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Convenient synthesis of N^2-isobutyryl-2′-*O*-methyl guanosine by efficient alkylation of O^6-trimethylsilylethyl-3′,5′-di-*tert*-butylsilanediyl guanosine

Ivan Zlatev, Jean-Jacques Vasseur and François Morvan*

Institut des Biomolécules Max Mousseron UMR 5247 CNRS-UM1-UM2, Université Montpellier 2, CC1704, Place E. Bataillon, 34095 Montpellier Cedex 5, France

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Abstract—We present a novel route for the synthesis of N^2-isobutyryl-2′-*O*-methyl guanosine, introducing 3′,5′-di-*tert*-butylsilyl and O^6-trimethylsilylethyl groups as efficient protections during the 2′-*O*-methylation step with NaH/CH_3I. These protections were then removed simultaneously in a single step with TBAF. The eight-step synthesis is easy to perform, employing convenient commercially available reagents; crude mixtures are of satisfying purity, so only three chromatography purifications were required. Title compound was obtained in 25% overall yield from guanosine.

1. Introduction

2′-*O*-Methyl oligoribonucleotides (2′-*O*-Me RNA) are an attractive class of analogues for the antisense approach because of their physiological stability, resistance to nucleases and high hybridization properties.[1] Indeed, the thermal stability of their hybrids with complementary RNA is greater than that of the RNA–DNA duplexes.[2] More recently some new applications in RNA splicing were reported[3,4] and 2′-*O*-Me RNA were also used for the synthesis of nuclease resistant hammerhead ribozymes.[5] Finally, due to the increasing interest in RNA interference phenomena, the use of 2′-*O*-Me RNA was evaluated as a modification in siRNA.[6–10] In some cases fully modified 2′-*O*-Me RNA displayed enhanced enzymatic stability and similar or increased in vitro potency compared to the unmodified siRNA.[6,8] These applications have made the synthetic research area to develop the 2′-*O*-methyl nucleosides of great interest all along until today.

Most of the methods for the preparation of 2′-*O*-methyl nucleosides were recently reviewed (Ref. 11 and references cited in). Direct alkylation of non-protected ribose moiety using diazomethane seems to be the shortest and the most popular way of synthesis and a better regioselectivity can be obtained when the alkylation is tin directed.[12–14] However, use of hazardous diazomethane is the major inconvenience of these methods. Even if it could be replaced by less dangerous $(CH_3)_3SiCHN_2$[15] this method is still not convenient. Use of classical NaH/MeI methylation conditions[16] hampers the regioselectivity. So better results are obtained when 3′ and 5′ hydroxyl groups are simultaneously protected by a silyl containing cyclic protection.[11] The regioselectivity problem becomes even more complex when one considers the nature of aglycone moiety. Indeed guanosine and uridine are known to undergo base methylation due to the presence of an acidic lactam type proton.[16,17] In the present work, we focused our attention on the synthesis of 2′-*O*-methyl guanosine so further considerations will not refer to uridine. A solution to avoid alkylation of N^1 of guanine moiety is to use 2-amino-6-chloropurine or 2,6-diaminopurine analogues that must be transformed into guanine analogues after the alkylation step.[11,13,14,18] Unfortunately, these methods are far more expensive than those starting from guanosine. Recently a selective methylation of 3′,5′-*O*-protected guanosine was reported.[19] However, although it provided good yield and a good selectivity, this method is not convenient since it requires a non-commercial disilyl cyclic protection and the use of gaseous MeCl, difficult to handle. Otherwise, if we consider guanosine as starting material, the way to avoid N^1 alkylation is to introduce a protecting group on the O^6 of guanosine. Hence, O^6-nitrophenylethyl[12,16] and O^6-nitrophenyl[20] have been reported for successful guanosine alkylation while diphenylcarbamoyl protection gave unsatisfying results.[20] Moreover, Grotli et al.[21,20] reported O^6-*tert*-butyldiphenylsilyl protection, which was used in

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* Corresponding author. Tel.: +33 467 144 961; fax: +33 467 042 029; e-mail: morvan@univ-montp2.fr

