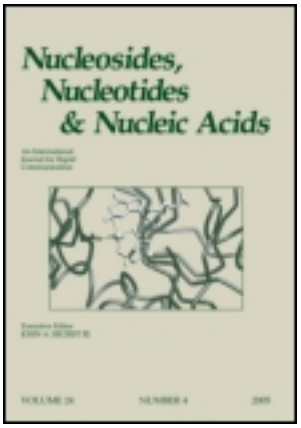

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Synthesis of A New Intercalating Nucleic Acid 6H-INDOLO[2,3-b] Quinoxaline Oligonucleotides to Improve Thermal Stability Of Hoogsteen-Type Triplexes

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SYNTHESIS OF A NEW INTERCALATING NUCLEIC ACID 6*H*-INDOLO[2,3-*b*] QUINOXALINE OLIGONUCLEOTIDES TO IMPROVE THERMAL STABILITY OF HOOGSTEEN-TYPE TRIPLEXES

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□ *A new intercalating nucleic acid monomer* ***X*** *was obtained in high yield starting from alkylation of 4-iodophenol with (S)-(+)-2-(2,2-dimethyl-1,3-dioxolan-4-yl)ethanol under Mitsunobu conditions followed by hydrolysis with 80% aqueous acetic acid to give a diol which was coupled under Sonogashira conditions with trimethylsilylacetylene (TMSA) to achieve the TMS protected (S)-4-(4-((trimethylsilyl)ethynyl)phenoxy)butane-1,2-diol. Tetrabutylammonium flouride was used to remove the silyl protecting group to obtain (S)-4-(4-ethynylphenoxy)butane-1,2-diol which was coupled under Sonogashira conditions with 2-(9-bromo-6H-indolo[2,3-b]quinoxalin-6-yl)-N,N-dimethylethanamine to achieve (S)-4-(4-((6-(2-(dimethylamino)ethyl)-6H-indolo[2,3-b]quinoxalin-9-yl)ethynyl)phenoxy)butane-1,2-diol. This compound was tritylated with 4,4′-dimethoxytrityl chloride followed by treatment with 2-cyanoethyltetraisopropylphosphordiamidite in the presence of N,N-diisopropyl ammonium tetrazolide to afford the corresponding phosphoramidite. This phosphoramidite was used to insert the monomer* ***X*** *into an oligonucleotide which was used for thermal denaturation studies of a corresponding parallel triplex.*

Keywords Intercalating nucleic acid; 6*H*-indolo[2,3-*b*]quinoxaline; hoogsteen base pairing; thermal stability; triplex forming oligonucleotides

INTRODUCTION

There is an interest for high-affinity and sequence specific recognition of double stranded DNA for diagnostic and therapeutic purposes.[1,2] Therefore, there is an interest in the design and synthesis of new triplex forming

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oligonucleotides (TFOs) that can hybridize efficiently and selectively to double stranded DNA at neutral pH. The so formed DNA triple helix consists of a third strand of nucleic acid of appropriate sequence bound in the major groove of an intact duplex.[3] These structures form most readily in polypurine·polypyrimidine tracts and they are stabilized by hydrogen bonds between the third strand bases and the duplex purines.[4] Depending on the target sequence the TFO might consists of purines or pyrimidines. In the pyrimidine motif, a (C,T) containing oligonucleotides binds parallel to the target oligopurine strand through Hoogsteen hydrogen bonds, and forms canonical C.GxC$^+$ and T.AxT base triplets. Due to the requirement for cytosine protonation, the stability of a C.GxC$^+$ motif triple helix is pH dependent. In the purine motif, the (G,A) containing oligonucleotide binds antiparallel to the oligopurine strand of the duplex by forming C.GxG and T.AxA base triplets through reverse Hoogsteen hydrogen bonds.[5–10] The insertions of conjugated intercalators in the TFO have been shown to be an effective way to stabilize the triplexes. The twisted intercalating nucleic acids (TINA) inserted as a bulge in a triple-helix forming oligonucleotidde (TFO), stabilize parallel Hoogsteen triplexes (Figure 1).[11] In addition, Paramavisam et al.[12] have recently shown that incorporation of TINA monomers in G-rich oligonucleotides interferes with quadruplex formation at physiological potassium concentrations releasing the oligonucleotide for antiparallel triplex formation.

Heterocyclic rings with nitrogen atoms constitute the core structures of molecules having various biological activities. The chemistry of indoloquinoxalines is of considerable interest possessing a broad spectrum of biological activities. Derivatives of indolo[2,3-b]quinoxaline possess diverse

