Neomycin-Neomycin Dimer: An All Carbohydrate Scaffold with High Affinity for AT Rich DNA Duplexes

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Supporting Information

Appendices

1. Characterization of synthesized compounds

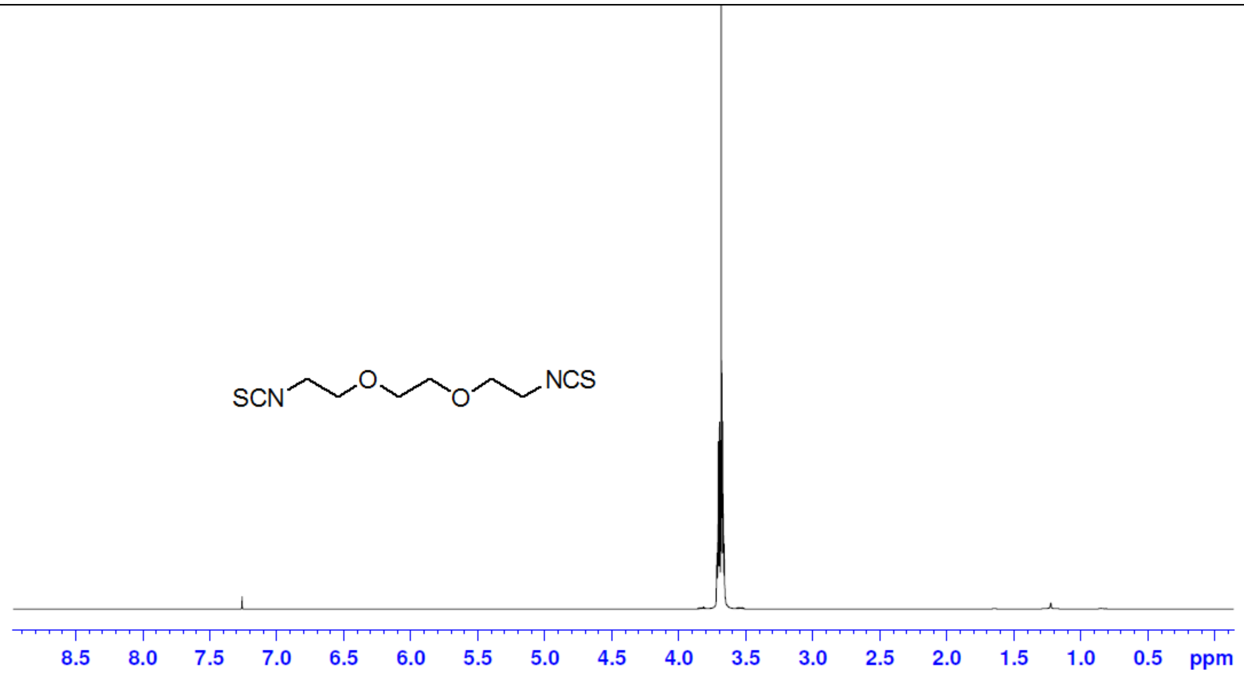


Figure S1. [1]H-NMR spectrum of 2,2'-(ethylenedioxy)bis(ethylisothiocyante) in $CDCl_3$.

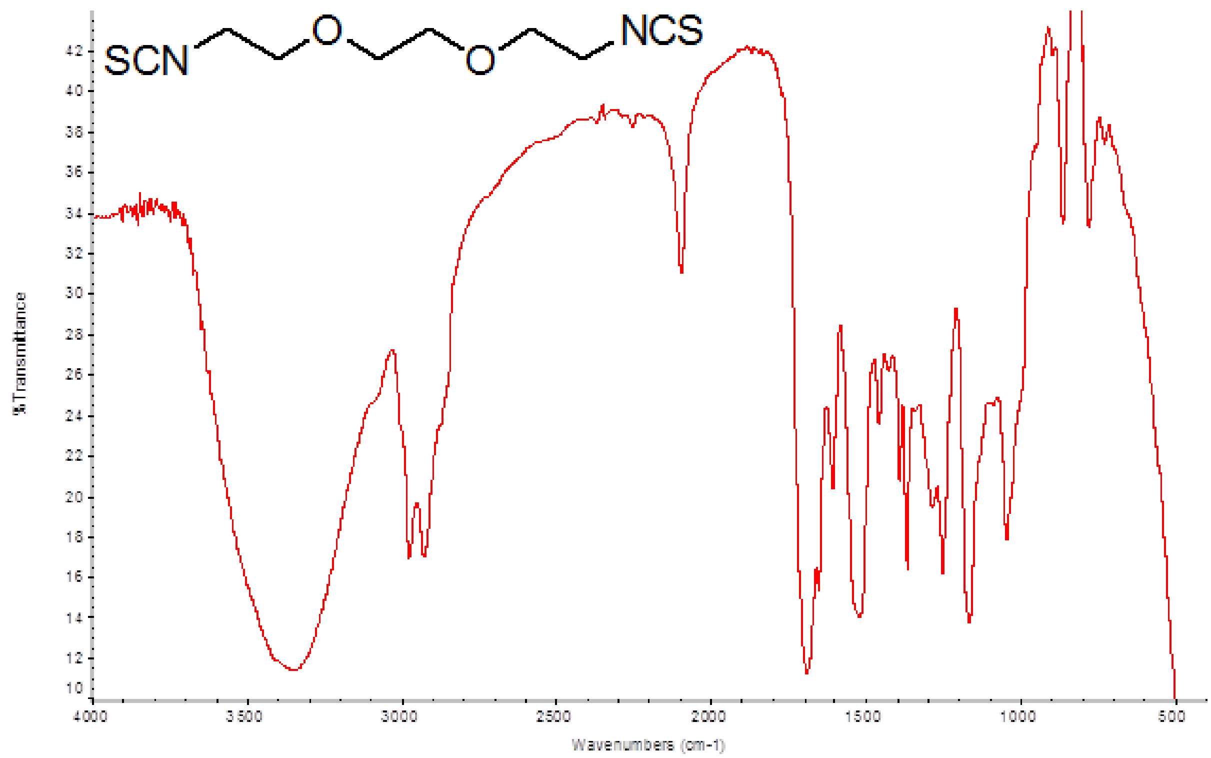


Figure S2. IR spectrum of 2,2'-(ethylenedioxy)bis(ethylisothiocyante).

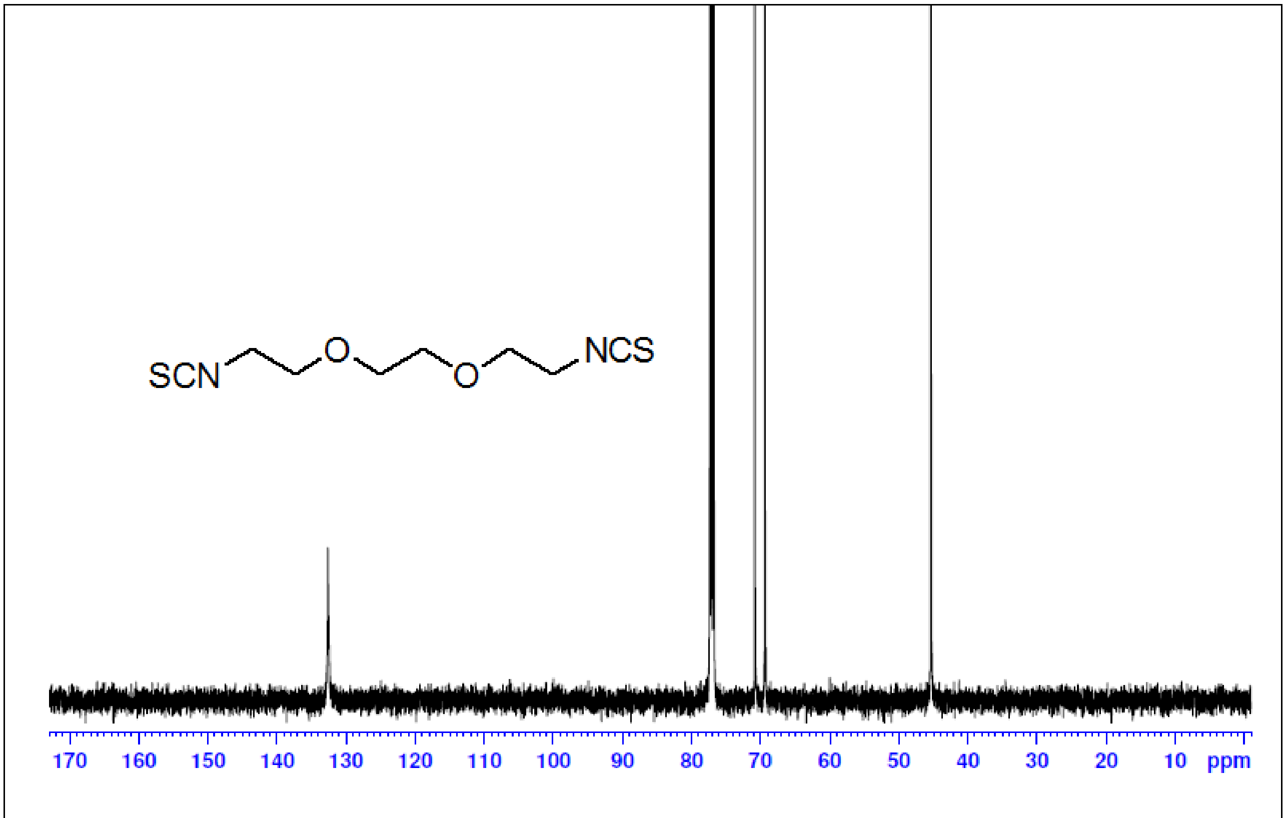


Figure S3. ^{13}C-NMR spectrum of 2,2'-(ethylenedioxy)bis(ethylisothiocyante) in $CDCl_3$.

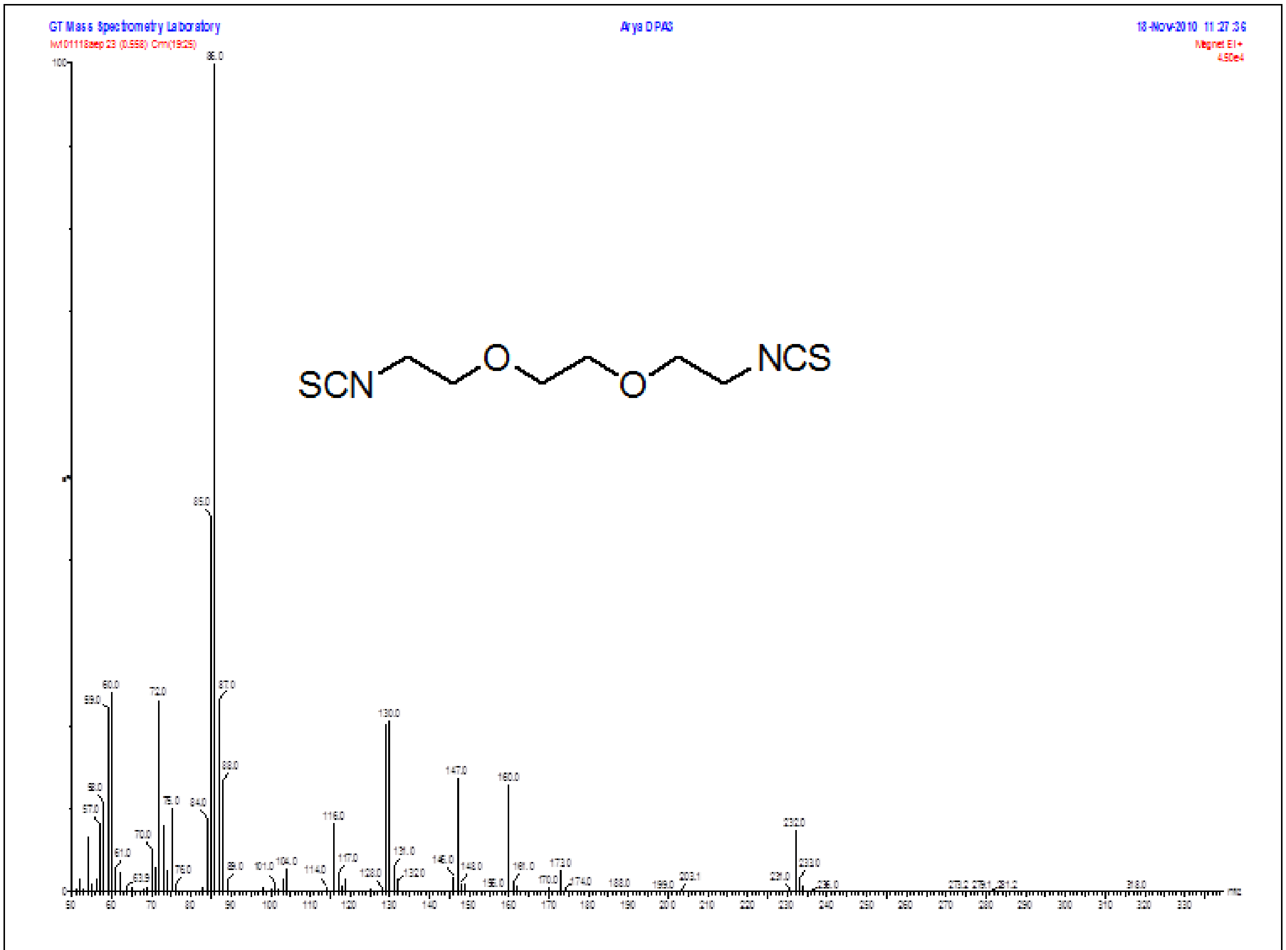


Figure S4. MS-ESI spectrum of 2,2'-(ethylenedioxy)bis(ethylisothiocyante).

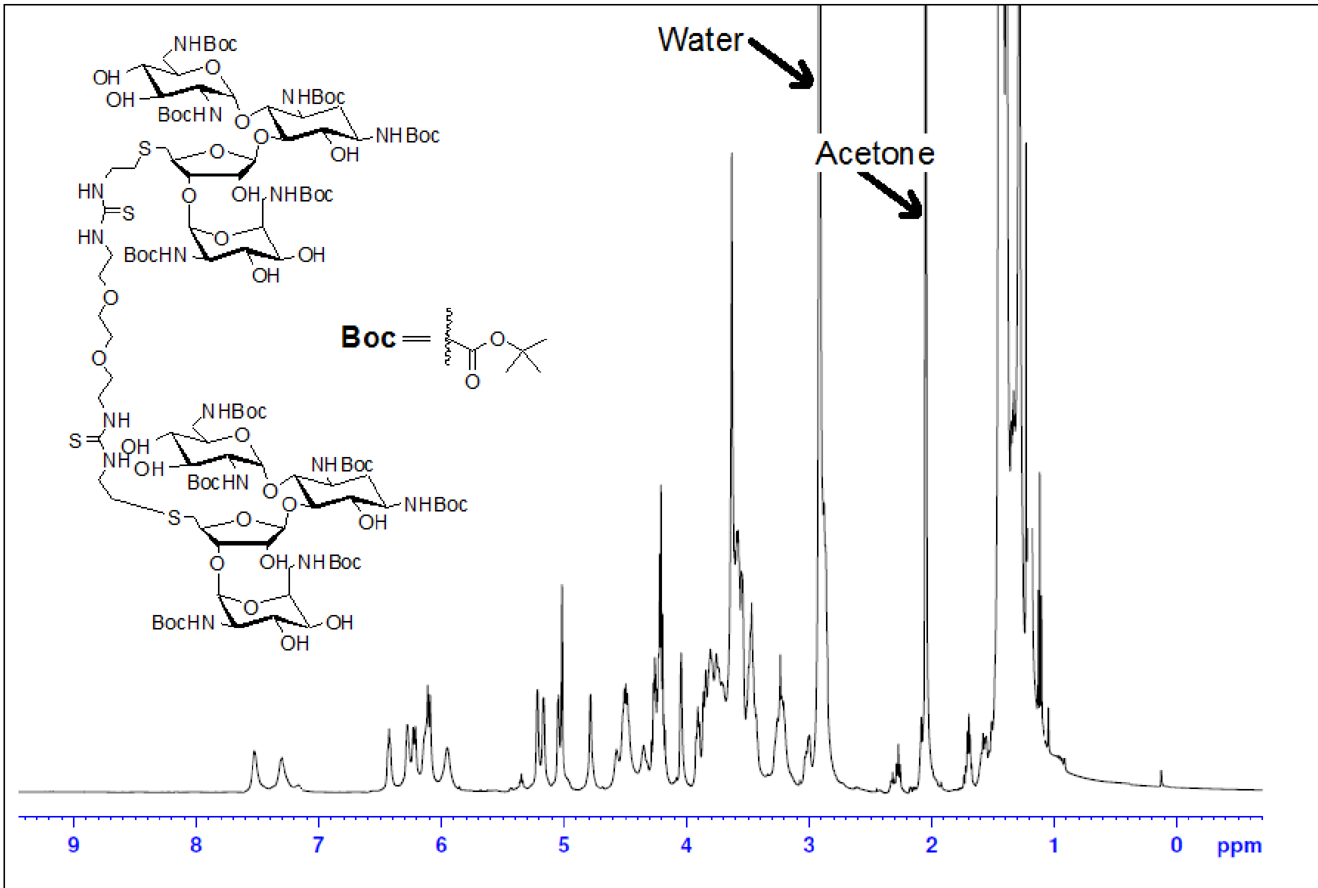


Figure S5. [1]H-NMR spectrum of **3a** in CD_3COCD_3. T = 22 °C.

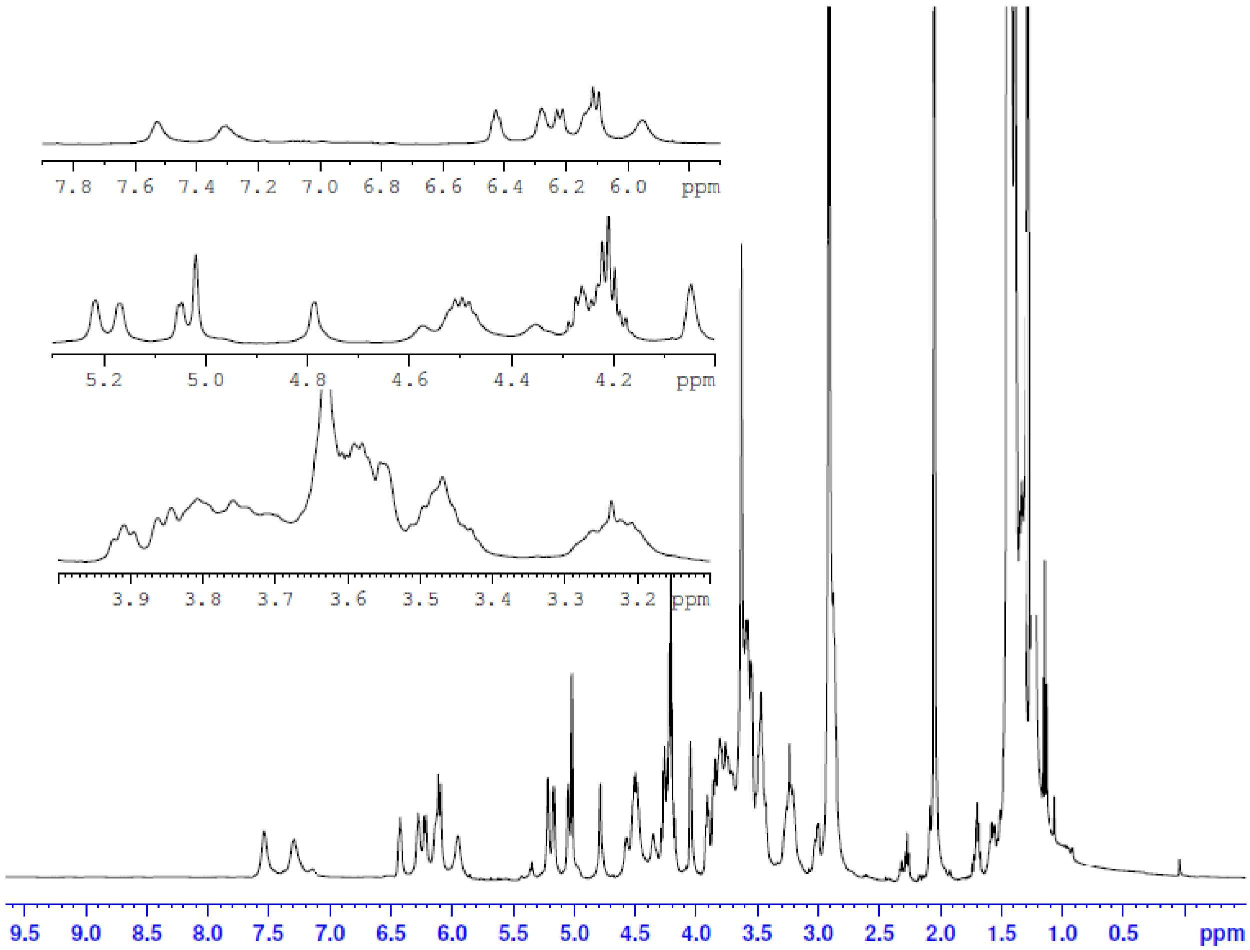


Figure S5A. Expansion of certain regions of ^{1}H-NMR spectrum of **3a** in CD_3COCD_3. T = 22 $^\circ$C.

