Reliable Chemical Synthesis of Oligoribonucleotides (RNA) with 2′-*O*-[(Triisopropylsilyl)oxy]methyl(2′-*O*-tom)-Protected Phosphoramidites

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A method for the introduction of the 2′-*O*-[(triisopropylsilyl)oxy]methyl (= tom) group into *N*-acetylated, 5′-*O*-dimethoxytritylated ribonucleosides is presented. The corresponding 2′-*O*-tom-protected phosphoramidite building blocks were obtained in pure form and were successfully employed for the routine synthesis of oligoribonucleotides on DNA synthesizers. Under DNA coupling conditions (2.5 min coupling time for a 1.5-μmol synthesis scale) and with 5-(benzylthio)-1*H*-tetrazole (BTT) as activator, 2′-*O*-tom-protected phosphoramidites exhibited average coupling yields > 99.4%. The combination of *N*-acetyl and 2′-*O*-tom protecting groups allowed a reliable and complete two-step deprotection, first with $MeNH_2$ in $EtOH/H_2O$ and then with Bu_4NF in THF, without concomitant destruction of the product RNA sequences.

1. Introduction. – The chemical synthesis of nucleic acids has been automated and is carried out on a solid phase by stepwise addition of appropriately activated and protected nucleotide building blocks until the desired sequence has been obtained. Subsequently, all protecting groups required during the assembly procedure are removed and the product is cleaved from the solid support. Length and uniformity of the product are limited by the coupling and deprotection efficiencies.

During the last decades, many research groups have successfully contributed to the development of a reliable chemical synthesis of nucleic acids. As a consequence, the synthesis of DNA oligonucleotides is probably the most evolved chemical process known so far and has almost reached perfection in terms of efficiency and automation. More than 10^7 DNA couplings/year are performed worldwide, and defined DNA sequences are now available commercially, at low cost and within a very short time. Currently, so-called phosphoramidite chemistry is carried out almost exclusively. It is based on nucleoside 3′-(2-cyanoethyldiisopropylphosphoramidite) building blocks, which are activated *in situ* by N-acids (usually 1*H*-tetrazoles); their coupling to the 5′-OH group of the growing oligonucleotide chains results in phosphorous acid triesters which are subsequently oxidized to the corresponding phosphoric acid triesters. This concept was introduced 1975 by *Letsinger et al.* [1] and subsequently improved by *Caruthers et al.* [2], *Adams et al.* [3], *McBride* and *Caruthers* [4], and *Sinha et al.* [5]. The 5′-*O*-positions of the phosphoramidite building blocks are usually protected by the acid-labile 4,4′-dimethoxytrityl ($(MeO)_2Tr$) group, which was introduced 1962 by *Khorana* and co-workers [6], and which allows a convenient optical in-line monitoring of the coupling efficiencies. The nucleophilic NH_2 groups of the adenine, guanine and cytosine nucleosides are typically masked by acylation. These nucleobase-protecting

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groups are removed after the assembly of the oligonucleotide, usually by treatment with aqueous NH_3 solution; simultaneously, the 2-cyanoethyl groups are removed by β-elimination and the sequence is detached from the support.

This extremely powerful methodology can, in principle, also be applied to the synthesis of the structurally very similar RNA oligonucleotides. Compared to DNA, however, each nucleotide unit within an RNA strand contains an additional 2′-OH group, which is responsible for the instability of RNA under basic conditions (pH > 12, 25°) and which has to be protected during the assembly. Since the RNA products are base-labile, the removal of these supplementary protecting groups is carried out separately, after removing the 2-cyanoethyl groups, the nucleobase-protecting groups, and the cleavage from the support under basic, nucleophilic conditions. As a consequence, the choice of these additional 2′-*O*-protecting groups has to meet several stringent requirements: their removal at the end of the synthesis must occur quantitatively without partial destruction of the base-labile RNA sequence; they have to be completely stable under all reaction conditions, specifically under acidic conditions (removal of the $(MeO)_2Tr$ group within each coupling step) and under the basic, nucleophilic deprotection conditions; they should be compatible and not interfere with the phosphoramidite coupling reactions; their introduction into monomers should be straightforward.

