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Tethered Naphthalene Diimide Intercalators Enhance DNA Triplex Stability

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Abstract—Naphthalene diimides function as effective intercalators and when tethered to the 5′-terminus of a pyrimidine-rich oligonucleotide can contribute significantly to the overall stabilization of DNA triplexes. This stabilization can be further enhanced by alterations to the linker tethering the DNA sequence and the intercalator. Less flexible linkers, and particularly one with a phenyl ring present, appear to permit the stabilization afforded by the bound intercalator to be transferred more effectively to the three-stranded complex. The conjugate containing the phenyl linker exhibits a T_M value that is increased by 28 °C relative to the unconjugated triplex. That the linker itself contributes to the observed stabilization is clear since introduction of the phenyl linker increases the observed T_M by 11 °C relative to a simple flexible linker.

Introduction

DNA triplexes were observed in the 1950s to result from the disproportionation of polymers composed of poly A and poly U[1] and subsequently similar observations were made for other polymer compositions.[2,3] In 1987, Dervan and coworkers[4] and Helene and coworkers,[5] demonstrated that purine-rich sequences of duplex DNA could be intermolecularly targeted in a sequence-specific manner with an oligonucleotide rich in pyrimidines. In the following year, similar duplex targeting was effected using a purine-rich strand.[6] A number of studies have subsequently explored the possibility of more generalized sequence targeting using a variety of triplex motifs[7,8] and including modified base residues[9–12] with varying levels of success.[13]

DNA triplexes, composed of three strands of DNA are generally less stable than corresponding duplexes and this observation may in part result from the additional charge–charge repulsive forces present when three negatively charged DNA strands are brought together in a single complex. A number of techniques, modified nucleosides, and ligands have been examined to increase the stability of three-stranded structures with a view to developing effective anti-gene or anti-sense therapeutics.[13] Tethering a ligand with the ability to provide added binding interactions between the third strand and the target duplex (or product triplex) can result in additional complex stabilization. Studies of this type have been reported for tethered intercalators,[14,15] groove binding agents,[16–18] and a variety of other ligands,[19,20] all of which can provide significant, or at least moderate enhancements in complex stability.

The tetracarboxylic diimides of naphthalene and perylene are effective intercalating agents,[21] and when tethered to one or both termini of a third strand,[22] the center of the third strand,[23] or in the center of a hairpin triplex,[24] they confer significant additional complex stability upon the DNA triplex. In the present work, we have continued these studies to explore the nature of the linker between the naphthalene diimide (NDI) intercalator and the oligonucleotide used in DNA triplex formation.

Results and Discussion

We have shown in previous studies that tethered NDI intercalators can be effective in stabilizing triple helical complexes formed from a third strand of DNA and a target duplex. The originally reported NDI-intercalator was tethered to one terminus of the third strand through a 2-hydroxyethyl-2-ethoxy linker. In that case, denaturation of the complex appeared to occur in two distinct

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steps when observed at 260 and 383 nm. Similar two-step transitions are observed for the current linkers as illustrated in Figure 1. The T_M values obtained at 260 nm have been interpreted as representing the dissociation of the third strand from the duplex while the values obtained at 383 nm were interpreted as reflecting the unstacking of the NDI residue from the duplex. The mid-point of the triplex to duplex transition as monitored at 260 nm does not correlate with the mid-point of the corresponding NDI unstacking event as monitored at 383 nm (Fig. 1). The fact that the values obtained at 383 nm are substantially higher than those observed at 260 nm (Fig. 1, Table 1) suggests a two-step process with an intermediate complex that could be characterized as having the NDI still intercalated into the duplex, but with the third strand no longer bound to the target. This intermediate state might in part be a result of the length and flexibility of the linker. Modifications to the linker to reduce its flexibility might be effective transferring the added stability of the intercalator to the entire complex and in doing so, enhance the T_M values measured at 260 nm.