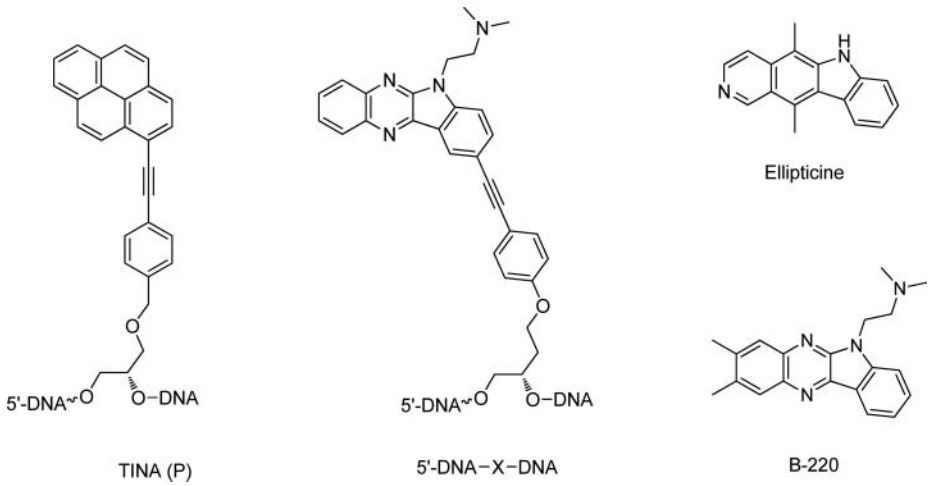


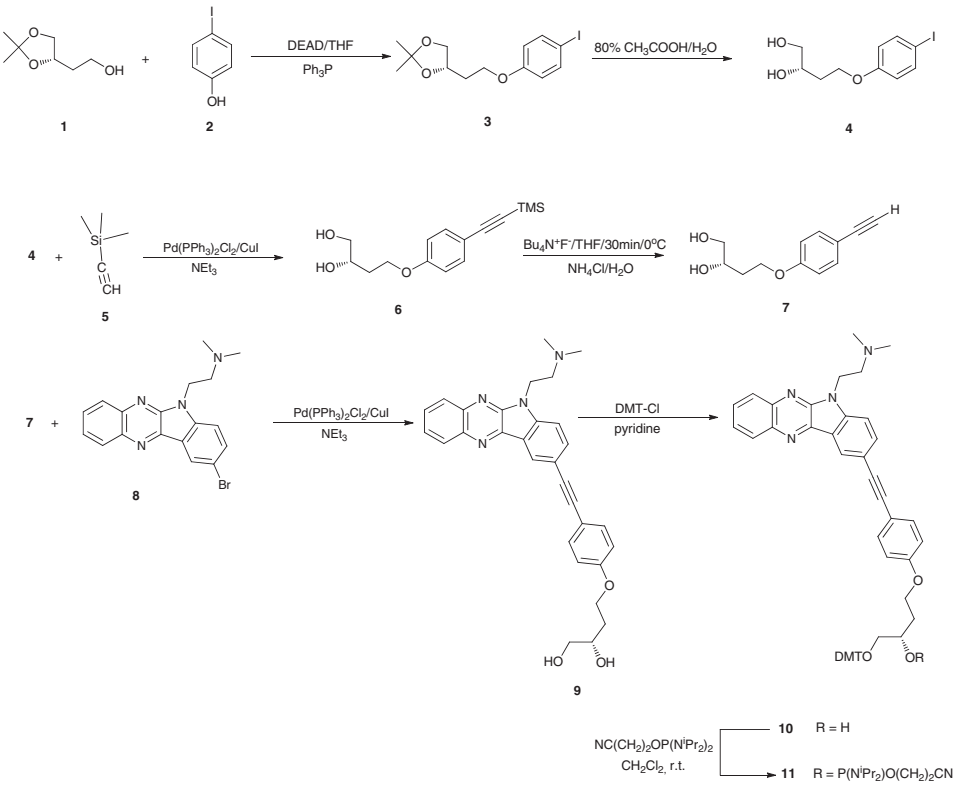
FIGURE 1 Intercalating nucleic acids TINA and 5′-DNA-X-DNA and the intercalators ellipticine and B-220.

biological activities such as anticancer, anticonvulsant and antibacterial. However, several condensed ring systems containing indole have antiviral activity.[13] Such compounds are distantly related to ellipticine and quinacrines, which are known to intercalate in DNA.[14] Intercalation of planar aromatic molecules was discovered in the early 1960s, when it was observed that acridine derivatives have the capacity to insert between two consecutive DNA base pairs.[15–17] Rapidly, this mode of binding to nucleic acids was found to occur with many compounds, in particular dyes like ethidium bromide and methylene blue. Later, it was realized that drugs could also exert a therapeutic effect via intercalation into DNA. Nowadays, numerous intercalating agents are being used as therapeutics for the treatment of cancer as well as for parasitic and viral diseases.[18–20] For example, the antitumor drugs Daunomycin, mitoxantrone and amsacrine contain a planar chromophore that intercalates into DNA. 6H-Indolo[2,3-b]quinoxaline compounds represent an important series of DNA intercalating agents endowed with antiviral and cytotoxic activities.[21] Their effect on herpes viruses was assayed in different cells. For example, compound B-220 (2,3-dimethyl-6-(2-dimethylaminoethyl)-6*H*-indolo[2,3-b]quinoxaline) has shown remarkable activity against herpes virus.[22, 23] Both the dimethylaminoethyl side chain and the tetracyclic chromophore contribute to the interaction with DNA.[24–26] Compound B-220 has a structural analogy with the antitumor alkaloid ellipticine which is a well-known DNA intercalator.[27] In our ongoing research, we are looking for new analogues of intercalating nucleic acids and we considered it interesting to replace the pyrene moiety in TINA with a moiety identical to the compound B-220 because this moiety is most likely a good intercalator and also because compound B-220 itself shows low in vivo toxicity when tested in rats.[22]

