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Improved free-energy parameters for predictions of RNA duplex stability

SUSAN M. FREIER*, RYSZARD KIERZEK†, JOHN A. JAEGER*, NAOKI SUGIMOTO*, MARVIN H. CARUTHERS‡, THOMAS NEILSON§¶, AND DOUGLAS H. TURNER*‖

*Department of Chemistry, University of Rochester, Rochester, NY 14627; †Institute of Bioorganic Chemistry, Polish Academy of Sciences, 60-704 Poznan, Noskowskiego 12/14, Poland; ‡Department of Chemistry and Biochemistry, University of Colorado, Boulder, CO 80309; and §Department of Biochemistry, McMaster University, Hamilton, ON L8N 3Z5, Canada

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ABSTRACT **Thermodynamic parameters for prediction of RNA duplex stability are reported. One parameter for duplex initiation and 10 parameters for helix propagation are derived from enthalpy and free-energy changes for helix formation by 45 RNA oligonucleotide duplexes. The oligomer sequences were chosen to maximize reliability of secondary structure predictions. Each of the 10 nearest-neighbor sequences is well-represented among the 45 oligonucleotides, and the sequences were chosen to minimize experimental errors in ΔG° at 37°C. These parameters predict melting temperatures of most oligonucleotide duplexes within 5°C. This is about as good as can be expected from the nearest-neighbor model. Free-energy changes for helix propagation at dangling ends, terminal mismatches, and internal G·U mismatches, and free-energy changes for helix initiation at hairpin loops, internal loops, or internal bulges are also tabulated.**

Stabilities of RNA duplexes and secondary structures of RNAs are often predicted by using free-energy parameters from a nearest-neighbor model (1–5). Sometimes, however, predictions are inconsistent with experimental data (6–10). One factor hindering successful predictions is that the reliability of parameters was limited by the availability of model oligonucleotides (2). Recent breakthroughs in synthesis of RNA oligoribonucleotides (11–16) permit design of oligonucleotides to provide improved parameters. This paper presents thermodynamic parameters derived from data on 45 complementary RNA duplexes. The parameters are able to predict the stabilities of RNA duplexes within the limits of the nearest-neighbor model.

MATERIALS AND METHODS

Choice of Sequences. Sequences were selected to minimize errors in the free-energy change for duplex formation at 37°C, ΔG°_{37} (17, 18). Thus, as much as possible, melting temperatures at 0.1 mM are near 37°C to minimize extrapolation. The oligomers were also chosen to independently represent all 10 nearest-neighbor sequences comprising Watson–Crick base pairs.

Oligonucleotide Synthesis. Oligonucleotides not reported elsewhere were synthesized on solid support using phosphoramidite procedures and purified as described (11, 19). Purities were confirmed by high-performance liquid chromatography for all oligomers.

Thermodynamic Parameters. Absorbance vs. temperature melting curves were measured in 1 M NaCl/0.005 M Na_2HPO_4/0.5 mM EDTA (disodium salt), pH 7, as described (11). Concentrations were determined from the high-temperature absorbance using extinction coefficients calculated as described (20). In units of 0.1 $mM^{-1}\cdot cm^{-1}$, calculated high-temperature extinction coefficients at 280 nm not reported elsewhere are as follows: GUGCAC, 2.77; GUCUAGAC, 3.66; GAUAUAUC, 3.05; GUAUAUAC, 3.00. Thermodynamic parameters of helix formation were obtained by two methods. (*i*) Individual melting curves were fit to a two-state model with sloping baselines and the enthalpy and entropy changes derived from the fits were averaged (21), and (*ii*) reciprocal melting temperature, t_m^{-1}, vs. log (C_T) was plotted as suggested by a rearrangement of Eq. 2 to yield enthalpy and entropy changes (3). On the basis of reproducibility, estimated error limits are ±5% for the enthalpy and entropy changes, ΔH° and ΔS°, and ±2% for the free energy change, ΔG°, at the t_m (19).

