Synthesis of 3′,5′-Cyclic Phosphate and Thiophosphate Esters of 2′-*C*-Methyl Ribonucleosides

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2′-*C*-Methylnucleosides are known to exhibit antiviral activity against Hepatitis C virus. Since the inhibitory activity depends on their intracellular conversion to 5′-triphosphates, dosing as appropriately protected 5′-phosphates or 5′-phosphorothioates appears attractive. For this purpose, four potential prodrugs of 2′-*C*-methylguanosine, *i.e.*, 3′,5′-cyclic phosphorothioate of 2′-*C*-methylguanosine and 2′-*C*,O^6-dimethylguanosine, **1** and **2**, respectively, the *S*-[(pivaloyloxy)methyl] ester of 2′-*C*,O^6-dimethylguanosine 3′,5′-cyclic phosphorothioate and the *O*-methyl ester of 2′-*C*,O^6-dimethylguanosine 3′,5′-cyclic phosphate, **3** and **4**, respectively, have been prepared.

Introduction. – Inhibitors of RNA-dependent RNA polymerase of Hepatitis C virus (HCV) constitute a class of potential drugs to combat against this worldwide viral infection [1–3]. Several of the known inhibitors are derivatives of 2′-*C*-methyl ribonucleosides, including 2′-*C*-methyl-3′-*O*-(L-valinyl)cytidine (*Vabilopicitabine*) [4], 4′-azido-3′,5′-bis-*O*-(2-methylpropanoyl)cytidine [5], and 7-deaza-2′-*C*-methyladenosine in addition to unsubstituted 2′-*C*-methyl ribonucleosides [6]. All these nucleosides need, however, to be converted to 5′-triphosphates to exhibit the desired inhibition. Since the first phosphorylation giving a nucleoside 5′-monophosphate usually limits the rate of this conversion, appropriately protected nucleoside 5′-monophosphates expectedly exhibit enhanced antiviral activity [1]. For example, the potency of 2′-*C*-methylguanosine in cell lines is dramatically increased by conversion to 5′-(*O*-arylphosphoramidates) derived from L-alanine alkyl esters [7]. Similarly, various alkyl [8], aryl [8], acyloxymethyl [9] and 2-(acylthio)ethyl esters of 3′,5′-cylic phosphates of base-modified 2′-*C*-methyl ribonucleosides appear to be more potent inhibitors of HCV RNA replication than the parent cyclic phosphodiester. Encouraged by these results, we now report on syntheses of the 3′,5′-cyclic phosphorothioates of 2′-*C*-methylguanosine, 2′-*C*,O^6-dimethylguanosine, **1** and **2**, respectively, the *S*-(pivaloyloxymethyl), and the *O*-methyl ester of 2′-*C*,O^6-dimethylguanosine 3′,5′-cyclic phosphate, **3** and **4**, respectively (*Fig.*). While all these nucleotides may exhibit antiviral activity, compound **3** expectedly is the most potent prodrug candidate. The (pivaloyloxy)methyl group is removed by esterase-dependent deacylation and the remaining *S*-(hydroxymethyl) group by subsequent chemical hydrolysis. The exposed 3′,5′-cyclic phosphorothioate diester may then undergo diesterase-catalyzed ring-opening to a 5′-phosphorothioate.

