Supplementary Information

for

Probing the stabilizing effects of modified nucleotides in the bacterial decoding region of 16S ribosomal RNA

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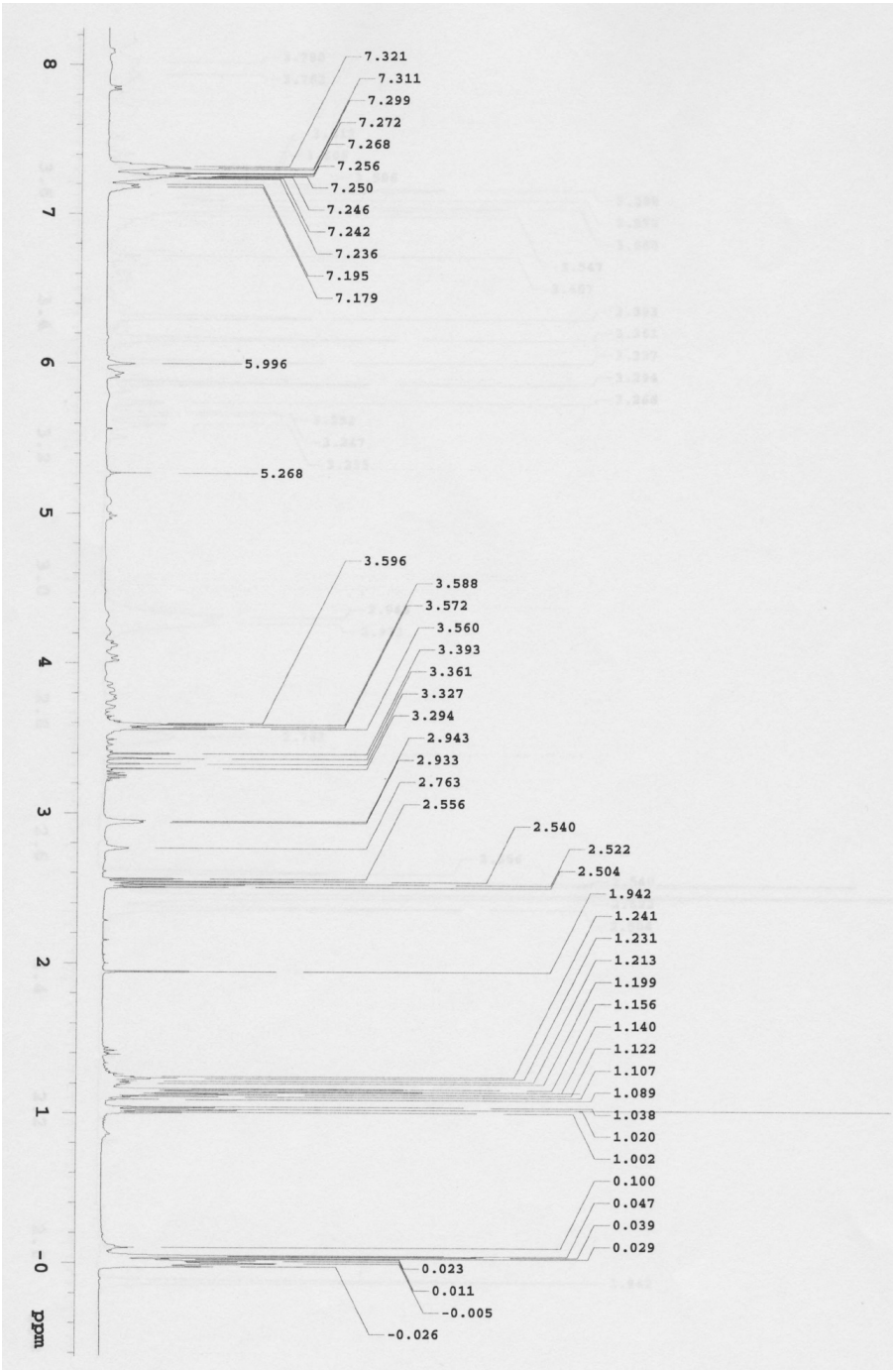


Figure S1. ^{1}H NMR spectrum of the m^{4}Cm phosphoramidite (**2**) is shown.

