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An *Octakis*-Intercalating Molecule

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Abstract—Herein we report the synthesis and characterization of a polyintercalator with eight potential intercalating 1,4,5,8-naphthalenetetracarboxylic diimide (NDI) units linked in a head-to-tail arrangement via a peptide linker. UV spectroscopy and viscometry measurements indicated the molecule binds to double-stranded DNA with all eight NDI units intercalated simultaneously. Competition dialysis and DNAse 1 footprinting studies revealed a preference for GC-rich regions of DNA, and circular dichroism studies revealed significant distortion of B-form DNA upon binding. Our so-called 'octamer' represents, to the best of our knowledge, the first intercalator that binds as an *octakis*-intercalator, capable of spanning at least 16 base pairs of DNA.

Introduction

The quest for gene specific binding agents has been fueled by the desire to modulate gene expression. A requisite property of such an agent is DNA sequence selectivity sufficient to recognize chosen sites in a target genome. For example, it has been estimated that a molecule must have the ability to recognize at least 16 base pairs to bind a unique site in human DNA.[1] We have been investigating a new polyintercalation strategy for the recognition and binding of long DNA sequences.[2] Herein is reported the synthesis and characterization of what is, to the best of our knowledge, the first *octakis*-intercalating molecule, spanning at least 16 base pairs of DNA when bound.

Attempts to create sequence-selective DNA binding agents have culminated in the production of minor groove-binding hairpin polyamides that can specifically recognize 16 base pairs or more of DNA in a programmable fashion.[3–5] This approach uses pyrrole and imidazole units to discriminate between the different base pair combinations in duplex DNA.[6]

Polyintercalation represents an alternative strategy for the sequence specific recognition of DNA, and there has been significant interest in the design and synthesis of high affinity, sequence selective DNA intercalators.[7–9] Several synthetic *bis*-intercalators have been described with high affinity,[6,10–13] and a few reports of *tris*-intercalators have appeared. A particularly noteworthy example is the ultra-high-affinity *tris*-acridine synthesized by Laguaa et al., which features a long, flexible linker between intercalating units.[14] Most previous polyintercalator designs involved aromatic intercalating groups pendant off of a central backbone, reminiscent of clothes hanging on a clothesline. Such an arrangement may encourage significant self-stacking of the aromatic units, thereby interfering with DNA-binding. An alternative *tris*-intercalator design was investigated by Takenaka et al. in which the central intercalator was linked on both ends to other intercalators, analogous to three beads on a string.[15] A threading mode of intercalation was proposed for the central aromatic unit, leading to a model of *tris*-intercalation in which linkers resided in both DNA grooves.

We have described a new class of polyintercalators intended to provide a modular framework and facile synthesis.[2] Our polyintercalator design (Fig. 1) consists of 1,4,5,8-naphthalenetetracarboxylic diimide (NDI) units linked in a head-to-tail arrangement via a peptide linker.[2] This design enabled the synthesis of the first known *tetrakis*-intercalator, spanning at least eight base pairs. The NDI moiety has been shown previously to be a threading intercalator, and substituents even as large

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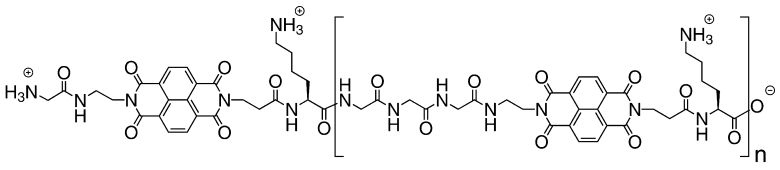

n	Compound
0	1 (monomer)
1	2 (dimer)
3	3 (tetramer)
7	4 (octamer)

Figure 1. General structure of polyintercalators.

as adamantyl groups were found in both the major and minor grooves.[16]

A long threading polyintercalator would 'snake' back and forth through the helix, causing the linkers to reside in the minor and major grooves in an alternating fashion. Having both grooves of the DNA occupied simultaneously represents a particularly attractive feature of a threading polyintercalator design. A threading polyintercalator might also be expected to exhibit relatively high affinity, due to the 'tangled' nature of the binding mode.

Importantly, a combinatorial study has shown that sequence specificity of an NDI-peptide-based *bis*-intercalator can be altered dramatically by changing the linker between intercalating NDI groups.[17] In addition, we have recently used NMR to derive the structure of a *bis*-intercalator with four amino acids between NDI units. In the structure, the molecule binds sequence specifically to DNA in a threading manner by spanning four base pairs, not the expected two base pairs (personal communication). This new information, coupled with the modular design and facile solid-phase synthesis of our NDI polyintercalators, implies a general strategy for creating polyintercalating molecules capable of high-affinity and sequence-selective binding to relatively long stretches of DNA.

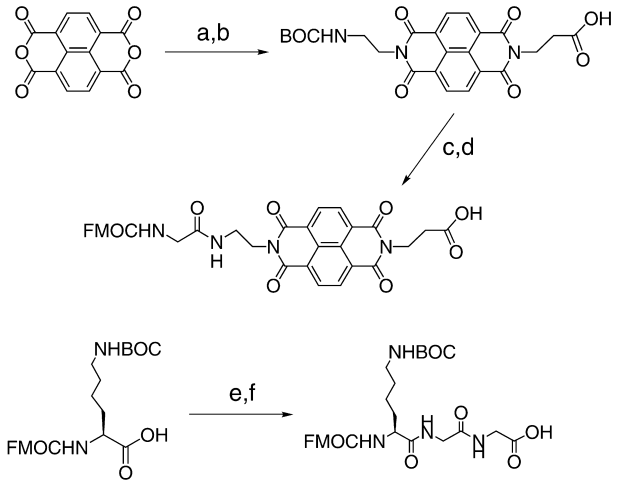

Figure 2. Synthesis of intermediates. Reagents: (a) β-alanine benzyl ester *p*-toluenesulphonate salt, BOCNH–$(CH_2)_2$–NH_2, *i*-Pr_2Net; (b) H_2, Pd/C; (c) TFA/CH_2Cl_2; (d) Fmoc-Gly-OC_6F_5, HOBT, 2,6-lutidine; (e) Gly-Gly-OBzl *p*-tosylate, PyBOP, *i*-Pr_2NEt; (f) H_2, Pd/C.