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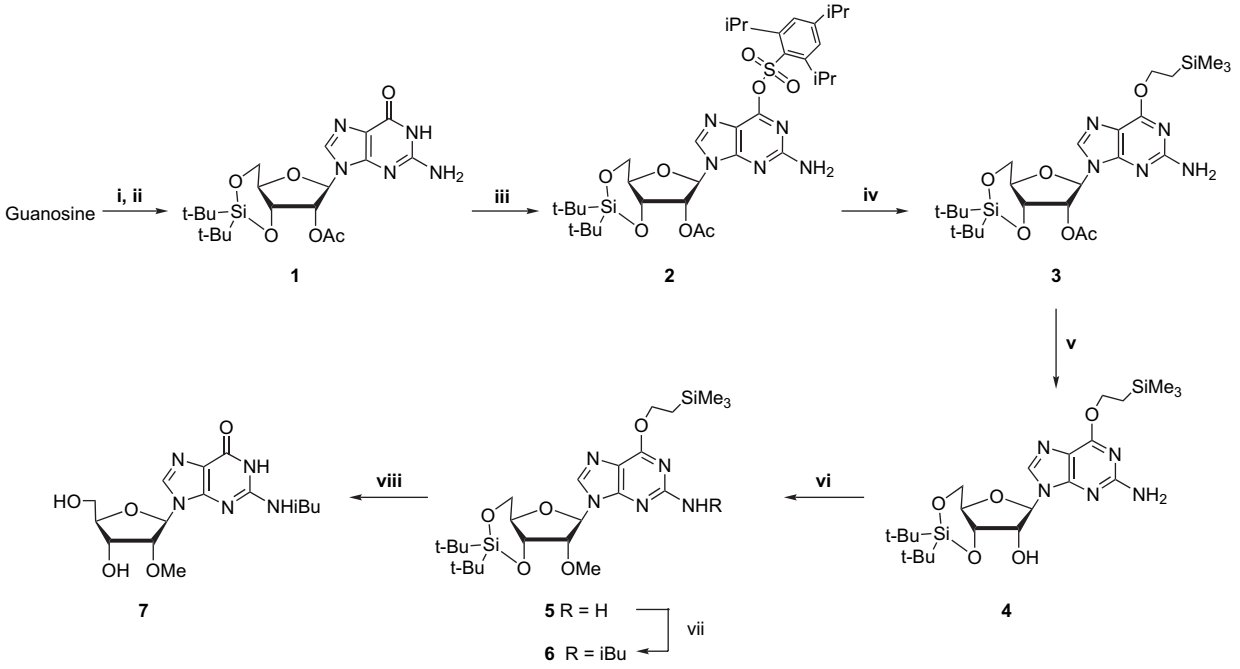
combination with silyl 3′,5′-TIPDS bridge. However, the O^6-silyl protecting group was partly unstable during chromatography on silica gel.[20] While working on the development of some dinucleotide 2′-*O*-alkylated guanosine analogues, we developed the idea to use the stable O^6-trimethylsilylethyl (TSE) protection. This protection was recently used in our group as a transient oligonucleotide protection.[22,23]

We describe herein an easy and efficient route to synthesize N^2-isobutyryl-2′-*O*-methyl guanosine using stable 3′,5′-*O* and O^6-silyl protecting groups and a classical NaH/MeI alkylation procedure. This synthesis requires a minimum of purification steps.

