Supporting Information

Sheng et al. 10.1073/pnas.1317799111

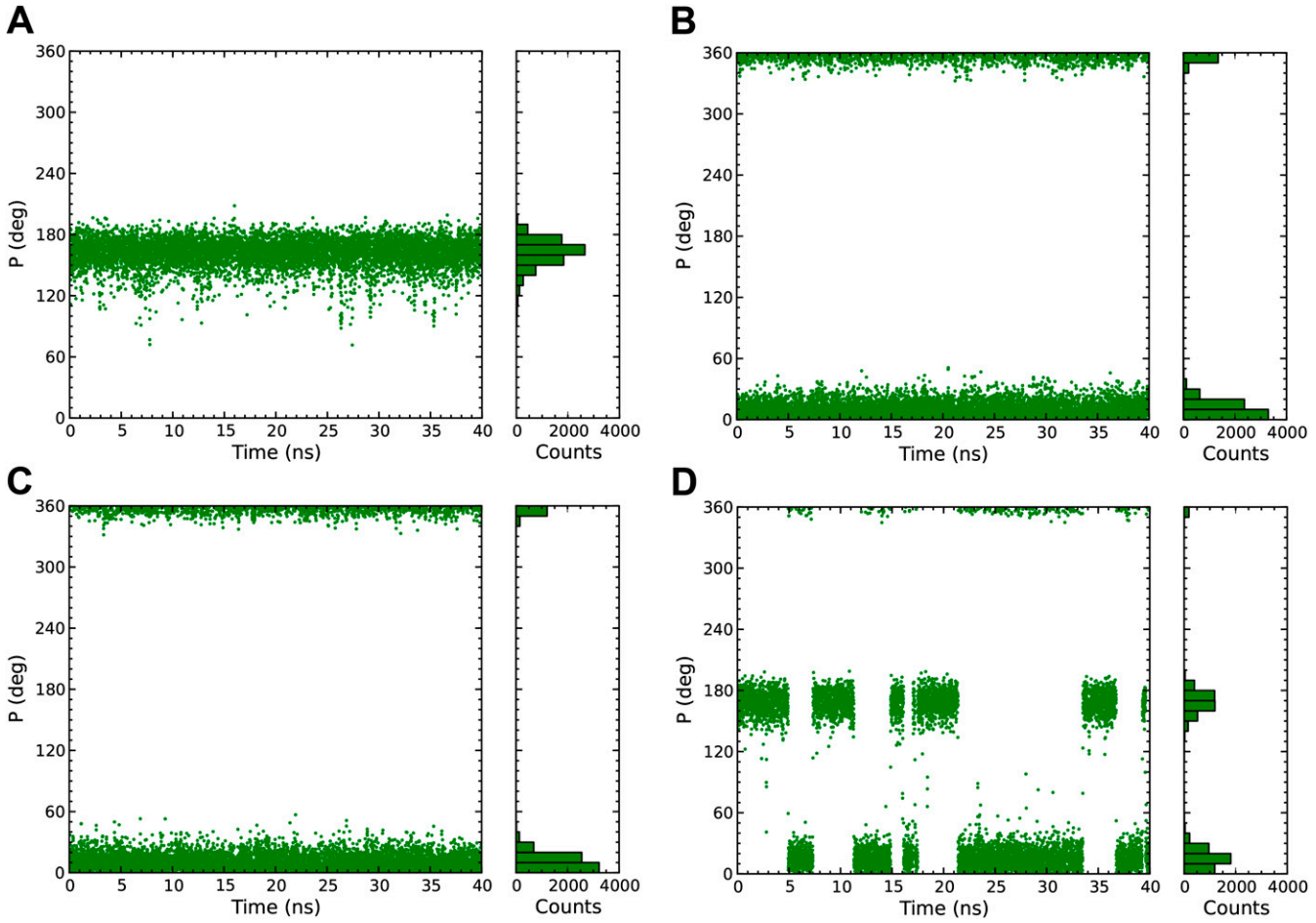

Fig. S1. Equilibrium distribution of pseudorotation angles calculated by molecular dynamics (MD) simulation. A total of 50-ns MD simulation was performed for the singly 2′-5′-linked RNA duplex (5′-CCGGC*GCCGG-3′), and the last 40 ns was used in the analysis. Pseudorotation angles of C5 in strand A (*A*), G6 in strand A (*B*), G6 in strand B (*C*), and G10 in strand A (*D*) were calculated from snapshots taken every 5 ps.