Results

Synthesis

The polyintercalators (Fig. 1) were synthesized according to FMOC-based solid-phase peptide synthesis protocols using two alternating building blocks, a peptide linker and a 1,4,5,8-naphthalenetetracarboxylic diimide fragment (Fig. 2), with FMOC-Lys(BOC)-functionalized Tenta-Gel™ resin as the solid support (Fig. 3). The structure of **4** was confirmed by high-field NMR and mass spectral analysis. The synthesis and characterization of the related compounds **1**–**3** have been reported previously.[2]

Hypochromicity

The absorbance spectra of the dimer **2**, tetramer **3**, and octamer **4** were measured in aqueous buffer, in 2% SDS solution, and in the presence of calf thymus (CT) DNA (Table 1). The absorbance of the intercalators in aqueous buffer was less than in 2% SDS. This hypochromism in aqueous buffer is attributed to partial self-stacking of the NDI units, an interaction that is presumably disrupted by 2% SDS detergent.[18] It is worth

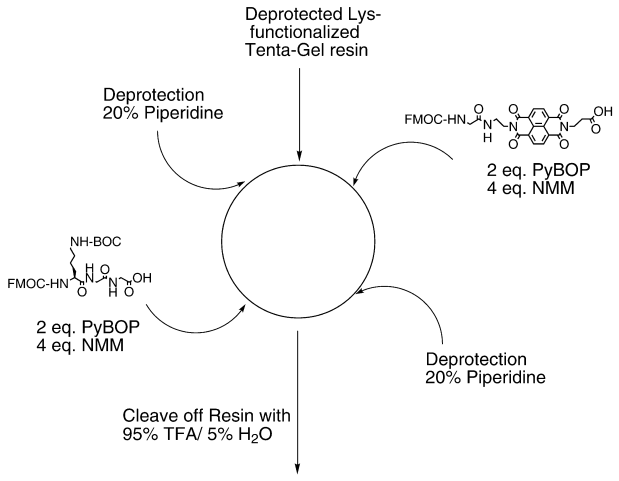

Figure 3. Solid-phase peptide synthesis cycle.

Table 1. Extinction coefficients of intercalators at 386 nm

	ε_F[a]	ε_{SDS}[b]	ε_{CT}[c]	ε_{GC}[d]	ε_{AT}[e]
Dimer	32,000	44,000	24,000	25,000	24,000
Tetramer	58,000	96,000	50,000	47,000	49,000
Octamer	104,500	190,000	92,000	98,000	89,000

[a]Measured in 10 mM Tris, 50 mM NaCl, 1 mM EDTA.
[b]Measured in same buffer with 2% SDS.
[c]In the presence of a large excess of calf thymus DNA.
[d]In the presence of a large excess of poly(dGdC)$_2$.
[e]In the presence of large excess of poly(dAdT)$_2$.

noting that in 2% SDS solution, the molar extinction coefficient of the octamer **4** was twice that of the tetramer **3**, and 4 times that of the dimer **2**, as expected for a homologous series of molecules containing non-interacting chromophores.

In the presence of CT DNA, poly(dGdC)$_2$ and poly(dAdT)$_2$, all molecules exhibited even further hypochromism compared to the absorbance observed in buffer alone. Such hypochomism is consistent with intercalation, a result of having the intercalating aromatic units stacked among DNA base pairs. Significantly, the molar extinction coefficients of the DNA-bound species were again multiples of each other, with the octamer **4** exhibiting a molar extinction coefficient twice that of the tetramer **3** and 4 times that of the dimer **2**. The dimer **2** and tetramer **3** were previously shown to be fully intercalated.[2] Any NDI units not intercalated when **4** was bound to DNA would be expected to lead to an increase in molar extinction coefficient above that seen. Thus, these extinction coefficient measurements are consistent will full intercalation by the octamer **4**.

Viscometry

The extent of intercalation by **2**, **3** and **4** were further probed using viscometry, according to the method of Cohen and Eisenberg.[19] Measurements were taken 15 min after mixing with CT DNA, then repeated 24 h later to confirm equilibrium had been reached. Both sets of data were identical. Figure 4 shows the concentration-dependence of the viscosity measured for each molecule. The observed slope of the data for **4** is precisely twice that of **3** and 4 times that of **2**. Thus, the viscometry experiments are also consistent with the octamer **4** being an *octakis*-intercalating molecule.

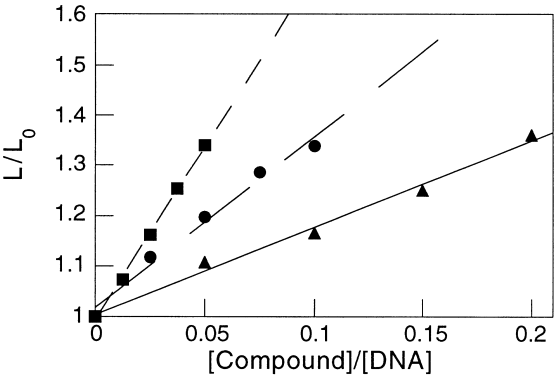

Figure 4. Viscometric data of dimer (▲, m = 1.7), tetramer (●, m = 3.4), and octamer (■, m = 6.9) with sonicated calf thymus (CT) DNA in TE buffer (10 nM Tris, 50 mM NaCl, 1 mM EDTA).

