Synthesis of 3′,5′-Cylic Phosphate and Thiophosphate Esters of 2′-*C*-Methylribonucleosides

by Anna Leisvuori,[a] Zafar Ahmed,[a] Mikko Ora,[a]* Leonid Beigelman,[b] Lawrence Blatt,[b] Harri Lönnberg[a]

[a]University of Turku, Department of Chemistry, FIN-20014 Turku Finland (phone: +358-2-3336706; fax: +358-2-3336700; e-mail: mikora@utu.fi)

[b]AliosBiopharma, 260 E. Grand Ave, 2nd Floor, South San Francisco, CA 94080, U.S.A

Abstract

Introduction. - Inhibitors of RNA-dependent RNA polymerase of Hepatitis C virus (HCV) constitute a class of potential drugs with which to combat against this worldwide viral infection [1]-[3]. Several of the known inhibitors are derivatives of 2′-*C*-methylribonucleosides, including 2′-*C*-methyl-3′-*O*-(L-valinyl)cytidine (Valopicitabine) [4] [5], 4′-azido-3′,5′-di-*O*-(2-methylpropanoyl)cytidine [6] and 7-deaza-2′-*C*-methyladenosine in addition to unsubstituted 2′-*C*-methylribonucleosides [7]. 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 [8]. Similarly, various alkyl [9], aryl [9], acyloxymethyl [10] and 2-(acylthio)ethyl esters of 3′,5′-cylic phosphates of base modified

2´-*C*-methylribonucleosides 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 (**1**) and 2´-*C*,O^6-dimethylguanosine (**2**), and the *S*-(pivaloyloxymethyl) ester **2** (**3**) and and the *O*-methyl ester of 2´-*C*,O^6-dimethylguanosine 3´,5´-cyclic phosphate (**4**). While all these nucleotides may exhibit antiviral activity, compound **3** expectedly is the most potent pro-drug candidate. The pivaloyloxymethyl group is expectedly 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.

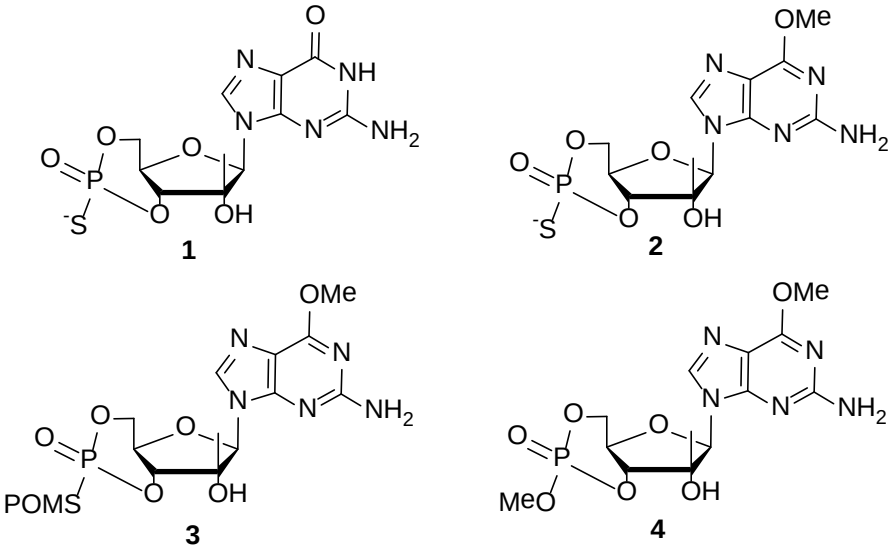
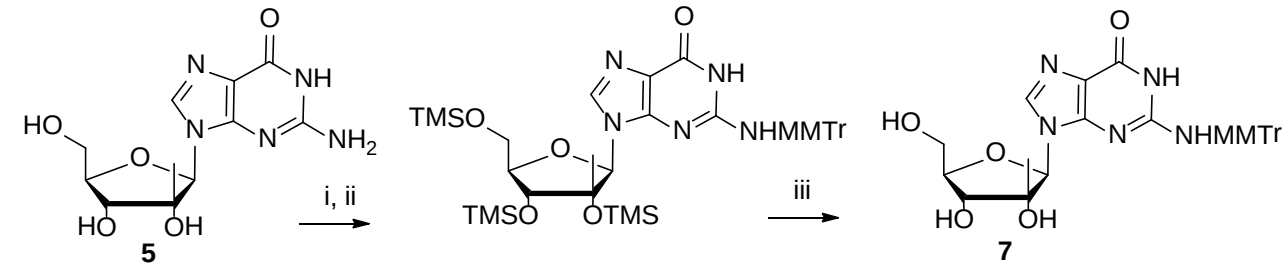


Fig. 1. *Structures of potential pro-drugs of HCV antiviral 2´-*C*-methylguanosine*