RESULTS AND DISCUSSION

Chemistry

The synthetic route for obtaining the desired acyclic amidite **11** is shown in (Scheme 1). The synthesis of (*S*)-4-(4-iodophenoxy)butane-1,2-diol **4** was achieved under Mitsunobu conditions as previously described.[28] In this way, (*S*)-2-(2,2-dimethyl-1,3-dioxolan-4-yl)ethanol **1**, diethyl azodicarboxylate (DEAD), triphenylphosphine, and 4-iodophenol **2** were reacted in tetrahydrofuran (THF) to afford (*S*)-4-(2-(4-iodophenoxy)ethyl)-2,2-dimethyl-1,3-dioxolane **3**. Subsequent treatment with 80% aqueous acetic acid gave the diol **4** in satisfactory yield which was used in the next step without further purification. The subsequent Sonogashira coupling with trimethylsilylacetylene (TMSA) was done with $Pd(PPh_3)_2Cl_2$, CuI,



SCHEME 1

NEt_3, under Ar affording the TMS protected (*S*)-4-(4-((trimethylsilyl)-ethynyl)phenoxy)butane-1,2-diol **6** in 95% yield. The silyl protecting group was removed by addition of tetrabutylammonium flouride (TBAF) in dry THF to obtain (*S*)-4-(4-ethynylphenoxy)butane-1,2-diol **7** in 81% yield, which was coupled under Sonogashira conditions with 2-(9-bromo-6H-indolo[3,2-b]quinoxalin-6-yl)-N,N-dimethylethanamine **8** prepared by method of Bergman and Åkerfeldt [29], $Pd(PPh_3)_2Cl_2$, CuI, and NEt_3 under Ar in a micro wave cavity to achieve (*S*)-4-(4-((6-(2-(dimethylamino) ethyl)-6H-indolo[3,2-b]quinoxalin-9-yl)ethynyl)phenoxy) butane-1,2-diol **9** in 89% yield. The primary hydroxyl group was protected with 4,4′-dimethoxytrityl chloride in dry pyridine to give (*S*)-1-(bis(4-methoxyphenyl) (phenyl)methoxy)-4-(4-((6-(2-(dimethylamino)ethyl)-6H-indolo[3,2-b]quinoxalin-9-yl)ethynyl)phenoxy)butan-2-ol **10** in 52% yield. Finally, the corresponding phosphoramidite **11** was obtained in 87% yield after treatment with 2-cyanoethyl *N,N,N′,N′*-tetraisopropylphosphordiamidite in the presence of diisopropylammonium tetrazolide in dry CH_2Cl_2. The obtained phosphoramidite **11** was incorporated into a 14-mer oligonucleotide by a standard phosphoramidite protocol on an automated DNA synthesizer.

However, an extended coupling time (10 minutes) in the oligonucleotide synthesis as was used for the amidite **11**. The modified ODN was purified by reversed-phase HPLC, and its mass was confirmed by MALDI-TOF-MS analysis. The purity of the final sequence was determined by ion-exchange HPLC (IE-HPLC) to be more than 95%.

Thermal Stability Studies

The thermal stability of parallel triplex containing the intercalating monomer **X** was evaluated by thermal denaturation experiments. The melting temperatures T_m (°C) were determined as the first derivatives of melting curves. Since protonation of cytosine is required to form stable Hoogsteen bonds, thermal stability of parallel triplexes using the synthesized oligonucleotides toward the duplex (**D1**).[30] was assessed both at pH 6.0 and pH 7.2, the ultimate goal being to find parallel Hoogsteen-type triplex at physiological pH conditions. Stabilization of parallel Hoogsteen-type triplex was found in **ON2** and compared with the wild type **ON1** at pH 6.0 and pH 7.2. For the incorporation of the intercalating monomer **X** in the 14-mer oligonucleotide at pH 6.0, the triplex stability of **ON2/D1** ($T_m = 39.8$°C) is increased compared to the unmodified triplex **ON1/D1** ($T_m = 26.5$°C) (Table 1 and Figure 2). For the incorporation of the intercalating monomer X at pH 7.2, the triplex stability of **ON2/D1** ($T_m = 26.9$°C) is increased compared to the unmodified triplex **ON1/D1** ($T_m < 5.0$°C). In both cases, the stabilization confirms that the intercalating properties of **X** contribute to stabilize the triplexes. Although the stabilization is lower than the one previously observed for TINA, we find it useful to add a new intercalator to the library of intercalating nucleic acids for studies of their bioactivity.[12]

TABLE 1 T_m (°C) data for parallel triplex, evaluated from UV melting curves ($\lambda = 260$ nm)

		3′-CTGCCCCTTTCTTTTTT-5′ 5′-GACGGGGAAAGAAAAAA-3′ **(D1)**	
Entry	TFO[a]	T_m (°C) pH 6.0	T_m (°C) pH 7.2
ON1	5′-CCCCTTTCTTTTTT-3′	26.5	<5.0
ON2	5′-CCCCTT**X**TCTTTTTT-3′	39.8	26.9
ON3[b]	5′-CCCCTT**P**TCTTTTTT-3′	45.5	28.0

[a]C = 1.5 μM of **ON1–ON2** and 1.0 μM of each strand of dsDNA **(D1)** in 20 mM sodium cacodylate, 100 mM NaCl, 10 mM $MgCl_2$, pH 6.0, and 7.2. Duplex $T_m = 56.7$°C (pH 6.0) and 56.2°C (pH 7.2). T_m values determined at 260 nm.

