General Procedures. Methylene chloride, pyridine, acetonitrile, and toluene were dried by refluxing with CaH_2 followed by distillation. Dioxane was distilled from sodium/benzophenone ketyl. Reactions were carried out in oven (150 ^oC) dried glassware under an atmosphere of dry nitrogen. NMR spectra were recorded on a Varian Mercury 300 MHz spectrometer at ambient temperature. CD spectra were recorded on a Jasco 715 spectropolarimeter in the same buffers as used for UV melting. Thin layer chromatography (TLC) was performed on Scientific Adsorbents Inc. 0.25 mm silica gel 60-F_{254} plates. Column chromatography was done on 230-400 mesh silica gel (60 Å, Scientific Adsorbents).

5-(1-Tritylimidazol-4-yl)uridine (3). A solution of 5-iodouridine[1] (0.74 g, 2 mmol), 4-tributylstanyl-1-trityl-1H-imidazole[2] (1.38 g, 2.3 mmol) and $(Ph_3P)_4Pd$ (0.116 g, 0.1 mmol) in dioxane (60 mL) was stirred at 80 ºC under an nitrogen atmosphere for 46 h. The solvent was evaporated, residue was washed with ether (3 × 30 mL), dried and purified by silica gel column chromatography (0-2.5% of methanol in dichloromethane) to give **1** as a white powder. Yield: 0.822 g, 74%, TLC R_f= 0.5, MeOH/CH_2Cl_2 (1:9). MS (FAB+) calculated for $C_{31}H_{28}N_4O_6$ 552.2, found [M+H] 552.7. ^{1}H NMR ($CDCl_3$, 300 MHz) δ 8.31 (s, 1H), 7.60 (s, 1H), 7.43 (s, 1H), 7.31-7.28 (m, 9H), 7.12-7.09 (m, 6H), 5.88 (d, *J*= 5 Hz, 1H), 4.28 (s, 2H), 4.03 (s, 1H), 3.89 (d, *J*= 1.4 Hz, 1H), 3.69 (d, *J*= 1.4 Hz, 1H). ^{13}C NMR ($(CD_3)_2CO$, 75.4 MHz) δ, 161.21, 150.14, 142.51, 138.81, 136.11, 132.86, 129.72, 128.43, 128.35, 120.19, 108.53, 89.03, 85.72, 75.57, 74.25, 70.37, 61.24.

5'-O-(4-methoxytrityl)-5-(1-tritylimidazol-4-yl)uridine (4). 5-(1-Tritylimidazol-4-yl)uridine (534 mg, 0.967 mmol) was co-evaporated with dry pyridine (3 × 10 mL), dissolved in dry pyridine (10 mL), and cooled on ice. 4-Methoxytrityl chloride (328 mg, 1.063 mmol) was added and the mixture was stirred overnight (left on slowly melting ice). Methanol (1 mL) was added and the mixture was evaporated. The residue was dissolved in CH_2Cl_2 (20 mL) and extracted with saturated $NaHCO_3$ aq. (20 mL) and water (20 mL). The organic layer was separated, dried over Na_2SO_4, evaporated and co-evaporated with toluene (2 × 20 mL) to remove traces of pyridine. The residue was chromatographed on silica gel (0-2% of MeOH in CH_2Cl_2 containing 0.05% of triethylamine) to give **4** as a white powder. Yield: 0.573 g, 72%, TLC R_f = 0.35, MeOH/CH_2Cl_2 (1:19). MS (FAB+) calculated for $C_{51}H_{44}N_4O_7$ 824.3, found [M+H] 825.0. ^{1}H NMR ($CDCl_3$, 300 MHz) δ 8.37 (s, 1H), 7.55 (s, 1H), 7.40-7.05 (m, 29 H), 6.73 (d, *J*= 10 Hz, 2H), 5.80 (d, *J*= 5 Hz, 1H), 4.41 (t, *J*= 5 Hz, 1H), 4.18 (d, *J*= 4 Hz, 1H), 4.11 (t, *J*= 5 Hz, 1H), 3.65 (s, 3H), 3.18 (d, *J*= 1.4 Hz, 1H), 3.16 (d, *J*= 1.4 Hz, 1H). ^{13}C NMR ($CDCl_3$, 75.4 MHz) δ 161.58, 158.70, 150.82, 144.49, 144.45, 142.47, 138.70, 135.80, 135.48, 131.91, 130.70, 130.00, 128.64, 128.344, 128.30, 128.02, 127.04, 121.05, 113.35, 109.63, 91.93, 87.12, 84.67, 77.74, 77.32, 76.89, 75.82, 74.73, 71.66, 64.05, 55.35.

[1] Sy, W. W. *Synth. Commun.* **1990,** *20,* 3391-3394.
[2] Jetter, M. C.; Reitz, A. B. *Synthesis* **1998,** 829-831.

2'-O-(2-Chlorobenzoyl)-5'-O-(4-methoxytrityl)-5-(1-tritylimidazol-4-yl)uridine-3'-yl hydrogenphosphonate triethylammonium salt (5). 5'-O-(4-methoxytrityl)-5-(1-tritylimidazol-4-yl)uridine (484 mg, 0.587 mmol) was co-evaporated with dry pyridine (10 mL) and dissolved in dry CH_2Cl_2 (8 mL). Pyridine (0.37 mL) was added and the mixture was cooled to -78 °C (acetone-dry ice). A solution of 2-chlorobenzoyl chloride (0.8 mL, 0.66 mmol) in CH_2Cl_2 (10 mL) was added under cooling and stirring during 15 min. The mixture was stirred at -78 °C for 30 min and added dropwise to a stirred and cooled (-78 °C) mixture of imidazole (0.439 g, 6.45 mmol), PCl_3 (0.18 mL, 2.1 mmol) and triethylamine (0.94 mL, 6.75 mmol) in dry CH_2Cl_2 (15 mL). The reaction mixture was stirred at -78 °C for 1 h and extracted with 2.0 M TEAB aq. pH 7.5 (2 × 25 mL). The organic layer was separated, dried over Na_2SO_4, and evaporated. The residue was chromatographed on silica gel (0-8% of MeOH in CH_2Cl_2 containing 0.1% of triethylamine) to give **5** as a white powder. Yield: 0.42 g, 63%, TLC R_f = 0.2, MeOH/CH_2Cl_2 (1:19). MS (FAB+) calculated for $C_{58}H_{48}ClN_4O_{10}P$ 1026.3, found [M+H] 1027. ^{1}H NMR ($CDCl_3$, 300 MHz) δ 8.28 (s, 1H), 8.01 (d, *J*= 4.7 Hz, 1H), 7.54-7.06 (m, 33 H), 6.79 (d, *J*= 630 Hz, 1H), 6.76 (d, *J*= 10 Hz, 2H), 6.25 (s, 1H), 5.74 (s, 1H), 5.09 (m, 1H), 4.39 (1H, s), 3.68 (s, 3H), 3.49 (m, 2H), 2.87 (q, *J*= 7 Hz, 6H), 1.14 (t, *J*= 7 Hz, 9H). ^{13}C NMR ($CDCl_3$, 75.4 MHz) δ 164.34, 161.24, 158.68, 150.73, 150.17, 144.67, 144.48, 142.64, 138.49, 135.53, 134.18, 133.13, 132.67, 132.06, 131.10, 130.90, 130.00, 129.31, 128.82, 128.76, 128.27, 128.23, 128.03, 127.98, 127.03, 126.92, 120.76, 113.34, 110.69, 87.21, 75.57, 75.01, 71.09, 63.37, 55.36, 45.53, 8.63.

Synthesis of oligoribonucleotides was done following our previously reported procedures.[3,4] Long chain alkylamino controlled pore glass support derivatized with protected cytidine[5] (~20 mg, 40 μmol/g, ~0.8 μmol) was placed in a gastight glass syringe (1 mL) equipped with a porous glass filter. All reactions and washings were done by manual intake of an appropriate solvent or reagent as shown in Table S1. In the coupling step, monomer **5** and the previously described protected ribonucleoside H-phosphonates[4] were used.

Table S1. Solid phase synthesis protocol

Step	Reagent or solvent	Volume	Time
Coupling	0.1 M H-phosphonate in pyridine, mix with 0.5 M pivaloyl chloride in MeCN	0.1 mL 0.1 mL	3 min
Washing	MeCN CH_2Cl_2	4 × 0.5 mL 4 × 0.5 mL	4 min
Deprotection	3% dichloroacetic acid in CH_2Cl_2	5 × 0.5 mL	2 min
Washing	CH_2Cl_2 MeCN	4 × 0.5 mL 4 × 0.5 mL	4 min

After the assembly of the desired sequence was complete, the support was treated with 2% I_2 in pyridine/water (98:2, 30 min, rt), washed with acetonitrile (5 × 1 mL) and CH_2Cl_2 (4 × 1 mL), and the N-trityl group was removed using a mixture of trifluoroacetic acid/triethylsilane/ CH_2Cl_2 (3:12:85, 1 mL, 30 min, rt). Oligoribonucleotides were cleaved from the support and deprotected using concentrated aqueous ammonia/ethanol (3:1, 8 h, rt) to cleave N-acyl and 2'-O-(2-chlorobenzoyl) groups. Oligonucleotides were purified by reverse phase HPLC as described previously (Figure S1).[6] The composition of the synthesized sequences was confirmed by MALDI-TOF mass spectroscopy (Table S2). Oligoribonucleotides **7-9** were purchased from Dharmacon.

[3] Westman, E.; Sigurdsson, S.; Stawinski, J.; Strömberg, R. *Nucleic Acids Sym Ser.* **1994**, *31*, 25. Strömberg, R., Stawinski, J. *Current Protocols in Nucleic Acid Chemistry*, unit 3.4. John Wiley & Sons, Inc.: New York, 2000.
[4] Rozners, E.; Renhofa, R.; Petrova, M.; Popelis, Y.; Kumpins, V.; Bizdena, E. *Nucleosides Nucleotides* **1992,** *11,* 1579-1593. Rozners, E.; Stromberg, R.; Bizdena, E. *Nucleosides Nucleotides* **1995,** *14,* 855-857.
[5] Sigurdsson, S.; Rozners, E.; Westner, E.; Bizdena, E.; Strömberg, R. *Nucleosides Nucleotides* **1995,** *14,* 875-878.
[6] Rozners, E.; Moulder, J. *Nucleic Acids Res.* **2004,** *32,* 248-254.

