Supporting information:

^{13}C-direct detected NMR experiments for the sequential J-based resonance assignment of RNA oligonucleotides

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Figure S1: Effect of temperature on S/N of the (H)CC-TOCSY-H1'C1' experiment:

Figure S1 shows the (H)CC-TOCSY-H1'C1' experiment running on the 14mer RNA sample at 298K in comparison with spectra recorded at 278K. The correlation time at T=298K has been determined previously. The correlation time at T=278K has been calculated using hydronmr. The experimental S/N-ratios are indicated. The experiment at T=278K has been recorded with factor 2 more scans that the experiment at T=298K.

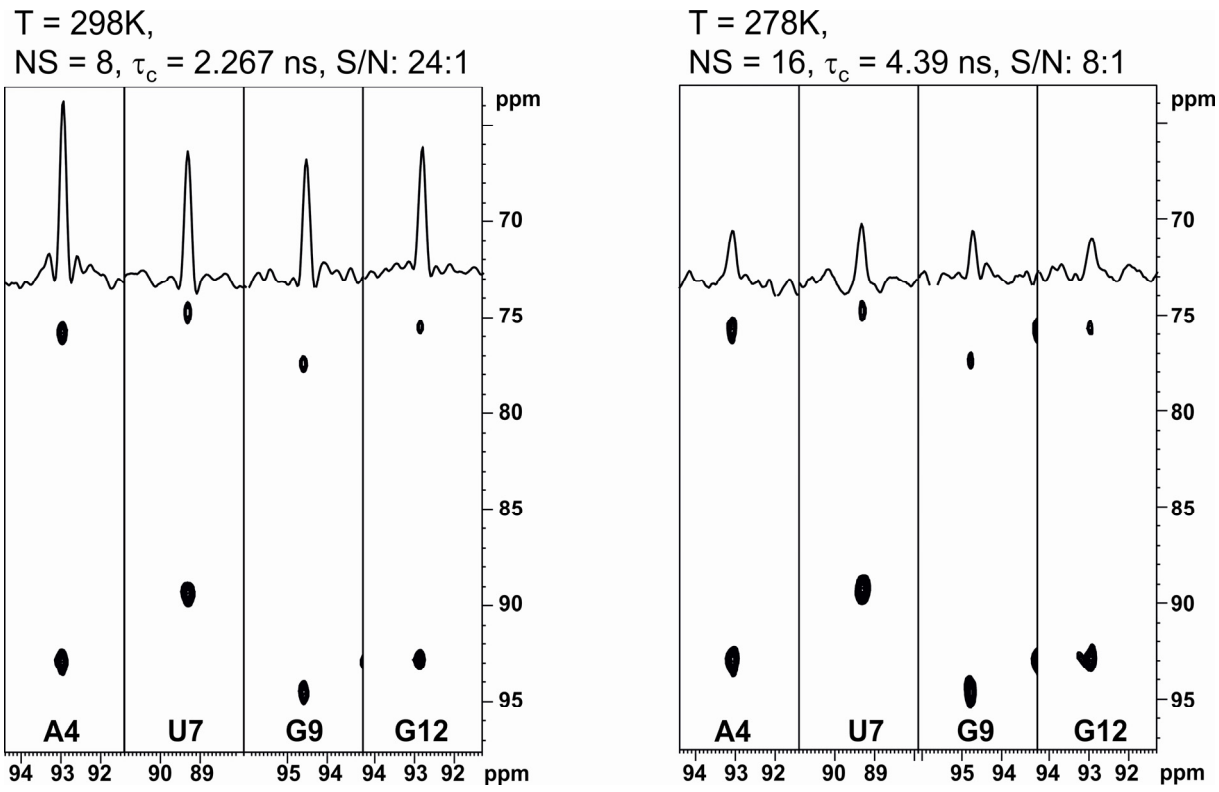

Figure S1. *2D strips of the 3D (H)CC-TOCSY-H1'C1' experiment for the uniformly $^{13}C,^{15}N$-labelled 14mer RNA with a CC-TOCSY mixing period of τ_M=3 ms (left) for 298 K and (right) 278 K, NS= 16. All experiments were recorded at a field strength of 14.4 T (600 MHz ^{1}H frequency) using a cryogenic probe with cooled carbon preamplifier. The rf-field strengths for ^{1}H and ^{13}C pulses were 22.7 and 20.3 kHz, respectively. During acquisition, GARP decoupling was applied at field strengths of 3.6 kHz and 1 kHz for ^{1}H and ^{15}N, respectively. The FLOPSY mixing sequence was applied at a field strength of 8.3 kHz. All 3D NMR experiments were recorded with 8 (16) scans over a period of 17 h (36 h) with 28, 28 and 1k complex points in t_1, t_2 and t_3. The acquisition time was set to 204 ms, t_2^{max} was 28 ms and t_1^{max} was 5.3 ms. A relaxation delay of 0.95 s was used.*

