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Full Paper

Radical Cascade Protocol for the Synthesis of (5′*S*)- and (5′*R*)-5′,8-Cyclo-2′-deoxyguanosine Derivatives*

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The reaction of the appropriately substituted 8-bromo-2′-deoxyguanosine with Bu_3SnH/2,2′-azobisisobutyronitrile (AIBN) can be favourably tuned to give the analogous 5′,8-cyclo-2′-deoxyguanosine derivatives in good yields, thus providing easy access to modified nucleosides that constitute an important DNA lesion. A large excess of AIBN is necessary. The creation of the new C5′–C8 bond is a non-chain radical cascade protocol.

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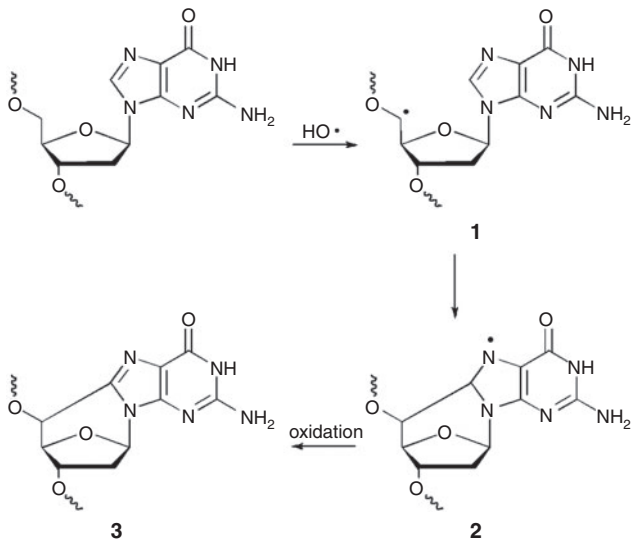
Introduction

Chemical studies play a central role in the identification and measurement of the main classes of DNA oxidative damage.[1,2] Purine 5′,8-cyclonucleosides are an important class of ionizing radiation-induced tandem DNA lesions.[3] Their potential to become free-radical stress markers is a matter of fundamental research.[4] Scheme 1 shows the case of the guanine moiety. The attack at H5′ of DNA by HO• radicals is estimated to be 55 % of the H-atom abstraction occurs at the sugar moieties.[5] After C5′ radical (**1**) formation, intramolecular addition to the C8–N7 double bond of the purine base and subsequent oxidation of the intermediate create the lesion. Therefore, as well as the usual glycosidic bond, another covalent bond between the C5′ sugar and the C8 purine carbon atoms is present. Recently, the rate constant for the cyclization step **1** → **2** at the nucleoside level was measured to be $6.1 \times 10^5\ s^{-1}$.[6] Lesion **3** exists in two diastereomeric forms, i.e. (5′*R*)- and (5′*S*)-cdGuo (5′,8-cyclo-2′-deoxyguanosine).

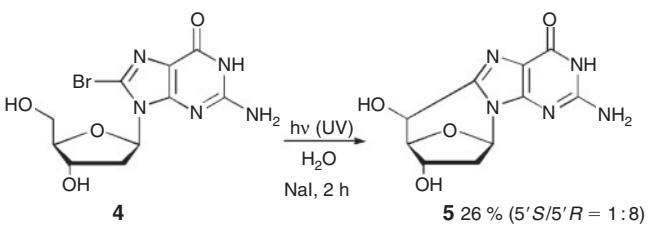


Scheme 1. Mode of formation of 5′,8-cyclo-2′-deoxyguanosine (cdGuo) in DNA.

Synthetic access to both (5′*R*)-cdGuo and (5′*S*)-cdGuo lesions and their phosphoramidites for incorporation in oligonucleotides models is still a challenge and limits the full development of biochemical, biological, and biophysical studies.[3] Unprotected (5′*R*)-cdGuo and (5′*S*)-cdGuo have been synthesized by photolysis of 8-bromo-2′-deoxyguanosine in aqueous solutions in 26 % yield and an 5′*S* : 5′*R* ratio of 1 : 8 (Scheme 2).[7] Apart from the problem of low yield, several attempts to selectively protect the two secondary OH groups in both diastereomeric forms also failed. Here, we report an *ex novo* synthetic strategy based on a radical cascade reaction that provides easy access to these building blocks for DNA modification.

