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C8-alkynyl- and alkylamino substituted 2′-deoxyguanosines: a universal linker for nucleic acids modification

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Abstract

Incorporation of modified nucleosides with a flexible universal linker is of great value for post-synthetic modification of nucleic acids. Thus, C8-alkynyl- and alkylamino substituted 2′-deoxyguanosines were synthesized for the first time and incorporated into short oligonucleotide sequences. The preference for *syn* conformation of these C8-substituted 2′-deoxyguanosines and the stability of the duplexes were discussed. The stabilizing effect of Z-DNA has also been examined.

1. Introduction

Modified nucleosides are of great interest due to their remarkable biological activity and wide range of applicability.[1] Among these, substituted guanosines are of greatest importance, specially because of its highly 'narcissistic' properties of forming important supramolecules, a variety of self-assembled structures including G-ribbons, and tetrameric complexes of high biological impact.[2] A particularly dramatic example is G-quadruplexes, which are connected with telomere stabilization and present in a region in HIV-1 genome.[3] Recently, modified guanine has been used for the formation of liquid crystalline phases,[4a] in separation of amino acid enantiomers[4b] and to create self-assembled ionophores.[4c–e] It was also reported that trivalent lanthanide metal ions assist in the formation of stable G-quartet.[4f]

Steric manipulations, especially, modification at C8 position can allow guanosines to modulate its electronic properties as well as to give novel self-assembled suprastructures with a considerable promise. For example, Sessler et al. have shown that a large C8-substituent forced the guanosine into a *syn* conformation resulting to a stable G-quartet with an empty cavity (Fig. 1).[5a] Gottarelli et al. has recently shown that 8-oxoguanine forms helical structures, resulting in a liquid crystalline phase.[5b] That the incorporation of a methyl group at guanine C8 position stabilizes the Z-form of oligonucleotides was also reported.[5c,d] Thus, it is clear that recent research efforts related to guanosine modification have demonstrated how base-pairing modes can be used to prepare higher ordered self-assembled ensembles to be used as building blocks in supramolecular chemistry.[6]

There exist very few reports, however, in the literature in which C8-position of 2′-deoxyguanosines is linked by a flexible linker possessing a post-synthetically modifiable functional group.[7] In connection with our recent development of microenvironment sensitive base-discriminating fluorescent (BDF) oligonucleotide probes[8] for SNPs typing, we were interested to prepare post-synthetically modifiable oligonucleotides. Thus, we required C8-functionalized 2′-deoxyguanosines having universal linker for attaching signaling molecules like fluorescent dyes or other reporter molecules via a post-synthetic modification. In this communication we report the first synthesis of C8 alkylamino substituted

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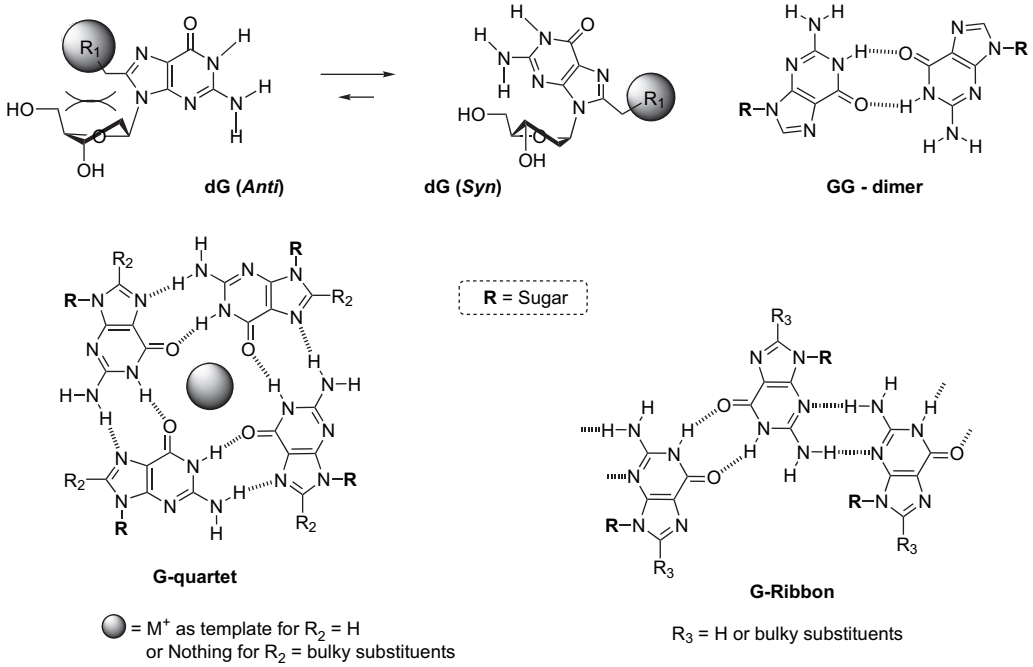


Figure 1. Conformational preference and the generated supramolecular architectures by C8-substituted/unsubstituted guanosines.

2′-deoxyguanosines and the oligonucleotides containing C8 modified guanosines.

2. Result and discussion

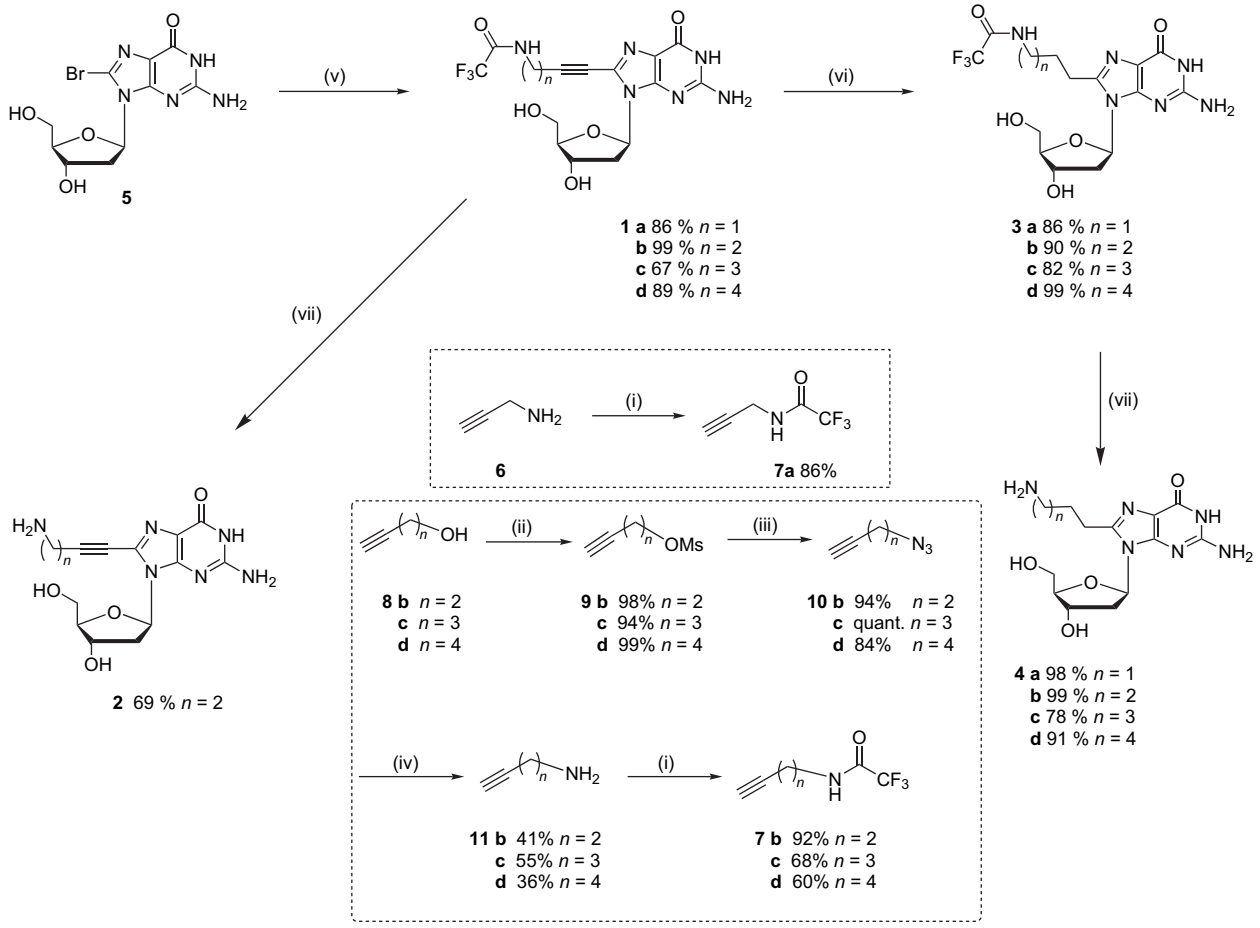
To introduce C8-alkynylamino side chain, 8-bromo-2′-deoxyguanosine was coupled with *N*-alkynyltrifluoroacetamide (**7a**–**d**) via Pd(0)-mediated Sonogashira coupling.[9] Thus, a coupling with *N*-propargyltrifluoroacetamide (**7a**) afforded protected propargylamino substituted nucleoside **1a** in 86% yield. Higher homologous alkynylamino substituted 2′-deoxyguanosines were prepared according to Scheme 1 in a similar way.[6d] Thus, the homopropargyl alcohol **8b** and its higher homologous **8c**,**d** were converted to their corresponding mesylates **9b**–**d**, respectively, in very good yields. The mesylates, **9b**–**d**, were then reacted with sodium azide in dry dimethylformamide to afford azides **10b**–**d** in very high yields, which were then converted to the corresponding amines **11b**–**d** by reduction with triphenylphosphine and water and were protected with trifluoroacetyl derivatives to afford the final linkers, **7b**–**d**. The protected amino linkers were then subjected to Pd(0)-mediated Sonogashira coupling to afford C8-substituted 2′-deoxyguanosines **1b**–**d** in good yield.

