Perylene- and Naphthalene-Based Linkers for Duplex and Triplex Stabilization

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Small molecule linkers have often been used to tether sequences of nucleic acids in place of nucleotide loops in duplexes,[1,2] triplexes,[3] and more complex structures,[4,5] to tether two independently hybridizing DNA/RNA probes,[6,7] or to tether ligands to DNA sequences.[8,9] Ethylene glycol-based linkers have been popular since they are hydrophilic and readily available in a variety of lengths.[3] Such linkers do not generally provide any stabilizing interactions themselves, but serve simply to tether two sites that are involved in stabilizing interactions. One exception is the recently described stilbene linker.[10]

In the present study, perylene was chosen for development into a planar π-stacking linker owing to the availability of the 3,4,9,-10-dianhydride and the presence of a potentially large area for π-stacking interactions. When bridging the two pyrimidine strands of DNA in a pyrimidine−purine−pyrimidine triplex, an aromatic perylene-based linker has the capability of providing base stacking interactions with all three of the base residues present in the terminal base triplet (Figure 1). The corresponding naphthalene derivative offers a similar although slightly smaller area for π-stacking interactions. Both perylene and naphthalene tetracarboxylic acid dianhydrides can be functionalized quite easily to form the corresponding diimides. Simple diimides formed from 1,4,5,8-naphthalene dianhydride appear to interact with DNA by intercalation,[11,12] while a simple 3,4,9,10-perylene dimide formed from an alkyl diamine may bind by a nonintercalative mechanism.[13] Naphthalene dimides have also been used as peroxide-based DNA cleavage agents,[14] and as the basis of a new class of peptide-linked polyintercalating agents.[15]

Both linkers for this study were prepared by reaction of the corresponding tetracarboxylic acid dianhydride with 2-aminoethoxyethanol (or its *t*BDMS derivative). The silyl protecting groups were critical to the solubility of the perylene diimide in most organic solvents. The protecting groups were then removed one at a time and replaced in the first case with 4,4′-dimethoxytrityl, and in the second case the unmasked hydroxyl was converted to the *N*,*N*-diisopropyl-β-cyanoethoxy phosphoramidite.

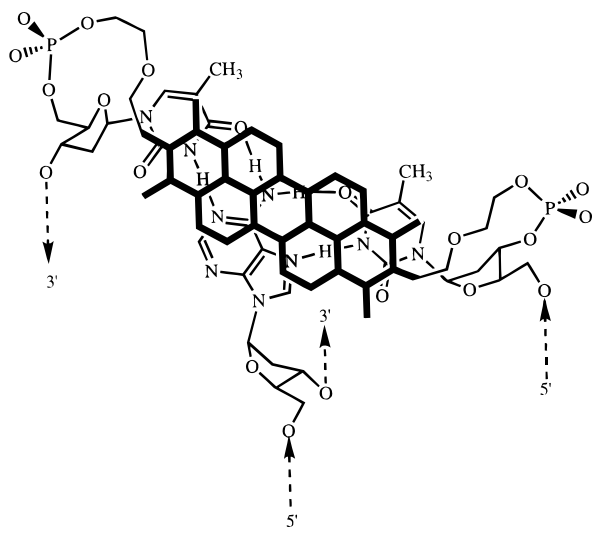

Figure 1. The terminal T-dA-T base triplet (behind) of a DNA triplex containing a perylene tetracarboxylic acid diimide linker (in bold). The linker bridges the terminal 3′-hydroxyl of the Watson−Crick strand (T) and the terminal 5′-hydroxyl of the Hoogsteen strand (T) used to target the third strand (dA).

We have examined the thermal stability of both DNA duplexes and triplexes. With the duplexes we prepared a nine-base pair stem with a simple hexa(ethylene glycol) linker (similar complexes have been described previously[3]) and compared this tethered duplex with the corresponding complexes containing a naphthalene or perylene tetracarboxylic acid diimide linker (see Table 1). In the triplex series, we examined the ability of the pyrimidine-rich sequence 5′-TCTTTTCTT−linker−TTCTTTTCT to target two nine-residue polypurine strands containing the sequence 5′-AGAAAAGAA. In one complex the purine sequence was limited to the minimal nine-residue target, while in the second complex a 19-mer target with the polypurine sequence embedded in the central region was employed (Table 1). In all of the complexes described, absorbance vs temperature plots gave a single transition.

The T_M was 23 °C for the simple 9-mer duplex and 64 °C for the duplex tethered by hexa(ethylene glycol), while the duplex containing the naphthalene-based linker exhibited a further 8 °C increase (Table 1). By comparison, the larger perylene-based linker resulted in only a moderate increase of 3−4 °C. These differences may simply reflect the results of model building studies which suggest that the perylene-based linker is significantly larger than necessary to bridge the phosphate residues at the terminus of a B-form helix, while the naphthalene-based linker can be more optimally positioned at the end of a duplex.

