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The Synthesis of 2′-*O*-[(Triisopropylsilyl)oxy] methyl (*TOM*) Phosphoramidites of Methylated Ribonucleosides (m^1G, m^2G, $m^2{}_2G$, m^1I, m^3U, m^4C, m^6A, $m^6{}_2A$) for Use in Automated *RNA* Solid-Phase Synthesis

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Summary. The straightforward synthesis of eight methylated ribonucleoside phosphoramidites is described. These building blocks allow for incorporation of the naturally occuring nucleosides 1-methylguanosine (m^1G), N^2-methylguanosine (m^2G), N^2,N^2-dimethylguanosine ($m^2{}_2G$), 1-methylinosine (m^1I), 3-methyluridine (m^3U), N^4-methylcytidine (m^4C), N^6-methyladenosine (m^6A), and N^6,N^6-dimethyladenosine ($m^6{}_2A$) into oligoribonucleotides by automated *RNA* solid-phase synthesis. In all cases, the ribose 2′-hydroxyl group of the building blocks is masked by the recently introduced [(triisopropylsilyl)oxy]methyl (*TOM*) group.

Keywords. Phosphoramidites; *TOM*-chemistry; *RNA* solid-phase synthesis; Methylation; Modified nucleosides.

Introduction

The rapid growth of demand for chemically synthesized *RNA*s is unequivocally associated with the biological phenomenon of *RNA* interference (*RNA*i) [1]. Thereby, double-stranded *RNA* effects the silencing of genes which are homologous in sequence to either of the *RNA* strands in the duplex [2]. The phenomenon results

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from degradation of the corresponding m*RNA* and can also be induced efficiently by very short duplex *RNA* of 21- to 23-base pairs, so-called small interfering *RNA*s (si*RNA*) [3]. si*RNA* is the upcoming gene silencing methodology and the key components of this new technology are short chemically synthesized oligoribonucleotides [4].

Most approaches for the chemical synthesis of *RNA* oligonucleotides have focused on retaining the established *DNA* protecting group concept of the acid-labile

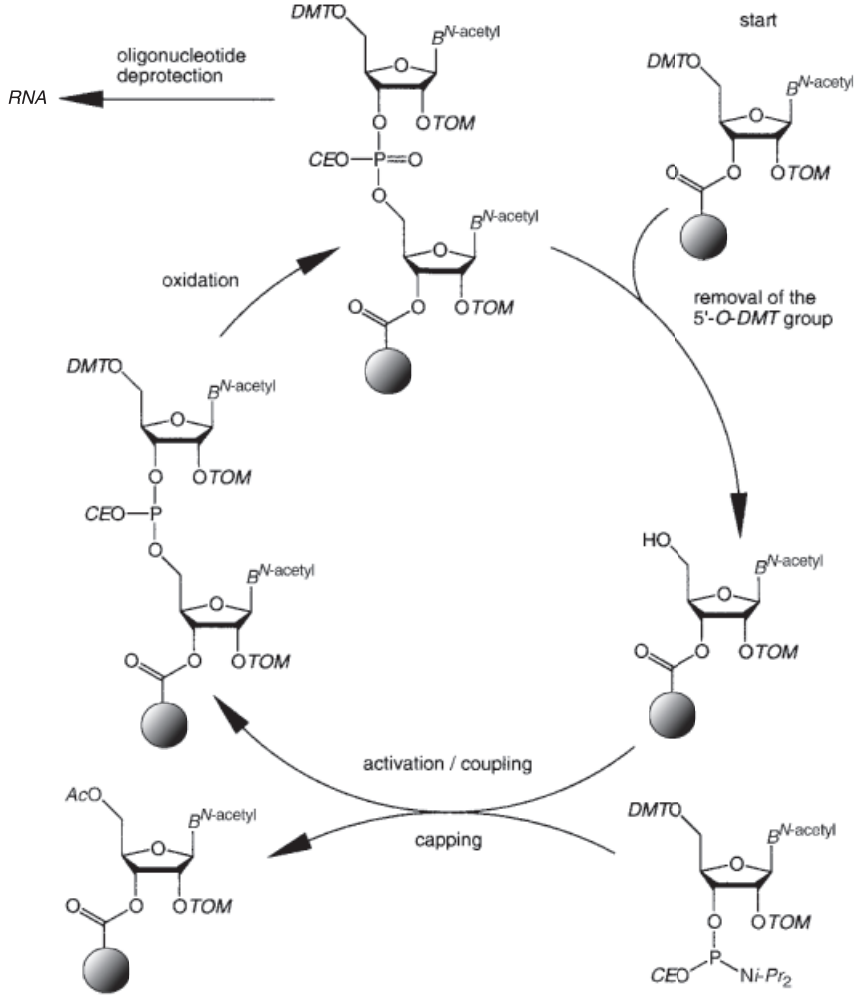

Fig. 1. Scheme of the automated *RNA* solid-phase synthesis using *N*-acetyl-2′-*O*-[(triisopropylsilyl)oxy]methyl (*TOM*) protected phosphoramidites; *synthesis cycle*: (1) removal of the 5′-*O*-*DMT* group: 4% dichloroacetic acid in 1,2-dichloroethane, 90 s; (2) activation and coupling: 0.25 *M* benzylthiotetrazole (65 eq)/0.1 *M* cyanoethyl phosphoramidite (6 eq) in acetonitrile, 90 s; (3) capping: Ac_2O/2,6-lutidine/*THF* (1/1/8, *v*/*v*) and *N*-methylimidazole/*THF* (16/84, *v*/*v*), 60 s; (4) oxidation: I_2/H_2O/pyridine/*THF* (3/2/20/75, *w*/*w*), 45 s; *oligonucleotide deprotection*: (1) 10 *M* *Me*NH_2 in *Et*OH/H_2O (1/1), 1–24 h, 25–33°C; (2) 1 *M* $Bu_4NF \cdot 3H_2O$ in *THF*, 1–50 h, 25°C; (3) 1 *M* *Tris* · HCl, H_2O, *pH* 7.4; 4,4′-dimethoxytrityl (*DMT*), 2-cyanoethyl (*CE*), α,α,α-tris(hydroxymethyl)methylamin (*Tris*)

4,4′-dimethoxytrityl group (*DMT*) at the ribose 5′-hydroxyl group combined with masking of the nucleophilic exocyclic amino groups of adenine, cytosine, and guanine by base-labile acyl protecting groups [5]. From the large number of documented ribose 2′-*O* protecting groups, the fluoride-labile *tert*-butyldimethylsilyl (*TBDMS*) group has found the widest application [6]. In 1998, the [(triisopropylsilyl)oxy]methyl (*TOM*) protection of the ribose 2′-OH was introduced by *S. Pitsch et al.* [7–9]. The high performance of this protecting group in automated *RNA* solid-phase synthesis has soon resulted in wide acceptance for the '*TOM*-chemistry' (Fig. 1) [4].

Here, we describe the synthesis and characterization of eight 2′-*O*-*TOM* nucleoside phosphoramidite building blocks that are methylated at the nucleobases according to naturally occuring methylation patterns. These building blocks allow for the incorporation of 1-methylguanosine (m^1G), N^2-methylguanosine (m^2G), N^2,N^2-dimethylguanosine ($m^2{}_2G$), 1-methylinosine (m^1I), 3-methyluridine (m^3U), N^4-methylcytidine (m^4C), N^6-methyladenosine (m^6A), and N^6,N^6-dimethyladenosine ($m^6{}_2A$) into oligoribonucleotides by standard automated *RNA* solid-phase synthesis (Fig. 2).

Following nucleosides with 2′-*O* methylation, nucleosides that are methylated at their nucleobases account for the second largest number of naturally occuring nucleoside modifications. They are encountered in all major *RNA* species, such as t*RNA*, r*RNA*, sn*RNA*, and m*RNA* [10]. The function of the majority of nucleoside modifications is far from being well understood. Straightforward synthetic procedures that guarantee the unlimited availability of modified nucleotide building blocks for their use in *RNA* solid-phase synthesis enable detailed investigation of structure-function relations of unmodified *versus* modified oligoribonucleotides. Studies of these kind contribute to rationalizing the impact of *RNA* modifications

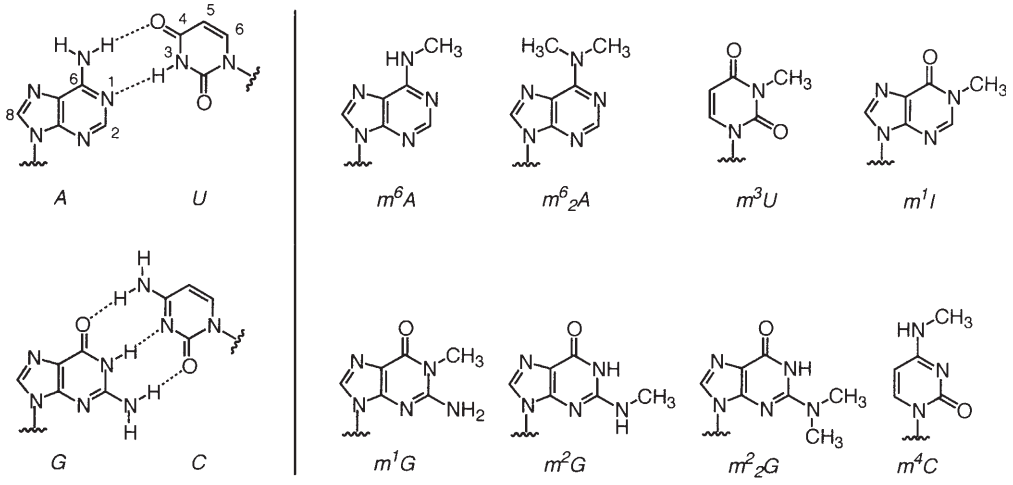

Fig. 2. Selection of naturally occuring, methylated ribonucleosides that were elaborated to 2′-*O*-*TOM* protected phosphoramidite building blocks for *RNA* solid-phase synthesis; 1-methylguanosine (m^1G), N^2-methylguanosine (m^2G), N^2,N^2-dimethylguanosine ($m^2{}_2G$), 1-methylinosine (m^1I), 3-methyluridine (m^3U), N^4-methylcytidine (m^4C), N^6-methyladenosine (m^6A), N^6,N^6-dimethyladenosine ($m^6{}_2A$)

on important cellular processes such as m*RNA* and r*RNA* maturation, ribosome assembly, r*RNA* processing, translation of the genetic code, recognition of t*RNA*s, *RNA* folding, and many more [11].

Results and Discussion

General Strategy for the Synthesis of Nucleobase-Methylated Ribonucleotide Phosphoramidites

Starting from the inexpensive ribonucleosides adenosine, cytidine, guanosine, uridine, and inosine, the synthesis of the corresponding methylated nucleoside phosphoramidites was carried out by chemical manipulation at the nucleobase first, introducing the methyl and the base protecting groups, followed by stepwise introduction of the 5′-*O-DMT* group, the 2′-*O-TOM* group, and finally the 3′-*O*-(2-cyanoethyl diisopropylphosphoramidite) (*CEP*) moiety (Fig. 3). Only in the case of m^4C we prefered an alternative route starting from the prefunctionalized derivative 5′-*O-DMT*-2′-*O-TOM*-uridine with subsequent transformation of the nucleobase to obtain higher overall yields.

5′-*O*-Tritylation

The conditions we prefered for tritylation of the 5′-OH were 1.1–1.6 equivalents of *DMT*-Cl added in portions to a 0.2–0.8 *M* solution of the corresponding methylated nucleoside in anhydrous pyridine. By-products such as 2′-*O* and 3′-*O* tritylated

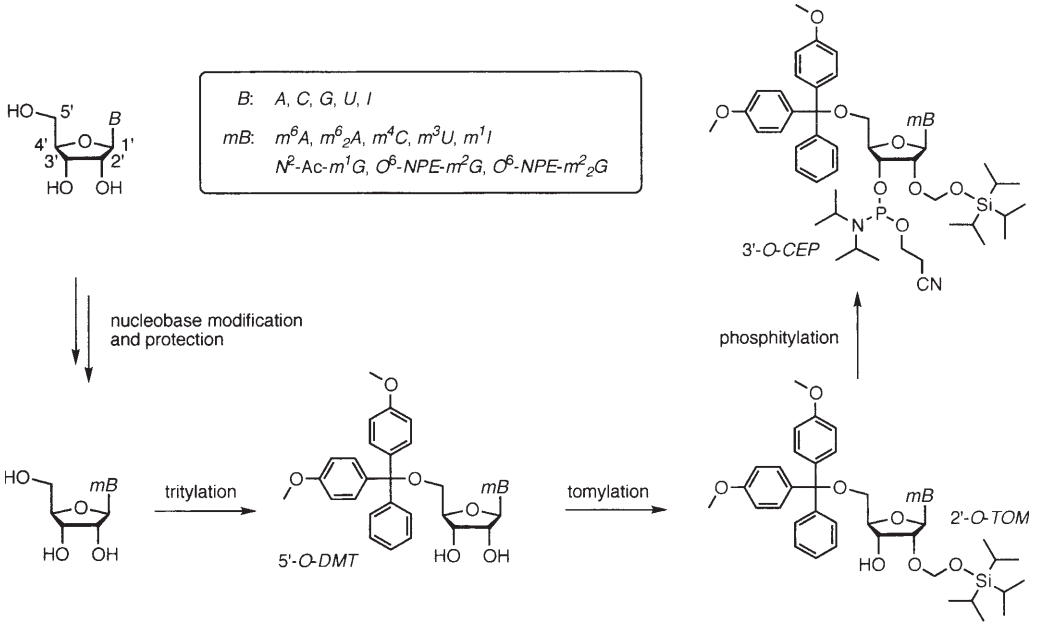

Fig. 3. Synthetic concept for the preparation of methylated 2′-*O-TOM* protected nucleoside phosphoramidites; 2-(4-nitrophenyl)ethyl (*NPE*), 2-cyanoethyl diisopropylphosphoramidite (*CEP*)

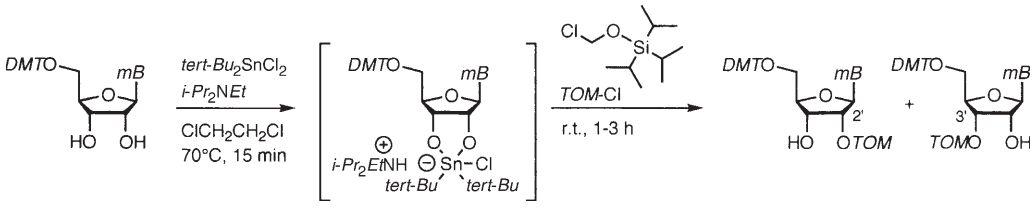

Fig. 4. Alkylation of 5′-*O*-tritylated nucleoside derivatives with [(triisopropylsilyl)oxy]methylchloride (*TOM*-Cl) *via* cyclic 2′,3′-*O*-di-*tert*-butylstannylidene intermediates according to a procedure by *S. Pitsch* [9]

regioisomers, and double tritylated nucleotides were separated by column chromatography on silica gel. 5′-*O* tritylation yields ranged from 50–90%.