Figure S2: Effect of molecular weight and size on S/N of the (H)CC-TOCSY-H1'C1' experiment:

We applied the (H)CC-TOCSY-H1'C1' experiment on the 2'-deoxyguanosine-dependent riboswitch (dGswitch), a 70mer RNA (Figure S2 A), results are shown in Figure S2. For the riboswitch RNA, the experiment starts with direct carbon excitation and for the detection, the IPAP step has been removed.

Figure S2 B shows the standard proton detected [^{13}C, ^{1}H]-2D HSQC. In this experiment, real time evolution is utilized and homonuclear ^{1}J(C,C) couplings evolve in the t_1. By using a constant time (CT) evolution period, the effect of ^{1}J(C,C) coupling can be suppressed. The required additional 25 ms delay τ ($\tau=1/^{1}$J(C,C)) dramatically decreased the sensitivity of the ^{1}H,^{13}C correlation. In the CT-HSQC obtained with 32 scans (factor 16 more than the real time experiment, data not shown), weak signals are still missing. However, for the selectively ^{13}C, ^{15}N-cytidine-labelled dGswitch RNA, the resolution in the real time HSQC is sufficient to detect 20 C1'H1' correlations: 18 cytidine signals from the dGswitsch, 2 residues from the bound and unbound ^{13}C, ^{15}N-labelled 2'-deoxyguanosine.

Figure S2 C and D show the results for the carbon detected 3D CC-TOCSY-C1'H1' experiment. Figure S2 C presents the 2D [^{1}H, ^{13}C] positive projection of the 3D experiment and Figure S2 D shows 2D [^{13}C, ^{13}C]-strips of the 3D experiment. A slight increase in sensitivity can be obtained by starting directly on the carbon coherence. The comparison of the 2D [^{13}C, ^{1}H] HSQC (Figure S2 B) with the 2D [^{1}H, ^{13}C] positive projection (Figure S2 C) shows that all possible diagonal signals can be observed in the 3D experiment with a reasonable sensitivity. Removing of the 12.5 ms IPAP step increases the sensitivity comparable to the differences of HSQC vs. CT-HSQC for larger RNAs. For the most signals in the selectively labelled dGswitch RNA the multiplet splitting due to J(C,C) in the direct carbon dimension are less problematic as the resolution for the strips is mainly based on the proton resolution.