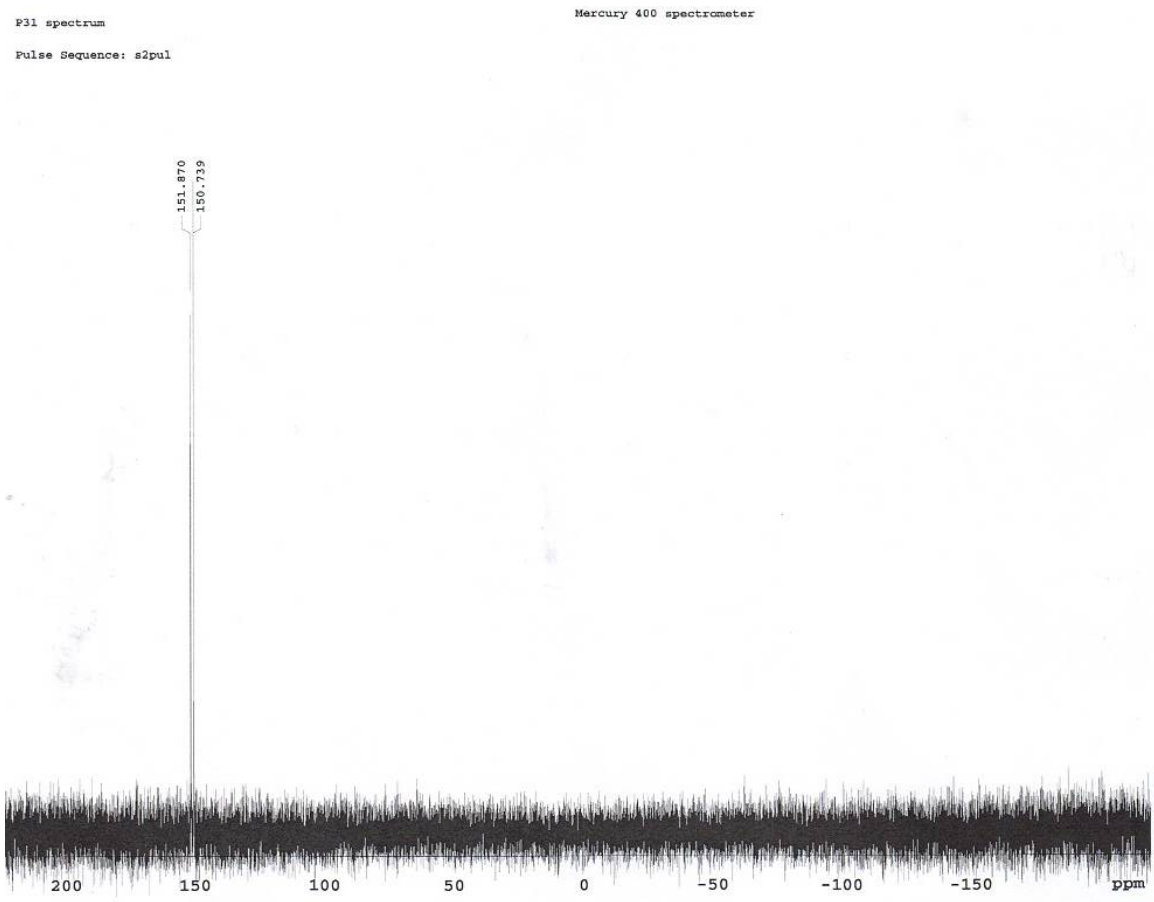


Figure S2. ^{31}P NMR spectrum of the m^{4}Cm phosphoramidite (**2**) is shown.

