Supporting Information (General Section, 2 pages; Supporting Figures 1-5, 5 pages)

Ribose-2'-*F* labeling: A simple tool for the characterization of RNA secondary structure equilibria by ^{19}F-NMR spectroscopy

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General Section

Oligonucleotide synthesis

Cyanoethylphosphoramidites of 2'-*F* adenosine, 2'-*F* cytidine, 2'-*F* guanosine, and 2'-*F* uridine, were obtained from GlenResearch, Sterling, VA. All oligoribonucleosides with site-specific 2'-*F* labels were synthesized based on the 2'-*O*-TOM methodology on CPG supports on a Pharmacia Gene Assembler Special. 2'-*F* modified nucleoside cyanoethylphosphoramidites were coupled using standard protocols.

Detritylation (2.0 min): dichloroacetic acid/1,2-dichloroethane (4/96); coupling (3.0 min): phosphoramidites/acetonitrile (0.1 M x 120 µL) were activated by benzylthiotetrazole/acetonitrile (0.30 M x 360 µL); capping (2 x 0.4 min): A: Ac_2O/*sym*-collidine/acetonitrile (2/3/5), B: 4-(dimethylamino)pyridine/acetonitrile (0.5 M), A/B = 1/1; oxidation (1.0 min): I_2 (10 mM) in acetonitrile/*sym*-collidine/H_2O (10/1/5). Amidite solutions, tetrazole solutions, and aceonitrile were dried over activated molecular sieves overnight. All sequences were synthesized trityl-off.

Deprotection and cleavage of 2'-*F* modified oligonucleotides from solid support were achieved under standard conditions (CH_3NH_2 in EtOH, 8M, 650 µL and CH_3NH_2 in water, 40%, 650 µL; 6 h, room temperature). 2'-*O*-silyl-ethers were removed by treatment with tetrabutylammonium fluoride trihydrate in anhydrous THF (1M, 950 µL) for at least 12 hours at room temperature. After addition of 950 µL triethylammonium acetate buffer (pH 6.8) the volume was reduced to approximately 1 mL and directly applied on a Sephadex G10 column (30 x 1.5 cm). The product was eluted with water and subsequently evaporated to dryness.

Further purification steps were carried out on a semipreparative Dionex DNAPac column at 80 °C (flow rate 2 mL min^{-1}; A 25 mM Tris.HCl, 6 M urea in water, pH 8.0; B 25 mM Tris.HCl, 6 M urea in water, 500 mM sodium perchlorate, pH 8.0). Fractions containing the purified oligonucleotide were desalted by loading on a C18 SepPak cartridge (Waters/Millipore), followed by elution with 100 mM triethylammonium bicarbonate buffer, water, and then water/acetonitrile (1/1, v/v). Combined fractions of the oligonucleotide were lyophilized to dryness. The molecular weights of the synthesized oligoribonucleotides were confirmed by ESI mass spectrometry.

Thermal denaturation studies

Absorbance *versus* temperature profiles were recorded at 250 nm, 260 nm, 270 nm, and 280 nm on a Cary-1 spectrophotometer equipped with a multiple cell holder and a Peltier temperature-control device. Data were collected after a complete cooling and heating cycle at a rate of 0.7 °C/minute. Melting transitions were essentially the same with respect to the four different wavelengths.

Sample preparation:

Oligonucleotides (triethylammonium salts) were lyophilized to dryness, dissolved in the corresponding buffer from stock solutions and subsequently degassed. A layer of silicon oil was placed on the surface of the solution. Values of ΔH^0 and of ΔS^0 for monomolecular melting transitions were derived from a two-state van't Hoff analysis by fitting the shape of the individual α *versus* temperature curve as described.[1,2] Errors for ΔH^0 and ΔS^0 arising from non-infinite cooperativity of two-state transitions and from the assumption of a temperature independent enthalpy are typically 10–15%. Additional error is introduced when free energies are extrapolated far away from the melting transitions; errors for ΔG^0 are typically 3–5%.