Nearest-Neighbor Thermodynamic Parameters. Enthalpy and free-energy changes for helix initiation and propagation were obtained by multiple linear regression (22) to observed oligonucleotide thermodynamic parameters upon the nearest-neighbor model (3, 18). Parameters derived from t_m^{-1} vs. log (C_T) plots were used because they are the most reproducible between different laboratories. For an oligomer to be included in the regression analysis, the average ΔH° from shapes of melting curves and the ΔH° from the t_m^{-1} vs. log (C_T) plot had to agree within 15%. This indicates the transitions are close to two state, so the thermodynamic analysis is reasonable (21, 23, 24). UCAUGA fit the linear regression poorly and is significantly less stable than UGAUCA, which contains the same nearest neighbors. This suggests a nearest-neighbor model is not adequate for UCAUGA, so it was omitted from the analysis. No correction was made for the 3′-terminal phosphate present on some oligomers. This correction is ≈0.2 kcal/mol and therefore negligible (19). The experimental data were weighted (25) assuming a ±5% error in the experimental enthalpy changes and an uncertainty of ±2% in the free-energy changes at the t_m (at 0.1 mM). These errors were propagated to obtain weights for ΔG°_{37}.

RESULTS

Measured enthalpy and entropy changes for helix formation are listed in Table 1. They were used to calculate ΔG°_{37} according to the equation

$$\Delta G^\circ_{37} = \Delta H^\circ - t\Delta S^\circ = \Delta H^\circ - 310.15\ \Delta S^\circ, \qquad [1]$$

where 310.15 is 37°C in Kelvin. According to the nearest-neighbor model (3), the standard-state free energy of helix formation for an oligonucleotide is the sum of three terms: (*i*) a free-energy change for helix initiation associated with

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Abbreviation: eu, entropy units.

¶Deceased, November 1985.
‖To whom reprint requests should be addressed.

Table 1. Thermodynamic parameters for duplex formation in 1 M NaCl

Sequence	Source or ref.	$-\Delta H^\circ$, kcal/mol	$-\Delta S^\circ$, eu	$-\Delta G^\circ_{37}$, kcal/mol	t_m at 0.1 mM, °C Measured	Predicted
		Two-state transitions				
AGAUAUCU	11	64.5	186.8	6.58	41.4	44.2
AUCUAGAU	11	59.9	169.9	7.20	45.1	44.2
AACUAGUU	11	54.6	153.0	7.16	45.7	42.6
AGUUAACU	11	52.4	148.5	6.36	41.1	42.6
ACUUAAGU	11	47.2	132.4	6.16	40.2	42.6
GAACGUUC	11	77.0	218.3	9.30	52.3	51.9
GUUCGAAC	11	74.2	211.0	8.76	50.4	51.9
UCUAUAGA	11	62.1	177.7	6.96	43.6	45.0
UAGAUCUA	11	60.2	170.6	7.25	45.3	45.0
GUCGAC	11	53.6	150.1	7.09	45.4	44.8
GACGUC	11	58.1	163.5	7.35	46.2	44.8
GCCGGCp	18	62.7	166.0	11.2	67.2	66.0
GGCGCCp	26	67.8	182.0	11.3	65.2	66.0
CAGCUGp	*	51.6	144.7	6.68	43.2	42.8
CUGCAGp	*	55.4	155.7	7.11	45.3	42.8
ACUAUAGU	11	59.2	168.4	6.98	44.0	44.0
UGAUCA	11	44.7	128.0	5.05	32.7	35.1
GCAUGC	*	62.3	177.2	7.38	45.7	48.0
GUGCAC	This work	59.6	167.5	7.65	47.6	47.5
ACCGGUp	21	59.8	164.5	8.51	52.4	53.4
UCCGGAp	19	51.9	142.3	7.79	50.1	53.6
AGGCCUp	19	52.0	139.9	8.63	55.7	56.2
AGCGCU	26	50.1	135.7	7.99	52.1	53.9
$CA_6G + CU_6G$	27	53.8	158.7	4.61	26.3	25.5
$CA_7G + CU_7G$	27	59.8	175.1	5.47	31.6	31.3
GUCUAGAC	This work	76.0	212.5	10.1	56.2	54.7
GAUAUAUC	This work	62.0	180.4	6.09	39.1	37.4
GUAUAUAC	This work	63.4	185.1	5.94	38.3	36.8
AUGCGCAUp	28	64.4	174.8	10.2	60.3	60.3
$(GA)_3 + (UC)_3$	†	62.1	178.1	6.95	39.1	38.7
$(AG)_4 + (CU)_4$	†	73.7	201.7	11.1	57.6	53.9
CCGG	21	34.2	95.6	4.55	27.1	22.6
GGCC	20	35.8	98.1	5.37	34.4	35.4
GCGC	18	30.5	83.4	4.61	26.5	31.4
CGGCCGp	18	54.1	142.6	9.90	63.3	60.4
GCGCGCp	18	66.0	178.5	10.6	62.1	64.2
CGCGCGp	26	54.5	146.4	9.12	57.9	58.2
UGCGCA	26	51.5	139.7	8.22	53.1	54.0
AUGUACAUp	28	55.9	159.3	6.49	41.6	44.2
AUACGUAU	28	54.4	154.2	6.53	41.9	41.8
GCUAGC	28	59.1	165.1	7.92	49.3	48.0
AAUGCAUUp	‡	59.8	169.7	7.2	45.0	43.2
UAUGCAUAp	‡	67.7	195.0	7.3	44.5	45.4
GAUGCAUCp	‡	72.8	201.9	10.1	57.2	54.8
CAUGCAUGp	‡	73.7	206.3	9.7	54.8	51.7
		Anomalous two-state transition				
UCAUGA	11	49.1	145.9	3.86	25.9	35.1
		Non-two-state transitions				
AGUAUACU	11	53.1	149.1	6.80	43.7	44.0
UGGCCAp	19	59.9	164.1	8.99	55.2	56.1
AUGCAUp	28	41.7	119.2	4.71	30.0	33.1
A_7U_7p	24	77.9	229.7	6.66	41.0	44.8