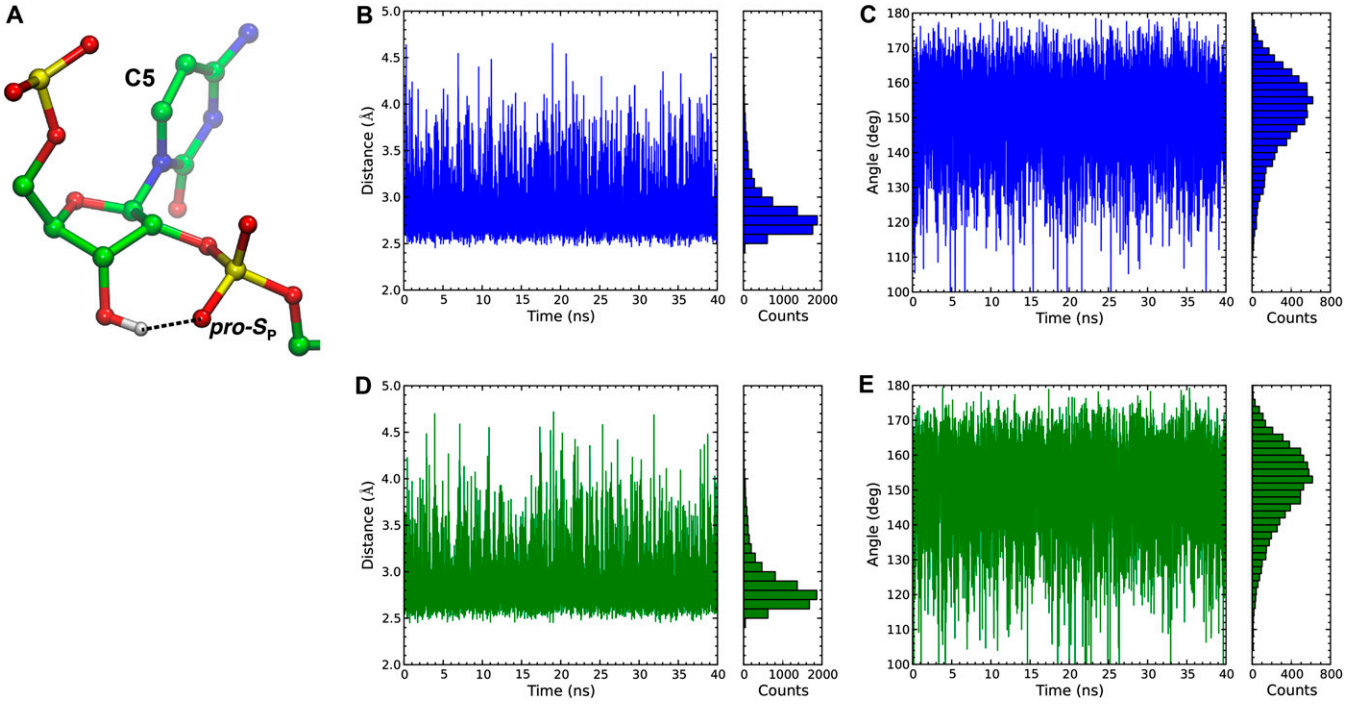

Fig. S2. MD simulations reveal a stable intramolecular hydrogen bond between 3′-OH and *pro-*S_P-oxygen. (*A*) Representative snapshot from the MD simulation illustrates the hydrogen bond (dashed line). All hydrogen atoms except the one in the hydrogen bond are omitted for clarity. The evolution of the distance between O3′ and *pro-*S_P-oxygen and the O-H-O angle during the 40-ns production run is plotted for C5 in chain A (*B* and *C*) and chain B (*D* and *E*), respectively.