A flexible linker might allow fluorophore to survey microenvironment very nicely along the DNA groove and hence it is necessary to synthesize nucleosides possessing flexible alkylamino linker. Thus, nucleosides **1a**–**d** were subjected to 10% Pd/C catalyzed hydrogenation in methanol to afford protected alkylamino substituted 2′-deoxyguanosines **3a**–**d** in high yields. Treatment of trifluoroacetyl protected nucleosides **1b** and **3a**–**d** with aqueous ammonia at room temperature for 17 h afforded the corresponding alkynylamino nucleoside **2** and alkylamino nucleosides **4a**–**d**, respectively, in good yield. At present, other homologous of alkynylamino substituted guanosines could not be isolated in pure.

After getting all the C8-substituted 2′-deoxyguanosines in hand, we next studied the conformation of the monomer base around the glycosidic bond, which plays a crucial role in the structures of oligonucleotides and in a variety of enzymatic reactions. Moreover, the *syn*–*anti* equilibrium is a key event in going from a right-handed B-DNA to a left-handed Z-DNA involving a conformational change of guanine base residues from *anti* to *syn* (Fig. 1).[5c,10]

It is known that the steric bulkiness of C8-substituents destabilizes the normally preferred *anti* conformation of 2′-deoxyguanosines (dG) and hence the dG:dC base pairs, which was reflected in the decrease of melting temperature of the oligonucleotide duplexes.[10,11d] To know the effect of steric bulkiness of C8-substituents on the destabilization of the favorable *anti* conformation of dG, deprotected nucleosides, Br-dG (**5**), alkynylamino-dG (**2**), and alkylamino-dG (**4a**–**d**), were tested for their inherent glycosidic bond conformation using NMR spectroscopy. It is well established that an *anti* to *syn* conformational change results in a downfield shift of C1′, C3′, C4′, and H2′ signals, as well as an upfield shift of C2′ signal.[11d] As shown in Table 1, such shifts were observed for all C8-substituted 2′-deoxyguanosines as compared with that of Br-dG, indicating their preference for the *syn* conformation.[10,11d] NOE between anomeric H and methylene proton of alkylamino linker has also been observed in **4b** supporting a preference for *syn* conformation.[12]

Next we examined the effect of C8-substituted dG on the thermal duplex stability. Thus, the trifluoroacetyl protected nucleosides **1b** and **3a**–**d** were converted to the corresponding phosphoramidites **14** and **17a**–**d**, following the standard protocol adopted in our laboratory according to Scheme 2. In



Scheme 1. Synthesis of C8-substituted 2′-deoxyguanosines. *Reagents and conditions*: (i) ethyl trifluoroacetate/MeOH; (ii) MsCl/Et_2O; (iii) NaN_3/DMF; (iv) PPh_3/Et_2O, H_2O; (v) **7a–d**, $Pd(PPh_3)_4$, CuI, Et_3N, DMF; (vi) MeOH, Pd/C, H_2; (vii) NH_3/H_2O.

Table 1
Chemical shifts of nuclei of dG, Br-dG, and other substituted 2'-deoxyguanosines

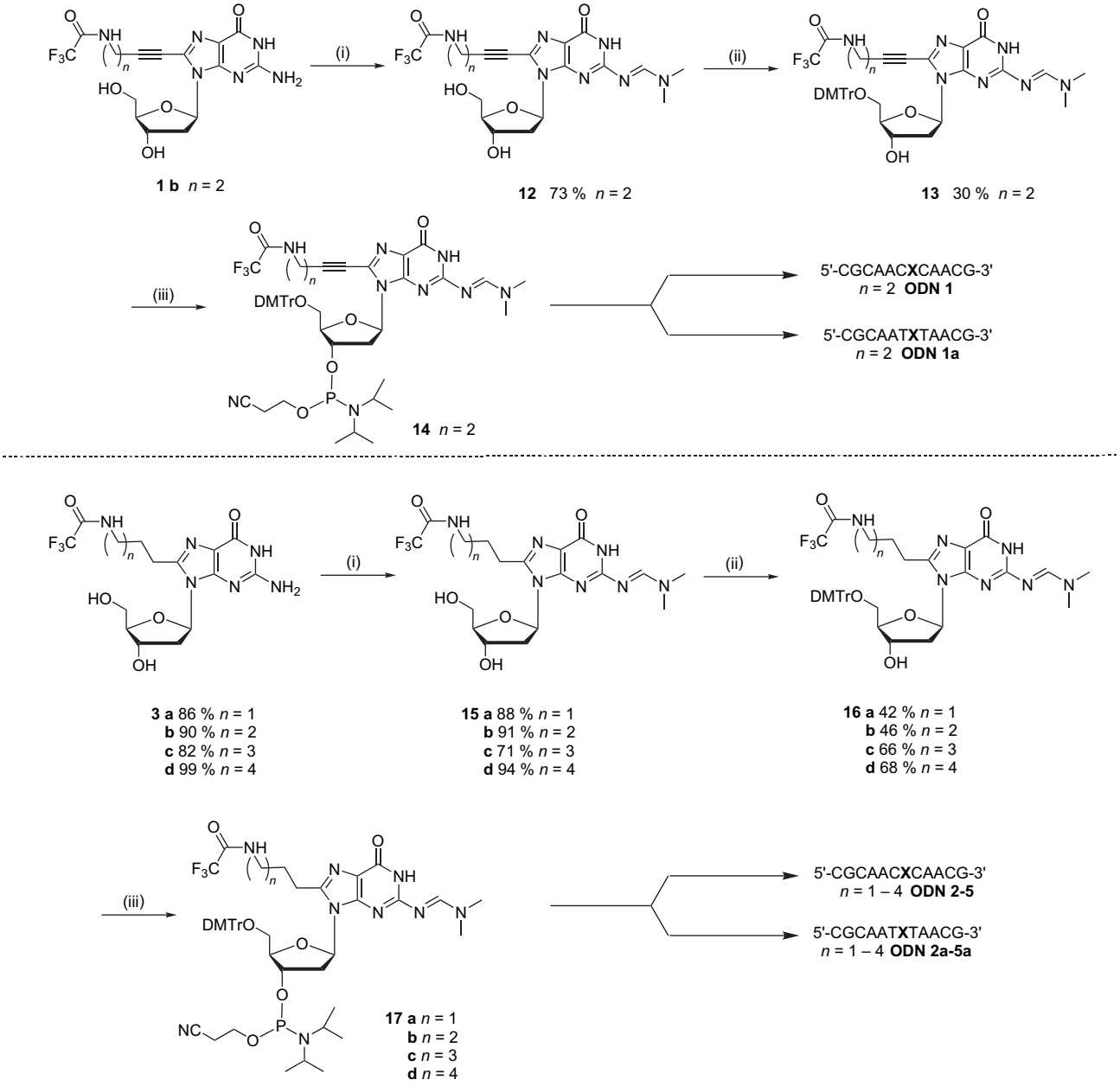
Nucleosides	C2′	H2′	C1′	C3′	C4′
dG	39.5	2.50	82.5	70.7	87.5
Br-dG (**5**)	36.4	3.17	85.0	71.0	87.8
2 (*n*=2)	37.1	3.07	83.7	70.9	87.7
4a (*n*=1)	37.6	2.83	83.1	70.7	87.4
4b (*n*=2)	37.6	2.89	83.1	70.8	87.3
4c (*n*=3)	37.5	2.91	83.2	70.9	87.5
4d (*n*=4)	37.4	2.91	83.2	70.9	87.5

Nucleoside (0.04 M) in DMSO-d_6 with 0.1% tetramethylsilane (TMS).

order to investigate the effects of C8 modifications on the stability of DNA duplex, phosphoramidites **14** and **17a–d** were employed in standard oligonucleotide synthesis, using automated DNA synthesizer. Thus, two 13mer ODNs containing C8 modified 2′-deoxyguanosines at the center were synthesized. While the ODN [5′-dCGCAAC**X**CAACGC-3′; **X**=**2**, **4a–d**] contains cytosine as the flanking bases, thymines are flanking bases in case of ODN [5′-dCGCAAT**X**TAACGC-3′; **X**=**2**, **4a–d**] (Scheme 2). Crude oligonucleotides containing **2** and **4a–d** were purified by reverse phase HPLC and detritylated using 3% trichloroacetic acid, repurified by HPLC and characterized by MALDI-TOF mass spectrometry.

Single stranded oligonucleotides containing C8-modified 2′-deoxyguanosines were hybridized with complimentary DNA strands and tested for their thermal stabilities (Table 2). From the thermal melting temperature experiment, it was found that, duplexes with modified 2′-deoxyguanosines are less stable than that of unmodified 2′-deoxyguanosines (dG). Thus, the duplexes containing C8-alkylamino modified 2′-deoxyguanosines showed a decrease in thermal stability as the number of carbons increases. It was also found that the duplexes (ODN**1**, ODN**1a**) containing C8-alkynylamino modified 2′-deoxyguanosine were more stable as compared with the duplexes containing the corresponding C8-alkylamino modified 2′-deoxyguanosine (ODN**2**, ODN**2a**). The decrease in stability with increasing number of carbon might well be explained if we consider both the steric bulkiness of the substituents and the unfavorable interaction of the appended free amino functionality within the duplexes.[11]

Z-form DNA is one of the characteristic and significant local structures of DNA and has been extensively studied in connection to transcription, methylation, and DNA supercoiling.[13] Recently, the study of possible biological roles of Z-DNA has drawn much interest. Thus, it was reported that Z-DNA-forming sequences are required for chromatin-dependent activation of CSF1 promoter.[14] Therefore, the modified nucleoside stabilizing the Z-DNA is of immense interest in recent years. Several groups have come forward to synthesize C8-substituted dG and studied the Z-DNA stabilization at physiological salt



Scheme 2. Synthesis of oligonucleotides containing modified 2′-deoxyguanosines. *Reagents and conditions*: (i) DMF-diethylacetal, DMF, 55 °C, 3 h; (ii) DMTrCl, DMAP, pyridine, rt, 12 h; (iii) $(N^{i}Pr_2)_2PO(CH_2)_2CN$, tetrazole, DCM, rt, 1 h.

concentrations if at all.[5d,15] However, the design of more powerful and chemically stable new types of Z-DNA stabilizers is still desirable.