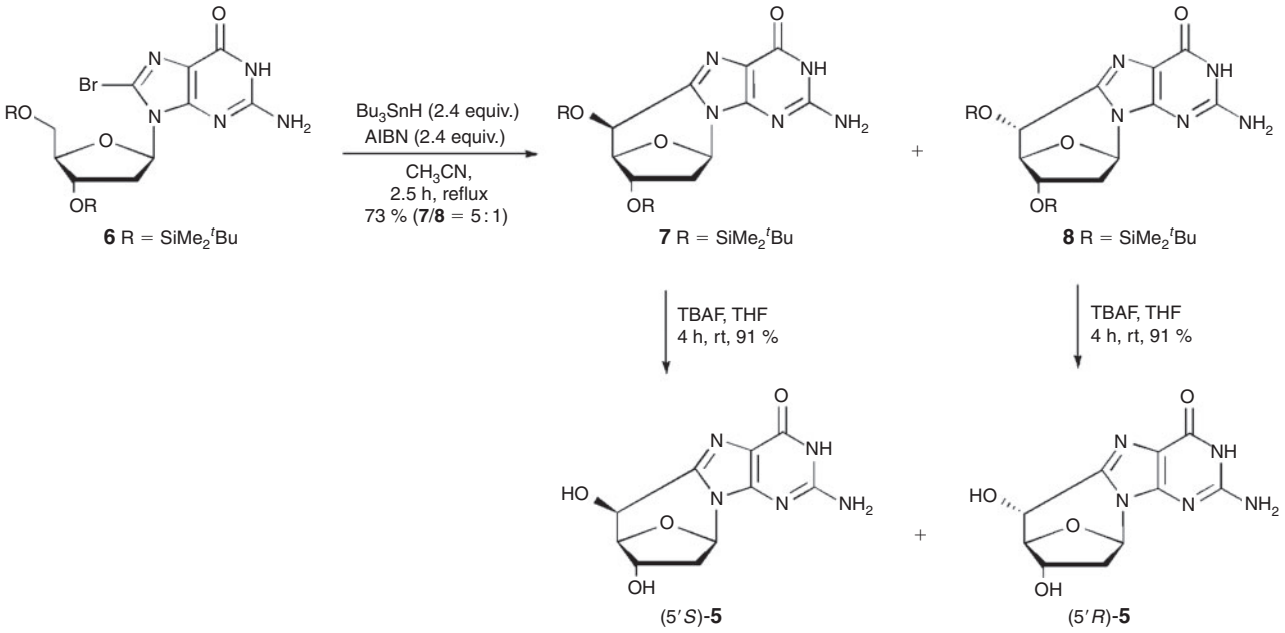
Results and Discussion

Our approach was based on previous experience in our laboratory with the reactivity of 8-bromopurine derivatives.[7,8] An initial choice was the cyclization reaction in organic medium of compound **6**, obtained from 8-bromo-2′-deoxyguanosine and ^{t}BuMe$_2$SiCl following a standard procedure in 98 % yield.[8]



Scheme 2.

*This work is dedicated to the memory of Athel Beckwith, an inspiring teacher and a friend.



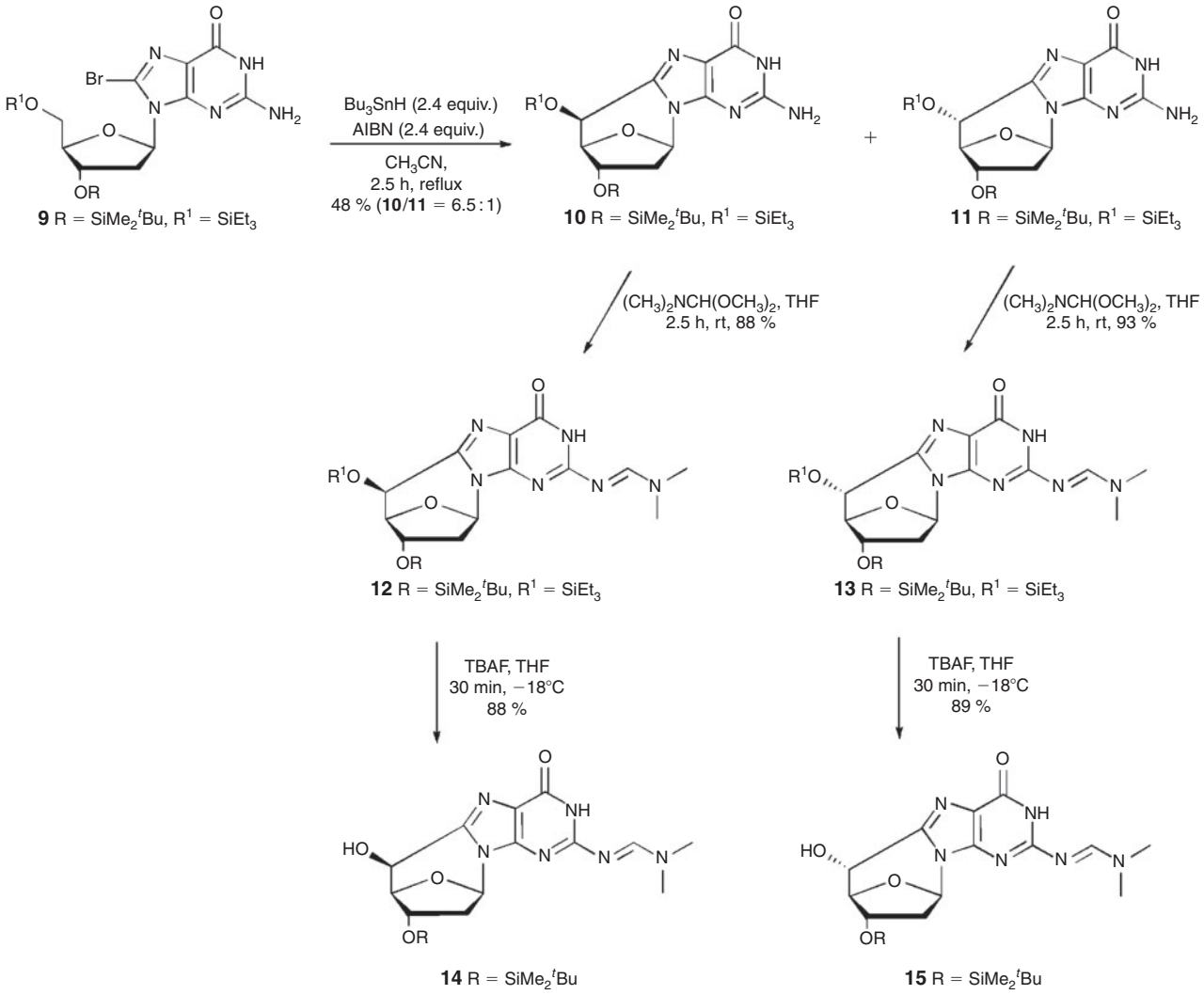
Scheme 3.