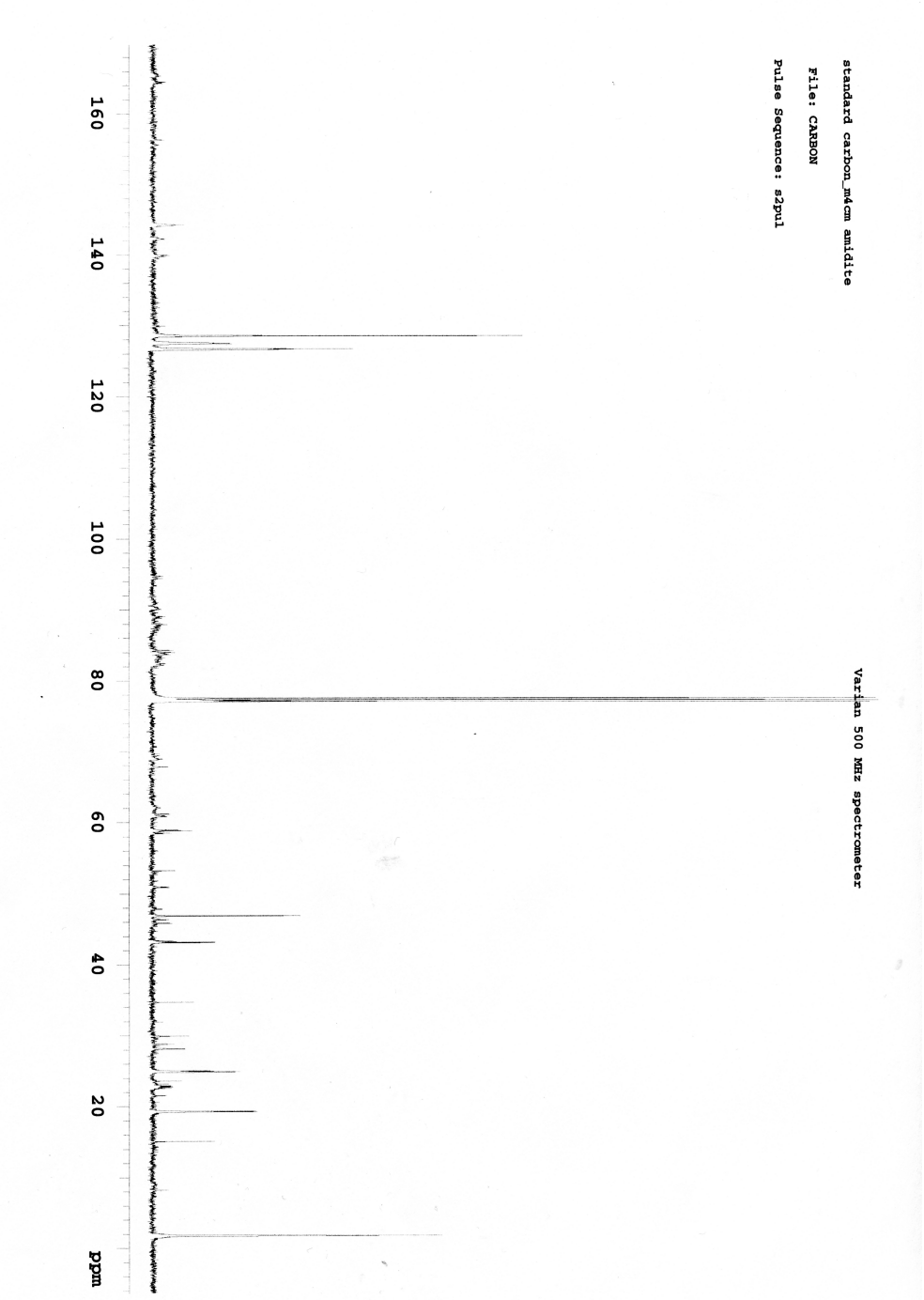


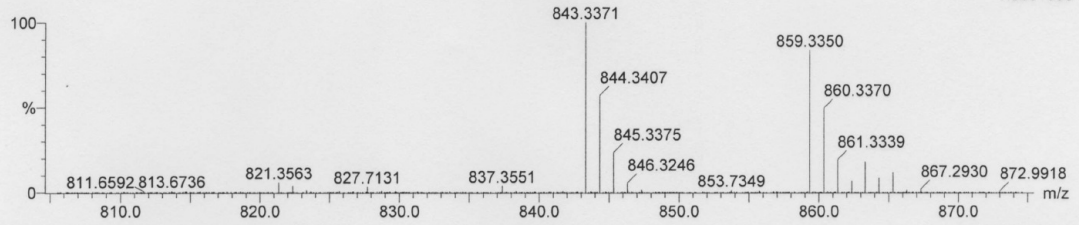
Figure S3. ^{13}C NMR spectrum of the m^4Cm phosphoramidite (**2**) is shown

Elemental Composition Report Page 1

Single Mass Analysis
Tolerance = 5.0 PPM / DBE: min = -1.5, max = 50.0
Element prediction: Off
Number of isotope peaks used for i-FIT = 3

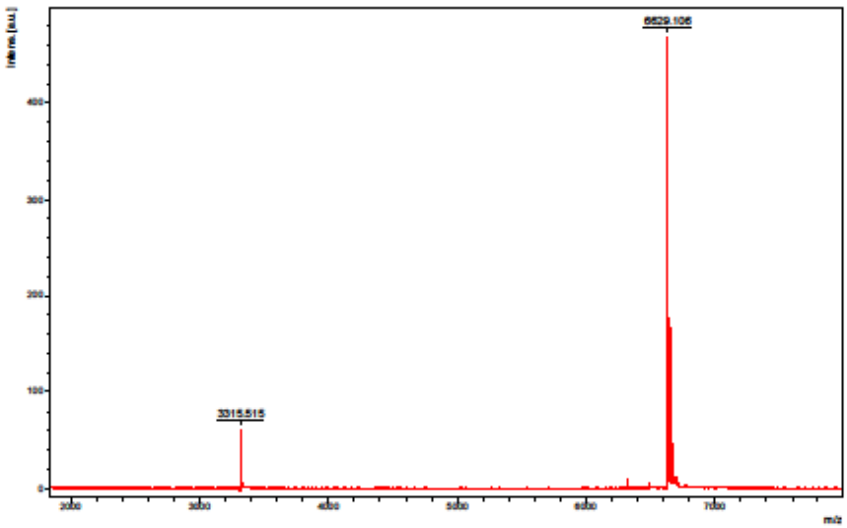
Monoisotopic Mass, Odd and Even Electron Ions
18667 formula(e) evaluated with 261 results within limits (up to 50 best isotopic matches for each mass)
Elements Used:
C: 0-50 H: 0-75 N: 0-10 O: 0-20 23Na: 0-1 Si: 0-3 P: 0-1

2009_0806_0527 13 (0.284) Cm (11:16-(1:6+39:46))
LCT Premier 06-Aug-2009 14:48:57 SANTOSH MAHTO SKM-I-m4Cm Phosphoramidite
1: TOF MS ES+ 1.82e+003

