Measurement of ^{1}H-^{15}N and ^{1}H-^{13}C residual dipolar couplings in nucleic acids from TROSY intensities

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Supporting Information

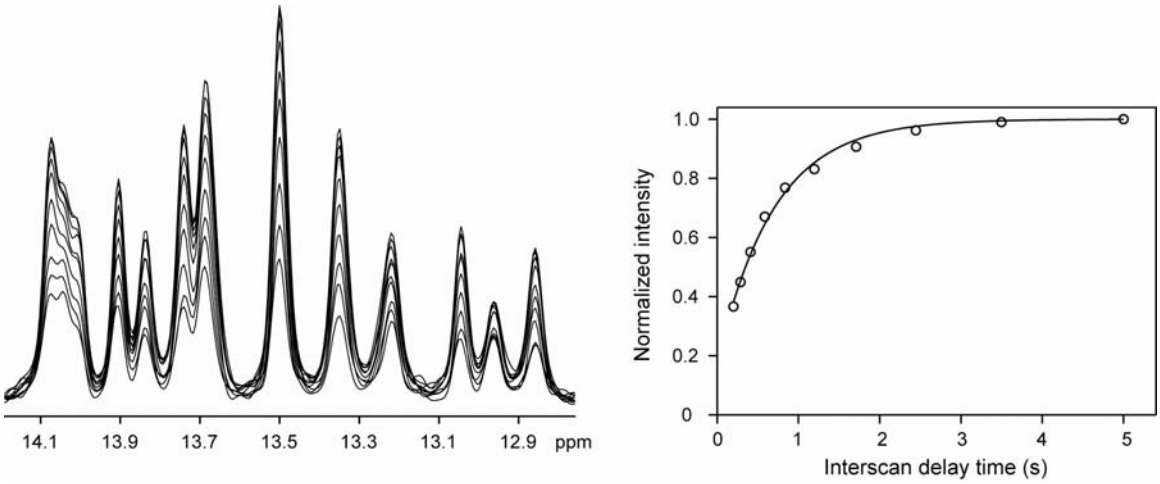

Fig. S1 Recovery of representative imino proton magnetization as a function of interscan delay in the N-H ARTSY experiment of Fig. 1. The imino regions of the 1D spectra shown (left) correspond to the first FID of the 900 MHz ARTSY reference experiment, and have been recorded for interscan delays ranging from 0.2-5 s. Recovery of the overlapping ^{1}H resonances is not fully exponential, indicating a range of recovery rates but can be fitted with $I(t) = I_{eq}[1 - 0.83\exp(-t/0.69s)]$ (right panel).

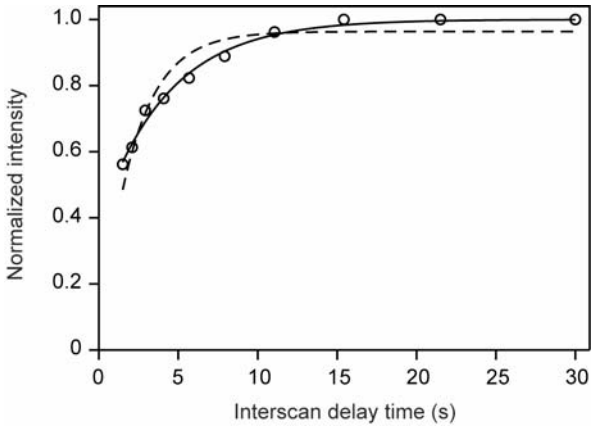

Fig. S2 Recovery curve of H_2, H_6 and H_8 magnetization as a function of interscan delay in the C-H ARTSY experiment of Fig. 3 for riboA in ~7 mg/mL Pf1 in D_2O, with normalized intensity obtained from the first FID of the 900 MHz ARTSY reference experiment, recorded for interscan delays ranging from 1.5-30 s. Note that for a sample in D_2O, the band selective ^{1}H pulses are unnecessary, but the same pulse sequence used for the H_2O sample in Fig. 3 was still used for generating this recovery profile. Recovery of the overlapping ^{1}H resonances is not fully exponential, indicating a substantial range of recovery rates which can be fitted with $I(t) = I_{eq}[1 - 0.61\exp(-t/4.28s)]$ (solid line). Assuming a single exponential recovery process, the same data is best fitted with $I(t) = 0.96I_{eq}[1 - \exp(-t/2.15s)]$, reflecting contributions from rapidly relaxing internally mobile nucleotides.

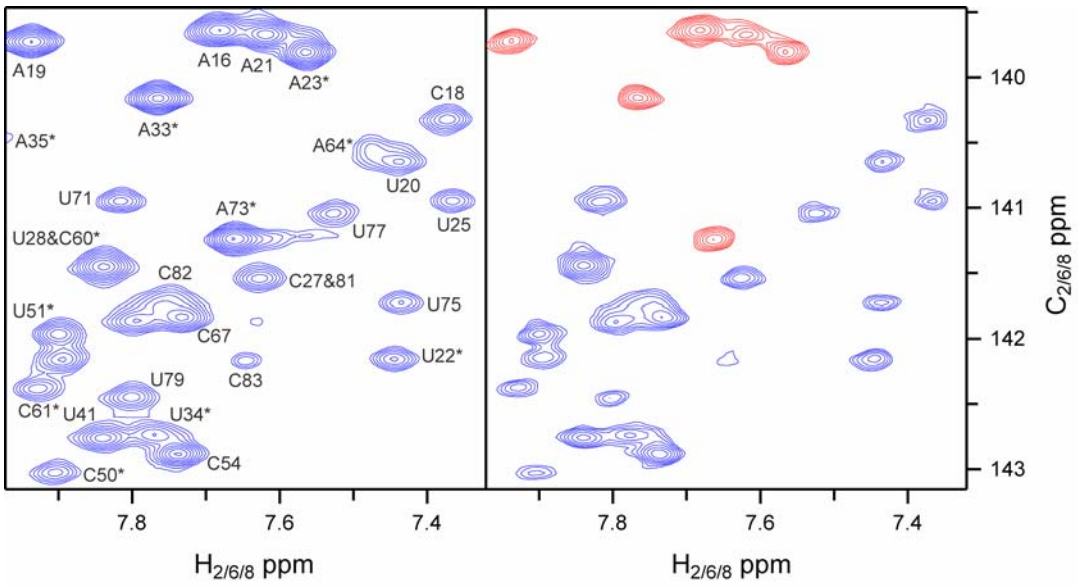

Fig. S3 Small region of the reference (left) and attenuated (right) ARTSY spectra of the $^{13}C_{6,8}$-^{1}H moieties in isotropic RiboA, recorded at 900 MHz ^{1}H frequency with an ARTSY dephasing time T = 5.1 ms. See Fig. 5 for data acquisition parameters. Contour levels for the attenuated spectrum are at a level 4 times lower than shown for the reference spectrum. Assignments marked with asterisks are tentative and correspond to nucleotides outside the helical stem regions.

