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Cellular activity of siRNA oligonucleotides containing synthetic isomorphic nucleoside surrogates

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S1. Oligonucleotides: Synthesis and Purification

Solid-phase oligonucleotide synthesis was performed on an Expedite 8909 synthesizer using commercially available reagents and phosphoramidites (Glen Research). The modified phosphoramidites were chemically synthesized (full synthetic details will be separately published in due course) and incorporated into oligonucleotide with coupling efficiency comparable to the commercially available phosphoramidites. The solution of the modified phosphoramidite was dried overnight over molecular sieve 3A (dried for 2 days at 300 ^{o}C under high vacuum) and was filtered using syringe filter right before use. Oligonucleotides were synthesized (with trityl-off) on a 500 Å CPG solid support column (1 µmol scale). Cleavage from the solid support and deprotection were accomplished with AMA (ammonium hydroxide/methylamine in water = 1/1) at 65 ^{o}C for 30 min. The 2'-TOM or TBDMS group was removed by TEA·3HF at 65 ^{o}C for 3 h and the residue was desalted by precipitation (Glen Report 19-20, Glen Research). All oligonucleotides were purified by preparative polyacrylamide gel electrophoresis (PAGE) using the crush and soak method; the desired band was cut out, pulverized, extracted with 50 mM TEAA (pH 7.0) for a minimum of 12 h (while shaking) and decanted. The buffer containing the purified oligonucleotide was lyophilized and the residue was taken up in 0.2 M TEAB (pH 7.0) buffer and desalted on a Sep-pek C-18 (Waters). The oligonucleotides were eluted with 40 v% acetonitrile in water. The purified oligonucleotides were quantified by UV absorbance at 260 nm at 70 °C by Shimadzu UV 2450 and confirmed by MALDI-TOF mass spectrometry. Oligonucleotide concentration was then determined using Beer's Law with the following extinction coefficients: rCMP, 7200; rUMP, 9900; rGMP, 11500; rAMP, 15400; and thA,7160; thU, 8200; thG, 5500, and thC, 11500.

S2. Oligonucleotides: MALDI-TOF MS

The MW of the modified oligonucleotides was determined *via* MALDI-TOF MS. An aliquot of 400 pmol of the oligonucleotide was lyophilized and then dissolved in 1 uL of water and combined with 1 µL of 100 mM ammonium citrate buffer (PE Biosystems), and 4 µL of saturated 3-hydroxypicolinic acid. The sample was spotted onto a gold-coated plate and air dried. MALDI-TOF spectra were recorded on a PE Biosystems Voyager-DE STR MALDI-TOF spectrometer in negative-ion, delayed-extraction mode.

NO.	RNA	Calculated MS	Found MS	ε_{260}
5	G4	6775.99	6775.86	218157
6	G7	6775.99	6781.40	218157
7	G13	6775.99	6782.40	218157
8	G16	6775.99	6775.60	218157
9	G19	6775.99	6778.50	218157
10	G4·5	6792.10	6794.77	212174
11	5Gs	6840.20	6845.76	194225
12	U2	6816.02	6816.00	222450
13	U6	6816.02	6871.29 [M+K+NH_3]	222450
14	U9	6816.02	6817.19	222450
15	U14	6816.02	6826.28 [M+NH_3]	222450
16	U2·6	6872.11	6911.34 [M+K]	220760
17	C8	6816.01	6830.91 [M+NH_3]	228458
18	C10	6816.01	6836.42 [M+Na]	228458
19	C8·10	6872.09	6890.96 [M+Na]	232776
20	A11	6775.99	6779.50	215846
21	A15	6775.99	6781.40 [M+Li]	215846
22	A11·15	6792.05	6799.00 [M+Li]	207552

Table S1. MALDI-Tof MS and extinction coefficient of siRNA at 260 nm.