2′-*O*-Alkylation

The alkylation reagent [(triisopropylsilyl)oxy]methylchloride (*TOM*-Cl) was synthesized as described by *S. Pitsch et al.* [9]. Alkylation of the 2′,3′-diol moieties of the tritylated ribonucleosides was achieved *via* the corresponding cyclic 2′,3′-*O*-di-*tert*-butylstannylidene derivatives formed *in situ* in the presence of ethyldiisopropylamine and *tert*-Bu_2SnCl_2 in 1,2-dichloroethane at 70°C (Fig. 4). Subsequent treatment with 1.1–1.5 equivalents of *TOM*-Cl at room temperature afforded mixtures of 2′-*O*- and 3′-*O*-alkylated regioisomers in a ratio between 5:4 and 9:1. The desired 2′-*O*-alkylated derivatives were easily isolated in pure form by chromatography on silica gel as the first-eluting isomers. Alkylation yields for the mixture of isomers ranged up to 70%.

In general, transformation of the methylated nucleosides into their 2′-*O*-*TOM* derivatives is more robust against further nucleobase alkylation with *TOM*-Cl than the same reaction employed to the four standard ribonucleosides. In case of the $m^2{}_2G$, $m^6{}_2A$, m^3U, and m^1I precursors, even a larger excess of *TOM*-Cl is applicable. Moreover, as mentioned by *S. Pitsch et al.* [9], the use of Bu_2SnCl_2 instead of *tert*-Bu_2SnCl_2 results in lower yields (~10%). We can confirm this, especially, if addition of *TOM*-Cl is performed at low temperatures.

3′-*O*-Phosphitylation

The 5′-*O*-*DMT*-2′-*O*-*TOM* protected intermediates were converted into the phosphoramidite building blocks with 1.5–2.3 equivalents of 2-cyanoethyl diisopropylphosphoramidochloridite preferably in the presence of a ten-fold excess of ethyldimethylamine. Product yields usually exceeded 80%.

Synthesis of the Individual Building Blocks

Building Block of 1-Methylguanosine (m^1G)

Guanosine was methylated with high regioselectivity at the amido nitrogen by treatment with 1 equivalent of NaH in *DMSO* followed by additon of 1 equivalent of methyl iodide (Fig. 5). Evaporation of the reaction mixture and subsequent

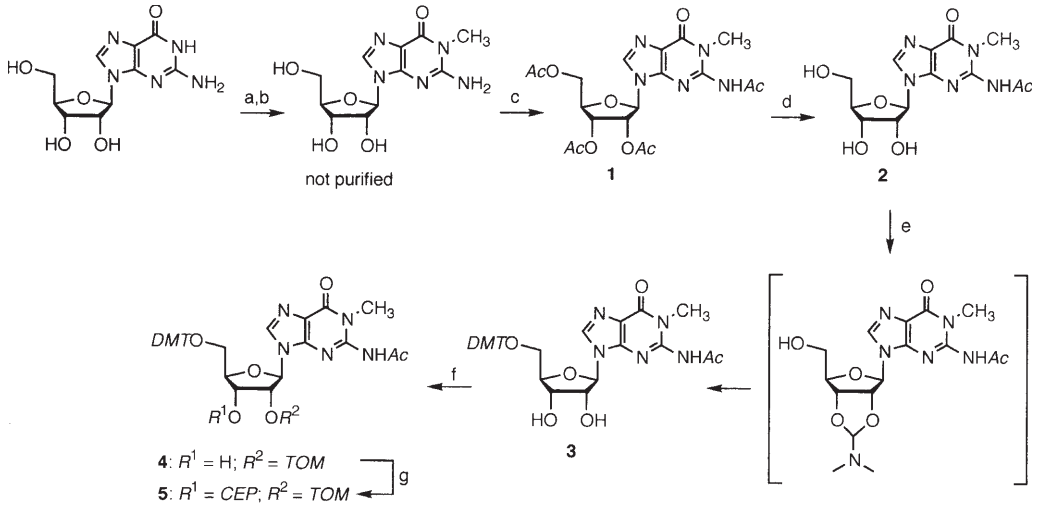

Fig. 5. Synthesis of the m^1G phosphoramidite **5**; (a) 1.0 eq NaH, *DMSO*, rt, 2 h; (b) 1.0 eq *Me*I, rt, 5 h; (c) Ac_2O/DMF/pyridine (1/1/1), 140°C, 10 h (**1**: 85% over (a)–(c)); (d) 1 *M* NaOH in $THF/MeOH/H_2O$ (5/4/2), rt, 15 min (**2**: 81%); (e) 1.2 eq dimethylformamide dimethylacetal, pyridine/*DMSO* (5/1), rt, 2 h, then 1.4 eq *DMT*-Cl, overnight (**3**: 45%); (f) 4.0 eq ethyldiisopropylamine, 1.2 eq *tert-Bu*$_2$$SnCl_2$, $ClCH_2CH_2Cl$, 70°C, then 1.2 eq *TOM*-Cl, rt, 1 h, separation of 2′-*O*-isomer by chromatography (**4**: 45%); (g) 10 eq ethyldimethylamine, 1.5 eq 2-cyanoethyl diisopropylphosphoramidochloridite, CH_2Cl_2, rt, 2 h (**5**: 85%)

refluxing of the crude product with acetic anhydride in pyridine/*DMF* at 140°C bath temperature resulted after adsorption on silica gel and chromatography in **1**. Selective hydrolysis of the *O*-acetyl groups was optimized to obtain N^2-acetyl-1-methyl guanosine **2**. Because of significant insolubility of **2** in pyridine, the introduction of the trityl group according to the general procedure described gave only poor yields (20%) and had to be improved. Therefore, **2** was reacted with *N*,*N*-dimethylformamide dimethylacetal to transiently form the corresponding nucleoside 2′,3′-*O*-acetal [12]. Solubility was enhanced and 5′-*O* tritylation yield increased to 45%. Derivative **3** was then alkylated (**4**) and phosphitylated (**5**) as described in the general section.

Building Blocks of N^2-Methylguanosine (m^2G) and N^2,N^2-Dimethylguanosine ($m^2{}_2G$)

Guanosine was transformed into 2′,3′,5′-*O*-triacetyl-O^6-[2-(4-nitrophenyl)ethyl]-guanosine by a method of *Pfleiderer* (compare Fig. 6) [13, 14]. Further synthesis of m^2G and $m^2{}_2G$ was achieved by a modified procedure of *Eritja et al.* [15]. Transformation of the exocyclic amino group proceeded under *Schiemann* conditions *via* diazotation and fluoride displacement with sodium nitrite and tetrafluoroboric acid in acetone/water at -20°C. After neutralization of the reaction mixture, extraction with CH_2Cl_2 resulted in sufficiently pure 2-fluoro nucleoside to be further treated with 8 *M* CH_3NH_2 in ethanol. Substitution of the 2-fluoro group and simultaneous cleavage of the *O*-acetyl groups gave O^6-*NPE*-m^2G (**6**) after column chromatography.

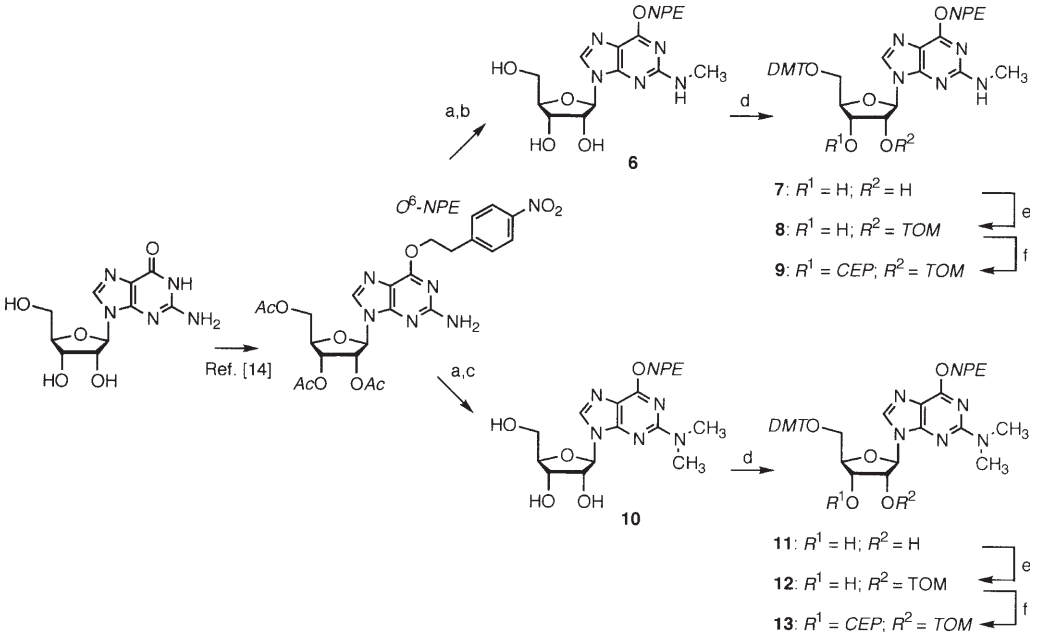

Fig. 6. Synthesis of the m^2G and $m^2{}_2G$ phosphoramidites **9** and **13**; (a) 100 eq HBF_4, 2.5 eq $NaNO_2$, acetone/water, $-20°C$ to rt, 3 h; (b) 8 *M* CH_3NH_2, ethanol, 7 h (**6**: 47% over (a) and (b)); (c) $(CH_3)_2NH$ in ethanol/water, rt, 3 h (**10**: 51% over (a) and (c)); (d) 1.1 eq *DMT*-Cl, 0.35 eq *DMAP* (0.1 eq for **11**), pyridine, rt, overnight (**7**: 70%, **11**: 83%); (e) 4.0 eq ethyldiisopropylamine, 1.2 eq *tert*-Bu_2SnCl_2, $ClCH_2CH_2Cl$, 70°C, then 1.1 eq *TOM*-Cl, rt, 3 h; separation of 2′-*O*-isomer by chromatography (**8**: 35%, **12**: 33%); (f) 10 eq ethyldimethylamine, 1.5 eq 2-cyanoethyl diisopropylphosphoramidochloridite (1.1 eq for **13**), CH_2Cl_2, rt, 2 h (**9**: 72%, **13**: 87%)

Likewise, the 2-fluoro nucleoside intermediate was converted into O^6-*NPE*-$m^2{}_2G$ (**10**) by a 1:1 mixture of 33% $(CH_3)_2NH$ in ethanol and 40% $(CH_3)_2NH$ in water. It has to be pinpointed that treatment with 33% $(CH_3)_2NH$ in ethanol alone gave 5′-*O*-acetyl-O^6-*NPE*-$m^2{}_2G$ exclusively. From **6** and **10**, the corresponding phosphoramidite building blocks **9** and **13** were synthesized in good overall yields as described in the general section above.

Building Blocks of 1-Methylinosine (m^1I) and 3-Methyluridine (m^3U)

In comparable manner to the preparation of 1-methylguanosine, methylation of inosine at nitrogen-1 and methylation of uridine at nitrogen-3 was achieved regioselectively by stepwise treatment with NaH/*DMSO* and methyl iodide (Fig. 7). Subsequent evaporation of the reaction mixture allowed direct tritylation without prior purification to furnish **14** and **17** in fair to good yields. The tritylated derivatives were alkylated (**15**, **18**) and phosphitylated (**16**, **19**) as described in the general section.

Building Block of N^4-Methylcytidine (m^4C)

The synthesis of the m^4C phosphoramidite has been accomplished by two different routes. In analogy to a procedure by *Verdine* [16], 2′,3′,5′-*O*-triacetyluridine was

trisylated at O^4. Subsequent substitution with CH_3NH_2 gave m^4C ready to be tritylated, tomylated, and phosphitylated. However, better overall yields were obtained using the prefunctionalized 5′-*O*-*DMT*-2′-*O*-*TOM*-uridine derivative [9]

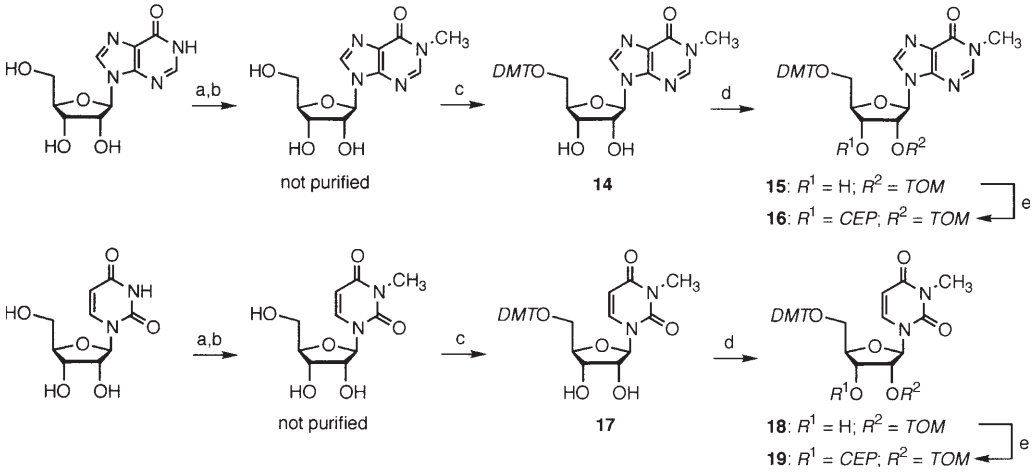

Fig. 7. Synthesis of the m^1I and m^3U phosphoramidites **16** and **19**; (a) 1.0 eq NaH, *DMSO*, rt, 2 h; (b) 1.0 eq *Me*I, rt, 4 h; (c) 1.1 eq *DMT*-Cl (1.6 eq for **17**), pyridine, rt, overnight (**14**: 47%, **17**: 55% over (a)–(c)); (d) 4.0 eq ethyldiisopropylamine, 1.2 eq *tert*-Bu_2SnCl_2, $ClCH_2CH_2Cl$, 70°C, then 1.1 eq *TOM*-Cl, rt, 1 h, separation of 2′-*O*-isomer by chromatography (**15**: 45%, **18**: 47%); (e) 10 eq ethyldimethylamine, 2.3 eq 2-cyanoethyl diisopropylphosphoramidochloridite (1.5 eq for **19**), CH_2Cl_2, rt, 2 h (**16**: 92%, **19**: 89%)

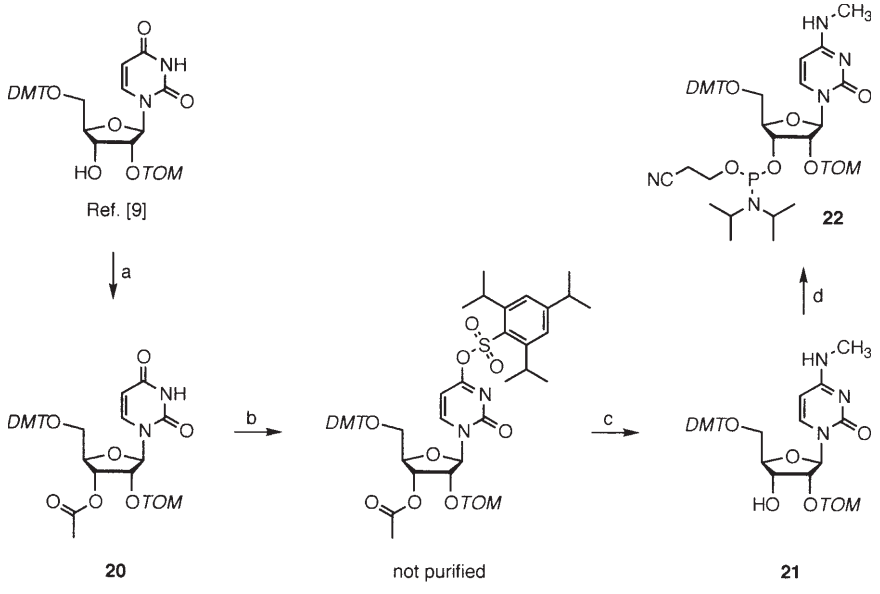

Fig. 8. Synthesis of the m^4C phosphoramidite **22**; (a) 1.1 eq Ac_2O, 0.1 eq *DMAP*, pyridine, 0°C to rt, 1 h (**20**: 96%); (b) 1.5 eq *TRIS*-Cl, 10 eq N*Et*$_3$, 0.1 eq *DMAP*, rt, 1 h; (c) 8 *M* CH_3NH_2 in ethanol, rt, overnight; (**21**: 81% over (b) and (c)); (d) 10 eq ethyldimethylamine, 1.5 eq 2-cyanoethyl diisopropylphosphoramidochloridite, CH_2Cl_2, rt, 2 h (**22**: 64%); 2,4,6-triisopropylbenzenesulfonyl (*TRIS*)

as starting material (Fig. 8). After 3′-*O*-acetylation, **20** was trisylated and directly converted into 5′-*O*-*DMT*-2′-*O*-*TOM*-N^4-methylcytidine (**21**) upon treatment with 8 *M* CH_3NH_2 in ethanol. Phosphitylation to **22** was then accomplished according to the general procedure.