Thus, we were interested to investigate whether our C8-modified dG is capable of stabilizing Z-DNA or not. We have incorporated modified nucleoside **3d** into a sequence

Table 2
Thermal melting behavior of ODNs

Duplexes [5′-d(CGCAAC **X**CAACGC-3′)]/[5′-d(GCGTTG **C**GTTGCG-3′)]; **X**=**2**, **4a**–**d**	T_m	ΔT_m	Duplexes [5′-d(CGCAAT **X**TAACGC-3′)]/[5′-d(GCGTTA **C**ATTGCG-3′)]; **X**=**2**, **4a**–**d**	T_m	ΔT_m
dG	63.8	—	dG	58.7	—
ODN**1** (*n*=2)	61.6	−2.2	ODN**1a** (*n*=2)	56.1	−2.6
ODN**2** (*n*=1)	59.3	−4.5	ODN**2a** (*n*=1)	54.0	−4.7
ODN**3** (*n*=2)	59.1	−4.7	ODN**3a** (*n*=2)	53.7	−5.0
ODN**4** (*n*=3)	58.5	−5.3	ODN**4a** (*n*=3)	51.2	−7.5
ODN**5** (*n*=4)	57.2	−6.6	ODN**5a** (*n*=4)	51.6	−7.1

Condition: All T_ms of the ODNs (2.5 μM, final duplex concentration) were taken in 50 mM sodium phosphate buffers (pH 7.0) containing 100 mM sodium chloride.

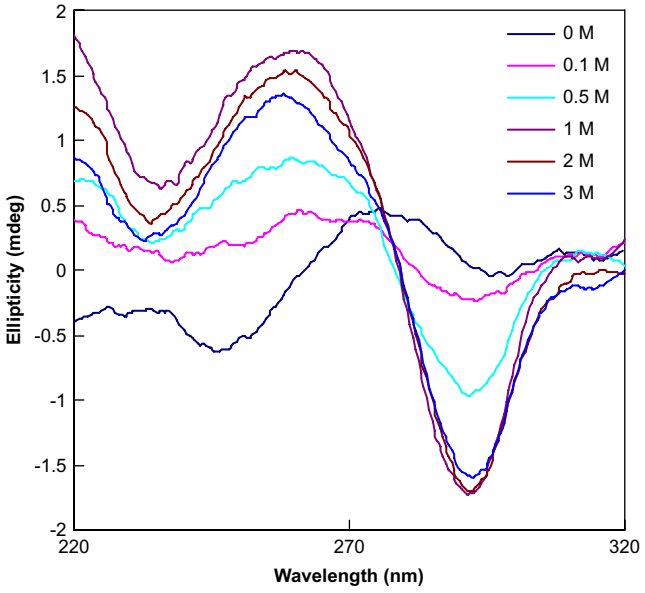


Figure 2. CD spectra of d(CGC**X**CG)$_2$ [**X**=**4c**] in 5 mM Na-cacodylate buffer, pH 7.0, at room temperature at various NaCl concentrations.

ODN **6** [5′-d(CGC**X**CG)-3′ (**X**=**3d**)] and studied the circular dichroism (CD) of salt behavior in the presence of different concentration. The appearance of an intense negative band at 295 nm clearly indicates the B–Z transition as the salt concentration increases (Fig. 2).[16] The mid point salt concentration was calculated about 300 mM [for natural dG, it is 2600 mM and for natural G, it is 800 mM], indicating that the modified alkylamino 2′-deoxyguanosine effectively stabilizes the Z-form DNA.

3. Conclusion

In conclusion, we have successfully synthesized the C8-alkynylamino and alkylamino substituted 2′-deoxyguanosines both in protected and unprotected form. To the best of our knowledge, except the propargylamino 2′-deoxyguanosine, none of these C8-substituted 2′-deoxyguanosines has been reported. It is demonstrated that as the number of carbon in the linker increases, the duplex stability decreases, mainly because of the steric clash. As an application of our synthesized nucleosides, we were also able to show that our first synthesized C8-alkylamino nucleoside is efficient in stabilizing Z-DNA. For post-synthetic modification, the alkynyl- and alkylamino modified oligonucleotides are very useful. Currently we are introducing highly solvofluorochromic fluorophores via post-synthetic modification techniques for DNA sequence analysis and applying them in the study of B–Z transition. The detail results will be published elsewhere in due course.

4. Experimental

4.1. General

^{1}H NMR spectra were measured with Varian Mercury 400 (400 MHz) and ^{13}C NMR spectra were measured with Bruker Avance 400F (100 MHz) spectrometer. Coupling constant (J value) are reported in hertz. The chemical shifts are shown in parts per million downfield from tetramethylsilane, using residual chloroform (δ 7.24 in ^{1}H NMR, δ 77.0 in ^{13}C NMR) and dimethyl sulfoxide (δ 2.48 in ^{1}H NMR, δ 39.5 in ^{13}C NMR) as an internal standard. FAB masses were recorded on a JEOL JMS HX-110A spectrometer.

The reagents for DNA synthesis were purchased from Glen Research. Mass spectra of oligodeoxynucleotides were determined with a MALDI-TOF MS (Shimadzu AXIMA-LNR, acceleration voltage 20 kV, positive mode) with 2′,3′,4′-trihydroxyacetophenone as a matrix. Calf intestinal alkaline phosphatase (Promega), Crotalus adamanteus venom phosphodiesterase I (USB), and *Penicillium citrinum* nuclease P1 (Roche) were used for the enzymatic digestion of ODNs. All aqueous solutions utilized purified water (Millipore, Milli-Q sp UF). Reversed-phase HPLC was performed on CHEMCO-BOND 5-ODS-H columns (10×150 mm, 4.6×150 mm) with a JASCO Chromatograph, Model PU-2080, using a UV detector, Model UV-2075 plus, at 254 nm.

4.1.1. General procedure for the synthesis of protected alkynylamino linkers

*4.1.1.1. Pent-4-yne-1-mesylate (**9c**).* Methanesulfonylchloride (7.27 g, 63.5 mmol) was added dropwise to a stirred solution of 4-pentyn-1-ol **8c** (5.20 ml, 52.9 mmol) and Et_3N (8.84 ml, 63.4 mmol) in anhydrous diethyl ether (80 ml) at 0 °C under argon atmosphere. After 3 h, water (100 ml) was added to the reaction mixture. The organic layer was separated, washed with water (4×100 ml), dried over anhydrous Na_2SO_4 and evaporated to yield **9c** (8.07 g, 49.8 mmol, 94%) as a pale yellow liquid. Without further purification we used this compound for the next step. ^{1}H NMR (CD_3OD, 400 MHz) δ 1.92 (tt, 2H, J=6.2, 6.2 Hz), 2.30–2.35 (complex, 3H), 3.07 (s, 3H), 4.33 (t, 2H, J=6.2 Hz); ^{13}C NMR (CD_3OD, 100 MHz) δ 15.3, 29.2, 37.0, 70.0, 70.7, 83.2; ESI-MS m/z 163.0 $[M+H]^+$.

*4.1.1.2. Hex-5-yne-1-mesylate (**9d**).* Compound **9d** (6.27 g, 35.6 mmol, 99%) was prepared from **8d** (4.0 ml, 35.9 mmol), methanesulfonylchloride (6.17 g, 53.9 mmol), and Et_3N (7.51 ml, 53.9 mmol) in anhydrous diethyl ether (100 ml) by the method described for **9c** as a pale yellow liquid. Without further purification we used this compound for the next step. ^{1}H NMR ($CDCl_3$, 400 MHz) δ 1.66 (m, 2H), 1.89 (m, 2H), 1.99 (m, 1H), 2.26 (m, 2H), 4.27 (m, 2H); ^{13}C NMR ($CDCl_3$, 100 MHz) δ 17.8, 24.3, 28.0, 37.4, 69.2, 69.5, 83.4; ESI-MS m/z 177.0 $[M+H]^+$.

*4.1.1.3. Pent-4-yne-1-azide (**10c**).* Sodium azide (8.90 g, 137 mmol) was added to a solution of mesylate **9c** (8.85 g, 54.6 mmol) in dry DMF (40 ml) under argon. The mixture was stirred at 70 °C for 3 h. The reaction mixture was poured into water (200 ml) and extracted with diethyl ether (3×100 ml). The solution was then dried over anhydrous Na_2SO_4 and evaporated to yield **10c** (6.32 g, 57.9 mmol, quant.) as a pale yellow liquid. Without further purification

we used this compound for the next step. ^{1}H NMR ($CDCl_3$, 400 MHz) δ 1.80 (tt, 2H, J=6.8, 6.8 Hz), 2.00 (t, 1H, J=2.8 Hz), 2.32 (dt, 2H, J=2.8, 6.8 Hz), 3.44 (t, 2H, J=6.8 Hz); ^{13}C NMR ($CDCl_3$, 100 MHz) δ 15.8, 27.7, 50.1, 69.4, 82.7; ESI-MS m/z 110.1 $[M+H]^+$.

*4.1.1.4. Hex-5-yne-1-azide (**10d**).* Compound **10d** (3.53 g, 28.7 mmol, 84%) was prepared from **9d** (5.98 g, 34.0 mmol), and sodium azide (5.51 g, 84.6 mmol) in dry DMF (30 ml) by the method described for **10c** as a pale yellow liquid. Without further purification we used this compound for the next step. ^{1}H NMR ($CDCl_3$, 400 MHz) δ 1.59 (m, 2H), 1.74 (m, 2H), 1.98 (t, 1H, J=2.8 Hz), 2.24 (dt, 2H, J=2.8, 6.8 Hz), 3.31 (t, 2H, J=6.8 Hz); ^{13}C NMR ($CDCl_3$, 100 MHz) δ 18.0, 25.5, 27.7, 51.0, 68.9, 83.7; ESI-MS m/z 124.1 $[M+H]^+$.

*4.1.1.5. Pent-4-yne-1-amine (**11c**).* To a solution of azide **10c** (5.00 g, 45.9 mmol) in anhydrous diethyl ether (30 ml) at 0 °C, PPh_3 (18.0 g, 68.6 mmol) was added and allowed to stirred for 3 h. Water (3 ml) was then added to the reaction mixture and stirred for another 17 h. The reaction mixture was poured into 10% aq HCl and extracted with diethyl ether (3×50 ml). The aqueous layer was then adjusted to basic solution (pH 10) with aq NaOH and extracted with diethyl ether (5×50 ml). The organic layer was dried over anhydrous Na_2SO_4 and evaporated to yield **11c** (2.10 g, 25.3 mmol, 55%) as a pale yellow liquid. Without further purification we used this compound for the next step. ^{1}H NMR ($CDCl_3$, 400 MHz) δ 1.19–1.28 (complex, 4H), 2.18 (s, 1H), 3.48 (t, 2H, J=7.2 Hz); ^{13}C NMR ($CDCl_3$, 100 MHz) δ 15.8, 32.1, 41.0, 65.9, 84.0; ESI-MS m/z 84.0 $[M+H]^+$.

