

Supporting methods

Equations to obtain GTP analog K_d s from competition binding experiments

The dissociation constant for a complex is defined by the following equation:

$$K_d = \frac{[R]_f[L]_f}{[RL]} \quad (1)$$

where:

[R]_f: concentration of free RNA

[L]_f: concentration of free ligand (GTP in this case)

[RL]: complex of RNA bound to its ligand

When the total concentration of RNA ([R]_t) is much higher than the concentration of the ligand, [R]_f is not significantly reduced when some of the RNA becomes complexed with the ligand¹. Because of this, when [R]_t >> [L]_t equation (1) can be rearranged (after ref. 2) to give:

$$\frac{[RL]}{[L]_t} = \frac{[R]_t}{[R]_t + K_d} \quad (2)$$

where:

[R]_t: total RNA concentration

[L]_t: total GTP concentration

[RL]/[L]_t: fraction of labeled GTP bound

Further rearrangement yields:

$$\frac{[RL]}{[L]_t} = \frac{1}{1 + \frac{K_d}{[R]_t}} \quad (3)$$

When the aptamer binds to the analog (I) the concentration of free RNA available to bind to the GTP is reduced by an amount equal to the concentration of the RNA-analog complex [RI] so that equation (3) becomes:

$$\frac{[RL]}{[L]_t} = \frac{1}{1 + \frac{K_d}{[R]_t - [RI]}} \quad (4)$$

At equilibrium, the apparent $K_{d(I)}$ of the aptamer for the analog can be described as:

$$K_{d(I)} = \frac{[R]_f [I]_f}{[RI]} \quad (5)$$

$K_{d(I)}$: K_d of the analog

$[R]_f$: concentration of free RNA = $[R]_t - [RI]$

$[I]_f$: concentration of free analog = $[I]_t - [RI]$

$[RI]$: concentration of RNA-analog complex

Substitution and rearrangement gives:

$$K_{d(I)} = \frac{([R]_t - [RI]) \cdot ([I]_t - [RI])}{[RI]} = \frac{[R]_t [I]_t - [I]_t [RI] - [R]_t [RI] + [RI]^2}{[RI]} \quad (6)$$

Setting the equation equal to zero results in a quadratic equation of the form

$$0 = ax^2 + bx + c$$

where:

$$a = 1$$

$$b = -([I]_t + [R]_t + K_{d(I)})$$

$$c = [R]_t [I]_t$$

After rearrangement:

$$[RI] = \frac{([R]_t + [I]_t + K_{d(I)}) \pm \sqrt{([R]_t + [I]_t + K_{d(I)})^2 - 4 [R]_t [I]_t}}{2} \quad (7)$$

Next, equation (4) is normalized for the condition of no competitor present by dividing by equation (3):

$$\frac{[RL]}{[L]_t} = \frac{1 + \frac{K_d}{[R]_t - [RI]}}{1 + \frac{K_d}{[R]_t}} \quad (8)$$

Finally, the right side of equation (7) is substituted into equation (8) and rearranged to yield:

$$\frac{[RL]}{[L]_t} = \frac{1 + \frac{K_d}{[R]_t}}{1 + \frac{K_d}{[R]_t - \frac{([R]_t + [I]_t + K_d) - \sqrt{([R]_t + [I]_t + K_d)^2 - 4 [R]_t [I]_t}}{2}}} \quad (9)$$

[R]_t: total RNA concentration

[I]_t: total inhibitor concentration

[RL]/[L]_t: fraction of labeled GTP bound

K_d: K_d of the analog obtained by non-linear least squares fit of the fraction of labeled GTP bound ([RL]/[L]_t) versus the concentration of unlabeled analog ([I]_t) in the sample .

Supporting References

1. Davis, J. H.; Szostak, J. W. Isolation of high-affinity GTP aptamers from partially structured RNA libraries. *Proc. Natl. Acad. Sci. U.S.A.* 2002, *99*, 11616-11621.
2. Langmuir, I. The Adsorption of Gases in Plane Surface of Glass, Mica and Platinum. *J. Am. Chem. Soc.* 1918, *40*, 1361-1402.

Supporting Figure 1. Chemical analogs of GTP employed in this study. (A) analogs used to assess the effect of bulky ligand substitutions on aptamer binding affinity (B) analogs used to assess the effect of removed or altered ligand functional groups on aptamer binding affinity. The modifications relative to GTP are highlighted. The number of aptamers with $\log_{10} K_d(\text{analog}) / K_d(\text{GTP}) < 2$ for each analog are shown in parentheses.

Supporting Figure 2. Aptamer-analog competition binding curves. (A-K) The normalized and base-line corrected fraction of α ³²P-GTP bound is plotted as a function of the concentration of analog competitor. The line represents the ideal fit of the data.

Supporting Figure 3. Each aptamer has a unique pattern of binding specificity. For each aptamer, ligand positions where chemical modification resulted in large reductions in aptamer binding affinity relative to GTP (i.e. $\log_{10} K_d(\text{Analog}) / K_d(\text{GTP}) > 1$) are colored red. Ligand positions where chemical modifications did not reduce binding affinity relative to

GTP are colored green. The large shaded circles represent results for analogs with bulky modifications while the smaller inset circles represent results obtained with modified or deleted functional groups. Only the GMP moiety of the ligand is shown here. However, note that the Class II aptamer has $\log_{10} K_d(\text{Analog}) / K_d(\text{GTP})$ values >1 for GDP, GMP, GTP- γ -s and GTP- α -S. Also see Figure 4 of the main text.

Figure 1A.

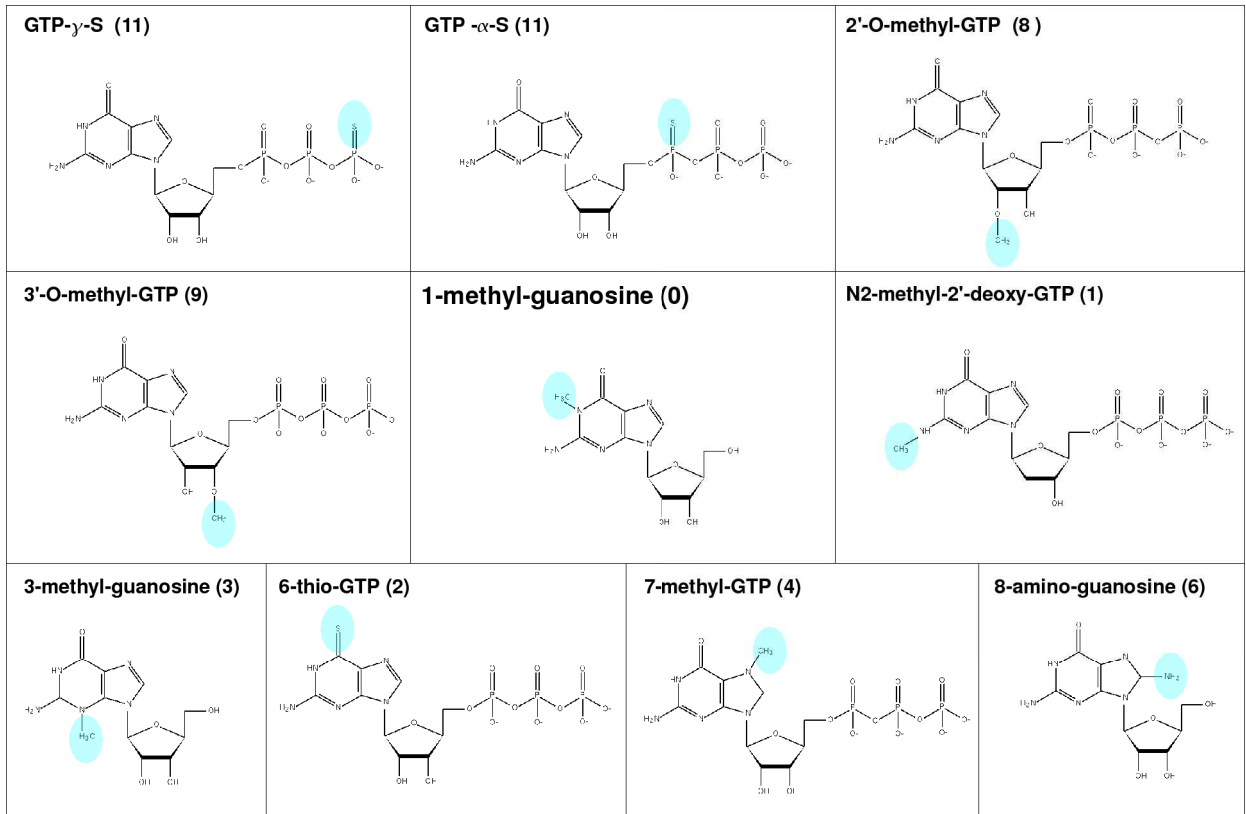


Figure 1B.