Two linkers were examined in this study, one was prepared from 3-hydroxypropylamine containing *gem*-dimethyl groups on the central carbon atom (Scheme 1) and the second was derived from *p*-(2-hydroxyethyl)aniline. In both cases, the corresponding NDI derivatives (Fig. 2) were obtained simply by heating equivalent amounts of the linker, *N,N*-dimethylaminoethylamine, and the tetracarboxylic dianhydride derivative of naphthalene. Although this approach also generates significant amounts of both of the corresponding symmetrical diimides, in each case roughly a third of the product mixture was the desired unsymmetrical diimide that could easily be resolved by column chromatography. The phosphoramidite derivatives of the diimides proved to be quite unstable and could not be stored effectively even at −20 °C. Instead of employing the conventional approach, we tethered the NDI derivatives to the 5′-terminus of the DNA strands using a reverse coupling protocol. In this procedure, the 5′-terminal DMT-protecting group was removed and the unmasked hydroxyl

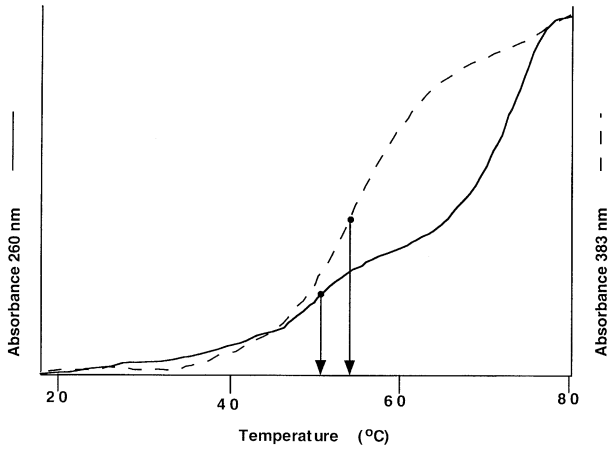

Figure 1. Thermally induced transitions foe the DMe–NDI conjugate measured at 260 nm (solid line) and 383 nm (dashed line). The mid-points of the transitions (arrows) do not coincide suggesting a biphasic transtion.

was converted to a terminal phosphoramidite (Scheme 2). Addition of the desired NDI derivative containing a free terminal hydroxyl group in the presence of tetrazole facilitated the conjugation of the NDI derivatives to the termini of the synthesized oligonucleotide strands. While this reaction does not occur with the high yields typical of nucleoside phosphoramidite couplings, the conjugation can be achieved in yields of about 30%, and in selected cases with yields of 50%. Conformation of the purity and composition of the conjugates could be accomplished by a combination of MALDI-TOF spectrometry, UV spectroscopy, and HPLC analyses.

Table 1. T_M value for NDI-conjugated triplexes

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5′   NDI-TTTCTTTTCTCTCTT      3′
 5′  GCGCGAAAGAAAAGAGAGAACCCGG  3′
 3′  CGCGCTTTCTTTTCTCTCTTGGGCC  5′
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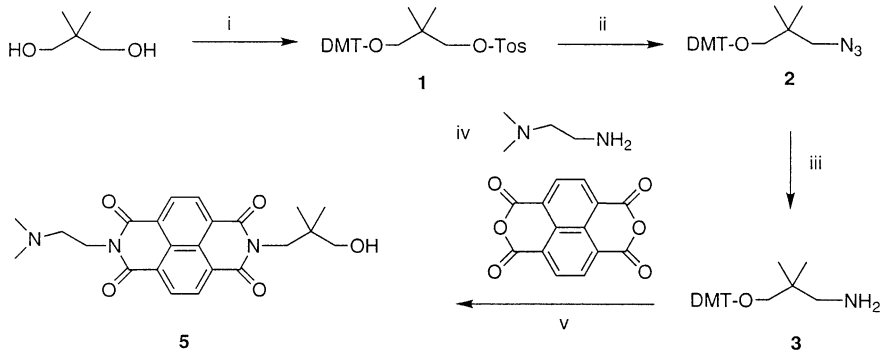
Sequence	Triplex T_M values (°C) measured at 260 nm		NDI unstacking T_M values (°C) measured at 383 nm
	pH 6.4	pH 7.0	pH 6.4
Native	30	22	—
NDI	47	22	59
DMe–NDI	52	40	54
Phen–NDI	58	48	61