Thermodynamic parameters were determined from plots of t_m^{-1} vs. log C_T. Although estimated errors in ΔH° and ΔS° are ± 5%, additional significant figures are given to allow accurate calculation of t_m. Predicted t_m values are based on nearest-neighbor parameters before rounding off. Transitions are classified as two-state if the enthalpy change obtained from the shapes of melting curves agrees within 15% with that obtained from plots of t_m^{-1} vs. log C_T. Thermodynamic parameters from shapes of melting curves that will not be reported elsewhere are GUGCAC (56.2, 156.8), GUCUAGAC (72.2, 200.6), GAUAUAUC (59.5, 172.1), GUAUAUAC (64.0, 187.3). Here the values in parentheses are ΔH° in kcal/mol and ΔS° in eu, respectively. The absorbance vs. temperature data of Nelson *et al.* (27) were reanalyzed using a model with sloping baselines to conform with the analysis of the other oligomers.

*D. Groebe, V. Cameron, S.M.F., D.H.T., and O. C. Uhlenbeck, unpublished data.

†J.A.J. and D.H.T., unpublished data.

‡N.S., R.K., and D.H.T., unpublished data.

forming the first base pair in the duplex, (*ii*) a sum of propagation free energies for forming each subsequent base pair, and (*iii*) a symmetry correction if the sequence is self-complementary (17, 29, 30). Therefore, the enthalpy and free-energy changes for the two-state transitions in Table 1 were used with the nearest-neighbor model to obtain parameters for helix initiation and propagation. These are listed in Table 2.

When the enthalpy change for helix initiation was included in the fit, a positive value with a large error estimate was obtained. The results were also sensitive to the oligomers included in the fit. Helix initiation does not involve stacking, and the enthalpy change for initiation is therefore often assumed to be zero (3). Since our results are ambiguous, we also assume zero enthalpy change for initiation.

DISCUSSION

Comparison of Predicted and Measured Duplex Stabilities. Measurements of thermodynamic parameters of oligomers with identical nearest neighbors but different sequences suggest the nearest-neighbor model should be able to predict ΔG°_{37}, ΔH°, and t_m (at 0.1 mM) with average deviations of roughly 6%, 8%, and 2 K, respectively (11). Thus, one test of the parameters in Table 2 is how well they are able to predict the experimental results in Table 1. For example, the predicted free-energy change of helix formation for GGCC is

$$\Delta G^\circ_{37}\ (\text{predicted}) = 2\Delta G^\circ_{37}\ (\overrightarrow{\text{GG}}/\overleftarrow{\text{CC}}) + \Delta G^\circ_{37}\ (\overrightarrow{\text{GC}}/\overleftarrow{\text{CG}}) + \Delta G^\circ_{37}\ (\text{initiation}) + \Delta G^\circ_{37}\ (\text{symmetry}) = 2\,(-2.9) + (-3.4) + 3.4 + 0.4 = -5.4\ \text{kcal/mol}.$$