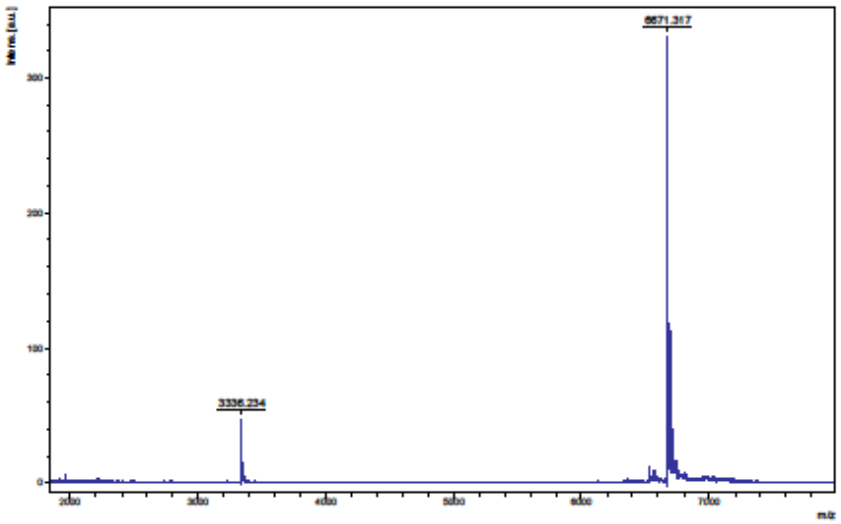


Minimum:				-1.5			
Maximum:		5.0	5.0	50.0			
Mass	Calc. Mass	mDa	PPM	DBE	i-FIT	i-FIT (Norm)	Formula
843.3371	843.3384	-1.3	-1.5	4.5	102.9	5.0	C27 H60 N8 O15 23Na Si3
	843.3352	1.9	2.3	12.0	102.7	4.7	C32 H58 N9 O10 Si3 P
	843.3355	1.6	1.9	13.5	102.3	4.4	C33 H57 N10 O7 23Na Si3 P
	843.3366	0.5	0.6	9.0	102.4	4.4	C33 H58 N5 O15 23Na Si2
	843.3376	-0.5	-0.6	12.5	101.7	3.7	C33 H55 N8 O14 Si2
	843.3379	-0.8	-0.9	14.0	101.0	3.1	C34 H54 N9 O11 23Na Si2
	843.3355	1.6	1.9	8.0	102.8	4.8	C34 H63 N3 O12 23Na Si3 P
	843.3366	0.5	0.6	11.5	102.4	4.4	C34 H60 N6 O11 Si3 P
	843.3376	-0.5	-0.6	7.0	102.4	4.4	C34 H61 N O19 Si2
	843.3368	0.3	0.4	13.0	102.3	4.3	C35 H59 N7 O8 23Na Si3 P
	843.3379	-0.8	-0.9	8.5	101.7	3.7	C35 H60 N2 O16 23Na Si2
	843.3390	-1.9	-2.3	12.0	101.7	3.7	C35 H57 N5 O15 Si2
	843.3379	-0.8	-0.9	16.5	102.6	4.6	C35 H56 N10 O7 Si3 P
	843.3368	0.3	0.4	7.5	102.7	4.7	C36 H65 O13 23Na Si3 P
	843.3379	-0.8	-0.9	11.0	102.9	4.9	C36 H62 N3 O12 Si3 P
	843.3403	-3.2	-3.8	17.0	101.6	3.6	C36 H53 N9 O11 Si2
	843.3392	-2.1	-2.5	13.5	101.2	3.2	C36 H56 N6 O12 23Na Si2
	843.3382	-1.1	-1.3	12.5	103.0	5.0	C37 H61 N4 O9 23Na Si3 P
	843.3406	-3.5	-4.2	18.5	101.5	3.5	C37 H52 N10 O8 23Na Si2
	843.3403	-3.2	-3.8	11.5	102.3	4.3	C37 H59 N2 O16 Si2
	843.3406	-3.5	-4.2	13.0	102.0	4.0	C38 H58 N3 O13 23Na Si2

Figure S4. Exact mass spectrum of the m^{4}Cm phosphoramidite (**2**) is shown.