Table S2. Yields and MS data for the synthesized oligoribonucleotides

No	Sequence	Yield (trityl)	Yield (isolated)	MALDI-TOF MS data	
				m/z calculated	*m/z* obtained
6	GGC**UG**GCC	29%	3.3 OD (5%)	2539.4	2539.0
6i	GGC**U^{Im}G**GCC	55%	5.3 OD (10%)	2605.4	2605.9
7i	GGC**GU^{Im}**GCC	35%	1.8 OD (3.5%)	2605.4	2605.4
8i	GAG**U^{Im}G**CUC	37%	3.4 OD (6.5%)	2590.4	2590.5
9i	GAG**GU^{Im}**CUC	21%	1.9 OD (2.5%)	2590.4	2591.1

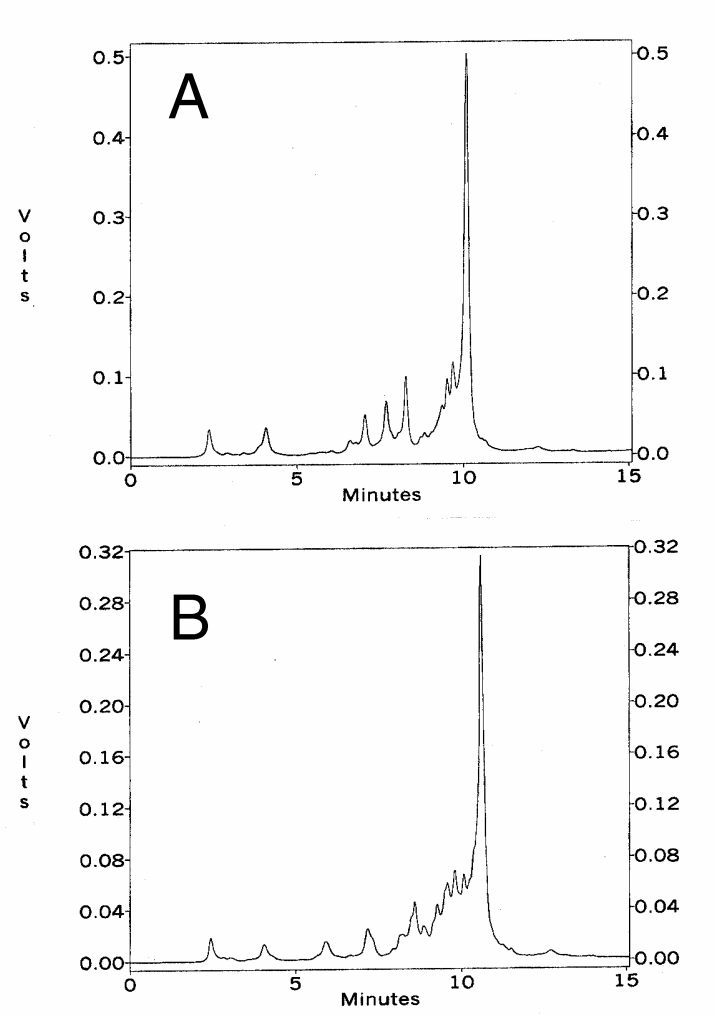


Figure S1. RP HPLC traces of crude synthesis mixtures: **A 6i** and **B 9i**. Conditions: Discovery® HS C18 column (4.6 × 250 mm, 3 μm) at 50 °C, linear gradient of acetonitrile (2-30 % in 30 min) in 50 mM triethylammonium acetate buffer (pH 6.5), flow rate 1 mL/min.

UV melting of each oligonucleotide (2 μM) was done in 10 mM sodium cacodylate, 0.1 mM EDTA, and 300 mM NaCl in presence of 0, 5, 10, 15, and 20 % weight/volume of each of the three osmolytes in Table 1. Oligonucleotide concentrations were determined using extinction coefficients (calculated from the nearest-neighbor approximation[7]) for **6** and **6i-9i** or data (OD per nmol) provided by Dharmacon for **7-9**. Absorbance vs. temperature profiles were measured at 280 nm on a Varian Bio 100 spectrometer equipped with a six-position Peltier temperature controller. The temperature was increased

[7] Puglisi, J. D.; Tinoco, I., Jr. *Methods Enzymol.* **1989**, *180*, 304-324.

at 0.5 °C per minute. Five samples were measured concurrently in the double-beam mode. To increase the accuracy of measurements the sixth position was used to record the temperature data points using a temperature probe directly in a cuvette. At temperatures below 15 °C the sample compartment was flushed with dry nitrogen gas.

The melting temperatures and thermodynamic parameters were obtained using Varian Cary software (Version 02.00). The experimental absorbance vs. temperature curves were converted into a fraction of strands remaining hybridized (α) vs. temperature curves by fitting the melting profile to a two-state transition model, with linearly sloping lower and upper base lines. The melting temperatures (t_m) were obtained directly from the temperature at α = 0.5. The final t_m was an approximation of usually seven or more measurements (Tables S3-S10). The thermodynamic parameters were determined using two different methods[8] as described bellow:

(a) from the van't Hoff plot of ln K vs $1/T_m$. For a bimolecular transition of self-complementary strands the equilibrium constant $K = \alpha/[2C(1-\alpha)^2]$ where C is the total strand concentration ($C = 2 \times 10^{-6}$ M). The van't Hoff plot (ln K vs. $1/T_m$) is linear with $-\Delta H/R$ as the slope and $\Delta S/R$ as the intercept (R is the universal gas constant 1.986 cal/mol·K). All fitting and calculation operations were done using Varian Cary software (Version 02.00) using settings for a bimolecular transition of self-complementary strands. The final $-\Delta H$ and $-\Delta S$ is the average of at least seven measurements (Table S3-S10, column 8).

(b) from the width at the half-height of differentiated melting curve. The fraction of strands remaining hybridized (α) vs. temperature curves were converted into differentiated melting curves ($\delta\alpha/\delta T_m^{-1}$) vs. T_m) using Varian Cary software (Version 02.00). The width of the of the differentiated melting curve at the half-height is inversely proportional to the van't Hoff transition enthalpy; for a bimolecular transition $\Delta H = 10.14/(T_1^{-1} - T_2^{-1})$ where T_1 is the lower temperature at one-half of ($\delta\alpha/\delta T_m^{-1}$)) and T_2 is the upper temperature at one-half of ($\delta\alpha/\delta(T_m^{-1})$).[8,9] The final $-\Delta H$ is the average of at least seven measurements (Table S3-S10, column 9).

Osmotic stressing monitors the depression of nucleic acid melting temperature upon addition of small organic molecules (ethylene glycol, glycerol, etc.).[10,11] Small molecules in solution are excluded from the immediate vicinity of the biopolymer, which leads to preferential hydration of its surface. The osmotic stressing method assumes that the effect of small molecules is to change the water activity only, the excluded co-solutes do not directly interact with the biopolymer. However, some interaction between the small molecules and the biopolymer cannot be completely ruled out. Record and co-workers[12] found that small solutes (including glycerol) are not completely excluded from the vicinity of proteins causing

[8] Breslauer, K. J. *Methods Enzymol.* **1995**, *259*, 221-242.
[9] Gralla, J.; Crothers, D.M. *J.Mol.Biol.* **1973**, *78*, 301-319.
[10] Parsegian, V. A.; Rand, R. P.; Rau, D. C. *Methods Enzymol.*, **1995**, *259*, 43-94.
[11] Spink, C. H.; Chaires, J. B. *Biochemistry* **1999,** *38,* 496-508.
[12] Courtenay, E. S.; Capp, M. W.; Anderson, C. F.; Record, M. T., Jr. *Biochemistry* **2000,** *39,* 4455-4471.

underestimation of the Δn_W in osmotic stressing experiments. Therefore, more studies are needed to establish the reliability of the *absolute values* of Δn_W for both proteins and nucleic acids. However, these uncertainties should not cause problems with our *relative comparison* of RNA **6** and **7**.

In our experiments the water activity was changed by addition of ethylene glycol, glycerol, and acetamide as the small organic co-solutes. The number of water molecules released upon melting of oligonucleotide duplexes, Δn_W, was calculated from the dependence of melting temperature on water activity and the enthalpy, both measured with UV thermal melting experiments.[11]

$$\Delta n_W = (-\Delta H/R)[d(T_m^{-1})/d(\ln a_w)],$$

where $-\Delta H$ is the enthalpy determined from the width at the half-height of differentiated melting curve (see above) in pure buffer and R is the universal gas constant (1.986 cal/mol·K). The experimentally determined values of water activity ($\ln a_w$) at given co-solute concentrations were provided by Professors Spink and Chaires. The slope of the plot of reciprocal temperature (in K) of melting vs. the logarithm of water activity ($\ln a_w$) at different concentrations (0, 5, 10, 15, and 20%) of small co-solutes gave the value of $d(T_m^{-1})/d(\ln a_w)$ (see Figures S3-S6). The final Δn_W were obtained by linear fitting using KaleidaGraph software (Version 3.51) with a confidence level usually better than 98%.

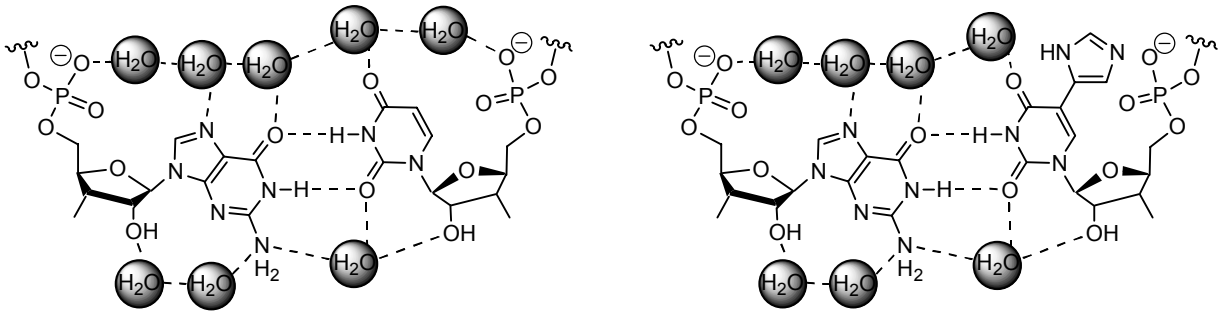
Error analysis: The uncertainties in the final Δn_W (Table 1) were estimated from standard deviations of experimental melting temperatures and ΔH according to standard procedures.[13] We prefer to use the standard deviations instead of the standard errors, which probably results in an overestimate of the uncertainties in Table 1, as a safer approach against false trends and misinterpretation of data. Even within the generous error estimates, the data were consistent within the general trends.

Uncertainties in the slopes of the $1/T_m$ vs. $\ln a_w$ plots ($\sigma d(T_m^{-1})/d(\ln a_w)$) were estimated by constructing alternative linear fits using error bars (standard deviations of $1/T_m$) as the data points. Two alternative plots were obtained by linear fits through $1/T_m$ plus or minus standard deviation at 0% and 20% co-solute, e.g., $1/T_m$ plus standard deviation at 0% co-solute and $1/T_m$ minus standard deviation at 20% co-solute gave one alternative whereas the $1/T_m$ minus standard deviation at 0% co-solute and the $1/T_m$ plus standard deviation at 20% co-solute gave the other alternative. The deviations of both alternative plots from the original plot were averaged. If necessary, to maintain the alternative plots inside the limits of error bars of the original plot, error bars at 5 or 15% co-solute were also included to construct the alternative plots. The final uncertainty in Δn_W was calculated as $\sigma\Delta n_W = \Delta n_W[(\sigma\Delta H/\Delta H)^2 + (\sigma\text{slope}/\text{slope})^2]^{0.5}$ where $\sigma\Delta H$ is the standard deviation and slope = $d(T_m^{-1})/d(\ln a_w)$.[11] For detailed input data and results of calculations see Tables S3-S10.

[13] Bewington, P. R.; Robinson, D. K. *Data reduction and error analysis for the physical sciences*, 3nd ed. WCB/McGraw-Hill: New York, 2003.

Hydration of G-U Base pairs: Water is an integral part of the nucleic acid structure.[14] It is conceivable that an extensive and well-ordered hydration is crucial for conformation and thermal stability of RNA's non-canonical structure, such as the wobble G-U base pairs. In the crystal structures, tandem G•U base pairs are stabilized by water mediated hydrogen bond bridges in both grooves (see cartoon in Figure S2).[15,16] In the minor groove, water molecules bridge the unpaired G amino group and the U O2 and 2'-OH. In the major groove, five water molecules link the phosphates on both strands through O6 of G and O4 of U.[16]

Figure S2. Cartoon of hydration scheme in wobble G-U base pairs (adopted from ref. 15).



Analysis of crystal structures of G•U wobble pairs suggest that the 5-imidazole modification may disrupt part of the major groove hydration network removing at least one water molecule per base pair. However, imidazole heterocycle can form hydrogen bonds that may stabilize the remaining hydration network leading to a relatively small net change.