Starting from inexpensive guanosine, di-*tert*-butylsilanediyl (DTBS)[24] was introduced first as a 3′,5′ cyclic protection (Scheme 1). It was chosen considering its greater stability to strong basic conditions compared to the Markiewicz TIPDS protection[25] and its lower cost.[26] Indeed the TIPDS protection is theorized to be fragile due to the inductive effect of the oxygen atom interconnecting the two silicon groups.[27] The DTBS protection was introduced by its bis-triflate derivative onto dried guanosine in dry DMF at 0 °C. In a one-pot reaction, acetyl group was then selectively introduced on the 2′-hydroxyl using acetic anhydride. It is well known that the amino function of guanine (in fact a guanidine function) has a low nucleophilicity, hence it could not react with acetic anhydride. It is worth noticing that the NH_2 group on position 2 must be kept free to avoid any undesired N^2-alkylation. Indeed, its acylation would yield an N^2 acid proton and hence alkylation would proceed on the both N^2 and $O^{2'}$ atoms. After work-up, the resulting crude mixture was pure enough and used in the next step without further purification. The conformationally restricted sugar due to the cyclic silanediyl protection was confirmed by NMR analysis showing a marked chemical shift difference between the 5′ and 5″ protons, and the anomeric $H_{1'}$ signal as a sharp singlet.[24] Subsequent O^6 alkylation was performed in two steps including sulfonate activation on the O^6 lactim (enol) form by tri-isopropylsulfonyl chloride activated by DMAP followed by a nucleophilic displacement of the latter by DABCO and 2-trimethylsilylethanol activated by DBU.[28] Crude O^6-TSE compound **3** was then deacetylated by 0.2 N NaOH, and the reaction was quenched with DOWEX 50W-X8 pyridinium form resin. After silica gel chromatography, the 2′-hydroxyl derivative **4** was obtained with a 40% overall yield from guanosine. The O^6-TSE protection was found to be stable during silica gel chromatography and minimal hydrolysis of the DTBS group was observed during the saponification step. Methylation reaction was performed in anhydrous conditions using iodomethane and NaH in DMF at 0 °C to afford desired compound **5** after work-up and silica gel chromatography with 70% yield (note that this chromatography is optional). It was then quantitatively protected on the N^2 position with isobutyryl chloride providing **6**. Finally the removal of silyl groups afforded the N^2-isobutyryl-2′-*O*-methyl guanosine **7** useful for further oligonucleotide construction (i.e., 5′-DMTr and 3′-phosphoramidite).

Elimination conditions of silyl groups were investigated in order to find a simple procedure leading directly to the desired final compound **7**. On one hand, treatment with the HF–pyridine complex in pyridine at room temperature gave a complete cleavage of the cyclic protection within 15 min while TSE group was only partially removed after 4 h. So, after aqueous work-up the mixture was treated with 0.1 N



Scheme 1. *Reagents and conditions*: (i) *t*-BuSi(OTf)$_2$, DMF, 0 °C to rt, 1 h; (ii) Ac_2O, DMAP, pyridine, rt, overnight; (iii) 2,4,6-tri-isopropylsulfonyl chloride, DMAP, NEt_3, CH_2Cl_2, rt, 2 h, 95%; (iv) DABCO, 2-trimethylsilylethanol, DBU, dioxane, 3 Å molecular sieve, rt, overnight; (v) 0.2 N NaOH in THF/MeOH/H_2O—5:3:1 (v/v/v), 0 °C, 5 min—then DOWEX 50W-X8-pyrH^+, 42%; (vi) NaH—60% in mineral oil, MeI, DMF, 3 Å molecular sieve, 0 °C, 1 h, 70%; (vii) isobutyryl chloride, pyridine, rt, 30 min, quant; (viii) 1 M TBAF in THF, rt, 1 h, 90%.

AcOH in methanol for 20 h providing nucleoside **7**. On the other hand, a more efficient treatment with 1 M TBAF in THF provided the expected N^2-isobutyryl-2′-*O*-methyl guanosine **7** within 1 h at room temperature. For each procedure, pure **7** was obtained in 90% yield after chromatography.

We have described a new multi-step synthetic method affording N^2-isobutyryl-2′-*O*-methyl guanosine **7**. For that purpose, we used two stable silyl protecting groups on the 3′,5′-hydroxyl (DTBS) and on the O^6 position (TSE), then a 2′-*O*-methylation was easily performed using classic NaH/iodomethane conditions. After N^2 protection, the both silyl groups were efficiently and simultaneously removed with TBAF affording the title compound. The reaction scheme is easy to perform, crude mixtures are of satisfying purity affording minimum chromatography purification steps and the overall yield starting from guanosine was 25% in eight steps. The three reactions 2′-methylation, N^2-protection and O^6,3′,5′-deprotection were performed without intermediate chromatography. Thus N^2-isobutyryl-2′-*O*-methyl guanosine **7** was obtained after only three chromatographies.

