am}U_{PS}U$ ^{31}P NMR spectra. ^{31}P NMR data from Figure S2 plotted as Δ(ppm) = [ppm(+Cd^{2+})-ppm(0 Cd^{2+})] and fit to simple 1:1 binding isotherm model described in Equation 1 (Methods). R_p isomer data are in blue and S_p isomer data are shown in red. $K_{d,obs}$ ($^{am}U_{PS}U$-R_p) = 46.9 mM, and $K_{d,obs}$ ($^{am}U_{PS}U$-S_p) = 21.6 mM for titration conditions of 15 ^{0}C, 5 mM Hepes pH 8.0, 100 mM NaCl.

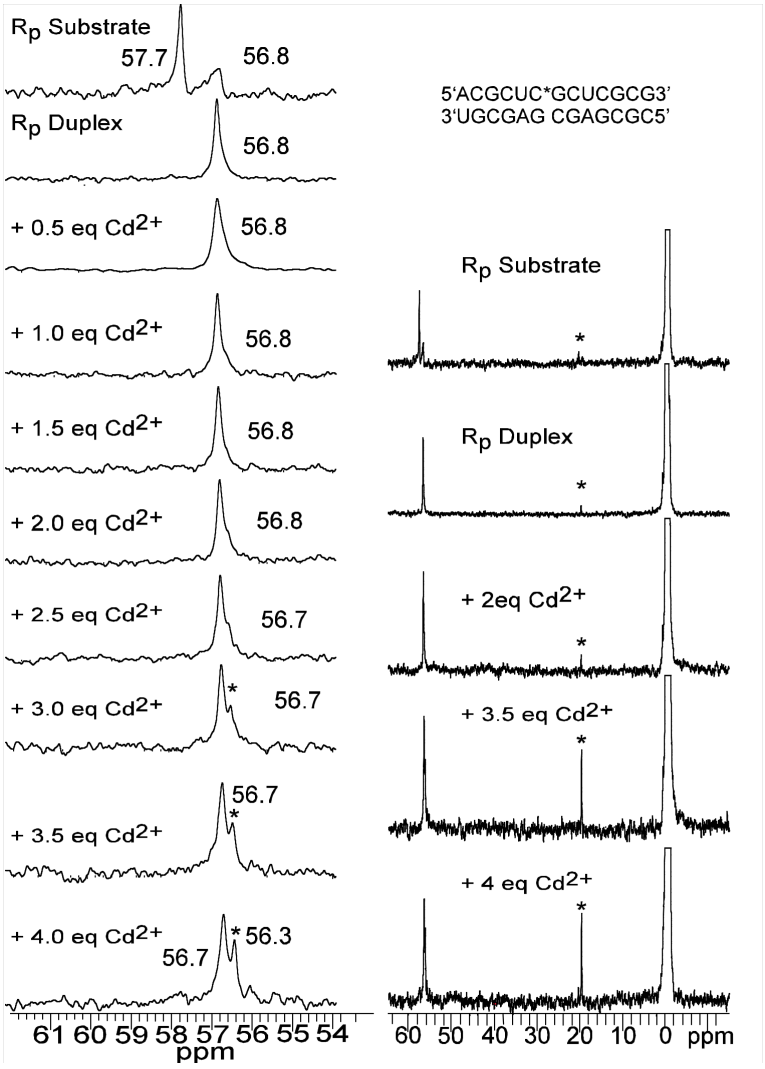


Figure S4a. ^{31}P NMR spectra of the RNA duplex including the mHHRz substrate sequence (blue) with the R_p diastereomer of a 2'-NH_2/PS substitution at the position indicated with the red C*. Addition of up to 4 equivalents of Cd^{2+} results in a <1 ppm shift of the phosphorothioate peak, indicating weak interactions with this site. These samples showed some non-specific RNA cleavage, as indicated by the appearance of a cyclic phosphate peak at 20 ppm (marked with asterisk in full ^{31}P spectrum shown at right) that correlates with appearance of a population with a slightly shifted phosphorothioate peak (asterisks on left side). Conditions: 15 ^{0}C, 5 mM Hepes pH 8.5, 100 mM NaCl.

