Supporting Information

for

Fragment screening against the thiamine pyrophosphate riboswitch *thiM*

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Constructs

RNA constructs

Sequence of RNAs used in the biophysical experiments; underlined regions correspond to the aptamers as defined elsewhere.[1,2]

E. coli thiM:
GGGCGAAUUGGGCCCGACGUCGCAUGCUCCCGGCCGCCAUGGCGGCCGCGGGAAUUCGAUUGAUCAUGAA
UUCGCAACCAAACGACUCGGGGUGCCCUUCUGCGUGAAGGCUGAGAAAUACCCGUAUCACCUGAUCUGGA
UAAUGCCAGCGUAGGGAAGU

B. subtilis lysC:
GGGCGAAUUGGGCCCGACGUCGCAUGCUCCCGGCCGCCAUGGCCGCGGGAUUUUUCAUAGUUAGAUCGU
GUUAUAUUGGUGAAGAUAGAGGUGCGAACUUCAAGAGUAUGCCUUUGGAGAAAGAUGGAUUCUGUGAAAAA
GGCUGAAAGGGGAGCGUCGCCGAAGCAAAUAAAACCCCAUCGGUAUUAUUUGCUGGCCGUGCAUUGAAUAA
AUGUAAGGCUGUCAAGAAAUCAUUUUCUUGGAGGGCUAUCUCGUU

DNA constructs used for *in vitro* transcription translation reporter assays

Primary sequences of the DNA templates used for the *in vitro* transcription translation (IVTT) assay. Both plasmids were constructed from the vector pBluescript II KS (-) and were transcribed from the T7 promoter. The Renilla *luc* gene was codon optimized for *C. reinhardtii.*[3]

Construct 1 (pKS- ThiM luc):
The sequence of the T7 promoter is highlighted in yellow, the *thiM* RS aptamer and expression platform are highlighted in green, the ribosome binding site (RBS) is typed in red and the translation start codon is highlighted in red. The restriction sites are highlighted in grey.

T7 promoter *ThiM*Riboswitch
GTAATACGACTCACTATAGGGCGATTGGAGCTCCACCGCGGTGGCGGCCGCTCTAGCGCACCAAACGACTC

GGGGTGCCCTTCTGCGTGAAGGCTGAGAAATACCCGTATCACCTGATCTGGATAATGCCAGCGTAGGGAAG
RBS Start
TCACGGACCACCAGGTCATTGCTTCTTCACGTTATGGCAGGAGCAAACTATGCAAGTCGACCTGCTGGGTT
SacI *Renilla luc*
CAGCGCAAGAGCTCATGGCCAGCAAGGTGTACGCCCCCGAGCAGCGCAAGCGCATGATCACCGGCCCTCAG

TGGTGGGCTCGCTGCAAGCAGATGAACGTGCTGGACAGCTTCATCAACTACTACGACAGCGAGAAGCACGC

CGAGAACGCCGTGATCTTCCTGCACGGCAACGCCGCCAGCAGCTACCTGTGGCGCCACGTGGTGCCCCACA

TCGAGCCCGTGGCCCGCTGCATCATCCCCGACCTGATCGGCATGGGCAAGAGCGGCAAGAGCGGCAACGGC

AGCTACCGCCTGCTGGACCACTACAAGTACCTGACCGCCTGGTTCGAGCTGCTGAACCTGCCCAAGAAGAT

CATCTTCGTGGGCCACGACTGGGGCGCCTGCCTGGCCTTCCACTACAGCTACGAGCNCCAGGACAAGATCA

AGGCCATCGTGCACGCCGAGAGCGTGGTGGACGTGATCGAGAGCTGGGACGAGTGGCCCGACATCGAGGAG

GACATCGCCCTGATCAAGAGCGAGGAGGGCGAGAAGATGGTGCTGGAGAACAACTTCTTCGTGGAGACCAT

GCTGCCCAGCAAGATCATGCGCAAGCTGGAGCCCGAGGAGTTCGCCGCCTACCTGGAGCCCTTCAAGGAGA

AGGGCGAGGTGCGCCGTCCCACCCTGAGCTGGCCTCGCGAGATCCCCCTGGTGAAGGGCGGCAAGCCCGAC

GTGGTGCAGATCGTGCGCAACTACAACGCCTACTTGCGCGCCAGCGACGACCTGCCCAAGATGTTCATCGA

GAGCGACCCCGGCTTCTTCAGCAACGCCATCGTGGAGGGCGCCAAGAAGTTCCCCAACACCGAGTTCGTGA

AGGTGAAGGGCCTGCACTTCAGCCAGGAGGACGCTCCCGACGAGATGGGCAAGTACATCAAGAGCTTCGTG

GAGCGCGTGCTGAAGAACGATACGGCCAGCCAGCCGGAGCTGGCCCCGGAGGATACGTAAGGATCCCCGCT

CCGTGTAAATGGAGGCGCTCGTTGATCTGAGCCTTGCCCCCTGACGAACGGCGGTGGATGGAAGATACTGC

TCTCAAGTGCTGAAGCGGTAGCTTAGCTCCCCGTTTCGTGCTGATCAGTCTTTTTCAACACGTAAAAAGCG

GAGGAGTTTTGCAATTTTGTTGGTTGTAACGATCCTCCGTTGATTTTGGCCTCTTTCTCCATGGGCGGGCT

HindIII

GGGCGTATTTGAAGCGAATTCAAGCTTATCGATACCGTCGACCTCGAGGGGGGGCCCGGTACCCAGCTTTT

GTTCCCTTTAGTG…

Construct 2 (pKS- luc): Control construct were the *Renilla luc* gene is not under control of the thiM riboswitch. The colour coding is the same described for the previous construct, except for the sequence in green, now contains only the last 41 nt of *thiM*-RS expression platform.