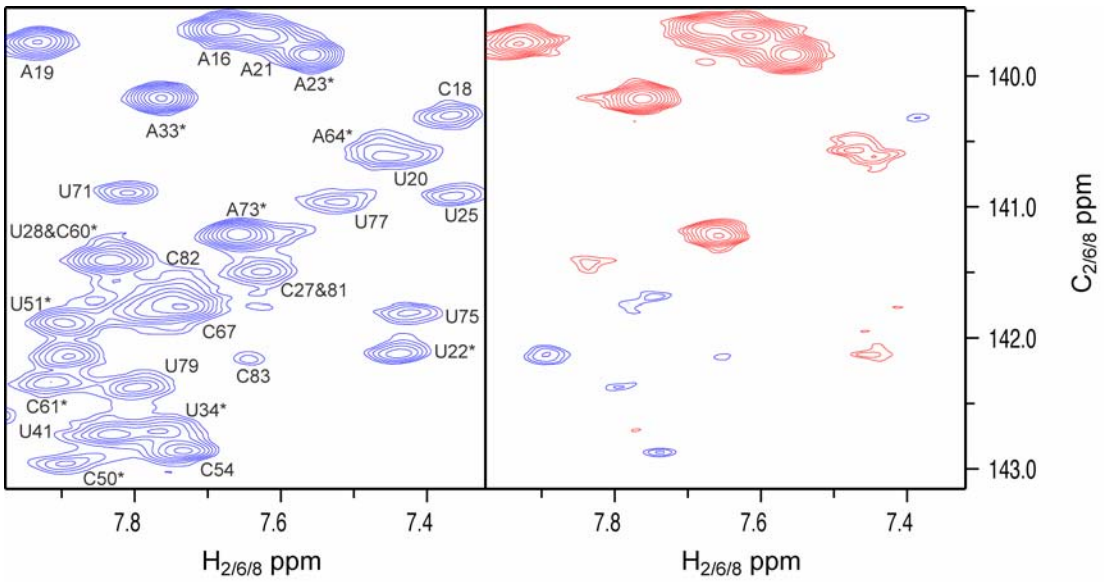

Fig. S4 Small region of the reference (left) and attenuated (right) ARTSY spectra of the $^{13}C_{6,8}$-^{1}H moieties in RiboA, recorded at 747 MHz ^{1}H frequency in the presence of 10 mg/mL Pf1. Spectra were recorded with 16 scans per FID, and an interscan delay of 1.5 s, and the total matrix for the interleaved set of spectra comprised 2 × 350* × 1024* datapoints, with N* referring to N complex data points, for acquisition times of 70 ms (t_1) and 42.9 ms (t_2). The delay T (Fig. 3) was set to 4.9 ms. Contour levels for the attenuated spectrum are at a level 5 times lower than shown for the reference spectrum. Assignments marked with asterisks are tentative and correspond to nucleotides outside the helical stem regions. The total measuring time for the two interleaved spectra was ca 10.5 h.

Table S1. $^1D_{CH}$ values (Hz) derived using C-H ARTSY for C_2, C_6, and C_8 of helical residues in RiboA measured using different dephasing times at 900 MHz (on a cryoprobe) as well as a measurement at 747 MHz (on a room-temperature probe), and the corresponding uncertainty (Hz) propagated from the spectral S/N. Helical residues whose resonances have not yet been assigned or whose RDCs cannot be determined accurately due to resonance overlap are excluded.

		$^1D_{CH,artsy,900}$								$^1D_{CH,artsy,747}$		avg[b]	std
		4.5m[a]	err	4.7m[a]	err	4.9m[a]	err	5.0m[a]	err	4.9m[a]	err		
A16	C2	19.9	0.6	20.3	0.5	20.1	0.5	19.8	0.7	20.0	0.7	20.0	0.2
A17	C2	11.8	0.5	11.8	0.5	12.8	0.5	11.6	0.7	12.9	0.6	12.2	0.6
A19	C2	15.8	0.7	16.2	0.6	16.0	0.6	15.4	0.9	17.7	0.7	16.2	0.9
A29	C2	39.4	1.0	39.6	1.0	39.6	1.0	40.5	1.3	39.6	0.8	39.7	0.4
A30	C2	35.1	0.6	34.7	0.6	35.2	0.6	34.1	0.9	32.3	0.8	34.3	1.2
A45	C2	35.1	0.7	34.3	0.6	33.3	0.6	33.5	1.0	33.4	0.8	33.9	0.7
A55	C2	8.0	1.6	7.4	1.3	5.4	1.3	9.6	1.7	7.1	0.9	7.5	1.5
A56	C2	19.7	0.6	19.7	0.5	19.4	0.5	19.7	0.7	18.9	0.7	19.5	0.3
A58	C2	44.6	0.7	43.0	0.7	43.1	0.7	43.7	1.1	42.6	0.9	43.4	0.8
A76	C2	40.3	1.0	41.5	1.0	41.9	1.0	40.2	1.7	41.9	1.3	41.1	0.8
A16	C8	17.6	1.0	16.6	1.0	17.1	1.0	18.1	1.4	17.8	1.6	17.4	0.6
A17	C8	17.3[c]	0.9	15.9[c]	0.9	13.3[c]	0.9	11.5[c]	1.2	17.7[c]	1.5	15.2[c]	2.7
A19	C8	26.6	1.1	27.3	1.0	26.5	1.1	26.3	1.6	28.6	1.9	27.0	0.9
A21	C8	44.6	1.3	42.2	1.2	42.1	1.3	42.2	2.0	43.6	2.2	43.0	1.1
A29	C8	29.9	1.1	30.4	1.1	29.4	1.2	28.9	1.7	30.3	2.2	29.8	0.6
A30	C8	14.1	1.0	12.6	0.9	12.2	0.9	12.6	1.3	13.1	1.7	12.9	0.7
A45	C8	25.9	1.0	25.1	0.9	25.5	1.0	24.3	1.4	28.4	1.7	25.9	1.5
A55	C8	23.7	1.0	23.8	0.9	23.2	1.0	23.2	1.4	25.6	1.7	23.9	1.0
A56	C8	38.6	1.5	39.1	1.4	40.2	1.5	38.6	2.3	37.7	2.7	38.9	0.9
A58	C8	39.9	0.7	40.6	0.7	39.3	0.7	40.1	1.2	40.4	1.5	40.1	0.5
A76	C8	39.8	1.9	46.1	1.8	38.6	1.8	38.9	3.0	44.6	3.1	41.6	3.5
G14	C8	24.1	3.0	19.8	2.9	16.1	2.8	19.7	4.3	22.9	4.9	20.5	3.1
G15	C8	19.9	1.4	20.0	1.3	18.9	1.4	21.8	2.0	19.4	2.4	20.0	1.1
G43	C8	38.1	1.0	39.1	1.0	37.8	1.1	36.0	1.6	38.6	2.1	37.9	1.2
G44	C8	34.8	1.0	34.5	0.9	36.8	1.0	33.8	1.4	31.1	1.7	34.2	2.0
G57	C8	43.6	1.4	44.7	1.4	44.2	1.6	41.0	2.4	46.6	3.0	44.0	2.0
G59	C8	22.0	1.6	18.9	1.5	19.4	1.5	19.7	2.0	21.3	1.7	20.3	1.3
G72	C8	12.2	1.2	12.4	1.1	14.6	1.2	14.1	1.6	10.1	2.1	12.7	1.8
G78	C8	25.5	1.1	26.0	1.0	24.4	1.1	26.2	1.6	25.4	1.9	25.5	0.7
U20	C6	38.8[c]	2.5	47.7[c]	2.3	43.7[c]	2.2	48.1[c]	3.3	25.4[c]	1.9	40.7[c]	9.4
U25	C6	22.7	3.2	26.5	2.7	22.5	2.7	32.8	3.6	35.4	3.6	28.0	5.9
U31	C6	10.9	2.5	10.9	2.2	7.3	2.1	10.0	3.0	10.8	3.0	10.0	1.5
U39	C6	14.0	2.2	13.2	1.9	14.6	1.8	12.0	2.6	13.8	2.4	13.5	1.0
U40	C6	27.0	2.5	24.4	2.3	21.1	2.2	26.4	3.2	24.0	2.9	24.6	2.4
U41	C6	26.6	1.9	26.8	1.6	30.0	1.6	27.6	2.2	23.4	2.2	26.9	2.4
U71	C6	32.6	2.5	30.3	2.2	36.7	2.2	34.4	3.0	37.5	2.9	34.3	2.9
U75	C6	42.2	3.0	42.9	2.7	40.7	2.8	40.6	4.0	45.2	4.1	42.3	1.9
U77	C6	33.9	2.9	28.7	2.7	34.8	2.6	32.0	3.5	31.8	3.6	32.2	2.4