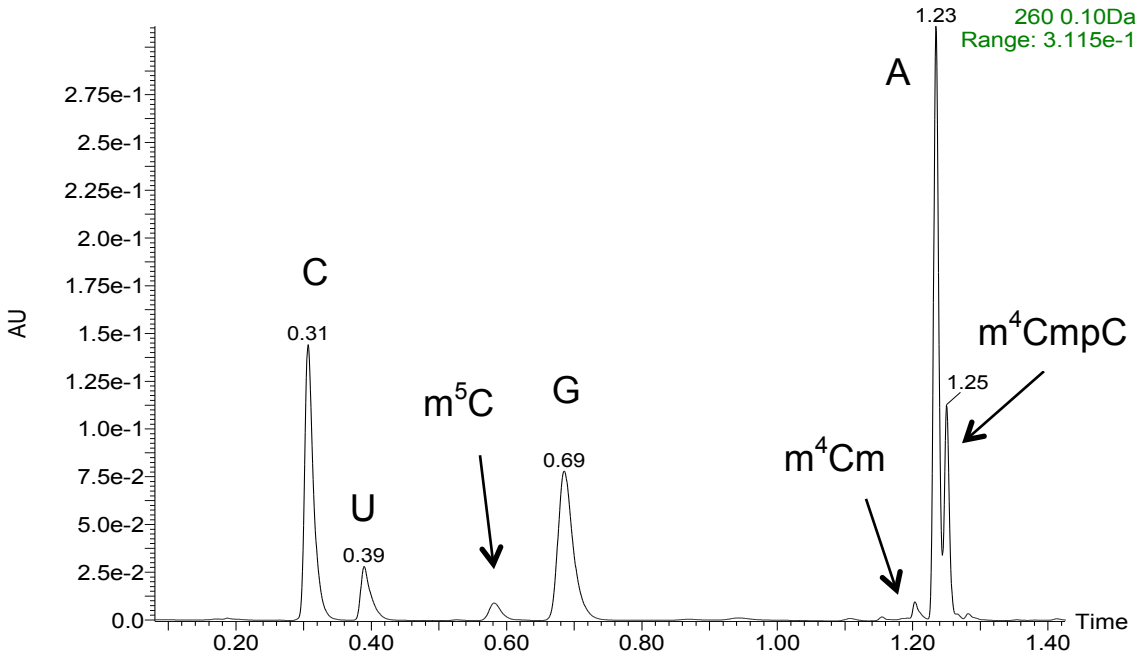


5' half decoding region unmodified (sequence 5'-GCA CAG ACC GCC CGU CAC ACC-3') calculated mass = 6628.0, observed mass = 6629.1 ($M+H^+$).



5' half decoding region modified (sequence 5'-GCA CAG ACC G (m^4Cm) C CGU (m^5C) AC ACC-3') calculated mass = 6670.1, observed mass = 6671.3 ($M+H^+$).

Figure S5. MALDI-TOF spectra of the bacterial decoding region analogues are shown.



Sequence: 5'-GCA CAG ACC G (m^4Cm) C CGU (m^5C) AC ACC-3'

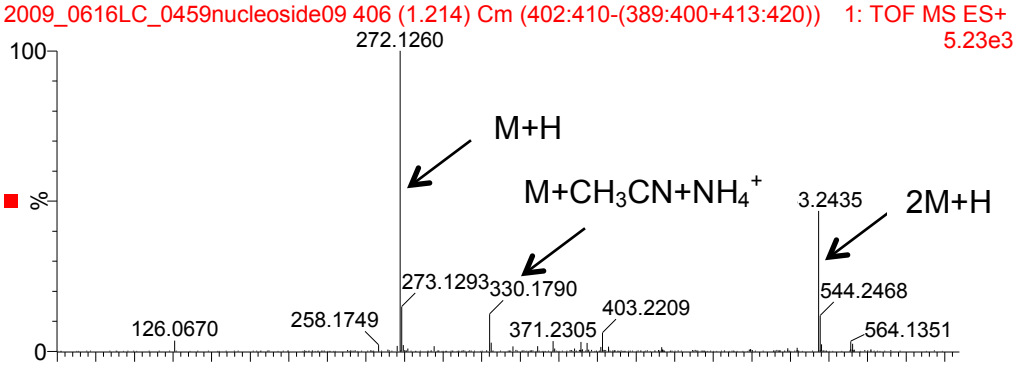


Figure S6. LCMS spectra of the digested bacterial decoding region analogue are shown. In the upper panel, the run of liquid chromatogram of the 5' modified decoding region digested sample is shown. In the lower panel, mass spectra data of the peak for modified nucleoside m^4Cm are given.