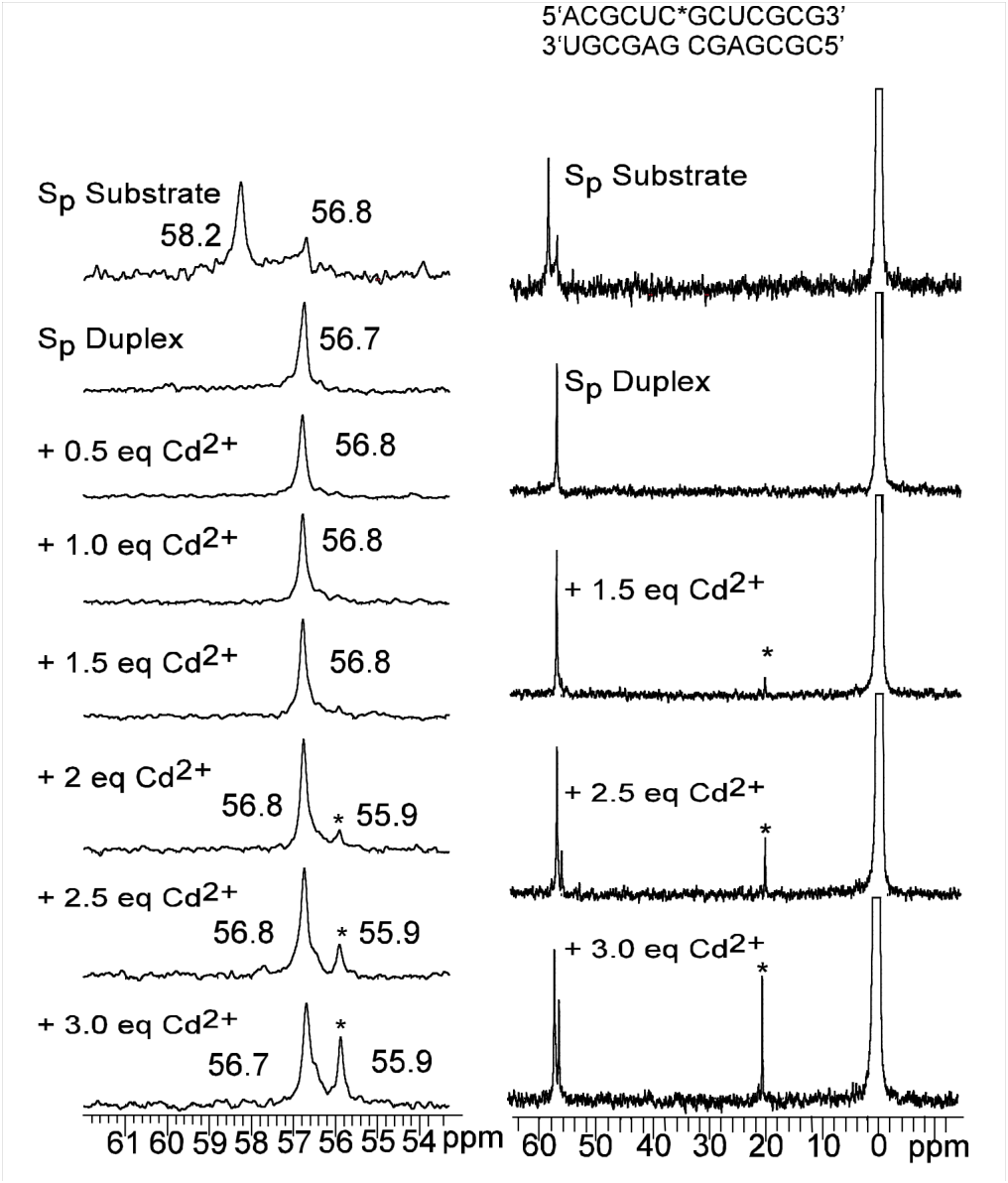