DNase I footprinting

DNase I footprinting experiments were carried out using **4** and a synthetic oligonucleotide target sequence that contained a 16 base-pair dG–dC sequence (Fig. 5).[2] The DNase I footprinting confirmed the expected dG–dC sequence preference for bound **4**. In addition, the lowest concentration of octamer **4** that produced a full footprint, namely 16 nM, can be used as an estimate of the dissociation constant of the octamer from these sequences. Consistent with full intercalation, the DNase I footprint spans the entire 16 base-pair dG–dC sequence.

Circular dichroism

A fully-bound, threading polyintercalator would likely cause a substantial distortion of the DNA duplex, so the circular dichroism (CD) of **4** bound to four different nucleic acid duplexes was investigated: poly(dAdT)$_2$, poly(dGdC)$_2$, CT DNA, and an RNA/DNA hybrid,

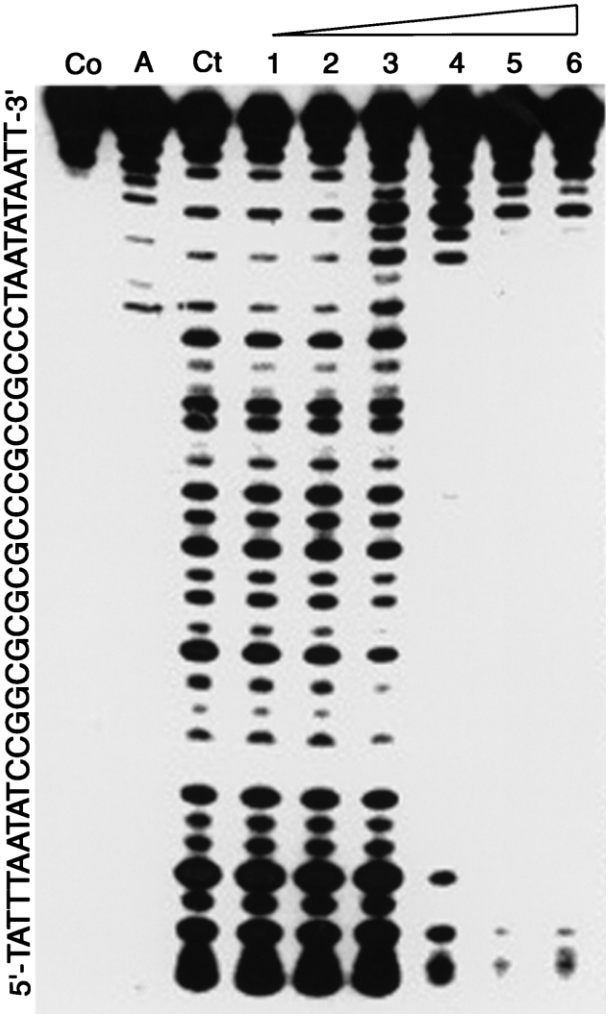

Figure 5. DNase I footprint of the octamer at increasing concentrations. Lanes 1–6 contain 2, 4, 7.8, 15.6, 31.25 and 62.5 nM octamer, respectively.

polyA/polydT. For comparison, the CD spectra of **2** and the NDI monomer **1** were also recorded with each type of DNA. Finally, ethidium bromide was used as a non-threading intercalator control in the experiments.

The CD spectra (Figs 6 and 7) show changes upon the octamer **4** binding to all four of the nucleic acid duplexes, with the CD spectrum of poly(dAdT)^2 showing the greatest alteration. The CD spectra of **4** bound to all four types of duplexes display unique features, yet the spectra can be placed into two general categories. The spectra taken with **4** bound to CT DNA and poly(dGdC)$_2$ have a negative peak near 235 nm, followed by a shoulder near 250 nm and positive peak of modest size at 275 and 285 nm, respectively. The spectra taken with poly(dAdT)2 and polyA/polydT have a more pronounced negative peak at 235 nm, followed by relatively intense positive peaks centered near 270 nm.

For each type of DNA, the spectra taken with bound dimer **2** or monomer **1** were similar to the spectra for the samples containing the octamer **4**. In contrast, the spectra of the samples with the non-threading intercalator ethidium bromide were quite distinct, resembling more the starting duplexes than the samples with bound **4**.