The 15-mer polypyrimidine strand of the conjugates was designed to target a 15-mer polypurine strand in a target DNA duplex (see Table 1). The native triplex was formed from the target duplex and the unconjugated third strand, and this complex resulted in two thermally induced transitions as we have reported previously. The early transition occurred at 30 °C (pH 6.4) or 22 °C (pH 7.0) depending upon the pH of the solution, and the later transition occurred at 71 °C regardless of pH.[22] The early transition was interpreted to reflect dissociation of the third strand from the duplex and the later transition the denaturation of the duplex. When the third strand tethers the NDI ligand through 2-hydroxyethyl-2-ethoxy linker, these two transitions were observed to increase by 17 and 13 °C, respectively (Table 1). Additionally, when the temperature versus absorbance data is monitored at 383 nm, a single transition was observed to occur at 59 °C (pH 6.4). The transition observed at 383 nm was only present with the NDI conjugates and was interpreted to reflect the unstacking of the NDI chromophore from a position of intercalation within the target duplex. The observation that the unstacking T_M (383 nm) and the triplex transition at pH 6.4 (260 nm) differ by some 12 °C for the simple NDI conjugate indicates that dissociation of the conjugated third strand from the duplex occurs by at least a two-step process.

We have examined T_M effects for two additional complexes with varying linkers tethering the NDI intercalator and the DNA third strand. The DMe–NDI conjugate contains a linker reduced from six atoms to four and additionally the central carbon of the linker is *gem*-dimethyl substituted (Fig. 2); these modifications should reduce the flexibility of the linker. The Phen–NDI conjugate has a linker of about the same length as



Scheme 1. (i) DMT-Cl, Tos-Cl, Py; (ii) NaN_3, DMF, 90 °C; (iii) H_2 Pd/C 10%, MeOH; (iv) isopropanol, Δ; (v) 3% trichloroacetic acid/CH_2Cl_2.

the NDI conjugate, but incorporates a phenyl ring into it. The T_M of the triplex formed with the DMe conjugate was 52 °C, some 5 °C higher than that observed with the NDI conjugate (Fig. 1), and 22 °C higher than the unconjugated triplex (Table 1). The unstacking T_M (383 nm) for the intercalator was reduced by 5 °C relative to that observed for the NDI conjugate. By comparison, the T_M for the Phen–NDI conjugate was increased a further 6 °C, a total of 11 °C higher (pH 6.4) than the NDI conjugate and some 28 °C higher than the unconjugated triplex (Table 1).

The enhanced T_M values for the DMe–NDI conjugate may result from reduced linker flexibility as a result of the shortened linker and the introduction of the dimethyl substituents. With reduced flexibility the linker may permit the bound NDI to better stabilize the three-stranded complex. Since the unstacking of the NDI from the duplex occurs at a higher temperature than the dissociation of the third-strand bases from the duplex, a less flexible linker may better transfer the binding effects of the intercalator and result in more effective overall complex stabilization, as observed. The reduction in the NDI unstacking T_M may also reflect the shortened nature of the linker. With a shorter linker, the intermediate complex, that with a denatured third strand and an intercalated NDI may experience enhanced charge–charge repulsion between the duplex and denatured third strand. This effect could then reduce the NDI unstacking T_M.