[b]P = TINA, data taken from;[31].

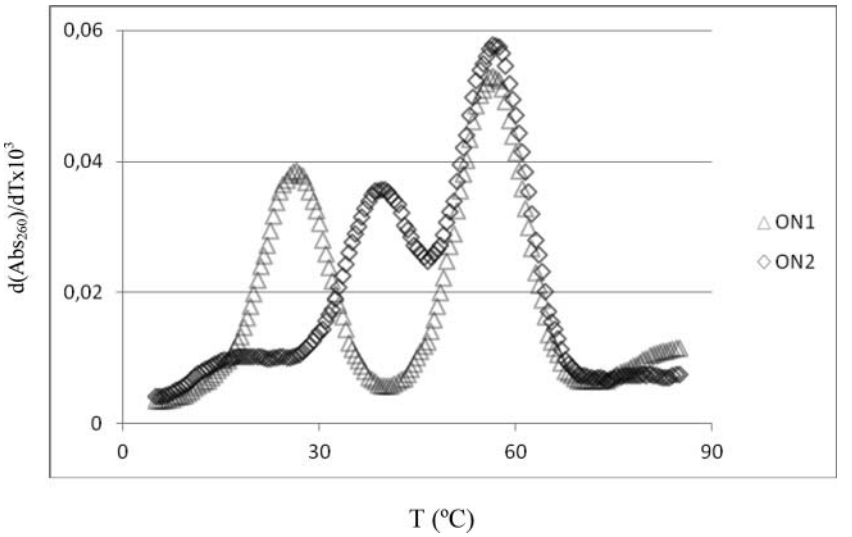


FIGURE 2 First derivatives plots of triplex melting for **ON1** wild-type triplex and **ON2** incorporating monomer X recorded at 260 nm versus increasing temperature (1°C/minute) in 20 mM sodium cacodylate, 100 mM NaCl, 10 mM $MgCl_2$ at pH 6.0.

CONCLUSIONS

We have synthesized a new intercalating nucleic acid **X** and shown its incorporation into 14-mer oligonucleotides using standard oligonucleotide synthesis procedures. Thermal melting studies showed that the intercalator **X** upon incorporation into a 14-mer oligonucleotide increase the thermal stability of a Hoogsteen-type triplex.

EXPERIMENTAL SECTION

General Information

NMR spectra were recorded on a Varian Gemini 2000 spectrometer at 300 MHz for ^{1}H, 75 MHz for ^{13}C and 121.5 MHz for ^{31}P with TMS as an internal standard for ^{1}H NMR, deuterated solvents $CDCl_3$ (δ 77.00 ppm), DMSO-d_6 (δ 39.44 ppm) for ^{13}C NMR, and 85% H_3PO_4 as an external standard for ^{31}P NMR. MALDI mass spectra of the synthesized compounds were recorded on a Fourier Transform Ion Cyclotron Resonance Mass Spectrometer (IonSpec, Irvine, CA). For accurate ion mass determinations, the (MH^+) or (MNa^+) ion was peak matched using ions derived from the 2,5-dihydroxybenzoic acid matrix. Electrospray ionization mass spectra (ESI-MS) were performed on a 4.7 T HiResESI Uitima (FT) mass spectrometer. Both spectrometers are controlled by the OMEGA Data System. Melting points were determined on a Büchi melting point apparatus. Silica gel (0.040–0.063 mm) used for column chromatography and analytical silica

gel TLC plates 60 F_{254} were purchased from Merck. Solvents used for column chromatography were distilled prior to use, while reagents were used as purchased.

Synthesis

(S)-4-(4-((trimethylsilyl)ethynyl)phenoxy)butane-1,2-diol (6)

To the solution of (*S*)-4-(4-Iodophenoxy)butane-1,2-diol **4** (0.5 g, 1.62 mmol), $Pd(PPh_3)_2Cl_2$ (81 mg, 0.12 mmol), and CuI (36 mg, 0.19 mmol) were dissolved in dry NEt_3 (20 mL). The reaction mixture was flushed with Ar for 10 minutes before TMSA **5** (0.32 g, 3.24 mmol) was added. The reaction mixture was stirred under Ar atmosphere for 24 hours. The solvent was removed under reduced pressure and the residue was dissolved in CH_2Cl_2 (50 mL), which was washed with 0.3 M aq. EDTA (2 × 50 mL). After back extraction with CH_2Cl_2 (50 mL), the organic phase was dried ($MgSO_4$), filtered, and concentrated under reduced pressure. The residue was purified by column chromatography (silica gel; CH_2Cl_2–MeOH 5%, v/v) to afford compound **6** as a brown oil. Yield 0.53 g (95%). ^{1}H NMR (300 MHz; $CDCl_3$): δ 0.19, 0.24, 0.26 (3 s, 9H) 1.90–1.96 (m, 2H), 2.82 (br.s, 2H), 3.50–3.56 (m, 1H), 3.69, 3.73 (dd, J = 3.3 Hz, 1H), 3.97–3.99 (m, 1H), 4.10–4.16 (m, 2H), 6.81 (d, J = 9.0 Hz, 2H), 7.39 (d, J = 8.7 Hz, 2H) ppm. ^{13}C NMR (75 MHz; $CDCl_3$): δ 0.00, 0.03, 0.07, 32.5, 65.0, 66.6, 69.8, 85.9, 92.6, 114.3, 115.6, 133.6, 158.7 ppm. EI-MS: Calcd for $C_{15}H_{22}O_3Si$ $[M]^+$ 278.42, found: 278.13.