[14] Makarov, V.; Pettitt, B. M.; Feig, M. *Acc. Chem. Res.* **2002,** *35,* 376-384. Berman, H. M.; Schneider, B. Nucleic acid hydration. In *Oxford Handbook of Nucleic Acid Structure*; Neidle, S.; Ed. Oxford University Press, New York, **1999**; pp 295-312. Westhof, E. *Annu. Rev. Biophys. Biophys. Chem.* **1988,** *17,* 125-144.

[15] Auffinger, P.; Westhof, E. *J. Biomol. Struct. Dyn.* **1998,** *16,* 693-707. Masquida, B.; Westhof, E. *RNA* **2000,** *6,* 9-15.

[16] Biswas, R.; Wahl, M. C.; Ban, C.; Sundaralingam, M. *J. Mol. Biol.* **1997,** *267,* 1149-1156. Holbrook, S. R.; Cheong, C.; Tinoco, I., Jr.; Kim, S. H. *Nature* **1991,** *353,* 579-581.

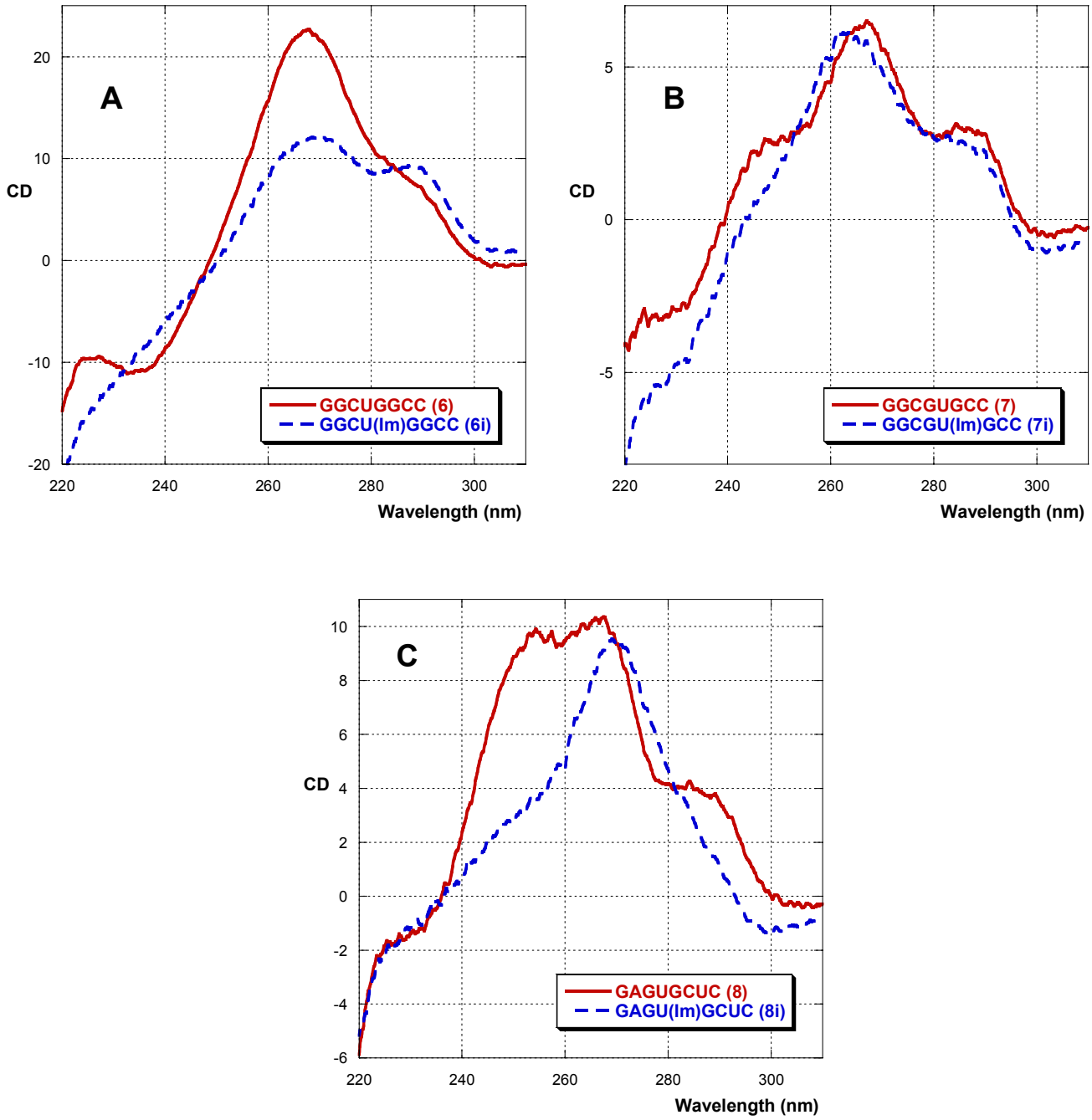


Figure S3. CD spectra of RNA duplexes: unmodified (red solid line) and having imidazole modification (blue broken line). **A 6** and **6i** (10 μM), **B 7** and **7i** (7 μM), **C 8** and **8i** (10 μM).

Table S3. Experimental t_m and thermodynamic data for melting of r(GGCUGGCC)

Experiment	0%	5%	10%	15%	20%	−ΔG(kcal /mol)	vant' Hoff −ΔH(cal/mol)	$\delta\alpha/\delta T_m$ −ΔH(cal/mol)	−ΔS (eu)
Ethylene Glycol									
er177B	51.5	50.4	49.3	48.5	47.7	11.9	87300	85195	243
er177C	51.8	50.7	49.9	48.6	47.5	12.1	91500	83955	256
er177C	51.6	50.7	49.6	48.8	47.2	11.9	85400	80414	237
er177C	51.7	50.9	49.7	48.6	47.4	12.0	87100	82880	242
er177D	51.6	50.8	49.6	48.5	47.3	12.1	87200	82988	242
er177D	51.7	50.7	49.9	48.4	47.2	12.0	89200	82977	249
er177D	51.8	50.6	49.9	48.7	47.6	12.1	87200	82869	242
er177D	51.8	50.8	49.9	48.7	47.5	12.0	86400	85791	240
Average	**51.7**	**50.7**	**49.7**	**48.6**	**47.4**	**12.0**	**87663**	**83384**	**244**
STDEV	**0.1**	**0.2**	**0.2**	**0.1**	**0.2**	**0.1**	**1878**	**1650**	**6**
Glycerol									
er188A	52.1	50.6	50	49.7	48.8	12.2	90400	85667	252
er188A	52	51	50.2	49.6	48.9	12.0	85500	82879	237
er188A	51.5	50.9	50.2	49.8	48.7	12.0	83000	80083	229
er188A	51.8	50.7	49.9	49.6	48.6	12.0	84900	84858	235
er188B	51.6	50.5	50.1	49.4	48.5	12.0	86100	81074	239
er188B	51.8	50.6	50.1	49.7	48.4	12.3	90100	87649	251
er188B	51.9	50.8	50.1	49.5	48.6	11.9	86600	88877	241
er188B	52.1	50.9	50.6	49.5	48.6	12.2	86900	83549	241
er188B	51.9	50.8	50.3	49.7	48.7	12.3	88900	83901	247
Average	**51.9**	**50.8**	**50.2**	**49.6**	**48.6**	**12.1**	**86933**	**84282**	**241**
STDEV	**0.2**	**0.2**	**0.2**	**0.1**	**0.2**	**0.2**	**2459**	**2857**	**8**
Acetamide									
er190A	51.8	49.7	47.5	45.9	43.8	12.1	89600	87170	250
er190A	51.6	49.7	47.7	45.7	43.6	11.9	84800	85315	235
er190A	52.0	49.6	47.5	45.6	43.9	12.2	88800	80447	247
er190A	51.8	49.7		45.7	43.4	12.2	88500	85929	246
er190B	51.7	49.6		45.8	43.7	12.3	89500	83767	249
er190B	51.8	49.7		45.9	43.9	12.1	88700	81926	247
er190B	51.8	49.7	47.8	45.6	43.6	12.0	88300	81378	246
er190C	52.0	49.8	47.7	45.9	43.7	12.2	90000	85259	251
er190C	51.9	49.9	47.8	45.7	43.6	12.0	87100	87368	242
er190C	51.8	49.9	47.7	45.8	43.9	11.9	88200	85536	246
er190C	51.7	49.9	47.8	45.9	43.8	11.9	85400	85686	237
Average	**51.8**	**49.7**	**47.8**	**45.8**	**43.7**	**12.1**	**88082**	**84525**	**245**
STDEV	**0.1**	**0.1**	**0.1**	**0.1**	**0.2**	**0.1**	**1675**	**2333**	**5**

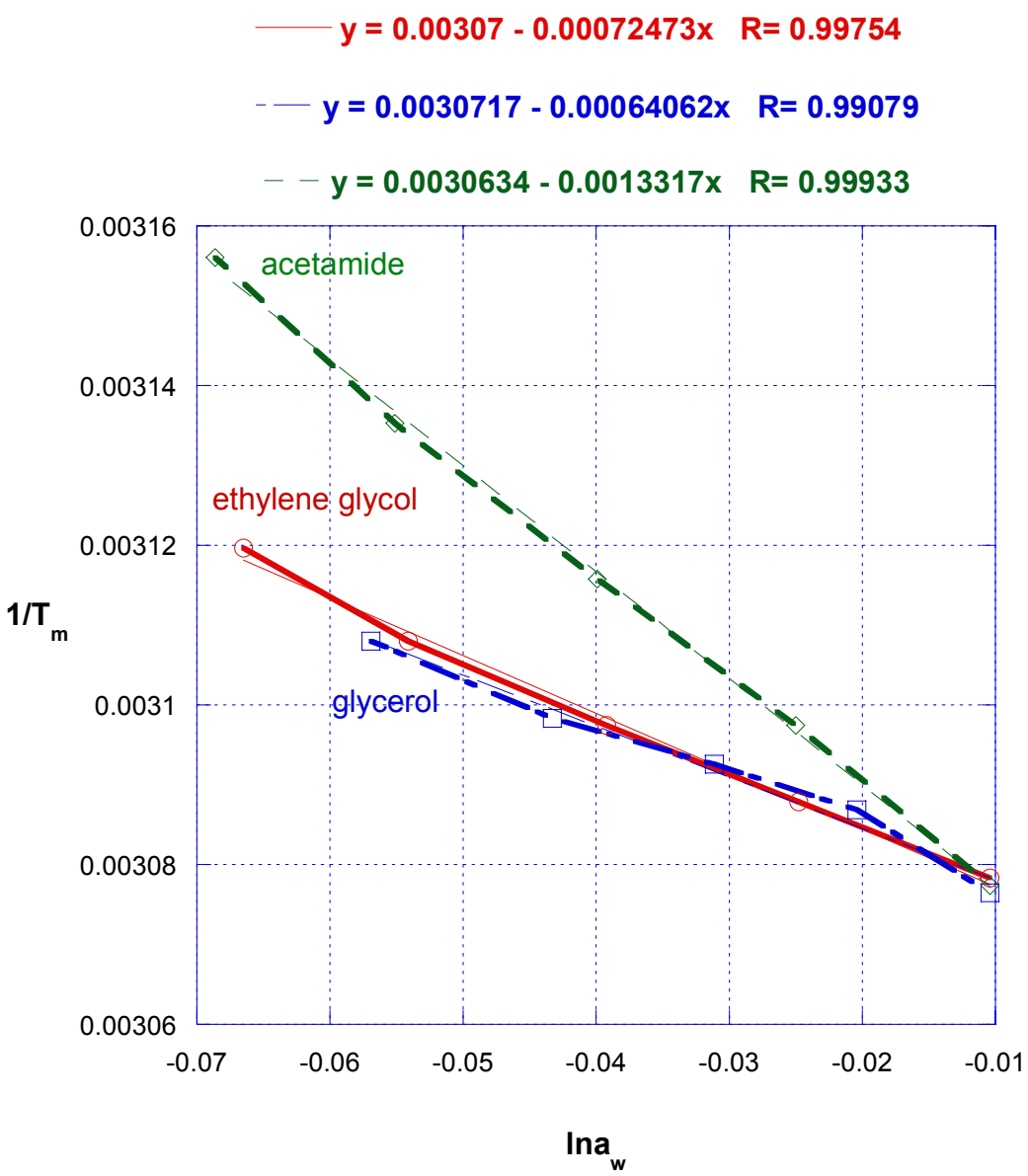


Figure S4. Reciprocal temperature of melting for r(GGCUGGCC) vs. the logarithm of water activity for small co-solutes