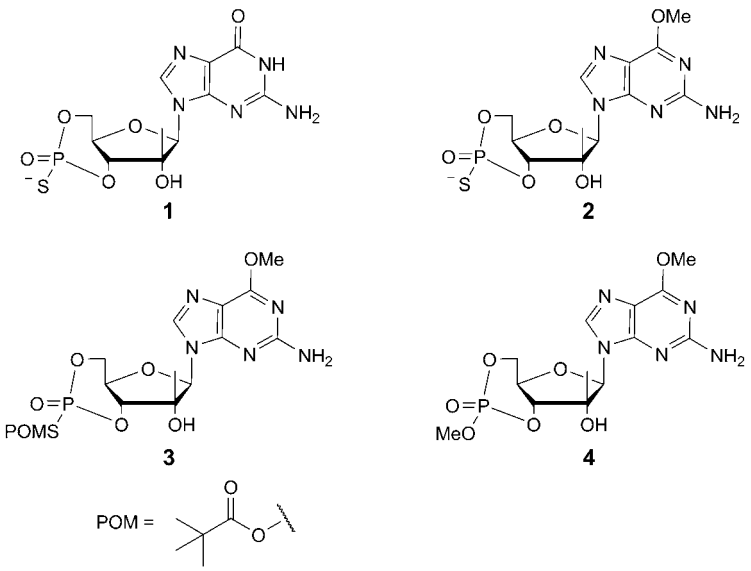


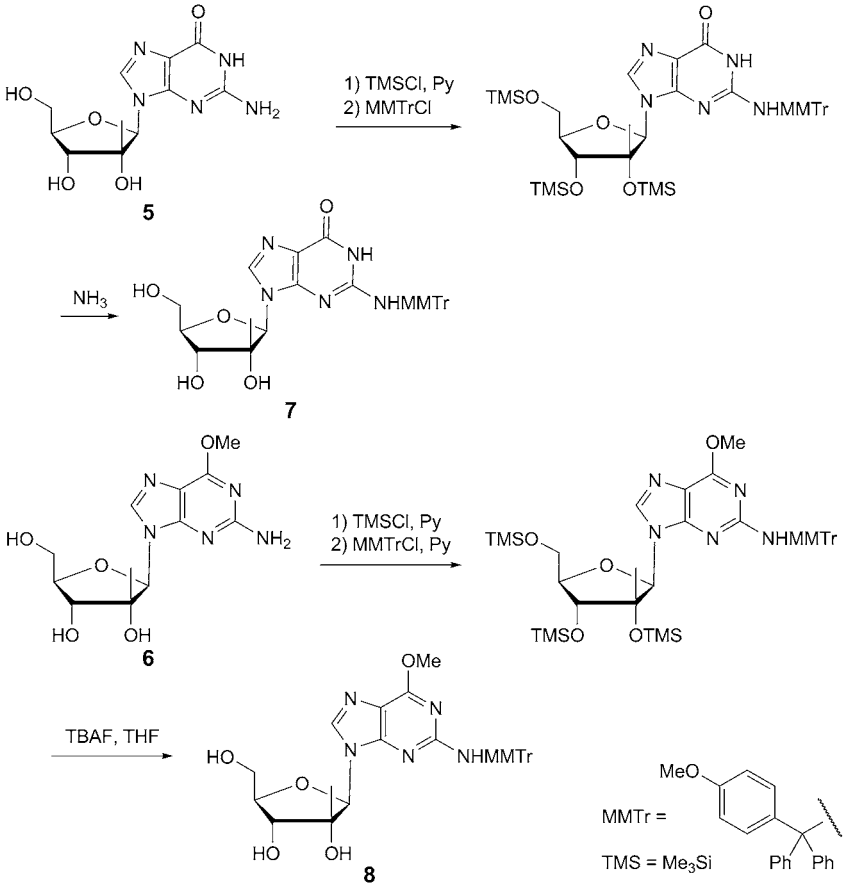
Figure. *Structures of potential prodrugs of HCV antiviral 2′-C-methylguanosine nucleotides*

Results and Discussion. – 2′-*C*-Methylguanosine (**5**) was prepared by glycosylation of persilylated N^2-acetylguanine, *i.e.*, 9-(trimethylsilyl)-6-(trimethylsilyloxy)-2-[(*N*-(trimethylsilyl)acetamido]purine, with 1,2,3,5-tetra-*O*-benzoyl-2-*C*-methylribofuranose, as described by *Li* and *Piccirilli* [10]. 2′-*C*,O^6-Dimethylguanosine (**6**) was, in turn, obtained by glycosylation of 6-chloroguanine with the same glycosyl donor, followed by treatment with MeONa in MeOH, as described previously by *McGuigan et al.* [7]. Conversion of 2′-*C*-methylguanosine (**5**) and 2′-*C*,O^6-dimethylguanosine (**6**) to N^2-(4-methoxytritylated) form, aimed at allowing their 5′-phosphitylation and subsequent intramolecular cyclization, is depicted in *Scheme 1*. Accordingly, 2′-, 3′-, and 5′-OH groups of **5** and **6** were protected with trimethylsilyl (TMS) groups, and the amino function was then alkylated with 4-methoxytrityl (=(4-methoxyphenyl)diphenyl, MMTr) chloride.

Finally, the TMS protecting groups were removed. With N^2-(4-methoxytrityl)-2′-*C*,O^6-dimethyl-2′,3′,5′-tris-*O*-(trimethylsilyl)guanosine, the removal of the 2′-*O*-$SiMe_3$ group turned out to be surprisingly difficult. Treatment with Bu_4NF (TBAF) in THF was required instead of the NH_3 treatment, which usually is sufficient to remove the TMS protecting groups from sugar OH functions.

Conversions of the tritylated 2′-*C*-methyl nucleosides **7** and **8** to 3′,5′-cyclic phosphorothioates **1** and **2**, respectively, and 3′,5′-cyclic (*S*-[(pivaloyloxy)methyl] phosphorothioate) and 3′,5′-cyclic *O*-methyl phosphate, **3** and **4**, respectively, are outlined in *Schemes 2–5*. Phosphitylation of N^2-(4-methoxytrityl)-2′-*C*-methylguanosine (**7**) with 2-cyanoethyl *N*,*N*-diisopropyl(chloro)phosphoramidite (*Scheme 2*) and subsequent 1*H*-tetrazole (TetH)-promoted intramolecular replacement of the 3′-OH group with the $(^iPr)_2N$ ligand gave a 3′,5′-cyclic phosphite triester, which was sulfurized