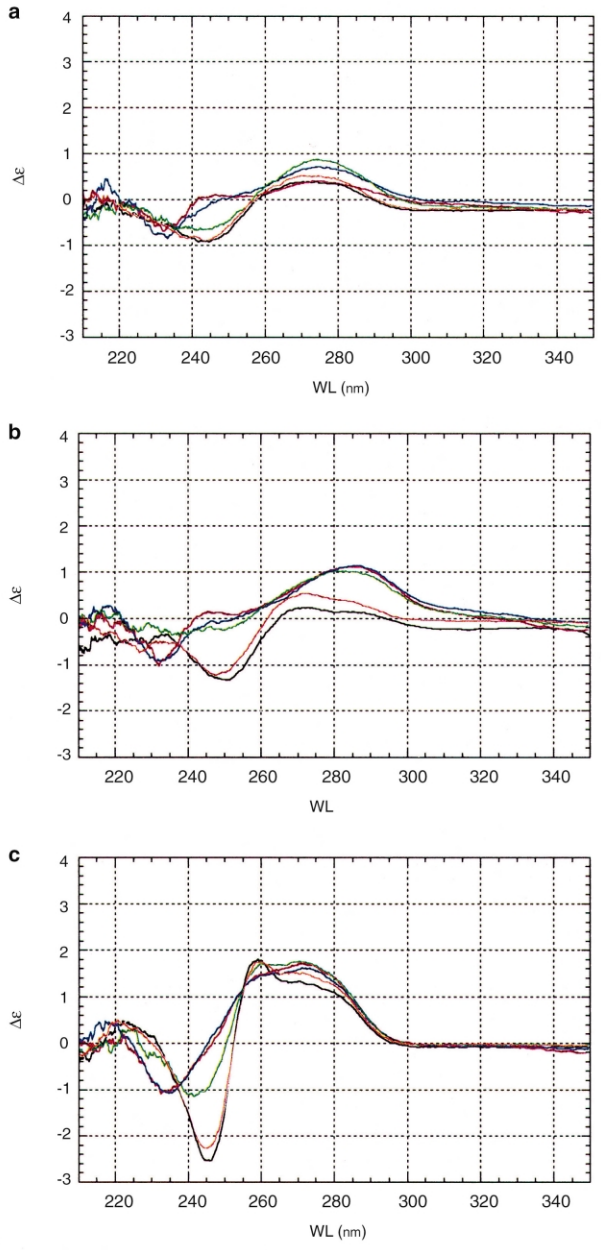

Figure 6. Circular dichroism traces of intercalators with DNA; a, calf thymus DNA; b, poly(dGdC)$_2$; c, poly(A):poly(dT); black, DNA alone; red, DNA and dimmer; blue, DNA and octamer; green, DNA and monomer; orange, DNA and ethidium bromide.

Competition dialysis

A competition dialysis technique was employed to determine the specificity of **4** for various nucleic acid species under equilibrium conditions.[20] The experiment consists of 13 dialysis tubes containing different nucleic acid samples, all at equal concentrations. These tubes were then dialyzed against a 1 μM solution of **4** and allowed to equilibrate. The amount of **4** bound to the different samples was determined by UV spectroscopy before and after addition of a 2% SDS solution

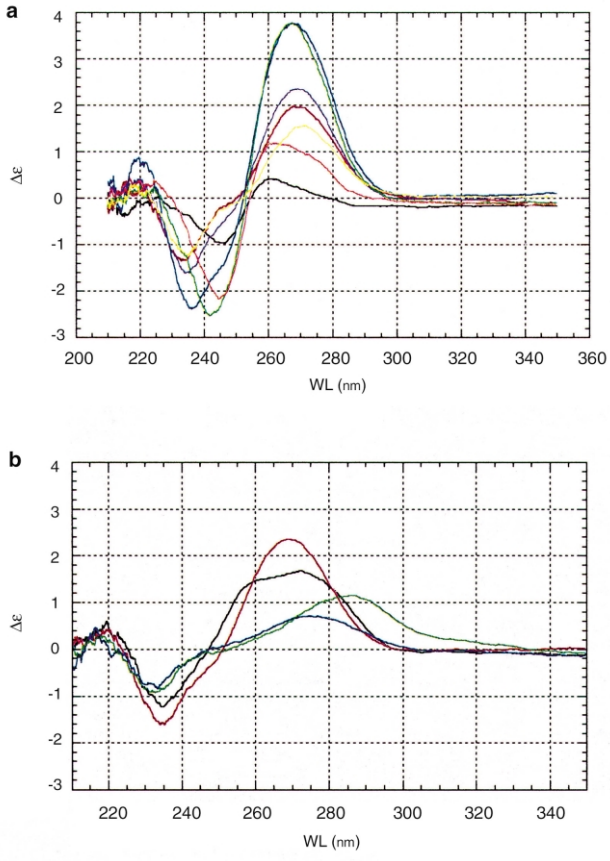

Figure 7. Circular dichroism traces of intercalators with DNA: a, poly(dAdT)$_2$; black, DNA alone; red, DNA and dimer; blue, DNA and octamer at 100% intercalation; yellow, DNA and octamer at 125% interacalation; purple, DNA and octamer at 80% intercalation; green, DNA and monomer; orange, DNA and ethidium bromide; b, comparison of octaner with different types of DNA; black, poly-(A)poly(dT); red, poly(dAdT)$_2$; blue, calf thymus DNA; green, poly(dGdC)$_2$.

to dissociate octamer from the DNA. The data in Table 2 show a preference for the high GC-containing nucleic acids CT DNA, *Micrococcus lysodeikticus* DNA, poly(dGdC)2, Z form DNA, and quadraplex DNA. Lower binding was observed for AT(U)-rich sequences, the exception being polyA/polydT, which showed higher binding. No binding to single-stranded poly(dA) or poly(dT) was detected.

Discussion

Degree of intercalation

The UV spectra and viscometry measurements are consistent with the octamer **4** being fully intercalated in double-stranded DNA. In particular, the extinction coefficient of **4** bound to calf thymus DNA is exactly twice that seen with the tetramer **3** and 4 times that of the dimer **2**. The viscometry results show the identical trend in which the slope of the viscometry plot of **4** is precisely twice that of **3** and 4 times that of **2**. Thus, it appears that our polyintercalator design, in which NDI units are linked in a head-to-tail fashion by polypeptide chains, successfully provides for full intercalation of even relatively long molecules. At this point, it is not clear if there is an upper limit to the number of NDI units that can be incorporated into a polyintercalator, while still maintaining full intercalation.

Assuming full intercalation, the octamer would be expected to cover at least 16 base pairs.[21] The observed DNase I footprint is fully consistent with such a large binding site. A recent NMR study has found that certain NDI dimers with the same linker length between NDI units as the octamer, namely four amino acids, can actually span four base pairs between intercalators if the intervening DNA sequence is appropriate (personal communication). This opens up the possibility of expanding an octamer binding site well beyond 16 base pairs, and future studies will investigate this possibility.

