Supplementary Data
Discovering Ligands for a microRNA Precursor with Peptoid Microarrays

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Characterization of Compounds: Each compound, **1-13**, was characterized by Matrix Assisted Laser Desorption Ionization (MALDI) mass spectrometry (Voyager DE Pro, ABI) using α-cyano-4-hydroxycinnamic acid as matrix. Each compound was purified to a single peak by HPLC using the method described in Experimental Procedures.

Table S1. Compound characterization by MALDI-MS

	Mass	
Compound	Calculated	Found (m + H^+)
1	673.34	674.40
2	725.41	726.40
3	636.75	637.58
4	287.17	288.45
5	616.32	617.45
6	559.63	560.47
7	588.27	589.38
8	525.27	526.50
9	596.31	597.30
10	643.30	644.45
11	607.44	608.54
12	673.34	674.40
13	673.34	674.41

2-Aminopurine Fluorescence, Isothermal Titration Calorimetry, and In-Line Probing Yield Equivalent Values for Dissociation Constants:

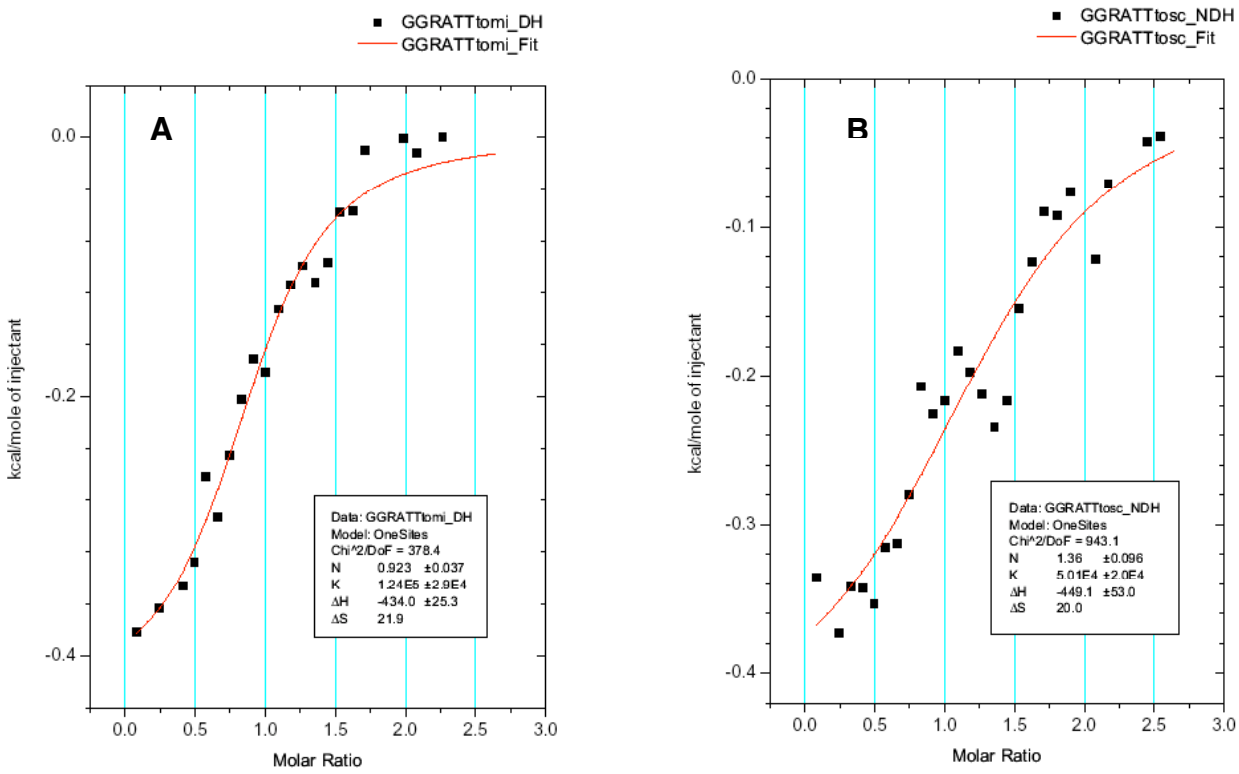

Figure S1. Isothermal titration calorimetry binding curves. ITC was carried out and data were analyzed as described in Experimental Procedures. (A) Association of RNA I with compound **2**. (B) Association of RNA II with compound **2**.

Dissociation constants for compound **2** with miR-21hp (RNA I) and control hp (RNA II) measured by monitoring 2-aminopurine fluorescence are equal, within experimental uncertainty, to those measured by ITC.

Table S2. Comparison of K_d Values Measured by ITC and 2-Aminopurine Fluorescence.

RNA	MiR-21hp (RNA I)		Control hp (RNA II)	
Method	ITC	fluorescence	ITC	fluorescence
K_d (μM)	8±2	10.1±0.4	20±8	19±1

Binding of Compounds **1** and **2** to miR-21hp was also assessed by the effects of the peptoids on Mg^{2+}-induced hydrolytic cleavage of the RNA (Figure 5C). The dissociation constants measured for **1** and **2** with miR-21hp using this analysis are compared to the dissociation constants measured by the 2-aminopurine fluorescence assay under the same conditions (10 mM KCl, 50 mM Tris, pH 8.5, 1 mM $MgCl_2$) in Table S3. Measurements made for both compounds by the two different methods agree within experimental uncertainty.

Table S3. Comparison of K_d Values Measured by In-Line Probing (Mg^{2+}-Induced Cleavage) and 2-Aminopurine Fluorescence for Binding of **1** and **2** to MiR-21hp.

Compound	**1**		**2**	
Method	In-line	fluorescence	In-line	fluorescence
Kd (μM)	251±52	249±22[a]	164±13	128±60[a]

[a]See representative binding curves in Figure S2.

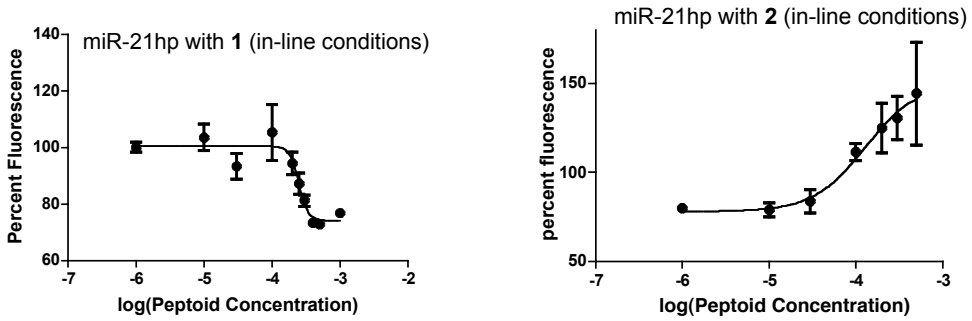

Figure S2. Representative binding curves for determination of dissociation constants by monitoring 2-aminopurine fluorescence as a function of peptoid concentration (M) under conditions of the in-line cleavage assay.

Thus, in all cases measured by two or more of the three assays, with both compounds **1** and **2** and RNA I and RNA II, the assays are in agreement. This agreement of multiple methods supports the validity of each. Most importantly, it supports the assumption that substitution of 2-aminopurine for adenine in the RNAs of this study does not perturb the binding interaction.