Supporting Information:

A Ribose Sugar Conformational Switch in the LTR-Retrotransposon Ty3 Polypurine Tract-containing RNA/DNA Hybrid

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Sample Preparation: The 20mer Ty3 RNA and DNA strands were synthesized using standard phosphoramidite chemistry. The RNA strand was purchased from Dharmacon Research (Boulder, CO) and the DNA strand purchased from Integrated DNA Technologies (Coralville, IA). Individual RNA and DNA strands were purified using preparative-scale denaturing polyacrylamide gel electrophoresis (PAGE), electroeluted from excised bands and desalted by dialysis against sterile ddH_2O followed by NMR buffer (10 mM phosphate [pH=7.0], 80 mM NaCl). RNA and DNA strand concentrations were determined by measuring the absorbance at 260 nm using an extinction coefficient of 218 $mM^{-1}cm^{-1}$ for the RNA strand and 168.2 $mM^{-1}cm^{-1}$ for the DNA strand. The polypurine-containing Ty3 RNA/DNA hybrid was formed by mixing the RNA and DNA strands at a 1:1 stoichiometric ratio, heating the mixture to 90 ºC and allowing it to slow cool to favor duplex annealing.

NMR Spectroscopy: NMR experiments were collected using a 1.2 mM sample of the 20mer Ty3 RNA/DNA duplex in 10 mM phosphate [pH=7.0], 80 mM NaCl dissolved in either 90% H_2O/10% D_2O or 99.96% D_2O. The spectra were processed using NMRPipe[1] on either an SGI/UNIX or PC/LINUX workstation. Other acquisition parameters and experimental details are provided in the supplemental figure legends.

Supporting Figure S1: (A) Native gel electrophoresis analysis of the Ty3 RNA/DNA hybrid NMR sample. Sample of the hybridized duplex for thermal melting profile and single strand DNA, 20 μM and 40 μM respectively, were loaded onto a 15% non-denaturing polyacrlyamide gel (19:1 acrylamide:bisacrylamide) and electrophoresed in 0.5x Tris-borate EDTA at 4°C. It was then stained with EtBr and visualized on Bio-Rad Gel Doc 2000 transilluminator. Lane 1 is the individual DNA strand and Lane 2 is the 1:1 RNA/DNA hybrid duplex. Bands are labeled on the right. The observation of a single band for the NMR sample indicates that the two complementary strands adopt a single hybrid species when mixed in a 1:1 stoichiometry. (B) UV-detected thermal melt for the 20bp RNA/DNA hybrid duplex. For measurement of melting temperatures (T_m), a 10 μg/ml solution of RNA/DNA hybrid in 10 mM phosphate [pH=7.0], 80 mM NaCl was analyzed in a Beckman DU 640 spectrophotometer. E_{260} was measured at 1 °C intervals from 30 to 85 °C. The DNA/RNA hybrid duplex melting curve could be fit to a single transition and a T_M of 69 ºC determined using the "first derivative" method described by the manufacturer. The first derivative of the Ty3 hybrid melting curve is shown in the inset.