*4.1.1.6. Hex-5-yne-1-amine (**11d**).* Compound **11d** (1.00 g, 10.3 mmol, 36%) was prepared from **10d** (3.53 g, 28.7 mmol) and PPh_3 (9.10 g, 34.7 mmol) in anhydrous diethyl ether (30 ml) by the method described for **11c** as a pale yellow liquid. Without further purification we used this compound for the next step. ^{1}H NMR ($CDCl_3$, 400 MHz) δ 1.53–1.60 (complex, 4H), 1.96 (t, 1H, J=2.8 Hz), 2.37 (m, 2H), 2.72 (m, 2H); ^{13}C NMR ($CDCl_3$, 100 MHz) δ 18.3, 25.8, 32.7, 41.6, 68.4, 84.3; ESI-MS m/z 98.1 $[M+H]^+$.

*4.1.1.7. N-Trifluoroacetyl pentynylamide (**7c**).* To a stirred solution of amine **11c** (2.10 g, 25.3 mmol) in anhydrous methanol (30 ml) at 0 °C was added ethyl trifluoroacetate (2.0 ml) dropwise and stirred at room temperature for 17 h. Thereafter, the solvent was evaporated and the crude product was purified by silica gel column chromatography ($CHCl_3$) to yield **7c** (3.10 g, 17.3 mmol, 68%) as a colorless liquid. ^{1}H NMR ($CDCl_3$, 400 MHz) δ 1.83 (tt, 2H, J=6.8, 6.8 Hz), 2.04 (t, 1H, J=2.4 Hz), 2.30 (dt, 2H, J=2.4, 6.8 Hz), 3.51 (td, 2H, J=6.8, 6.8 Hz), 7.00 (br 1H); ^{13}C NMR ($CDCl_3$, 100 MHz) δ 16.1, 27.1, 39.3, 69.8, 82.8, 115.8 (q, J=285.8 Hz), 157.4 (q, J=36.7 Hz); ESI-MS m/z 180.1 $[M+H]^+$.

*4.1.1.8. N-Trifluoroacetyl hexynylamide (**7d**).* Compound **7d** (1.20 g, 6.21 mmol, 60%) was prepared from **11d** (1.00 g, 10.3 mmol) and ethyl trifluoroacetate (2.0 ml) in anhydrous methanol (30 ml) by the method described for **7c** as a colorless waxy solid. ^{1}H NMR ($CDCl_3$, 400 MHz) δ 1.61 (m, 2H), 1.74 (m, 2H), 1.99 (t, 1H, J=2.8 Hz), 2.26 (dt, 2H, J=2.8, 6.8 Hz), 3.41 (dt, 2H, J=6.8, 6.8 Hz), 6.53 (br, 1H); ^{13}C NMR ($CDCl_3$, 100 MHz) δ 18.0, 25.4, 27.9, 39.5, 69.1, 83.6, 115.9 (q, J=285.9 Hz), 157.3 (q, J=36.6 Hz); ESI-MS m/z 194.1 $[M+H]^+$.

4.1.2. General procedure for Sonogashira coupling

*4.1.2.1. 8-[N-Trifluoroacetyl propynylamido]-2′-deoxyguanosine (**1a**).* Compound **5** (331 mg, 0.956 mmol) was dissolved in dry DMF (30 ml). Compound **7a** (513 mg, 3.42 mmol), $Pd(PPh_3)_4$ (119 mg, 0.103 mmol), CuI (95.5 mg, 0.501 mmol), and Et_3N (200 μl, 1.43 mmol) were added to the reaction mixture and was stirred at 55 °C under argon. After 3.5 h of stirring, the reaction mixture was evaporated and purified by silica gel column chromatography ($CHCl_3$/MeOH=10:1 to 5:1) to yield **1a** (342 mg, 0.822 mmol, 86%) as a colorless powder. ^{1}H NMR (DMSO-d_6, 400 MHz) δ 2.09 (ddd, 1H, J=2.0, 6.8, 13.2 Hz), 3.05 (m, 1H), 3.50 (ddd, 1H, J=5.6, 5.6, 12.0 Hz), 3.62 (ddd, 1H, J=5.6, 5.6, 12.0 Hz), 3.79 (ddd, 1H, J=2.8, 5.6, 5.6 Hz), 4.37–4.41 (complex, 3H), 4.87 (br, 1H), 5.26 (d, 1H, J=4.0 Hz), 6.23 (dd, 1H, J=6.8, 8.0 Hz), 6.58 (s, 2H), 10.20 (br, 1H), 10.84 (br, 1H); ^{13}C NMR (CD_3OD, 100 MHz) δ 29.4, 37.4, 62.0, 71.0, 72.8, 83.6, 87.8, 89.3, 115.7 (q, J=286.3 Hz), 117.1, 128.6, 150.7, 153.8, 156.0, 156.3 (q, J=36.7 Hz); ESI-MS m/z 417.1 $[M+H]^+$.

*4.1.2.2. 8-[N-Trifluoroacetyl butynylamido]-2′-deoxyguanosine (**1b**).* Compound **1b** was prepared from **5** (339 mg, 0.979 mmol), **7b** (460 mg, 2.79 mmol), $Pd(PPh_3)_4$ (119 mg, 0.103 mmol), CuI (58.2 mg, 0.306 mmol), and Et_3N (150 μl, 1.08 mmol) in dry DMF (30 ml) by the method described for **1a**. Purification by silica gel column chromatography ($CHCl_3$/MeOH=10:1 to 5:1) gave **1b** (420 mg, 0.976 mmol, 99%) as a colorless powder. ^{1}H NMR (DMSO-d_6, 400 MHz) δ 2.10 (ddd, 1H, J=3.6, 6.8, 13.2 Hz), 2.80 (t, 2H, J=6.8 Hz), 3.01 (m, 1H), 3.46 (t, 2H, J=6.8 Hz), 3.50 (dd, 1H, J=6.0, 11.6 Hz), 3.61 (dd, 1H, J=5.2, 11.6 Hz), 3.79 (ddd, 1H, J=3.6, 5.2, 6.0 Hz), 4.40 (m, 1H), 4.87 (br, 1H), 5.24 (d, 1H, J=4.4 Hz), 6.24 (dd, 1H, J=6.8, 7.2 Hz), 6.54 (br, 2H), 9.71 (br, 1H), 10.77 (br, 1H); ^{13}C NMR (DMSO-d_6, 100 MHz) δ 18.8, 37.0, 37.5, 61.9, 70.8, 71.8, 83.5, 87.6, 92.0, 115.8 (q, J=286.4 Hz), 116.9, 129.3, 150.5, 153.5, 155.9, 156.3 (q, J=36.0 Hz); ESI-MS m/z 431.2 $[M+H]^+$.

*4.1.2.3. 8-[N-Trifluoroacetyl pentynylamido]-2′-deoxyguanosine (**1c**).* Compound **1c** was prepared from **5** (200 mg, 0.578 mmol), **7c** (250 mg, 1.40 mmol), $Pd(PPh_3)_4$ (134 mg, 0.116 mmol), CuI (22.0 mg, 0.116 mmol), and Et_3N (300 μl, 2.15 mmol) in dry DMF (10 ml) by the method described for **1a**. Purification by silica gel column chromatography ($CHCl_3$/MeOH=10:1 to 5:1) gave **1c** (173 mg, 0.390 mmol, 67%) as a colorless powder. ^{1}H NMR (DMSO-d_6, 400 MHz) δ 1.81 (tt, 2H, J=6.8, 7.2 Hz), 2.10 (ddd, 1H, J=3.2, 6.8,

13.2 Hz), 2.57 (t, 2H, *J*=7.2 Hz), 3.06 (m, 1H), 3.32 (t, 2H, *J*=6.8 Hz), 3.51 (ddd, 1H, *J*=5.6, 6.0, 12.0 Hz), 3.60 (ddd, 1H, *J*=5.6, 6.0, 12.0 Hz), 3.79 (ddd, 1H, *J*=3.2, 5.6, 5.6 Hz), 4.39 (m, 1H), 4.87 (dd, 1H, *J*=6.0, 6.0 Hz), 5.24 (d, 1H, *J*=4.4 Hz), 6.25 (dd, 1H, *J*=6.8, 7.6 Hz), 6.51 (s, 2H), 9.53 (br, 1H), 10.79 (s, 1H); ^{13}C NMR (DMSO-d_6, 100 MHz) δ 16.2, 26.8, 36.9, 38.3, 62.0, 71.0, 71.2, 83.6, 87.6, 94.3, 115.8 (q, *J*=286.0 Hz), 116.8, 129.5, 150.4, 153.6, 155.9, 156.2 (q, *J*=36.0 Hz); ESI-MS *m/z* 445.1 $[M+H]^+$.

*4.1.2.4. 8-[N-Trifluoroacetyl hexynylamido]-2′-deoxyguanosine (**1d**).* Compound **1d** was prepared from **5** (200 mg, 0.578 mmol), **7d** (223 mg, 1.16 mmol), $Pd(PPh_3)_4$ (66.7 mg, 0.0577 mmol), CuI (17.3 mg, 0.0908 mmol), and Et_3N (121 μl, 0.867 mmol) in dry DMF (20 ml) by the method described for **1a**. Purification by silica gel column chromatography ($CHCl_3$/MeOH=10:1 to 5:1) gave **1d** (237 mg, 0.517 mmol, 89%) as a colorless powder. ^{1}H NMR (DMSO-d_6, 400 MHz) δ 1.54−1.69 (complex, 4H), 2.09 (ddd, 1H, *J*=3.2, 6.4, 12.8 Hz), 2.56 (t, 2H, *J*=6.8 Hz), 3.05 (ddd, 1H, *J*=6.4, 8.0, 12.8 Hz), 3.24 (dt, 2H, *J*=6.1, 6.3 Hz), 3.50 (ddd, 1H, *J*=6.0, 6.0, 11.2 Hz), 3.60 (ddd, 1H, *J*=5.6, 5.6, 11.2 Hz), 3.79 (ddd, 1H, *J*=3.2, 5.6, 6.0 Hz), 4.38 (m, 1H), 4.88 (dd, 1H, *J*=5.6, 6.0 Hz), 5.24 (d, 1H, *J*=4.0 Hz), 6.24 (dd, 1H, *J*=6.4, 8.0 Hz), 6.49 (s, 2H), 9.47 (t, 1H, *J*=6.1 Hz), 10.77 (s, 1H); ^{13}C NMR (DMSO-d_6, 100 MHz) δ 18.1, 24.7, 27.4, 36.9, 38.5, 62.0, 71.0, 71.2, 83.6, 87.6, 94.9, 115.9 (q, *J*=286.2 Hz), 116.8, 129.6, 150.5, 153.6, 155.9, 156.1 (q, *J*=35.0 Hz); ESI-MS *m/z* 459.2 $[M+H]^+$.