T7 promoter *XbaI* RBS Start

GTAATACGACTCACTATAGGGCGATTGGAGCTCCACCGCGGTGGCGGCCGCTCTAGAAGGAGCAAACTATG

SacI *Renilla luc*

CAAGTCGACCTGCTGGGTTCAGCGCAAGAGCTCATGGCCAGCAAGGTGTACGCCCCCGAGCAGCGCAAGCG

CATGATCACCGGCCCTCAGTGGTGGGCTCGCTGCAAGCAGATGAACGTGCTGGACAGCTTCATCAACTACT

ACGACAGCGAGAAGCACGCCGAGAACGCCGTGATCTTCCTGCACGGCAACGCCGCCAGCAGCTACCTGTGG

CGCCACGTGGTGCCCCACATCGAGCCCGTGGCCCGCTGCATCATCCCCGACCTGATCGGCATGGGCAAGAG

CGGCAAGAGCGGCAACGGCAGCTACCGCCTGCTGGACCACTACAAGTACCTGACCGCCTGGTTCGAGCTGC

TGAACCTGCCCAAGAAGATCATCTTCGTGGGCCACGACTGGGGCGCCTGCCTGGCCTTCCACTACAGCTAC

GAGCNCCAGGACAAGATCAAGGCCATCGTGCACGCCGAGAGCGTGGTGGACGTGATCGAGAGCTGGGACGA

GTGGCCCGACATCGAGGAGGACATCGCCCTGATCAAGAGCGAGGAGGGCGAGAAGATGGTGCTGGAGAACA

ACTTCTTCGTGGAGACCATGCTGCCCAGCAAGATCATGCGCAAGCTGGAGCCCGAGGAGTTCGCCGCCTAC

CTGGAGCCCTTCAAGGAGAAGGGCGAGGTGCGCCGTCCCACCCTGAGCTGGCCTCGCGAGATCCCCCTGGT

GAAGGGCGGCAAGCCCGACGTGGTGCAGATCGTGCGCAACTACAACGCCTACTTGCGCGCCAGCGACGACC

TGCCCAAGATGTTCATCGAGAGCGACCCCGGCTTCTTCAGCAACGCCATCGTGGAGGGCGCCAAGAAGTTC

CCCAACACCGAGTTCGTGAAGGTGAAGGGCCTGCACTTCAGCCAGGAGGACGCTCCCGACGAGATGGGCAA

GTACA[illegible]GATACGGCCAGCCAGCCGGAGCTGGCCCCGGAGG

ATACGTAAGGATCCCCGCTCCGTGTAAATGGAGGCGCTCGTTGATCTGAGCCTTGCCCCCTGACGAACGGC

GGTGGATGGAAGATACTGCTCTCAAGTGCTGAAGCGGTAGCTTAGCTCCCCGTTTCGTGCTGATCAGTCTT

TTTCAACACGTAAAAAGCGGAGGAGTTTTGCAATTTTGTTGGTTGTAACGATCCTCCGTTGATTTTGGCCT

HindIII

CTTTCTCCATGGGCGGGCTGGGCGTATTTGAAGCGAATTCAAGCTTATCGAT…

Supplementary Figures and Tables

Figure S1: Fluorescence melting curve of *thiM*-RS (0.5 µM), with the dye EvaGreen ± 50 µM TPP.

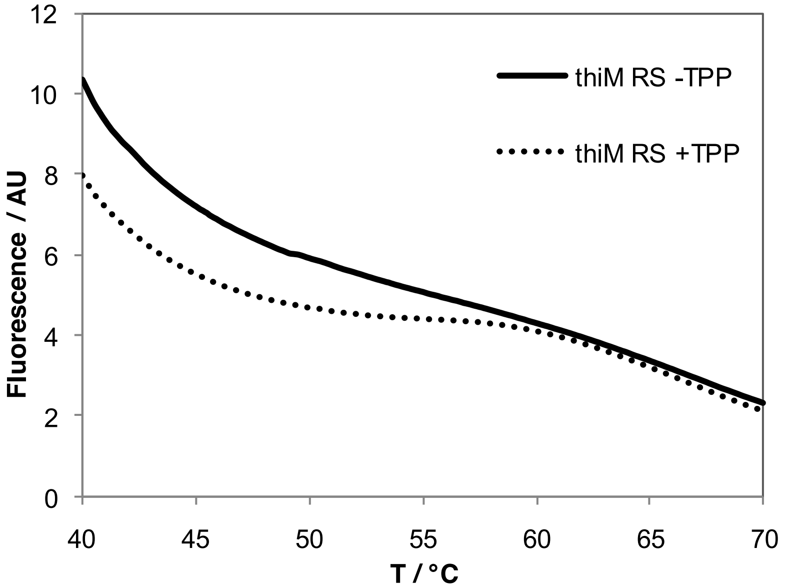


Table S1: Structures and equilibrium dialysis [^{3}H]-thiamine displacement percentages of fragments **S1-S3** for which K_D could not be determined by ITC.

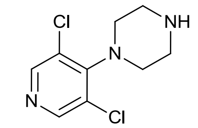
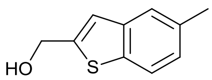
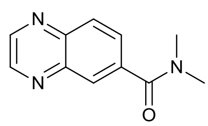
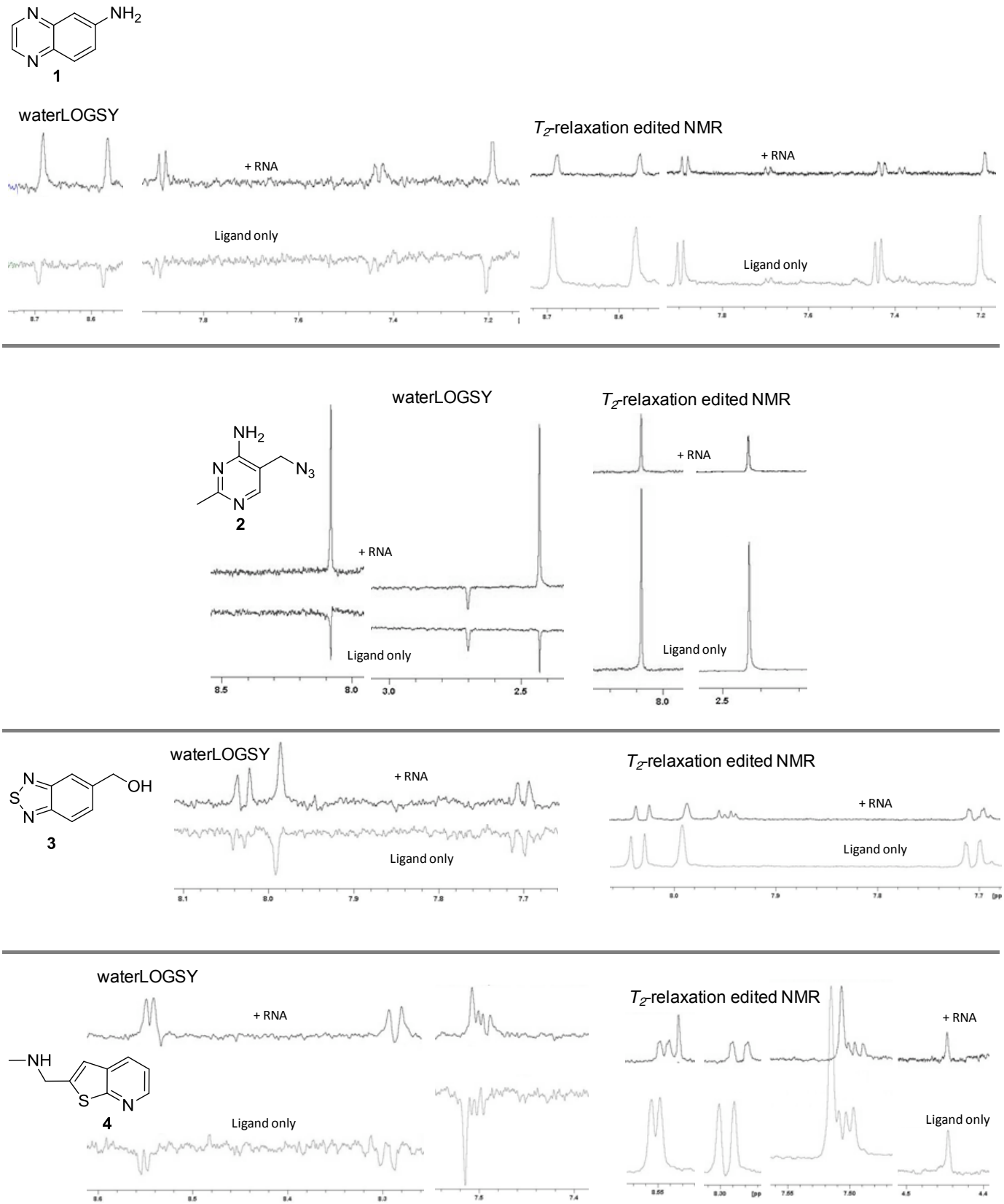
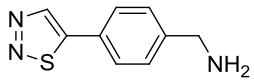
Fragment	Structure	% thiamine displacement
S1		41
S2		31
S3		31

Table S2: WaterLOGSY (left panels) and T_2 relaxation-edited (right panels) NMR spectra of fragments **1-17, S1-S3, 24-26.** The chemical shift δ (ppm) scale is indicated for each spectrum.