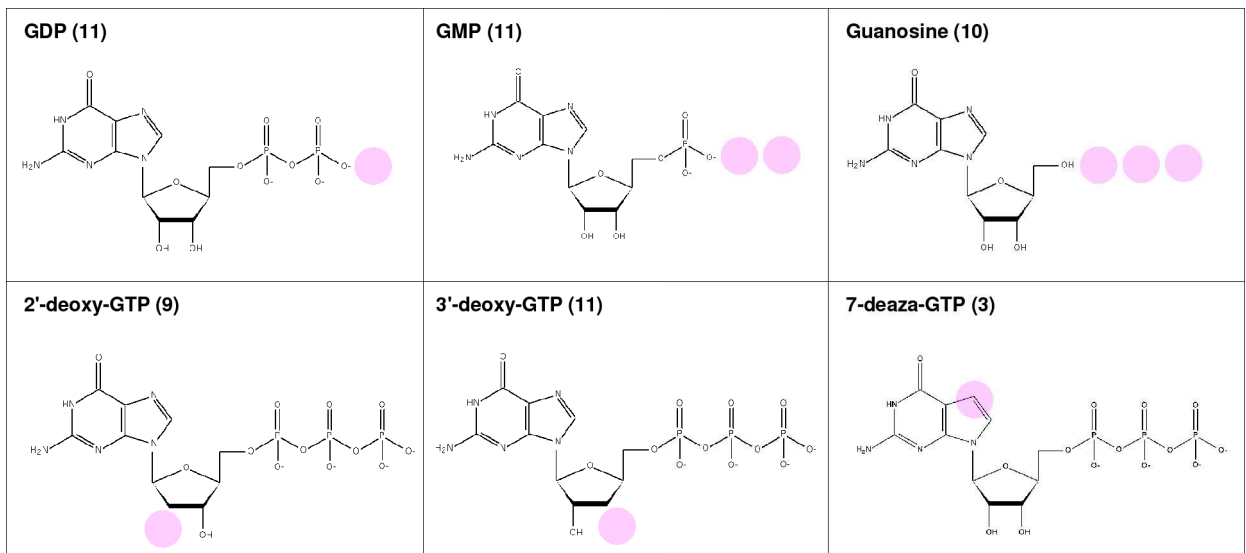


Figure 2A. 9-4 analog binding curves.

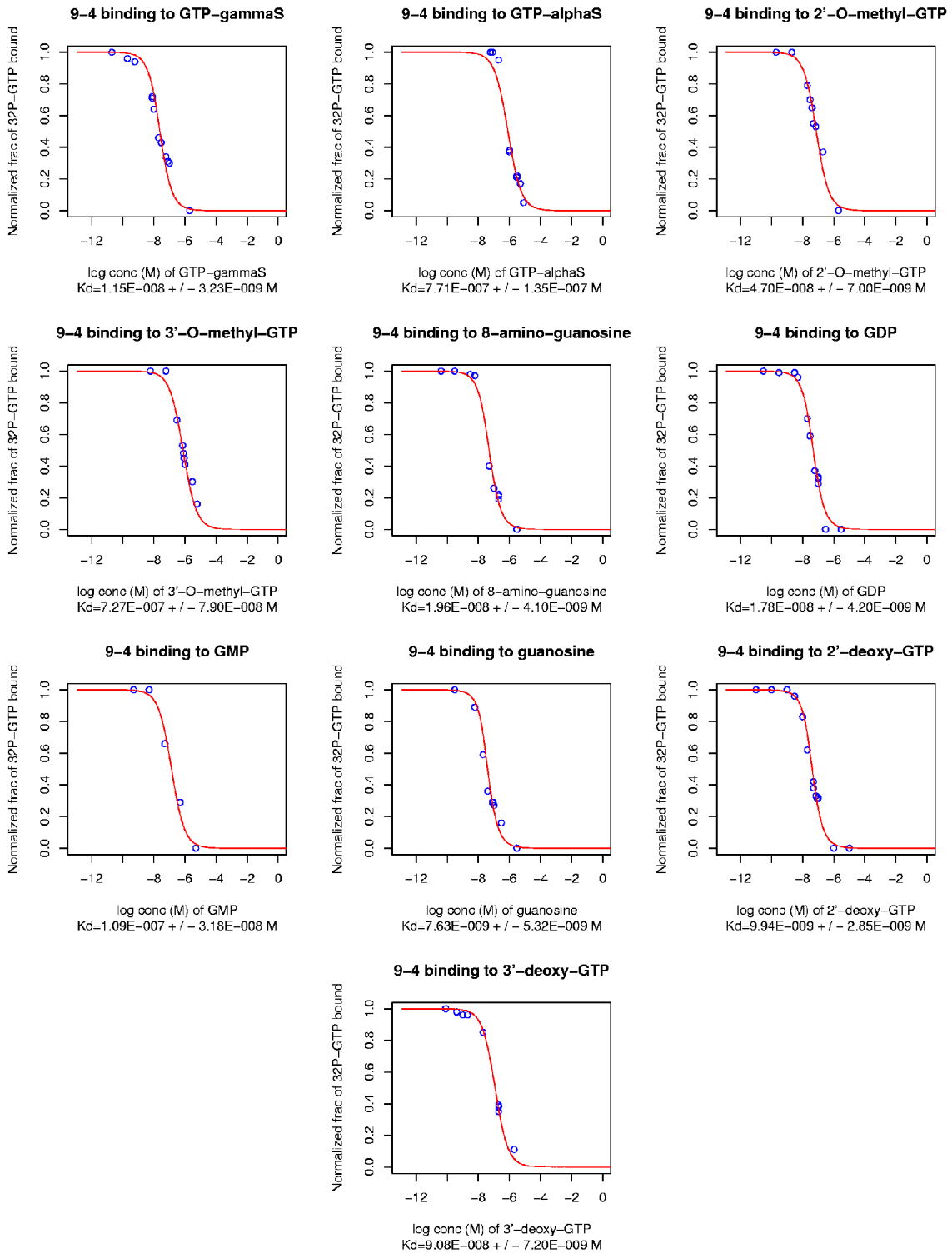


Figure 2B. Class V analog binding curves.