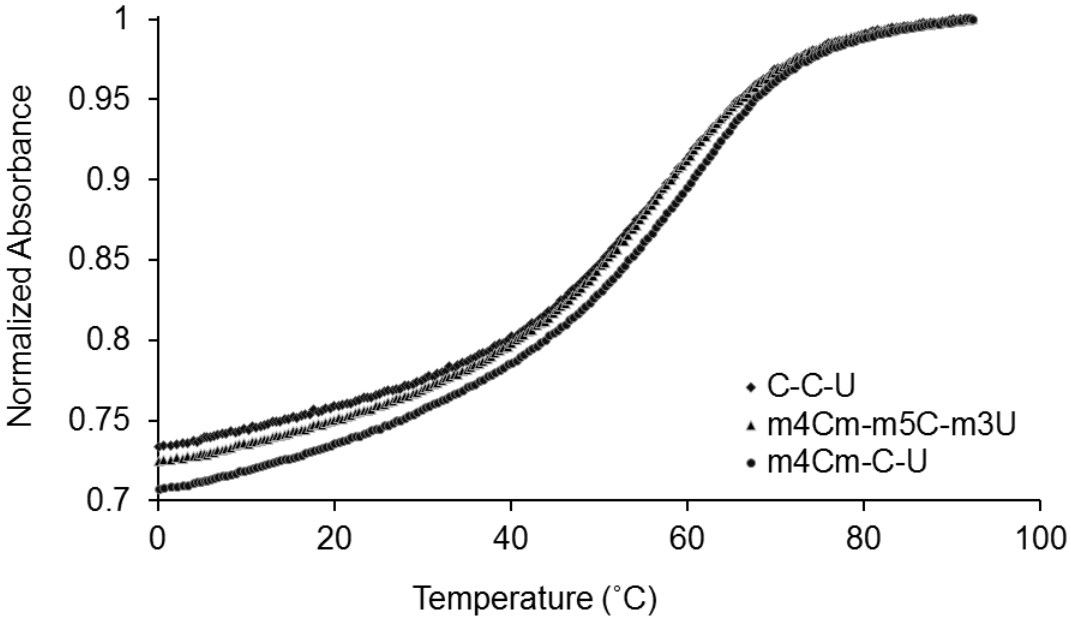


Figure S7. Representative normalized UV melting curves for unmodified decoding region (C-C-U), modified decoding region (m^4Cm-m^5C-m^3U), and singly modified decoding region (m^4Cm-C-U) are shown. All melting curves were normalized at 95 ˚C. Buffer: 20 mM sodium cacodylate, 1 M NaCl, 0.5 mM Na_2EDTA, pH 7.0.

