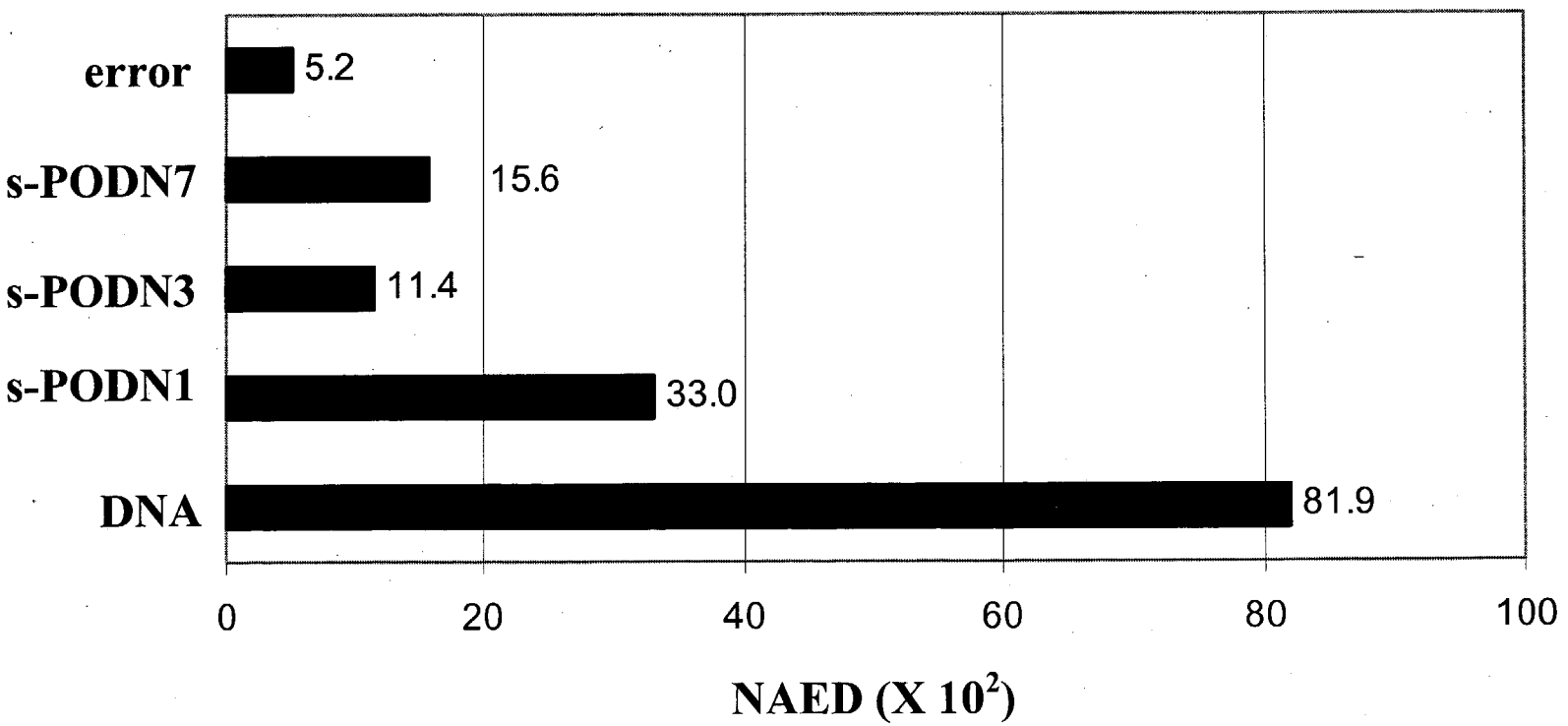
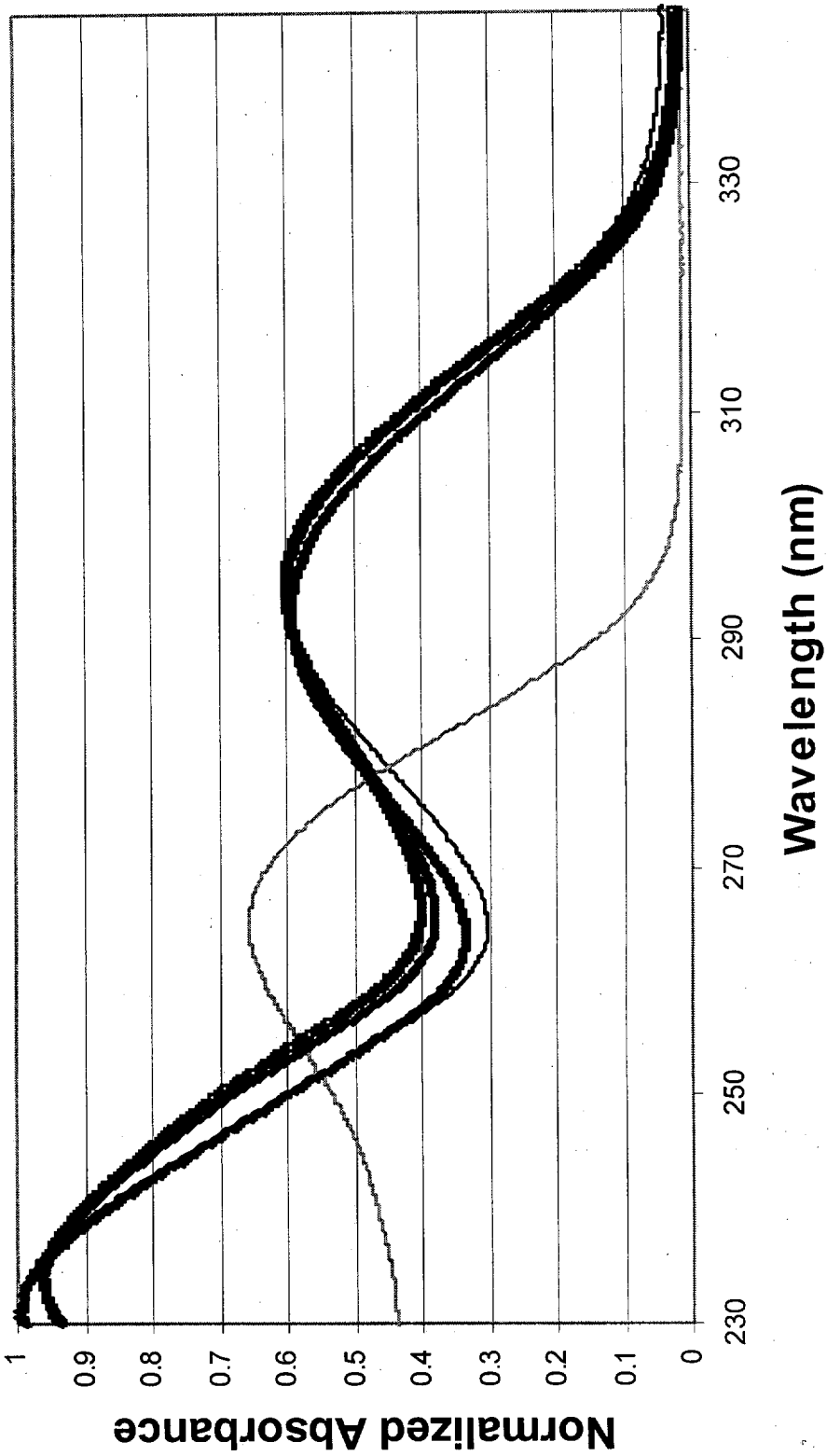
Supporting Material Figure 1: The Normalized Absolute Ellipticity Differences (NAEDs) at 20 °C between the single strand PODN and other unpropynylated/propynylated single strands. For example, the bar with a value of 81.9 and labeled DNA quantifies the differences between the CD spectra of 5'-dCCUCCUU-3' and 5'-dC^{P}C^{P}U^{P}C^{P}C^{P}U^{P}U^{P}-3'.