U79	C6	11.4	2.5	10.5	2.1	8.3	2.1	6.6	2.9	16.4	2.8	10.6	3.7
U80	C6	16.0	2.4	13.8	2.1	16.1	2.0	17.1	2.7	14.5	3.0	15.5	1.3
C18	C6	20.4	2.3	19.5	2.1	16.4	2.0	16.9	2.8	14.3	3.0	17.5	2.5
C54	C6	17.4	1.4	18.5	1.2	19.8	1.2	18.8	1.7	17.5	3.0	18.4	1.0
C67	C6	21.5	1.5	22.1	1.3	19.5	1.2	17.4	1.7	19.2	3.0	20.0	1.9
C69	C6	39.4	2.6	42.0	2.3	43.0	2.3	36.4	3.3	43.5	3.0	40.9	3.0
C83	C6	15.5	4.2	15.2	3.6	12.6	3.6	11.1	4.5	7.1	3.0	12.3	3.4

[a] ARTSY dephasing time T in ms used for determining $^{1}J_{CH} + {}^{1}D_{CH}$.

[b] Average of all 5 sets of RDCs with an equal weight for each set of data. Averaged RDCs were used for optimizing the helix 1 orientation relative to the remainder of the structure.

[c] U20 C_6-H_6, and A64 C_8-H_8 correlations partially overlap; A17 C_8-H_8 partially overlaps with an unassigned resonance.

Table S2. $^1D_{CH}$ values (Hz) measured for C_5 of helical residues in RiboA, using C_5-H_5 (at 747 MHz on a room-temperature probe) or C_5-H_6 (at 600 MHz on a cryoprobe) correlation spectra, as well as the corresponding uncertainty propagated from the spectral S/N. Helical residues whose resonance has not been assigned or whose RDCs cannot be determined accurately due to resonance overlap are excluded.

	$^1D_{CH,artsy}$, H_5 detected				$^1D_{CH,artsy}$, H_6 detected				weighted average[c]	err
	H_2O at 747		D_2O at 600[b]		H_2O at 600					
	5.1[a]	err	5.34[a]	err	5.2[a]	err	5.1[a]	err		
C18[d]	9.4	1.3	9.1	1.7	7.7	3.4	9.9	3.1	9.2	1.0
C54	14.1	2.2	10.4	2.9	29.1	4.9	8.7	5.4	13.8	1.6
C67[d]	37.6	2.4	45.7	3.0	40.7	5.1	36.6	4.1	40.2	1.6
C82	13.2	1.3	16.7	1.7	6.3	3.4	2.2	3.5	12.5	0.9
U20	19.9	0.9	16.6	1.5	25.8	2.6	15.2	2.6	19.2	0.7
U25[d]	29.5	0.9	28.9	1.5	15.0	2.5	19.1	2.4	27.2	0.7
U28	30.2	0.8	32.5	1.4	24.9	2.2	26.3	2.1	30.0	0.6
U31	14.8	0.8	17.1	1.5	9.2	2.1	14.1	2.1	14.8	0.7
U39	38.3	0.8	41.1	1.3	36.6	1.8	37.9	1.7	38.8	0.6
U40[d]	27.9	0.8	30.2	1.4	30.8	2.4	31.2	2.4	28.9	0.7
U41	14.9	0.7	17.2	1.4	13.7	2.0	17.7	2.0	15.6	0.6
U68[d]	23.3	0.7	23.4	0.2	26.8	2.0	30.9	1.9	24.3	0.5
U70[d]	27.5	0.7	28.0	1.3	24.9	1.5	20.4	1.4	26.8	0.6
U71[d]	27.5	1.0	29.1	1.7	20.0	2.9	10.3	2.6	25.7	0.8
U77	43.7	1.0	41.4	1.5	37.9	2.6	42.9	2.6	42.6	0.8
U79	34.3	0.7	35.5	1.2	33.4	2.1	35.3	2.1	34.6	0.5
U80	18.3	0.9	17.3	1.5	28.2	2.3	32.1	2.3	20.1	0.7

[a] ARTSY dephasing time T in ms used for determining $^1J_{CH}+^1D_{CH}$.

[b] RDCs were measured for a RiboA sample containing ca 7 mg/mL Pf1 in D_2O (using an experiment similar to Fig. 3 with all the ^1H band-selective pulse replaced by non-selective hard pulses except the REBUPR pulse in the middle of the ARTSY dephasing delay T), and then scaled up to match the alignment strength in the H_2O sample by the ratio of the solvent ^2H quadrupole splitting (ca 10.1 Hz and 6.9 Hz in H_2O and D_2O, respectively).

[c] Weighted average of all 4 sets of RDCs. The weight is the inverse of the square of the averaged error for each nucleoside, i.e. C and U's. The averaged RDCs were used in modeling the helix 1 structure

[d] Residues whose $^1D_{C5H5}$ were not used in modeling the structure of helix 1 and in optimizing its relative orientation to the remainder of the structure.