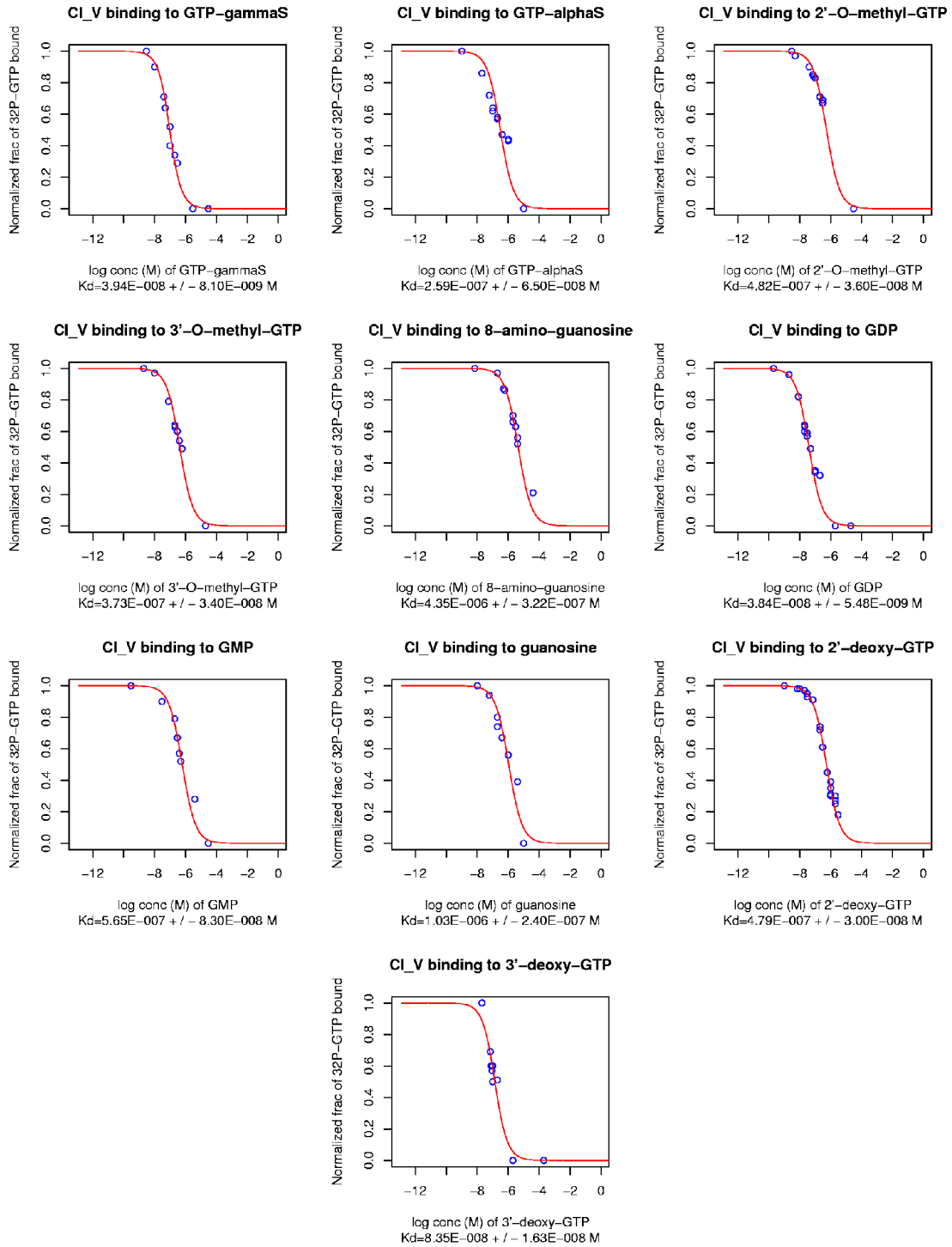


Figure 2C. 10-10 analog binding curves.

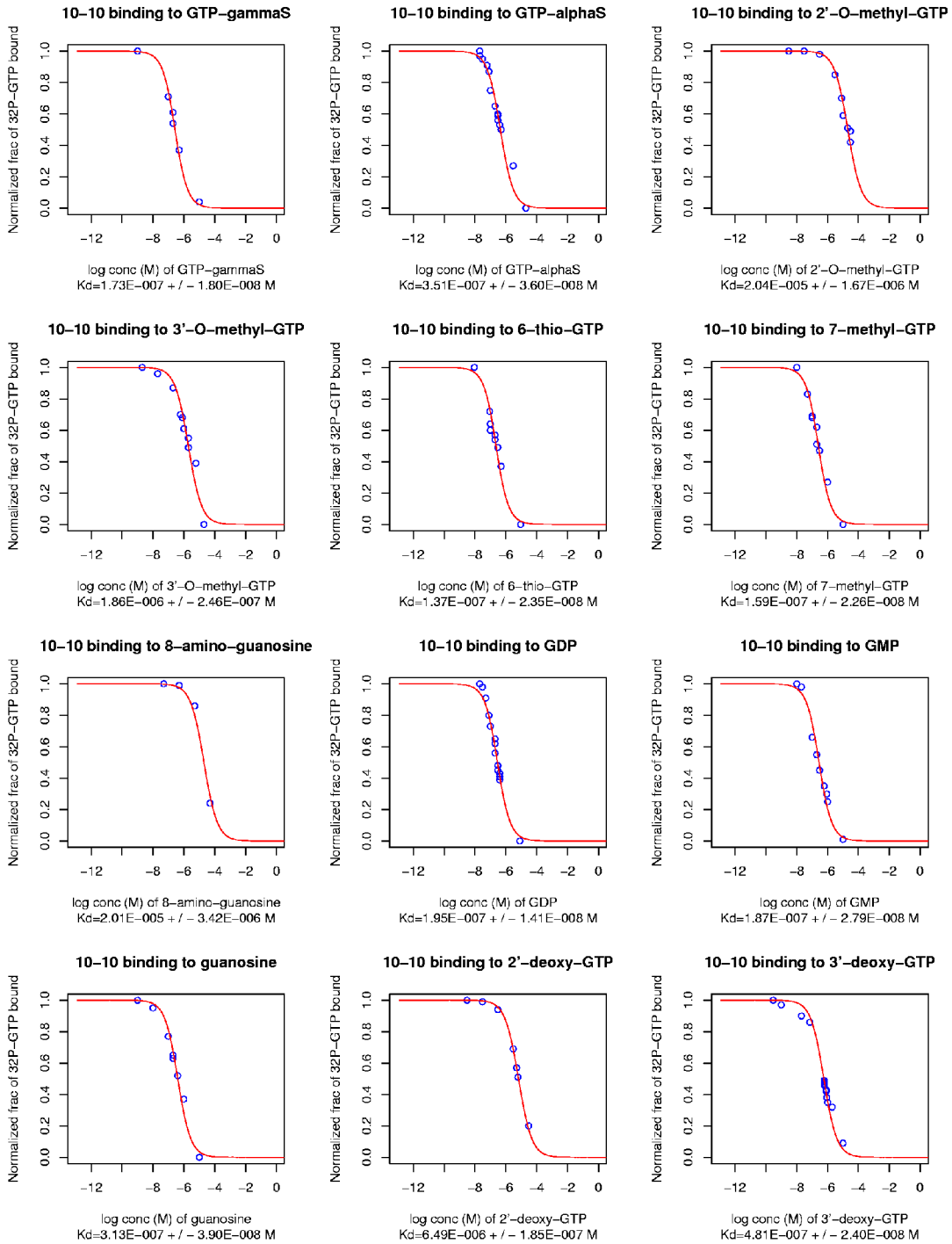


Figure 2C. 10-10 analog binding curves cont'd.

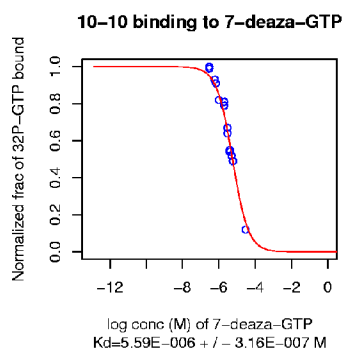


Figure 2D. Class I analog binding curves.