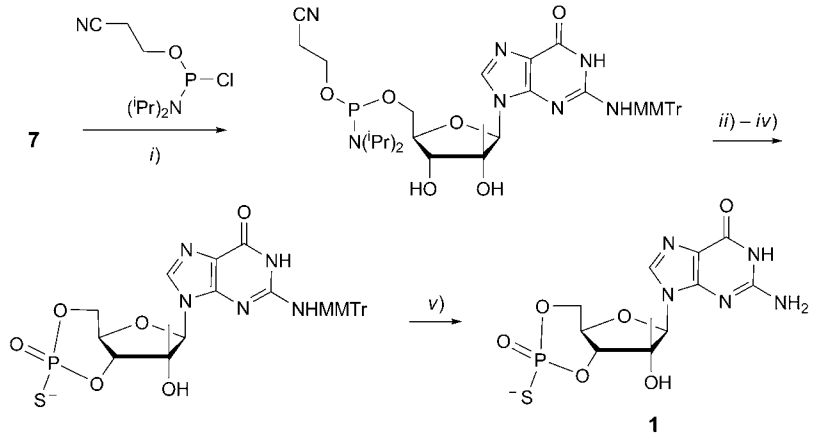
Scheme 1. *Preparation of 2′-C-Methyl- and 2′-C,O⁶-Dimethylguanosine*



with 3*H*-1,2-benzodithiol-3-one 1,1-dioxide (*Beaucage* reagent) in MeCN to N^2-(4-methoxytrityl)-2′-*C*-methylguanosine cyclic 3′,5′-(*O*-(2-cyanoethyl) phosphorothioate). The 2-cyanoethyl group was cleaved during chromatographic purification (SiO_2; CH_2Cl_2 containing 1% Et_3N). The MMTr group was then removed by treatment with aqueous 80% AcOH to give 2′-*C*-methylguanosine 3′,5′-cyclic phosphorothioate (**1**).

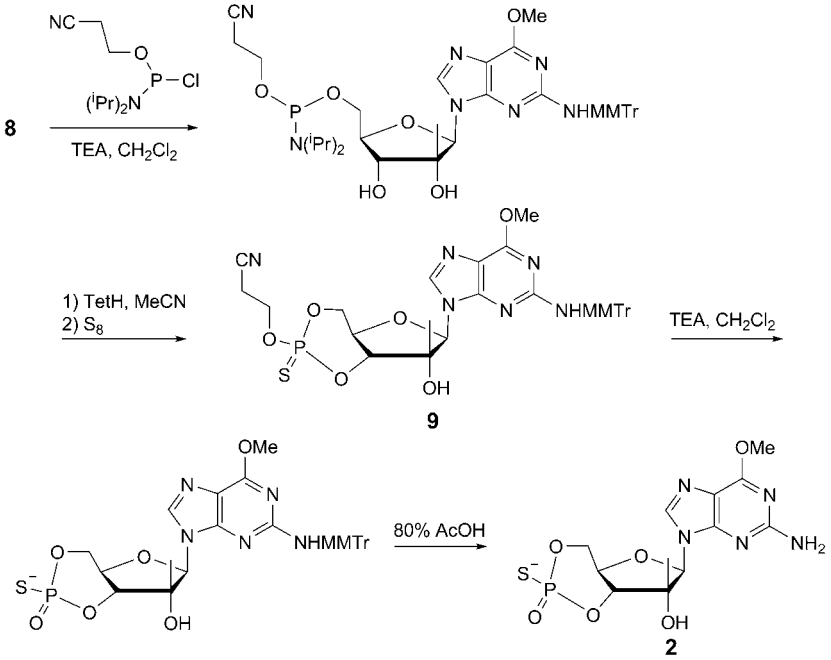
The same method was applied for preparation of N^2-(4-methoxytrityl)-2′-*C*,O^6-dimethylguanosine 3′,5′-cyclic phosphorothioate (**2**). Accordingly, N^2-(4-methoxytrityl)-2′-*C*,O^6-dimethylguanosine (**8**) was phosphitylated with 2-cyanoethyl *N*,*N*-diisopropylchlorophosphoramidite and the 3′-OH group was replaced with $(^iPr)_2N$ ligand using tetrazole as an activator (*Scheme 3*). The phosphite triester obtained was sulfurized to thiophosphate ester **9** with elemental sulfur. The desired fully deprotected 3′,5′-cyclic phosphorothioate **2** was obtained by removal of the 2-cyanoethyl group with Et_3N in CH_2Cl_2 and of the MMTr group with aq. AcOH.

Scheme 2. *Preparation of 2′-C-Methylguanosine 3′,5′-Cyclic Phosphorothioate*



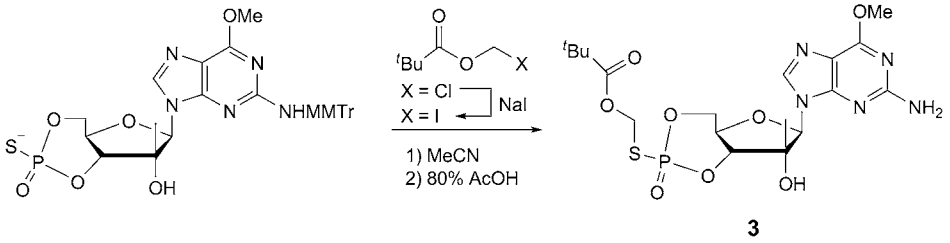
i) Et_3N, CH_2Cl_2. *ii*) TetH, MeCN. *iii*) 3*H*-1,2-Benzodithiol-3-one 1,1-dioxide. *iv*) SiO_2 Chromatography with CH_2Cl_2/Et_3N. *v*) 80% AcOH.