Supporting Material Figure 2: The absorbance spectra of single strands (~0.01 mM) at 90 °C: 5'-dCCUCCUU-3' (grey), 5'-dC^{p}C^{p}U^{p}C^{p}C^{p}U^{p}U^{p}-3' (black), 5'-d**C**C^{p}U^{p}C^{p}C^{p}U^{p}U^{p}-3' (△), 5'-dC^{p}C^{p}**U**C^{p}C^{p}U^{p}U^{p}-3' (□), and 5'-dC^{p}C^{p}U^{p}C^{p}C^{p}U^{p}**U**-3' (◇).

Supporting Table 1: Thermodynamic Parameters of DNA:RNA and PODN:RNA Hybrid Duplexes Containing Various 5' and 3' Dangling Ends[a]

		1/Tm Plots				Curve-Fit Parameters			
d(5'CCUCCUU3')	reference symbol	$-\Delta G^{o}_{37}$ (kcal/mol)	$-\Delta H^{o}$ (kcal/mol)	$-\Delta S^{o}$ (kcal/mol)	T_m (°C)[b]	$-\Delta G^{o}_{37}$ (kcal/mol)	$-\Delta H^{o}$ (kcal/mol)	$-\Delta S^{o}$ (kcal/mol)	T_m (°C)[b]
r(5'AAGGAGG3')	7-mer	-7.6 ± 0.1	-53.3 ± 2.0	-147.3 ± 6.4	43.3	-7.7 ± 0.2	-60.0 ± 4.8	-168.7 ± 15.5	43.1
r(5'AAGGAGG**A**3')	8-mer	-8.9 ± 0.1	-61.2 ± 1.8	-168.6 ± 5.7	49.3	-9.1 ± 0.2	-68.4 ± 3.9	-191.2 ± 11.8	49.3
r(5'**A**AAGGAGG**A**3')	9-mer	-9.4 ± 0.2	-62.0 ± 2.4	-169.7 ± 7.1	52.0	-9.6 ± 0.3	-68.7 ± 2.9	-190.5 ± 8.3	51.8
d(5'C^{p}C^{p}U^{p}C^{p}C^{p}U^{p}U^{p}3')									
r(5'AAGGAGG3')	7-mer	-15.3 ± 0.4	-80.9 ± 4.0	-211.6 ± 11.3	74.5	-14.6 ± 0.1	-74.6 ± 1.9	-193.5 ± 5.7	74.7
r(5'AAGGAGG**A**3')	8-mer	-16.2 ± 0.6	-79.0 ± 5.2	-200.4 ± 14.7	80.2	-16.0 ± 0.5	-77.6 ± 4.1	-198.5 ± 11.6	80.1
r(5'**A**AAGGAGG**A**3')	9-mer	-18.2 ± 0.3	-89.0 ± 3.1	-228.5 ± 10.8	83.6	-17.9 ± 0.3	-86.4 ± 5.3	-220.8 ± 8.3	84.1
r(5'AAGGAGG3')									
d(5'**C**CCUCCUU3')	DNA-5'C	-8.3 ± 0.1	-56.1 ± 3.0	-154.2 ± 9.6	46.9	-8.4 ± 0.2	-60.4 ± 4.9	-167.5 ± 15.3	47.1
d(5'CCUCCUU**C**3')	DNA-3'C	-7.8 ± 0.1	-51.4 ± 2.1	-140.7 ± 6.8	44.8	-7.8 ± 0.1	-53.8 ± 4.3	-148.3 ± 13.8	44.5
d(5'**C**^{p}C^{p}C^{p}U^{p}C^{p}C^{p}U^{p}U^{p}3')	PODN-5'C^p	-15.6 ± 0.4	-79.6 ± 3.4	-206.2 ± 9.9	76.9	-16.0 ± 1.0	-82.4 ± 9.3	-214.2 ± 26.6	75.0
d(5'C^{p}C^{p}U^{p}C^{p}C^{p}U^{p}U^p**C**^{p}3')	PODN-3'C^p	(-16.0 ± 0.4)	(-88.0 ± 3.6)	(-232.3 ± 10.5)	74.4	(-14.3 ± 0.5)	(-71.6 ± 4.4)	(-184.5 ± 12.9)	77.0

