Quadruplex Self-Assembly

DOI: 10.1002/anie.200701883

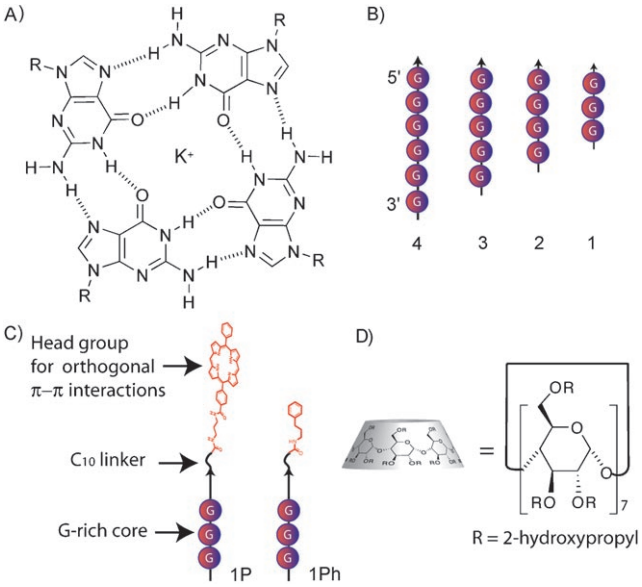
Allosteric Control of Self-Assembly: Modulating the Formation of Guanine Quadruplexes through Orthogonal Aromatic Interactions**

*Janarthanan Jayawickramarajah, Debarati M. Tagore, Lun K. Tsou, and Andrew D. Hamilton**

Aromatic interactions are critical noncovalent forces that help stabilize the self-assembly of oligonucleotide (ODN) structures including duplex and quadruplex DNA.[1] Much current work has focused on the development of ODN-tethered affinity-enhancing groups that make intramolecular contact and further stabilize duplex DNA through additional aromatic interactions.[2] Recently, Berova and co-workers have demonstrated that porphyrin macrocycles linked to the 5′-terminus of ODNs can be used to stabilize, non-complementary, non-Watson–Crick duplexes through aromatic interactions with the terminal base pairs.[3] However, there has been no exploration of the potential of these ODN-linked synthetic units with more complex DNA aggregates, such as guanine quadruplexes which self-assemble through sequential stacking of metal-templated guanine quartets (Scheme 1 A).[4] Given the importance of quadruplex structures in telomere stabilization,[5a,b] oncogene activation,[5c] regulation of the insulin gene,[5d] as well as in the development of structural and functional supramolecular assemblies,[6] we were keen to introduce synthetic agents capable of stabilizing guanine quadruplexes through designed orthogonal aromatic interactions.

Herein we report a novel strategy that can dramatically enhance the stability of parallel tetramolecular quadruplexes by harnessing the self-stacking interactions of porphyrin macrocycles in water. Furthermore, we demonstrate that addition of (2-hydroxypropyl)-β-cyclodextrin (HP-β-CD), a porphyrin complexation agent, can attenuate porphyrin–porphyrin stacking interactions and thus modulate the self-assembly of quadruplexes.

Initially, a series of ODNs were prepared to identify the minimum length of guanine-rich sequences necessary to form parallel tetramolecular quadruplexes under previously established conditions.[7] Thus, parent ODNs **1**–**4** containing contiguous tracts of guanines (with sequences d(TG_nT_4) where $n = 3$–6 for ODNs **1**–**4**, respectively) were obtained (Scheme 1 B). Each of these ODNs included a flanking T_4 sequence at the 3′-terminus to further destabilize the association of the tetramolecular quadruplexes whilst also serving to minimize longitudinal stacking between individual quadruplexes.[8]



Scheme 1. A) Structure of a cyclic guanine quartet formed by potassium ion templation and Hoogsteen hydrogen-bonding interactions. B) Sequence of guanine-rich ODNs (**1**–**4**) with no head groups. C) Sequence of ODNs tethered at the 5′-termini with porphyrin **1P** or phenyl **1Ph** head groups. D) Illustration of (2-hydroxypropyl)-β-cyclodextrin (HP-β-CD).