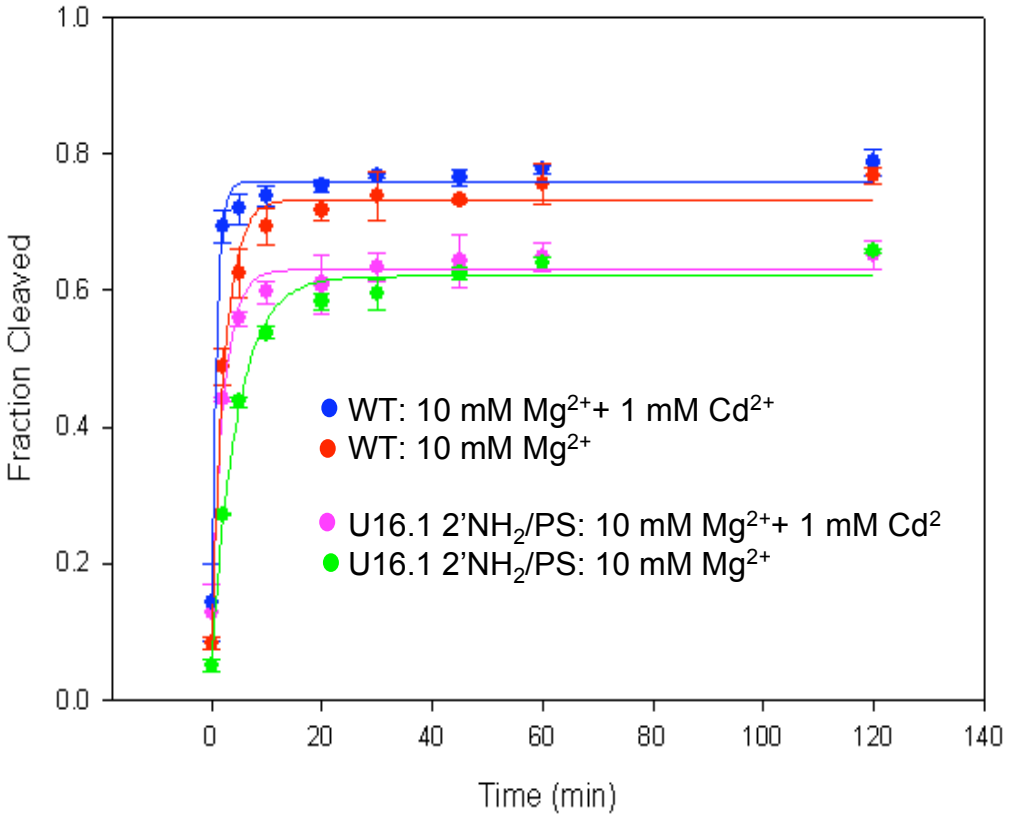