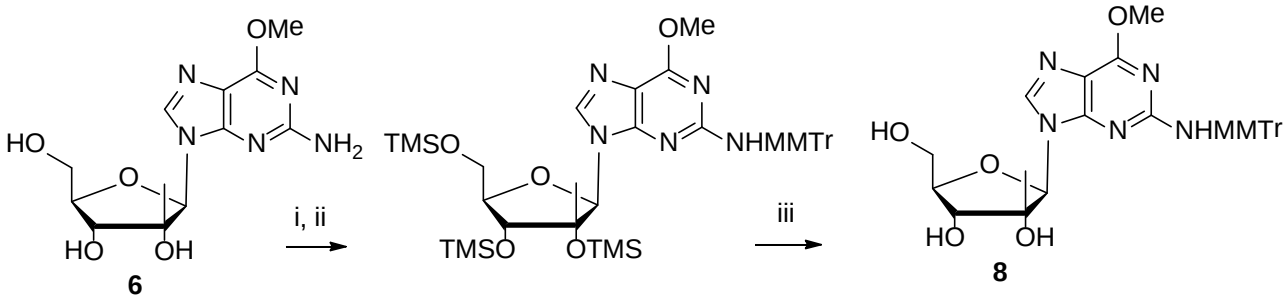
Results and Discussion. - 2'-*C*-Methylguanosine (**5**) was prepared by glycosylation of persilylated N^2-acetylguanine, *i. e.* 6-(trimethylsilyloxy)-2-(*N*-trimethylsilylacetamido)-9-

(trimethylsilyl)purine, with 1,2,3,5-tetra-*O*-benzoyl-2-*C*-methylribofuranose, as described previously by Piccirilli *et al* [11]. 2´-*C*,O^6-Dimethylguanosine (**6**) was, in turn, obtained by glycosylation of 6-chloroguanine with the same glycosyl donor, followed by treatment with NaOMe in MeOH, as described previously by McGuigan *et al* [5]. 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 groups and the amino function was then alkylated with 4-methoxytrityl chloride.

Scheme 1. *Preparation of 2´*-C-*methyl- and 2´*-C,O^6-*dimethyl-guanosine.*



i) TMSCl, Py; ii) MMTrCl; iii) NH_3



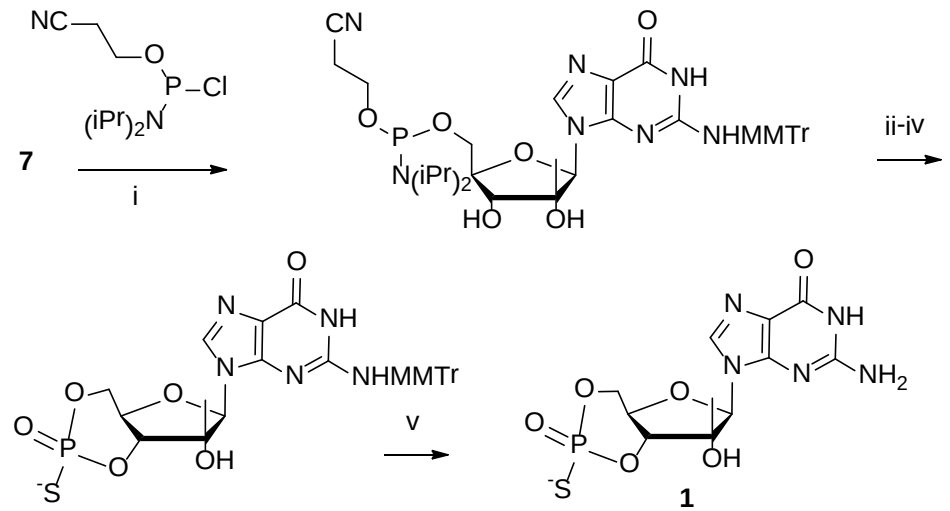
i) TMSCl, Py; ii) MMTrCl, Py; iii) TBAF, THF

Finally, the silyl protections were removed. With 2´,3´,5´-tri-*O*-(trimethylsilyl)-N^2-(4-methoxytrityl)-2´-*C*,O^6-dimethylguanosine, the removal of the 2´-O-$SiMe_3$ group turned out to be surprisingly difficult. Treatment with TBAF in THF was required instead of the ammonia treatment, which usually is sufficient to remove the trimethylsilyl protecting groups from sugar hydroxy functions.