Building Blocks of N^6-Methyladenosine (m^6A) and N^6,N^6-Dimethyladenosine ($m^6{}_2A$)

Inosine was used as starting material for the N^6-methylated adenosine building blocks (Fig. 9). After *O*-acetylation and reaction with chloromethylenedimethyliminiumchloride (*Vilsmeier* reagent) 2′,3′,5′-*O*-triacetyl-6-chloroinosine (**23**) was obtained in good yields. Substitution of the 6-chloro group with 8 *M* CH_3NH_2 in ethanol and simultaneous cleavage of the *O*-acetyl groups gave m^6A. After evaporation of the solvents the crude product was tritylated without prior purification to furnish **24**.

Likewise, the 6-chloro nucleoside intermediate was converted into $m^6{}_2A$ by reaction with $(CH_3)_2NH$ in ethanol/water. After evaporation of the solvents the crude product was tritylated without prior purification and gave **27** in good yields.

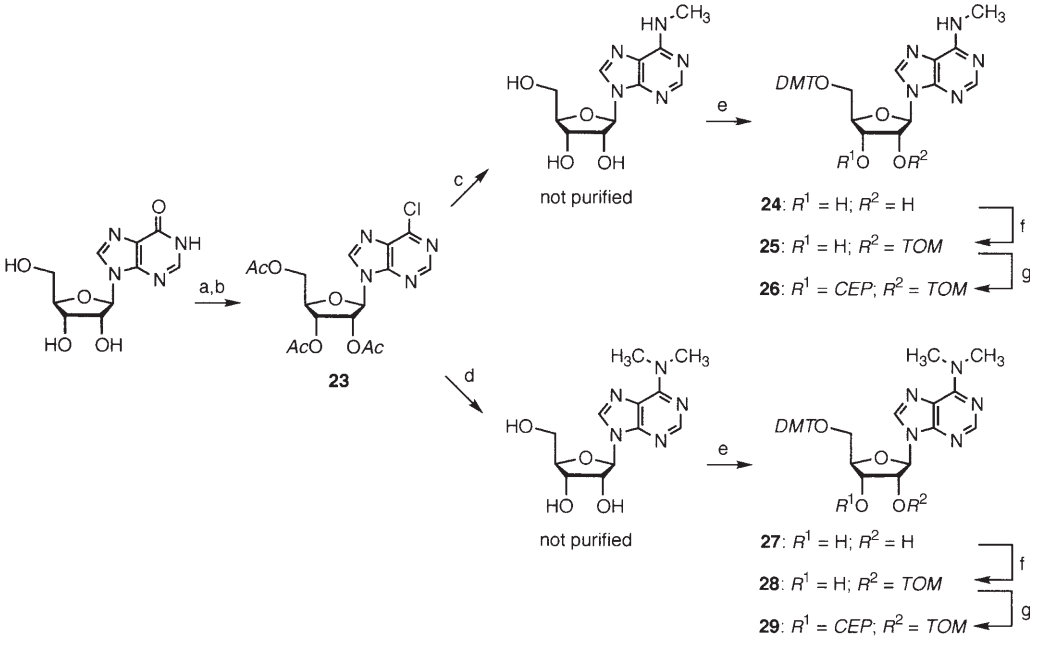

Fig. 9. Synthesis of the m^6A and $m^6{}_2A$ phosphoramidites **26** and **29**; (a) 10 eq Ac_2O, pyridine, rt, overnight; (b) 2 eq chloromethylenedimethyliminiumchloride, $CHCl_3$, reflux, 4 h (**23**: 93% over (a) and (b)); (c) CH_3NH_2 in ethanol/water, rt, 12 h; (d) $(CH_3)_2NH$ in ethanol/water, rt, 9 h; (e) 1.6 eq *DMT*-Cl (1.5 eq for **27**), pyridine, rt, overnight (**24**: 74% over (c) and (e), **27**: 61% over (d) and (e)); (f) 4.0 eq ethyldiisopropylamine, 1.2 eq *tert*-Bu_2SnCl_2, $ClCH_2CH_2Cl$, 70°C, then 1.2 eq *TOM*-Cl (1.1 eq for **28**), rt, 3 h, separation of 2′-*O*-isomer by chromatography (**25**: 23%, **28**: 20%); (g) 10 eq ethyldimethylamine, 1.5 eq 2-cyanoethyl diisopropylphosphoramidochloridite, CH_2Cl_2, rt, 2 h (**26**: 86%, **29**: 85%)

From **24** and **27**, the corresponding phosphoramidite building blocks **26** and **29** were conveniently synthesized as described in the general section.

Oligoribonucleotides

The incorporation of the m^1G, m^2G, $m^2{}_2G$, m^1I, m^3U, m^4C, m^6A, and $m^6{}_2A$ phosphoramidites into oligoribonucleotides has already been documented by our group [17–21]. All methylated building blocks mentioned here have been reproducibly coupled under standard conditions with an average coupling yield between 97 and 99.5%. They are compatible to standard oligoribonucleotide two-step deprotection with ethanolic/aqueous CH_3NH_2, followed by treatment with 1 *M TBAF/THF*. Noteably, deprotection of the *NPE*-groups (m^2G and $m^2{}_2G$) does not require an additional step involving *DBU* in acetonitrile. The *NPE*-groups are simultaneously cleaved during cleavage of the *TOM* protecting groups in 1 *M TBAF/THF*.

Conclusion

The straight-forward synthesis of the methylated phosphoramidite building blocks for m^1G, m^2G, $m^2{}_2G$, m^1I, m^3U, m^4C, m^6A, and $m^6{}_2A$ from standard ribonucleosides generates the basis to study structural effects of these methylations on *RNA*. In this sense, we have documented the impact of *RNA* methylations on duplex-hairpin equilibria [17], the possible role of methylation in the ribosomal helix 45 with respect to secondary structure formation [18], and the stabilizing effect of a methylated guanosine on codon-anticodon pairing by cyclic model compounds [19–21]. Other studies on the structural impact of *RNA* methylations are currently in progress in our laboratory. Due to the lack of an appropriate biosynthetic system the chemical synthesis is the method of choice for site-specifically introducing modified nucleosides into *RNA* oligoribonucleotides. Moreover, the novel 2′-*O*-*TOM* phosphoramidite building blocks presented in this paper should further document the great flexibility of the still young '*RNA*-*TOM*-chemistry' towards applications involving modified nucleosides.

Experimental

^1H, ^{13}C, and ^{31}P NMR spectra were recorded on a Bruker DRX 500 MHz, Bruker DRX 300 MHz, or Varian Unity 500 MHz instrument. The chemical shifts are reported relative to *TMS* and referenced to the residual proton signal of the deuterated solvents: $CDCl_3$ (7.26 ppm), d_6-*DMSO* (2.49 ppm) or d_8-toluene (7.08 ppm) for ^1H NMR spectra; $CDCl_3$ (77.0 ppm) or d_6-*DMSO* (39.5 ppm) for ^{13}C NMR spectra. ^{31}P shifts are relative to external 85% phosphoric acid. Multiplicity of ^1H-NMR resonance signals: doublet (d), triplet (t), and quartet (q); if no coupling constant is given, the abbreviations refer to signal appearance and not to theoretical multiplicity. UV spectra were recorded on a Varian Cary 100. Mass spectra were obtained from the service facilities at ETH Zürich. Elemental analyses for all 2′-*O*-*TOM*-nucleoside derivatives and the corresponding phosphoramidites were obtained from service facilities (ETH Zürich); their values agreed favourably with the calculated ones.

Analytical thin-layer chromatography (TLC) was carried out on silica 60F-254 plates. Flash column chromatography was carried out with silica gel 60 (230–400 mesh). Packing of silica gel columns was performed with 1% Et_3N added to the first 100 cm^3 of the corresponding starting eluent.

All reactions were carried out under Ar atmosphere. Workup implies partitioning of the reaction mixture between CH_2Cl_2 and semi-saturated aqueous $NaHCO_3$ solution, drying of the organic layer

($MgSO_4$ or Na_2SO_4), and evaporation. NaH from paraffine suspensions was washed three times with hexane and dried under vacuum.

Chemical reagents and solvents were purchased from commercial suppliers and used without further purification. Solvents for reactions were dried overnight over freshly activated molecular sieves (4 Å).

N^2,2′,3′,5′-O-Tetraacetyl-1-methylguanosine (**1**, $C_{19}H_{23}N_5O_9$)

Guanosine (3 g, 10.6 mmol) was dissolved in 20 cm^3 of anhydrous *DMSO* and treated with 254 mg of NaH (10.6 mmol). The mixture was stirred until H_2 evolution ceased. Methyliodide (1.5 g, 10.6 mmol) dissolved in 1 cm^3 of *DMSO* was added dropwise. The mixture was stirred for 5 h at room temperature, evaporated to dryness under reduced pressure at 80°C and dissolved in pyridine/*DMF*/acetic anhydride (50 cm^3 each). The mixture was heated to 140°C for 5 to 10 h and evaporated again. The resulting solid was dissolved in 200 cm^3 of *Me*OH and 50 g of silica gel were added. *Me*OH was evaporated, the silica gel coated by the crude product was dried overnight under reduced pressure, and applied to column chromatography (silica gel, CH_2Cl_2:CH_3OH = 40:1 to 25:1) resulting in **1** (4.2 g, 85%). TLC (silica gel, CH_2Cl_2:CH_3OH = 25:1): R_f = 0.5; ^{1}H NMR (500 MHz, $CDCl_3$, 25°C): δ = 2.07, 2.08, 2.13, 2.33 (4s, $COCH_3$), 3.60 (s, 1-CH_3), 4.40–4.46 (m, H^1–C(5′), H–C(4′)), 4.53 (dd, J = 5.2, 11.0 Hz, H^2–C(5′)), 5.67 (t, H–C(3′)), 5.91 (t, H–C(2′)), 5.99 (d, J = 5.2 Hz, H–C(1′)), 7.80 (s, H–C(8)), 8.76 (br s, NH) ppm; ^{13}C NMR (125 MHz, $CDCl_3$, 25°C): δ = 20.37, 20.54, 20.79, 23.78 (CO*C*H_3), 31.90 (1-CH_3), 63.19 (C(5′)), 70.72 (C(3′)), 72.95 (C(2′)), 80.23 (C(4′)), 87.00 (C(1′)), 122.90, 138.80 (C(8)), 145.73, 146.90, 157.29, 169.34, 169.62, 170.10, 171.04 ppm.

N^2-Acetyl-1-methylguanosine (**2**, $C_{13}H_{17}N_5O_6$)

Compound **1** (2.0 g, 4.3 mmol) was dissolved in 24 cm^3 of *THF*/*Me*OH/H_2O (5/4/2). Aqueous NaOH (2 cm^3, 10 *M*) was added under vigorous stirring. The reaction was monitored by TLC ($CHCl_3$:*Me*OH = 1:1) and was completed typically after 15 min. The mixture was quenched with ~2 cm^3 of acetic acid to give a final *pH* of 6.5. Product **2** (1.18 g, 81%) precipitated as pale yellow solid, was collected by filtration and washed two times with 10 cm^3 of cold *THF*/*Me*OH/H_2O (−20°C). TLC (silica gel, $CHCl_3$:CH_3OH = 8:5): R_f = 0.5; ^{1}H NMR (300 MHz, d_6-*DMSO*, 27°C): δ = 2.13 (s, $COCH_3$), 3.41 (s, 1-CH_3), 3.63 (dd, J = 3.9, 11.9 Hz, H^1–C(5′)), 3.53 (dd, J = 4.0, 11.9 Hz, H^2–C(5′)), 3.91 (q, H–C(4′)), 4.12 (t, H–C(3′)), 4.45 (t, H–C(2′)), 4.99 (br s, HO–C(5′)), 5.15, 5.44 (2br s, HO–C(2′), HO–C(3′)), 5.79 (d, J = 5.8 Hz, H–C(1′)), 8.31 (s, H–C(8)) ppm; ^{13}C NMR (75 MHz, d_6-*DMSO*, 27°C): δ = 23.8 (CO*C*H_3), 31.9 (1-CH_3), 62.1 (C(5′)), 71.2 (C(3′)), 74.9 (C(2′)), 86.5 (C(4′)), 87.8 (C(1′)), 139.8 (C(8)), 122.0, 147.3, 148.0, 157.7, 171.0 ppm; UV (H_2O): $\lambda(\varepsilon)$ = 202 (max, 22400), 254 (max, 9880), 260 (8860) nm ($mol^{-1}\,dm^3\,cm^{-1}$).

N^2-Acetyl-5′-O-(4,4′-dimethoxytrityl)-1-methylguanosine (**3**, $C_{34}H_{35}N_5O_8$)

Method A: A suspension of 2.0 g of **2** (5.89 mmol) in 14 cm^3 of *DMSO* and 25 cm^3 of pyridine was heated to 60°C and treated with 2.19 g of 4,4′-dimethoxytritylchloride (6.48 mmol) in three portions over a period of 3 h. Stirring was continued overnight. Unreacted educt was recovered by filtration of the reaction mixture. Addition of *Me*OH, evaporation to dryness, workup, and column chromatography (silica gel, CH_2Cl_2:CH_3OH = 20:1) yielded 0.76 g of **3** as pale yellow foam (20%). The educt recovered can be directly used for repeated tritylation.