4.1.3. General procedure for hydrogenation

*4.1.3.1. 8-[N-Trifluoroacetyl propylamido]-2′-deoxyguanosine (**3a**).* Pd/C (10%, 58 mg) was added to a solution of **1a** (294 mg, 0.707 mmol) in anhydrous methanol (15 ml). Then, the reaction mixture was stirred at room temperature for 24 h under H_2 atmosphere. Thereafter, the reaction mixture was filtered, evaporated and purified by silica gel column chromatography ($CHCl_3$/MeOH=5:1) to yield **3a** (255 mg, 0.607 mmol, 86%) as a colorless powder. ^{1}H NMR (DMSO-d_6, 400 MHz) δ 1.94 (m, 2H), 2.06 (ddd, 1H, *J*=2.5, 6.2, 13.0 Hz), 3.31 (t, 2H, *J*=6.8 Hz), 2.70−2.95 (complex, 3H), 3.52 (m, 1H), 3.62 (m, 1H), 3.79 (ddd, 1H, *J*=3.1, 3.1, 5.0 Hz), 4.36 (m, 1H), 4.98 (dd, 1H, *J*=5.4, 5.4 Hz), 5.24 (d, 1H, *J*=4.2 Hz), 6.14 (dd, 1H, *J*=6.2, 8.4 Hz), 6.33 (s, 2H), 9.52 (t, 1H, *J*=5.3 Hz), 10.60 (s, 1H); ^{13}C NMR (DMSO-d_6, 100 MHz) δ 24.7, 25.6, 37.6, 38.8, 61.8, 70.5, 83.0, 87.4, 115.3, 115.9 (q, *J*=286.5 Hz), 147.2, 151.5, 152.8, 156.2 (q, *J*=36.0 Hz), 156.2; ESI-MS *m/z* 421.2 $[M+H]^+$.

*4.1.3.2. 8-[N-Trifluoroacetyl butylamido]-2′-deoxyguanosine (**3b**).* Compound **3b** was prepared from **1b** (813 mg, 1.89 mmol) and 10% Pd/C (200 mg) in anhydrous methanol (40 ml) by the method described for **3a**. Purification by silica gel column chromatography ($CHCl_3$/MeOH=5:1) gave **3b** (743 mg, 1.71 mmol, 90%) as a colorless powder. ^{1}H NMR (DMSO-d_6, 400 MHz) δ 1.58 (m, 2H), 1.69 (m, 2H), 2.05 (ddd, 1H, *J*=2.4, 6.4, 12.8 Hz), 2.77 (m, 2H), 2.89 (ddd, 1H, *J*=6.4, 8.0, 12.8 Hz), 3.22 (td, 2H, *J*=6.4, 6.4 Hz), 3.53 (m, 1H), 3.62 (m, 1H), 3.79 (ddd, 1H, *J*=3.2, 4.8, 4.8 Hz), 4.37 (m, 1H), 5.00 (dd, 1H, *J*=5.2, 5.2 Hz), 5.25 (d, 1H, *J*=4.4 Hz), 6.14 (dd, 1H, *J*=6.4, 8.0 Hz), 6.32 (s, 2H), 9.47 (t, 1H, *J*=5.5 Hz), 10.58 (br, 1H); ^{13}C NMR (DMSO-d_6, 100 MHz) δ 23.9, 26.8, 27.7, 37.6, 38.9, 61.8, 70.8, 83.1, 87.4, 115.4, 115.9 (q, *J*=286.5 Hz), 147.6, 151.4, 152.7, 156.1 (q, *J*=35.7 Hz), 156.2; ESI-MS *m/z* 435.2 $[M+H]^+$.

*4.1.3.3. 8-[N-Trifluoroacetyl pentylamido]-2′-deoxyguanosine (**3c**).* Compound **3c** was prepared from **1c** (63.7 mg, 0.143 mmol) and 10% Pd/C (30 mg) in anhydrous methanol (5.0 ml) by the method described for **3a**. Purification by silica gel column chromatography ($CHCl_3$/MeOH=5:1) gave **3c** (52.3 mg, 0.117 mmol, 82%) as a colorless powder. ^{1}H NMR (CD_3OD, 400 MHz) δ 1.41 (m, 2H), 1.61 (tt, 2H, *J*=7.3, 7.5 Hz), 1.76 (tt, 2H, *J*=7.5, 7.5 Hz), 2.21 (ddd, 1H, *J*=2.0, 6.1, 13.3 Hz), 2.83 (t, 2H, *J*=7.5 Hz), 3.09 (ddd, 1H, *J*=6.1, 8.7, 13.3 Hz), 3.27 (t, 2H, *J*=7.3 Hz), 3.74 (dd, 1H, *J*=3.7, 12.1 Hz), 3.85 (dd, 1H, *J*=3.1, 12.1 Hz), 4.03 (ddd, 1H, *J*=3.1, 3.7, 5.6 Hz), 4.59 (ddd, 1H, *J*=2.0, 5.6, 6.1 Hz), 6.14 (dd, 1H, *J*=6.1, 8.7 Hz); ^{13}C NMR (DMSO-d_6, 100 MHz) δ 25.7, 26.3, 27.2, 27.9, 37.5, 40.1, 61.8, 70.9, 83.1, 87.4, 115.4, 115.9 (q, *J*=287 Hz), 147.8, 151.4, 152.7, 156.0 (q, *J*=35.6 Hz), 156.2; ESI-MS *m/z* 449.7 $[M+H]^+$.

*4.1.3.4. 8-[N-Trifluoroacetyl hexylamido]-2′-deoxyguanosine (**3d**).* Compound **3d** was prepared from **1d** (153 mg, 0.334 mmol) and 10% Pd/C (60 mg) in anhydrous methanol (15 ml) by the method described for **3a**. Purification by silica gel column chromatography ($CHCl_3$/MeOH=5:1) gave **3d** (152.1 mg, 0.329 mmol, 99%) as a colorless powder. ^{1}H NMR (CD_3OD, 400 MHz) δ 1.33−1.47 (complex, 4H), 1.57 (tt, 2H, *J*=7.1, 7.1 Hz), 1.74 (tt, 2H, *J*=7.5, 7.5 Hz), 2.19 (ddd, 1H, *J*=2.0, 6.1, 13.4 Hz), 2.82 (t, 2H, *J*=7.5 Hz), 3.09 (ddd, 1H, *J*=6.1, 8.8, 13.4 Hz), 3.26 (t, 2H, *J*=7.1 Hz), 3.74 (dd, 1H, *J*=3.6, 12.1 Hz), 3.85 (dd, 1H, *J*=3.1, 12.1 Hz), 4.03 (m, 1H), 4.60 (m, 1H), 6.23 (dd, 1H, *J*=6.1, 8.8 Hz); ^{13}C NMR (DMSO-d_6, 100 MHz) δ 25.8, 26.7, 27.2, 28.0, 28.1, 37.5, 39.0, 61.8, 70.9, 83.1, 87.4, 115.4, 115.9 (q, *J*=286.5 Hz), 147.9, 151.4, 152.7, 156.0 (q, *J*=36.0 Hz), 156.2; ESI-MS *m/z* 463.2 $[M+H]^+$.

4.1.4. General procedure for deprotection of TFA group

*4.1.4.1. 8-Butynylamino-2′-deoxyguanosine (**2**).* A solution of **1b** (70.3 mg, 0.163 mmol) in 28% aq NH_4OH (20 ml) was stirred at room temperature for 17 h. Then, ammonia was evaporated and the residue was triturated with Et_2O to give **2** (37.9 mg, 0.113 mmol, 69%) as a colorless powder. ^{1}H NMR (DMSO-d_6, 400 MHz) δ 2.11 (ddd, 1H, *J*=2.8, 6.8, 13.2 Hz), 2.87 (t, 1H, *J*=6.8 Hz), 3.00−3.09 (complex, 3H), 3.50 (dd, 1H, *J*=5.6, 11.6 Hz), 3.63 (dd, 1H, *J*=5.2, 11.6 Hz), 3.80 (ddd, 1H, *J*=3.2, 5.2, 5.6 Hz), 4.40 (m, 1H), 4.90 (br, 1H), 5.29 (br, 1H), 6.26 (dd, 1H, *J*=6.8, 7.2 Hz), 6.61 (s, 2H), 8.53 (br, 2H); ^{13}C NMR (DMSO-d_6, 100 MHz)

δ 17.8, 37.1, 38.8, 62.0, 71.0, 72.6, 83.7, 87.7, 90.5, 117.0, 129.1, 150.5, 153.7, 156.0; HRMS (FAB) calcd for $[C_{14}H_{18}N_6O_4+H]^+$ 335.1462, found 335.1452.