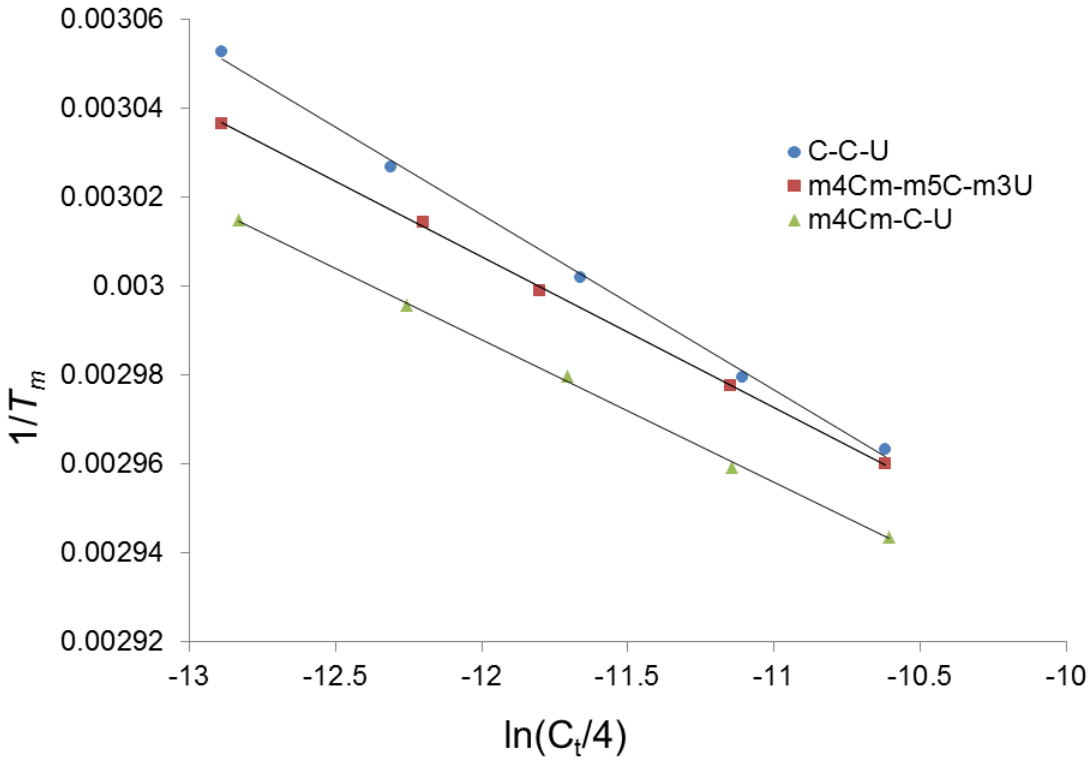


Figure S8. Representative reciprocal melting temperature(T_m) vs. ln (Total strand concentration/4) plots for unmodified decoding region (C-C-U), modified decoding region (m^4Cm-m^5C-m^3U), and singly modified decoding region (m^4Cm-C-U) are shown. Buffer: 20 mM sodium cacodylate, 1 M NaCl, 0.5 mM Na_2EDTA, pH 7.0.

Analogues	**$\Delta G°_{37}$ (kcal/mol)**	**$\Delta H°$ (kcal/mol)**	**$\Delta S°$ (cal/K• mol)**
C-C-U	-10.9	-49	-122
m^4Cm-C-U	-12.0	-62	-161
m^4Cm-m^5C-m^3U	-11.4	-59	-153

Table S1. Sequences and modification patterns of decoding region analogues.

Name and sequence	Calculated mass	Observed mass $(M+H)^+$
5' half of the unmodified decoding region (**C-C**): 5'-GCA CAG ACC GCC CGU CAC ACC-3'	6628.0	6629.1
5' half of *E. coli* decoding region (**m^4Cm-m^5C**): 5'-GCA CAG ACC G (m^4Cm) C CGU (m^5C) AC ACC-3'	6670.1	6671.3
5' half of *C. acetobutylicum* decoding region (**m^4Cm-m^5C**): 5'-GCA CAG ACC G (m^4Cm) C CGU CA(m^5C) ACC-3'	6670.1	6671.2
5' half of *T. Thermophilus* decoding region (**m^5C-m^4Cm- m^5C-m^5C**): 5'-GCA CAG A(m^5C)C G (m^4Cm) C (m^5C)GU (m^5C) AC ACC-3'	6698.2	6699.5
5' half of the decoding region (**m^4Cm-C**): 5'-GCA CAG ACC G (m^4Cm) C CGU CAC ACC-3'	6656.1	6657.2
5' half of the decoding region (**C-m^5C**): 5'-GCA CAG ACC GCC CGU m^5C AC ACC-3'	6642.0	6643.1
5' half of the decoding region (**m^4C-C**): 5'-GCA CAG ACC G (m^4C) C CGU CAC ACC-3'	6642.0	6643.1
5' half of the decoding region (**Cm-C**): 5'-GCA CAG ACC G (Cm) C CGU CAC ACC-3'	6642.0	6643.0
3' half of the bacterial unmodified decoding region (**U**): 5'-GGU GAA GUC GUA ACA AGG CUG UGC-3'	7771.7	7772.9
3' half of the bacterial modified decoding region (**m^3U**): 5'-GGU GAA GUC G (m^3U) A ACA AGG CUG UGC-3'	7785.7	7787.0