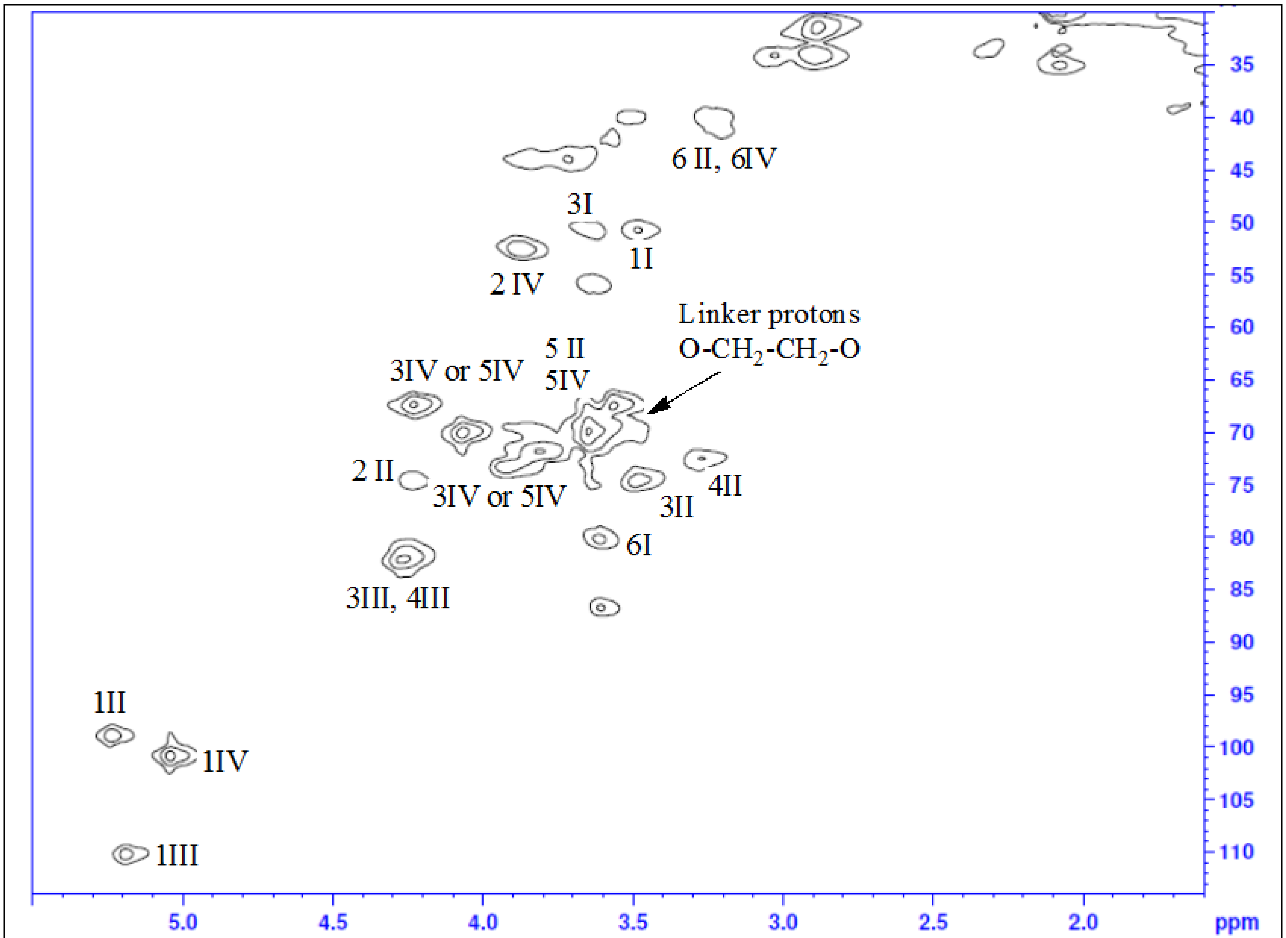


Figure S5B. HMQC spectrum of **3a** in CD_3COCD_3. T = 22 °C. Assignments were made with the help of TOCSY, HMQC, and COSY spectra.

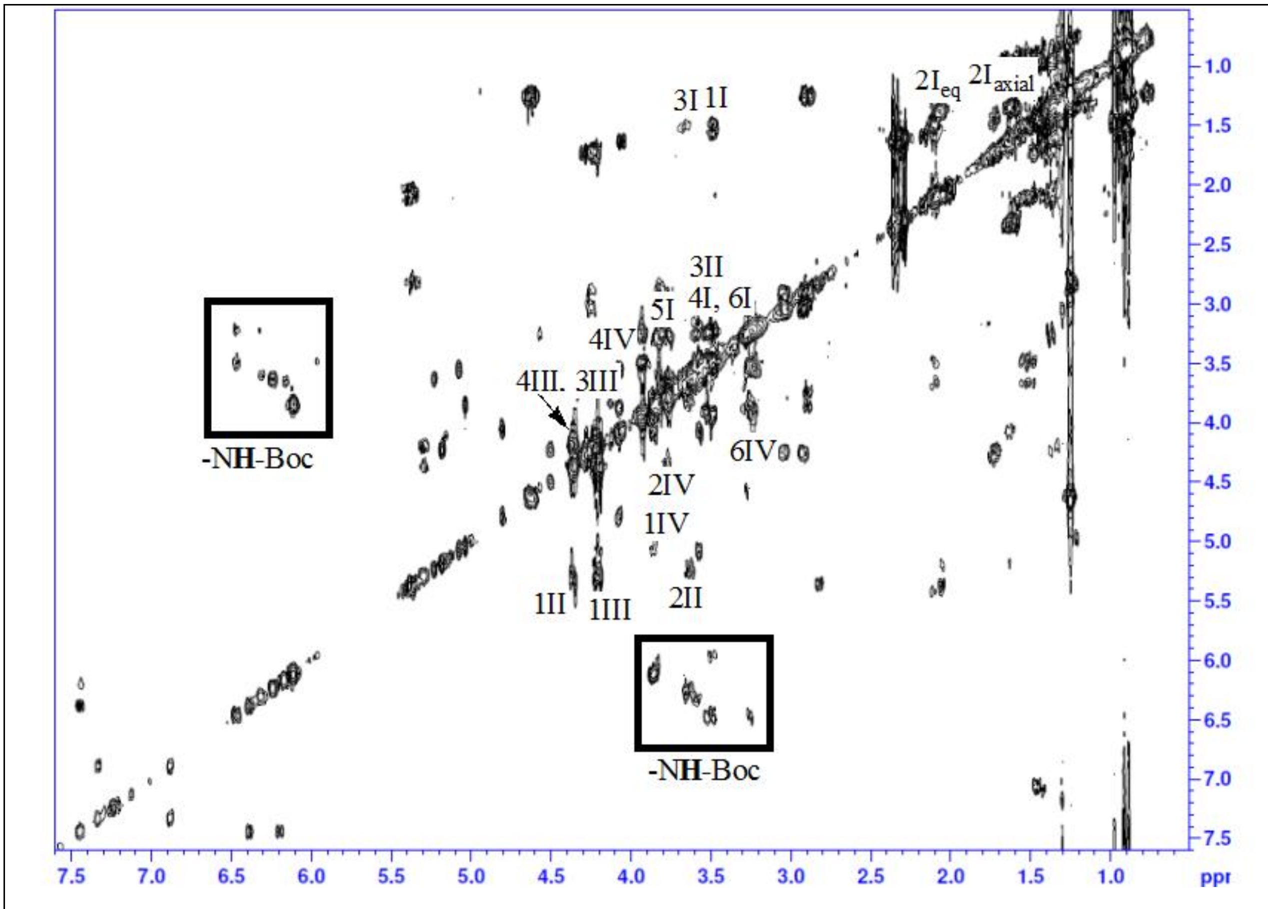


Figure S5C. COSY spectrum of **3a** in CD_3COCD_3. T = 22 °C. Assignments were made with the help of TOCSY, HMQC, and COSY spectra.

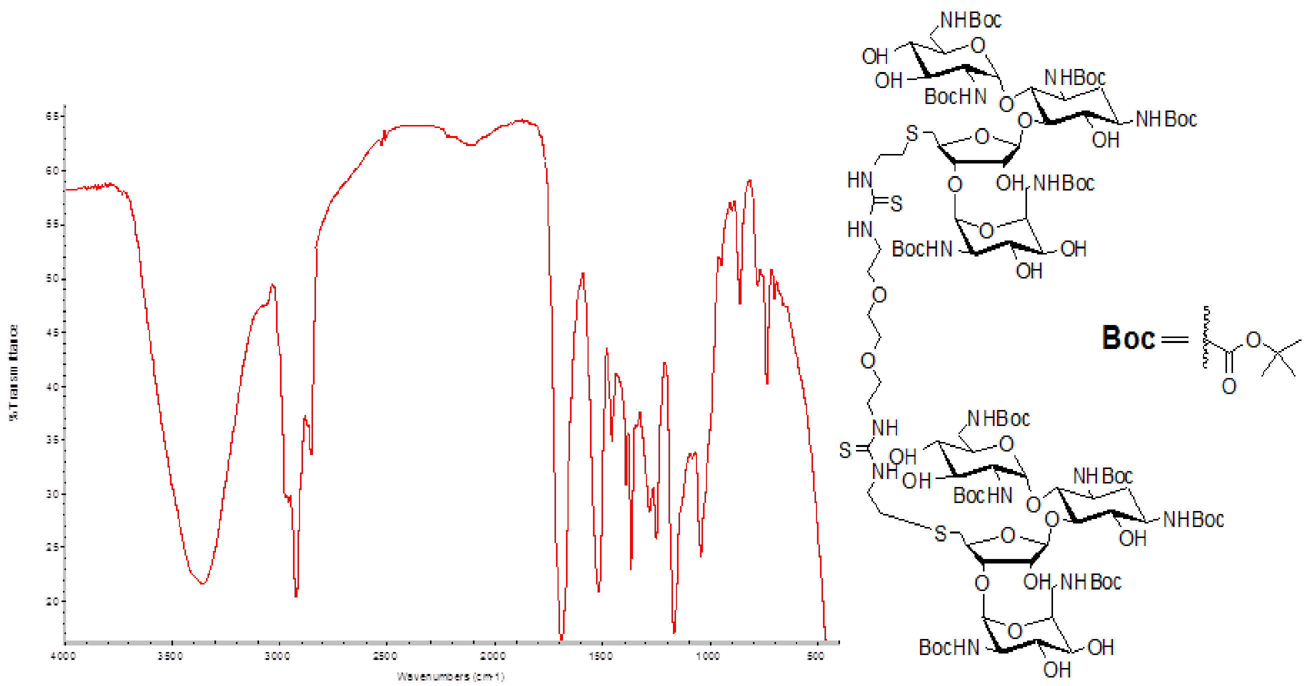


Figure S5D. IR spectrum of **3a**.

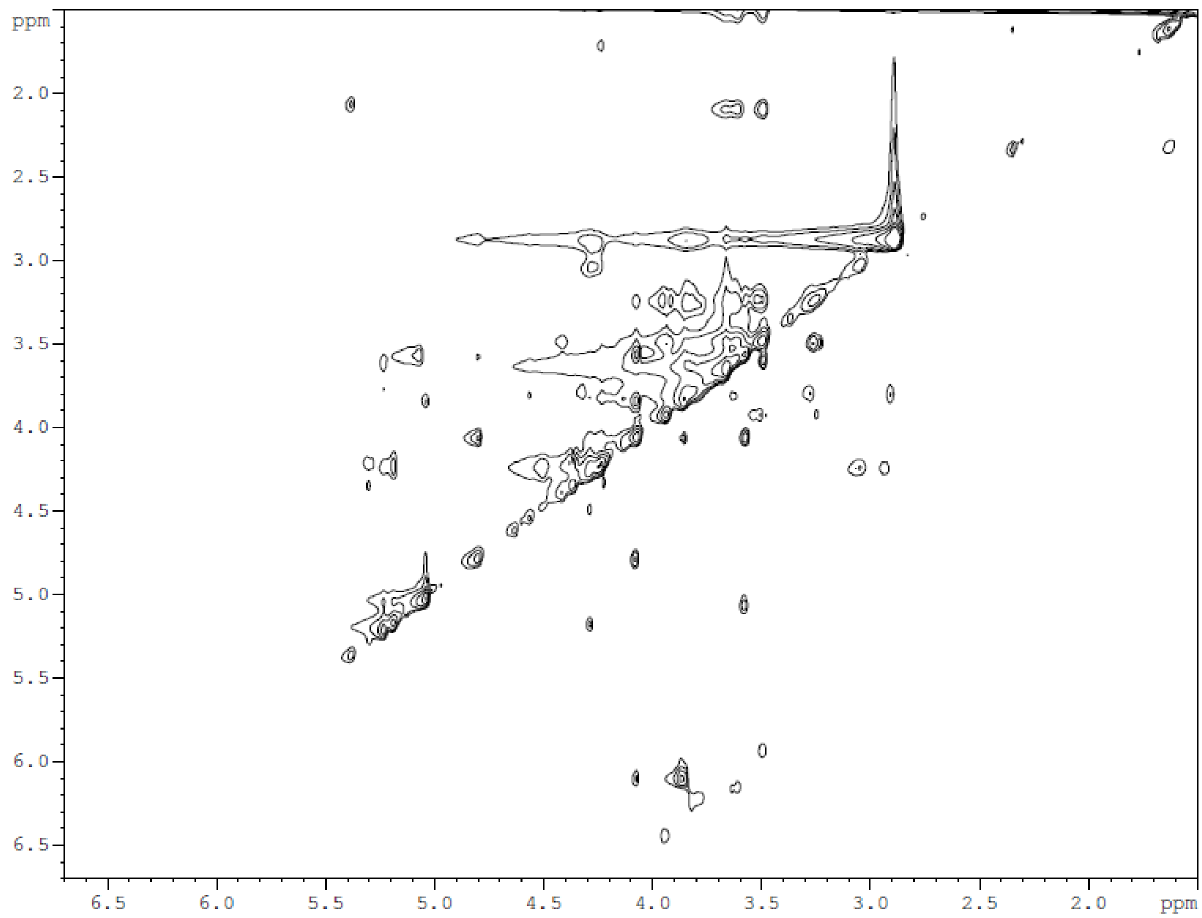


Figure S5E. TOCSY spectrum of **3a** in CD_3COCD_3. T = 22 $^{\circ}$C.

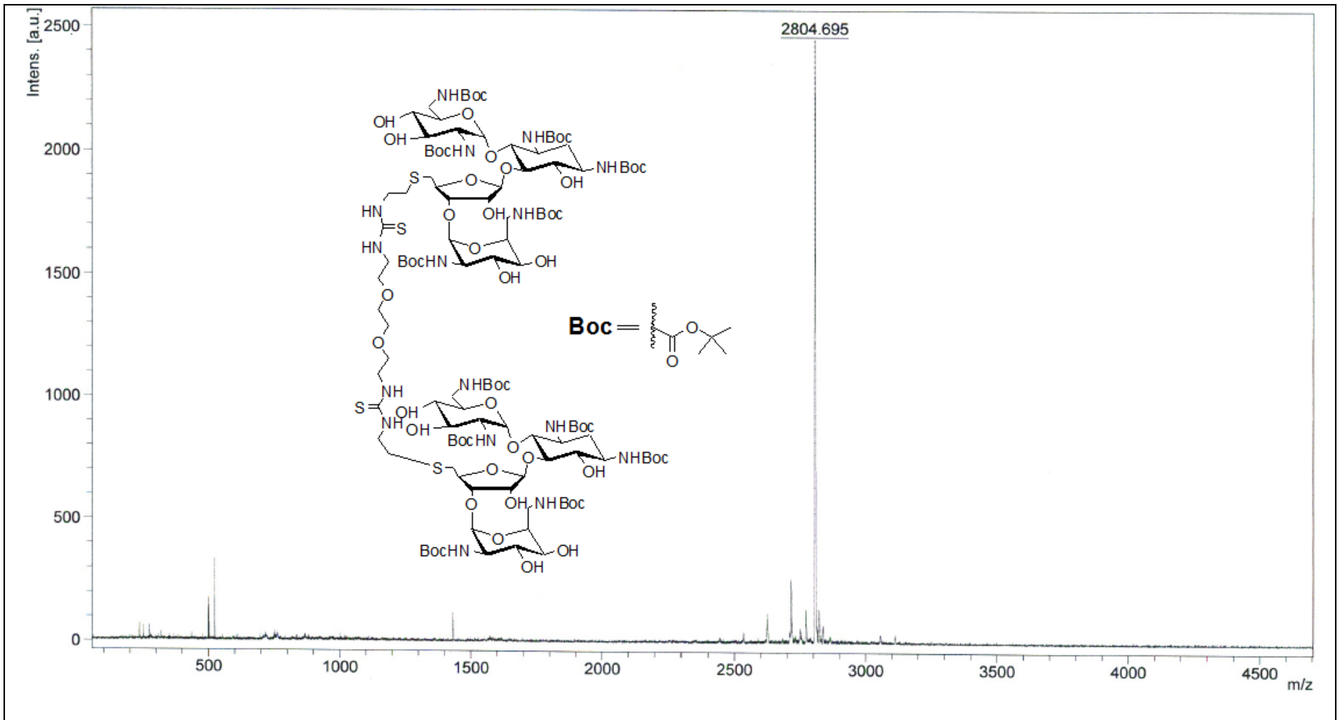


Figure S6. MALDI-TOF spectrum of **3a**.

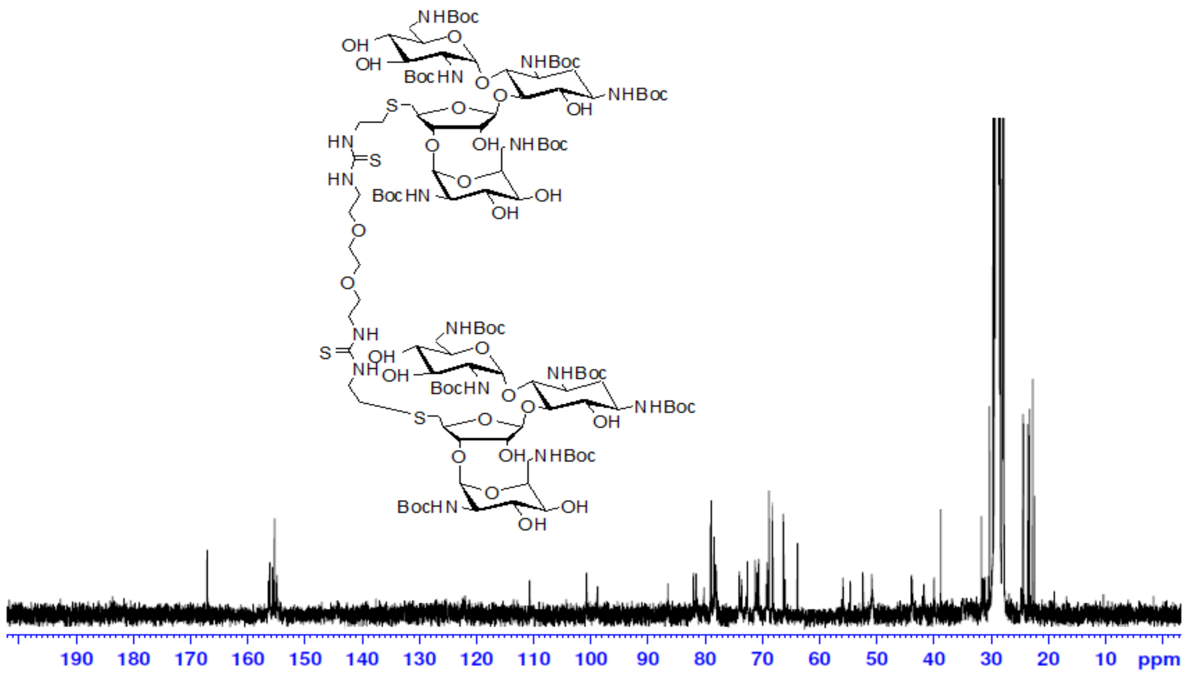


Figure S7. ^{13}C-NMR spectrum of **3a** in CD_3COCD_3. T = 22 $^{\circ}$C.

