Synthesis of 2′-*O*-[(Triisopropylsilyl)oxy]methyl (=tom)-Protected Ribonucleoside Phosphoramidites Containing Various Nucleobase Analogues

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The first results of a study aiming at an efficient preparation of a large variety of 2′-*O*-[(triisopropylsilyl)-oxy]methyl(=tom)-protected ribonucleoside phosphoramidite building blocks containing modified nucleobases are reported. All of the here presented nucleosides have already been incorporated into RNA sequences by several other groups, employing 2′-*O*-tbdms- or 2′-*O*-tom-protected phosphoramidite building blocks (tbdms=(*tert*-butyl)dimethylsilyl). We now optimized existing reactions, developed some new and shorter synthetic strategies, and sometimes introduced other nucleobase-protecting groups. The 2′-*O*-tom, 5′-*O*-(dimethoxytrityl)-protected ribonucleosides N^2-acetylisocytidine **5**, O^2-(diphenylcarbamoyl)-N^6-isobutyrylisoguanosine **8**, N^6-isobutyryl-N^2-(methoxyacetyl)purine-2,6-diamine ribonucleoside (=N^8-isobutyryl-2-[(methoxyacetyl)amino]adenosine) **11**, 5-methyluridine **13**, and 5,6-dihydrouridine **15** were prepared by first introducing the nucleobase protecting groups and the dimethoxytrityl group, respectively, followed by the 2′-*O*-tom group (*Scheme 1*). The other presented 2′-*O*-tom, 5′-*O*-(dimethoxytrityl)-protected ribonucleosides inosine **17**, 1-methylinosine **18**, N^6-isopent-2-enyladenosine **21**, N^6-methyladenosine **22**, N^6,N^6-dimethyladenosine **23**, 1-methylguanosine **25**, N^2-methylguanosine **27**, N^2,N^2-dimethylguanosine **29**, N^6-(chloroacetyl)-1-methyladenosine **32**, N^6-{{{(1*S*,2*R*)-2-{[(tert-butyl)dimethylsilyl]oxy}-1-{[2-(4-nitrophenyl)ethoxy]carbonyl}propyl}amino}carbonyl}}-adenosine **34** (derived from L-threonine) and N^4-acetyl-5-methylcytidine **36** were prepared by nucleobase transformation reactions from standard, already 2′-*O*-tom-protected ribonucleosides (*Schemes 2–4*). Finally, all these nucleosides were transformed into the corresponding phosphoramidites **37**–**52** (*Scheme 5*), which are fully compatible with the assembly and deprotection conditions for standard RNA synthesis based on 2′-*O*-tom-protected monomeric building blocks.

1. Introduction. – All tRNAs found in Nature contain modified nucleosides in an extent of up to 25%, and more than 80 different modifications have been identified so far [1][2]. During their biosynthesis, tRNAs are undergoing different levels of processing, and the introduction of the modified nucleosides by tRNA-modifying enzymes can occur at all of these levels[1]). Some biological studies about the role of these modifications have been carried out after labor-intensive selection of modification-defective tRNA mutants, revealing the involvement of these modifications in the correct (functional) folding [3], in the maintenance of the reading frame [4] and in the proper interaction with aminoacyl-tRNA synthetases [5]. However, for a exhaustive study of their function, it would also be necessary to incorporate one or several modifications (natural or not) at selected positions [6]. Apart from a pioneering synthesis of the $tRNA^{Ala}$ from yeast, containing m^5U, Ψ, and D^2) reported in 1992 [7] and the enzymatic incorporation of a single m^1A into a tRNA transcript [8], no other attempt to prepare such compounds has been reported. Whereas a number of unnatural base-modified nucleosides have been introduced into ribozymes [9] and siRNAs [10], some of the naturally occurring modifications have been incorporated chemically into short (< 20 mer)

[1]) Interestingly, about 1% of the *E. coli* genom is coding for such enzymes [2].

model RNA sequences, thereby minimizing the difficulties resulting from incompatible assembly and deprotection conditions: m^1G [11], m_2^2G [11], m^2G [11], m^1I [11], m^3U [11], m^4C [11], m^6A [11], m_2^6A [11], m^1A [12], I [13], Ψ [14], $m^3Ψ$ [15], s^4U [16], mcm^5U [17], mcm^5s^2U [17], s^2U [18], mnm^5U [18], i^6A [19], ms^2i^6A [19], mnm^5s^2U [20] and t^6A [20][21][2]). The preparation of correspondingly modified full-length tRNA sequences, containing several modified nucleotides, however, still represents a challenge and requires an adaptation of protecting groups and deprotection conditions.

A couple of years ago, we have introduced a reliable RNA synthesis method based on 2′-*O*-[(triisopropylsilyl)oxy]methyl(=tom)-protected ribonucleoside phosphoramidites, which allows the preparation of long RNA sequences under DNA coupling conditions [22][23]. In contrast to the usually employed 2′-*O*-[(*tert*-butyl)dimethylsilyl] (=tbdms) protecting group, the 2′-*O*-tom group is stable towards a multitude of reagents and reaction conditions and offers the unique possibility to carry out base transformations in its presence [24][25]. Here we report novel and efficient syntheses of a variety of fully compatible, 2′-*O*-tom protected ribonucleoside phosphoramidites containing appropriately protected modified nucleobases[3]).

2. Synthesis of 2′-*O*-tom-Protected Nucleosides. – 2.1. *Isocytidine (isoC)*. In the context of attempts to expand the genetic code, a number of unnatural, orthogonal base pairs have been prepared. Among them, the isoG·isoC· base pair has been extensively investigated in the DNA series [27–29]. In the RNA series, both the 2′-*O*-tbdms- and N^2-[(dialkylamino)methylidene] (=dialkylformamidine)-protected isocytidine [29] [30] and 5-methylisocytidine phosphoramidite [31], respectively, have been prepared and incorporated[4]).

Uridine was first transformed into its cyclic derivative **1** under *Mitsunobu* conditions as reported [32], and then ring-opened under optimized conditions, with MeOH in the presence of Et_3N, resulting in the formation of O^2-methyluridine, which was directly transformed into its 4,4′-dimethoxytrityl ($(MeO)_2Tr$) derivative **2** under standard conditions (60% yield from **1**) (*Scheme 1*). Substitution of the MeO group of **2** with liquid NH_3 at 65° gave the isocytidine derivative **3**. After evaporation,

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[2]) Nucleoside abbreviations: m^5U=5-methyluridine, Ψ=pseudouridine, D=5,6-dihydrouridine, m^1G=1-methylguanosine, $m_2^2G=N^2,N^2$-dimethylguanosine, $m^2G=N^2$-methylguanosine, m^1I=1-methylhypoxanthosine, m^3U=3-methyluridine, $m^4C=N^4$-methylcytidine, $m^6A=N^6$-methyladenosine, $m_2^6A=N^6,N^6$-dimethyladenosine, m^1A=1-methyladenosine, I=hypoxanthosine, $m^3Ψ$=3-methylpseudouridine, s^4U=4-thiouridine, mcm^5U=5-(carboxymethyl)uridine methyl ester, mcm^5s^2U=2-thio-5-(carboxymethyl)uridine methyl ester, s^2U=2-thiouridine, mnm^5U=5-[(methylamino)methyl]uridine, $i^6A=N^6$-isopent-2-enyladenosine ms^2i^6A=2-(methylthio)-N^6-isopent-2-enyladenosine, mnm^5s^2U=2-thio-5-[(methylamino)methyl]uridine, $t^6A=N^6$-[(L-threonin-N^2-yl)carbanoyl]adenosine, DAP=purine-2,6-diamine ribonucleoside=n^2A=2-aminoadenosine.

[3]) All of the here presented modified nucleotides have already been prepared as 2′-*O*-tbdms- or 2′-*O*-tom protected phosphoramidite building blocks and were incorporated into RNA sequences. A publication about the preparation and introduction of modified ribonucleoside phosphoramidites of the nucleosides pseudouridine, 4-thiouridine, wyosine, and 5-methyl-4-demethylwyosine is in preparation [26].

[4]) The 5-methylisocytidine has been systematically used in place of isocytidine, both in the preparation of correspondingly modified DNA and RNA sequences, to avoid loss of the nucleobase during oligonucleotide deprotection, initially observed in the DNA series (for a discussion, see [28]). Meanwhile, the first RNA sequences containing isocytidine have been prepared in the context of thermodynamic investigations [30].

the N^2-acetyl protecting group was introduced by first forming the 2′,3′-bis-*O*-(trimethylsilyl) derivative with Me_3SiCl in pyridine, *N*-acetylation with AcCl, extractive workup, and treatment of the intermediate with Bu_4NF in THF (→ **4**, 63% yield, based on **2**)[5]). Introduction of the 2′-*O*-tom group into **4** was quite difficult, and even by employing optimized conditions ($^tBu_2SnCl_2$ at 25°, instead of Bu_2SnCl_2 at 70°), the 2′-*O*-tom-protected isocytidine building block **5** could be obtained only in a low yield of 12%.

2.2. *Isoguanosine (isoG).* Several syntheses of 2′-deoxyisoguanosine and isoguanosine phosphoramidites have been published [28–30][33][34]. These reports contain contradictory statements about isoguanosine nucleobase protecting groups that are compatible with the synthesis of the building blocks, and their introduction into oligonucleotide sequences, respectively. Furthermore, due to synthetic problems encountered during attempts to protect the N–C(6) with acyl-type groups [34], (dialkylformamidine)-type protecting groups have been chosen for this position, together with the diphenylcarbamoyl protecting group for O–C(2) [30]. Despite these reports, we were able to efficiently prepare N^6-isobutyrylisoguanosine (**6**) by treating carefully dried isoguanosine (24 h at 50°/0.01 mbar, followed by treatment with 4 Å molecular sieves in pyridine for 2 h at 25°) first with 10 equiv. of Me_3SiCl and 1.2 equiv. of isobutyryl chloride (=2-methylpropanoyl chloride), followed by extraction at 4° and subsequent hydrolysis of the Me_3Si ethers with AcOH in MeOH[6]) (*Scheme 1*). The crude product **6**, obtained after evaporation, was sufficiently pure (^{1}H-NMR: >90%) to be used directly for the next one-pot reaction sequence. First, the O–C(2) position was protected with diphenylcarbamoyl chloride in pyridine, and then the O–C(5′) position with $(MeO)_2TrCl$ (→ **7**, 45% yield from isoguanosine). The 2′-*O*-tom-protected derivative **8** was then prepared under standard conditions (23% yield).

2.3. *Purine-2,6-diamine Ribonucleoside (DAP=2-Aminoadenosine=n^2A).* So far, three syntheses of 2′-*O*-tbdms-protected 2-aminoadenosine phosphoramidites have been reported, carrying as nucleobase protecting groups two benzoyl [34], two diphenoxyacetyl [35], or two acetyl groups [36]. To be fully compatible with our standard deprotection protocol [23], we have chosen to introduce an N^6-isobutyryl and an N^2-(methoxyacetyl) protecting group, respectively, in analogy to the corresponding 3′-deoxyribopyranosyl building block [37]. The 2-[(methoxyacetyl)amino]adenosine **9** was obtained by treating the nucleoside in pyridine first with Me_3SiCl, and then with methoxyacetyl chloride, followed by extraction and subsequent treatment with NH_3 in $MeOH/H_2O$/THF (86% yield after crystallization; *Scheme 1*). A similar procedure was then employed to introduce the N^6-isobutyryl group. The intermediate **9** in pyridine was treated first with Me_3SiCl and then with isobutyryl chloride; after extraction, the remaining Me_3Si ethers were cleaved by AcOH in MeOH according to [23]. After evaporation, the crude N^6-acylated 2-[(methoxyacetyl)amino]adenosine was treated with $(MeO)_2TrCl$ in pyridine (→ **10**, 54% yield). The 2′-*O*-tom group was finally introduced under standard conditions (→ **11**, 37% yield)[7]).

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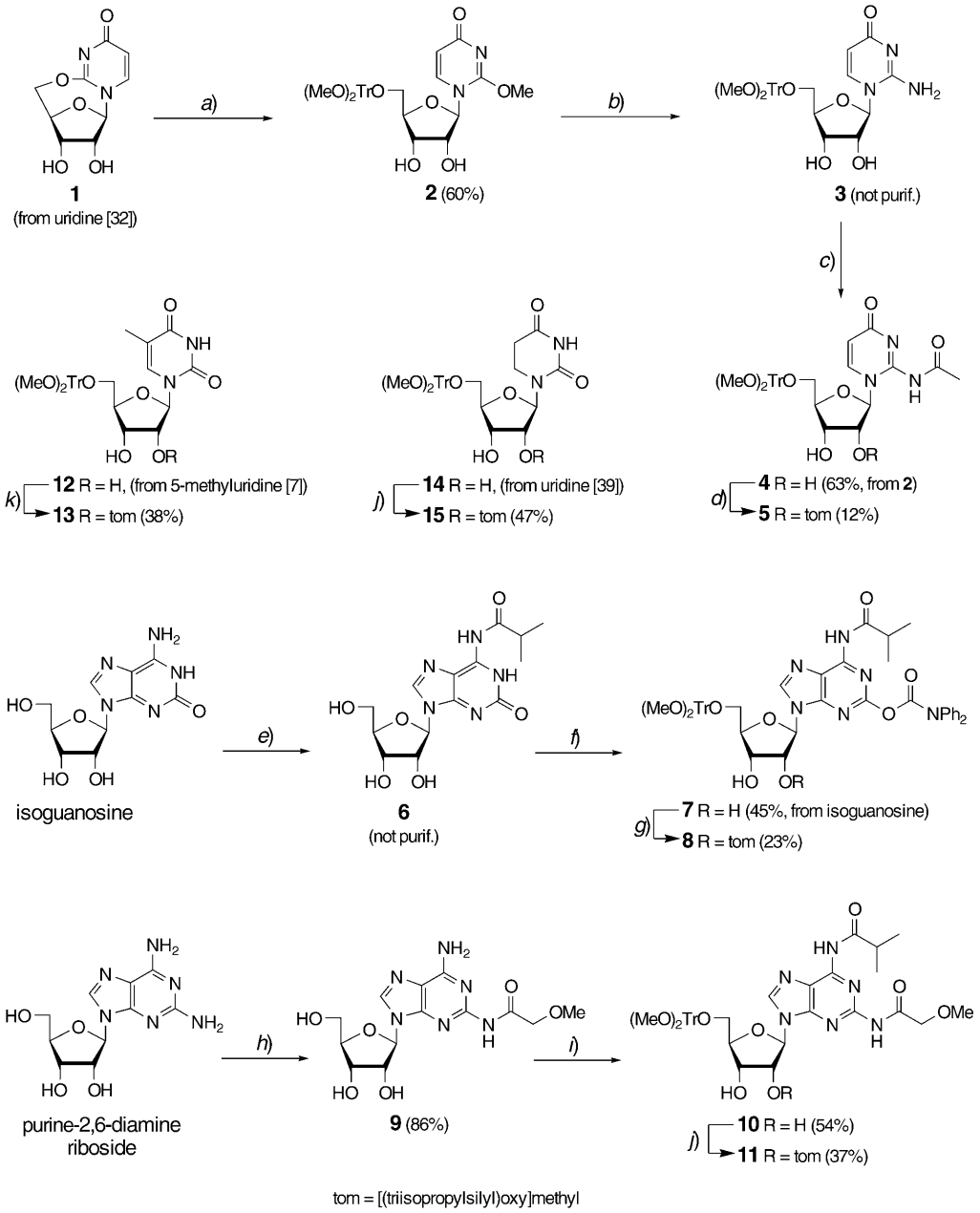
[5]) The traditional, less efficient synthesis involves first preparation of isocytidine (treatment of **1** with NH_3), followed by sequential protection of positions N(2), O–C(5′), and O–C(2′) [28].

[6]) This reaction sequence was carried out in analogy to the preparation of N^6-acetyladenosine [23].

[7]) Carried out by *L. Reymond* (Ph. D. Thesis EPFL, in preparation)

Scheme 1



a) 1. Et_3N, MeOH, 65°; 2. $(MeO)_2TrCl$, pyridine, 20°. *b*) NH_3, 65°. *c*) 1. Me_3SiCl, pyridine, 4°; then AcCl, DMAP (= *N*,*N*-dimethylpyridin-4-amine), 20°; 2. Bu_4NF, THF, 20°. *d*) $^tBu_2SnCl_2$, $^iPr_2NEt$, $(CH_2Cl)_2$, 20°; then tom-Cl, 20°. *e*) 1. Me_3SiCl, pyridine, 4°; then DMAP, isobutyryl chloride, 20°; 2. AcOH, MeOH, 20°. *f*) $Ph_2NC(=O)Cl$, $^iPr_2NEt$, pyridine, 20°; then $(MeO)_2TrCl$, 20°. *g*) Bu_2SnCl_2, $^iPr_2NEt$, $(CH_2Cl)_2$, 20°; then tom-Cl, 75°. *h*) 1. Me_3SiCl, pyridine, 4°; then methoxyacetyl chloride, MeCN, 4°; 2. NH_3, MeOH, THF, H_2O, 20°. *i*) 1. Me_3SiCl, pyridine, 4°; then isobutyryl chloride, 20°; 2. AcOH, MeOH, 20°; 3. $(MeO)_2TrCl$, pyridine, 20°. *j*) Bu_2SnCl_2, $^iPr_2NEt$, $(CH_2Cl)_2$, 20°; then tom-Cl, 80°. *k*) Bu_2SnCl_2, ^iA_2NEt, $(CH_2Cl)_2$, 20°; then tom-Cl, 20°.

2.4. *5-Methyluridine (m^5U)*. The 5-methyluridine is one of the most conserved modified nucleosides found in tRNAs and is usually located at position 54 in the TΨC loop [1]. We prepared the 2′-*O*-tom protected 5-methyluridine **13** from the parent nucleoside in two steps, by first introducing the dimethoxytrityl group with $(MeO)_2TrCl$ in pyridine according to [7], followed by alkylation of the product **12** with tom-Cl under standard conditions [23] (→ **13**, 38% yield; *Scheme 1*).

2.5. *5,6-Dihydrouridine (D)*. This nucleoside is extensively conserved in the D-loop of bacterial and eukaryotic tRNAs. Its saturated C(5)–C(6) bond prevents this uracil derivative from stacking[8]). We prepared the protected nucleoside **14** in analogy to the reported approach [39], consisting in hydrogenation of uridine with Rh/H_2, followed by introduction of the $(MeO)_2Tr$ group. The 2′-*O*-tom group was introduced under standard conditions [23] (→ **15**, 47% yield; *Scheme 1*)[9]).

2.6. *Inosine (I)*. This nucleoside was one of the first modifications found at position 34 of certain tRNAs, and its unique pairing properties (it is able to recognize A, U, and C) was the foundation of the wobble hypothesis postulated by *Crick* [1]. The incorporation of the 2′-*O*-tbdms-protected inosine phosphoramidite into RNA sequences permitted several biological investigations [13]. We prepared its 2′-*O*-tom protected analogue **16** earlier, as a common intermediate for the synthesis of ^{15}N-labelled adenosine and guanosine phosphoramidites by nucleobase transformation reactions [24]. Treatment of this intermediate **16** with NH_3 in MeOH, followed by evaporation and dimethoxytritylation in pyridine under standard conditions provided **17** (67% yield; *Scheme 2*).

2.7. *1-Methylinosine (m^1I)*. The 2′-*O*-tom protected phosphoramidite of this nucleoside, found at position 37 of tRNAs, has already been prepared by the introduction of the tom group into the corresponding nucleoside [11]. As an alternative to this procedure, we efficiently methylated the 2′-*O*-tom 5′-*O*-$(MeO)_2Tr$ protected inosine derivative **17** with MeI/K_2CO_3 in DMF, obtaining **18** in 98% yield (*Scheme 2*).