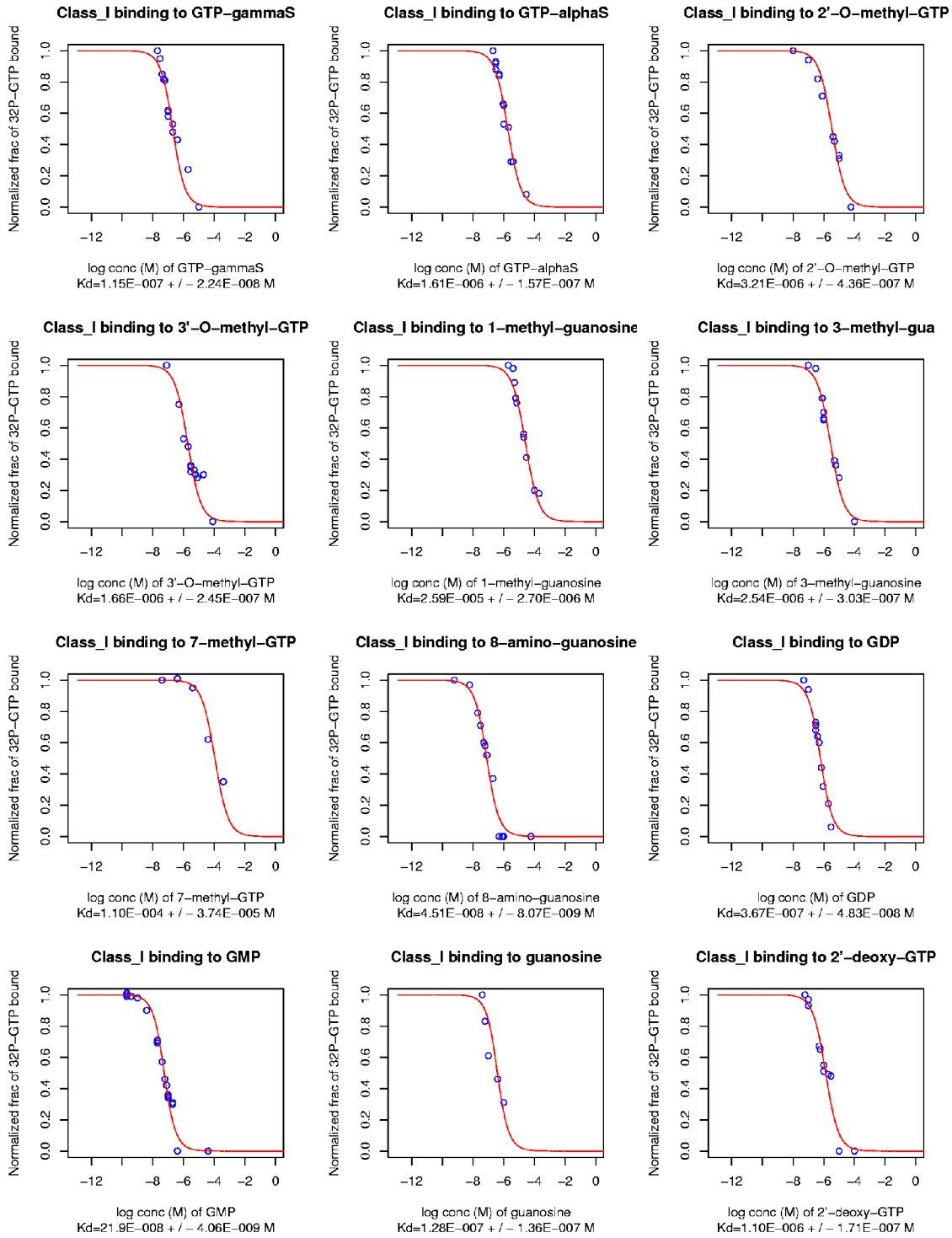


Figure 2D. Class I analog binding curves cont'd.

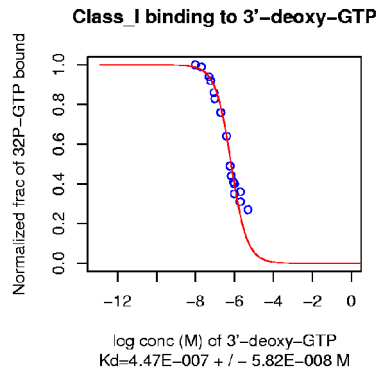


Figure 2E. 10-59 analog binding curves.

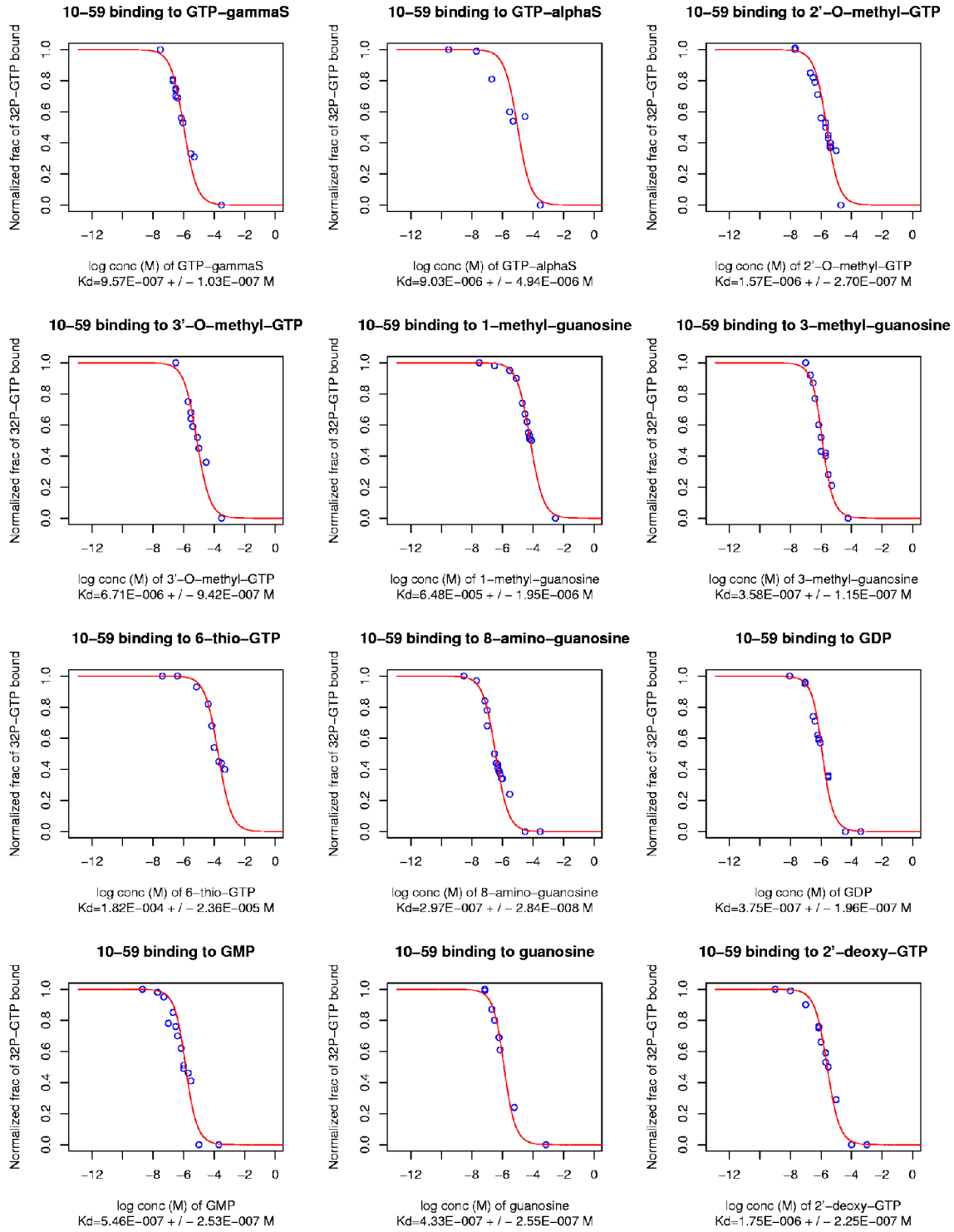


Figure 2E. 10-59 analog binding curves cont'd.

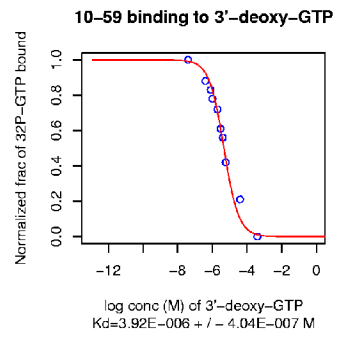


Figure 2F. 9-12 analog binding curves.