S3. UV-monitored Thermal Denaturation Experiments

Melting temperatures were determined by measuring changes in absorbance at 260 nm as a function of temperature using a Beckman-Coulter DU 640 spectrophotometer equipped with a high performance temperature controller and micro auto six-cell holder. Absorbance was recorded in the forward and reverse direction at temperatures from 25 to 95 °C at a rate of 1 °C/min. The melting samples were denatured at 95 ^{o}C for 5 min and annealed slowly to RT then stored at 4 ^{o}C until experiments were initiated. All melting samples were prepared in a total volume of 500 µL containing 1 µM of each strand oligonucleotide, 20 mM sodium cacodylate (pH 7.0), 20 mM NaCl and 0.5 mM EDTA. Melting curves were fit with the nonlinear least-squares program described by Turner et al.,[s1,s2] and van't Hoff analyses were obtained from these melting curves.[s3] All calculations were performed by PeakFit v4.0 (SPSS Inc.).

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P     3'-TUG ACC CAC GAG UCC AUC ACC   -5'
G     5'-  C UGG GUG CUC AGG UAG UGG UT-3'
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NO.	Duplex [a]	Tm (°C) [b]	stdev.	ΔTm [c]	$-\Delta G^{o}_{37}$ (kcal/mol)	stdev.	$-\Delta H^{o}$ (kcal/mol)	stdev.	$-\Delta S^{o}$ (cal/mol)	stdev.
2	WT	74.8	0.07	0.0	15.7	0	69.1	1.1	0.172	0.004
5	G4	74.3	0.71	-0.5	14.2	0.4	55.5	1.5	0.133	0.004
6	G7	75.4	0.64	0.6	17.9	0.2	86.3	1.1	0.221	0.003
7	G13	75.3	0.64	0.5	16.5	0.5	74.0	4.0	0.185	0.011
8	G16	75.3	0.64	0.5	18.6	0	93.3	0.4	0.241	0.001
9	G19	75.8	0.07	1.0	18.2	0	89.6	0.7	0.230	0.002
10	G4.5	75.8	0.07	1.0	16.5	0.8	76.1	4.3	0.192	0.011
11	5GS	71.8	0.14	-2.9	15.5	0.3	71.3	0.9	0.181	0.004
12	U2	74.4	0.64	-0.4	14.4	1.1	57.3	7.8	0.138	0.022
13	U6	73.9	0.07	-0.9	15.4	0.1	70.9	1.8	0.179	0.005
14	U9	71.9	0.00	-2.8	14.7	0.1	64.6	1.6	0.161	0.005
15	U14	73.0	0.07	-1.8	16.0	0.2	73.4	1.1	0.185	0.003
16	U2.6	71.8	0.00	-3.0	15.8	0.6	73.5	4.2	0.186	0.012
17	C8	72.4	0.78	-2.4	14.6	0.1	63.1	2.3	0.156	0.007
18	C10	73.9	0.07	-0.9	14.8	0.6	62.1	4.3	0.152	0.012
19	C8.10	72.9	0.00	-1.8	15.5	0.4	71.1	4.6	0.179	0.013
20	A11	75.3	0.71	0.5	15.7	0.1	67.1	1.1	0.166	0.001
21	A15	75.3	0.71	0.5	14.8	0.2	60.0	0.0	0.146	0.001
22	A11.15	77.9	0.07	3.1	17.3	0.1	77.3	1.2	0.194	0.003

Table S2. siRNA Duplexes with Melting Temperatures; [a] Passenger strand are listed on top and guide strands below. [b] T_m values were measured at duplex concentrations of 1 μM in 20 mM sodium carcodylate, pH 7.0, 20 mM NaCl, and 0.5 mM EDTA. Errors of at least two independent T_m measurements were less than 0.8 °C.; [c] Difference in T_m compared to WT.

S4. Hybridization and Transfection

Human H1299 lung adenocarcinoma cells containing an integrated, constitutively expressed destabilized GFP (dGFP) reporter gene (~2 h half-life) were cultured in medium which contains DMEM (Invitrogen) supplemented with 5% fetal bovine serum (Life Technologies), 100 units/ml penicillin, and 100 units/ml streptomycin (Invitrogen).Transfection mixtures were prepared in 100 ul Optimem-I® with 2 ul Lipofectamine® 2000 (Invitrogen), and left at room temperature for 20 minutes prior to treatment. Cells were reverse transfected overnight in 24-well plates at 80,000 cells/well in 400 ul medium to which 100 ul transfection mixtures was added. After transfection cells were trypsinized and replated. Knockdown of GFP was analyzed by flow cytometry on a BD Biosciences LSRII (BD Sciences, San Jose, CA) at time point of 24, 48 and 72 hour. All experiments were performed triple times or more. Three different set of experiments, which are 1) thG-modified, 2) thU- and thC-modified and 3) thA-modified siRNAs, was performed and the data were normalized by each WT results.

