DOI: 10.1002/ejoc.200700991

Guanosine Analog with Respect to Z-DNA Stabilization: Nucleotide with Combined C8-Bromo and C2′-Ethynyl Modifications

André Nadler[a] and Ulf Diederichsen*[a]

Keywords: Circular dichroism / Conformation / Nucleotide modification / Oligonucleotides / Z-DNA

The stabilization of left-handed Z-DNA double strands by chemical modifications is especially of interest regarding investigations of its biological role. Incorporation of modified nucleotide building blocks in DNA allows recognition studies under physiological conditions without the need for a Z-DNA inducing environment. Approaches to enforce the Z-DNA double helix using guanosine derivatives are the introduction of sterically demanding groups at guanine C8 or of 2′-ribosyl substitutions determining the ribosyl conformation. 8-Bromo-2′-ethynyl-*arabino*-deoxyguanosine was synthesized as phosphoramidite building block. Incorporated in suitable oligonucleotides the potential to induce the Z-form DNA was investigated by CD spectroscopy. With a nucleotide containing the 8-bromo and the 2′-ethynyl group a synergistic effect of the two modifications was expected to provide stronger stabilization of Z-DNA.

Introduction

The potential of DNA to adopt various double-strand topologies besides B-DNA is crucial for protein and small molecule recognition.[1] Effective DNA binding often depends on the respective oligonucleotide conformation which is influenced by the environment and can also be induced by an interacting small molecule or protein.[2] Especially Z-DNA as the only left-handed double helix is of interest since only few small molecules[3] or proteins[4,5] are known for specific Z-DNA binding. The transient stability of Z-DNA conformation might be an explanation for the small number of structurally characterized Z-DNA complexes. The low population of the higher-energy Z-DNA conformation is caused by the required *syn*-conformation for every other purinyl nucleotide which is sterically disfavored compared to the usual *anti*-conformation.[6] Further, phosphodiester distances in the backbone are lowered by incorporation of *syn*-nucleotides thereby enhancing the electrostatic repulsion. For studying the impact of the Z-DNA topology on specific recognition phenomena and regulative processes, double strands stabilized in Z-DNA conformation are desirable.

Typical nucleoside modifications favoring the *syn*-conformation are the introduction of substituents like a methyl group[7] and a bromo substituent at C8 of the purine[8] or CH_3 at C5 of cytosine, respectively.[9] These substituents are sterically demanding and thereby competing with the nucleobase for avoiding backbone interactions. A second approach for Z-DNA stabilization takes advantage of an *arabino*-configured ribosyl modification of deoxyguanosine introducing an ethynyl group at C2′ (dG^{Et}).[10] Oligomer $d(CG^{Et}CG^{Et}CG)_2$ exhibits a left-handed helical structure even under salt free conditions,[10] whereas its natural counterpart $d(CGCGCG)_2$ requires 4 M NaCl concentration to adopt Z-form DNA.[11] The ethynyl substitution affects the DNA topology by inducing a C2′-*exo* sugar conformation which is closely related to the C3′-*endo* conformation required for Z-DNA. So far, 8-methylguanosine is the most powerful Z-DNA stabilizing nucleotide combining the steric demand of the methyl group at C8 with an 2′-OH group in the solvent exposed area of the Z-DNA double helix.[12] Furthermore, the ribosyl conformation of the modified nucleotide is affected by the 2′-OH group towards Z-form stabilization.

In order to further investigate the synergistic effect of two nucleotide modifications with respect to the induction of Z-DNA conformation, a nucleotide with a bromo substituent at C8 and the ethynyl group at C2′ was prepared and introduced in appropriate DNA oligomers to induce Z-DNA (Figure 1).

It was expected that the combination of these approaches for Z-DNA stabilization should provide nucleotides with increased efficiency based on a large nucleobase substituent and the preference for the deoxyribosyl C2′-*exo* conformation. This hypothesis was validated comparing oligonucleotides containing 8-bromo-2′-ethynyl-*arabino*-deoxyguanosine modifications with the respective 8-bromo-deoxyguanosine (dG^{Br}) oligonucleotides. The synthesis of 8-bromo-2′-ethynyl-*arabino*-deoxyguanosine phosphoramidite (**1**) is described and the potential of the artificial nucleoside for Z-DNA induction was investigated by CD spectroscopy.

[a] Institut für Organische und Biomolekulare Chemie, Georg August Universität Göttingen,
Tammannstr. 2, 37077 Göttingen, Germany
Fax: +49-551-392944
E-mail: udieder@gwdg.de