NMR spectroscopy

^{1}H-NMR-spectra were recorded on a Varian Inova 500 MHz NMR spectrometer equipped with a 5 mm *Indirect Detection PFG* probe applying a selective excitation refocusing sequence employing selective pulses shaped according to the G4 (excitation;[3] 2.62 ms, rf amplitude 1.74 kHz) or REBURP (refocusing;[4] 1.4 ms, rf amplitude 4.47 kHz) profile, respectively. Both shaped pulses were centered at 13 ppm.

^{19}F-NMR spectra without ^{1}H-decoupling were recorded at a frequency of 470.3 MHz on the same instrumentation (Varian Inova 500 MHz). Typical experimental parameters were chosen as follows: spectral width 7.5 kHz, ^{19}F excitation pulse 12 µs, acquisition time 1 s, relaxation delay 1.5 s, number of scans 2 K. Prior to Fourier transformation all time domain data was processed with a shifted sine bell window function.

^{19}F-NMR spectra with ^{1}H-decoupling were recorded at a frequency of 376.5 MHz on a Bruker Avance DRX 400 MHz WB NMR spectrometer equipped with a 5 mm QNP probe (^{1}H/^{13}C/^{19}F/^{31}P). Typical experimental parameters were chosen as follows: spectral width 7.5 kHz, ^{19}F excitation pulse 18 µs, acquisition time 2 s, relaxation delay 2 s, number of scans 2 K, proton decoupling using Waltz-16 with γB_1 = 1 kHz. Prior to Fourier transformation all time domain data was processed with an exponential window function using a line broadening factor of 2 Hz.

^{19}F-resonances were referenced relative to external CCl_3F.

Sample preparation:

Oligonucleotides (triethylammonium salts) together with the adequate amount of sodium phosphate buffer were lyophilized to dryness, and subsequently dissolved in H_2O/D_2O (9/1, v/v, 500 µL). All samples were heated to 90 °C for two minutes, then rapidly cooled to room temperature and equilibrated for one hour before measurements.

Supporting Material References

1. Marky, L.; Breslauer, K. Calculating thermodynamic data for transitions of any molecularity from equilibrium melting curves. *Biopolymers* **1987**, 26, 1601-1620.

2. Xia, T.; Mathews, D. H.; Turner, D. H. Thermodynamics of RNA secondary structure formation. In *Comprehensive Natural Product Chemistry*, Vol. 8 (Söll, D., Nishimura, S. & Moore, P., eds), Elsevier, Oxford, **1999**, pp. 21-47.

3. Emsley, L.; Bodenhausen, G. Gaussian pulse cascades: new analytical functions for rectangular selective inversion and in-phase excitation in NMR. *Chem. Phys. Lett*. **1990**, *165*, 469-476.