The measured value is −5.4 kcal/mol. Similarly, the predicted enthalpy change for GGCC is ΔH° (predicted) = 2(−12.2) + (−14.2) + 0 = −38.6 kcal/mol. The measured ΔH° is −35.8 kcal/mol. Note that ΔH° for initiation is assumed to be 0 kcal/mol, and there is no symmetry term for ΔH°. The predicted entropy change for GGCC is ΔS° (predicted) = 2(−29.7) + (−34.9) + (−10.8) + (−1.4) = −106.5 eu. The measured ΔS° is −98.1 eu. Note that for this self-complementary sequence, ΔG°_{37} (symmetry) and ΔS° (symmetry) are 0.4 kcal/mol and −1.4 eu, respectively. For non-self-complementary sequences, the ΔG° (symmetry) and ΔS° (symmetry) are zero.

For self-complementary sequences, the t_m in °C is predicted from (3)

$$t_m = \frac{\Delta H^\circ}{\Delta S^\circ + R \ln(C_T)} - 273.15. \qquad [2]$$

Here, R is 1.987 cal·K^{-1}·mol^{-1}, and C_T is the total strand concentration. For non-self-complementary sequences, C_T in Eq. **2** is replaced by $C_T/4$. Use of Eq. **2** can be simplified by including all the changes between self-complementary and non-self-complementary oligomers in a constant A:

$$t_m = \frac{\Delta H^\circ}{A + \Delta S^\circ_{NN} + R \ln(C_T)} - 273.15. \qquad [3]$$

Here, ΔS°_{NN} is the entropy change without any symmetry term, C_T is always the total strand concentration, and A is −1.4 and −2.8 eu for self- and non-self-complementary oligomers, respectively. Note that in Eqs. **1–3**, if the units for ΔH° are kcal/mol, they must be multiplied by 1000 if ΔS° is in eu. For GGCC, the predicted t_m at 0.1 mM is 36.1°C, close to the experimental value of 34.4°C (18). Part of this difference is attributable to round-off errors present in Table 2. If the parameters from the linear regression are not rounded off, the predicted t_m is 35.4°C, as listed in Table 1. When similar comparisons are made for the 45 sequences in Table 1 used to derive the parameters in Table 2, the largest difference between predicted and measured t_m values at 0.1 mM is 5°C with an average deviation of 1.6°C. For ΔG°, the largest difference is 11%, with an average of 4%. For ΔH°, the largest difference is 17%, with an average of 7%. Thus, the predictive capability of the parameters in Table 2 is about equal to that expected for the nearest-neighbor model (11).

Predictions of thermodynamic properties for oligomers containing only A·U base pairs require a ΔS° and ΔG° for helix initiation at an A·U base pair. Since all two-state oligomers in Table 1 contain G·C pairs, the data cannot provide a ΔG° for helix initiation at A·U base pairs. Values derived previously for initiation at A·U or dA·dT pairs, however, range from 3 to 4 kcal/mol at 37°C (31–35). It therefore seems reasonable to use for initiation at both A·U and G·C pairs, the ΔG° of 3.4 kcal/mol listed in Table 2.

The prediction of thermodynamic parameters is based on a two-state model for the transitions. Presumably, transitions that are not two state require a statistical model for accurate predictions (5). Four oligomers with non-two-state transitions are listed in Table 1. When the ΔG°_{37}, ΔH°, and t_m of these oligomers are predicted with the parameters in Table 2, the average differences relative to measured values are 7%, 7%, and 2°C, respectively. This suggests the two-state model can also provide reasonable approximations for oligomers that do not have strictly two-state transitions.