Conversion of the tritylated 2´-*C*-methylnucleosides, **7** and **8,** to 3´,5´-cyclic phosphorothioates (**1**,**2**), 3´,5´-cyclic *S*-pivaloyloxymethyl phosphorothioate (**3**) and 3´,5´-cyclic *O*-methyl phosphate (**4**) is depicted in *Schemes 2-5*. Phosphitylation of N^2-(4-methoxytrityl)-2´-*C*-methylguanosine (**7**) with 2-cyanoethyl-*N*,*N*-diisopropylchlorophosphoramidite (*Scheme 2*) and subsequent tetrazole promoted intramolecular displacement of the diisopropylamino ligand by the 3´-hydroxy group gave a 3´,5´-cyclic phosphite triester, which was sulfurized with 3*H*-1,2-benzodithiol-3-one 1,1-dioxide (Beaucage reagent) in MeCN to N^2-(4-methoxytrityl)-2´-*C*-methylguanosine 3´,5´-cyclic *O*-(2-cyanoethyl) phosphorothioate. The 2-cyanoethyl group was cleaved during silica gel chromatographic purification on eluting with DCM containing 1% triethylamine. The 4-methoxytrityl group was then removed by treatment with aq. 80% AcOH, giving 2´-*C*-methylguanosine 3´,5´-cyclic phosphorothioate (**1**).

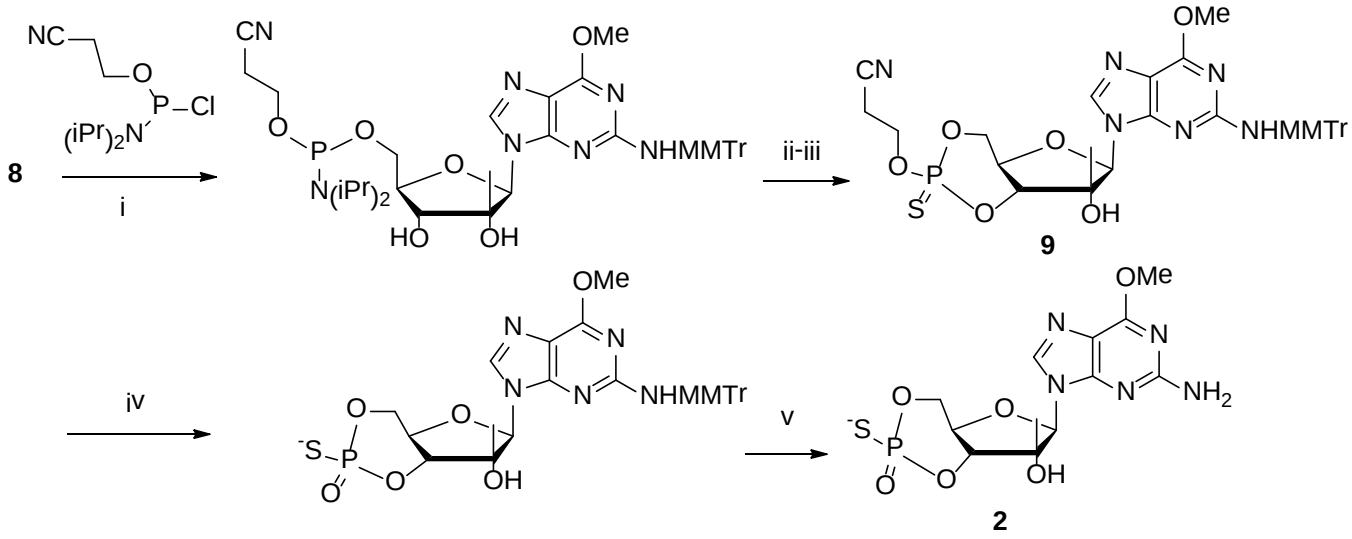
The same method was applied to preparation of N^2-(4-methoxytrityl)-2´-*C*,O^6-dimethylguanosine 3´,5´-cyclic phosphorothioate (**2**). Accordingly, N^2-(4-methoxytrityl)-2´-*C*,O^6-dimethylguanosine (**8**) was phosphitylated with 2-cyanoethyl-*N*,*N*-diisopropylchlorophosphoramidite and the *N*,*N*-diisopropylamino ligand was replaced with 3´-OH group using tetrazole as an activator (*Scheme 3*). The phosphite triester obtained was sulphurized to thiophosphate ester **9** with elemental sulfur. The desired fully deprotected 3´,5´-

Scheme 2. *Preparation of 2´-*C*-methylguanosine 3´,5´-cyclic phosphorothioate.*



i) TEA, DCM; ii) TetH, MeCN; iii) 3H-1,2-benzodithiol-3-one 1,1-dioxide; iv) Silica gel chromatography eluting with DCM/TEA; v) 80% AcOH

Scheme 3. *Preparation of 2´-*C*,O^6-dimethylguanosine 3´,5´-cyclic phosphorothioate.*