Table S3. ^{1}H-^{15}N couplings (Hz) of imino groups in isotropic and aligned RiboA measured at 900 MHz with dephasing times of T = 11.8 and 10.1 ms, respectively, using the experiment of Fig. 1.

	$^{1}J_{NH}$ [a]	error	$^{1}J_{NH}+^{1}D_{NH}$	error	$^{1}D_{NH}$	error
G14	-85.4	0.3	-99.8	0.4	-14.3	0.5
G15	-86.4	0.3	-98.1	0.2	-11.7	0.3
U20	-81.7	0.2	-100.5	0.2	-18.8	0.3
U22	-84.0	0.7	-104.1	0.8	-20.2	1.1
U25	-84.0	0.1	-95.9	0.1	-11.9	0.2
U28	-87.2	0.0	-104.9	0.1	-17.7	0.1
U31	-87.5	0.1	-91.7	0.1	-4.2	0.1
U34	-83.6	0.8	-104.1	0.8	-20.5	1.1
G37	-84.6	0.2	-102.9	0.2	-18.3	0.3
G38	-85.4	0.1	-101.7	0.2	-16.2	0.2
U39	-86.7	0.1	-95.1	0.1	-8.4	0.1
U40	-82.9	0.1	-90.7	0.2	-7.8	0.2
U41	-85.1	0.3	-96.1	0.3	-11.0	0.4
G42	-86.2	0.1	-106.2	0.2	-20.1	0.2
G43	-84.4	0.1	-102.1	0.1	-17.8	0.1
G44	-85.0	0.1	-97.7	0.1	-12.7	0.1
G46	-86.0	0.1	-104.8	0.2	-18.7	0.3
U47	-89.0	0.1	-105.7	0.1	-16.7	0.1
U49	-89.2	0.1	-93.1	0.1	-3.9	0.1
U51	-83.4	0.1	-97.7	0.1	-14.3	0.1
G57	-85.0	0.1	-100.8	0.1	-15.8	0.2
G59	-85.0	0.1	-94.0	0.2	-9.0	0.3
U68	-82.6	0.1	-92.7	0.1	-10.1	0.1
U70	-82.7	0.1	-102.4	0.1	-19.7	0.2
U71	-83.0	0.1	-98.6	0.1	-15.7	0.2
G72	-85.2	0.1	-89.5	0.2	-4.3	0.2
U74	-83.1	0.1	-94.5	0.1	-11.4	0.2
U75	-81.9	0.1	-100.7	0.2	-18.9	0.2
U77	-83.7	0.2	-99.4	0.2	-15.7	0.3
G78	-86.1	0.1	-94.9	0.2	-8.8	0.2
U79	-85.3	0.1	-90.5	0.2	-5.2	0.2
U80	-86.4	0.1	-90.5	0.1	-4.1	0.1

[a] Note that $^{1}J_{NH}$ values listed here include a small RDC contribution due to weak magnetic susceptibility anisotropy alignment of RiboA at 21 Tesla.

Table S4. The angle θ between the $H_{8/6}$-$H_2'_{i-1}$ and $C_{8/6}$-$H_{8/6}$ dipole vectors and the long range $^{n}D_{HH}$ predicted using the alignment tensors from a joined SVD fit of $^{1}D_{NH}$ and $^{1}D_{CH}$ to the core of RiboA.

Residue	Residue No.	$\theta_{HH\text{-}CH}$ (°)	$^{n}D_{HH}$ (Hz)
GUA	14	115.6	-0.6
GUA	15	112.0	4.2
ADE	16	112.1	5.9
ADE	17	115.1	1.2
CYT	18	120.9	-11.5
ADE	19	110.3	-24.3
URI	20	114.1	-23.8
ADE	21	110.4	-5.1
URI	25	138.8	0.8
CYT	26	114.4	-7.2
CYT	27	118.3	-11.3
URI	28	122.4	4.3
ADE	29	102.3	-14.0
ADE	30	120.2	6.1
URI	31	127.1	-0.7
URI	39	151.8	0.9
URI	40	116.9	-9.8
URI	41	124.9	2.7
GUA	42	125.0	8.8
GUA	43	123.9	-3.3
GUA	44	123.3	-7.9
ADE	45	122.8	-19.3
CYT	54	133.7	1.1
ADE	55	113.4	-13.1
ADE	56	97.5	-28.7
GUA	57	118.3	-23.5
ADE	58	112.7	-34.7
GUA	59	115.6	-25.3
CYT	67	104.1	-1.2
URI	68	109.5	-42.3
CYT	69	112.5	-25.7
URI	70	122.9	-41.5
URI	71	101.1	-21.8
GUA	72	107.9	-1.9
URI	75	115.0	-12.1
ADE	76	109.4	-28.1
URI	77	107.0	-29.3
GUA	78	112.0	-1.6
URI	79	113.6	-5.0
URI	80	106.5	-4.0
CYT	81	101.7	-3.2
CYT	82	107.4	-4.6
CYT	83	108.7	-12.7

Table S5. Nucleotide-type specific target values for the torsion angles that define an ideal A-form helix.

Torsion angle	A	G	C	U
α [a]	298[b]	294	295	295
β	174	173	173	175
γ	51	54	52	51
δ	81	80	79	80
ε	209	208	209	207
ζ	286	289	288	288
χ	82	78	81	82
χ_1	-29	-29	-29	-29
χ_2	41	42	42	42
χ_3	-39	-41	-41	-41
χ_4	22	24	24	24
χ_5	4	3	4	3

[a] Average torsion angles extracted from the crystallographic database for A form helical structures, solved at a resolution of 2.51 Å or higher. The following PDB depositions: 1M5K, 2EZ6, 2A43, 1ZCI, 1ZEV, 1TFW, 1T0D, 1SJ3, 1RC7, 1R3E, 1Q96, 1Q93, 1Q9A, 1NUJ, 1FEU, 1VC7, 2FGP, 2BCZ, 1ZFX, 2GOZ, 1X9C, 2B57, 1Y26, 1U8D, 2GDI, 402D, 405D, 409D, 413D, 420D, 433D, 434D, 438D, 439D, 466D, 1QCU, 1QC0, 472D, 1CSL, 1D4R, 1DQH, 1DUQ, 1I9X, 1KD3, 1KD5, 1L3Z, 1NLC, 377D, 1RXB, 259D, 255D, 157D, 1RNA, 1F27, 1MWL, 1MSW, 1DRZ, 1CX0, 1DFU, 1C0A, 1DI2, 1DUL, 1EC6, 1F7U, 1FFY, 1G2E, 1HQ1, 1E7X, 1JBR, 1K8W, 1LNG, 1M5O, 1M8W, 1M8X, 1MJI, 1N78, 1A34, 1QTQ, 1A9N, 1EHZ, 437D, 462D, 483D, 1HR2, 1J8G, 1L2X, 1MSY, 280D, 1GID, 353D, 1JJ2. Torsion angles of canonical A-form RNA were averaged from a set of hydrogen bonded A_i-U_j or G_i-C_j base pairs surrounded by Watson-Crick i-1/j+1 and i+1/j-1 base pairs.