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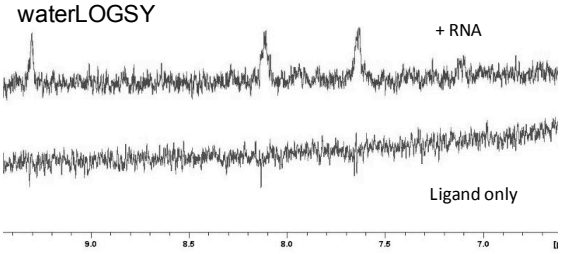
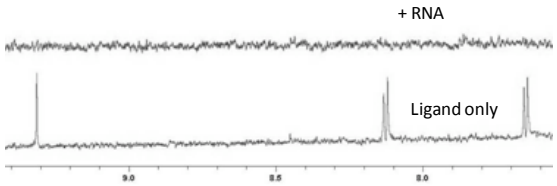
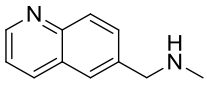
waterLOGSY



T_2-relaxation edited NMR





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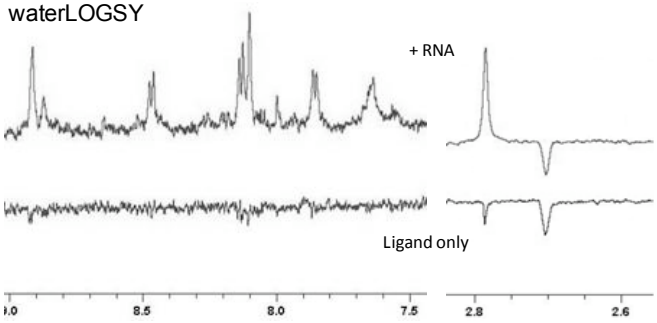
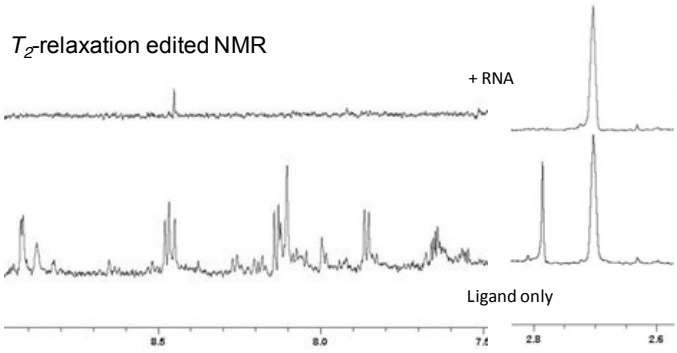
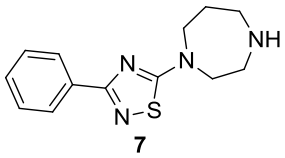
waterLOGSY



T_2-relaxation edited NMR





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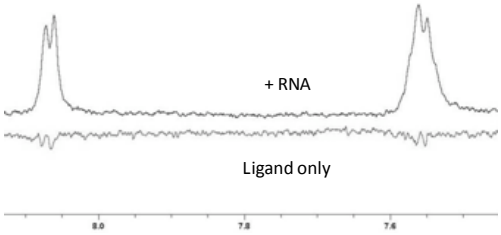
waterLOGSY



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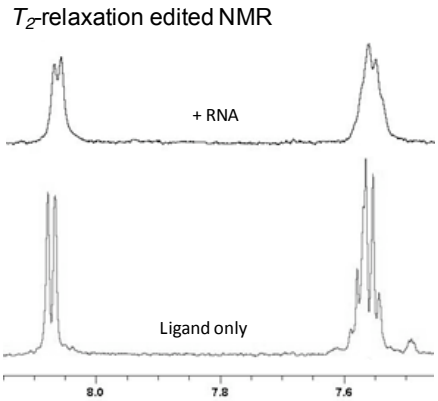

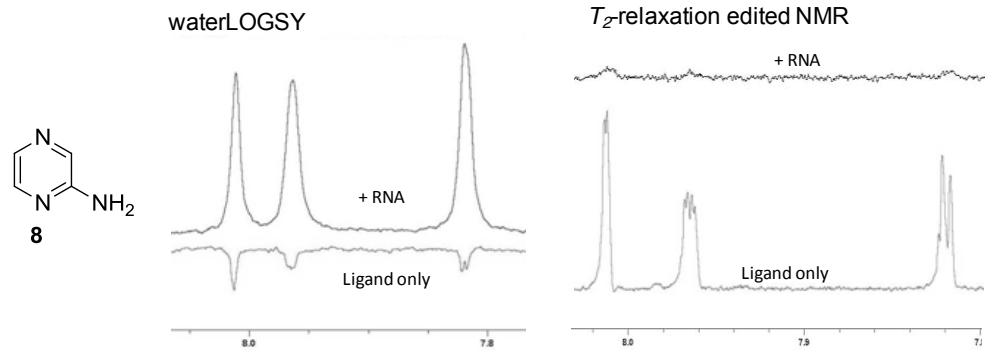
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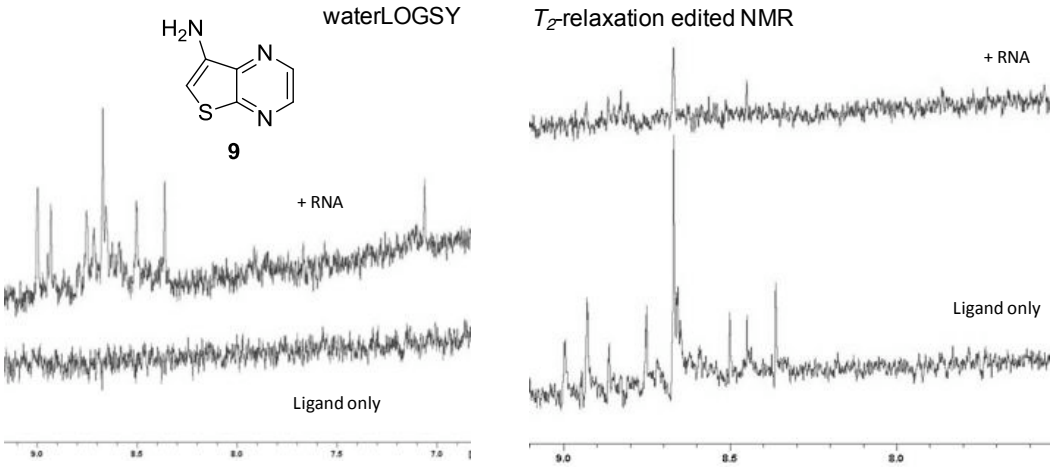
Ligand only
+ RNA
Ligand only
8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T_2-relaxation edited NMR
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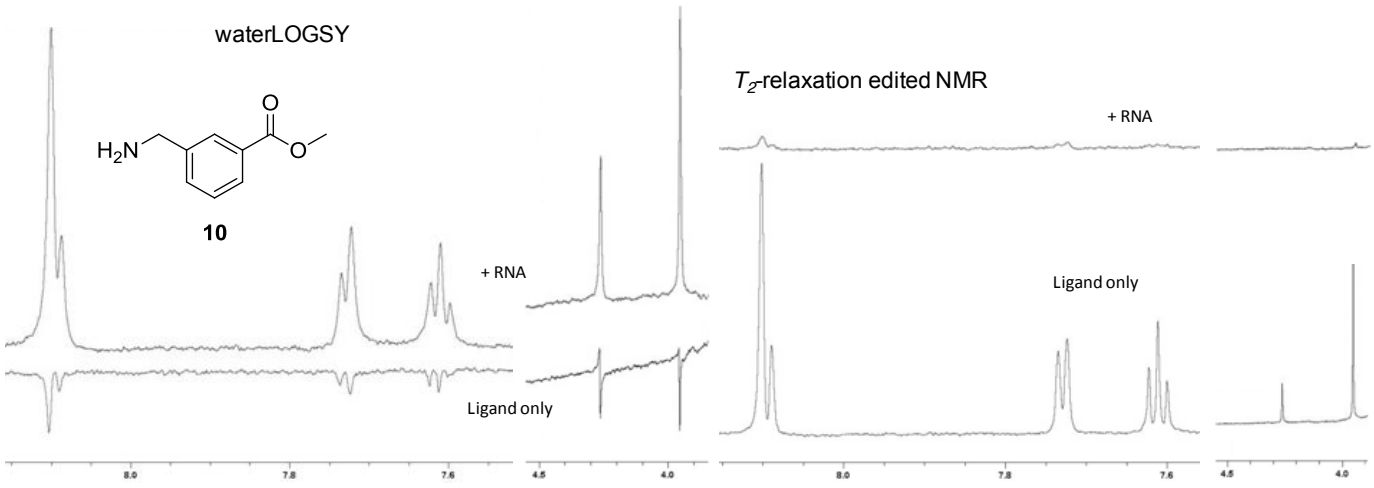
+ RNA
Ligand only
+ RNA
Ligand only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T_2-relaxation edited NMR
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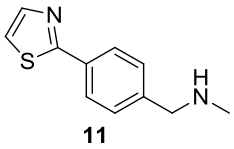
+ RNA
Ligand only
+ RNA
Ligand only