Affinity for DNA

The maximum possible binding constant of a *bis*-intercalator can theoretically be estimated as the square of the corresponding *mono*-intercalators, corrected for a tethering effect of the linker.[22] The NDI monomer 1, with attached amino acids and an overall charge of +1, has a dissociation constant of 5×10^{-6} M with CT DNA, corresponding to a ΔG of −7.2 kcal/mol at 25 °C. The dimer **2** exhibited a ΔG of −9.0 kcal/mol indicating that the second NDI unit/positive charge added only −1.8 kcal/mol binding energy.[23] As more NDI/positive charge units are added past the dimer, a consistent yet relatively small contribution of −0.2 to −0.3 kcal/mol was made to the total ΔG of binding (Table 3). These latter free-energy increments are smaller in magnitude than expected even from an electrostatic point of view, since, under these solution conditions (50 mM NaCl), a free-energy increment of $\Delta G_{pe} = RT(0.88)\ln[0.05] = -1.6$ kcal/mol would be expected from the addition of a single positive charge to the ligand.[24]

Table 2. Competition dialysis experiment data

Type of DNA	(μM)[a]
PolydT	0
PolydA	0
C. perfringens DNA	4.4
Calf thymus DNA	6.5
M. lysodeikticus	7.3
PolydA/polydT	0.1
$Poly(dAdT)_2$	3.3
$Poly(dGdC)_2$	6.2
PolyA/polydT	4.0
PolyA/polyU	0.4
Z DNA (Br-$poly(dGdC)_2$)	5.6
Triplex ($PolydA/polydT)_2$)	2.0
Quadraplex ($(5'TG_{20}T)_4$)	5.3

[a]The numbers represent the amount of intercalator, measured in units of μM, bound to each kind of DNA.

The lower than expected contribution of additional linker/NDI units, especially for **3** and **4**, could have a number of possible explanations that are perhaps acting in concert. For example, there is expected to be an unfavorable contribution from the rotation restriction of at least some of the linker bonds when bound.[25] To a first approximation, it is reasonable to expect that this rotational effect should be consistent as the number of linker/NDI units are added in a molecule, so that each additional linker/NDI unit of the dimer, tetramer, and octamer are influenced in a similar fashion. Thus, the relatively large difference observed between the dimer and the longer derivatives indicate other factors must be important, especially for **3** and **4**.

Self-stacking of the NDI units, indicated by the hypochromism observed in aqueous buffer compared to 2% SDS solution (Table 1), is occurring with the polyintercalators. Self-stacking must be disrupted prior to DNA binding, so it could be preventing the additional NDI units from making more of a contribution to overall octamer binding. The ratios of extinction coefficients in buffer versus 2% SDS for **2**, **3** and **4** are 0.73, 0.60, and 0.55, respectively, indicating that **3** and **4** are considerably more stacked compared to the dimer. Thus, self-stacking is likely inhibiting binding for these longer systems to a larger degree than the dimer **2**, perhaps explaining in part the observed relative affinities.

In addition, it is also possible that structural features of the linker are inhibiting DNA binding. Linker effects could be particularly important for a threading polyintercalator of repeating structure, since an optimum linker residing in the minor groove would be expected to

Table 3. Free energy contribution to binding for each additional chromophore in the homologous series of compounds

Molecule	ΔG (kcal/mol)	K_D (M)	Contribution from each additional NDI unit (kcal/mol)
Monomer	−7.2	5×10^{-6}	N/A
Dimer	−9.0	2.5×10^{-7}	−1.8
Tetramer	−9.4	1.25×10^{-7}	−0.2
Octamer	−10.24	1.56×10^{-8}	−0.3

be different from the optimum linker residing in the major groove. Such groove-specific linker effects should be manifest only in the tetramer **3** and octamer **4**, in which a threading binding mode would necessarily place linkers in both grooves. The dimer **2** would presumably place the linker only in the more favorable groove, and thus avoid unfavorable groove interactions. Thus, like the self-stacking effects described above, linker groove specificity could help explain the observed large change seen between energetic contributions from additional linker/NDI units in the dimer relative to the longer polyintercalators. Linker restricted rotation, NDI unit self-stacking, and groove specificity are currently being addressed in next-generation linker designs.

Nucleic acid preference

Results of the octamer **4** competition dialysis experiments show a clear preference for poly(dGdC)$_2$ over poly(dAdT)$_2$. Additionally, in the footprinting analysis there is a strong preference for binding the GC-rich region. Consistent with a general GC preference, the other nucleic acids with appreciable GC content; CT DNA, *M. lysodeikticus* DNA, Z form DNA, and quadraplex DNA, all showed relatively strong binding. In fact, for the heterogeneous DNAs, the amount of binding was correlated to GC content, being highest for *M. lysodeikticus* DNA (72% GC), lowest for *Clostridium perfringens* DNA (31% GC) and intermediate for CT DNA (42% GC).[20]

DNA structure recognition/distortion

At this time it is not clear whether the relatively high affinities seen with the Z-form sample used in the equilibrium dialysis experiment is due to the unique three-dimensional structures of this system, or simply the high GC content following an allosteric conversion back to the right-handed helical form upon polyintercalator binding. However, the observed enhanced binding to polyA/polydT compared to poly(dAdT)$_2$ could very well have a structural base, since the former duplex adopts an A-form helix in solution. Consistent with having a preference for A-form helices, the CD-spectra recorded for the CT DNA, poly(dGdC)$_2$ and poly(dAdT)$_2$ samples show an increase in the maxima at 270–290 nm, suggesting a change toward an A-like conformation. This is further supported by the observation that there was but a modest perturbation in the CD signal upon octamer binding to polyA/polydT.