2. Experimental

2.1. General

TLC was performed on Merck silica coated plates $60F_{254}$ (art. 5554). Compounds were revealed on UV light (254 nm) and after spraying with 10% sulfuric acid/ethanol solution. Column chromatography was performed on silica gel Merck 60 (art. 9385). Reverse-phase liquid chromatography was performed using silica C_{18} Merck LiChroprep® RP-18 (art. 9303), ISCO TRIS Pump and UA-6 detector (254 nm). Ion exchange reactions were performed using DOWEX 50W-X8 H^+ resin (Aldrich); different ion forms were obtained after washing with corresponding 2 M aqueous solutions and then water until neutrality.

All moist-sensitive reactions were carried out under anhydrous conditions using dry glassware, anhydrous solvents and argon atmosphere. Acetonitrile was distilled from CaH_2 after one night stirring at room temperature and 2 h stirring under reflux, and then stored over 3 Å molecular sieves. Pyridine and triethylamine were distilled from CaH_2 after one night stirring at room temperature and 2 h stirring under reflux. Dichloromethane was distilled from P_2O_5 after stirring 2 h under reflux. Dioxane was dried over Al_2O_3, and then stored over 3 Å molecular sieves. All other commercially available anhydrous solvents were used as received. Molecular sieves (3 Å) were dried in a 300 °C oven during 3 h prior to use.

FAB-MS and FAB-HRMS spectra were recorded on a JEOL SX102 mass spectrometer using a mixture of glycerol/thioglycerol [50:50, v/v (GT)] as a matrix. Sugar (Su) and base (B) fragments are given if observed. ^1H and ^{13}C NMR spectra are recorded at room temperature on a Brüker spectrometer at 400 or 200 MHz (^1H) and 100 MHz (^{13}C). Chemical shifts are given in parts per million referenced to the solvent residual peak ($CDCl_3$—7.27 and 77.0 ppm; DMSO-d_6—2.49 and 39.5 ppm). Coupling constants are given in hertz. Signal assignments were based on 2D homo-$^1H/^1H$ or heteronuclear-$^1H/^{13}C$ correlations and D_2O exchange.

2.1.1. 2′-*O*-Acetyl-3′,5′-*O*-di-*tert*-butylsilanediyl guanosine 1. To a suspension of guanosine hydrate (1.4 g, 5 mmol, 1 equiv), dried by azeotropic distillation with anhydrous pyridine, in anhydrous DMF (10 mL) at 0 °C was added dropwise di-*tert*-butylsilyl-bistriflate (1.8 mL, 5.5 mmol, 1.1 equiv). Solution was stirred for an hour at room temperature and then DMAP (122 mg, 1 mmol, 0.2 equiv), 8 mL of anhydrous pyridine and 1.2 mL of acetic anhydride (12.5 mmol, 2.5 equiv) were added. Mixture was stirred overnight at room temperature. Reaction was quenched with 20 mL of methanol. Solvents were removed under reduced pressure, and residue dissolved in ethyl acetate and washed three times with saturated bicarbonate solution. Organic layers were combined, dried over Na_2SO_4 and evaporated to dryness. Product **1** was then used in the next step without further purification. R_f: 0.69—CH_2Cl_2/MeOH—9:1 (v/v). ^1H NMR: (DMSO-d_6, 200 MHz) δ 10.72 (1H, s, br, H_1); 7.98 (1H, s, H_8); 6.50 (2H, s, br, NH2); 5.90 (1H, s, $H_{1'}$); 5.65 (1H, d, $H_{2'}$, $^3J_{H2'-H3'}$=5.5 Hz); 4.55 (1H, dd, $H_{3'}$, $^3J_{H2'-H3'}$=5.5 Hz); 4.40 (1H, dd, $H_{5'}$, $^3J_{H4'-H5'}$=4.3 Hz); 4.05 (2H, m, $H_{4'}$, $H_{5''}$); 2.15 (3H, s, C*H*3$_{acetyl}$); 1.05–1.10 (18H, 2s, C*H*3$_{DTBS}$). FAB^+ (GT) *m*/*z* 466 $(M+H)^+$; 315 $(Su)^+$; 152 $(BH_2)^+$; FAB^- (GT) *m*/*z* 464 $(M-H)^-$; 150 $(B)^-$.