Table S4. Calculations for r(GGCUGGCC)

		$-\Delta H$(cal/mol)	$-\sigma H$(cal/mol)	−slope	$-\sigma$slope	Δn_w	σn_w
er177	Ethylene glycol	83384	1650	0.0007247	0.00004955	**30.4**	**2.2**
er188	Glycerol	84282	2857	0.0006406	0.00009107	**27.2**	**4.0**
er190	Acetamide	84525	2333	0.0013317	0.00005050	**56.7**	**2.7**

Table S5. Experimental t_m and thermodynamic data for melting of r(GGCU^{Im}GGCC)

Experiment	0%	5%	10%	15%	20%	$-\Delta G$(kcal/mol)	vant' Hoff $-\Delta H$(cal/mol)	$\delta\alpha/\delta T_m$ $-\Delta H$(cal/mol)	$-\Delta S$(eu)
Ethylene Glycol									
er186A	49.5	48.8	47.6	46.7	45.6	11.4	85500	79277	239
er186B	49.5	48.1	47.5	46.7	45.9	11.6	88200	82813	247
er186B	49.4	48.5	47.6	46.5	45.5	11.3	86000	78067	241
er186C	49.3	48.6	47.7	46.4		11.4	85800	81945	240
er186D	49.4	48.6	47.2	46.7	45.5	11.4	85500	83509	239
er186D	49.5	48.6	47.7	46.4	45.3	11.2	86300	82158	242
er186D	49.7	48.6	47.9	46.5	45.5	11.2	83800	81089	234
Average	**49.5**	**48.5**	**47.6**	**46.6**	**45.6**	**11.3**	**85871**	**81266**	**240**
STDEV	**0.1**	**0.2**	**0.2**	**0.1**	**0.2**	**0.1**	**1303**	**1954**	**4**
Glycerol									
er186E	49.8	48.5	47.9	47.6		11.5	85000	79411	237
er186E	49.7	48.8	48.1	47.5	47.1	11.5	85300	84082	238
er186F	49.2		47.9	47.5	46.7	11.2	83200	82566	232
er186F	49.6	48.9	48.2	47.9	46.7	11.5	87500	79260	245
er186F	49.5	48.9	47.9	47.5	47.2	11.3	85700	82684	240
er186F	49.6	48.7	48	47.6	47.3	11.3	86400	84484	242
er186F	49.8	48.9	48.4	47.7	47	11.4	86500	79869	242
er186G	49.7		48.4	47.9	47	11.5	84100	78820	234
Average	**49.6**	**48.8**	**48.1**	**47.7**	**47.0**	**11.4**	**85463**	**81397**	**239**
STDEV	**0.2**	**0.2**	**0.2**	**0.2**	**0.2**	**0.1**	**1382**	**2307**	**4**
Acetamide									
er191A	49.6	48.4	46.6	45.1	43.5	11.4	85200	77206	238
er191B	49.5	48.1		45.0	43.5	11.0	80200	76648	223
er191B	49.6	48.5	46.7	45.2	43.4	11.2	83800	81833	234
er191B	49.9	48.3	46.6	45.3	43.4	11.3	83300	81160	232
er191B	49.9	48.4	46.6	45.3		11.4	84600	76927	236
er191C	49.4	48.5	46.5			11.1	78100	79489	216
er191C	49.5	48.3	46.6		43.2	11.3	82900	78798	231
er191D	49.6	48.1	46.5	45.0	43.3	11.7	89200	85848	250
er191E	49.4	48.4	46.3	44.9	43.3	11.3	86400	81434	242
er191E	49.5	47.9	46.3	44.7	43.2	11.3	87000	82343	244
er191E	49.6	48.3	46.3	45.1	43.0	11.2	83200	79435	232
er191E	49.5	48.1	46.3	44.8	43.2	11.7	89500	84911	251
Average	**49.6**	**48.3**	**46.5**	**45.0**	**43.3**	**11.3**	**84450**	**80503**	**236**
STDEV	**0.2**	**0.2**	**0.2**	**0.2**	**0.2**	**0.2**	**3345**	**2985**	**10**

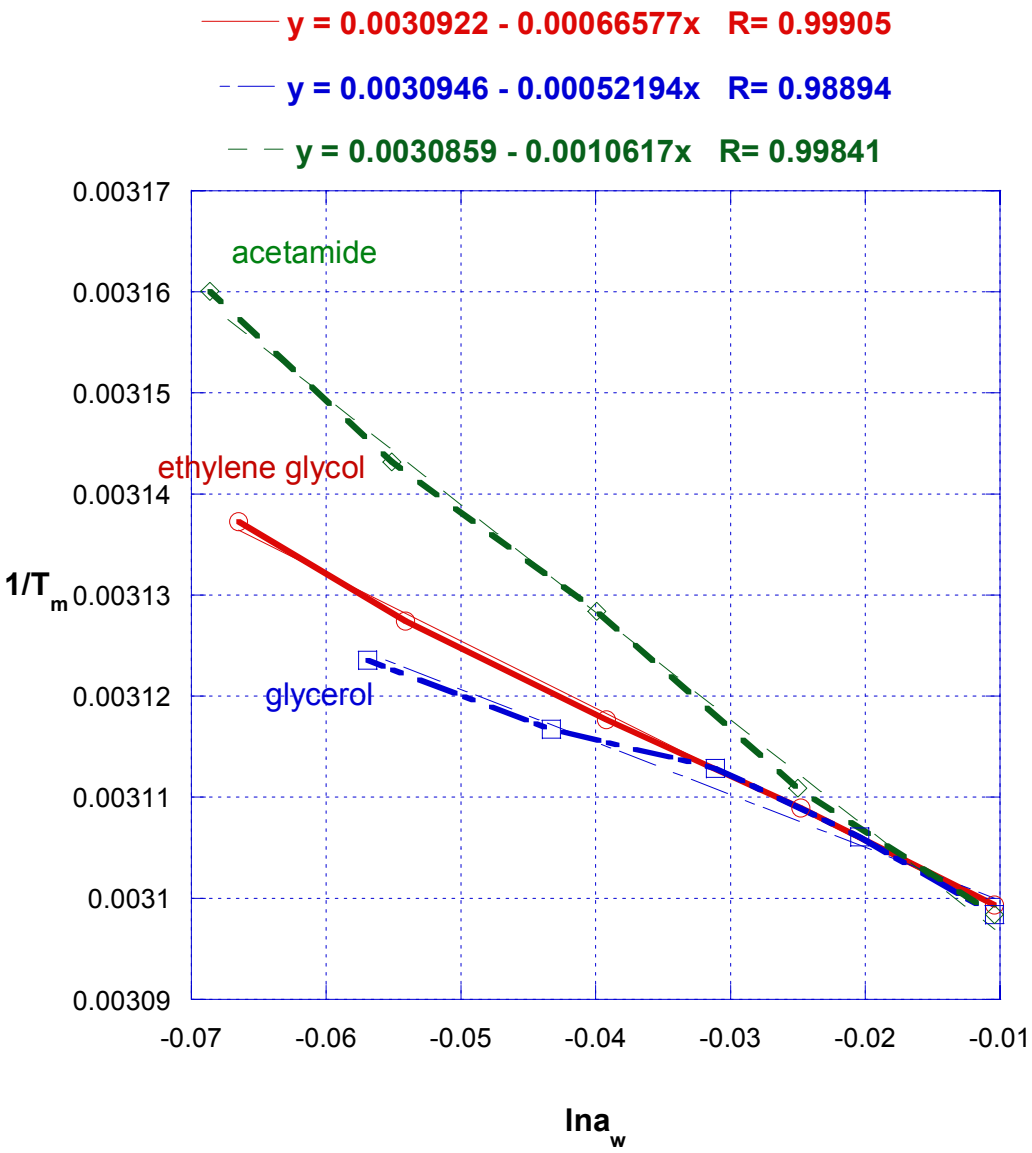


Figure S5. Reciprocal temperature of melting for r(GGCU^{Im}GGCC) vs. the logarithm of water activity for small co-solutes

Table S6. Calculations for r(GGCU^{Im}GGCC)

		$-\Delta H$(cal/mol)	$-\sigma H$(cal/mol)	−slope	$-\sigma$slope	Δn_w	σn_w
er186A-D	Ethylene glycol	81266	1954	0.0006658	0.00005222	**27.2**	**2.2**
er186E-G	Glycerol	81397	2307	0.0005219	0.00008946	**21.4**	**3.7**
er191	Acetamide	80503	2985	0.0010617	0.00006733	**43.0**	**3.2**

Table S7. Experimental t_m and thermodynamic data for melting of r(GGCGUGCC)

Experiment	0%	5%	10%	15%	20%	–ΔG(kcal/mol)	vant' Hoff –ΔH(cal/mol)	δα/δT_m –ΔH(cal/mol)	–ΔS(eu)
Ethylene Glycol									
er229A	42.7	42.0	42.0	40.1	38.9	9.4	80700		230
er229A	42.5	42.5	42.0	40.2	38.6	9.5	78400	71862	222
er229A		42.7	41.3	40.7	39.1				
er229A	42.9	41.8	41.7	40.1	38.7	9.4	75800		214
er229B	42.6	41.9	41.3	40.2	38.8				
er229C	43.0	42.1	41.6	40.2	39.3	9.7	76700	73896	216
er229C	43.2	42.3	41.8	40.5	39.4	9.4	73300	74071	206
er229C	42.9	42.6	41.5	40.4	38.8	9.7	79500	75055	225
er229D	42.5	42.0	41.4	40.6	38.8	9.3	73800	73425	208
er229D	42.6	42.0	41.4	40.4	39.0	9.7	79500	76847	225
er229D	42.8	42.1	42.1	40.0	38.9	9.4	76100	69900	215
er229D	42.8	42.2	41.8	40.1	38.7	9.6	77200	75570	218
er229D	43.0	42.4		40.2	38.8	9.6	75000	76752	211
er229E	42.7	42.0	41.4	40.1	38.7	9.3	79100	70994	225
er229E	43.1	42.7	42.0	40.4	39.2	9.4	73300	72201	206
Average		**42.2**	**41.7**	**40.3**	**38.9**				
STDEV		**0.3**	**0.3**	**0.2**	**0.2**				
Glycerol									
er230A	42.6	42.2	41.8	39.9	38.9	9.7	79800	76165	226
er230B	43.2	42.2	41.8	39.8	38.8	9.6	77800	74023	220
er230C	43.1	42.5		40.3	39.6	9.4	73900		208
er230C	43.0	42.7	41.8	40.2	39.8	9.6	75700		213
er230C	42.7	42.4	41.8	40.1	39.7	9.6	80300	77756	228
er230C	42.7	42.3	42.1	40.0	39.0	9.5	81100	76510	231
er230D	42.6	42.3	41.6	39.9	39.6	9.5	77700	78921	220
er230D		42.0	41.7	39.9	39.3				
er230E		42.4	41.6	40.0	39.4				
er230E		42.5	41.8	40.1	39.2				
er230E		42.6	41.3	39.7	39.2				
er230E		42.6		40.3	39.4				
er230E		42.6	41.3	40.2	39.9				
Average		**42.4**	**41.7**	**40.0**	**39.4**				
STDEV		**0.2**	**0.2**	**0.2**	**0.3**				