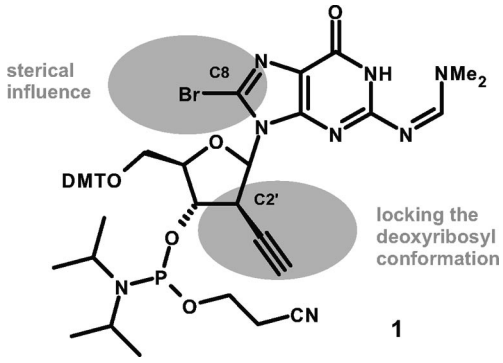


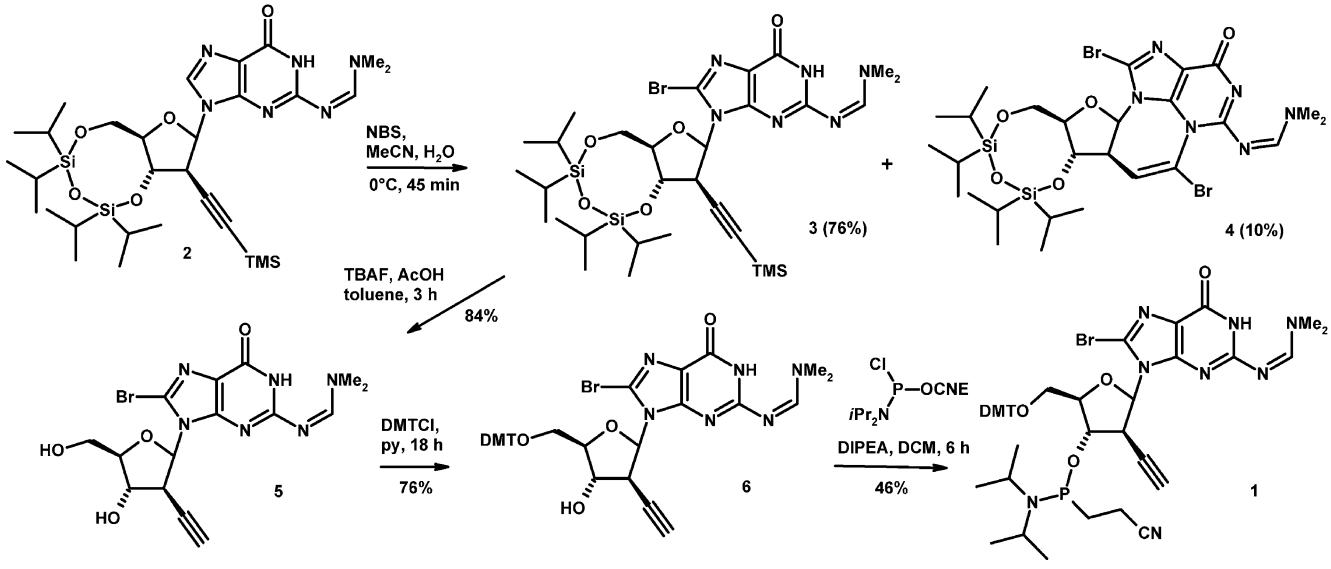
Figure 1. Guanosine phosphoramidite **1** for solid phase synthesis containing the 8-bromo and 2′-ethynyl modifications.

Results and Discussion

The synthesis of **1** started from guanosine following the procedure of Buff and Hunziker to obtain the 2′-ethynyl-nucleoside **2**.[10] Protection of the 5′- and 3′-OH with the 1,1,3,3-tetraisopropyldisiloxane-1,3-diyl (TIPDS) group and of the nucleobase N2-amino group with the dimethylaminomethylene group was followed by oxidation of the 2′-OH functionality with Dess–Martin periodinane. Lithium acetylide addition to the ketone was succeeded by radical deoxygenation providing the 2′-ethynyl-nucleoside **2** (Scheme 1). For introduction of bromine at C8 of the 2′-ethynyl-nucleoside **2** a mild protocol was applied using *N*-bromosuccinimide (NBS) at 0 °C in MeCN/H_2O, 4:1 (Scheme 1). The desired bromonucleoside **3** was obtained in 76% yield. Nevertheless, separation from a cyclic byproduct **4** was required that was obtained in 10% yield likely due to bromine addition at the alkyne followed by nucleophilic attack of the guanine N1. Changing to more reactive reaction conditions (3 h and 10 equiv. NBS) the cyclic product **4** can be obtained predominantly (50%). Complete desilylation to nucleoside **5** was accomplished using TBAF in toluene/AcOH. The temporary DMT-protecting group required at the 5′-OH for solid phase synthesis was attached in good yield. The phosphoramidite building block **1** was synthesized using standard procedures.[13] The 8-bromo-2′-deoxyguanosine phosphoramidite required for comparison of Z-form DNA induction was synthesized according to literature.[14]

Compound **1** (dG*) was incorporated in the oligonucleotides d(CGCG*CG) (**7**), d(GCG*CGC) (**8**), d(CG*CATG*TG) (**9**), and d(CTACG*TAG) (**10**). Oligomers **7**, **8**, and **10** are self-complementary providing two dG* modifications in the respective double strands whereas oligomer **9** requires a counter strand d(CACATGCG) (**11**) for duplex formation. The regular oligonucleotides $d(CG)_3$ (**12**), $d(GC)_3$ (**13**), and d(CTACGTAG) (**14**) as well as the 8-bromo-deoxyguanosine modified oligomers $d(CGCG^{Br}CG)$ (**15**) and $d(GCG^{Br}CGC)$ (**16**) were synthesized to evaluate the synergistic effect of the bromo-ethynyl modification with respect to Z-DNA induction.

The modified oligomers **7** and **8** are derived from DNA double strands $[d(CG)_3]_2$ (**12**) and $[d(GC)_3]_2$ (**13**) containing the alternation of guanosine and cytidine nucleosides that is necessary for facile induction of Z-DNA. The C/G-alternating sequences serve as reference since they crystallize in left-handed Z-DNA conformation and are known to form the Z-DNA at high salt concentrations.[6] The double helix conformation of modified oligomers is conveniently analyzed by CD spectroscopy deriving the helical sense and using spectra for pure B-DNA and Z-DNA for comparison.[15] The transition from B to Z-DNA is accompanied by a complete inversion of the CD spectrum: A positive Cotton effect around 280 nm for B-DNA is replaced by a more intense negative band at 295 nm in the Z-form. Similarly, the negative Cotton effect around 250 nm detected with B-DNA converts into a positive signal at 260 nm with Z-DNA.