The cyclization reaction was initially tested using typical radical chain conditions, i.e. a deoxygenated solution of **6** with Bu_3SnH (2.4 equiv.) and 2,2′-azobisisobutyronitrile (AIBN, 0.2 equiv.) in refluxing CH_3CN. After 2 h, low conversion of the starting bromide was observed. By increasing the amount of AIBN, the reaction outcome improved.[9] When the bis-protected derivative **6** was treated with stoichiometric amounts of Bu_3SnH (2.4 equiv.) and AIBN (2.4 equiv.) in CH_3CN under reflux for 2.5 h, the protected cyclopurine diastereomers **7** and **8** were obtained in 73 % yield and in a ratio 5′*S*/5′*R* of 5 : 1 (Scheme 3), in addition to 25 % yield of debrominated product. When replacing Bu_3SnH with $(TMS)_3SiH$, the cyclonucleosides were obtained in much lower yields (**7** + **8**, 15 %).[10] Compounds **7** and **8** were separated by reverse-phase chromatography and fully characterized. Bis-deprotection of the two secondary silyloxy groups afforded the compounds (5′*S*)-**5** and (5′*R*)-**5** respectively in high yields (Scheme 3).[7] In order to use the cyclopurines as building blocks for the synthesis of oligonucleotides, thus obtaining the insertion of the lesion in a DNA sequence, selective mono-deprotection of the 5′ secondary sililoxy group in **7** and **8** is necessary. Several attempts at this reaction failed.

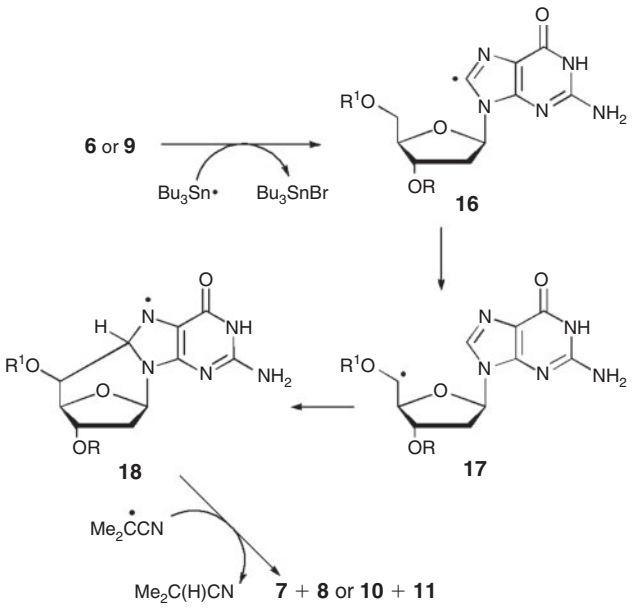
Therefore, we changed strategy, introducing two different protections for the OH groups before the cyclization step. For example, compound **9** was prepared in two steps from **6** by primary 5′OH deprotection followed by a new 5′-protection, in 92 and 91 % yield respectively. Derivative **9** under the cyclization conditions described above afforded the protected cdGuo diastereomers **10** and **11** in 48 % yield and in a 5′*S*/5′*R* ratio of ~6.5 : 1, together with a 50 % yield of debrominated product. Column chromatography using reverse phase silica gel afforded **10** and **11** as pure compounds. The selective deprotection of the Et_3SiO moiety was achieved for each diastereomer after coupling the amino group with DMF (Scheme 4).

It is worth recalling that in the presence of small amounts of AIBN (0.1–0.2 equiv. with respect to Bu_3SnH), in a manner typical of radical chain reactions, the reaction does not proceed. Stoichiometric amounts of AIBN and Bu_3SnH are necessary, which means nearly twice the amount of $Me_2C(^{\bullet})CN$ radicals with respect to hydride concentration, in order to quantitatively convert the starting material into the products. Based on previous findings,[9,11] these results suggest the reaction mechanism drawn in Scheme 5 for the cyclization step. The $Bu_3Sn^{\bullet}$ radical, initially generated from the reaction of the $Me_2C(^{\bullet})CN$ radical with tin hydride, abstracts a bromine atom from **6** or **9** to give an aryl-type radical **16**. Radical **16** abstracts a hydrogen atom either intramolecularly from the 5′ position to generate the C5′ radical **17** or from Bu_3SnH to give the reduction product. Cyclization of **17** is expected to produce radical **18** (a mixture of 5′*S* and 5′*R* isomers). In the end, the oxidized products **7** + **8** or **10** + **11** derive from the reaction of radical **18** with $Me_2C(^{\bullet})CN$ in accordance with the previous findings on Bu_3SnH-mediated intramolecular-homolytic substitution.[9,11] In other words, the reaction mechanism in Scheme 5 is an unusual non-chain radical cascade protocol involving 1,6-H shift and 6-*exo*-trig cyclization.[12] The reason for the low yield with $(TMS)_3SiH$ as mediator is unknown; the interference of $(TMS)_3SiBr$ as by-product with the exocyclic amino group of the guanine moiety could be play a role.