*4.1.4.2. 8-Propylamino-2′-deoxyguanosine (**4a**).* Compound **4a** (24.5 mg, 0.0756 mmol, 98%) was prepared from **3a** (32.5 mg, 0.0774 mmol) in methanol (6.4 ml) and 28% aq NH_4OH (1.0 ml) by the method described for **2** as a colorless powder. ^{1}H NMR (DMSO-d_6, 400 MHz) δ 1.90–2.90 (complex, 3H), 2.79–3.00 (complex, 5H), 3.53 (dd, 1H, *J*=4.8, 11.6 Hz), 3.62 (dd, 1H, *J*=4.8, 11.6 Hz), 3.79 (m, 1H), 4.38 (m, 1H), 5.01 (br, 1H), 5.28 (d, 1H, *J*=3.6 Hz), 6.15 (dd, 1H, *J*=6.4, 8.4 Hz), 6.43 (s, 2H), 7.79 (br, 2H); ^{13}C NMR (DMSO-d_6, 100 MHz) δ 24.2, 24.4, 37.6, 38.4, 61.7, 70.7, 83.0, 87.4, 115.2, 146.8, 151.6, 152.9, 156.2; HRMS (FAB) calcd for $[C_{13}H_{20}N_6O_4+H]^+$ 325.1619, found 325.1601.

*4.1.4.3. 8-Butylamino-2′-deoxyguanosine (**4b**).* Compound **4b** (71.5 mg, 0.211 mmol, 99%) was prepared from **3b** (91.8 mg, 0.211 mmol) in methanol (20 ml) and 28% aq NH_4OH (10 ml) by the method described for **2** as a colorless powder. ^{1}H NMR (DMSO-d_6, 400 MHz) δ 1.64 (m, 2H), 1.76 (m, 2H), 2.06 (ddd, 1H, *J*=2.4, 6.2, 12.9 Hz), 2.72–2.92 (complex, 5H), 3.52 (m, 1H), 3.62 (dd, 1H, *J*=4.4, 11.7 Hz), 3.79 (ddd, 1H, *J*=3.0, 4.4, 4.8 Hz), 4.38 (m, 1H), 5.01 (br, 1H), 5.28 (d, 1H, *J*=4.2 Hz), 6.15 (dd, 1H, *J*=6.2, 8.4 Hz), 6.40 (s, 2H), 7.75 (br, 2H), 10.72 (br, 1H); ^{13}C NMR (DMSO-d_6, 100 MHz) δ 23.3, 26.5, 26.7, 37.6, 38.6, 61.8, 70.8, 83.1, 87.4, 115.3, 147.4, 151.5, 152.8, 156.2; HRMS (FAB) calcd for $[C_{14}H_{22}N_6O_4+H]^+$ 339.1775, found 339.1783.

*4.1.4.4. 8-Pentylamino-2′-deoxyguanosine (**4c**).* Compound **4c** (11.2 mg, 0.0318 mmol, 78%) was prepared from **3c** (18.2 mg, 0.0406 mmol) in 28% aq NH_4OH (10 ml) by the method described for **2** as a colorless powder. ^{1}H NMR (DMSO-d_6, 400 MHz) δ 1.40 (m, 2H), 1.58 (tt, 2H, *J*=7.2, 7.6 Hz), 1.71 (tt, 2H, *J*=7.6, 7.8 Hz), 2.06 (ddd, 1H, *J*=2.4, 6.4, 12.8 Hz), 2.67–2.83 (complex, 4H), 2.90 (ddd, 1H, *J*=6.0, 8.4, 12.8 Hz), 3.51 (dd, 1H, *J*=5.2, 11.6 Hz), 3.62 (dd, 1H, *J*=5.2, 11.6 Hz), 3.79 (ddd, 1H, *J*=5.2, 5.2, 7.6 Hz), 4.37 (m, 1H), 5.27 (br, 1H), 6.13 (dd, 1H, *J*=6.4, 8.4 Hz), 6.38 (s, 2H); ^{13}C NMR (DMSO-d_6, 100 MHz) δ 25.2, 25.8, 26.7, 27.0, 37.5, 38.6, 61.8, 70.9, 83.1, 87.4, 115.3, 147.7, 151.4, 152.8, 156.2; HRMS (FAB) calcd for $[C_{15}H_{24}N_6O_4+H]^+$ 353.1932, found 353.1946.

*4.1.4.5. 8-Hexylamino-2′-deoxyguanosine (**4d**).* Compound **4d** (15.2 mg, 0.0416 mmol, 91%) was prepared from **3d** (21.2 mg, 0.0459 mmol) in 28% aq NH_4OH (10 ml) by the method described for **2** as a colorless powder. ^{1}H NMR (DMSO-d_6, 400 MHz) δ 1.35–1.37 (complex, 4H), 1.55 (m, 2H), 1.69 (m, 2H), 2.06 (ddd, 1H, *J*=2.4, 6.4, 13.2 Hz), 2.68–2.82 (complex, 4H), 2.90 (ddd, 1H, *J*=6.4, 8.4, 13.2 Hz), 3.52 (dd, 1H, *J*=4.8, 11.6 Hz), 3.63 (dd, 1H, *J*=4.8, 11.6 Hz), 3.80 (ddd, 1H, *J*=2.4, 4.8, 4.8 Hz), 4.38 (ddd, 1H, *J*=2.4, 2.4, 6.4 Hz), 5.30 (br, 1H), 6.13 (dd, 1H, *J*=6.4, 8.4 Hz), 6.42 (s, 2H), 8.49 (br, 2H); ^{13}C NMR (DMSO-d_6, 100 MHz) δ 25.3, 26.3, 26.7, 27.0, 27.8, 37.4, 38.6, 61.7, 70.8, 83.1, 87.3, 115.2, 147.8, 151.3, 152.7, 156.2; HRMS (FAB) calcd for $[C_{16}H_{26}N_6O_4+H]^+$ 367.2122, found 367.2105.

4.1.5. General procedure for the protection of C2-amino group

*4.1.5.1. 8-(N-Trifluoroacetyl butynylamido)-N^2-(N′,N′-dimethylformamidine)-2′-deoxyguanosine (**12**).* To a solution of **1b** (201 mg, 0.467 mmol) in dry DMF (10 ml) was added *N*,*N*-dimethylformamide diethylacetal (1.0 ml), and the solution was stirred at 55 °C for 1.5 h. After evaporation of the solvent, the residue was purified by silica gel column chromatography ($CHCl_3$/MeOH=8:1) to afford **12** (166 mg, 0.342 mmol, 73%) as a colorless powder. ^{1}H NMR (CD_3OD, 400 MHz) δ 2.28 (ddd, 1H, *J*=3.0, 6.8, 13.4 Hz), 2.83 (t, 2H, *J*=6.7 Hz), 3.10 (s, 3H), 3.11–3.19 (complex, 4H), 3.57 (t, 2H, *J*=6.7 Hz), 3.72 (dd, 1H, *J*=4.1, 12.0 Hz), 3.82 (dd, 1H, *J*=3.4, 12.0 Hz), 3.99 (ddd, 1H, *J*=3.0, 3.4, 4.1 Hz), 4.65 (m, 1H), 6.47 (dd, 1H, *J*=6.8, 7.7 Hz), 8.52 (s, 1H); ^{13}C NMR (CD_3OD, 100 MHz) δ 20.3, 35.3, 39.1, 39.4, 41.5, 63.6, 71.7, 73.0, 86.9, 89.3, 95.1, 117.5 (q, *J*=285.0 Hz), 121.1, 133.7, 151.2, 159.2 (q, *J*=37.0 Hz), 159.5, 159.5, 159.9; ESI-MS *m*/*z* 486.1 $[M+H]^+$.

*4.1.5.2. 8-(N-Trifluoroacetyl propylamido)-N^2-(N′,N′-dimethylformamidine)-2′-deoxyguanosine (**15a**).* Compound **15a** was prepared from **3a** (229 mg, 0.545 mmol) and *N*,*N*-dimethylformamide diethylacetal (1.0 ml) in dry DMF (10 ml) by the method described for **12**. Purification by silica gel column chromatography ($CHCl_3$/MeOH=5:1) gave **15a** (229 mg, 0.482 mmol, 88%) as a colorless powder. ^{1}H NMR (CD_3OD, 400 MHz) δ 2.05 (tt, 2H, *J*=7.2 Hz), 2.28 (ddd, 1H, *J*=2.8, 6.4, 13.4 Hz), 2.92 (t, 2H, *J*=7.2 Hz), 3.08 (s, 3H), 3.14–3.26 (complex, 4H), 3.40 (t, 2H, *J*=7.2 Hz), 3.73 (dd, 1H, *J*=4.0, 12.0 Hz), 3.83 (dd, 1H, *J*=3.3, 12.0 Hz), 4.01 (ddd, 1H, *J*=3.3, 4.0, 6.6 Hz), 4.65 (m, 1H), 6.29 (dd, 1H, *J*=6.4, 8.0 Hz), 8.50 (s, 1H); ^{13}C NMR (CD_3OD, 100 MHz) δ 26.0, 27.6, 35.2, 39.5, 40.0, 41.4, 63.6, 73.0, 86.0, 89.1, 117.5 (q, *J*=285.0 Hz), 119.9, 151.4, 152.4, 158.4, 159.0 (q, *J*=36.0 Hz), 159.6, 159.7; ESI-MS *m*/*z* 476.1 $[M+H]^+$.

*4.1.5.3. 8-(N-Trifluoroacetyl butylamido)-N^2-(N′,N′-dimethylformamidine)-2′-deoxyguanosine (**15b**).* Compound **15b** was prepared from **3b** (229 mg, 0.527 mmol) and *N*,*N*-dimethylformamide diethylacetal (1.0 ml) in dry DMF (10 ml) by the method described for **12**. Purification by silica gel column chromatography ($CHCl_3$/MeOH=10:1) gave **15b** (235 mg, 0.480 mmol, 91%) as a colorless powder. ^{1}H NMR (CD_3OD, 400 MHz) δ 1.66 (m, 2H), 1.79 (m, 2H), 2.24 (ddd, 1H, *J*=2.7, 6.4, 13.2 Hz), 2.90 (t, 2H, *J*=7.2 Hz), 3.09 (s, 3H), 3.13–3.23 (complex, 4H), 3.32 (m, 2H), 3.71 (dd, 1H, *J*=3.3, 12.0 Hz), 3.81 (dd, 1H, *J*=3.8, 12.0 Hz), 3.99 (ddd, 1H, *J*=2.7, 3.3, 3.8 Hz), 4.64 (ddd, 1H, *J*=2.7, 2.7, 5.9 Hz), 6.29 (dd, 1H, *J*=6.4, 8.4 Hz), 8.49 (s, 1H); ^{13}C NMR (CD_3OD, 100 MHz) δ 26.0, 28.2, 29.2, 35.2, 39.5, 40.3,

41.4, 63.6, 73.1, 86.1, 89.2, 117.6 (q, J=284.0 Hz), 119.9, 152.1, 152.4, 158.5, 159.0 (q, J=36.0 Hz), 159.7, 159.7; ESI-MS m/z 490.2 $[M+H]^+$.

