

Supplementary Material for

The hydration and unusual hydrogen bonding in the crystal structure of an RNA duplex containing alternating CG base pairs

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Supplementary Table 1. Sugar, backbone and glycosidic torsion angles[§] calculated for the [CGCG(5-FC)G]₂ structures. Each column contains data for strands A, B and K, L from the triclinic form, strands A and B from the rhombohedral form, their average value and standard deviation (*italics*) and the average value and standard deviation for the previously reported 2'-O-Me(CGCGCG)₂ structures (PDB id 310d and 1i7j).^{6a,b}

Residue	α	β	γ	δ	ϵ	ζ	χ	Φ	P	pucker	
C1			64.0	87.5	-153.5	-72.1	-173.2	37.9	2.9	C3'-endo	
			59.7	79.8	-152.7	-74.5	-169.0	42.5	9.2	C3'-endo	
				78.7	-146.5	-75.3	-162.2	46.1	9.1	C3'-endo	
			53.8	75.2	-149.3	-74.4	-160.6	44.0	12.7	C3'-endo	
			89.9	86.3	-135.3	-92.5	-176.6	37.8	12.1	C3'-endo	
			55.3	75.5	-154.3	-75.5	-160.3	42.5	10.7	C3'-endo	
			<i>64.5±14.7</i>	<i>80.5±5.3</i>	<i>-148.6±7.1</i>	<i>-77.4±7.5</i>	<i>-167.0±7.0</i>	<i>41.8±3.3</i>	<i>5.4±9.2</i>		
			<i>42.5±7.8</i>	<i>87.0±8.9</i>	<i>-147.8±7.9</i>	<i>-76.9±3.7</i>	<i>-162.0±3.7</i>	<i>47.1±4.7</i>	<i>14.6±7.9</i>	<i>C3'-endo</i>	
G2	-77.9	-179.7	57.1	78.8	-154.7	-76.7	-159.8	45.7	8.5	C3'-endo	
	-65.7	179.6	50.7	76.3	-144.9	-68.2	-165.0	44.8	14.3	C3'-endo	
	-63.2	166.4	54.5	80.2	-154.5	-70.7	-168.7	44.6	12.3	C3'-endo	
	-65.5	175.6	50.2	79.8	-141.4	-72.2	-158.5	44.1	14.0	C3'-endo	
	-65.4	-172.5	47.8	82.4	-145.0	-71.2	-156.5	44.5	359.2	C2'-exo	
	-66.9	177.2	56.3	81.2	-144.6	-73.4	-161.8	43.4	9.3	C3'-endo	
	<i>-67.4±5.3</i>	<i>177.8±6.9</i>	<i>52.8±3.7</i>	<i>79.8±2.1</i>	<i>-147.5±5.6</i>	<i>72.1±2.9</i>	<i>-161.7±4.5</i>	<i>44.5±0.8</i>	<i>9.6±5.6</i>		
		<i>-67.2±3.2</i>	<i>177.7±1.3</i>	<i>49.2±3.7</i>	<i>83.6±7.6</i>	<i>-144.8±8.7</i>	<i>-73.4±4.1</i>	<i>-165.3±1.4</i>	<i>40.1±8.1</i>	<i>13.5±15.8</i>	<i>C3'-endo</i>
C3	-62.8	169.5	52.8	79.3	-147.6	-61.6	-155.9	45.8	9.8	C3'-endo	
	-64.5	168.0	54.5	79.7	-158.9	-76.3	-163.6	44.1	8.8	C3'-endo	
	-60.9	174.4	47.0	81.8	-161.7	-74.0	-162.3	46.1	9.1	C3'-endo	
	-65.1	167.9	59.8	77.8	-160.6	-74.6	-157.0	44.1	15.5	C3'-endo	
	-61.2	166.5	55.2	55.2	-154.3	-66.7	-158.9	50.1	19.2	C3'-endo	
	-59.8	172.7	47.2	79.4	-157.9	-69.3	-158.0	48.3	11.6	C3'-endo	
