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5′-Tethered Stilbene Derivatives as Fidelity- and Affinity-Enhancing Modulators of DNA Duplex Stability

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Oligonucleotides serve as hybridization probes, primers, and biomedical agents. DNA chips are arrays of immobilized oligonucleotides for massively parallel hybridization.[1] The sequence selectivity of oligonucleotides themselves is limited, and cross hybridization between strands with partial complementarity limits the usefulness of DNA chips.[2] The termini of duplexes are hot spots of low base pairing fidelity due to fraying and few neighboring base pairs affected. Even for short oligonucleotides, a mismatch at the terminus lowers the UV-melting point by only a few degrees and the free energy of binding by <1 kcal/mol, both for DNA[3] and for RNA.[4] This is less than the variation in binding constants between strands with different sequence and G/C content.[5] Because in a typical DNA chip experiment,[2] over 10^5 probe sequences compete for their targets, the low selectivity at the termini is significant.

Since nature did not evolve DNA to serve as a hybridization probe, but as a polymeric carrier of information read by fidelity-enhancing polymerases, chemically modifying oligonucleotides may improve fidelity. Some polymerase primers employ short sequences with fidelity-enhancing molecular appendages to achieve improved selectivity,[6] but routine protocols for DNA chips do not employ fidelity-enhancing elements for the termini of DNA probes. Wider searches for such elements have been limited to acylamido substituents[7] that have to be introduced through peptide coupling to aminodeoxynucleotides. Here, we report on fidelity-enhancing 5′-caps that can be introduced via automated phosphoramidite chemistry.

The present caps are stilbenes, a class of compounds known to bind to DNA.[8,9] Stilbene-DNA conjugates form very stable bridged hairpins[10] and are well known from elegant work on electron transfer.[11] To our knowledge, the effect of singly appended stilbenes on base pairing fidelity has not been studied. However, Letsinger, Lewis, Egli, and co-workers have identified diethers[12] and stilbenecarboxamides[13] as optimized bridges for hairpins. Singly linked stilbenecarboxamide **a** (Figure 1) was the lead for our study. To exclude possible (but unlikely) intercalation of the stilbene ring system, tricyclic **b** was prepared, which cannot intercalate. Six different carboxylic acids were coupled on-support to protected DNA with a 5′-aminopropanol residue (Supporting Information), followed by deprotection, producing **1a**−**g**.

UV-melting points of self-complementary **1a**−**g** showed strong interactions between stilbenes and DNA, as evidenced by melting point (T_m) increases (Table 1). For $(\mathbf{1e})_2$, the highest melting points were detected, followed by **1a**, and electron deficient pentafluoride **1d**. Difluoride **1c**, aminomethylstilbene **1f**, and **1g** each gave T_m increases in the range of 6−7 °C per modification, whereas tricyclic **1b** showed only half that effect. For the most duplex-stabilizing cap **e**, phosphoramidite **5** was synthesized, which can be used directly on DNA synthesizers. With **5**, modified sequences **2Ae**−**Te** were prepared. Mismatch discrimination at the terminus was improved in the presence of **e**, both for the terminal and for the penultimate base pair of sequence **2T** (Table 2 and Table S1, Supporting Information). The affinity- and fidelity-enhancing effect was also found for A:T, C:G, and G:C as terminal base pairs (Tables 1 and 2), with ΔT_m's of up to −23.4 °C ($\Delta\Delta G°$ of 6.3 kcal/mol) for a terminal C:A mismatch. An overhang in the target strand (sequence **11**), a situation typical for hybridization to longer DNA, did not reduce the duplex-stabilizing effect. In fact, the +10.2 °C ΔT_m over control for **2Te**:**11** at 1 M salt is one of the higher ΔT_m's found for cap **e** and octamer **2**. It is greater than that for **2Te**:**7**.