2.1.2. O^6-[(2,4,6-Tri-isopropyl)-benzenesulfonyl]-2′-*O*-acetyl-3′,5′-*O*-di-*tert*-butylsilanediyl guanosine 2. To a solution of crude **1** (5 mmol, 1 equiv), dried by azeotropic distillation with anhydrous acetonitrile, in 30 mL of anhydrous dichloromethane were added DMAP (122 mg, 1 mmol, 0.2 equiv), triethylamine (3 mL, 20 mmol, 4 equiv) and 2,4,6-tri-isopropylbenzenesulfonyl chloride (5 g, 16 mmol, 3 equiv). After 2 h of stirring at room temperature the mixture was diluted with 100 mL of ethyl acetate, washed three times with saturated bicarbonate solution and three times with brine. The organic layers were combined, dried over Na_2SO_4 and evaporated to dryness. Resulting oil was quickly purified by column chromatography (isocratic cyclohexane/AcOEt—7:3 (v/v)) and pure compound **2** obtained as a yellow foam (3.5 g, 95% from guanosine). R_f: 0.33—cyclohexane/AcOEt—7:3 (v/v). ^1H NMR: ($CDCl_3$, 200 MHz) δ 7.70 (1H, s, H_8); 7.22 (2H, s, H_{arom}); 5.85 (1H, s, $H_{1'}$); 5.75 (1H, d, $H_{2'}$, $^3J_{H2'-H3'}$=5.3 Hz); 4.90 (1H, dd, $H_{3'}$, $^3J_{H2'-H3'}$=5.3 Hz, $^3J_{H3'-H4'}$=9.1 Hz); 4.45 (1H, m, $H_{5'}$); 4.30 (2H, septet, C*Ho*–^{i}Pr$_{arom}$, ^3J=6.7 Hz); 4.05 (2H, m, $H_{4'}$ and $H_{5''}$); 2.91 (1H, septet, C*Hp*–^{i}Pr$_{arom}$, ^3J=6.9 Hz); 2.20 (3H, s, C*H*3$_{acetyl}$); 1.20–1.25 (18H, 2d, C*H*3–^{i}Pr$_{arom}$, ^3J=6.7 Hz); 1.05 (18H, 2 s, C*H*3$_{DTBS}$). FAB^+ (GT) *m*/*z* 732 $(M+H)^+$; 418 $(BH_2)^+$; 315 $(Su)^+$.

2.1.3. O^6-[(2-Trimethylsilyl)-ethyl]-2′-*O*-acetyl-3′,5′-*O*-di-*tert*-butylsilanediyl guanosine 3. To a solution of **2** (3.5 g, 4.8 mmol, 1 equiv) in anhydrous dioxane (80 mL) were added DABCO (1.2 g, 10.6 mmol, 2 equiv) and 3 g of dry 3 Å molecular sieves. After 30 min of stirring at room temperature were added 2-(trimethylsilyl)ethanol (4 mL, 26.6 mmol, 5 equiv) and DBU (2 mL, 13.8 mmol, 2.5 equiv). Mixture was stirred overnight under argon, filtered off and filtrate was then diluted with 250 mL of ethyl acetate and washed three times with brine. Organic layers

were combined, dried over Na_2SO_4 and evaporated to dryness. Product **3** was used in next step without further purification.

R_f: 0.44—CH_2Cl_2/AcOEt—9:1 (v/v). ^{1}H NMR: ($CDCl_3$, 200 MHz) δ 7.65 (1H, s, H_8); 5.85 (1H, s, $H_{1'}$); 5.80 (1H, d, $H_{2'}$, $^3J_{H2'-H3'}$=5.3 Hz); 4.95 (1H, m, $H_{3'}$); 4.60 (2H, m, O–C*H*2$_{TSE}$); 4.45 (1H, pd, $H_{5'}$); 4.10 (2H, m, $H_{4'}$ and $H_{5''}$); 2.20 (3H, s, C*H*3$_{acetyl}$); 1.20 (2H, m, TMS–C*H*2$_{TSE}$); 1.05–1.10 (18H, 2s, C*H*3$_{DTBS}$); 0.10 (9H, s, Si–C*H*3$_{TSE}$). FAB^+ (GT) *m/z* 566 $(M+H)^+$; 315 $(Su)^+$; 252 $(BH_2)^+$; FAB^- (GT) *m/z* 464 $(M-H-TSE)^-$; 250 $(B)^-$.