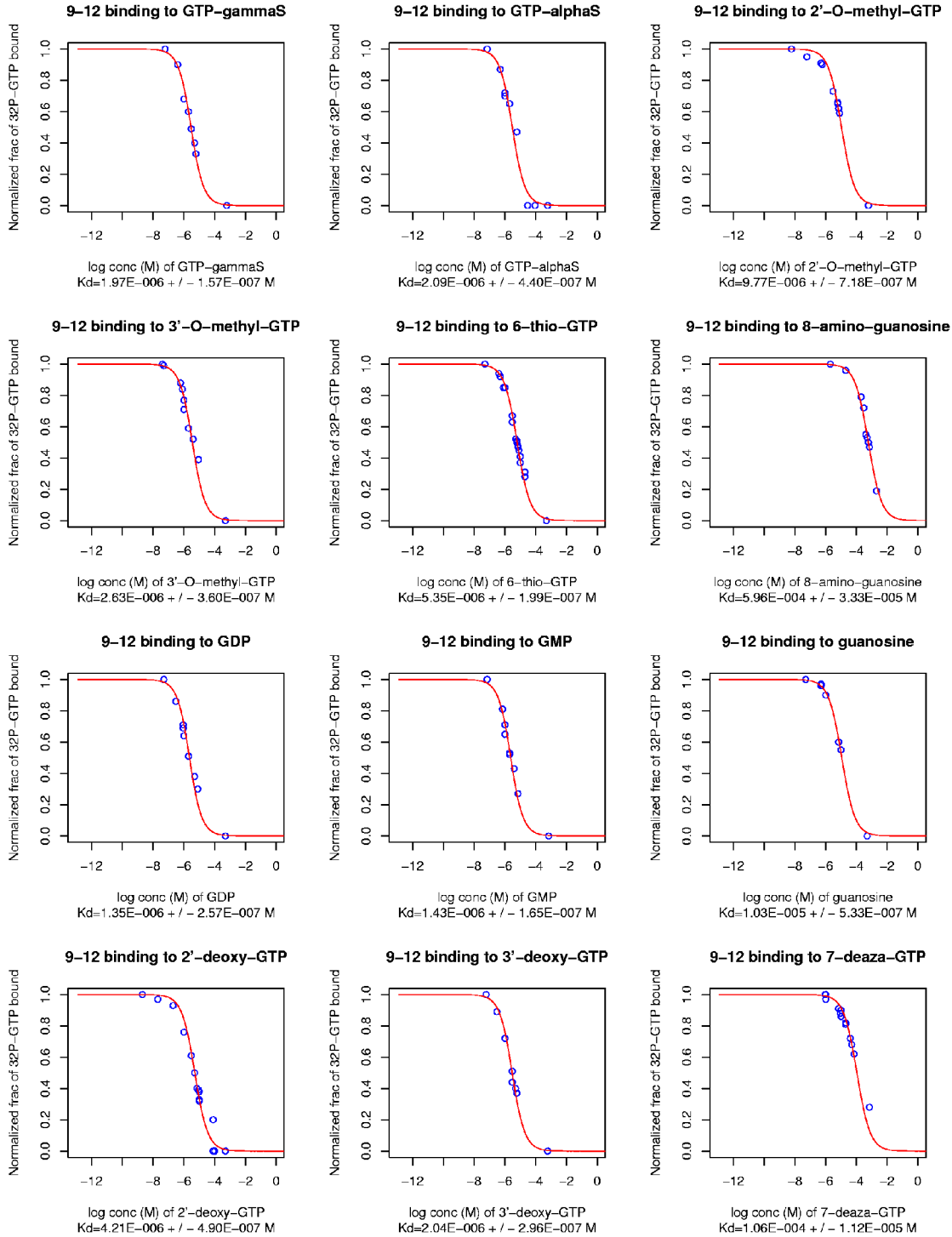


Figure 2G. 10-24 analog binding curves.

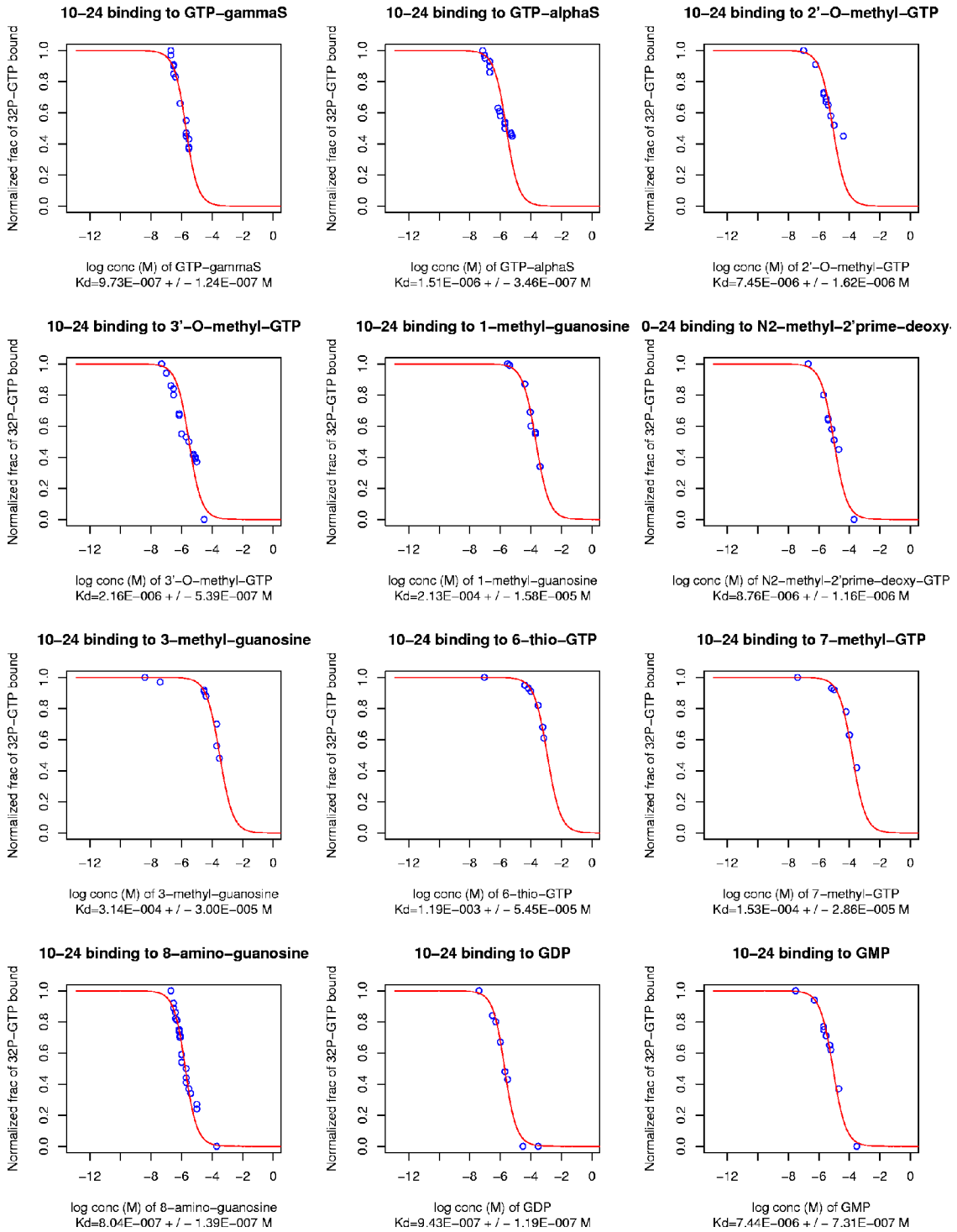


Figure 2G. 10-24 analog binding curves cont'd.

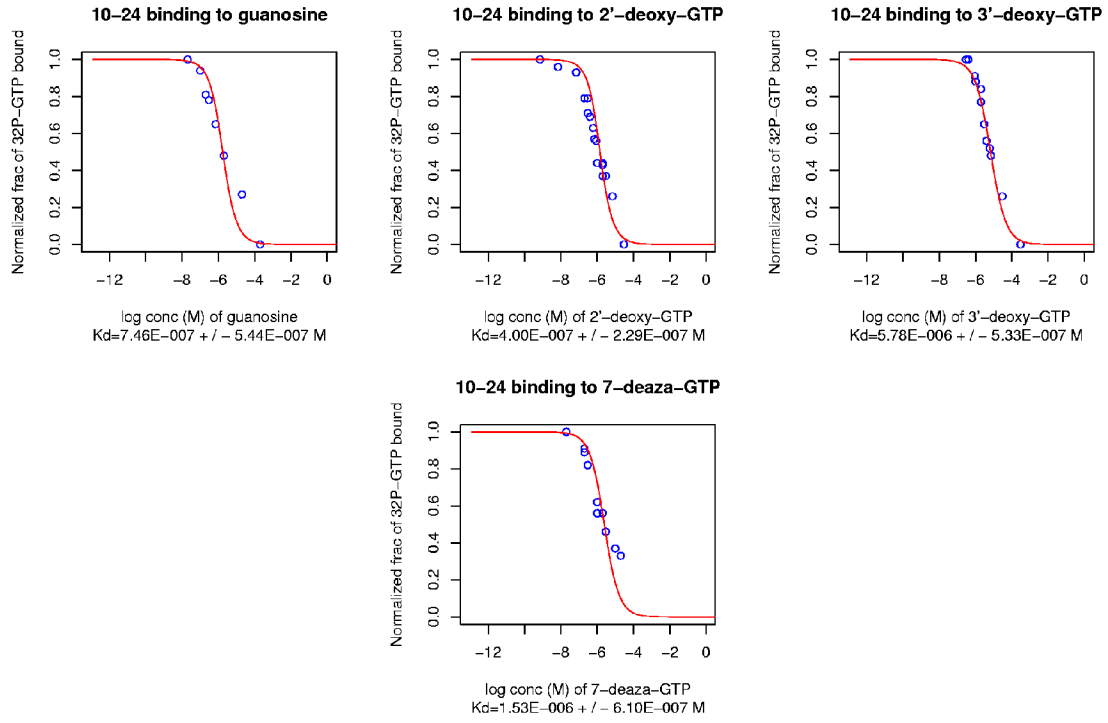


Figure 2H. 10-6 analog binding curves.