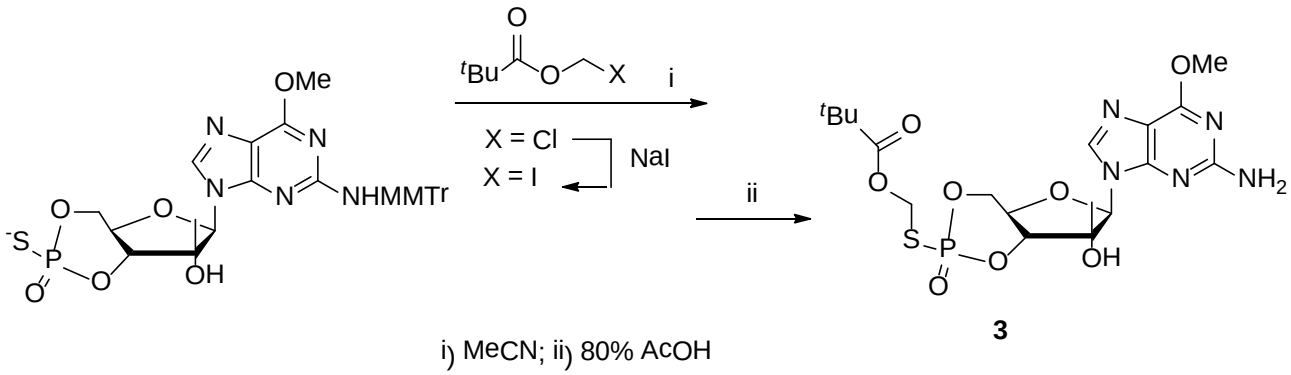


i) TEA, DCM; ii) TetH, MeCN; iii) S_8; iv) TEA, DCM; vii) MeCN; vi) 80% AcOH

cyclic phosphorothioate **2** was obtained by removal of the 2-cyanoethyl group with TEA in DCM and the 4-methoxytrityl group with aqueous acetic acid.

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 pivaloyloxymethyl group by treating **2** with iodomethyl pivalate in MeCN (*Scheme 4*). The 4-methoxytrityl group was then removed by treatment with aq. 80% AcOH to afford 2´-*C*,O^6-dimethylguanosine 3´,5´-cyclic *O*-pivaloyloxymethyl phosphorothioate (**3**).

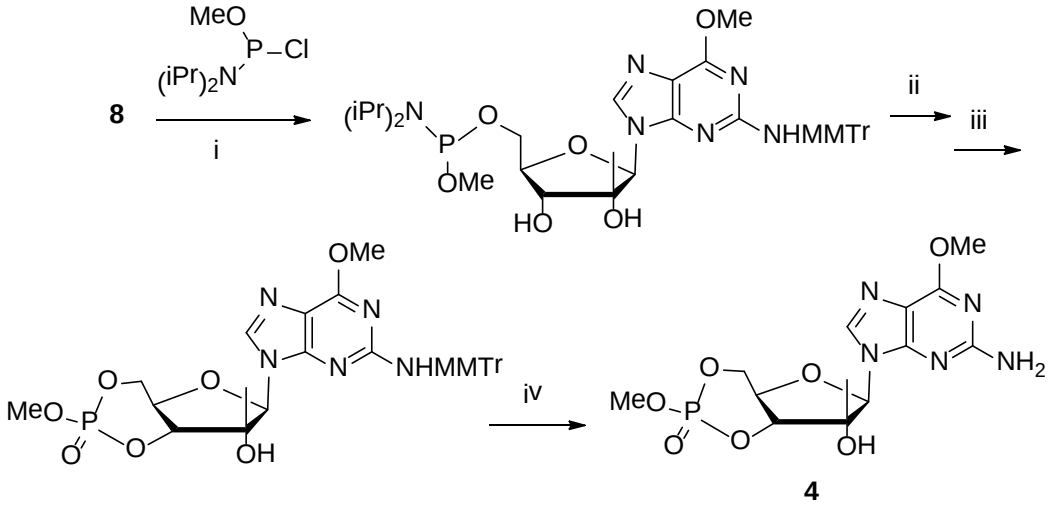
Scheme 4. *Preparation of the* S-*pivaloyloxymethyl ester of 2´*-C,O^6-*dimethylguanosine 3´,5´-cyclic phosphorothioate.*



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*-diisopropylchlorophosphoramidite and the diisopropylamino ligand was then displaced with the 3´-OH group, as described above. The resulting cyclic phosphite triester was oxidized to

phosphate ester with iodine in aqueous THF containing 2,6-lutidine, and the 4-methoxytrityl group was removed by acetic acid treatment to afford 2´-*C*,O^6-dimethylguanosine 3´,5´-cyclic *O*-methyl phosphate (**4**; Scheme 5).