The triplex formed with the 9-mer target and the hexa(ethylene glycol) linker exhibited a T_M of 39 °C at pH 5.5 (Table 1), and this value decreased with increasing pH as would be expected due to the presence of two C^+-G-C base triplets in the 9-mer complex. When the corresponding 19-mer target was used, a slight decrease in T_M was observed. This effect was noted previously[3] with triplexes containing glycol linkers and presumably reflects a slightly unfavorable interaction between the linker and the target as the target sequence extends beyond the three-stranded complex. When the hexa(ethylene glycol) linker was replaced by the naphthalene tetracarboxylic acid diimide a 10 °C increase in T_M was observed at pH 5.5 for the simple 9-mer triplex, and similar increases were present over the pH range examined. With the perylene tetracarboxylic acid diimide the T_M was increased

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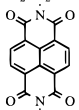
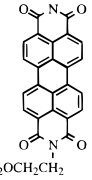
Table 1. T_M Values for Duplexes and Triplexes Containing Naphthalene- and Perylene-Based Linkers

Duplex	Triplex/9-mer	Triplex/19-mer					
3' AGAAAAGAA L 5' TCTTTTCTT	3' TCTTTTCTT 5' AGAAAAGAA L 5' TCTTTTCTT	3' TCTTTTCTT 5' CTCCCAGAAAAGAA L CTCCC 3' 5' TCTTTTCTT					
			pH Value[a]				
Linker = L		Complex[b]	5.5	6.4	7.0	7.5	8.0
-O(CH$_2$CH$_2$O)$_5$CH$_2$CH$_2$O-		Duplex	–	64	64	–	–
		Triplex/9-mer	39	35	33	30	27
		Triplex/19-mer	34	31	27	25	23
-OCH$_2$CH$_2$OCH$_2$CH$_2$  -OCH$_2$CH$_2$OCH$_2$CH$_2$		Duplex	–	72	72	–	–
		Triplex/9-mer	49	46	43	39	36
		Triplex/19-mer	51	45	41	39	35
-OCH$_2$CH$_2$OCH$_2$CH$_2$  -OCH$_2$CH$_2$OCH$_2$CH$_2$		Duplex	–	68	67	–	–
		Triplex/9-mer	50	48	43	41	37
		Triplex/19-mer	53	49	44	42	38

[a] Buffers employed: MES (pH 5.5), PIPES (pH 6.4 and 7.0), and HEPES pH 7.5 and 8.0). [b] T_M values were obtained for duplexes or complexes containing a 1:1 mixture of oligonucleotide−linker−oligonucleotide and single-stranded target in the noted buffer at oligonucleotide concentrations of 1 μM. Solutions were heated in 1 deg C steps and absorbances were recorded after temperature stabilization with an AVIV 14DS spectrophotometer. Absorbance and temperature readings were plotted with Igor Pro software. T_M values were determined from first-order derivatives as well as graphically from absorbance vs temperature plots. T_M values are the average of at least two determinations reproducible to ±1 °C.

by 16 °C at pH 5.5. The most significant T_M enhancements were those that occurred with the 19-mer complex. The presence of the naphthalene-based linker in the 19-mer complex results in essentially the same T_M as obtained for the 9-mer complex—but it is some 14−17 °C higher than that obtained with the glycol linker. The perylene-based linker in the 19-mer complex raises the T_M slightly more such that relative to the glycol linker, a 19 °C increase is observed at pH 5.5. The perylene-based linker is likely more effective in triplex stabilization since, as noted, it can potentially provide stacking interactions with all three residues (Figure 1). With the 19-mer complexes there may be an additional benefit in that the first base residue of the target strand that extends beyond the triplex could be stacked onto either the naphthalene- or perylene-based linker; the linker would then be intercalated between the last base triplet and the first single-stranded base residue in the 19-mer complex. In the illustrated complex this residue is a dC (note arrow, Table 1), but when this residue in the perylene 19-mer complex was replaced by dA, an ~3 °C increase in T_M was observed, consistent with an intercalation-type model.

The presence of base-stacking interactions between the perylene-based linker and the DNA triplex is suggested by three experiments. (i) Absorbance vs temperature plots obtained at 537 nm for duplexes or triplexes containing the perylene-based linker exhibited cooperative increases in hyperchromicity with a midpoint in the transitions that corresponded exactly to those obtained at 260 nm. (ii) Asorbance vs temperature plots obtained in solutions containing 10 and 30% ethanol exhibited T_M values that were reduced by 3 and 7 °C, respectively. (iii) The fluorescence of the 5′-TCTTTTCTT−perylene−TTCTTTTCT sequence (λ_{ex} = 500 nm, λ_{em} = 520−570 nm) is quenched upon complex formation with either the 9-mer or the 19-mer target, and the duplex containing the perylene linker exhibits a very low quantum yield.

The T_M value (pH 7.0) obtained for the perylene-based triplex composed of nine base triplets within a 19-mer target (44 °C) is comparable with that obtained for triplexes from circular oligonucleotides containing a hexa(ethylene glycol)-based loop and as many as 12-base triplets (43.5 °C).[3]

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Note Added in Proof: After this manuscript had been accepted for publication, a paper appeared in which it was shown[16] that *N*,*N*′-bis[2-(1-piperidino)ethyl]-3,4,9,10-perylenetetracarboxylic diimide (PIPER) will bind to G-tetraplexes by a threading intercalation mode.

Supporting Information Available: Linker preparation procedures, conditions for thermal denaturation experiments, and sample absorbance vs temperature plots (17 pages, print/PDF). See any current masthead page for ordering information and Web access instructions.

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