Supplementary Material (ESI) for Chemical Communications

Experiment	0%	5%	10%	15%	20%	$-\Delta G$(kcal /mol)	vant' Hoff $-\Delta H$(cal/mol)	$\delta\alpha/\delta T_m$ $-\Delta H$(cal/mol)	$-\Delta S$(eu)
Acetamide									
er230F	42.4	40.3	38.2		34.4	9.4	78900	78399	224
er230G	42.7	40.4	38.3	36.3	33.9	9.3	71000	77119	199
er230G		40.9	38.4	36.5	33.9				
er230G		40.7	38.5	36.0	33.9				
er230G		40.5	38.4	36.3	33.9				
er230H		40.2	38.3	36.1	34.2				
er230H	43.0	40.8	38.6	36.4	34.1	9.5	79000	78790	224
er230H	42.8	40.4	38.4	35.8	34.3	9.6	75000	75599	211
er230H	42.9	40.7	38.3	36.2		9.7	79500	73850	225
er230G	43.2	40.7	38.4	36.3	34.0	9.7	84100	74496	240
er230G		40.4		36.1	34.2				
er230G	43.1	40.2	38.1	36.0	34.2	9.6	78500	72563	222
er230G		40.6	38.1	36.2	34.3				
er230J	43.3		38.3		34.2	9.9	81200	71974	230
er230J	43.2	40.6	38.2	36.3		9.8	80200	79756	227
Average	**42.9**	**40.5**	**38.3**	**36.2**	**34.1**	**9.5**	**77659**	**75060**	**220**
STDEV	**0.2**	**0.2**	**0.1**	**0.2**	**0.2**	**0.2**	**2966**	**2651**	**9**

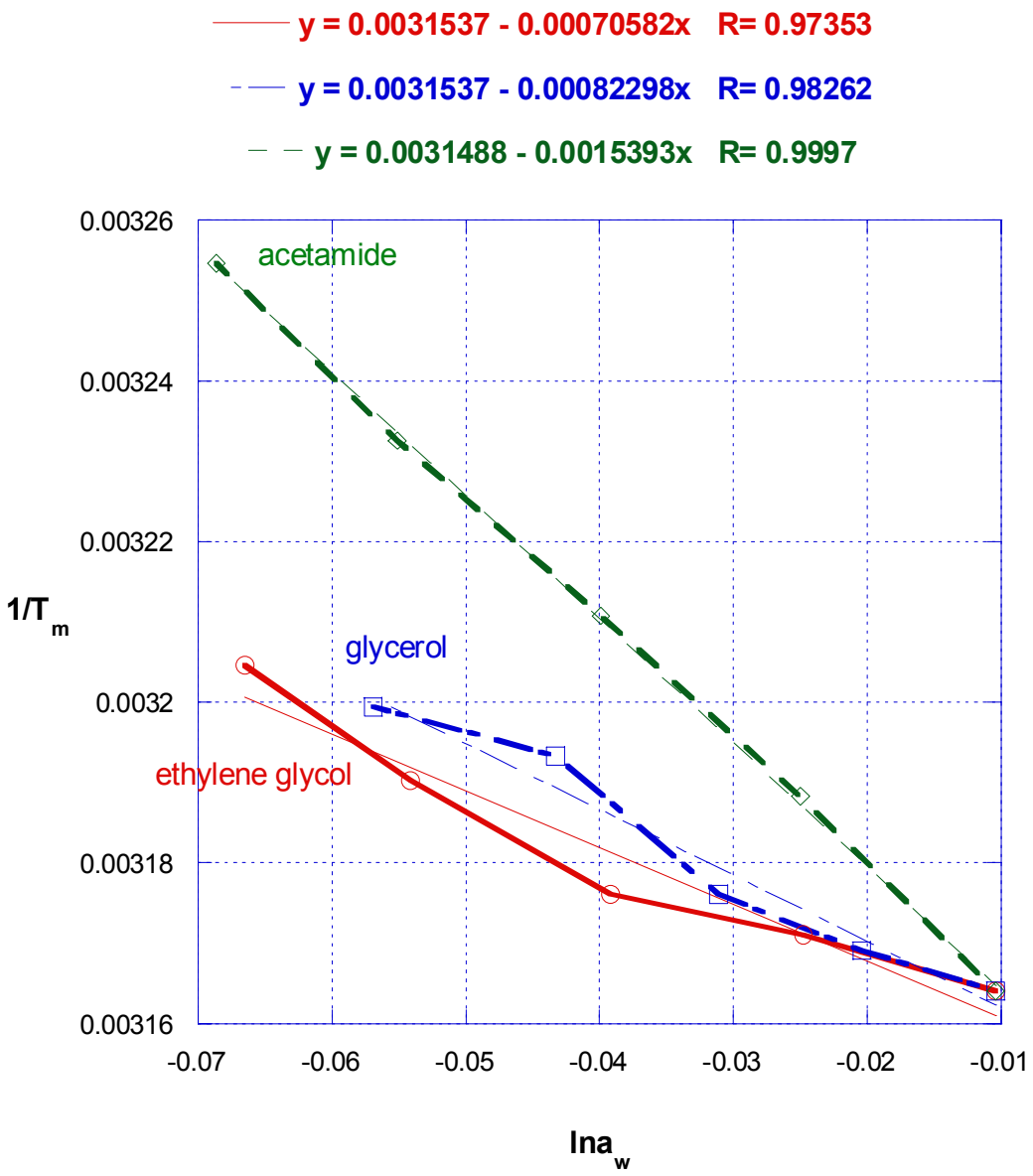


Figure S6. Reciprocal temperature of melting for r(GGCGUGCC) vs. the logarithm of water activity for small co-solutes

Table S8. Calculations for r(GGCGUGCC)

		$-\Delta H$(cal/mol)	$-\sigma H$(cal/mol)	−slope	$-\sigma$slope	Δn_w	σn_w
er229	Ethylene glycol	75060	2651	0.0007058	0.00009346	26.7	**3.7**
er230A-E	Glycerol	75060	2651	0.0008230	0.00012271	31.1	**4.8**
er230F-J	Acetamide	75060	2651	0.0015393	0.00007080	58.2	**3.4**

Table S9. Experimental t_m and thermodynamic data for melting of r(GGCGU^{Im}GCC)

Experiment	0%	5%	10%	15%	20%	$-\Delta G$(kcal/mol)	vant' Hoff $-\Delta H$(cal/mol)	$\delta\alpha/\delta T_m$ $-\Delta H$(cal/mol)	$-\Delta S$(eu)
Ethylene Glycol									
er221A	41.2	39.8	38.9	37.7	35.9	9.0	63900		177
er221B	41.2	39.4	38.7	37.4	36.5	9.3	74400		210
er221B	41.4	39.7	38.8	38.1	35.8	8.9	63500	59867	176
er221C	41.3	39.2	39.5	37.3	35.5	8.7	64490	61024	180
er221C	40.9	39.2	39.1	37.4	36.0	9.1	73300	65164	207
er221C	40.8	39.3	39.0	37.6	35.9	8.9	67500	63865	189
er221C	41.0	39.6		37.9	36.5	9.2	73700	74240	208
er221D	40.7	39.2	39.1	38.0	36.0	9.0	69800	63922	196
er221D	41.6	39.7	38.8	37.8	35.9	9.2	81800	72501	234
Average		**39.5**	**39.0**	**37.7**	**36.0**				
STDEV		**0.2**	**0.3**	**0.3**	**0.3**				
Glycerol									
er221E		40.2	39.2	37.0	37.7				
er221E	41.5	40.6	39.5	37.4	37.3	9.2	73400	67381	207
er221E		40.2	38.6	37.8	36.9				
er221E	40.8	39.9	38.6	37.7	37.0	8.8	72400	72368	205
er221F		40.1	39.6	37.8					
er221F		40.7	39.6	37.9	37.3				
er221F	41.5	40.6	39.5	37.6	37.0	9.1	64600	69795	179
er221F	41.5	40.5	38.9	38.1	37.7	9.2	67500	65292	188
er221F		40.6	39.5	37.5	36.9				
Average		**40.4**	**39.2**	**37.6**	**37.2**				
STDEV		**0.3**	**0.4**	**0.3**	**0.3**				
Acetamide									
er223A	41.0	38.3	36.3	34.1		9.0	68500		192
er223B	40.7	38.2	36.6	34.2	31.4	8.8	69900	61757	197
er223C		38.8	37.1	34.8	31.8				
er223C	41.3	38.9	37.0	34.4		9.2	70600	67201	198
er223C	40.7	38.8	36.9			8.9	72500	70526	205
er223C	41.1	38.7	36.4	34.4		8.8	65600	73019	183
er223D		38.2	36.3	34.6	31.6				
er223E		38.5	36.5		31.1				
Average	**41.1**	**38.6**	**36.6**	**34.4**	**31.5**	**9.0**	**69855**	**67195**	**196**
STDEV	**0.3**	**0.3**	**0.3**	**0.3**	**0.3**	**0.2**	**4727**	**4685**	**15**