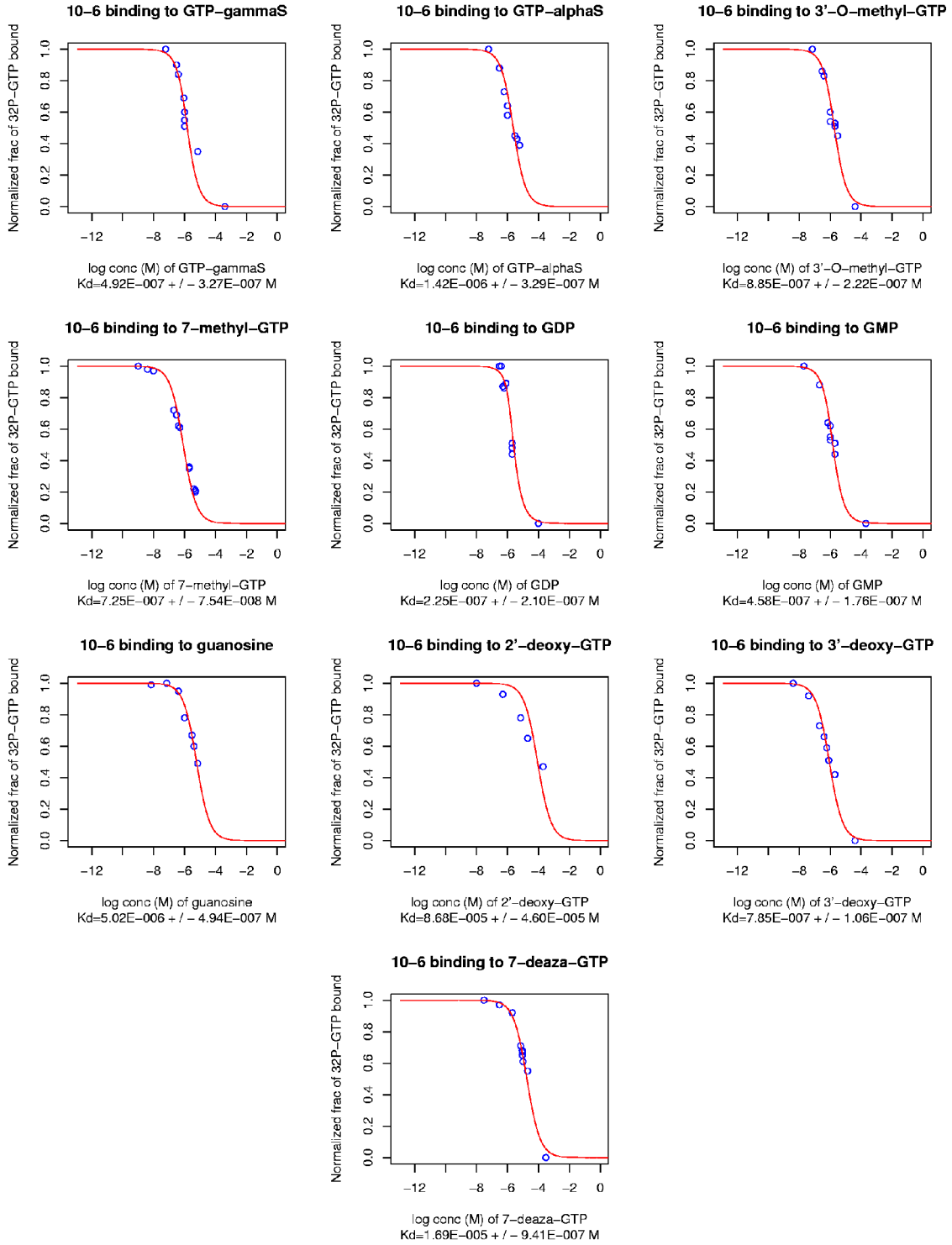


Figure 2I. Class II analog binding curves.

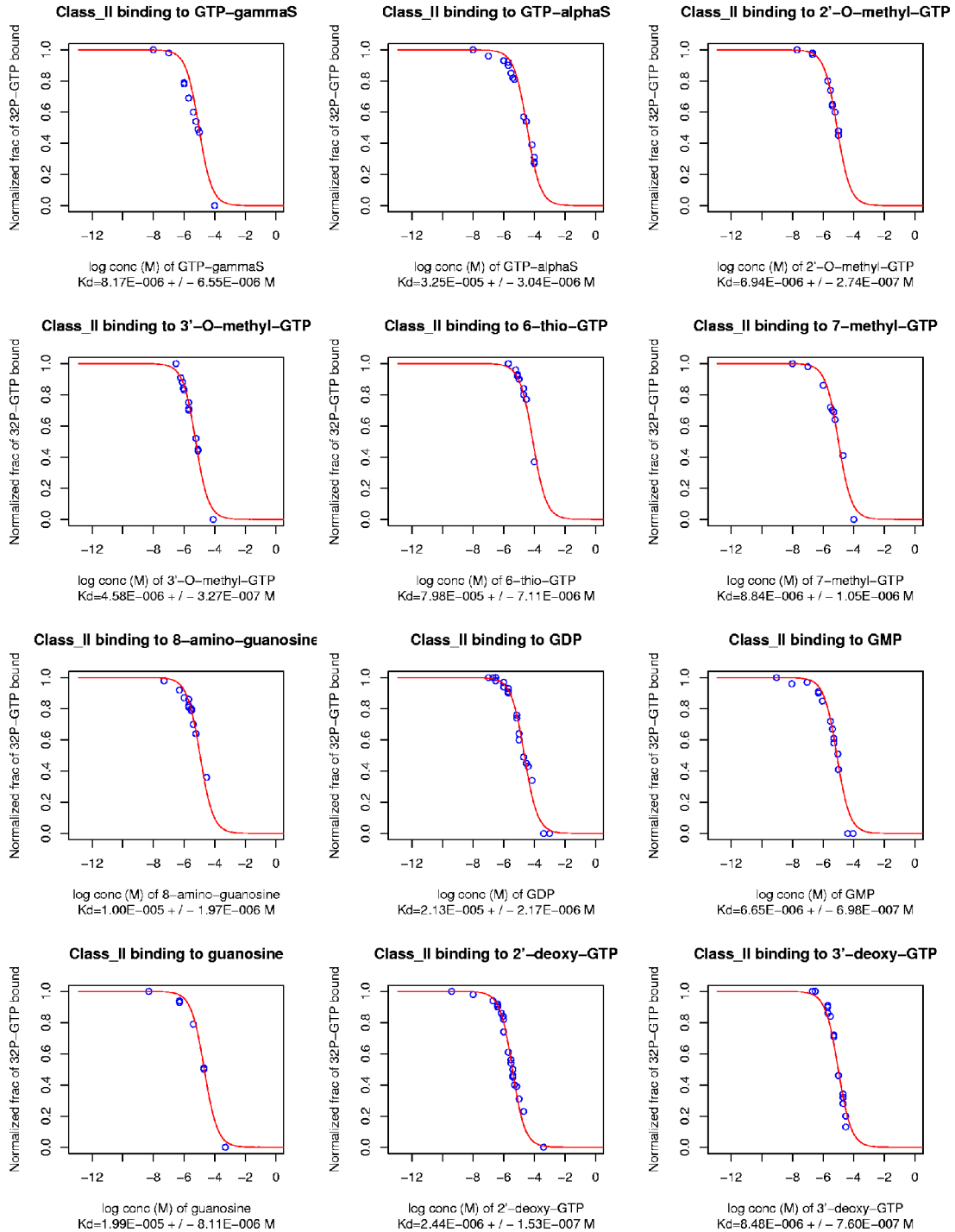


Figure 2I. Class II analog binding curves cont'd.

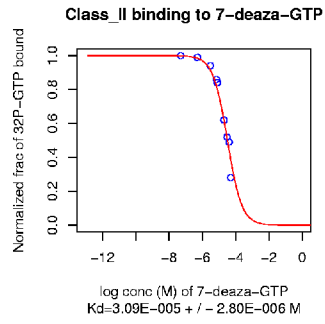


Figure 2J. Class IV analog binding curves.