Guanine-containing ODNs **1**–**4** were exposed to quadruplex self-assembly protocols (80 mM KCl, 10 mM Tris-HCl (Tris = tris(hydroxymethyl)aminomethane), pH 7.5) with incubation times of 48 h at 4 °C.[7] The secondary structure of the ensuing complexes was assessed by circular dichroism spectroscopy. Incubation of ODNs **4** or **3** (Figure 1) both gave rise to a high-intensity circular dichroism profile, with a positive ellipticity at 266 nm and a negative peak at 243 nm. This profile is characteristic of parallel tetramolecular quadruplexes (namely, $(\mathbf{4})_4$ and $(\mathbf{3})_4$).[9] In contrast, the incubation of shorter ODNs **2** or **1**, under the time frame of the experiment, displayed weak circular dichroism bands as the nucleobase units are not stacked to any appreciable extent. This latter result is not surprising since quadruplexes formed by ODNs **2** and **1** can only be stabilized by four and three guanine quartets, respectively.

Among the ODN sequences used in this study, parent sequence **1** was anticipated to have the lowest propensity for the formation of a quadruplex. Thus, we focused on using orthogonal aromatic interactions to stabilize quadruplexes

[*] Dr. J. Jayawickramarajah, Dr. D. M. Tagore, L. K. Tsou, Prof. A. D. Hamilton
Department of Chemistry
Yale University
P. O. Box 208107, New Haven, CT 06520-8107 (USA)
Fax: (+1) 203-432-3221
E-mail: andrew.hamilton@yale.edu
Homepage: http://ursula.chem.yale.edu/~hamgrp

[**] This study has been partially supported by the National Science Foundation and the National Institutes of Health (GM 35208).

Supporting information for this article is available on the WWW under http://www.angewandte.org or from the author.

