Synthesis of 13*H*-Benzo[6,7]- and 13*H*-Benzo[4,5]indolo[3,2-*c*]-quinolines: A New Series of Potent Specific Ligands for Triplex DNA

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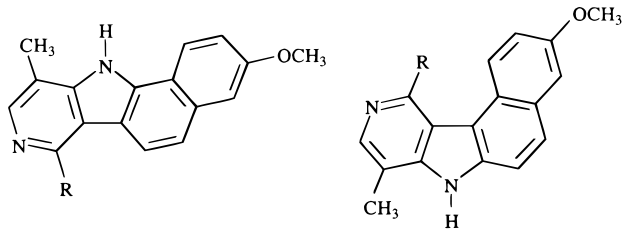
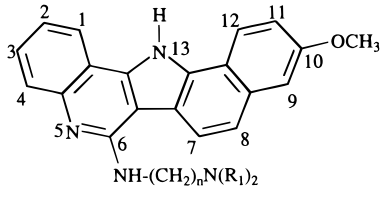
Abstract: Triple-helical complexes formed upon binding of oligonucleotides to oligopyrimidine·oligopurine sequences of double-helical DNA can be stabilized by intercalating ligands such as benzopyridoindole derivatives (Mergny et al. *Science* **1992**, *256*, 1681). Based on molecular modeling studies, it was predicted that better stacking interactions could be achieved between the intercalator and base triplets by extending the size of the aromatic ring system. Here we describe the synthesis of pentacyclic aromatic molecules which exhibit a highly selective binding to triplex structures. The thermal Fischer indolization of hydrazones resulting from 4-hydrazinoquinolin-2(1*H*)-one and 6-methoxy-1 (and -2) -tetralones led to the expected cyclized intermediates. After complete aromatization, these compounds were transformed by phosphorus oxychloride giving 6-chloro-10-methoxy-13*H*-benzo[6,7]- (and 6-chloro-9-methoxy-13*H*-benzo[4,5]-) indolo[3,2-*c*]quinolines. Usual substitution by various diamines provided derivatives of these pentacyclic ring systems which are, so far, the most potent DNA triplex-specific ligands ever described in our studies.

Introduction

In the past few years, several ligands have been reported for their ability to stabilize triple-helical structures with various sequences.[1−14] Among them, the benzo- and benzo[*e*]pyrido-[4,3-*b*]indoles (BgPI and BePI, **1** and **2**) have been synthe-





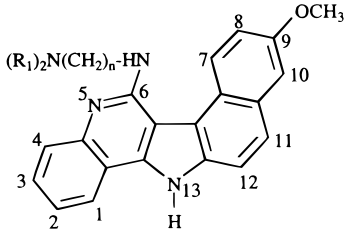


Figure 1. Chemical structure of BgPI (**1**), BePI(**2**), B[6,7]IQ (**3**), and B[4,5]IQ (**4**).

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(1) Scaria, P. V.; Shafer, R. H. *J. Biol. Chem.* **1991**, *266*, 5417.
(2) Mergny, J. L.; Collier, D.; Rougée, M.; Montenay-Garestier, T.; Hélène, C. *Nucleic Acids Res.* **1991**, *19*, 1521.
(3) Mergny, J. L.; Duval-Valentin, G.; Nguyen, C. H.; Perrouault, L.; Faucon, B.; Rougée, M.; Montenay-Garestier, T.; Bisagni, E.; Hélène, C. *Science* **1992**, *256*, 1681.
(4) Pilch, D. S.; Waring, M. J.; Sun, J. S.; Rougée, M.; Nguyen, C. H.; Bisagni, E.; Garestier, T.; Hélène, C. *J. Mol. Biol.* **1993**, *232*, 926.
(5) Pilch, D. S.; Martin, M. T.; Nguyen, C. H.; Sun, J. S.; Bisagni, E.; Garestier, T.; Hélène, C. *J. Am. Chem. Soc.* **1993**, *115*, 9942.
(6) Lee, J. S.; Latimer, L. J. P.; Hampel, K. J. *Biochemistry* **1993**, *32*, 5591.
(7) Wilson, W. D.; Tanious, F. A.; Mizan, S.; Yao, S.; Kiselyov, A. S.; Zon, G.; Strekowski, L. *Biochemistry* **1993**, *32*, 10614.
(8) Escudé, C.; Nguyen, C. H.; Mergny, J. L.; Sun, J. S.; Bisagni, E.; Garestier, T.; Hélène, C. *J. Am. Chem. Soc.* **1995**, *117*, 10212.
(9) Fox, K. R.; Polucci, P.; Jenkins, T. C.; Neidle, S. *Proc. Natl. Acad. Sci. U.S.A.* **1995**, *92*, 7887.
(10) Latimer, L. J. P.; Payton, N.; Forsyth, G.; Lee, J. S. *Biochem. Cell Biol.* **1995**, *73*, 11.
(11) Strekowski, L.; Gulevich, Y.; Van Aken, K.; Wilson, D. W.; Fox, K. R. *Tetrahedron Lett*. **1995**, *36*, 225.
(12) Park, Y. W.; Breslauer, K. J. *Proc. Natl. Acad. Sci. U.S.A.* **1992**, *89*, 6653.
(13) Durand, M.; Thuong, N. T.; Maurizot, J. C. *J. Biol. Chem.* **1992**, *267*, 24394.
(14) Pilch, D. S.; Breslauer, K. J. *Proc. Natl. Acad. Sci. U.S.A.* **1994**, *91*, 9332.