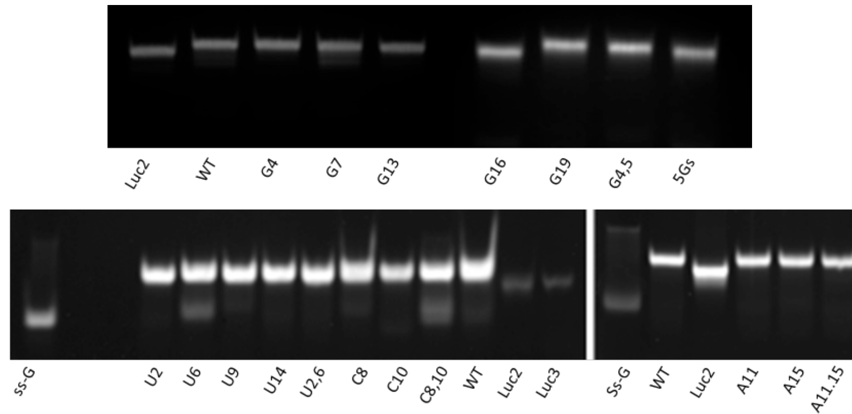

Figure S1. Representations of siRNA hybridization prior to transfection

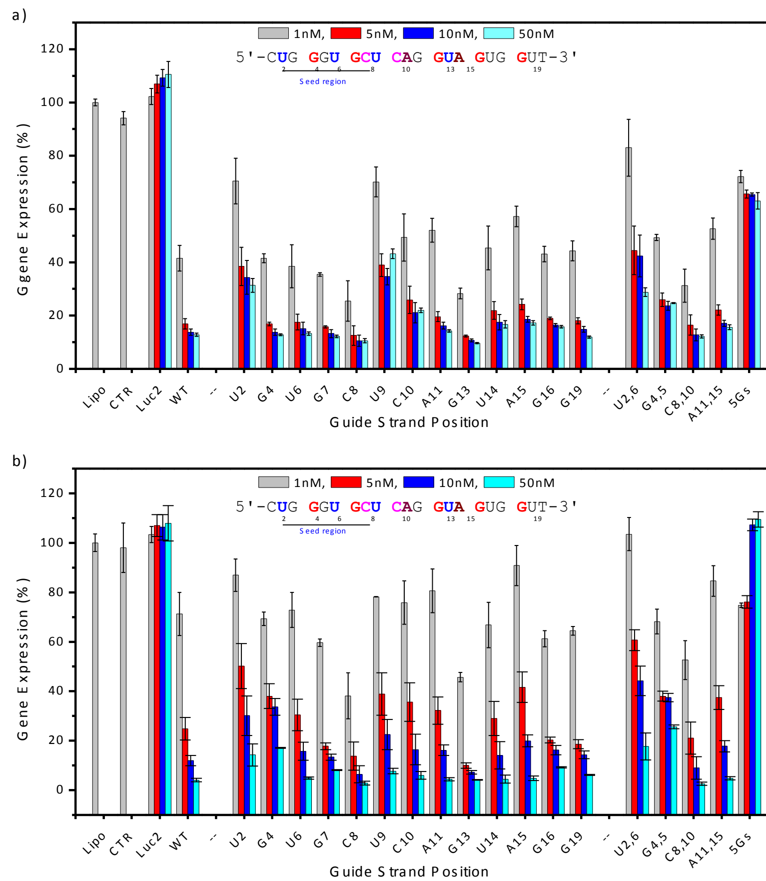

Figure S2. Gene expression of dGFP; a) 24 h; b) 72 hour; Lipo = lipofectamin only; CTR = buffer; Luc2 = luciferase2; WT = wild type; grey = 1 nM, red = 5 nM, blue = 10 nM, cyan = 50 nM.

References

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