[a]Measured in 1.0 M NaCl, 0.05 mM Na_2EDTA, 20 mM sodium cacodylate, pH 7. Values in parentheses indicate that the ΔH^{o} values determined from T_M^{-1} vs $\ln(C_T/4)$ plots and from curve fitting differ by more than 15%, thus indicating non-two-state melting. Errors are based on the standard deviations of the thermodynamic parameters and were calculated as described in (12). Significant figures are given beyond error estimates to allow accurate calculation of T_M and other parameters. [b]T_M for a total strand concentration of 1×10^{-4} M.

Supporting Table 2: Thermodynamic Parameters of the DNA,s-DNAs, PODN, and s-PODNs when Bound to, 5'-rUA**AAGGAGG**AG-3.[a]									
		1/Tm Plots				Curve-Fit Parameters			
	reference symbol	$-\Delta G^{\circ}_{37}$ (kcal/mol)	$-\Delta H^{\circ}$ (kcal/mol)	$-\Delta S^{\circ}$ (eu)	T_m (°C)	$-\Delta G^{\circ}_{37}$ (kcal/mol)	$-\Delta H^{\circ}$ (kcal/mol)	$-\Delta S^{\circ}$ (eu)	T_m (°C)
d(5'CCUCCUU3')	DNA	-9.6 ± 0.1	-62.80 ± 1.7	-171.4 ±4.9	53.0	-9.85 ± 0.2	-69.0 ± 2.1	-190.7 ±6.5	52.7
d(5'**C**^{p}CUCCUU3')	s-DNA1	-9.6 ± 0.1	-65.62 ± 2.7	-180.66 ± 8.4	52.2	-9.79 ± 0.1	-70.5 ± 3.7	-195.7 ± 11.7	52.0
d(5'C**C**^{p}UCCUU3')	s-DNA2	-10.0 ± 0.1	-61.0 ± 2.0	-164.4 ±6.0	55.7	-10.5 ± 0.2	-70.0 ± 4.0	-191.8 ± 12.43	55.6
d(5'CC**U**^{p}CCUU3')	s-DNA3	-10.1 ± 0.1	-64.0 ± 1.5	-173.7 ± 4.68	55.2	-10.2 ± 0.2	-66.6 ± 5.6	-182.0 ± 17.3	55.0
d(5'CCU**C**^{p}CUU3')	s-DNA4	-10.6 ± 0.2	-68.2 ± 3.7	-185.7 ± 11.4	56.7	-10.7 ± 0.3	-70.1 ± 3.5	-191.4 ± 10.4	56.7
d(5'CCUC**C**^{p}UU3')	s-DNA5	-10.2 ± 0.1	-61.6 ± 2.3	-165.7 ± 7.1	56.6	-10.7 ± 0.3	-70.4 ± 3.4	-192.5 ± 10.2	56.5