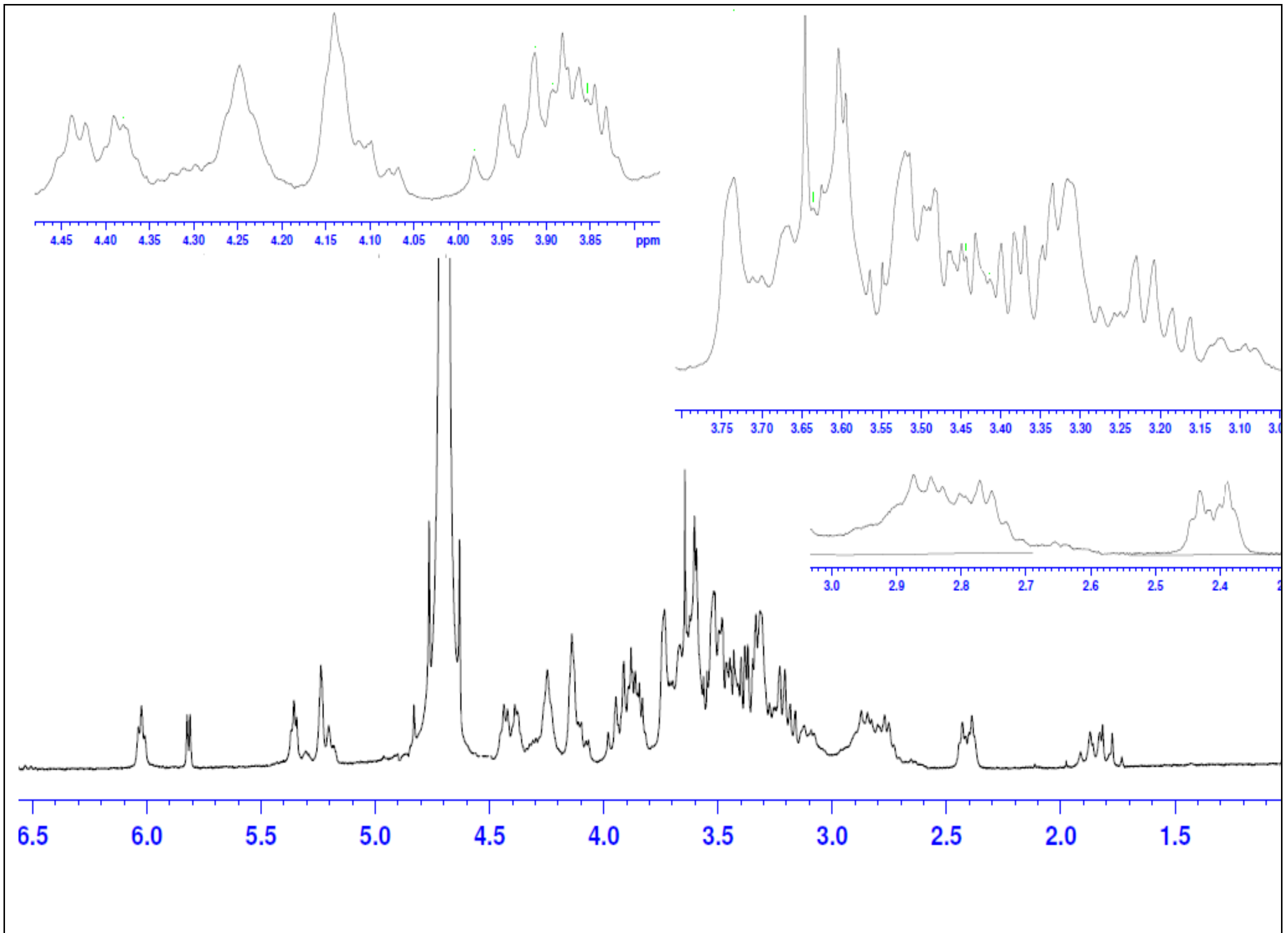


Figure S8A. Expansion of certain regions of ^{1}H-NMR spectrum of **3** in D_2O. T = 22 $^{\circ}$C.

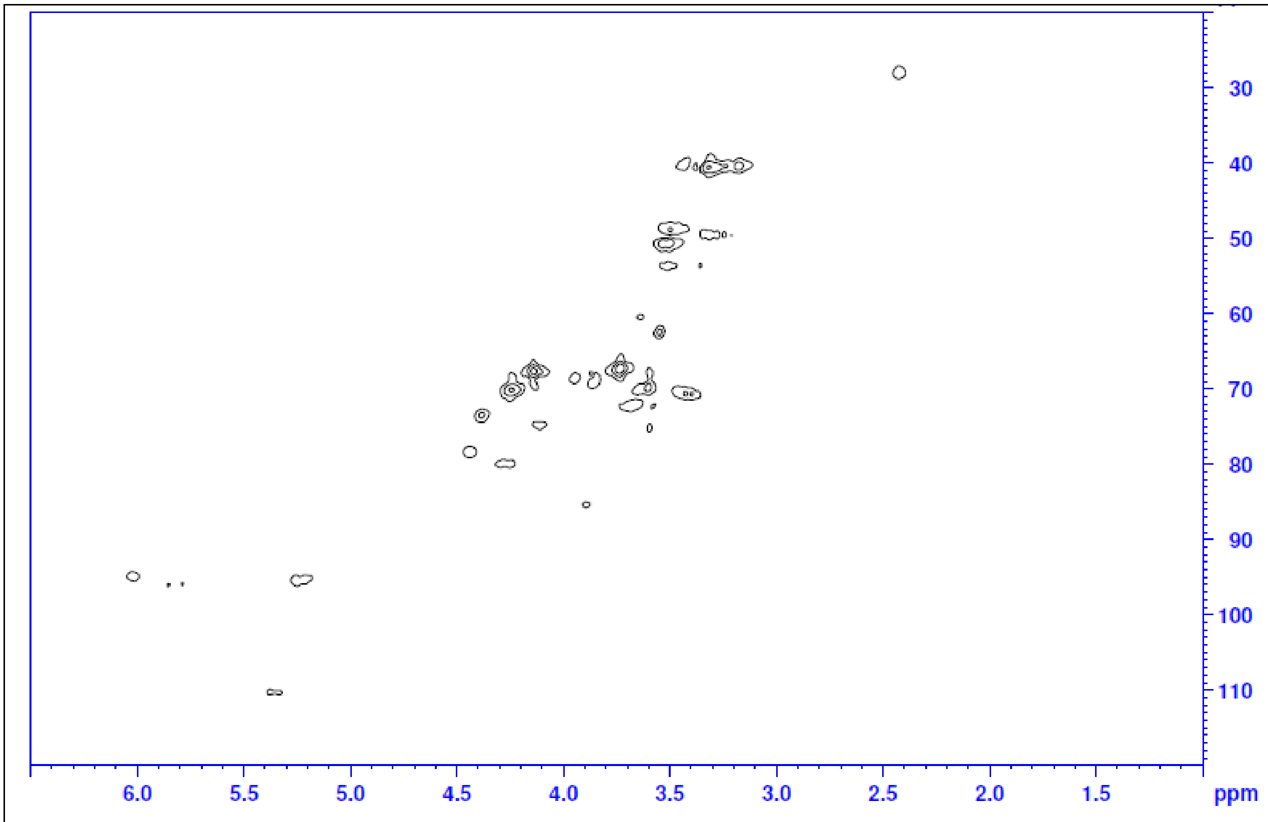


Figure S8B. HMQC spectrum of **3** in D_2O. T = 22 °C.

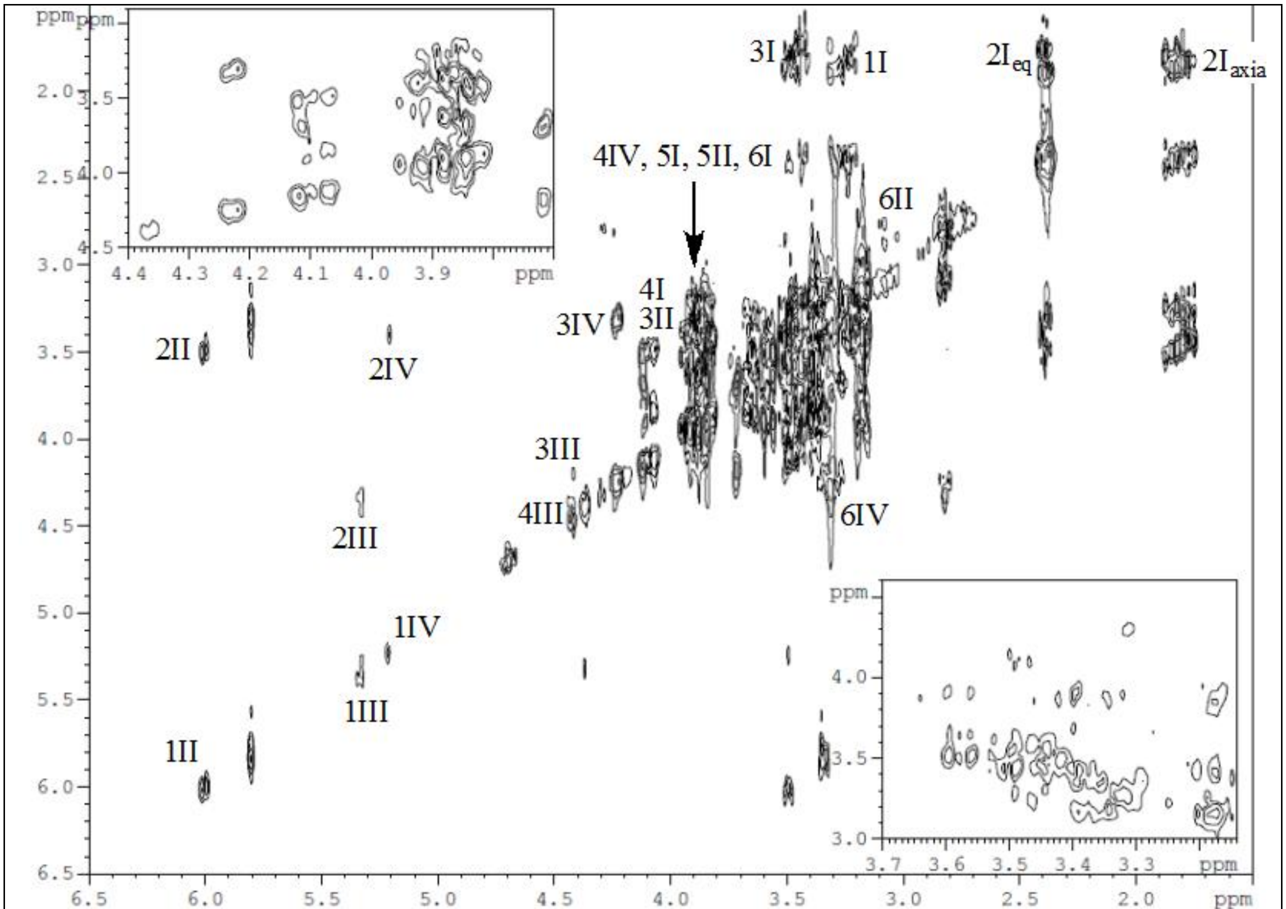


Figure S8C. DFQT-COSY spectrum of **3** in D_2O (The inset shows the heavily crowded region of cross peaks at low intensity). T = 22 °C. Peak Assignments were made with the help of TOCSY, HMQC, and COSY spectra.

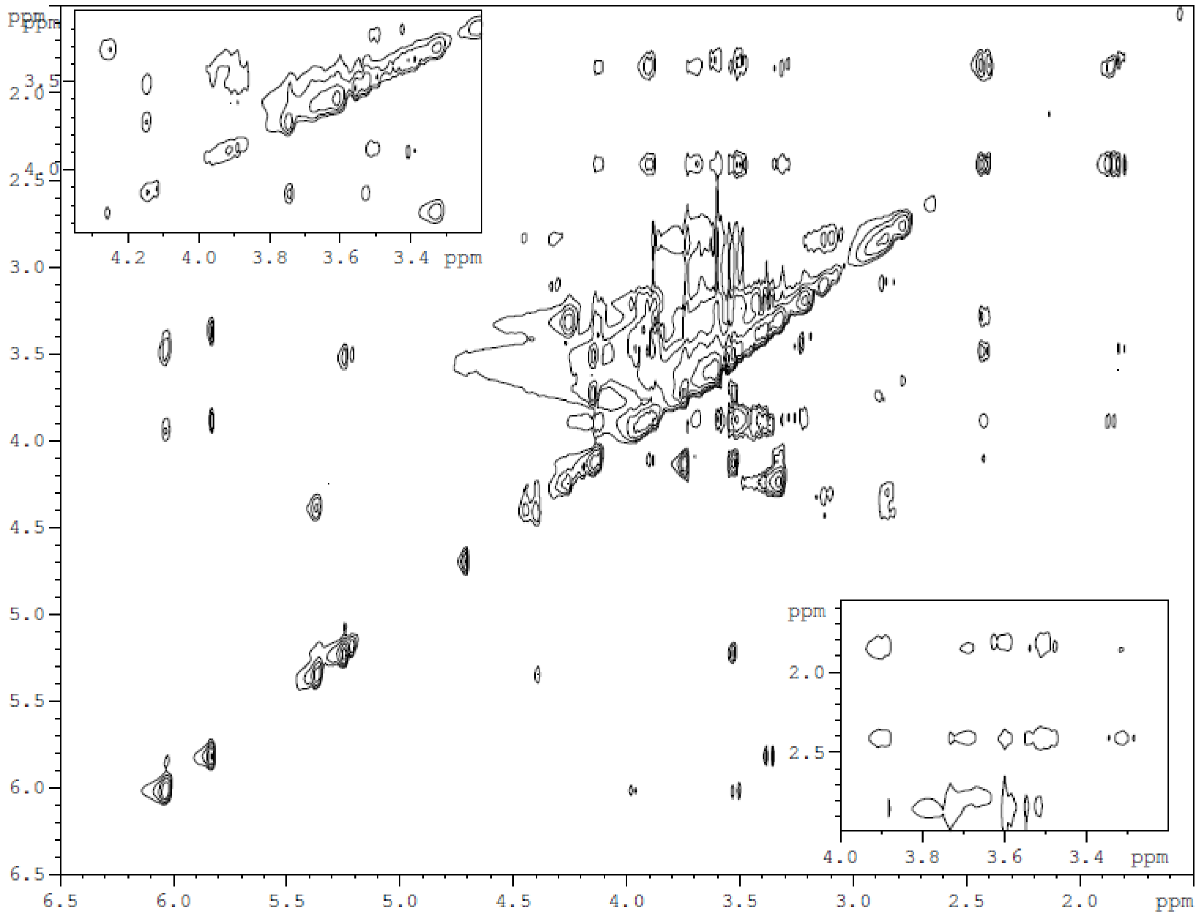


Figure S8D. TOCSY spectrum of **3** in D_2O (The inset shows the heavily crowded region of cross peaks at low intensity). T = 22 °C.

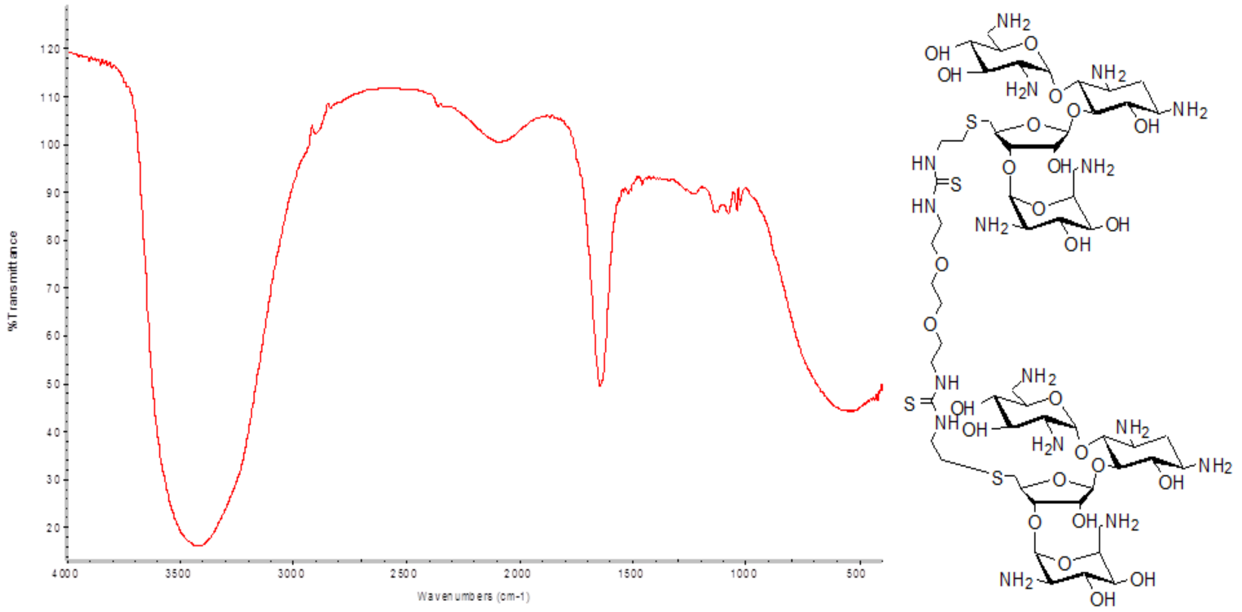


Figure S8E. IR spectrum of **3.**

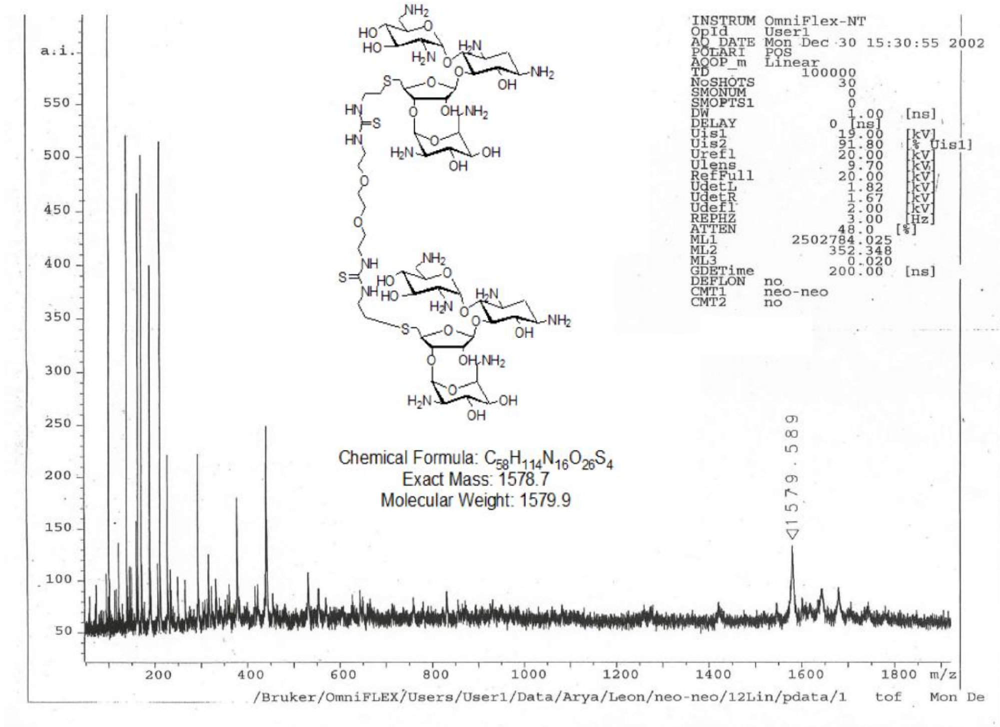


Figure S9. MALDI-TOF spectrum of **3**.

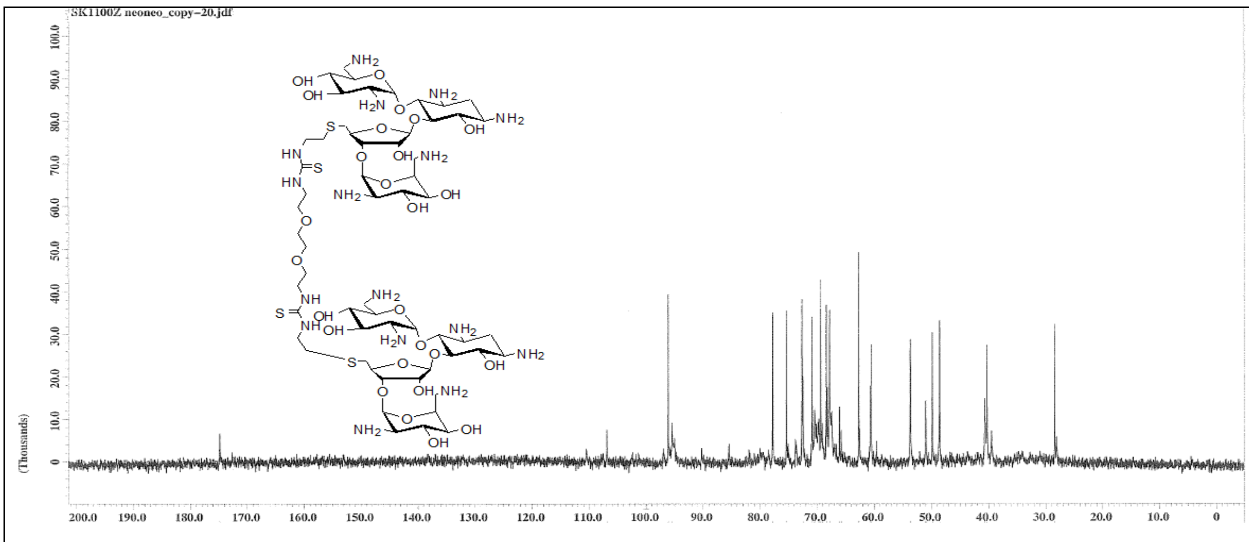


Figure S10. ^{13}C-NMR spectrum of **3** in D_2O. T = 22 °C.