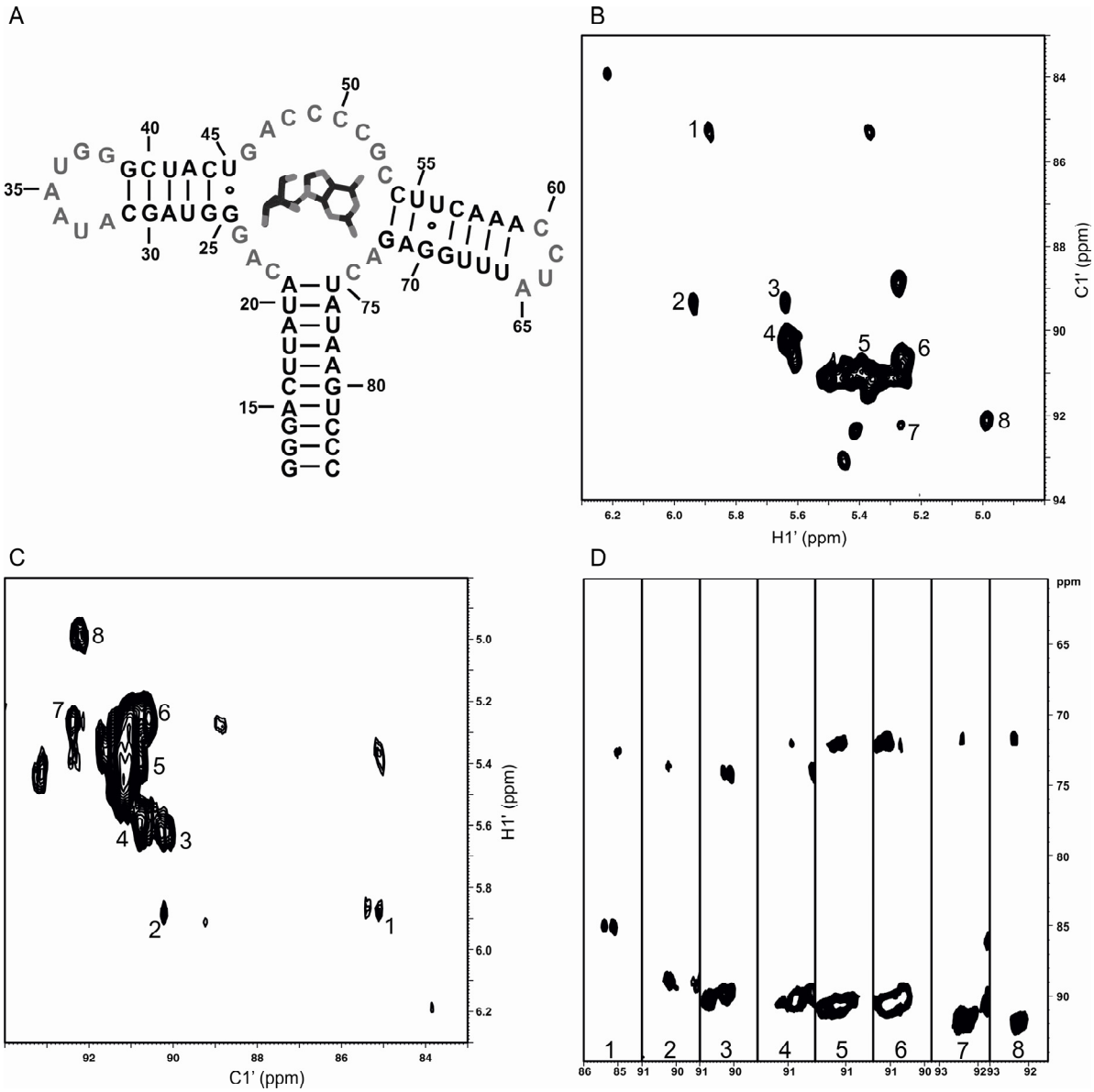

Figure S2. *A: Secondary structure of the aptamer domain of a 2'-deoxyguanosine-dependent riboswitch RNA (dGswitch). B: proton detected standard 2D [^{13}C, ^{1}H]-HSQC experiment with real time evolution in t_1 of ^{13}C, ^{15}N-cytidine labelled 70mer dGswitch recorded with 2 scans and 128 complex points in t_1. The numbers indicate the peaks which were used later for the 2D strips. C: 2D positive projection of the carbon direct detected 3D CC-TOCSY-H1'C1' experiment. D: 2D strips of the carbon direct detected 3D CC-TOCSY-H1'C1' experiment for the selectively ^{13}C, ^{15}N-cytidine labelled 70mer dGswitch with a CC-TOCSY mixing period of τ_M=3 ms at 298 K. All experiments were recorded at a field strength of 22.3 T (950 MHz ^{1}H frequency) using a triple resonance cryogenic probe with ^{1}H, ^{13}C and ^{15}N. The field strengths for ^{1}H and ^{13}C pulses were 19.6 and 23.8 kHz respectively. During acquisition, GARP decoupling was applied at field strengths of 3.6 kHz and 1 kHz for ^{1}H and ^{15}N, respectively. The FLOPSY mixing sequence was applied at a field strength of 8.3 kHz. The 3D NMR experiments were recorded with 128 scans in 50 h with 16, 16 and 1k complex points in t_1, t_2 and t_3. The acquisition time was set to 100 ms, t_2^{max} was 11.2 ms and t_1^{max} was 1.92 ms. A relaxation delay of 1s was used. Direct start with carbon coherence yields spectra with slightly better signal-to-noise. In order to reduce signal-to-noise loss due to time of transversal magnetization (T_2-relaxation), we removed the 12.5 ms IPAP scheme from the pulse sequence and detected directly after the proton refocusing delay.*