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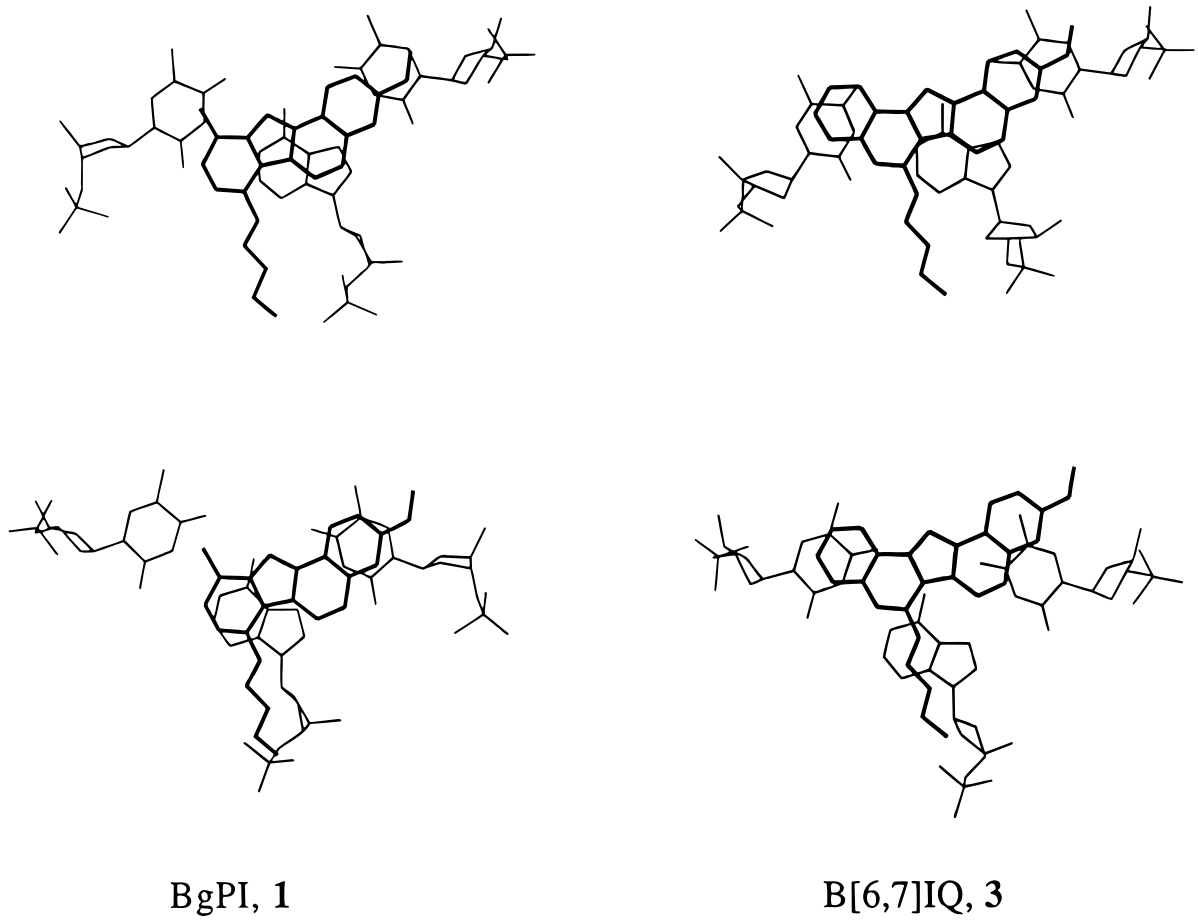


Figure 2. Energy-minimized models for the intercalation of BgPI (**1**, left part) and B[6,7]IQ (**3**, right part) between two adjacent T·AxT base triplets. BgPI and B[6,7]IQ molecules (shown in bold line) are shown stacked with the base triplet above (top) or below (bottom) the intercalation site. Hydrogen atoms were not drawn for clarity.

sized[15,16] and studied[3−5,8] (Figure 1). Whereas their important antitumor properties in various experimental rodent tumor models[15−18] are mainly attributed to their capacity to inhibit topoisomerases (II and/or I), the same compounds turned out to be very interesting triple helix-specific ligands.[3−5,8]

The propensity of these compounds to stabilize DNA triple helices suggest that these drugs might also induce H-DNA structures and therefore interfere with the transcription and replication machineries. These findings could represent a new mechanism of action of antitumor drugs.[3] Moreover, due to the potential applications of triplex technology, it seemed of interest to obtain other triplex specific ligands which further stabilize DNA triplex structures. All the preceding series that we have studied (BgPI, BePI) were tetracyclic derivatives. Molecular modeling and experimental studies have suggested that an additional aromatic ring could increase the stacking interaction between ligands and base triplets.

Here we report the design, synthesis and DNA triplex stabilization of new pentacyclic derivatives, namely 13*H*-benzo-[6,7]- (and 13*H*-benzo[4,5]) -indolo[3,2-*c*]quinolines (**3** and **4** on Figure 1), two new heterocyclic series related to benzo[*g*]- and benzo[*e*]pyridoindoles, respectively (**1** and **2** on Figure 1).

(15) Nguyen, C. H.; Lhoste, J.-M.; Lavelle, F.; Bissery, M. C.; Bisagni, E. *J. Med. Chem.* **1990**, *33*, 1519.

(16) Nguyen, C. H.; Lavelle, F.; Riou, J.-F.; Bissery, M. C.; Huel, C.; Bisagni, E. *Anticancer Drug Design* **1992**, *7*, 235.

(17) Bissery, M. C.; Nguyen, C. H.; Bisagni, E.; Vrignaud, P.; Lavelle, F. *Invest. New Drugs* **1993**, *11*, 263.

(18) Riou, J. F.; Fossé, P.; Nguyen, C. H.; Larsen, A. K.; Bissery, M. C.; Grondard, L.; Saucier, J. M.; Bisagni, E.; Lavelle, F. *Cancer Res.* **1993**, *53*, 5987.

Results

Design of 13*H*-Benzo[6,7]- (and 13*H*-Benzo[4,5]) -indolo-[3,2-*c*]quinoline Ligands. A molecular modeling method was previously developed to characterize the complexes of benzo-[*e*]- (or [*g*]) -pyridoindole (BePI or BgPI) derivatives with triplex structures.[8] These models which were in agreement with experimental data[8,20] showed that (1) the tetracyclic ring system of BgPI and BePI ligands were well stacked with Hoogsteen AxT base pairs; (2) the aminoalkylamine side chains of BgPI and BePI were located in the minor groove and in the major groove, respectively (Figures 2 and 3, left part).

The structure of the triplex-intercalated ligands BgPI and BePI suggested that it should be possible to further enhance binding by extending stacking interactions to the pyrimidines which are already engaged in Watson−Crick base pairing. This could be achieved by adding a benzene ring adjacent to the pyridine ring of benzopyridoindole derivatives. The lowest energy-minimized model of the pentacyclic crescent-shaped 13*H*-benzo[6,7]indolo-[3,2-*c*]quinoline (B[6,7)IQ) derivative **3** (Figure 2, right part) showed that this molecule could significantly increase stacking interactions with both the base triplets adjacent to the intercalation site as compared to the parent BgPI molecule. For the S-shaped 13*H*-benzo[4,5]indolo[3,2-*c*]quinolines (B[4,5]IQ, **4**), the pentacyclic ring system could stack with the Watson−Crick

(19) Escudé,C.; Nguyen, C.-H.; Kukreti, S.; Sun, J. S.; Bisagni, E.; Garestier, T.; Hélène, C. submitted.