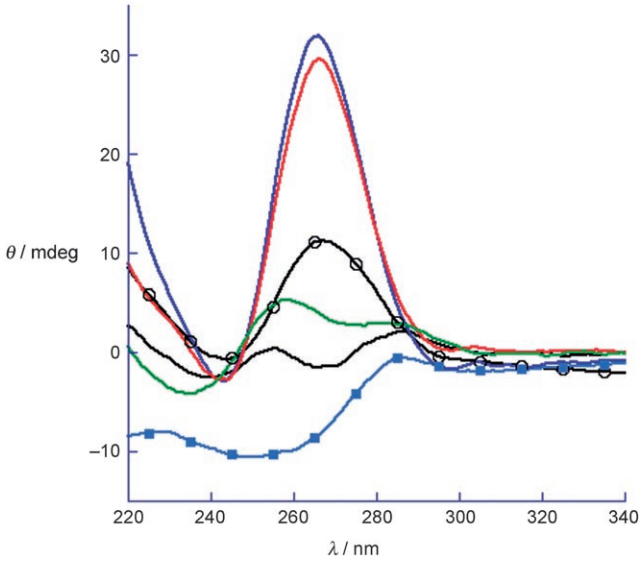


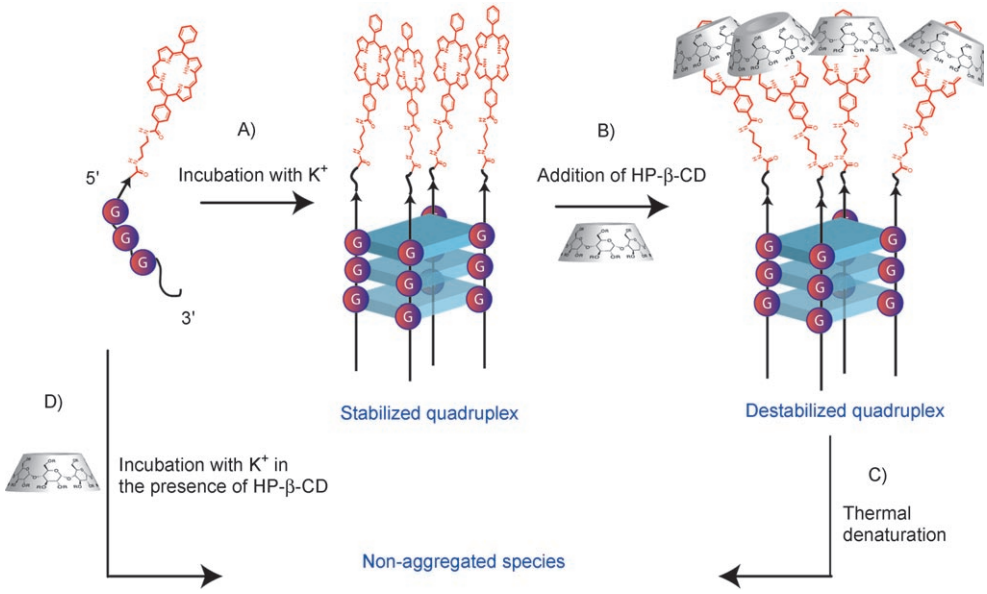
Figure 1. Circular dichroism spectra of ODNs after self-assembly: **4** (——), **3** (——), **2** (——), **1** (——), **1P** (○), and **1Ph** (■). All measurements were carried out in 80 mM KCl and 10 mM Tris-HCl (pH 7.5). Concentrations of all ODNs were 25 μM (single strand).

derived from this sequence. Accordingly, congener **1P** (with sequence **P**d(TG_3T_4) containing a 5,15-diphenylporphyrin module (**P**) tethered to the 5′-terminus) was prepared (Scheme 1C). Our expectation was that aromatic stacking interactions between the porphyrin chromophores should enhance quadruplex self-assembly.[10] A C_{10} spacer was included to minimize interactions between the porphyrin macrocycles and the nucleobase core. A circular dichroism study of the resultant self-assembly for ODN **1P** clearly showed significant guanine stacking with a profile consistent with the presence of the parallel quadruplex $(\mathbf{1P})_4$, which is in sharp contrast to what was observed for **1**.[11] This enhancement in quadruplex formation is thought to occur as a result of aromatic interactions between the large hydrophobic surfaces of the 5′-tethered porphyrin macrocycles (Scheme 2A).

The quadruplex-forming capability of an ODN incorporating a smaller, phenyl head group (**1Ph**) was also explored (Scheme 1C).[12] Here, a profile lacking appreciable secondary structure was observed (Figure 1). Although not rigorously established, a possible rationale for why porphyrin-based self-association leads to clean formation of a quadruplex as opposed to the ODN with the smaller, phenyl group lies in the large hydrophobic component for porphyrin aggregation in water (Log P for **P** = 6.95 versus Log P for **Ph** = 2.03).

The reversibility of the noncovalent interactions that promote self-association of porphyrin rings suggested a simple route to controling the assembly of the quadruplex. Addition of HP-β-CD (Scheme 1D), a receptor that is capable of binding to phenylporphyrins through the formation of an inclusion complex,[13] should remove the stabilizing effect of the porphyrin head groups and thus promote the disassembly of $(\mathbf{1P})_4$. To test this hypothesis, a solution of self-assembled $(\mathbf{1P})_4$ was mixed for 48 h with 7.2% (w/v) HP-β-CD (Scheme 2B). Indeed, analysis of the resultant complex by circular dichroism spectroscopy showed destabilization of $(\mathbf{1P})_4$, as judged by both a decrease in the intensity as well as a bathochromic shift of the maximum ellipticity.[14]