Scheme 3. *Preparation of 2′-C,O⁶-Dimethylguanosine 3′,5′-Cyclic Phosphorothioate*



The intranucleosidic 3′,5′-phosphorothioate linkage of N^2-(4-methoxytrityl)-2′-*C*,O^6-dimethylguanosine 3′,5′-cyclic phosphorothioate (**2**) was protected with an esterase-labile (pivaloyloxy)methyl group by treating **2** with iodomethyl pivalate in MeCN (*Scheme 4*). The MMTr group was then removed by treatment with aq. 80%

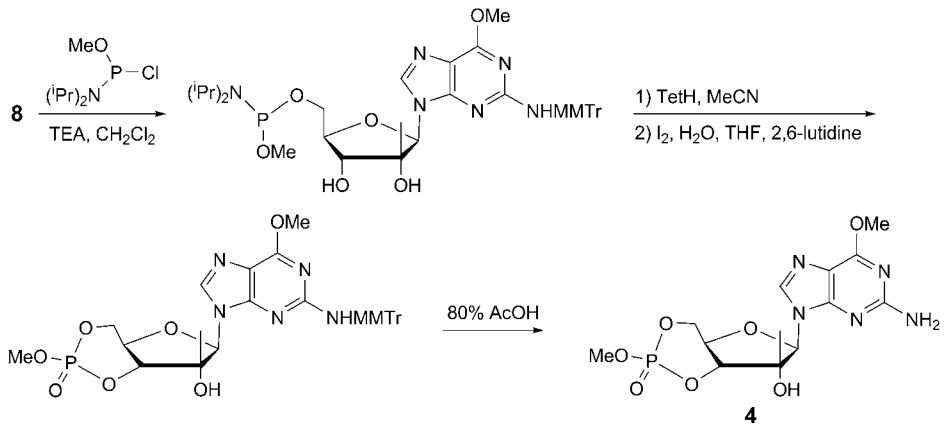
Scheme 4. *Preparation of the of 2′-*C,O^6*-Dimethylguanosine 3′,5′-Cyclic (*S*-(Pivaloyloxy)methyl Phosphorothioate)*



AcOH to afford 2′-*C*,*O*^{6}-dimethylguanosine cyclic 3′,5′-(*O*-[(pivaloyloxy)methyl] phosphorothioate) (**3**).

To prepare 2′-*C*,*O*^{6}-dimethylguanosine 3′,5′-cyclic (*O*-methyl phosphate) (**4**), *N*^{2}-(4-methoxytrityl)-2′-*C*,*O*^{6}-dimethylguanosine (**8**) was phosphitylated with methyl-*N*,*N*-diisopropyl(chloro)phosphoramidite, and the (^{i}Pr)$_2$N ligand then resplaced the 3′-OH group, as described above. The resulting cyclic phosphite triester was oxidized to phosphate ester with I_2 in aqueous THF containing 2,6-lutidine, and the MMTr group was removed by AcOH treatment to afford 2′-*C*,*O*^{6}-dimethylguanosine cyclic 3′,5′-(*O*-methyl phosphate) (**4**; *Scheme 5*).

Scheme 5. *Preparation of the of 2′-*C,O^6*-Dimethylguanosine 3′,5′-Cyclic (Methyl Phosphate)*



Experimental Part

General. Chemicals were purchased from *Sigma–Aldrich*, *Fluka*, and *Glen Research*. MeCN, CH_2Cl_2, THF, and pyridine were dried over 4-Å molecular sieves. Et_3N was dried by refluxing over CaH_2 and distilled before use. Column chromatography (CC): silica gel *60* (SiO_2; 230–400 mesh; *Fluka*). HPLC: *Perkin-Elmer LC 250* with *LC-290* UV detector. ^{1}H-, ^{13}C-, and ^{31}P-NMR spectra: *Bruker Avance 500* or *400* NMR spectrometer; δ in ppm rel. to Me_4Si as internal standard, *J* in Hz. HR-ESI-MS: *Bruker Daltonics micrOTOF-Q*; in *m*/*z*. LC-ESI-MS: *Perkin-Elmer Sciex-API-365* triple-quadrupole spectrometer; in *m*/*z*.