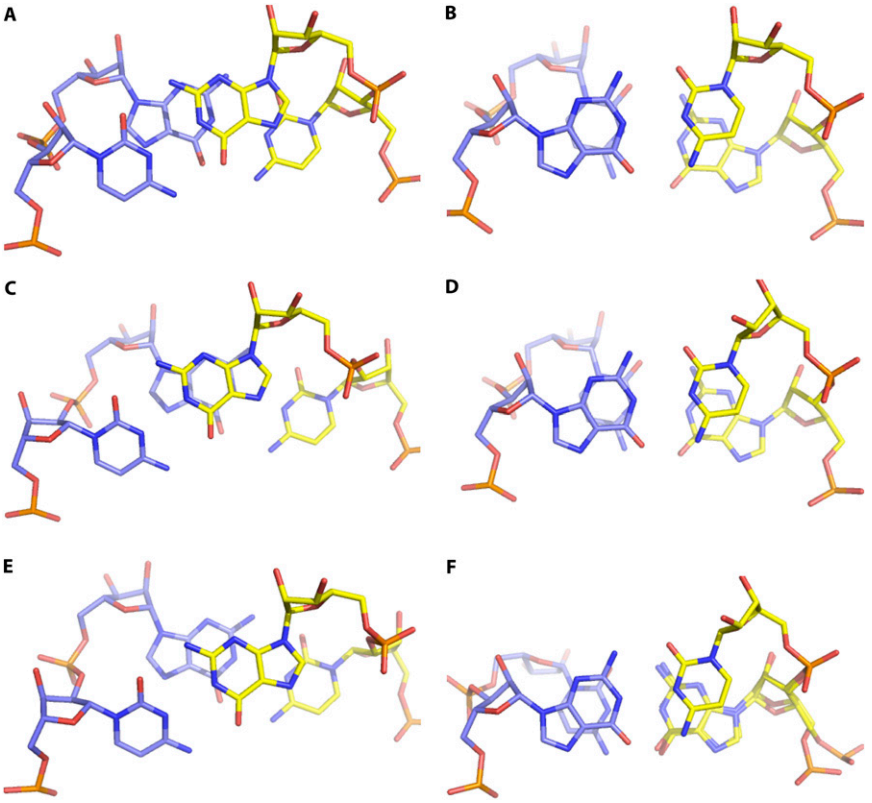

Fig. S3. Top views of base pair steps overlap. (*A*) C5-G6 step and (*B*) G6-C7 step in native 10-mer structure. (*C*) C5-G6 step and (*D*) G6-C7 step in single 2′-5′ 10mer structure. (*E*) C5-G6 step and (*F*) G6-C7 step in triple 2′-5′ 10-mer structure.