Scheme 1. Synthesis of 8-bromo-2′-ethynyl-*arabino*-deoxyguanosine phosphoramidite (**1**).

First indication for the capability of **1** (dG*) to induce the Z-conformation was obtained by comparison of oligomer **7** containing only one modification with the natural counterpart **12** and the oligonucleotide containing only the 8-bromo-2′-deoxyguanosine modification (dG^{Br}) (Figure 2). At 100 mM NaCl concentration the self pairing oligomer **12** formed a B-DNA-like duplex, whereas for the modified hexamers **7** and **15** a left-handed helix was indicated. The modified nucleoside **1** (dG*) had the expected Z-form inducing effect on double-stranded DNA providing an increased Z-DNA helix propensity with the double modification dG* incorporated instead of dG^{Br}.

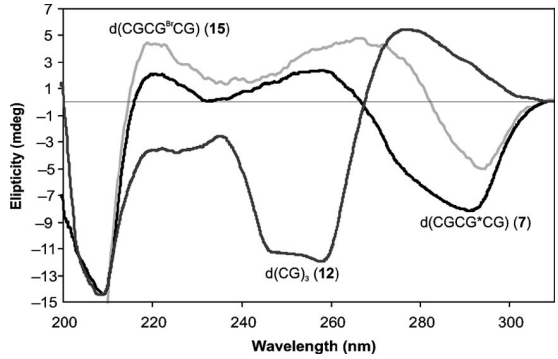


Figure 2. CD spectra of oligomers d(CGCG*CG) (**7**), d(CGCG^{Br}CG) (**15**), and of oligomer $d(CG)_3$ (**12**) all at 100 mM NaCl concentration in 10 mM phosphate buffer, pH 7.

The double strand stability of oligomer **7** (T_m = 28 °C, 8% hyperchromicity, 100 mM NaCl, 6 μM) determined by temperature dependent UV spectroscopy indicated that two artificial nucleosides **1** (dG*) significantly effected the duplex stability since the natural counterpart **12** had a stability of T_m = 44 °C (3% hyperchromicity, 100 mM NaCl, 6 μM). The thermal stability of the duplex formed by oligomer **7** was also followed by CD spectroscopy measured at various temperatures without NaCl addition (Figure 3). A left-handed helix was found at temperatures between 5 and 20 °C. The helix propensity decreased with rising temperature indicating a single strand without conformational preference at higher temperatures.

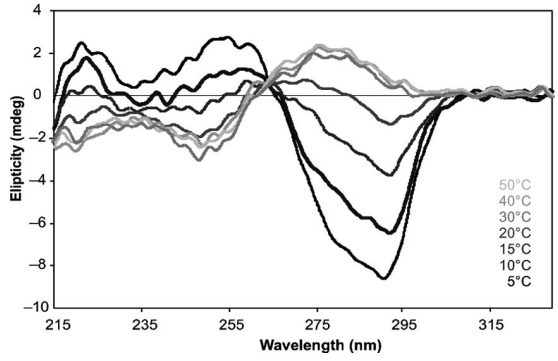


Figure 3. CD spectra of oligomer d(CGCG*CG) (**7**) in 10 mM phosphate buffer, pH 7 without NaCl at various temperatures: 5, 10, 15, 20, 30, 40, and 50 °C.

Oligonucleotide $d(CG)_3$ (**12**) has a higher Z-DNA forming capability compared to oligomer $d(GC)_3$ (**13**) due to stabilizing stacking and sugar interactions based on nucleotide dimers required in Z-DNA double strands.[16] Therefore, it was expected that the induction of Z-DNA conformation would also be stronger in oligomer **7** compared to the double strand of the oligomer with inversed sequence **8**. From the comparison of CD spectra of oligomers **8**, **13**, and **16** at 100 mM NaCl concentration (Figure 4) it can be concluded that the guanosine modification **1** is at least as effective with respect to Z-DNA induction in oligomer **8** compared to duplex **7**. The strong Z-form induction of dG* in $d(GC)_3$ oligomer **8** is quite remarkable since one 8-bromoguanosine modification (dG^{Br}) was not sufficient for Z-DNA induction in this sequence.

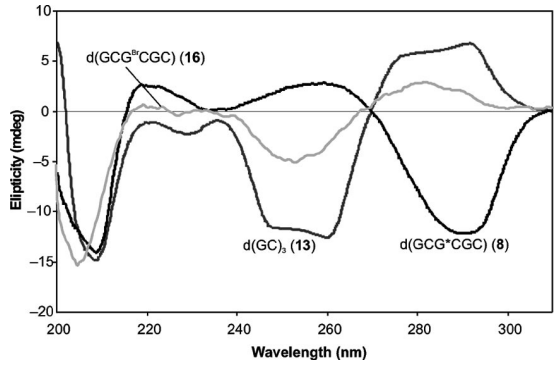


Figure 4. CD spectra of oligomers d(GCG*CGC) (**8**), d(GCG^{Br}CGC) (**16**), and of oligomer $d(GC)_3$ (**13**) all at 100 mM NaCl concentration in 10 mM phosphate buffer, pH 7.