[b] All values are in degrees. Angle definitions are as follows:
α: $O3'_{i-1}/P_i/O5'_i/C5'_i$, β: $P_i/O5'_i/C5'_i/C4'_i$, γ: $O5'_i/C5'_i/C4'_i/C3'_I$, δ: $C5'_i/C4'_i/C3'_i/O3'_I$, ε: $C4'_i/C3'_i/O3'_i/P_{i+1}$, ζ: $C3'_i/O3'_i/P_{i+1}/O5'_{i+1}$, χ: $C2'_i/C1'_i/N1(9)_i/C4(2)_i$,
χ_1: $O4'_i/C1'_i/C2'_i/C3'_i$, χ_2: $C1'_i/C2'_i/C3'_i/C4'_i$, χ_3: $C2'_i/C3'_i/C4'_i/O4'_i$,
χ_4: $C3'_i/C4'_i/O4'_i/C1'_i$, χ_5: $C4'_i/O4'_i/C1'_i/C2'_i$

Table S6. Nucleotide pair-type specific target values and uncertainties for the heavy atom inter-atomic distances that define base stacking characteristic of an ideal A-form helix. All distances are in Å.

A_i/A_{i+1}	N9/C8	C8/C8	N7/N7	C5/N7	C6/C5	N6/N6	N1/C5	C2/C4	N3/N9	C4/N7
	4.037[a] ± 0.160	4.559 ± 0.221	4.066 ± 0.231	3.555 ± 0.159	3.634 ± 0.114	3.353 ± 0.213	3.488 ± 0.169	3.613 ± 0.212	3.734 ± 0.212	3.652 ± 0.121
A_i/G_{i+1}	N9/C8	C8/N7	N7/N7	C5/N7	C6/C5	N6/O6	N1/C5	C2/C4	N3/N9	C4/C8
	3.995 ± 0.159	4.276 ± 0.270	3.994 ± 0.267	3.518 ± 0.148	3.605 ± 0.243	3.257 ± 0.188	3.479 ± 0.227	3.603 ± 0.198	3.741 ± 0.186	3.648 ± 0.143
A_i/C_{i+1}	N9/C6	C8/C5	N7/C5	C5/C5	C6/C4	N6/N4	N1/N3	C2/C2	N3/N1	C4/C6
	3.900 ± 0.184	3.929 ± 0.255	3.760 ± 0.316	3.543 ± 0.222	3.376 ± 0.114	3.284 ± 0.185	3.364 ± 0.177	3.517 ± 0.202	3.677 ± 0.138	3.685 ± 0.160
A_i/U_{i+1}	N9/C6	C8/C5	N7/C5	C5/C5	C6/C4	N6/O4	N1/N3	C2/N3	N3/C6	C4/C5
	3.956 ± 0.305	3.949 ± 0.407	3.753 ± 0.409	3.503 ± 0.254	3.377 ± 0.154	3.285 ± 0.217	3.453 ± 0.240	3.747 ± 0.424	3.826 ± 0.378	3.615 ± 0.242

G_i/A_{i+1}	N9/C8	C8/C8	N7/N7	C5/N7	C6/C5	O6/N6	N1/C5	C2/C4	N2/C4	N3/N9	C4/C8
	4.002 ± 0.232	4.420 ± 0.306	3.892 ± 0.331	3.454 ± 0.199	3.428 ± 0.210	3.240 ± 0.284	3.412 ± 0.166	3.539 ± 0.197	3.775 ± 0.357	3.659 ± 0.188	3.585 ± 0.169
G_i/G_{i+1}	N9/C8	C8/C8	N7/N7	C5/N7	C6/C5	O6/C6	N1/C5	C2/C4	N2/N3	N3/N9	C4/C8
	4.120 ± 0.231	4.591 ± 0.361	4.051 ± 0.340	3.497 ± 0.212	3.541 ± 0.222	3.577 ± 0.321	3.371 ± 0.166	3.588 ± 0.182	3.769 ± 0.288	3.712 ± 0.212	3.626 ± 0.203
G_i/C_{i+1}	N9/C6	C8/C5	N7/C5	C5/C5	C6/C4	O6/N4	N1/N3	C2/C2	N2/O2	N3/N1	C4/C6
	4.016 ± 0.289	4.116 ± 0.470	3.865 ± 0.423	3.503 ± 0.192	3.361 ± 0.141	3.264 ± 0.303	3.380 ± 0.188	3.556 ± 0.222	3.604 ± 0.363	3.679 ± 0.210	3.646 ± 0.186
G_i/U_{i+1}	N9/C6	C8/C5	N7/C5	C5/C5	C6/C4	O6/O4	N1/N3	C2/C2	N2/O2	N3/N1	C4/C6
	4.010 ± 0.255	4.088 ± 0.252	3.882 ± 0.302	3.515 ± 0.123	3.353 ± 0.088	3.265 ± 0.172	3.356 ± 0.112	3.526 ± 0.123	3.557 ± 0.243	3.661 ± 0.132	3.650 ± 0.163

C_i/A_{i+1}	N1/N7	C2/N7	O2/C8	N3/N7	C4/C6	N4/N6	C5/C6	C6/N7
	4.018 ± 0.224	3.576 ± 0.212	3.729 ± 0.276	3.322 ± 0.254	4.223 ± 0.195	3.357 ± 0.273	5.268 ± 0.227	4.202 ± 0.242
C_i/G_{i+1}	N1/C8	C2/C8	O2/C8	N3/N7	C4/N7	N4//O6	C5/N7	C6/N7
	3.901 ± 0.229	3.520 ± 0.238	3.716 ± 0.350	3.296 ± 0.223	3.468 ± 0.370	3.571 ± 0.350	3.978 ± 0.353	4.239 ± 0.271
C_i/C_{i+1}	N1/C6	C2/C6	O2/N1	N3/C4	C4/C5	N4/N4	C5/C5	C6/C6
	4.030 ± 0.189	3.717 ± 0.192	3.930 ± 0.272	3.549 ± 0.225	3.663 ± 0.229	3.256 ± 0.218	3.956 ± 0.268	4.503 ± 0.231
C_i/U_{i+1}	N1/C6	C2/C6	O2/C6	N3/C5	C4/C5	N4/C5	C6/C5	C6/C5
	3.967 ± 0.153	3.645 ± 0.194	3.794 ± 0.324	3.465 ± 0.169	3.570 ± 0.199	4.129 ± 0.297	3.854 ± 0.237	3.998 ± 0.207