(20) Escudé, C., Mohammadi, S., Sun, J. S., Nguyen, C.-H., Bisagni, E., Liquier, J., Taillandier, E., Garestier, T.; Hélène, C. *Chem. Biol.* **1996**, *3*, 57−65.

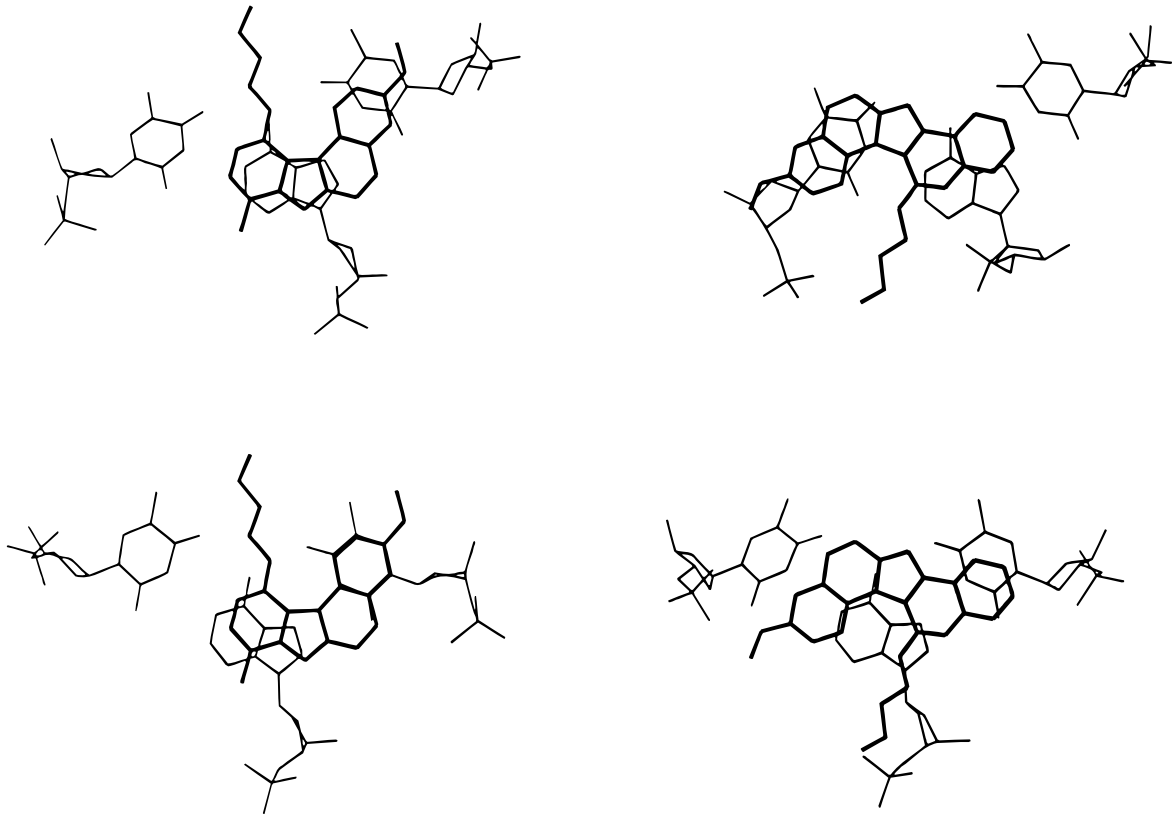


Figure 3. Energy-minimized models for the intercalation of BePI (**2**, left part) and B[4,5]IQ (**4**, right part) between two adjacent T·AxT base triplets. BePI and B[4,5]IQ molecules (shown in bold line) are shown stacked with the base triplet above (top) or below (bottom) the intercalation site. Hydrogen atoms were not drawn for clarity.

part of the base triplet on one side and the Hoogsteen part of the base triplet on the other side (Figure 3, right). These results suggested that B[4,5]IQ could afford stronger stacking interactions with neighboring base triplets than the parent BePI molecule but should be less efficient than the crescent-shaped B[6,7]IQ molecule. It should be noted that the aminoalkyl side chains of both B[4,5]IQ and B[6,7]IQ are located in the minor groove as is that of BgPI. Only BePI is predicted to have its side chain in the Watson−Hoogsteen groove of the triplex.