A salt dependency of the Z-form stabilization was observed for oligomer **8** (Figure 5) showing a decreasing helix propensity at 50 mM NaCl concentration compared to oligomer **8** at 100 mM NaCl. Without NaCl a complete loss of double helix formation was observed. The CD spectra of oligomer **8** without NaCl addition were also measured at various temperatures; in contrast to DNA **7** even at low temperatures only non-structured oligonucleotides have been detected (data not shown).

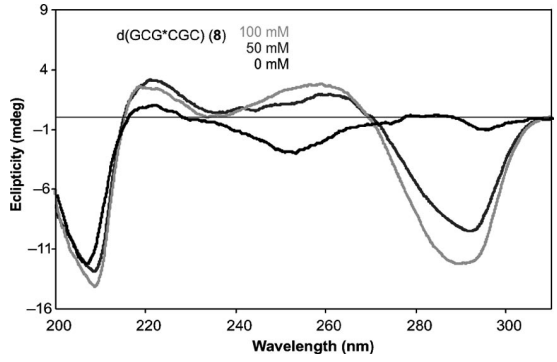


Figure 5. CD spectra of oligomer d(GCG*CGC) (**8**) at 0, 50 and 100 mM NaCl concentration in 10 mM phosphate buffer, pH 7.

DNA with a C/G-alternating sequence is destined for Z-DNA formation under the appropriate conditions. Since it is much more difficult to induce Z-form DNA with oligonucleotides that also contain A-T base pairs, oligomer d(CG*CATG*TG) (**9**) was investigated that together with its counter strand d(CACATGCG) (**11**) still provides the purine/pyrimidine alternation but includes three A-T base pairs. It was expected that two modified nucleotides dG* (**1**) might be sufficient to also facilitate the B to Z-DNA transition in the A-T containing duplex **9** + **11**. Nevertheless, it was not possible to induce Z-form DNA by NaCl addition up to 4 M concentration. The CD spectra measured in aqueous buffer at pH 7 at 300 mM NaCl concentration provided a negative Cotton effect around 288 nm at temperatures below 5 °C that would be in line with a left-handed helix (Figure 6). Anyhow, positive Cotton effects below 260 nm that are typical for Z-DNA were missing and at higher temperatures the DNA double strand **9** + **11** was likely to be right-handed but deviating from a B-DNA double strand. The power of the guanosine analog **1** for Z-DNA induction seems to be restricted to C/G-alternating oligomers. Already by A/T incorporation the double helix seems to be locked in a right-handed form that differs from B-DNA.

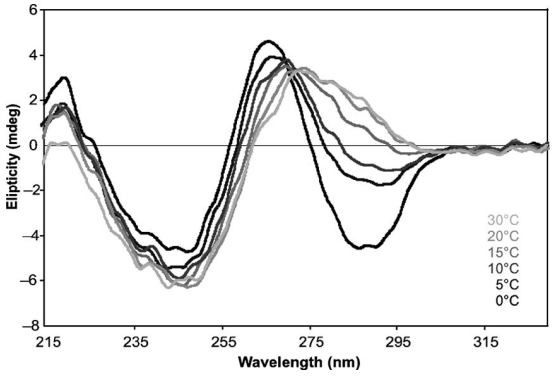


Figure 6. CD spectra of an equimolar mixture of oligomers **9** and **11** in 10 mM phosphate buffer, pH 7 at 300 mM NaCl concentration with increasing temperature 0, 5, 10, 15, 20, and 30 °C.

Inducing the Z-DNA conformation is even more difficult in case of the self pairing oligomer d(CTACG*TAG) (**10**) which besides containing four A-T base pairs has no strict purine/pyrimidine alternation. Therefore, it was not surprising that transition from B- to Z-DNA was not accomplished at 300 mM or even at 4 M NaCl concentration (spectra not shown). As already observed for the double strand formed by oligomers **9** + **11**, the incorporation of the guanosine modification dG* (**1**) in self pairing DNA **10** provided a right-handed helix. The guanosine modification dG* (**1**) incorporated in a DNA oligomer that has not a typical Z-DNA forming sequence is not strong enough to induce a left-handed helix but leads to a defined right-handed B-DNA like double strand.

Conclusions

The stabilization of DNA in its left-handed Z-form by incorporation of modified nucleotides is of significance to investigate the biological role of Z-DNA. Two approaches to induce Z-DNA by incorporation of modified nucleotides were combined in one derivative by synthesis of 8-bromo-2′-ethynyl-*arabino*-deoxyguanosine phosphoramidite building block **1**. The nucleoside carrying the bromine for steric hindrance at C8 of the nucleobase and an ethynyl residue at C2′ to adjust the conformation of the ribosyl sugar unit was incorporated as phosphoramidite into DNA sequences. Within C/G-alternating oligomers that are likely to be transformed to the Z-form DNA the guanosine derivative with combined modifications strongly induced Z-DNA transition. Compared to oligomers with an 8-bromoguanosine (dG^{Br}) or a 2′-ethynylguanosine modification (dG^{Et})[10] only, a synergistic effect was detected for the combined modifications (dG*). Nevertheless, the C/G alternation seems to be a prerequisite for 8-bromo-2′-ethynyl modification containing DNA double strands to adopt Z-form DNA. A purine/pyrimidine alternating DNA double strand including A-T base pairs was not converted into Z-DNA.