*4.1.5.4. 8-(N-Trifluoroacetyl pentylamido)-N^2-(N′,N′-dimethylformamidine)-2′-deoxyguanosine (**15c**).* Compound **15c** was prepared from **3c** (57.3 mg, 0.128 mmol) and *N*,*N*-dimethylformamide diethylacetal (1.0 ml) in dry DMF (3.0 ml) by the method described for **12**. Purification by silica gel column chromatography ($CHCl_3$/MeOH=10:1) gave **15c** (46.0 mg, 0.0914 mmol, 71%) as a colorless powder. ^{1}H NMR (CD_3OD, 400 MHz) δ 1.42 (m, 2H), 1.63 (tt, 2H, J=7.6 Hz), 1.78 (tt, 2H, J=7.6 Hz), 2.24 (ddd, 1H, J=2.6, 6.4, 13.4 Hz), 2.86 (t, 2H, J=7.6 Hz), 3.09 (s, 3H), 3.17–3.23 (complex, 4H), 3.27 (t, 2H, J=7.6 Hz), 3.71 (dd, 1H, J=3.8, 12.0 Hz), 3.81 (dd, 1H, J=3.4, 12.0 Hz), 4.00 (ddd, 1H, J=2.6, 3.4, 3.8 Hz), 4.63 (ddd, 1H, J=2.6, 2.6, 5.8 Hz), 6.28 (dd, 1H, J=6.4, 8.4 Hz), 8.48 (s, 1H); ^{13}C NMR (CD_3OD, 100 MHz) δ 27.2, 28.6, 28.6, 29.4, 35.2, 39.5, 40.5, 41.4, 63.7, 73.2, 86.2, 89.2, 117.6 (q, J=285.0 Hz), 120.0, 152.3, 152.4, 158.5, 158.9 (q, J=37.0 Hz), 159.6, 159.7; ESI-MS m/z 504.2 $[M+H]^+$.

*4.1.5.5. 8-(N-Trifluoroacetyl hexylamido)-N^2-(N′,N′-dimethylformamidine)-2′-deoxyguanosine (**15d**).* Compound **15d** was prepared from **3d** (83.5 mg, 0.181 mmol) and *N*,*N*-dimethylformamide diethylacetal (1.0 ml) in dry DMF (5.0 ml) by the method described for **12**. Purification by silica gel column chromatography ($CHCl_3$/MeOH=10:1) gave **15d** (88.2 mg, 0.171 mmol, 94%) as a colorless powder. ^{1}H NMR (CD_3OD, 400 MHz) δ 1.36–1.45 (complex, 4H), 1.56 (tt, 2H, J=7.2, 7.2 Hz), 1.75 (tt, 2H, J=6.8, 6.8 Hz), 2.23 (ddd, 1H, J=2.8, 6.4, 13.6 Hz), 2.86 (t, 2H, J=7.2 Hz), 3.09 (s, 3H), 3.16–3.24 (complex, 4H), 3.26 (t, 2H, J=6.8 Hz), 3.71 (dd, 1H, J=3.6, 12.0 Hz), 3.81 (dd, 1H, J=3.6, 12.0 Hz), 4.00 (ddd, 2H, J=3.6, 3.6, 3.6 Hz), 4.63 (ddd, 1H, J=2.8, 3.6, 6.4 Hz), 6.27 (dd, 1H, J=6.4, 8.4 Hz), 8.48 (s, 1H); ^{13}C NMR (CD_3OD, 100 MHz) δ 27.4, 28.6, 29.0, 29.6, 29.7, 35.2, 39.5, 40.7, 41.4, 63.7, 73.2, 86.2, 89.2, 119.0 (q, J=284.6 Hz), 120.0, 152.3, 152.5, 158.5, 158.9 (q, J=37.0 Hz), 159.6, 159.7; ESI-MS m/z 518.3 $[M+H]^+$.

4.1.6. General procedure for 5′-O-tritylation

*4.1.6.1. 8-(N-Trifluoroacetyl butynylamido)-N^2-(N′,N′-dimethylformamidine)-5′-O-(dimethoxytrityl)-2′-deoxyguanosine (**13**).* To a solution of **12** (138 mg, 0.284 mmol) and *N*,*N*-dimethylaminopyridine (catalytic amount) in anhydrous pyridine (3.0 ml) was added 4,4′-dimethoxytrityl chloride (115 mg, 0.341 mmol) and stirred at room temperature for 17 h. After completion of the reaction, methanol (1.0 ml) was added to the reaction mixture. The solvent was then evaporated and the crude mixture was purified by silica gel column chromatography ($CHCl_3$/MeOH/Et_3N=40:2:1) to yield **13** (66.9 mg, 0.0850 mmol, 30%) as a colorless powder. ^{1}H NMR (CD_3OD, 400 MHz) δ 2.36 (ddd, 1H, J=5.9, 8.0, 13.8 Hz), 2.76 (t, 2H, J=6.6 Hz), 3.01 (s, 3H), 3.06–3.14 (complex, 4H), 3.20 (dd, 1H, J=3.2, 10.1 Hz), 3.27 (m, 1H), 3.54 (t, 2H, J=6.6 Hz), 3.73 (s, 3H), 3.74 (s, 3H), 4.06 (m, 1H), 4.78 (m, 1H), 6.48 (dd, 1H, J=4.8, 8.0 Hz), 6.65–6.76 (complex, 4H), 7.10–7.29 (complex, 7H), 7.31–7.36 (complex, 2H), 8.36 (s, 1H); ^{13}C NMR (CD_3OD, 100 MHz) δ 20.3, 35.4, 38.2, 39.2, 41.6, 55.7 (×2), 65.4, 72.2, 72.8, 86.2, 87.3, 87.7, 95.0, 113.8 (×4), 117.5 (q, J=285.0 Hz), 121.0, 127.7, 128.6 (×2), 129.3 (×2), 131.1 (×2), 131.3 (×2), 134.1, 137.2, 137.3, 146.5, 151.1, 158.9, 159.2 (q, J=37.0 Hz), 159.5, 159.6, 159.9, 160.0; ESI-MS m/z 788.3 $[M+H]^+$.

*4.1.6.2. 8-(N-Trifluoroacetyl propylamido)-N^2-(N′,N′-dimethylformamidine)-5′-O-(dimethoxytrityl)-2′-deoxyguanosine (**16a**).* Compound **16a** was prepared from **15a** (215 mg, 0.453 mmol), *N*,*N*-dimethylaminopyridine (catalytic amount), and 4,4′-dimethoxytrityl chloride (184 mg, 0.543 mmol) in anhydrous pyridine (5.0 ml) by the method described for **13**. Purification by silica gel column chromatography ($CHCl_3$/MeOH/Et_3N=40:2:1) gave **16a** (146 mg, 0.188 mmol, 42%) as a colorless powder. ^{1}H NMR (CD_3OD, 400 MHz) δ 2.07 (m, 2H), 2.34 (m, 1H), 2.88–3.08 (complex, 9H), 3.22–3.44 (complex, 4H), 3.73 (s, 3H), 3.75 (s, 3H), 4.05 (m, 1H), 4.81 (m, 1H), 6.29 (dd, 1H, J=5.1, 7.8 Hz), 6.63–6.76 (complex, 4H), 7.08–7.35 (complex, 9H), 8.37 (s, 1H); ^{13}C NMR (CD_3OD, 100 MHz) δ 26.1, 27.5, 35.3, 38.8, 40.1, 41.5, 55.7 (×2), 65.1, 72.6, 85.2, 87.2, 87.3, 113.9 (×4), 117.5 (q, J=285.0 Hz), 119.8, 127.3, 128.6 (×2), 129.2 (×2), 131.0 (×2), 131.2 (×2), 137.1, 137.3, 146.4, 151.9, 152.4, 157.8, 158.9 (q, J=37.3 Hz), 159.2, 159.8, 159.9, 160.0; ESI-MS m/z 778.4 $[M+H]^+$.

*4.1.6.3. 8-(N-Trifluoroacetyl butylamido)-N^2-(N′,N′-dimethylformamidine)-5′-O-(dimethoxytrityl)-2′-deoxyguanosine (**16b**).* Compound **16b** was prepared from **15b** (111 mg, 0.226 mmol), *N*,*N*-dimethylaminopyridine (catalytic amount), and 4,4′-dimethoxytrityl chloride (92.0 mg, 0.272 mmol) in anhydrous pyridine (3.0 ml) by the method described for **13**. Purification by silica gel column chromatography ($CHCl_3$/MeOH/Et_3N=40:2:1) gave **16b** (82.2 mg, 0.104 mmol, 46%) as a colorless powder. ^{1}H NMR (CD_3OD, 400 MHz) δ 1.69 (m, 2H), 1.88 (m, 2H), 2.38 (ddd, 1H, J=2.4, 7.6, 13.6 Hz), 2.98 (t, 2H, J=7.2 Hz), 3.05 (s, 3H), 3.11 (s, 3H), 3.25–3.45 (complex, 5H), 3.78 (s, 3H), 3.79 (s, 3H), 4.08 (m, 1H), 4.84 (m, 1H), 6.35 (dd, 1H, J=5.2, 7.6 Hz), 6.70–6.81 (complex, 4H), 7.15–7.25 (complex, 7H), 7.34–7.39 (complex, 2H), 8.40 (s, 1H); ^{13}C NMR (CD_3OD, 100 MHz) δ 25.8, 28.3, 29.2, 35.3, 38.9, 40.3, 41.5, 55.7 (×2), 65.2, 72.7, 85.2, 87.2, 87.4, 113.9 (×4), 117.6 (q, J=285.0 Hz), 119.8, 127.7, 128.6 (×2), 129.3 (×2), 131.1 (×2), 131.2 (×2), 137.2, 137.3, 146.4, 152.3, 152.6, 157.7, 159.0 (q, J=37.0 Hz), 159.2, 159.8, 159.9, 160.0; ESI-MS m/z 792.3 $[M+H]^+$.