N-*[(4-Methoxyphenyl)(diphenyl)methyl]-2′*-C-*methylguanosine* (**7**). *2′*-C-*Methylguanosine* (**5**; 0.520 g, 1.74 mmol) was co-evaporated 3 times with dry pyridine and dissolved in the same solvent (10.0 ml). The mixture was cooled on an ice bath, and TMSCl (1.99 ml, 15.74 mmol) was added. The ice bath was removed, and the mixture was stirred at r.t. for 2 h. The mixture was cooled on an ice bath and (4-methoxyphenyl)(diphenyl)methyl chloride (MMTrCl; 2.70 g, 8.74 mmol) in pyridine was added. After stirring overnight (20 h) at r.t., the mixture was cooled on an ice bath, and cold H_2O (3 ml) was added. After 5 min stirring, concentrated aq. NH_3 (33%; 5 ml) was added, and stirring was continued for 30 min. The solvent was removed by evaporation under reduced pressure. The residue was dissolved in CH_2Cl_2, and the org. phase was washed with H_2O, dried (Na_2SO_4) and evaporated to dryness. The product was purified by CC (SiO_2; MeOH/CH_2Cl_2 1:9) to yield **7** (0.37 g, 38%). Solid. ^{1}H-NMR (400 MHz, CD_3OD): 8.16 (*s*, H–C(8)); 7.37–7.19 (12 H, MMTr); 6.85 (*d*, *J* = 8.91, MMTr); 5.30 (*s*, H–C(1′)); 3.97 (*d*, *J* = 9.1, H–C(3′)); 3.92 (*dd*, *J* = 12.5, 2.1, H–C(5′)); 3.87 (*m*, H–C(4′)); 3.75–3.72 (*m*, Me of MMTr, H–C(5″)); 0.55 (*s*, 2′-Me). ^{13}C-NMR (101 MHz, CD_3OD): 158.48 (C(6)); 157.97 (MMTr); 151.07 (C(4)); 150.11 (C(2)); 144.94 and 144.85 (MMTr); 136.72 (MMTr); 136.28 (C(8)); 130.04; 128.64, 127.45, 126.45 (MMTr); 116.10 (C(5)); 112.76 (MMTr); 90.17 (C(1′)); 82.36 (C(4′)); 78.89 (C(2′)); 71.56 (C(3′)); 70.38 (Spiro C_q of MMTr); 59.23 (C(5′)); 54.32 (MeO of MMTr); 18.48 (2′-Me). HR-ESI-MS: 570.2381 ($[M+H]^+$, $C_{31}H_{32}N_5O_6^+$; calc. 570.2353).

N^2-*(4-Methoxytrityl)-2′*-C,O^6-*dimethylguanosine* (=*6-Methoxy*-N-*[(4-methoxyphenyl)(diphenyl)-methyl]-9-(2*-C-*methyl-β*-D-*ribofuranosyl)-9*H-*purin-2-amine*; **8**). To a precooled soln. of *2′*-C,O^6-*dimethylguanosine* (**6**; 1.30 g, 4.17 mmol) in dry pyridine, TMSCl (2.65 ml, 20.88 mmol) was added, and the mixture was stirred at r.t. for 2 h. MMTrCl (1.28 g, 4.17 mmol) was added and the mixture was kept at 50° for 20 h. The solvent was removed by evaporation under reduced pressure. The residue was dissolved in CH_2Cl_2 and washed twice with sat. aq. $NaHCO_3$ and brine, and evaporated to dryness. To remove the TMS groups, the residue, N^2-(4-methoxytrityl)-2′,O^6-dimethyl-2′,3′,5′-tris-*O*-(trimethylsilyl)-guanosine (ESI-MS (pos.): 800.9 ($[M+H]^+$)) was dissolved in dry THF, and Bu_4NF hydrate was added. The soln. was stirred for 90 min at r.t. The mixture was evaporated to dryness, and the residue was dissolved in CH_2Cl_2 and treated with sat. aq. $NaHCO_3$. The org. phase was evaporated to dryness. The residue was purified by CC (SiO_2; CH_2Cl_2 containing 5% MeOH) to give **8** (1.27 g, 53%). White solid. ^{1}H-NMR (500 MHz, CD_3OD): 8.27 (*s*, H–C(8)); 7.41–7.16 (MMTr); 6.80 (*d*, *J* = 5.0, MMTr); 5.77 (br. *s*, H–C(1′)); 4.11 (*d*, *J* = 10.0, H–C(3′)); 3.98–3.96 (*m*, H–C(4′), H–C(5″)); 3.79 (*d*, *J* = 15.0, H–C(5′)); 3.74 (*s*, MeO); 3.31 (*s*, MeO of MMTr); 0.79 (br. *s*, 2′-Me). ^{13}C-NMR (500 MHz, CD_3OD): 160.0 (C(6)); 158.30 (MMTr); 152.59 (C(2)); 145.90 and 145.85 (C(4) and MMTr); 137.93 and 139.91 (MMTr and C(8)); 130.04, 128.64, 127.45 and 126.15 (MMTr); 113.79 (C(5)); 112.49 (MMTr); 90.17 (C(1′)); 82.36 (C(4′)); 78.91 (C(2′)); 71.87 (C(3′)); 70.35 (C_q of MMTr); 59.45 (C(5′)); 54.28 (MeO of MMTr); 53.02 (MeO); 18.79 (2′-Me). HR-ESI-MS: 584.2363 ($[M+Na]^+$, $C_{32}H_{34}N_5O_6^+$; calc. 584.2509).