	<i>-62.4±2.1</i>	<i>169.8±3.1</i>	<i>52.8±5.0</i>	<i>75.5±10.0</i>	<i>-156.8±5.2</i>	<i>-70.4±5.6</i>	<i>-159.3±3.0</i>	<i>46.4±2.4</i>	<i>12.3±4.2</i>		
		<i>-74.7±9.0</i>	<i>172.1±1.3</i>	<i>52.2±7.7</i>	<i>74.0±4.9</i>	<i>-146.6±4.9</i>	<i>-69.2±1.5</i>	<i>-161.7±1.8</i>	<i>40.9±7.2</i>	<i>18.3±7.7</i>	<i>C3'-endo</i>
G4	-88.5	159.1	90.0	71.9	-150.6	-75.6	-155.9	47.8	17.3	C3'-endo	
	-67.3	173.1	55.4	78.8	-143.0	-73.7	-160.9	44.9	13.5	C3'-endo	
	-59.3	172.1	54.7	78.4	-155.4	-78.0	-161.8	44.1	12.6	C3'-endo	
	161.5	-171.2	176.8	85.2	-152.0	-71.4	-178.5	43.3	10.4	C3'-endo	
	-73.2	168.6	63.0	78.8	-154.8	-70.6	-161.4	42.1	7.3	C3'-endo	
	-68.4	176.4	54.2	77.6	-138.4	-78.7	-164.3	47.5	9.1	C3'-endo	
	<i>-56.4±37.9</i>	<i>173.0±9.7</i>	<i>82.4±48.2</i>	<i>78.5±4.2</i>	<i>-149.0±6.8</i>	<i>-74.7±3.4</i>	<i>-163.8±7.7</i>	<i>45.0±2.3</i>	<i>11.7±3.6</i>		
		<i>-70.1±2.9</i>	<i>178.1±1.73</i>	<i>51.4±3.7</i>	<i>77.5±2.1</i>	<i>-145.9±0.7</i>	<i>-71.8±2.3</i>	<i>-162.3±1.2</i>	<i>45.4±2.7</i>	<i>13.6±9.1</i>	<i>C3'-endo</i>
5-FC5	-70.0	173.1	49.8	80.3	-151.2	-64.8	-160.7	46.5	9.9	C3'-endo	
	-61.8	171.1	47.6	75.1	-154.6	-69.7	-160.5	44.4	13.0	C3'-endo	
	-67.0	172.3	50.4	76.4	-155.4	-74.8	-159.7	46.0	11.8	C3'-endo	
	-65.8	177.3	54.8	81.2	-156.3	-74.3	-165.0	45.3	19.0	C3'-endo	
	-65.6	171.3	53.8	82.3	-148.1	-69.5	-160.3	47.2	10.3	C3'-endo	
	-51.9	169.2	37.6	85.0	-157.9	-71.4	-160.3	50.7	8.4	C3'-endo	
	<i>-63.7±6.3</i>	<i>172.4±2.7</i>	<i>49.0±6.2</i>	<i>80.1±3.7</i>	<i>-153.9±3.6</i>	<i>-70.8±3.7</i>	<i>-161.0±1.9</i>	<i>46.7±2.2</i>	<i>12.1±3.7</i>		
		<i>-66.6±1.1</i>	<i>171.9±1.0</i>	<i>51.6±2.0</i>	<i>74.3±3.5</i>	<i>-151.4±3.3</i>	<i>-66.5±2.1</i>	<i>-165.7±2.3</i>	<i>41.9±2.5</i>	<i>11.1±3.4</i>	<i>C3'-endo</i>
G6	-69.9	179.6	57.1	77.9			-160.7	39.3	18.0	C3'-endo	
	-60.9	165.7	62.9	89.1			-168.2	35.4	2.5	C3'-endo	
	-60.7	165.2	61.5	82.2			-162.8	39.9	19.1	C3'-endo	
	-64.5	-179.6	50.4	83.2			-153.2	42.7	12.9	C3'-endo	
	-65.9	175.8	53.4	82.2			-160.5	41.0	22.6	C3'-endo	
	-58.5	167.3	58.9	66.2			-164.2	47.5	30.3	C3'-endo	
	<i>-63.4±4.2</i>	<i>172.3±7.1</i>	<i>57.4±4.8</i>	<i>80.1±7.7</i>			<i>-161.6±5.0</i>	<i>41.0±4.0</i>	<i>17.6±9.4</i>		
		<i>-65.9±2.5</i>	<i>179.5±3.7</i>	<i>56.9±7.5</i>	<i>80.6±10.0</i>		<i>-158.3±1.7</i>	<i>40.0±8.5</i>	<i>16.4±3.5</i>	<i>C3'-endo</i>	
A-RNA	-68	178	54	82	-153	-71	-158			C3'-endo	