Figure S4b. ^{31}P NMR spectra of the RNA duplex including the trHHRz substrate sequence (blue) with the S_p diastereomer of a 2'-NH_2/PS substitution at the position indicated with the red C*. Addition of up to 3 equivalents of Cd^{2+} results in a <1 ppm shift of the phosphorothioate peak, indicating weak interactions with this site. These samples showed some non-specific RNA cleavage, as indicated by the appearance of a cyclic phosphate peak at 20 ppm (marked with asterisk in full ^{31}P spectrum shown at right) that correlates with appearance of a population with a slightly shifted phosphorothioate peak (asterisks on left side). Conditions: 15 ^{0}C, 5 mM Hepes pH 8.5, 100 mM NaCl.



	k_{obs} (min^{-1})
10 mM Mg^{2+}	
WT	0.43 ± 0.05
U16.1 2' NH_2/PS	0.22 ± 0.02
10 mM Mg^{2+}/1 mM Cd^{2+}	
WT	1.1 ± 0.18
U16.1 2' NH_2/PS	0.44 ± 0.05

Figure S5. Activity of the mHHRz with a U16.1 2'-NH_2/PS substitution in comparison with WT. Kinetics were measured in single-turnover conditions (10:1 enzyme:substrate) in 25 mM MOPS pH 7.0, 10 mM NaCl, and the indicated divalent metal ion concentrations. Data are plotted as (fraction cleaved) vs. time, and fit to a single-exponential rate k_{obs} as described in Methods.

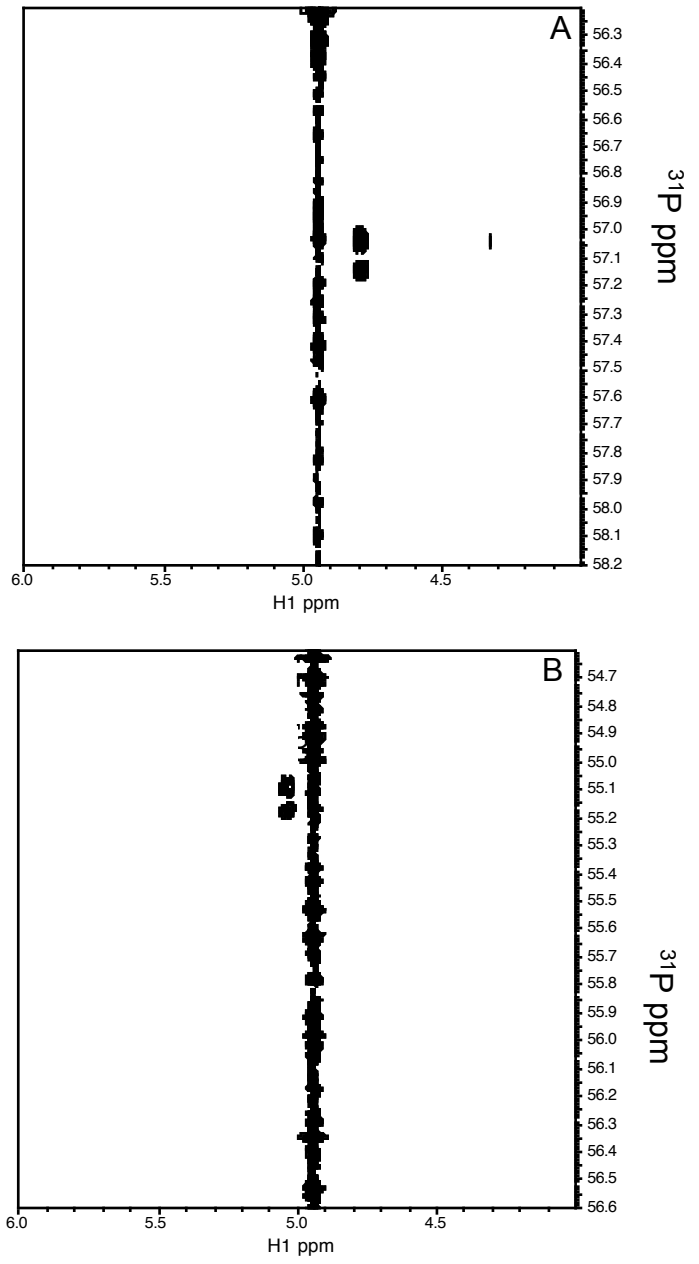


Figure S6a. ^{1}H-^{31}P COSY spectra of $^{am}U_{PS}U$ R_p diastereomer in absence (A) and presence (B) of 40 mM $CdCl_2$. A ^{1}H-^{31}P crosspeak appears between between the H3' of the 2'-NH_2 ribose and the phosphorothioate. Sample conditions: 10 mM phosphate/D_2O buffer pH 7.0 (corrected), 100 mM NaCl, 10^0C.