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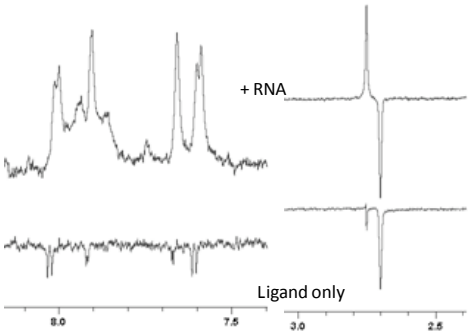

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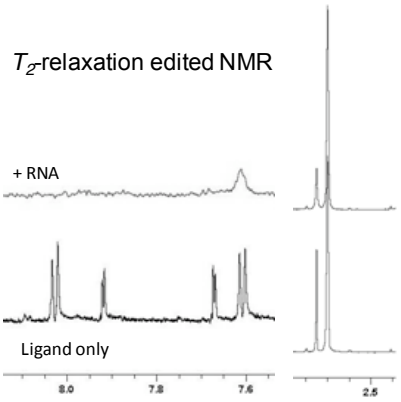

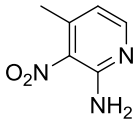

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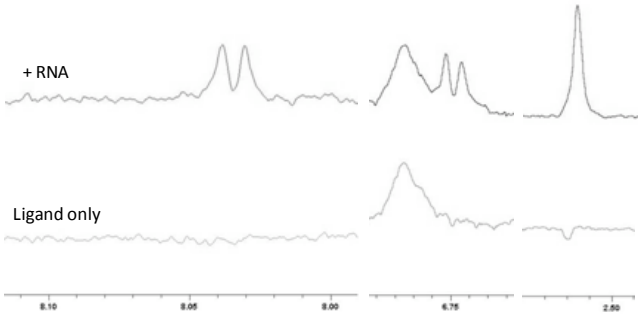

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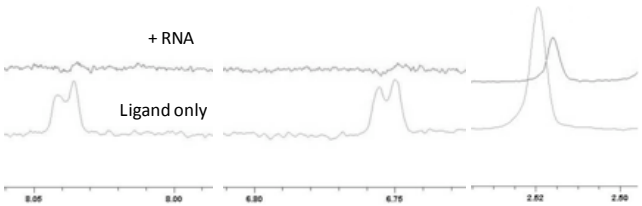

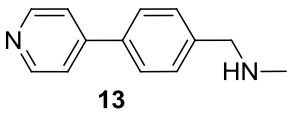

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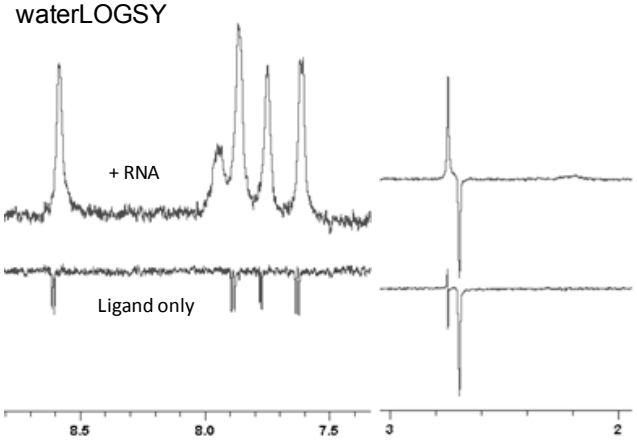

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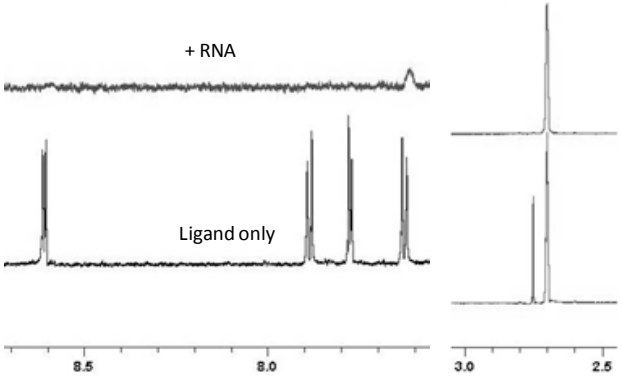