2. Biophysical characterization of 3 with Nucleic acids.

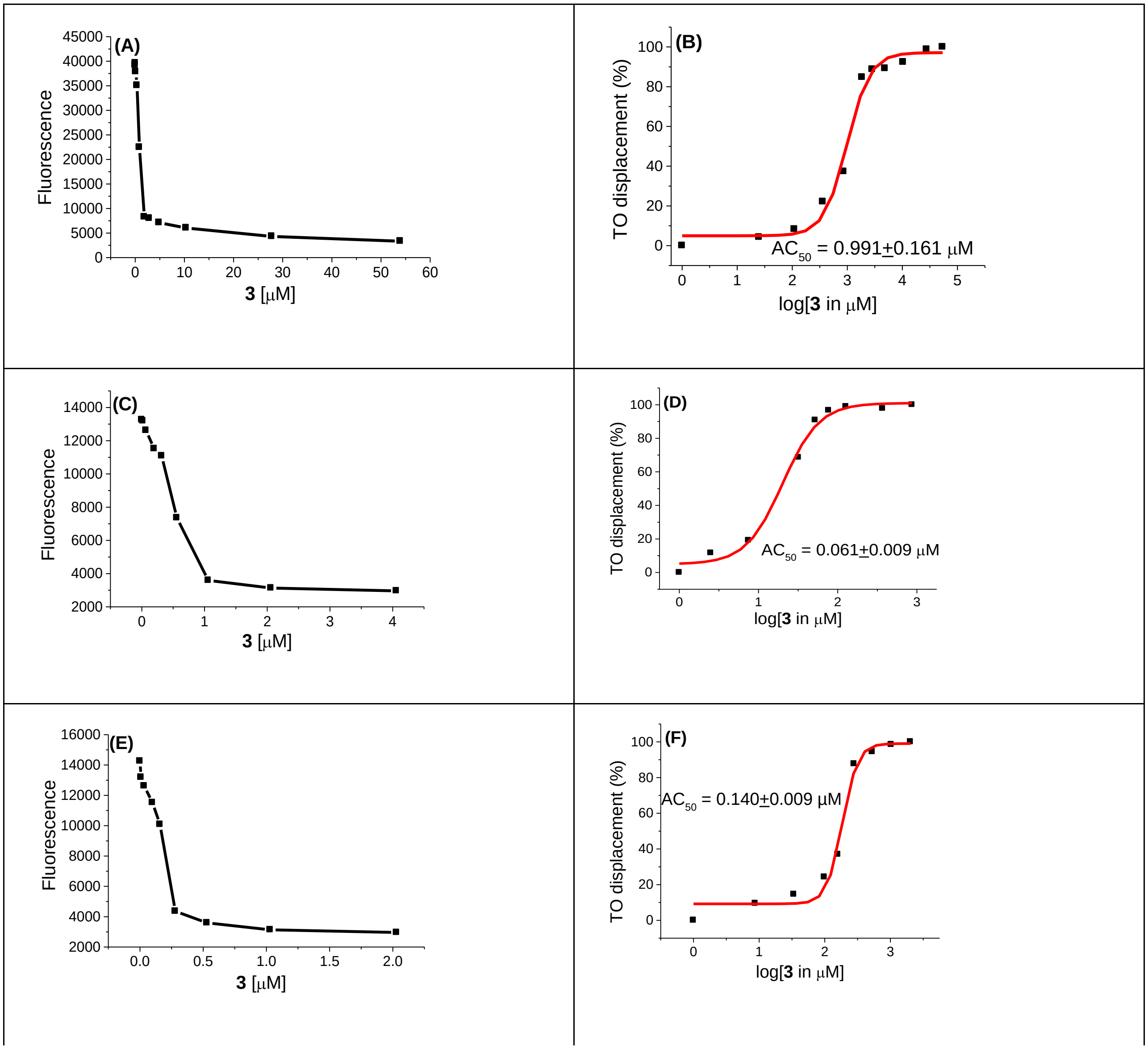


Figure S11. A graphical representation of FID assay to calculate the AC_{50} value of **3** with various polynucleotides. (A) Plot showing decrease in the fluorescence intensity of poly(dA).2poly(dT)−TO complex as a function of increasing concentration of **3**. (B) A sigmoidal fit between percentage of TO displaced from poly(dA).2poly(dT) as a function of log[**3**]. (C) Plot showing decrease in the fluorescence intensity of poly(dA.dT)$_2$−TO complex as a function of increasing concentration of **3**. (D) A sigmoidal fit between percentage of TO displaced from poly(dA.dT)$_2$ as a function of log[**3**]. (E) Plot showing decrease in the fluorescence intensity of poly(dG).poly(dC)−TO complex as a function of increasing concentration of **3**. (F) A sigmoidal fit between percentage of TO displaced from poly(dG).poly(dC) as a function of log[**3**]. Buffer conditions: 100 mM KCl, 10 mM SC, 0.5 mM EDTA, pH 6.8. [polynucleotide] = 0.88 μM/bp, [TO] = 1.25 μM. The reported AC_{50} value in each case is an average of three separate experiments. The AC_{50} value for each experiment was calculated with Origin 5.0.

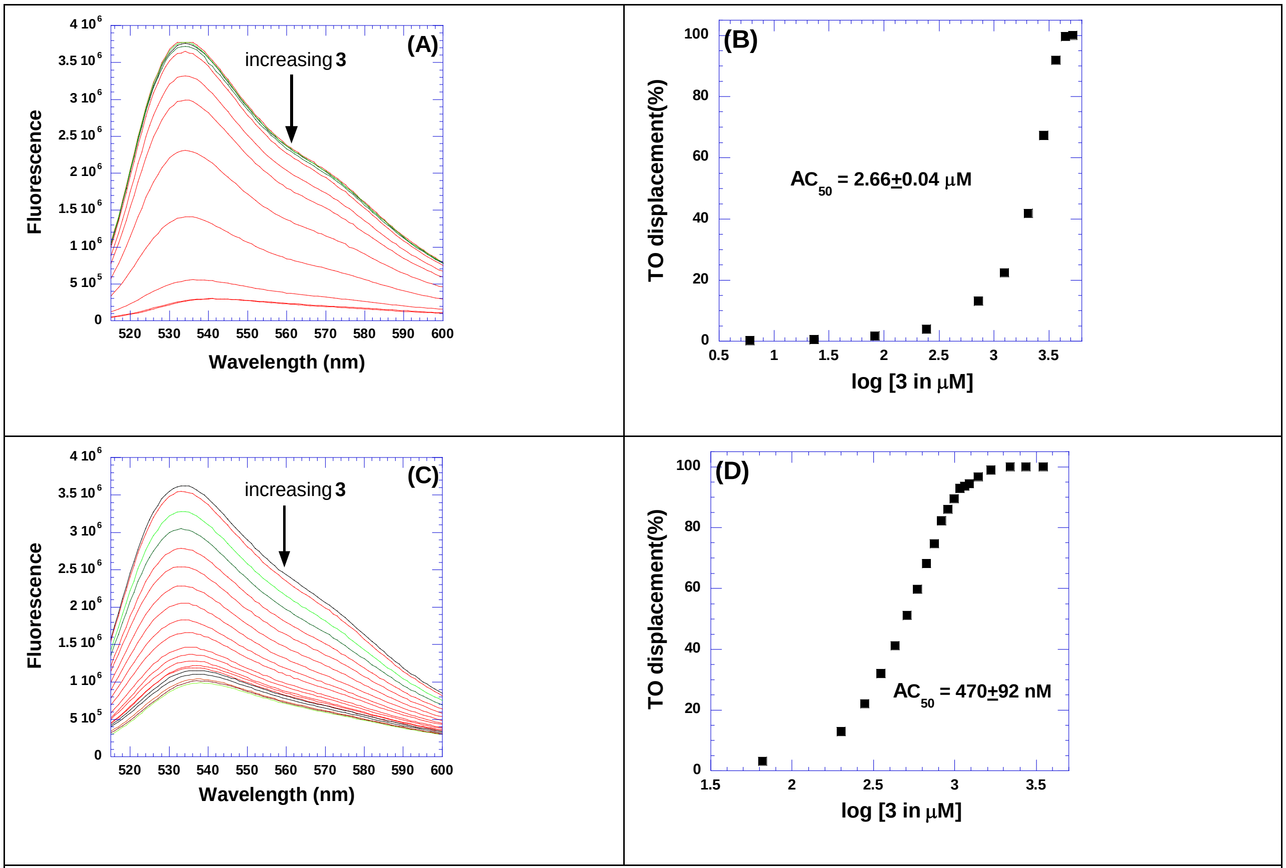
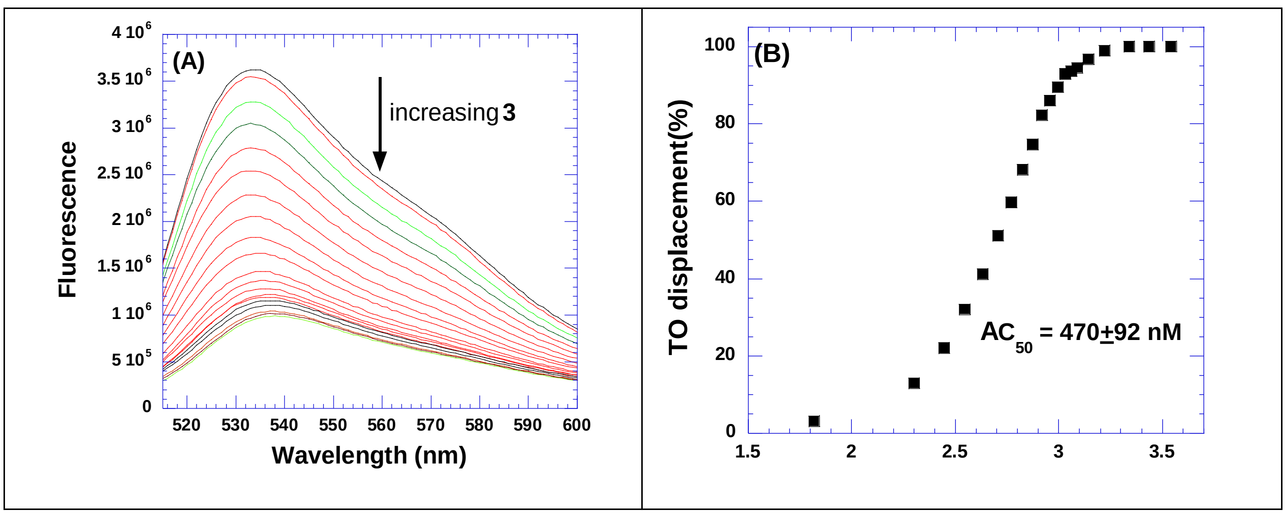


Figure S12. FID titration of **3** with intramolecular duplex d[5'-A_{12}-x-T_{12}-3'] and triplex d[5'-A_{12}-x-T_{12}-x-T_{12}-3']. Raw fluorescence emission spectrum of (A) intramolecular triplex d[5'-A_{12}-x-T_{12}-x-T_{12}-3'] and (C) intramolecular duplex d[5'-A_{12}-x-T_{12}-3']. A sigmoidal fit between percentage of TO displaced from (B) triplex d[5'-A_{12}-x-T_{12}-x-T_{12}-3'] and (D) intramolecular duplex d[5'-A_{12}-x-T_{12}-3'] as a function of log[**3**]. Buffer conditions: 100 mM KCl, 10 mM SC, 0.5 mM EDTA, pH 6.8. d[5'-A_{12}-x-T_{12}-3'] = 1 μM/strand. [TO] = 6 μM. The reported AC_{50} value in each case is an average of three separate experiments. The AC_{50} value for each experiment was calculated with Origin 5.0.

Compound name	AC_{50} values (nM)
Neomycin	$(471\pm28)\times10^{2}$
3	470±92

Table S1. AC_{50} values of **3** and neomycin with d[5'-A_{12}-x-T_{12}-3']. The solutions were equilibrated for 5 min. before taking the fluorescence emission scans. Buffer conditions: 100 mM KCl, 10 mM SC, 0.5 mM EDTA, pH 6.8. d[5'-A_{12}-x-T_{12}-3'] = 1 μM/strand. [TO] = 6 μM. The reported AC_{50} value is an average of three separate experiments.



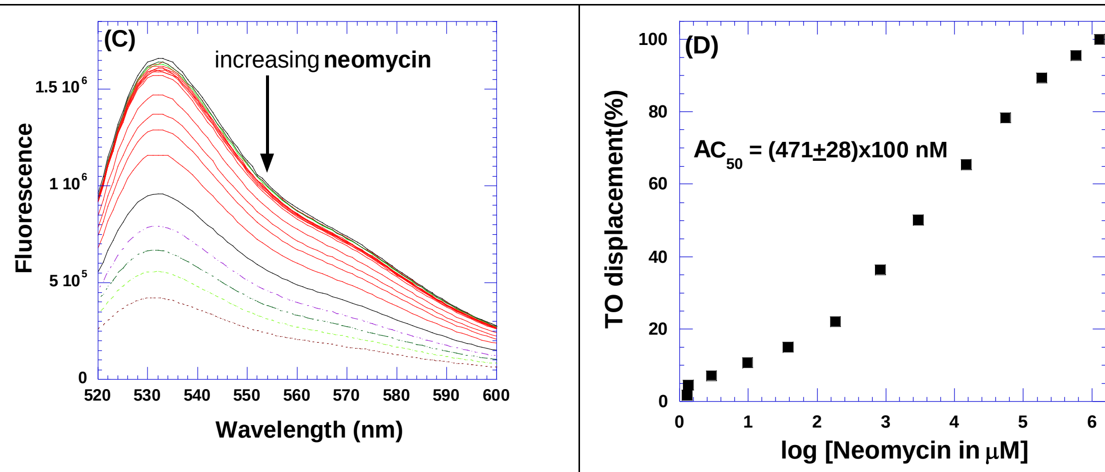


Figure S13. FID titration of ligands (**3** and neomycin) with intramolecular duplex d[5'-A_{12}-x-T_{12}-3']. (A) Raw fluorescence emission spectrum in the presence of increasing concentration of **3**. (B) A sigmoidal fit between percentage of thiazole orange displaced from intramolecular duplex d[5'-A_{12}-x-T_{12}-3'] as a function of log[**3**]. (C) Raw fluorescence emission spectrum in the presence of increasing concentration of neomycin. (D) A sigmoidal fit between percentage of thiazole orange displaced from intramolecular duplex d[5'-A_{12}-x-T_{12}-3'] as a function of log[neomycin]. The solutions were equilibrated for 5 min. before taking the fluorescence emission scans. Buffer conditions: 100 mM KCl, 10 mM SC, 0.5 mM EDTA, pH 6.8. d[5'-A_{12}-x-T_{12}-3'] = 1 μM/strand. [TO] = 6 μM. The reported AC_{50} value in each case is an average of three separate experiments. The AC_{50} value for each and every experiment was calculated by using Origin 5.0 with sigmoidal fit. The lowest and highest values during fit were constrained as 0% and 100% respectively.

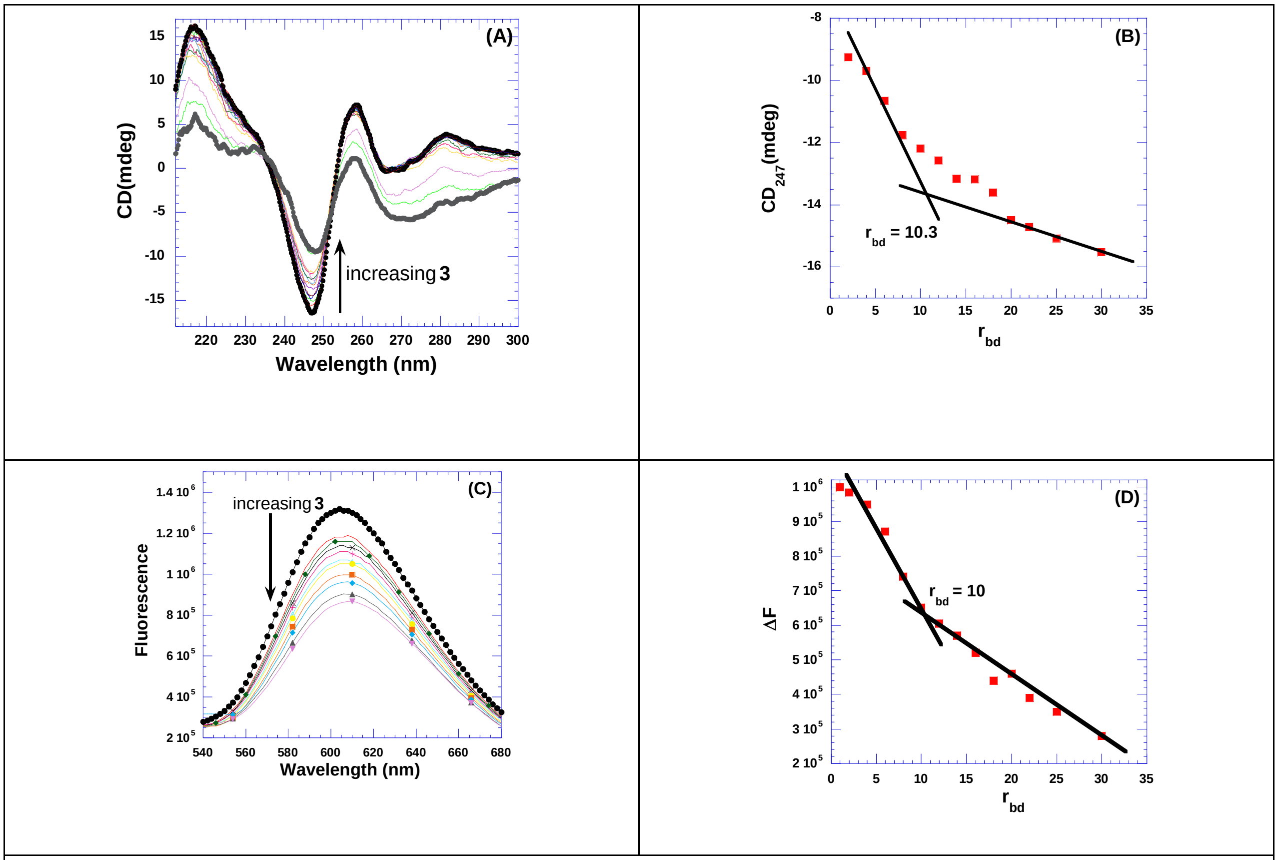


Figure S14. (A) CD titration of poly(dA.dT)$_2$ with increasing concentration of **3**. The continuous changes in the CD spectra correspond to the incremental amount of **3** ranging from a r_{bd} of 0 to 30. (B) A plot of normalized molar ellipticity versus r_{bd} for CD titration curve of **3** with poly(dA.dT)$_2$. Molar ellipticity is per molar base pairs and r_{bd} = ratio of the base pair/drug. (C) Raw fluorescence emission spectrum of poly(dA.dT)$_2$–EtBr complex with the increasing concentration of **3**. (D) A plot of change in the EtBr fluorescence versus r_{bd} to obtain the binding site size of **3** with poly(dA.dT)$_2$. The CD solution was incubated for 1 h at 20 °C before titrating it with **3**. The FID solutions were equilibrated for 5 min. before taking the fluorescence emission scans. Buffer conditions: 100 mM KCl, 10 mM SC, 0.5 mM EDTA, pH 6.8. [DNA] = 10 µM/bp (for fluorescence titration) and 40 µM/bp (for CD spectroscopy).