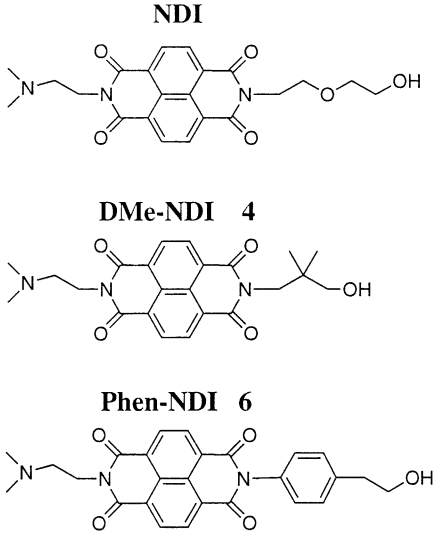
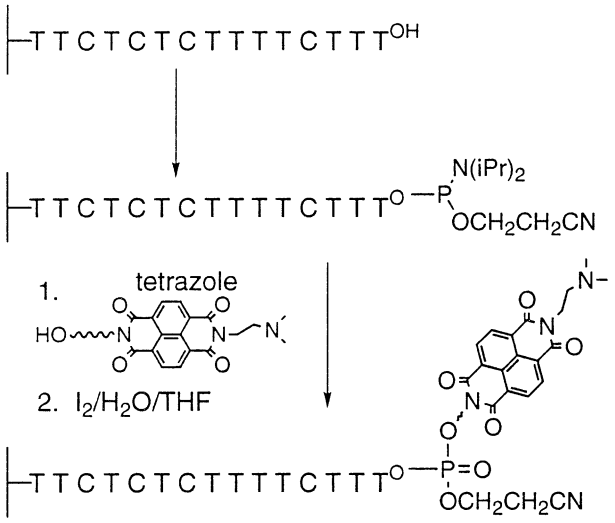


Figure 2. Structures of three naphthalene diimide based intercalators with linkers based upon 2-hydroxy-2-ethoxyethylamine (NDI), 3-hydroxy-2,2-dimethylpropylamine (DMe–NDI) and 2-hydroxyethylaniline (Phen–NDI).

A similar argument for increased overall stability, and a reduced unstacking T_M can be made for the Phen–NDI conjugate since the introduction of the aromatic ring into the linker should also reduce flexibility relative to the NDI conjugate. This effect is likely present since the triplex T_M value for the Phen–NDI conjugate increases by 11 °C relative to the NDI conjugate and is 5 °C higher than the DMe–NDI conjugate (pH 6.4). However, the unstacking T_M is also observed to increase relative to the DMe–NDI conjugate, and is even slightly higher than that of the NDI conjugate (Table 1). In fact, this complex exhibits the smallest difference (3 °C) between the T_M value obtained at 260 nm (58 °C) and the unstacking T_M obtained at 383 nm (61 °C). This observation suggests that the Phen–NDI is not only better able to intercalate into the duplex, but is also better able to transfer that stabilizing effect to the binding by the third strand. In addition to reducing flexibility in the linker, the presence of the phenyl ring in the Phen–NDI conjugate could provide additional stabilizing base stacking interactions. Simple model building suggests that the Phen–NDI ligand might be better able to position itself such that it cold benefit from base stacking interactions with all three base residues at the final position in the triplex (Fig. 3). The additional base stacking interactions resulting from the phenyl-based linker would not only enhance the unstacking T_M of the ligand, but could provide an additional stabilizing force for the DNA triplex itself. In a study of hairpin triplexes, we have also observed that increasing the size of the aromatic portion of the linker (from naphthalene to perylene) enhanced complex stability presumably through increased base stacking interactions with all three residues of the terminal base triplet. Other studies have also shown that ligands designed to interact with all three residues of the base triplet are more effective in triplex stabilization than simple DNA duplex intercalators.



Scheme 2.

Conclusions

Selected ligands capable of stacking with the base triplets present in three-stranded DNA triplexes can significantly enhance the overall stability of the complex. In the present study, the introduction of a NDI intercalator tethered to the DNA third strand with a phenyl-containing linker results in a 28 °C increase in the observed T_M value for the simple triplex. That the linker itself can contribute to such dramatic increases in T_M values is clear from the observation that the presence of the phenyl-containing linker enhances the observed T_M value by 11 °C relative to a simple 2-hydroxyethyl-2-ethoxy linker.