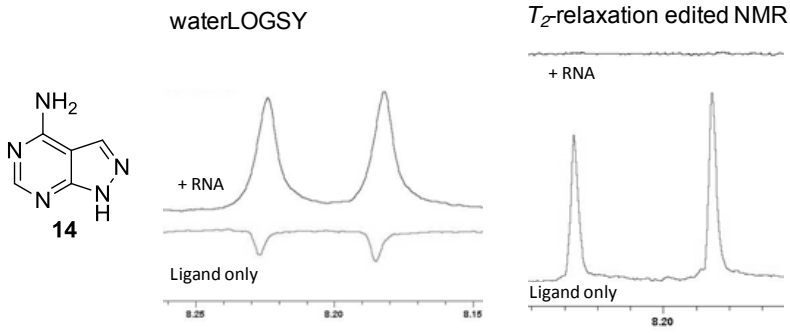
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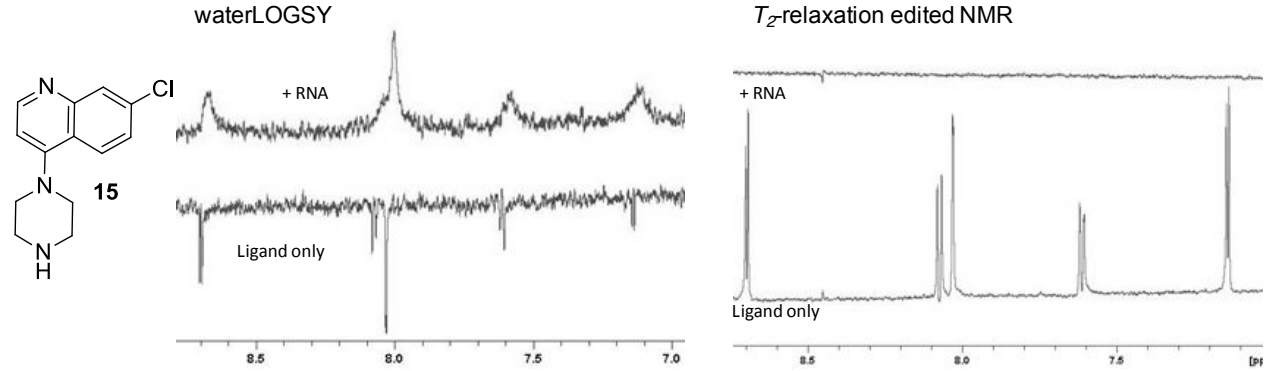
+ RNA
Ligand only
8.20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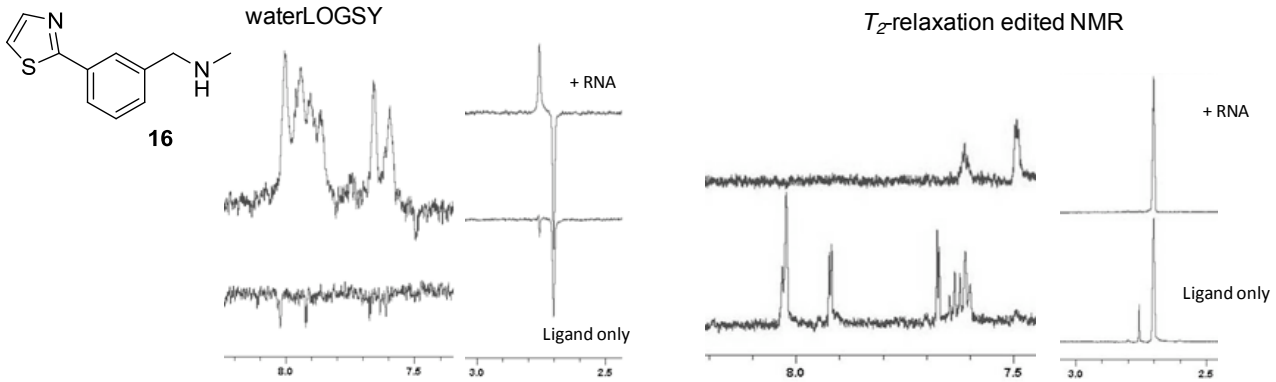
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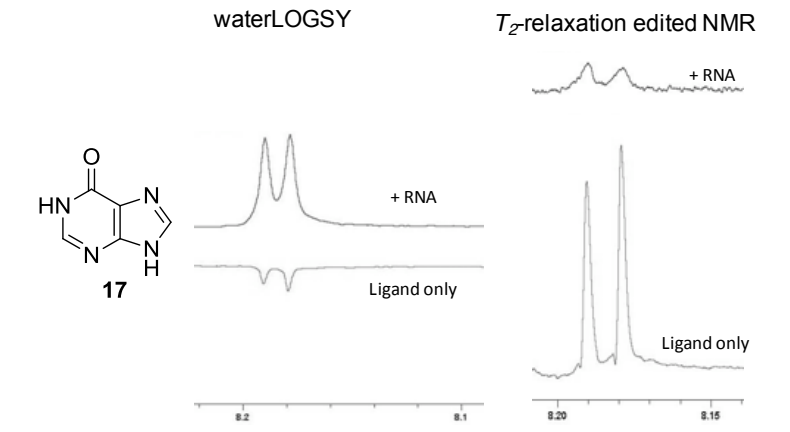
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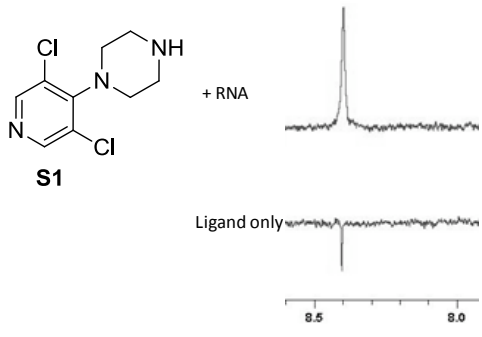

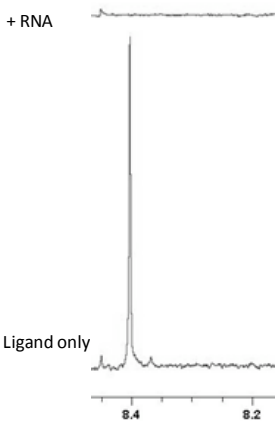

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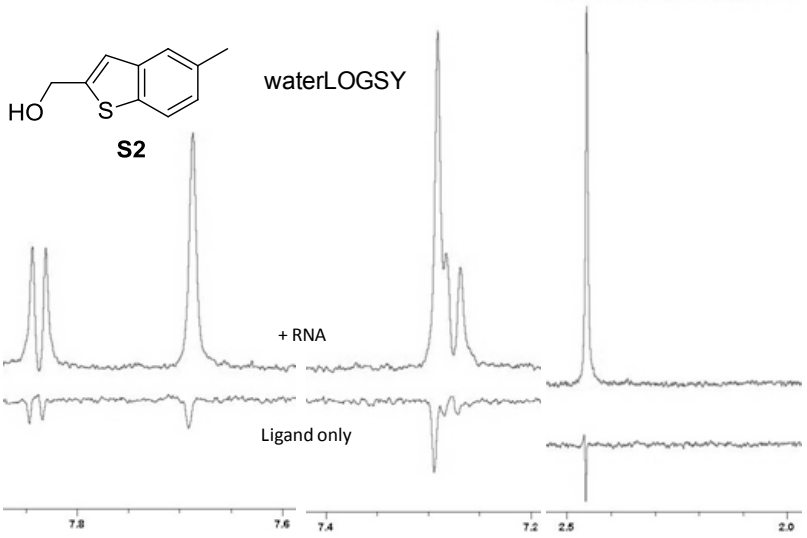

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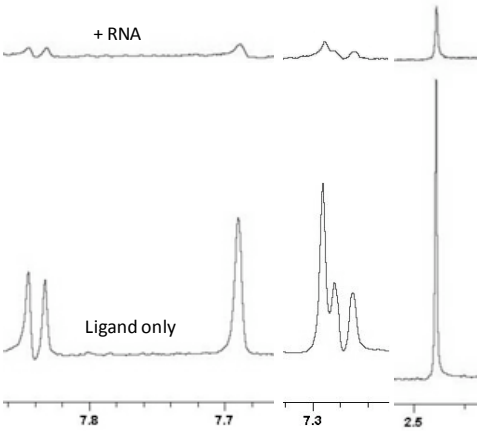

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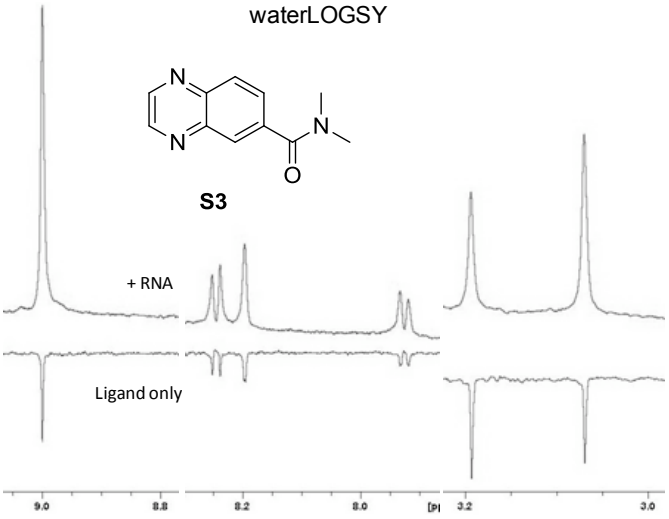