Experimental Section

General Remarks: Infrared spectra were recorded with a Perkin–Elmer type 1600 Series FT-IR infrared spectrophotometer. ^{1}H and ^{13}C NMR spectra were recorded with a Varian Unity 300 spectrometer. UV spectra were recorded with a Perkin–Elmer type Lambda 10 UV/Vis spectrophotometer. ESI-MS data were obtained with a Finnigan (type LGC or TSQ 7000) spectrometer, high resolution spectra were obtained with a Bruker (type Apex-Q IV 7T). Flash chromatography was performed using Merck silica gel 60. HPLC purification of the oligonucleotides was carried out by IBA using a DuPont Series 8800 Chromatograph and a Gemini 5µ C18 150×4.6 mm column. A 0–60 gradient of 50 mM TEAA/50 mM TEAA 70% MeCN was utilized. CD spectra were recorded with a Jasco J-810 spectropolarimeter. All samples were measured in 10 mM phosphate buffer at pH 7.

8-Bromo-9-{2′-deoxy-3′,5′-*O*-(tetraisopropyldisiloxan-1,3-diyl)-*N*^{2}-[(dimethylamino)methylene]-2′C-[(trimethylsilyl)ethynyl]-β-D-arabinofuranosyl}guanine (3): *N*-Bromosuccinimide (1.81 g, 9.63 mmol) was added in portions at 0 °C to a stirred solution of **2** (2.27 g, 3.43 mmol) in MeCN/H_2O, 4:1 (80 mL). The reaction was quenched by addition of saturated $Na_2S_2O_3$ solution (20 mL) after 45 min. The solvent was removed in vacuo and the residue was dissolved in ethyl acetate and washed with water (2×50 mL). The organic layer was dried with Na_2SO_4 and the solvent evaporated. The residue was purified by flash chromatography using the eluent CH_2Cl_2/MeOH, 92:8 to yield a yellowish oil (1.92 g, 76%). R_f(CH_2Cl_2/MeOH, 9:1) = 0.54. UV/Vis: λ_{max} = 236.5, 281.0, 307.5 nm. IR (KBr): $\tilde{\nu}$ = 2946, 2895, 2180, 1694, 1632, 1535, 1348, 1290, 1249, 1206, 1068, 1036, 958, 885, 761, 701 cm^{-1}. ^{1}H NMR (300 MHz, [D$_6$]DMSO, 100 °C): δ = 11.06 (br. s, 1 H, NH), 8.43–8.50 (m, 1 H, N=CH), 8.58 (d, ^3J = 9.0 Hz, 1 H, H1′), 5.03–5.11 (m, 1 H, H3′), 4.19 (dd, ^2J = 12.3, ^3J = 6.3 Hz, 1 H, H5′), 4.08 (dd, ^2J = 12.3, ^3J = 3.3 Hz, 1 H, H5′), 3.85 (m$_z$, 1 H, H4′), 3.74 (t, ^3J = 8.7 Hz, 1 H, H2′), 3.15 (s, 3 H, NCH_3), 3.05 (s, 3 H, NCH_3),

1.01–1.11 (m, 28 H, *i*Pr-H), –0.09 [s, 9 H, $Si(CH_3)_3$] ppm. ^{13}C NMR (75 MHz, [D$_6$]DMSO, 100 °C): δ = 157.2 (Ar-C), 156.7 (Ar-C), 155.6 (Ar-C), 150.9 (Ar-C), 100.5 (C≡C), 85.1 (C≡C), 82.5 (C4′), 77.4 (C1′), 44.2 (C2′), 39.3 (NCH_3), 34.1 (NCH_3), 16.7, 16.6, 16.6, 16.5, 16.5, 16.4, 16.3, 16.3 (*i*Pr-CH), 12.3, 12.1, 12.0, 11.7 (*i*Pr-CH), –1.4 [$Si(CH_3)_3$] ppm. ESI: *m*/*z* (rel.%) = 739 (100) $[M + H]^+$, 741 (90) $[M + H]^+$. HR-ESI-MS: *m*/*z* $[M + H]^+$ = calcd. 739.24849 for $C_{30}H_{51}BrN_6O_5Si_3$, found 727.24820.

Analytical Data of Cyclic Byproduct 4: In addition to **3** the annulated nucleoside **4** was isolated as yellowish oil (280 mg, 10%). R_f(CH_2Cl_2/MeOH, 9:1) = 0.50. ^1H NMR (300 MHz, [D$_6$]DMSO, 100 °C): δ = 8.73 (s, 1 H, N=CH), 6.26 (d, ^3J = 6.2 Hz, 1 H, H1′), 4.85 (dd, ^2J = 6.0, ^2J = 6.3 Hz, 1 H, H2′), 4.38 (dd, ^2J = 6.8, ^2J = 5.3 Hz, 1 H, H3′), 4.13–4.17 (m, 1 H, H4′), 3.80 (dd, ^2J = 12.3, ^3J = 3.7 Hz, 1 H, H5′), 3.26 (s, 3 H, NCH_3), 3.04 (s, 3 H, NCH_3), 2.98 (dd, ^2J = 12.3, ^3J = 6.7 Hz, 1 H, H5′), 0.98–1.07 (m, 28 H, *i*Pr-H), 0.09 [s, 9 H, $Si(CH_3)_3$] ppm. ^{13}C NMR (75 MHz, [D$_6$]DMSO, 100 °C): δ = 160.1 (Ar-C or N=CH), 159.9 (Ar-C or N=CH), 153.5 (Ar-C or N=CH), 138.7 (C=C), 132.5 (C=C), 119.1 (C8), 117.1 (C4), 86.0 (C4′), 84.5 (C1′), 74.4 (C3′), 62.8 (C5′), 53.4 (C2′), 40.7 (NCH_3), 34.6 (NCH_3), 16.7, 16.7, 16.5, 16.4, 16.4, 16.3, 16.3, 16.2 (*i*Pr-CH_3), 12.6, 12.0, 11.9, 11.8 (*i*Pr-CH), –1.3 [$Si(CH_3)_3$] ppm. ESI-MS: *m*/*z* (rel.%) = 817 (50) $[M + H]^+$, 819 (90) $[M + H]^+$, 821 (50) $[M + H]^+$. HR-ESI-MS: *m*/*z* = $[M + H]^+$ = calcd. 817.15900 for $C_{30}H_{51}BrN_6O_5Si_3$, found 817.15890.

