Online Supplementary Material

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Part A.

Methods

UV melting and circular dichroism studies

These experiments were carried out as described previously (27) at an oligonucleotide concentration of ~ 4.5 μM in 0.01 M Na_2HPO_4, 0.1 mM Na_2EDTA, buffer, pH 7.00 ± 0.02. The melting temperature was calculated according to the base line method assuming a two-state (all-or-none) transition. Single-strand molar extinction coefficients were calculated from those of mononucleotides and dinucleotides using the nearest-neighbor approximation method (ref. 30). 2',5'-RNA, and RNA:2',5'-RNA chimeras were assumed to have the same molar extinction coefficient as RNA. Single-strand concentration was determined from UV absorbance at high temperature.

All CD spectra were recorded at 5 °C and were normalized by subtraction of the background scan with buffer. Taking the known oligonucleotide concentration into account, the normalized spectra were converted to molar ellipticities.

5'-End [^{32}P]-labeling of oligonucleotides

The oligonucleotide (100 pmol) was dissolved in 5 μl of 10 x T4 PNK Buffer (0.5 M Tris-HCl, pH 7.6, 100 mM $MgCl_2$, 100 mM 2-mercaptoethanol) to which 10 pmol of Gamma-ATP and 9 units (3-6 μl, diluted with 1 x T4 PNK buffer) of T4 Polynucleotide Kinase (PNK) were added. The assay volume was adjusted to 50 μl in total volume with dd water. After the contents were mixed well and centrifuged briefly, they were incubated at 37 ºC for 1.5 h. The reaction was terminated by heating to 65 ºC for 5 min followed by evaporation (under vacuum) until dryness.

Just prior to loading onto a gel, the dried sample was dissolved in 16 μl of loading buffer and subsequently heated to 100 ºC for about 10 min to effect denaturation of any secondary structure. The gel was run at 2000 V for 3 h. After removal of the glass plates, the major and slowest moving band was excised from the gel and crushed and soaked in 0.5 ml of doubled distilled water overnight at 37 ºC. The sample was then desalted by loading on a Nap™-5 column containing Sephadex® G-25 Medium (0.9 x 2.8 cm, Pharmacia Biotech) and elution with 1 ml dd water. The obtained solution was lyophilized to dryness and subsequently quantitated for radioactive content by using a Bioscan/QC 2000 counter (Amersham).

Part B. Characterization and Purification of Oligonucleotides

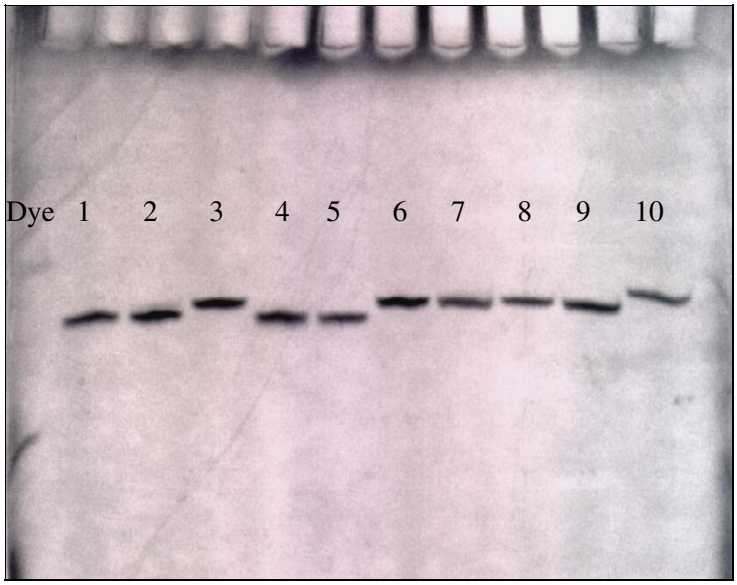
Reported yields from solid-phase synthesis