Scheme 5. *Preparation of the methyl ester of 2´-*C,O^6*-dimethylguanosine 3´,5´-cyclic phosphate.*



i) TEA, DCM; ii) TetH, MeCN; iii); iv) 80% AcOH

Experimental

***N*^{2}-(4-Methoxytrityl)-2´-*C*-methylguanosine (7).** 2'-*C*-Methylguanosine (**5;** 0.520 g, 1.74 mmol) was coevaporated 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 room temperature for 2 hours. The mixture was cooled on an ice bath and MMTrCl (2.70 g, 8.74 mmol) in pyridine was added. After stirring overnight (20 h) at room temperature, the mixture was cooled on an ice bath and cold water (3 mL) was added. After 5 min stirring, concentrated aqueous ammonia (33%; 5 mL) was added

and the stirring was continued for 30 minutes. The solvent was removed by evaporation under reduced pressure. The residue was dissolved in DCM and the organic phase was washed with water, dried over Na_2SO_4 and evaporated to dryness. The product was purified by Silica gel chromatography eluting with a 1:9 mixture of MeOH and DCM. Compound **7** was obtained as solid in 38% yield (0.37 g). ^{1}H-NMR (400 MHz, CD_3OD) δ: 8.16 (s, 1H, H8), 7.37-7.19 (12H, MMTr), 6.85 (d, 2H, *J* = 8.91 Hz, MMTr), 5.30 (s, 1H, H1´), 3.97 (d, *J* = 9.1 Hz, 1H, H3´), 3.92 (dd, 1H, *J* = 12.5 Hz and 2.1 Hz, H5´), 3.87 (m, 1H, H4´), 3.75-3.72 (m, 4H, CH_3 of MMTr and H5´´), 0.55 (s, 3H, 2´-CH_3). ^{13}C-NMR (101 MHz, CD_3OD) δ: 158.48 (C6), 157.97 (MMTr); 151.07 (C4), 150.11 (C2), 144.94 and 144.85 (MMTr), 136.72 (MMTr), 136.28 (C8), 130.04, 128.64, 127.45 and 126.45 (MMTr), 116.10 (C5); 112.76 (MMTr), 90.17 (C1´), 82.36 (C4´), 78.89 (C2´), 71.56 (C3´), 70.38 (spiro C); 59.23 (C5´), 54.32 (OMe), 18.48 (2´-Me). HRMS: $[M+H]^+$ obsd. 570.2381, calcd. 570.2353.

***N*^{2}-(4-Methoxytrityl)-2´-*C*,*O*^{6}-dimethylguanosine (8).** To a precooled solution 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 room temperature for 2 hours. MMTrCl (1.28 g, 4.17 mmol) was added and the mixture was kept at 50 °C for 20 hours. The solvent was removed by evaporation under reduced pressure. The residue was dissolved in DCM and washed twice with saturated aq $NaHCO_3$ and brine, and evaporated to dryness. To remove the trimethylsilyl groups, the residue, *N*^{2}-(4-methoxytrityl)-2´,3´,5´-tri-*O*-trimethylsilyl-2´,*O*^{6}-dimethylguanosine (MS-ESI$^+$ *m/z* $[M+H]^+$ = 800.9), was dissolved in dry THF and Bu_4NF hydrate was added. The solution was stirred for 90 min at room temperature. The mixture was evaporated to dryness and the residue was dissolved in DCM and treated with saturated aq $NaHCO_3$. The organic phase was

evaporated to dryness. The residue was purified by Silica gel chromatography using DCM containing 5% MeOH as eluent. Compound **8** was obtained as white solid in 53% yield (1.27 g). ^{1}H-NMR (500 MHz, CD_3OD) δ: 8.27 (s, 1H, H8), 7.41-7.16 (12H, MMTr), 6.80 (d, 2H, *J* = 5.0 Hz, MMTr), 5.77 (s broad, 1H, H1´), 4.11 (1H, H3´), 3.98-3.96 (m, 2H, H4´, H5´´), 3.79 (1H, 54´), 3.74 (OMe),3.31 (OMe of MMTr), 0.79 (s broad, 3H, Me). ^{13}C-NMR (500 MHz, CD_3OD) δ: 160.0 (C6), 158.30 (MMTr), 152.59 (C2); 145.90 and 145.85 (C4 and MMTr), 137.93 and 139.91 (MMTr and C8), 130.04, 128.64, 127.45 and 126.15 (MMTr), 113.79 (C5), 112.49 (MMTr), 90.17 (C1´), 82.36 (C4´), 78.91 (C2´), 71.87 (C3´), 70.35 (spiro C of MMTr); 59.45 (C5´), 54.28 (OMe of MMtr), 53.02 (OMe), 18.79 (2´-Me). HR-ESI-MS: $[M+Na]^+$ obsd. 584.2363, calcd. 584.2504.