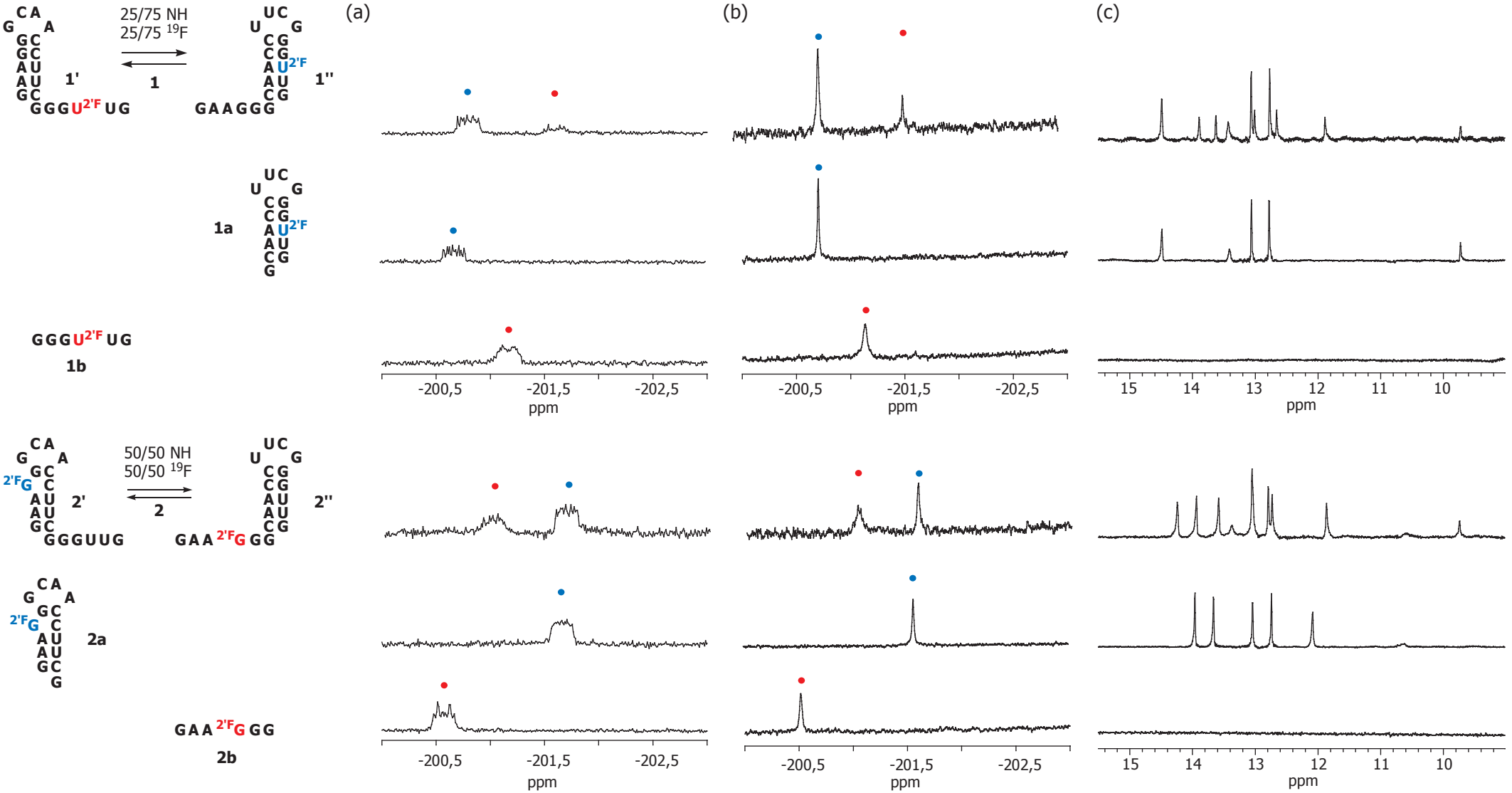
4. Geen, H.; Freeman, R. Band-selective radiofrequency pulses. *J. Magn. Reson*. **1991**, *93*, 93-141.

5. Deng, J.; Xiong, Y.; Sundaralingam, M. X-ray analysis of an RNA tetraplex $(UGGGGU)_4$ with divalent Sr^{2+} ions at subatomic resolution (0.61 Å). *Proc. Nat. Acad. Sci. USA* **2001**, *98*, 13665-13670.