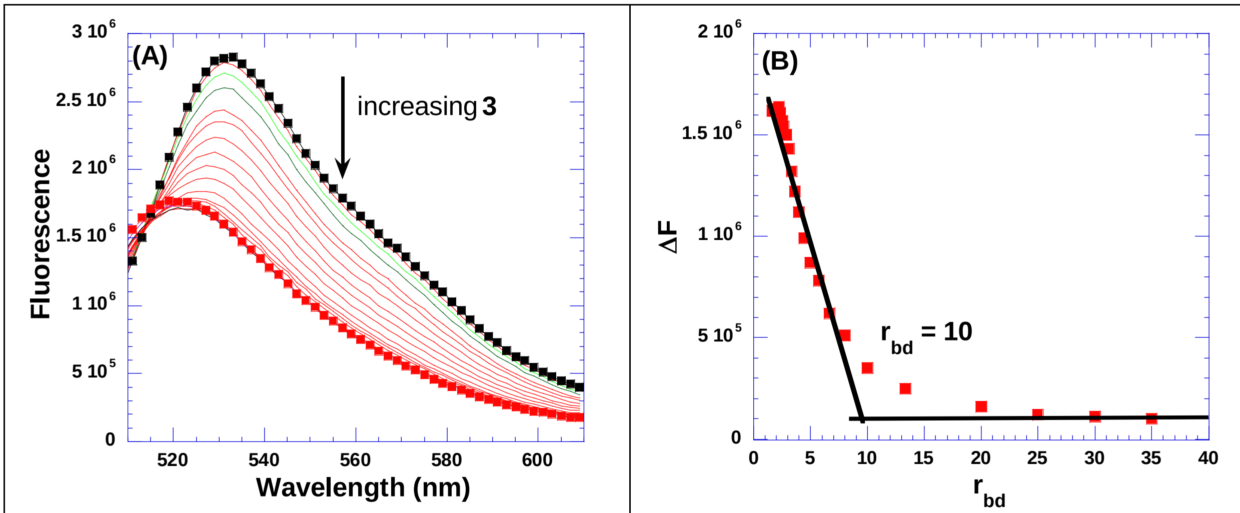


Figure S15. TO displacement titration between d[5'-$A_{30}T_{30}$-3'] and **3**. (Left) Raw fluorescence emission spectrum of d[5'-$A_{30}T_{30}$-3']–TO complex with increasing concentration of **3** showing the decrease of fluorescence intensity (at 532 nm) of d[5'-$A_{30}T_{30}$-3']-TO complex. (Right) A plot of change in TO fluorescence and r_{bd} to determine the binding site size of **3** with d[5'-$A_{30}T_{30}$-3']. The solution was incubated for 1 h at 20 °C before titrating it with **3**. The solutions were equilibrated for 5 min. before taking the fluorescence emission scans. Buffer conditions: 100 mM KCl, 10 mM SC, 0.5 mm EDTA, pH 6.8. T = 20 °C. d[5'-$A_{30}T_{30}$-3'] = 0.5 μM/duplex, [TO] = 15 μM.

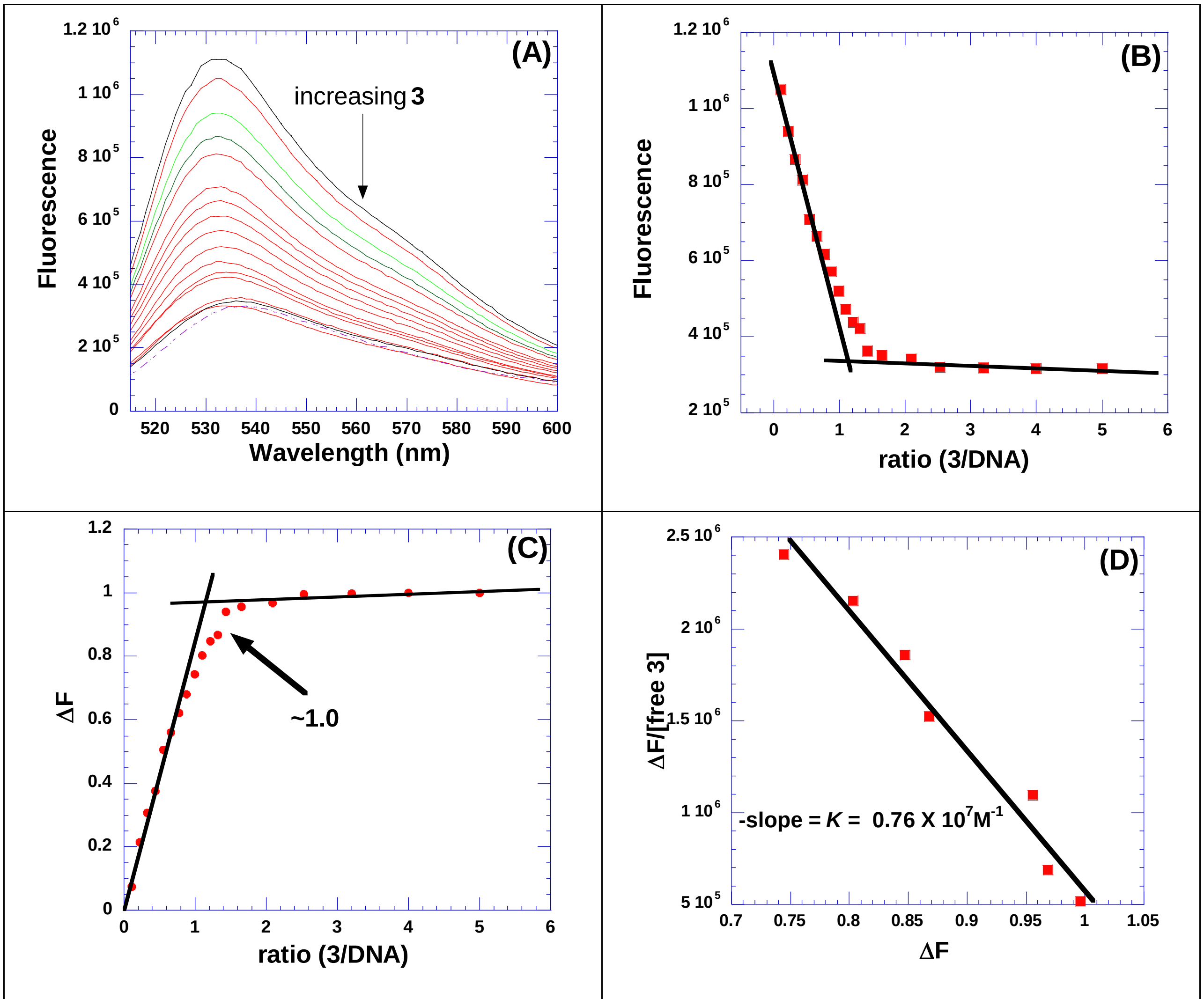


Figure S16. FID titration of **3** with intramolecular hairpin duplex d[5'-A_{12}-x-T_{12}-3']. (A) Raw fluorescence emission spectrum in the presence of increasing concentration of **3**. (B) The decrease of fluorescence intensity (at 532 nm) of DNA-TO complex with increasing concentration of **3**. (C) A plot of fraction of TO displaced versus increase in the concentration of **3**. (D) Scatchard analysis of **3** with duplex d[5'-A_{12}-x-T_{12}-3']. The solution was incubated for 1 h at 15 °C before titrating it with **3**. The solution was equilibrated for 5 min. before taking the fluorescence emission scans. Buffer conditions: 150 mM KCl, 10 mM SC, 0.5 mM EDTA, pH 6.8. d[5'-A_{12}-x-T_{12}-3'] = 1 μM/strand, [TO] = 6 μM.

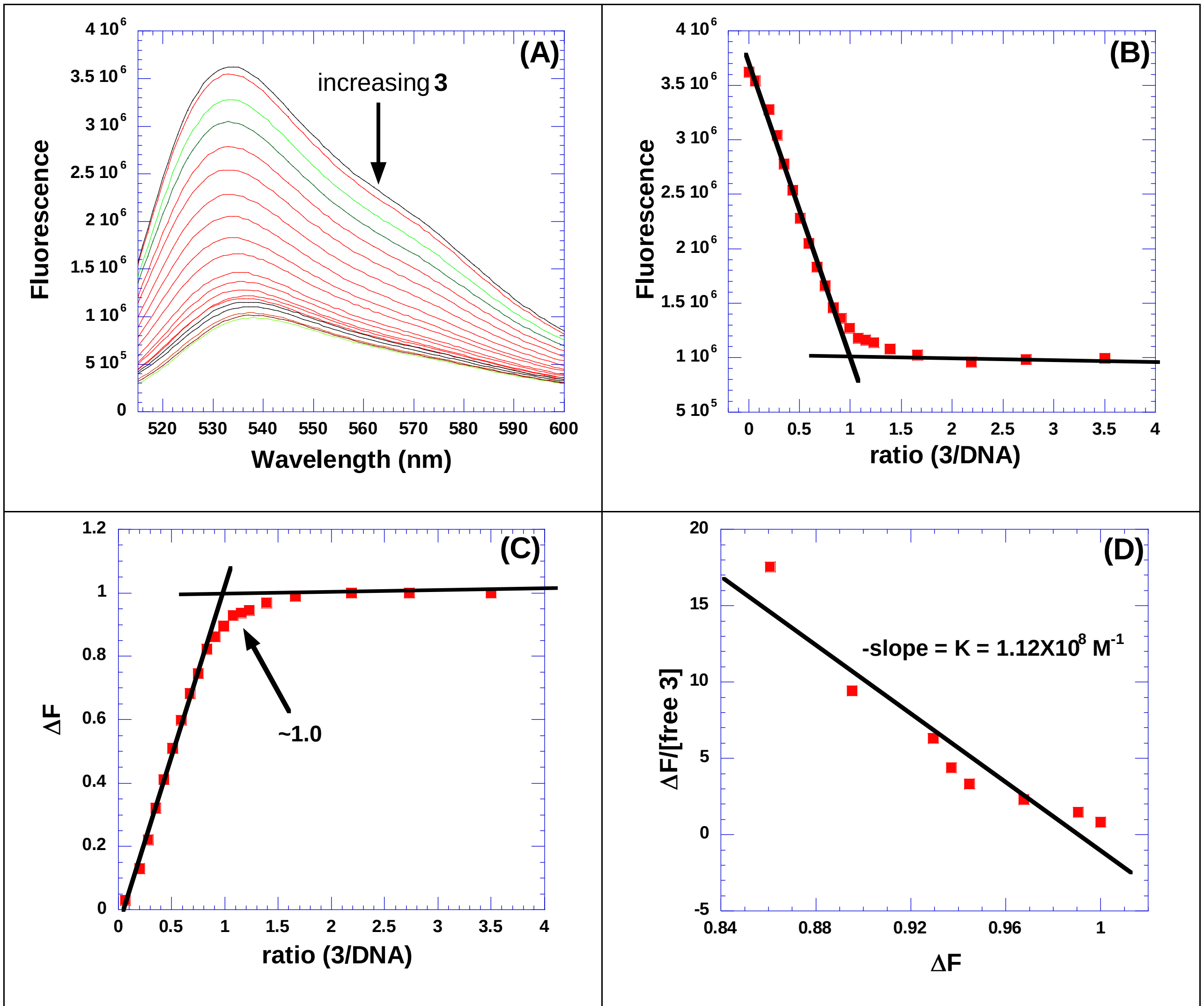


Figure S17. FID titration of **3** with intramolecular hairpin duplex d[5'-A_{12}-x-T_{12}-3']. (A) Raw fluorescence emission spectrum in the presence of increasing concentration of **3**. (B) The decrease of fluorescence intensity (at 532 nm) of DNA-TO complex with increasing concentration of **3**. (C) A plot of fraction of TO displaced versus increase in the concentration of **3**. (D) Scatchard analysis of **3** with duplex d[5'-A_{12}-x-T_{12}-3']. The solution was incubated for 1 h at 15 °C before titrating it with **3**. The solutions were equilibrated for 5 min. before taking the fluorescence emission scans. Buffer conditions: 100 mM KCl, 10 mM SC, 0.5 mM EDTA, pH 6.8. d[5'-A_{12}-x-T_{12}-3'] = 1 μM/strand, [TO] = 6 μM.

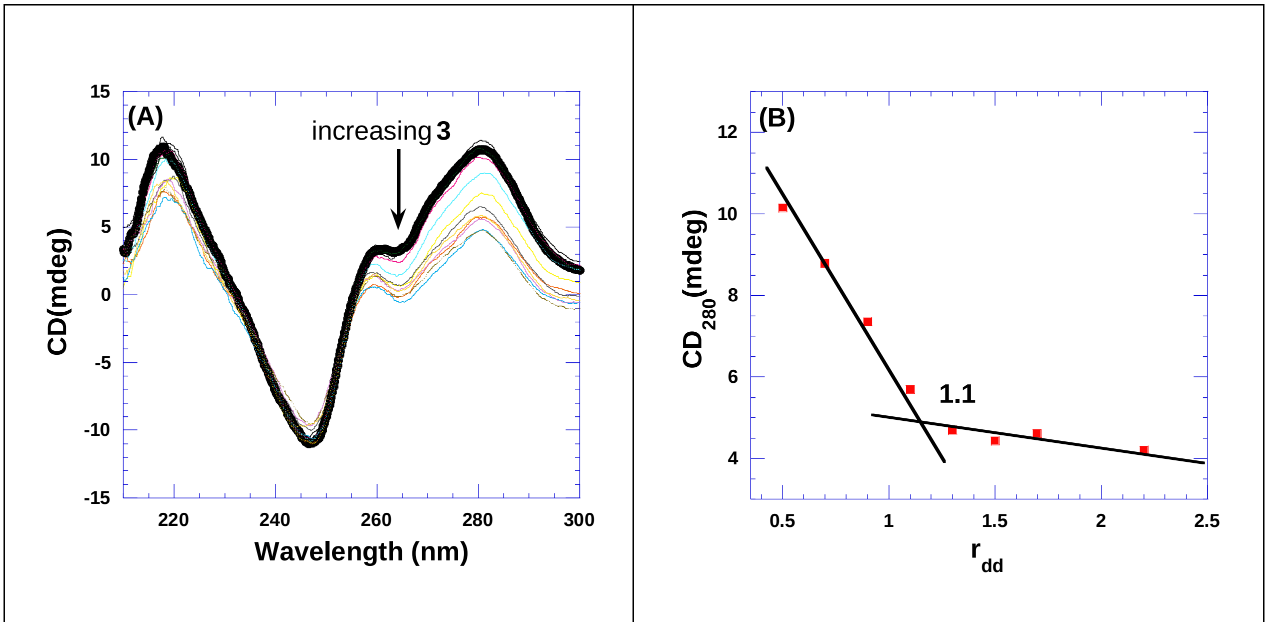


Figure S18. (A) CD titration of d[5'-A_{12}-x-T_{12}-3'] with increasing concentration of **3**, ranging from a ratio (**3**/DNA) of 0 to 2.5. (B) A plot of normalized molar ellipticity versus r_{dd} (ratio of drug to DNA duplex). Molar ellipticity is per molar DNA duplex and r_{dd} = ratio of the drug/DNA duplex. Buffer condition: 100 mM KCl, 10 mM sodium cacodylate, 0.5 mM EDTA, pH 6.8. T = 25 °C. [DNA] = 4 μM/duplex.

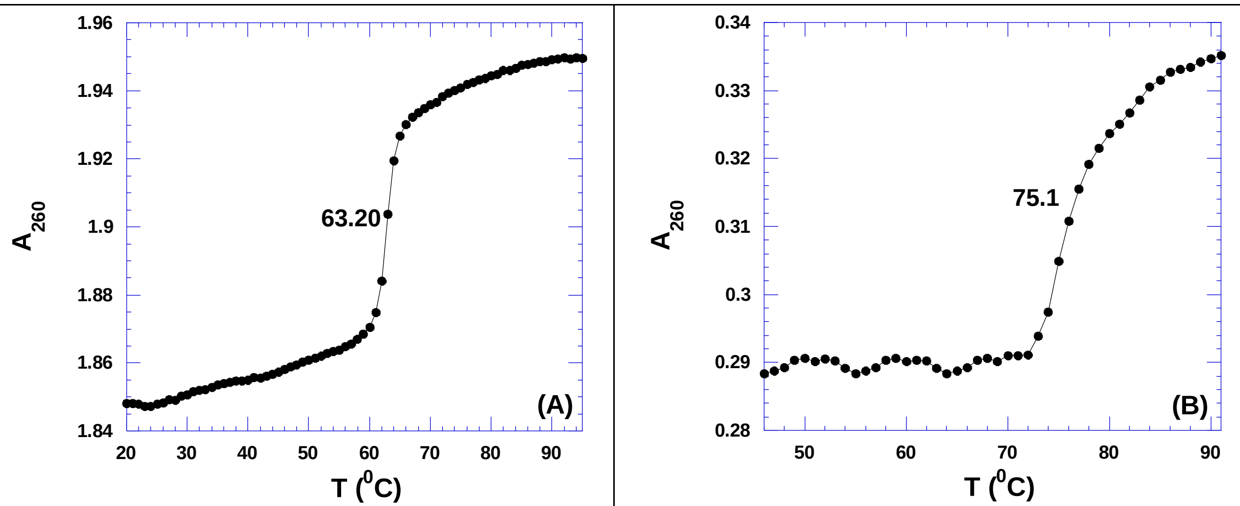


Figure S19. UV thermal denaturation profile of poly(dA.dT)$_2$ in the absence (A) and presence (B) of **3** (1.5 μM, r_{bd} = 10). Buffer conditions: 100 mM KCl, 10 mM SC, 0.5 mM EDTA, pH 6.8. [DNA] = 15 μM/bp.

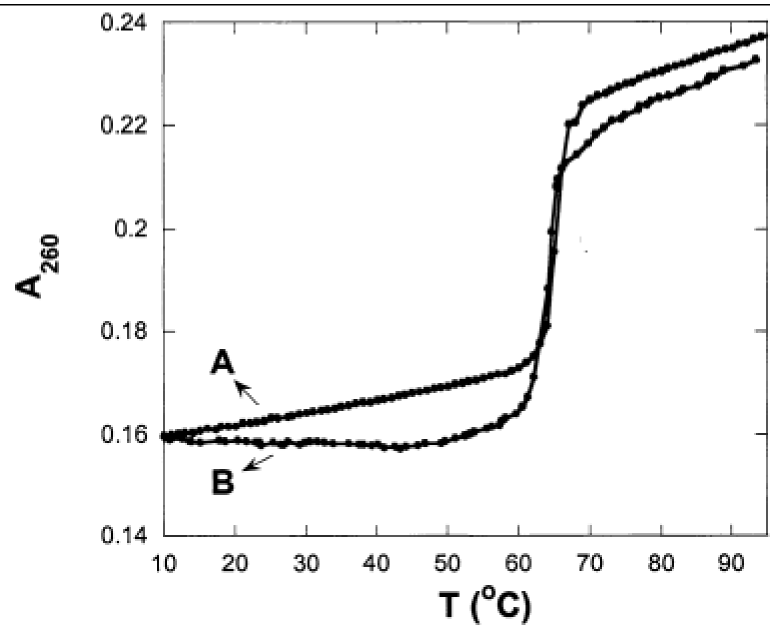
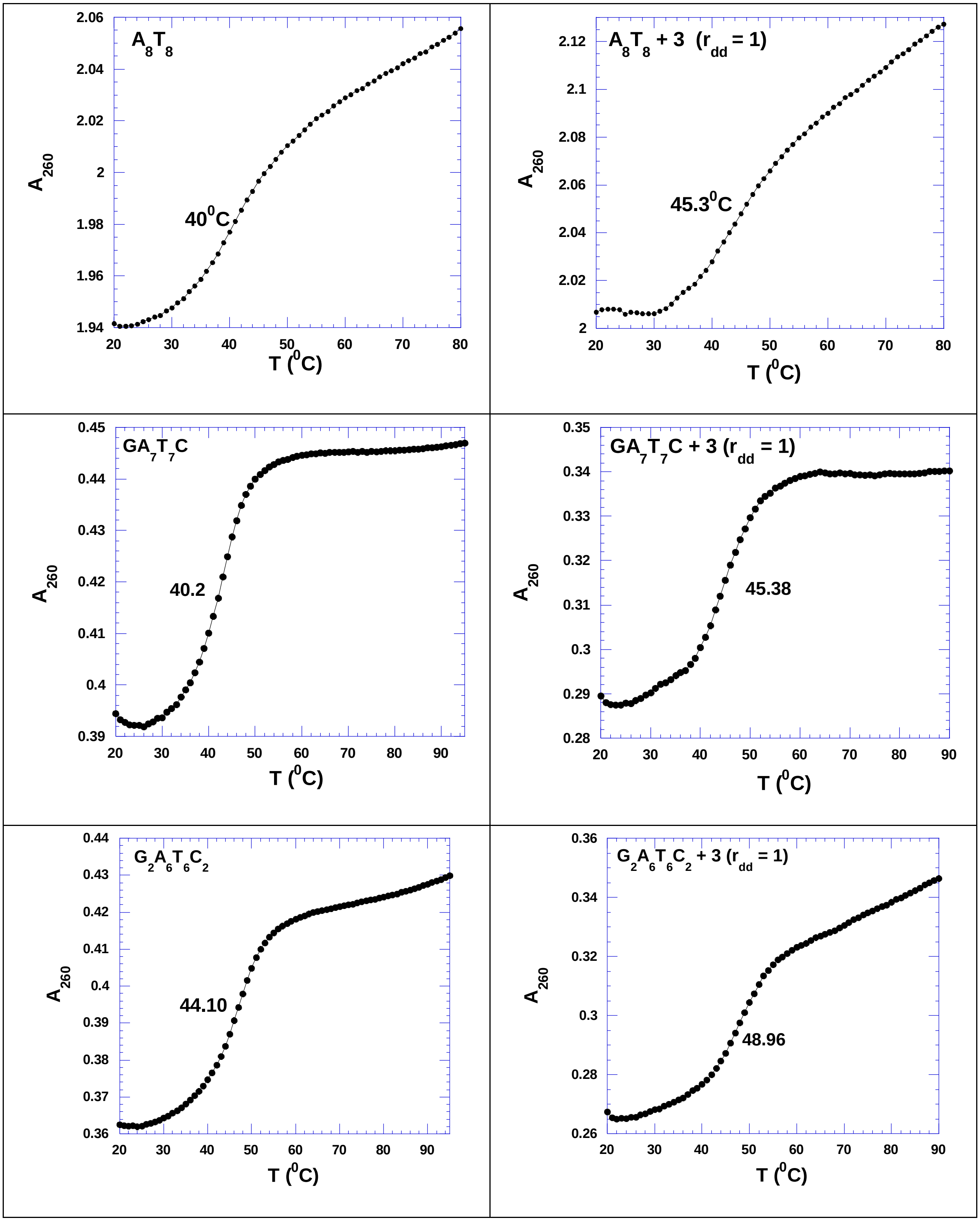


Figure S20. UV thermal denaturation profile of poly(dA.dT)$_2$ in the absence (A) and presence (B) of neomycin (1.5 μM, r_{bd} = 10). Buffer conditions: 100 mM KCl, 10 mM SC, 0.5 mM EDTA, pH 6.8. [DNA] = 15 μM / bp.