(S)-4-(4-ethynylphenoxy)butane-1,2-diol (7)

Dissolve compound **6** (0.5 g, 1.8 mmol) in dry THF (15 ml) and added 1.2 eq. TBAF tetrabutylammoniumflouride (2.16 ml, 2.16 mmol) slowly to this solution. Cooled at 0°C. The resulting solution was stirred at this temperature for 30 minutes. The reaction was quenched by addition of saturated aq. NH_4Cl and the mixture extracted with EtOAC (30 ml). After back extraction with EtOAC (30 ml), the combined organic phase was washed with water (2 × 50 ml) and brine (2 × 50 ml), dried ($MgSO_4$), filtered and concentrated under reduced pressure. The residue was purified by column chromatography (silica gel; 5% MeOH in CH_2Cl_2, v/v) to afford compound **7** as brown oil. Yield 0.30 g (81%). ^{1}H NMR (300 MHz; $CDCl_3$): δ 1.87–1.93 (m, 2H), 3.01 (s, 1H), 3.53–3.55 (m, 1H), 3.65–3.72 (m, 3H), 4.09– 4.14 (m, 1H), 6.83 (d, J = 8.7 Hz, 2H), 7.39 (d, J = 8.7 Hz, 2H) ppm. ^{13}C NMR (75 MHz; $CDCl_3$): δ 32.5, 65.0, 66.7, 69.4, 75.7, 83.6, 113.9, 114.8, 114.9, 133.4, 159.2 ppm. EI-MS: Calcd for $C_{12}H_{14}O_3$ $[M]^+$ 206.24, found: 206.09.

2-(9-bromo-6H-indolo[2,3-b]quinoxalin-6-yl)-N,N-dimethylethanamine (8)

^{1}H NMR (300 MHz; $CDCl_3$): δ 2.36 (s, 6H), 2.81 (t, 2H), 4.56 (t, J = 6.9 Hz, 2H), 7.37 (d, J = 8.4 Hz, 1H), 7.67–7.76 (m, 3H), 8.12 (d, J = 8.4 Hz,

1H), 8.26 (d, $J = 8.1$ Hz, 1H), 8.57 (s, 1H) ppm. ^{13}C NMR (75 MHz; $CDCl_3$): δ 40.0, 45.8, 57.2, 110.9, 113.6, 121.2, 125.4, 126.3, 127.9, 129.1, 129.4, 133.4, 139.5, 140.8, 142.9, 145.5 ppm. EI-MS: Calcd for $C_{18}H_{17}BrN_4$ $[M]^+$ 369.0709, found: 369.0726.

(S)-4-(4-((6-(2-(dimethylamino)ethyl)-6H-indolo[2,3-b]quinoxalin-9-yl) ethynyl)phenoxy)butane-1,2-diol (9)

A solution of 2-(9-bromo-6H-indolo[3,2-b]quinoxalin-6-yl)-N,N-dimethylethan-amine **8** (0.23 g, 0.62 mmol), $Pd(PPh_3)_2Cl_2$ (31 mg, 0.04 mmol), CuI (14 mg, 0.07 mmol), and powdered PPh_3 (43 mg, 0.16 mmol) were dissolved in dry NEt_3 (25 ml) and DMF (2 ml). The reaction mixture was flushed with Ar for 30 minutes before (*S*)-4-(4-ethynylphenoxy)butane-1,2-diol **7** (0.20 g, 0.93 mmol) was added. The reaction mixture was stirred under Ar in a heavy walled smith process vial at 120°C for 25 minutes in the micro wave cavity. The reaction mixture was washed with 0.3 M aq. EDTA (2 × 50 ml) to afford compound **9** as brown solid without purification. Yield 0.33 g (89%). Mp 150–152°C. ^{1}H NMR (300 MHz; DMSO-d_6): δ 1.68 (m, 1H), 1.93 (m, 1H), 2.73 (s, 3H), 2.81 (t, 2H), 2.89 (s, 3H), 3.17 (m, 2H), 3.65 (m, 1H), 4.12 (m, 2H), 4.61(m, 2H), 7.01 (m, 2H), 7.54 (m, 2H), 7.76–7.89 (m, 3H), 7.95 (m, 1H), 8.12 (d, $J =$ 8.4 Hz, 1H), 8.26 (d, $J = 8.1$ Hz, 1H), 8.47 (m, 1H) ppm. ^{13}C NMR (75 MHz; DMSO-d_6): δ 32.9, 45.2, 56.6, 57.5, 64.7, 65.9, 67.9, 88.0, 88.7, 111.1, 114.6, 118.8, 124.6, 126.3, 127.6, 128.9, 131.5, 133.9, 134.3, 138.8, 140.0, 143.6, 145.3, 158.9 ppm. HRMS (MALDI) Calcd for $C_{30}H_{30}N_4O_3$ $[M + H]^+$ 495.2391, found: 495.2401.