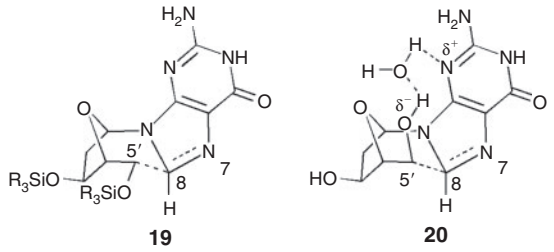
The radical cyclization **17** → **18** reported in Scheme 5 is worthy of an additional comment. The rate constant for the cyclization has been measured to be $6.1 \times 10^5\ s^{-1}$ at 20°C by time-resolved spectroscopy for the unprotected radical[6] and $\sim 1 \times 10^6\ s^{-1}$ at 30°C by competitive kinetics for the ^{t}BuMe$_2$Si-diprotected radical.[13] Scheme 6 shows the chair transition states in the *pro*-(5′*S*) and *pro*-(5′*R*) conformers of the cyclization. The cyclization of the *pro*-(5′*S*) conformer of unprotected 2′-deoxyguanosin-5′-yl radicals was also studied theoretically using the DFT B3LYP formalism with the 6-311++G(d,p) basis set and including Conductor Polarizable Continuum Model bulk solvation effects.[14] It was found that the solvation effects have a little influence on reaction and activation energies. We found that the solvent and nature of the substituent have a profound effect on the stereoselectivity of the C5′ radical cyclization. Indeed, the 5′*S*/5′*R* isomer ratio varies from 1 : 8 for the unprotected radical in water at room temperature (Scheme 2) to 5 : 1 or 6.5 : 1 for the bulky 5′-OSiR$_3$ substituent in CH_3CN at 80°C (see Schemes 3 and 4). We suggest that a bulky silyloxy



Scheme 4.



Scheme 5. Reaction mechanism for the radical cascade protocol.



Scheme 6. Chair transition states in the *pro*-(5′*S*) and *pro*-(5′*R*) conformers of the cyclization.

substituent in the equatorial position favours the conformer **19** in the transition state, thus affording a diastereomeric ratio favouring the 5′*S* isomer, whereas in aqueous solution, only the *pro*-(5′*R*) conformer can be stabilized by hydrogen bonding, involving either the N3 of the base (such as in **20**) or the oxygen of the sugar ring.

Conclusions

We have described a short and efficient synthetic sequence for building blocks **14** and **15**. The key step is a non-chain radical

cascade involving bromine atom abstraction and formation of a C8 radical, radical translocation to the C5′ position, and radical cyclization to the aromatic ring, with a final oxidation step. Incorporation of building blocks **14** and **15** into oligonucleotides can be obtained following previously published procedures.[15] Our efficient route to (5′*R*)- and (5′*S*)-5′,8-cyclo-2′-deoxyguanosine lesions represents an important facilitation for further biochemical, biological, and biophysical studies. Indeed, it was recently found that these tandem lesions accumulate with aging in a tissue-specific manner (liver > kidney > brain), providing evidence that DNA repair mechanisms are inadequate to preserve the genetic material from these lesions.[16,17] Results from competitive transcription and adduct bypass assays revealed that (5′*S*)-cdGuo strongly inhibits transcription in vitro and in mammalian cells and induces transcriptional mutagenesis both in vitro and in vivo.[18]

Experimental

8-Bromo-3′,5′-O-bis(tert-*butyldimethylsilyl)-2′-deoxyguanosine* ***6***

8-Bromo-2′-deoxyguanosine (3.115 g, 9 mmol), imidazole (1.84 g, 27 mmol), and 4-dimethylaminopyridine (269 mg, 2.2 mmol) were mixed in dry DMF (40 mL) and stirred until homogenization. Next, *tert*-butyldimethylsilyl chloride (4.07 g, 27 mmol) was added and the reaction mixture was stirred at room temperature under an Ar atmosphere. After 18 h, the reaction mixture was poured into ice water (100 mL) and the precipitate was filtered and dried under vacuum. After chromatography on silica gel (AcOEt/CH_2Cl_2 6 : 4 v/v), 5.06 g (98 %) of a light yellow foam was obtained. δ_H (400 MHz, $CDCl_3$) 11.87 (bs, 1H), 6.23 (t, *J* 6.9, 1H), 6.12 (bs, 2H), 4.78–4.69 (m, 1H), 3.92 (dd, *J* 9.2, 5.5, 1H), 3.85 (dd, *J* 10.7, 6.6, 1H), 3.71 (dd, *J* 10.9, 4.8, 1H), 3.45 (dt, *J* 13.0, 6.5, 1H), 2.16 (ddd, *J* 12.8, 6.8, 3.8, 1H), 0.93 (s, 9H), 0.87 (s, 9H), 0.14 (s, 6H), 0.03 (s, 3H), 0.01 (s, 3H). δ_C (101 MHz, $CDCl_3$) 157.8, 153.2, 152.5, 122.2, 118.1, 87.8, 86.0, 72.8, 62.6, 36.6, 25.8, 18.1, 6.7, 4.4, −4.8. *m/z* (ESI+) 576, 574 $[M+H]^+$. *m/z* (MS/MS) 230, 232.