2.1.4. O^6-[(2-Trimethylsilyl)-ethyl]-3′,5′-*O*-di-*tert*-butylsilanediyl guanosine 4. To a solution of crude **3** (4.8 mmol, 1 equiv) in THF/MeOH/H_2O 5:3:1 (v/v/v) (180 mL) was added at 0 °C an aqueous 2 N sodium hydroxide solution (12 mL, 24 mmol, 5 equiv). After 5 min of stirring at 0 °C the reaction mixture was neutralized by adding pyridinium DOWEX 50W-X8 resin, and stirred for five more minutes. The resin was then filtered off and rinsed with water and THF. Filtrates were evaporated to dryness and the residue purified by column chromatography (gradient: cyclohexane with AcOEt 30–50%). Pure **4** was obtained as colourless vitreous solid (1.1 g, 42% from **2**).

R_f: 0.43—cyclohexane/AcOEt—5:5 (v/v). ^{1}H NMR: (DMSO-d_6, 200 MHz) δ 8.02 (1H, s, H_8); 6.35 (2H, s, br, NH2); 5.80 (1H, s, $H_{1'}$); 5.75 (1H, d, br, $OH_{2'}$, $^3J_{H2'-OH2'}$= 3.9 Hz); 4.50 (2H, m, $H_{3'}$ and $H_{2'}$); 4.40 (2H, m, O–C*H*2$_{TMSE}$); 4.30 (1H, m, $H_{5'}$); 4.00 (2H, m, $H_{4'}$ and $H_{5''}$); 1.10 (2H, m, TMS–C*H*2$_{TSE}$); 1.00–1.05 (18H, 2s, C*H*3$_{DTBS}$); 0.10 (9H, s, Si–C*H*3$_{TSE}$). FAB^+ (GT) *m/z* 524 $(M+H)^+$; 252 $(BH_2)^+$; FAB^- (GT) *m/z* 522 $(M-H)^-$; 422 $(M-H-TSE)^-$; 250 $(B)^-$. HRMS:FAB^+ (GT) *m/z* calcd for $(M+H)^+$: 524.2725, found: 524.2707.

2.1.5. O^6-[(2-Trimethylsilyl)-ethyl]-2′-*O*-methyl-3′,5′-*O*-di-*tert*-butylsilanediyl guanosine 5. To a solution of **4** (0.19 g, 0.35 mmol, 1 equiv), dried by azeotropic distillation with anhydrous acetonitrile, in 2.5 mL of anhydrous DMF at 0 °C were added molecular sieves 3 Å, iodomethane (65 μL, 1 mmol, 3 equiv) and sodium hydride 60% dispersion in mineral oil (16 mg, 0.4 mmol, 1.1 equiv). The mixture was stirred for 1 h at 0 °C and then the same quantity of NaH was added. Stirring was followed for 30 more minutes, and then the mixture was hydrolyzed with 10 mL of absolute ethanol, diluted with 50 mL of cold ethyl acetate and finally washed with saturated ammonium chloride solution. The organic layers were combined, dried over Na_2SO_4 and evaporated to dryness. Crude product was then purified by column chromatography (gradient: cyclohexane with AcOEt 0–30%) and pure compound **5** obtained as colourless vitreous solid (0.13 g, 70%). This chromatography could be omitted. R_f: 0.33—cyclohexane/AcOEt—7:3 (v/v). ^{1}H NMR: ($CDCl_3$, 400 MHz) δ 7.50 (1H, s, H_8); 5.72 (1H, s, $H_{1'}$); 4.59 (1H, dd, $H_{3'}$, ^3J=4.7 Hz); 4.48 (2H, m, O–C*H*2$_{TSE}$); 4.35 (1H, dd, $H_{5'}$, $^3J_{H5'-H4'}$=4.9 Hz, $^2J_{H5'-H5''}$=9.0 Hz); 4.12 (1H, d, $H_{2'}$, $^3J_{H2'-H3'}$=4.7 Hz); 4.00 (1H, m, $H_{4'}$, $^3J_{H4'-H5'}$=4.9 Hz, $^3J_{H4'-H5''}$=10.4 Hz); 3.91 (1H, dd, $H_{5''}$, $^2J_{H5'-H5''}$=9.1 Hz, $^3J_{H4'-H5''}$=10.4 Hz); 3.55 (3H, s, 2′-O–C*H*3); 1.12 (2H, m, TMS–C*H*2$_{TSE}$); 1.00 (18H, 2s, C*H*3$_{DTBS}$); 0.05 (9H, s, Si–C*H*3$_{TSE}$). ^{13}C NMR: ($CDCl_3$, 100 MHz) δ 161.5 (C_6); 159.3 (C_2); 152.9 (C_4); 137.6 (C_8); 116.4 (C_5); 89.2 ($C_{1'}$); 82.1 ($C_{2'}$); 77.2 ($C_{3'}$); 74.4 ($C_{4'}$); 67.6 ($C_{5'}$); 65.0 (O–*C*H2$_{TSE}$); 59.3 (2′-O–*C*H3); 27.4; 26.9 (*C*H3$_{DTBS}$); 22.8; 20.3 (C_{DTBS}); 17.6 (*C*H2–TMS_{TSE}); −1.4 (*C*H3–Si_{TSE}). FAB^+ (GT) *m/z* 538 $(M+H)^+$; 252 $(BH_2)^+$. HRMS:FAB^+ (GT) *m/z* calcd for $(M+H)^+$: 538.2881, found: 538.2878.