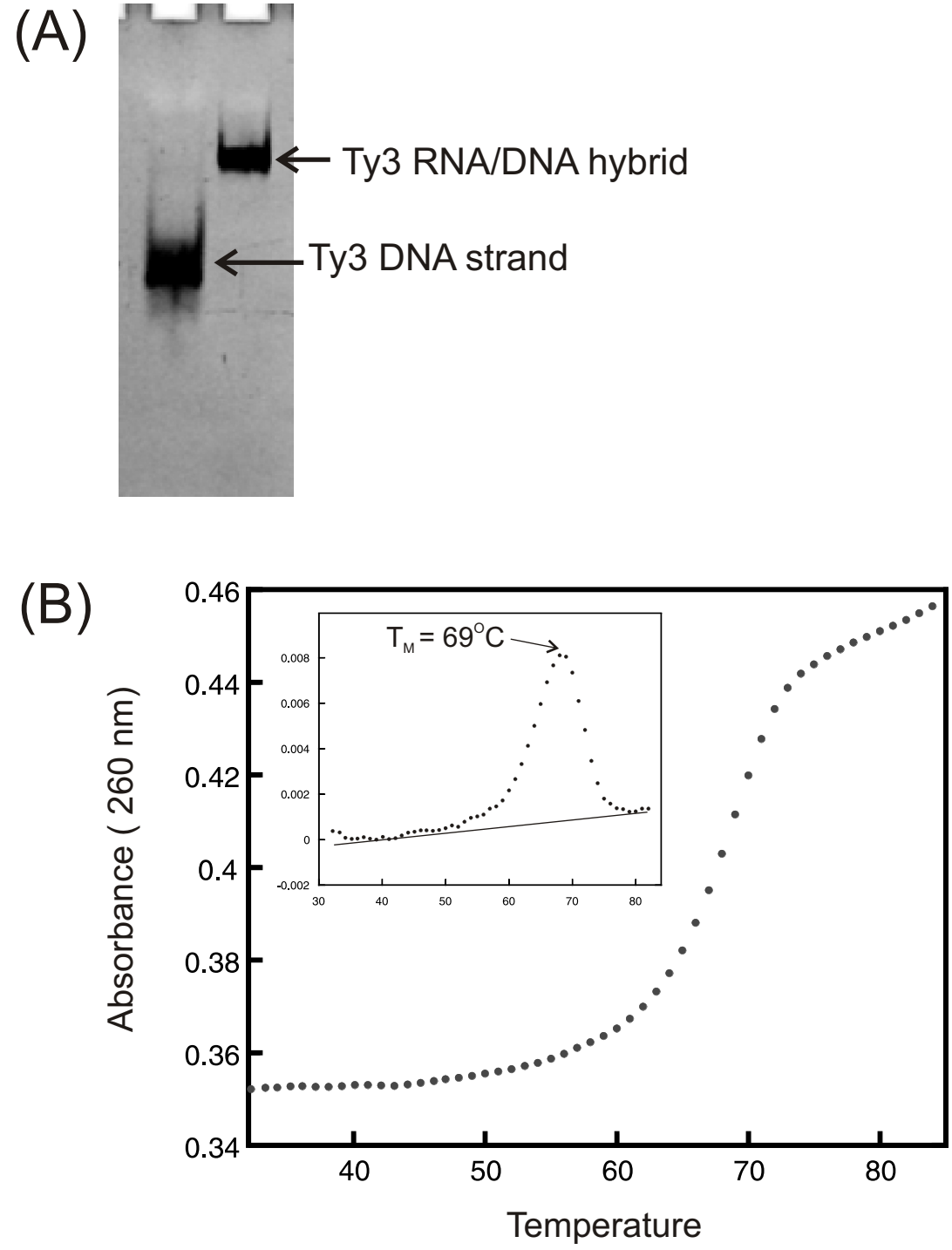
Supporting Figure S2: (A) An expansion of the pyrimidine H5/H6 correlated region of a DQF-COSY experiment applied to the 1.2 mM sample of the 20mer Ty3 RNA/DNA duplex in 99.96% D_2O at 30 ºC. The residual HDO signal was suppressed using 1s presaturation pulse. Positive contours of the COSY cross peaks are shown in blue and negative contours in red. This spectrum was acquired using a Bruker Biospin DMX500 spectrometer equipped with an actively shielded triple-axis gradient, triple resonance TXI probe. The proton carrier frequency was set to 4.704 ppm. The spectrum was acquired with sweep widths of 5,000 Hz in both dimensions, 2K complex points in t_2, 480 complex points in t_1 and 48 scans per increment. The assigned RNA strand H5/H6 cross peaks are indicated by lower case letters and assigned DNA strand H5/H6 cross peaks are indicated in upper case letters. (B) An expansion of the ribose H1' to C1' correlated region of a natural abundance ^{13}C-heteronuclear multiple quantum coherence (HMQC) experiment applied to the 1.2 mM sample of the 20mer Ty3 RNA/DNA hybrid duplex in 99.96% D_2O. This spectrum was acquired using a Bruker Biospin DMX600 spectrometer equipped with an actively shielded triple-axis gradient, triple resonance 600 MHz TXI probe at 30 ºC. The proton carrier frequency was set to 4.754 ppm and the carbon frequency to 108 ppm. The spectrum was acquired with sweep widths of 6,000 Hz in both dimensions, 1K complex points in t_2, 180 complex points in t_1 and 256 scans per increment. The RNA H1'/C1' cross peaks are labeled with assignments.

Supporting Figure S3: An expansion of the anomeric (H1') to aromatic region of a NOESY (τ_{NOE} = 250 ms) experiment applied to the 1.2 mM sample of the 20mer Ty3 RNA/DNA duplex in 99.96% D_2O at 30 ºC. The spectrum was acquired using four-channel Varian Inova 800 MHz spectrometers equipped with an actively shielded Z-axis gradient, triple resonance probe. The residual HDO signal was suppressed using 1s presaturation pulse. The proton carrier frequency was set to 4.725 ppm. The spectrum was acquired with sweep widths of 8,000 Hz in both dimensions, 1K complex points in t_2, 512 complex points in t_1 and 64 scans per increment. The sequential assignment 'walk' for residues a1 through a20 on the RNA strand is indicated by blue lines. Dashed green lines are used to annotate correlations of adenosine H2 protons with 5' sequential, and 3' cross strand anomeric (H1') protons. The H5, H6 cross peaks from the RNA strand pyrimidine residues c3, c4, c5 and u6 are labeled and marked with blue X's. The two observed sets of correlations for the C2 H6 proton are highlighted by a red box and dashed red lines. The position of the sequential cross peak between g19 H1' and a20 H8, which is not observed, is shown by a black X.