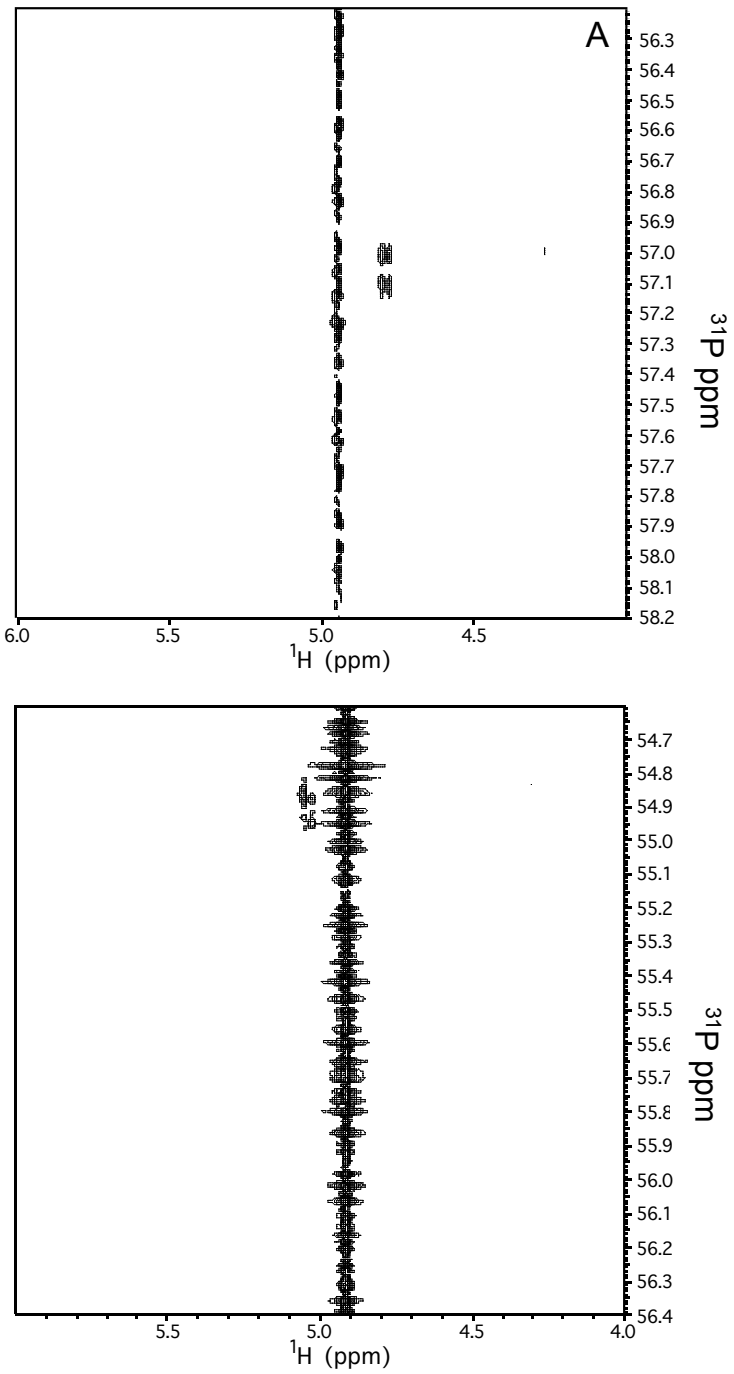


Figure S6b. ^{1}H-^{31}P COSY spectra of $^{am}U_{PS}U$ S_p diastereomer in absence (A) and presence (B) of 40 mM $CdCl_2$. A ^{1}H-^{31}P crosspeak appears between between the H3' of the 2'-NH_2 ribose and the phosphorothioate. Sample conditions: 10 mM phosphate/D_2O buffer pH 7.0 (corrected), 100 mM NaCl, 10^0C.