Experimental

NMR spectra were obtained on a Varian spectrometer (400 MHz) and contained trimethylsilane as an internal standard. HRMS and LRMS (FAB) mass spectra were obtained from the Mass Spectrometry Laboratory, School of Chemical Sciences, University of Illinois, Urbana, IL, USA. MALDI-TOF mass spectra were obtained from the Mass Spectrometry Laboratory, Merkert Chemistry Center, Boston College, MA, USA. Rotary evaporations were performed under reduced pressure utilizing Buchi systems. Thin layer chromatography (TLC) was performed on Silica Gel 60 F_{254} precoated on aluminum sheets (EM Separations Technology). Anhydrous solvents and starting materials were purchased from Aldrich Chemical Company and used without further purification. UV measurements were obtained using a Beckman DU 640 spectrometer. Oligodeoxyribonucleotides were synthesized on an Applied Biosystems 381A DNA Synthesizer. 2′-Deoxyribonucleotide phosphoramidites, fast deprotecting *N*-acetyl-2′-deoxycytidine phosphoramidite (Ac-dC), and 3′ terminal nucleoside controlled pore glass support (CPG), were purchased from Glen Research (Sterling, VA, USA). Fast flow high performance liquid chromatography (HPLC) was performed on a Waters 600 Pump Control Unit HPLC system, using Oligo R3 columns (Perseptive Biosytems, Framingham, MA, USA) with detection at 260 and 383 nm. Oligodeoxyribonucleotides were desalted with Econo-Pac 10DG disposable chromatography columns (Bio Rad, Hercules, CA, USA). T_M measurements were performed on an AVIV Spectrometer, Model 14DS UV/Vis.

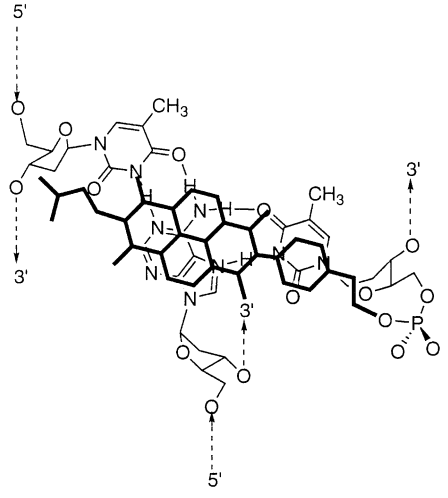


Figure 3. Possible relationship of the Phen–NDI intercalator (bold) and the terminal base triplet of a DNA triplex.

***O*-Dimethoxytrityl-*O′*-tosylneopentyl glycol (1).** To 4.0 g (0.04 mol) of neopentyl glycol, evaporated twice from freshly distilled pyridine and under nitrogen atmosphere, was added 50 mL of freshly distilled pyridine, followed by 13.6 g (0.04 mol) of dimethoxytrityl chloride, 7.6 g (0.04 mol) of tosyl chloride, and 2.4 g (0.02 mol) of dimethylaminopyridine. The mixture was stirred at room temperature overnight. The solvent was removed in vacuo until an oily residue was obtained, the desired product was subsequently purified by silica gel column chromatography, using a gradient of dichloromethane in hexane (0–40%) resulting in 11.2 g (0.02 mol) of 1, 48% yield, as a yellowish oil. R_f (chloroform): 0.68. ^{1}H NMR (DMSO-d_6): δ 0.78 (s, 6H, CH_3); 2.38 (s, 3H, Tosyl–CH_3); 2.70 (s, 2H, CH_2); 3.73 (s, 6H, –OCH_3); 3.83 (s, 2H, CH_2); 6.83–7.74 (m, 13H, Ar–H) ppm. ^{13}C NMR ($CDCl_3$): δ 22.9, 23.2, 37.2, 56.4, 68.7, 76.9, 86.7, 114.0, 127.6, 128.7, 129.0, 129.1, 129.4, 130.8, 131.1, 131.3, 137.0, 145.5, 146.0, 159.3 ppm. HR-MS calcd for $C_{33}H_{36}O_6S_1$ (M): 560.2233, found: 560.2233.