U_i/A_{i+1}	N1/C8	C2/C8	O2/C8	N3/N7	C4/N7	O4/N6	C5/N7	C6/N7
	3.981 ± 0.149	3.672 ± 0.189	3.862 ± 0.345	3.383 ± 0.259	3.575 ± 0.400	3.366 ± 0.322	3.988 ± 0.322	4.195 ± 0.208
U_i/G_{i+1}	N1/C8	C2/C8	O2/C8	N3/N7	C4/N7	O4/N7	C5/N7	C6/N7
	3.908 ± 0.177	3.532 ± 0.223	3.703 ± 0.287	3.287 ± 0.210	3.521 ± 0.286	3.917 ± 0.359	4.016 ± 0.346	4.242 ± 0.235
U_i/C_{i+1}	N1/C6	C2/C5	O2/C6	N3/C5	C4/C4	O4/N4	C5/C5	C6/C5
	4.003 ± 0.209	3.554 ± 0.192	3.704 ± 0.304	3.469 ± 0.220	3.712 ± 0.276	3.273 ± 0.299	4.052 ± 0.350	4.094 ± 0.266
U_i/U_{i+1}	N1/C5	C2/C4	O2/N1	N3/C4	C4/O4	O4/O4	C5/C5	C6/C5
	3.942 ± 0.213	4.083 ± 0.158	3.755 ± 0.198	3.470 ± 0.175	3.530 ± 0.323	3.259 ± 0.345	3.979 ± 0.409	4.104 ± 0.346

[a] Inter-atomic distances between the heavy atoms of the sequential stacked bases were extracted from the i/j and i+1/j-1 base pairs. The statistics were limited to all such sets that exhibited canonical A-form torsion angles for all 4 nucleotides. For each heavy atom of base i, only one distance to a heavy atom of base j was retained, with the smallest standard deviation. For each such distance, the allowed range is reported as half the distance between the largest and the smallest value in the set.

Structure calculation protocol

Coordinates of the mutated RiboA were modeled based on the 1Y26 crystal structure, using Xplor-NIH. The region encompassing nucleotides 18-78, where the sequences of the modified RiboA and 1Y26 match, was restrained by a tight (9000 kcal/Å^2) non-crystallographic symmetry term to ensure a close match (~0.01 Å rmsd) between the heavy atom coordinates of the modeled RiboA and 1Y26. The remaining part of the structure was free to move subject to the energy terms that included bond lengths, angles, improper torsions, repulsive-only non-bonded terms, as well as database-extracted torsion and base stacking distance restraints that kept the region 13-18 & 79-83 close to the ideal A-form RNA (Tables S5 and S6). Planarity and distance restraints were used to ensure proper geometry across the hydrogen-bonded base pairs. A slow-cooling simulated annealing protocol of 100,000 1-fs steps was followed, with the temperature decreasing from 2000K to 1K, followed by 100 steps of conjugate gradient minimization. The script and restraint files used can be downloaded from http://spin.niddk.nih.gov/bax/pp/

Pulse sequences and parameters

All pulse sequences, acquisition parameters, NMRPipe conversion and processing scripts, can be downloaded from http://spin.niddk.nih.gov/bax/pp/ Pulse sequence code is also provided below.

Bruker pulse sequence of Fig. 1 for imino $^{1}D_{NH}$ determination:

```
#include "bits.jfy"
#include "Delay.incl"

"d0=105u+p7*0.635-p3*2"
"d11=50m"
"d25=p22+100u"
"d22=2.75m-p21-p12*0.5-5u-d25*0.5"
"d23=1.28m-p24-p12*0.5-10u"

"d21=10.1m"       ; needs to be set to ~1/J or 1/(J+D)
"d29=p7*4.44"
"DELTA1=d21-d29*3-80u"

1       ze
        1m
        1m
2       1m do:C1
        d11 LOCKH_OFF
        2m
3       3m
4       3m
5       3m
6       1m
7       1m
        10u pl7:N
        10u pl0:H
        d1
        1m UNBLKGRAD
        (p7 ph0):N
         10u
         p20:gp1
         10m
       (p10:sp0 ph0):H ;eburp2
        5u
        5u gron12
        DELTA1*0.25
        10u groff
        d29*2
        5u
        5u gron12
        DELTA1*0.25
        10u groff

    if "l6==1" goto 20
```

```
      (p12:sp2 ph0):H
      (p7 ph0 p7*2.44 ph1 p7 ph0):N
       5u
       5u gron12
       DELTA1*0.25
       10u groff
       d29
     goto 25

20    (p12:sp2 ph0):H
      d29*0.75
       5u
       5u gron12
       DELTA1*0.25
       10u groff
      (p7 ph0 p7*2.44 ph1 p7 ph0):N
       d29*0.25

25     5u
       5u gron12
       DELTA1*0.25
       10u groff
      (p10:sp1 ph11):H ;eburp2-TimeReversed
        5u pl0:H
        p20:gp0
        50u pl0:H

;****************************************
       if "l1==1" goto 88
      (p7 ph17):N
       10u
       p23:gp6
       200u pl7:N
      (p7*2 ph17):N
       p23:gp5
       goto 89

88    (p7 ph7):N
       10u
       p23:gp5
       200u pl7:N
      (p7*2 ph7):N
       p23:gp6
89     d0 pl4:C1
      (p3 ph0 p3*2 ph1 p3 ph0):C1
       d0
```

```
      (p10:sp1 ph2):H  ;eburp2, time reversed
       10u
       p24:gp2
       d23
      (center (p12:sp2 ph0):H (p7*2 ph0):N)
       10u
       p24:gp2
       d23
      (p10:sp0 ph0):H
      (p7 ph4):N
       5u
       p21:gp3
       d22
      (center (d25 p12:sp2 ph0):H (p7*2 ph0):N)
       5u
       p21:gp3
       d22
      (p7 ph0):N
       p22:gp4
999  95u pl31:C1
       5u BLKGRAMP
      go=2 ph31 cpd4:C1
       1m do:C1
       1m LOCKH_OFF
       d11 wr #0 if #0 zd
       1m iu6
      lo to 3 times 2
       0.5m ru6
       0.5m iu1
       1m ip2*2
       1m ip4*2
      lo to 4 times 2
       600u ip7*2
       600u ip17*2
       600u ip31*2
       600u id0
       600u ru1
      lo to 5 times l3
       10u rd0
1m do:C1
1m do:N
exit

ph0=0
ph1=1
ph2=1
```

ph4=1
ph5=2
ph11=1 3
ph7=1 1 0 0 3 3 2 2
ph17=1 1 2 2 3 3 0 0
ph20=0
ph31=1 3 2 0 3 1 0 2