A8T8
40°C
A260
T (°C)
A8T8 + 3 (rdd = 1)
45.3°C
GA7T7C
40.2
GA7T7C + 3 (rdd = 1)
45.38
G2A6T6C2
44.10
G2A6T6C2 + 3 (rdd = 1)
48.96

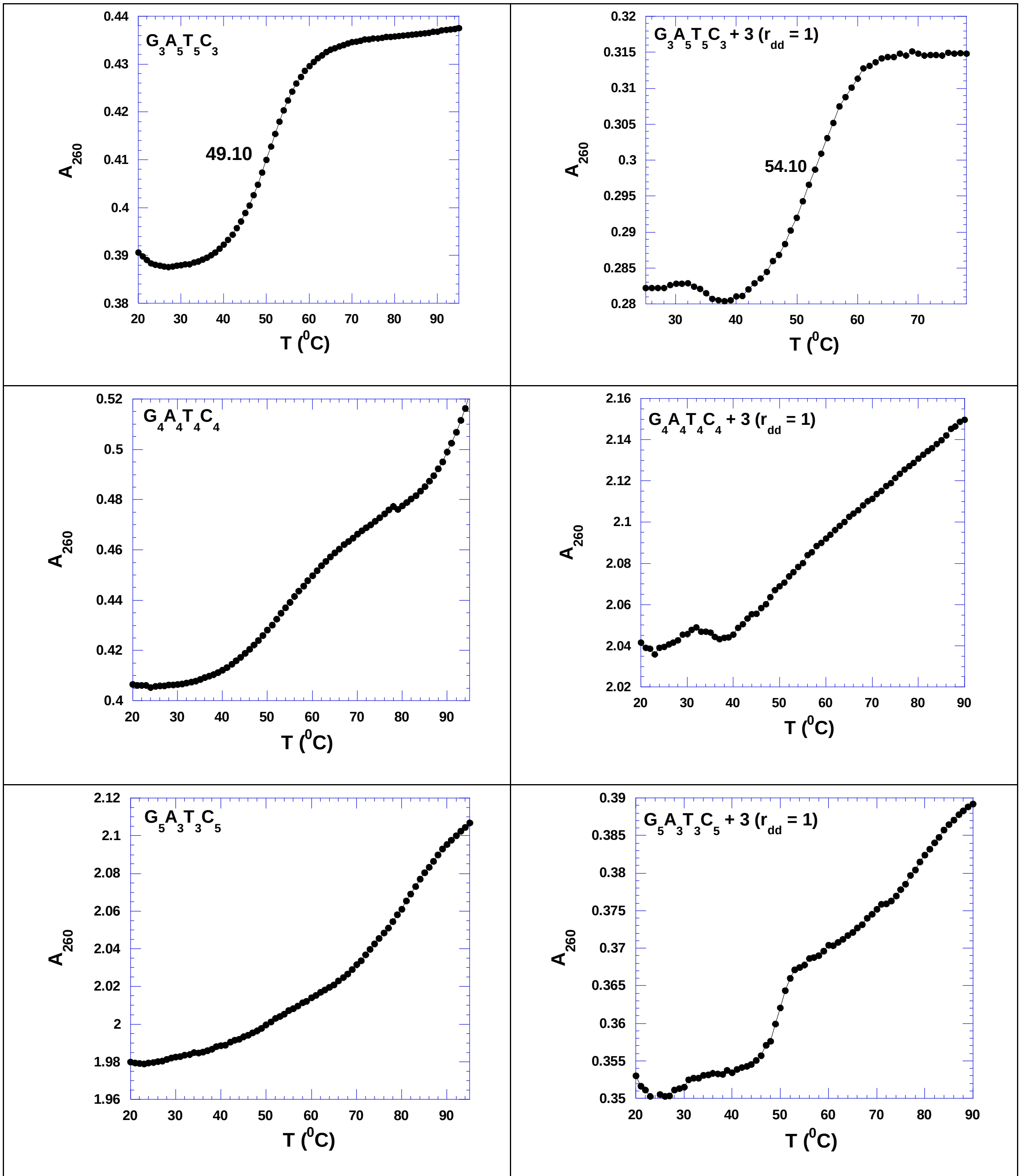


Figure S21. UV thermal denaturation profiles of different deoxyoligonucleotides in the absence (left) and presence (right) of **3** at r_{dd} = 1 (r_{dd} = ratio of drug to DNA duplex) at 260 nm. Buffer conditions: 100 mM KCl, 10 mM SC, 0.5 mM EDTA, pH 6.8. [DNA] = 1 μM/duplex.

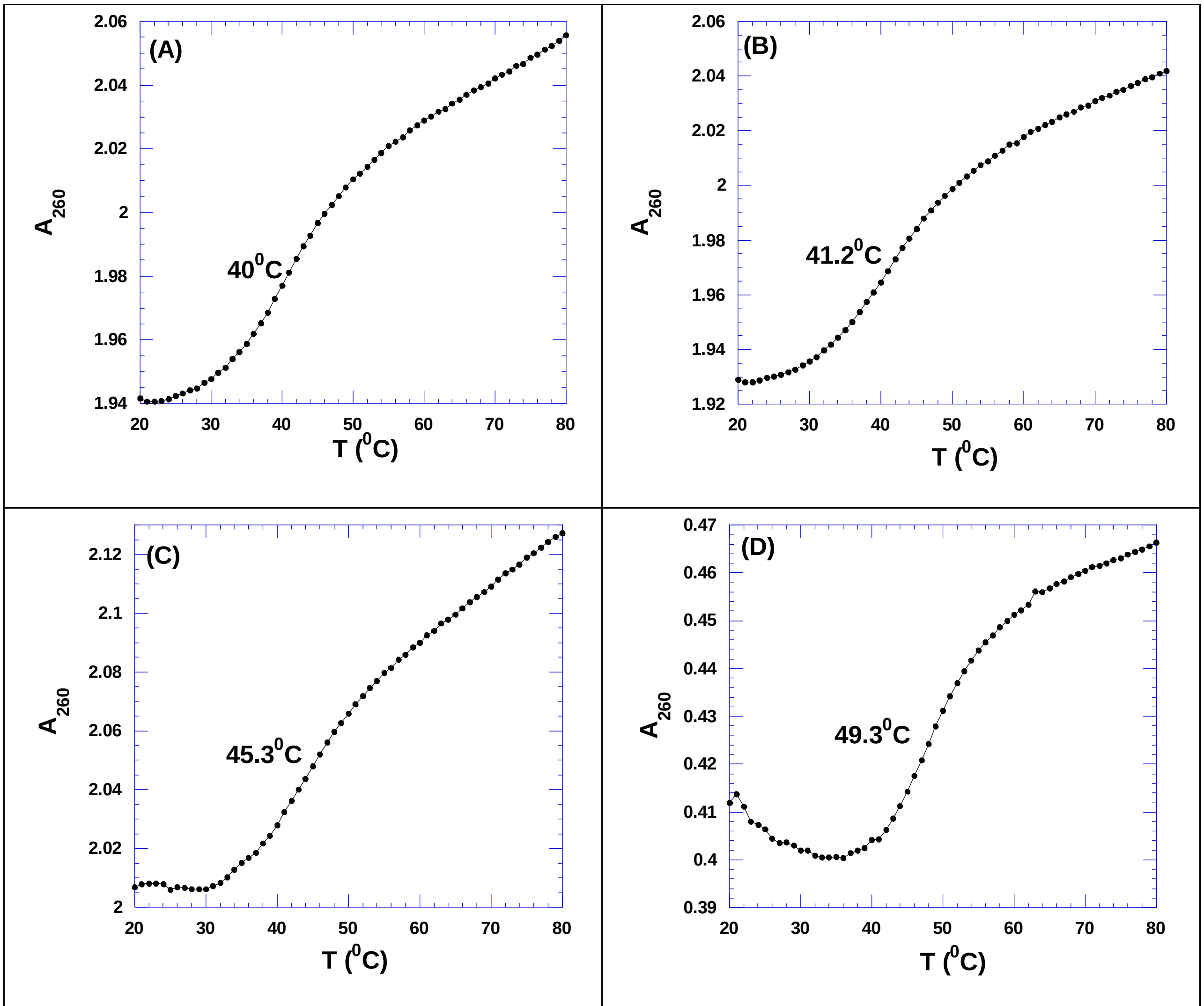
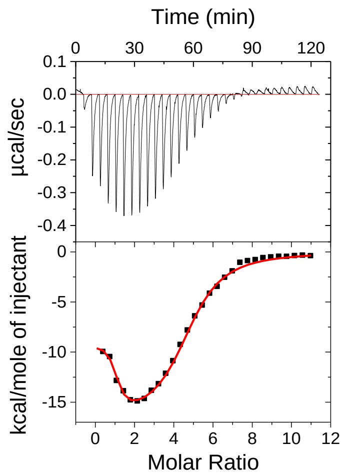


Figure S22. UV thermal denaturation profiles of d[5'-A_8T_8-3'] in the absence (A) and presence of **3** at r_{dd} ratio (rdd = ratio of drug to DNA duplex) of (A) 0.0, (B) 0.5 (0.5 µM), (C) 1.0 (1.0 µM), and (D) 2 (2.0 µM). Buffer conditions: 100 mM KCl, 10 mM SC, 0.5 mM EDTA, pH 6.8.

(A)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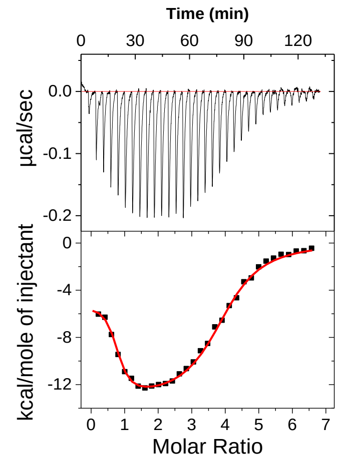
µcal/sec
0.1 0.0 -0.1 -0.2 -0.3 -0.4
kcal/mole of injectant
0 -5 -10 -15
0 2 4 6 8 10 12
Molar Ratio

(B)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µcal/sec
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kcal/mole of injectant
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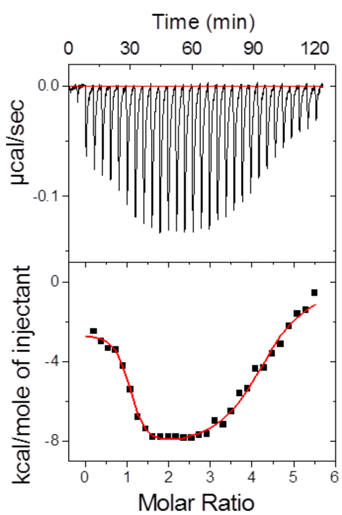
Molar Ratio

(C)


Time (min)
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µcal/sec
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kcal/mole of injectant
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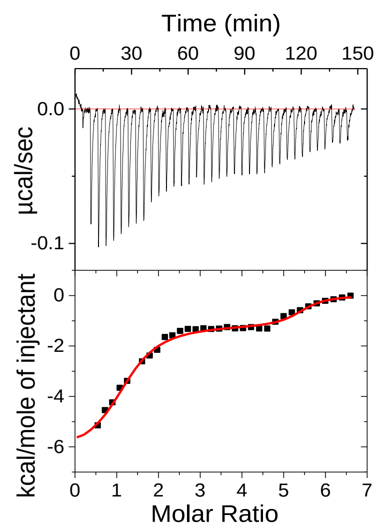
0 1 2 3 4 5 6
Molar Ratio

(D)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kcal/mole of injectant
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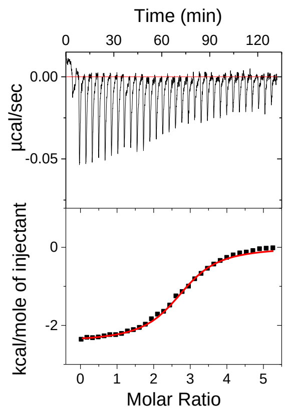
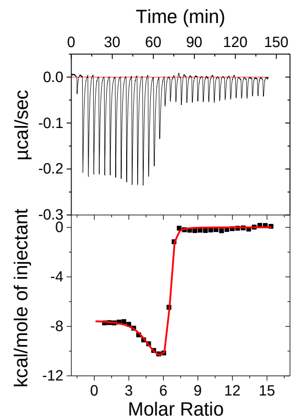
Molar Ratio

(E)



(F)



(G)

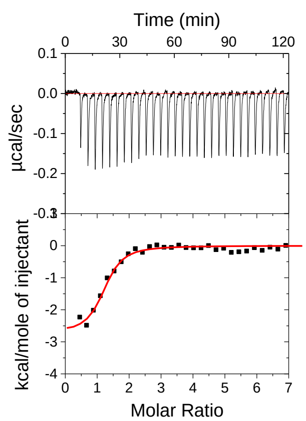
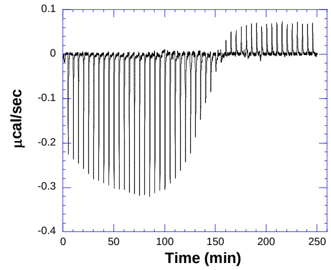
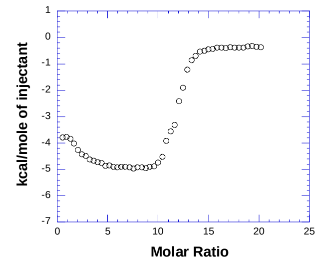


Figure S23. ITC profiles of **3** with (A) d[5'-$(AT)_8$-3'], (B) d[5'-$G_2A_6T_6C_2$-3'], (C) d[5'-$G_3A_5T_5C_3$-3'], (D) d[5'-A_{12}-x-T_{12}-3'], (E) d[5'- $A_3G_5C_5T_3$-3'], (F) d[5'-$A_{30}T_{30}$-3'], and (G) d[5'-$G_3(AT)_5C_3$-3']. Top Panel: ITC titrations represent the heat burst curves and each heat burst curve is a result of 9 µL injection of 125 µM of **3**, into the duplex DNA. The area under each heat burst curve was calculated by integration and yields the associated injection heats which were then plotted as a function of molar ratio of drug to DNA in the lower panel in each figure.. The corrected injection heats were derived by integration of the ITC profiles, followed by subtraction of the corresponding dilution heats obtained from titration of ligand into buffer. Buffer conditions: 100 mM KCl, 10 mM SC, 0.5 mM EDTA, pH 6.8. T = 25°C. [DNA] = 4 µM/duplex. [**3**] = 125 µM. d[5'-$A_{30}T_{30}$-3'] = 2 µM/duplex

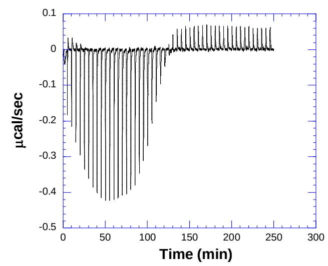
(A)



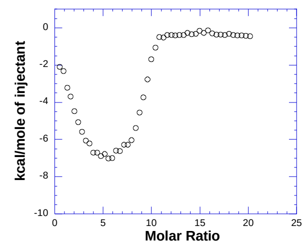
(B)



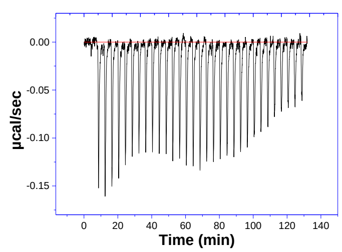
(C)



(D)



(E)



(F)

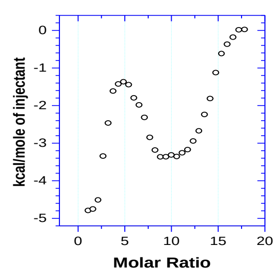


Figure S24. (A) ITC profile of sequence D8 (4μM in strand) titrated with **3** (440 μM) in 150 mM KCl, 10 mM SC, 0.5 mM EDTA, pH 6.8. T = 25 °C. (B) Corrected injection heats plotted as a function of the [drug]/[sequence D8] ratio. (C) ITC profile of sequence D7 (4μM in strand) titrated with **3** (440 μM) in 150 mM KCl, 10 mM SC, 0.5 mM EDTA, pH 6.8, T = 25 °C. (D) Corrected injection heats plotted as a function of the [drug]/[sequence D7] ratio. (E) ITC profile of sequence D10 (4μM in strand) titrated with **3** (440 μM) in 150 mM KCl, 10 mM SC, 0.5 mM EDTA, pH 6.8, T = 25 °C. (F) Corrected injection heats plotted as a function of the [drug]/[sequence D10] ratio. The corrected injection heats were derived by integration of the ITC profiles, followed by subtraction of the corresponding dilution heats derived from titration of ligand into buffer.

Sequence D8:

5’-AAGAGGAGAGAAGAGAGGAGAA-3’

3’-TTCTCCTCTCTTCTCTCCTCTT-5’

Sequence D7:

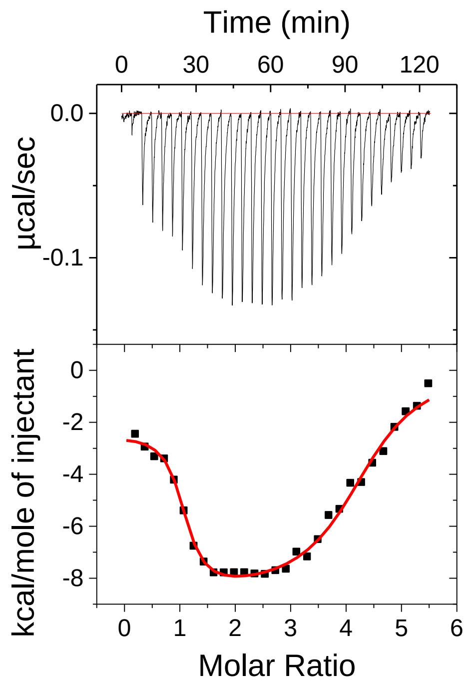
5’-AAGGGAAAAAAAAAAAAGGGAA-3’

3’-TTCCCTTTTTTTTTTTTCCCTT-5’

Sequence D10:

5’-AAGGGATATATATATATGGGAA-3’

3’-TTCCCTATATATATATACCCTT-5’



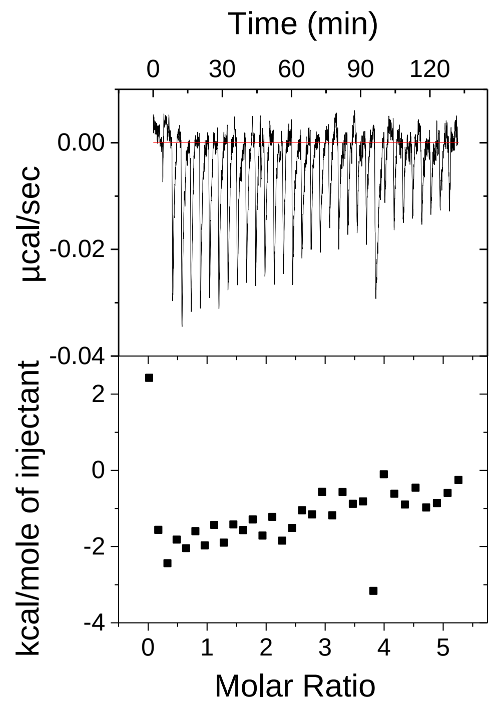


Figure S25. ITC-Derived thermodynamic profiles for the binding of **3** (left) and neomycin (right) with d[5'-$G_3A_5T_5C_3$-3']. Top Panel: ITC titration represents the heat burst curves and each heat burst curve is a result of 9 µL injections of 125 µM of **3** (left) and neomycin (right). The area under each heat burst curve was calculated by integration and yields the associated injection heats which are plotted as a function of molar ratio of drug to DNA in the lower panel in each figure. Bottom panel: Corrected injection heats plotted as a function of the [drug]/DNA ratio. The corrected injection heats were derived by integration of the ITC profiles, followed by subtraction of the corresponding dilution heats derived from titration of ligand into buffer. Buffer conditions: 100 mM KCl, 10 mM SC, 0.5 mM EDTA, pH 6.8. T = 25^{o}C. [DNA] = 4 µM/duplex. [**3**] = 125 µM. [neomycin] = 125 µM. As opposed to dimer **3**, neomycin shows very weak binding as evident from the differences in heat evolved.