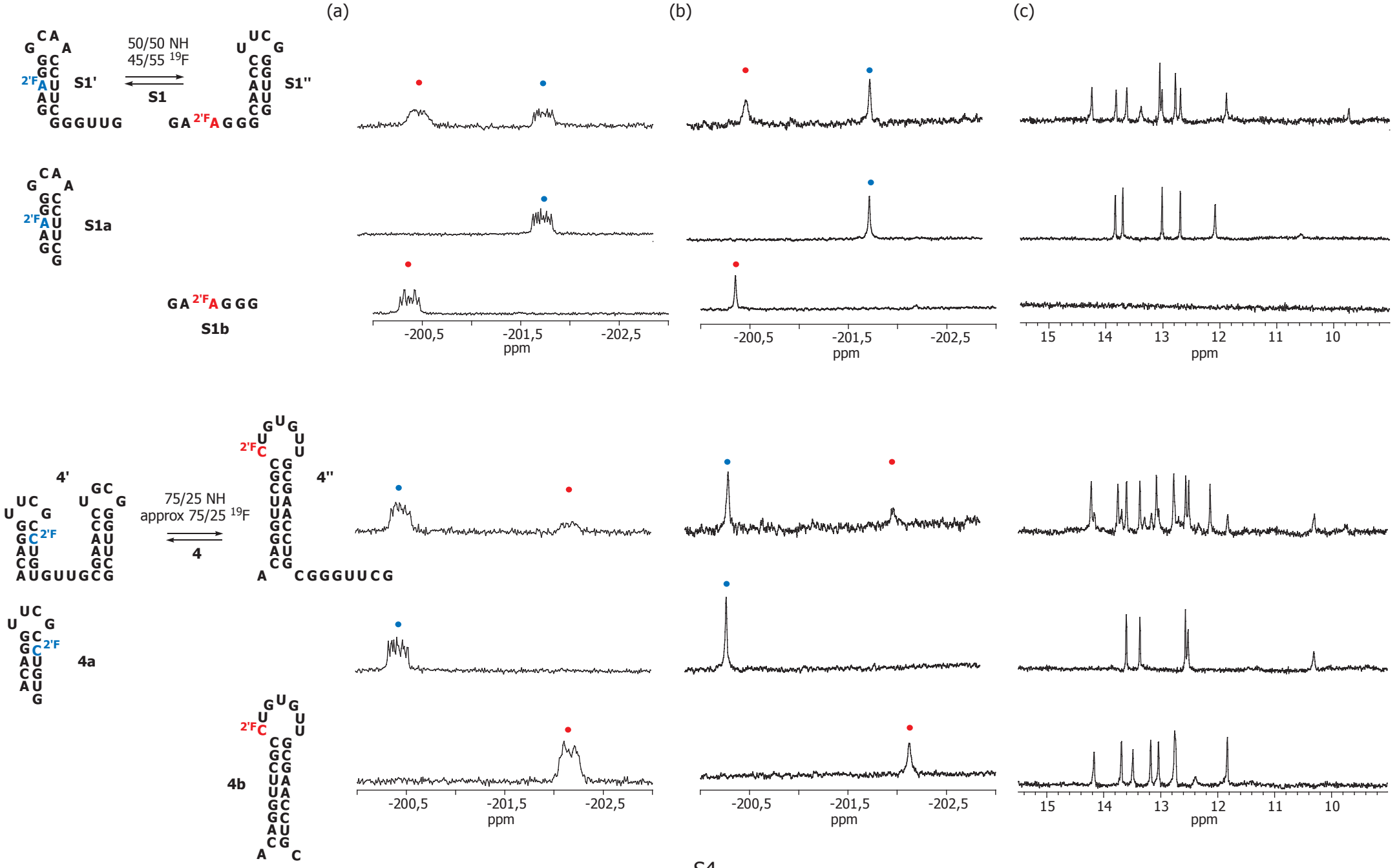
6. Pan, B.; Xiong, Y.; Shi, K.; Sundaralingam, M. An eight-stranded helical fragment in RNA crystal structure: implications for tetraplex interaction. *Structure* **2003**, *11*, 825-831.

7. Pan, B.; Xiong, Y.; Shi, K.; Deng, J.; Sundaralingam, M. Crystal structure of an RNA purine-rich tetraplex containing adenine tetrades: implications for specific binding in RNA tetraplexes. *Structure* **2003**, *11*, 815-823.