Bruker pulse sequence of Fig. 3 for base $^{1}D_{C2\text{-}H2}$, $^{1}D_{C6\text{-}H6}$ and $^{1}D_{C8\text{-}H8}$ measurement in H_2O:

```
#include "bits.jfy"
#include "Delay.incl"


"d2=1.1m"
"d13=d2-p22-p10*0.6"
"d11=50m"
"d12=20u"
"d0=p3*0.64"
"d14=d2-p24-p14*0.5"
"d15=d14-p25+p24"

"d27=4.9m"              ;needs to be set to ~1/J or 1/(J+D)
"d29=p3*4.44"
"DELTA1=d27-d29*3-80u"

"d3=p10-p12+p13"
"d4=p10-d3-p3"


1     ze
      1m
      1m
2     10u do:C1 do:N
      10u
      d11
      10u LOCKH_OFF ;LOCK_ON
      d12
3     d12*2 do:H
4     d12*2 do:C1
5     d12 do:N
6     d12
7     1m
     d1 do:N
     1m UNBLKGRAD
     10u pl1:H
     10u pl3:C1
    (p3 ph0):C1
    5u
    p20:gp1
    10m
; ***************** INEPT and ARTSY intensity encoding
10   (p10:sp0 ph0):H
```

```
    5u
    5u gron2
    DELTA1*0.25
    10u groff
    d29*2
    5u
    5u gron2
    DELTA1*0.25
    10u groff

  if "l6==1" goto 20
    (p12:sp2 ph0):H
    (p3 ph0 p3*2.44 ph1 p3 ph0):C1
    5u
    5u gron2
    DELTA1*0.25
    10u groff
    d29
  goto 25

 20  (p12:sp2 ph0):H
    d29*0.75
    5u
    5u gron2
    DELTA1*0.25
    10u groff
    (p3 ph0 p3*2.44 ph1 p3 ph0):C1
    d29*0.25

25   5u pl1:H
    5u gron2
    DELTA1*0.25
    10u groff
    (p10:sp1 ph11):H
    5u
    p20:gp0
    0.1m pl7:N
;**************** start C evolution
   (p3 ph17):C1
   (p3 ph0 p3*2.44 ph1 p3 ph0):C1
   d0
   (p3 ph0 p3*2.44 ph1 p3 ph0):C1
   10u
   p27:gp27*EA
   90u pl0:C1
   (center (p13:sp13 ph4):C1 (p7 ph0 p7*2.4 ph1 p7 ph0):N)
```

```
   d0
   p26:gp26*EA
   50u pl1:H
   50u ;pl3:f4
;--end C evolution
;--Rance Kay transfer back
      (p10:sp1 ph8):H
      5u
      p22:gp22
      d13
      (p14:sp4 ph1):H (p13:sp13 ph6):C1
      5u
      p22:gp22
      d13 pl3:C1
      (p10:sp0 ph2):H (d3 p3 ph9):C1
      10u
      p24:gp24
      d14
      50u
      (center (p14:sp4 ph0):H (p3 ph1 p3*2 ph0 p3 ph1):C1)
      5u pl30:N
      p25:gp25
      d15
      45u
      3u BLKGRAMP
      3u pl31:C1
      4u
      go=2 ph31 cpd4:C1 cpd2:N
      10u do:C1 do:N
      10u do:N
      d11 wr #0 if #0 zd
      10u LOCKH_OFF
d12 iu6
lo to 3 times 2
d12*0.25 ru6
d12*0.25 ip8
d12*0.25 ip8
d12*0.25 igrad EA
d12 iu1
lo to 4 times 2
d12 ru1
d12*0.25 ip17*2
d12*0.25 ip31*2
d12*0.25 id0
d12*0.25 dd10
lo to 5 times l3
```

1m
exit

ph0=0
ph1=1
ph2=2
ph3=3
ph4=0
ph6=0
ph8=1
ph9=1 1 1 1 3 3 3 3
ph10=0
ph11=1 3
ph17=0 0 2 2
ph31=0 2 2 0 2 0 0 2

Bruker pulse sequence of Fig. 6 for base $^{1}D_{C5\text{-}H5}$ measurement in H_2O on a probe with triple axis gradients:

```
#include "bits.jlb"


"d2=1.2m"
"d3=d2*0.5-100u"
"d13=d2-p22-p15*0.4"
"d11=50m"
"d12=20u"
"d0=3u"
"d14=d2-p24-p15*0.4-60u"
"d15=100u-p3+p1"

"d27=5.1m"      ; needs to be set to ~1/J or 1/(J+D)
"d29=p3*4.44"
"DELTA1=d27-d29*3-80u-p1*1.27"


1 ze
  1m
     1m
2    10u do:C1 do:N
     10u
     d11
     10u LOCKH_OFF
     d12
3    d12*2 do:H
4    d12*2 do:C1
5    d12 do:N do:H
6    d12
7    1m
     d1
     1m UNBLKGRAD
     10u pl1:H
     10u pl3:C1
    (p3 ph0):C1
    10u
    p20:gp1
    1m
; ********** INEPT
10   (p1 ph0):H
    5u
    5u gron2
    DELTA1*0.25
```

```
    10u groff
    d29*2
    5u pl0:H
    5u gron2
    DELTA1*0.25
    10u groff

  if "l6==1" goto 20
    (p12:sp2 ph0):H
    (p3 ph0 p3*2.44 ph1 p3 ph0):C1
    5u
    5u gron2
    DELTA1*0.25
    10u groff
    d29
  goto 25

 20  (p12:sp2 ph0):H
    d29*0.75
    5u
    5u gron2
    DELTA1*0.25
    10u groff
    (p3 ph0 p3*2.44 ph1 p3 ph0):C1
    d29*0.25

25   5u pl1:H
    5u gron2
    DELTA1*0.25
    10u groff
    (p1 ph11):H
    (p3 ph7):C1
     3u
     3u gron3
     d3
     94u groff
    (center (p3*2 ph0):C1 (p1 ph0 p1*2 ph1 p1 ph0):H)
     3u
     3u gron3
     d3
     94u groff
    (p3 ph1):C1 ;put the trosy pk on z
    10m
    p20:gp0
    100u pl7:N
;**************** start C evolution
```

```
   (p3 ph17):C1
   (p3:sp3 ph0 p3*2.44:sp3 ph1 p3:sp3 ph0):C1
   d0
   (p3:sp3 ph0 p3*2.44:sp3 ph1 p3:sp3 ph0):C1
   6u
   p27:gp27*EA
   100u pl0:C1
   (center (p15:sp15 ph4):C1 (p7 ph0 p7*2.4 ph1 p7 ph0):N)
   d0
   p26:gp26*EA
   50u pl1:H
   50u pl3:C1
;--end C evolution
;--Rance Kay transfer back
      (p1 ph0):H (p3 ph8):C1
      5u
      p22:gp22
      d13 pl0:C1
      (center (p1*2 ph0):H (p15:sp15 ph0):C1)
      5u
      p22:gp22
      d13 pl3:C1
      (ralign (p1 ph1):H (p3 ph1):C1)
      10u
      p24:gp24
      d14
      50u pl0:C1
      (center (p1*2 ph0):H (p15:sp15 ph0):C1)
      10u
      p24:gp24
      d14 pl3:C1
      50u
      (p1 ph0):H (p3 ph0):C1
      5u
      p25:gp28
      d15
      (p1*2 ph0):H
      5u
      p25:gp25
      90u pl30:N
      3u BLKGRAMP
      3u pl31:C1
      4u pl30:N
      go=2 ph31 cpd4:C1 cpd2:N
      10u do:C1 do:N
      10u do:N
```

```
      d11 wr #0 if #0 zd
      10u LOCKH_OFF
d12 iu6
lo to 3 times 2
d12*0.25 ru6
d12*0.25 ip8
d12*0.25 ip8
d12*0.25 igrad EA
d12 iu1
lo to 4 times 2
d12 ru1
d12*0.25 ip17*2
d12*0.25 ip31*2
d12*0.25 id0
d12*0.25 dd10
lo to 5 times l3
d12
exit


ph0=0
ph1=1
ph2=2
ph3=3
ph4=0
ph6=0
ph7=(8) 1 1 1 1 5 5 5 5
ph8=0
ph10=0
ph11=1 3
ph17=0 0 2 2
ph31=0 2 2 0 2 0 0 2
```