2′-C-*Methylguanosine 3′,5′-Cyclic Phosphorothioate* (=*(4a*R,*6*R,*7*R,*7a*R*)-6-(2-Amino-1,6-dihydro-6-oxo-9*H-*purin-9-yl)-tetrahydro-7-hydroxy-7-methyl-4*H-*furo[3,2-*d*][1,3,2]dioxaphosphinine-2-thiolate 2-Oxide*; **1**). Compound **7** (250 mg, 0.438 mmol), dried (P_2O_5) overnight, was dissolved in dry CH_2Cl_2 (3 ml) under Ar. Dry Et_3N (0.288 ml, 2.06 mmol) and 2-cyanoethyl *N*,*N*-diisopropyl(chloro)phosphoramidite (0.103 ml, 0.46 mmol) were added, and the mixture was stirred at r.t. for 40 min. The product was isolated by passing the mixture through a short SiO_2 column with dry AcOEt containing 0.5% Et_3N. The solvent was removed under reduced pressure. The product was co-evaporated with dry MeCN (2 × 20 ml) to remove the traces of Et_3N. The phosphitylated nucleoside was dissolved in dry MeCN (60 ml) under Ar, and 1*H*-tetrazole (1.03 mmol, 2.25 ml of 0.45 mol/dm^3 soln. in MeCN) was added. The mixture was stirred for 3 h, and then 3*H*-1,2-benzodithiol-3-one 1,1-dioxide (185 mg, 0.877 mmol) was added, and stirring was continued overnight. The mixture was evaporated to dryness. The crude was purified by CC (SiO_2; CH_2Cl_2 containing 15% MeOH and 0.5% Et_3N). The 2-cyanoethyl group was cleaved during CC and, hence, N^2-(4-methoxytrityl)-2′-*C*-methylguanosine 3′,5′-cyclic phosphorothioate was obtained as white solid in 32% yield (90 mg).

To remove the MMTr group, N^2-(4-methoxytrityl)-2′-*C*-methylguanosine 3′,5′-cyclic phosphorothioate (71 mg, 0.08 mmol) was treated with aq. 80% AcOH (2.0 ml) at r.t., and the mixture was stirred overnight. The mixture was evaporated to dryness, and the residue was co-evaporated twice with H_2O. The crude was purified CC(SiO_2;CH_2Cl_2 containing 10% MeOH). The triethylammonium salt of **1** was

obtained as solid form in 73% (38 mg). The ^{13}C-NMR spectra of **1** could not be obtained due to a poor solubility. For the same reason, the triethylammonium cation could not be exchanged by cation exchange chromatography. ^{1}H-NMR (400 MHz, D_2O): 7.90 and 7.84 (br. *s*, H–C(8)); 5.90 (br. *s*, H–C(1′)); 4.97 (H–C(3′)); 4.50–4.35 (H–C(4′), H–C(5″)); 4.21 (H–C(5′)); 3.37 (br. *s*, 2′-OH) and 1.00 (br. *s*, 2′-Me). ^{31}P-NMR (162 MHz, DMSO): 56.28 and 55.06. HR-ESI-MS: 398.0299 ([M + Na]$^+$, $C_{11}H_{14}N_5NaO_6PS^+$; calc. 398.0300).

N^2-*(4-Methoxytrityl)-2′,*O^6*-dimethylguanosine 3′,5′-Cyclic* O*-(2-Cyanoethyl) Phosphorothioate* (= *3-{[(4a*R*,6*R*,7*R*,7a*R*)-Tetrahydro-7-hydroxy-6-(6-methoxy-2-{[(4-methoxyphenyl)(diphenyl)methyl]amino}-9*H*-purin-9-yl)-7-methyl-2-sulfido-4*H*-furo[3,2-*d*][1,3,2]dioxaphosphinin-2-yl]oxy}propanenitrile*; **9**). Compound **8** (0.625 g, 1.07 mmol) was dried (P_2O_5) overnight and dissolved in dry CH_2Cl_2 (5 ml). Anh. Et_3N (0.75 ml, 5.39 mmol) and 2-cyanoethyl *N*,*N*-diisopropylphosphoramidochloridite (0.26 ml, 1.17 mmol) were added, and the mixture was stirred for 60 min under Ar at r.t. The phosphitylated product was isolated by passing the mixture through a short SiO_2 column with dry AcOEt containing 0.5% Et_3N. The solvent was removed under reduced pressure, and the crude was co-evaporated twice with dry MeCN. The residue was dissolved in dry MeCN (60 ml) and 0.45 mol/dm^3 soln. of 1*H*-tetrazole in MeCN (5.95 ml, 5.80 mmol) was added. After 2.5 h stirring at r.t., dry pyridine (80 ml) and elemental sulfur (245 mg, 7.65 mmol) were added, and stirring was continued overnight. After filtration, the mixture was evaporated to dryness. The residue was dissolved in CH_2Cl_2, and the org. layer was washed with aq. $NaHCO_3$ and brine, dried (Na_2SO_4), and evaporated to dryness. The crude was purified by CC (SiO_2; CH_2Cl_2 containing 3% MeOH and 0.5% pyridine). Compound **9** was obtained as a mixture of (R_P)- and (S_P)-diastereoisomers in 21% yield (156 mg by repeating the synthesis). ^{1}H-NMR (500 MHz, $CDCl_3$): 7.58 (*s*, H–C(8)); 7.38–7.20 (*m*, MMTr); 6.85–6.78 (*m*, MMTr); 6.53 (*s*, H–C(1′)); 5.76 (br. *s*, NH); 4.55–4.37 (*m*, H–C(3′), H–C(5′), H–C(5″), CH_2O); 3.90 (*dd*, J = 6.3 and 6.3, H–C(4′)); 3.83 (*s*, MMTr, 6-MeO); 2.82–2.75 (*m*, CH_2CN); 1.09 (*s*, 2′-Me). ^{13}C-NMR (126 MHz, $CDCl_3$): 160.50 (C(6)); 158.20 and 158.20–158.13 (MMTr); 158.10 (C(4)); 152.17 (C(2)); 145.80 (C(8)); 144.46 (MMTr); 135.69 (MMTr); 130.5–126.5 (MMTr); 118.32 (CN); 116.62 (C(5)); 113.5–113.0 (MMTr); 94.70 (C(1′)); 86.76 (C_q of MMTr); 80.90 (C(3′)); 70.61 and 70.05 (C(4′), C(2′)); 57.67 (C(5′)); 55.29 (MeO of MMTr); 54.05 (MeO); 51.99 (CH_2O); 19.73 (2′-Me); 19.58 (CH_2CN). ^{31}P-NMR (202 MHz, CD_3OD): 83.57 and 67.84. HR-ESI-MS: 715.2087 ([M + H]$^+$, $C_{35}H_{36}N_6O_7PS^+$; calc. 715.2104).