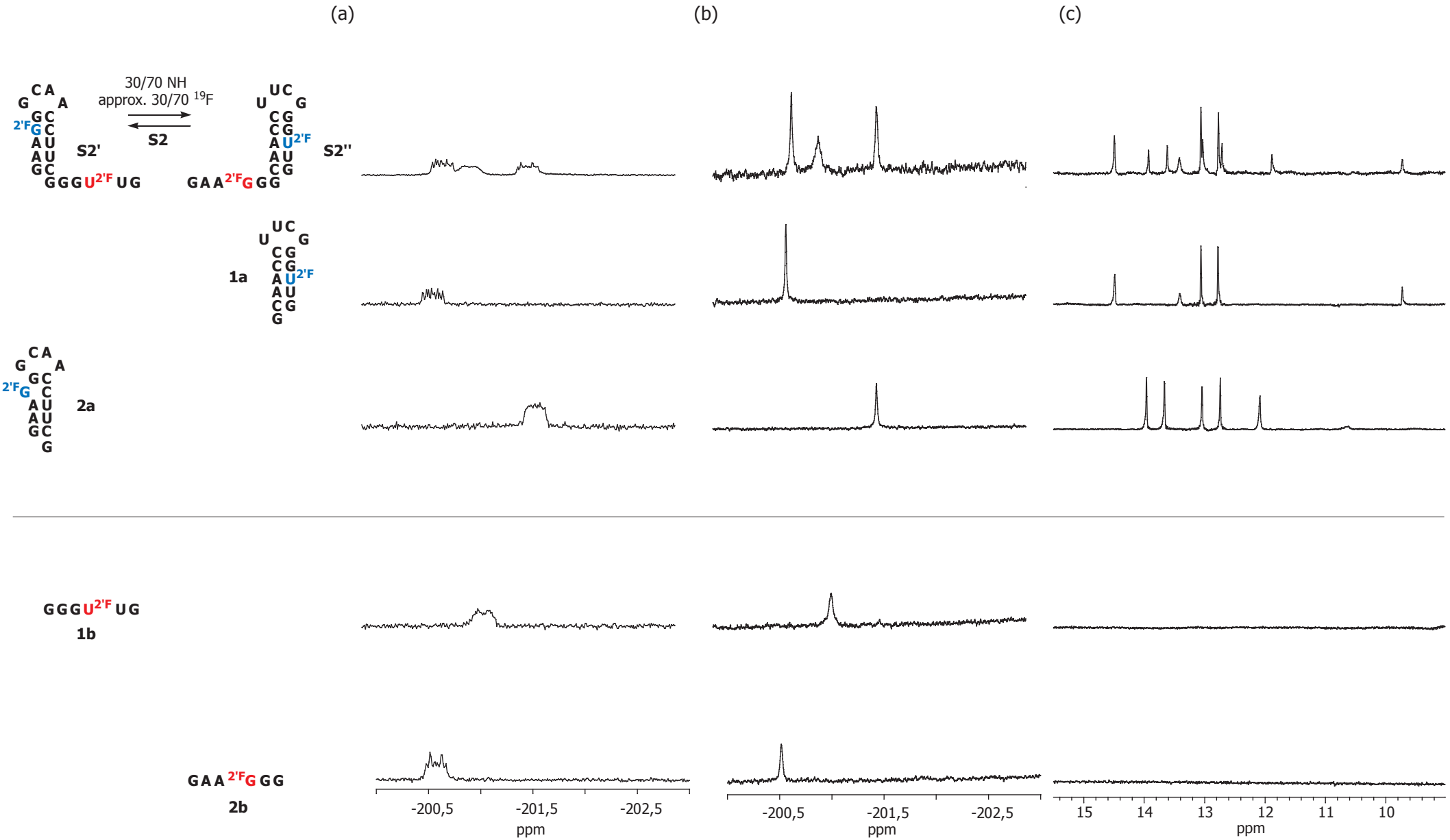
Supporting Figure 1. ^{19}F- and ^{1}H-NMR spectra of bistable RNAs **1** and **2** and of the corresponding reference RNAs **1a**, **1b**, **2a**, and **2b**; (a) ^{19}F-NMR-spectra without ^{1}H-decoupling; (b) ^{1}H-decoupled ^{19}F-NMR spectra; (c) ^{1}H-NMR NH spectra; conditions: 0.3 mM RNA, 25 mM sodium phosphate buffer, pH 7.0, 298 K.



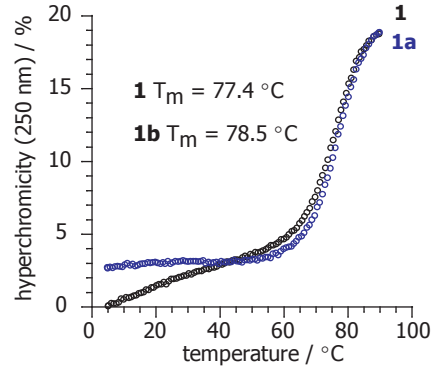
Supporting Figure 2. ^{19}F- and ^{1}H-NMR spectra of bistable RNAs **S1** and **4** and of the corresponding reference RNAs **S1a**, **S1b**, **4a**, and **4b**; (a) ^{19}F-NMR-spectra without ^{1}H-decoupling; (b) ^{1}H-decoupled ^{19}F-NMR spectra; (c) ^{1}H-NMR NH spectra; conditions: 0.3 mM RNA, 25 mM sodium phosphate buffer, pH 7.0, 298 K.