d(5'CCUCC**U**^{p}U3')	s-DNA6	-9.8 ± 0.1	-61.2 ± 1.8	-165.7 ± 5.5	54.6	-10.0 ± 0.23	-65.2 ± 4.8	-177.7 ± 14.7	54.7
d(5'CCUCCU**U**^{p}3')	s-DNA7	-9.6 ± 0.1	-62.0 ± 2.0	-169.6 ± 6.2	52.0	-9.7 ± 0.1	-69.1 ± 3.4	-191.5 ± 10.9	51.8
d(5'CCUCCUU3')	DNA 6-mer	-6.7 ± 0.1	-52.8 ± 1.9	-148.5 ± 6.3	38.1	-6.7 ± 0.1	-57.7 ± 4.8	-164.4 ± 15.3	38.2
d(5'C^{p}U^{p}C^{p}C^{p}U^{p}U^{p}3')	PODN 6-mer	-13.8 ± 0.3	-75.8 ± 2.8	-199.9 ± 8.1	70.1	-13.3 ± 0.6	-70.5 ± 6.4	-184.4 ± 18.8	70.0
d(5'C^{p}C^{p}U^{p}C^{p}C^{p}U^{p}U^{p}3')	PODN	-18.4 ± 0.6	-89.3 ± 5.4	-228.7 ± 15.5	84.3	-18.2± 1.1	-88.3 ± 9.9	-226.1 ± 28.6	84.1
d(5'**C**C^{p}U^{p}C^{p}C^{p}U^{p}U^{p}3')	s-PODN1	-15.2 ± .3	-71.1 ± 2.9	-180.2 ± 8.4	79.9	-15.4 ± 0.4	-73.0 ± 3.0	-185.6 ± 8.3	79.9
d(5'C^{p}**C**U^{p}C^{p}C^{p}U^{p}U^{p}3')	s-PODN2	-14.8 ± 0.2	-70.8 ± 2.0	-180.6 ± 4.9	78.2	-14.9 ± 0.4	-71.4 ± 4.1	-182.3 ± 10.3	78.2
d(5'C^{p}C^{p}**U**C^{p}C^{p}U^{p}U^{p}3')	s-PODN3	-14.4 ± 0.2	-67.4 ± 3.9	-171.0 ± 11.0	77.8	-14.7 ± 0.5	-69.9 ± 4.5	-178.2 ± 12.9	77.8
d(5'C^{p}C^{p}U^{p}**C**C^{p}U^{p}U^{p}3')	s-PODN4	-14.9 ± 0.2	-74.3 ± 1.8	-191.5 ± 5.1	76.5	-15.0 ± 0.5	-74.9 ± 5.1	-193.2 ± 14.7	76.4
d(5'C^{p}C^{p}U^{p}C^{p}**C**U^{p}U^{p}3')	s-PODN5	-14.7 ± 0.2	-73.8 ± 1.4	-190.7 ± 4.2	75.3	-15.7 ± 0.4	-83.1 ± 3.6	-217.4 ± 10.3	75.4
d(5'C^{p}C^{p}U^{p}C^{p}C^{p}**U**U^{p}3')	s-PODN6	-16.3 ± 0.3	-83.7 ± 2.4	-217.4 ± 6.9	78.0	-15.4 ± 0.1.1	-76.2 ± 9.1	-195.9 ± 25.9	78.1
d(5'C^{p}C^{p}U^{p}C^{p}C^{p}U^{p}**U**3')	s-PODN7	-16.4 ± 0.3	-79.7 ± 3.0	-204.1 ± 5.2	80.9	-15.7 ± 0.8	-73.6 ± 5.8	-186.9 ± 16.3	80.9