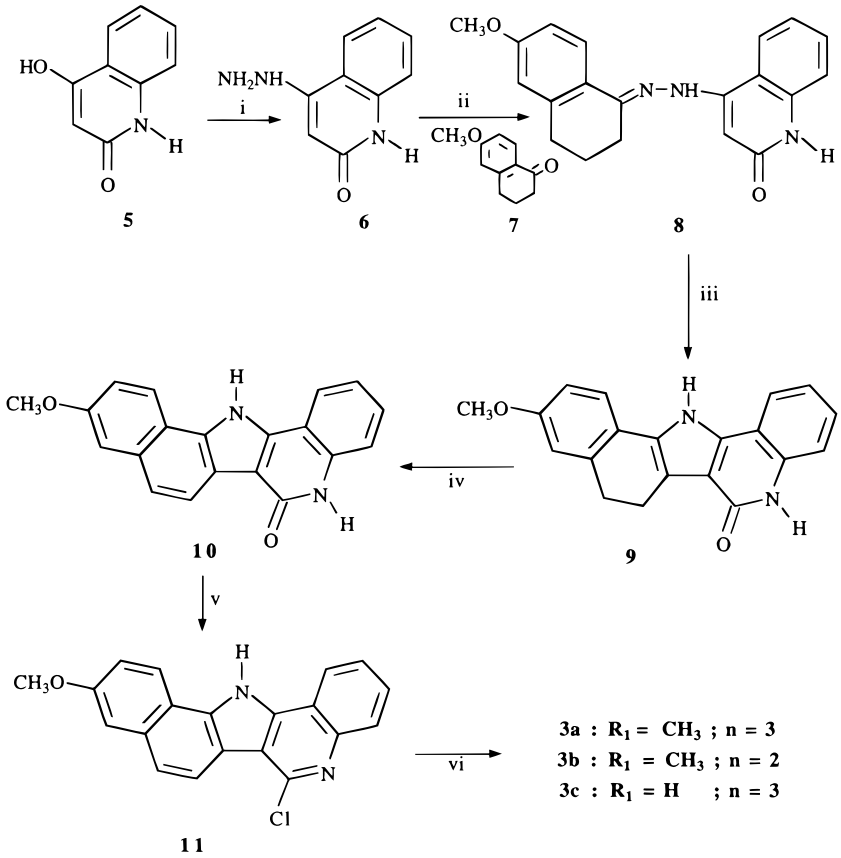
Synthetic Chemistry. According to the technique which allowed us to prepare 4-hydrazino-6-methyl (and 5-methyl)-pyridin-2(1*H*)-ones,[21,22] 4-hydroxyquinolin-2(1*H*)-one **5** led to 4-hydrazinoquinolin-2-(1*H*)-one **6** in 77% yield. In contrast to what was described earlier for the preparation of BePI and BgPI,[15,16] due to the poor solubility of the hydrazine derivative **6** in boiling ethanol or 2-ethoxyethanol, the condensation of **6** with 6-methoxy-1-tetralone **7** gave very poor yields in these solvents. In the present case, the expected hydrazone **8** was obtained in 88% yield by using acetic acid as solvent at 20 °C. The hydrazone **8** was submitted to the thermal Fischer indolization reaction giving dihydrobenzoindoloquinolone **9** which was in part directly dehydrogenated into the corresponding aromatized compound **10**. After complete aromatization in the presence of 10% palladium on charcoal, pure 10-methoxy-5*H*,13*H*-benzo[6,7]indolo[3,2-*c*]quinolin-6-one **10** was obtained in 44% yield, and these two transformations were done in the same vessel (Scheme 1).

After chlorination[23] of **10** to **11** (42% yield), chlorine atom substitution provided 6-aminosubstituted-10-methoxy-13*H*-benzo[6,7]indolo[3,2-*c*]quinolines **3a**−**c** in 66−93% yields. When 1-tetralone **7** was replaced by its isomer 2-tetralone **12**, the same general pathway successively led to **13** (not isolated), **14** (65% yield from **6**), **15** (74%), and 6-aminosubstituted-9-methoxy-13*H*-benzo[4,5]indolo[3,2-*c*]quinoline derivatives **4a**−**c** in 71−75% yields (Scheme 2).

Triple Helix Stabilization. Triple helix stabilization by various ligands was investigated by thermal denaturation experiments which were carried out by monitoring the absorbance at 260 nm as a function of temperature. The ability of compounds **3a**−**c** and **4a**−**c** as well as their parent molecules **1** and **2** to stabilize triple helices as well as double helices were studied by measuring the change in melting temperatures of triplexes and duplexes in the presence and in the absence of ligands. Triple helices were formed upon binding of a 14-mer pyrimidine oligonucleotide to a 36-bp DNA fragment containing a 14-bp oligopyrimidine·oligopurine target sequence. One of the triplexes contains three positively charged C·GxC$^+$ triplets (triplex **14C3**), and the other one has five (triplex **14C5**). When the temperature was increased, two transitions were observed, as previously described.[8] The first one corresponds to the transition triplex ↔ duplex + third strand oligonucleotide; half-dissociation temperature of the triplex is referred to as $Tm^{3\rightarrow 2}$. The second transition corresponds to the transition duplex ↔ single strands, and its half-dissociation temperature is referred to as $Tm^{2\rightarrow 1}$. Due to the high triplex stabilization afforded by

(21) Bisagni, E.; Ducrocq, C.; Civier, A. *Tetrahedron* **1976**, *32*, 1383.
(22) Nguyen, C. H., Bisagni, E. *Tetrahedron* **1986**, *42*, 2303.
(23) Robins, M. J.; Uznanski, B. *Can. J. Chem.* **1981**, *59*, 2601.

Scheme 1. Synthetic Pathway for B[6,7]IQ Compounds (**3a**−**c**)[a]



[a] Conditions: (i) NH_2-NH_2, $EtO(CH_2)_2OH$, N_2, reflux, 48 h; (ii) AcOH, room temperature, 36 h; (iii) Ph_2O, N_2, reflux, 30 min; (iv) Pd/C, Ph_2O, N_2, reflux 1 h; (v) $Et_2NC_6H_5$, $C_6H_5CH_2NEt_3Cl$, CH_3CN, $POCl_3$, N_2, reflux, 48 h; (vi) diamine (large excess), N_2, reflux, 24 h.

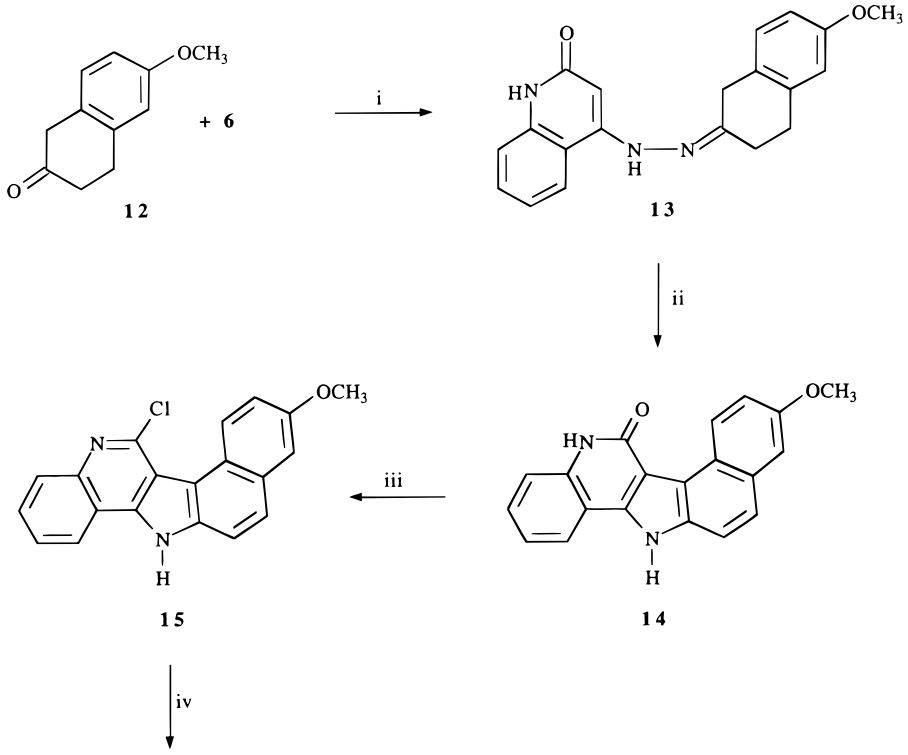
compounds **3a**−**c**, the values of $\Delta Tm^{3\rightarrow2}$ were obtained by using two 36-bp hairpin duplexes in triplex assays (as shown at the top of Table 1) in order to separate the triplex-to-duplex (3→2) and duplex-to-single strands (2→1) transitions. The values of $\Delta Tm^{2\rightarrow1}$ were determined by using the corresponding 36-bp duplexes without hairpin. Results are summarized in Table 1 where $\Delta Tm^{3\rightarrow2}$ and $\Delta Tm^{2\rightarrow1}$ stand for the difference of melting temperatures (Tm) of the triplex-to-duplex and the duplex-to-single strands transitions, respectively, in the presence and in the absence of ligands.