Bruker pulse sequence of Fig. 9 for base $^{1}D_{C5\text{-}H5}$ measurement in H_2O on a cryogenic probe:

```
#include "bits.jlb"


"d2=1.1m"
"d11=50m"
"d12=20u"

"d3=0.65m-100u-p15*0.5"

"d7=d2-p14*0.2"

"d9=4m-p13*0.5-10u"
"d8=d9+10u-p14-d7"
"d14=d2-p24-p14*0.25-60u"

"d27=5.2m"     ;needs to be set to ~1/J or 1/(J+D)
"d29=p3*4.44"
"DELTA1=d27-d29*3-80u-p1*1.27"

"d6=3u"
"d20=3u"
"d18=3u"
"d30=4m-p19*0.5-6u-p13"
"d5=d30+6u+p3*0.635"
"d17=p13-p7*4.44"


"in30=in20"
"in6=in20"
"in17=in20"
"in18=in20"
"in19=in20"

1 ze
  1m
     1m
2     10u do:C1 do:N
      10u
      d11
      10u LOCKH_OFF
      d12
3     d12*2 do:H
4     d12*2 do:C1
```

```
5     d12*2 do:N do:H
6     d12
7     d12
      d1 do:N do:H
      1m UNBLKGRAD
      10u fq=cnst0(bf ppm):C1 ;100ppm for C5
      10u pl0:H
      10u pl3:C1
     (p3 ph0):C1 (p10:sp0 ph0):H
      5u
      p20:gp1
      1m pl1:H
; ********** INEPT and ARTSY intensity encoding
10   (p1 ph0):H  ;(p10:sp0 ph0):H
     5u
     5u
     DELTA1*0.25
     10u
     d29*2
     5u pl0:H
     5u
     DELTA1*0.25
     10u

  if "l6==1" goto 20
     (p12:sp2 ph0):H
     (p3 ph0 p3*2.44 ph1 p3 ph0):C1
     5u
     5u
     DELTA1*0.25
     10u
     d29
   goto 25

 20  (p12:sp2 ph0):H
     d29*0.75
     5u
     5u
     DELTA1*0.25
     10u
     (p3 ph0 p3*2.44 ph1 p3 ph0):C1
     d29*0.25

25   5u pl1:H
     5u
     DELTA1*0.25
```

```
     10u
     (p1 ph11):H
      3u pl3:C1
     (p3 ph7):C1  ;45 deg shift for each of the 2 cmp.
      3u
      3u
      d3 pl0:C1
      94u
     (center (p15:sp15 ph0):C1 (p1 ph0 p1*2 ph1 p1 ph0):H)
      3u
      3u
      d3 pl3:C1
      94u
     (p3 ph8):C1 ;put the trosy pk on z
      3u
      3u pl0:C1
      10u pl7:N
;*********************************
     (p16:sp5 ph3):C1 ;eburp2 on C5
      d6                    ; d6 = ξ1 in Fig. 9
     (p15:sp15 ph13):C1 ;reburp on C5
      6u pl0:H
     (p19:sp9 ph5):C1 ;hsec on C4/C5/C6
; d20 = ξ2 in Fig. 9
; d18 = ξ3 in Fig. 9
; d17 = pulse width offset that includes ξ1 in Fig. 9
     (d20 p13:sp7 ph0):C1 (d18 p7 ph0 p7*2.44 ph1 p7 ph0 d17):N ;decoupling
      d5 pl0:C1
     (p19:sp9 ph4):C1 ;hsec on C4/C5/C6
      3u
     (p13:sp7 ph0):C1 ;C4 iburp2 pulse, B.S. compensation
      d30
      3u pl3:C1
;****************C5->C6 transfer
    (p3 ph17):C1
    d7
    (p14:sp8 ph0):H  ;H6 inversion
    d8 pl0:C1
    (p13:sp13 ph6):C1 ;C5C6
    d9
    10u pl3:C1
    (p3 ph0):C1
       5u
       p22:gp22
       100u fq=cnst6(bf ppm):C1
;----C6-->H6
```

```
      (p10:sp0 ph0):H
      10u
      p24:gp24
      d14
      50u pl3:C1
      (center (p14:sp4 ph2):H (p3 ph0 p3*2 ph1 p3 ph0 1m):C1)
      5u
      p24:gp24
      d14
      45u
999 3u pl30:N
      3u pl31:C1
      4u BLKGRAMP
      go=2 ph31 cpd4:C1 cpd2:N
      10u do:C1 do:N
      10u
      d11 wr #0 if #0 zd
      10u LOCKH_OFF
d12 iu6
lo to 3 times 2
d12 ru6
d12 ip3
lo to 4 times 2
if "d30 > in30"
{
  d12*0.4 id20
  d12*0.4 id18
  d12*0.4 dd30
  d12*0.4
}
else
{
  d12*0.4 id20
  d12*0.4
  d12*0.4 id17
  d12*0.4 id6
}
d12*0.4 ip31*2
lo to 5 times l4
d12
exit


ph0=0
ph1=1
ph2=0 0 1 1
```

ph3=1 1 1 1 1 1 1 1 3 3 3 3 3 3 3 3
ph4=0 0 0 0 0 0 0 0 2 2 2 2 2 2 2 2
ph5=0
ph6=0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 1 1 1 1 1 1 1 1 1 1 1 1 1 1 1 1
ph7=(8) 1 1 1 1 5 5 5 5
ph8=1
ph11=1 3
ph13=0
ph17=0
ph31=0 2 2 0 2 0 0 2 2 0 0 2 0 2 2 0 2 0 0 2 0 2 2 0 0 2 2 0 2 0 0 2