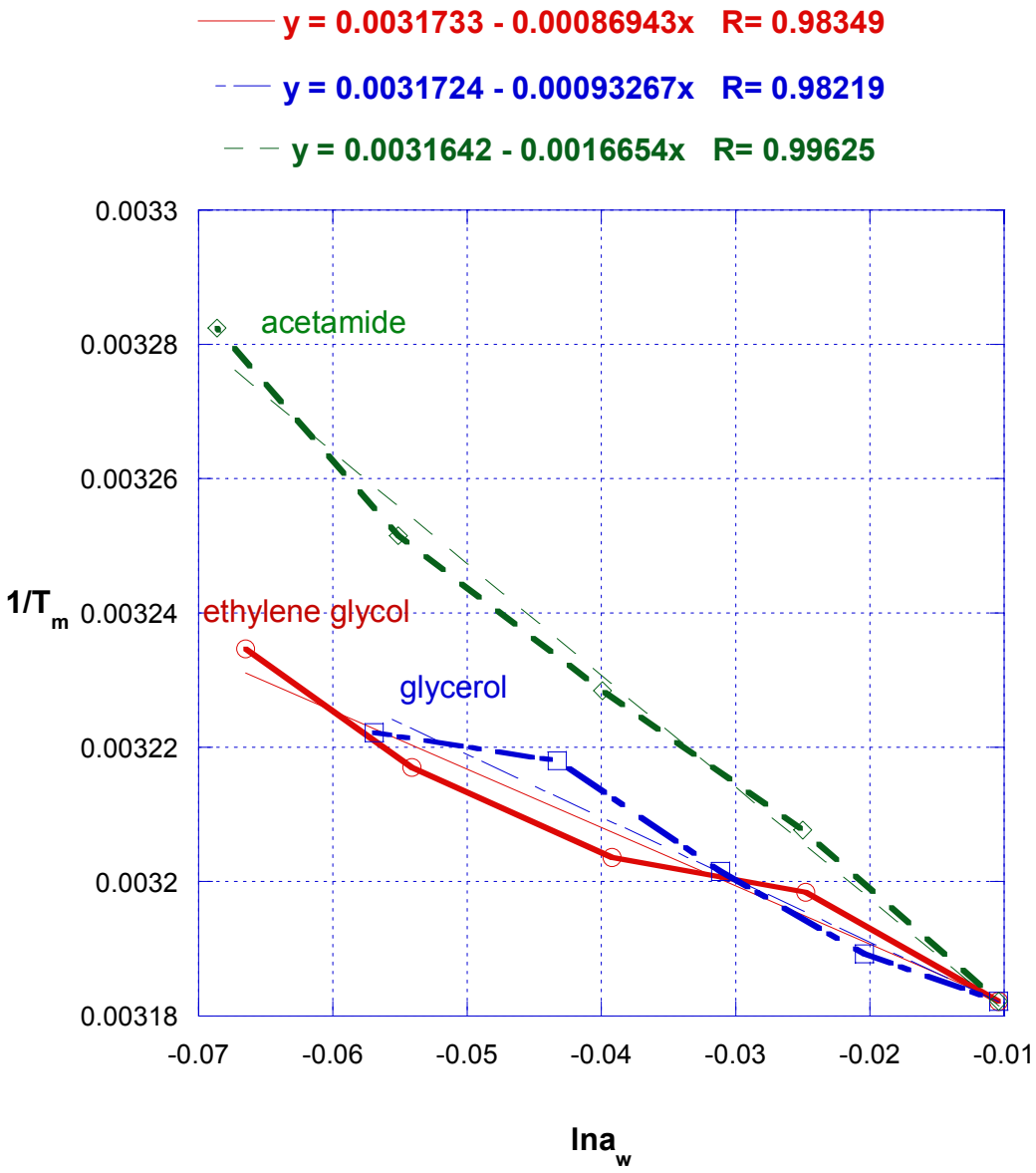


Figure S7. Reciprocal temperature of melting for r(GGCGU^{Im}GCC) vs. the logarithm of water activity for small co-solutes

Table S10. Calculations for r(GGCGU^{Im}GCC)

		–ΔH(cal/mol)	–σH(cal/mol)	–slope	–σslope	Δn_w	σn_w
er221A-D	Ethylene glycol	67195	4685	0.0008694	0.00012436	29.4	**4.7**
er221E-F	Glycerol	67195	4685	0.0009327	0.00015859	31.6	**5.8**
er223	Acetamide	67195	4685	0.0016654	0.00011555	56.3	**5.5**

Table S11. Experimental t_m and thermodynamic data for melting of r(GAGUGCUC)

Experiment	0%	5%	10%	15%	20%	$-\Delta G$(kcal/mol)	vant' Hoff $-\Delta H$(cal/mol)	$\delta\alpha/\delta T_m$ $-\Delta H$(cal/mol)	$-\Delta S$(eu)
Ethylene Glycol									
er231A		38.6	37.8	36.3	35.6				
er231A	39.6	38.6		36.4	35.9	8.6	82100	75994	237
er231A	39.5	38.7	37.5	35.9	35.3	8.7	76300		218
er231A	39.1	38.8	37.8	36.6	35.5	8.6	81800	72964	236
er231B	39.5	38.4	37.5	36.3	35.4	8.7	83800	72376	242
er231B	39.7	38.7	37.8	36.0		8.9	75600	69917	215
er231C		38.9	37.6	36.2	35.8				
er231C	39.0	38.9	37.5	36.0	35.5	7.8	74200	71689	214
er231D	38.9	38.4	37.5	36.5	35.6	8.7	86200	78643	250
er231D	39.0	38.4	37.3	36.2	35.3	8.7	86500	75600	251
Average		**38.6**	**37.6**	**36.2**	**35.5**				
STDEV		**0.2**	**0.2**	**0.2**	**0.2**				
Glycerol									
er231E	39.0	38.5	38.4	36.9	35.9	8.5	87900	81523	256
er231E	39.2			37.0		8.6	87100	77520	253
er231E	39.4	38.4	38.6	37.1		8.9	84560	78509	244
er231E	39.1	38.9	38.6	37.2		8.8	81070	72954	233
er231F	39.1	38.5	38.0	37.5	36.3	8.6	80550	72544	232
er231F	39.2	38.3	38.1	38.0	36.2	8.6	83000	79961	240
er231G	39.3				36.1	8.8	83500	75678	241
er231G	38.8	38.0	38.7			8.4	78500	69279	226
er231G	39.1	38.3	37.8		36.5	8.6	75900	74781	217
er231G	39.5	38.3		37.3	36.4	8.5	74300	75496	212
er231G	39.5	38.8			36.3	8.6	77500	75169	222
er231H		39.3	38.6	38.1					
er231H		38.3		38.1	35.9				
er231H	39.9		38.1	37.4	35.9	8.8	80800	82686	232
er231H	39.5			38.0	36.4	8.9	78700	73089	225
er231H	39.5		38.8	37.6	36.3	8.8	74200	73047	211
Average		**38.5**	**38.4**	**37.5**	**36.2**				
STDEV		**0.4**	**0.3**	**0.4**	**0.2**				
Acetamide									
er232A			36.3	33.8	32.0				
er232A	39.2		36.2	33.3	31.4	8.7	86200	71480	250
er232A	39.3		35.8			8.4	83800	78244	243
er232A	39.3		36.1	33.3	31.6	8.6	76200	76552	218
er232B			35.9	33.1	32.2				
er232B		37.9	35.9		32.0				
er232B	39.0	37.4	36.4	33.7		8.8	82300	74103	237
er232B	39.0	37.7		33.6	32.0	8.7	79700	74321	229
Average	**39.3**	**37.7**	**36.1**	**33.5**	**31.9**	**8.6**	**80825**	**75158**	**233**
STDEV	**0.3**	**0.3**	**0.2**	**0.3**	**0.3**	**0.2**	**4269**	**3373**	**14**

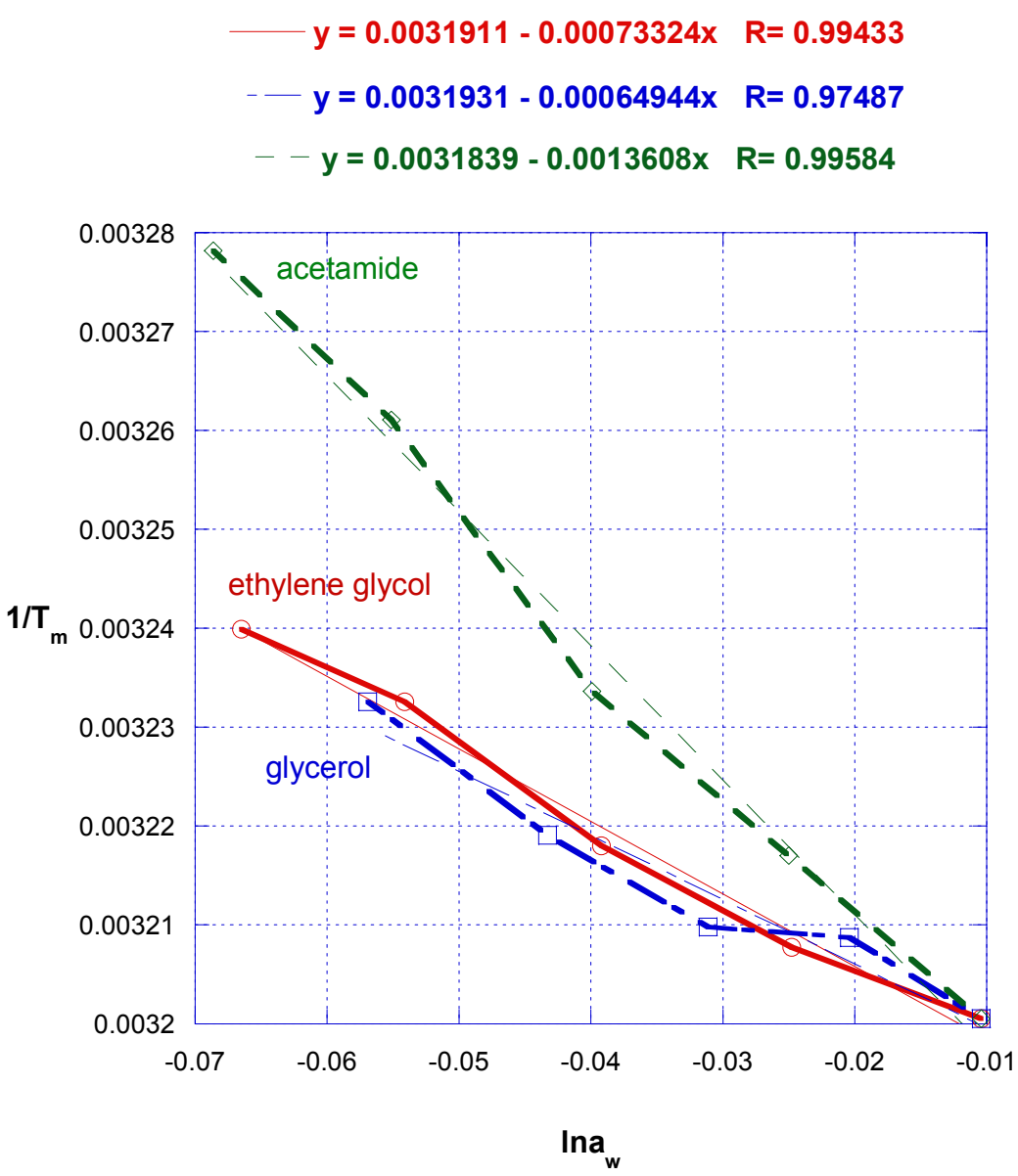


Figure S8. Reciprocal temperature of melting for r(GAGUGCUC) vs. the logarithm of water activity for small co-solutes

Table S12. Calculations for r(GAGUGCUC)

		$-\Delta H$(cal/mol)	$-\sigma H$(cal/mol)	−slope	$-\sigma$slope	Δn_w	σn_w
er231A-D	Ethylene glycol	75158	3373	0.0007332	0.00010298	27.7	**4.1**
er231E-H	Glycerol	75158	3373	0.0006494	0.00015873	24.6	**6.1**
er232	Acetamide	75158	3373	0.0013609	0.00011670	51.5	**5.0**

Table S13. Experimental t_m and thermodynamic data for melting of r(GAGU^{Im}GCUC)

Experiment	0%	5%	10%	15%	20%	$-\Delta G$(kcal/mol)	vant' Hoff $-\Delta H$(cal/mol)	$\delta\alpha/\delta T_m$ $-\Delta H$(cal/mol)	$-\Delta S$(eu)
Ethylene Glycol									
er220B	29.9	29.1	28.0	27.9	27.4	6.0	85100	77126	255
er220B	30.2	29.3	28.5	27.6	26.9	6.2	84400	71575	252
er220B	30.3	29.3	28.1	27.8	27.2	6.4	77100	67096	228
er220B	30.0	28.9	28.0	27.5	27.1	7.2	85700	69166	253
er220C	30.0	28.5		27.8	26.9	6.4	73400	62969	216
er220C		29.2	28.2	27.8	26.9				
er220C		29.4	28.1	27.4	27.2				
Average		**29.1**	**28.2**	**27.7**	**27.1**				
STDEV		**0.3**	**0.2**	**0.2**	**0.2**				
Glycerol									
er220D	30.3		28.7	27.4	27.3	6.3	82300	63648	245
er220D	30.2	29.6	28.5	28.2	27.3	6.5	76600	63596	226
er220D	29.8	29.4	28.6	27.8	27.0	6.4	76800	67918	227
er220D	30.2	29.2		28.0	27.6	6.2	90300	75153	271
er220E	30.3		28.9	28.2	27.5	6.5	74100	65961	218
er220E	29.9	29.7	29.4	27.6	27.1	6.4	76200	73997	225
er220F	30.3		28.7	27.7	27.1	6.2	78500	72907	233
er220F		29.2	28.4	28.1	27.3				
er220F			28.6	27.5	27.1				
er220F	29.9	29.4	28.3	27.8	27.2	6.0	82600	69470	247
Average		**29.4**	**28.7**	**27.8**	**27.3**				
STDEV		**0.2**	**0.3**	**0.3**	**0.2**				
Acetamide									
er222A		28.8	27.9	26.2	25.6				
er222B		28.8	28.0	26.7	25.6				
er222B		29.4	28.2	26.6					
er222C		28.9	28.3	26.1	24.8				
er222D		28.8	28.4	26.5	25.0				
er222E		29.5	27.7	26.7	24.9				
er222E		29.6	27.6	26.6	25.1				
er222E		28.9	28.2	26.2					
er222E			28.5	26.8	25.0				
Average	**30.1**	**29.1**	**28.1**	**26.5**	**25.1**	**6.4**	**80238**	**69276**	**238**
STDEV	**0.2**	**0.3**	**0.3**	**0.3**	**0.3**	**0.3**	**5168**	**4639**	**17**

