Table S1: Enthalpy Comparison Between Duplexes Containing I·U, A-U, and G-U Pairs

Duplex[a]	-ΔHº (kcal/mol)[b]	-NN A-U (kcal/mol)[c]	ΔA-U (kcal/mol)[d]	-NN G-U (kcal/mol)[e]	ΔG-U (kcal/mol)[f]
Internal					
GCAICGC CGUUGCG	71.5	65.45	-6.05	63.03	-8.47
GCAIGGC CGUUCCG	74.2	67.28	-6.92	65.30	-8.90
GCCIAGC CGGUUCG	70.0	67.28	-2.72	68.46	-1.54
GCCICGC CGGUGCG	73.7	72.02	-1.68	68.38	-5.32
GCCIGGC CGGUCCG	85.2	73.85	-11.35	70.65	-14.55
GCCIUGC CGGUACG	68.7	69.80	1.10	70.18	1.48
GCGIAGC CGCUUCG	68.8	66.53	-2.27	68.43	-0.37
GCGICGC CGCUGCG	72.8	71.27	-1.53	68.35	-4.45
GCGIGGC CGCUCCG	72.4	73.10	0.70	70.62	-1.78
GCGIUGC CGCUACG	75.6	67.56	-8.04	64.37	-11.23
GCUIAGC CGAUUCG	64.6	61.62	-2.98	66.93	2.33
GCUICGC CGAUGCG	76.1	66.36	-9.74	66.85	-9.25
GCUIGGC CGAUCCG	71.2	68.19	-3.01	69.12	-2.08
GCUIUGC CGAUACG	75.4	64.14	-11.26	62.87	-12.53
GUCUAGIC CIGAUCUG	72.1[g]	72.72	0.62	66.88	-5.22
Average			-4.34		-5.46

Duplex[a]	-ΔHº (kcal/mol)[b]	-NN A-U (kcal/mol)[c]	ΔA-U (kcal/mol)[d]	-NN G-U (kcal/mol)[e]	ΔG-U (kcal/mol)[f]
Terminal					
GCGCAI CGCGUU	58.8	50.33	-8.47	46.72	-12.08
GCGCCI CGCGGU	64.7	56.90	-7.80	52.07	-12.63
GCGCGI CGCGCU	61.6	56.15	-5.45	52.04	-9.56
GCGCUI CGCGAU	50.9	51.24	0.34	50.54	-0.36
IAGCGC UUCGCG	53.1	50.37	-2.73	56.38	3.28
ICGCGC UGCGCG	63.3	55.11	-8.19	56.30	-7.00
IGGCGC UCCGCG	64.8	56.94	-7.86	58.57	-6.23
IUGCGC UACGCG	67.1	52.89	-14.21	52.32	-14.78
ICCGGU UGGCCI	55.7[g]	49.17	-6.53	51.55	-4.15
IGAGGG UCUCCCGG	54.8[g]	52.05	-2.75	53.68	-1.12
Average			-6.37		-6.46
Tandem					
GUCUIGAC CAGIUCUG	75.8[g]	72.72	-3.08	77.55	1.75
GGCIUGCC CCGUICGG	59.9[h]	83.19	23.29	78.74	18.84
GGCUIGCC CCGIUCGG	68.6[h]	81.58	12.98	86.41	17.81
GGCIIGGC CCGUUCCG	72.9[h]	80.67	7.77	84.12	11.22
Average			10.24		12.41

[a] For each duplex, the top strand is written 5' to 3' and the bottom strand is written 3' to 5'.
[b] The measured -ΔHº of the listed duplex, using values from the log C_T plots, if available.
[c] -ΔHº calculated using the nearest neighbor model *(18)* for the duplex if the I•U pair was an A-U pair.
[c] The difference in -ΔHº between the -NN A-U value and the measured -ΔHº.
[e] -ΔHº calculated using the nearest neighbor model (*18*) for the duplex if the I•U pair was a G-U pair.
[f] The difference in -ΔHº between the -NN G-U value and the measured -ΔHº.
[g] Ref *(17)* .
[h] Ref *(16)* .

Table S2: Entropy Comparison Between Duplexes Containing I·U, A-U, and G-U Pairs

Duplex[a]	-ΔHº (kcal/mol)[b]	-NN A-U (kcal/mol)[c]	ΔA-U (kcal/mol)[d]	-NN G-U (kcal/mol)[e]	ΔG-U (kcal/mol)[f]
Internal					
GCAICGC CGUUGCG	71.5	65.45	-6.05	63.03	-8.47
GCAIGGC CGUUCCG	74.2	67.28	-6.92	65.30	-8.90
GCCIAGC CGGUUCG	70.0	67.28	-2.72	68.46	-1.54
GCCICGC CGGUGCG	73.7	72.02	-1.68	68.38	-5.32
GCCIGGC CGGUCCG	85.2	73.85	-11.35	70.65	-14.55
GCCIUGC CGGUACG	68.7	69.80	1.10	70.18	1.48
GCGIAGC CGCUUCG	68.8	66.53	-2.27	68.43	-0.37
GCGICGC CGCUGCG	72.8	71.27	-1.53	68.35	-4.45
GCGIGGC CGCUCCG	72.4	73.10	0.70	70.62	-1.78
GCGIUGC CGCUACG	75.6	67.56	-8.04	64.37	-11.23
GCUIAGC CGAUUCG	64.6	61.62	-2.98	66.93	2.33
GCUICGC CGAUGCG	76.1	66.36	-9.74	66.85	-9.25
GCUIGGC CGAUCCG	71.2	68.19	-3.01	69.12	-2.08
GCUIUGC CGAUACG	75.4	64.14	-11.26	62.87	-12.53
GUCUAGIC CIGAUCUG	72.1[g]	72.72	0.62	66.88	-5.22
Average			-4.34		-5.46