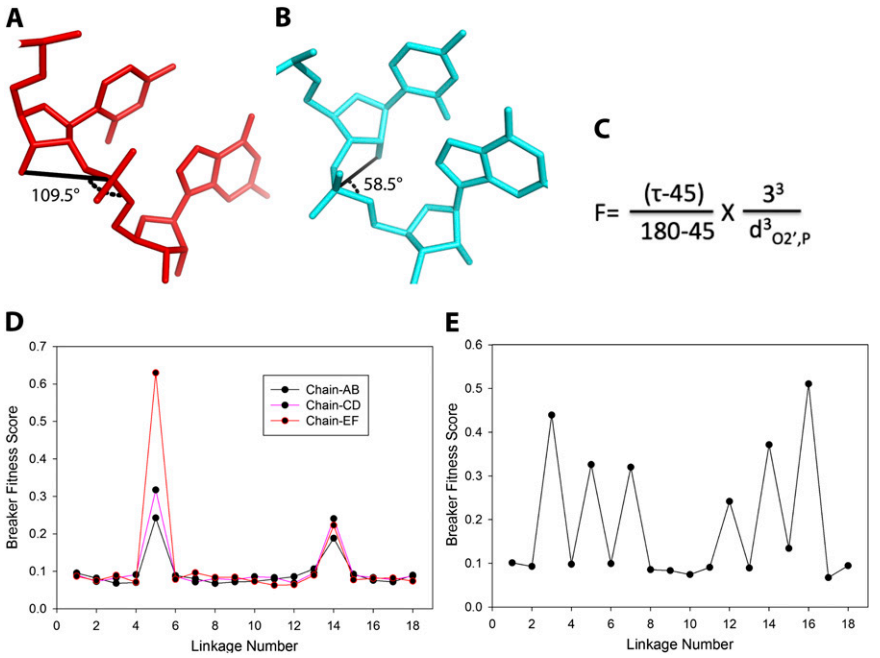

Fig. S4. Hydrolytic destabilization of native and 2′-5′-linked RNA. (*A*) Structure of 2′-5′ linkage at C5-G6 step in the crystal structure, showing an O3′-P-O5′ angle of 109.5°. (*B*) The corresponding O2′-P-O5′ angle in the native C5-G6 step is 58.5°. (*C*) In-line fitness score formula as described by Soukup and Breaker (1). (*D*) In-line fitness scores of all of the linkages in the single 2′-5′ 10-mer (5′-CCGGC*GCCGG-3′, the linkage number starts from the 5′-end of one strand and then continues with the 5′-end of the other strand; there are three duplexes in one asymmetric unit). (*E*) In-line fitness scores of all of the linkages in the triple 2′-5′ 10mer, with the same naming of linkages.

1. Soukup GA, Breaker RR (1999) Relationship between internucleotide linkage geometry and the stability of RNA. *RNA* 5(10):1308–1325.

Table S1. X-ray data collection and structural refinement statistics of native 10mer (5′-CCGGCGCCGG-3′), single 2′-5′ 10mer (5′-CCG GC*GCCGG-3′) and triple 2′-5′ 10mer (5′-CCG*GC*GC*CGG-3′)[1]

Scaling	Native 10mer	Single 2′-5′ 10mer	Triple-2′-5′ 10mer
Space group	$P3_121$	C2	$C222_1$
Unit cell parameters (Å, °)	22.71, 22.71, 77.72 90, 90, 120	96.15, 25.89, 69.72 90, 112.6, 90	24.22, 45.50, 81.62 90, 90, 90
Resolution range, Å (last shell)	50–1.32 (1.37–1.32)	30–1.55 (1.61–1.55)	30–1.20 (1.24–1.20)
Unique reflections	5,742 (480)	22,386 (1,611)	13,957 (5,280)
Completeness, %	96.0 (84.7)	95.0 (69.0)	95.7 (74.9)
R_{merge}, %	5.0 (21.3)	4.0 (21.7)	6.2 (28.9)
<I/σ(I)>	33.2 (8.3)	36.2 (3.4)	24.4 (4.2)
Redundancy	11.8 (10.4)	5.9 (2.9)	9.2 (4.9)
Refinement			
Molecules per asymmetric unit	Single strand	Three duplexes	One duplex
Resolution range, Å	25.91–1.32	30–1.55	22.75–1.20
Number of reflections	5,463	21,233	13,223
Completeness, %	97.1	95.0	95.8
R_{work}, %	18.8	18.2	17.6
R_{free}, %	22.8	24.7	21.7
Bond length r.m.s. Å	0.015	0.005	0.017
Bond angle r.m.s., °	1.71	1.53	1.99

These RNA duplexes yielded crystals that diffracted to higher resolution than duplexes containing A:U base pairs. $R_{merge} = \Sigma|I - \langle I\rangle|/\Sigma I$.

Table S2. Base pair helical and step parameters of the three structures labeled as native, s-2′-5′ (single 2′-5′-linked 10mer) and t-2′-5′ (triple 2′-5′-linked 10mer in which G3 in strand A is in C2′-*endo* conformation)