Further evidence for HP-β-CD-induced destabilization of $(\mathbf{1P})_4$ came from thermal denaturation studies. For example, the melting temperature ($T_{1/2}$) for $(\mathbf{1P})_4$ was found to be 77 °C (Figure 2, inset) while incubation of $(\mathbf{1P})_4$ with HP-β-CD for 48 h resulted in a $T_{1/2}$ of 65 °C. In addition to the decrease in the $T_{1/2}$ value, the thermal denaturation profile for $(\mathbf{1P})_4$ incubated with HP-β-CD indicates that simple heating above the $T_{1/2}$ value can be used to disaggregate the quadruplex structure back to a monomeric form (Scheme 2C). This result is significant because in many cases quadruplexes stabilized in potassium-containing buffer are still substantially folded even upon heating at 95 °C.[15]



Scheme 2. A) Self-assembly of ODN **1P** in potassium-containing buffer leads to the formation of the parallel guanine quadruplex $(\mathbf{1P})_4$ stabilized by porphyrin-based orthogonal aromatic interactions. B) The resultant quadruplex can be destabilized by addition of HP-β-CD through host–guest complexation. C) Thermal denaturation of the destabilized quadruplex leads to disassembly. D) Self-assembly of **1P** in the presence of HP-β-CD inhibits formation of the quadruplex.

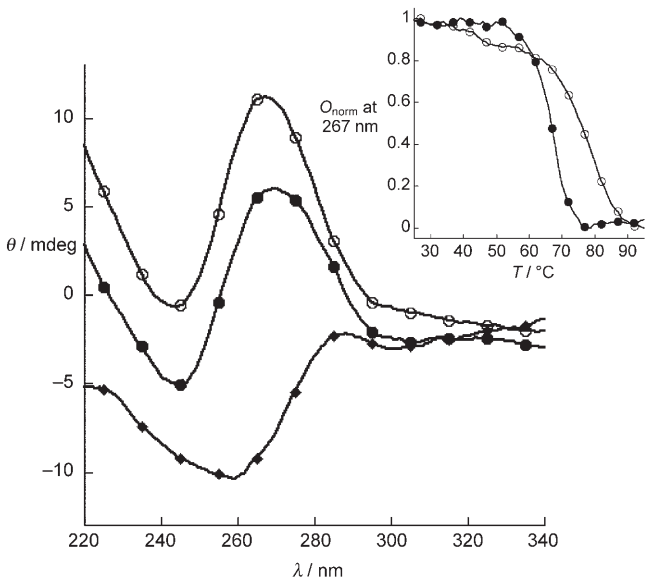


Figure 2. Circular dichroism spectra of ODN **1P** after formation of the quadruplex (○), as well as subsequent to mixing with 7.2% (w/v) HP-β-CD for 48 h (●). Also shown is ODN **1P** wherein HP-β-CD was added prior to self-assembly of the quadruplex (◆). Inset: Thermal denaturation profiles of quadruplex $(\mathbf{1P})_4$ in the absence and presence of 7.2% (w/v) HP-β-CD. All measurements were carried out in 80 mM KCl and 10 mM Tris-HCl (pH 7.5). Concentrations of all ODNs were 25 μM (single strand).

To confirm that porphyrin-based aromatic interactions are responsible for the enhancement in stability of $(\mathbf{1P})_4$, electronic absorption studies were carried out. The results from these experiments (Figure 3) substantiate the presence of porphyrin self-association through cofacial π–π interactions. For example, the presence of a broad, blue-shifted shoulder (centered at 350 nm) in the Soret region is consistent with H-type, π–π interactions between the porphyrin macrocycles.[16] In contrast, a porphyrin-tethered single-stranded control (**SSP**; [**P**d(CGTCATATCTTA)]), which is incapable of forming a tetraplex, displayed no aggregation behavior. Importantly, UV/Vis studies of quadruplex $(\mathbf{1P})_4$ upon mixing with excess HP-β-CD showed a decrease in the porphyrin aggregation band. These findings suggest that a large fraction of the porphyrin head groups on $(\mathbf{1P})_4$ are capped with HP-β-CD.