(S)-1-(bis(4-methoxyphenyl)(phenyl)methoxy)-4-(4-((6-(2-(dimethylamino) ethyl)-6H-indolo[2,3-b]quinoxalin-9-yl)ethynyl)phenoxy)butan-2-ol (10)

(*S*)-4-(4-((6-(2-(dimethylamino)ethyl)-6H-indolo[3,2-b]quinoxalin-9-yl) ethynyl)phenoxy)-butane-1,2-diol **9** (0.3 g, 0.61 mmol) was dissolved in dry pyridine (20 ml) before 4,4′-dimethoxytrityl chloride (0.25 g, 0.73 mmol) was added under N_2. The reaction was stirred at room temperature overnight; an extra portion of DMT-Cl (0.12 g, 0.37 mmol) was added before it was quenched with MeOH (2 ml). The reaction mixture was diluted with EtOAc (150 ml) before washing with saturated aq. $NaHCO_3$ (2 × 40 ml). After back extraction with EtOAc (2 × 20 ml), the combined organic phase was dried (Na_2SO_4), filtered, and concentrated under reduced pressure. The residue was coevaporated with toluene–EtOH (2 × 25 ml, 1: 1, v/v) before it was purified by column chromatography (silica gel; 0.5% Et_3N v/v, 30–70% EtOAc in cyclohexane) to afford **10** as a yellow foam. Yield 0.25 g (52%). ^{1}H NMR (300 MHz; DMSO-d_6): δ 1.83 (m, 2H), 2.26 (s, 6H), 2.71 (m, 2H), 3.14 (m, 2H), 3.67 (s, 6H), 3.97 (m, 2H), 4.20 (m, 1H), 4.42 (m, 2H), 6.72 (d, $J = 8.7$ Hz, 5H), 7.16–7.25 (m, 7H),

7.30–7.37 (m, 5H), 7.55–7.66 (m, 4H), 8.01 (d, $J = 8.1$ Hz, 1H), 8.15 (d, $J = 8.1$ Hz, 1H), 8.49 (m, 1H) ppm. ^{13}C NMR (75 MHz; DMSO-d_6): δ 33.1, 45.7, 46.0, 55.1, 57.0, 64.7, 67.4, 68.1, 86.0, 88.0, 88.7, 109.4, 113.0, 114.5, 116.1, 119.6, 125.8–130.8, 132.8, 133.9, 135.9, 139.4, 140.6, 143.4, 144.8, 145.7, 158.4, 158.7 ppm. HRMS (ESI) Calcd for $C_{51}H_{48}N_4O_5$ $[M + H]^+$ 797.3697, found: 797.3680.

(S)-1-(bis(4-methoxyphenyl)(phenyl)methoxy)-4-(4-((6-(2-(dimethylamino) ethyl)-6H-Indolo[2,3-b]quinoxalin-9-yl)ethynyl)phenoxy)butan-2-yl (2-cyanoethyl) diisopropylphosphoramidite (11)

(*S*)-1-(bis(4-methoxyphenyl)(phenyl)methoxy)-4-(4-((6-(2-(dimethylamino)ethyl)-6H-indolo[2,3-b]quinoxalin-9-yl)ethynyl)phenoxy)butan-2-ol **10** (0.18 g, 0.23 mmol) and diisopropyl ammonium tetrazolide (0.06 g, 0.35 mmol) were dissolved under Ar in dry CH_2Cl_2 (20 ml), followed by drop wise addition of 2-cyanoethyl *N*,*N*,*N*′,*N*′-tetraisopropylphosphordiamidite (0.21 g, 0.69 mmol) via a syringe at 0°C. The reaction mixture was stirred under Ar at room temperature overnight before the reaction was quenched with H_2O (50 ml) and the organic phase was washed with H_2O (2 × 50 ml). After back extraction with CH_2Cl_2 (50 ml), the combined organic phase was dried ($MgSO_4$), filtrated, and concentrated under reduced pressure. The residue was purified by dry column vacuum chromatography (silica gel; Et_3N 0.5% v/v, 50–50% EtOAc in cyclohexane) to afford **11** as a yellow foam. Yield 0.2 g (87%). ^{1}H NMR (300 MHz; $CDCl_3$): δ 1.15–1.18 (m, 12H), 1.19 (m, 2H), 2.35 (s, 6H), 2.73 (t, $J = 6.3$ Hz, 2H), 2.81 (m, 2H), 3.44–3.55 (m, 6H), 3.78 (s, 6H), 4.09–4.17 (m, 3H), 4.53 (m, 2H), 6.84 (m, 5H), 7.19–7.51 (m, 12H), 7.62–7.79 (m, 4H), 8.15 (d, $J = 8.1$ Hz, 1H), 8.23 (d, $J = 8.1$ Hz, 1H), 8.56 (m, 1H) ppm. ^{13}C NMR (75 MHz; DMSO-d_6): δ 20.0, 24.1, 24.2, 24.3, 24.4, 33.2, 42.7, 42.9, 44.9, 45.0, 54.9, 56.8, 57.8, 57.9, 63.9, 65.5, 70.6, 85.7, 87.8, 88.5, 109.3, 112.7, 114.3, 116.7, 119.3, 125.4–129.8, 132.5, 132.6, 135.8, 139.2, 140.3, 143.2, 144.7, 145.5, 158.1, 158.5 ppm. ^{31}P NMR (300 MHz; $CDCl_3$): δ 149.53, 149.95 in a 4:3 ratio. HRMS (ESI) Calcd for $C_{60}H_{65}N_6O_6P$ $[M + Na]^+$ 1019.4696, found: 1019.4669.