Because the sequences were designed to provide reliable

Table 2. Thermodynamic parameters for RNA helix initiation and propagation in 1 M NaCl

Propagation sequence	ΔH°, kcal/mol	ΔS°, eu	ΔG°_{37}, kcal/mol
$\overrightarrow{\text{AA}}/\overleftarrow{\text{UU}}$	−6.6	−18.4	−0.9
$\overrightarrow{\text{AU}}/\overleftarrow{\text{UA}}$	−5.7	−15.5	−0.9
$\overrightarrow{\text{UA}}/\overleftarrow{\text{AU}}$	−8.1	−22.6	−1.1
$\overrightarrow{\text{CA}}/\overleftarrow{\text{GU}}$	−10.5	−27.8	−1.8
$\overrightarrow{\text{CU}}/\overleftarrow{\text{GA}}$	−7.6	−19.2	−1.7
$\overrightarrow{\text{GA}}/\overleftarrow{\text{CU}}$	−13.3	−35.5	−2.3
$\overrightarrow{\text{GU}}/\overleftarrow{\text{CA}}$	−10.2	−26.2	−2.1
$\overrightarrow{\text{CG}}/\overleftarrow{\text{GC}}$	−8.0	−19.4	−2.0
$\overrightarrow{\text{GC}}/\overleftarrow{\text{CG}}$	−14.2	−34.9	−3.4
$\overrightarrow{\text{GG}}/\overleftarrow{\text{CC}}$	−12.2	−29.7	−2.9
Initiation	(0)	−10.8	3.4
Symmetry correction (self-complementary)	0	−1.4	0.4
Symmetry correction (non-self-complementary)	0	0	0

Arrows point in a 5′ to 3′ direction. For example, $\overrightarrow{\text{GU}}/\overleftarrow{\text{CA}}$ is the duplex between GpU and ApC. Values were derived by fitting thermodynamic parameters determined from t_m^{-1} vs. log C_T plots for the two-state transitions in Table 1. The enthalpy change for helix initiation was assumed to be zero.

Table 3. Free-energy increments for unpaired terminal nucleotides

	X					X			
	A	C	G	U		A	C	G	U
	3′ dangling ends					5′ dangling ends			
$\overrightarrow{AX}$ / U ←	−0.8	−0.5	−0.8	−0.6	$\overrightarrow{XA}$ / U ←	−0.3	−0.3	−0.4	−0.2
$\overrightarrow{CX}$ / G ←	−1.7	−0.8	−1.7	−1.2	$\overrightarrow{XC}$ / G ←	−0.5	−0.2	−0.2	−0.1
$\overrightarrow{GX}$ / C ←	−1.1	−0.4	−1.3	−0.6	$\overrightarrow{XG}$ / C ←	−0.2	−0.3	−0.0	−0.0
$\overrightarrow{UX}$ / A ←	−0.7	−0.1	−0.7	−0.1	$\overrightarrow{XU}$ / A ←	−0.3	−0.2	−0.2	−0.2

Free-energy parameters, in kcal/mol, for RNA at 37°C in 1 M NaCl. From refs. 18, 20, 21, and 26, and N.S. and D.H.T., unpublished data.

parameters near 37°C, thermodynamic properties near 37°C will be predicted most accurately. For example, it is encouraging that the ΔH° for an AA stack is essentially identical to the ΔH° measured calorimetrically for poly(A)·poly(U) at 37°C (24, 36–38). Uncertainties in predicted enthalpy changes along with heat capacity effects omitted from the model (19, 21, 39), however, will lead to less-reliable predictions at temperatures far from 37°C.

Comparison with Results of Borer *et al.* (3). In the pioneering work of Borer *et al.* (3), six parameters for nearest-neighbor interactions were determined. Table 2 contains parameters for all 10 nearest neighbors. Several of the parameters for ΔH° and ΔS° differ by about a factor of 2 from those determined previously. Differences in ΔG°_{37} are much less, however, because the differences in ΔH° and ΔS° compensate. The largest changes in ΔG°_{37} are for GG nearest neighbors and for helix initiation (18).

Prediction of Secondary Structure. The prediction of RNA secondary structure from sequence is a major application of the parameters in Table 2. For these parameters to be useful for predicting secondary structure, they must be combined with thermodynamic parameters for hairpin loops, internal loops and bulges, mismatches, unpaired terminal nucleotides, and other structures. Although parameters for such structures have been tabulated (2, 10, 40), they are correlated to the helix propagation parameters and should be recalculated with the parameters in Table 2 (41). In addition, previous compilations of loop free-energy changes are at 25°C and free-energy changes at 37°C are necessary for use with the values in Table 2. Unfortunately, for many loop structures experimental data are limited. To obtain reliable parameters, it will be necessary to design and study more model compounds. For use until such data are available, we have combined the limited data in the literature with data recently obtained in our laboratory and calculated updated free-energy parameters for common RNA structures. These are listed in Tables 3–6. For several parameters, the experimental data are so limited that assumptions were made without good justification. These parameters are marked with an asterisk. Parameters for unpaired terminal nucleotides and