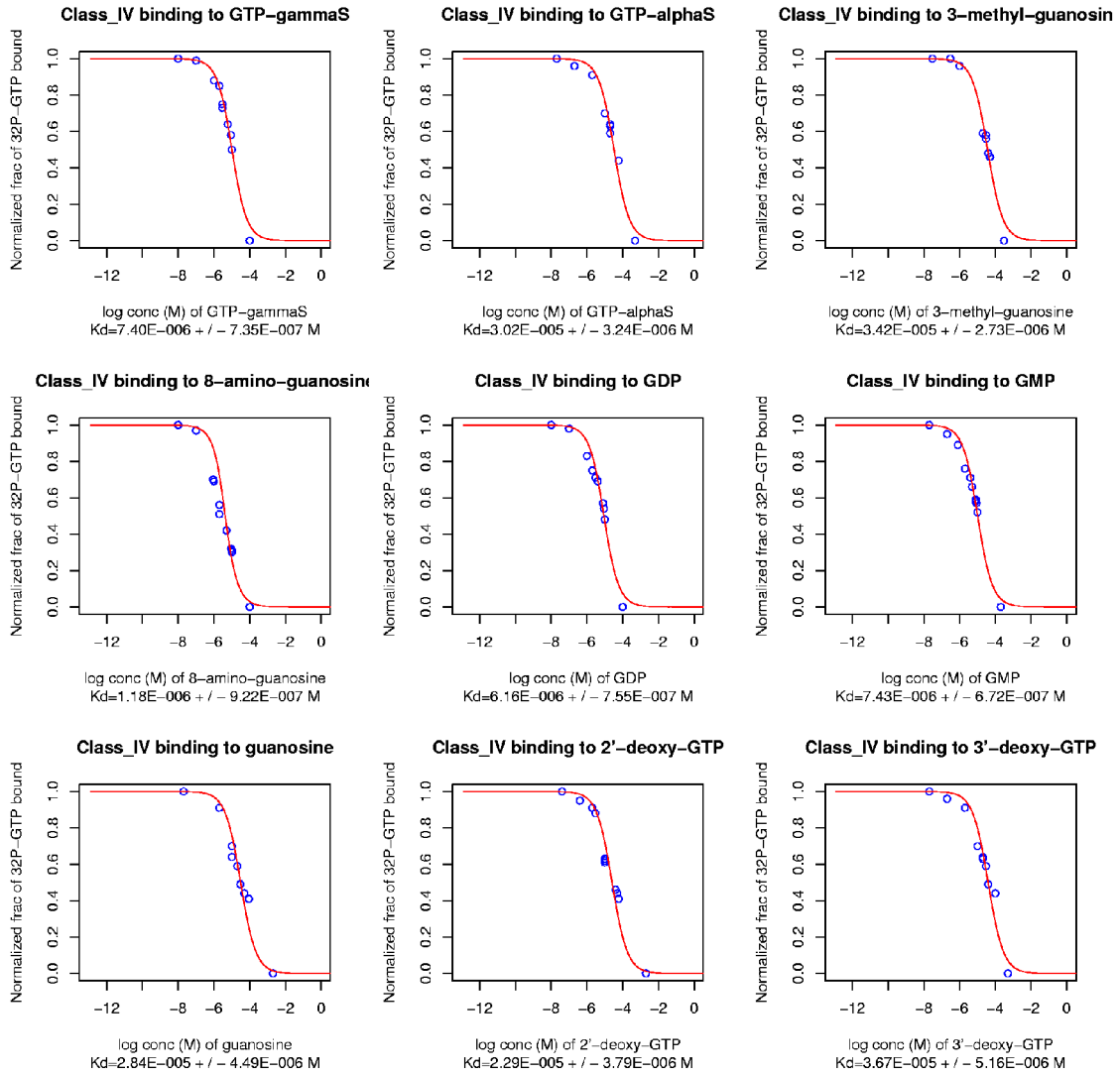
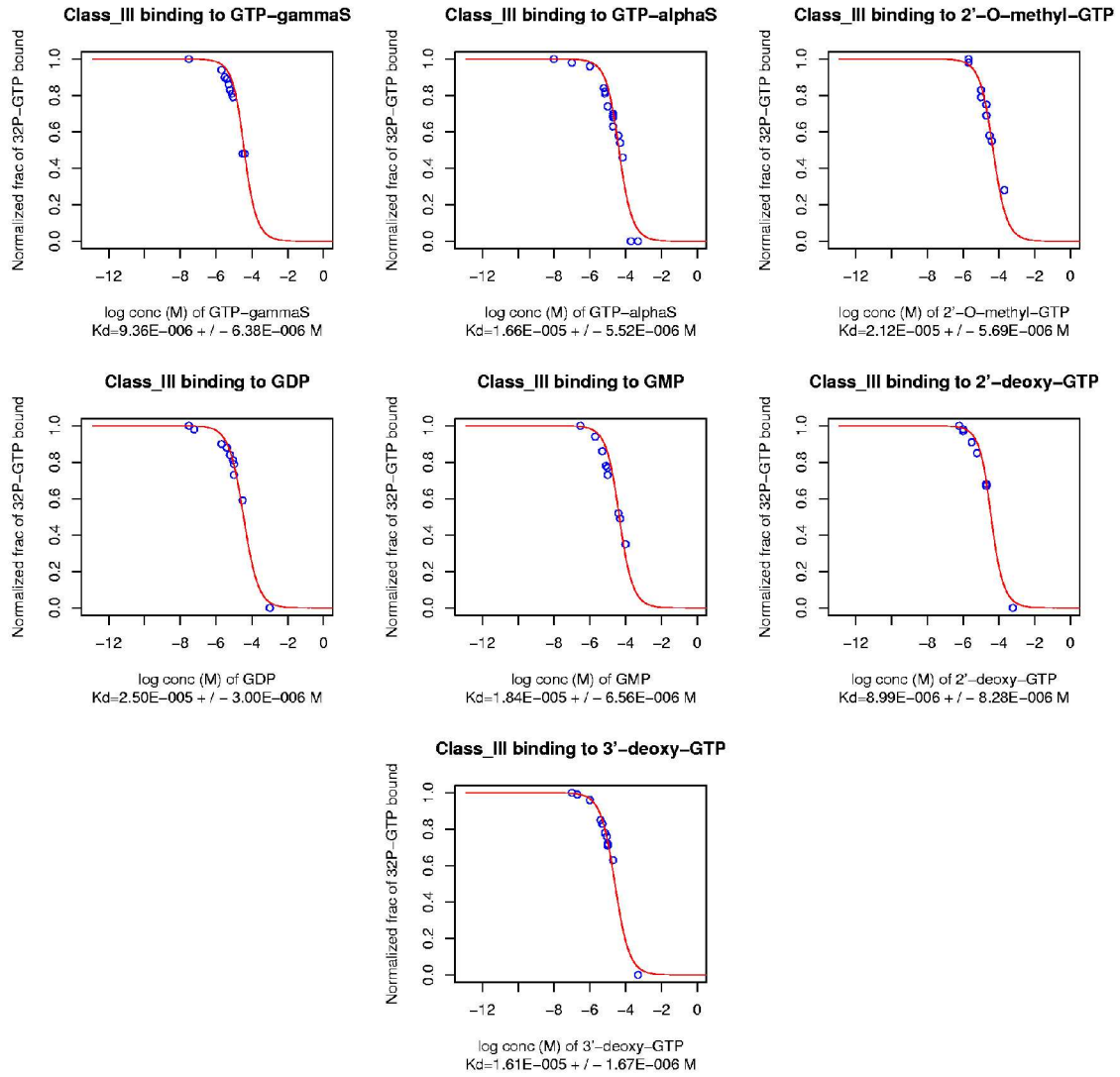
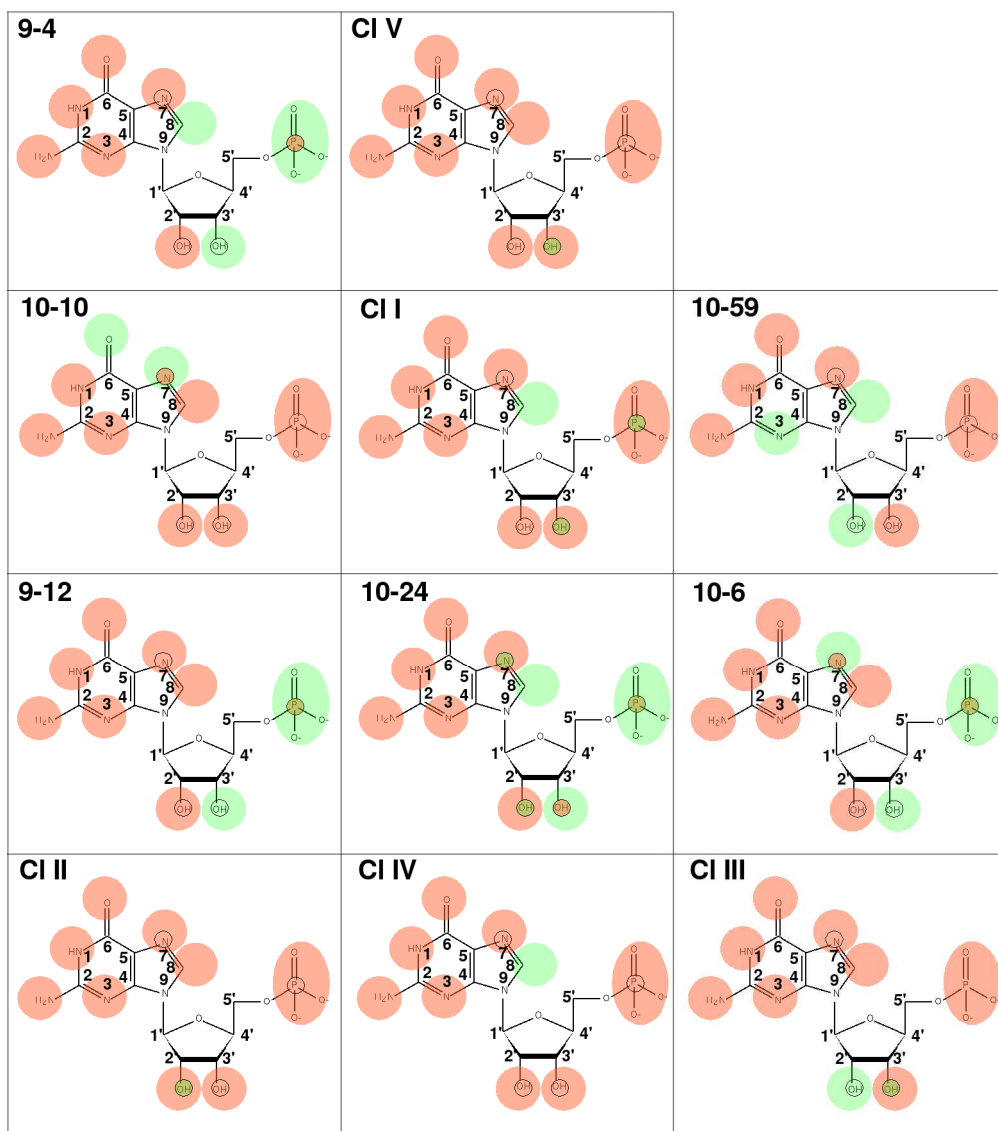