2.8. *N^6-Isopent-2-enyladenosine (i^6A)*. This nucleoside acts as a growth factor (cytokinin) in plants [40] and is also found at position 37 of tRNAs [1]. Only recently, it was incorporated enzymatically into tRNAs for studying anticodon stem structuration [41], and chemically into short RNA sequences for thermodynamic investigations [19][42]. The reported methodology involved the preparation of a phosphoramidite building block with a reactive, 'convertible' purine derivative, which was converted into the N^6-isopentenyladenosine upon treatment of the immobilized RNA sequence with isopentenylamine[10]). We recently reported an optimized method for the preparation of the 2′-*O*-tom-protected, 6-(nitrotriazolyl)-substituted adenosine derivative **19** [24], which served as convenient precursor for the N^6-alkylated adenosine derivatives **20**, **22**, and **23** (*Scheme 2*). By treatment of **19** with isopent-2-enylamine (=3-methylbut-2-en-1-amine) in pyridine/Et_3N, the corresponding N^6-isopent-2-enyl derivative **20** was obtained in 89% yield. Deacetylation of this intermediate with NH_3 in MeOH, fol-

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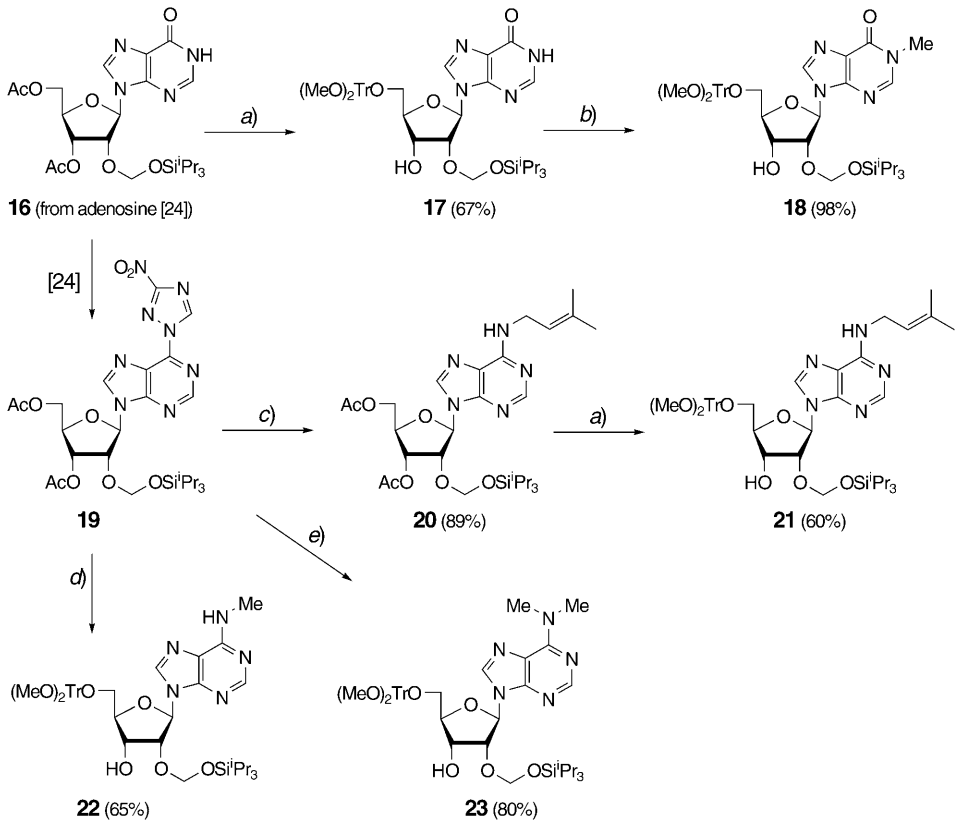
[8]) Revealed by an NMR investigation of a short synthetic D-containing oligomer, which was prepared from the 2′-*O*-tbdms-protected phosphoramidite [38].

[9]) Hydrogenation of 2′-*O*-tom protected uridine provided the corresponding 5,6-dihydrouridine derivative in excellent yield. This reaction, however, could not be carried out in the presence of a 5′-*O*-$(MeO)_2Tr$ group.

[10]) The same strategy was used for the preparation of a m^6A containing RNA sequence [43].

Scheme 2



a) 1. NH_3, MeOH, 20°; 2. $(MeO)_2TrCl$, pyridine, 20°. *b*) 1. K_2CO_3, DMF, 20°; then MeI, −15°. *c*) Isopent-2-enylamine·HCl, Et_3N, pyridine, 20°. *d*) 1. $MeNH_2$, EtOH, 20°; 2. $(MeO)_2TrCl$, pyridine, 20°. *e*) 1. Me_2NH, EtOH, 20°; 2. $(MeO)_2TrCl$, pyridine, 20°.

lowed by evaporation and dimethoxytritylation with $(MeO)_2TrCl$ in pyridine gave the fully protected N^6-isopent-2-enyladenosine **21** in 60% yield.

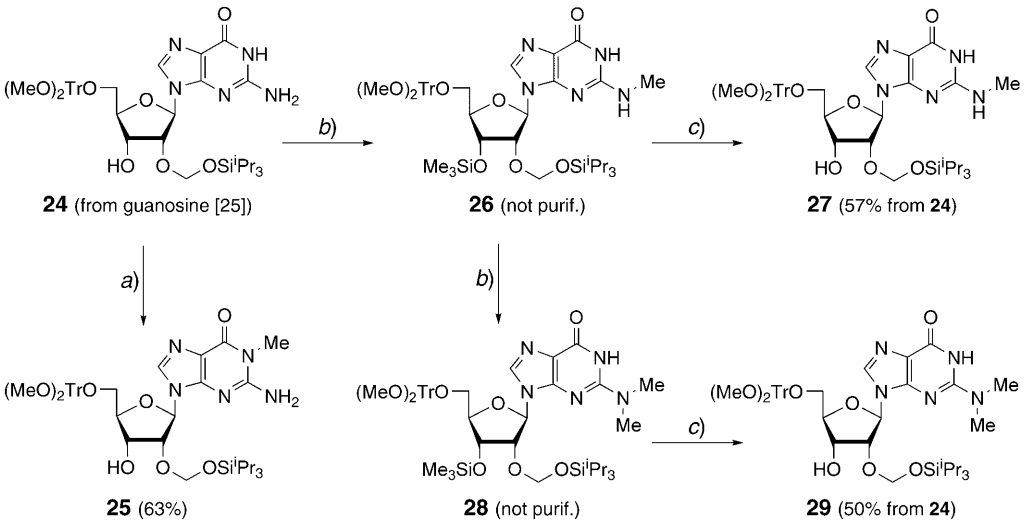
2.9. *N^6-Methyladenosine (m^6A).* Often adjacent to a cytidine at position 37 in some bacterial tRNAs, this nucleoside is involved in a proper codon/anticodon recognition [1]. In analogy to the preparation of **21** (see *Sect. 2.8*), we treated the 2′-*O*-tom-protected purine derivative **19** with $MeNH_2$ in EtOH, followed by introduction of the $(MeO)_2Tr$ group under standard conditions, and obtained **22** in 65% yield (*Scheme 2*).

2.10. *N^6,N^6-Dimethyladenosine (m^6_2A).* This adenosine derivative is found at the 5′-terminal cap sequence of some mRNAs and is related to the antibiotic nucleoside analogue puromycin. The 2′-*O*-tom, 5′-*O*-$(MeO)_2Tr$ protected N^6,N^6-dimethyladenosine **23** was obtained again from the intermediate **19**, upon treatment with Me_2NH in EtOH and $(MeO)_2TrCl$ in pyridine (80% yield, *Scheme 2*).

2.11. *1-Methylguanosine (m^1G).* This abundant guanosine derivative is found at position 37 of many tRNAs and is correlated with the presence of adenosine in position 36 [1], preventing frame shift by ‘quadruplet translocation’ [44]. A multi-step synthesis

of the 2′-*O*-tom, *N*-acetyl-protected 1-methylguanosine phosphoramidite has been reported [11]. We prepared the corresponding nucleoside **25** from the readily available intermediate **24** [25] by treatment with MeI/K_2CO_3 in DMF at −15° (63% yield; *Scheme 3*)[11]).

Scheme 3



a) 1. K_2CO_3, DMF, 20°; then MeI, −15°. b) 1. Me_3Si-Cl, pyridine, 20°; then 1,3-benzodithiolylium tetrafluoroborate; 2. $(SiMe_3)_3SiH$/AIBN, benzene, reflux. *c*) NH_3, MeOH, THF, 20°.

2.12. N^2-*Methylguanosine* (*m^2G*). This modified nucleoside, present at different positions within tRNAs, has been already incorporated into RNA sequences as its 2′-*O* tom/*O^6*-(nitrophenylethyl)-protected phosphoramidite building block. The methylamino substituent was thereby introduced into a protected 2-fluoroinosine derivative by a nucleophilic aromatic substitution reaction with $MeNH_2$ [11]. In contrast, we introduced the Me group into the protected guanosine derivative **24** by adopting a one-pot method developed by *Sekine* and *Satoh* [45]. First, the nucleoside **24** in pyridine was treated with Me_3SiCl (→ silylation of O−C(6) and O−C(3′)) and then with 1,3-benzodithiolylium tetrafluoroborate (*Scheme 3*). The resulting *N^2*-(1,3-benzodithiol-2-yl) derivative was filtered on silica gel and treated with $(Me_3Si)_3SiH$ [46]/2,2′-azobis[isobutyronitrile] (AIBN) in refluxing benzene (→ **26**, not isolated)[12]). Finally, the remaining Me_3Si group of **26** was removed with NH_3 in MeOH, and the nucleoside **27** was obtained in a 57% yield (based on **24**).

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[11]) The modified nucleoside **25** was already incorporated by employing a corresponding *N^2*-acetylated buidling block. We found, however, that this protecting group is not required, since the N(2) position of guanosine derivatives is inert under standard coupling conditions (see also [25] for the incorporation of the parent *N^2*-unprotected guanosine phosphoramidite into RNA sequences).

[12]) This reduction was originally carried out with Bu_3SnH/AIBN according to [45]; however, it was difficult to separate the tin-containing by-products from **26** and **27**.

2.12. N^2,N^2-*Dimethylguanosine* (m_2^2G). This dimethylated guanosine is a rare tRNA modification, occurring at different positions. The corresponding 2′-*O*-tom,5′-*O*-$(MeO)_2$-Tr,6-*O*-[(4-nitrophenyl)ethyl]-protected phosphoramidite has been prepared earlier in analogy to the related N^2-monomethylguanosine derivative [11] (see also *Sect. 2.11*). We prepared the protected N^2,N^2-dimethylguanosine nucleoside **28** from the crude N^2-monomethylated intermediate **26** (see *Sect. 2.11*) by repeating the sequential treatment with 1,3-benzodithiolylium tetrafluoroborate [45] and $(Me_3Si)_3SiH$ [46]/AIBN (→ **28**, not isolated; *Scheme 3*). After cleavage of the Me_3Si group with NH_3 in MeOH, the fully protected nucleoside **29** was obtained in 50% yield (from **24**).

2.13. *1-Methyladenosine* (m^1A). This modified nucleoside is found at position 58 of *ca*. 25% of all tRNAs, at position 14 in the D-loop and at position 9 of some mitochondrial tRNAs, and is required for a proper functional folding of human mitochondrial $tRNA^{Lys}$ [8]. The corresponding 2′-*O*-tbdms-protected phosphoramidite has been prepared by stepwise introduction of all protecting groups (1. chloroacetyl, 2. $(MeO)_2$Tr, 3. tbdms) into 1-methyladenosine [12]. In contrast, we first prepared the 1-methyl, 2′-*O*-tom-protected adenosine derivative **31** by treatment of the easily accessible nucleoside **30** [25] with MeI in DMF (98% yield; *Scheme 4*). The chloroacetyl protecting group was then introduced with $(ClCH_2CO)_2O$ in pyridine according to [12], and the fully protected 1-methyladenosine nucleoside **32** was obtained in a yield of 64% after cleavage of the concomitantly formed 3′-*O*-(chloroacetyl) group with NH_3 in MeOH according to [12].

2.14. N-*[(9-β-D-Ribofuranosyl-9*H*-purin-6-yl)carbamoyl]-*L*-threonine* ($t^6A = N^6$*[(*L*-Threonin-*N^α*-yl)carbamoyl]adenosine)*. This is one of the most extensively modified nucleosides found at the position 37 of tRNAs which recognize codons starting with an adenosine; it is also the most conserved modification within all phylogenetic domains of life [1]. The corresponding 2′-*O*-tbdms-protected phosphoramidite has been prepared already twice (with different protecting groups for the L-threonine moiety), by first adding the protected amino acid derivative to an activated N^6-carbamoyladenosine [20] or an N^6-isocyanato adenosine [21], followed by stepwise introduction of the 5′-*O*-$(MeO)_2$Tr and the 2′-*O*-tbdms protecting group [20][21]. In 1999, we have introduced a method for the preparation of *N*-carbamoylated nucleosides, which was applied to the synthesis of ribonucleosides containing photolabile [47] and fluoride labile [25] nucleobase protecting groups. Meanwhile, we have further optimized this method[13]), which now allowed the straightforward synthesis of nucleoside **34** from the adenosine derivative **30**. Acetylation at the 3′-*O* position of **30** with Ac_2O in pyridine gave the derivative **33** in a yield of 88% (*Scheme 4)*. This intermediate was treated first with 1,1′-carbonylbis[(1*H*-1,2,4-triazole] and Et_3N in 1,2-dichloroethane, followed by addition of *O*-[(*tert*-butyl)dimethylsilyl]-L-threonine 2-(4-nitrophenyl)ethyl ester (prepared according to [21]). After extraction, the remaining 3′-*O*-acetyl group was cleaved with NH_3 in MeOH, and the fully protected t^6A derivative **34** was isolated in of 56% yield (based on **33**).

2.15. *5-Methylcytidine* (m^5C). Present at position 49 of some tRNAs, this nucleoside interestingly is also found at the wobble position 34 of some eukaryotic tRNA, where it

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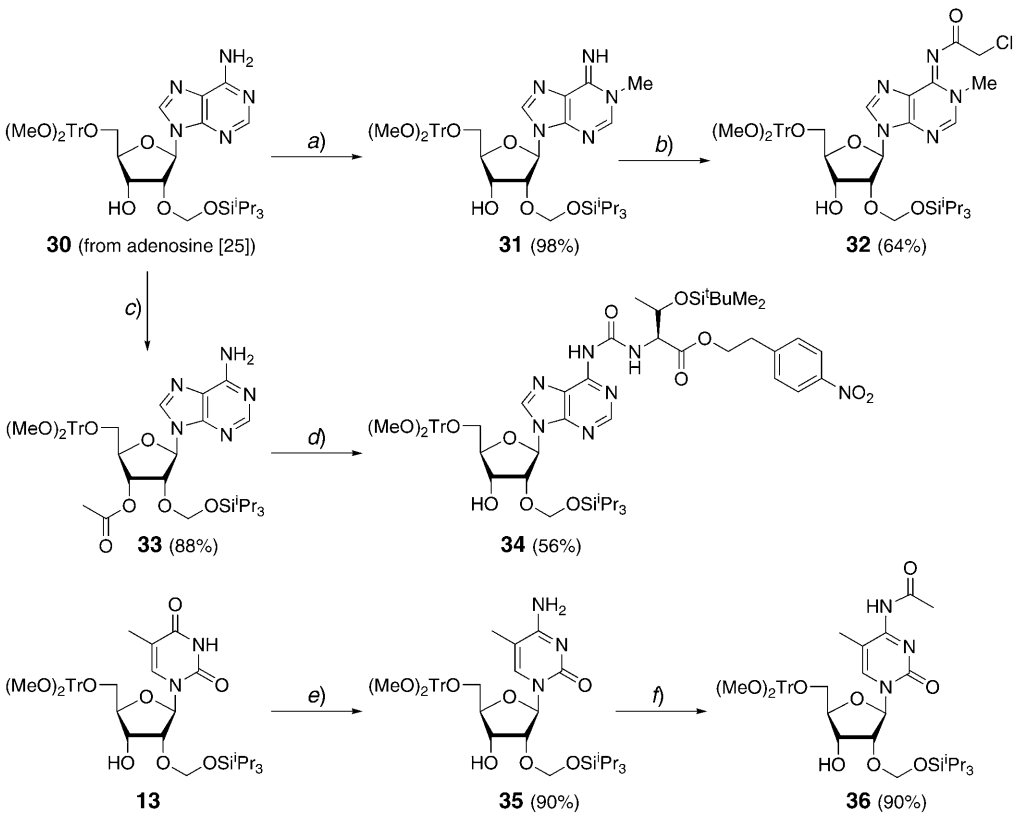
[13]) Carried out by *M. Meyappan* (Postdoc EPFL).

Scheme 4

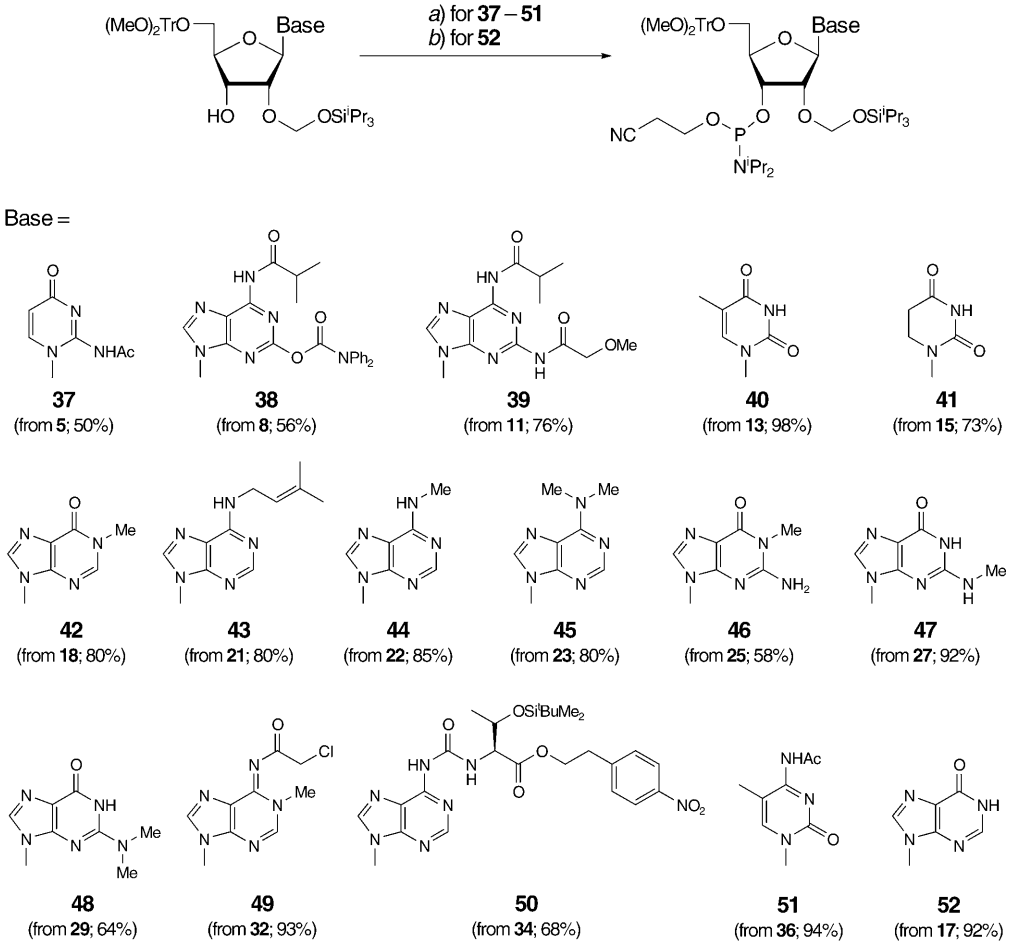


a) MeI, DMF, 20°. *b*) 1. $(ClCH_2CO)_2O$, $(CH_2Cl)_2$, pyridine, −15°; 2. NH_3, MeOH, −15°. *c*) 1. Ac_2O, DMAP, pyridine, 20°. *d*) 1. 1,1′-Carbonylbis[1*H*-(1,2,4 triazole], Et_3N, $(CH_2Cl)_2$, 70°, then O^3-[(*tert*-butyl)dimethylsilyl]-L-threonine 2-(4-nitrophenyl)ethyl ester, 70°; 2. NH_3, MeOH, 20°. *e*) 1. Ac_2O, DMAP, pyridine, 20°; 2. 1*H*-1,2,4-triazole, 4-chlorophenyl phosphorodichloridate, $^iPr_2NEt$, MeCN, 20°; 3. NH_3, dioxane, MeCN, H_2O, 20°; 4. NaOH, THF, MeOH, H_2O, 4°. f) Ac_2O, DMF, 20°.

replaces 2′-*O*-methylcytidine [1]. We prepared the corresponding 2′-*O*-tom protected nucleoside **36** by adapting one of our recently reported, optimized base-transformation methods [24]. The 5-methyluridine nucleoside **13** (see *Scheme 1*) was first acetylated at the 3′-OH group with Ac_2O in pyridine, then treated with $(ClC_6H_4O)P(O)Cl_2$, 1*H*-1,2,4-triazole, and $^iPr_2NEt$ in MeCN (→ formation of the 4-triazolide derivative), and finally with NH_3 in dioxane/H_2O; after extraction and deacetylation with NaOMe in MeOH, the cytidine nucleoside **35** was isolated in 90% yield. Finally, the latter was transformed into its N^4-acetylated derivative **36** by selective *N*-acetylation with Ac_2O in DMF and isolated in 90% yield.

3. Preparation of Phosphoramidites. – The protected nucleosides **5**, **8**, **11**, **13**, **15**, **18**, **21**–**23**, **25**, **27**, **29**, **32**, **34**, and **36** were finally converted with $^iPr_2NEt$/2-cyanoethyl diisopropylphosphoramidochloridite in CH_2Cl_2 into the corresponding phosphoramidites

Scheme 5



a) 2-Cyanoethyl diisopropylphosphoramidochloridite, ^{i}Pr$_2$NEt, CH_2Cl_2, 25°. *b*) 2-Cyanoethyl tetraisopropylphosphoramidite, 5-benzyl-1*H*-tetrazole, MeCN, 25°.