Oligonucleotide Synthesis, Purification, and Melting Temperature Determination

DMT-on oligodeoxynucleotides were carried out at 0.2 μmol scales on CPG supports with an *Expedite*™ Nucleic Acid Synthesis System Model 8909 from (Applied Biosystems), using 1*H*- tetrazole as an activator for coupling reaction and a 0.05 mM solution of the amidite (**11**) dissolved in anhydrous CH_2Cl_2. And inserted into the growing oligonucleotides using a syringe attached to the CPG-support and the solution was pressed through the support giving a coupling time of 10 minutes. DMT-on oligonucleotides bound to CPG supports were treated with aq. NH_3 (32%, 1 cm^3) at room temperature

and then at 55°C over night. Purification of 5′-*O*-DMT-on ONs was accomplished by reversed-phase semipreparative HPLC on a *waters Xterra MS C_{18}* column with a waters *Delta Prep 4000* Preparative Chromatography System {Buffer A [0.05_M triethylammonium acetate in H_2O (pH 7.4)] and Buffer B (75% CH_3CN in H_2O)}. Flow 2.5 cm^3/ minute. Gradients: 2 minutes 100% A, linear gradient to 70% B in 38 minutes, Linear gradient to 100% B in 3 minutes, and then 100% A in 10 minutes). ODNs were DMT deprotected in 100 μl 80% acetic acid for 20 minutes. afterwards, H_2O (100 μl), and aq. AcONa ($3_{M,}$ 50 μl) were added, and sodium per chlorate (15 μl) and the ONs were precipitated from acetone (500 μl). All modified ODNs were confirmed by MALDI-TOF analysis on a *Voyager Elite Bio* spectroscopy Research Station from PerSeptive Biosystems. The purity of the final ODNs was found to be over 90%, checked by ion-exchange chromatography using LaChrom system from Merck Hitachi on *Genpak-Fax* column (Waters). Melting temperature measurments were performed on a *perkin–Elmer* UV/VIS spectrometer *lambda 35* fitted with a PTP-6 temperature Programmer. The triplexes were formed by first mixing the two strands of the Watson–Crick duplex, each at a concentration of 1.0 μM, followed by addition of the third (TFO) strand at a concentration of 1.5 μM in a buffer consisting of sodium cacodylate (20 mM), NaCl (100 mM), and $MgCl_2$ (10 mM) at pH 6.0 or 7.2. ON2 m/z found: 4676.3; calcd: 4677.3. The melting temperature (T_m, °C) was determined as the maximum of the first derivative plots of the melting curves obtained by absorbance at 260 nm against increasing temperature (1.0°C/minute). All melting temperatures are within the uncertainty ±1.0°C as determined by repetitive experiments.

REFERENCES

1. Duca, M.; Vekhoff, P.; Oussedik, K.; Halby, L.; Arimondo, P.B. The triple helix: 50 years later, the outcome. *Nucleic Acids Res.* **2008**, 36, 5123–5138.
2. Mukherjee, A.; Vasquez, K.M. Triplex technology in studies of DNA damage, DNA repair, and mutagenesis. *Biochimie* **2011**, 93, 1197–1208.
3. Felsenfeld, G.; Davies, D.R.; Rich, A. Formation of a three-stranded polynucleotide molecule. *J. Am. Chem. Soc.* **1957**, 79, 2023–2024.
4. Frank-Kamenetskii, M.D.; Mirkin, S.M. Triplex and structures. *Annu. Rev. Biochem.* **1995**, 64, 65–95.
5. Pilch, D.S.; Levenson, C.; Shafer, R.H. Structure, stability, and thermodynamics of a short intermolecular purine-purine-pyrimidine triplex. *Biochemistry* **1991**, 30, 6081–6087.
6. Cheng, A.-J.; Dyke, M.W.V. Monovalent cation effect on intermolecular purine-purine-pyrimidine triple-helix formation. *Nucleic Acids Res.* **1993**, 21, 5630–5635.
7. Cheng, A.-J.; Vandyke, M.W. Oligodeoxyribonucleotide length and sequence effects on intermolecular purine-purine-pyrimidine triple-helix formation. *Nucleic Acids Res.* **1994**, 22, 4742–4747.
8. Milligan, J.F.; Matteucci, M.D.; Martin, J.C. Current concepts in antisense drug design. *J. Med. Chem.* **1993**, 36, 1923–1937.
9. Durland, R.H.; Kessler, D.J.; Gunnell, S.; Duvic, M.; Pettitt, B.M.; Hogan, M.E. Binding of triple helix forming oligonucleotides to sites in gene promoters. *Biochemistry* **1991**, 30, 9246–9255.
10. Beal, P.A.; Dervan, P.B. Second structural motif for recognition of DNA by oligonucleotide-directed triple-helix formation. *Science* **1991**, 251, 1360–1363.