It can be seen that the $\Delta Tm^{3\rightarrow2}$ values characterizing the stabilization of the triplex-to-duplex transition of the newly synthesized 13*H*-benzo[6,7]indolo[3,2-*c*]quinolines (B[6,7]IQ, compounds **3a**−**c**) are in the range of 50−54 °C for the **14C3** triplex and 27−34 °C for the **14C5** triplex, whereas the $\Delta Tm^{2\rightarrow1}$ values characterizing the stabilization of the duplex-to-single strands transitions are in the range of 15−21 °C and 12−19 °C for the systems **14C3** and **14C5**, respectively. For the 13*H*-benzo[4,5]indolo[3,2-*c*]quinolines (B[4,5]IQ) compounds (**4a**−**c**), the $\Delta Tm^{3\rightarrow2}$ values are significantly lower than those observed for their B[6,7]IQ analogues by 9−19 °C for both triplexes. It should be pointed out that the lower triplex stabilization for triplex **14C5** as compared to triplex **14C3** could be explained by the unfavorable charge repulsion between positively charged ligands and C·GxC$^+$ base triplets, as previously described.[3]

Discussion

The newly synthesized pentacyclic aromatic compounds, 6-amino benzoindoloquinoline derivatives, (B[6,7]IQ and B[4,5]-IQ, **3a**−**c** and **4a**−**c**), are significantly more potent at stabilizing triplexes than their parent BgPI and BePI molecules (**1** and **2**, respectively), whereas their stabilizing effects on duplexes are similar to those of BgPI and BePI. The improved triplex stabilization could be ascribed to a greater stacking interaction as the $\pi-\pi$ electronic orbital overlap was increased between base triplets and the pentacyclic ring system of BIQ (Figures 2 and 3). The observation of a higher triplex stabilization by the crescent-shaped B[6,7]IQ derivatives which have better shape to fit with that of base triplets than the S-shaped B[4,5]IQ derivatives is consistent with energy-minimized models. It should be noted that the values of $\Delta Tm^{3\rightarrow2}$ measures a difference in binding for triplex versus duplex structures and not the strength of binding to the triplex alone. Similarly $\Delta Tm^{2\rightarrow1}$ measures a difference in binding to the duplex versus single strands. The most interesting property of this new series of molecules is their ability to discriminate between triplex and duplex structures, as judged by the comparison of ΔTm values between the triplex-to-duplex and the duplex-to-single strands transitions ($\Delta Tm^{3\rightarrow2}$ versus $\Delta Tm^{2\rightarrow1}$). The B[6,7]IQ derivatives (**3a**−**c**) not only stabilize the triplex-to-duplex transition of the **14C3** system by 50−54 °C (22−26 °C more than the parent BgPI molecule) but also exhibit a high selectivity toward triple-helical structures (with $\Delta Tm^{2\rightarrow1}$ values similar to that of the parent molecule BgPI). It turns out that one of the B[6,7]IQ derivatives (**3b**) is the most potent triplex-specific ligand ever described in our various works.[3−5,8] Triplex ligands with high selectivity stabilize intermolecular triple helices; they could also induce and trap intramolecular triple helices (H-DNA) in vivo. The prospect of delineating the role of triple helices in cells with the help of the highly specific triplex ligand (**3b**) motivates us to carry out further developments starting from this new

Scheme 2. Synthetic Pathway for B[4,5]IQ Compounds (**4a**−**c**)[a]



[a] Conditions: (i) AcOH, room temperature, 3.5 h; (ii) Ph_2O, reflux, 1 h then 10% Pd/C, N_2 reflux, 1.5 h; (iii) $Et_2NC_6H_5$, $C_6H_5CH_2NEt_3Cl$, CH_3CN, $POCl_3$, N_2, reflux 20 h; (iv) diamine, large excess, reflux, 20 h.

promising lead compound. Further synthetic studies are now in progress in our laboratories in order to improve the triplex stabilizing properties and the triplex/duplex discrimination of these ligands. Conjugates of these triplex-specific intercalating agents with triplex-forming oligonucleotides should lead to major groove ligands of DNA with high affinity and specificity.

Experimental Section

Molecular Modeling. A DNA triplex structure was constructed by molecular modeling techniques using coordinates that correctly take into account the sugar conformation of (T,C)-motif triple helices.[25] This structure is closer to a B-form DNA as reported by NMR studies[26,27] than the structure previously proposed by Arnott[28] based on fiber X-ray diffraction. The JUMNA program allows us to construct DNA structures according to their helical parameters.[29] An intercalation site can be easily created in the triplex by doubling the rise parameter for two adjacent T·AxT base triplets (rise = 6.8 Å) and subsequently decreasing the twist parameter between these two triplets from 34° to 16° in order to reduce bond distance constraints. Molecular structures for BgPI (**1**), BePI (**2**), B[6,7]IQ (**3**), and B[4,5]IQ (**4**) (see Figure 1) were constructed using the builder module of the Insight II package (MSI, San Diego) and minimized using the discover module. These molecules were docked into the triplex intercalation site, in various orientations with the aminoalkyl side chain located either in the minor groove or in the major groove of triple helices. Then, energy minimization was performed in order to generate the conformations of lowest energy. Energies were also calculated for the free ligand molecules and the triplex structure in the absence of ligand. Interaction energies were then estimated from these separate calculations. Solvent and counterions were not explicitly included in these calculations. Instead, a sigmoidal distance-dependent dielectric constant was used, and each phosphate group was assigned half an electronic charge.