37–**51** (*Scheme 5*). However, due to a significant side reaction at O–C(6), the protected inosine phosphoramidite **52** was prepared by a different method, by treating the nucleoside **17** with 2-cyanoethyl tetraisopropylphosphoramidite and 5-(benzylthio)-1*H*-tetrazole [22] [23] in MeCN (conditions adapted from [48]). After chromatography on silica gel, these building blocks were isolated in yields between 50 and 98%[14]).

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[14]) The lower yields were obtained from small-scale reactions. High yields of phosphoramidites (>85%) can usually be obtained only from reactions carried out on a >0.5-mmol scale. Since for small-scale reactions more equivalents of silica gel have to be employed, the decomposition of these reactive compounds occurs more efficiently.

We are grateful to *Luc Reymond* and *Muthulaniappan Meyyappan* for developing the synthesis of compounds **11** and a method for the preparation of **34**, respectively, and to *Philipp Wenter* for his continuous support in carrying out NMR experiments. This work was generously supported by the EPFL and by the *Swiss National Science Foundation* (Grant Nr. 2000-06890).

Experimental Part

General. Reagents and solvents (highest purity): from various suppliers, used without further purification; [(triisopropylsilyl)oxy]methyl chloride (=tom-Cl) was prepared according to [23]. The 4 Å molecular sieves (4 Å MS) were activated overnight at 180°/0.01 mbar. Room temperature (r.t.) means *ca.* 20°. Workup implies distribution of the reaction mixture between CH_2Cl_2 and sat. aq. $NaHCO_3$ soln., drying of the org. layer ($MgSO_4$), and evaporation. TLC: precoated silica gel plates from *Merck*, stained by dipping into a soln. of anisaldehyde (10 ml), H_2SO_4 (10 ml), and AcOH (2 ml) in EtOH (180 ml) and subsequent heating with a heat gun. CC (column chromatography): silica gel *60* (230–400 mesh) from *Fluka*. NMR: *Bruker spectrometers;* chemical shift δ in ppm rel. to external standards (^{1}H- and ^{13}C: Me_4Si; ^{31}P: 85% aq. H_3PO_4 soln.), *J* in Hz. ESI-MS (pos. mode): *Finnigan SSQ-710* system; measurements in MeCN/H_2O/AcOH 50:50:1; in *m*/*z* (rel.%).

*5′-*O*-(4,4′-Dimethoxytrityl)-*O^2*-methyluridine* (**2**). A soln. of **1** (4.67 g, 20.6 mmol, prepared according to [32]) in MeOH (100 ml) was treated with Et_3N (10 ml, 58.4 mmol) and stirred overnight at 70° in an autoclave. Evaporation gave a white solid which was dissolved in pyridine (75 ml), treated with $(MeO)_2TrCl$ (6.93 g, 20.3 mmol), and stirred for 12 h at r.t. Workup and CC (SiO_2 (140 g), CH_2Cl_2/MeOH 19:1 → 17:3) gave **2** (6.15 g, 60%). Yellow foam. TLC (CH_2Cl_2/MeOH 19:1): R_f 0.15. ^{1}H-NMR (400 MHz, $CDCl_3$): 3.44 (*m*, CH_2(5′)); 3.49 (*s*, OH); 3.78 (*s*, 2 MeO); 3.96 (*s*, MeO–C(2)); 4.19 (*d*, *J*=3.9, H–C(4′)); 4.41 (*t*, *J*=4.8, H–C(2′)); 4.48 (*t*, *J*=5.1, H–C(3′)); 5.59 (*d*, *J*=7.7, H–C(5)); 5.95 (*d*, *J*=5.5, H–C(1′)); 6.85 (*d*, *J*=8.8, 4 arom. H); 7.21–7.41 (*m*, 9 arom. H); 7.77 (*d*, *J*=7.7, H–C(6)). ^{13}C-NMR (100 MHz, $CDCl_3$): 55.3 (*q*, *Me*O); 55.9 (*q*, MeO–C(2)); 63.0 (*t*, C(5′)); 71.1 (*d*, C(3′)); 74.7 (*d*, C(2′)); 83.9 (*d*, C(4′)); 87.2 (*s*, arom. C); 89.7 (*d*, C(1′)); 113.4 (*d*, arom. C); 127.1, 128.1, 128.2, 130.1, 130.2 (5*d*, arom. C); 135.1, 135.3 (2*s*, arom. C); 138.4 (*d*, C(6)); 144.3 (*s*, arom. C); 156.3 (*s*, C(2)); 158.7 (*s*, arom. C); 164.1 (*s*, C(4)); 171.6 (*s*, arom. C). ESI-MS: 561.27 (100, $[M+H]^+$).

*5′-*O*-(4,4′-Dimethoxytrityl)isocytidine* (**3**). NH_3 (50 ml) was condensed into an autoclave containing **2** (2.50 g, 4.4 mmol). After sealing, the mixture was stirred for 12 h at 65°. Evaporation gave crude **3** (2.07 g) as yellow oil. An anal. sample was obtained by prep. TLC (CH_2Cl_2/MeOH 9:1). TLC (CH_2Cl_2/MeOH 19:1): R_f 0.18. ^{1}H-NMR (400 MHz, (D_6)DMSO): 3.20–3.26 (*m*, CH_2(5′)); 3.73 (*s*, 2 MeO); 4.00 (*d*, *J*=3.0, H–C(4′)); 4.07–4.11 (*m*, H–C(3′), H–C(2′)); 5.27 (*d*, *J*=5.1, OH–C(3′)); 5.35 (*d*, *J*=7.4, H–C(5)); 5.52 (*d*, *J*=5.1, OH–C(2′)); 5.60 (*d*, *J*=4.4, H–C(1′)); 6.89 (*d*, *J*=8.8, 4 arom. H); 7.22–7.37 (*m*, 9 arom. H); 7.53 (*d*, *J*=7.3, H–C(6)). ^{13}C-NMR (100 MHz, (D_6)DMSO): 55.6 (*q*, MeO); 63.4 (*t*, C(5′)); 69.9 (*d*, C(2′)); 74.2 (*d*, C(3′)); 83.7 (*d*, C(4′)); 86.4 (*s*, arom. C); 90.7 (*d*, C(1′)); 107.4 (*d*, C(5)); 113.7 (*d*, arom. C); 127.3, 128.1, 128.4, 130.2 (4*d*, arom. C); 135.6 (*s*, arom. C); 137.4 (*s*, arom. C); 145.0 (*d*, C(6)); 155.2 (*d*, C(2)); 158.6 (*s*, arom. C); 169.92 (*s*, C(4)). ESI-MS: 546.29 (100, $[M+H]^+$).

N^2*-Acetyl-5′-*O*-(4,4′-dimethoxytrityl)isocytidine* (**4**). A soln. of crude **3** (2.30 g, ca. 4.2 mmol) in pyridine (18 ml) was treated with Me_3SiCl (2.6 ml, 21.0 mmol). After 90 min at 4°, DMAP (250 mg, 2.1 mmol) and AcCl (0.3 ml, 4.2 mmol) were added. After 14 h at r.t., and workup, the residue was treated 1M with Bu_4NF in THF (20 ml) at r.t. for 5 min. Addition 0.1M of aq. Na-phosphate buffer (100 ml; pH 7), workup, and CC (SiO_2 (60 g), CH_2Cl_2/acetone 9:1 → 1:1) gave **4** (1.81 g, 63% based on **2**). Yellow foam. TLC (CH_2Cl_2/MeOH 19:1): R_f 0.23. ^{1}H-NMR (400 MHz, $CDCl_3$): 2.24 (*s*, MeCO); 3.29–3.33 (*m*, CH_2(5′)); 3.80 (*s*, 2 MeO); 3.37–3.46 (*m*, H–C(3′), H–C(4′)); 4.40–4.43 (*m*, H–C(2′), OH); 5.62 (*d*, *J*=7.8, H–C(5)); 6.02 (*s*, H–C(1′)); 6.84 (*d*, *J*=7.0, 4 arom. H); 7.24–7.38 (*m*, 9 arom. H); 7.92 (*d*, *J*=8.6, H–C(6)); 8.62 (*d*, *J*=3.9, NH–C(2)). ^{13}C-NMR (100 MHz, $CDCl_3$): 24.5 (*q*, *Me*CO); 55.7 (*q*, MeO); 59.4 (*t*, C(5′)); 63.4 (*d*, C(2′)); 72.7 (*d*, C(3′)); 86.7 (*d*, C(4′)); 87.6 (*s*, arom. C); 93.5 (*d*, C(1′)); 105.7 (*d*, C(5)); 113.7 (*d*, arom. C); 124.2, 127.6, 128.4, 128.5, 130.4, (5*d*, arom. C); 135.4, 135.5 (2*s*, arom. C); 139.9 (*d*, C(6)); 144.5 150.1 (2*s*, arom. C); 153.6 (*d*, C(2)); 159.1 (*s*, arom. C); 159.7 (*s*, C(4)); 184.7 (*s*, Me*C*O). ESI-MS: 588.36 (100, $[M+H]^+$).

N^2*-Acetyl-5′-*O*-(4,4′-dimethoxytrityl)-2′-*O*-{[(triisopropylsilyl)oxy]methyl}isocytidine* (**5**). A soln. of **4** (3.50 g, 5.9 mmol) and ^{i}Pr_2NEt (3.50 ml, 20.8 mmol) in $ClCH_2CH_2Cl$ (35 ml) was treated with ^{t}Bu_2SnCl_2 (1.99 g, 6.6 mmol), stirred for 30 min at r.t., treated with tom-Cl (1.46 g, 6.6 mmol), and stirred at r.t. for 25 min. Workup and CC (SiO_2 (35 g), hexane/AcOEt 9:1 → 1:1) gave **5** (552 mg, 12%). Light yellow foam. TLC (hexane/AcOEt 1:1): R_f 0.62. ^{1}H-NMR (400 MHz, $CDCl_3$): 1.00–1.22 (*m*, ^{i}Pr_3Si); 2.20 (*s*, MeCO); 3.02 (*d*, *J*=7.8,

OH–C(3′)); 3.59 (br. *s*, CH_2(5′)); 3.82 (*s*, 2 MeO); 4.09 (*d*, *J* = 7.8, H–C(4′)); 4.35 (*d*, *J* = 3.9, H–C(2′)); 4.51 (*d*, *J* = 4.7, H–C(3′)); 5.26 (*d*, *J* = 3.9, 1 H, OCH_2O); 5.29 (*d*, *J* = 3.9, 1 H, OCH_2O); 5.35 (*d*, *J* = 7.8, H–C(5)); 6.27 (*s*, H–C(1′)); 6.86 (*d*, *J* = 8.6, 4 arom. H); 7.29–7.45 (*m*, 9 arom. H); 8.29 (*d*, *J* = 8.5, H–C(6)); 13.0 (br. *s*, NH–C(2)). ^{13}C-NMR (100 MHz, $CDCl_3$): 12.3 (*d*, Me_2CH); 18.2 (*q*, Me_2CH); 28.8 (*q*, *Me*CO)); 55.7 (*q*, MeO); 61.5 (*t*, C(5′)); 68.6 (*d*, C(2′)); 82.3 (*d*, C(3′)); 84.0 (*d*, C(4′)); 87.6 (*s*, arom. C); 89.4 (*d*, C(1′)); 90.4 (*t*, OCH_2O); 105.6 (*d*, C(5)); 113.7 (*d*, arom. C); 127.6, 128.4, 128.6, 130.5, 130.6 (5*d*, arom. C); 135.4 (*s*, arom. C); 140.2 (*d*, C(6)); 144.7 (*s*, arom. C); 152.4 (*d*, C(2)); 159.2 (*s*, arom. C); 160.2 (*s*, C(4)); 185.4 (*s*, Me*C*O). ESI-MS: 774.29 (100, $[M+H]^+$).

N^6-*Isobutyrylisoguanosine* (= N^6-*(2-Methyl-1-oxopropyl)isoguanosine;* **6**). Carefully dried (0.01 mbar, 24 h at 50°) isoguanosine (= 1,2-dihydro-2-oxoadenosine; 2.83 g, 10 mmol) was suspended in pyridine (50 ml) containing 4 Å MS, stirred 2 h at r.t., treated with Me_3SiCl (10 ml, 80 mmol), and stirred for 2 h at 4°. Then, DMAP (980 mg, 7 mmol) and isobutyryl chloride (1.2 ml, 11 mmol) were added. After 12 h at r.t., workup, and evaporation, the yellow oil was dissolved in MeOH/AcOH 9 : 1 (50 ml) and stirred for 3 h at r.t. Evaporation gave crude **6** (3.41 g). White solid. TLC (CH_2Cl_2/MeOH 1 : 1): R_f 0.10. ^{1}H-NMR (400 MHz, (D_6)DMSO): 1.12 (*d*, *J* = 6.8, Me_2CHCO); 3.00 (*sept.*, *J* = 6.8, Me_2CHCO); 3.55–3.65 (*m*, CH_2(5′)); 3.92 (*dd*, *J* = 6.7, 3.4, H–C(4′)); 4.10 (*dd*, *J* = 8.2, 4.7, H–C(3′)); 4.48 (*dd*, *J* = 5.8, 11.2, H–C(2′)); 5.18 (*d*, *J* = 4.8, OH–C(3′)); 5.25 (*m*, OH–C(5′)); 5.47 (*d*, *J* = 5.9, OH–C(2′)); 5.72 (*d*, *J* = 5.9, H–C(1′)); 8.32 (*s*, H–C(8)); 11.92 (br. *s*, NH). ^{13}C-NMR (100 MHz, (D_6)DMSO): 22.0 (*q*, Me_2CHCO); 35.1 (*d*, Me_2CHCO); 62.3 (*t*, C(5′)); 71.3 (*d*, C(3′)); 74.1 (*d*, C(2′)); 86.6 (*d*, C(4′)); 88.3 (*d*, C(1′)); 110.0 (*s*, C(5)); 139.6 (*d*, C(8)); 152.2 (*s*, C(4)), 154.7 (*s*, C(6)); 157.0 (*s*, C(2)); 179.9 (*s*, Me_2CHCO). ESI-MS: 354.29 (100, $[M+H]^+$).

5′-O-*(4,4′-Dimethoxytrityl)*-O^2-*(diphenylcarbamoyl)*-N^6-*isobutyrylisoguanosine* (**7**). A soln. of crude **6** (5.63 g, ca. 15.9 mmol) in pyridine (250 ml) was treated at r.t. with ^{i}Pr$_2$NEt (3.0 ml, 17.5 mmol) and then with diphenylcarbamic chloride (4.06 g, 17.5 mmol). After 30 min at r.t., the mixture was treated with $(MeO)_2$TrCl (5.40 g, 15.9 mmol). Workup after 1 h at r.t. and CC (SiO_2 (90 g), AcOEt → AcOEt/MeOH 99 : 1) afforded **7** (6.09 g, 45% from isoguanosine). Yellow foam. TLC (CH_2Cl_2/MeOH 17 : 3): R_f 0.88. ^{1}H-NMR (400 MHz, $CDCl_3$): 1.28–1.32 (*d*, *J* = 7.3, Me_2CHCO); 3.23–3.33 (*m*, Me_2CHCO, OH–C(3′), H–C(5′)); 3.40 (*dd*, *J* = 3.7, 11.0, H'–C(5′)); 3.77 (*s*, 2 MeO); 4.37 (*d*, *J* = 3.0, H–C(3′)); 4.43 (br. *s*, H–C(4′)); 4.77 (br. *s*, H–C(2′)); 5.32 (br. *s*, OH–C(2′)); 6.01 (*d*, *J* = 5.1, H–C(1′)); 6.75–6.79 (*m*, 4 arom. H); 7.16–7.39 (*m*, 19 arom. H); 8.12 (*s*, H–C(8)). ^{13}C-NMR (100 MHz, $CDCl_3$): 19.1 (*q*, Me_2CHCO); 35.8 (*d*, Me_2CHCO); 55.2 (*q*, MeO); 63.5 (*t*, C(5′)); 72.4 (*d*, C(2′)); 75.9 (*d*, C(3′)); 76.9 (*d*, C(4′)); 85.7 (*d*, C(1′)); 90.1 (*s*, arom. C); 113.2 (*d*, arom. C); 120.5 (*s*, C(5)); 126.6, 126.7, 126.8, 126.9, 127.0, 127.1, 127.8, 127.9, 128.2, 129.2, 135.4, 136.6 (12*d*, arom. C); 141.2 (*d*, C(8)); 144.3 (*s*, C(4)); 150.3 (*s*, arom. C); 152.0 (*s*, CO); 155.5 (*s*, C(6)); 158.6 (*s*, C(2)); 176.3 (*s*, Me_3-CH*C*O). ESI-MS: 851.36 (100, $[M+H]^+$).

5′-O-*(4,4′-Dimethoxytrityl)*-O^2-*(diphenylcarbamoyl)*-N^6-*isobutyryl-2′*-O-*{[(triisopropylsilyl)oxy]methyl}isoguanosine* (**8**). A soln. of **7** (5.90 g, 6.9 mmol) and ^{i}Pr$_2$NEt (4.2 ml, 24.3 mmol) in $ClCH_2CH_2Cl$ (28 ml) was treated with Bu_2SnCl_2 (2.32 g, 7.6 mmol), stirred for 10 min at r.t., heated to 75°, treated with tom-Cl (1.85 g, 8.3 mmol), and heated for 25 min at 75°. Workup and CC (SiO_2 (150 g), hexane/AcOEt 9 : 1 → 3 : 2) gave **8** (1.64 g, 23%). Light yellow foam. TLC (hexane/AcOEt 1 : 1): R_f 0.27. ^{1}H-NMR (400 MHz, $CDCl_3$): 0.92–1.21 (*m*, ^{i}Pr$_3$Si); 1.32 (*d*, *J* = 7.3, Me_2CHCO); 3.19 (*s*, OH–C(3′)); 3.35–3.51 (*m*, Me_2CHCO); 3.74–3.82 (*m*, H–C(5′)); 3.82 (*s*, 2 MeO); 3.99 (*d*, *J* = 13.2, H′–C(5′)); 4.38 (*s*, H–C(3′)); 4.58 (*d*, *J* = 4.4, H–C(4′)); 4.84 (*d*, *J* = 5.1, 1 H, OCH_2O); 4.86–4.89 (*m*, H–C(2′)); 5.04 (*d*, *J* = 5.1, 1 H, OCH_2O); 5.93 (*d*, *J* = 7.4, H–C(1′)); 6.85 (*d*, *J* = 4.4, 4 arom. H); 7.10–7.45 (*m*, 19 arom. H); 8.57 (*s*, H–C(8)). ^{13}C-NMR (100 MHz, $CDCl_3$): 11.8 (*d*, Me_2CH); 17.8 (*q*, Me_2CH, Me_2CHCO); 35.8 (*d*, Me_2CHCO); 55.3 (*q*, MeO); 63.3 (*t*, C(5′)); 72.0 (*d*, C(2′)); 81.9 (*d*, C(3′)); 86.5 (*d*, C(4′)); 87.8 (*d*, C(1′)); 89.6 (*s*, arom. C); 90.8 (*t*, OCH_2O); 113.2 (*d*, arom. C); 121.9 (*s*, C(5)); 126.6, 126.7, 126.8, 126.9, 127.0, 127.1, 127.8, 127.9, 128.2, 130.0, 130.1, 135.7, 136.6 (12*d*, arom. C); 139.5 (*d*, C(8)); 143.2 (*s*, C(4)); 150.7 (*s*, arom. C); 151.3 (*s*, CO), 155.4 (*s*, C(6)); 158.6 (*s*, C(2)); 176.4 (*s*, Me_2CHCO). ESI-MS: 1037.36 (100, $[M+H]^+$).