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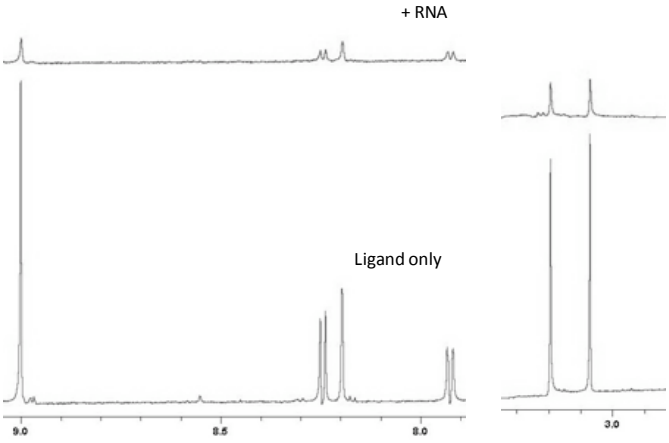

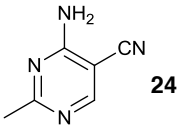

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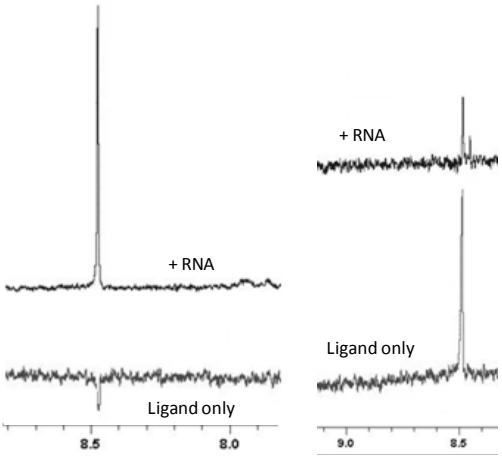
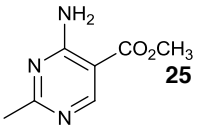
waterLOGSY

T_2-relaxation edited NMR





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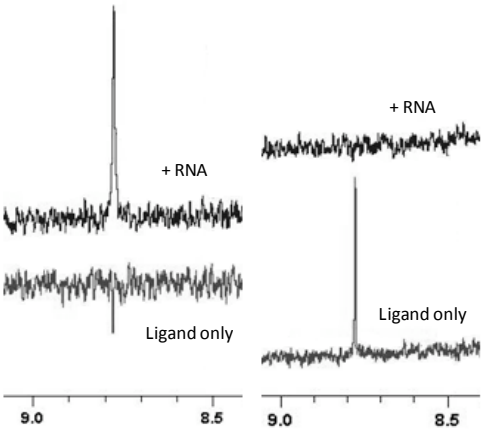

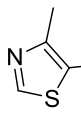

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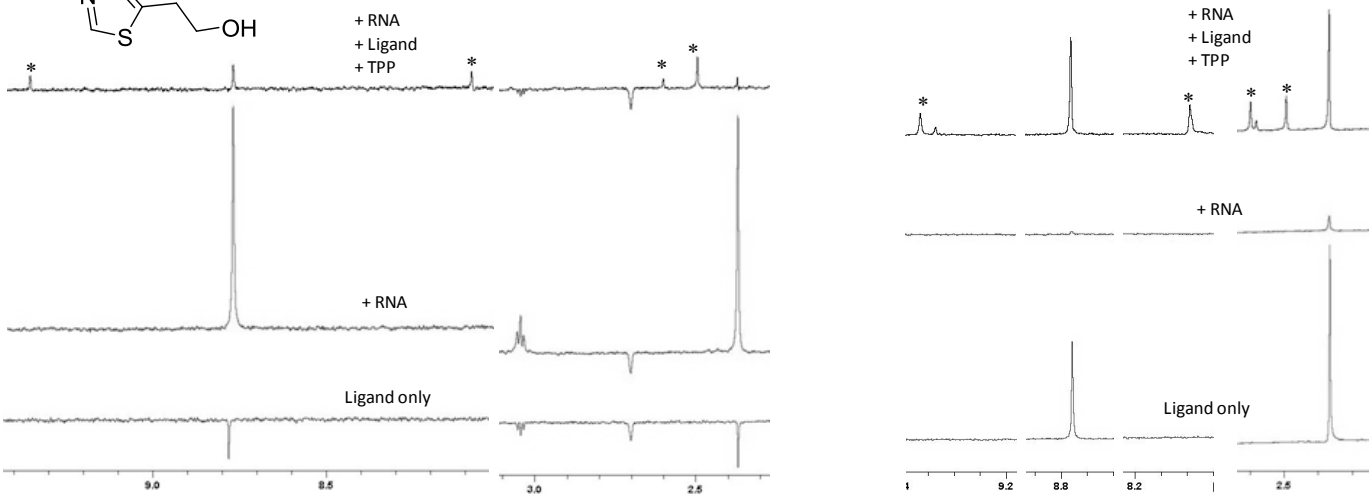
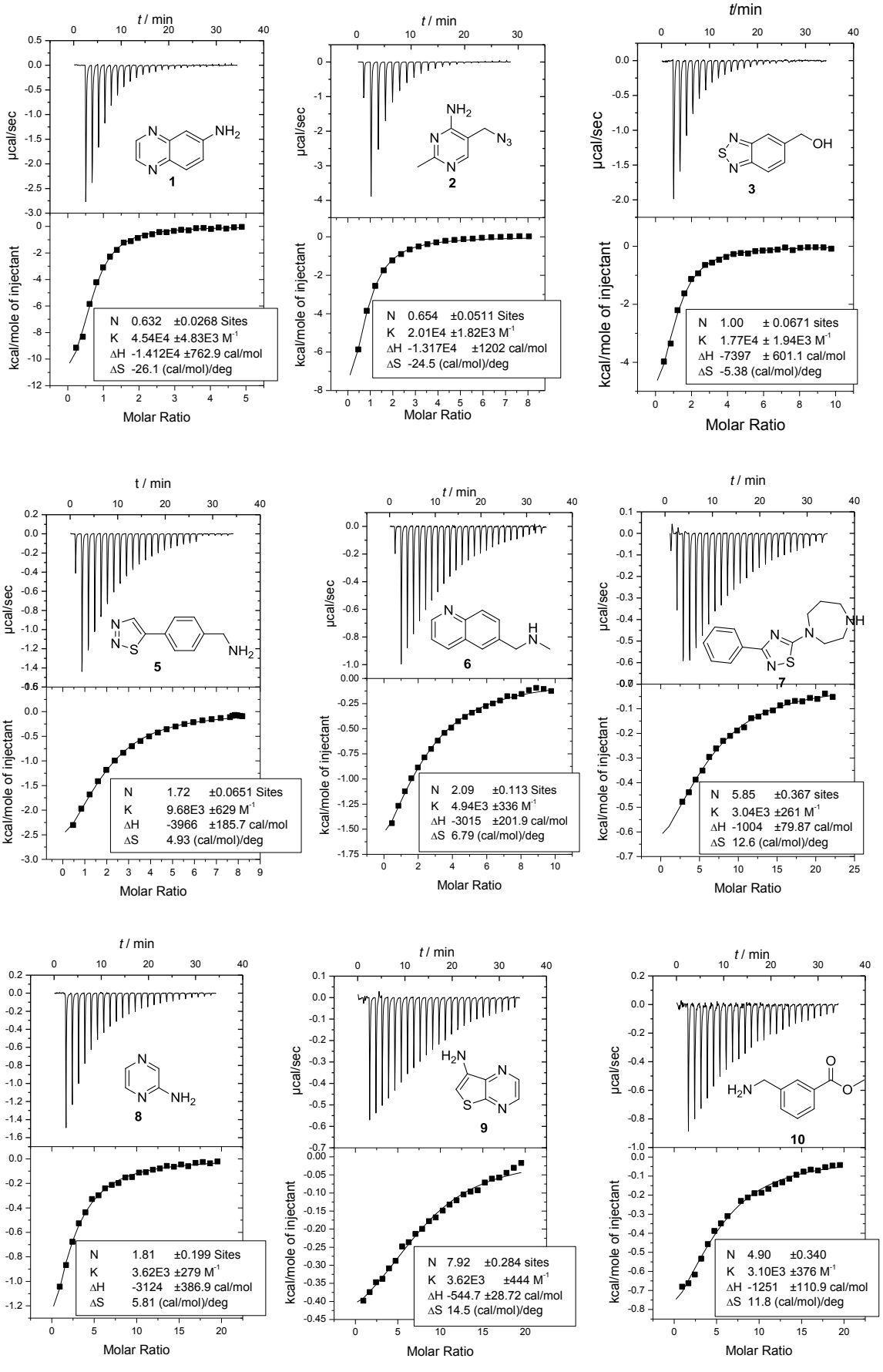
waterLOGSY