Chemistry. Melting points (mp) are uncorrected and were measured using an Electrothermal IA9200 melting point apparatus. NMR spectroscopy were performed on a Bruker AC 200 spectrometer in DMSO-d_6. ^{1}H chemical shifts δ were referenced to residual solvent peak DMSO-d_6 (2.54 ppm). Mass spectra (MS) were performed using a Kratos MF80 mass spectrometer by the electron ionization procedure (70 ev). Elemental analysis were performed by Service Central de Microanalyses du CNRS, 91190 Gif sur Yvette, France.

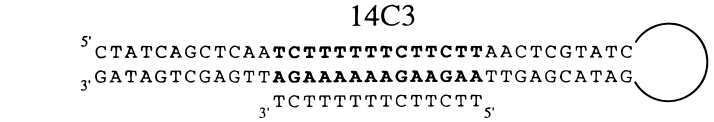
4-Hydrazinoquinolin-2(1*H*)-one (6). The mixture of 4-hydroxyquinolin-2(1*H*)-one **5** (10 g, 62 mmol), 2-ethoxyethanol (30 mL) and hydrazine hydrate (14 mL, 288 mmol) was heated at reflux under nitrogen for 48 h and then cooled to 0 °C. The resulting precipitate was filtered, taken up in boiling absolute ethanol (100 mL), and then filtered to provide 8.4 g (77%) of beige microcrystals of **6**, mp 259−261 °C (dec). ^{1}H NMR: δ 10.72 (1 H, br s), 8.19 (1 H, s), 7.86 (1 H, d, J = 8 Hz), 7.50−7.35 (1 H, m), 7.21 (1 H, d, J = 7.6 Hz), 7.15−6.95 (1 H, m), 5.72 (1 H, s), 4.28 (2 H, s). Anal. Calcd for $C_9H_9N_3O$: C, 61.70; H, 5.18; N, 23.99. Found. C, 61.83; H, 5.18; N, 24.10.

***N*-[(Quinolin-2(1*H*)-one)-4-yl]-*N*′-[1-(6-methoxy-1,2,3,4-tetrahydronaphthylidene)]hydrazine (8).** To a solution of 4-hydrazinoquinolone **6** (5.1 g, 29 mmol) in acetic acid (750 mL) was added a solution of 6-methoxy-1-tetralone (5.7 g, 32 mmol) in the same solvent (50 mL). The mixture was stirred at room temperature under nitrogen for 36 h, and the resulting precipitate was collected, washed with ethanol, and dried to give colorless microcrystals of **8** (8.6 g, 88%) mp 276 °C.

(24) Cantor, C. R.; Warshaw, M. M. *Biopolymers* **1970**, *9*, 1059.
(25) Ouali, M.; Letellier, R.; Adnet, F.; Liquier, J.; Sun, J. S.; Lavery, R.; Taillandier, E. *Biochemistry* **1993**, *32*, 2098.
(26) Macaya, R. F.; Schultze, P.; Feigon, J. *J. Am. Chem. Soc.* **1992**, *114*, 781.
(27) Radhakrishnan, I.; Patel, D. J. *Biochemistry* **1994**, *33*, 11405.
(28) Arnott, S., Bond, P. J., Selsing, E.; Smith, P. J. C. *Nucl. Acids Res.* **1976**, *3*, 2459.
(29) Lavery, R.; Sklenar, H. *J. Biomol. Struct. Dyn.* **1988**, *6*, 63.

Table 1. Two Triplex Systems Used in the Present Work[a] and ΔTm Values (±1 °C) of Triplex and Duplex in the Presence of 15 μM Ligands (**3a**−**c** and **4a**−**c)** as well as Their Parent Compounds (**1** and **2**), Respectively[b]

14C3



14C5

	triplex			
	14C3		14C5	
compd	$\Delta Tm^{3\rightarrow2}$	$\Delta Tm^{2\rightarrow1}$	$\Delta Tm^{3\rightarrow2}$	$\Delta Tm^{2\rightarrow1}$
1	+28	+16	+12	+13
3a	+54	+21	+32	+19
3b	+54	+15	+34	+12
3c	+50	+18	+27	+17
2	+20	+12	+3	+10
4a	+35	+12	+13	+12
4b	+36	+13	+15	+12
4c	+41	+7	+18	+6

[a] They differ by the number of C·GxC$^+$ base triplets (3 in **14C3** and 5 in **14C5**). In each system, a 14-mer triple helix-forming oligopyrimidine can bind to the 36-bp DNA duplex containing a 14-bp oligopyrimidine·oligopurine target sequence. [b] In the absence of ligands, the Tm of the triplexes **14C3** and **14C5** are 18 and 26 °C, respectively, whereas the Tm of the corresponding target duplexes are 58 and 60 °C, respectively. A hairpin duplex was used to measure $\Delta Tm^{3\rightarrow2}$ and an intermolecular duplex for $\Delta Tm^{2\rightarrow1}$ measurements. The thermal denaturation experiments were carried out in 10 mM cacodylate buffer (pH 6.2) containing 0.1 M NaCl, for both triple and double helix at a concentration of 1.5 μM (see Experimental Section for details).

^{1}H NMR: δ 11.05 (1 H, br s), 9.20 (1 H, s), 8.15−7.95 (2 H, m), 7.60−7.45 (1 H, m), 7.30 (1 H, d, J = 7.9 Hz), 7.20−7.10 (1 H, m), 6.88 (1 H, d, J = 8.7 Hz), 6.81 (1 H, s), 6.27 (1 H, s), 3.80 (3 H, s), 2.95−2.70 (4 H, m), 1.90−1.80 (2 H, m). MS: 32 (100%); 333 (41%). Anal. Calcd for $C_{20}H_{19}N_3O_2$·0.25H_2O: C, 71.11; H, 5.77; N, 12.74; O, 10.38. Found. C, 71.00; H, 5.88; N, 12.55; O, 10.57.