Base pair helical and step parameters	RNA duplex	1 CC/GG	2 CG/CG	3 GG/CC	4 GC/GC	5 CG/CG	6 GC/GC	7 CC/GG	8 CG/CG	9 GG/CC	Average
X-disp (Å)	Native	−4.72	−5.02	−3.58	−2.51	−4.94	−2.51	−3.58	−5.03	−4.72	−4.07
	s-2′-5′	−3.71	−5.51	−3.80	−2.91	**−6.12**	−3.06	−4.90	−5.39	−4.42	−4.43
	t-2′-5′	−4.40	−4.45	**−4.27**	−2.85	**−4.78**	−2.94	**−5.32**	−5.47	−5.51	−4.44
Inclination(°)	Native	11.13	20.60	11.35	5.64	17.72	5.64	11.35	20.60	11.13	12.80
	s-2′-5′	18.94	26.68	15.63	13.67	**17.08**	11.28	13.52	20.77	15.73	17.03
	t-2′-5′	22.15	21.57	**8.05**	22.18	**23.26**	17.32	**19.40**	26.32	16.65	19.66
Shift (Å)	Native	−0.66	−0.81	0.72	−0.63	0.00	0.63	−0.72	0.81	0.66	0.00
	s-2′-5′	−0.05	0.06	−0.50	0.23	**0.28**	0.33	0.18	0.07	0.55	0.13
	t-2′-5′	−0.77	−0.28	**0.28**	−0.61	**0.76**	0.67	**−0.34**	0.32	0.09	0.01
Slide (Å)	Native	−1.98	−1.74	−1.51	−1.21	−1.76	−1.21	−1.50	−1.74	−1.98	−1.63
	s-2′-5′	−1.41	−1.76	−1.53	−1.02	**−3.06**	−1.24	−1.90	−1.83	−1.78	−1.73
	t-2′-5′	−1.69	−1.61	**−2.61**	−0.45	**−1.93**	−0.98	**−2.45**	−1.77	−2.04	−1.73
Rise (Å)	Native	3.3	3.40	3.31	3.13	3.05	3.13	3.31	3.40	3.30	3.26
	s-2′-5′	3.28	3.18	3.14	3.05	**3.44**	3.01	3.16	3.36	3.23	3.21
	t-2′-5′	3.49	2.98	**3.71**	2.79	**3.36**	2.79	3.45	3.45	3.39	3.27
Tilt (°)	Native	−4.09	−2.74	2.03	−1.56	0.00	1.56	−2.03	2.74	4.09	0.00
	s-2′-5′	−1.22	−0.12	−1.07	2.63	**2.41**	−1.72	2.05	1.49	2.99	0.83
	t-2′-5′	−6.28	−1.64	**4.64**	−1.79	**1.85**	3.64	**−2.46**	2.20	0.13	0.03
Roll (°)	Native	6.00	11.23	6.64	3.36	9.13	3.37	6.64	11.23	6.00	7.07
	s-2′-5′	11.83	13.89	9.33	7.86	**10.97**	6.61	7.30	10.80	9.05	9.74
	t-2′-5′	13.96	12.14	**5.77**	11.02	**14.53**	10.10	**12.24**	14.32	8.66	11.42
Twist (°)	Native	30.72	30.26	33.55	34.58	28.94	34.57	33.56	30.26	30.72	31.91
	s-2′-5′	35.16	28.07	34.03	32.73	**35.67**	33.04	30.28	29.09	32.40	32.27
	t-2′-5′	34.65	31.17	**41.59**	27.34	**34.48**	32.81	**35.39**	29.36	29.32	32.90

Significant structural perturbations are shown in bold.

Table S3. Summary of MD simulations

System	Simulation method	Simulation time (ns)
Native duplex	Unbiased	50
Single 2′-5′-linked duplex	Unbiased	50
Triple 2′-5′-linked duplex (conformer A*)	Unbiased	50
Triple 2′-5′-linked duplex (conformer B†)	Unbiased	50
Native duplex	Umbrella sampling	$0.4 \times 16 \times 5 = 32$
Single 2′-5′-linked duplex	Umbrella sampling	$0.4 \times 16 \times 5 = 32$
Triple 2′-5′-linked duplex (conformer A*)	Umbrella sampling	$0.4 \times 16 \times 5 = 32$
Total		296

*Conformer A corresponds to the structure in which G3 in strand A is in C2′-*endo* conformation.
†Conformer B corresponds to the structure in which G3 in strand A is in C3′-*endo* conformation.