DNA binding mode

NDI monomers are threading intercalators,[16] and an NDI dimer has been found to be threading in our recent detailed NMR analysis (pers. commun.). In the CD experiments reported here, the octamer **4** displayed spectra that were very similar to those of these known threading intercalators, and quite distinct from the spectra produced by the non-threading control ethidium bromide. Thus, to the extent that CD spectra can be used to assign bound structure, a threading mode for the octamer **4** is indicated. In addition, the similar spectra among the series suggests the linker does not introduce any large distortions in the bound complex compared to the NDI monomers or dimers. A detailed NMR or X-ray structure will be required to further refine the structure of an octamer-bound duplex and thereby definitively establish the mode of polyintercalation.

Conclusion

A polyintercalating octamer based on the NDI-peptide linker design was synthesized that exhibits a preference for binding to GC-rich regions of DNA. The available spectroscopic evidence is most consistent with full intercalation of all eight NDI units. To the best of our knowledge, the tetramer based on the same design was previously the longest polyintercalator to display full intercalation. Thus, the octamer **4** has established the new standard of *octakis* polyintercalation. The preliminary DNAse I footprint analysis indicated that the octamer was capable of spanning at least 16 base pairs of GC-rich sequence when bound. A more detailed footprinting analysis will be required to establish the largest binding site size that can be accommodated by a single bound octamer.

Recent studies in the lab have found that alteration in linker amino acid sequence can have a profound influence on DNA sequence selectivity, due to some specific interactions between groups on the linker and the DNA base pairs.[17] Furthermore, the same type of amino acid-based linkers used between NDI units in the octamer can span up to four base pairs of duplex DNA (pers. commun.). Future NDI-peptide linker designs will take advantage of both the altered linker sequences and four base-pair sites between NDI units to create polyintercalators that recognize extremely long stretches of DNA in a sequence-specific manner. In addition, attempts will be made to find designs that maximize the energetic contributions of additional linker/NDI units, so that the promise of extraordinarily high-affinity polyintercalators with programmable sequence specificity may soon be realized.

Experimental

General

NMR spectra were recorded on a Varian 300 MHz instrument. Starting materials and solvents were used without purification, unless otherwise stated. Nucleic acids were purchased from Sigma (St. Louis, MO) and Pharmacia (Piscataway, NJ), and organic chemicals were purchased from Aldrich (St. Louis, MO). DNAse 1 footprinting experiments were carried out as described.[2]

***N*-(2-*tert*-Butoxycarbonylaminoethyl)-*N*-(2-carboxyethyl)-1,4,5,8-napthalenetetracarboxylic diimide.** 1,4,5,8-Napthalenetetracarboxylic dianhydride (5.9 g, 22.0 mmol) was suspended in *i*-PrOH. β-Alanine benzyl ester *p*-toluenesulfonate salt, mono-*tert*-butoxycarbonylaminoethylamine, and *N*,*N*-diisopropylethylamine were added

and the solution heated to reflux for 18 h. The mixture was concentrated in vacuo and partitioned between CH_2Cl_2 and 0.1 M sodium citrate buffer (pH 4.5). The organic layer was extracted with buffer (2×) saturated $NaHCO_3$ (2×) and brine and dried over sodium sulfate, filtered, and concentrated in vacuo. The resulting solid was suspended in absolute ethanol and purged with nitrogen. Pd on C (10%, 5 g) was added and the flask charged with H_2 at atmospheric pressure and stirred 48 h. The solution was concentrated to 100 mL and 10% triethylamine in CH_2Cl_2 was added and the solution filtered through a pad of Celite and washed with 10% triethyl amine. The filtrate was concentrated and purified by silica gel chromatography with 0–10% MeOH in 10% Et_3N in CH_2Cl_2. The desired product was concentrated, redissolved in 10% MeOH in CH_2Cl_2 and 20 mL of HOAc was added to precipitate the product and the suspension was stirred at room temperature overnight. The precipitate was filtered, rinsed with MeOH and dried in vacuo to yield the product (3.03 g, 28%) as a tan solid. ^{1}H NMR (DMSO-d_6) δ 8.58 (s, 4H), 6.9 (br t, 1H), 4.25 (t, J = 7.5 Hz, 2H), 4.12 (br t, 2H), 3.27 (br q, 2H), 2.62 (t, J = 7.4 Hz, 2H), 1.20 (s, 9H); ^{13}C NMR (DMSO-d_6) δ 172.4, 162.7, 162.4, 155.8, 130.4, 130.3, 126.4, 126.1, 77.5, 37.6, 36.1, 32.0, 28.1; HRMS (FAB) m/z 482.1547 (482.1563 calcd for $C_{24}H_{24}N_3O_8$).

N-2-(N^{α}-9-Fluorenylmethoxycarbonylglycyl)aminoethyl-N'-(2-carboxyethyl)-1,4,5,8-napthalenetetracarboxylic diimide[1] (compound A). The above product (2 g, 4.16 mmol) was suspended in 15 mL CH_2Cl_2 and 15 mL TFA was added slowly. After standing for 10 min, the solution was evaporated and the residual TFA removed by azeotropic evaporation (2×) from heptane. The resulting solid was triturated with ether, filtered, and dried in vacuo. The solid was suspended in DMF (15 mL) and N-9-fluorenylmethoxycarbonylglycine pentafluorophenyl ester (1.92 g, 4.16 mmol) was added, followed by 1-hydroxybenztriazole (HOBT, 561 mg, 4.16 mmol) and 2,6-lutidine (890 mg, 8.32 mmol). After stirring for 4 h, the mixture was poured slowly into rapidly stirred H_2O (150 mL), and the resulting suspension was allowed to stand for several hours. The mixture was filtered and the resulting yellow solid was rinsed with H_2O and dried in the presence of P_2O_5 in a vacuum desiccator overnight. The crude product was triturated with Et_2O several times (to remove the residual 1-hydroxybenztriazole and pentafluorophenol), filtered, and dried in vacuo to the product (77%) as a yellow powder. ^{1}H NMR (DMSO-d_6) δ 8.58 (s, 4H), 6.90 (br t, 1H), 4.25 (t, J = 7.5 Hz, 2H), 4.12 (br t, 2H), 3.27 (br q, 2H), 2.62 (t, J = 7.4 Hz, 2H), 1.20 (s, 9H); ^{13}C NMR (DMSO-d_6) 172.4, 162.7, 162.4, 155.8, 130.4, 130.3, 126.4, 126.1, 77.5, 37.6, 36.1, 32.0, 28.1; HRMS (FAB) m/z 482.1547 (482.1563 calcd for $C_{24}H_{24}N_3O_8$).