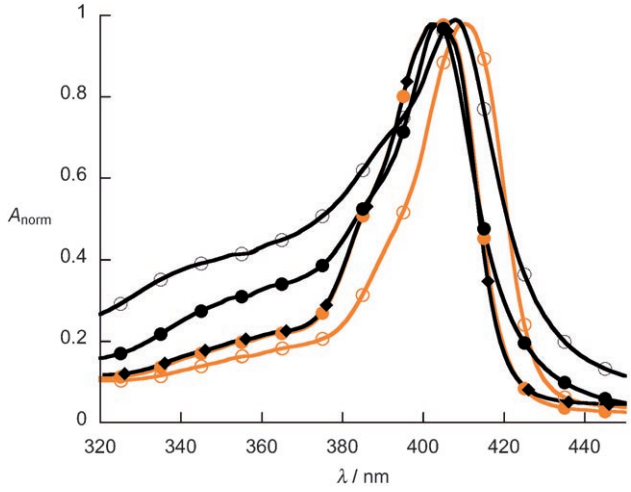


Figure 3. Normalized electronic absorption spectra of ODN **1P** after formation of the quadruplex (○), as well as after mixing with 7.2% (w/v) HP-β-CD for 48 h (●). Also shown is ODN **1P** wherein HP-β-CD was added prior to self-assembly of the quadruplex (◆). For the sake of comparison, single-stranded ODN **SSP** in the absence (○) and presence (●) of 7.2% (w/v) HP-β-CD is also included. All measurements were carried out in 80 mM KCl and 10 mM Tris-HCl (pH 7.5). Concentrations of all ODNs were 25 μM (single strand).

The aforementioned experiments suggested that aromatic porphyrin–porphyrin interactions are important in the formation and subsequent stability of quadruplex $(\mathbf{1P})_4$. Thus, self-assembly of **1P** in the presence of HP-β-CD should preclude the formation of quadruplex $(\mathbf{1P})_4$ (Scheme 2D). In fact, addition of 7.2% (w/v) HP-β-CD to a solution of ODN **1P** (80 mM KCl, 10 mM Tris-HCl, pH 7.5) prior to quadruplex self-assembly[7] (that is, exposure to a heating and annealing cycle) resulted in no quadruplex formation. Furthermore, the normalized UV/Vis spectrum of the resulting species is strikingly similar to that observed for control ODN **SSP** in the presence of HP-β-CD. Taken together, these results demonstrate that the formation of cyclodextrin–porphyrin host–guest complexes can minimize porphyrin–porphyrin self-association and thus inhibit the formation of quadruplex $(\mathbf{1P})_4$, which is dependent on these engineered π–π interactions.

In conclusion, we have detailed the use of allosteric porphyrin-based aromatic interactions that can stabilize the formation of a tetramolecular DNA quadruplex which otherwise does not readily form. An important versatility of this system is that addition of HP-β-CD can be used to modulate the porphyrin-stacking interactions thereby allowing for control over the self-assembly of the quadruplex. This approach can potentially be used to fine-tune the aggregation of relevant tetramolecular quadruplexes important in materials applications. Furthermore, a similar strategy can be envisaged for the modulation of biologically relevant intramolecular quadruplexes. For example, porphyrins appended to the 3′- and 5′-ends of a telomeric repeat sequence $d(T_2G_4)_2$ may lead to the stablization of an intramolecular quadruplex (through porphyrin-based π–π interactions) that can be destabilized in the presence of HP-β-CD. Work towards this goal is currently in progress.