2.1.6. N^2-Isobutyryl-O^6-[(2-trimethylsilyl)-ethyl]-2′-*O*-methyl-3′,5′-*O*-di-*tert*-butylsilanediyl guanosine 6. To a solution of **5** (0.21 g, 0.39 mmol, 1 equiv) dried by azeotropic distillation with anhydrous pyridine, in 5 mL of anhydrous pyridine at 0 °C was added dropwise isobutyryl chloride (82 μL, 0.78 mmol, 2 equiv). After 1 h of stirring at room temperature the reaction was quenched by adding 10 mL of methanol. Solvents were then removed under reduced pressure, resulting residue dissolved in 20 mL of ethyl acetate and washed with saturated bicarbonate solution. Organic layers were combined, dried over Na_2SO_4 and evaporated to dryness. Product **6** was used in next step without further purification.

R_f: 0.36—cyclohexane/AcOEt—7:3 (v/v). ^{1}H NMR: ($CDCl_3$, 400 MHz) δ 7.72 (1H, s, H_8); 7.68 (1H, s, exch, NH_{ibu}); 5.81 (1H, s, $H_{1'}$); 4.53 (2H, 2dt, ^2J=9.8 Hz, O–C*H*2$_{TSE}$); 4.38 (1H, pt, $H_{3'}$, ^3J=4.8 Hz); 4.36 (1H, m, $H_{5'}$); 4.15 (1H, d, $H_{2'}$, $^3J_{H2'-H3'}$=4.6 Hz); 4.06 (1H, m, $H_{4'}$, ^3J=4.9 Hz); 3.92 (1H, dd, $H_{5''}$, $^2J_{H5'-H5''}$=9.2 Hz, $^3J_{H4'-H5''}$=10.5 Hz); 3.57 (3H, s, 2′-O–C*H*3); 2.85 (1H, septet, C*H*$_{ibu}$); 1.18 (6H, d, C*H*3$_{ibu}$); 1.12 (2H, m, TMS–C*H*2$_{TSE}$); 1.00 (18H, 2s, C*H*3$_{DTBS}$); 0.05 (9H, s, Si–C*H*3$_{TSE}$). ^{13}C NMR: ($CDCl_3$, 100 MHz) δ 180.4 (CO_{ibu}); 161.8 (C_6); 161.2 (C_2); 152.2 (C_4); 139.5 (C_8); 118.8 (C_5); 89.5 ($C_{1'}$); 82.1 ($C_{2'}$); 77.3 ($C_{3'}$); 74.6 ($C_{4'}$); 67.5 ($C_{5'}$); 65.9 (O–*C*H2$_{TSE}$); 59.7 (2′-O–*C*H3); 35.5 (*C*H$_{ibu}$); 27.3; 27.3 (*C*H3$_{DTBS}$); 19.3 (*C*H3$_{ibu}$); 18.9; 18.3 (C_{DTBS}); 17.5 (*C*H2-TMS_{TSE}); −1.39 (*C*H3–Si_{TSE}). FAB^+ (GT) *m/z* 608 $(M+H)^+$; 508 $(M+H-TSE)^+$; FAB^- (GT) *m/z* 606 $(M-H)^-$.