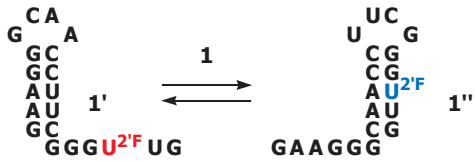


Supporting Figure 3. ^{19}F- and ^{1}H-NMR spectra of bistable RNAs **S2** and of the corresponding reference RNAs **1a**, **1b**, **2a**, and **2b**; (a) ^{19}F-NMR-spectra without ^{1}H-decoupling; (b) ^{1}H-decoupled ^{19}F-NMR spectra; (c) ^{1}H-NMR NH spectra; conditions: 0.3 mM RNA, 25 mM sodium phosphate buffer, pH 7.0, 298 K.



Supporting Figure 4. Thermal denaturation studies and thermodynamic analysis of the bistable RNAs **1**, **2**, **3**, and **S1**, and of the corresponding reference hairpins **1a**, **2a**, **3a**, **3b**, and **S1a**; conditions: 2 μM RNA, 150 mM NaCl, 10 mM sodium phosphate buffer, pH 7.0.





Tm= 78.5 °C
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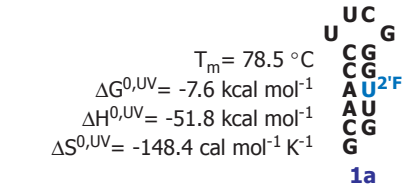
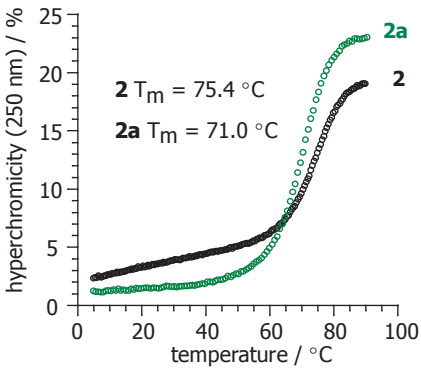
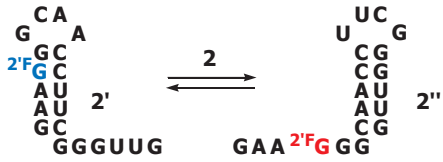
ΔH0,UV= -51.8 kcal mol-1
ΔS0,UV= -148.4 cal mol-1 K-1







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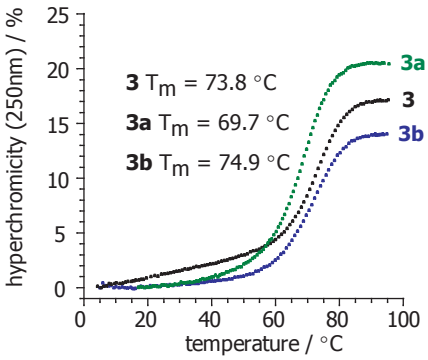
ΔS0,UV= -150.8 cal mol-1 K-1



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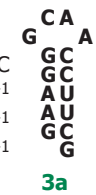
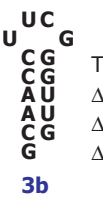
ΔS0,UV= -128.7 cal mol-1 K-1





Tm= 74.9 °C
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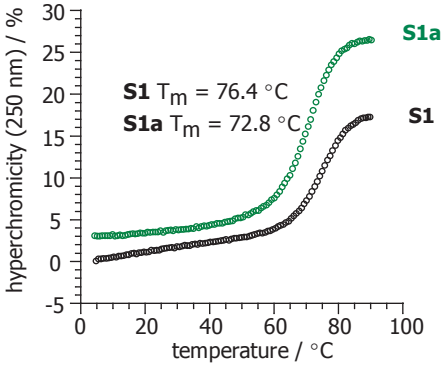
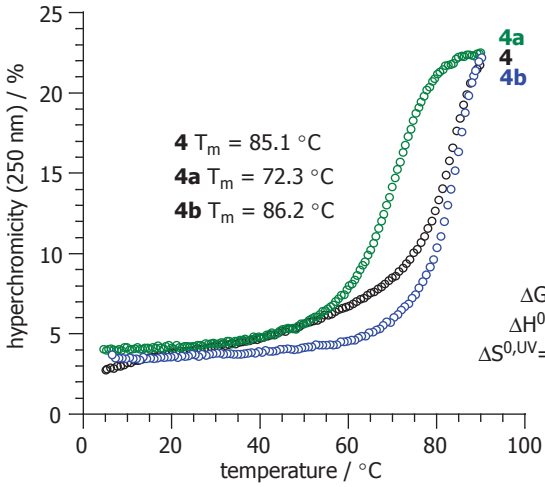
ΔS0,UV= -142.0 cal mol-1 K-1