Received: April 28, 2007
Revised: July 10, 2007
Published online: September 6, 2007

Keywords: π interactions · cyclodextrins · DNA structures · porphyrinoids · supramolecular chemistry

[1] For general reviews on aromatic interactions, see a) C. A. Hunter, K. R. Lawson, J. Perkins, C. J. Urch, *J. Chem. Soc. Perkin Trans. 2* **2001**, 651–669; b) M. L. Waters, *Curr. Opin. Chem. Biol.* **2002**, *6*, 736–741; c) E. A. Meyer, R. K. Castellano, F. Diederich, *Angew. Chem.* **2003**, *115*, 1244–1287; *Angew. Chem. Int. Ed.* **2003**, *42*, 1210–1250; Corrigendum: E. A. Meyer, R. K. Castellano, F. Diederich, *Angew. Chem.* **2003**, *115*, 4254;

Angew. Chem. Int. Ed. **2003**, *42*, 4120; for references pertinent to aromatic interactions in DNA, see d) C. M. Olsen, W. H. Gmeiner, L. A. Marky, *J. Phys. Chem. B* **2006**, *110*, 6962 – 6969; e) E. T. Kool, *Annu. Rev. Biophys. Biomol. Struct.* **2001**, *30*, 1 – 22; f) C. Brotschi, A. Haberli, C. J. Leumann, *Angew. Chem.* **2001**, *113*, 3101 – 3103; *Angew. Chem. Int. Ed.* **2001**, *40*, 3012 – 3014.

[2] a) J. Tuma, R. Paulini, J. A. Rojas Stutz, C. Richert, *Biochemistry* **2004**, *43*, 15680 – 15687; b) K. M. Guckian, B. A. Schweitzer, R. X.-F. Ren, C. J. Sheils, P. L. Paris, D. C. Tahmassebi, E. T. Kool, *J. Am. Chem. Soc.* **1996**, *118*, 8182 – 8183; c) Z. Dogan, R. Paulini, J. A. Rojas Stutz, S. Narayanan, C. Richert, *J. Am. Chem. Soc.* **2004**, *126*, 4762 – 4763.

[3] M. Balaz, B. C. Li, S. Jockusch, G. A. Ellestad, N. Berova, *Angew. Chem.* **2006**, *118*, 3610 – 3613; *Angew. Chem. Int. Ed.* **2006**, *45*, 3530 – 3533.

[4] For reviews on DNA quadruplexes, see a) J. R. Williamson, *Annu. Rev. Biophys. Biomol. Struct.* **1994**, *23*, 703 – 730; b) T. Simonsson, *Biol. Chem.* **2001**, *382*, 621 – 628; c) J. T. Davis, *Angew. Chem.* **2004**, *116*, 684 – 716; *Angew. Chem. Int. Ed.* **2004**, *43*, 668 – 698; d) *Quadruplex Nucleic Acids* (Eds.: S. Neidle, S. Balasubramanian), RSC, Cambridge, **2006**, pp. 1 – 301.

[5] a) W. I. Sundquist, A. Klug, *Nature* **1989**, *342*, 825 – 829; b) H. Han, L. H. Hurley, *Trends Protein Sci.* **2000**, *21*, 136 – 141; c) T. Simonsson, P. Pecinka, M. Kubista, *Nucleic Acid Res.* **1998**, *26*, 1167 – 1172; d) M. C. Hammond-Kosack, M. W. Kilpatrick, K. Doucherty, *J. Mol. Endocrinol.* **1992**, *9*, 221 – 225.