CAUTION: Azides are potentially explosive.[28,29]

***O*-Dimethoxytrityl-2-dimethyl-3-hydroxypropyl azide (2).** To 4.9 g (8.7 mmol) of **1** dissolved in 25 ml of dimethylformamide (DMF), was added 1.4 g (22 mmol) of sodium azide. The reaction flask was put on an oil bath and heated up to 90 °C, and the mixture was stirred for 2 days. The contents of the flask were then poured into a separatory funnel and the desired product was extracted with dichloromethane from water. The organic phase was collected, dried on $NaSO_4$ and fil-

tered. The solvent was removed in vacuo, and the desired product was subsequently purified by silica gel column chromatography, using a gradient of chloroform in hexane (0–40%) resulting in 3.2 g (7.5 mmol) of 2, 86% yield, as a yellowish oil. R_f (dichloromethane/hexane, 1/1): 0.60. ^{1}H NMR ($CDCl_3$): δ 0.97 (s, 6H, CH_3); 2.89 (s, 2H, CH_2); 3.34 (s, 2H, CH_2); 3.82 (s, 6H, –OCH_3); 6.86–7.49 (m, 13H, Ar–H) ppm. ^{13}C NMR ($CDCl_3$): δ 24.3, 38.1, 56.4, 61.1, 69.7, 86.7, 114.1, 127.7, 128.8, 129.2, 131.2, 137.3, 146.2, 159.3 ppm. HR-MS calcd for $C_{26}H_{29}N_3O_3$ (M): 431.2209, found: 431.2208.

***O*-Dimethoxytrityl-2-dimethyl-3-hydroxypropyl amine (3).** The primary amine was obtained by hydrogenation of the azide. 2.65 g (6.2 mmol) of **2** were put in a pressure resistant glass bottle, dissolved in 25 mL of methanol, and 0.9 g of Pd/C 10% w/w was added. The glass bottle was filled with H_2 to a pressure of 50 psi, and the mixture was shaken vigorously overnight. The contents of the glass bottle were filtered on Celite and washed with methanol. The filtrate was collected, concentrated and the desired product was subsequently purified by silica gel column chromatography, using a gradient of methanol in chloroform (0–5%) resulting in 1.3 g (3.4 mmol) of **3**, 54% yield, as an oil. R_f (methanol/dichloromethane, 1/19): 0.15. ^{1}H NMR ($CDCl_3$): δ 0.90 (s, 6H, CH_3); 2.58 (s, 2H, CH_2); 2.82 (s, 2H, CH_2); 3.79 (s, 6H, –OCH_3); 6.81–7.45 (m, 13H, Ar–H) ppm. ^{13}C NMR ($CDCl_3$): δ 23.2, 49.9, 55.5, 70.8, 86.5, 113.5, 127.1, 128.2, 128.3, 130.3, 135.9, 144.9, 158.7 ppm.

***N*-(*O*-Dimethoxytrityl-2-dimethyl-3-hydroxypropyl)-*N*′-[2-(*N*,*N*-dimethylamino)-ethyl]-1,4,5,8-naphthalene tetracarboxylic diimide (4) (DM e–NDI).** The synthesis of **4** was performed following a general procedure for the preparation of unsymmetrical naphthalene diimides.[21,22] To 680 mg (2.6 mmol) of 1,4,5,8-naphthalenetetracarboxylic dianhydride was added 1.03 g of **3** and 280 μL (2.6 mmol) of *N,N*-dimethylethylenediamine in 25 mL of isopropanol. The reaction mixture refluxed overnight. The volatiles were removed by rotary evaporation and the desired product was purified by silica gel column chromatography using a gradient of methanol in chloroform (0–3%). The unsymmetrical diimide eluted as the 'middle' compound between the two symmetric secondary products, obtained roughly in a 1:2:1 ratio. For 4, 465 mg (0.64 mmol) of product was obtained (yield 25%) as an orange solid. R_f (methanol/dichloromethane, 1/19): 0.55; mp 96–99 °C. ^{1}H NMR ($CDCl_3$): δ 1.05 (s, 6H, CH_3), 2.35 (s, 6H, N–CH_3); 2.68 (t, J = 6.6 Hz, 2H, CH_2); 2.98 (s, 2H, CH_2); 3.74 (s, 6H, –OCH_3); 4.25 (s, 2H, CH_2); 4.36 (t, J = 6.6 Hz, 2H, CH_2); 6.65–7.38 (m, 13H, Ar–H); 8.65–8.73 (m, 4H, Ar-H) ppm. ^{13}C NMR ($CDCl_3$): δ 25.0, 30.0, 38.8, 46.0, 48.0, 55.4, 57.2, 71.4, 113.0, 126.7, 126.8, 127.0, 127.8, 128.6, 130.4, 131.1, 136.5, 158.4, 163.3, 163.6 ppm. HR-MS calcd for $C_{44}H_{44}N_3O_7$ (M + H^+): 726.3179, found: 726.3182.