10-Methoxy-5*H*,13*H*-benzo[6,7]indolo[3,2-*c*]quinolin-6-one (10). The solution of **8** (2.23 g, 6.7 mmol) in diphenyl ether (200 mL) was stirred under N_2 and heated under reflux for 30 min. The mixture was allowed to cool to ca. 150 °C, and 10% palladium on charcoal (600 mg) suspended in diphenyl ether (20 mL) was added cautiously. The new mixture was heated under reflux for 1 h, hexane (100 mL) was added to the cooled mixture, and the resulting precipitate was filtered and washed with hexane (2 × 50 mL). It was taken up in boiling 2-ethoxyethanol (200 mL) and filtered for elimination of palladized charcoal, and the solvent was evaporated under reduced pressure. After purification by heating in boiling ethanol (200 mL), the solid was collected to provide pale-yellow microcrystals of **10** (0.93 g, 44%) mp > 270 °C. ^{1}H NMR: δ 12.80 (1 H, s), 11.49 (1 H, s), 8.58 (1 H, d, J = 8.9 Hz), 8.39 (1 H, d, J = 8.0 Hz), 8.28 (1 H, d, J = 8.6 Hz), 7.68 (1 H, d, J = 8.6 Hz), 7.60−7.45 (3 H, m), 7.45−7.30 (2 H, m), 3.94 (3 H, s). MS: 28 (100%); 314 (65%). Anal. Calcd for $C_{20}H_{14}N_2O_2$·0.25H_2O: C, 75.35; H, 4.55; N, 8.79; O, 11.31. Found. C, 75.34; H, 4.83; N, 8.96; O, 11.30.

6-Chloro-10-methoxy-13*H*-benzo[6,7]indolo[3,2-*c*]quinoline (11). A mixture of benzoindoloquinolone **10** (490 mg, 1.6 mmol), acetonitrile (20 mL), benzyltriethylammonium chloride (1.45 g, 6.4 mmol), diethylaniline (1 mL, 6.3 mmol), and phosphorus oxychloride (7.5 mL, 80 mmol) was heated at reflux under nitrogen for 48 h and evaporated under reduced pressure. Ice (100 g) was added to the residue, and the mixture was stirred at room temperature for 2 h. The resulting solid

Table 2. Characterization of the 6-Amino Substituted Benzo[6,7] (and Benzo[4,5])indolo[3,2-*c*]quinoline **3a**−**c** and **4a**−**c** Derivatives

	preparation						
compd	time of reactn (h)	purifctn method of free base[a]	yield[b] (%)	formula[c]	mp (°C)	mass spectra	^{1}H NMR (DMSO-d_6) δ (ppm)
3a	20	A	93	$C_{25}H_{26}N_4O$; $2C_4H_4O_4$; H_2O	234−236	54 (100%); 398 (15%)	12.83 (1 H, s), 8.67 (1 H, d, J = 9.1 Hz), 8.46 (1 H, d, J = 6.7 Hz), 8.37 (1 H, d, J = 8.8 Hz), 7.8−7.3 (6 H, m), 7.21 (1 H, br s), 3.95 (3 H, s), 3.9−3.7 (2 H, m), 2.6−2.4 (2 H, m), 2.29 (6 H, s), 2.0−1.8 (2 H, m)
3b	24	B	67	$C_{24}H_{24}N_4O$; $2C_4H_4O_4$; H_2O	177−179 dec	28 (100%); 58 (87%); 313 (67%)	12.86 (1 H, s), 8.67 (1 H, d, J = 9.1 Hz), 8.47 (1 H, d, J = 7.9 Hz), 8.35 (1 H, d, J = 8.9 Hz), 7.8−7.3 (6 H, m), 6.60 (1 H, t, J = 6.0 Hz), 3.95 (3 H, s), 3.9−3.8 (2 H, m), 2.68 (2 H, t, J = 6.7 Hz), 2.31 (6 H, s)
3c	26	C	66	$C_{23}H_{22}N_4O$	243−245	327 (100%); 370 (15%)	13.2−12.5 (2 H, br s), 8.67 (1 H, d, J = 9.0 Hz), 8.6−8.4 (2 H, m), 7.8−7.0 (7 H, m), 3.95 (3 H, s), 3.9−3.7 (2 H, m), 2.9−2.7 (2 H, m), 2.0−1.8 (2 H, m)
4a	17	A	75	$C_{25}H_{26}N_4O$; $2C_4H_4O_4$	205−207 dec	326 (100%); 398 (12%)	12.83 (1 H, br s), 9.13 (1 H, d, J = 9.2 Hz), 8.31 (1 H, d, J = 7.5 Hz), 8.0−7.75 (2 H, m), 7.71 (1 H, d, J = 8.2 Hz), 7.6−7.4 (2 H, m), 7.4−7.2 (2 H, m), 6.24 (1 H, br s), 3.94 (3 H, s), 3.8−3.6 (2 H, m), 2.6−2.4 (2 H, m), 2.15 (6 H, s), 2.05−1.9 (2 H, m)
4b	20	A	71	$C_{24}H_{24}N_4O$; $2C_4H_4O_4$	173−175	313 (100%); 385 (3%); 384 (1%)	12.84 (1 H, s), 9.09 (1 H, d, J = 9.2 Hz), 8.31 (1 H, d, J = 7.8 Hz), 7.95−7.8 (2 H, m), 7.71 (1 H, d, J = 8.2 Hz), 7.6−7.4 (2 H, m), 7.35−7.25 (2 H, m), 6.49 (1 H, br s), 3.94 (3 H, s), 3.8−3.6 (2 H, m), 2.8−2.7 (2 H, m), 2.30 (6 H, s)
4c	18	C	71	$C_{23}H_{22}N_4O$	201−203	326 (100%); 370 (11%)	9.14 (1 H, d, J = 9.2 Hz), 8.31 (1 H, d, J = 7.1 Hz), 7.95−7.8 (2 H, m), 7.71 (1 H, d, J = 7.9 Hz), 7.6−7.4 (2 H, m), 7.4−7.25 (2 H, m) 6.81 (1 H, br s), 3.94 (3 H, s), 3.73 (1 H, t, J = 6.5 Hz) 2.83 (1 H, t, J = 6.5 Hz), 2.05−1.85 (1 H, m)

[a] Method A: flash chromatography was performed on a neutral alumina column with (i) pure methylene chloride and (ii) 99:1 methylene chloride−ethanol mixture as eluents. Method B: as method A, followed by a second alumina column with ethyl acetate as eluent. Method C: recrystallization from toluene. [b] Yield of maleate salts (**3a**, **3b**, **4a**, and **4b**) or free bases (**3c** and **4c**) from chloro derivatives. [c] Results of elemental analysis are within ±0.4% of the theoretical values corresponding to the mentioned formulas for the C, H, N, and O elements.