2´-*C*-Methylguanosine 3´,5´-cyclic phosphorothioate (1). N^2-(4-Methoxytrityl)-2´-*C*-methylguanosine (**7**) (250 mg, 0.438 mmol) dried over P_2O_5 overnight, was dissolved in dry DCM (3 mL) under argon. Dry triethylamine (0.288 mL, 2.06 mmol) and 2-cyanoethyl-*N*,*N*-diisopropylchlorophosphoramidite (0.103 mL, 0.46 mmol) were added and the reaction mixture was stirred at room temperature for 40 min. The product was isolated by passing the mixture through a short silica gel column with dry EtOAc containing 0.5% Et_3N. The solvent was removed under reduced pressure. The product was coevaporated with dry MeCN (2 × 20 mL) to remove the traces of Et_3N. The phosphitylated nucleoside was dissolved in dry MeCN (60 mL) under argon and tetrazole (1.03 mmol, 2.25 mL of 0.45 mol L^{-1} solution in MeCN) was added. The mixture was stirred for 3 hours and then 3H-1,2-benzodithiol-3-one 1,1-dioxide (185 mg, 0.877 mmol) was added and the stirring was continued overnight. The reaction mixture was evaporated to dryness. The crude was purified by silica gel chromatography eluting with DCM

containing 15% MeOH and 0.5% TEA. The 2-cyanoethyl group was cleaved during the silica gel chromatography 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 monomethoxytrityl group, N^2-(4-methoxytrityl)-2´-*C*-methylguanosine 3´,5´-cyclic phosphorothioate (71 mg, 0.08 mmol) was treated with aq 80% acetic acid (2.0 mL) at room temperature and the mixture was stirred overnight. The mixture was evaporated to dryness and the residue was coevaporated twice with water. The crude was purified by silica gel chromatography eluting with DCM containing 10% MeOH. The triethylammonium salt of compound **1** was obtained as solid form in 73% (38 mg). The ^{13}C-NMR spectra of compound **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) δ: ^{1}H-NMR (400 MHz, D_2O) δ: 7.90 and 7.84 (s broad, 1H, H8), 5.90 (s broad, 1H, H1´), 4.97 (1H, H3´), 4.50-4.35 (2H, H4´, H5´´), 4.21 (1H, H5´), 3.37 (s broad, 1H, 2´OH) and 1.00 (s broad, 3H, 2´-CH_3). ^{31}P-NMR (162 MHz, DMSO) δ: 56.28 and 55.06. HRMS: $[M+Na]^+$ obsd. 398.0299, calcd. 398.0299.

N^2-(4-Methoxytrityl)-2´,O^6-dimethylguanosine 3´,5´-cyclic *O*-(2-cyanoethyl) phosphorothioate (9). Compound **8** (0.625 g, 1.07 mmol) was dried over P_2O_5 overnight and dissolved in dry DCM (5 mL). Anhydrous 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 argon at room temperature. The phosphitylated product was isolated by passing the mixture through a short silica gel column with dry EtOAc containing 0.5% triethylamine. The solvent was removed under reduced pressure and the crude was coevaporated

twice with dry MeCN. The residue was dissolved in dry MeCN (60 mL) and 0.45 M solution of tetrazole in MeCN (5.95 mL, 5.80 mmol) was added. After 2.5 hours stirring at room temperature, dry pyridine (80 mL) and elemental sulphur (245 mg, 7.65 mmol) were added and the stirring was continued overnight. After filtration, the reaction mixture was evaporated to dryness. The residue was dissolved in DCM and the organic layer was washed with aq $NaHCO_3$ and brine, dried over Na_2SO_4 and evaporated to dryness. The crude was purified by silica gel chromatography eluting with DCM containing 3% MeOH and 0.5% pyridine. Compound **9** was obtained as a mixture of R_P- and S_P-diastereomers in 21% yield (156 mg by repeating the synthesis). ^{1}H-NMR (500 MHz, $CDCl_3$) δ: 7.58 (s, 1H, H8); 7.38-7.20 (m, 12H, MMTr), 6.85-6.78 (m, 2H, MMTr), 6.53 (s, 1H, H1´), 5.76 (s broad 1H, NH), 4.55-4.37 (m, 5H, H3´, H5´, H5´´, CH_2O), 3.90 (dd, J = 6.3 and 6.3 Hz, H4´), 3.83 (s, 6H, MMTr and 6-OMe), 2.82-2.75 (m, 2H, CH_2CN), 1.09 (s, 3H, 2´-Me). ^{13}C-NMR (126 MHz, $CDCl_3$) δ: 160.50 (C6), 158.20, 158.20-158.13 (MMTr), 158.10 (C4), 152.17 (C2), 145.80 (C8), 144.46 (MMTr), 135.69 (MMTr), 130.5-126.5 (MMTr), 118.32 (CN), 116.62 (C5), 113.5-113.0 (MMTr), 94.70 (C1´), 86.76 (spiro C of MMTr), 80.90 (C3´), 70.61 and 70.05 (C4´ and C2´), 57.67 (C5´), 55.29 (OMe of MMTr), 54.05 (OMe), 51.99 (CH_2O), 19.73 (2´-Me), 19.58 (CH_2CN). ^{31}P-NMR (202 MHz, CD_3OD) δ: 83.57 and 67.84. HRMS: HR-ESI-MS: $[M+H]^+$ obsd. 715.2087, calcd. 715.2098.