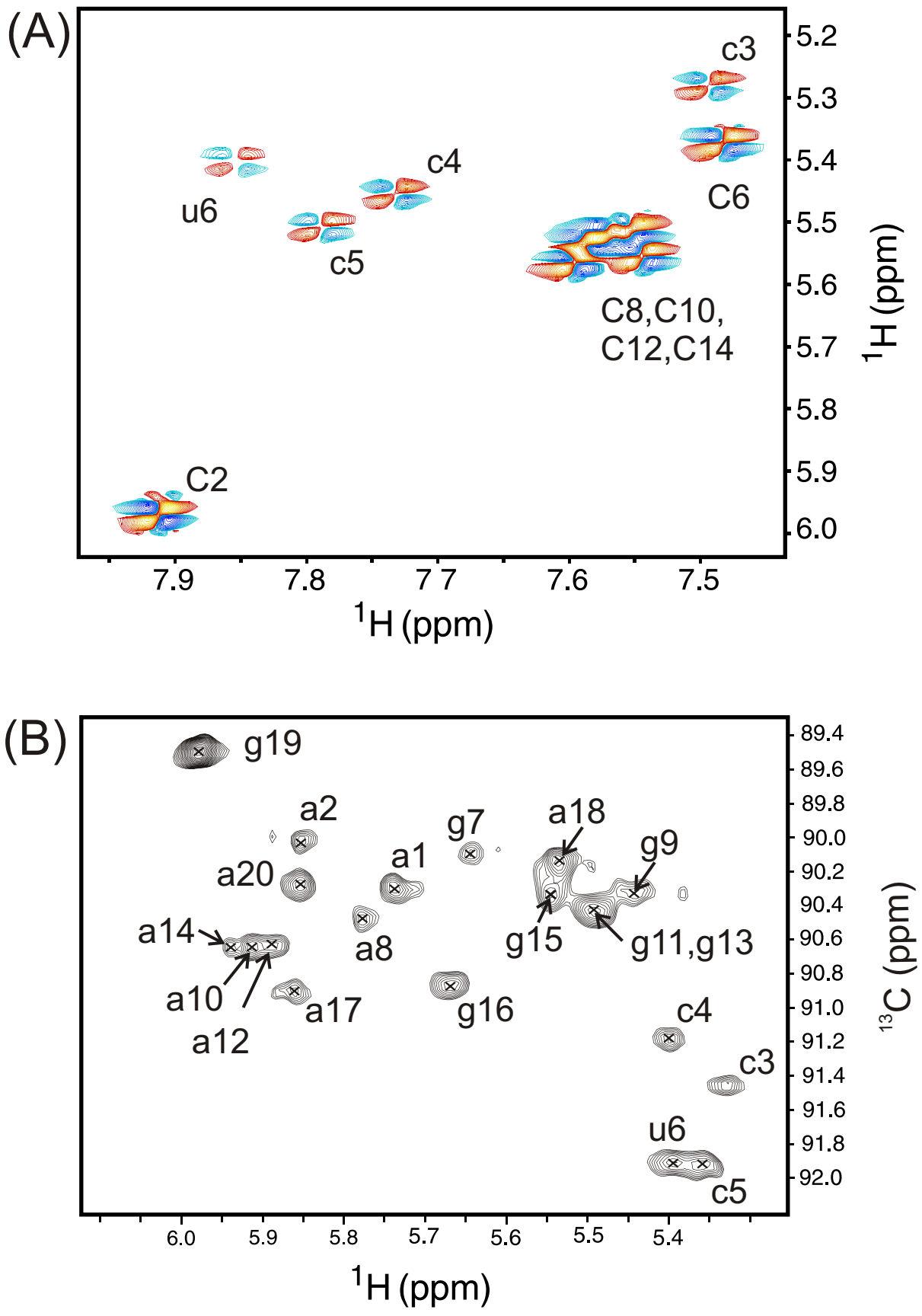
Supporting Figure S4: Structural model of the effect of a sugar pucker switch on the RNA backbone geometry of a RNA/DNA hybrid. The RNA strand aga step at the PPT/U3 junction of the Ty3 sequence is shown with standard A-form geometry (all ribose sugars have a C3-endo conformation) as a blue stick representation. The yellow stick representation of the aga step is modeled with the ribose of residue g(+1) in a C2'-endo sugar pucker conformation and a(-1) and a(+2) riboses in C3'-endo conformations. In both models, backbone 3'-oxygens and phosphates are shown in green and red, respectively. Scissile bond and g(+1) sugar switch are indicated.