[6] For reviews on nanomachines based on guanine quadruplexes, see a) P. Alberti, A. Bourdoncle, B. Sacca, L. Lacroix, J.-L. Mergny, *Org. Biomol. Chem.* **2006**, *4*, 3383 – 3391; b) M. K. Beissenhirtz, I. Willner, *Org. Biomol. Chem.* **2006**, *4*, 3392 – 3401; for references on quadruplex-based nanoparticle assemblies, see c) F. Seela, A. M. Jawalekar, L. Chi, D. Zhong, *Chem. Biodiversity* **2005**, *2*, 84 – 91; d) Z. Li, C. A. Mirkin, *J. Am. Chem. Soc.* **2005**, *127*, 11568 – 11569; for a reference on protein denaturants based on guanine quadruplexes, see e) D. M. Tagore, K. I. Sprinz, S. Fletcher, J. Jayawickramarajah, A. D. Hamilton, *Angew. Chem.* **2007**, *119*, 227 – 229; *Angew. Chem. Int. Ed.* **2007**, *46*, 223 – 225; f) for a recent reference on using ODN-tethered pyrene reporter groups to probe for tetramolecular quadruplex formation, see H. Zhu, F. D. Lewis, *Bioconjugate. Chem.* **2007**, DOI: 10.1021/bc060279u.

[7] It is important to note that quadruplex formation is highly sensitive to the ODN and potassium concentrations as well as incubation times. Thus, constant experimental conditions were used (see the Supporting Information) for the quadruplexes prepared in this work. See also reference [6e].

[8] Q. Guo, M. Lu, N. R. Kallenbach, *Biochemistry* **1993**, *32*, 3596 – 3603.

[9] a) V. Dapic, V. Abdomerovic, R. Marrington, J. Peberdy, A. Rodger, J. O. Trent, P. J. Bates, *Nucleic Acids Res.* **2003**, *31*, 2097 – 3107; b) M. Luo, Q. Guo, N. R. Kallenbach, *Biochemistry* **1992**, *31*, 2455 – 2459.

[10] For examples of porphyrin self-association through aromatic interactions, see a) R. F. Pasternack, P. R. Huber, P. Boyd, G. Engasser, L. Francesconi, E. Gibbs, P. Fasella, G. Cerio Venturo, L. deC. Hinds, *J. Am. Chem. Soc.* **1972**, *94*, 4511 – 4517; b) E. Ojadi, R. Selzer, H. Linschitz, *J. Am. Chem. Soc.* **1985**, *107*, 7783 – 7784.

[11] The porphyrin-containing ODN **2P** [**P**d(TG_4T_4)] also forms a parallel quadruplex (see the Supporting Information) in contrast to parent ODN **2**.

[12] **1Ph** = **Ph**d(TG_3T_4), where **Ph** = a phenyl head group. An identical C10 spacer was used as for the porphyrin version.

[13] For examples of β-CD derivatives that bind to meso-phenyl-substituted porphyrins in water through host–guest complexation, see a) J. M. Ribó, J.-A. Farrera, M. L. Valero, A. Virgili, *Tetrahedron* **1995**, *51*, 3705 – 3712; b) K. Kano, R. Nishiyabu, R. Doi, *J. Org. Chem.* **2005**, *70*, 3667 – 3673; c) T. Konishi, A. Ikeda, M. Asai, T. Hatano, S. Shinkai, M. Fujitsuka, O. Ito, Y. Tsuchiya, J. Kikuchi, *J. Phys. Chem. B* **2003**, *107*, 11261 – 11266; importantly, it has been previously established that β-CDs do not bind to guanine or thymine nucleobases, and do not interact with well-folded nucleic acid helices, see d) J. L. Hoffman, R. M. Bock, *Biochemistry* **1970**, *9*, 3542 – 3550.

[14] In contrast, addition of HP-β-CD to control $(\mathbf{4P})_4$ (**4P** = **P**d(TG_6T_4), which contains six guanine residues, resulted in no appreciable change in the circular dichroism profile for the ODN region (see the Supporting Information).

[15] For example, the thermal denaturation of $(\mathbf{4P})_4$ shows minimal disaggregation at 95 °C (see the Supporting Information); see also reference [9b].

[16] a) R. F. Khairutdinov, N. Serpone, *J. Phys. Chem. B* **1999**, *103*, 761 – 769; b) M. Kasha, *Radiat. Res.* **1963**, *20*, 55 – 70.