Entry	Code	Hairpin 5'→2'/3'direction	Crude Yield (ODU)	Isolated Yield (ODU)	Purification Technique
1		ggac(UUCG)gtcc	90.2	23.8	
2	RRR	GGAC(UUCG)GUCC	61.3	18.6	HPLC/ppt.
3	RRR	GGAC(UUCG)GUCC	67.7	16.9	Gel/Seph.
4	RRR	GGAC(UUCG)GUCC	63.6	8.34	HPLC/ppt.
5	RRR	GGAC(UUCG)GUCC	53.5	17.2	Gel/Seph.
6	RRD	GGAC(UUCG)gtcc	63.8	19.86	Gel/Seph.
7	DRD	ggac(UUCG)gtcc	47.1	19.4	HPLC/ppt.
8	DRR	ggac(UUCG)GUCC	52.8	17.5	Gel/Seph.
9		GGAC(UUCG)GUCC	56.0	9.8	Gel/Seph.
10	RRR	GGAC(UUCG)GUCC	58.0	13.9	HPLC/ppt.
11	DRR	ggac(UUCG)GUCC	49.1	12.4	Gel/Seph.
12	RRD	GGAC(UUCG)gtcc	66.1	14.3	Gel/Seph.
13	RRR	GGAC(UUCG)GUCC	61.7	9.8	Gel/Seph.
14	RRR	GGAC(UUCG)GUCC	63.7	12.9	Gel/Seph.
15	R_CRR	GGAC(UUCG)GUCC	61.4	9.3	Gel/Seph.
16	RR_GR	GGAC(UUCG)GUCC	65.0	11.4	Gel/Seph.
17	TRT	tttt(UUCG)tttt	60.7	12.3	Gel/Seph.
18	RR^1R	GGAC(UACG)GUCC	53.6	4.4	HPLC/ppt.
19	RR^2R	GGAC(UUUG)GUCC	51.3	3.7	HPLC/ppt.
20	RR^3R	GGAC(UUUU)GUCC	34.2	2.7	HPLC/ppt.
21	DR^1D	ggac(UACG)gtcc	45.8	6.1	Gel/Seph.
22	DR^2D	ggac(UUUG)gtcc	79.0	10.5	HPLC/ppt.
23	DR^3D	ggac(UUUU)gtcc	86.0	6.6	HPLC/ppt.
24	DDD	ggac(uucg)gtcc	88.8	19.2	Gel/Seph.
25	RDR	GGAC(uucg)GUCC	80.8	5.5	Gel/Seph.
26		ggac(tttt)gtcc	90.5	22.3	Gel/Seph.
27	DUD	ggac(UUUU)gtcc	64.1	15.6	Gel/Seph.
28	DRR	ggac(UUCG)GUCC	60.0	12.4	Gel/Seph.
29	DRR	ggac(UUCG)GUCC	58.9	10.1	Gel/Seph.
30	R_CRR	GGAC(UUCG)GUCC	55.6	14.0	Gel/Seph.
31	RR_UR	GGAC(UUCG)GUCC	65.8	19.1	Gel/Seph.

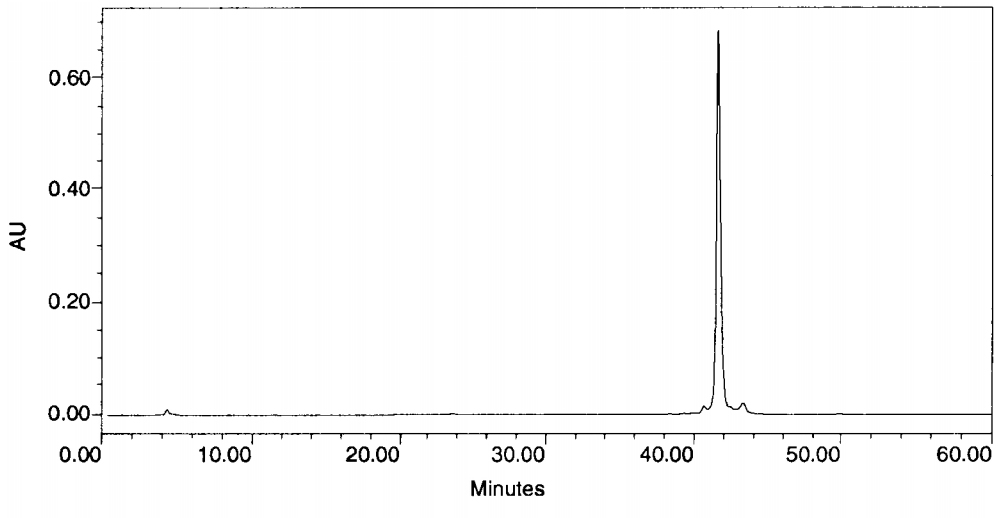
Crude yield represents the total amount of oligonucleotide obtained after solid-phase synthesis. The isolated yield represents that total amount of oligonucleotide obtained after purification/desalting. “Seph.” denotes Sephadex; “ppt.” refers to propanol precipitation.

Purity Check

Purity of all purified desalted oligonucleotides was checked by using either analytical 24% denaturing acrylamide gels or analytical ion-exchange HPLC, and was found to be > 90% in most cases.