*2′-C,*O^6*-Dimethylguanosine 3′,5′-Cyclic Phosphorothioate* (= *(4a*R*,6*R*,7*R*,7a*R*)-6-(2-Amino-6-methoxy-9*H*-purin-9-yl)-tetrahydro-7-hydroxy-7-methyl-4*H*-furo[3,2-*d*][1,3,2]dioxaphosphinine-2-thiolate 2-Oxide*; **2**). To remove the 2-cyanoethyl group, **9** (230 mg, 0.32 mmol) was dissolved in a mixture of dry CH_2Cl_2 (1.5 ml) and dry Et_3N (2.0 ml). The mixture was stirred at r.t. for 18 h, evaporated to dryness, and co-evaporated with dry MeCN. *N*^{2}-(4-Methoxytrityl)-2′-*C*,*O*^{6}-dimethylguanosine 3′,5′-cyclic phosphorothioate was obtained as white solid foam and used without further purification. The residue was dissolved in 80% aq. AcOH (10 ml), and the mixture was stirred at r.t. for 5 h and evaporated to dryness. The crude product was purified by HPLC eluting with 15% MeOH in H_2O. The diastereoisomers were not separated. The product was dissolved in H_2O and passed through *Dowex* Na^+-form (50 W, 100–200 mesh). The product was obtained as white solid in 32% yield (42 mg). ^{1}H-NMR (500 MHz, CD_3OD): 7.95 (*s*, H–C(8)); 5.94 (*s*, H–C(1′)); 4.61–4.22 (H–C(3′), H–C(4′), H–C(5″), H–C(5′)); 4.07 (s, 6-MeO); 1.06 (*s*, 2′-Me). ^{13}C-NMR (126 MHz, CD_3OD): 161.34 (C(6)); 160.34 (C(2)); 153.00 (C(4)); 138.12 (C(8)); 114.25 (C(5)); 94.33 (C(1′)); 80.40 (C(3′)); 77.06, 77.29 (C(2′)); 72.50 (C(4′)); 66.90, 69.85 (C(5′)); 52.80 (MeO); 18.51 (2′-Me). ^{31}P-NMR (202 MHz, DMSO): 55.97. HR-ESI-MS: 388.0482 ([M – H]$^-$, $C_{12}H_{15}N_5O_6PS^-$; calc. 388.0486).

*2′-C,*O^6*-Dimethylguanosine Cyclic* S*-3′,5′-([(Pivaloyloxy)methyl] Phosphorothioate)* (= *{[(4a*R*,6*R*,7*R*,7a*R*)-6-(2-Amino-6-methoxy-9*H*-purin-9-yl)-tetrahydro-7-hydroxy-7-methyl-2-oxido-4*H*-furo[3,2-*d*][1,3,2]dioxaphosphinin-2-yl]sulfanyl}methyl 2,2-Dimethylpropanoate*; **3**). Chloromethyl pivalate (1.0 ml, 6.90 mmol) was added to a mixture of NaI (2.08 g, 13.80 mmol) and dry MeCN (10 ml). The mixture was stirred at r.t. overnight in dark. The mixture was evaporated to dryness, dissolved in CH_2Cl_2, and washed with 5% aq. $NaHSO_3$ and brine. The org. layer was dried (Na_2SO_4) and evaporated to dryness. The iodomethyl pivalate obtained was used as such for the next step. *N*^{2}-(4-Methoxytrityl)-2′-*C*,*O*^{6}-dimethylguanosine 3′,5′-cyclic phosphorothioate was dissolved in dry MeCN (3 ml), and the iodomethyl pivalate (56 mg, 0.23 mmol) was added. The mixture was stirred for 2.5 h at r.t. Sat. aq.