8-Bromo-9-(2′-deoxy-2′C-ethynyl-β-D-arabinofuranosyl)-*N*^{2}-[(dimethylamino)methylene]guanine (5): TBAF × 3 H_2O (2.20 g, 6.98 mmol) and AcOH (0.5 mL) were added to a solution of **3** (1.64 g, 2.26 mmol) in toluene (50 mL). The reaction mixture was stirred at room temp. for 3 h. The solvent was evaporated and the residue purified by flash chromatography using the eluent $CHCl_3$/MeOH, 9:1 to afford **5** (800 mg, 1.89 mmol, 84%) as a colorless oil. R_f($CHCl_3$/MeOH, 9:1) = 0.30. UV/Vis: λ_{max} = 235.5, 307.0 nm. IR (KBr): $\tilde{\nu}$ = 3130 (br), 2946, 2897, 2175, 1756, 1682, 1505, 1465, 1385, 1251, 1199, 1115, 1008, 957, 921, 814, 786, 636 cm^{-1}. ^1H NMR (300 MHz, [D$_6$]DMSO, 35 °C): δ = 11.44 (br. s, 1 H, NH), 8.58 (s, 1 H, N=CH), 6.37 (d, ^3J = 8.4 Hz, 1 H, H1′), 5.81 (br. s, 1 H, 3′-OH), 4.80–4.75 (m, 2 H, H3′, 5′-OH), 3.78–3.66 (m, 3 H, H4′, H5′), 3.59 (dd, ^3J = 9.3, ^4J = 2.7 Hz, 1 H, H2′), 3.17 (s, 3 H, NCH_3), 3.06 (s, 3 H, NCH_3), 2.88 (d, ^4J = 2.4 Hz, 1 H, C≡C-H) ppm. ^{13}C NMR (75 MHz, [D$_6$]DMSO, 35 °C): δ = 158.0 (Ar-C or N=CH), 156.9 (Ar-C or N=CH), 156.3 (Ar-C or N=CH), 150.8, (C4), 121.2 (C8), 120.1 (C5), 85.4 (C4′), 84.3 (C1′), 79.3 (C≡C), 75.5 (C≡C), 74.0 (C3′), 60.7 (C5′), 42.6 (C2′), 40.8 (NCH_3), 34.6 (NCH_3) ppm. ESI-MS: *m*/*z* (%) = 447.0, 449.0 (10) $[M + Na]^+$, 870.9, 872.9, 874.8 (100) $[2M + Na]^+$. HR-ESI-MS: *m*/*z* $[M + H]^+$ = calcd. 425.05674 for $C_{15}H_{18}BrN_6O_4$, found 425.05705.

8-Bromo-9-(2′-deoxy-2′C-ethynyl-5′*O*-dimethoxytrityl-β-D-arabinofuranosyl)-*N*^{2}-[(dimethylamino)methylene]guanine (6): DMTCl (1.60 g, 4.73 mmol) was added to a solution of **5** (770 mg, 1.82 mmol) in dry pyridine under an argon atmosphere. The reaction mixture was stirred for 18 h and then poured into a mixture of CH_2Cl_2 (100 mL) and saturated $NaHCO_3$ solution (100 mL). The organic layer was dried with Na_2SO_4 and the solvent evaporated. The residue was purified by flash chromatography using the eluent CH_2Cl_2/MeOH/Et_3N, 92:7:1 to afford **6** (998 mg, 1.37 mmol, 76%) as yellowish foam. R_f(CH_2Cl_2/MeOH, 9:1) = 0.40. UV/Vis: λ_{max} = 235.0, 283.0, 310.0 nm. IR (KBr): $\tilde{\nu}$ = 3377 (br), 3001 (br), 2930, 2045, 1685, 1632, 1534, 1422, 1346, 1249, 1176, 1114, 829, 704 cm^{-1}. ^1H NMR (300 MHz, [D$_6$]DMSO, 35 °C): δ = 11.43 (br. s, 1 H, NH), 8.12 (br. s, 1 H, N=CH), 7.31 (dd, ^3J = 7.8, ^4J = 1.8 Hz, 2 H, Ph-H), 7.15–7.21 (m, 7 H, DMT-H), 6.72–6.80 (m, 4 H, DMT-H), 6.44 (d, ^3J = 8.7 Hz, 1 H, 3′-OH), 5.84 (d, ^3J = 6.0 Hz, 1 H, H1′), 4.67–4.76 (m, 1 H, H3′), 3.95 (m_z, 1 H, H4′), 3.71 (s, 6 H, OCH_3), 3.70 (s, 6 H, OCH_3), 3.56–3.67 (m, 2 H, H5′), 3.20 (m_z, 1 H, H2′), 2.98 (s, 3 H, NCH_3), 2.93 (s, 3 H, NCH_3), 2.86 (d, ^4J = 2.4 Hz, 1 H, ≡CH) ppm. ^{13}C NMR (75 MHz, [D$_6$]DMSO, 35 °C): δ = 157.9 (Ar-C or N=CH), 157.9 (Ar-C or N=CH), 156.3 (Ar-C or N=CH), 149.5 (Ar-C), 144.8 (Ar-C), 136.0 (Ar-C), 135.4 (Ar-C), 129.6 (Ar-C), 129.4 (Ar-C), 127.6 (Ar-C), 127.5 (Ar-C), 123.8 (Ar-C), 112.9 (Ar-C), 112.9 (Ar-C), 85.4 (C≡C), 84.7 (C1′), 83.6 (C4′), 79.2 (C≡C), 74.8 (C3′), 63.6 (C5′), 54.9 (OCH_3), 54.9 (OCH_3), 42.6 (C2′), 40.7 (NCH_3) 34.5 (NCH_3) ppm. ESI-MS: *m*/*z* (%) = 727 (100) $[M + H]^+$, 729 (90) $[M + H]^+$. HR-ESI-MS: *m*/*z* $[M + H]^+$ = calcd. 727.18742 for $C_{36}H_{35}BrN_6O_6$, found 727.18749.