Analytical polyacrylamide (24%) denaturing gel showing representative purified oligonucleotides. Lane 1: RRR; Lane 2: RRR; Lane 3: DRR; Lane 4: RRD; Lane 5: RRR; Lane 6: RRR; Lane 7: R_CRR; Lane 8: RR_GR; Lane 9: TRT; Lane 10: C_5RC_5.



Representative anion-exchange HPLC profile for a pure sample. Analysis was done at 55 ºC with a flow rate of 1 ml/min and a linear gradient of 0–23% $NaClO_4$ in H_2O.

MALDI-TOF Mass Spectrometry

The molecular weights of the purified oligonucleotides were confirmed by MALDI-TOF mass spectrometry (for representative examples, see below). The matrix used was 6-aza-2-thiothymine (Aldrich) at a concentration of 10 mg/ml in 20 mM ammonium citrate (Fluka) (1:1 acetonitrile/water, v/v) buffer. The machine was run in either the positive reflecton or negative linear mode. This gave correct molecular weight signals for the desired oligonucleotides with excellent signal to noise ratios. Typically, 20 μM solution of purified oligomer in water was prepared, from which 2 μl was pipetted into a tube containing 2 μl of matrix. After this solution was vortexed, 1 μl was pipetted, applied to a metal plate and subsequently air-dried. A brief pulse of nitrogen laser was directed to the sample in order to ionize it prior to analysis by the Kratos Kompact instrument.

MALDI-TOF MS of representative hairpins and calculated extinction coefficients

Entry	Code	Calculated $\varepsilon \times 10^{-4}$ ($M^{-1}.cm^{-1}$)	Calculated Mass (g/mol)	Observed Mass (g/mol)	Observed Molecular Ion
1	DRD	11.448	3682.4	3682.1	$(M-3H+2Li)^-$
2	RRR	11.595	3810.2	3810.6	$(M-3H+4Li)^+$
3	<u>R</u>RR	11.595	3802.3	3802.3	$(M-4H+3Li)^-$
4	RR<u>R</u>	11.595	3792.4	3792.8	$(M+Li)^+$
5	<u>R</u>R<u>R</u>	11.595	3792.4	3792.7	$(M+Li)^+$
6	<u>R</u>RD	11.448	3756.5	3755.5	$(M-2H+Na)^-$
7	D<u>R</u>D	11.448	3688.3	3686.9	$(M-4H+3Li)^-$
8	DR<u>R</u>	11.453	3748.4	3748.8	$(M-3H+Na+Li)^-$
9	<u>RRR</u>	11.595	3806.4	3804.3	$(M-2H+Na)^-$
10	R<u>R</u>R	11.595	3806.4	3806.2	$(M-2H+Na)^-$
11	D<u>RR</u>	11.453	3742.5	3740.0	$(M-2H+Na)^-$
12	<u>RR</u>D	11.448	3752.3	3752.1	$(M-4H+3Li)^-$
13	<u>RR</u>R	11.595	3802.3	3801.3	$(M-4H+3Li)^-$
14	R<u>RR</u>	11.595	3784.5	3786.7	$(M-H)^-$
15	$R_{\underline{C}}RR$	11.595	3802.3	3801.3	$(M-4H+3Li)^-$
16	$RR_{\underline{G}}R$	11.595	3802.3	3802.3	$(M-4H+3Li)^-$
17	TRT	10.690	3650.2	3648.5	$(M-4H+3Li)^-$
18	$R\underline{R}^1R$	11.918	3807.5	3807.6	$(M-H)^-$
19	$R\underline{R}^2R$	11.778	3803.2	3803.3	$(M-4H+3Li)^-$
20	$R\underline{R}^3R$	11.709	3764.2	3763.3	$(M-4H+3Li)^-$
21	$D\underline{R}^1D$	11.771	3693.6	3694.8	$(M-H)^-$
22	$D\underline{R}^2D$	11.631	3689.3	3689.8	$(M-4H+3Li)^-$
23	$D\underline{R}^3D$	11.562	3650.3	3648.5	$(M-4H+3Li)^-$

24	DDD	11.448	3618.4	3621.8	$(M-3H+2Li)^-$
25	RDR	11.595	3736.4	3736.6	$(M-3H+2Na)^-$
26	DTD	11.124	3624.6	3626.5	$(M-H)^-$
27	DUD	11.562	3632.5	3632.2	$(M-H)^-$
28	DRR	11.453	3720.5	3720.6	$(M-H)^-$
29	D<u>R</u>R	11.453	3720.5	3720.6	$(M-H)^-$
30	$R_{\underline{C}}$<u>R</u>R	11.595	3784.5	3783.5	$(M-H)^-$
31	$RR_{\underline{U}}R$	11.595	3784.5	3785.4	$(M-H)^-$

Molar Extinction coefficients (ε) for the various oligonucleotides were calculated using the nearest-neighbor approximation method (ref. 30). 2',5'-RNA, and RNA:2',5'-RNA chimeras were assumed to have the same molar extinction coefficient as RNA.