*4.1.6.4. 8-(N-Trifluoroacetyl pentylamido)-N^2-(N′,N′-dimethylformamidine)-5′-O-(dimethoxytrityl)-2′-deoxyguanosine (**16c**).* Compound **16c** was prepared from **15c** (46.0 mg, 0.0914 mmol), *N*,*N*-dimethylaminopyridine (catalytic amount), and 4,4′-dimethoxytrityl chloride (36.2 mg, 0.107 mmol) in anhydrous pyridine (3.0 ml) by the method described for **13**. Purification by silica gel column chromatography ($CHCl_3$/MeOH/

Et_3N=40:2:1) gave **16c** (48.5 mg, 0.0602 mmol, 66%) as a colorless powder. ^{1}H NMR (CD_3OD, 400 MHz) δ 1.40 (m, 2H), 1.58 (m, 2H), 1.84 (m, 2H), 2.34 (m, 1H), 2.90 (t, 2H, J=7.5 Hz), 3.00 (s, 3H), 3.06 (s, 3H), 3.17–3.41 (complex, 5H), 3.72–3.73 (complex, 6H), 4.04 (m, 1H), 4.78 (m, 1H), 6.31 (dd, 1H, J=5.4, 7.6 Hz), 6.64–6.73 (complex, 4H), 7.13–7.32 (complex, 9H), 8.34 (s, 1H); ^{13}C NMR (CD_3OD, 100 MHz) δ 27.3, 28.5, 28.7, 29.5, 35.3, 38.9, 40.6, 41.5, 55.7 (2), 65.3, 72.8, 85.2, 87.2, 87.4, 113.9 (4), 117.6 (q, J=285.0 Hz), 119.8, 127.7, 128.6 (2), 129.3 (2), 131.1 (2), 131.3 (2), 137.2, 137.3, 146.4, 152.3, 153.0, 157.7, 158.9 (q, J=36.0 Hz), 159.2, 159.8, 160.0, 160.0; ESI-MS m/z 806.4 $[M+H]^+$.

*4.1.6.5. 8-(N-Trifluoroacetyl hexylamido)-N^2-(N′,N′-dimethylformamidine)-5′-O-(dimethoxytrityl)-2′-deoxyguanosine (**16d**).* Compound **16d** was prepared from **15d** (78.0 mg, 0.151 mmol), *N*,*N*-dimethylaminopyridine (catalytic amount), and 4,4′-dimethoxytrityl chloride (62.3 mg, 0.184 mmol) in anhydrous pyridine (3.0 ml) by the method described for **13**. Purification by silica gel column chromatography ($CHCl_3$/MeOH/Et_3N=40:2:1) gave **16d** (83.5 mg, 0.102 mmol, 68%) as a colorless powder. ^{1}H NMR (CD_3OD, 400 MHz) δ 1.18–1.28 (complex, 4H), 1.37 (tt, 2H, J=7.2, 7.2 Hz), 1.67 (m, 2H), 2.18 (ddd, 1H, J=2.8, 6.4, 13.6 Hz), 2.75 (m, 2H), 2.86 (s, 3H), 2.92 (s, 3H), 3.04–3.24 (complex, 5H), 3.58 (s, 3H), 3.59 (s, 3H), 3.89 (dd, 1H, J=3.2, 5.2 Hz), 4.62 (dd, 1H, J=2.0, 5.2 Hz), 6.16 (dd, 1H, J=5.2, 7.6 Hz), 6.52–6.58 (complex, 4H), 6.98–7.05 (complex, 7H), 7.16 (complex, 2H,), 8.33 (s, 1H); ^{13}C NMR (CD_3OD, 100 MHz) δ 27.5, 28.8, 28.8, 29.7, 29.8, 35.3, 38.9, 40.7, 41.5, 55.7 (×2), 65.3, 72.8, 85.2, 87.2, 87.4, 113.9 (×4), 117.6 (q, J=285.0 Hz), 119.8, 127.7, 128.6 (×2), 129.3 (×2), 131.1 (×2), 131.3 (×2), 137.2, 137.3, 146.4, 152.3, 153.1, 157.7, 158.9 (q, J=37.0 Hz), 159.17, 159.8, 159.9, 160.0; ESI-MS m/z 820.4 $[M+H]^+$.

4.1.7. General procedure for 3′-O-cyanoethylphosphoramidite reaction

*4.1.7.1. 8-(N-Trifluoroacetyl butynylamido)-N^2-(N′,N′-dimethylformamidine)-5′-O-(dimethoxytrityl)-3′-O-cyanoethyl phosphoramidate-2′-deoxyguanosine (**14**).* To a solution of **13** (66.9 mg, 0.0849 mmol) and 1*H*-tetrazol (0.45 M in CH_3CN, 282 μl) in anhydrous CH_2Cl_2 (670 μl) was added 2-cyanoethyl tetraisopropylphosphoro diamidate (50 μl) under argon atmosphere. The resulting mixture was stirred at room temperature for 1 h and was then evaporated to dryness. The residue was dissolved in $CHCl_3$ and was washed with aq $NaHCO_3$ and brine. The organic layer was separated and used for oligodeoxynucleotide synthesis without further purification.

*4.1.7.2. 8-(N-Trifluoroacetyl propylamido)-N^2-(N′,N′-dimethylformamidine)-5′-O-(dimethoxytrityl)-3′-O-cyanoethyl phosphoramidate-2′-deoxyguanosine (**17a**).* Compound **17a** was prepared from **16a** (52.0 mg, 0.0669 mmol), 1*H*-tetrazol (0.45 M in CH_3CN, 226 μl), and 2-cyanoethyl tetraisopropylphosphoro diamidate (32 μl) by the method described for **14**.

*4.1.7.3. 8-(N-Trifluoroacetyl butylamido)-N^2-(N′,N′-dimethylformamidine)-5′-O-(dimethoxytrityl)-3′-O-cyanoethyl phosphoramidate-2′-deoxyguanosine (**17b**).* Compound **17b** was prepared from **16b** (73.4 mg, 0.928 mmol), 1*H*-tetrazol (0.45 M in CH_3CN, 310 μl), and 2-cyanoethyl tetraisopropylphosphoro diamidate (50 μl) by the method described for **14**.

*4.1.7.4. 8-(N-Trifluoroacetyl pentylamido)-N^2-(N′,N′-dimethylformamidine)-5′-O-(dimethoxytrityl)-3′-O-cyanoethyl phosphoramidate-2′-deoxyguanosine (**17c**).* Compound **17c** was prepared from **16c** (56.2 mg, 0.0698 mmol), 1*H*-tetrazol (0.45 M in CH_3CN, 310 μl), and 2-cyanoethyl tetraisopropylphosphoro diamidate (50 μl) by the method described for **14**.

*4.1.7.5. 8-(N-Trifluoroacetyl hexylamido)-N^2-(N′,N′-dimethylformamidine)-5′-O-(dimethoxytrityl)-3′-O-cyanoethyl phosphoramidate-2′-deoxyguanosine (**17d**).* Compound **17d** was prepared from **16d** (82.1 mg, 0.100 mmol), 1*H*-tetrazol (0.45 M in CH_3CN, 500 μl), and 2-cyanoethyl tetraisopropylphosphoro diamidate (50 μl) by the method described for **14**.

4.1.8. Oligonucleotide synthesis and characterization

All the reagents for DNA synthesis were purchased from Glen Research. ODNs were synthesized by a conventional phosphoramidite method by using an Applied Biosystems 392 DNA/RNA synthesizer. ODNs were purified by reverse phase HPLC on a 5-ODS-H column (10×150 mm, elution with 50 mM ammonium formate buffer (AF), pH 7.0, linear gradient over 45 min from 3 to 20% acetonitrile at a flow rate 2.0 ml/min). ODNs containing modified nucleotides were fully digested with calf intestine alkaline phosphatase (50 U/ml), snake venom phosphodiesterase (0.15 U/ml), and P1 nuclease (50 U/ml) at 37 °C for 3 h. Digested solutions were analyzed by HPLC on a COSMOSIL (4.6×150 mm), elution with a solvent mixture of 50 mM ammonium formate buffer (AF), pH 7.0, flow rate 1.0 ml/min. The concentration of each ODNs was determined by comparing peak areas with a standard solution containing dA, dC, dG, and dT at a concentration of 0.1 mM. Mass spectra of ODNs purified by HPLC were determined with a MALDI-TOF mass spectrometer.

4.1.9. MALDI-TOF mass spectral data for the ODNs

Table 3
MALDI-TOF mass spectral data for the ODNs

ODNs	MALDI-TOF mass calcd $[M+H]^+$	MALDI-TOF mass found $[M+H]^+$
ODN**1**	3981.72	3981.94
ODN**2**	3971.72	3971.78
ODN**3**	3985.75	3985.96
ODN**4**	3999.78	4000.49
ODN**5**	4013.80	4012.93
ODN**1a**	4011.74	4010.39
ODN**2a**	4001.75	4001.70
ODN**3a**	4015.77	4015.56
ODN**4a**	4029.80	4031.40
ODN**5a**	4043.83	4042.13
ODN**6**	1880.39	1880.95

4.1.10. Melting temperature (T_m) measurements

All T_ms of the ODNs (2.5 μM, final duplex concentration) were taken in 50 mM sodium phosphate buffers (pH 7.0) containing 100 mM sodium chloride. Absorbance versus temperature profiles were measured at 260 nm using a Shimadzu UV-2550 spectrophotometer equipped with a Peltier temperature controller using 1 cm path length cell. The absorbance of the samples was monitored at 260 nm from 4 to 90 °C with a heating rate of 1 °C/min. From these profiles, first derivatives were calculated to determine T_m values.

4.1.11. Circular dichroism (CD) measurements

CD spectra were recorded with a JASCO J-805 CD spectrophotometer. CD spectra of oligonucleotide solutions (0.15 mM base concentration in 5 mM sodium cacodylate buffer, pH 7.0, at 10 °C at various NaCl concentrations) were measured using 1 cm path length cell (Fig. 2).

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Supplementary data

Copies of ^{1}H, ^{13}C NMR, and NOESY spectra of selected compounds. This material is available free of charge via the internet at http://elsevier.com. Supplementary data associated with this article can be found in the online version, at doi:10.1016/j.tet.2008.01.091.

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