2.1.7. N^2-Isobutyryl-2′-*O*-methyl guanosine 7. *Method A*: To a solution of crude product **6** (0.34 mmol, 1 equiv) in 2 mL of anhydrous dichloromethane in a Teflon flask at 0 °C was added 60 μL of HF·pyridine diluted in 0.37 mL of anhydrous pyridine. The reaction mix was stirred for 1 h at 0 °C, then diluted with 10 mL of ethyl acetate and washed with 1 M TEAB solution. Aqueous layers containing fully desilylated nucleoside were kept away and the organic layers, containing O^6-TSE nucleoside were evaporated to dryness and treated with 3 mL of 0.1 M aqueous acetic acid in 3 mL of methanol for 20 h at room temperature. After completion of the reaction the mixture was neutralized with 1 M TEAB and evaporated to dryness. The two fractions containing deprotected **7** were put together and purified by column chromatography (gradient: dichloromethane with methanol 0–10%). Pure **7** was obtained after lyophilization from water as a white spongy solid (0.11 g, 90%).

Method B: To a solution of crude **6** (0.48 mmol, 1 equiv) in 2.5 mL of anhydrous THF in a Teflon flask was added 5.2 mL (5.2 mmol, 10 equiv) of a 1 M TBAF solution in THF. Mixture was stirred 1 h at room temperature and then neutralized with 9 mL of 1 M TEAB and solvents were removed under reduced pressure. The resulting brown residue

was purified by flash chromatography on reverse-phase RP-18 (gradient: water with acetonitrile 0–50%) and the fractions containing nucleoside **7** after evaporation were lyophilized from water giving a white spongy solid (0.16 g, 90%).

R_f: 0.44—CH_2Cl_2/MeOH—9:1 (v/v). ^{1}H NMR: (DMSO-d_6, 400 MHz) δ 12.09 (1H, s, exch, NH_1); 11.65 (1H, s, exch, NH_{ibu}); 8.32 (1H, s, H_8); 5.91 (1H, d, $H_{1'}$, $^3J_{H1'-H2'}$= 6.1 Hz); 5.26 (1H, d, exch, $OH_{3'}$, $^3J_{OH3'-H3'}$=4.5 Hz); 5.09 (1H, t, exch, $OH_{5'}$, $^3J_{OH5'-H5'}$=5.1 Hz); 4.31 (1H, pd, $H_{3'}$, $^3J_{H3'-H4'}$=3.2 Hz); 4.23 (1H, pt, $H_{2'}$, $^3J_{H2'-H1'}$=5.6 Hz, $^3J_{H2'-H3'}$=4.8 Hz); 3.93 (1H, pd, $H_{4'}$, $^3J_{H4'-H3'}$=3.0 Hz); 3.50–3.65 (2H, m, $H_{5'}$ and $H_{5''}$); 3.30 (3H, s, 2′-O–C*H*3); 2.77 (1H, septet, CH_{ibu}, ^3J=6.9 Hz); 1.16 (6H, d, C$H3_{ibu}$, ^3J=7.2 Hz). ^{13}C NMR: (DMSO-d_6, 100 MHz) δ 180.1 (CO_{ibu}); 154.8 (C_6); 148.2 (C_2); 106.5 (C_8); 86.1 ($C_{4'}$); 84.4 ($C_{1'}$); 82.9 ($C_{2'}$); 68.6 ($C_{3'}$); 61.1 ($C_{5'}$); 57.5 (2′-O–*C*H3); 34.7 (*C*H_{ibu}); 18.8 (*C*$H3_{ibu}$). FAB$^+$ (GT) *m*/*z* 735 $(2M+H)^+$; 368 $(M+H)^+$; 222 $(BH_2)^+$; 152 $(BH_2-ibu)^+$; FAB$^-$ (GT) *m*/*z* 733 $(2M-H)^-$; 366 $(M-H)^-$; 220 $(B)^-$. HRMS:FAB$^+$ (GT) *m*/*z* calcd for $(M+H)^+$: 368.1570, found: 368.1558.

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