Method B: To a suspension of 200 mg of **2** (0.59 mmol) in 3 cm^3 of pyridine, 84.3 mg of *N*,*N*-dimethylformamide dimethylacetal (0.71 mmol) were added. The mixture was stirred for 2 h, heated to 50°C, treated with 0.1 cm^3 of *DMSO*, and concentrated to a volume of 1.5 cm^3. Addition of 3 cm^3 of pyridine/*DMSO* (5/1) resulted in a clear solution and 280 mg of 4,4′-dimethoxytritylchloride

(0.83 mmol) were added in three portions over a period of 24 h. Addition of 1 cm^3 of *Me*OH, evaporation of pyridine, workup, and column chromatography (silica gel, CH_2Cl_2:CH_3OH = 20:1; 1% Et_3N) yielded 171 mg of **3** as pale yellow foam (45%). TLC (silica gel, CH_2Cl_2:CH_3OH = 10:1): R_f = 0.4; ^{1}H NMR (500 MHz, $CDCl_3$, 25°C): δ = 1.84 ($COCH_3$), 3.22, 3.42 (2d, H_2–C(5′)), 3.45 (1-CH_3), 3.71, 3.72 (2s, 2 OCH_3), 4.29 (t, H–C(4′)), 4.42 (t, H–C(3′)), 4.88 (t, H–C(2′)), 5.86 (d, J = 6.1 Hz, H–C(1′)), 6.25 (br q, NH), 6.72–6.80 (m, 4H, trityl-H), 7.10–7.42 (m, 9H, trityl-H), 7.85 (s, H–C(8)) ppm; ^{13}C NMR (125 MHz, $CDCl_3$, 25°C): 23.62 ($COCH_3$), 31.30 (1-CH_3), 55.20 (2 CH_3O), 63.68 (C(5′)), 71.91 (C(3′)), 74.88 (C(2′)), 85.27 (C(4′)), 86.40, 89.58 (C(1′)), 113.19 (trityl-C), 121.2, 126.96, 127.90, 128.03, 129.99, 130.05 (trityl-C), 135.55, 135.63, 138.93 (C(8)), 144.66, 146.05, 146.43, 157.35, 158.54, 170.25 ppm; UV ($CHCl_3$): $\lambda(\varepsilon)$ = 260 (11500), 277 (max, 11000) nm ($mol^{-1}\,dm^3\,cm^{-1}$); FAB-MS: m/z = 642.2 (100, $[M+H]^+$), 303.1 (39, $[(MeO)_2Tr]^+$).

N^2-Acetyl-5′-O-(4,4′-dimethoxytrityl)-1-methyl-2′-O-[[(triisopropylsilyl)oxy]methyl] guanosine (**4**, $C_{44}H_{57}N_5O_9Si$)

To a stirred solution of 345 mg of **3** (0.54 mmol) and 278 mg of ethyldiisopropylamine (2.15 mmol) in 3 cm^3 of 1,2-dichloroethane, 198 mg of di-*tert*-butyltindichloride (0.65 mmol) were added. The mixture was heated to 70°C for 15 min, allowed to cool to rt again, and treated with 134 mg of [(triisopropylsilyl)oxy]methylchloride (0.6 mmol). Stirring was continued for 60 min, followed by addition of 0.2 cm^3 of *Me*OH and workup. Column chromatography (silica gel, CH_2Cl_2:CH_3OH = 70:1 to 50:1) afforded 200 mg of **4** as white, solid foam (45%). The regioselectivity of 2′-*O*-alkylated over 3′-*O*-alkylated product was approximately 7:2. TLC (silica gel, CH_2Cl_2:CH_3OH = 20:1): R_f = 0.4; ^{1}H NMR (500 MHz, $CDCl_3$, 25°C): δ = 0.98–1.07 (m, $^iPr_3Si$), 1.42 (s, $COCH_3$), 3.00 (d, J = 1.5 Hz, HO–C(3′)), 3.17 (dd, J = 3.2, 10.5 Hz, H^1–C(5′)), 3.52 (s, 1-CH_3), 3.55 (dd, J = 1.8, 10.5 Hz, H^2–C(5′)), 3.78, 3.79 (2s, 2 OCH_3), 4.25 (m, H–C(4′)), 4.52 (m, H–C(3′)), 4.94 (q, H–C(2′)), 4.90, 5.11 (2d, J = 4.7 Hz, OCH_2O), 5.93 (d, J = 7.3 Hz, H–C(1′)), 6.80 (m, 4H, trityl-H), 6.98 (br s, NH), 7.17–7.54 (m, 9H, trityl-H), 7.83 (s, H–C(8)) ppm; ^{13}C NMR (125 MHz, $CDCl_3$, 25°C): δ = 11.80 ($(CH_3)_2CH$), 17.75 ($(CH_3)_2CH$), 22.89 ($COCH_3$), 31.79 (1-CH_3), 55.28 (2 CH_3O), 63.78 (C(5′)), 70.90 (C(3′)), 81.96 (C(2′)), 84.37 (C(4′)), 86.37 (C(1′)), 86.50, 91.07 (OCH_2O), 113.26, 113.32 (trityl-C), 123.05, 127.16, 128.01, 128.08, 129.97, 130.12 (trityl-C), 135.68, 135.89, 139.58 (C(8)), 145.12, 146.24, 157.30, 158.72, 169.68 ppm; UV ($CHCl_3$): $\lambda(\varepsilon)$ = 260 (12600), 276 (max, 11200) nm ($mol^{-1}\,dm^3\,cm^{-1}$); FAB-MS: m/z = 828.2 (83, $[M+H]^+$), 303.1 (100, $[(MeO)_2Tr]^+$).

N^2-Acetyl-5′-O-(4,4′-dimethoxytrityl)-1-methyl-2′-O-[[(triisopropylsilyl)oxy]methyl] guanosine 3′-(2-cyanoethyl diisopropylphosphoramidite) (**5**, $C_{53}H_{74}N_7O_{10}PSi$)

A solution of 183 mg of **4** (0.22 mmol) in 2 cm^3 of CH_2Cl_2 was treated consecutively with 160 mg of ethyldimethylamine (2.2 mmol) and 79 mg of 2-cyanoethyl diisopropylphosphoramidochloridite (0.33 mmol). The mixture was stirred for 2 h, quenched with 0.3 cm^3 of *Me*OH, and evaporated to dryness. Workup and column chromatography (silica gel, CH_2Cl_2:CH_3OH = 60:1 (+2% Et_3N)) afforded 193 mg of **5** as white, solid foam (85%, 1:1 mixture of diastereoisomers). TLC (silica gel, CH_2Cl_2:CH_3OH = 30:1): R_f = 0.6; ^{1}H NMR (500 MHz, $CDCl_3$, 25°C): δ = 0.86–1.19 (m, 42H, $^iPr_3Si$, 24H, $((CH_3)_2CH)_2N$), 1.44, 1.66 (br s, 6H, $COCH_3$), 2.27, 2.69 (2m, 4H, CH_2CN), 3.21 (m, 2H, H^1–C(5′)), 3.50–3.55 (m, 4H, $((CH_3)_2CH)_2N$), 3.51, 3.52 (2s, 6H, 1-CH_3), 3.53, 3.59 (m, 2H, $POCH_2$), 3.51, 3.62 (m, 2H, H^2–C(5′)), 3.77, 3.78 (2s, 12H, OCH_3), 3.90, 3.97 (2m, 2H, $POCH_2$), 4.27, 4.34 (2q, 2H, H–C(4′)), 4.52, 4.57 (2m, 2H, H–C(3′)), 4.89–4.96 (4d, 4H, J = 5.2 Hz, OCH_2O), 5.00, 5.06 (t, q, 2H, H–C(2′)), 5.89, 5.99 (2d, 2H, J = 7.1 Hz, H–C(1′)), 6.76–6.82, 7.20–7.31, 7.35–7.53 (m, 26H, trityl-H), 7.82, 7.84 (2s, 2H, H–C(8)) ppm; ^{31}P NMR (200 MHz, $CDCl_3$, 27°C): δ = 149.98, 150.50 ppm; UV (*Me*OH): $\lambda(\varepsilon)$ = 260 (13300), 274 (max, 12800), 281 (sh, 12000) nm ($mol^{-1}\,dm^3\,cm^{-1}$); FAB-MS: m/z = 1028.7 (44, $[M+H]^+$), 821.5 (100), 303.1 (35, $[(MeO)_2Tr]^+$).

N^2-Methyl-O^6-[2-(4-nitrophenyl)ethyl]guanosine (**6**, $C_{19}H_{22}N_6O_7$)

2′,3′,5′-*O*-Triacetyl-O^6-[2-(4-nitrophenyl)ethyl]guanosine [13, 14] (6.6 g, 11.7 mmol) in 45 cm^3 of acetone was cooled to -20°C and treated with 111 cm^3 of 50% HBF_4 (1.2 mol). Under vigorous stirring 2.04 g of $NaNO_2$ (29.4 mmol) in 28 cm^3 of water were added dropwise. The reaction mixture was allowed to warm to room temperature, stirred for 3 h, and neutralized with 50% NaOH (or $NaHCO_3$). The reaction mixture was extracted two times with CH_2Cl_2, the organic layers were dried with Na_2SO_4 and evaporated to dryness. The crude material was dissolved in 8 *M* CH_3NH_2 in ethanol and stirred for 7 h. The reaction mixture was evaporated to dryness. Column chromatography (silica gel, CH_2Cl_2:*Me*OH = 15:1) afforded 2.46 g of **6** (47%). TLC (silica gel, $CHCl_3$:CH_3OH = 10:1): $R_f = 0.5$; ^{1}H NMR (500 MHz, d_6-*DMSO*, 50°C): $\delta = 2.82$ (d, $J = 4.8$ Hz, N^2-CH_3), 3.26 (t, $J = 6.8$ Hz, CH_2–C_6H_4–NO_2), 3.55, 3.63 (2m, H_2–C(5′)), 3.90 (q, H–C(4′)), 4.14 (q, H–C(3′)), 4.57 (q, H–C(2′)), 4.71 (t, $J = 6.8$ Hz, O^6–CH_2), 4.84 (t, HO–C(5′)), 4.99 (d, $J = 4.9$ Hz, HO–C(3′)), 5.23 (d, $J = 6.1$ Hz, HO–C(2′), 5.79 (d, $J = 5.9$ Hz, H–C(1′)), 6.75 (br q, NH), 7.61 (d, $J = 8.5$ Hz, 4-nitrophenyl H–C(2)/H–C(6)), 8.02 (s, H–C(8)), 8.16 (d, $J = 8.5$ Hz, 4-nitrophenyl H–C(3)/H–C(5)) ppm; ^{13}C NMR (125 MHz, d_6-*DMSO*, 40°C): $\delta = 28.65$ (N^2–CH_3), 34.82 (CH_2–C_6H_4–NO_2), 62.05 (C(5′)), 65.79 (O^6–CH_2), 70.96 (C(3′)), 73.56 (C(2′)), 85.74 (C(4′)), 87.49 (C(1′)), 123.78 (4-nitrophenyl C(3)/C(5)), 114.33, 130.63 (4-nitrophenyl C(2)/C(6)), 138.73, 138.79 (C(8)), 146.74, 147.08, 154.67, 159.76, 160.34 ppm.

5′-O-(4,4′-Dimethoxytrityl)-N^2-methyl-O^6-[2-(4-nitrophenyl)ethyl]guanosine (**7**, $C_{40}H_{40}N_6O_9$)

Compound **6** (1.40 g, 3.14 mmol) was coevaporated three times with anhydrous pyridine to be finally dissolved in 15 cm^3. Then, 1.17 g of 4,4′-dimethoxytritylchloride (3.45 mmol) were added in three portions over a period of 3 h. *DMAP* (0.14 g, 1.11 mmol) was added and stirring was continued overnight. Addition of *Me*OH, evaporation to dryness, workup, and column chromatography (silica gel, CH_2Cl_2:CH_3OH = 20:1) yielded 1.63 g of **7** as pale yellow foam (70%). TLC (silica gel, CH_2Cl_2:CH_3OH = 10:1): $R_f = 0.4$; ^{1}H NMR (500 MHz, d_6-*DMSO*, 60°C): $\delta = 2.73$ (d, $J = 4.8$ Hz, N^2-CH_3), 3.22 (d, $J = 4.7$ Hz, H_2–C(5′)), 3.25 (t, $J = 6.7$ Hz, CH_2–C_6H_4–NO_2), 3.71, 3.72 (2s, 2 OCH_3), 4.01 (q, H–C(4′)), 4.32 (q, H–C(3′)), 4.64 (q, H–C(2′)), 4.71 (t, $J = 6.7$ Hz, O^6–CH_2), 4.97 (d, $J = 5.8$ Hz, HO–C(3′)), 5.28 (d, $J = 5.0$ Hz, HO–C(2′)), 5.82 (d, $J = 4.6$ Hz, H–C(1′)), 6.65 (br q, NH), 6.81 (m, 4H, trityl-H), 7.17–7.27, 7.33–7.37 (m, 9H, trityl-H), 7.60 (d, $J = 8.7$ Hz, 4-nitrophenyl H–C(2)/H–C(6)), 7.90 (s, H–C(8)), 8.15 (d, $J = 8.7$ Hz, 4-nitrophenyl H–C(3)/H–C(5)) ppm; ^{13}C NMR (125 MHz, d_6-*DMSO*, 60°C): $\delta = 28.54$ (N^2-CH_3), 34.78 (CH_2–C_6H_4–NO_2), 55.45 (2 CH_3O), 64.46 (C(5′)), 65.81 (O^6–CH_2), 70.98 (C(3′)), 73.26 (C(2′)), 83.46 (C(4′)), 85.98, 88.25 (C(1′)), 113.56 (trityl-C), 114.49, 123.73 (4-nitrophenyl C(3)/C(5)), 126.98, 128.05, 128.17 (trityl-C), 130.03, 130.08, 130.58 (trityl-C, 4-nitrophenyl C(2)/C(6)), 136.08, 138.71 (C(8)), 145.22, 146.83, 147.06, 158.52, 158.55, 159.80, 160.40 ppm.

5′-O-(4,4′-Dimethoxytrityl)-N^2-methyl-O^6-[2-(4-nitrophenyl)ethyl]-2′-O-[[(triisopropylsilyl)oxy]methyl]guanosine (**8**, $C_{50}H_{62}N_6O_{10}Si$)

To a stirred solution of 1.63 g of **7** (2.18 mmol) and 1.13 g of ethyldiisopropylamine (8.71 mmol) in 20 cm^3 of anhydrous 1,2-dichloroethane, 0.79 g of di-*tert*-butyltindichloride (2.61 mmol) were added. The mixture was heated to 70°C for 15 min, allowed to cool to rt again and treated with 0.53 g of [(triisopropylsilyl)oxy]methylchloride (2.40 mmol). Stirring was continued for 3 h, followed by addition of 0.5 cm^3 of *Me*OH, evaporation to dryness and workup. Column chromatography (silica gel, hexanes:*Et*OAc = 3:1 to 1:1) afforded 0.70 g of **8** as white solid foam (35%). The regioselectivity of 2′-*O*-alkylated over 3′-*O*-alkylated product was approximately 3:2. TLC (silica gel, hexanes:*Et*OAc = 1:1): $R_f = 0.4$; ^{1}H NMR (500 MHz, $CDCl_3$, 30°C): $\delta = 0.98$–1.10 (m, $^iPr_3$Si), 2.86

(d, $J = 5.1$ Hz, N^2-CH_3), 2.98 (d, $J = 4.1$ Hz, HO–C(3′)), 3.28 (t, $J = 6.9$ Hz, CH_2–C_6H_4–NO_2), 3.36 (dd, $J = 4.5$, 10.5 Hz, H^1–C(5′)), 3.46 (dd, $J = 3.9$, 10.5 Hz, H^2–C(5′)), 3.78, 3.79 (2s, 2 OCH_3), 4.22 (q, H–C(4′)), 4.58 (q, H–C(3′)), 4.67 (br s, NH), 4.73 (t, $J = 6.9$ Hz, O^6–CH_2), 4.95 (t, H–C(2′)), 4.98, 5.13 (2d, $J = 4.8$ Hz, OCH_2O), 6.03 (d, $J = 5.4$ Hz, H–C(1′)), 6.79 (m, 4H, trityl-H), 7.19–7.32, 7.42–7.43 (m, 9H, trityl-H), 7.47 (d, $J = 8.7$ Hz, 4-nitrophenyl H–C(2)/H–C(6)), 7.71 (s, H–C(8)), 8.16 (d, $J = 8.7$ Hz, 4-nitrophenyl H–C(3)/H–C(5)) ppm; ^{13}C NMR (75 MHz, $CDCl_3$, 27°C): $\delta = 11.85$ ($(CH_3)_2CH$), 17.74 ($(CH_3)_2CH$), 28.59 (N^2-CH_3), 35.24 (CH_2–C_6H_4–NO_2), 55.16 (2 CH_3O), 63.55 (C(5′)), 65.83 (O^6–CH_2), 70.99 (C(3′)), 81.22 (C(2′)), 83.74 (C(4′)), 86.42, 86.70 (C(1′)), 90.79 (OCH_2O), 113.13 (trityl-C), 115.33, 123.68 (4-nitrophenyl C(3)/C(5)), 126.81, 127.79, 128.20 (trityl-C), 129.88, 130.08 (trityl-C, 4-nitrophenyl C(2)/C(6)), 135.77, 135.83, 138.02 (C(8)), 144.64, 146.11, 146.84, 154.10, 158.52, 159.64, 160.51 ppm; UV (*Me*OH): $\lambda(\varepsilon) = 260$ (20100), 275 (max, 18300), 284 (max, 17900) nm ($mol^{-1}\,dm^3\,cm^{-1}$); MALDI-FTICR-MS: $m/z = 957.4189$ (5, $[M + Na]^+$), 303.1399 (100, $[(MeO)_2Tr]^+$).