[§] P ^α O5' ^β C5' ^γ C4' ^δ C3' ^ε O3' ^ζ P

Supplementary Table 2. Helical parameters for [CGCG(5-FC)G]₂ structures, for duplexes A/B and K/L from the triclinic form, duplex A/B from the rhombohedral form and the values (*italics*) for the previously reported 2'-O-Me(CGCGCG)₂ structures (PDB ids 310d and 1i7j). ^{6a,b} The parameters were calculated with the program 3DNA. ²⁶

Local base-pair parameters

bp	Shear	Stretch	Stagger	Buckle (°)	Propeller (°)	Opening (°)
1 C-G	0.08 0.24 0.01 <i>0.21</i> <i>0.03</i>	-0.22 -0.16 -0.22 <i>0.08</i> <i>-0.07</i>	0.06 -0.01 0.02 <i>-0.11</i> <i>-0.06</i>	-3.42 5.91 9.01 <i>7.21</i> <i>9.03</i>	-8.99 -8.08 -18.27 <i>-14.14</i> <i>-16.36</i>	1.72 0.10 1.85 <i>-0.79</i> <i>-2.31</i>
2 G-C	-0.28 -0.11 -1.14 <i>-0.13</i> <i>-0.18</i>	-0.14 -0.15 -0.06 <i>0.04</i> <i>-0.14</i>	0.11 0.19 0.25 <i>0.03</i> <i>0.27</i>	-4.04 1.79 -0.33 <i>3.19</i> <i>6.93</i>	-10.16 -13.01 -14.26 <i>-12.47</i> <i>-13.36</i>	1.14 1.41 6.18 <i>0.23</i> <i>-0.44</i>
3 C-G	0.17 0.26 0.18 <i>0.18</i> <i>0.19</i>	-0.14 -0.20 -0.19 <i>0.02</i> <i>-0.15</i>	0.09 0.03 0.09 <i>-0.09</i> <i>-0.07</i>	-0.76 0.65 3.69 <i>2.48</i> <i>3.68</i>	-9.91 -10.75 -17.99 <i>-14.10</i> <i>-15.82</i>	-2.02 0.45 -1.65 <i>-1.67</i> <i>-1.23</i>
4 G-C	-0.28 -0.17 -0.08 <i>-0.18</i> <i>-0.15</i>	-0.14 -0.11 -0.14 <i>0.03</i> <i>-0.18</i>	0.15 0.05 0.02 <i>0.01</i> <i>-0.04</i>	-0.30 -2.12 -3.83 <i>-1.10</i> <i>-3.04</i>	-10.33 -10.53 -13.58 <i>-13.05</i> <i>-15.39</i>	-1.20 0.10 -1.68 <i>-1.70</i> <i>-1.54</i>
5 FC-G	0.22 0.16 0.21 <i>0.28</i> <i>0.15</i>	-0.22 -0.11 -0.26 <i>0.05</i> <i>-0.14</i>	0.04 -0.02 0.15 <i>0.03</i> <i>0.12</i>	0.99 2.87 -3.41 <i>-3.51</i> <i>-5.49</i>	-13.20 -14.72 -15.20 <i>-12.62</i> <i>-14.40</i>	-2.69 -0.38 -1.10 <i>0.27</i> <i>-1.41</i>
6 G-C	-0.05 -0.08 -0.09 <i>0.06</i> <i>-0.04</i>	-0.13 -0.15 -0.13 <i>0.08</i> <i>-0.12</i>	-0.06 0.08 0.02 <i>-0.08</i> <i>-0.01</i>	-3.03 -2.63 -9.76 <i>-6.86</i> <i>-8.04</i>	-7.49 -15.12 -14.00 <i>-15.80</i> <i>-17.14</i>	-1.14 -0.34 -1.98 <i>-1.78</i> <i>-3.63</i>
Ave., st.dev.	-0.02±0.22 0.05±0.19 -0.15±0.50 <i>0.07±0.19</i> <i>-0.00±0.16</i>	-0.16±0.04 -0.15±0.03 -0.17±0.07 <i>0.05±0.03</i> <i>-0.13±0.04</i>	0.06±0.07 0.05±0.08 0.09±0.09 <i>-0.03±0.06</i> <i>0.03±0.14</i>	-1.76±2.02 1.08±3.20 -0.77±6.53 <i>0.24±5.07</i> <i>0.51±7.01</i>	-10.01±1.88 -12.03±2.73 -15.55±2.07 <i>-13.70±1.25</i> <i>-15.41±1.37</i>	-0.70±1.76 0.22±0.66 0.27±3.22 <i>-0.91±0.97</i> <i>-1.76±1.09</i>