2-[(Methoxyacetyl)amino]adenosine (= N^2*(Methoxyacetyl)purine Ribonucleoside* = N^2-*(Methoxyacetyl)-9-β*-D-*ribofuranosyl-9*H-*purine-2,6-diamine*; **9**). At 4°, a soln. of 2-aminoadenosine (18.5 g, 65.6 mmol) in pyridine (130 ml) was treated with Me_3SiCl (82.5 ml, 656 mmol), stirred for 2 h at r.t., diluted with MeCN (195 ml), cooled to 4°, treated with methoxyacetyl chloride (21 ml, 230 mmol), and finally stirred for 40 min at 4°. After addition of CH_2Cl_2 (400 ml), the soln. was poured into H_2O (400 ml). The org. phase was evaporated, and the residue was co-evaporated with toluene (3×50 ml). The resulting foam was dissolved in MeOH/THF/25% aq. NH_3 soln. 1 : 1 : 1 (300 ml). After 1 h at r.t., evaporation, co-evaporation with toluene (3×50 ml), and crystallization (AcOEt), **9** (19.9 g, 86%). Light yellow powder. TLC (MeOH/CH_2Cl_2 3 : 17): R_f 0.18. ^{1}H-NMR (400 MHz, (D_6)DMSO): 3.42 (*d, J* = 3.7, CH_2OMe); 3.55 (*td*, *J* = 4.9, 12.2, H–C(5′)); 3.65 (*td*, *J* = 4.7, 11.9,

H′–C(5′)); 4.58 (*dd*, *J* = 5.7, 11.2, H–C(4′)); 4.16 (*dd*, *J* = 4.7, 8.2, H–C(3′)); 4.18 (*s*, MeO); 4.58 (*dd*, *J* = 5.7, 11.2, H–C(2′)); 5.13 (*t*, *J* = 5.6, OH–C(5′)); 5.13 (*d*, *J* = 4.3, OH); 5.44 (*d*, *J* = 5.9, OH); 5.81 (*d*, *J* = 5.9, H–C(1′)); 7.30 (br. *s*, NH_2–C(6)); 8.23 (*s*, H–C(8)); 9.63 (*s*, NH–C(2)). ^{13}C-NMR (100 MHz, (D_6)DMSO): 58.9 (*q*, MeO); 61.9 (*t*, C(5′)); 70.9 (*t*, CH_2OMe); 72.3 (*d*, C(2′)); 73.9 (*d*, C(3′)); 86.0 (*s*, C(5)); 87.6 (*d*, C(4′)); 116.7 (*d*, C(1′)); 139.5 (*d*, C(8)); 150.6 (*s*, C(4)); 152.6 (*s*, C(6)), 156.5 (*s*, C(2)); 168.9 (*s*, CO). ESI-MS: 355.34 (100, $[M+H]^+$).

*5′-*O*-(4,4′-Dimethoxytrityl)-*N^6*-isobutyryl-2-[(methoxyacetyl)amino]adenosine* (**10**). At 4°, a soln. of **9** (25.4 g, 72 mmol) in pyridine (140 ml) was treated with Me_3Si-Cl (90 ml, 720 mmol), stirred for 2 h at r.t., diluted with MeCN (215 ml), cooled to 4°, treated with isobutyryl chloride (9.1 ml, 86.2 mmol), and finally stirred for 40 min at 4°. After addition of CH_2Cl_2 (400 ml), the soln. was poured into H_2O (400 ml). The org. phase was evaporated, and the residue was co-evaporated with toluene (3×50 ml). The resulting foam was dissolved in MeOH/AcOH 3 : 1 (200 ml). After 1 h at r.t., evaporation, co-evaporation with toluene (3×50 ml), and co-evaporation with pyridine (50 ml), a yellow foam was obtained, which was dried (0.1 mbar) overnight. The foam was dissolved in pyridine (250 ml), treated with $(MeO)_2$TrCl (25.0 g, 74.0 mmol), and stirred for 2 h at r.t. After workup, evaporation, and CC (SiO_2 (400 g), $CH_2Cl_2 \rightarrow CH_2Cl_2$/MeOH 94 : 6), **10** (27.4 g, 54%) was obtained. Yellow foam. TLC (CH_2Cl_2/MeOH 19 : 1): R_f 0.44. ^{1}H-NMR (400 MHz, $CDCl_3$): 1.42 (*d*, *J* = 7.0, *Me*$_2$CH); 2.35 (br. *s*, OH–C(3′)); 3.01 (*sept.*, *J* = 7.0, Me$_2$C*H*); 3.19 (*dd*, *J* = 2.4, 10.5, H–C(5′)); 3.39 (*dd*, *J* = 3.3, 10.6, H'–C(5′)); 3.53 (br. *s*, MeO); 3.58 (br. *s*, OH–C(2′)); 3.60 (*s*, MeO); 4.08 (*s*, CH_2CO); 4.41 (*d*, *J* = 5.0, H–C(2′)); 4.45 (br. *s*, H–C(3′)); 5.03 (*dd*, *J* = 5.4, 9.0, H–C(4′)); 5.97 (*d*, *J* = 6.1, H–C(1′)); 6.70 (*d*, *J* = 8.7, 4 arom. H); 7.10–7.31 (*m*, 9 arom. H); 8.19 (*s*, H–C(8)); 8.68 (*s*, NH–C(6)); 9.33 (*s*, NH–C(2)). ^{13}C-NMR (100 MHz, $CDCl_3$): 19.6 (*d*, Me$_2$*C*H); 36.9 (*q*, *Me*$_2$CH); 53.9 (*q*, *Me*OCH$_2$); 59.8 (*q*, MeO); 64.3 (*t*, C(5′)); 72.5 (*t*, MeO*C*H$_2$); 74.5 (*d*, C(2′)); 87.0 (*d*, C(3′)); 87.6 (*s*, C(5)); 92.8 (*d*, C(4′)); 119.7 (*d*, C(1′)); 127.2, 128.1, 128.2, 130.3 (4*d*, arom. C); 135.6 (*d*, C(8)); 135.7 (*s*, arom. C); 144.8 (*s*, arom. C); 150.3 (*s*, C(4)); 151.1 (*s*, C(6)), 151.5 (*s*, C(2)); 158.9 (*s*, arom. C); 168.8 (*s*, CH_2*C*O); 175.9 (*s*, Me$_2$CH*C*O). ESI-MS: 727.82 (100, $[M+H]^+$).

*5′-*O*-(4,4′-Dimethoxytrityl)-*N^6*-isobutyryl-2-[(methoxyacetyl)amino]-2′-*O*-{[triisopropylsilyl)oxy]methyl}-adenosine* (**11**). A soln. of **10** (625 mg, 0.9 mmol) and ^{i}Pr$_2$NEt (0.51 ml, 3.0 mmol) in $ClCH_2CH_2Cl$ (3.5 ml) was treated with Bu_2SnCl_2 (313 mg, 1.0 mmol), stirred for 30 min at r.t., treated with tom-Cl (229 mg, 1.03 mmol), and stirred for 25 min at 80°. Workup and CC (SiO_2 (10 g), hexane/AcOEt 3 : 1 → AcOEt) gave **11** (290 mg, 37%). Light yellow foam. TLC (AcOEt): R_f 0.58. ^{1}H-NMR (400 MHz, $CDCl_3$): 1.08–1.16 (*m*, ^{i}Pr$_3$Si); 1.32 (*d*, *J* = 6.4, *Me*$_2$CHCO); 2.03 (br. *s*, CH_2(5′)); 3.28–3.97 (*m*, MeO, Me$_2$C*H*CO); 3.14 (*d*, *J* = 5.4, OH–C(3′)); 3.79 (*s*, MeO); 4.06 (br. *s*, CH_2CO); 4.27 (br. *d*, *J* = 2.5, H–C(3′)); 4.59 (*dd*, *J* = 3.8, 8.2, H–C(4′)); 4.88 (*t*, *J* = 5.1, H–C(2′)); 5.08 (*d*, *J* = 4.5, 1 H, OCH_2O); 5.18 (*d*, *J* = 4.9, 1 H, OCH_2O); 6.19 (*d*, *J* = 5.1, H–C(1′)); 6.80 (*d*, *J* = 8.3, 4 arom. H); 7.19–7.45 (*m*, 10 arom. H); 8.09 (*s*, H–C(8)); 8.59 (*s*, NH–C(6)); 8.84 (*s*, NH–C(2)). ^{13}C-NMR (100 MHz, $CDCl_3$): 12.2 (*d*, Me$_2$*C*H); 18.2 (*q*, *Me*$_2$CH); 19.6 (*q*, *Me*$_2$CHCO); 36.3 (*d*, Me$_2$*C*HCO); 55.7 (*q*, *Me*OCH$_2$); 59.7 (*q*, MeO); 63.9 (*t*, C(5′)); 71.0 (*t*, MeO*C*H$_2$); 72.8 (*d*, C(2′)); 82.8 (*d*, C(3′)); 84.7 (*s*, C(5)); 87.5 (*d*, C(4′)); 91.5 (*t*, OCH_2O); 113.6 (*d*, arom. C); 119.9 (*d*, C(1′)); 127.4, 128.3, 128.6, 130.5 (4*d*, arom. C); 136.1 (*d*, C(8)); 136.2 (*s*, arom. C); 145.0 (*s*, arom. C); 150.0 (*s*, C(4)); 152.0 (*s*, C(6)), 152.6 (*s*, C(2)); 158.9 (*s*, arom. C); 167.7 (*s*, CH_2*C*O); 176.8 (*s*, Me$_2$CH*C*O). ESI-MS: 913.34 (100, $[M+H]^+$).

*5′-*O*-(4,4′-Dimethoxytrityl)-5-methyl-2′-*O*-{[(triisopropylsilyl)oxy]methyl}uridine* (**13**). A soln. of **12** (1.00 g, 1.8 mmol; obtained according to [7]) and ^{i}Pr$_2$NEt (0.78 ml, 4.6 mmol) in $ClCH_2CH_2Cl$ (35 ml) was treated with Bu_2SnCl_2 (560 mg, 2.0 mmol), stirred for 30 min at r.t., treated with tom-Cl (450 mg, 2.0 mmol), and stirred for 12 h at r.t. Workup and CC (SiO_2 (30 g), hexane/AcOEt 9 : 1 → 1 : 4) gave **13** (500 mg, 38%). Light yellow foam. TLC (hexane/AcOEt 1 : 1): R_f 0.47. ^{1}H-NMR (400 MHz, $CDCl_3$): 1.04–1.16 (*m*, ^{i}Pr$_3$Si); 1.38 (*s*, Me–C(5)); 3.13 (br. *s*, OH–C(3′)); 3.40 (*dd*, *J* = 1.5, 11.4, H–C(5′)); 3.53 (*dd*, *J* = 1.9, 11.5, H'–C(5′)); 3.81 (*s*, 2 MeO); 4.19 (br. *s*, H–C(4′)); 4.38 (*t*, *J* = 5.1, H–C(2′)); 4.48 (br. *s*, H–C(3′)); 5.03 (*d*, *J* = 4.5, 1 H, OCH_2O); 5.24 (*d*, *J* = 4.5, 1 H, OCH_2O); 6.14 (*d*, *J* = 8.3, H–C(1′)); 6.84–6.86 (*m*, 4 arom. H); 7.26–7.43 (*m*, 9 arom. H); 7.66 (br. *s*, H–C(6)); 8.47 (br. *s*, H–N(3)). ^{13}C-NMR (100 MHz, $CDCl_3$): 12.1 (*d*, Me$_2$*C*H); 12.3 (*q*, *Me*–C(5)); 18.2 (*q*, *Me*$_2$CH); 55.7 (*q*, MeO); 63.5 (*t*, C(5′)); 70.8 (*d*, C(2′)); 82.9 (*d*, C(3′)); 84.3 (*d*, C(4′)); 86.2 (*s*, arom. C); 87.4 (*d*, C(1′)); 91.2 (*t*, OCH_2O); 111.7 (*s*, C(5)); 113.6 (*d*, arom. C); 127.6, 128.2, 128.5, 130.6 (4*d*, arom. C); 135.6, 135.8 (2*s*, arom. C); 144.7 (*s*, C(6)); 150.7 (*s*, C(2)); 159.2 (*s*, arom. C); 163.9 (*s*, C(4)). ESI-MS: 373.29 (100, $[M+H]^{2+}$).

*5′-*O*-(4,4′-Dimethoxytrityl)-5,6-dihydro-2′-*O*-{[(triisopropylsilyl)oxy]methyl}uridine* (**15**). A soln. of **14** (7.20 g, 13.0 mmol; obtained according to [39]) and ^{i}Pr$_2$NEt (7.9 ml, 46.0 mmol) in $ClCH_2CH_2Cl$ (50 ml) was treated with Bu_2SnCl_2 (4.39 g, 14.4 mmol), stirred for 30 min at r.t., treated with tom-Cl (0.45 g, 2.0 mmol), and stirred for 25 min at 80°. Workup and CC (SiO_2 (100 g), hexane/AcOEt 9 : 1 → 1 : 1) gave **15** (4.37 g, 47%). Light yellow foam. TLC (hexane/AcOEt 1 : 1): R_f 0.61. ^{1}H NMR (400 MHz, $CDCl_3$): 1.09–1.28 (*m*, ^{i}Pr$_3$-Si); 2.39–2.57 (*m*, CH_2(5)); 3.04 (*d*, *J* = 3.1, OH–C(3′)); 3.31–3.44 (*m*, CH_2(6), CH_2(5′)); 3.66–3.72 (*m*, H–C(4′)); 3.81 (*s*, 2 MeO); 4.26 (*t*, *J* = 5.5, H–C(2′)); 4.39–4.40 (*m*, H–C(3′)); 5.01 (*d*, *J* = 4.7, 1 H, OCH_2O);

5.22 (*d*, *J* = 4.7, 1 H; OCH_2O); 6.03 (*d*, *J* = 6.2, H–C(1′)); 6.80–6.82 (*d*, *J* = 9.4, 4 arom. H); 7.22–7.44 (*m*, 9 arom. H). ^{13}C NMR (100 MHz, $CDCl_3$): 12.3 (*d*, Me_2CH); 18.2 (*q*, Me_2CH); 31.5 (*t*, C(5)); 36.8 (*t*, C(6)); 55.7 (*q*, MeO); 63.9 (*t*, C(5′)); 71.4 (*d*, C(3′)); 79.8 (*d*, C(2′)); 83.2 (*d*, C(4′)); 86.9 (*s*, arom. C); 87.1 (*d*, C(1′)); 90.9 (*t*, OCH_2O); 113.6 (*d*, arom. C); 127.4, 128.3, 128.6, 128.7, 130.5 (5*d*, arom. C); 135.9, 136.0 (2*s*, arom. C); 144.9 (*s*, arom. C); 152.5 159.0 (2*s*, C(2), C(4)); 169.9 (*s*, arom. C). ESI-MS: 757.38 (100, $[M+H]^+$).

5′-O-(4,4′-Dimethoxytrityl)-2′-O-{[(triisopropylsilyl)oxy]methyl}inosine (**17**). A soln. of **16** (1.0 g, 1.9 mmol, prepared according to [24]) in MeOH (2 ml) was treated with a sat. NH_3 soln. in MeOH (6 ml) and stirred for 3 h at r.t. After evaporation, the residue was dissolved in pyridine (5.5 ml), treated with $(MeO)_2TrCl$ (0.78 g, 2.3 mmol), and stirred for 2 h at r.t. Workup and CC (SiO_2 (25 g), hexane/AcOEt 1:1, then CH_2Cl_2/MeOH 99:1 → 9:1) gave **17** (939 mg, 67%). Yellow foam. TLC (CH_2Cl_2/MeOH 1:9): R_f 0.50. ^{1}H-NMR (400 MHz, $CDCl_3$) 0.90–1.10 (*m*, $^iPr_3Si$); 3.10 (*d*, *J* = 3.1, OH–C(3′)); 3.42 (*dd*, *J* = 3.9, 10.2, H–C(5′)); 3.47 (*dd*, *J* = 3.9, 10.2, H'–C(5′)); 3.80 (*s*, 2 MeO); 4.32 (*m*, H–C(4′)); 4.55 (*m*, H–C(3′)); 4.84 (*t*, *J* = 4.7, H–C(2′)); 4.98 (*d*, *J* = 4.7, 1 H, OCH_2O); 5.17 (*d*, *J* = 4.7, 1 H OCH_2O); 6.17 (*d*, *J* = 5.4, H–C(1′)); 6.82 (*d*, *J* = 8.8, 4 arom. H); 7.30–7.46 (*m*, 9 arom. H); 7.81 (*s*, H–C(8)); 7.94 (*s*, H–C(2)); 12.98 (br. *s*, NH). ^{13}C-NMR (400 MHz, $CDCl_3$): 12.2 (*d*, Me_2CH); 18.2 (*q*, Me_2CH); 55.6 (*q*, MeO); 63.9 (*t*, C(5′)); 71.4 (*d*, C(2′)); 82.9 (*d*, C(3′)); 84.8 (*d*, C(4′)); 87.1 (*d*, C(1′)); 87.4 (*s*, arom. C); 91.3 (*t*, OCH_2O); 113.6 (*d*, arom. C); 125.7 (*s*, C(5)); 127.4, 128.3, 128.6, 129.5, 130.5 (5*d*, arom. C), 136.0, 136.1 (2*s*, arom. C); 139.5 (*d*, C(8)); 144.9 (*s*, arom. C); 145.2 (*s*, C(4)); 149.3 (*s*, C(2)); 158.9 (*s*, C(6)); 159.6 (*s*, arom. C). ESI-MS: 757.34 (100, $[M+H]^+$).

5′-O-(4,4′-Dimethoxytrityl)-1-methyl-2′-O-{[(triisopropylsilyl)oxy]methyl}inosine (**18**). A soln. of **17** (76 mg, 0.1 mmol) in DMF was treated with K_2CO_3 (15 mg, 0.1 mmol), stirred for 1.5 h at −15°, treated with MeI (31 mg, 0.2 mmol), and stirred for 2 h at r.t. Workup, evaporation, and CC (SiO_2 (2 g), hexane/AcOEt 3:2 → AcOEt) gave **17** (77 mg, 98%). White solid. TLC (CH_2Cl_2/MeOH 19:1): R_f 0.50. ^{1}H-NMR (400 MHz, $CDCl_3$): 1.00–1.12 (*m*, $^iPr_3Si$); 2.99 (*d*, *J* = 4.7, OH–C(3′)); 3.39 (*dd*, *J* = 4.2, 10.4, H–C(5′)); 3.43 (*dd*, *J* = 3.4, 10.4, H′–C(5′)); 3.61 (*s*, Me–N(1)); 3.78 (*s*, 2 MeO); 4.29 (*q*, *J* = 4.1, H–C(4′)); 4.53 (*q*, *J* = 4.2, H–C(3′)); 4.82 (*t*, *J* = 5.0, H–C(2′)); 4.94, 5.13 (2*d*, *J* = 4.8, OCH_2O); 6.11 (*d*, *J* = 4.9, H–C(1′)); 6.81 (*m*, 4 arom. H); 7.18–7.35, 7.41–7.47 (*m*, 9 arom. H); 7.83 (*s*, H–C(8)); 7.93 (*s*, H–C(2)). ^{13}C-NMR (100 MHz, $CDCl_3$): 12.2 (*d*, Me_2CH); 18.1 (*q*, Me_2CH); 34.5 (*q*, MeN(1)); 55.6 (*q*, MeO); 64.0 (*t*, C(5′)); 71.5 (*d*, C(3′)); 82.8 (*d*, C(2′)); 84.8 (*d*, C(4′)); 86.8 (*s*, arom. C); 87.0 (*d*, C(1′)); 91.3 (*t*, OCH_2O); 113.6 (*d*, arom. C); 125.5 (*s*, C(5)); 127.29, 128.23, 128.56, 130.48, 130.51 (5*d*, arom. C); 136.0, 136.1 (2*s*, arom. C); 139.2 (*d*, C(8)); 145.0 (*s*, arom. C); 147.5 (*s*, C(4)); 148.1 (*s*, C(2)); 157.4 (*s*, C(6)); 159.0 (*s*, arom. C). ESI-MS: 771.40 (100, $[M+H]^+$).

3′,5′-Di-O-acetyl-N^6-isopent-2-enyl-2′-O-{[(triisopropylsilyl)oxy]methyl}adenosine (**20**). A soln. of isopent-2-enylamine·HCl (264 mg, 2.2 mmol) in pyridine (5 ml) was treated with Et_3N (0.6 ml, 4.3 mmol) and **19** (200 mg, 0.3 mmol; prepared according to [24]), and stirred for 1 h at r.t. Workup and CC (SiO_2 (5 g), hexane/AcOEt 9:1 → 3:7) gave **20** (200 mg, 89%). Yellow foam. TLC (hexane/AcOEt 1:1): R_f 0.50. ^{1}H-NMR (100 MHz, $CDCl_3$): 0.89–1.05 (*m*, $^iPr_3Si$); 1.76 (*s*, Me); 1.78 (*s*, Me); 2.13 (*s*, MeCO); 2.18 (*s*, MeCO); 4.23 (br. *s*, CH_2NH); 4.37–4.50 (*m*, H–C(3′), H–C(4′), CH_2(5′)); 4.86 (*d*, *J* = 4.7, 1 H, OCH_2O); 4.92 (*d*, *J* = 4.7, 1 H, OCH_2O); 5.22 (*t*, *J* = 6.3, C*H*=C); 5.64 (br. *s*, H–C(2′)); 6.13 (*d*, *J* = 5.7, H–C(1′)); 7.88 (*s*, H–C(8)); 8.47 (br. *s*, H–C(2)). ^{13}C-NMR (100 MHz, $CDCl_3$): 12.1 (*q*, *Me*C=); 12.2 (*d*, Me_2CH); 18.0 (*q*, Me_2CH); 18.4 (*q*, *Me*′C=); 21.2 (*q*, 2 *Me*CO); 26.1 (*t*, CH_2NH); 63.9 (*t*, C(5′)); 71.9 (*d*, C(3′)); 76.7 (*d*, C(2′)); 80.9 (*d*, C(4′)); 87.8 (*d*, C(1′)); 89.9 (*t*, OCH_2O); 107.6 (*s*, CH=C); 120.6 (*s*, C(5)); 137.4 (*s*, Me_2C=); 138.9 (*d*, C(8)); 152.9 (*s*, C(4)); 153.8 (*d*, C(2)); 155.1 (*s*, C(6)); 170.5 (*s*, MeCO); 170.8 (*s*, MeCO). ESI-MS: 606.80 (100, $[M+H]^+$).