***N*-(2-dimethyl-3-hydroxypropyl)-*N*′-[2-(*N*,*N*-dimethylamino)-ethyl]-1,4,5,8-naphthalene tetracarboxylic diimide (5).** To 290 mg (0.41 mmol) of **4** dissolved in 2 mL of dichloromethane and stirred on ice for 5 min was added dropwise 2 mL of 3% trichloroacetic acid in dichloromethane and the reaction was stirred for an additional 5 min. The volatiles were removed by rotary evaporation and the desired product was purified by silica gel column chromatography using a gradient of methanol in dichloromethane (0–5%). For 5, 130 mg (0.31 mmol) of product was obtained (yield 76%) as a yellow solid. R_f (methanol/dichloromethane, 1/9): 0.50; mp 244–247 °C ^{1}H NMR (DMSO-d_6): δ 1.01 (s, 6H, CH_3), 2.35 (s, 6H, N–CH_3); 2.68 (t, J = 7.2 Hz, 2H, CH_2); 3.16 (s, 2H, CH_2); 4.19 (s, 2H, CH_2); 4.36 (t, J = 6.8 Hz, 2H, CH_2); 8.78 (s, 4H, Ar-H) ppm. ^{13}C NMR ($CDCl_3$): δ 24.6, 39.6, 39.9, 47.0, 58.1, 69.7, 128.0, 132.0, 132.5, 163.7, 165.2 ppm. HR-MS calcd for $C_{23}H_{26}N_3O_5$ (M + H^+): 424.1872, found: 424.1871.

***N*-[4-(2-Hydroxyethyl)phen]-*N*′-[2-(*N*,*N*-dimethylamino)-ethyl]-1,4,5,8-naphthalene tetracarboxylic diimide (6) (Phen-NDI).** The synthesis was performed following a procedure similar to that described for 4.[24,25] To 3.26 g (12.2 mmol) of 1,4,5,8-naphthalenetetracarboxylic dianhydride was added 2 g (14.6 mmol) of 4-aminophenylethyl alcohol, 1.6 mL (14.6 mmol) of *N,N*-dimethylethylenediamine, and 2.68 g (12.2 mmol) of zinc acetate dihydrate in 50 mL of pyridine. The reaction mixture refluxed overnight, the volatiles were removed by rotary evaporation and the desired product was purified by silica gel column chromatography using a gradient of methanol in chloroform (0–4%). The unsymmetrical diimide eluted as the 'middle' compound between the two symmetric secondary products. For **6** 1.12 g (2.44 mmol) of product was obtained (yield 20%) as a light yellow solid. R_f (methanol/dichloromethane, 1/9): 0.41; mp 246–248 °C. ^{1}H NMR (DMSO-d_6): δ 2.21 (s, 6H, N–CH_3); 2.54 (t, J = 7.2 Hz, 2H, CH_2); 2.81 (t, J = 7.2, 2H, CH_2); 3.68 (q, J = 6.8, 2H, CH_2); 4.17 (t, J = 6.8 Hz, 2H, CH_2); 4.74 (t, J = 5.2 Hz, 1H, OH); 7.29–7.38 (m, 4H, Ar–H); 8.67–8.68 (m, 4H, Ar–H) ppm. ^{13}C NMR ($CDCl_3$): δ 40.0, 40.2, 47.0, 58.2, 64.6, 127.6, 127.9, 129.5, 131.3, 132.1, 132.3, 163.8 ppm. HR-MS calcd for $C_{26}H_{24}N_3O_5$ (M + H^+): 458.1716, found: 458.1717.