[a]Bases at which systematic substitutions and deletions occur are in bold. Errors are based on the standard deviations od the thermodynamic parameters and were calculated as described in (12). Significant figures are given beyond error estimates to allow accurate calculation of T_M.

Supporting Table 3: Thermodynamic Parameters of m-DNA Strands Bound to 5'-UA**AAGGAGG**AG-3'.[a]									
	1/Tm Plots					Curve-Fit Parameters			
	reference symbol	$-\Delta G^{\circ}_{37}$ (kcal/mol)	$-\Delta H^{\circ}$ (kcal/mol)	$-\Delta S^{\circ}$ (eu)	T_m (°C)	$-\Delta G^{\circ}_{37}$ (kcal/mol)	$-\Delta H^{\circ}$ (kcal/mol)	$-\Delta S^{\circ}$ (eu)	T_m (°C)
d(5'CCUCC**U^{p}U^{p}**3')	m-DNA6,7	-10.4 ± 0.1	-64.6 ± 1.7	-174.6 ± 5.3	56.9	-10.8 ± 0.3	-70.9 ± 3.5	-193.8 ± 10.3	56.8
d(5'**C^{p}C^{p}**UCCUU3')	m-DNA1,2	-11.1 ± 0.2	-68.3 ± 3.1	-184.4 ± 9.4	59.3	-11.6 ± 0.4	-74.6 ± 6.1	-203.2 ± 12.3	59.4
d(5'C**C^{p}**UC**C^{p}**UU3')	m-DNA2,5	-11.4 ± 0.2	-67.3 ± 2.3	-180.4 ± 6.9	61.0	-11.2 ± 0.3	-65.06 ± 3.9	-173.6 ± 11.7	61.1
d(5'CCU**C^{p}C^{p}**UU3')	m-DNA4,5	-11.8 ± 0.3	-77.2 ± 4.7	-211.1 ± 14.2	59.6	-11.6 ± 0.3	-75.5 ± 3.5	-205.9 ± 10.4	59.5
d(5'**C^{p}C^{p}**UC^{p}C^{p}UU3')	m-DNA1,2,4,5	-12.6 ± 0.1	-71.6 ± 1.7	-190.2 ± 5.1	65.8	-13.5 ± 0.4	-82.7 ± 5.2	-223.2 ± 15.6	65.5
d(5'**C^{p}C^{p}U^{p}C^{p}C^{p}**UU3')	m-DNA1,2,3,4,5	-13.4 ± 0.3	-70.1 ± 3.0	-183.0 ± 8.7	70.6	-13.7 ± 0.2	-74.7 ± 7.7	-196.7 ± 12.8	70.0

[a]Bases at which C5-1-propynyl substitutions occur are in bold. Errors are based on the standard deviations of the thermodynamic paramters and were calculated as described in (12). Significant figures are given beyond error estimates to allow accurate calculation of T_M.

Supporting Table 4: Modeling the Stability of Y^p- containing DNA:RNA Hybrid Duplexes. The RNA strand is 3'-rGAGGAGGAAAU-5'

	ΔG^o_i(Y^p-containing duplex) (kcal/mol)	$\Delta\Delta G^o_i$ (kcal/mol)	Internal Y^p bonus (x_i)	5' Dangling end bonus (y_i)	cooperative bonus (z_i)	Predicted $\Delta\Delta G^{o\,a}_i$ (kcal/mol)	$\Delta\Delta\Delta G^{o\,b}_i$ (kcal/mol)
Antisense							
d(5'**C^p**CUCCUU3')	-9.6	0.00	0	0	0	0.17	0.170
d(5'C**C^p**UCCUU3')	-10	-0.40	1	0	0	-0.85	-0.450
d(5'CC**U^p**CCUU3')	-10.1	-0.50	1	0	0	-0.85	-0.350
d(5'CCU**C^p**CUU3')	-10.6	-1.00	1	0	0	-0.85	0.150
d(5'CCUC**C^p**UU3')	-10.2	-0.60	1	0	0	-0.85	-0.250
d(5'CCUCCU**^{p}**U3')	-9.8	-0.20	1	0	0	-0.85	-0.650
d(5'CCUCCUU**^{p}**3')	-9.6	0.00	0	0	0	0.17	0.170
d(5'**C^{p}U^{p}C^{p}C^{p}U^{p}U^p**3')	-13.8	-7.10	4	1	1	-6.60	0.500
d(5'**CC^{p}U^{p}C^{p}C^{p}U^{p}U^p**3')	-15.2	-5.60	5	1	0	-5.98	-0.380
d(5'C**C^{p}U^{p}C^{p}C^{p}U^{p}U^p**3')	-14.8	-5.20	4	1	0	-4.95	0.246
d(5'C^p**UC^{p}C^{p}U^{p}U^p**3')	-14.9	-5.30	4	1	0	-4.95	0.350
d(5'C^{p}C^{p}U^p**CC^{p}U^{p}U^p**3')	-14.4	-4.80	4	1	0	-4.95	-0.150
d(5'C^{p}C^{p}U^{p}C^p**CU^{p}U^p**3')	-14.7	-5.10	4	1	0	-4.95	0.150
d(5'C^{p}C^{p}U^{p}C^{p}C^p**UU^p**3')	-16.3	-6.70	4	1	1	-6.60	0.100
d(5'C^{p}C^{p}U^{p}C^{p}C^{p}U^p**U**3')	-16.4	-6.80	5	0	1	-6.60	0.200
d(5'CCUCC**U^{p}U^p**3')	-10.4	-0.80	1	0	0	-0.85	-0.050
d(5'**C^{p}C^p**UCCUU3')	-11.1	-1.50	1	0	0	-0.85	0.650
d(5'C**C^p**UCC^{p}UU3')	-11.4	-1.80	2	0	0	-1.88	-0.080
d(5'CCU**C^{p}C^p**UU3')	-11.8	-2.20	2	0	0	-1.88	0.320
d(5'**C^{p}C^p**U**C^{p}C^p**UU3')	-12.6	-3.00	3	0	0	-2.90	0.100
d(5'**C^{p}C^{p}U^{p}C^{p}C^p**UU3')	-13.4	-3.80	4	0	0	-3.92	-0.120
CCUCCUU	-9.6	0.00	0	0	0	0.17	0.170
Fitted Value (kcal/mol)			(-0.99)	(-1.17)	(-1.94)		