8-Bromo-9-[2′-deoxy-2′C-ethynyl-3′*O*-[cyanoethyl(diisopropylamino)phosphanyl]-5′*O*-dimethoxytrityl-β-D-arabinofuranosyl]-*N*^{2}-[(dimethylamino)methylene]guanine (1): Cyanoethyl-*N*,*N*-diisopropylphosphoramidite chloride (40.0 mg, 0.21 mmol) was added to a solution of **6** (100 mg, 0.14 mmol) and DIPEA (53 mg, 0.41 mmol) in dry CH_2Cl_2 (1.50 mL) under an argon atmosphere. The reaction mixture was stirred for 6 h and the reaction quenched by addition of a saturated $NaHCO_3$ solution (5 mL). The resulting mixture was extracted with CH_2Cl_2 (3 × 10 mL) and the combined organic layers were dried with Na_2SO_4. The solvent was evaporated and the residue purified by flash chromatography using the eluent CH_2Cl_2/acetone/Et_3N, 1:1:0.01. The raw phosphoramidite was dissolved in a minimum amount of $CHCl_3$ and the solution was added dropwise to pentane (100 mL) at –17 °C. The resulting precipitate was collected and dried in vacuo to give **1** (59 mg, 0.06 mmol, 46%) as a colorless powder. R_f(CH_2Cl_2/MeOH, 9:1) = 0.44. UV/Vis: λ_{max} = 234.5, 282.5, 310.0 nm. IR (KBr): $\tilde{\nu}$ = 2966, 2245, 1695, 1632, 1532, 1423, 1347, 1286, 1250, 1178, 1115, 1031, 980, 830, 703 cm^{-1}. ^1H NMR (300 MHz, [D$_6$]DMSO, 35 °C): δ = 11.95 (br. s, 1 H, NH), 8.17 (s, 0.5 H, N=CH), 8.13 (s, 0.5 H, N=CH), 7.28–7.33 (m, 2 H, Ph-H), 7.14–7.22 (m, 7 H, DMT-H), 6.68–6.78 (m, 4 H, DMT-H), 6.50 (d, ^3J = 4.2 Hz, 0.5 H, H1′), 6.48 (d, ^3J = 4.2 Hz, H1′, 0. H), 4.96–5.11 (m, 1 H, H3′), 4.11 (m_z, 1 H, H4′), 3.87 (m_z, 1 H, H2′), 3.70–3.78, (m, 1 H, H5′), 3.72 (s, 6 H, OMe), 3.70 (s, 6 H, OMe), 3.71 (s, 6 H, OMe), 3.43–3.66 (m, 4 H, *i*Pr-CH, CH_2CN), 3.33 (dd, ^2J = 9.6, ^3J = 1.8 Hz, 0.5 H, H5′), 3.27 (dd, ^2J = 9.6, ^3J = 1.8 Hz, 0.5 H, H5′), 2.76 (d, ^4J = 2.7 Hz, 0.5 H, ≡CH), 2.74 (d, ^4J = 2.7 Hz, 0.5 H, ≡CH), 2.47–2.51 (m, 2 H, PCH_2), 1.11 (d, ^3J = 7.2 Hz, 6 H, *i*Pr-CH_3) ppm. ^{31}P NMR (122 MHz, [D$_6$]DMSO, 35 °C): δ = 152.07 (br. s), 151.04 ppm. ESI-MS: *m*/*z* (%) = 927 (100) $[M + H]^+$, 929 (90) $[M + H]^+$. HR-ESI-MS: *m*/*z* $[M + H]^+$ = calcd. 927.29527 for $C_{45}H_{53}BrN_8O_7P$, found 927.29576.