Table 4. Free-energy increments for terminal mismatches and base pairs

	Y					Y			
X	A	C	G	U	X	A	C	G	U
			$\overrightarrow{GX}$ / CY ←					$\overrightarrow{CX}$ / GY ←	
A	−1.1	−1.3	−1.3	−2.3	A	−1.9	−2.0	−1.9	−1.8
C	−1.1	−0.6	−3.4	−0.5	C	−1.0	−1.1	−2.9	−0.8
G	−1.6	−2.9	−1.4	−1.4	G	−1.9	−2.0	−1.9	−1.6
U	−2.1	−0.8	−2.3	−0.7	U	−1.7	−1.5	−1.9	−1.2
	Y					Y			
X	A	C	G	U	X	A	C	G	U
			$\overrightarrow{AX}$ / UY ←					$\overrightarrow{UX}$ / AY ←	
A	−0.8	−1.0	−1.0	−0.9	A	−1.0	−0.8	−1.1	−1.1
C	−0.7	−0.7	−2.1	−0.7	C	−0.7	−0.6	−2.3	−0.5
G	−0.8	−1.7	−1.0	−0.9	G	−1.1	−1.8	−1.2	−0.9
U	−0.9	−0.8	−0.9	−0.8	U	−0.9	−0.6	−1.0	−0.5

Free-energy parameters, in kcal/mol, for RNA at 37°C in 1 M NaCl. From ref. 42, and N.S. and D.H.T., unpublished data.

Table 5. Free-energy increments for internal G·U pairs

X	Y	$\overrightarrow{XG}$ / YU ←	$\overrightarrow{XU}$ / YG ←
A	U	−0.5*	−0.7
C	G	−1.5	−1.5
G	C	−1.3	−1.9
U	A	−0.7	−0.5*
G	U	−0.5*	−0.5*
U	G	−0.6	−0.5*

Free-energy parameters, in kcal/mol, for RNA at 37°C in 1 M NaCl. From ref. 28.
*Parameters are based on untested assumptions and are particularly unreliable.

Table 6. Free-energy increments for loops

Loop size	Internal loop*†	Bulge loop*‡	Hairpin loop*§
1	—	+3.3	—
2	+0.8	+5.2	—
3	+1.3	+6.0	+7.4
4	+1.7	+6.7	+5.9
5	+2.1	+7.4	+4.4
6	+2.5	+8.2	+4.3
7	+2.6	+9.1	+4.1
8	+2.8	+10.0	+4.1
9	+3.1	+10.5	+4.2
10	+3.6	+11.0	+4.3
12	+4.4	+11.8	+4.9
14	+5.1	+12.5	+5.6
16	+5.6	+13.0	+6.1
18	+6.2	+13.6	+6.7
20	+6.6	+14.0	+7.1
25	+7.6	+15.0	+8.1
30	+8.4	+15.8	+8.9

Free-energy parameters, in kcal/mol, for RNA at 37°C in 1 M NaCl.
*Parameters are based on untested assumptions and are particularly unreliable.
†Calculated from parameters in Table 2 and data from refs. 5 and 43 and N.S. and D.H.T., unpublished data. When experimental data were not available, increments were interpolated or derived from ref. 10.
‡Calculated from parameters in Table 2 and data from refs. 44 and 45, and C. E. Longfellow and D.H.T., unpublished data. When experimental data were not available, increments were interpolated or derived from ref. 10.
§Calculated from parameters in Table 2 and data from refs. 4 and 45–47. When experimental data were not available, increments were interpolated or derived from ref. 10.

terminal mismatches are not included in most algorithms for structure prediction (48, 49). In spite of the inability to include some parameters and the uncertainties in others, when the parameters were used with the program of Zuker and Stiegler (9, 48) to predict secondary structures for 142 randomly chosen tRNA sequences, 82% of the four major stems of the cloverleaf model were predicted within 2 base pairs. The parameters listed by Salser (40) predicted 67% of these stems. Thus, the parameters in Tables 2–6 predict almost half of the major stems missed by the most commonly used set of parameters.

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