5′-O-(4,4′-Dimethoxytrityl)-N^2-methyl-O^6-[2-(4-nitrophenyl)ethyl]-2′-O-[[(triisopropylsilyl)oxy]methyl]guanosine 3′-(2-cyanoethyl diisopropylphosphoramidite) (**9**, $C_{59}H_{79}N_8O_{11}PSi$)

A solution of 137 mg of **8** (0.14 mmol) in 3 cm^3 of anhydrous CH_2Cl_2 was treated consecutively with 103 mg of ethyldimethylamine (1.4 mmol) and 50 mg of 2-cyanoethyl diisopropylphosphoramidochloridite (0.21 mmol). The mixture was stirred for 2 h, quenched with 0.15 cm^3 of *Me*OH, and evaporated. Workup and column chromatography (silica gel, hexane:*Et*OAc = 2:1 (+ 1% Et_3N)) afforded 120 mg of **9** as white, solid foam (72%, 1:1 mixture of diastereoisomers). TLC (silica gel, hexane:*Et*OAc) = 1:1): $R_f = 0.5$; ^{1}H NMR (500 MHz, $CDCl_3$, 25°C): $\delta = 0.89$–1.24 (m, 42H, $^iPr_3Si$, 24H, $((CH_3)_2CH)_2N$), 2.34, 2.65 (2m, 4H, CH_2CN), 2.85 (2d, 6H, $J = 5.1$ Hz, N^2-CH_3), 3.27 (t, 4H, $J = 6.9$ Hz, CH_2–C_6H_4–NO_2), 3.35–3.40 (m, 2H, H^1–C(5′)), 3.45–3.66 (m, 2H, H^2–C(5′), 2H, $POCH_2$, 4H, $((CH_3)_2CH)_2N$), 3.77, 3.78 (2s, 12H, OCH_3), 3.82–3.96 (2m, 2H, $POCH_2$), 4.31, 4.36 (2q, 2H, H–C(4′)), 4.64 (br q, NH), 4.70 (m, 2H, H–C(3′)), 4.73 (t, $J = 6.9$ Hz, O^6–CH_2), 4.89–4.96 (m, 4H, OCH_2O), 5.12 (t, 2H, H–C(2′)), 6.00, 6.05 (2d, 2H, $J = 6.0$ Hz, H–C(1′)), 6.75–6.83, 7.18–7.35, 7.40–7.44 (m, 26H, trityl-H), 7.48 (d, $J = 7.5$ Hz, 4-nitrophenyl H–C(2)/H–C(6)), 7.69 (s, 2H, H–C(8)), 8.15 (d, $J = 7.5$ Hz, 4-nitrophenyl H–C(3)/H–C(5)) ppm; ^{31}P NMR (200 MHz, $CDCl_3$, 27°C): $\delta = 150.09$; 150.63 ppm; UV (*Me*OH): $\lambda(\varepsilon) = 260$ (19800), 275 (max, 17100), 282 (max, 17500) nm ($mol^{-1}\,dm^3\,cm^{-1}$); MALDI-FTICR-MS: $m/z = 1157.5268$ (8, $[M + Na]^+$), 303.1395 (100, $[(MeO)_2Tr]^+$).

N^2,N^2-Dimethyl-O^6-[2-(4-nitrophenyl)ethyl]guanosine (**10**, $C_{20}H_{24}N_6O_7$)

2′,3′,5′-*O*-Triacetyl-O^6-[2-(4-nitrophenyl)ethyl]guanosine [13, 14] (3.0 g, 5.3 mmol) in 20 cm^3 of acetone was cooled to −20°C and treated with 50 cm^3 of 50% HBF_4 (0.55 mol). Under vigorous stirring 0.92 g of $NaNO_2$ (13.4 mmol) in 13 cm^3 of water were added dropwise. The reaction mixture was allowed to warm to rt, stirred for 3 h, and neutralized with 50% NaOH (or $NaHCO_3$). The reaction mixture was extracted twice with CH_2Cl_2, the organic layers dried with Na_2SO_4 and evaporated to dryness. The crude material was dissolved in a stirred solution of 33% $(CH_3)_2NH$ in 20 cm^3 of ethanol. After 1 h a solution of 40% $(CH_3)_2NH$ in 20 cm^3 of water was added and stirring was continued for another 3 h. The reaction mixture was evaporated to dryness and coevaporated three times with *Me*OH/toluene/CH_2Cl_2. Column chromatography (silica gel, CH_2Cl_2:*Me*OH = 15:1) afforded 1.24 g of **10** (51%). TLC (silica gel, $CHCl_3$:CH_3OH = 10:1): $R_f = 0.5$; ^{1}H NMR (500 MHz, *d*$_6$-*DMSO*, 25°C): $\delta = 3.12$ (s, N^2-$(CH_3)_2$), 3.27 (t, $J = 6.7$ Hz, CH_2–C_6H_4–NO_2), 3.50, 3.61 (2m, H_2–C(5′)), 3.87 (q, H–C(4′)), 4.14 (q, H–C(3′)), 4.59 (q, H–C(2′)), 4.73 (t, $J = 6.7$ Hz, O^6–CH_2), 4.90 (t, $J = 5.6$ Hz, HO–C(5′)), 5.15 (d, $J = 4.9$ Hz, HO–C(3′)), 5.35 (d, $J = 6.2$ Hz, HO–C(2′), 5.80 (d, $J = 5.9$ Hz, H–C(1′)),

7.60 (d, $J = 8.7$ Hz, 4-nitrophenyl H–C(2)/H–C(6)), 8.08 (s, H–C(8)), 8.16 (d, $J = 8.7$ Hz, 4-nitrophenyl H–C(3)/H–C(5)) ppm; ^{13}C NMR (125 MHz, *d*$_6$-*DMSO*, 25°C): $\delta = 34.77$ (*C*H$_2$–C$_6$H$_4$–NO$_2$), 37.53 (N^2-(CH$_3$)$_2$), 61.99 (C(5′)), 65.81 (O^6–CH$_2$), 70.89 (C(3′)), 73.41 (C(2′)), 85.58 (C(4′)), 87.40 (C(1′)), 113.69, 123.85 (4-nitrophenyl C(3)/C(5)), 130.64 (4-nitrophenyl C(2)/C(6)), 139.29, 139.31 (C(8)), 146.68, 147.12, 154.71, 158.87, 159.86 ppm.

5′-O-(4,4′-Dimethoxytrityl)-N^2,N^2-dimethyl-O^6-[2-(4-nitrophenyl)ethyl]guanosine (**11**, C$_{41}$H$_{42}$N$_6$O$_9$)

Compound **10** (500 mg, 1.09 mmol) was coevaporated three times with anhydrous pyridine to be finally dissolved in 4 cm^3. Then, 410 mg of 4,4′-dimethoxytritylchloride (1.21 mmol) were added in three portions over a period of 3 h. *DMAP* (10 mg, 0.08 mmol) was added and stirring was continued overnight. Addition of *Me*OH, evaporation to dryness, workup, and column chromatography (silica gel, CH_2Cl_2:$CH_3OH = 35{:}1$) yielded 690 mg of **11** as pale yellow foam (83%). TLC (silica gel, CH_2Cl_2:$CH_3OH = 20{:}1$): $R_f = 0.4$; ^{1}H NMR (500 MHz, $CDCl_3$, 30°C): $\delta = 3.04$ (s, HO–C(3′)), 3.18 (s, N^2-(CH$_3$)$_2$), 3.24 (dd, $J = 3.5$, 10.4 Hz, H^1–C(5′)), 3.31 (t, $J = 6.8$ Hz, C*H*$_2$–C$_6$H$_4$–NO$_2$), 3.41 (dd, $J = 3.8$, 10.4 Hz, H^2–C(5′)), 3.76, 3.77 (2s, 2 OCH$_3$), 4.39 (d, H–C(3′)), 4.44 (t, H–C(4′)), 4.68 (t, H–C(2′)), 4.78 (t, $J = 6.8$ Hz, O^6–CH$_2$), 5.83 (d, $J = 6.6$ Hz, H–C(1′)), 6.73–6.79 (m, 4H, trityl-H), 7.16–7.22, 7.26–7.30 (m, 9H, trityl-H), 7.49 (d, $J = 8.9$ Hz, 4-nitrophenyl H–C(2)/H–C(6)), 7.84 (s, H–C(8)), 8.16 (d, $J = 8.9$ Hz, 4-nitrophenyl H–C(3)/H–C(5)) ppm; ^{13}C NMR (125 MHz, $CDCl_3$, 30°C): $\delta = 35.16$ (*C*H$_2$–C$_6$H$_4$–NO$_2$), 37.78 (N^2-(CH$_3$)$_2$), 55.10 (2 CH$_3$O), 63.73 (C(5′)), 65.89 (O^6–CH$_2$), 72.84 (C(3′)), 76.21 (C(2′)), 86.34 (C(4′), 86.38, 90.41 (C(1′)), 113.06 (trityl-C), 114.10, 123.70 (4-nitrophenyl C(2)/C(6)), 126.77, 127.76, 127.97 (trityl-C), 129.82, 129.83, 129.89, 129.93 (trityl-C and 4-nitrophenyl C(3)/C(5)), 135.38, 135.55, 136.83 (C(8)), 144.27, 145.90, 146.81, 153.22, 158.45, 160.10 ppm; UV (*Me*OH): $\lambda(\varepsilon) = 259$ (max, 12400), 260 (12300), 275 (sh, 9400), 281 (sh, 9100) nm (mol^{-1} dm^3 cm^{-1}); MALDI-FTICR-MS: $m/z = 785.2855$ (6, $[M + Na]^+$), 303.1 (100, $[(MeO)_2Tr]^+$).

5′-O-(4,4′-Dimethoxytrityl)-N^2,N^2-dimethyl-O^6-[2-(4-nitrophenyl)ethyl]-2′-O-[[(triisopropylsilyl)oxy]methyl]guanosine (**12**, C$_{51}$H$_{64}$N$_6$O$_{10}$Si)

To a stirred solution of 540 mg of **11** (0.71 mmol) and 366 mg of ethyldiisopropylamine (2.84 mmol) in 6 cm^3 of anhydrous 1,2-dichloroethane, 258 mg of di-*tert*-butyltindichloride (0.85 mmol) were added. The mixture was heated to 70°C for 15 min, allowed to cool to rt again and treated with 173 mg of [(triisopropylsilyl)oxy]methylchloride (0.8 mmol). Stirring was continued for 3 h, followed by addition of 0.5 cm^3 of *Me*OH, evaporation to dryness and workup. Column chromatography (silica gel, hexane:*Et*OAc = 3:1 to 1:1) afforded 222 mg of **12** as white solid foam (33%). The regioselectivity of 2′-*O*-alkylated over 3′-*O*-alkylated product was approximately 5:3. TLC (silica gel, hexane:*Et*OAc = 1:1): $R_f = 0.4$; ^{1}H NMR (500 MHz, $CDCl_3$, 30°C): $\delta = 1.02$–1.12 (m, ^{i}*Pr*$_3$Si), 2.99 (d, $J = 4.7$, HO–C(3′)), 3.10 (s, N^2-(CH$_3$)$_2$), 3.30 (t, $J = 6.9$ Hz, C*H*$_2$–C$_6$H$_4$–NO$_2$), 3.46 (d, H$_2$–C(5′)), 3.78 (s, 2 OCH$_3$), 4.20 (q, H–C(4′)), 4.57 (q, H–C(3′)), 4.76 (t, $J = 6.9$ Hz, O^6–CH$_2$), 4.92 (t, H–C(2′)), 4.99, 5.14 (2d, $J = 4.8$ Hz, OCH$_2$O), 6.05 (d, $J = 4.9$ Hz, H–C(1′)), 6.78 (m, 4H, trityl-H), 7.19–7.33, 7.38–7.42 (m, 9H, trityl-H), 7.48 (d, $J = 8.4$ Hz, 4-nitrophenyl C(2)/C(6)), 7.71 (s, H–C(8)), 8.17 (d, $J = 8.4$ Hz, 4-nitrophenyl H–C(3)/H–C(5)) ppm; ^{13}C NMR (125 MHz, $CDCl_3$, 30°C): $\delta = 11.78$ ((CH$_3$)$_2$*C*H), 17.66 ((*C*H$_3$)$_2$CH), 35.23 (*C*H$_2$–C$_6$H$_4$–NO$_2$), 37.33 (N^2-(CH$_3$)$_2$), 55.08 (2 CH$_3$O), 63.59 (C(5′)), 65.57 (O^6–CH$_2$), 70.80 (C(3′)), 81.27 (C(2′)), 83.49 (C(4′)), 86.34, 86.75 (C(1′)), 90.72 (OCH$_2$O), 113.06 (trityl-C), 114.16, 123.65 (4-nitrophenyl C(3)/C(5)), 126.71, 127.71, 128.11 (trityl-C), 129.81, 129.99 (trityl-C, 4-nitrophenyl C(2)/C(6)), 135.70, 135.79, 137.74 (C(8)), 144.56, 146.11, 146.79, 154.13, 158.43, 159.04, 159.86 ppm; UV (*Me*OH): $\lambda(\varepsilon) = 260$ (18100) nm (mol^{-1} dm^3 cm^{-1}); MALDI-MS: $m/z = 949.2$ (10, $[M + H]^+$), 303.1 (100, $[(MeO)_2Tr]^+$).