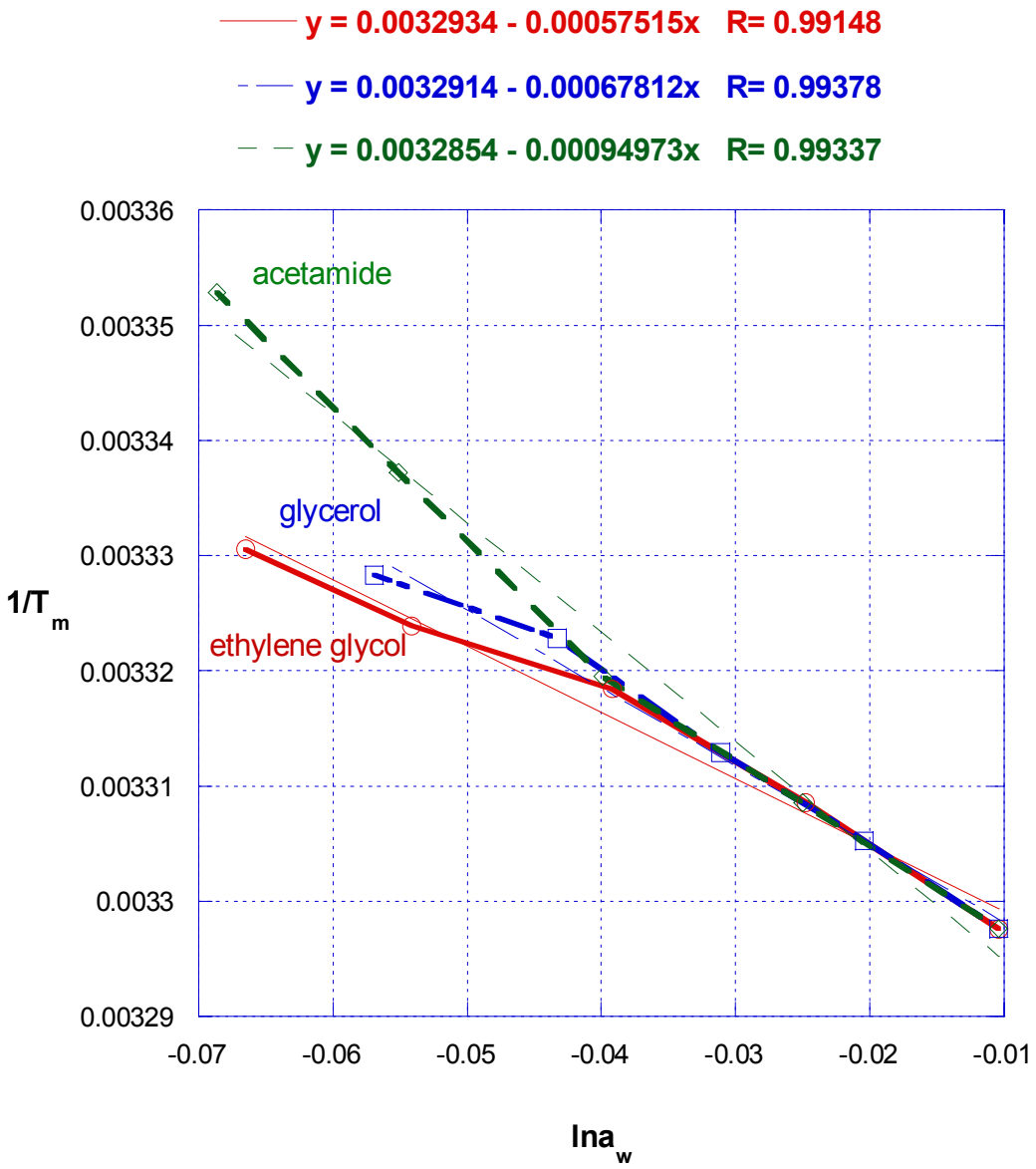


Figure S9. Reciprocal temperature of melting for r(GAGU^{Im}GCUC) vs. the logarithm of water activity for small co-solutes

Table S14. Calculations for r(GAGU^{Im}GCUC)

		$-\Delta H$(cal/mol)	$-\sigma H$(cal/mol)	−slope	$-\sigma$slope	Δn_w	σn_w
er220B-C	Ethylene glycol	69276	4639	0.0005752	0.00009031	20.1	**3.4**
er220D-F	Glycerol	69276	4639	0.0006781	0.00010782	23.7	**4.1**
er222	Acetamide	69276	4639	0.00094973	0.00010851	33.1	**4.4**

Table S15. Experimental t_m and thermodynamic data for melting of r(GAGGUCUC)

Experiment	0%	5%	10%	15%	20%	$-\Delta G$(kcal/mol)	vant' Hoff $-\Delta H$(cal/mol)	$\delta\alpha/\delta T_m$ $-\Delta H$(cal/mol)	$-\Delta S$(eu)
Ethylene Glycol									
er198A	33.1	32.4	31.8	31.2		6.8	88100	82997	262
er198B	32.4	32	31.3	30.3	29.9	6.9	83800	82433	248
er198C	32.4	33	31.2	30.8	29.3	7.1	78100	73039	229
er198C	32.9	32.7	31.5	30.3	30.2	7.0	81700	76987	241
er198C	32.6	33	31.8	31.1	30.1	6.9	80700	78937	238
er198C	32.5	32.9	32.2	31.2	29.6	6.9	82600	75928	244
er198D	32.5	32.4	31.7	30.7	29.2	6.9	86600	76191	257
er198D	32.9	32.5	31.3	30.9	30.2	6.9	91600	73991	273
er198D	32.7	32.4	31.7	30.9	29.8	6.8	84300	81164	250
er198D	32.6	32.8	31.8	31	29.7	6.9	82900	73876	245
er198E	32.9	33	31.7	30.7	29.9	6.9	86000	82013	255
er198E	33.1	32.5	31.7	31	29.5	6.9	90600	75041	270
er198E	32.8	33.1	31.8	31.1	29.6	6.9	85700	73101	254
er198E	32.7		31.4	30.9	29.8	6.8	85900	78294	255
er198F	32.4	32.1	31.6	30.2		7.0	84500	72258	250
er198F	32.7	31.9	31.7	30.5		7.0	85200	74251	252
er198F	33	32.1	31.6	30.1		6.9	89700	80181	267
Average	**32.7**	**32.6**	**31.6**	**30.8**	**29.8**	**6.9**	**85176**	**77099**	**252**
STDEV	**0.2**	**0.4**	**0.2**	**0.4**	**0.3**	**0.1**	**3538**	**3612**	**12**
Glycerol									
er200A	32.5	32.4	31.7	31.1	30.4	6.7	86100		256
er200B	32.3	32.2	31.8	31.4	30.3	6.9	86900		258
er200B	32.6	32.8	32	31.1	30.6	6.9	83200	75467	246
er200B	32.6	32.5	32.3	31.2	30.1	7.0	85200	77985	252
er200B	32.6	32.8	31.8	31.6	30.7	6.7	82700	74734	245
er200C	32.2	32.8	31.8	31.5	30.5	6.9	85100	79530	252
er200D	32.7		31.8	31.8	30.9	6.8	88400	83448	263
er200E	32.8		32.6	31.7	30.6	6.9	92800	81746	277
er200F	32.3	32.6		31.4	30.9	6.9	90000	80768	268
er200G	33.0	32.4		31.2	30.2	6.8	91800	81668	274
er200H	32.6	32.9		31.4	30.6	6.8	85000		252
Average	**32.6**	**32.6**	**32.0**	**31.4**	**30.5**	**6.9**	**87018**	**79418**	**258**
STDEV	**0.2**	**0.2**	**0.3**	**0.2**	**0.3**	**0.1**	**3350**	**3120**	**11**
Acetamide									
er203A	32.3	30.5	29.5	26.9	25.5	6.7	86700	77398	258
er203A	32.3	31	29.1	26.8	25.9	6.8	85900	80747	255
er203B	32.8	30.9	29	27	25.7	6.8	91500	82631	273
er203B	32.4	31.2	29.4	26.9	25.8	6.7	89500	79075	267
er203B	32.8	31	29.6	27.6	25.1	6.8	95200	76495	285
er203B	32.5	30.5	29.4	27.7	25.7	6.9	90000	82098	268
er203C	33.1	31.5	29.2	27.7	25.3	6.9	94100	75359	281
er203D	32.5	30.5	29.1	27.6	26	7.0	83300	76170	246
Average	**32.6**	**30.9**	**29.3**	**27.3**	**25.6**	**6.8**	**89525**	**78747**	**267**
STDEV	**0.3**	**0.4**	**0.2**	**0.4**	**0.3**	**0.1**	**4091**	**2814**	**13**