Table 1. UV-Melting Points[a] (°C) of DNA Duplexes

duplex	150 mM[b]	1 M[b]	ΔT_m to control (**v**)[c]
$(\mathbf{1v})_2$	31.6 ± 1.7	33.5 ± 0.5	−/−
$(\mathbf{1a})_2$	50.6 ± 0.4	55.3 ± 0.7	19.0/21.8
$(\mathbf{1b})_2$	39.1 ± 3.9	n.d.	7.5/−
$(\mathbf{1c})_2$	43.6 ± 1.1	47.1 ± 0.5	12.0/13.6
$(\mathbf{1d})_2$	48.9 ± 0.5	54.1 ± 0.3	17.3/20.6
$(\mathbf{1e})_2$	51.9 ± 0.4	55.8 ± 0.2	20.3/22.3
$(\mathbf{1f})_2$	43.9 ± 0.7	47.7 ± 0.3	12.3/14.2
$(\mathbf{1g})_2$	42.9 ± 1.3	46.1 ± 0.5	11.3/12.6
2Tv:**7**	29.8 ± 0.5	36.1 ± 0.3	−/−
2Te:**7**	38.2 ± 0.5	45.5 ± 0.6	8.4/9.4
2Cv:**9**	32.7 ± 0.3	38.8 ± 0.9	−/−
2Ce:**9**	44.9 ± 0.8	50.3 ± 0.9	12.2/11.5
2Av:**10**	31.5 ± 0.8	35.9 ± 0.6	−/−
2Ae:**10**	36.0 ± 0.9	42.9 ± 0.5	4.5/7.0
2Gv:**8**	30.0 ± 1.0	38.5 ± 3.2	−/−
2Ge:**8**	41.2 ± 0.3	46.9 ± 0.4	11.2/8.4
2Tv:**11**	27.2 ± 0.7	35.2 ± 0.5	−/−
2Te:**11**	36.9 ± 0.7	45.4 ± 1.7	9.7/10.2
3v:**12**	<15	19.0 ± 0.5	−/−
3h:**12**	17.3 ± 0.8	25.2 ± 0.7	−/6.2
3i:**12**	22.6 ± 0.7	33.0 ± 0.4	−/14.0
3j:**12**	34.8 ± 1.0	43.5 ± 0.9	−/24.5
3k:**12**	19.1 ± 0.9	27.4 ± 0.9	−/8.4
3l:**12**	41.9 ± 0.8	51.7 ± 1.4	−/32.7
3m:**12**	n.d.	24.6 + 1.3	−/5.6

[a] Mean of three T_m's ± SD at pH 7, 3.0 ± 0.8 μM strand concentration (entries 1−8; 19−25), or 1.5 ± 0.2 μM strand concentration (entries 9−18). [b] [salt]; entries 1−18, NH_4OAc; entries 19−25, NaCl in 10 mM phosphate buffer. [c] At 150 mM/1 M salt.

Table 2. Melting Points for Duplexes with Terminal Mismatches

duplex	mismatch	T_m (°C)[a]	ΔT_m[b]	ΔT_m for control duplex[b]
2Te:**8**	T:C	35.8 ± 0.9	−9.7	−2.1
2Te:**9**	T:G	39.0 ± 0.9	−6.5	−1.4
2Te:**10**	T:T	36.5 ± 0.8	−9.0	−1.9
2Ae:**7**	A:A	29.5 ± 1.5	−13.4	−2.2
2Ce:**7**	C:A	26.9 ± 0.7	−23.4	−6.1
2Ge:**7**	G:A	30.5 ± 0.9	−16.4	−4.2
3l:**13**	T:C	34.9 ± 1.5	−7.0	−[c]
3l:**14**	T:G	37.7 ± 2.4	−4.2	−[c]
3l:**15**	T:T	37.4 + 3.7	−4.5	−[c]

[a] Mean of three T_m's ± SD; 1.5 μM strands and 1 M NH_4OAc (entries 1−6), or 3 μM strands, 150 mM NaCl, 10 mM phosphate (entries 7−9). [b] To perfectly matched duplex. [c] Control too low for accurate determination.