5′-O-(4,4′-Dimethoxytrityl)-N^2,N^2-dimethyl-O^6-[2-(4-nitrophenyl)ethyl]-2′-O-[[(triisopropylsilyl)oxy]methyl]guanosine 3′-(2-cyanoethyl diisopropylphosphoramidite) (**13**, $C_{60}H_{81}N_8O_{11}PSi$)

A solution of 444 mg of **12** (0.47 mmol) in 4 cm^3 of anhydrous CH_2Cl_2 was treated consecutively with 343 mg of ethyldimethylamine (4.7 mmol) and 116 mg of 2-cyanoethyl diisopropylphosphoramidochloridite (0.49 mmol). The mixture was stirred for 2 h, quenched with 0.15 cm^3 of *Me*OH, and evaporated to dryness. Workup and column chromatography (silica gel, hexane:*Et*OAc = 5:2 (+ 2% Et_3N)) afforded 469 mg of **13** as white, solid foam (87%, 1:1 mixture of diastereoisomers). TLC (silica gel, hexane:*Et*OAc = 1:1): $R_f = 0.7$; ^{1}H NMR (500 MHz, $CDCl_3$, 26°C): $\delta = 0.86$–1.28 (m, 42H, $^iPr_3Si$, 24H, $((CH_3)_2CH)_2N$), 2.32, 2.64 (2m, 4H, CH_2CN), 3.06, 3.07 (2s, 12H, N^2-$(CH_3)_2$), 3.29 (t, 4H, $J = 6.4$ Hz, CH_2–C_6H_4–NO_2), 3.35–3.47 (m, 4H, H_2–C(5′)), 3.48–3.65 (m, 4H, $((CH_3)_2CH)_2N$, 2H, $POCH_2$), 3.77, 3.78 (2s, 12H, OCH_3), 3.84–3.96 (2m, 2H, $POCH_2$), 4.30, 4.33 (2q, 2H, H–C(4′)), 4.62 (m, 2H, H–C(3′)), 4.75 (t, $J = 6.4$ Hz, O^6–CH_2), 4.88–4.97 (q, 4H, $J \sim 6$ Hz, OCH_2O), 5.06 (m, 2H, H–C(2′)), 6.05, 6.09 (2d, 2H, $J = 5.9$ Hz, H–C(1′)), 6.75–6.79, 7.20–7.40 (m, 26H, trityl-H), 7.48 (2d, $J \sim 8$ Hz, 4-nitrophenyl H–C(2)/H–C(6)), 7.70 (s, 2H, H–C(8)), 8.16 (d, $J \sim 8$ Hz, 4-nitrophenyl H–C(3)/H–C(5)) ppm; ^{31}P NMR (200 MHz, $CDCl_3$, 27°C): $\delta = 150.22$, 150.59 ppm; UV (*Me*OH): $\lambda(\varepsilon) = 260$ (max, 26400), 281 (sh, 18200) nm ($mol^{-1}\,dm^3\,cm^{-1}$); MALDI-FTICR-MS: $m/z =$ 1171.5763 (6, $[M + Na]^+$), 303.1412 (100, $[(MeO)_2Tr]^+$).

5′-O-(4,4′-Dimethoxytrityl)-1-methylinosine (**14**, $C_{32}H_{32}N_4O_7$)

Inosine (276 mg, 1.0 mmol) was dissolved in 2.7 cm^3 of anhydrous *DMSO* and treated with 25 mg of NaH (1.0 mmol). The mixture was stirred until H_2 evolution ceased followed by dropwise addition of 142 mg of methyliodide (1.0 mmol). The mixture was stirred for 4 h at rt and evaporated to dryness. The pasty solid was coevaporated with MeOH and three times with anhydrous pyridine to be finally dissolved in 1.5 cm^3. Then, 360 mg of 4,4′-dimethoxytritylchloride (1.06 mmol) were added in three portions over a period of 3 h. Stirring was continued for one more hour at 60°C. Addition of *Me*OH, evaporation to dryness, workup, and column chromatography (silica gel, CH_2Cl_2:CH_3OH = 25:1 to 20:1) yielded 270 mg of **14** as pale yellow foam (47%). TLC (silica gel, CH_2Cl_2:CH_3OH = 15:1): $R_f = 0.4$; ^{1}H NMR (500 MHz, *d_6-DMSO*, 25°C): $\delta = 3.17$ (m, H^1–C(5′)), 3.23 (m, H^2–C(5′)), 3.49 (s, 1-CH_3), 3.71, 3.72 (2s, 2 OCH_3), 4.06 (q, H–C(4′)), 4.20 (q, H–C(3′)), 4.56 (q, H–C(2′)), 5.22 (d, $J = 5.8$ Hz, HO–C(3′)), 5.56 (d, $J = 5.7$ Hz, HO–C(2′)), 5.88 (d, $J = 4.7$ Hz, H–C(1′)), 6.83 (m, 4H, trityl-H), 7.16–7.27, 7.33–7.36 (m, 9H, trityl-H), 8.20 (s, H–C(8)), 8.32 (s, H–C(2)) ppm; MALDI-FTICR-MS: $m/z = 607.2150$ (8, $[M + Na]^+$), 303.1379 (100, $[(MeO)_2Tr]^+$).

5′-O-(4,4′-Dimethoxytrityl)-1-methyl-2′-O-[[(triisopropylsilyl)oxy]methyl]inosine (**15**, $C_{42}H_{54}N_4O_8Si$)

To a stirred solution of 250 mg of **14** (0.43 mmol) and 220 mg of ethyldiisopropylamine (1.7 mmol) in 3 cm^3 of 1,2-dichloroethane, 156 mg of di-*tert*-butyltindichloride (0.51 mmol) were added. The mixture was heated to 70°C for 15 min, allowed to cool to rt again and treated with 105 mg of [(triisopropylsilyl)oxy]methylchloride (0.47 mmol). Stirring was continued for 3 h, followed by addition of 0.5 cm^3 of *Me*OH, evaporation to dryness and workup. Column chromatography (silica gel, CH_2Cl_2: *Me*OH = 45:1 to 30:1) afforded 148 mg of **15** as white, solid foam (45%). The regioselectivity of 2′-*O*-alkylated over 3′-*O*-alkylated product was approximately 5:4. TLC (silica gel, CH_2Cl_2:CH_3OH = 20:1): $R_f = 0.5$; ^{1}H NMR (500 MHz, $CDCl_3$, 25°C): $\delta = 1.00$–1.12 (m, $^iPr_3Si$); 2.99 (d, $J = 4.7$ Hz, HO–C(3′)), 3.39 (dd, $J = 4.2$, 10.4 Hz, H^1–C(5′)), 3.43 (dd, $J = 3.4$, 10.4 Hz, H^2–C(5′)), 3.61 (s, 1-CH_3), 3.78 (s, 2 OCH_3), 4.29 (q, H–C(4′)), 4.53 (q, H–C(3′)), 4.82 (t, H–C(2′)), 4.94, 5.13 (2d, $J = 4.8$ Hz, OCH_2O), 6.11 (d, $J = 4.9$ Hz, H–C(1′)), 6.81 (m, 4H, trityl-H), 7.18–7.35, 7.41–7.47 (m, 9H, trityl-H), 7.83 (s, H–C(8)), 7.93 (s, H–C(2)) ppm; ^{13}C NMR (75 MHz,

$CDCl_3$, 28°C): $\delta = 12.25$ ((CH_3)$_2$*C*H), 18.14 ((CH$_3$)$_2$CH), 34.46 (1-CH_3), 55.61 (2 CH_3O), 64.00 (C(5′)), 71.52 (C(3′)), 82.79 (C(2′)), 84.78 (C(4′) and trityl-C), 87.03 (C(1′)), 91.27 (OCH_2O), 113.57 (trityl-C), 125.45, 127.29, 128.23, 128.56, 130.48, 130.51 (trityl-C), 136.04, 136.15, 139.22 (C(8)), 144.97, 147.48 (C(2)), 148.09, 157.37, 159.00 ppm; UV (*Me*OH): $\lambda(\varepsilon) = 235$ (max, 31700), 260 (plateau, 8800), 281 (sh, 6600) nm ($mol^{-1}\,dm^3\,cm^{-1}$); MALDI-FTICR-MS: $m/z = 793.3797$ (42, $[M + Na]^+$), 303.1413 (100, $[(MeO)_2Tr]^+$).

5′-O-(4,4′-Dimethoxytrityl)-1-methyl-2′-O-[[(triisopropylsilyl)oxy]methyl]inosine 3′-(2-cyanoethyl diisopropylphosphoramidite) (**16**, $C_{51}H_{71}N_6O_9PSi$)

A solution of 300 mg of **15** (0.39 mmol) in 3 cm^3 of anhydrous CH_2Cl_2 was treated consecutively with 286 mg of ethyldimethylamine (3.9 mmol) and 208 mg of 2-cyanoethyl diisopropylphosphoramidochloridite (0.88 mmol). The mixture was stirred for 2 h, quenched with 0.15 cm^3 of *Me*OH, and evaporated to dryness. Workup and column chromatography (silica gel, hexane:*Et*OA*c* = 7:3 (+ 2% Et_3N)) afforded 348 mg of **16** as white, solid foam (92%, 1:1 mixture of diastereoisomers). TLC (silica gel, hexane:*Et*OA*c* (2% *TEA*) = 4:1): $R_f = 0.5$; ^{1}H NMR (500 MHz, $CDCl_3$, 25°C): $\delta = 0.86$–1.19 (m, 42H, ^{i}*Pr*$_3$Si, 24H, ((CH_3)$_2$CH)$_2$N), 2.38, 2.65 (2m, 4H, CH_2CN), 3.32–3.40 (m, 2H, H^1–C(5′)), 3.41–3.52 (m, 2H, H^2–C(5′)), 3.60, 3.61 (2s, 6H, 1-CH_3), 3.60–3.70 (m, 2H, $POCH_2$, 4H, ((CH_3)$_2$C*H*)$_2$N), 3.78, 3.79 (2s, 12H, OCH_3), 3.84–3.97 (m, 2H, $POCH_2$), 4.35, 4.41 (2q, 2H, H–C(4′)), 4.57–4.65 (m, 2H, H–C(3′)), 4.91–4.98 (4d, 4H, $J = 5.0$ Hz, OCH_2O), 5.00–5.04 (2t, 2H, H–C(2′)), 6.05, 6.09 (2d, 2H, $J = 6.1$ Hz, H–C(1′)), 6.75–6.83, 7.18–7.35, 7.40–7.44 (m, 26H, trityl-H), 7.78 (s, 2H, H–C(8), 7.92, 7.93 (2s, 2H, H–C(2)) ppm; ^{31}P NMR (200 MHz, $CDCl_3$, 27°C): $\delta = 150.06$, 150.73 ppm; UV (*Me*OH): $\lambda(\varepsilon) = 235$ (max, 26000), 260 (plateau, 6900), 281 (sh, 5000) nm ($mol^{-1}\,dm^3\,cm^{-1}$); MALDI-FTICR-MS: $m/z = 993.4972$ (24, $[M + Na]^+$), 303.1414 (100, $[(MeO)_2Tr]^+$).

5′-O-(4,4′-Dimethoxytrityl)-3-methyluridine (**17**, $C_{31}H_{32}N_2O_8$)

Uridine (10.0 g, 41 mmol) was dissolved in 100 cm^3 of anhydrous *DMSO* and treated with 985 mg of NaH (41 mmol). The mixture was stirred until H_2 evolution ceased. Methyliodide (5.8 g, 41 mmol) was added dropwise. The mixture was stirred for 5 h at rt and evaporated to dryness. The pasty solid was coevaporated with *Me*OH and three times with anhydrous pyridine to be finally dissolved in 50 cm^3. Then, 22.2 g of 4,4′-dimethoxytritylchloride (66 mmol) were added in four portions over a period of 4 h. Stirring was continued for one more hour at 60°C. Addition of *Me*OH, evaporation to dryness, workup, and column chromatography (silica gel, CH_2Cl_2:CH_3OH = 30:1) yielded 12.7 g of **17** as pale yellow foam (55%). TLC (silica gel, CH_2Cl_2:CH_3OH = 20:1): $R_f = 0.4$; ^{1}H NMR (500 MHz, $CDCl_3$, 30°C): $\delta = 3.35$ (s, 3-CH_3), 3.40 (dd, $J = 3.4$, 10.9 Hz, H^1–C(5′)), 3.49 (dd, $J = 2.9$, 10.9 Hz, H^2–C(5′)), 3.79 (s, 2 OCH_3), 4.28 (q, H–C(4′)); 4.34 (m, 2H, H–C(3′) and H–C(2′)), 5.56 (d, $J = 8.2$ Hz, H–C(5)); 5.80 (d, $J = 3.6$ Hz, H–C(1′)), 6.83 (m, 4H, trityl-H), 7.23–7.36 (m, 9H, trityl-H), 7.75 (d, $J = 8.2$ Hz, H–C(6)) ppm; ^{13}C NMR (125 MHz, $CDCl_3$, 27°C): $\delta = 27.47$ (3-CH_3), 55.16 (2 CH_3O), 62.52 (C(5′)), 71.25 (C(3′)), 76.49 (C(2′)), 84.92 (C(4′)), 87.01, 92.06 (C(1′)), 101.37 (C(5)), 113.20, 127.06, 127.89, 127.95, 129.97 (trityl-C), 135.06, 135.15, 137.30 (C(6)), 144.15, 152.12, 158.66, 162.53 ppm; UV (*Me*OH): $\lambda(\varepsilon) = 233$ (max, 24400), 260 (10500) nm ($mol^{-1}\,dm^3\,cm^{-1}$); MALDI-FTICR-MS: $m/z = 583.2051$ (3, $[M + Na]^+$); 303.1388 (100, $[(MeO)_2Tr]^+$).

5′-O-(4,4′-Dimethoxytrityl)-3-methyl-2′-O-[[(triisopropylsilyl)oxy]methyl]uridine (**18**, $C_{41}H_{54}N_2O_9Si$)

To a stirred solution of 3.5 g of **17** (6.25 mmol) and 3.23 g of ethyldiisopropylamine (25 mmol) in 30 cm^3 of 1,2-dichloroethane, 2.28 g of di-*tert*-butyltindichloride (7.5 mmol) were

added. The mixture was heated to 70°C for 15 min, allowed to cool to rt and treated with 1.53 g of [(triisopropylsilyl)oxy]methylchloride (6.88 mmol). Stirring was continued for 60 min, followed by addition of 2 cm^3 of *Me*OH and workup. Column chromatography (silica gel, hexane:*Et*OAc = 4:1 to 1:1) afforded 2.18 g of **18** as white, solid foam (47%). The regioselectivity of 2′-*O*-alkylated over 3′-*O*-alkylated product was approximately 3:2. TLC (silica gel, hexane:*Et*OAc = 2:1): $R_f = 0.5$; ^{1}H NMR (500 MHz, $CDCl_3$, 30°C): $\delta = 1.07$–1.27 (m, $^iPr_3$Si), 3.16 (d, $J = 6.3$ Hz, HO–C(3′)), 3.32 (s, 3-CH_3), 3.51 (q, H_2–C(5′)), 3.80 (s, 2 OCH_3), 4.10 (m, H–C(4′)), 4.26 (m, H–C(2′)), 4.44 (q, H–C(3′)), 5.06, 5.24 (2d, $J = 4.7$ Hz, OCH_2O), 5.39 (d, $J = 8.2$ Hz, H–C(5)), 6.03 (d, $J = 3.1$ Hz, H–C(1′)), 6.83 (m, 4H, trityl-H), 7.24–7.40 (m, 9H, trityl-H), 7.90 (d, $J = 8.2$ Hz, H–C(6)) ppm; ^{13}C NMR (75 MHz, $CDCl_3$, 27°C): $\delta = 11.87$ ((CH_3)$_2$*C*H), 17.76 ((*C*H$_3$)$_2$CH), 27.51 (3-CH_3), 55.21 (2 CH_3O), 62.07 (C(5′)), 69.19 (C(3′)), 82.84 (C(2′)), 83.54 (C(4′)), 87.05 (C(1′)), 88.65, 90.58 (OCH_2O), 101.58 (C(5)), 113.26, 113.27, 127.09, 127.94, 128.17, 130.11, 130.15 (trityl-C), 135.18, 135.42, 137.71 (C(6)), 144.36, 151.04, 158.69, 158.72, 162.80 ppm; UV (*Me*OH): $\lambda(\varepsilon) = 234$ (max, 23600), 260 (10400) nm ($mol^{-1}\,dm^3\,cm^{-1}$); MALDI-FTICR: $m/z = 769.3489$ (5, $[M + Na]^+$); 303.1388 (100, $[(MeO)_2Tr]^+$).