*(5′*S*)-3′,5′-O-Bis*(tert-*butyldimethylsilyl)-5′,8-cyclo-2′-deoxyguanosine* ***7*** *and (5′*R*)-3′,5′-O-Bis*(tert-*butyldimethylsilyl)-5′,8-cyclo-2′-deoxyguanosine* ***8***

8-Bromo-3′,5′-*O*-bis(*tert*-butyldimethylsilyl)-2′-deoxyguanosine (2.87 g, 5 mmol) was added to a round-bottom flask (1 L) containing acetonitrile (500 mL) (previously flushed with Ar for 30 min) to give a 10 mM concentration. The mixture was heated to boiling point and stirred until the solid was fully dissolved. Next, AIBN (1.97 g, 12 mmol) and tributyltin hydride (3228 µL, 12 mmol) were added and the reaction mixture was stirred at reflux temperature under an Ar atmosphere. The reaction was monitored with HPLC-UV and after consumption of the starting material (2.5 h), the reaction mixture was cooled, the solvent was removed by rotary evaporation and the crude residue was chromatographed on silica gel (AcOEt/hexane 5 : 1) to give 1.799 g (73 %) of the (5′*S*)- and (5′*R*)-3′,5′-*O*-bis(*tert*-butyldimethylsilyl)-5′,8-cyclo-2′-deoxyguanosine in a 5 : 1 mixture (based on HPLC-UV analysis) as a white foam. A second fraction of 3′,5′-*O*-bis(*tert*-butyldimethylsilyl)-2′-deoxyguanosine (620 mg, 25 %) was isolated as a white solid. The (5′*S*)- and (5′*R*)-3′,5′-*O*-bis(*tert*-butyldimethylsilyl)-5′,8-cyclo-2′-deoxyguanosine mixture was dissolved in a small quantity of warm acetonitrile and subjected to column chromatography on silica C18 (CH_3CN/H_2O 7 : 3) to afford the two pure diastereomers. Flash chromatography using the automated system CombiFlash® also works well (see Supplementary Material).

7: δ_H (400 MHz, $CDCl_3$) 11.46 (bs, 1H), 6.17 (d, *J* 4.7, 1H), 6.04 (bs, 2H), 5.12 (d, *J* 6.1, 1H), 4.84 (dd, *J* 7.1, 4.2, 1H), 4.51 (d, *J* 6.1, 1H), 2.50 (dd, *J* 13.1, 7.3, 1H), 2.15 (dt, *J* 13.0, 4.6, 1H), 1.00 (s, 9H), 0.88 (s, 9H), 0.30 (s, 3H), 0.26 (s, 3H), 0.07 (s, 3H), 0.04 (s, 3H). δ_C (101 MHz, $CDCl_3$) 158.4, 153.5, 149.4, 144.7, 116.8, 87.1, 84.8, 69.6, 66.2, 46.5, 26.0, 25.7, 18.6, 17.8, −4.4, −4.4, −4.6, −4.8. *m/z* (ESI+) 494 $[M+H]^+$. *m/z* (MS/MS) 362, 294, 264, 230, 187. Anal. Calc. for $C_{22}H_{39}N_5O_4Si_2$: C 53.52, H 7.96, N 14.18. Found: C 53.50, H 7.95, N 14.22 %.

8: δ_H (400 MHz, $CDCl_3$) 11.72 (bs, 1H), 6.27 (d, *J* 4.5, 1H), 6.03 (bs, 2H), 4.68 (d, *J* 0.8, 1H), 4.51 (s, 1H), 4.25 – 4.13 (m, 1H), 2.38 (dd, *J* 13.1, 7.1, 1H), 2.21–2.09 (m, 1H), 0.93 (s, 9H), 0.89 (s, 9H), 0.19 (s, 3H), 0.12 (s, 3H), 0.07 (s, 3H), 0.05 (s, 3H). δ_C (101 MHz, $CDCl_3$) 159.0, 153.6, 149.2, 142.9, 116.8, 71.4, 67.0, 45.5, 25.9, 25.8, 18.4, 18.1, 1.1, −4.2, −4.7, −4.7, −4.9. *m/z* (ESI+) 494 $[M+H]^+$. *m/z* (MS/MS) 362, 294, 264, 230, 187. Anal. Calc. for $C_{22}H_{39}N_5O_4Si_2$: C 53.52, H 7.96, N 14.18. Found: C 53.46, H 7.95, N 14.20 %.

*(5′*S*)- or (5′*R*)-5′,8-Cyclo-2′-deoxyguanosine* ***5***

Compound **7** or **8** (49.4 mg, 0.1 mmol) was dissolved in THF (5 mL). A solution of TBAF in THF (1 M, 15 µL, 0.3 mmol) was then added, and the mixture was stirred at room temperature. After 2 h, the starting material was consumed and the solvent was removed. The resulting residue was chromatographed on C18 reverse-phase silica (CH_3CN/H_2O 2 : 8) to give 91 % yield of (5′*S*)-**5** or (5′*R*)-**5**. Spectroscopic and analytical data are identical with the previously described reference compounds.[7]