5′-O-(4,4′-Dimethoxytrityl)-N^6-isopent-2-enyl-2′-O-{[(triisopropylsilyl)oxy]methyl}adenosine (**21**). A soln. of **20** (100 mg, 0.16 mmol), was treated with a sat. NH_3 soln. in MeOH (3 ml) for 6 h at r.t. After evaporation, the residue was dissolved in pyiridine (0.7 ml), treated with $(MeO)_2TrCl$ (67 mg, 0.19 mmol), and stirred for 4 h at r.t. Workup and CC (SiO_2 (2 g), hexane/AcOEt 8:2 → 3:7) gave **21** (81 mg, 60%). Yellow foam. TLC (hexane/AcOEt 1:1): R_f 0.53. ^{1}H-NMR (400 MHz, $CDCl_3$): 0.90–1.06 (*m*, $^iPr_3Si$); 1.73 (*s*, Me); 1.78 (*s*, Me); 2.84 (*s*, OH–C(3′)); 3.39 (*dd*, *J* = 4.7, 10.2, H–C(5′)); 3.47 (*dd*, *J* = 3.9, 10.2, H′–C(5′)); 3.79 (*s*, 2 MeO); 4.01 (*d*, *J* = 4.7, H–C(4′)); 4.22–4.24 (*m*, CH_2NH); 4.69 (*d*, *J* = 4.7, H–C(3′)); 5.10 (*s*, OCH_2O); 5.22 (*t*, *J* = 4.7, C*H*=C); 5.42–5.44 (*m*, H–C(2′)); 6.17 (*d*, *J* = 4.7, H–C(1′)); 6.83–6.87 (*m*, 4 arom. H); 7.22–7.49 (*m*, 9 arom. H); 8.13 (*s*, H–C(8)); 8.19 (br. *s*, H–C(2)); 12.98 (br. *s*, NH). ^{13}C-NMR (100 MHz, $CDCl_3$): 12.1 (*d*, Me_2CH); 17.6 (*q*, Me_2CH); 28.8 (*q*, *Me*C=); 29.9 (*q*, *Me*′C=); 38.4 (*t*, CH_2NH); 54.9 (*q*, MeO); 64.0 (*t*, C(5′)); 70.9 (*d*, C(3′)); 79.7 (*d*, C(2′)); 84.5 (*d*, C(4′)); 86.5 (*d*, C(1′)); 87.5 (*s*, arom. C); 90.2 (*t*, OCH_2O); 109.4 (*s*, CH=C); 113.4 (*d*, arom. C); 122.2 (*s*, C(5)); 127.0, 128.0, 128.5, 130.4, 130.5 (5*d*, arom. C), 134.6, 136.4 (2*s*, arom. C, Me_2C=); 139.8 (*d*, C(8)); 145.2 (*d*, C(4)); 145.6 (*s*, arom. C); 153.1 (*d*, C(2)); 155.3 (*s*, C(6)); 159.1 (*s*, arom. C). ESI-MS: 824.37 (100, $[M+H]^+$).

5′-O-(4,4′-Dimethoxytrityl)-N^6-methyl-2′-O-{[(triisopropylsilyl)oxy]methyl}adenosine (**22**). A soln. of **19** (150 mg, 0.2 mmol; prepared according to [24]) was treated with a 33% $MeNH_2$ soln. in EtOH (3 ml) for 3 h

at r.t. After evaporation, the residue was dissolved in pyridine (2 ml), treated with $(MeO)_2TrCl$ (89 mg, 0.3 mmol) and stirred for 3 h at r.t. Workup and CC (SiO_2 (3 g), hexane/AcOEt 3:2 → AcOEt) gave **22** (120 mg, 65%). Yellow foam. TLC (hexane/AcOEt 1:9): R_f 0.60. ^{1}H-NMR (400 MHz, $CDCl_3$): 0.96–1.05 (*m*, ^{i}Pr$_3$Si); 3.06 (*d*, *J* = 3.9, OH–C(3′)); 3.19 (*d*, *J* = 4.5, *Me*NH); 3.38 (*dd*, *J* = 4.2, 10.2, H–C(5′)); 3.50 (*dd*, *J* = 3.6, 10.2, H′–C(5′)); 3.78 (*s*, 2 MeO); 4.26 (*q*, *J* = 4.0, H–C(4′)); 4.52 (*q*, *J* = 4.2, H–C(3′)); 4.93 (*t*, *J* = 5.0, H–C(2′)); 4.98 (*d*, *J* = 4.8, 1 H, OCH_2O); 5.14 (*d*, *J* = 4.8, 1 H, OCH_2O); 5.75 (br. *s*, NH); 6.14 (*d*, *J* = 5.4, H–C(1′)); 6.78 (*m*, 4 arom. H); 7.22–7.44 (*m*, 9 arom. H); 7.93 (*s*, H–C(8)); 8.33 (*s*, H–C(2)). ^{13}C-NMR (100 MHz, $CDCl_3$): 11.8 (*d*, Me_2*C*H); 17.7 (*q*, *Me*$_2$CH); 27.7 (*q*, MeNH); 55.1 (*q*, MeO); 63.4 (*t*, C(5′)); 70.8 (*d*, C(3′)); 81.8 (*d*, C(2′)); 84.1 (*d*, C(4′)); 86.5 (*d*, C(1′)); 87.0 (*s*, arom. C); 90.7 (*t*, OCH_2O); 113.4 (*d*, arom. C); 120.4 (*s*, C(5)); 126.8, 127.8, 128.2, 130.0, 135.8 (5*d*, arom. C); 138.6 (*d*, C(8)); 144.6 (*d*, C(4)); 153.3 (*d*, C(2)); 155.5 (*s*, C(6)); 158.5 (*s*, arom. C). ESI-MS: 769.91 (100, $[M+H]^+$).

*5′-*O*-(4,4′-Dimethoxytrityl)-*N^6,N^6*-dimethyl-2′-*O*-{[(triisopropylsilyl)oxy]methyl}adenosine* (**23**). A soln. of **19** (0.15 g, 0.2 mmol, prepared according to [24]) was treated with a 33% Me_2NH soln. in EtOH (3 ml) for 3 h at r.t. After evaporation, the residue was dissolved in pyridine (2 ml), treated with $(MeO)_2TrCl$ (89 mg, 0.3 mmol), and stirred for 3 h at r.t. Workup and CC (SiO_2 (3 g), hexane/AcOEt 3:2 → AcOEt) gave **23** (148 mg, 80%). Yellow foam. TLC (hexane/AcOEt 1:1): R_f 0.70. ^{1}H-NMR (400 MHz, $CDCl_3$): 1.00–1.11 (*m*, ^{i}Pr$_3$-Si); 3.05 (*d*, *J* = 4.1, OH–C(3′)); 3.36 (*dd*, *J* = 4.3, 10.5, H–C(5′)); 3.50 (*dd*, *J* = 3.0, 10.5, H′–C(5′)); 3.45–3.58 (br. *s*, Me_2N); 3.78, 3.79 (2*s*, 2 MeO); 4.26 (*q*, *J* = 3.7, H–C(4′)); 4.48 (*q*, *J* = 4.4, H–C(3′)); 4.87 (*t*, *J* = 5.0, H–C(2′)); 4.99 (*d*, *J* = 4.7, 1 H, OCH_2O); 5.15 (*d*, *J* = 4.7, 1 H, OCH_2O); 6.17 (*d*, *J* = 5.2, H–C(1′)); 6.78–6.82 (*m*, 4 arom. H); 7.18–7.46 (*m*, 9 arom. H); 7.93 (*s*, H–C(8)); 8.27 (*s*, H–C(2)). ^{13}C-NMR (100 MHz, $CDCl_3$): 11.8 (*d*, Me_2*C*H); 17.7 (*q*, *Me*$_2$CH); 38.4 (*q*, 2 Me_2N); 55.1 (*q*, MeO); 63.4 (*t*, C(5′)); 70.7 (*d*, C(3′)); 81.8 (*d*, C(2′)); 83.8 (*d*, C(4′)); 86.4 (*d*, C(1′)); 86.8 (*s*, arom. C); 90.7 (*t*, OCH_2O); 113.1 (*d*, arom. C); 120.6 (*s*, C(5)); 126.7, 127.7, 128.1, 130.0, 135.8 (5*d*, arom. C); 136.8 (*d*, C(8)); 144.6 (*d*, C(4)); 144.6, 150.3 (2*s*, arom. C); 152.4 (*d*, C(2)); 154.9 (*s*, C(6)); 158.4 (*s*, arom. C). ESI-MS: 785.41 (100, $[M+H]^+$).

*5′-*O*-(4,4′-Dimethoxytrityl)-1-methyl-2′-*O*-{[(triisopropylsilyl)oxy]methyl}guanosine* (**25**). A soln. of **24** (400 mg, 0.5 mmol, prepared according to [25]) in DMF (4 ml) was treated with K_2CO_3 (79 mg, 0.6 mmol), stirred for 2 h at r.t., treated with MeI (162 mg, 1.1 mmol), and stirred for 14 h at −15°. Workup and CC (SiO_2 (10 g), CH_2Cl_2/MeOH 99:1 → 9:1) gave **25** (0.26 g, 63%). Colorless foam. TLC (CH_2Cl_2/MeOH 9:1): R_f 0.72. ^{1}H-NMR (400 MHz, $CDCl_3$): 0.97–1.12 (*m*, ^{i}Pr$_3$Si); 1.87 (br. *s*, NH_2); 3.08 (br. *s*, OH–C(3′)); 3.34 (*dd*, *J* = 3.2, 10.2, H–C(5′)); 3.52 (br. *s*, H′–C(5′), Me–N(1)); 3.79 (*s*, 2 MeO); 4.27 (br. *s*, H–C(4′)); 4.58 (br. *s*, H–C(3′)); 4.92 (br. *s*, H–C(2′)); 4.96 (*d*, *J* = 4.4, 1 H, OCH_2O); 5.14 (*d*, *J* = 5.1, 1 H, OCH_2O); 5.94 (*d*, *J* = 6.6, H–C(1′)); 6.80–6.82 (*m*, 4 arom. H); 7.20–7.36 (*m*, 9 arom. H); 7.65 (*s*, H–C(8)). ^{13}C-NMR (100 MHz, $CDCl_3$): 11.9 (*d*, Me_2*C*H); 17.8 (*q*, *Me*$_2$CH); 28.1 (*q*, Me–N(1)); 55.3 (*q*, MeO); 63.6 (*t*, C(5′)); 71.2 (*d*, C(2′)); 81.6 (*d*, C(3′)); 83.9 (*d*, C(4′)); 85.9 (*s*, arom. C); 86.5 (*d*, C(1′)); 90.9 (*t*, OCH_2O); 113.2 (*d*, arom. C); 118.2 (*s*, C(5)); 126.9, 127.9, 128.2, 130.1 (4*d*, arom. C); 135.7 (*s*, arom. C); 136.6 (*d*, C(8)); 144.6 (*s*, arom. C); 148.8, 153.1 (2*s*, C(2), C(4)); 158.6 (*s*, C(6)). ESI-MS: 786.82 (100, $[M+H]^+$).

*5′-*O*-(4,4′-Dimethoxytrityl)-*N^2*-methyl-2′-*O*-{[triisopropylsilyl)oxy]methyl}guanosine* (**27**). A soln. of **24** (501 mg, 0.66 mmol, prepared according to [25]) in pyridine (8 ml) was treated with Me_3SiCl (200 mg, 2.0 mmol), stirred for 40 min at r.t., treated with 1,3-benzodithiolylium tetrafluoroborate (275 mg, 1.0 mmol), and stirred for 6 h at r.t. Workup and filtration (SiO_2 (11 g), hexane/AcOEt 3:7 → AcOEt) gave a mixture of 3′-O-Me_3Si and 3′-OH derivatives as a yellow foam (576 mg, *ca.* 88%; TLC (CH_2Cl_2/MeOH 9:1): R_f 0.48 and 0.45), which was dissolved in benzene (6 ml), treated with AIBN (49 mg, 0.3 mmol), 1,1,1,3,3,3-hexamethyl-2-(trimethylsilyl)trisilane (718 mg, 3.0 mmol) and heated to reflux for 8 h. After evaporation, the residue (crude **26** and **27**) was dissolved in MeOH (0.3 ml), treated with a sat. NH_3 soln. in MeOH (2.7 ml), and stirred for 4 h at r.t. Workup and CC (SiO_2 (10 g), hexane/AcOEt 2:3 → AcOEt, then AcOEt/MeOH 95:5 → 9:1) gave **27** (304 mg, 57% from **24**). Pink foam. TLC (CH_2Cl_2/MeOH 9:1): R_f 0.39. ^{1}H-NMR (400 MHz, $CDCl_3$): 0.88–1.08 (*m*, ^{i}Pr$_3$Si); 2.78 (br. *s*, MeNH–C(2)); 3.09 (*d*, *J* = 3.1, OH–C(3′)); 3.44 (*m*, CH_2(5′)); 3.77 (*s*, 2 MeO); 4.24 (*m*, H–C(4′)); 4.55 (*m*, H–C(3′)); 4.82 (*m*, H–C(2′)); 5.04 (*d*, *J* = 3.9, 1 H, OCH_2O); 5.20 (*d*, *J* = 3.9, 1 H, OCH_2O); 6.06 (*d*, *J* = 4.7, H–C(1′)); 6.81 (*d*, *J* = 8.6, 4 arom. H); 7.30–7.46 (*m*, 9 arom. H); 7.69 (*s*, H–C(8)); 12.06 (br. *s*, NH). ^{13}C-NMR (100 MHz, $CDCl_3$): 12.3 (*d*, Me_2*C*H); 18.2 (*q*, *Me*$_2$CH); 28.2 (*q*, *Me*NH–C(2)); 55.6 (*q*, MeO); 63.8 (*t*, C(5′)); 71.2 (*d*, C(2′)); 82.4 (*d*, C(3′)); 84.1 (*d*, C(4′)); 86.9 (*d*, C(1′)); 87.1 (*s*, arom. C); 91.3 (*t*, OCH_2O); 113.6 (*d*, arom. C); 127.3 (*s*, C(5)); 128.3, 128.6, 128.8, 130.5 (4*d*, arom. C), 136.1, 136.2 (2*s*, arom. C); 136.3 (*d*, C(8)); 145.0 (*s*, arom. C); 152.3 (*s*, C(4)); 153.9 (*s*, C(2)); 158.9 (*s*, C(6)); 159.9 (*s*, arom. C). ESI-MS: 787.74 (100, $[M+H]^+$).

*5′-O-(4,4′-Dimethoxytrityl)-*N^2,N^2*-dimethyl-2′-O-{[(triisopropylsilyl)oxy]methyl}guanosine* (**29**). A soln. of crude **26** and **27** (260 mg, 0.3 mmol, obtained from **24** as described above) in pyridine (2 ml) was treated with

Me_3SiCl (46 mg, 0.8 mmol), stirred for 40 min at r.t., treated with 1,3-benzodithiolylium tetrafluoroborate (137 mg, 0.5 mmol), and stirred for 6 h at r.t. Workup and filtration (SiO_2 (11 g), hexane/AcOEt 3:7 → AcOEt) gave a mixture of 3′-*O*-Me_3Si and 3′-OH derivatives as a yellow foam (288 mg, ca. 94%; TLC (CH_2Cl_2/MeOH 9:1): R_f 0.54 and 0.50), which was dissolved in benzene (3 ml), treated with AIBN (25 mg, 0.15 mmol) and 1,1,1,3,3,3-hexamethyl-2-(trimethylsilyl)trisilane (359 mg, 1.5 mmol) and heated to reflux for 8 h. After evaporation, the mixture (crude **28** and **29**) was dissolved in MeOH (0.3 ml), treated with a sat. NH_3 soln. in MeOH (2.7 ml), and stirred for 4 h at r.t. Workup and CC (SiO_2 (5 g), hexane/AcOEt 2:3 → AcOEt then AcOEt/MeOH 95:5 → 9:1) gave **29** (144 mg, 50% from **24**). Pink foam. TLC (CH_2Cl_2/MeOH 9:1): R_f 0.45. ^{1}H-NMR (400 MHz, $CDCl_3$): 0.90–1.28 (*m*, ^{i}Pr$_3$Si); 3.03 (*d*, *J* = 3.9, OH–C(3′)); 3.16 (*s*, Me_2N); 3.41 (*d*, *J* = 3.9, CH_2(5′)); 3.79 (*s*, 2 MeO); 4.24 (br. *d*, *J* = 4.7, H–C(4′)); 4.52 (br. *d*, *J* = 4.7, H–C(3′)); 4.78 (*t*, *J* = 5.5, H–C(2′)); 4.98 (*d*, *J* = 4.7, 1 H, OCH_2O); 5.16 (*d*, *J* = 4.7, 1 H, OCH_2O); 6.03 (*d*, *J* = 5.4, H–C(1′)); 6.81 (*d*, *J* = 9.4, 4 arom. H); 7.19–7.45 (*m*, 9 arom. H); 7.66 (*s*, H–C(8)); 10.64 (br. *s*, NH). ^{13}C-NMR (100 MHz, $CDCl_3$): 12.3 (*d*, Me_2CH); 18.6 (*q*, *Me*$_2$CH); 38.5 (*q*, Me_2N); 55.6 (*q*, MeO); 64.1 (*t*, C(5′)); 71.4 (*d*, C(2′)); 82.3 (*d*, C(3′)); 84.1 (*d*, C(4′)); 86.7 (*d*, C(1′)); 86.9 (*s*, arom. C); 91.2 (*t*, OCH_2O); 113.6 (*d*, arom. C); 127.3 (*s*, C(5)); 128.3, 128.6, 128.7, 130.5 (4*d*, arom. C), 136.1, 136.2 (2*s*, arom. C); 136.6 (*d*, C(8)); 144.9 (*s*, arom. C); 151.9 (*s*, C(4)); 153.2 (*s*, C(2)); 158.9 (*s*, arom. C); 159.2 (*s*, C(6)). ESI-MS: 800.33 (100, $[M+H]^+$).

5′-O-(4,4′-Dimethoxytrityl)-1-methyl-2′-O-{[(triisopropylsilyl)oxy]methyl}adenosine (**31**). A soln. of **30** (1.00 g, 1.3 mmol; prepared according to [22][25]) in DMF (15 ml) was treated with MeI (0.08 ml, 1.3 mmol) and stirred for 5 days at r.t. Workup, evaporation, and crystallization (acetone) gave **31** (1.02 g, 98%). White solid. M.p. 160° (dec.). TLC (CH_2Cl_2/MeOH 95:5): R_f 0.05. ^{1}H-NMR (400 MHz, $CDCl_3$): 0.89–1.15 (*m*, ^{i}Pr$_3$-Si); 1.27 (br. *s*, HN=C(6)); 3.03 (br. *s*, OH–C(3′)); 3.38 (*dd*, *J* = 3.7, 11.0, H–C(5′)); 3.45 (*dd*, *J* = 3.7, 10.2, H′–C(5′)); 3.66 (*s*, Me–N(1)); 3.80 (*s*, 2 MeO); 4.29 (*d*, *J* = 2.9, H–C(4′)); 4.53 (*m*, H–C(3′)); 4.81 (*t*, *J* = 5.9, H–C(2′)); 4.96, 5.14 (2*d*, *J* = 5.0, OCH_2O); 6.03 (*d*, *J* = 6.6, H–C(1′)); 6.82 (*d*, *J* = 8.8, 4 arom. H); 7.20–7.46 (*m*, 9 arom. H); 7.60 (*s*, H–C(2)); 7.85 (*s*, H–C(8)). ^{13}C-NMR (100 MHz, $CDCl_3$): 11.8 (*d*, Me_2CH); 17.8 (*q*, *Me*$_2$CH); 35.9 (*q*, Me–N(1)); 55.3 (*q*, MeO); 63.6 (*t*, C(5′)); 71.0 (*d*, C(2′)); 82.4 (*d*, C(3′)); 84.3 (*d*, C(4′)); 86.6 (*d*, C(1′)); 86.8 (*s*, arom. C); 90.8 (*t*, OCH_2O); 113.2 (*d*, arom. C); 121.8 (*s*, C(5)); 126.9, 127.9, 128.0, 128.2, 130.1 (5*d*, arom. C); 135.63, 135.69 (2*s*, arom. C); 139.3 (*d*, C(8)); 144.6 (*s*, arom. C); 145.6 (*s*, C(4)); 147.3 (*s*, C(2)); 149.1 (*s*, C(6)); 158.6 (*s*, arom. C). ESI-MS: 770.36 (100, $[M+H]^+$).