5′-O-(4,4′-Dimethoxytrityl)-3-methyl-2′-O-[[(triisopropylsilyl)oxy]methyl]uridine 3′-(2-cyanoethyl diisopropylphosphoramidite) (**19**, $C_{50}H_{71}N_4O_{10}PSi$)

A solution of 1.6 g of **18** (2.14 mmol) in 7 cm^3 of CH_2Cl_2 was treated consecutively with 1.6 g of ethyldimethylamine (21.5 mmol) and 760 mg of 2-cyanoethyl diisopropylphosphoramidochloridite (3.21 mmol). The mixture was stirred for 2 h, quenched with 0.3 cm^3 of *Me*OH, and evaporated to dryness. Workup and column chromatography (silica gel, hexane:*Et*OAc = 3:1 (+ 2% Et_3N)) afforded 1.80 g of **19** as white, solid foam (89%, 1:1 mixture of diastereoisomers). TLC (silica gel, hexane:*Et*OAc = 2:1): $R_f = 0.5$; ^{1}H NMR (500 MHz, $CDCl_3$, 25°C): $\delta = 0.99$–1.08 (m, $^iPr_3$Si), 1.12–1.26 (m, ((CH_3)$_2$CH)$_2$N), 2.39, 2.64 (2m, 4H, CH_2CN), 3.31 (2s, 6H, 3-CH_3), 3.39 (m, 2H, H^1–C(5′)), 3.53–3.60 (m, 2H, H^2–C(5′), 2H, $POCH_2$, 4H, ((CH_3)$_2$C*H*)$_2$N), 3.78, 3.79 (2s, 12H, OCH_3), 3.81–3.96 (m, 2H, $POCH_2$), 4.24, 4.29 (2q, 2H, H–C(4′)), 4.40–4.46 (m, 4H, H–C(2′)), H–C(3′)), 4.99–5.07 (4d, 4H, $J = 5.0$ Hz, OCH_2O), 5.41, 5.46 (2d, $J = 8.1$ Hz, H–C(5)), 6.16, 6.18 (2d, 2H, $J = 4.7$ Hz, H–C(1′)), 6.82–6.85, 7.26–7.37, 7.35–7.53 (m, 26H, trityl-H), 7.78, 7.81 (d, $J = 8.1$ Hz, H–C(6)) ppm; ^{31}P NMR (200 MHz, $CDCl_3$, 27°C): $\delta = 149.90$, 150.48 ppm; UV (*Me*OH): $\lambda(\varepsilon) = 260$ (11100) nm ($mol^{-1}\,dm^3\,cm^{-1}$); MALDI-FTICR-MS: $m/z = 969.4564$ (8, $[M + Na]^+$), 303.1394 (100, $[(MeO)_2Tr]^+$).

3′-O-Acetyl-5′-O-(4,4′-dimethoxytrityl)-2′-O-[[(triisopropylsilyl)oxy]methyl]uridine (**20**, $C_{42}H_{54}N_2O_{10}Si$)

A stirred solution of 430 mg of 5′-*O*-(4,4′-dimethoxytrityl)-2′-*O*-[[(triisopropylsilyl)oxy]methyl]uridine (0.59 mmol) [9] and 7 mg of *DMAP* (0.06 mmol) in 0.75 cm^3 of anhydrous pyridine under Ar atmosphere was cooled to 0°C and treated with 0.06 cm^3 of acetic anhydride (0.65 mmol). The mixture was stirred for 1 h at room temperature, quenched with 0.1 cm^3 of *Me*OH, and evaporated to dryness. Workup and column chromatography (silica gel, hexane:*Et*OAc = 6:4 (+ 1% Et_3N)) afforded 440 mg of **20** as white, solid foam (96%). TLC (silica gel, CH_2Cl_2:*Me*OH = 98:2): $R_f = 0.5$; ^{1}H NMR (300 MHz, $CDCl_3$, 27°C): $\delta = 0.97$–1.08 (m, $^iPr_3$Si), 2.10 (s, $COCH_3$), 3.44 (dd, $J = 2.5$, 11.0 Hz, H^1–C(5′)), 3.53 (dd, $J = 2.5$, 11.0 Hz, H^2–C(5′)), 3.79 (s, 2 OCH_3), 4.21 (q, H–C(4′)), 4.56 (t, H–C(2′)), 4.93 (t, H–C(3′)), 4.95 (s, OCH_2O), 5.39 (d, $J = 8.2$ Hz, H–C(5)), 6.13 (d, $J = 5.9$ Hz, H–C(1′)), 6.84 (m, 4H, trityl-H), 7.24–7.39 (m, 9H, trityl-H), 7.75 (s, H–C(6)), 8.54 (s, NH) ppm; ^{13}C NMR (75 MHz, $CDCl_3$, 27°C): $\delta = 11.86$ ((CH_3)$_2$*C*H), 17.74 ((*C*H$_3$)$_2$CH), 27.77 (CO*C*H$_3$), 55.22 (2 CH_3O), 62.67 (C(5′)), 71.18 (C(3′)), 77.20 (C(2′)), 81.90 (C(4′)), 86.64 (C(1′)), 87.44, 89.39 (OCH_2O), 102.65 (C(5)), 113.35, 127.20, 128.04, 128.14, 130.07, 130.12 (trityl-C), 135.04, 135.15, 140.05 (C(6)),

144.12, 150.21, 158.77, 162.68, 170.05 ppm; UV (*Me*OH): $\lambda(\varepsilon)) = 235$ (max, 23300), 260 (11300) nm ($mol^{-1}\,dm^3\,cm^{-1}$).

5′-O-(4,4′-Dimethoxytrityl)-N^4-methyl-2′-O-[[(triisopropylsilyl)oxy]methyl]cytosine (**21**, $C_{41}H_{55}N_3O_8Si$)

Method A: To a stirred solution of 105 mg of **20** (0.14 mmol) and 1.2 mg of *DMAP* (0.01 mmol) in anhydrous 1.5 cm^3 of 1,2-dichloroethane, 142 mg of triethylamine (1.4 mmol) and subsequently, 62 mg of triisopropylbenzenesulfonylchloride (0.21 mmol) were added. The solution was stirred for 1 h. After workup the crude product was dissolved in 33% CH_3NH_2 in ethanol and stirred overnight at rt. The solvents were evaporated and column chromatography (silica gel, CH_2Cl_2:*Me*OH = 99:1 to 96:4)) afforded 85 mg of **21** as white solid foam (81%).

Method B: To a stirred solution of 490 mg of 5′-*O*-(4,4′-dimethoxytrityl)-N^4-methylcytosine (0.88 mmol) [16] and 455 mg of ethyldiisopropylamine (3.52 mmol) in 3.5 cm^3 of 1,2-dichloroethane, 320 mg of di-*tert*-butyltindichloride (1.06 mmol) were added. The mixture was heated to 70°C for 15 min, allowed to cool to rt again and treated with 235 mg of [(triisopropylsilyl)oxy] methylchloride (1.06 mmol). Stirring was continued for 3 h, followed by addition of 0.5 cm^3 of *Me*OH, evaporation to dryness and workup. Column chromatography (silica gel, *EtOAc*) afforded 100 mg of **21** as white solid foam (15%). The regioselectivity of 2′-*O*-alkylated over 3′-*O*-alkylated product was approximately 5:4. TLC (silica gel, *EtOAc*): $R_f = 0.5$; ^{1}H NMR (300 MHz, d_6-*DMSO*, 27°C): $\delta = 0.92$–1.13 (m, $^iPr_3Si$), 2.74 (d, $J = 4.5$ Hz, N^4-CH_3), 3.21 (m, H_2–C(5′)), 3.73 (s, 2 OCH_3), 3.94 (q, H–C(4′)), 4.14–4.19 (q, H–C(2′), H–C(3′)), 4.95–5.02 (m, OCH_2O, HO–C(3′)), 5.53 (d, $J = 7.5$ Hz, H–C(5)), 5.93 (d, $J = 3.5$ Hz, H–C(1′)), 6.88, 7.27–7.39 (d, 4H, m, 9H, trityl-H), 7.60 (d, $J = 7.5$ Hz, H–C(6)), 7.67 (q, NH) ppm; ^{13}C NMR (75 MHz, d_6-*DMSO*, 27°C): $\delta = 11.17$ ($(CH_3)_2CH$), 17.59 ($(CH_3)_2CH$), 26.77 (N^4-CH_3), 54.96 (2 CH_3O), 63.00 (C(5′)), 68.72 (C(3′)), 78.02 (C(2′)), 82.46 (C(4′)), 85.82, 87.59 (C(1′)), 88.44 (OCH_2O), 94.71 (C(5)), 113.14 (trityl-C), 117.63, 126.69, 127.68, 127.77, 129.67 (trityl-C), 135.27, 135.39, 139.51 (C(6)), 144.59, 154.89, 158.07, 163.68 ppm; UV (*Me*OH): $\lambda(\varepsilon) = 233$ (max, 26400), 260 (10200), 273 (max, 11600) nm ($mol^{-1}\,dm^3\,cm^{-1}$); MALDI-TOF-MS: $m/z = 746.0$ (23, $[M + H]^+$), 303.1 (100, $[(MeO)_2Tr]^+$).

5′-O-(4,4′-Dimethoxytrityl)-N^4-methyl-2′-O-[[(triisopropylsilyl)oxy]methyl]cytosine 3′-(2-cyanoethyl diisopropylphosphoramidite) (**22**, $C_{50}H_{72}N_5O_9PSi$)

A solution of 95 mg of **21** (0.13 mmol) in 3 cm^3 of CH_2Cl_2 was treated consecutively with 95 mg of ethyldimethylamine (1.3 mmol) and 47 mg of 2-cyanoethyl diisopropylphosphoramidochloridite (0.20 mmol). The mixture was stirred for 1 h, quenched with 0.1 cm^3 of *Me*OH, and evaporated to dryness. Workup and column chromatography (silica gel, hexane:*EtOAc* = 1:1 (+ 1% Et_3N)) afforded 78 mg of **22** as white foam (64%, 1:1 mixture of diastereoisomers). TLC (silica gel, hexane: *EtOAc* = 2:8): $R_f = 0.4$; ^{1}H NMR (500 MHz, $CDCl_3$, 26°C, four species: mixture of 2 diastereomers and 2 rotamers): $\delta = 0.92$–1.27 (m, 42H, $^iPr_3Si$, 24H, $((CH_3)_2CH)_2N$), 2.36 (t, $J = 6.5$ Hz, 2H, CH_2CN), 2.61 (m, 2H, CH_2CN), 2.81, 2.99 (2br s, 3:1 (rotamers), 6H, N^4-CH_3), 3.34–3.37 (m, 2H, H^1–C(5′)), 3.45–3.70 (m, 8H, $((CH_3)_2CH)_2N$, H^2–C(5′), $POCH_2$), 3.78, 3.79 (2s, 12H, 2 OCH_3), 3.82–3.96 (m, 2H, $POCH_2$), 4.12–4.30 (4 m, 2H, H–C(4′), 2H, H–C(2′)), 4.40, 4.49 (2m, 2H, H–C(3′)), 4.81 (br, 2H, NH), 5.12–5.20 (m, 4H, OCH_2O, 2H, H–C(5)), 6.12, 6.17 (2br s, 2H, H–C(1′)), 6.80–6.84, 7.18–7.43 (m, 26H, trityl-H), 7.88, 7.97, 8.07, 8.11 (2d, 2br s, 3:3:1:1, 2H, H–C(6)) ppm; the additional splitting of some signals (N^4-CH_3, H–C(6)) is tentatively assigned to rotamers around the glycosidic bond or around the C(4)-N^4-bond; at 40°C ($CDCl_3$) the rotamer ratio changes to 4:1 (judged by the signal of N^4-CH_3); at 60°C (d_8-toluene) only a single rotamer of each P-diastereomer is observed; ^{31}P NMR (200 MHz, $CDCl_3$, 27°C): $\delta = 149.89$, 150.58 ppm; UV (*Me*OH): $\lambda(\varepsilon) = 233$ (max, 24800), 260 (10100), 273 (max, 11300) nm ($mol^{-1}\,dm^3\,cm^{-1}$); MALDI-TOF-MS: $m/z =$ 946.3 (10, $[M + H]^+$), 303.1 (100, $[(MeO)_2Tr]^+$).

2′,3′,5′-O-Triacetyl-6-chloroinosine (**23**, $C_{16}H_{17}N_4O_7Cl$)

2′,3′,5′-*O*-Triacetylinosine (5.41 g, 13.7 mmol) in 70 cm^3 of $CHCl_3$ was treated with 3.51 g of chloromethylenedimethyliminiumchloride (27.4 mmol) and refluxed for 4 h. The reaction mixture was transferred into a separatory funnel and slowly dropped into 100 cm^3 of a stirred, semi-saturated aqueous $NaHCO_3$ solution. The organic layer was washed twice with water, dried with Na_2SO_4, and evaporated to dryness. Column chromatography (silica gel, CH_2Cl_2:*Me*OH = 20:1) yielded 5.24 g of **23** (93%). TLC (silica gel, CH_2Cl_2:CH_3OH = 20:1): $R_f = 0.7$; ^{1}H NMR (500 MHz, $CDCl_3$, 30°C): $\delta = 2.10$, 2.13, 2.17 (3s, $COCH_3$), 4.40 (dd, $J = 4.4$, 12.2 Hz, H^1–C(5′)), 4.44–4.50 (m, H^2–C(5′), H–C(4′)), 5.65 (t, H–C(3′)), 5.96 (t, H–C(2′)), 6.24 (d, $J = 5.2$ Hz, H–C(1′)), 8.29 (s, H–C(8)), 8.79 (s, H–C(2)) ppm; ^{13}C NMR (125 MHz, $CDCl_3$, 30°C): $\delta = 20.24$, 20.40, 20.62 ($COCH_3$), 62.79 (C(5′)), 70.42 (C(3′)), 73.05 (C(2′)), 80.51 (C(4′)), 86.81 (C(1′)), 132.32, 143.40 (C(8)), 151.65, 151.65, 152.25 (C(2)), 169.19, 169.40, 170.10 ppm.

5′-O-(4,4′-Dimethoxytrityl)-N^6-methyladenosine (**24**, $C_{32}H_{33}N_5O_6$)

Compound **23** (960 mg, 2.32 mmol) was dissolved in a mixture of 33% CH_3NH_2 in 8 cm^3 of ethanol and 40% $(CH_3)NH_2$ in 8 cm^3 of water. The solution was stirred for 12 h, then evaporated to dryness, three times coevaporated with anhydrous pyridine to be finally dissolved in 5 cm^3. Then, 1.30 g of 4,4′-dimethoxytritylchloride (3.83 mmol) were added in three portions over a period of 6 h. Stirring was continued overnight. Addition of *Me*OH, evaporation to dryness, workup, and column chromatography (silica gel, CH_2Cl_2:CH_3OH = 50:1 to 10:1) yielded 1.0 g of **24** as pale yellow foam (74%). TLC (silica gel, CH_2Cl_2:CH_3OH = 20:1): $R_f = 0.5$; ^{1}H NMR (300 MHz, $CDCl_3$, 27°C): $\delta = 3.20$ (br d, N^6-CH_3), 3.26 (dd, $J = 3.3$, 10.5 Hz, H^1–C(5′)), 3.44 (dd, $J = 3.3$, 10.5 Hz, H^2–C(5′)), 3.76 (s, 2 OCH_3), 4.38–4.42 (m, H–C(4′), H–C(3′)), 4.77 (t, H–C(2′)), 5.97 (d, $J = 5.7$ Hz, H–C(1′)), 6.15 (q, NH), 6.73 (m, 4H, trityl-H), 7.16–7.30 (m, 9H, trityl-H), 8.02 (s, H–C(8)), 8.34 (H–C(2)) ppm; ^{13}C NMR (75 MHz, $CDCl_3$, 27°C): $\delta = 27.45$ (br, N^6-CH_3), 55.15 (2 CH_3O), 63.60 (C(5′)), 72.55 (C(3′)), 75.95 (C(2′)), 86.13, 86.49 (C(4′)), 90.64 (C(1′)), 113.11 (trityl-C), 120.22, 126.84, 127.78, 128.03, 129.93, 129.94 (trityl-C), 135.50, 135.63, 137.96 (C(8)), 144.33, 152.62 (C(2)), 155.56, 158.52 ppm; UV (*Me*OH): $\lambda(\varepsilon) = 234$ (max, 20800), 260 (14500), 265 (max, 15600) nm ($mol^{-1}\,dm^3\,cm^{-1}$).