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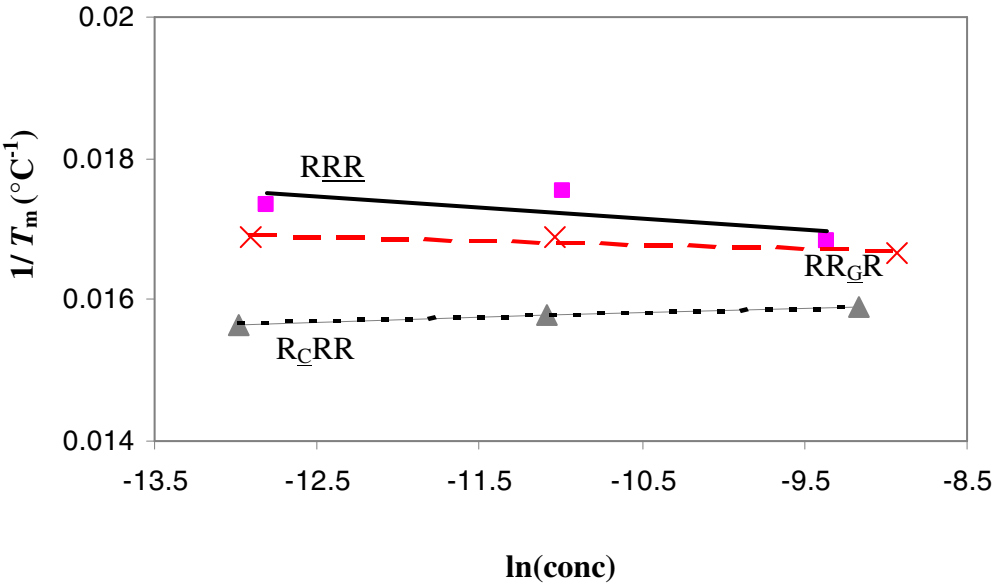
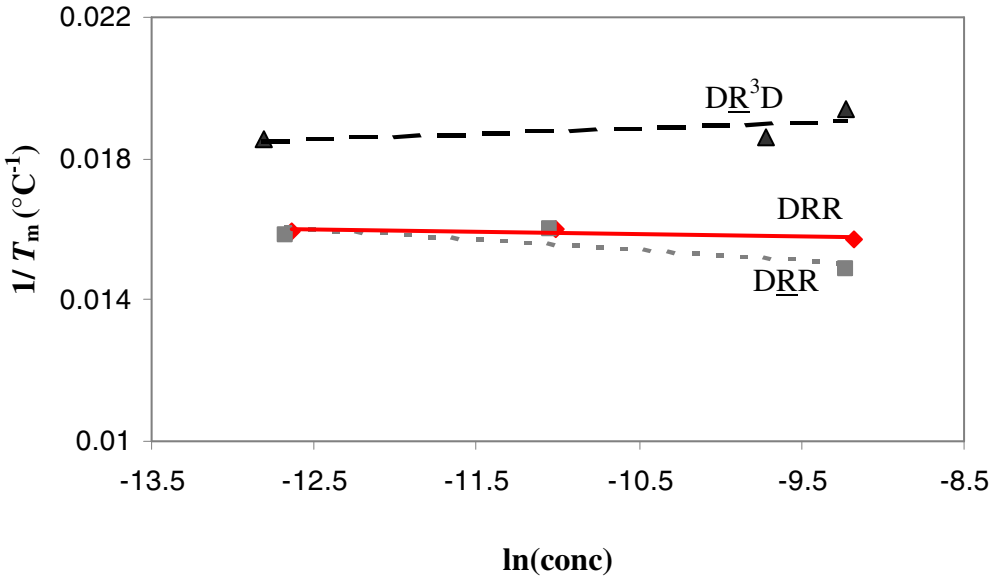
Part C.





Van't Hoff plots of representative hairpin library members. The spectra show concentration independence over a 50-fold range, consistent with a *unimolecular* transition process. UV thermal melting measurements were recorded at 260 nm in 0.01 M Na_2HPO_4, 0.1 mM Na_2EDTA, pH 7.0 buffer.

