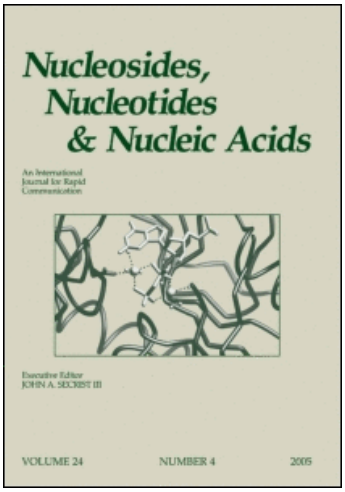

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Endcaps for Stabilizing Short DNA Duplexes

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ABSTRACT

The syntheses of endcaps for covalently linking the 3′ and 5′ hydroxyl groups of blunt end double-stranded DNA are described. Endcap diols were converted into DMTr protected phosphoramidites and incorporated between nucleotides 4 and 5 of a self-complementary octamer. The stabilizing effect of the endcaps on duplex DNA was determined by T_m experiments on the self-complementary octamer.

Key Words: Endcap; Amide; Terthiophene; Stabilization; DNA duplexes.

We have developed a series of endcaps, both aromatic hydrophobic and aliphatic hydrophilic, for stabilizing very short DNA duplexes. The aromatic hydrophobic endcaps are based on either a naphthalene diimide core (**1**)[1] which provides the greatest stability when incorporated adjacent to a CG base pair; or a terthiophene core (**2**) that results in the highest stability when incorporated adjacent to an AT base

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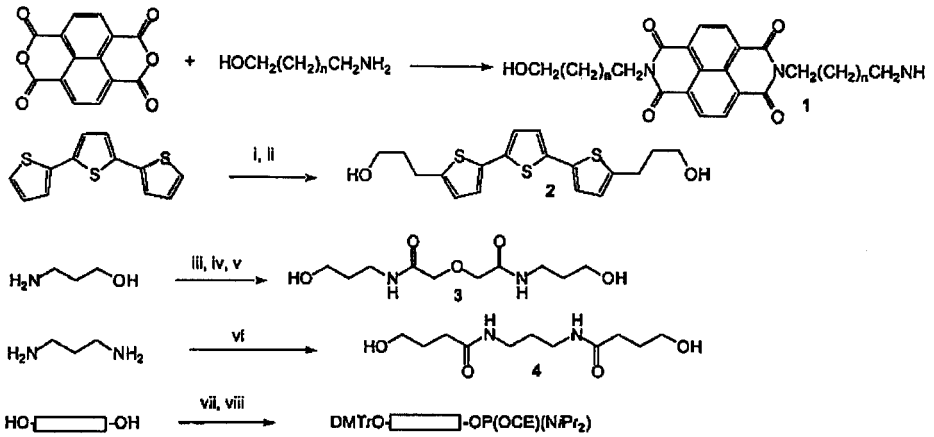

Figure 1. Synthesis of endcap spacers. **1** N,N′-bis(3-hydroxypropyl)naphthalene-1,4,5,8-tetracarboxylic diimide **2** 5, 5″-bis(3-hydroxypropyl)-2,2′ : 5′,2″-terthiophene: i) nBuLi, ii) $BF_3 \cdot Et_2O$,oxetane **3** N,N′-bis(3-hydroxypropyl)-2, 2′-oxyacetamide: iii) CH_2Cl_2, Et_3N, *t*-butyldimethylsilyl chloride, iv) CH_2Cl_2, Et_3N, diglycolyl chloride, v) 2.9% HCl in EtOH; **4** 4-Hydroxy-N-[3-(4-hydroxybutyrylamino)propyl]butyramide: vi) dimethylaminopyridine (DMAP), γ-butyrolactone, methanol DMTr-phosphoramidite synthesis: vii) Dimethoxytrityl chloride, DMAP, pyridine, viii) 2-cyanoethyl N,N′-diisopropylchlorophosphoramidite or 2-cyanoethyl bis(N,N′ diisopropyl)phosphoramidite, tetrazole.

pair. The hydrophilic endcaps are based on aliphatic diamides (**3**, **4**) that provide a more rigid structure than the commonly used poly(ethylene glycol) linkers.[2] Endcaps can be visualized as replacements for the loop region of stem-loop hairpin oligonucleotides. Endcaps are synthesized as dimethoxytrityl protected phosphoramidite derivatives that can be incorporated into the oligonucleotide during automated synthesis (Fig. 1). The distance spanned by the endcaps can be readily tuned by using an appropriate amino alcohol or carboxylic acid during synthesis of the endcap. We have investigated the thermal melting characteristics of endcapped four base-pair duplexes comprising one of each type of base-pair (AT, TA, GC and

Table 1. Melting temperatures (T_m) of complementary sequences linked by spacers **1–4** (**Π**).

	Spacer T_m°C				
Sequence	**1**, n = 3	**1**, n = 4	**2**	**3**	**4**
GCTA-**Π**-TAGC	62	53	66	41	44
GCAT-**Π**-ATGC				48	53
TACG-**Π**-CGTA				45	50
CGTA-**Π**-TACG				44	48
ATGC-**Π**-GCAT	75	66	62	51	56

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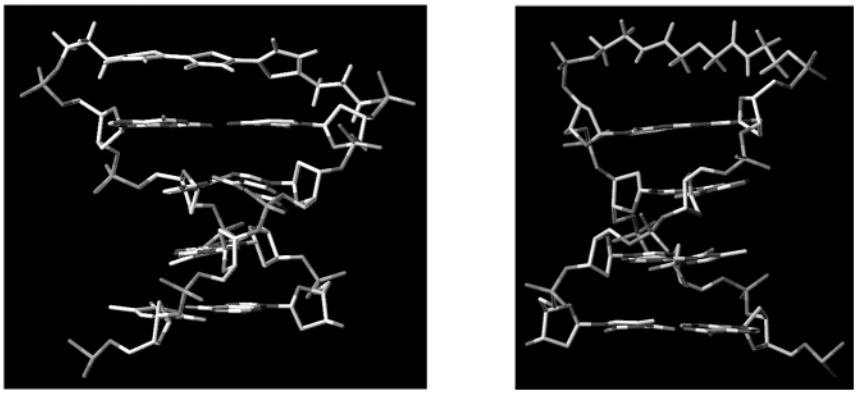

Figure 2. Models of complementary tetramers linked by endcaps **2** and **3**.

CG). The aromatic hydrophobic endcaps result in duplexes that have T_m values in the range of 53 to 75°C (Table 1). The aliphatic hydrophilic endcaps provide duplexes that have T_m values in the range of 41 to 56°C (Table 1). Molecular modeling indicates that the endcaps do not result in significant distortion of the helical duplex structure (Fig. 2). Endcapped duplexes are being studied as components of protein-DNA complexes for structural and biological studies.

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