5′-O-(4,4′-Dimethoxytrityl)-N^6-methyl-2′-O-[[(triisopropylsilyl)oxy]methyl]adenosine (**25**, $C_{42}H_{55}N_5O_7Si$)

To a stirred solution of 970 mg of **24** (1.66 mmol) and 858 mg of ethyldiisopropylamine (6.64 mmol) in 6 cm^3 of 1,2-dichloroethane, 605 mg of di-*tert*-butyltindichloride (1.99 mmol) were added. The mixture was heated to 70°C for 15 min, allowed to cool to rt and treated with 443 mg of [(triisopropylsilyl) oxy]methylchloride (1.99 mmol). Stirring was continued for 60 min, followed by addition of 0.4 cm^3 of *Me*OH and workup. Column chromatography (silica gel, hexane:*Et*OAc = 6:4 to 1:9) afforded 290 mg of **25** (23%) as white, solid foam. The regioselectivity of 2′-*O*-alkylated over 3′-*O*-alkylated product was approximately 5:4. TLC (silica gel, hexane:*Et*OAc = 1:9): $R_f = 0.6$; ^{1}H NMR (300 MHz, $CDCl_3$, 27°C): $\delta = 0.96$–1.05 (m, $^iPr_3$Si), 3.06 (d, $J = 3.9$ Hz, HO–C(3′)), 3.19 (d, $J = 4.5$ Hz, N^6-CH_3), 3.38 (dd, $J = 4.2$, 10.2 Hz, H^1–C(5′)), 3.50 (dd, $J = 3.6$, 10.2 Hz, H^2–C(5′)), 3.78 (s, 2 OCH_3), 4.26 (q, H–C(4′)), 4.52 (q, H–C(3′)), 4.93 (t, H–C(2′)), 4.98, 5.14 (2d, $J = 4.8$ Hz, OCH_2O), 5.75 (br, NH), 6.14 (d, $J = 5.4$ Hz, H–C(1′)), 6.78 (m, 4H, trityl-H), 7.22–7.44 (m, 9H, trityl-H), 7.93 (s, H–C(8)), 8.33 (s, H–C(2)) ppm; ^{13}C NMR (75 MHz, $CDCl_3$, 27°C): $\delta = 11.80$ ($(CH_3)_2CH$), 17.70 ($(CH_3)_2CH$); 27.70 (br s, N^6-CH_3), 55.13 (2 CH_3O), 63.37 (C(5′)), 70.80 (C(3′)), 81.80 (C(2′)), 84.06 (C(4′)), 86.48, 87.05 (C(1′)), 90.72 (OCH_2O), 113.10 (trityl-C), 120.41, 126.79, 127.76, 128.18, 130.05 (trityl-C), 135.77, 135.78, 138.55 (C(8)), 144.59, 153.27 (C(2)), 155.46, 158.50 ppm; UV (*Me*OH): $\lambda(\varepsilon) = 232$ (max, 28700), 260 (17300), 265 (max, 18400) nm ($mol^{-1}\,dm^3\,cm^{-1}$); MALDI-TOF-MS: $m/z = 769.9$ (20, $[M+H]^+$), 303.1 (100, $[(MeO)_2Tr]^+$).

5′-O-(4,4′-Dimethoxytrityl)-N^6-methyl-2′-O-[[(triisopropylsilyl)oxy]methyl]adenosine 3′-(2-cyanoethyl diisopropylphosphoramidite) (**26**, $C_{51}H_{72}N_7O_8PSi$)

A solution of 200 mg of **25** (0.26 mmol) in 3 cm^3 of CH_2Cl_2 was treated consecutively with 336 mg of ethyldimethylamine (2.6 mmol) and 92 mg of 2-cyanoethyl diisopropylphosphoramidochloridite (0.39 mmol). The mixture was stirred for 2 h, quenched with 0.3 cm^3 of *Me*OH, and evaporated to dryness. Workup and column chromatography (silica gel, hexane:*Et*OA*c* = 3:1 to 3:2 (+ 1% Et_3N)) afforded 216 mg of **26** as pale yellow, solid foam (86%, 1:1 mixture of diastereoisomers). TLC (silica gel, hexane:*Et*OA*c* = 1:1): $R_f = 0.5$; ^{1}H NMR (500 MHz, $CDCl_3$, 26°C): $\delta = 0.88$–1.26 (m, 42H, $^iPr_3Si$, 24H, $((CH_3)_2CH)_2N$), 2.37 (t, $J = 6.5$ Hz, 2H, CH_2CN), 2.65 (m, 2H, CH_2CN), 3.20 (br s, 6H, N^6-CH_3), 3.30–3.33 (m, 2H, H^1–C(5′)), 3.49–3.70 (m, 4H, $((CH_3)_2CH)_2N$, 2H, H^2–C(5′), 2H, $POCH_2$), 3.77, 3.78 (2s, 12H, 2 OCH_3), 3.86–3.97 (m, 2H, $POCH_2$), 4.32, 4.37 (2q, 2H, H–C(4′)), 4.67–4.75 (m, 2H, H–C(3′)), 4.91–5.01 (4d, $J = 5.0$ Hz, 4H, OCH_2O), 5.16–5.21 (m, 2H, H–C(2′)), 5.66 (br, 2H, NH), 6.11, 6.13 (2d, $J = 5.5$ Hz, 2H, H–C(1′)), 6.75–6.79, 7.17–7.41 (m, 26H, trityl-H), 7.90, 7.92 (2s, 2H, H–C(8)), 8.28, 8.30 (2s, 2H, H–C(2)) ppm; ^{31}P NMR (200 MHz, $CDCl_3$, 27°C): $\delta = 149.89$, 150.58 ppm; UV (*Me*OH): $\lambda(\varepsilon) = 232$ (max, 23200), 260 (15400), 265 (max, 16400) nm ($mol^{-1}\,dm^3\,cm^{-1}$); MALDI-TOF-MS: 970.2 (45, $[M + H]^+$), 303.1 (100, $[(MeO)_2Tr]^+$).

5′-O-(4,4′-Dimethoxytrityl)-N^6,N^6-dimethyladenosine (**27**, $C_{33}H_{35}N_5O_6$)

Compound **23** (950 mg, 2.30 mmol) was dissolved in a solution of 33% $(CH_3)_2NH$ in 6.0 cm^3 of ethanol. After 1 h a solution of 40% $(CH_3)_2NH$ in 3.0 cm^3 of water was added and stirring was continued for another 8 h. The reaction mixture was evaporated to dryness and coevaporated three times with anhydrous pyridine to be finally dissolved in 2.3 cm^3. Then, 1.16 g of 4,4′-dimethoxytritylchloride (3.41 mmol) were added in three portions over a period of 3 h. *DMAP* (45 mg, 0.36 mmol) was added and stirring was continued overnight. Addition of *Me*OH, evaporation to dryness, workup, and column chromatography (silica gel, CH_2Cl_2:CH_3OH = 25:1) yielded 840 mg of **27** as white foam (61%). TLC (silica gel, CH_2Cl_2:CH_3OH = 15:1): $R_f = 0.4$; ^{1}H NMR (500 MHz, $CDCl_3$, 30°C): $\delta = 3.14$ (s, OH), 3.20 (dd, $J = 3.6$, 10.6 Hz, H^1–C(5′)), 3.44 (dd, $J = 3.3$, 10.6 Hz, H^2–C(5′)), 3.45–3.65 (br s, N^6-$(CH_3)_2$), 3.76, 3.77 (2s, 2 OCH_3), 4.35 (d, H–C(4′)), 4.47 (t, H–C(3′)), 4.71 (t, H–C(2′)), 5.91 (d, $J = 6.4$ Hz, H–C(1′)), 6.72–6.75 (m, 4H, trityl-H), 7.04–7.28 (m, 9H, trityl-H), 8.01 (s, H–C(8)), 8.28 (H–C(2)) ppm; ^{13}C NMR (75 MHz, $CDCl_3$, 27°C): $\delta = 38.53$ (br s, N^6-$(CH_3)_2$), 55.15 (2 CH_3O), 63.64 (C(5′)), 72.94 (C(3′)), 76.33 (C(2′)), 86.46 C(4′)), 86.48, 91.00 (C(1′)), 113.13 (trityl-C), 120.54, 126.82, 127.81, 128.03, 129.92, 129.94 (trityl-C), 135.50, 135.67, 136.17 (C(8)), 144.33, 149.42, 151.58 (C(2)), 151.59, 154.96, 158.51 ppm; UV (*Me*OH): $\lambda(\varepsilon) = 260$ (13400), 274 (max, 19400) nm ($mol^{-1}\,dm^3\,cm^{-1}$); MALDI-FTICR-MS: $m/z = 620.2482$ (3, $[M + Na]^+$), 303.1390 (100, $[(MeO)_2Tr]^+$).

5′-O-(4,4′-Dimethoxytrityl)-N^6,N^6-dimethyl-2′-O-[[(triisopropylsilyl)oxy]methyl]adenosine (**28**, $C_{43}H_{57}N_5O_7Si$)

To a stirred solution of 3.23 g of **27** (5.41 mmol) and 2.79 g of ethyldiisopropylamine (21.6 mmol) in 35 cm^3 of anhydrous 1,2-dichloroethane, 1.81 g of di-*tert*-butyltindichloride (5.95 mmol) were added. The mixture was heated to 70°C for 15 min, allowed to cool to rt and treated with 1.32 g of [(triisopropylsilyl)oxy]methylchloride (5.95 mmol). Stirring was continued for 3 h, followed by addition of 0.5 cm^3 of *Me*OH, evaporation to dryness and workup. Column chromatography (silica gel, hexane: *Et*OA*c* = 3:1 to 1:1) afforded 831 mg of **28** as white, solid foam (20%). The regioselectivity of 2′-*O*-alkylated over 3′-*O*-alkylated product was approximately 5:4. TLC (silica gel, hexane: *Et*OA*c* = 1:1): $R_f = 0.7$; ^{1}H NMR (300 MHz, $CDCl_3$, 27°C): $\delta = 1.00$–1.11 (m, $^iPr_3Si$), 3.05 (d, $J =$ 4.1 Hz, HO–C(3′)), 3.36 (dd, $J = 4.3$, 10.5 Hz, H^1–C(5′)), 3.50 (dd, $J = 3.0$, 10.5 Hz, H^2–C(5′), 3.45–3.58 (br s, N^6-$(CH_3)_2$), 3.78, 3.79 (2s, 2 OCH_3), 4.26 (q, H–C(4′)), 4.48 (q, H–C(3′)), 4.87 (t, H–C(2′)),

4.99, 5.15 (2d, $J = 4.7$ Hz, OCH_2O), 6.17 (d, $J = 5.2$ Hz, H–C(1′)), 6.78–6.82 (m, 4H, trityl-H), 7.18–7.32, 7.37–7.46 (m, 9H, trityl-H), 7.93 (s, H–C(8)), 8.27 (s, H–C(2)) ppm; ^{13}C NMR (125 MHz, $CDCl_3$, 30°C): $\delta = 11.77$ ((CH_3)$_2$*C*H), 17.66, 17.71 ((CH_3)$_2$CH), 38.42 (br, N^6-(CH_3)$_2$), 55.10 (2 CH_3O), 63.36 (C(5′)), 70.68 (C(3′)), 81.85 (C(2′)), 83.84 (C(4′)), 86.38, 86.82 (C(1′)), 90.70 (OCH_2O), 113.07 (trityl-C), 120.63, 126.72, 127.73, 128.14, 130.01 (trityl-C), 135.78, 135.81, 136.78 (C(8)), 144.59, 150.35, 152.40 (C(2)), 154.87, 158.43 ppm; UV (*Me*OH): $\lambda(\varepsilon) = 260$ (12800), 274 (max, 18200) nm (mol^{-1} dm^3 cm^{-1}); MALDI-FTICR-MS: $m/z = 806.3910$ (9, $[M + Na]^+$), 303.1392 (100, $[(MeO)_2Tr]^+$).

5′-O-(4,4′-Dimethoxytrityl)-N^6,N^6-dimethyl-2′-O-[[(triisopropylsilyl)oxy]methyl]adenosine 3′-(2-cyanoethyl diisopropylphosphoramidite) (**29**, $C_{52}H_{74}N_7O_8PSi$)

A solution of 713 mg of **28** (0.91 mmol) in 8.5 cm^3 of anhydrous CH_2Cl_2 was treated consecutively with 697 mg of ethyldimethylamine (9.53 mmol) and 324 mg of 2-cyanoethyl diisopropylphosphoramidochloridite (1.37 mmol). The mixture was stirred for 2 h, quenched with 0.15 cm^3 of *Me*OH, and evaporated to dryness. Workup and column chromatography (silica gel, hexane:*Et*OA*c* = 5:2 (+ 2% Et_3N)) afforded 780 mg of **29** as white, solid foam (85%, 1:1 mixture of diastereoisomers). TLC (silica gel, hexane:*Et*OA*c*) = 2:1): $R_f = 0.6$; ^{1}H NMR (500 MHz, $CDCl_3$, 28°C): $\delta = 0.88$–1.20 (m, 42H, ^{i}*Pr*$_3$Si, 24H, ((CH_3)$_2$CH)$_2$N), 2.37, 2.65 (2m, 4H, CH_2CN), 3.30 (m, 2H, H^1–C(5′)), 3.45–3.68 (m, 2H, H^2–C(5′), 12H, N^6-(CH_3)$_2$, 4H, ((CH_3)$_2$C*H*)$_2$N, 2H, $POCH_2$), 3.77, 3.78 (2s, 12H, 2 CH_3O), 3.81–3.98 (2m, 2H, $POCH_2$), 4.32, 4.38 (2q, 2H, H–C(4′)), 4.68, 4.73 (2m, 2H, H–C(3′)), 4.92–5.01 (2m, 4H, $J \sim 5$ Hz, OCH_2O), 5.16 (m, 2H, H–C(2′)), 6.15 (2d, 2H, $J = 5.5$ Hz, H–C(1′)), 6.78, 7.20–7.40 (m, 26H, trityl-H), 7.88, 7.90 (2s, 2H, H–C(8)), 8.22, 8.24 (2s, 2H, H–C(2)) ppm; ^{31}P NMR (200 MHz, $CDCl_3$, 27°C): $\delta = 149.86$, 150.54; UV (*Me*OH): $\lambda(\varepsilon) = 232$ (max, 23100), 260 (13700), 274 (max, 19700) nm (mol^{-1} dm^3 cm^{-1}); MALDI-FTICR-MS: $m/z = 1006.4996$ (12, $[M + Na]^+$), 303.1394 (100, $[(MeO)_2Tr]^+$).

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