[a]The stability of each duplex predicted by the parameters dertermined by linear regression according to eq 2. $\Delta\Delta G^o_i$ is the free energy increment due to addition of propynyl groups. The fitted parameters are listed in the appropriate column at the bottom of the Table.

[b]$\Delta\Delta\Delta G^o_i$ is the difference between the experimental $\Delta\Delta G^o_i$ and that predicted by the long-range cooperative model.

Supporting Table 5: Thermodynamic Parameters of DNA, PODN, and s-PODNs Bound to r(5'UA**AAGGA/G**AG3')[a]

		1/Tm Plots				Curve-Fit Parameters			
Antisense Strand	reference symbol	$-\Delta G^{\circ}_{37}$ (kcal/mol)	$-\Delta H^{\circ}$ (kcal/mol)	$-\Delta S^{\circ}$ (eu)	T_m (°C)	$-\Delta G^{\circ}_{37}$ (kcal/mol)	$-\Delta H^{\circ}$ (kcal/mol)	$-\Delta S^{\circ}$ (eu)	T_m (°C
d(5'C^{p}C^{p}U^{p}C^{p}C^{p}U^{p}U^{p}U^{p}3')	PODN	-14.5 ± 0.7	-75.5 ± 6.2	-196.7 ± 8.0	73.8	-14.6 ± 0.5	-75.8 ± 4.1	-197.4 ± 11.7	73.9
d(5'CCUCCUU3')	DNA	(-7.9 ± 0.1)	(-62.7 ± 3.7)	(-176.6 ± 11.0)	44.3	(-7.9 ± 0.2)	(-51.5 ± 5.9)	(-140.7 ± 8.6)	45.2
d(5'C^{p}C^{p}U^p**C**C^{p}U^{p}U^{p}3')	s-PODN4	-13.9 ± 0.4	-81.5 ± 3.7	-217.9 ± 10.9	67.9	-13.7 ± 0.6	-79.2 ± 6.4	-211.2 ± 18.6	67.7
d(5'C^{p}C^{p}U^{p}C^p**C**U^{p}U^{p}3')	s-PODN5	-13.5 ± 0.3	-77.2 ± 3.2	-205.5 ± 9.5	67.8	-13.5 ± 0.6	-78.0 ± 6.3	-207.8 ± 18.6	67.6

[a]Positions of C5-(1-propyne) deletions within the PODN strand are in bold. Values in parentheses indicate that the ΔH° values ditermined from T_M^{-1} vs. $\ln(C_T/4)$ plots and from curve fitting differ by more than 15%, thus inidcating non-two-state melting. Errors are based on the standard diviations of the thermodynamic parameters and were calculated as described in (12). Significant figures are given beyond error estimates to allow accurate calculation of T_M and other parameters.