$NaHCO_3$ soln. was added, and the crude product was extracted with CH_2Cl_2. The org. layer was dried (Na_2SO_4) and evaporated to dryness. The residue was dissolved in 80% aq. AcOH (2.0 ml), and the mixture was stirred at r.t. for 20 h. The mixture was evaporated to dryness and, the residue was co-evaporated twice with H_2O. The crude product **3** was purified by CC (SiO_2;CH_2Cl_2 containing 10% MeOH) to give **3** (15 mg, 14%). White solid. ^{1}H-NMR (500 MHz, CD_3OD): 7.95 (*s*, H–C(8)); 5.93 (*s*, H–C(1′)); 5.58–5.54 (*m*, SCH_2); 4.80–4.69 (*m*, H–C(3′), H–C(4′), H–C(5″)); 4.45 (*m*, H–C(5′)); 4.06 (*s*, MeO); 1.20 (*s*, $C(Me)_3$); 1.10 (*s*, 2′-Me). ^{13}C-NMR (126 MHz, CD_3OD): 177.48 (C=O); 161.50 (C(6)); 160.22 (C(2)); 152.66 (C(4)); 139.14 (C(8)); 129.34 (C(5)); 95.15 (C(1′)); 81.87 (C(3′)); 76.76 and 76.70 (C(2′)); 71.00, 70.93, 70.80 and 70.81 (C(4′), C(5′)); 60.23, 60.20 (SCH_2); 52.87 (MeO); 38.52 (Me_3C); 25.85 (Me_3C); 18.18 (2′-Me). ^{31}P-NMR (202 MHz, CD_3OD): 23.13. HR-ESI-MS: 504.1323 ($[M+H]^+$, $C_{18}H_{27}N_5O_8PS^+$; calc. 504.1318).

2′-C,O^6-Dimethylguanosine Cyclic 3′,5′-(O-Methyl Phosphate) (=*(4a*R,*6*R,*7*R,*7a*R*)-6-(2-Amino-6-methoxy-9*H*-purin-9-yl)-tetrahydro-2-methoxy-7-methyl-4*H*-furo[3,2*-d*][1,3,2]dioxaphosphinin-7-ol 2-Oxide*; **4**). Compound **8** (1.80 mmol, 1.05 g) dried (P_2O_5) overnight was dissolved in dry CH_2Cl_2 (8 ml) under N_2. Dry Et_3N (9.00 mmol, 1.25 ml) and methyl *N,N*-diisopropylchlorophosphoramidite (1.99 mmol, 385 µl) were added, and the mixture was stirred at r.t. for 30 min. The product was isolated by passing the mixture through a short SiO_2 with dry hexane containing 30% dry AcOEt and 0.5% dry Et_3N. The solvent was removed under reduced pressure, and the residue was co-evaporated three times with dry MeCN to remove the traces of Et_3N. The phosphitylated nucleoside was dissolved in dry MeCN (70 ml) under N_2, and 1*H*-tetrazole (4.50 mmol, 10.00 ml of 0.45 mol/dm^3 soln. in MeCN) was added. The mixture was stirred for 3 h. After 2 h stirring at r.t., I_2 (0.1M) in a mixture of THF, H_2O, and 2,6-lutidine (4:2:1 (*v*/*v*/*v*); 7 ml) was added, and stirring was continued overnight (16 h). The 3′,5′-cyclic *O*-methyl phosphate of *N*^{2}-(4-methoxytrityl)-2′-*C*,*O*^{6}-dimethylguanosine was isolated by CH_2Cl_2/5% aq. $NaHSO_3$ workup and subsequent CC(SiO_2; CH_2Cl_2 containing 5% MeOH). The identity of the product was verified by HR-ESI-MS (660.2207 ($[M+H]^+$; calc. 660.2218)). To remove the MMTr group, the product (0.53 mmol, 0.35 g) was dissolved in aq. 80% AcOH (5.0 ml), and the mixture was stirred overnight at r.t. (24 h). The mixture was evaporated to dryness, and the residue was co-evaporated twice with H_2O. The diastereoisomers were separated by HPLC eluting with 39% aq. MeOH. The product was obtained in 60% yield (60 mg + 63 mg). ^{1}H-NMR (500 MHz, CD_3OD): 8.00 (*s*, H–C(8)); 6.02 (*s*, H–C(1′)); 4.71–4.66 (*m*, H–C(3′), H–C(4′), H–C(5″)); 4.40 (*m*, H–C(5′)); 4.07 (*s*, COMe); 3.94, 3.91 (2*s*, POMe); 1.09 (*s*, 2′-Me). ^{13}C-NMR (126 MHz, CD_3OD): 161.40 (C(6)); 160.52 (C(2)); 153.00 (C(4)); 138.14 (C(8)); 114.32 (C(5)); 94.53 (C(1′)); 82.56, 82.49 (C(3′)); 76.64, 76.59 (C(2′)); 70.75, 70.71, 69.99, 69.89 (C(4′), C(5′)); 53.65, 53.60 (POMe); 52.90 (COMe); 18.29 (2′-Me). ^{31}P-NMR (202 MHz, CD_3OD): −3.56. HR-ESI-MS: 388.0992 ($[M+H]^+$, $C_{13}H_{19}N_5O_7P^+$; calc. 388.1022).

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