N^6-(*Chloroacetyl*)-*5′-O-(4,4′-dimethoxytrityl)-1-methyl-2′-O-{[(triisopropylsilyl)oxy]methyl}adenosine* (**32**). A soln. of **31** (1.00 g, 1.3 mmol) in pyridine/$ClCH_2CH_2Cl$ 1:9 (55 ml) was treated with chloroacetic anhydride (890 mg, 5.2 mmol) and stirred for 1 h at −15°. After workup and evaporation, the residue was dissolved in MeOH (2 ml) and treated with a sat. NH_3 soln. (7 ml) for 4 h at −15°. Workup and CC (SiO_2 (20 g), hexane/AcOEt 9:1 → AcOEt) gave **32** (0.70 g, 64%). Light yellow foam. TLC (CH_2Cl_2/MeOH 19:1): R_f 0.74. ^{1}H-NMR (400 MHz, $CDCl_3$): 0.99–1.08 (*m*, ^{i}Pr$_3$Si); 3.03 (*d*, *J* = 2.9, OH–C(3′)); 3.35 (*dd*, *J* = 4.4, 10.3, H–C(5′)); 3.46 (*dd*, *J* = 4.4, 10.3, H′–C(5′)); 3.64 (*s*, Me–N(1)); 3.81 (*s*, 2 MeO); 4.29 (*m*, H–C(4′)); 4.44 (*s*, $ClCH_2$); 4.52 (*m*, H–C(3′)); 4.76 (*t*, *J* = 5.1, H–C(2′)); 4.93, 5.14 (2*d*, *J* = 5.1, OCH_2O); 6.08 (*d*, *J* = 6.6, H–C(1′)); 6.82 (*d*, *J* = 8.8, 4 arom. H); 7.20–7.34 (*m*, 9 arom. H); 7.81 (*s*, H–C(2)); 7.94 (*s*, H–C(8)). ^{13}C-NMR (100 MHz, $CDCl_3$): 11.8 (*d*, Me_2CH); 17.8 (*q*, *Me*$_2$CH); 36.8 (*q*, Me-N(1)); 46.0 (*t*, CH_2Cl); 55.3 (*q*, MeO); 63.4 (*t*, C(5′)); 71.0 (*d*, C(2′)); 82.7 (*d*, C(3′)); 84.4 (*d*, C(4′)); 86.5 (*d*, C(1′)); 86.7 (*s*, arom. C); 91.0 (*t*, OCH_2O); 113.2 (*d*, arom. C); 122.4 (*s*, C(5)); 127.6, 127.9, 128.1, 128.3, 130.1 (5*d*, arom. C), 135.63, 135.69 (2*s*, arom. C); 139.3 (*d*, C(8)); 144.6 (*s*, arom. C); 145.6 (*s*, C(4)); 147.3 (*s*, C(6)); 149.1 (*d*, C(2)); 158.6 (*s*, arom. C); 178.09 (s, CO). ESI-MS: 846.74 (70, $[M+H]^+$), 848.74 (30, $[M+H]^+$).

3′-O-Acetyl-5′-O-(4,4′-dimethoxytrityl)-2′-O-{[(triisopropylsilyl)oxy]methyl}adenosine (**33**). A soln. of **30** (1.0 g, 1.16 mmol; prepared according to [22][25]) in pyridine (9.3 ml) was treated with DMAP (14 mg, 0.16 mmol) and Ac_2O (143 mg, 1.4 mmol). Workup (1. 10% citric acid, 2. $NaHCO_3$ soln.) after 30 min at r.t. and CC (SiO_2 (25 g), hexane/AcOEt 2:3 → 1:9 (+3% Et_3N)) gave **33** (0.88 g, 88%). Colorless foam. TLC (hexane/AcOEt 1:9): R_f 0.57. ^{1}H-NMR (100 MHz, $CDCl_3$): 0.88–1.05 (*m*, ^{i}Pr$_3$Si); 2.13 (*s*, MeCO); 3.43 (*dd*, *J* = 6.5, 10.4, H–C(5′)); 3.52 (*dd*, *J* = 5.6, 10.4, H′–C(5′)); 3.78 (*s*, 2 MeO); 4.34 (*m*, H–C(4′)); 4.87 (*s*, OCH_2-O); 5.19 (*dd*, *J* = 5.3, 6.9, H–C(2′)); 5.51 (*dd*, *J* = 2.5, 5.3, H–C(3′)); 5.80 (br. *s*, NH_2); 6.16 (*d*, *J* = 6.9, H–C(1′)); 6.78–6.83 (*m*, 4 arom. H); 7.20–7.45 (*m*, 9 arom. H); 7.97 (*s*, H–C(2)); 8.25 (*s*, H–C(8)). ^{13}C-NMR (100 MHz, $CDCl_3$): 11.7 (*d*, Me_2CH); 17.6 (*q*, *Me*$_2$CH); 20.9 (*q*, *Me*CO); 55.3 (*q*, MeO); 63.4 (*t*, C(5′)); 72.1 (*d*, C(3′)); 77.0 (*d*, C(2′)); 82.5 (*d*, C(4′)); 86.2 (*d*, C(1′)); 86.8 (*s*, arom. C); 89.6 (*t*, OCH_2O); 113.2 (*d*, arom. C); 120.1 (*s*, C(5)); 127.0, 127.9, 128.2, 130.1 (4*d*, arom. C), 135.6 (*s*, arom. C); 139.2 (*d*, C(8)); 144.4 (*s*, arom. C); 150.2 (*s*, C(4)); 153.2 (*d*, C(2)); 155.4 (*s*, C(6)); 158.6 (*s*, arom. C); 170.1 (*s*, CO). ESI-MS: 868.35 (100, $[M+Na]^+$).

N^6-*{{{(1S,2R)-2-{[(*tert*-Butyl)dimethylsilyl]oxy}-1-{[2-(4-nitrophenyl)ethoxy]carbonyl}propyl}amino}carbonyl}-5′*-O-*(4,4′-dimethoxytrityl)-2′*-O-*{[triisopropylsilyl)oxy]methyl}adenosine* (O^3-*[(*tert*-Butyl)dimethylsilyl]*-N-*{{9-[5′*-O-*(4,4′-dimethoxytrityl)-2′*-O-*{[(triisopropylsilyl)oxy]methyl}-β-*D-*ribofuranosyl}-9*H*-purin-6-yl}carbonyl}-*L*-threonine*; **34**). A soln. of **33** (400 mg, 0.5 mmol) in $ClCH_2CH_2Cl$ (2 ml) was treated with Et_3N (250 mg, 2.5 mmol) and 1,1′-carbonylbis[1*H*-1,2,4-triazole] (120 mg, 0.7 mmol), stirred for 10 min at 70°, treated with *O*^{3}-[(*tert*-butyl)dimethylsilyl]-L-threonine 2-(4-nitrophenyl)ethyl ester (280 mg, 0.7 mmol, prepared according to [21]), and stirred for 15 min at 70°. After workup and evaporation, the residue was treated with a sat. NH_3 soln. in MeOH (5 ml) for 3 h at r.t. Workup and CC (SiO_2 (7 g), hexane/AcOEt 3:2 → AcOEt) gave **34** (326 mg, 56%). Colorless foam. TLC (CH_2Cl_2/MeOH 9:1): R_f 0.25. ^{1}H-NMR (400 MHz, $CDCl_3$): −0.05, −0.03 (2*s*, 2 MeSi); 0.91 (s, ^{t}BuSi); 0.95–1.06 (*m*, ^{i}Pr$_3$Si); 1.09 (*d*, *J* = 4.2, Me(γ)); 3.06 (*t*, *J* = 5.7, *CH*$_2$CH$_2$O); 3.15 (*d*, *J* = 3.7, OH–C(3′)); 3.14–3.55 (*m*, CH_2(5′)); 3.80 (*s*, 2 MeO); 4.30–4.60 (*m*, H–C(4′), H–C(3′), CH(α), CH(β), CH$_2$*CH*$_2$O); 4.97 (*t*, *J* = 5.1, H–C(2′)); 5.04 (*d*, *J* = 4.7, 1 H, OCH$_2$O); 5.19 (*d*, *J* = 4.7, 1 H, OCH$_2$O); 6.24 (*d*, *J* = 6.2, H–C(1′)); 6.81 (*d*, *J* = 8.7, 4 arom. H); 7.22–7.44 (*m*, 9 arom. H); 7.22–7.46 (*m*, 13 arom. H); 7.98 (*d*, *J* = 8.8, 2 arom. H); 8.19 (*s*, H–C(8)); 8.42 (*s*, H–C(2)); 9.99 (d, *J* = 8.8, NH–C(α)); 10.02 (br. *s*, NH–C(6)). ^{13}C-NMR (100 MHz, $CDCl_3$): −5.4, −4.3 (*q*, MeSi); 11.8 (*d*, Me$_2$*C*H); 17.8 (*q*, *Me*$_2$CH); 21.1 (Me(γ)); 25.5 (*q*, *Me*$_3$CSi); 34.8 (*t*, *CH*$_2$CH$_2$O); 55.2 (*q*, MeO); 59.6 (*t*, CH$_2$*CH*$_2$O); 63.4 (*t*, C(5′)); 64.6 (*d*, C(α)); 68.6 (*d*, C(β)); 70.8 (*d*, C(3′)); 76.6 (*d*, C(2′)); 82.4 (*d*, C(4′)); 84.4 (*d*, C(1′)); 87.4 (*s*, arom. C); 90.9 (*t*, OCH$_2$O); 113.2 (*d*, arom. C); 120.9 (*s*, C(5)); 123.6, 126.9, 127.8, 128.2, 129.7 (5*d*, arom. C); 130.1 (*s*, arom. C); 135.7 (*s*, arom. C); 141.7 (*d*, C(8)); 144.6 (*d*, C(2)); 145.5 (*s*, arom. C); 150.0 (*s*, C(4)); 150.2 (*s*, C(6)); 154.2 (*s*, NH*C*ONH); 158.6 (*s*, arom. C); 170.9 (*s*, *C*OOCH$_2$). ESI-MS: 1164.37 (100, $[M+H]^+$).

5′-O-*(4,4′-Dimethoxytrityl)-5-methyl-2′*-O-*{[(triisopropylsilyl)oxy]methyl}cytidine* (**35**). A soln. of **13** (0.65 g, 0.9 mmol) in pyridine (3.5 ml) was treated with DMAP (22 mg, 0.17 mmol) and Ac_2O (0.17 ml, 1.77 mmol) and stirred for 3 h at r.t. Workup and evaporation gave a yellow solid foam (0.63 g), which was carefully dried (14 h at 60°/0.05 mbar) and dissolved in MeCN (3 ml). Meanwhile, under Ar and at 4°, 4-chlorophenyl phosphorodichloridate (0.65 g, 2.65 mmol) was added dropwise to a suspension of finely powdered 1*H*-1,2,4-triazole (1.07 g, 15.4 mmol, dried by sublimation) in dry MeCN (6 ml). After 15 min at 4°, ^{i}Pr$_2$NEt (2.3 ml, 13.3 mmol) was added, and after 40 min at r.t., the mixture was again cooled to 4° and treated with the MeCN soln. obtained before (3 ml, containing 0.65 g of the intermediate nucleoside). After 6 h at r.t., the soln. was diluted with dioxane (9 ml), treated with sat. aq. NH_3 soln. (13 ml), and stirred for another 3 h at r.t. Extraction with CH_2Cl_2/10% aq. citric acid and sat. aq. $NaHCO_3$ soln. gave a yellow solid foam (0.62 g) which was dissolved in THF/MeOH 5:4 (33 ml), cooled to 4°, and treated with 2N NaOH (3.7 ml). After 30 min at 4°, the soln. was treated with AcOH (0.43 ml) and concentrated to 30 ml. Workup and CC (SiO_2 (8 g), CH_2Cl_2 → CH_2Cl_2/MeOH 19:1) gave **35** (0.60 g, 90%). Colorless foam. TLC (CH_2Cl_2/MeOH 15:185): R_f 0.50. ^{1}H-NMR (400 MHz, (D$_6$)DMSO): 1.00–1.14 (*m*, ^{i}Pr$_3$Si); 1.49 (*s*, Me–C(5)); 3.26 (br. *s*, CH$_2$(5′)); 3.74 (*s*, 2 MeO); 3.96 (*dd*, *J* = 5.6, 3.2, H–C(4′)); 4.22 (*q*, *J* = 5.2, H–C(3′)); 4.27 (*t*, *J* = 5.4, H–C(2′)); 4.96 (*d*, *J* = 5.2, 1 H, OCH$_2$O); 4.99 (*d*, *J* = 5.1, 1 H, OCH$_2$O); 5.05 (*d*, *J* = 5.0, OH–C(3′)); 5.99 (*d*, *J* = 5.8, H–C(1′)); 6.89 (*d*, *J* = 8.8, 4 arom. H); 7.22–7.42 (*m*, 9 arom. H, H–C(6)). ^{13}C-NMR (100 MHz, (D$_6$)DMSO): 11.9 (*d*, Me$_2$*C*H); 13.3 (*q*, *Me*–C(5)); 18.1 (*q*, *Me*$_2$CH); 55.5 (*q*, MeO); 63.9 (*t*, C(5′)); 67.1 (*d*, C(2′)); 78.2 (*d*, C(3′)); 83.4 (*d*, C(4′)); 86.4 (*s*, arom. C); 87.4 (*d*, C(1′)); 88.8 (*t*, OCH$_2$O); 102.2 (*s*, C(5)); 113.7 (*d*, arom. C); 127.3, 128.2, 128.4 130.2 (4*d*, arom. C); 135.8, 135.9, 138.4 (3*s*, arom. C); 145.1 (*d*, C(6)); 155.6 (*s*, C(2)); 158.6 (*s*, arom. C); 165.8 (*s*, C(4)). ESI-MS: 1491.80 (100, $[2M+1]^+$).

N^4-*Acetyl-5′*-O-*(4,4′-dimethoxytrityl)-5-methyl-2′*-O-*{[(triisopropylsilyl)oxy]methyl}cytidine* (**36**). A soln. of **35** (0.6 g, 0.80 mmol) in DMF (3.3 ml) was treated with Ac_2O (113 mg, 1.1 mmol) for 8 h at r.t. Workup and CC (SiO_2 (8 g), hexane/AcOEt 4:1 → 3:7) gave **36** (0.55 g, 90%). Colorless solid foam. TLC (hexane/AcOEt 1:9): R_f 0.52. ^{1}H-NMR (400 MHz, (D$_6$)DMSO): 0.87–1.08 (*m*, ^{i}Pr$_3$Si); 1.13 (*s*, Me–C(5)); 2.25 (*s*, *Me*CO); 3.26 (br. *s*, CH$_2$(5′)); 3.74 (*s*, 2 MeO); 4.05 (br. *d*, *J* ≈ 4.6, H–C(4′)); 4.25 (*q*, *J* = 4.5, H–C(3′)); 4.33 (*t*, *J* = 4.7, H–C(2′)); 4.99 (*d*, *J* = 5.2, 1 H, OCH$_2$O); 5.04 (*d*, *J* = 5.1, 1 H, OCH$_2$O); 5.15 (*d*, *J* = 5.0, OH–C(3′)); 5.99 (*d*, *J* = 4.4, H–C(1′)); 6.89 (*d*, *J* = 8.8, 4 arom. H); 7.23–7.41 (*m*, 9 arom. H); 7.82 (br. *s*, H–C(6)); 9.82 (br. *s*, NH–C(4)). ^{13}C-NMR (100 MHz, (D$_6$)DMSO): 11.9 (*d*, Me$_2$*C*H); 13.8 (*q*, *Me*-C(5)); 18.1 (*q*, *Me*$_2$CH); 25.3 (*q*, *Me*CO); 55.5 (*q*, MeO); 63.6 (*t*, C(5′)); 69.2 (*d*, C(2′)); 78.7 (*d*, C(3′)); 83.9 (*d*, C(4′)); 86.4 (*d*, C(1′)); 88.9 (*s*, arom. C); 90.4 (*t*, OCH$_2$O); 113.7 (*d*, arom. C); 127.3 (*s*, C(5)); 128.2, 128,4, 130.2, 135.7, 135.9 (5*d*, arom. C); 142.5, 145.0 (2*s*, arom. C); 152.5 (*d*, C(6)); 155.5 (*s*, C(2)); 158.6 (*s*, arom. C); 162.9 (*s*, C(4)); 171.2 (*s*, Me*C*O). ESI-MS: 788.34 (100, $[M+H]^+$).

N^2-*Acetyl-5′*-O-*(4,4′-dimethoxytrityl)-2′*-O-*{[(triisopropylsilyl)oxy]methyl}isocytidine 3′-(2-Cyanoethyl Diisopropylphosphoramidite)* (**37**). A soln. of **5** (100 mg, 0.13 mmol) in CH_2Cl_2 (2 ml) was treated consecutively with ^{i}Pr$_2$NEt (0.05 ml, 0.32 mmol) and cyanoethyl diisopropylphosphoramidochloridite (43 mg, 0.15 mmol).

After stirring for 14 h at r.t., the mixture was subjected to CC (SiO_2 (3 g), hexane/AcOEt 9:1 → 4:6 (+ 3% Et_3N)): **37** (60 mg, 50%, 1:1 mixture of diastereoisomers). Colorless foam. TLC (hexane/AcOEt 1:1): R_f 0.64. ^{1}H-NMR (400 MHz, $CDCl_3$): 0.98–1.07 (*m*, ^{i}Pr$_3$Si); 1.09, 1.18 (2*d*, *J* = 7.0 (Me_2CH)$_2$N); 2.20, 2.21 (2*s*, MeCO); 2.45 (*t*, *J* = 5.7, 1 H, CH_2CN); 2.67 (*q*, *J* = 6.3, 1 H, CH_2CN); 3.44–3.69 (*m*, 3.5 H, $POCH_2$, (Me$_2$C*H*)$_2$N, H–C(5′)); 3.81 (*s*, 2 MeO); 3.94 (*m*, 1 H, $POCH_2$); 4.25 (*d*, *J* = 6.2, 0.5 H, H–C(4′)); 4.30 (*d*, *J* = 6.2, 0.5 H, H–C(4′)); 4.34–4.47 (*m*, H–C(2′), H–C(3′)); 5.17 (br. *s*, 1 H, OCH_2O); 5.28 (*dd*, *J* = 4.6, 11.2, 1 H, OCH_2O); 5.34, 5.40 (2*d*, *J* = 8.1, H–C(5)); 6.54, 6.59 (2*d*, *J* = 3.1, H–C(1′)); 6.81–6.88 (*m*, 4 arom. H); 7.32–7.44 (*m*, 9 arom. H); 8.17, 8.23 (2*d*, *J* = 8.6, H–C(6)); 13.03, 13.06 (2 br. *s*, NH–C(2)). ^{31}P-NMR (162 MHz, $CDCl_3$): 151.2, 151.6. MALDI-MS: 974.30 (100, $[M+H]^+$).

*5′-*O*-(4,4′-Dimethoxytrityl)-*O^2*-(diphenylcarbamoyl)-*N^6*-isobutyryl-2′-*O*-{[(triisopropylsilyl)oxy]methyl}isoguanosine 3′-(2-Cyanoethyl Diisopropylphosphoramidite)* (**38**). As described for **37**, with **8** (100 mg, 0.096 mmol), CH_2Cl_2 (0.7 ml), ^{i}Pr$_2$NEt (0.04 ml, 0.24 mmol), and cyanoethyl diisopropylphosphoramidochloridite (34 mg, 0.14 mmol). CC (SiO_2 (3 g), hexane/AcOEt 9:1 → 1:1 (+3% Et_3N)) gave **38** (70 mg, 56%; 1:1 mixture of diastereoisomers). Colorless foam. TLC (hexane/AcOEt 1:1): R_f 0.49. ^{1}H-NMR (400 MHz, $CDCl_3$): 0.85–1.00 (*m*, ^{i}Pr$_3$Si); 1.05 (*d*, *J* = 6.2, (Me_2CH)$_2$N); 1.12–1.29 (*m*, (Me_2CH)$_2$N); 1.33 (*d*, *J* = 7.0, Me_2CHCO); 2.34 (*t*, *J* = 7.0, 0.5 H, CH_2CN); 2.48–2.70 (*m*, 1.5 H, CH_2CN); 3.32 (*dd*, *J* = 5.9, 10.2, 0.5 H, H–C(5′)); 3.44–3.73 (*m*, 4.5 H, (MeC*H*)$_2$N, MeC*H*CO, H–C(5′), $POCH_2$); 3.78, 3.79 (2*s*, 2 MeO); 3.82–3.92 (*m*, 1 H, $POCH_2$); 4.30 (*d*, *J* = 3.1, 0.5 H, H–C(4′)); 4.35 (*d*, *J* = 3.9, 0.5 H, H–C(4′)); 4.57–4.64 (*m*, 1 H); 4.85–5.02 (*m*, 3H, OCH_2O, H–C(2′), H–C(3′)); 6.23 (*t*, *J* = 4.7, H–C(1′)); 6.78–6.83 (*m*, 4 arom. H); 7.20–7.42 (*m*, 9 arom. H); 8.14, 8.19 (2*s*, H–C(8)). ^{31}P-NMR (162 MHz, $CDCl_3$): 151.7, 151.8. MALDI-MS: 1237.83 (100, $[M+H]^+$).