N^{α}-9-Fluorenylmethyoxycarbonyl-(N^{ε}-*tert*-butoxycarbonyl)lysylglycylglycine (compound B). N^{α}-9-Fluorenylmethoxycarbonyl-(N^{ε}-tert-butoxycarbonyl)lysine (3.0 g, 6.4 mmol) and glycylglycine benzyl ester *p*-toluenesulfonate salt (1.42 g, 6.4 mmol) were dissolved in CH_2Cl_2. (Benzotriazole-1-yloxy)tripyrrolidinophosphonium hexafluorophospate (PyBOP, 5.0 g, 9.6 mmol) and N,N-diisopropylethylamine (3.57 mL, 20.5 mmol) were added and the solution stirred at room temperature for 3 h, diluted with CH_2Cl_2 and washed with 0.1 M citric acid buffer (pH 4) (2×) and water (2×). The solution was dried over sodium sulfate, filtered, and concentrated in vacuo. The resulting oil was dissolved in absolute EtOH and purged with Ar. Pd on C (10%) was added and the flask charged with H2 at atmospheric pressure and stirred at room temperature for 3 h. Filter the reaction through Celite and rinse with 10% MeOH in CH_2Cl_2. The filtrate was concentrated and purified by silica gel chromatography (2–10% MeOH in CH_2Cl_2). The product was concentrated and crystallized with 30% ethyl acetate/CH_2Cl_2 and pentane, and the white solid filtered. ^{1}H NMR (300 MHz, DMSO-d_6) δ 8.17 (t, 1H, J = 5.4 Hz), 8.09 (t, 1H, J = 5.8 Hz) 7.87 (d, 2H, J = 7.4 Hz), 7.72 (dd, 2H, J = 7.2, 4.3 Hz), 7.53 (d, 1H, J = 7.8 Hz), 7.41 (t, 2H, J = 6.9 Hz), 7.32 (t, 2H, J = 7.0 Hz), 6.76 (t, 1H, J = 5.3 Hz), 4.32.19 (m, 3H), 3.96 (br q, 1H), 3.74 (t, 4H, J = 6.1 Hz), 2.88 (q, 2H, J = 4.5 Hz), 1.7.1 (c, 6H), 1.35 (s, 9H); ^{13}C NMR (300 MHz, DMSO-d_6) δ 172.3, 171.1, 169.1, 156.1, 155.6, 143.9, 143.8, 140.7, 127.6, 127.1, 125.3, 120.1, 77.3, 65.6, 54.7, 46.7, 40.6, 39.7, 31.4, 29.2, 28.3, 22.8; HRMS (FAB) m/z 583.2793 (583.2768 calcd for $C_{30}H_{39}N_4O_8$, MH).

Solid-phase peptide synthesis. Fmoc-Lys(BOC) functionalized Tenta-Gel Resin (NovaBiochem, 0.75 g, 0.13 mmol/g loading capacity) was added to 10 mL fritted filter flask with a screw cap and Teflon stopcock. The resin was washed with DMF (3×5 mL), *i*POH (3×3 mL), and DMF (3×5 mL), and then deprotected with 5 mL of 20% piperidine in DMF for 20 min. The resin was washed with DMF/*i*POH/DMF. A 0.195 mM stock solution of PyBOP was made in DMF and a 0.195 mM solution of N-methyl morpholine in DMF. Compound A (128.8 mg, 0.195 mmol) was dissolved in 1 mL of the PyBOP stock solution and added to the resin followed by 2 mL of the NMM stock solution. The coupling reacted for 45 min, was filtered, and the resin washed with DMF/*i*POH/DMF. This coupling was repeated, and then the resin was capped with a solution of 20% acetic anhydride with 1 mL NMM stock solution for 20 min and the resin rinsed. The resin was deprotected, washed and then the coupling was repeated with compound B (113.6 mg, 0.195 mmol). This process was repeated with alternating couplings until the octamer had been synthesized. After the final piperidine deprotection the resin was rinsed with MeOH and CH_2Cl_2 and dried in vacuum dessicator for 2 h. The compound was then cleaved off the resin with 95% TFA/5% water for 12 h and the resin beads filtered off. The filtrate was concentrated in vacuo and ether was added to precipitate the product. The resulting reddish brown solid was purified by reverse-phase C18 column (PrepRPC 15m, Pharmacia) with a 2-h gradient (0–100% 0.07% TFA in ACN in 0.07% TFA in water). The product fractions were combined and lyopholized to yield the octamer (15 mg, 2.6%) as a light-yellow solid.

Octamer 4. ^{1}H NMR (500 MHz, D_2O) δ 8.24.14 (m, 32H), 4.30.50 (m, 100H), 3.02.64 (m, 32H), 1.83.44 (m, 48H) MS (ESI) m/z 744.9 (744.5 calcd for (M + 7H)7 +),

868.8 (868.5 calcd for (M + 6H)6 +), 1042.2 (1042.0 calcd for (M + 5H)5 +), 1302.4 (1302.3 calcd for (M + 4H)4 +), 1735.8 (1736.0 calcd for (M + 3H)3 +).