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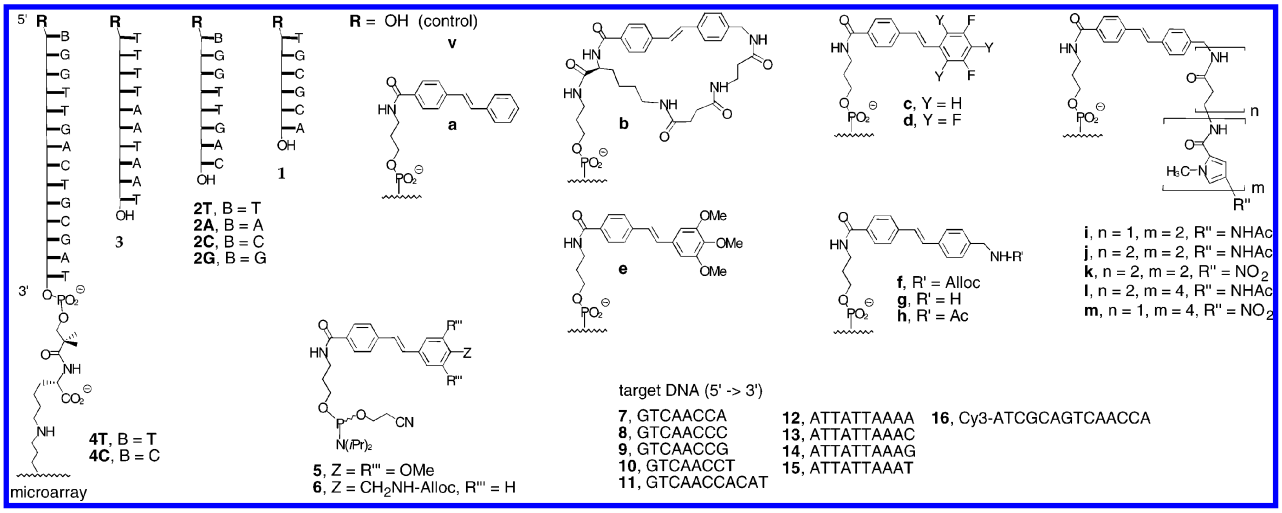

Figure 1.

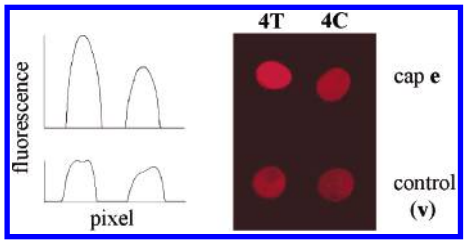

Figure 2. Hybridization of **4Te**/**4Ce**/**4Tv**/**4Cv** with Cy3-labeled **16**; (left) integration of fluorescence of spots, (right) fluorescence scan.

This encouraged us to attach the trimethoxystilbene to hybridization probes immobilized on glass surfaces as part of a small DNA microarray.[14] Incubation with target **16** gave a more than 2-fold stronger signal for **4Te** over **4Tv** (Figure 2). Even without optimizing hybridization conditions and without picking a well discriminating base pair, there was a clearly detectable discrimination against a terminal mismatch (**4Ce**), which was almost absent for the unmodified controls (**4Tv**/**4Cv**).

Next, it was tested whether stilbene caps can bind synergistically with oligopyrrolamides, when incorporated in DNA-minor groove binder conjugates.[15] When netropsin-derived pyrrolamides[16] were linked to all-A/T sequence **3** via aminomethyl stilbene **g**, introduced through phosphoramidite **6**, the resulting caps were strongly duplex stabilizing (Table 1, entries 19−25). On the level of dimers, two β-alanine linkers were better than one (caps **i** and **j**). A terminal nitro group (cap **k**), which interferes with proper hydrogen bonding in the minor groove by displaying an N−O moiety where an N−H hydrogen bond donor is required, gave lower duplex stability. Tetramer **l**, when linked through 2 β-alanines, gave a melting point increase of 32.7 °C, shifting the T_m of the all-A/T duplex **3** into a range typical for duplexes with higher G/C content. Control tetramer **m** again had almost no effect on the T_m. Also, the base pairing fidelity at the terminal base pair capped by the stilbene portion of the DNA decamer is satisfactory (Table 2, entries 7−9).

Our data suggest that trimethoxystilbene caps selectively stabilize duplexes with a matched terminal base pair. As binding elements they may be called "orthogonal", since they increase the affinity for target strands without themselves offering binding motives for other DNA sequences (as an elongation of the sequences would). Molecular modeling shows that the methyl group of the 4′-methoxy substituent protrudes out of the plane of the stilbene ring system and may thus "gauge" the size of base pairs. The aminomethylstilbene combined with minor groove binders produces "embracing caps" that induce a very substantial melting point increase for A/T-rich sequences. More elaborate minor groove binders[17] should bind other sequences. Modified hybridization probes may thus alleviate both poor base pairing fidelity at the termini and the low duplex stability of A/T-rich sequences, two issues central to increasing the fidelity of DNA chips. High fidelity DNA chip data are needed,[18] and they may be obtained, if better biostatistics are combined with improved molecular recognition on the chip.

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Supporting Information Available: Experimental protocols, NMR or MS spectra for all new compounds (PDF). This material is available free of charge via the Internet at http://pubs.acs.org.