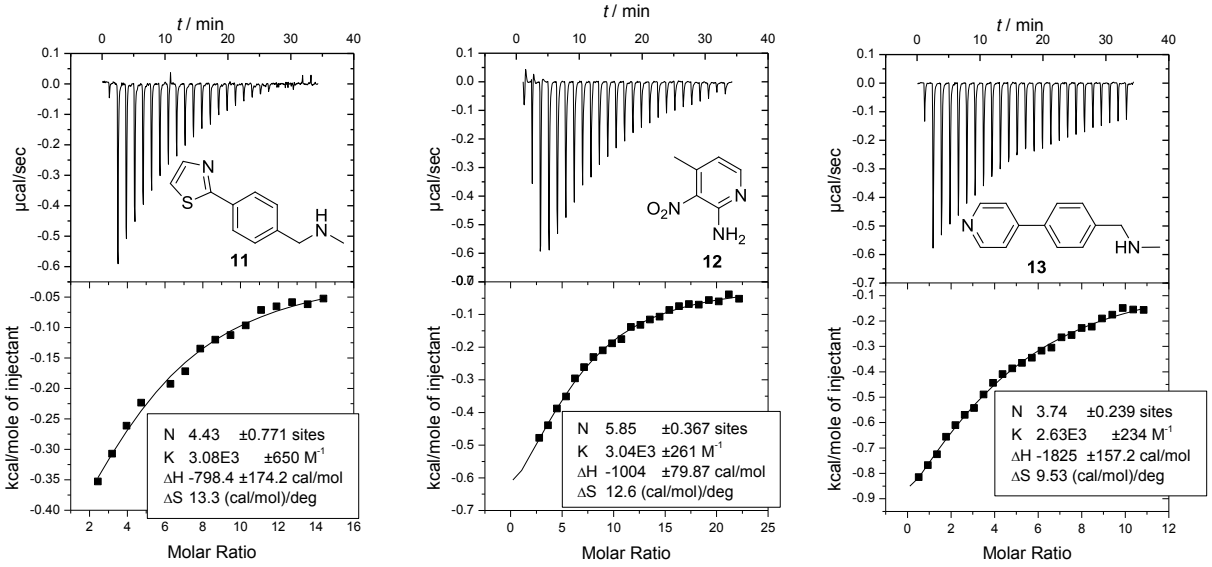
T_2-relaxation edited NMR

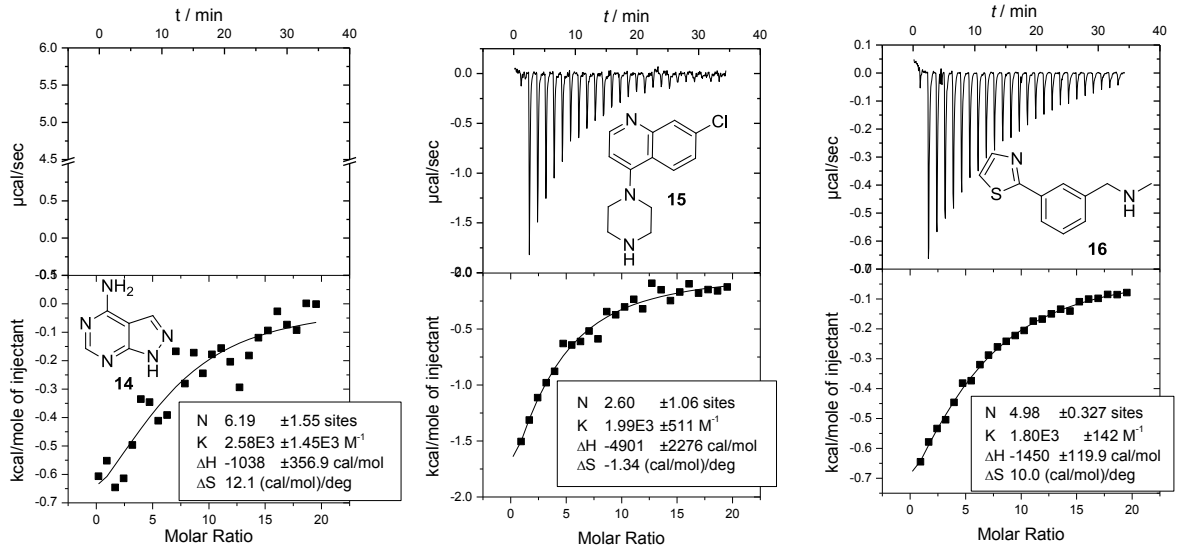


∗ Asterixs denote TPP peaks

Table S3: Isothermal titration thermograms of fragments **1-3, 5-17, 24, 25.** Isothermal titration thermogram of fragment **4** is shown in Figure 4a. The thermodynamic parameters for each titration are indicated in the boxes. N is the calculated number of ligand molecules that bind to one RNA molecule, K (M^{-1}) is the thermodynamic association constant, ΔH and ΔS are the enthalpy and entropy variation respectively.