Duplex[a]	-ΔHº (kcal/mol)[b]	-NN A-U (kcal/mol)[c]	ΔA-U (kcal/mol)[d]	-NN G-U (kcal/mol)[e]	ΔG-U (kcal/mol)[f]
Terminal					
GCGCAI CGCGUU	58.8	50.33	-8.47	46.72	-12.08
GCGCCI CGCGGU	64.7	56.90	-7.80	52.07	-12.63
GCGCGI CGCGCU	61.6	56.15	-5.45	52.04	-9.56
GCGCUI CGCGAU	50.9	51.24	0.34	50.54	-0.36
IAGCGC UUCGCG	53.1	50.37	-2.73	56.38	3.28
ICGCGC UGCGCG	63.3	55.11	-8.19	56.30	-7.00
IGGCGC UCCGCG	64.8	56.94	-7.86	58.57	-6.23
IUGCGC UACGCG	67.1	52.89	-14.21	52.32	-14.78
ICCGGU UGGCCI	55.7[g]	49.17	-6.53	51.55	-4.15
IGAGGG UCUCCCGG	54.8[g]	52.05	-2.75	53.68	-1.12
Average			-6.37		-6.46
Tandem					
GUCUIGAC CAGIUCUG	75.8[g]	72.72	-3.08	77.55	1.75
GGCIUGCC CCGUICGG	59.9[h]	83.19	23.29	78.74	18.84
GGCUIGCC CCGIUCGG	68.6[h]	81.58	12.98	86.41	17.81
GGCIIGGC CCGUUCCG	72.9[h]	80.67	7.77	84.12	11.22
Average			10.24		12.41

[a] For each duplex, the top strand is written 5' to 3' and the bottom strand is written 3' to 5'.
[b] The measured -ΔHº of the listed duplex, using values from the log C_T plots, if available.
[c] -ΔHº calculated using the nearest neighbor model *(18)* for the duplex if the I•U pair was an A-U pair.
[c] The difference in -ΔHº between the -NN A-U value and the measured -ΔHº.
[e] -ΔHº calculated using the nearest neighbor model (*18*) for the duplex if the I•U pair was a G-U pair.
[f] The difference in -ΔHº between the -NN G-U value and the measured -ΔHº.
[g] Ref *(17)* .
[h] Ref *(16)* .

Table S3: I·U Contribution to Thermodynamics[a]

Duplex[b]	ΔH° (kcal/mol)	ΔS° (eu)	$\Delta G°_{37}$ (kcal/mol)
GCAICGC CGUUGCG	-24.27	-74.8	-1.12
GCAIGGC CGUUCCG	-24.22	-74.6	-1.08
GCCIAGC CGGUUCG	-19.98	-62.5	-0.60
GCCICGC CGGUGCG	-23.52	-69.5	-2.03
GCCIGGC CGGUCCG	-32.27	-94.7	-2.87
GCCIUGC CGGUACG	-18.72	-60.3	-0.02
GCGIAGC CGCUUCG	-21.53	-67.2	-0.72
GCGICGC CGCUGCG	-25.37	-76.6	-1.61
GCGIGGC CGCUCCG	-22.22	-62.3	-2.96
GCGIUGC CGCUACG	-28.37	-85.0	-2.00
GCUIAGC CGAUUCG	-17.49	-59.7	0.98
GCUICGC CGAUGCG	-28.83	-89.2	-1.21
GCUIGGC CGAUCCG	-21.18	-65.5	-0.94
GCUIUGC CGAUACG	-28.33	-92.6	0.36
GCGCAI CGCGUU	-11.57	-28.8	-2.62
GCGCCI CGCGGU	-14.52	-40.6	-1.98
GCGCGI CGCGCU	-14.17	-39.8	-1.92
GCGCUI CGCGAU	-3.63	-8.5	-0.97
IAGCGC UUCGCG	-5.83	-15.6	-1.02
ICGCGC UGCGCG	-15.87	-42.5	-2.67
IGGCGC UCCGCG	-14.62	-41.5	-1.83
IUGCGC UACGCG	-19.87	-53.0	-3.42
ICCGGU UGGCCI	-21.85[c]	-57.1[c]	-4.14[c]
IGAGGG UCUCCCGG	-9.51[c]	-23.1[c]	-2.44[c]
GUCUAGIC CIGAUCUG	-36.00[c]	-99.0[c]	-5.33[c]
GUCUIGAC CAGIUCUG	-21.04[c]	-66.1[c]	-0.64[c]
GGCIUGCC CCGUICGG	-6.97[d]	-29.1[d]	2.04[d]
GGCUIGCC CCGIUCGG	-15.67[d]	-51.5[d]	0.24[d]
GGCIIGGC CCGUUCCG	-19.97[d]	-66.7[d]	0.67[d]

[a] Calculated by subtracting the Watson-Crick nearest neighbor contributions *(18)* from the measured value of the duplex containing the I·U pair(s). Note: For duplexes containing multiple I·U pairs, these values listed are the total I·U contribution, not the contribution per I·U pair.

[b] For each duplex, the top strand is written 5' to 3' and the bottom strand is written 3' to 5'.

[c] Raw data from Ref (*17*).

[d] Raw data from Ref (*16*).