Table S4. Structural parameters of base pair steps in RNA duplexes calculated from MD simulations

Base pair step parameter	RNA duplex	Base pair step index						
		2	3	4	5	6	7	8
Shift (Å)	N	0.0 ± 0.7	0.1 ± 0.7	−0.1 ± 0.6	−0.1 ± 0.7	0.1 ± 0.6	−0.1 ± 0.6	0.0 ± 0.7
	S	−0.0 ± 0.7	0.1 ± 0.6	−0.2 ± 0.5	0.0 ± 0.5	0.3 ± 0.6	−0.2 ± 0.6	0.0 ± 0.7
	TA	−0.5 ± 0.7	0.1 ± 0.4	0.2 ± 0.4	0.0 ± 0.5	−0.1 ± 0.5	−0.1 ± 0.4	0.8 ± 1.0
	TB	−0.6 ± 0.7	0.0 ± 0.4	0.2 ± 0.4	−0.1 ± 0.6	−0.1 ± 0.5	−0.0 ± 0.5	0.9 ± 1.0
Slide (Å)	N	−1.7 ± 0.4	−1.9 ± 0.3	−1.5 ± 0.5	−1.8 ± 0.4	−1.4 ± 0.5	−1.9 ± 0.3	−1.7 ± 0.4
	S	−1.7 ± 0.4	−1.9 ± 0.4	**−1.0 ± 0.5**	**−2.6 ± 0.4**	**−1.0 ± 0.5**	−1.9 ± 0.3	−1.7 ± 0.4
	TA	−1.4 ± 0.4	**−2.3 ± 0.3**	**−0.8 ± 0.4**	**−2.4 ± 0.3**	**−0.9 ± 0.5**	**−2.3 ± 0.3**	−1.5 ± 0.5
	TB	−1.4 ± 0.4	**−2.3 ± 0.3**	**−0.9 ± 0.4**	**−2.5 ± 0.4**	**−0.9 ± 0.5**	**−2.3 ± 0.3**	−1.5 ± 0.5
Rise (Å)	N	3.7 ± 0.4	3.2 ± 0.3	3.2 ± 0.2	3.6 ± 0.4	3.2 ± 0.2	3.2 ± 0.3	3.7 ± 0.4
	S	3.7 ± 0.4	3.1 ± 0.3	**3.0 ± 0.3**	**4.0 ± 0.4**	**3.0 ± 0.2**	3.1 ± 0.3	3.7 ± 0.4
	TA	3.4 ± 0.4	3.4 ± 0.3	**2.9 ± 0.3**	**3.9 ± 0.4**	**2.9 ± 0.3**	3.4 ± 0.3	3.4 ± 0.5
	TB	3.4 ± 0.4	3.4 ± 0.3	**2.9 ± 0.3**	**3.9 ± 0.4**	**2.9 ± 0.3**	3.4 ± 0.3	3.4 ± 0.6
Tilt (°)	N	0 ± 4	−2 ± 4	1 ± 4	0 ± 5	−1 ± 4	2 ± 4	0 ± 4
	S	0 ± 4	−3 ± 4	3 ± 4	0 ± 6	3 ± 4	3 ± 4	0 ± 4
	TA	−2 ± 4	−2 ± 4	2 ± 5	1 ± 6	−2 ± 5	2 ± 5	0 ± 6
	TB	−2 ± 4	−2 ± 4	2 ± 5	0 ± 6	−2 ± 5	2 ± 5	2 ± 6
Roll (°)	N	18 ± 8	9 ± 5	5 ± 5	16 ± 7	5 ± 4	9 ± 5	17 ± 7
	S	18 ± 8	8 ± 5	7 ± 5	17 ± 7	6 ± 5	8 ± 5	18 ± 8
	TA	22 ± 6	10 ± 5	7 ± 4	16 ± 6	7 ± 5	9 ± 5	21 ± 8
	TB	21 ± 6	9 ± 5	8 ± 4	17 ± 6	9 ± 5	10 ± 5	22 ± 8
Twist (°)	N	30 ± 4	28 ± 4	32 ± 4	28 ± 4	33 ± 4	28 ± 4	29 ± 4
	S	29 ± 4	28 ± 4	34 ± 4	**37 ± 4**	33 ± 4	28 ± 4	30 ± 4
	TA	29 ± 5	**37 ± 3**	36 ± 4	**37 ± 4**	35 ± 4	**36 ± 4**	27 ± 7
	TB	29 ± 5	**37 ± 3**	36 ± 4	**36 ± 4**	35 ± 4	**36 ± 4**	28 ± 7

Significant structural perturbations are shown in bold. N, native duplex; S, single 2′-5′-linked 10mer; TA, triple 2′-5′-linked 10mer starting with conformer A; TB, triple 2′-5′-linked 10mer starting with conformer B.