Table S2. Thiazole orange FID Assay for 512-Member Deoxyoligonucleotide Library. The 12 base-pair duplex has 10 variable AT base pairs flanked by two conserved CG base pairs. Sequence 1 corresponds to the sequence with little displacement of TO upon binding of **3**, suggesting it is the weakest binder of all. Sequence 512 showed the maximum displacement of TO upon binding of **3**. Buffer conditions: 100 mM KCl, 10 mM SC, 0.5 mM EDTA, pH 6.8. [DNA] = 1 μM/strand. [**3**] = 1 μM.

S.No.	Sequence name	% Fluorescence
1	5'-CATATTTATATCAAAAAGATATAAATATG-3'	99.852
2	5'-CATATATATATCAAAAAGATATATATATG-3'	99.731
3	5'-CATAAATAAATCAAAAAGATTTATTTATG-3'	99.718
4	5'-CATATAATATTCAAAAAGAATATTATATG-3'	99.62
5	5'-CATAATTAATTCAAAAAGAATTAATTATG-3'	99.509
6	5'-CATTTTAATTTCAAAAAGAAATTAAAATG-3'	99.458
7	5'-CATATATATTTCAAAAAGAAATATATATG-3'	99.257
8	5'-CATATTTTAATCAAAAAGATTAAAATATG-3'	99.05
9	5'-CATAAAATATTCAAAAAGAATATTTTATG-3'	98.946
10	5'-CATATATTTATCAAAAAGATAAATATATG-3'	98.783
11	5'-CATATAAAAATCAAAAAGATTTTTATATG-3'	98.718
12	5'-CATAATAAAATCAAAAAGATTTTATTATG-3'	98.685
13	5'-CATTAAATATTCAAAAAGAATATTTAATG-3'	98.368
14	5'-CATTATAAAATCAAAAAGATTTTATAATG-3'	98.081
15	5'-CAAAATATTAACAAAAAGTTAATATTTTG-3'	98.076
16	5'-CATAAAATTATCAAAAAGATAATTTTATG-3'	98.068
17	5'-CATAATATTATCAAAAAGATAATATTATG-3'	97.968
18	5'-CATAAATTTTTCAAAAAGAAAAATTTATG-3'	97.82

19	5'-CATAAAATTTTCAAAAAGAAAATTTTATG-3’	97.447
20	5'-CATTATAAATTCAAAAAGAATTTATAATG-3’	97.395
21	5'-CATTATTTTTTCAAAAAGAAAAAATAATG-3’	97.315
22	5'-CATTAAAATTTCAAAAAGAAATTTTAATG-3’	97.17
23	5'-CATAATATATTCAAAAAGAATATATTATG-3’	96.986
24	5'-CATTAATTTATCAAAAAGATAAATTAATG-3’	96.652
25	5'-CATAAATAAAACAAAAAGTTTTATTTATG-3’	96.54
26	5'-CATATTAAAATCAAAAAGATTTTAATATG-3’	96.47
27	5'-CATATAATTTTCAAAAAGAAAATTATATG-3’	96.331
28	5'-CATTATAATATCAAAAAGATATTATAATG-3’	96.298
29	5'-CATTATATTTTCAAAAAGAAAATATAATG-3’	96.137
30	5'-CATTAATATTTCAAAAAGAAATATTAATG-3’	96.011
31	5'-CATAAAAATATCAAAAAGATATTTTTATG-3’	95.996
32	5'-CATATTTAATTCAAAAAGAATTAAATATG-3’	95.753
33	5'-CATATTTAAATCAAAAAGATTTAAATATG-3’	95.751
34	5'-CATATTAAATTCAAAAAGAATTTAATATG-3’	95.677
35	5'-CATAAATATATCAAAAAGATATATTTATG-3’	95.525
36	5'-CATATTATATTCAAAAAGAATATAATATG-3’	95.412
37	5'-CATTTAAAAAACAAAAAGTTTTTTAAATG-3’	95.396
38	5'-CATAATTTAATCAAAAAGTAATTTAATAG-3’	95.32
39	5'-CATAAATTAATCAAAAAGATTAATTTATG-3’	95.05
40	5'-CATATAAATATCAAAAAGATATTTATATG-3’	94.939
41	5'-CATTATATAATCAAAAAGATTATATAATG-3’	94.811

42	5'-CATTATTAAATCAAAAAGATTTAATAATG-3’	94.751
43	5'-CATATATTTTTCAAAAAGAAAAATATATG-3’	94.627
44	5'-CATAATTAAATCAAAAAGATTTAATTATG-3’	94.571
45	5'-CAATAAAATATCAAAAAGATATTTTATTG-3'	94.55
46	5'-CATAAAATAATCAAAAAGATTATTTTATG-3’	94.539
47	5'-CATTAAAATATCAAAAAGATATTTTAATG-3’	94.489
48	5'-CATAATAATATCAAAAAGATATTATTATG-3’	94.409
49	5'-CATATAATAATCAAAAAGATTATTATATG-3’	94.284
50	5'-CATATATAATACAAAAAGTATTATATATG-3’	94.208
51	5'-CATTATATTATCAAAAAGATAATATAATG-3’	94.111
52	5'-CATTAATAATTCAAAAAGAATTATTAATG-3’	93.997
53	5'-CATATTATAATCAAAAAGATTATAATATG-3’	93.895
54	5'-CATATATATTACAAAAAGTAATATATATG-3’	93.872
55	5'-CAATAAAAATTCAAAAAGAATTTTTATTG-3’	93.743
56	5'-CATAATTATATCAAAAAGATATAATTATG-3’	93.735
57	5'-CATTATTTAATCAAAAAGATTAAATAATG-3’	93.525
58	5'-CATAATTTTATCAAAAAGATAAAATTATG-3’	93.505
59	5'-CATAAATTTATCAAAAAGATAAATTTATG-3’	93.158
60	5'-CAAAAATAATACAAAAAGTATTATTTTTG-3’	93.146
61	5'-CATTAATTATTCAAAAAGAATAATTAATG-3’	93.118
62	5'-CAAATTTTAATCAAAAAGATTAAAATTTG-3’	93.03
63	5'-CATAATAAATTCAAAAAGAATTTATTATG-3’	92.746
64	5'-CATAAAAATTTCAAAAAGAAATTTTTATG-3’	92.653

65	5'-CAATAAAATAACAAAAAGTTATTTTATTG-3'	92.45
66	5'-CATAATATAATCAAAAAGATTATATTATG-3’	92.404
67	5'-CATTATATATTCAAAAAGAATATATAATG-3’	92.335
68	5'-CATTATTTATTCAAAAAGAATAAATAATG-3’	92.26
69	5'-CATATATTATTCAAAAAGAATAATATATG-3’	92.049
70	5'-CATAAATAATTCAAAAAGAATTATTTATG-3’	91.864
71	5'-CAAAATTTAAACAAAAAGTTTAAATTTTG-3'	91.8
72	5'-CATATTAATTTCAAAAAGAAATTAATATG-3’	91.728
73	5'-CAAATAAAAAACAAAAAGTTTTTTATTTG-3'	91.56
74	5'-CATATAAAATTCAAAAAGAATTTTATATG-3’	91.49
75	5'-CATTAAATTATCAAAAAGATAATTTAATG-3’	91.3
76	5'-CATATTTTATTCAAAAAGAATAAAATATG-3’	91.165
77	5'-CATATATTAATCAAAAAGATTAATATATG-3’	91.14
78	5'-CATTAATAAATCAAAAAGATTTATTAATG-3’	91.12
79	5'-CATTATTATATCAAAAAGATATAATAATG-3’	90.951
80	5'-CATTAATTTTTCAAAAAGAAAAATTAATG-3’	90.882
81	5'-CATATATAAATCAAAAAGATTTATATATG-3’	90.782
82	5'-CATTATAATTTCAAAAAGAAATTATAATG-3’	90.66
83	5'-CATATAAATTTCAAAAAGAAATTTATATG-3’	90.622
84	5'-CATTAAATTTTCAAAAAGAAAATTTAATG-3’	90.601
85	5'-CATATTATTATCAAAAAGATAATAATATG-3’	90.543
86	5'-CATAATTTTTTCAAAAAGAAAAAATTATG-3’	90.354
87	5'-CATATTATTTTCAAAAAGAAAATAATATG-3’	90.318

88	5'-CATATTAATATCAAAAAGATATTAATATG-3’	90.313
89	5'-CATATATAATTCAAAAAGAATTATATATG-3’	90.299
90	5'-CATAATATTTTCAAAAAGAAAATATTATG-3’	90.161
91	5'-CATATTTTTATCAAAAAGATAAAAATATG-3’	90.157
92	5'-CATTAAATAATCAAAAAGATTATTTAATG-3’	89.813
93	5'-CATATAATTATCAAAAAGATAATTATATG-3’	89.748
94	5'-CATAAATTATTCAAAAAGAATAATTTATG-3’	89.631
95	5'-CATTAATATATCAAAAAGATATATTAATG-3’	89.472
96	5'-CATATTTATTTCAAAAAGAAATAAATATG-3’	89.44
97	5'-CATTAAAAAATCAAAAAGATTTTTTAATG-3’	89.026
98	5'-CAATATATAATCAAAAAGATTATATATTG-3’	89.005
99	5'-CAATATTTAATCAAAAAGATTAAATATTG-3’	88.948
100	5'-CATTTAATAAACAAAAAGTTTATTAAATG-3’	88.881
101	5'-CATAAATATTTCAAAAAGAAATATTTATG-3’	88.819
102	5'-CATTAATTAATCAAAAAGATTAATTAATG-3’	88.715
103	5'-CAATAATATAACAAAAAGTTATATTATTG-3’	88.654
104	5'-CATTTATTATTCAAAAAGAATAATAAATG-3’	88.478
105	5'-CATATATATAACAAAAAGTTATATATATG-3’	88.344
106	5'-CAAATAAATTTCAAAAAGAAATTTATTTG-3’	88.257
107	5'-CATAATTTATTCAAAAAGAATAAATTATG-3’	88.247
108	5'-CAAATATATATCAAAAAGATATATATTTG-3’	88.23
109	5'-CATTTATATATCAAAAAGATATATAAATG-3’	88.087
110	5'-CATATTTTTTTCAAAAAGAAAAAAATATG-3’	88.077

111	5'-CAAAATTTAATCAAAAAGATTAAATTTTG-3'	87.9
112	5'-CATTAAAAATTCAAAAAGAATTTTTAATG-3’	87.879
113	5'-CAAAAATATAACAAAAAGTTATATTTTTG-3’	87.837
114	5'-CAATATATATACAAAAAGTATATATATTG-3’	87.82
115	5'-CAAAAAAAATACAAAAAGTATTTTTTTTG-3’	87.697
116	5'-CATTATTATTTCAAAAAGAAATAATAATG-3’	87.493
117	5'-CAAATAAAATACAAAAAGTATTTTATTTG-3’	87.486
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189	5'-CATTTTATAATCAAAAAGATTATAAAATG-3’	82.647
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192	5'-CAATTATTAATCAAAAAGATTAATAATTG-3’	82.409
193	5'-CAATTTATTAACAAAAAGTTAATAAATTG-3’	82.371
194	5'-CAAAAATAAAACAAAAAGTTTTATTTTTG-3’	82.285
195	5'-CAATATATATTCAAAAAGAATATATATTG-3’	82.281
196	5'-CATTAATTTAACAAAAAGTTAAATTAATG-3’	82.258
197	5'-CAATTTTAAAACAAAAAGTTTTAAAATTG-3’	82.214
198	5'-CAAATTATAATCAAAAAGATTATAATTTG-3’	82.127
199	5'-CAAATATATTACAAAAAGTAATATATTTG-3’	82.07
200	5'-CAATTATATTACAAAAAGTAATATAATTG-3’	81.994
201	5'-CATATATAAAACAAAAAGTTTTATATATG-3’	81.856
202	5'-CATTATTTTTACAAAAAGTAAAAATAATG-3’	81.779

203	5'-CATTTAAAATACAAAAAGTATTTTAAATG-3’	81.71
204	5'-CATTAATAAAACAAAAAGTTTTATTAATG-3’	81.7
205	5'-CATAAAATTAACAAAAAGTTAATTTTATG-3’	81.696
206	5'-CAATTTATAATCAAAAAGATTATAAATTG-3’	81.651
207	5'-CATATATTAAACAAAAAGTTTAATATATG-3’	81.556
208	5'-CAATTAATAATCAAAAAGATTATTAATTG-3’	81.518
209	5'-CAATATTATATCAAAAAGATATAATATTG-3’	81.464
210	5'-CATAAAATAAACAAAAAGTTTATTTTATG-3’	81.448
211	5'-CATTAAATTTACAAAAAGTAAATTTAATG-3’	81.315
212	5'-CATTTTTTATACAAAAAGTATAAAAAATG-3’	81.23
213	5'-CAATAATTTAACAAAAAGTTAAATTATTG-3’	81.208
214	5'-CATTAAAATTACAAAAAGTAATTTTAATG-3’	81.201
215	5'-CAATTAATTATCAAAAAGATAATTAATTG-3’	81.173
216	5'-CAATAATTATTCAAAAAGAATAATTATTG-3’	81.166
217	5'-CAATTAATATACAAAAAGTATATTAATTG-3’	81.141
218	5'-CAAATAATTATCAAAAAGATAATTATTTG-3’	81.052
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222	5'-CAATATATTTACAAAAAGTAAATATATTG-3’	81.011
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224	5'-CAAAAAATTTACAAAAAGTAAATTTTTTG-3’	80.916
225	5'-CATATTATATACAAAAAGTATATAATATG-3’	80.914

226	5'-CAATTTTTATTCAAAAAGAATAAAAATTG-3’	80.899
227	5'-CAATAAATTAACAAAAAGTTAATTTATTG-3’	80.886
228	5'-CAATTAAATATCAAAAAGATATTTAATTG-3’	80.844
229	5'-CAATTTTATAACAAAAAGTTATAAAATTG-3’	80.813
230	5'-CATTTTATTTTCAAAAAGAAAATAAAATG-3’	80.812
231	5'-CAATTTAATAACAAAAAGTTATTAAATTG-3’	80.807
232	5'-CAATTAAAAATCAAAAAGATTTTTAATTG-3’	80.63
233	5'-CAAAATTATATCAAAAAGATATAATTTTG-3’	80.625
234	5'-CATTTATAATACAAAAAGTATTATAAATG-3’	80.543
235	5'-CAATATAAATACAAAAAGTATTTATATTG-3’	80.485
236	5'-CAATTATATATCAAAAAGATATATAATTG-3’	80.456
237	5'-CAAATTTTATACAAAAAGTATAAAATTTG-3’	80.351
238	5'-CAATATTAAATCAAAAAGATTTAATATTG-3’	80.293
239	5'-CAAAATAATAACAAAAAGTTATTATTTTG-3’	80.286
240	5'-CATTTTTAAATCAAAAAGATTTAAAAATG-3’	80.22
241	5'-CATAAAAATTACAAAAAGTAATTTTTATG-3’	80.205
242	5'-CAATTAAAAAACAAAAAGTTTTTTAATTG-3’	80.181
243	5'-CAATTAATTTTCAAAAAGAAAATTAATTG-3’	80.181
244	5'-CATTAAAAATACAAAAAGTATTTTTAATG-3’	80.168
245	5'-CAATAAATATACAAAAAGTATATTTATTG-3’	80.164
246	5'-CATTAAAAAAACAAAAAGTTTTTTTAATG-3’	80.136
247	5'-CAATATTAAAACAAAAAGTTTTAATATTG-3’	80.109
248	5'-CAAAAATATATCAAAAAGATATATTTTTG-3’	79.997

249	5'-CATAATATAAACAAAAAGTTTATATTATG-3’	79.989
250	5'-CATAAAAAAATCAAAAAGATTTTTTTATG-3’	79.95
251	5'-CAATTTAAAATCAAAAAGATTTTAAATTG-3’	79.947
252	5'-CATATATTTAACAAAAAGTTAAATATATG-3’	79.901
253	5'-CATATTTTATACAAAAAGTATAAAATATG-3’	79.896
254	5'-CAAAATAATTACAAAAAGTAATTATTTTG-3’	79.889
255	5'-CATAATTTAAACAAAAAGTTTAAATTATG-3’	79.851
256	5'-CAATATTATAACAAAAAGTTATAATATTG-3’	79.836
257	5'-CAATAATTTTTCAAAAAGAAAAATTATTG-3’	79.793
258	5'-CAATAATTAAACAAAAAGTTTAATTATTG-3’	79.784
259	5'-CATTTTAAATTCAAAAAGAATTTAAAATG-3’	79.747
260	5'-CATATTTTAAACAAAAAGTTTAAAATATG-3’	79.726
261	5'-CAAATATTAAACAAAAAGTTTAATATTTG-3’	79.664
262	5'-CAATAAATATTCAAAAAGAATATTTATTG-3’	79.571
263	5'-CAATTTATTTACAAAAAGTAAATAAATTG-3’	79.473
264	5'-CATATTTAATACAAAAAGTATTAAATATG-3’	79.45
265	5'-CAAATTTTTATCAAAAAGATAAAAATTTG-3’	79.444
266	5'-CATAAAATATACAAAAAGTATATTTTATG-3’	79.4
267	5'-CATATTATAAACAAAAAGTTTATAATATG-3’	79.391
268	5'-CAAAAATTTATCAAAAAGATAAATTTTTG-3’	79.359
269	5'-CATTTATTAAACAAAAAGTTTAATAAATG-3’	79.35
270	5'-CAAATATTATTCAAAAAGAATAATATTTG-3’	79.324
271	5'-CAAATTAAATACAAAAAGTATTTAATTTG-3’	79.246

272	5'-CAATTAATTAACAAAAAGTTAATTAATTG-3’	79.236
273	5'-CAATAAATTTTCAAAAAGAAAATTTATTG-3’	79.219
274	5'-CATATATTATACAAAAAGTATAATATATG-3’	79.166
275	5'-CAATTATTATACAAAAAGTATAATAATTG-3’	79.146
276	5'-CAATTATAATACAAAAAGTATTATAATTG-3’	79.144
277	5'-CAAATATTTAACAAAAAGTTAAATATTTG-3’	79.14
278	5'-CAAATTAATTTCAAAAAGAAATTAATTTG-3’	79.067
279	5'-CATATTTAAAACAAAAAGTTTTAAATATG-3’	79.045
280	5'-CATATTTATTACAAAAAGTAATAAATATG-3’	79.013
281	5'-CATTTTTATTTCAAAAAGAAATAAAAATG-3’	78.987
282	5'-CAAAAAATTATCAAAAAGATAATTTTTTG-3’	78.954
283	5'-CATAATTTTAACAAAAAGTTAAAATTATG-3’	78.944
284	5'-CAATATTTAAACAAAAAGTTTAAATATTG-3’	78.931
285	5'-CAATTTTAATACAAAAAGTATTAAAATTG-3’	78.877
286	5'-CAATAATATTTCAAAAAGAAATATTATTG-3’	78.798
287	5'-CATAAAAATAACAAAAAGTTATTTTTATG-3’	78.683
288	5'-CATTAAATAAACAAAAAGTTTATTTAATG-3’	78.678
289	5'-CATAATAATAACAAAAAGTTATTATTATG-3’	78.667
290	5'-CAATATTTTAACAAAAAGTTAAAATATTG-3’	78.658
291	5'-CAAAAATTTTACAAAAAGTAAAATTTTTG-3’	78.543
292	5'-CATTAAATATACAAAAAGTATATTTAATG-3’	78.533
293	5'-CATATTTTTAACAAAAAGTTAAAAATATG-3’	78.49
294	5'-CAATTTATATACAAAAAGTATATAAATTG-3’	78.472