*8-Bromo-3′-O-*tert-*butyldimethylsilyl-2′-deoxyguanosine*

8-Bromo-3′,5′-*O*-bis(*tert*-butyldimethylsilyl)-2′-deoxyguanosine (5.96 g, 10 mmol) was taken up in THF (125 mL). The mixture was stirred until all material had dissolved and then water (62.5 mL) and acetic acid (62.5 mL) were added sequentially (final ratio of THF/H_2O/AcOH 2 : 1 : 1). The reaction mixture was stirred at room temperature and was monitored with HPLC-UV, reaching after 20 h a bis-protected/mono-protected/unprotected ratio of 29 : 62 : 7. Dichloromethane (150 mL) was added to the reaction mixture and subsequently the mixture was quenched carefully with saturated $NaHCO_3$ solution to pH 7.5. Next, the mixture was extracted with dichloromethane (2 × 200 mL), the organic layer was dried over Na_2SO_4 and the solvent was removed by rotary evaporation. The crude reaction product was purified by column chromatography on silica gel (AcOEt/CH_2Cl_2 6 : 4, changed to AcOEt/CH_2Cl_2/MeOH 2 : 1 : 1) and 2.76 g (60 %, or 92 % based on starting material recovered) of 8-bromo-3′-*O*-*tert*-butyldimethylsilyl-2′-deoxyguanosine was obtained as a light-brown foam. δ_H (400 MHz, $CDCl_3$) 12.00 (bs, 1H), 6.66 (bs, 2H), 6.28 (dd, *J* 9.5, 5.6, 1H), 4.64 (d, *J* 5.1, 1H), 4.11 (s, 1H), 3.97 (d, *J* 12.0, 1H), 3.71 (d, *J* 12.3, 1H), 3.00–2.81 (m, 1H), 2.14 (dd, *J* 12.8, 5.3, 1H), 1.88 (s, 1H), 0.93 (s, 9H), 0.12 (s, 3H), 0.12 (s, 3H). δ_C (101 MHz, $CDCl_3$) 157.7, 153.5, 151.1, 121.2, 118.3, 90.0, 88.2, 63.2, 25.8, 18.0, −4.7, −4.7. *m/z* (ESI+) 482, 484 $[M+Na]^+$. *m/z* (MS/MS) 230, 232.

*8-Bromo-3′-*O*-*tert*-butyldimethylsilyl-5′-*O*-triethylsilyl-2′-deoxyguanosine* ***9***

8-Bromo-3′-*O*-*tert*-butyldimethylsilyl-2′-deoxyguanosine (2.3 g, 5 mmol) and imidazole (1.02 g, 15 mmol) were taken up in dry dichloromethane (30 mL) and stirred until homogenization. Next, triethylsilyl chloride (2.517 mL, 15 mmol) was added dropwise and the reaction mixture was stirred at room temperature under an Ar atmosphere. After 45 min, TLC showed the consumption of the starting material, so the mixture was quenched with saturated $NaHCO_3$ solution, extracted with dichloromethane and washed with 5 % $NaHCO_3$ solution (2×50 mL). The organic layer was dried over Na_2SO_4 and the solvent was removed by rotary evaporation. The crude reaction mixture was chromatographed on silica gel (AcOEt/CH_2Cl_2/MeOH/TEA 5 : 3 : 1 : 0.05) to give 2.248 g (91 %) of 8-bromo-3′-*O*-*tert*-butyldimethylsilyl-5′-*O*-triethylsilyl-2′-deoxyguanosine as a light-brown foam. δ_H (400 MHz, $CDCl_3$) 11.85 (bs, 1H), 6.48 (bs, 2H), 6.21 (t, *J* 7.0, 1H), 4.77–4.65 (m, 1H), 3.93 (ddd, *J* 6.8, 5.3, 3.2, 1H), 3.83 (dd, *J* 10.7, 6.9, 1H), 3.69 (dd, *J* 10.7, 5.2, 1H), 3.48 (dt, *J* 13.1, 6.5, 1H), 2.16 (ddd, *J* 13.0, 6.7, 3.5, 1H), 1.00–0.83 (m, 18H), 0.56 (q, *J* 7.9, 6H), 0.13 (s, 6H). δ_C (101 MHz, $CDCl_3$) 157.8, 153.2, 152.5, 122.2, 118.1, 87.8, 86, 72.8, 62.6, 36.6, 25.8, 18.1, 6.7, 4.3, −4.6, −4.7. *m/z* (ESI+) 576, 574 $[M + H]^+$. *m/z* (MS/MS) 230, 232.

*(5′*S*)-3′-*O*-tert-Butyldimethylsilyl-5′-*O*-triethylsilyl-5′,8-cyclo-2′-deoxyguanosine* ***10*** *and (5′*R*)-3′-*O*-tert-Butyldimethylsilyl-5′-*O*-triethylsilyl-5′,8-cyclo-2′-deoxyguanosine* ***11***

Starting from **9**, 2.87 g (5 mmol) and applying the same conditions described for the synthesis of (5′*R*)- and (5′*S*)-3′,5′-*O*-bis(*tert*-butyldimethylsilyl)5′,8-cyclo-2′-deoxyguanosine, the synthesis of (5′*S*)- and (5′*R*)-3′-*O*-*tert*-butyldimethylsilyl-5′-*O*-triethylsilyl-5′,8-cyclo-2′-deoxyguanosine was achieved. After purification by silica gel chromatography (AcOEt/hexanes 5 : 1), 1.11 g (45 %) of a diastereomeric mixture of (5′*S*) and (5′*R*) in a 6.5 : 1 ratio (based on HPLC-UV analysis) was obtained as a white foam along with 1.24 g (50 %) reduced product. The diastereomeric mixture was separated by C18 reverse-phase silica gel chromatography (CH_3CN/H_2O 7 : 3).