DNA synthesis

The native 25-mer target sequences, 5′-d(GCGCGAAAGAAAAGAGAGAACCCGG)-3′, 5′-d(CCGGGTTCTCTCTTTTCTTTCGCGC)-3′, and the control 15-mer sequence, 5′-d(TTTCTTTTCTCTCTT)-3′ were prepared by solid-phase DNA synthesis using conventional protocols.[26] The products were characterized by MALDI-TOF mass spectrometry. For example, 5′-d(TTTCTTTTCTCTCTT)-3′: calculated 15-mer: 4442, found 15-mer: 4444.

Introduction of the NDI intercalators at the 5′ -terminus The phosphoramidite derivatives of the synthesized naphthalene diimides (NDIs) were difficult to store, or to use effectively in coupling reactions owing to their relatively rapid decomposition. The synthesis of the 15-mers functionalized at the 5′ terminus with the naphthalene intercalators was performed following the described reverse coupling procedure.[22,27]

The oligomers were synthesized in the normal fashion using the fast-deprotecting Ac-dC and dT phosphoramidites, and the DMT protecting group was removed after assembly of the 15-mers. Subsequently 135 μL (0.425 mmol) of 2-cyanoethyl tetraisopropylphosphorbisamidite diluted in 300 μL of freshly distilled acetonitrile was delivered to the columns to react with the free 5′-OH of the 15-mers over a 1 h period of time. The columns were then flushed with acetonitrile for 1 min and then removed from the synthesizer and the following step was performed manually. For the DMe-NDI-15-mer, 22 mg (50 μmol) of **5**, dissolved in 250 μL of anhydrous dichloromethane, were delivered together with 300 μL of a solution of sublimed 1H-tetrazole (as the activating agent) in acetonitrile using a syringe. The coupling reaction between the naphthalene moiety and the terminal phosphoramidite was allowed to occur for 1 h. The column was then reconnected to the synthesizer, rinsed with acetonitrile, and oxidized in the normal manner. Phen-NDI-15mer, was prepared in a similar fashion, using 22 mg of **6** (50 μmol), dissolved in 250 μL of anhydrous DMF and allowing the coupling reaction to occur overnight (~18 h).

Upon completion of the DNA syntheses, the beads were dried in vacuo and deprotected in 5 mL of 0.05 M K_2CO_3 in methanol for 2 h at ambient temperature. The pH was then adjusted to approximately 6.5 with 2% acetic acid. The solutions were filtered to remove the CPG beads, and concentrated in vacuo to remove methanol. Purification of the conjugated oligonucleotides from failed sequences and unconjugated 15-mer was accomplished by fast flow HPLC (4.6×100 mm column), starting with 0% B for 1 min, then using a linear gradient 0–40% B over 6.0 min, and then 40% B for 2 min (A: 50 mM triethylammonium acetate, adjusted to pH = 7.0 with glacial acetic acid; B: 50 mM triethylammonium acetate in 70% acetonitrile). The desired sequences were collected as the latest eluting peaks with a yield of roughly 30% each. These products were then desalted (Sephadex G-10), and stored at −20 °C.

MALDI-TOF MS calcd for 5′ DMe–NDI-TTTCTTTTCTCTCTT 3′ (15-mer$^+$): 4928, found: 4938.

MALDI-TOF MS calcd for 5′ Phen–NDI-TTTCTTTTCTCTCTT 3′ (15-mer$^+$): 4962, found: 4972.

Thermal melting analyses

T_M values were obtained for complexes containing a 1:1:1 mixture of oligonucleotides at a concentration of 1 μM in 50 mM NaCl, 10 mM $MgCl_2$ and 10 mM Pipes (pH 6.4 and 7.0). Solutions were heated in 1 °C steps and absorbances were recorded after temperature stabilization using an AVIV 14DS spectrophotometer. Absorbance and temperature readings were plotted using Igor Pro software. T_M values were determined from first order derivatives as well as graphically from absorbance versus temperature plots. Differences greater than ±1 °C were not observed when using the two procedures.

Acknowledgements

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