Local base-pair step parameters

Step	Shift (Å)	Slide (Å)	Rise (Å)	Tilt	Roll	Twist
1 CG/(5-FC)G	-0.63 0.45 -0.68 <i>-0.54</i> <i>-0.37</i>	-1.88 -1.64 -1.90 <i>-1.93</i> <i>-1.82</i>	3.16 3.28 3.36 <i>3.39</i> <i>3.13</i>	-0.12 -2.09 -4.44 <i>-4.33</i> <i>-7.43</i>	7.66 9.30 9.48 <i>15.45</i> <i>15.00</i>	29.71 30.29 33.56 <i>30.53</i> <i>32.30</i>
2 G(5-FC)/GC	-0.46 0.25 -0.20 <i>-0.41</i> <i>-0.58</i>	-1.30 -1.96 -1.30 <i>-1.41</i> <i>-1.42</i>	3.24 3.27 3.15 <i>3.44</i> <i>3.29</i>	0.47 1.92 0.89 <i>-0.85</i> <i>0.45</i>	6.97 0.22 6.47 <i>10.27</i> <i>10.37</i>	33.21 34.98 33.76 <i>33.55</i> <i>34.92</i>
3 CG/CG	0.97 -0.63 0.15 <i>-0.04</i> <i>-0.02</i>	-1.80 -2.25 -1.59 <i>-1.84</i> <i>-1.67</i>	3.13 3.24 3.31 <i>3.34</i> <i>3.23</i>	0.86 -0.80 1.55 <i>-1.01</i> <i>-0.41</i>	11.41 9.09 13.87 <i>18.06</i> <i>19.90</i>	27.28 23.60 30.80 <i>29.29</i> <i>30.77</i>
4 GC/G(5-FC)	-0.61 -0.75 -0.03 <i>0.43</i> <i>0.54</i>	-1.42 -1.34 -1.37 <i>-1.40</i> <i>-1.41</i>	3.23 3.20 3.22 <i>3.48</i> <i>3.28</i>	-0.68 -2.04 0.52 <i>1.56</i> <i>1.11</i>	4.66 5.30 3.95 <i>10.36</i> <i>10.49</i>	35.54 32.20 35.39 <i>34.67</i> <i>34.37</i>
5 (5-FC)G/CG	0.65 0.37 0.49 <i>0.42</i> <i>0.39</i>	-1.91 -1.54 -1.63 <i>-1.96</i> <i>-1.82</i>	3.27 3.24 3.27 <i>3.34</i> <i>3.16</i>	1.80 0.15 4.44 <i>4.05</i> <i>5.84</i>	9.52 16.75 12.04 <i>16.27</i> <i>15.42</i>	31.74 30.75 32.15 <i>31.80</i> <i>32.31</i>
Ave., st.dev.	-0.02±0.77 -0.06±0.58 -0.06±0.43 <i>-0.03±0.45</i> <i>-0.01±0.48</i>	-1.66±0.28 -1.75±0.36 -1.56±0.24 <i>-1.71±0.28</i> <i>-1.63±0.20</i>	3.21±0.06 3.25±0.03 3.26±0.08 <i>3.40±0.06</i> <i>3.22±0.07</i>	0.47±0.95 -0.57±1.68 0.59±3.21 <i>-0.12±3.13</i> <i>-0.09±4.76</i>	8.04±2.56 8.13±6.07 9.16±4.03 <i>14.08±3.57</i> <i>14.24±3.97</i>	31.49±3.17 30.36±4.20 33.13±1.74 <i>31.97±2.19</i> <i>32.93±1.69</i>