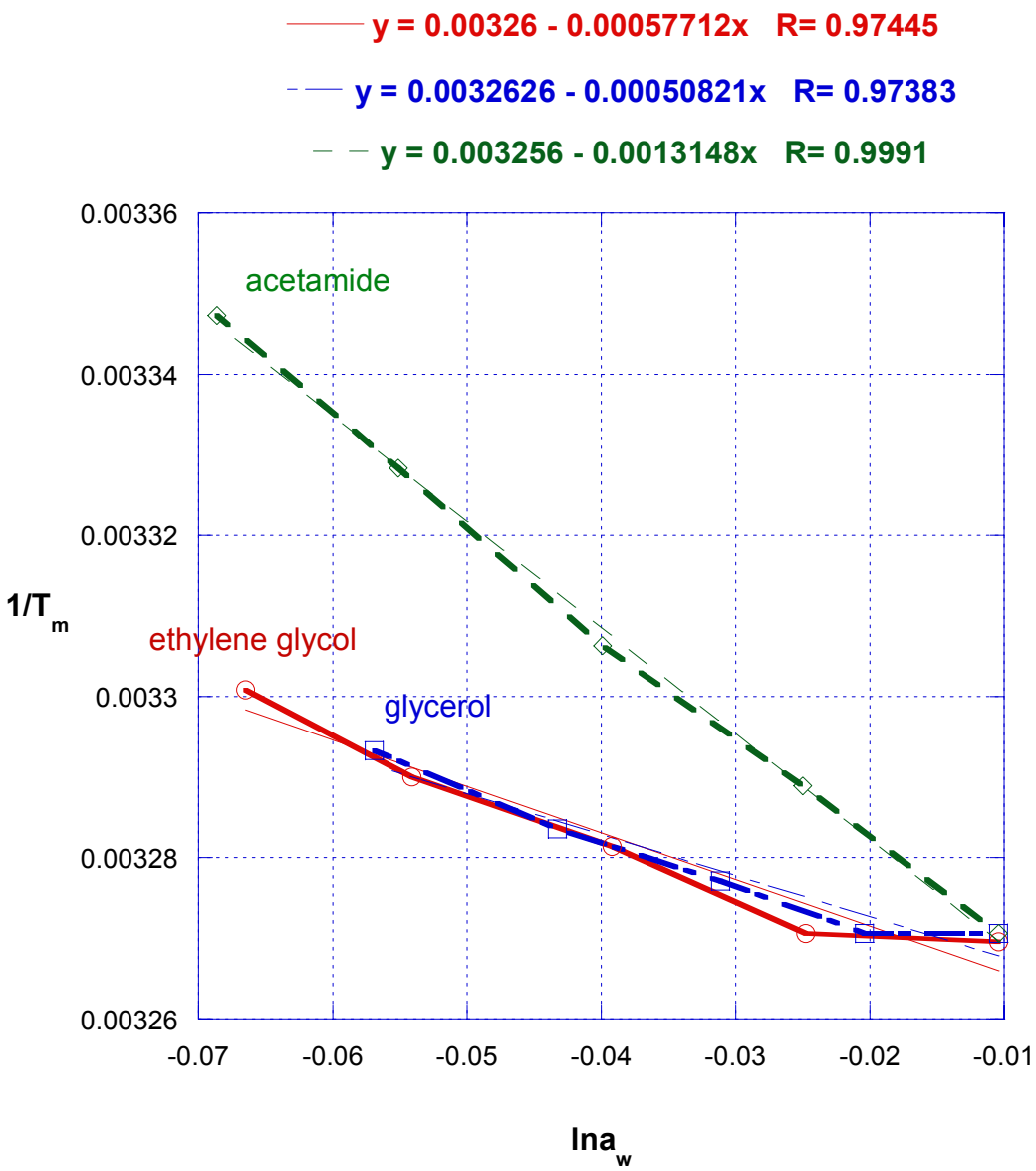


Figure S10. Reciprocal temperature of melting for r(GAGGUCUC) vs. the logarithm of water activity for small co-solutes

Table S16. Calculations for r(GAGGUCUC)

		$-\Delta H$(cal/mol)	$-\sigma H$(cal/mol)	−slope	$-\sigma$slope	Δn_w	σn_w
er198	Ethylene glycol	77099	3612	0.0005771	0.00011529	**22.4**	**4.6**
er200	Glycerol	79418	3120	0.0005082	0.00012453	**20.3**	**5.0**
er203	Acetamide	78747	2814	0.0013148	0.00011285	**52.1**	**4.8**

Table S17. Experimental t_m and thermodynamic data for melting of r(GAGGU^{Im}CUC)

Experiment	0%	5%	10%	15%	20%	−ΔG(kcal /mol)	vant' Hoff −ΔH(cal/mol)	δα/δT$_m$ −ΔH(cal/mol)	−ΔS(eu)
Ethylene Glycol									
er207A	29.3	28.3	28	26.7	25.7	6.2	81900		244
er207A	29.4	27.9	28.2	26.2	25.9	6.2	80900	77678	241
er207A	29.2	28.6	28.3	26.3	25.7	6.1	76800	75093	228
er207A	29	28.4	27.1	26.4	25.1	6.1	80800	78110	241
er207B		28	27.7	26.8	25				
er207B	28.6	28	27.6	26.3	25.8	5.8	79600	73999	238
er207B	29.3			26.8	25.3	6.2	80900		241
er207B	29.1	28.8		26.2	25.6	5.9	81600	73279	244
er207C	28.7	28.5	28.3	26.8	25.2	6.0	73900	69892	219
er207C	28.8	28.5	28.1	26.8	25.8	5.9	80000	72583	239
er207D	29.4	28.7	27.3	26.7	25.3	5.8	88000	79538	265
er207D	29.1	28.5	27.7	26.7	25.1	6.0	78900	76048	235
er207D	28.6	27.9	28	26.4	25.4	5.9	77900	75212	232
er207D	29.2	28.1		26	24.8	6.0	80700	75667	241
Average	**29.1**	**28.3**	**27.8**	**26.5**	**25.4**	**6.0**	**80146**	**75191**	**239**
STDEV	**0.3**	**0.3**	**0.4**	**0.3**	**0.3**	**0.1**	**3239**	**2730**	**11**
Glycerol									
er208A		27.7	27.5		26				
er208A	28.8	27.2	28	26.5	26	6.0	82600	69348	247
er208A	29.2	28	28.4	27.5	26	6.0	81700	70329	244
er208A	29.5	28.3	27.7	27.5	26.6	6.1	82400	71042	246
er208B	28.8	28.1	27.6	26.4	26.2	5.8	87100		262
er208B	28.9	28	27.6	27.6	26.6	5.7	87600		264
er208C	29.2	28.5	27.4	26.6	26.9	6.3	76100	68592	225
er208C	29.2	28.1	28.5	26.8	26.7	6.0	79200	70534	236
er208C	29.4	28.2	27.4		26.6	6.2	81300	78694	242
er208C	29.2	28.6	28.2	26.7	26.8	6.1	82700	72560	247
er208D	29.2	28.2	27.4	26.6	26.9	6.1	83900	75409	251
er208D		28.2	28.3	27.1	26.5				
er208D	28.8	27.5		26.8	26.5	6.3	69300	73587	203
er208D		27.8		26.6	26.2				
Average	**29.1**	**28.0**	**27.8**	**26.9**	**26.5**	**6.1**	**81264**	**72233**	**242**
STDEV	**0.2**	**0.4**	**0.4**	**0.4**	**0.3**	**0.2**	**5104**	**3226**	**17**

Experiment	0%	5%	10%	15%	20%	$-\Delta G$(kcal /mol)	vant' Hoff $-\Delta H$(cal/mol)	$\delta\alpha/\delta T_m$ $-\Delta H$(cal/mol)	$-\Delta S$(eu)
Acetamide									
er209A		27.1	24.7	22.2	19.6				
er209B	29.1	26.6	24.6	21.9		6.1	80500	77832	240
er209C	29.1	26.9	24.5	21.8	19.3	6.2	74400	77759	220
er209D	28.9	26.5	24.7	21.9	19.2	6.0	78900	71475	235
er209D	29.2	26.5	25.4			5.9	81000	73587	242
er209D	29.2	27.1	25.1			6.0	81100	75005	242
er209D	29.4	27	24.8		19.3	5.9	81300	71081	243
er209E	29.5	26.7	25.2		19	5.9	81000	77148	242
er209F	29.4		24.2		19.7	6.1	78400	73755	233
er209F	29.2	27.6	24.2			5.9	79100	73354	236
er209F	29.3	27.5	24.7		20.0	6.2	72300	71948	213
er209F		27.6	24.9		19.5				
Average	**29.2**	**27.0**	**24.8**	**22.0**	**19.5**	**6.0**	**78800**	**74294**	**235**
STDEV	**0.2**	**0.4**	**0.4**	**0.2**	**0.3**	**0.1**	**3091**	**2552**	**10**

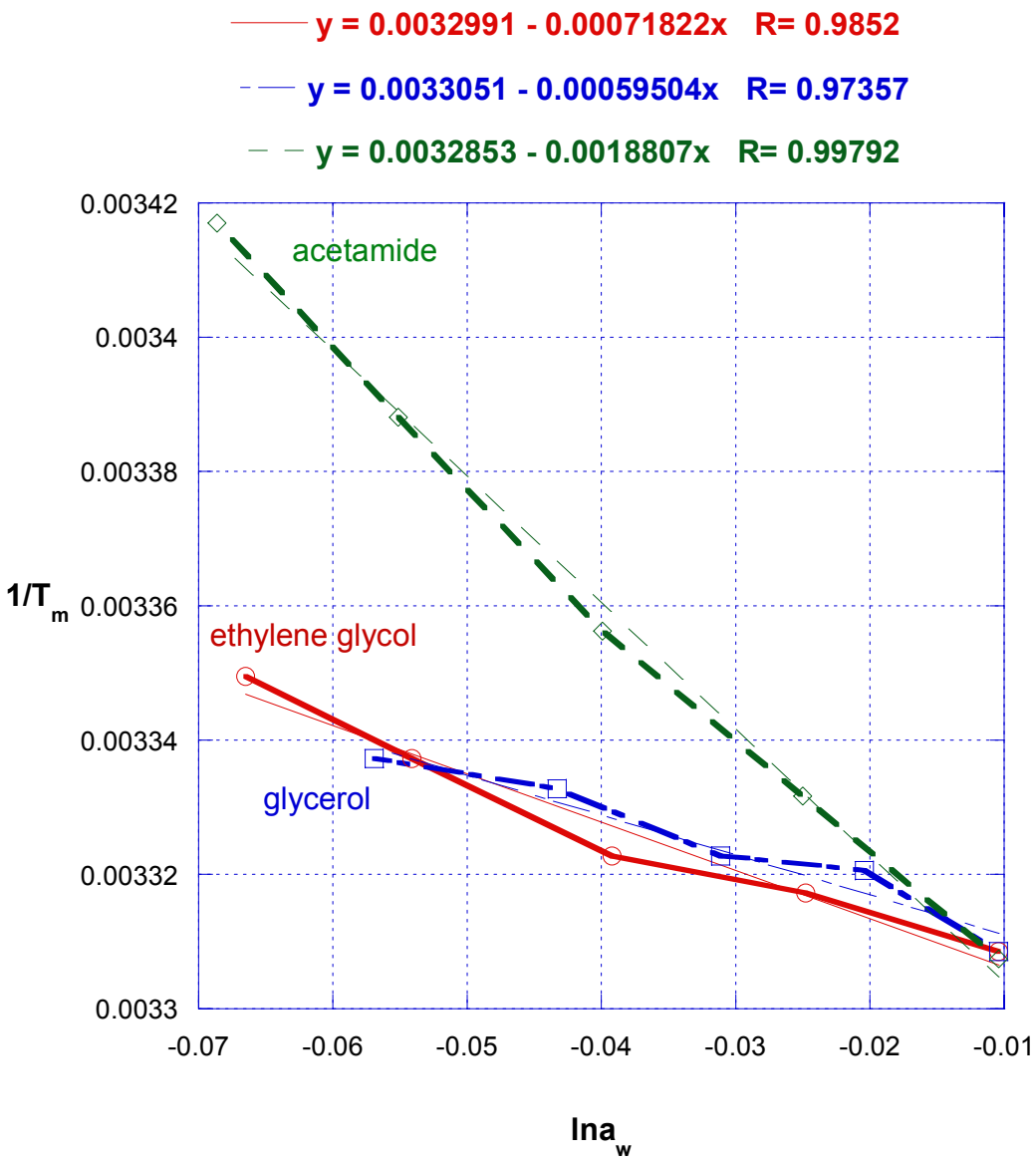


Figure S11. Reciprocal temperature of melting for r(GAGGU^{Im}CUC) vs. the logarithm of water activity for small co-solutes

Table S18. Calculations for r(GAGGU^{Im}CUC)

		–ΔH(cal/mol)	–σH(cal/mol)	–slope	–σslope	Δn_w	σn_w
er207	Ethylene glycol	75191	2730	0.0007182	0.00012722	**27.2**	**4.9**
er208	Glycerol	72233	3226	0.0005950	0.00017152	**21.6**	**6.3**
er209	Acetamide	74294	2552	0.0018807	0.00009775	**70.4**	**4.4**