295	5'-CATTAAAATAACAAAAAGTTATTTTAATG-3’	78.4
296	5'-CAATAAATTATCAAAAAGATAATTTATTG-3’	78.356
297	5'-CAAATTATTTTCAAAAAGAAAATAATTTG-3’	78.306
298	5'-CATTATATTTACAAAAAGTAAATATAATG-3’	78.257
299	5'-CAATAAATAATCAAAAAGATTATTTATTG-3’	78.25
300	5'-CAAATTATAAACAAAAAGTTTATAATTTG-3’	78.249
301	5'-CAATTTAAATACAAAAAGTATTTAAATTG-3’	78.113
302	5'-CAAATTAAAAACAAAAAGTTTTTAATTTG-3’	78.069
303	5'-CATTAATAATACAAAAAGTATTATTAATG-3’	78.05
304	5'-CATATAAAATACAAAAAGTATTTTATATG-3’	78.006
305	5'-CATTATAATAACAAAAAGTTATTATAATG-3’	77.977
306	5'-CATTATATAAACAAAAAGTTTATATAATG-3’	77.946
307	5'-CAATTTATAAACAAAAAGTTTATAAATTG-3’	77.892
308	5'CAATTATTATTCAAAAAGAATAATAATTG-3’	77.872
309	5'-CAATTTAAATTCAAAAAGAATTTAAATTG-3’	77.829
310	5'-CAATTATTAAACAAAAAGTTTAATAATTG-3’	77.803
311	5'-CATATTTATAACAAAAAGTTATAAATATG-3’	77.768
312	5'-CAAATTTTAAACAAAAAGTTTAAAATTTG-3’	77.67
313	5'-CAATTTTATTACAAAAAGTAATAAAATTG-3’	77.646
314	5'-CATTATAAAAACAAAAAGTTTTTATAATG-3’	77.611
315	5'-CATAATTTATACAAAAAGTATAAATTATG-3’	77.558
316	5'-CATTTTTATAACAAAAAGTTATAAAAATG-3’	77.4
317	5'-CAAATTTATATCAAAAAGATATAAATTTG-3’	77.399

318	5'-CATTTATTTTTCAAAAAGAAAAATAAATG-3’	77.233
319	5'-CATAATAATTACAAAAAGTAATTATTATG-3’	77.173
320	5'-CAATTATAAAACAAAAAGTTTTATAATTG-3’	77.135
321	5'-CATAATTTTTACAAAAAGTAAAAATTATG-3’	77.088
322	5'-CAAAATAAATTCAAAAAGAATTTATTTTG-3’	76.995
323	5'-CAATAATTAATCAAAAAGATTAATTATTG-3’	76.995
324	5'-CATTTATAATTCAAAAAGAATTATAAATG-3’	76.994
325	5'-CATTATTATAACAAAAAGTTATAATAATG-3’	76.985
326	5'-CATTTTTTATTCAAAAAGAATAAAAAATG-3’	76.982
327	5'-CAATATAAAAACAAAAAGTTTTTATATTG-3’	76.96
328	5'-CATTAAATTAACAAAAAGTTAATTTAATG-3’	76.934
329	5'-CAAATTTTATTCAAAAAGAATAAAATTTG-3’	76.865
330	5'-CATTAATATTACAAAAAGTAATATTAATG-3’	76.827
331	5'-CATTAATTAAACAAAAAGTTTAATTAATG-3’	76.794
332	5'-CAATATAATTTCAAAAAGAAATTATATTG-3’	76.745
333	5'-CAAATAATTAACAAAAAGTTAATTATTTG-3’	76.725
334	5'-CAAAATTAATACAAAAAGTATTAATTTTG-3’	76.711
335	5'-CATTTATTATACAAAAAGTATAATAAATG-3’	76.623
336	5'-CAAAATATATTCAAAAAGAATATATTTTG-3’	76.614
337	5'-CAAAAATTAATCAAAAAGATTAATTTTTG-3’	76.56
338	5'-CAATTATATAACAAAAAGTTATATAATTG-3’	76.537
339	5'-CATTAATTATACAAAAAGTATAATTAATG-3’	76.517
340	5'-CAAAAATTATTCAAAAAGAATAATTTTTG-3’	76.412

341	5'-CATTTTTAATTCAAAAAGAATTAAAAATG-3’	76.403
342	5'-CATATAAAAAACAAAAAGTTTTTTATATG-3’	76.368
343	5'-CAATAAATAAACAAAAAGTTTATTTATTG-3’	76.327
344	5'-CAATATAATATCAAAAAGATATTATATTG-3’	76.291
345	5'-CATTTAATTTTCAAAAAGAAAATTAAATG-3’	76.199
346	5'-CAAAATAATATCAAAAAGATATTATTTTG-3’	76.166
347	5'-CAAATTTAAAACAAAAAGTTTTAAATTTG-3’	76.122
348	5'-CATAAAATTTACAAAAAGTAAATTTTATG-3’	76.031
349	5'-CAAATATTTTACAAAAAGTAAAATATTTG-3’	75.981
350	5'-CATTTTATTTACAAAAAGTAAATAAAATG-3’	75.979
351	5'-CATAAATTAAACAAAAAGTTTAATTTATG-3’	75.95
352	5'-CAATTTAATTTCAAAAAGAAATTAAATTG-3’	75.882
353	5'-CATTTTTATTACAAAAAGTAATAAAAATG-3’	75.861
354	5'-CAAAATTATTACAAAAAGTAATAATTTTG-3’	75.806
355	5'-CAAATTATTTACAAAAAGTAAATAATTTG-3’	75.795
356	5'-CAATTTTTAAACAAAAAGTTTAAAAATTG-3’	75.724
357	5'-CATATTAAAAACAAAAAGTTTTTAATATG-3’	75.71
358	5'-CATTAATATAACAAAAAGTTATATTAATG-3’	75.653
359	5'-CAATTATTTTTCAAAAAGAAAAATAATTG-3’	75.644
360	5'-CAAAATAAAAACAAAAAGTTTTTATTTTG-3’	75.616
361	5'-CAATATATAAACAAAAAGTTTATATATTG-3’	75.586
362	5'-CAATATATTATCAAAAAGATAATATATTG-3’	75.553
363	5'-CATAAAAAATTCAAAAAGAATTTTTTATG-3’	75.549

364	5'-CAAAAATAAATCAAAAAGATTTATTTTTG-3’	75.422
365	5'-CATTTATTTTACAAAAAGTAAAATAAATG-3’	75.361
366	5'-CATATTAATTACAAAAAGTAATTAATATG-3’	75.324
367	5'-CATTTTATAAACAAAAAGTTTATAAAATG-3’	75.208
368	5'-CAAATTTAATTCAAAAAGAATTAAATTTG-3’	75.144
369	5'-CAATATTTTATCAAAAAGATAAAATATTG-3’	75.076
370	5'-CAATTATTTTACAAAAAGTAAAATAATTG-3’	75.073
371	5'-CAAAAAAATTACAAAAAGTAATTTTTTTG-3’	75.029
372	5'-CATTATTAAAACAAAAAGTTTTAATAATG-3’	75.01
373	5'-CATATAATAAACAAAAAGTTTATTATATG-3’	75.003
374	5'-CAAATATTTATCAAAAAGATAAATATTTG-3’	74.98
375	5'-CATAATAAATACAAAAAGTATTTATTATG-3’	74.919
376	5'-CATTTTATTAACAAAAAGTTAATAAAATG-3’	74.912
377	5'-CATTTTATTATCAAAAAGATAATAAAATG-3’	74.815
378	5'-CATTTATATAACAAAAAGTTATATAAATG-3’	74.735
379	5'-CAAAAAATATTCAAAAAGAATATTTTTTG-3’	74.633
380	5'-CAATTTTTTTTCAAAAAGAAAAAAAATTG-3’	74.615
381	5'-CAATTATATTTCAAAAAGAAATATAATTG-3’	74.571
382	5'-CAAAATTTTATCAAAAAGATAAAATTTTG-3’	74.541
383	5'-CATAATTAAAACAAAAAGTTTTAATTATG-3’	74.541
384	5'-CATATAATTAACAAAAAGTTAATTATATG-3’	74.486
385	5'-CAAATAATATTCAAAAAGAATATTATTTG-3’	74.474
386	5'-CATAAATATAACAAAAAGTTATATTTATG-3’	74.401

387	5'-CAATATTTATACAAAAAGTATAAATATTG-3'	74.173
388	5'-CATAAAAAAAACAAAAAGTTTTTTTTATG-3'	74.173
389	5'-CATAAAAAATACAAAAAGTATTTTTTATG-3'	74.068
390	5'-CAAATTAAATTCAAAAAGAATTTAATTTG-3'	74.055
391	5'-CAAAAAATAAACAAAAAGTTTATTTTTTG-3'	73.86
392	5'-CATATTTTTTACAAAAAGTAAAAAATATG-3'	73.839
393	5'-CAATTATAAATCAAAAAGATTTATAATTG-3'	73.803
394	5'-CAATAATAAAACAAAAAGTTTTATTATTG-3'	73.661
395	5'-CAAATTTATTTCAAAAAGAAATAAATTTG-3'	73.637
396	5'-CATTTTTAAAACAAAAAGTTTTAAAAATG-3'	73.448
397	5'-CATTATATTAACAAAAAGTTAATATAATG-3'	73.434
398	5'-CAAATTTATAACAAAAAGTTATAAATTTG-3'	73.397
399	5'-CATTTATAAATCAAAAAGATTTATAAATG-3'	73.387
400	5'-CAATTAAATTTCAAAAAGAAATTTAATTG-3'	73.341
401	5'-CAAAATTATTTCAAAAAGAAATAATTTTG-3'	73.333
402	5'-CAATAAATTTACAAAAAGTAAATTTATTG-3'	73.33
403	5'-CATATTAATAACAAAAAGTTATTAATATG-3'	73.291
404	5'-CAAATATATAACAAAAAGTTATATATTTG-3'	73.26
405	5'-CAATTAATTTACAAAAAGTAAATTAATTG-3'	73.239
406	5'-CATATAAATAACAAAAAGTTATTTATATG-3'	73.143
407	5'-CAATTTAATTACAAAAAGTAATTAAATTG-3'	73.075
408	5'-CATAAATATTACAAAAAGTAATATTTATG-3'	73.029
409	5'-CATATAAATTACAAAAAGTAATTTATATG-3'	72.984

410	5'-CAAAATTAATTCAAAAAGAATTAATTTTG-3’	72.902
411	5'-CAATAAAATTACAAAAAGTAATTTTATTG-3’	72.867
412	5'-CATTTAATTAACAAAAAGTTAATTAAATG-3’	72.864
413	5'-CATAATTATTACAAAAAGTAATAATTATG-3’	72.837
414	5'-CATAATATATACAAAAAGTATATATTATG-3’	72.729
415	5'-CATAAATTTAACAAAAAGTTAAATTTATG-3’	72.652
416	5'-CAAAAATTTAACAAAAAGTTAAATTTTTG-3’	72.627
417	5'-CAATATTTTTTCAAAAAGAAAAAATATTG-3’	72.582
418	5'-CATTAATTTTACAAAAAGTAAAATTAATG-3’	72.508
419	5'-CAATTTTTTTACAAAAAGTAAAAAAATTG-3’	72.444
420	5'-CATTTAATTTACAAAAAGTAAATTAAATG-3’	72.411
421	5'-CAATTTTTAATCAAAAAGATTAAAAATTG-3’	72.387
422	5'-CAATATTATTACAAAAAGTAATAATATTG-3’	72.358
423	5'-CAAAATATTTTCAAAAAGAAAATATTTTG-3’	72.096
424	5'-CATTTTTTTAACAAAAAGTTAAAAAAATG-3’	72.08
425	5'-CATAAATAATACAAAAAGTATTATTTATG-3’	71.97
426	5'-CAAAATAATTTCAAAAAGAAATTATTTTG-3’	71.876
427	5'-CAATTTTAATTCAAAAAGAATTAAAATTG-3’	71.875
428	5'-CAAAAAAATAACAAAAAGTTATTTTTTTG-3’	71.874
429	5'-CAAAATTAAAACAAAAAGTTTTAATTTTG-3’	71.852
430	5'-CAAAATTTATTCAAAAAGAATAAATTTTG-3’	71.836
431	5'-CAAATTAATTACAAAAAGTAATTAATTTG-3’	71.81
432	5'-CAAAATATTATCAAAAAGATAATATTTTG-3’	71.778

433	5'-CAATATTTTTACAAAAAGTAAAAATATTG-3’	71.71
434	5'-CAATTTTAAATCAAAAAGATTTAAAATTG-3’	71.516
435	5'-CATTTTAAAAACAAAAAGTTTTTAAAATG-3’	71.492
436	5'-CAAATTTTTTTCAAAAAGAAAAAAATTTG-3’	71.4
437	5'-CATAAATTATACAAAAAGTATAATTTATG-3’	71.293
438	5'-CAAATTTTTTACAAAAAGTAAAAAATTTG-3’	71.135
439	5'-CATTATAATTACAAAAAGTAATTATAATG-3’	71.046
440	5'-CAAAAATATTTCAAAAAGAAATATTTTTG-3’	71.028
441	5'-CAATTTTATTTCAAAAAGAAATAAAATTG-3’	70.998
442	5'-CATTTAAATAACAAAAAGTTATTTAAATG-3’	70.997
443	5'-CAATTAAAATTCAAAAAGAATTTTAATTG-3’	70.759
444	5'-CAAATAATTTACAAAAAGTAAATTATTTG-3’	70.652
445	5'-CAAAATTTTTACAAAAAGTAAAAATTTTG-3’	70.619
446	5'-CAAAAAATAATCAAAAAGATTATTTTTTG-3’	70.617
447	5'-CATTTTAATAACAAAAAGTTATTAAAATG-3’	70.348
448	5'-CAAAATAAAATCAAAAAGATTTTATTTTG-3’	70.146
449	5'-CAATAATTTTACAAAAAGTAAAATTATTG-3’	70.138
450	5'-CATAATAAAAACAAAAAGTTTTTATTATG-3’	70.091
451	5'-CATAATTAATACAAAAAGTATTAATTATG-3’	70.032
452	5'-CAATTAAATTACAAAAAGTAATTTAATTG-3’	69.755
453	5'-CATAATTATAACAAAAAGTTATAATTATG-3’	69.75
454	5'-CATTTTAATTACAAAAAGTAATTAAAATG-3’	69.684
455	5'-CAATAATAATTCAAAAAGAATTATTATTG-3’	69.622

456	5'-CATAAATTTTACAAAAAGTAAAATTTATG-3’	69.548
457	5'-CAAAAAAATTTCAAAAAGAAATTTTTTTG-3’	69.421
458	5'-CATATAATTTACAAAAAGTAAATTATATG-3’	69.362
459	5'-CATTATTTAAACAAAAAGTTTAAATAATG-3’	69.315
460	5'-CATATATTTTACAAAAAGTAAAATATATG-3’	69.271
461	5'-CAAATTTAAATCAAAAAGATTTAAATTTG-3’	69.227
462	5'-CATATTAAATACAAAAAGTATTTAATATG-3’	69.118
463	5'-CAATTTTTTAACAAAAAGTTAAAAAATTG-3’	69.028
464	5'-CAAAATATAATCAAAAAGATTATATTTTG-3’	68.946
465	5'-CATTTTATATTCAAAAAGAATATAAAATG-3’	68.818
466	5'-CATAATATTAACAAAAAGTTAATATTATG-3’	68.669
467	5'-CATTTTAAATACAAAAAGTATTTAAAATG-3’	68.499
468	5'-CATTTATTTAACAAAAAGTTAAATAAATG-3’	68.455
469	5'-CATAATATTTACAAAAAGTAAATATTATG-3’	68.285
470	5'-CAAAAAAAAATCAAAAAGATTTTTTTTTG-3’	68.142
471	5'-CAAAATTAAATCAAAAAGATTTAATTTTG-3’	68.097
472	5'-CATTTATATTTCAAAAAGAAATATAAATG-3’	67.832
473	5'-CAAATTAAAATCAAAAAGATTTTAATTTG-3’	67.582
474	5'-CAAAAATTTTTCAAAAAGAAAAATTTTTG-3’	67.371
475	5'-CATATAATATACAAAAAGTATATTATATG-3’	67.336
476	5'-CAATAATAATACAAAAAGTATTATTATTG-3’	67.255
477	5'-CATTTTTTTTACAAAAAGTAAAAAAAATG-3’	67.035
478	5'-CAATTTTTTATCAAAAAGATAAAAAATTG-3’	66.377

479	5'-CAAAAATTATACAAAAAGTATAATTTTTG-3’	66.158
480	5'-CATTATTATTACAAAAAGTAATAATAATG-3’	65.9
481	5'-CAAAAAAAAAACAAAAAGTTTTTTTTTTG-3’	65.45
482	5'-CATTATTTATACAAAAAGTATAAATAATG-3’	65.389
483	5'-CATTTTTTTATCAAAAAGTAAAAAAAATG-3’	64.944
484	5'-CAAATATAAAACAAAAAGTTTTATATTTG-3’	63.478
485	5'-CAATAAAAAAACAAAAAGTTTTTTTATTG-3’	63.15
486	5'-CAAATTTATTACAAAAAGTAATAAATTTG-3’	62.583
487	5'-CAATTATTTATCAAAAAGATAAATAATTG-3’	62.225
488	5'-CAAAATTTATACAAAAAGTATAAATTTTG-3’	61.688
489	5'-CAATTTTTATACAAAAAGTATAAAAATTG-3’	61.036
490	5'-CAAAATTTTTTCAAAAAGAAAAAATTTTG-3’	60.89
491	5'-CATTTAAATATCAAAAAGATATTTAAATG-3’	60
492	5'-CAATTTATTTTCAAAAAGAAAATAAATTG-3’	59.784
493	5'-CATTTATAAAACAAAAAGTTTTATAAATG-3’	59.729
494	5'-CATTTTTTAATCAAAAAGATTAAAAAATG-3’	59.546
495	5'-CATTTATTTATCAAAAAGATAAATAAATG-3’	59.35
496	5'-CATTTAATATACAAAAAGTATATTAAATG-3’	59.326
497	5'-CATTTAATAATCAAAAAGATTATTAAATG-3’	58.75
498	5'-CATTTTTATATCAAAAAGATATAAAAATG-3’	57.265
499	5'-CAAATTAATAACAAAAAGTTATTAATTTG-3’	56.521
500	5'-CAATATATTTTCAAAAAGAAAATATATTG-3’	55.031
501	5'-CAATTTAAAAACAAAAAGTTTTTAAATTG-3’	53.252

502	5'-CATTTAAATTTCAAAAAGAAATTTAAATG-3’	52.038
503	5'-CATTTAATATTCAAAAAGAATATTAAATG-3’	48.761
504	5'-CAATAAAAATACAAAAAGTATTTTTATTG-3’	48.756
505	5'-CATTTTTTTTTCAAAAAGAAAAAAAAATG-3’	47.955
506	5'-CAATTTTATATCAAAAAGATATAAAATTG-3’	45.379
507	5'-CAATTTATTATCAAAAAGATAATAAATTG-3’	45.312
508	5'-CAAATTTTTAACAAAAAGTTAAAAATTTG-3’	44.013
509	5'-CAAAAAAATATCAAAAAGATATTTTTTTG-3’	43.936
510	5'-CAAATTATTAACAAAAAGTTAATAATTTG-3’	43.455
511	5'-CAATTAATAAACAAAAAGTTTATTAATTG-3’	42.366
512	5'-CAAAAAATTTTCAAAAAGAAAATTTTTTG-3’	39.385

The top 24 sequences:

CAATTTTTATACAAAAAGTATAAAAATTG	CAATTTAAAAACAAAAAGTTTTTAAATTG
CAAAATTTTTTCAAAAAGAAAAAATTTTG	CATTTAAATTTCAAAAAGAAATTTAAATG
CATTTAAATATCAAAAAGATATTTAAATG	CATTTAATATTCAAAAAGAATATTAAATG
CAATTTATTTTCAAAAAGAAAATAAATTG	CAATAAAAATACAAAAAGTATTTTTATTG
CATTTATAAAACAAAAAGTTTTATAAATG	CATTTTTTTTTCAAAAAGAAAAAAAAATG
CATTTTTTAATCAAAAAGATTAAAAAATG	CAATTTTATATCAAAAAGATATAAAATTG
CATTTATTTATCAAAAAGATAAATAAATG	CAATTTATTATCAAAAAGATAATAAATTG
CATTTAATATACAAAAAGTATATTAAATG	CAAATTTTTAACAAAAAGTTAAAAATTTG
CATTTAATAATCAAAAAGATTATTAAATG	CAAAAAAATATCAAAAAGATATTTTTTTG
CATTTTTATATCAAAAAGATATAAAAATG	CAAATTATTAACAAAAAGTTAATAATTTG
CAAATTAATAACAAAAAGTTATTAATTTG	CAATTAATAAACAAAAAGTTTATTAATTG
CAATATATTTTCAAAAAGAAAATATATTG	CAAAAAATTTTCAAAAAGAAAATTTTTTG

Table S3. List of the top 24 highest affinity sequences from the FID assay of 512 DNA hairpin oligonucleotides.

Derivation of p value for 86 transitions in the “top 24” sequences.

N = 216 (number of transitions in the top 24 = 9 x 24)

m = 86 / 216 = (probability of a switch in the top 24)

m_0 = 0.5 (expected probability if no correlation)

s = sqrt(m_0 (1-m_0) / N) = 0.034 (standard deviation of the mean)

z = |m_0 - m| / s = 2.99 (z score for the observed mean = # of standard deviations from the expected mean)

p = 1 - (1 + erf(z)) / 2 (p value for observing the z score, if transitions were random)=0.001%