Table S5. Structural parameters of base pairs in RNA duplexes calculated from MD simulations

Base pair parameter	RNA duplex	Base pair index 2	3	4	5	6	7	8	9
Shear (Å)	N	0.2 ± 0.4	−0.2 ± 0.4	−0.4 ± 0.5	0.3 ± 0.4	−0.4 ± 0.4	0.5 ± 0.4	0.3 ± 0.4	−0.3 ± 0.4
	S	0.3 ± 0.5	−0.3 ± 0.4	−0.5 ± 0.5	0.3 ± 0.3	−0.3 ± 0.3	0.5 ± 0.5	0.3 ± 0.4	−0.3 ± 0.4
	TA	0.5 ± 0.7	−0.3 ± 0.3	−0.4 ± 0.3	0.3 ± 0.3	−0.3 ± 0.4	0.4 ± 0.3	0.3 ± 0.3	−0.7 ± 1.1
	TB	0.4 ± 0.7	−0.3 ± 0.3	−0.5 ± 0.3	0.2 ± 0.3	−0.3 ± 0.4	0.4 ± 0.3	0.4 ± 0.4	−0.8 ± 1.1
Stretch (Å)	N	−0.1 ± 0.2	−0.2 ± 0.1	−0.1 ± 0.2	−0.1 ± 0.2	−0.1 ± 0.2	−0.1 ± 0.2	−0.1 ± 0.1	−0.1 ± 0.2
	S	−0.1 ± 0.2	−0.1 ± 0.1	**−0.1 ± 0.2**	**−0.1 ± 0.1**	**−0.1 ± 0.1**	−0.1 ± 0.2	−0.1 ± 0.1	−0.1 ± 0.1
	TA	**−0.1 ± 0.3**	**−0.2 ± 0.1**	**−0.1 ± 0.1**	**−0.1 ± 0.1**	**−0.1 ± 0.1**	**−0.1 ± 0.1**	**−0.2 ± 0.1**	**0.0 ± 0.5**
	TB	−0.1 ± 0.3	−0.1 ± 0.1	−0.2 ± 0.1	−0.1 ± 0.1	−0.1 ± 0.1	−0.1 ± 0.1	−0.2 ± 0.1	0.1 ± 0.5
Stagger (Å)	N	−0.3 ± 0.4	−0.2 ± 0.4	−0.1 ± 0.4	−0.2 ± 0.4	−0.2 ± 0.4	−0.0 ± 0.4	−0.2 ± 0.4	−0.3 ± 0.4
	S	−0.2 ± 0.4	−0.2 ± 0.4	**0.1 ± 0.4**	**−0.3 ± 0.4**	**−0.3 ± 0.4**	0.1 ± 0.4	−0.3 ± 0.4	−0.3 ± 0.4
	TA	−0.1 ± 0.5	−0.1 ± 0.3	**0.0 ± 0.4**	**−0.2 ± 0.4**	**−0.2 ± 0.4**	0.0 ± 0.4	−0.1 ± 0.3	−0.1 ± 0.6
	TB	−0.1 ± 0.5	−0.0 ± 0.3	0.0 ± 0.4	−0.2 ± 0.4	−0.2 ± 0.4	0.0 ± 0.4	−0.1 ± 0.4	0.0 ± 0.7
Buckle (°)	N	9 ± 10	−7 ± 9	−4 ± 10	6 ± 10	−6 ± 9	3 ± 9	7 ± 9	−8 ± 10
	S	8 ± 10	−7 ± 9	−2 ± 9	**15 ± 10**	**−16 ± 10**	3 ± 9	8 ± 9	−10 ± 10
	TA	11 ± 11	5 ± 8	−7 ± 9	**16 ± 10**	**−17 ± 10**	6 ± 9	−4 ± 10	−8 ± 13
	TB	10 ± 11	5 ± 9	−6 ± 9	**17 ± 10**	**−17 ± 10**	6 ± 9	−4 ± 10	−12 ± 15
Propeller (°)	N	−16 ± 9	−14 ± 8	−10 ± 8	−14 ± 8	−15 ± 8	−10 ± 8	−14 ± 8	−16 ± 9
	S	−16 ± 9	−15 ± 8	−10 ± 8	−10 ± 8	−9 ± 8	−9 ± 8	−15 ± 8	−17 ± 10
	TA	−25 ± 9	−9 ± 7	−9 ± 7	−11 ± 7	−12 ± 7	−7 ± 7	−8 ± 7	−24 ± 11