*5′-*O*-(4,4′-Dimethoxytrityl)-*N^6*-isobutyryl-2-[(methoxyacetyl)amino]-2′-*O*-{[(triisopropylsilyl)oxy]methyl}adenosine 3′-(2-Cyanoethyl Diisopropylphosphoramidite)* (**39**). As described for **37**, with **11** (270 mg, 0.30 mmol), CH_2Cl_2 (1.0 ml), ^{i}Pr$_2$NEt (0.13 ml, 0.75 mmol), and cyanoethyl diisopropylphosphoramidochloridite (85 mg, 0.36 mmol). CC (SiO_2 (6 g), hexane/AcOEt 4:1 → 2:3 (+ 3% Et_3N)) gave **39** (250 mg, 76%; 1:1 mixture of diastereoisomers). Colorless foam. TLC (hexane/AcOEt 1:9): R_f 0.29. ^{1}H-NMR (400 MHz, $CDCl_3$): 0.87–0.93 (*m*, ^{i}Pr$_3$Si); 1.05 (*d*, *J* = 6.8, (Me_2CH)$_2$N); 1.17–1.22 (*m*, (Me_2CH)$_2$N); 1.32 (*d*, *J* = 6.8, Me_2CHCO); 1.94 (*s*, *Me*OCH$_2$); 2.31–2.41 (*m*, 1 H, CH_2CN); 2.69 (*t*, *J* = 6.4, 1 H, CH_2CN); 3.37–3.84 (*m*, 6 H, (MeC*H*)$_2$N, CH_2(5′), Me$_2$C*H*CO, $POCH_2$); 3.79, 3.80 (2*s*, 2 MeO); 3.81–4.03 (*m*, 1.5 H, $POCH_2$); 4.09 (br. *s*, 2 H, MeOC*H*$_2$); 4.33 (br. *d*, *J* ≈ 3.1, 0.5 H, H–C(4′)); 4.38 (br. *d*, *J* ≈ 3.4, 0.5 H, H–C(4′)); 4.53–4.69 (*m*, 1 H, H–C(3′)); 4.91–5.08 (*m*, 3 H, OCH_2O, H–C(2′)); 6.15 (*d*, *J* = 5.8, 0.5 H, H–C(1′)); 6.21 (*d*, *J* = 6.1, 0.5 H, H–C(1′)); 6.78–6.82 (*m*, 4 arom. H); 7.28–7.35 (*m*, 9 arom. H); 8.08 (*s*, H–C(8)); 8.63 (2 br. *s*, NH–C(6)); 8.72–8.77 (*m*, NH–C(2)). ^{31}P-NMR (162 MHz, $CDCl_3$): 151.5, 151.8. MALDI-MS: 1113.30 (100, $[M+H]^+$).

*5′-*O*-(4,4′-Dimethoxytrityl)-5-methyl-2′-*O*-{[(triisopropylsilyl)oxy]methyl}uridine 3′-(2-Cyanoethyl Diisopropylphosphoramidite)* (**40**). As described for **37**, with **13** (400 mg, 0.13 mmol), CH_2Cl_2 (4 ml), ^{i}Pr$_2$NEt (0.23 ml, 1.35 mmol), and cyanoethyl diisopropylphosphoramidochloridite (139 mg, 0.59 mmol). CC (SiO_2 (12 g), hexane/AcOEt 9:1 → 1:4 (+3% Et_3N)) gave **40** (500 mg, 98%; 1:1 mixture of diastereoisomers). Colorless foam. TLC (hexane/AcOEt 1:1): R_f 0.71. ^{1}H-NMR (400 MHz, $CDCl_3$): 1.00–1.12 (*m*, ^{i}Pr$_3$Si); 1.16–1.32 (*m*, (Me_2CH)$_2$N); 1.46, 1.49 (2*s*, Me–C(5)); 2.36 (*t*, *J* = 6.9, 1 H, CH_2CN); 2.64–2.66 (*dt*, *J* = 3.1, 6.4, 1 H, CH_2-CN); 3.32 (*dt*, *J* = 2.5, 7.1, H–C(5′)); 3.46–3.70 (*m*, 4 H, (Me$_2$C*H*)$_2$N, H′–C(5′), $POCH_2$); 3.80, 3.81 (2*s*, 2 MeO); 3.82–3.97 (*m*, 1 H, $POCH_2$); 4.20 (br. *d*, *J* ≈ 1.5, 0.5 H, H–C(4′)); 4.30 (br. *d*, *J* ≈ 2.2, 0.5 H, H–C(4′)); 4.45–4.65 (*m*, H–C(2′), H–C(3′)); 4.96–5.06 (*m*, OCH_2O); 6.18 (*d*, *J* = 6.6, 0.5 H, H–C(1′)); 6.20 (*d*, *J* = 6.5, 0.5 H, H–C(1′)); 6.83–6.87 (*m*, 4 arom. H); 7.25–7.38 (*m*, 9 arom. H); 7.41 (br. *s*, H–C(6)); 7.43 (br. *d*, H–N(3)). ^{31}P-NMR (162 MHz, $CDCl_3$): 150.2, 150.9. MALDI-MS: 947.31 (100, $[M+H]^+$).

*5′-*O*-(4,4′-Dimethoxytrityl)-5,6-dihydro-2′-*O*-{[(triisopropylsilyl)oxy]methyl}uridine 3′-(2-Cyanoethyl Diisopropylphosphoramidite)* (**41**). As described for **37**, with **15** (400 mg, 0.54 mmol), CH_2Cl_2 (5 ml), ^{i}Pr$_2$NEt (0.24 ml, 1.35 mmol), and cyanoethyl diisopropylphosphoramidochloridite (160 mg, 0.65 mmol). CC (SiO_2 (10 g), hexane/AcOEt 4:1 → 1:9 (+3% Et_3N)) gave **41** (367 mg, 73%; 1:1 mixture of diastereoisomers). Colorless foam. TLC (hexane/AcOEt 7:3): R_f 0.73. ^{1}H-NMR (400 MHz, $CDCl_3$): 1.03–1.10 (*m*, ^{i}Pr$_3$Si); 1.15–1.31 (*m*, (Me_2CH)$_2$N); 2.37 (*t*, *J* = 6.6, 1 H, CH_2CN); 2.50–2.62 (*m*, CH_2(5)); 2.66 (*dt*, *J* = 1.8, 6.7, 1 H, CH_2CN); 3.22–3.27 (*m*, 1 H, H–C(6)); 3.37–3.52 (*m*, 2 H, H′–C(6), H–C(5′)); 3.52–3.77 (*m*, 4 H (Me$_2$C*H*)$_2$N, H′–C(5), $POCH_2$); 3.81, 3.82 (2*s*, 2 MeO); 3.82–3.97 (*m*, 1 H, POC*H*$_2$); 4.12 (br. *d*, *J* ≈ 2.2, 0.5 H, H–C(4′)); 4.19 (br. *d*, *J* ≈ 2.7, 0.5 H, H–C(4′)); 4.39–4.46 (*m*, H–C(2′), H–C(3′)); 4.97 (*d*, *J* = 5.0, 0.5 H, OCH_2O); 5.05 (*q*, *J* = 4.9, 1.5H, OCH_2O); 6.05 (*d*, *J* = 5.9, 0.5 H, H–C(1′)); 6.07 (*d*, *J* = 5.7, 0.5 H, H–C(1′)); 6.82–6.86 (*m*, 4 arom. H); 7.23–7.44 (*m*, 9 arom. H). ^{31}P-NMR (162 MHz, $CDCl_3$): 150.1, 150.4. MALDI-MS: 935.36 (100, $[M+H]^+$).

5′-O-(4,4′-Dimethoxytrityl)-1-methyl-2′-O-{[(triisopropylsilyl)oxy]methyl}inosine 3′-(2-Cyanoethyl Diisopropylphosphoramidite) (**42**). As described for **37**, with **18** (77 mg, 0.1 mmol), CH_2Cl_2 (2 ml), $^iPr_2NEt$ (0.04 ml, 0.25 mmol), and cyanoethyl diisopropylphosphoramidochloridite (30 mg, 0.12 mmol). CC (SiO_2 (2 g), hexane/AcOEt 4:1 → 3:2 (+ 3% Et_3N)) gave **42** (78 mg, 80%; 1:1 mixture of diastereoisomers). Colorless foam. TLC (hexane/AcOEt 8:2): R_f 0.50. TLC (hexane/AcOEt 1:1): R_f 0.50. ^{1}H-NMR (400 MHz, $CDCl_3$): 0.86–1.05 (*m*, $^iPr_3Si$); 1.16–1.19 (*m*, $(Me_2CH)_2N$); 2.39 (*t*, *J* = 6.4, 1 H, CH_2CN); 2.67 (*t*, *J* = 6.4, 1 H, CH_2CN); 3.32–3.40 (*m*, 1 H, H–C(5′)); 3.41–3.51 (*m*, 1 H, H′–C(5′)); 3.51–3.70 (*m*, 3 H, $(Me_2CH)_2N$, $POCH_2$); 3.60, 3.61 (2*s*, Me–N(1)); 3.79, 3.80 (2*s*, 2 MeO); 3.84–3.97 (*m*, 1 H, $POCH_2$); 4.35, 4.41 (2*q*, *J* = 3.1, H–C(4′)); 4.57–4.65 (*m*, H–C(3′)); 4.91–4.98 (*m*, OCH_2O); 5.00–5.04 (*m*, H–C(2′)); 6.05 (*d*, *J* = 6.1, 0.5 H, H–C(1′)); 6.09 (*d*, *J* = 6.1, 0.5 H, H–C(1′)); 6.75–6.83 (*m*, 4 arom. H); 7.18–7.44 (*m*, 9 arom. H); 7.78 (*s*, H–C(8)); 7.92, 7.93 (2*s*, H–C(2)). ^{31}P-NMR (162 MHz, $CDCl_3$): 150.06; 150.73. ESI-MS: 971.83 (100, $[M+H]^+$).

5′-O-(4,4′-Dimethoxytrityl)-N^6-isopent-2-enyl-2′-O-{[(triisopropylsilyl)oxy]methyl}adenosine 3′-(2-Cyanoethyl Diisopropylphosphoramidite) (**43**). As described for **37**, with **21** (90 mg, 0.12 mmol), CH_2Cl_2 (1.5 ml), $^iPr_2NEt$ (0.05 ml, 0.27 mmol), and cyanoethyl diisopropylphosphoramidochloridite (21 mg, 0.18 mmol). CC (SiO_2 (3 g), hexane/AcOEt 9:1 → 2:3 (+ 3% Et_3N)) gave **43** (90 mg, 80%; 1:1 mixture of diastereoisomers). Light yellow foam. TLC (hexane/AcOEt 1:1): R_f 0.70. ^{1}H-NMR (400 MHz, $CDCl_3$): 0.86–0.98 (*m*, $^iPr_3Si$); 1.11, 1.14 (2*d*, *J* = 6.6, $(Me_2CH)_2N$); 1.22 (*sept.*, *J* = 5.5, $(Me_2CH)_2N$); 1.76 (*s*, Me); 1.78 (*s*, Me); 2.39 (*t*, *J* = 6.5, 1 H, CH_2CN); 2.67 (*dt*, *J* = 2.3, 6.7, 1 H, CH_2CN); 3.32–3.72 (*m*, 1 H of $POCH_2$, $(MeCH)_2N$, H–C(5′)); 3.79 (*s*, 2 MeO); 3.84–3.99 (*m*, 1 H, $POCH_2$); 4.22 (br. *s*, CH_2–NH); 4.34 (*d*, *J* = 3.7, H–C(4′)); 4.40 (*t*, *J* = 4.0, H–C(4′)); 4.67–4.74 (*m*, H–C(3′)); 4.94, 4.96 (2*d*, *J* = 5.0, OCH_2O); 4.97–5.04 (*m*, OCH_2O); 5.16–5.23 (*m*, H–C(2′)); 5.40 (*t*, *J* = 4.7, H–C(11)); 6.13, 6.16 (2*d*, *J* = 5.7, H–C(1′)); 6.77–6.82 (*m*, 4 arom. H); 7.20–7.43 (*m*, 12 arom. H); 7.92, 7.94 (2*s*, H–C(8)); 8.28, 8.30 (2*s*, H–C(2)). ^{31}P-NMR (162 MHz, $CDCl_3$): 150.1, 150.8. MALDI-MS: 1024.36 (100, $[M+H]^+$).

5′-O-(4,4′-Dimethoxytrityl)-N^6-methyl-2′-O-{[(triisopropylsilyl)oxy]methyl}adenosine 3′-(2-Cyanoethyl Diisopropylphosphoramidite) (**44**). As described for **37**, with **22** (120 mg, 0.16 mmol), CH_2Cl_2 (3 ml), $^iPr_2NEt$ (0.07 ml, 0.39 mmol), and cyanoethyl diisopropylphosphoramidochloridite (39 mg, 0.19 mmol). CC (SiO_2 (3 g), hexane/AcOEt 4:1 → 2:3 (+3% Et_3N)) gave **44** (128 mg, 85%; 1:1 mixture of diastereoisomers). Colorless foam. TLC (hexane/AcOEt 1:1): R_f 0.50. ^{1}H-NMR (400 MHz, $CDCl_3$): 0.88–1.10 (*m*, $^iPr_3Si$); 1.16–1.26 (*m*, $(Me_2CH)_2N$); 2.37 (*t*, *J* = 6.5, 1 H, CH_2CN); 2.65 (*t*, *J* = 6.4, 1 H, CH_2CN); 3.20 (br. *s*, *Me*NH)); 3.30–3.33 (*m*, H–C(5′)); 3.49–3.70 (*m*, 4 H, H'–C(5′), $(Me_2CH)_2N$, $POCH_2$); 3.77, 3.78 (2*s*, 2 MeO); 3.86–3.97 (*m*, 1 H, $POCH_2$); 4.32, 4.37 (2*q*, *J* = 3.0, H–C(4′)); 4.67–4.75 (*m*, H–C(3′)); 4.91–5.01 (*m*, OCH_2O); 5.16–5.21 (*m*, H–C(2′)); 6.11 (*d*, *J* = 5.5, 0.5 H, H–C(1′)); 6.13 (*d*, *J* = 5.2, 0.5 H, H–C(1′)); 6.75–6.79 (*m*, 4 arom. H); 7.17–7.41 (*m*, 9 arom. H); 7.90, 7.92 (2*s*, H–C(8); 8.28, 8.30 (2*s*, H–C(2)). ^{31}P-NMR (162 MHz, $CDCl_3$): 149.89; 150.58. ESI-MS: 546.29 (100, $[M+H]^+$).

5′-O-(4,4′-Dimethoxytrityl)-N^6,N^6-dimethyl-2′-O-{[(triisopropylsilyl)oxy]methyl}adenosine 3′-(2-Cyanoethyl Diisopropylphosphoramidite) (**45**). As described for **37**, with **23** (148 mg, 0.19 mmol), CH_2Cl_2 (3 ml), $^iPr_2NEt$ (0.08 ml, 0.47 mmol), and cyanoethyl diisopropylphosphoramidochloridite (47 mg, 0.23 mmol). CC (SiO_2 (3 g), hexane/AcOEt 9:1 → 1:1 (+3% Et_3N)) gave **45** (148 mg, 80%; 1:1 mixture of diastereoisomers). Colorless foam. TLC (hexane/AcOEt 2:1): R_f 0.61. ^{1}H-NMR (400 MHz, $CDCl_3$): 0.88–1.10 (*m*, $^iPr_3Si$); 1.12–1.20 (*m*, $(Me_2CH)_2N$); 2.37 (*t*, *J* = 6.5, 1 H, CH_2CN); 2.65 (*t*, *J* = 6.4, 1 H, CH_2CN); 3.45–3.68 (*m*, 11 H, Me_2N, $CH_2(5′)$, $(Me_2CH)_2N$, $POCH_2$); 3.77, 3.78 (2*s*, 2 MeO); 3.81–3.98 (*m*, 1 H, $POCH_2$); 4.32, 4.38 (2*q*, *J* = 3.1, H–C(4′)); 4.67–4.75 (*m*, H–C(3′)); 4.92–5.01 (*m*, OCH_2O); 5.14–5.17 (*m*, H–C(2′)); 6.14 (*d*, *J* = 5.5, 0.5 H, H–C(1′)); 6.16 (*d*, *J* = 5.2, 0.5 H, H–C(1′)); 6.76–6.80 (*m*, 4 arom. H); 7.20–7.40 (*m*, 9 arom. H); 7.88, 7.90 (2*s*, H–C(8)); 8.22, 8.24 (2*s*, H–C(2)). ^{31}P-NMR (162 MHz, $CDCl_3$): 149.86; 150.54. ESI-MS: 546.29 (100, $[M+H]^+$).

5′-O-(4,4′-Dimethoxytrityl)-1-methyl-2′-O-{[(triisopropylsilyl)oxy]methyl}guanosine 3′-(2-Cyanoethyl Diisopropylphosphoramidite) (**46**). As described for **37**, with **25** (84 mg, 0.11 mmol), CH_2Cl_2 (0.5 ml), $^iPr_2NEt$ (0.03 ml, 0.27 mmol), and cyanoethyl diisopropylphosphoramidochloridite (22 mg, 0.19 mmol). CC (SiO_2 (1.5 g), hexane/AcOEt 2:3 → AcOEt (+ 3% Et_3N)) gave **46** (61 mg, 58%; 1:1 mixture of diastereoisomers). Light yellow foam. TLC (hexane/AcOEt 1:9): R_f 0.34. ^{1}H-NMR (400 MHz, $CDCl_3$): 0.90–1.00 (*m*, $^iPr_3Si$); 1.06–1.31 (4*d*, *J* = 6.5, $(Me_2CH)_2N$); 2.35 (*t*, *J* = 6.4, 1 H, CH_2CN); 2.69 (*t*, *J* = 6.7, 1 H, CH_2CN); 2.77 (*dd*, *J* = 1.6, 6.3, 0.5 H, H–C(5′)); 3.27–3.38 (*m*, 0.5 H, H–C(5′)); 3.46, 3.47 (2*s*, 2 Me-N(1)); 3.49–3.72 (*m*, 4 H, $(MeCH)_2N$, H–C(5′), $POCH_2$); 3.79 (*s*, 2 MeO); 3.86–4.25 (*m*, $POCH_2$); 4.30 (br. *q*, *J* ≈ 2, 0.5 H, H–C(4′)); 4.38 (br. *t*, *J* ≈ 3, 0.5 H, H–C(4′)); 4.58–4.68 (*m*, H–C(3′)); 4.73, 4.80 (2*s*, 1 H, OCH_2O); 4.88–5.15 (*m*, 1.5 H, H–C(2′), OCH_2O); 5.02–5.15 (*m*, 0.5 H, H–C(2′)); 5.89 (*d*, *J* = 6.5, 0.5 H, H–C(1′)); 5.96 (*d*, *J* = 7.0, 0.5 H,

H–C(1′)); 6.80–6.82 (*m*, 4 arom. H); 7.21–7.48 (*m*, 9 arom. H, H–C(8)); 7.64, 7.66 (2 br. *s*, NH–C(2)). ^{31}P-NMR (162 MHz, $CDCl_3$): 151.3; 151.6. MALDI-MS: 986.31 (100, $[M+H]^+$).

5′-O-(4,4′-Dimethoxytrityl)-N^2-methyl-2′-O-{[(triisopropylsilyl)oxy]methyl}guanosine 3′-(2-Cyanoethyl Diisopropylphosphoramidite) (**47**). As described for **37**, with **27** (75 mg, 0.09 mmol), CH_2Cl_2 (0.5 ml), ^{i}Pr$_2$NEt (0.04 ml, 0.23 mmol), and cyanoethyl diisopropylphosphoramidochloridite (27 mg, 0.11 mmol). CC (SiO_2 (2 g), CH_2Cl_2/MeOH 1:0 → 94:6 (+ 3% Et_3N)) gave **47** (87 mg, 92%; 1:1 mixture of diastereoisomers). Light pink foam. TLC (CH_2Cl_2/MeOH 9:1): R_f 0.37. ^{1}H-NMR (400 MHz, $CDCl_3$): 0.83–1.02 (*m*, ^{i}Pr$_3$Si); 1.06 (*d*, *J* = 6.0, *Me*$_2$CH)$_2$N); 1.17–1.24 (*m*, (*Me*$_2$CH)$_2$N); 1.38 (*t*, *J* = 6.6, (*Me*$_2$CH)$_2$N); 2.35 (*t*, *J* = 6.6, 1 H, CH_2CN); 2.61–2.74 (*m*, 1 H, CH_2CN); 3.15 (2*d*, *J* = 7.2, *Me*NH-C(2)); 3.26–3.70 (*m*, 4.5 H, (MeC*H*)$_2$N, H–C(5′), $POCH_2$); 3.777, 3.786 (2*s*, 2 MeO); 3.82–3.98 (*m*, 1.5 H, $POCH_2$); 4.32, 4.36 (2 br. *d*, *J* = 4.1, H–C(4′)); 4.58–4.65 (*m*, H–C(3′)); 4.92–5.06 (*m*, H–C(2′), OCH_2O); 6.07 (*d*, *J* = 5.8, 0.5 H, H–C(1′)); 6.10 (*d*, *J* = 5.9, 0.5 H, H–C(1′)); 6.79–6.84 (*m*, 4 arom. H); 7.16–7.50 (*m*, 9 arom. H); 7.67, 7.68 (2*s*, H–C(8)); 11.9 (br. *s*, H–N(1)). ^{31}P-NMR (162 MHz, $CDCl_3$): 150.4; 150.9. MALDI-MS: 986.29 (100, $[M+H]^+$).