Figure 2K. Class III analog binding curves.



Supporting Figure 3.



Supporting Table 1. Aptamer binding to GTP analogs bearing bulky substituents.

The ratio of $K_{d(\text{analog})} / K_{d(\text{GTP})} \pm \text{s.d.}$ is shown for each aptamer. NB indicates that no binding was detected.

aptamer	GTP- γ -S	GTP- α -S	2'-O-methyl-	3'-O-methyl-	1-methyl-	N2-methyl-
			GTP	GTP	guanosine	2'-deoxy guanosine
9-4	1.3 \pm 0.4	85.7 \pm 17.8	5.2 \pm 1.0	80.8 \pm 12.6	NB \pm	NB \pm
CI V	2.3 \pm 0.7	15.2 \pm 5.2	28.4 \pm 7.0	21.9 \pm 5.5	NB \pm	NB \pm
10-10	5.8 \pm 1.3	11.7 \pm 2.6	680.3 \pm 147.1	62.0 \pm 14.9	NB \pm	NB \pm
CI I	1.5 \pm 0.3	21.2 \pm 2.2	42.2 \pm 6.0	21.8 \pm 3.3	340.3 \pm 38.0	NB \pm
10-59	3.8 \pm 0.5	36.1 \pm 20.0	6.3 \pm 1.2	26.8 \pm 4.3	259.2 \pm 22.2	NB \pm
9-12	6.6 \pm 1.2	7.0 \pm 1.9	32.6 \pm 5.9	8.8 \pm 1.9	NB \pm	NB \pm
10-24	3.2 \pm 0.7	5.0 \pm 1.4	24.8 \pm 6.8	7.2 \pm 2.2	711.3 \pm 129.8	29.2 \pm 6.2
10-6	1.6 \pm 1.2	4.7 \pm 1.9	NB \pm	3.0 \pm 1.2	NB \pm	NB \pm
CI II	20.4 \pm 19.3	81.3 \pm 41.4	17.4 \pm 8.7	11.5 \pm 5.8	NB \pm	NB \pm
CI IV	8.2 \pm 2.0	33.6 \pm 8.3	NB \pm	NB \pm	NB \pm	NB \pm
CI III	1.2 \pm 0.1	2.1 \pm 0.3	2.6 \pm 0.3	NB \pm	NB \pm	NB \pm

aptamer	3'-methyl-	6-thio-	7-methyl-	8-amino-
	guanosine	GTP	GTP	guanosine
9-4	NB \pm	NB \pm	NB \pm	2.2 \pm 0.5
CI V	NB \pm	NB \pm	NB \pm	255.9 \pm 63.1
10-10	NB \pm	4.6 \pm 1.2	5.3 \pm 1.3	668.7 \pm 175.8
CI I	33.4 \pm 4.2	NB \pm	1449 \pm 496	0.6 \pm 0.2
10-59	1.4 \pm 0.5	726.0 \pm 110.8	NB \pm	1.2 \pm 0.2
9-12	NB \pm	17.8 \pm 3.0	NB \pm	1987 \pm 349.3
10-24	1048 \pm 201	3963 \pm 685	510 \pm 127.7	2.7 \pm 0.6
10-6	NB \pm	NB \pm	2.4 \pm 0.8	NB \pm
CI II	NB \pm	199.4 \pm 101.3	22.1 \pm 11.4	25.1 \pm 13.5
CI IV	38.0 \pm 9.0	NB \pm	NB \pm	1.3 \pm 1.1
CI III	NB \pm	NB \pm	4.4 \pm 0.5	NB \pm

Supporting Table 2. Aptamer binding to GTP analogs with modified or deleted functional groups.

The ratio of $K_{d(\text{analog})} / K_{d(\text{GTP})} \pm \text{s.d.}$ is shown for each aptamer. NB indicates that no binding was detected.

aptamer	GDP	GMP	guanosine	2'-deoxy-	3'-deoxy-	7-deaza-
				GTP	GTP	GTP
9-4	2.0 \pm 0.5	12.1 \pm 3.8	0.8 \pm 0.6	1.1 \pm 0.3	10.1 \pm 1.4	NB \pm
CI V	2.3 \pm 0.6	33.2 \pm 9.2	60.6 \pm 20.1	28.2 \pm 6.9	4.9 \pm 1.5	NB \pm
10-10	6.5 \pm 1.4	6.2 \pm 1.6	10.4 \pm 2.5	216.4 \pm 43.7	16.0 \pm 3.3	186.3 \pm 38.7
CI I	4.8 \pm 0.7	0.3 \pm 0.1	1.7 \pm 1.8	14.5 \pm 2.3	5.9 \pm 0.8	NB \pm
10-59	1.5 \pm 0.8	2.2 \pm 1.0	1.7 \pm 1.0	7.0 \pm 1.1	15.7 \pm 2.0	NB \pm
9-12	4.5 \pm 1.1	4.8 \pm 1.0	34.3 \pm 6.0	14.0 \pm 2.9	6.8 \pm 1.5	352.7 \pm 69.6
10-24	3.1 \pm 0.7	24.8 \pm 4.8	2.5 \pm 1.9	1.3 \pm 0.8	19.3 \pm 3.7	5.1 \pm 2.2
10-6	0.8 \pm 0.7	1.5 \pm 0.8	16.7 \pm 5.8	289.2 \pm 181.2	2.6 \pm 0.9	56.3 \pm 19.0
CI II	53.3 \pm 27.2	16.6 \pm 8.5	49.8 \pm 32.1	6.1 \pm 3.1	21.2 \pm 10.8	77.2 \pm 39.2
CI IV	6.8 \pm 1.7	8.3 \pm 2.0	31.6 \pm 8.6	25.4 \pm 7.0	40.8 \pm 10.7	NB \pm
CI III	2.1 \pm 0.5	2.3 \pm 0.3	NB \pm	1.1 \pm 0.1	2.0 \pm 0.3	NB \pm