11. Filichev, V.V.; Gaber, H.; Olsen, T.R.; Jorgensen, P.T.; Jessen, C.H.; Pedersen, E.B. Twisted intercalating nucleic acids - intercalator influence on parallel triplex stabilities. *Eur. J. Org. Chem.* **2006**, 3960–3968.
12. Paramasivam, M.; Cogoi, S.; Filichev, V.V.; Bomholt, N.; Pedersen, E.B.; Xodo, L.E. Purine twisted-intercalating nucleic acids: A new class of anti-gene molecules resistant to potassium-induced aggregation. *Nucleic Acids Res.* **2008**, 36, 3494–3507.
13. Gwaltney, J.M. Rhinovirus inhibition by 3-substituted triazinoindoles. *Proc. Soc. Exp. Biol. Med.***1970**, 133, 1148–1154.
14. Kansal, V.; Potier, P. The biogenetic, synthetic and biochemical aspects of ellipticine, an antitumor alkaloid. *Tetrahedron* **1986**, 42, 2389–2408.
15. Lerman, L.S. Structural considerations in the interaction of DNA and acridines. *J. Mol. Biol.* **1961**, 3, 18–30.
16. Lerman, L.S. The structure of the DNA-Acridine complex, *Proc. Natl. Acad. Sci. USA* **1963**, 49, 94–102.
17. Waring, M.J. Variation of the super coils in closed circular DNA by binding of antibiotics and drugs: Evidence for molecular models involving intercalation. *J. Mol. Biol.* **1970**, 54, 247–279.
18. Denny, W.A. DNA-intercalating ligands as anti-cancer drugs: Prospects for future design. *Anti-Cancer Drug Des.* **1989**, 4, 241–263.
19. Baguley, B.C. DNA intercalating anti-tumour agents. *Anti-Cancer Drug Des.* **1991**, 6, 1–35.
20. Wakelin, L.P.G.; Waring, M.J. DNA intercalating agents, in *Comprehensive Medicinal Chemistry*, ed. P.G. Sammes. Pergamon Press, Oxford, 1990, 2, pp. 703–717.
21. Zegar, I.; Gräslund, A.; Bergman, J.; Eriksson, M.; Nordén, B. Interaction of ellipticine and an indolo[2,3b]-quinoxaline derivative with DNA and synthetic polynucleotides. *Chem. Biol. Interact.* **1989**, 72, 277–293.
22. Harmenberg, J.; Wahren, B.; Bergman, J.; Akerfeldt, S.; Lundblad, L. Antiherpesvirus activity and mechanism of action of indolo-(2,3-b)quinoxaline and analogs. *Antimicrob. Agents Chemother.* **1988**, 32, 1720–1724.
23. Harmenberg, J.; Akesson-Johansson, A.; Gräslund, A.; Malmfors, T.; Bergman, J.; Wahren, B.; Akerfeldt, S.; Lundblad, L.; Cox, S. The mechanism of action of the anti-herpes virus compound 2,3-dimethyl-6(2-dimethylaminoethyl)-6H-indolo-(2,3-b)quinoxaline. *Antiviral Res.* **1991**, 15, 193–204.
24. Patel, N.; Bergman, J.; Gräslund, A. 1H HMR studies of the interaction between self-complementary deoxyoligonucleotide duplex and indolo[2,3-b]quinoxaline derivatives active against herpes virus. *Eur. J. Biochem.* **1991**, 197, 597–604.
25. Behravan, G.; Leijon, M.; Sehlstedt, U.; Nordén, B.; Vallberg, H.; Bergman, J.; Gräslund, A. The interaction of ellipticine derivatives with nucleic acids studied by optical and 1H-NMR spectroscopy: Effect of size of the heterocyclic ring system. *Biopolymers* **1994**, 34, 599–609.
26. Sehlstedt, U.; Aich, P.; Bergman, J.; Vallberg, H.; Nordén, B.; Gräslund, A. Interactions of the antiviral quinoxaline derivative 9-OH-B220 {2,3-dimethyl-6-(dimethylaminoethyl)-9-hydroxy-6H-indolo-[2,3-b]quinoxaline} with duplex and triplex forms of synthetic DNA and RNA. *J. Mol. Biol.* **1998**, 278, 31–56.
27. Kohn, K.W.; Waring, M.J.; Glaubiger, D.; Friedman, C.A. Intercalative Binding of Ellipticine to DNA. *Cancer Res.* **1975**, 35, 71–76.
28. Bomholt, N.; Osman, A.M.A.; Pedersen, E.B. High physiological thermal triplex stability optimization of twisted intercalating nucleic acids (TINA). *Org. Biomol. Chem.* **2008**, 6, 3714–3722.
29. Bergman, J.O.E.; Åkerfeldt, S. Substituted Indoloquinoxalines. PCT Int. Appl. WO 8704436A1 (30 July 1987).
30. Felsenfeld, G.; Miles, H.T. Physical and chemical properties of nucleic acids. *Annu. Rev. Biochem.* **1967**, 36, 407–448.
31. Filichev, V.V.; Pedersen, E.B. Stable and selective formation of Hoogsteen-type triplexes and duplexes using twisted intercalating nucleic acids (TINA) prepared via postsynthetic Sonogashira solid-phase coupling reactions. *J. Am. Chem. Soc.* **2005**, 127, 14849–14858.