References

(1) Pirrung, M. C. *Angew. Chem., Int. Ed.* **2002**, *41*, 1277−1289.
(2) (a) Evertsz, E. M.; Au-Young, J.; Ruvolo, M. V.; Lim, A.C.; Reynolds, M. A. *BioTechniques* **2001**, *31*, 1182−1192. (b) Zhang, L.; Miles, M. F.; Aldape, K. D. *Nat. Biotechnol.* **2003**, *21*, 818−821. (c) Siegmund, K.; Steiner, U. E.; Richert, C. *J. Chem. Inf. Comput. Sci.* **2003**, *43*, 2153−2162.
(3) See, for example: (a) Bleczinski, C. F.; Richert, C. *J. Am. Chem. Soc.* **1999**, *121*, 10889−10894. (b) Connors, W. H.; Narayanan, S.; Kryatova, O. P.; Richert, C. *Org. Lett.* **2003**, *5*, 247−250, and this work.
(4) Freier, S.M.; Kierzek, R.; Caruthers, M. H.; Neilson, T.; Turner, D. H. *Biochemistry* **1986**, *25*, 3209−3213.
(5) SantaLucia, J. *Proc. Natl. Acad. Sci. U.S.A.* **1998**, *95*, 1460−1465.
(6) Kutyavin, I. V.; Afonina, I. A.; Mills, A.; Gorn, V. V.; Lukhtanov, E. A.; Belousov, E. S.; Singer, M. J.; Walburger, D. K.; Lokhov, S. G.; Gall, A. A.; Dempcy, R.; Reed, M. W.; Meyer, R. B.; Hedgpeth, J. *Nucleic Acids Res.* **2000**, *28*, 655−661.
(7) (a) Altman, R. K.; Schwope, I.; Sarracino, D. A.; Tetzlaff, C. N.; Bleczinski, C. F.; Richert, C. *J. Comb. Chem.* **1999**, *1*, 493−508. (b) Mokhir, A. A.; Tetzlaff, C. N.; Herzberger, S.; Mosbacher, A.; Richert, C. *J. Comb. Chem.* **2001**, *3*, 374−386.
(8) Festy, B.; Daune, M. *Biochemistry* **1973**, *12*, 4827−4834.
(9) Fukuhara, K.; Miyata, N. *Bioorg. Med. Chem. Lett.* **1998**, *8*, 3187−3192.
(10) Herrlein, M. K.; Letsinger, R. L. *Angew. Chem., Int. Ed. Engl.* **1997**, *36*, 599−601.
(11) (a) Lewis, F. D.; Wu, T.; Zhang, Y.; Letsinger, R. L.; Greenfield, S. R.; Wasielewski, M. R. *Science* **1997**, *277*, 673−676. (b) Lewis, F. D.; Wu, Y. S.; Liu, X. Y. *J. Am. Chem. Soc.* **2002**, *124*, 12165−12173.
(12) Lewis, F. D.; Liu, X.; Wu, Y.; Miller, S. E.; Wasielewski, M. R.; Letsinger, R. L.; Sanishivili, R.; Joachimiak, A.; Tereshko, V.; Egli, M. *J. Am. Chem. Soc.* **1999**, *121*, 9905−9906.
(13) Lewis, F. D.; Wu, T. F.; Liu, X. Y.; Letsinger, R. L.; Greenfield, S. R.; Miller, S. E.; Wasielewski, M. R. *J. Am. Chem. Soc.* **2000**, *122*, 2889−2902.
(14) Dombi, K. L.; Griesang, N.; Richert, C. *Synthesis* **2002**, 816−824.
(15) (a) Sinyakov, A. N.; Lokhov, S. G.; Kutyavin, I. V.; Gamper, H. B.; Meyer, R. B. *J. Am. Chem. Soc.* **1995**, *117*, 4995−4996. (b) Szewcyk, J. W.; Baird, E. E.; Dervan, P. B. *Angew. Chem., Int. Ed. Engl.* **1996**, *35*, 1487−1489. (c) Robles, J.; Rajur, S. B.; McLaughlin, L. W. *J. Am. Chem. Soc.* **1996**, *118*, 5820−5821.
(16) Bailly, C.; Chaires, J. B. *Bioconjugate Chem.* **1998**, *9*, 513−538.
(17) Kielkopf, C. L.; White, S.; Szewczyk, J. W.; Turner, J. M.; Baird, E. E.; Dervan, P. B.; Rees, D. C. *Science* **1998**, *282*, 111−115.
(18) Tilstone, C. *Nature* **2003**, *424*, 610−612.

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