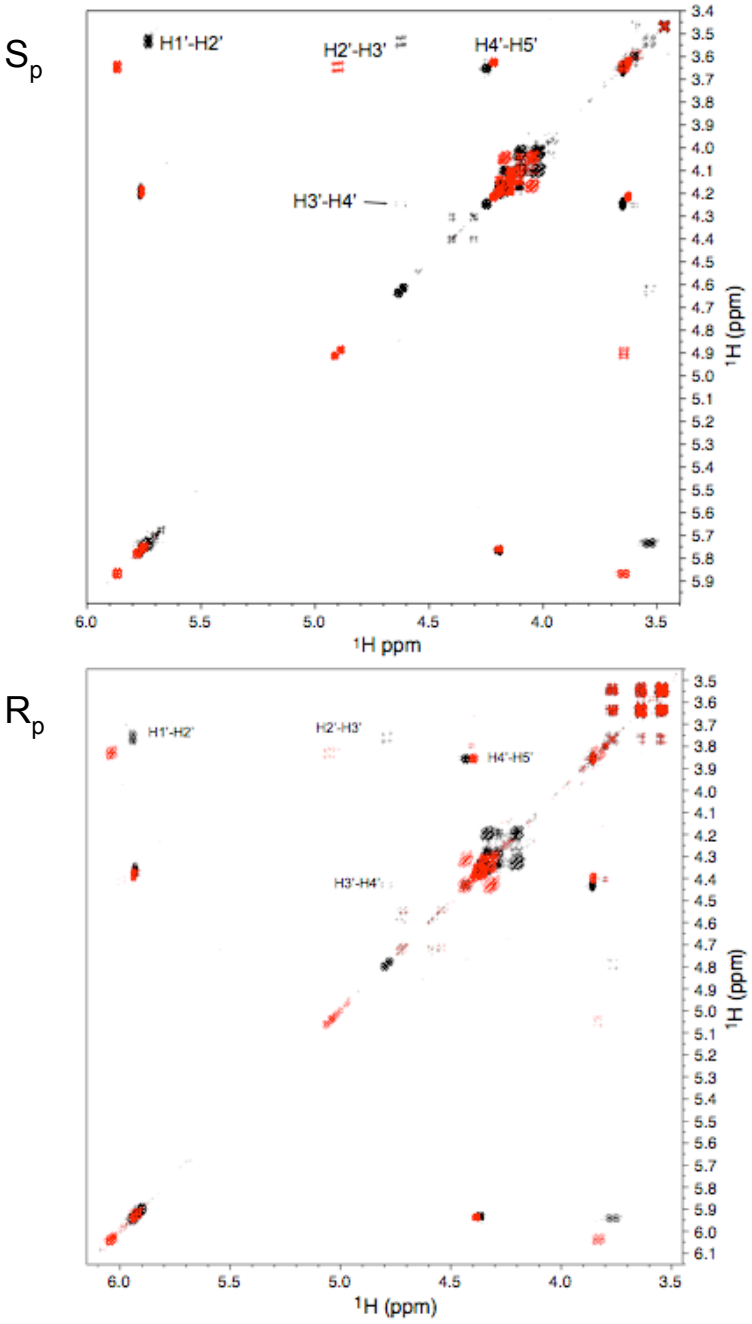


Fig. S7. DQF-COSY spectra of $^{am}U_{PS}U$ diastereomers in presence and absence of Cd^{2+}. Data are shown for S_p (upper) and R_p (lower) diastereomer in the absence (black) and presence (red) of 40 mM $CdCl_2$. Conditions: 10 mM sodium cacodylate/D_2O, pH 7.4 (corrected), 100 mM NaCl, 10^0C.