2´-*C*,*O*^{6}-Dimethylguanosine 3´,5´-cyclic phosphorothioate (2). To remove the 2-cyanoethyl group, compound **9** (230 mg, 0.32 mmol) was dissolved in a mixture of dry DCM (1.5 mL) and dry Et_3N (2.0 mL). The mixture was stirred at room temperature for 18 hours, evaporated to dryness and coevaporated 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 reaction mixture was stirred at room tempertature for 5 hours and evaporated to dryness. The crude product was purified by HPLC eluting with 15% MeOH in H_2O. The diastereomers were not separated. The product was dissolved in H_2O and passed through Dowex Na^+-form (50W, 100-200 mesh). The product was obtained as white solid in 32% yield (42 mg). ^{1}H-NMR (500 MHz, CD_3OD) δ: 7.95 (s, 1H, H8); 5.94 (s, 1H, H1´), 4.61-4.22 (4H, H3´, H4´, H5´´ and H5´), 4.07 (6-OMe), 1.06 (s, 1H, 2´-Me). ^{13}C-NMR (126 MHz, CD_3OD) δ: 161.34 (C6), 160.34 (C2), 153.00 (C4), 138.12 (C8), 114.25 (C5), 94.33 (C1´), 80.40 (C3´), 77.06 and 77.29 (C2´), 72.50 (C4´), 66.90 and 69.85 (C5´), 52.80 (OMe), 18.51 (2´-Me). ^{31}P-NMR (202 MHz, DMSO) δ: 55.97. HR-ESI-MS: $[M-H]^-$ obsd. 388.0482, calcd. 388.0486.

2´-*C*,*O*^{6}-Dimethylguanosine 3´,5´-cyclic *O*-pivaloyloxymethyl phosphorothioate (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 reaction mixture was stirred at room temperature overnight in dark. The mixture was evaporated to dryness, dissolved in DCM and washed with 5% aq $NaHSO_3$ and brine. The organic layer was dried over 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 g, 0.23 mmol) was added. The reaction mixture was stirred for 2.5 hours at room temperature. Saturated aq. $NaHCO_3$ was added and the crude product was extracted with DCM. The organic layer was dried over Na_2SO_4 and evaporated to dryness. The residue was dissolved in 80% aq. AcOH (2.0 ml) and the reaction mixture was stirred at RT for 20 hours. The reaction mixture was evaporated to dryness and the residue was coevaporated

twice with water. The crude product **3** was purified by silica gel chromatography eluting with DCM containing 10% MeOH. Compound **3** was obtained as white solid in 14% yield (15.0 mg). ^{1}H-NMR (500 MHz, CD_3OD) δ: 7.95 (s, 1H, H8); 5.93 (s, 1H, H1´), 5.58-5.54 (m, 2H, SCH_2), 4.80-4.69 (m, 3H, H3´, H4´, H5´´); 4.45 (m, 1H, H5´); 4.06 (s, 3H, OMe); 1.20 (s, 3H, $C(Me)_3$); 1.10 (s, 1H, 2´-Me). ^{13}C-NMR (126 MHz, CD_3OD) δ: 177.48 (C=O), 161.50 (C6), 160.22 (C2), 152.66 (C4), 139.14 (C8), 129.34 (C5), 95.15 (C1´), 81.87 (C3´), 76.76 and 76.70 (C2´), 71.00, 70.93, 70.80 and 70.81 (C4´ and C5´), 60.23 and 60.20 (SCH_2), 52.87 (OMe), 38.52 (spiro C of Piv), 25.85 ($C(Me)_3$), 18.18 (2´-Me). ^{31}P-NMR (202 MHz, CD_3OD) δ: 23.13 HRMS: HR-ESI-MS: $[M+H]^+$ obsd. 504.1323, calcd. 504.1312.

2´-*C*,*O*^{6}-Dimethylguanosine 3´,5´-cyclic *O*-methyl phosphate (4). *N*^{2}-(4-Methoxytrityl)-2´-*C*,*O*^{6}-dimethylguanosine (**8**) (1.80 mmol; 1.05 g) dried over P_2O_5 overnight was dissolved in dry DCM (8 mL) under nitrogen. Dry triethylamine (9.00 mmol; 1.25 ml) and methyl-*N*,*N*-diisopropylchlorophosphoramidite (1.99 mmol; 385 μl) were added and the reaction mixture was stirred at room temperature for 30 min. The product was isolated by passing the mixture through a short silica gel with dry hexane containing 30% dry EtOAc and 0.5% dry Et_3N. The solvent was removed under reduced pressure and the residue was coevaporated three times with dry MeCN to remove the traces of Et_3N. The phosphitylated nucleoside was dissolved in dry MeCN (70 mL) under nitrogen and tetrazole (4.50 mmol; 10.00 ml of 0.45 mol L^{-1} solution in MeCN) was added. The mixture was stirred for 3 hours. After 2 hours stirring at room temperature, iodine (15.30 mmol; 0.49 g) was added and the 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 DCM / 5% aq. $NaHSO_3$ workup and subsequent Silica gel chromatography eluting