was filtered, washed with water (3 × 10 mL), suspended in water (50 mL), and treated with an excess of ammonia, and the precipitate was collected after stirring at ambient temperature for 18 h. It was recrystallized from ethanol to give colorless microcrystals of **11** (220 mg, 42%) mp 281−283 °C (dec). ^{1}H NMR: δ 13.46 (1 H, s), 8.85−8.55 (2 H, m), 8.47 (1 H, d, J = 8.8 Hz), 8.25−8.00 (1 H, m), 7.95−7.70 (3 H, m), 7.59 (1 H, d, J = 2.4 Hz), 7.46 (1 H, dd, J = 8.9, 2.4 Hz), 3.97 (3 H, s). MS: 332 & 334 (100 & 42%); 289 (59%). Anal. Calcd for $C_{20}H_{13}ClN_2O$: C, 72.18; H, 3.91; N, 8.42; Cl, 10.68. Found. C, 72.22; H, 3.93; N, 8.35; Cl, 10.77.

9-Methoxy-5*H*,13*H*-benzo[4,5]indolo[3,2-*c*]quinolin-6-one (14). To a solution of 4-hydrazinoquinolone **6** (2.0 g, 11.4 mmol) in acetic acid (220 mL) was added 6-methoxy-2-tetralone **12** (2.25 g, 12.8 mmol). The mixture was stirred at room temperature under nitrogen for 3.5 h, and the solvent was evaporated to dryness under reduced pressure. After elimination of traces of acetic acid by coevaporation with dioxane (3 × 20 mL) under reduced pressure, diphenyl ether (300 mL) was added, and the new mixture was heated at reflux (245 °C) under nitrogen for 1 h. After cooling to ca. 150 °C, 10% palladium on charcoal (1.0 g) suspended in diphenyl ether (30 mL) was added cautiously and the heating at reflux was pursued for 1.5 h. Hexane (150 mL) was added to the cooled mixture, and the resulting precipitate was filtered and washed with hexane (2 × 50 mL). It was taken up in boiling acetic acid (150 mL) and filtered for elimination of palladized charcoal, and the solvent was concentrated to ca. 25 mL by evaporation under reduced pressure. The solid was collected, washed with hot toluene (80 mL), and dried to provide beige microcrystals of **14** (2.35 g, 65%) mp 298−301 °C dec. Unsatisfactory elemental analysis results were obtained for this compound due to the presence of traces of toluene (characterized by NMR) even after drying the sample in a hot vacuum desiccator for one night. However NMR and mass spectra are fully consistent with the assigned structure. ^{1}H NMR: δ 12.95 (1 H, s), 11.51 (1 H, br s), 10.31 (1 H, d, J = 9.2 Hz), 8.29 (1 H, d, J = 7.9 Hz), 7.90−7.75 (2 H, m), 7.60−7.50 (2 H, m), 7.48 (1 H, d, J = 2.6 Hz), 7.40−7.25 (2 H, m), 3.94 (3 H, s). MS: 28 (100%); 314 (72%).

6-Chloro-9-methoxy-13*H*-benzo[4,5]indolo[3,2-*c*]quinoline (15). The compound **15** was prepared (2 g scale, 74% yield) as described above for compound **11** (obtained from **10**) with a heating time of 20 h, mp > 270 °C. ^{1}H NMR: δ 12.34 (1 H, s), 9.70 (1 H, d, J = 9.4 Hz), 8.70−8.60 (1 H, m), 8.15−8.00 (2 H, m), 7.93 (1 H, d, J = 8.8 Hz), 7.85−7.70 (2 H, m), 7.62 (1 H, d, J = 2.8 Hz), 7.41 (1 H, dd, J = 7.9, 2.8 Hz), 3.97 (3 H, s). MS: 332 & 334 (100 & 36%). Anal. Calcd for $C_{20}H_{13}ClN_2O\cdot0.5C_2H_5OH\cdot0.5H_2O$: C, 68.95; H, 4.96; N, 7.66; Cl, 9.69; O, 8.75. Found. C, 69.36; H, 4.87; N, 7.44; Cl, 9.62; O, 8.71.

General Procedure for Obtaining 6-(Aminoalkyl)-amino-10-(and 9)-methoxy-13*H*-benzo[6,7](and [4,5])indolo[3,2-*c*]quinoline Derivatives (3a−c and 4a−c). The mixture of the chloro derivative (**11** or **15**, 10 mmol) and the required primary amine (30 mL, large excess) was heated at reflux under N_2 for the period indicated in Table 2. Excess amine was evaporated under reduced pressure; the residue was taken up in 5% ammonia (60 mL) and filtered after 15 min stirring, and the solid was suspended during 1 h in water (60 mL). Free bases **3a−c** and **4a−c** were collected by filtration, washed with water, air-dried, and purified using the method mentioned in Table 2. Maleate salts were prepared by treatment of the free bases with an excess of maleic acid (3 equiv) in acetone as solvent, then washed with acetone, and air-dried.

Other Materials. Oligonucleotides (OligoGold grade) were purchased from Eurogentec (Seraing, Belgium). They were ethanol precipitated, and their concentration was calculated using a nearest-neighbor model.[24]

Thermal Denaturation Experiments. All thermal denaturation studies were carried out on a Uvikon 940 spectrophotometer and interfaced to an IBM-AT personal computer for data collection and analysis. Temperature control of the cell holder was achieved by a Haake D8 circulating water bath. The temperature of the water bath was decreased from 90 to 0 °C and then increased back to 90 °C at a rate of 0.1 °C/min with a Haake PG 20 thermoprogrammer, and the absorbance at 260 nm was recorded every 10 min. The maxima of the first derivatives of the melting curves gave a good approximation of the half-dissociation temperature (Tm) and allowed us to characterize the stabilities of the complexes in a reproducible way. Due to the high triplex stabilization afforded by compunds **3a−c**, two hairpin 36-bp duplexes were used in triplex assays in order to separate the triplex-to-duplex and the duplex-to-single strands transitions. The DNA melting experiments were carried out in a 10 mM cacodylate buffer (pH 6.2) containing 0.1 M NaCl in the absence and in the presence of 15 μM ligands, for both the triple and double helices at a concentration of 1.5 μM.

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