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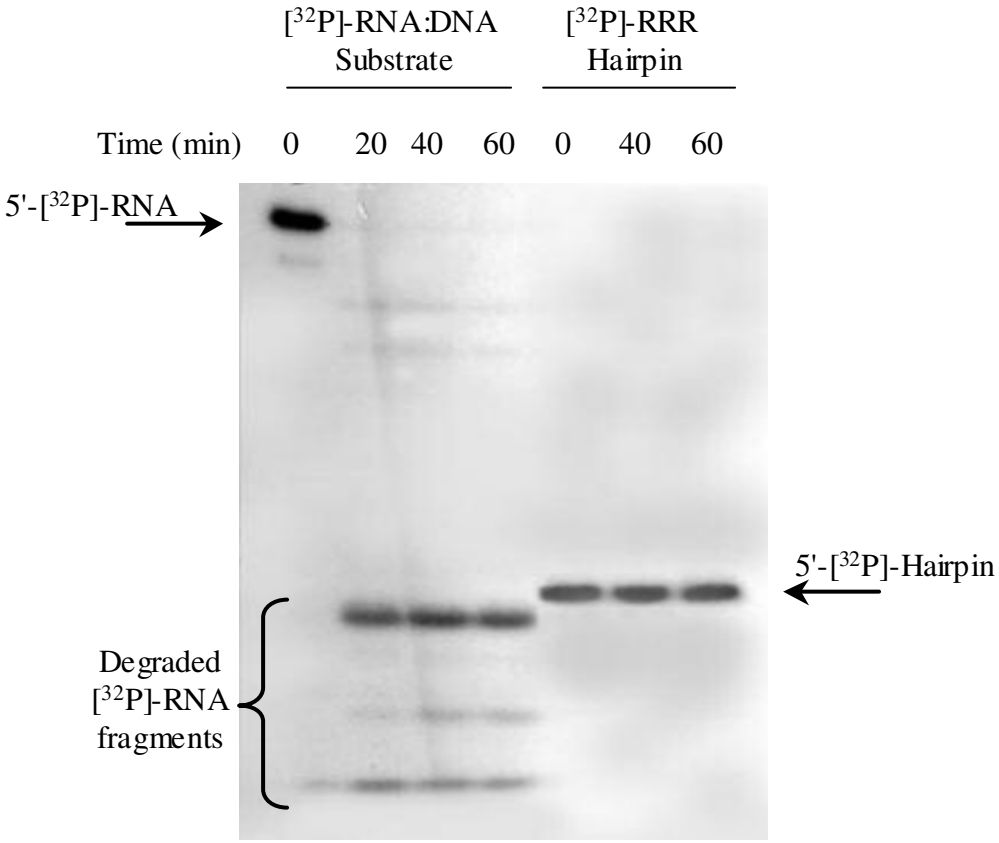
Part D.



Integrity of hairpin aptamers towards RNase H activity. A representative 5'-[^{32}P]-labeled potent hairpin aptamer was incubated with the enzyme in the absence of the natural RNA:DNA substrate. The gel shows no enzyme degradation of the hairpin aptamer, excluding the possibility that this might have been a reason for the observed decreased rate of degradation of the natural substrate in the inhibition assays performed earlier. The 5'-[^{32}P]-RNA strand in the RNA:DNA substrate, in the absence of hairpin inhibitor, is completely degraded within 20 min.

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Part E.

Test of RNase H inhibitory activity of short linear oligomers

Entry	5'-Sequence-3'	Code	Mixture	IC_{50} (μM)
1	GGAC	5'-R	5'-R + R-3'	n.i
2	GUCC	R-3'	5'-D + R-3'	n.i.
3	ggac	5'-D	5'-R + RR	n.i.
4	(UUCG)GUCC	RR	5'-R + $\underline{R}$R	n.i.
5	($\underline{UUCG}$)GUCC	$\underline{R}$R	5'-D + RR	n.i.
6			5'-D + $\underline{R}$R	n.i.

n.i. = no inhibition; IC_{50} is the oligomer concentration required to inhibit 50% RNase H activity of HIV-1 RT. Measurements were repeated twice. Capital letters represent RNA residues; underlined letters are 2',5'-RNA residues (*e.g.* $\underline{UC}$ = $U_{2'p5'}C_{2'p}$); DNA residues are represented by small letters. The appropriate oligomers were mixed at different molar concentrations, heated to 95 °C, and then left to anneal at 4 °C for 72 h. The inhibition assay was run as described in the Materials and Methods section.