Viscometry. Calf thymus DNA (activated, type XV, Sigma) was suspended in TE + 50 mM NaCl buffer in a concentration of approximately 1 mg per 1 mL and sonicated on ice at 35% power with a Fischer Sonic Dismembrator Model 300 for 50 min, with 5 min of sonication followed by a 5 min cool down. The length of the DNA was an average of 300 base pairs as determined by 1.2% agarose gel electrophoresis. The DNA was filtered through a 0.2-μ Acrodisk syringe filter into dialysis membrane (Spectropore CE, MW cutoff 10,000) and dialyzed against TE + 50 mM NaCl buffer. Concentration of the stock solution was determined spectrometrically using $\varepsilon_{260} = 12{,}824$. DNA and intercalator samples were prepared at double the final concentration in 0.5 mL of buffer each and mixed with a mixing apparatus (shown in Fig. 4). The DNA/intercalator solution were centrifuged for 4 min at 9000 rpm and then transferred to a Cannon-Ubblehold semi-micro viscometer which was cleaned with water, followed by acetone and then dried by blowing compressed nitrogen through the apparatus. The viscometer was emerged in a constant temperature water bath and allowed to equilibrate to constant temperature for 15 min before the flow times were measured.

Circular dichroism. DNA samples were used at a concentration of 0.025 mM (base pairs). Intercalator concentrations were calculated according to 100% intercalation using the nearest neighbor exclusion principle. For example, a dimer would be expected to cover four base pairs. Therefore the final concentration of dimer would be (0.025 mM/4) or 6.25 μM. Total volume used in each experiment was 1 mL. The samples were loaded into a quartz circular dichroism vessel, and the CD was measured by a Jas.Co J-600 Spectropolarimeter.

Competition dialysis. Competition dialysis experiments were performed in aqueous BPES buffer (0.01 M sodium phosphate, 0.001 M EDTA, 0.2 M NaCl). The DNA, RNA, and DNA/RNA hybrids were all used at an equal concentration of 75 μM (per base if single stranded, per base pair if double stranded, etc.), and were placed in separate dialysis tubes and dialyzed against a 1-μM intercalator solution. After 72 h at 25 °C, the concentration of free and total ligand in the dialysis tubes was determined by UV–vis spectrophotometry both before and after the addition of 2% SDS to dissociate the DNA–ligand complexes.[20]

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References and Notes

1. Dervan, P. B. *Science* **1986**, *232*, 464.
2. Lokey, R. S.; Kwok, Y.; Guelev, V.; Pursell, C. J.; Hurley, L. H.; Iverson, B. I. *J. Am. Chem. Soc.* **1997**, *119*, 7202.
3. Trauger, J. W.; Baird, E. E.; Dervan, P. B. *J. Am. Chem. Soc.* **1998**, *120*, 3534.
4. Turner, J. M.; Swalley, S. E.; Baird, E. E.; Dervan, P. B. *J. Am. Chem. Soc.* **1998**, *120*, 6219.
5. Walker, W. L.; Kopka, M. L.; Goodsell, D. S. *Biopolymers* **1998**, *44*, 323.
6. Leng, F.; Priebe, W.; Chaires, J. B. *Biochemistry* **1998**, *37*, 1743.
7. Wakelin, L. P. G. *Med. Res. Rev.* **1986**, *6*, 275.
8. Waring, M. J. In *Molecular Aspects of Anticancer Drug–DNA Interactions*; Niedle, S., Waring, M. J., Eds.; CRC: Boca Raton, 1993; Vol. 1, Chapter 7.
9. Chaires, J. B. *Curr. Opin. Struct. Biol.* **1998**, *8*, 314.
10. Bailly, C.; Braña, M.; Waring, M. J. *Eur. J. Biochem.* **1996**, *240*, 195.
11. Perez, J. M.; Lopez-Solera, I.; Montero, E. I., Jr. *J. Med. Chem.* **1999**, *42*, 5482.
12. Denny, W. A.; Atwell, G. J.; Willmott, G. A.; Wakelin, L. P. G. *Biophys. Chem.* **1985**, *22*, 17.
13. Onfelt, B.; Lincoln, P.; Norden, B. *J. Am. Chem. Soc.* **1999**, *121*, 10846.
14. Laugaa, P.; Markovitz, J.; Delbarre, A.; Le Pecq, J.; Roques, B. P. *Biochemistry* **1985**, *24*, 5567.
15. Takenaka, S.; Nishira, S.; Tahara, K.; Kondo, H.; Takagi, M. *Supramol. Chem.* **1993**, *2*, 41.
16. Yen, S.; Gabbay, E. J.; Wilson, W. D. *Biochemistry* **1982**, *21*, 2070.
17. Guelev, V. M.; Harting, M. T.; Lokey, R. S.; Iverson, B. L. *Chem. Biol.* **2000**, *7*, 1.
18. Steullet, V.; Dixon, D. W. *J. Chem. Soc. Perkin Trans 2* **1999**, *7*, 1547.
19. Cohen, G.; Eisenberg, H. *Biopolymers* **1969**, *8*, 45.
20. Ren, J.; Chaires, J. B. *Biochemistry* **2000**, *38*, 16067.
21. Crothers, D. M. *Biopolymers* **1968**, *6*, 575.
22. Jencks, W. P. *Proc. Natl. Acad. Sci. U.S.A.* **1981**, *78*, 4046.
23. Interestingly, other designed *bis*-intercalating systems with flexible linkers generally diaplay lower binding than expected as well. See, for example: Dervan, P. B.; Becker, M. M. *J. Am. Chem. Soc.* **1978**, *100*, 1968. Leng, F.; Priebe, W.; Chaires, J. B. *Biochemistry* **1998**, *37*, 1743.
24. Chaires, J. B. *Anti-Cancer Drug Des.* **1996**, *11*, 569.
25. Novatny, J.; Bruccoleri, R. E.; Saul, F. A. *Biochemistry* **1989**, *28*, 4735.