DNA Oligomer 7: ESI-MS: *m*/*z* (%) = 1894.26 [M], 1916.23 [M – H+Na], 1938.19 [M – 2H + 2Na]. R_t = 11.86 min; T_m = 28 °C, 8% hyperchromicity, 100 mM NaCl, 6 μM.

DNA Oligomer 8: ESI-MS: *m*/*z* (%) = 1894.25 [M], 1916.24 [M – H+Na], 1938.22 [M – 2H + 2Na], 1960.21 [M – 3H+3Na]. R_t = 12.15 min; T_m = 17 °C, 2% hyperchromicity, 100 mM NaCl, 6 μM.

DNA Oligomer 9: ESI-MS: *m*/*z* = 2628.29 [M], 2650.27 [M – H + Na], 2672.26 [M – 2H + 2Na]. R_t = 11.79 min; double-strand stability of oligomer **9** + **11**: T_m = 15 °C, 8% hyperchromicity, 100 mM NaCl, 6 μM.

DNA Oligomer 10: ESI-MS: *m*/*z* (%)= 2510.38 $[M – H]^-$, 2532.36 $[M – 2H + Na]^-$, 2554.34 $[M – 3H + 2Na]^-$, 2576.32 $[M – 4H + 3Na]^-$, 2598.301 $[M – 5H + 4Na]^-$, 2620.29 $[M – 6H + 5Na]^-$. R_t = 12.02 min; T_m = 28 °C, 8% hyperchromicity, 100 mM NaCl, 6 μM.

Acknowledgments

Generous support of the Deutsche Forschungsgemeinschaft (DFG) (Di 542/5) is gratefully acknowledged.

[1] N. R. Cozzarelli, J. C. Wang, *DNA Topology and Its Biological Effects*, Cold Spring Harbor, Laboratory Press, New York, **1990**.

[2] a) A. Rich, A. Nordheim, A. H.-J. Wang, *Annu. Rev. Biochem.* **1984**, *53*, 791–846; b) R. Allemann, M. Egli, *Chem. Biol.* **1997**, *9*, 643–650; c) C. Garvie, C. Wolberger, *Mol. Cell* **2001**, *8*, 937–946.

[3] a) B. Spingler, P. M. Antoni, *Chem. Eur. J.* **2007**, *13*, 6617–6622; b) X. Qu, J. O. Trent, I. Fokt, W. Priebe, J. B. Chaires, *Proc. Natl. Acad. Sci. USA* **2000**, *97*, 12032–12037; c) Y.-G. Kim, H.-J. Park, K. K. Kim, K. Lowenhaupt, A. Rich, *Nucleic Acids Res.* **2006**, *34*, 4937–4942.

[4] G. L. Wang, K. M. Vasquez, *Front. Biosci.* **2007**, *12*, 4424–4438.

[5] a) T. Schwartz, M. A. Rould, K. Lowenhaupt, A. Herbert, A. Rich, *Science* **1999**, *284*, 1841–1845; b) T. Schwartz, J. Behlke, K. Lowenhaupt, U. Heinemann, A. Rich, *Nat. Struct. Biol.* **2001**, *8*, 761–765; c) Y.-G. Kim, M. Muralinath, T. Brandt, M. Pearcy, K. Hauns, K. Lowenhaupt, B. L. Jacobs, A. Rich, *Proc. Natl. Acad. Sci. USA* **2003**, *100*, 6974–6979.

[6] A. H.-J. Wang, G. J. Quigley, F. J. Kolpak, J. L. Crawford, J. H. van Boom, G. van der Marel, A. Rich, *Nature* **1979**, *282*, 680–686.

[7] H. Sugiyama, K. Kawai, A. Matsunaga, K. Fujimoto, I. Saito, H. Robinson, A. H.-J. Wang, *Nucleic Acids Res.* **1996**, *24*, 1272–1278.

[8] S. Uesugi, T. Shida, M. Ikehara, *Biochemistry* **1982**, *21*, 3400–3408.

[9] M. Behe, G. Felsenfeld, *Proc. Natl. Acad. Sci. USA* **1981**, *78*, 1619–1623.

[10] a) R. Buff, J. Hunziker, *Bioorg. Med. Chem. Lett.* **1998**, *8*, 521–524; b) R. Buff, J. Hunziker, *Nucleosides Nucleotides* **1999**, *18*, 1387–1388; c) R. Buff, J. Hunziker, *Helv. Chim. Acta* **2002**, *85*, 224–254.

[11] W. Saenger, *Principles of Nucleic Acid Structure*, Springer, New York, **1984**.

[12] Y. Xu, R. Ikeda, H. Sugiyama, *J. Am. Chem. Soc.* **2003**, *125*, 13519–13524.

[13] M. J. Gait, *Oligonucleotide Synthesis – A Practical Approach*, IRL Oxford University Press, Oxford, **1984**.

[14] C. Fàbrega, M. J. Marcías, R. Eritja, *Nucleosides Nucleotides* **2001**, *20*, 251–260.

[15] F a) F. M. Pohl, T. M. Jovin, *J. Mol. Biol.* **1972**, *67*, 375–396; b) J. Curtis, *CD of Nucleic acids Circular Dichroism*, 2nd ed., Wiley-VCH, New York, **2000**.

[16] J. Šponer, H. A. Gabb, J. Leszczynski, P. Hobza, *Biophys. J.* **1997**, *73*, 76–87.

Received: October 18, 2007
Published Online: February 5, 2008