	TB	−26 ± 9	−8 ± 7	−8 ± 7	−11 ± 8	−12 ± 8	−7 ± 7	−9 ± 7	−29 ± 12
Opening (°)	N	−0 ± 5	−1 ± 6	1 ± 5	−0 ± 6	−1 ± 5	−0 ± 5	−1 ± 4	−1 ± 5
	S	−0 ± 6	−1 ± 4	0 ± 5	**−2 ± 4**	−2 ± 4	2 ± 6	−1 ± 4	−1 ± 5
	TA	4 ± 10	**−2 ± 3**	−2 ± 4	**−2 ± 4**	−2 ± 4	**−2 ± 4**	−2 ± 4	9 ± 17
	TB	5 ± 10	−2 ± 4	−2 ± 4	−1 ± 4	−1 ± 4	−2 ± 4	−2 ± 4	10 ± 17
Pseudorotation strand A (°)	N	19 ± 11	13 ± 8	10 ± 7	13 ± 9	12 ± 7	11 ± 8	13 ± 8	13 ± 7
	S	18 ± 11	13 ± 8	10 ± 7	14 ± 9	164 ± 13	8 ± 9	12 ± 8	13 ± 7
	TA	18 ± 11	13 ± 8	**165 ± 13**	10 ± 8	165 ± 12	8 ± 9	**164 ± 12**	9 ± 7
	TB	18 ± 11	13 ± 8	**165 ± 14**	10 ± 8	165 ± 12	8 ± 9	**164 ± 12**	9 ± 7
Pseudorotation strand B (°)	N	12 ± 7	13 ± 8	12 ± 8	11 ± 7	13 ± 9	10 ± 7	13 ± 8	19 ± 11
	S	13 ± 8	12 ± 8	8 ± 9	**164 ± 13**	15 ± 10	10 ± 7	13 ± 8	19 ± 11
	TA	8 ± 7	**166 ± 11**	8 ± 9	**165 ± 12**	11 ± 9	**163 ± 17**	14 ± 8	18 ± 11
	TB	8 ± 7	**166 ± 11**	9 ± 9	**164 ± 13**	12 ± 9	**164 ± 16**	14 ± 9	17 ± 10

Significant structural perturbations are shown in bold. N, native duplex; S, single 2′-5′-linked 10mer; TA, triple 2′-5′-linked 10mer starting with conformer A; TB, triple 2′-5′-linked 10mer starting with conformer B.

Table S6. Overlap area summary of each base pair steps in the three duplex structures

Step number	Step	Total overlap area Native	s-2′-5′	t-2′-5′
1	CC/GG	4.54	3.68	4.41
2	CG/CG	4.02	4.38	4.90
3	GG/CC	4.38	3.15	4.78
4	GC/GC	11.67	10.74	9.50
5	CG/CG	4.31	7.96	4.51
6	GC/GC	11.67	12.05	10.95
7	CC/GG	4.38	3.46	3.81
8	CG/CG	4.03	4.44	4.36
9	GG/CC	4.54	4.43	3.82
Overall		53.54	54.29	51.04

The total overlap area (in $Å^2$) includes both intrastrand and interstrand overlap within the four bases of each base pair step. s-2′-5′, single 2′-5′-linked 10mer; t-2′-5′, triple 2′-5′-linked 10mer in which G3 in strand A is in C2′-*endo* conformation.