10: δ_H (400 MHz, $CDCl_3$) 11.38 (bs, 1H), 6.17 (d, *J* 4.6, 3H), 5.09 (d, *J* 6.0, 1H), 4.83 (dd, *J* 7.0, 4.5, 1H), 4.48 (d, *J* 6.1, 1H), 2.47 (dd, *J* 13.1, 7.3, 1H), 2.12 (dt, *J* 12.9, 4.3, 1H), 1.03 (t, *J* 7.9, 9H), 0.87 (s, 9H), 0.78 (ddd, *J* 12.9, 7.9, 4.5, 6H), 0.06 (s, 3H), 0.03 (s, 3H). δ_C (101 MHz, $CDCl_3$) 158.3, 153.7, 149.3, 144.4, 116.7, 86.9, 84.7, 69.6, 65.8, 46.2, 25.7, 17.8, 6.8, 4.8, −4.7, −4.9. *m/z* (ESI+) 494 $[M + H]^+$. *m/z* (MS/MS) 362, 294, 264, 230, 230.

11: δ_H (400 MHz, $CDCl_3$) 11.65 (bs, 1H), 6.28 (d, *J* 4.5, 1H), 6.05 (bs, 2H), 4.68 (d, *J* 1.0, 1H), 4.52 (s, 1H), 4.20 (dd, *J* 6.9, 4.6, 1H), 2.37 (dd, *J* 13.1, 7.2, 1H), 2.2 −2.09 (m, 1H), 0.97 (t, *J* 7.9, 8H), 0.88 (s, 9H), 0.76–0.63 (m, 6H), 0.07 (s, 3H), 0.04 (s, 3H). δ_C (101 MHz, $CDCl_3$) 158.9, 153.7, 149.2, 142.3, 116.8, 90.2, 84.9, 71.4, 66.6, 45.5, 25.7, 18.0, 6.8, 4.9, −4.7, −4.9. *m/z* (ESI+) 494 $[M + H]^+$. *m/z* (MS/MS) 362, 294, 264, 230, 230.

*(5′*S*)-N-Dimethylformamidine-3′-*O*-tert-butyldimethylsilyl-5′-*O*-triethylsilyl-5′,8-cyclo-2′-deoxyguanosine* ***12***

(5′*S*)-3′-*O*-*tert*-Butyldimethylsilyl-5′-*O*-triethylsilyl-5′,8-cyclo-2′-deoxyguanosine (988 mg, 2 mmol) was dissolved in dry THF (15 mL) and stirred at room temperature. Dimethylformamide diethylacetal (1371 µL, 8 mmol) was then added dropwise and the reaction mixture was stirred under an Ar atmosphere. The reaction was monitored by TLC (CH_2Cl_2/MeOH 97 : 3). After consumption of the starting material (2.5 h), the solvent was removed by rotary evaporation and the mixture was subjected to silica gel chromatography (CH_2Cl_2/MeOH from 99 : 1 to 97 : 3) to give 966 mg (88 %) of (5′*S*)-N^6-dimethylformyl-3′-*O*-*tert*-butyldimethylsilyl-5′-*O*-triethylsilyl-2′-deoxyguanosine as a white foam. δ_H (400 MHz, $CDCl_3$) 9.55 (s, 1H), 8.55 (s, 1H), 6.21 (d, *J* 4.6, 1H), 5.12 (d, *J* 6.0, 1H), 4.87 (dd, *J* 7.0, 4.2, 1H), 4.50 (d, *J* 6.0, 1H), 3.18 (s, 3H), 3.09 (s, 3H), 2.49 (dd, *J* 13.0, 7.3, 1H), 2.14 (dt, *J* 12.7, 4.4, 1H), 1.03 (t, *J* 7.8, 9H), 0.87 (s, 9H), 0.80 (td, *J* 15.1, 7.3, 6H), 0.07 (s, 3H), 0.04 (s, 3H). δ_C (101 MHz, $CDCl_3$) 157.9, 157.9, 156.6, 147.9, 144.8, 119.9, 87.1, 84.6, 69.6, 65.8, 46.4, 41.3, 35.1, 25.7, 17.9, 6.9, 4.8, −4.7, −4.9. *m/z* (ESI+) 418 $[M + H]^+$. *m/z* (MS/MS) 349, 319, 285.