In gray: control titration

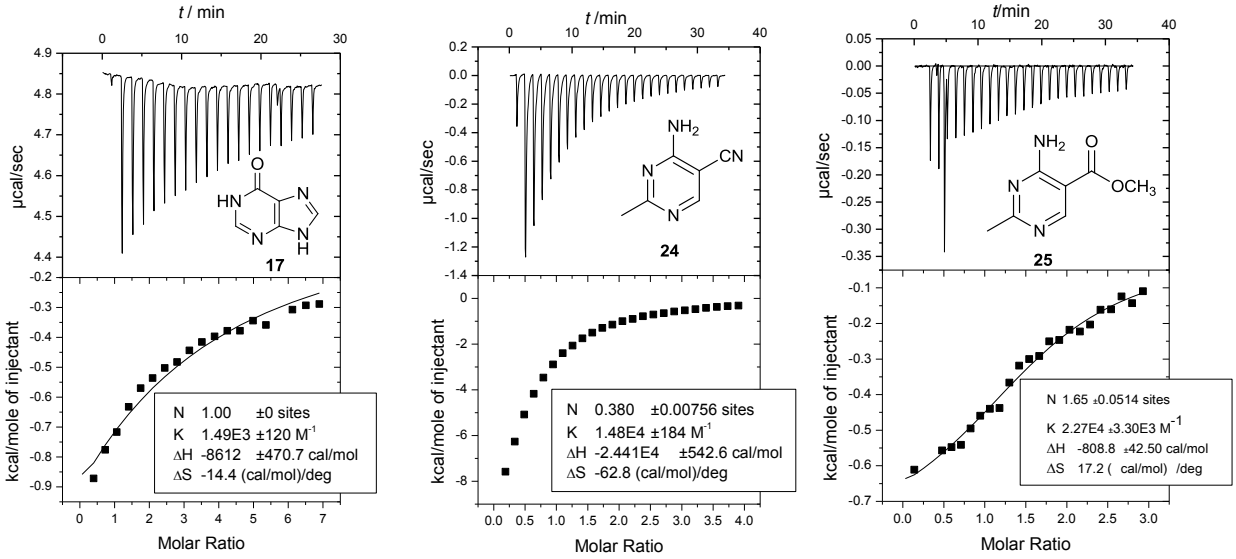


Figure S2: Linear correlation between the luminescence signal and the amount of IVTT reaction mix. A complete IVTT reaction was incubated at 37ºC for 1 hour, using the construct **1** as DNA template. The mix was then diluted 4 fold, and aliquots of different volumes were taken, diluted to 50 µL and assayed for luciferase activity as described in the Experimental Section. The x-axis shows the volume of the undiluted IVTT reaction mix used in the assays. The experimental values (red squares) were fitted with a line (black).

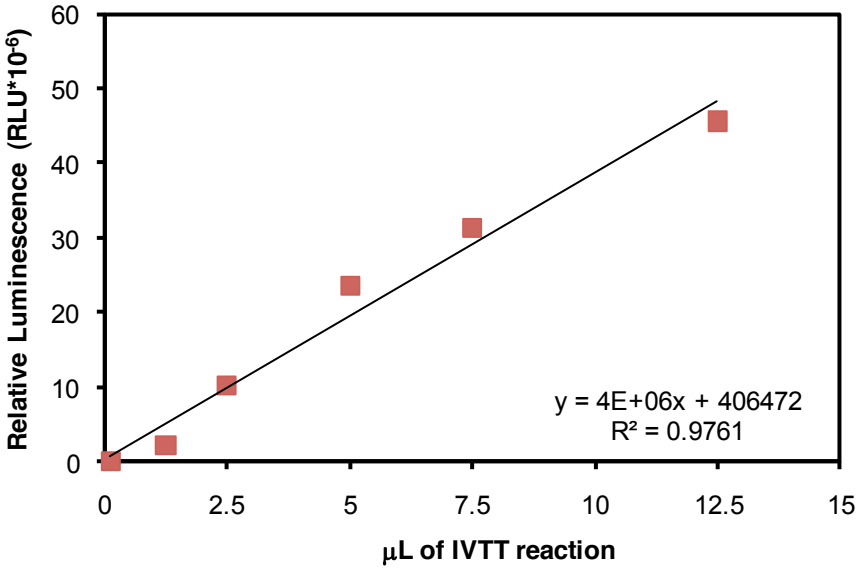


Figure S3: TPP (0.1 mM) effect on *Renilla* luciferase expression in IVTT reactions with construct **1**, containing the *thiM*-RS and construct **2** (pKS- luc) not containing the riboswitch *thiM*-RS. Red bars indicate refer to IVTT performed with 0.1 mM TPP, green bars to IVTTs without TPP. The experiments were performed in duplicate and the error bars were calculated from the experimental error on the duplicates.

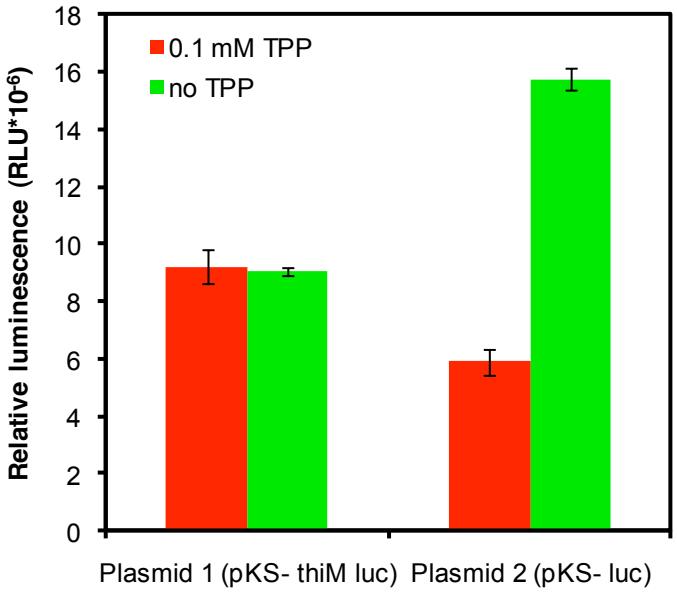
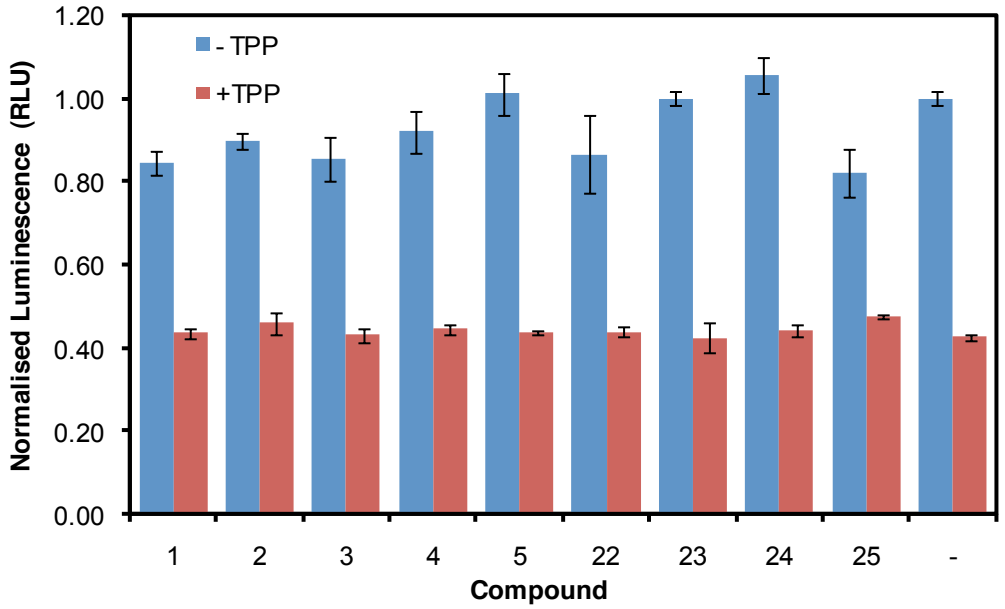


Figure S4: Effect of the compounds **1-5** and **22-25** on *Renilla* luciferase expression in IVTT systems. The compounds at 100 µM concentration were added to IVTT reactions in presence or absence of 100 µM TPP and incubated for 2 hours at 37ºC. The plot shows the normalised luminescence (obtained by dividing the luminescence of each incubation by that of the control reaction without any compound and without TPP). The incubations were performed in duplicate and the error bars were calculated from the experimental error on the duplicates.



References

1. W.C. Winkler, A. Nahvi, R.R. Breaker, *Nature* 2002, **419**, 952-956.
2. Sudarsan, J.K. Wickiser, S. Nakamura, M.S. Ebert, R.R. Breaker, *Genes Dev.* 2003, **17**, 2688-2697.
3. Fuhrmann, A. Hausherr, L. Ferbitz, T. Schodl, M. Heitzer, P. Hegemann *Plant Mol Biol.,* 2004, **55**, 869–881.