Local base-pair helical parameters

Step	X-disp	Y-disp	h-Rise	Incl.	Tip	h-Twist
1 CG/(5-FC)G	-4.91	1.18	2.61	14.63	0.22	30.66
	-4.59	1.18	2.63	17.27	3.88	31.72
	-4.51	0.49	2.80	15.94	7.47	35.11
	-5.46	0.30	2.24	27.11	7.61	34.40
	-4.72	-0.29	2.14	24.99	12.37	36.28
2 G(5-FC)/GC	-3.29	0.87	2.91	12.02	-0.81	33.92
	-3.28	-0.13	3.27	0.36	-3.19	35.03
	-3.13	0.47	2.85	11.01	-1.51	34.37
	-3.88	0.55	2.90	17.29	1.43	35.06
	-3.64	0.99	2.77	16.83	-0.73	36.38
3 CG/CG	-5.61	-1.74	2.24	22.95	-1.73	29.54
	-7.44	1.24	2.25	21.23	1.87	25.28
	-4.80	-0.03	2.40	24.60	-2.74	33.75
	-5.59	-0.06	1.92	32.15	1.80	34.32
	-5.00	-0.02	1.85	33.47	0.69	36.51
4 GC/G(5-FC)	-2.96	0.90	3.04	7.59	1.10	35.84
	-3.25	1.00	2.99	9.46	3.65	32.68
	-2.79	0.12	3.06	6.47	-0.85	35.60
	-3.72	-0.47	2.97	16.91	-2.55	36.18
	-3.71	-0.73	2.76	17.26	-1.82	35.91
5 (5-FC)G/CG	-4.83	-0.86	2.63	16.92	-3.20	33.15
	-4.81	-0.60	2.15	29.05	-0.26	34.92
	-4.47	-0.18	2.55	20.73	-7.64	34.56
	-5.28	-0.16	2.16	27.44	-6.83	35.85
	-4.80	0.09	2.14	25.72	-9.74	36.17
Ave., st. dev.	-4.32±1.14 -4.67±1.71 -3.94±0.91 -4.79±0.91 -4.37±0.65	0.07±1.29 0.06±1.03 0.17±0.30 0.03±0.40 0.01±0.63	2.69±0.31 2.66±0.48 2.73±0.26 2.44±0.47 2.33±0.41	14.82±5.72 15.47±11.01 15.75±7.28 24.18±6.76 23.65±6.89	-0.88±1.68 1.19±2.96 -1.05±5.46 0.29±5.38 0.16±7.94	32.62±2.53 31.93±3.98 34.68±0.71 35.16±0.84 36.25±0.23