*(5′*R*)-N-Dimethylformamidine-3′-*O*-tert-butyldimethylsilyl-5′-*O*-triethylsilyl-5′,8-cyclo-2′-deoxyguanosine* ***13***

The procedure described for (5′*S*)-3′-*O*-*tert*-butyldimethylsilyl-5′-*O*-triethylsilyl-5′,8-cyclo-2′-deoxyguanosine was followed starting from 140 mg (0.28 mmol) of the 5′*R* diastereomer. After purification, 142 mg (91 %) of (5′*R*)-N^6-dimethylformyl-3′-*O*-*tert*-butyldimethylsilyl-5′-*O*-triethylsilyl-2′-deoxyguanosine was obtained as a white foam. δ_H (400 MHz, $CDCl_3$) 9.63 (bs, 1H), 8.54 (s, 1H), 6.31 (d, *J* 4.5, 1H), 4.68 (d, *J* 1.3, 1H), 4.51 (s, 1H), 4.17 (dd, *J* 7.2, 4.4, 1H), 3.17 (s, 3H), 3.08 (d, *J* 0.4, 3H), 2.31 (dd, *J* 13.0, 7.3, 1H), 2.20–2.06 (m, 1H), 0.96 (t, *J* 7.9, 9H), 0.86 (s, 9H), 0.75–0.65 (m, 6H), 0.04 (s, 3H), 0.02 (s, 3H). δ_C (101 MHz, $CDCl_3$) 158.1, 158, 156.9, 147.8, 142.4, 119.9, 90.3, 84.8, 71.3, 66.5, 45.7, 41.4, 35.2, 25.8, 25.7, 18.0, 6.8, 4.8, −4.8, −5. *m/z* (ESI+) 418 $[M + H]^+$. *m/z* (MS/MS) 349, 319, 285.

*(5′*S*)-N-Dimethylformamidine-3′-*O*-tert-butyldimethylsilyl-5′,8-cyclo-2′-deoxyguanosine* ***14***

(5′*S*)-N^6-Dimethylformyl-3′-*O*-*tert*-butyldimethylsilyl-5′-*O*-triethylsilyl-2′-deoxyguanosine (549 mg, 1 mmol) was taken up in THF (50 mL). The solution was cooled down to −18°C, 1 M solution of TBAF in THF (0.52 mL, 0.52 mmol) was added, and the mixture was stirred at this temperature. The reaction was monitored by TLC (CH_2Cl_2/CH_3OH 93 : 7), and after 30 min, when the starting material was consumed, was quenched with a saturated solution of $NaHCO_3$ and extracted with ethyl acetate (2×200 mL). The organic layer was dried over anhydrous Na_2SO_4, filtered, and the solvent was subsequently removed by rotary evaporation. The resulting residue was subjected to column chromatography on silica gel (CH_2Cl_2/CH_3OH from 100 : 0 to 92 : 8) to give 382 mg (88 %) of (5′*S*)-N^6-dimethylformyl-3′-*O*-*tert*-butyldimethylsilyl-2′-deoxyguanosine as a white foam. δ_H (400 MHz, $CDCl_3$) 8.79 (bs, 1H), 8.56 (s, 1H), 6.24 (d, *J* 4.8, 1H), 5.23 (d, *J* 6.2, 1H), 4.87 (dd, *J* 7.4, 4.3, 1H), 4.68 (d, *J* 6.2, 1H), 4.36 (bs, 1H), 3.19 (s, 3H), 3.09 (s, 3H), 2.51 (dd, *J* 13.3, 7.4, 1H), 2.20 (dt, *J* 13.1, 4.6, 1H), 0.88 (s, 9H), 0.11 (s, 3H), 0.08 (s, 3H). δ_C (101 MHz, $CDCl_3$) 158, 157.4, 157.0, 147.7, 146.4, 119.5, 86.3, 69.4, 64.2, 46.5, 41.4, 35.2, 25.8, 18.0, −4.7, −5. *m/z* (ESI+) 435 $[M + H]^+$. *m/z* (MS/MS) 285, 235. Anal. Calc. for $C_{19}H_{30}N_6O_4Si$: C 52.51, H 6.96, N 19.34. Found: C 52.55, H 6.96, N 19.30 %.

*(5′*R*)-N-Dimethylformamidine-3′-*O*-tert-butyldimethylsilyl-5′,8-cyclo-2′-deoxyguanosine* ***15***

The same procedure as for (5′*S*)-N^6-dimethylformyl-3′-*O*-*tert*-butyldimethylsilyl-5′-*O*-triethylsilyl-2′-deoxyguanosine

was followed for the 5′*R* diastereomer. Starting from 110 mg (0.2 mmol) of (5′*R*)-N^6-dimethylformyl-3′-*O*-*tert*-butyldimethylsilyl-5′-*O*-triethylsilyl-2′-deoxyguanosine, 77 mg (89 %) of product was obtained as a white foam. δ_H (400 MHz, $CDCl_3$) 9.70 (bs, 1H), 8.54 (s, 1H), 6.29 (d, *J* 4.7, 1H), 4.71 (s, 1H), 4.67 (s, 1H), 4.43 (bs, 1H), 4.26 (dd, *J* 7.1, 4.3, 1H), 3.19 (s, 3H), 3.07 (s, 3H), 2.39 (dd, *J* 13.2, 7.2, 1H), 2.15 (dt, *J* 13.7, 4.5, 1H), 0.87 (s, 9H), 0.06 (s, 3H), 0.03 (s, 3H). δ_C (101 MHz, $CDCl_3$) 158.2, 157.9, 157.2, 147.7, 143.0, 119.5, 89.1, 84.6, 71.6, 65.6, 45.7, 41.5, 35.2, 25.7, 18.0, −4.8, −4.9. *m/z* (ESI+) 435 $[M+H]^+$. *m/z* (MS/MS) 285, 235. Anal. Calc. for $C_{19}H_{30}N_6O_4Si$: C 52.51, H 6.96, N 19.34. Found: C 52.50, H 6.95, N 19.35 %.

Supplementary Material

Further details about experimental procedures and data analysis are available on the Journal's website.

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