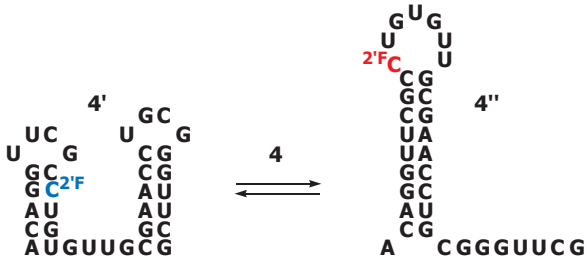


Tm= 72.8 °C
ΔG0,UV= -6.3 kcal mol-1
ΔH0,UV= -48.1 kcal mol-1
ΔS0,UV= -140.3 cal mol-1 K-1

Supporting Figure 5. Thermal denaturation studies and thermodynamic analysis of (a) the bistable RNA **4** and of the corresponding reference hairpins **4a** and **4b** (conditions: 2 μM RNA each; 150 mM NaCl, 10 mM sodium phosphate buffer, pH 7.0); (b) thermal denaturation studies of the 6 nt 2'-*F*-labeled sequence **1b** (conditions: 40 μM, 75 μM, and 100 μM RNA; 1 M NaCl, 10 mM sodium phosphate, pH 7.0); the hysteresis is indicative for a higher order structure such as a tetraplex (compare structures of rUGGGGU, rU(dG)AGGU, and dUrGAGGU).[5,6,7]

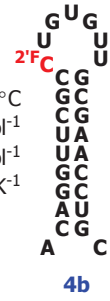
(a)





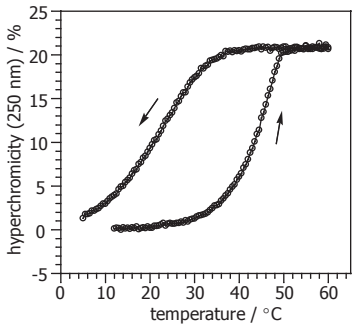
T_m= 72.3 °C
$\Delta G^{0,UV}$= -6.8 kcal mol^{-1}
$\Delta H^{0,UV}$= -52.7 kcal mol^{-1}
$\Delta S^{0,UV}$= -154.0 cal mol^{-1} K^{-1}

T_m= 86.2 °C
$\Delta G^{0,UV}$= -9.3 kcal mol^{-1}
$\Delta H^{0,UV}$= -58.1 kcal mol^{-1}
$\Delta S^{0,UV}$= -163.8 cal mol^{-1} K^{-1}

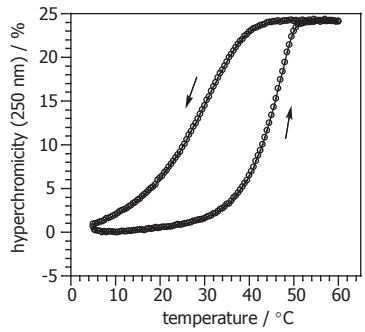


(b)

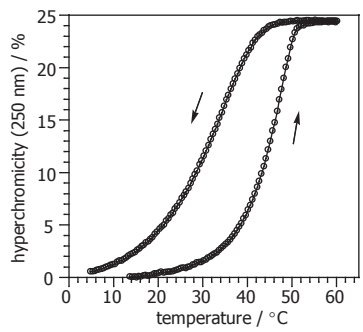
GGGU$^{2'F}$UG (1b)



c_T = 40 μM
T_m (heating) = 44.6 °C
T_m (cooling) = 23.1 °C



c_T = 75 μM
T_m (heating) = 45.5 °C
T_m (cooling) = 30.5 °C



c_T = 100 μM
T_m (heating) = 46.0 °C
T_m (cooling) = 34.1 °C