with DCM containing 5% MeOH. The identity of the product was verified by HR-ESI-MS: $[M+H]^+$ obsd. 660.2207, calcd. 660.2218. To remove the monomethoxytrityl 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 room temperature (24 h). The reaction mixture was evaporated to dryness and the residue was coevaporated twice with water. The diastereomers 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) δ: 8.00 (s, 1H, H8); 6.02 (s, 1H, H1´), 4.71-4.66 (m, 3H, H3´, H4´, H5´´); 4.40 (m, 1H, H5´); 4.07 (s, 3H, COMe); 3.94 and 3.91 (2 x s, 3H, POMe); 1.09 (s, 1H, 2´-Me). ^{13}C-NMR (126 MHz, CD_3OD) δ: 161.40 (C6), 160.52 (C2), 153.00 (C4), 138.14 (C8), 114.32 (C5), 94.53 (C1´), 82.56 and 82.49 (C3´), 76.64 and 76.59 (C2´), 70.75, 70.71, 69.99 and 69.89 (C4´ and C5´), 53.65 and 53.60 (POMe), 52.90 (COMe), 18.29 (2´-Me). ^{31}P-NMR (202 MHz, DMSO) δ: -3.56. HRMS: $[M+H]^+$ obsd. 388.0992, calcd. 388.1022.

References

1) Bobeck, D.R.; Schinazi, R. F.; Coats, S. J. *Antiviral Therapy* **2010**, *15*. 935.
2) De Francesco, R.; Migliaccio, G. *Nature* **2005**, *436*, 953-960.
3) Cheng, K.-C.; Gupta, S.; Wang, H.; Uss, A. S.; Njoroge, G. F.; Hughes, E. *J. Pharm. Pharmacol.* **2011**, *63*, 883–892
4) Afdhal, N.; O'Brien, C.; Godofsky, E.; Rodriguez-Torres, M.; Pappas, S. C.; Pockros, P.; Lawitz, E.; Bzowej, N.; Rustgi, V.; Sulkowski, M.; Sherman, K.; Jacobson, I.; Chao, G.; Knox, S.; Pietropaolo, K.; Brown, N. *Hepatology* **2006**, *44*, S19.
5) Dieterich, D. et al. *Hepatology* **2006**; *44*, S271.

6) Roberts, S. K.; Cooksley, G.; Dore, G. J.; Robson, R.; Shaw, D.; Berns, H.; Hill, G.; Klumpp, K.; Najera, I.; Washington, C. *Hepatology* **2008**, *48*, 398.

7) Eldrup, A. B.; Allerson, C. R.; Bennett, C. F.; Bera, S.; Bhat, N.; Bosserman, M. R.; Brooks, J.; Burlein, C.; Carroll, S. S.; Cook, P. D.; Getty, K. L.; MacCoss, M.; McMasters, D. R.; Olsen, D. B.; Prakash, T. P.; Prhavc, M.; Song, Q.; Tomassini, J. E.; Xia, J. *J. Med. Chem.* **2004**, *47*, 2283.

8) C. McGuigan, K. Madela, M. Aljarah, A. Gilles, A. Brancale, N. Zonta, S. Chamberlain, J. Vernachio, J. Hutchins, A. Hall, B. Ames, E. Gorovits, B. Ganguly, A. Kolykhalov, J. Wang, J. Muhammad, J. M. Patti, G. Henson, *Bioorg. Med. Chem. Lett***. 2010**, *20*, 4850-4854.

9) Reddy, P. G.; Bao, D.; Chang, W.; Chun, B.-K.; Du, J.; Nagarathnam, D; Rachakonda, S.; Ross, B. S.; Zhang, H.-R.; Bansal, S.; Espiritu, C. L.; Keilman, M.; Lam, A. M.; Niu, C.; Steuer, H. M.; Furman, P. A.; Otto, M. J.; Sofia, M. J. *Bioorg. Med. Chem. Lett.* **2010**, *20*, 7376-7380.

10) Gunic, E.; Girardet, J.-L.; Ramasamy, K.; Stoisavljevic-Petkov, V.; Chow, S.; Yeh, L.-T.; Hamatake, R. K.; Raney, A.; Hong, Z. *Bioorg. Med. Chem. Lett.* **2007**, *17*, 2452-2455.

11) Li, N.S.; Piccirillí, J. A. *J. Org. Chem.* **2006**, *71*, 4018-4020.