1) Delaglio, F., Grzesiek, S., Vuister, G. W., Zhu, G., Pfeifer, J. and Bax, A. (1995) *J. Biomol. NMR*, **6**(3), 277-293.

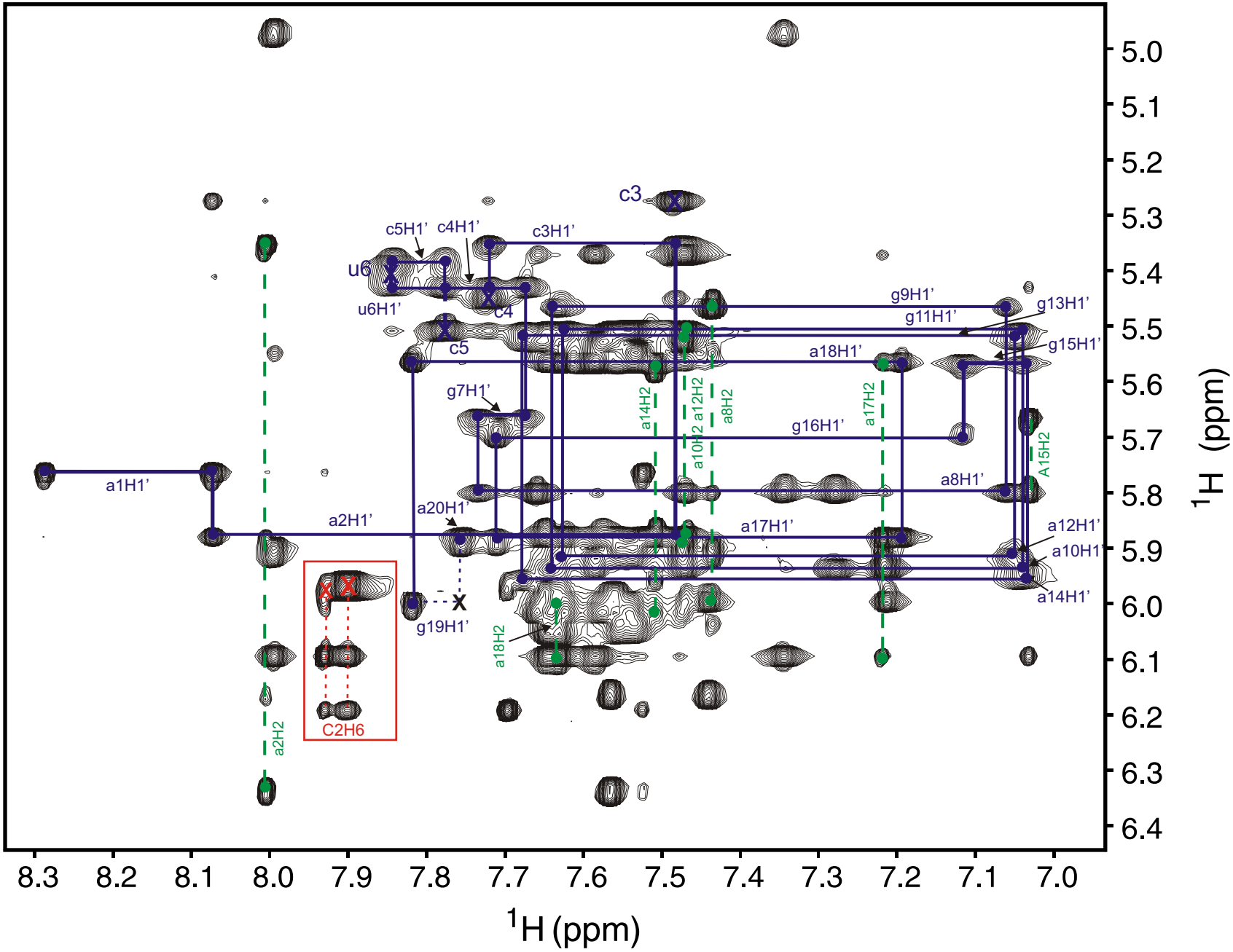
Supporting Figure S1, Yi-Brunozzi et al.:



Supporting Figure S2, Yi-Brunozzi et al.:



Supporting Figure S3, Yi-Brunozzi et al.:



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