5′-O-(4,4′-Dimethoxytrityl)-N^2,N^2-dimethyl-2′-O-{[(triisopropylsilyl)oxy]methyl}guanosine 3′-(2-Cyanoethyl Diisopropylphosphoramidite) (**48**). As described for **37**, with **29** (140 mg, 0.16 mmol), CH_2Cl_2 (0.7 ml), ^{i}Pr$_2$NEt (0.07 ml, 0.4 mmol), and cyanoethyl diisopropylphosphoramidochloridite (58 mg, 0.24 mmol). CC (SiO_2 (4 g), CH_2Cl_2 → CH_2Cl_2/MeOH 95:5 (+3% Et_3N)) gave **48** (104 mg, 64%; 1:1 mixture of diastereoisomers). Light yellow foam. TLC (CH_2Cl_2/MeOH 9:1): R_f 0.47. ^{1}H-NMR (400 MHz, $CDCl_3$): 0.95–0.97 (1*d*, *J* = 6.6, ^{i}Pr$_3$Si); 1.04–1.06 (1*d*, *J* = 6.6, (*Me*$_2$CH)$_2$N); 1.17–1.23 (*m*, (*Me*$_2$CH)$_2$N); 1.27–1.34 (*t*, *J* = 6.6, (*Me*$_2$CH)$_2$N); 2.34 (*t*, *J* = 6.6, 1 H, CH_2CN); 2.60–2.72 (*m*, 0.5 H, CH_2CN); 2.34 (*dt*, *J* ≈ 1, 4.4, 0.5 H, CH_2CN); 3.13, 3.14 (2*s*, Me_2N–C(2)); 3.31–3.70 (*m*, 5 H, (MeC*H*)$_2$N, H–C(5′), $POCH_2$); 3.786, 3.795 (2*s*, 2 MeO); 3.80–3.96 (*m*, 1.5 H, $POCH_2$); 4.11–4.27 (*m*, 1 H, $POCH_2$); 4.32 (br. *d*, *J* ≈ 3.7, 0.5 H, H–C(4′)); 4.36 (br. *d*, *J* ≈ 3.6, 0.5 H, H–C(4′)); 4.53–4.60 (*m*, H–C(3′)); 4.90–5.06 (*m*, H–C(2′), OCH_2O); 6.03 (*d*, *J* = 6.6, 0.5 H, H–C(1′)); 6.07 (*d*, *J* = 5.8, 0.5 H, H–C(1′)); 6.78–6.83 (*m*, 4 arom. H); 7.23–7.43 (*m*, 9 arom. H); 7.67 (br. *s*, H–C(8)); 10.9 (br. *s*, H–N(1)). ^{31}P-NMR (162 MHz, $CDCl_3$): 150.4; 150.9. ESI-MS: 1000.36 (100, $[M+H]^+$).

N^6-(*Chloroacetyl*)-*5′-O-(4,4′-dimethoxytrityl)-1-methyl-2′-O-{[(triisopropylsilyl)oxy]methyl}adenosine 3′-(2-Cyanoethyl Diisopropylphosphoramidite)* (**49**). As described for **37**, with **32** (400 mg, 0.48 mmol), CH_2Cl_2 (4 ml), ^{i}Pr$_2$NEt (0.21 ml, 1.23 mmol), and cyanoethyl diisopropylphosphoramidochloridite (134 mg, 0.56 mmol). CC (SiO_2 (10 g), hexane/AcOEt 4:1 → 1:4 (+3% Et_3N)) gave **49** (462 mg, 93%; 1:1 mixture of diastereoisomers). Colorless foam. TLC (hexane/AcOEt 1:9): R_f 0.75. ^{1}H-NMR (400 MHz, $CDCl_3$): 0.95–1.02 (*m*, ^{i}Pr$_3$-Si); 1.08, 1.19 (2*d*, *J* = 7.3, (Me$_2$CH)$_2$N); 2.37 (*t*, *J* = 6.6, 1 H, CH_2CN); 2.66 (*t*, *J* = 5.8, 1 H, CH_2CN); 3.34 (*dt*, *J* = 4.3, 10.5, 1 H, $POCH_2$); 3.57–3.94 (*m*, 1 H of $POCH_2$, (MeC*H*)$_2$N, H–C(5′)); 3.81 (*s*, MeO); 4.35 (*d*, *J* = 3.6, 0.5 H, H–C(4′)); 4.41 (*d*, *J* = 3.6, 0.5 H, H–C(4′)); 4.44 (*s*, $ClCH_2$); 4.62 (*dt*, *J* = 4.9, 10.9, H–C(3′)); 4.91–5.01 (*m*, H–C(2′), OCH_2O); 6.04, 6.07 (2*d*, *J* = 5.9, H–C(1′)); 6.79–6.84 (*m*, 4 arom. H); 7.22–7.38 (*m*, 12 arom. H); 7.74 (*s*, H–C(2)); 7.91, 7.93 (2*s*, H–C(8)). ^{31}P-NMR (162 MHz, $CDCl_3$) 150.3, 150.9. MALDI-MS: 1046.74 (70, $[M+H]^+$) 1048.74 (30, $[M+H]^+$).

N^6-*{{{(1S,2R)-2-{[(tert-Butyl)dimethylsilyl]oxy}-1-{[2-(4-nitrophenyl)ethoxy]carbonyl}propyl}amino}carbonyl}-5′-O-(4,4′-dimethoxytrityl)-2′-O-{[(triisopropylsilyl)oxy]methyl}adenosine 3′-(2-Cyanoethyl Diisopropylphosphoramidite)* (**50**). As described for **37**, with **34** (100 mg, 0.08 mmol), CH_2Cl_2 (1 ml), ^{i}Pr$_2$NEt (0.05 ml, 0.21 mmol), and cyanoethyl diisopropylphosphoramidochloridite (24 mg, 0.10 mmol). CC (SiO_2 (3 g), hexane/AcOEt 6:4 → AcOEt (+3% Et_3N)) gave **50** (80 mg, 68%; 1:1 mixture of diastereoisomers). Colorless foam. TLC (hexane/AcOEt 1:9): R_f 0.62. ^{1}H-NMR (400 MHz, $CDCl_3$): −0.03, 0.09 (2*s*, *Me*Si); 0.97–1.08 (*m*, ^{i}Pr$_3$Si, ^{t}BuSi); 1.09 (*d*, *J* = 6.6, Me(*γ*)); 1.10–1.30 (*m*, (*Me*$_2$CH)$_2$N); 2.40 (*t*, *J* = 5.9, 1 H, CH_2CN); 2.67 (*t*, *J* = 6.6, 1 H, CH_2CN); 3.07 (t, *J* = 6.6, *CH*$_2$CH$_2$O); 3.35–3.39 (*m*, 1 H, $POCH_2$); 3.51–3.66 (*m*, (Me$_2$C*H*)$_2$N, H–C(5′)); 3.80–4.00 (*m*, 1 H, $POCH_2$); 3.79 (*s*, 2 MeO); 4.32–4.44 (*m*, H–C(4′), CH_2CH_2O); 4.54 (*d*, *J* = 6.6, CH(*β*)); 4.58 (*d*, *J* = 8.8, CH(*α*)); 4.63–4.73 (*m*, H–C(3′)); 4.96, 4.97, 5.03 (3*d*, *J* = 4.4, OCH_2O); 5.08–5.13 (*m*, H–C(2′)); 6.24 (*d*, *J* = 5.1, 0.5 H, H–C(1′)); 6.26 (*d*, *J* = 5.8, 0.5 H, H–C(1′)); 6.79–6.84 (*m*, 4 arom. H); 7.24–7.54 (*m*, 15 arom. H); 8.06 (*d*, *J* = 8.1, 2 arom. H); 8.16, 8.19 (2*s*, H–C(8)); 8.43, 8.44 (2*s*, H–C(2)); 10.04 (*t*, *J* = 9.5, NN–C(6)). ^{31}P-NMR (162 MHz, $CDCl_3$): 150.8; 150.2. ESI-MS: 1364.30 (100, $[M+H]^+$).

N^4-*Acetyl-5′-O-(4,4′-dimethoxytrityl)-5-methyl-2′-O-{[(triisopropylsilyl)oxy]methyl}cytidine 3′-(2-Cyanoethyl Diisopropylphosphoramidite)* (**51**). As described for **37**, with **36** (1.20 g, 1.56 mmol), CH_2Cl_2 (6.3 ml), ^{i}Pr$_2$NEt (0.60 ml, 3.90 mmol), and cyanoethyl diisopropylphosphoramidochloridite (444 mg, 1.88 mmol). CC (SiO_2 (30 g), hexane/AcOEt 1:1 → AcOEt (+2% Et_3N)) gave **51** (1.42 g, 94%; 1:1 mixture of diastereoisomers). Colorless foam. TLC (hexane/AcOEt 3:7): R_f 0.75. ^{1}H-NMR (400 MHz, $CDCl_3$): 0.97–1.08 (*m*, ^{i}Pr$_3$Si); 1.08–1.13 (*m*, (*Me*$_2$CH)$_2$N); 2.25 (br. *s*, MeCO); 2.51 (br. *s*, Me–C(5)); 2.54 (*t*, *J* = 5.9, 1 H, CH_2CN); 2.75 (br. *q*, *J* = 5.9, 1 H, CH_2CN); 3.42–3.24 (*m*, 1 H of $POCH_2$, (Me$_2$C*H*)$_2$N, H–C(5′)); 3.66–3.82 (*m*, 1 H,

$POCH_2$); 3.73 (*s*, 2 MeO); 7.85 (2br. *s*, H–C(4′)); 4.32–4.48 (*m*, H–C(2′), H–C(3′)); 4.89–5.04 (*m*, OCH_2O); 6.00 (*d*, *J* = 4.4, H–C(1′)); 6.87–6.89 (*m*, 4 arom. H); 7.22–7.43 (*m*, 9 arom. H); 7.85 (*d*, *J* = 8.0, H–C(6)). ^{31}P-NMR (162 MHz, $CDCl_3$): 149.0, 149.5. ESI-MS: 988.30 (100, $[M+H]^+$).

5′-O-(4,4′-Dimethoxytrityl)-2′-O-{[(triisopropylsilyl)oxy]methyl}inosine 3′-(2-Cyanoethyl Diisopropylphosphoramidite) (**52**). A suspension of **17** (1.5 g, 2 mmol), 5-benzyl-1*H*-tetrazole (0.43 g, 2.2 mmol; prepared according to [23]), and 4 Å MS in MeCN (10 ml) was treated with 2-cyanoethyl tetraisopropylphosphoramidite (1.8 g, 6 mmol). After 4 h at r.t., the mixture was diluted with CH_2Cl_2 (100 ml) and poured into a well-stirred mixture of CH_2Cl_2 (200 ml) and sat. aq. $NaHCO_3$ soln. (250 ml). The org. phase was dried (Na_2SO_4) and evaporated. CC (SiO_2 (40 g), hexane/AcOEt 1:1 → AcOEt (+3% Et_3N)) gave **52** (1.75 g, 92%; 1:1 mixture of diastereoisomers). Colorless foam. TLC (hexane/AcOEt 1:9): R_f 0.48. ^{1}H-NMR (400 MHz, $CDCl_3$): 0.85–1.00 (*m*, ^{i}Pr$_3$Si); 1.18–1.31 (*m*, $(Me_2CH)_2N$); 2.40 (*t*, *J* = 6.5, 1 H, CH_2CN); 2.62–2.71 (*dt*, *J* = 1.5, 7.6, 1 H, CH_2-CN); 3.35–3.41 (*m*, $POCH_2$); 3.46–3.74 (*m*, 4 H $(Me_2CH)_2N$, H–C(5′), $POCH_2$); 3.79, 3.80 (2*s*, 2 MeO); 3.85–4.00 (*m*, 1 H, $POCH_2$); 4.38 (br. *d*, *J* = 3.4, 0.5 H, H–C(4′)); 4.43 (br. *d*, *J* = 3.6, 0.5 H, H–C(4′)); 4.58–4.64 (*m*, H–C(2′), H–C(3′)); 4.92–5.09 (*m*, OCH_2O); 6.13 (*d*, *J* = 6.0, 0.5 H, H–C(1′)); 6.16 (*d*, *J* = 6.0, 0.5 H, H–C(1′)); 6.79–6.84 (*m*, 4 arom. H); 7.19–7.47 (*m*, 9 arom. H); 7.94, 7.95 (2*s*, H-C(8)); 8.01, 8.04 (2*s*, H–C(2)). ^{31}P-NMR (162 MHz, $CDCl_3$): 151.3, 152.1. MALDI-MS: 957.36 (100, $[M+H]^+$).

REFERENCES

[1] 'Modification and Editing of RNA', Eds. H. Grosjean and R. Benne, ASM Press, Washington, 1998; D. Söll, U. RajBhandary, 'tRNA: Structure, Biosynthesis and Function', ASM Press, Washington 1995.
[2] G. R. Björk, 'Biosynthesis and Function of Modified Nucleosides', American Society for Microbiology, Washington, D.C. 2005, 1995.
[3] M. Helm, H. Brulé, F. Degoul, C. Cepanec, J.-P. Leroux, R. Giegé, C. Florentz, *Nucleic Acids Res.* **1998**, *26*, 1636.
[4] D. R. Liu, T. J. Magliery, M. Pastrnak, P. G. Schultz, *Proc. Natl. Acad. Sci. U.S.A.* **1997**, *94*, 10092; L. Wang, A. Brock, B. Herbrich, P. G. Schultz, *Science (Washington, D.C.)* **2001**, *292*, 498; A. K. Kowal, C. Kohrer, U. L. RajBhandary, *Proc. Natl. Acad. Sci. U.S.A.* **2001**, *98*, 2268.
[5] S. A. Connolly, A. E. Rosen, K. Musier-Forsyth, C. S. Francklyn, *Biochemistry* **2004**, *43*, 962.
[6] K. N. Nobles, C. S. Yarain, G. Liu, R. H. Guenther, P. F. Agris, *Nucleic Acids Res.* **2002**, *30*, 4751.
[7] D. Gasparutto, T. Livache, H. Bazin, A.-M. Duplaa, A. Guy, A. Khorlin, D. Molko, A. Roget, R. Téoule, *Nucleic Acids Res.* **1992**, *20*, 5159.
[8] M. Helm, R. Giegé, C. Florentz, *Biochemistry* **1999**, *38*, 13338.
[9] A. E. Kloepffer, J. W. Engels, *Nucleosides, Nucleotides, Nucleic Acids* **2003**, *22*, 1347.
[10] Y.-L. Chiu, T. M. Rana, *RNA* **2003**, *9*, 1034.
[11] C. Höbartner, C. Kreutz, E. Flecker, E. Ottenschläger, W. Pils, K. Grubmayr, R. Micura, *Monatsh. Chem.* **2003**, *134*, 851.
[12] S. N. Mikhailov, J. Rozenski, E. V. Efimtseva, R. Busson, A. Van Aerschot, P. Herdewijn, *Nucleic Acids Res.* **2002**, *30*, 1124.
[13] R. Green, J. W. Szostak, S. A. Benner, A. Rich, N. Usman, *Nucleic Acids Res.* **1991**, *19*, 4161.
[14] H. M.-P. Chui, M. Meroueh, S. A. Scaringe, C. S. Chow, *Biol. Med. Chem.* **2002**, *10*, 325.
[15] H. M.-P. Chui, J.-P. Desaulniers, S. A. Scaringe, C. S. Chow, *J. Org. Chem.* **2002**, *67*, 8847.
[16] R. K. Kumar, D. R. Davis, *Nucleic Acids Res.* **1997**, *25*, 1272.
[17] A. C. Bajii, D. R. Davis, *Org. Lett.* **2000**, *2*, 3865.
[18] P. F. Agris, A. Malkiewicz, A. Kraszewski, K. Everett, B. Nawrot, E. Sochacka, J. Jankowska, R. Guenther, *Biochimie* **1995**, *77*, 125.
[19] E. Kierzek, R. Kierzek, *Nucleic Acids Res.* **2003**, *31*, 4461.
[20] M. Sundaram, P. C. Durant, D. R. Davis, *Biochemistry* **2000**, *39*, 12575.
[21] V. Boudou, J. Langridge, A. Van Aerschot, C. Hendrix, A. Millar, P. Weiss, P. Herdewijn, *Helv. Chim. Acta* **2000**, *83*, 152.
[22] X. Wu, S. Pitsch, *Nucleic Acids Res.* **1998**, *26*, 4315; S. Pitsch, P. A. Weiss, X. Wu, D. Ackermann, T. Honegger, *Helv. Chim. Acta* **1999**, *82*, 1753.
[23] S. Pitsch, P. A. Weiss, L. Jenny, A. Stutz, X. Wu, *Helv. Chim. Acta* **2001**, *84*, 3773.
[24] P. Wenter, S. Pitsch, *Helv. Chim. Acta* **2003**, *86*, 3955.
[25] A. Stutz, C. Höbartner, S. Pitsch, *Helv. Chim. Acta* **2000**, *83*, 2477.
[26] S. Porcher, S. Pitsch, in preparation.

[27] A. Rich, in 'Horizons in Biochemistry', Eds. M. Kasha and B. Pullmann, Academic Press, New York, 1962, p. 103.
[28] S. C. Jurczyk, J. T. Kodra, D. Rozzell, S. A. Benner, T. R. Battersby, *Helv. Chim. Acta* **1998**, *81*, 793.
[29] C. Roberts, R. Bandaru, C. Switzer, *J. Am. Chem. Soc.* **1997**, *119*, 4640.
[30] X. Chen, R. Kierzek, D. H. Turner, *J. Am. Chem. Soc.* **2001**, *123*, 1267.
[31] S. A. Strobel, T. R. Cech, N. Usman, L. Beigelman, *Biochemistry* **1994**, *33*, 13224; S. A. Strobel, T. R. Cech, *Biochemistry* **1996**, *35*, 1201.
[32] J. S. Vyle, K. J. Young, J. A. Grasby, *Tetrahedron Lett.* **1998**, *39*, 5093.
[33] F. Seela, T. Fröhlich, *Helv. Chim. Acta* **1994**, *77*, 399.
[34] M. M. P. Ng, F. Benseler, T. Tuschl, F. Eckstein, *Biochemistry* **1994**, *33*, 12119.
[35] K. Hosono, H. Gozu, H. Hideo, K. Sakamoto, S. Shigeyuki, K. Takai, H. Takaku, *Biochim. Biophys. Acta* **1997**, *1354*, 211.
[36] K. Kikuchi, Y. Taniyama, R. Marumoto, *Z. Naturforsch. B* **1988**, *43*, 623.
[37] D. Ackermann, S. Pitsch, *Helv. Chim. Acta* **2002**, *85*, 1443.
[38] J. J. Dalluge, T. Hashizume, A. E. Sopchik, J. A. Mac Closkey, D. R. Davis, *Nucleic Acids Res.* **1996**, *24,* 1073.
[39] D. Flockerzi, G. Silber, R. Charubala, W. Schlosser, R. S. Varma, F. Creegan, W. Pfeiderer, *Liebigs Ann. Chem*. **1981**, 1568.
[40] F. Skoog, D. J. Armstrong, *Annu. Rev. Plant Physiol.* **1970**, *21*, 359.
[41] J. Cabello-Villegas, M. E. Winkler, E. P. Nikonowicz, *J. Mol. Biol.* **2002**, 319, 1015.
[42] E. Kierzek, R. Kierzek, *Biophys. Chem.* **2001**, *91*, 135.
[43] E. Kierzek, R. Kierzek, *Nucleic Acids Res.* **2003**, *31*, 4472.
[44] G. R. Björk, P. M. Wikström, A. S. Byström, *Science (Washington, D.C.)* **1989**, *244*, 986.
[45] M. Sekine, T. Satoh, *J. Org. Chem.* **1991**, *56*, 1224.
[46] M. Ballestri, C. Chatgilialoglu, K. B. Clark, D. Griller, B. Giese, B. Kopping, *J. Org. Chem.* **1991**, *56*, 678; I. Ryu, K. Nagahara, A. Kurihara, M. Komatsu, N. Sonoda, *J. Organomet. Chem.* **1997**, *548*, 105; Y. Apeloig, M. Nakash, *J. Am. Chem. Soc.* **1994**, *116*, 10781; C. Chatgilialoglu, *Acc. Chem. Res.* **1992**, *25*, 188.
[47] A. Stutz, S. Pitsch, *Synlett* **1999**, 930.
[48] Y. Jl, W. Bannwarth, B. Luu, *Tetrahedron* **1990**, *46*, 487.

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