The large number of reported 2′-*O*-ribonucleoside protecting groups can essentially be divided into acid- [7–9], photo- [10–13], and fluoride-labile groups [14–16]. Among them, the fluoride-labile (*tert*-butyl)dimethylsilyl (= tbdms) group, introduced in 1973 by *Ogilvie et al.* [14], has found the widest application. However, several factors, including the relatively low coupling yields of typically ≤ 98.0% obtained with corresponding building blocks (despite rather long coupling times of *ca.* 15 min), as compared to > 99.5% with DNA phosphoramidites (coupling times of *ca.* 2 min only), limited the length of routinely synthesized RNA sequences. A very attractive alternative was the photolabile [(2-nitrobenzyl)oxy]methyl (= nbm) group, introduced in 1992 by *Gough* and co-workers [11], which led to coupling yields of ≈ 99% under DNA coupling conditions. The superior coupling behavior of nbm-protected building blocks is presumably the result of the minimal steric demand of the nbm group, achieved by connecting the photocleavable 2-nitrobenzyl protecting group *via* a sterically nondemanding formaldehyde acetal linker to the 2′-*O*-position of the ribonucleosides [11][16]. In 1997, we published a convenient method for the introduction of the nbm group into 5′-*O*-dimethoxytritylated, base-protected ribonucleosides [12]. Subsequently, we applied our synthetic method also to the introduction of structurally related 2′-*O*-protecting groups yielding as a common feature a formaldehyde acetal moiety.

We now describe in detail the preparation of 2′-*O*-{[(triisopropylsilyl)oxy]methyl}-protected (= 2′-*O*-tom-protected) phosphoramidites of the four canonical ribonucleosides and summarize all necessary information required for their application in the routine synthesis of oligoribonucleotides. Preliminary results obtained with these novel RNA building blocks were published previously [13][17–21].

2. Results. – 2.1. *2′-O-tom-Protected Phosphoramidites.* The preparation of the phosphoramidite building blocks from the four ribonucleosides was carried out by

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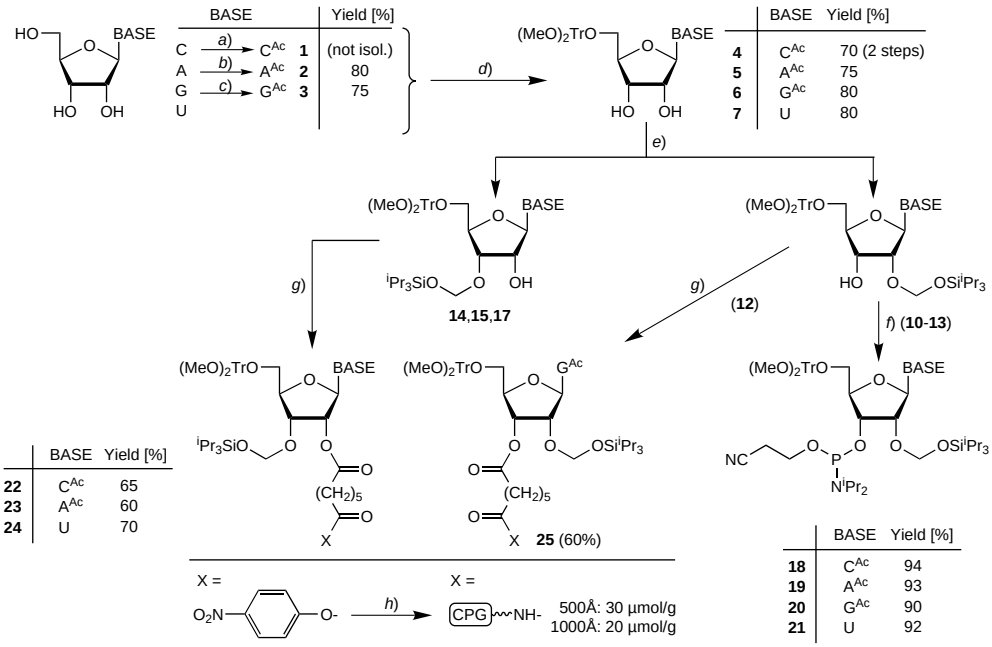
stepwise introduction of the base-protecting acetyl group, the 5′-*O*-$(MeO)_2Tr$ group, the tom group, and finally the 3′-(2-cyanoethyl diisopropylphosphoramidite) moiety (*Scheme 1*).

Scheme 1



a) Ac_2O, DMF, 25°. *b*) 1. Me_3SiCl, Py, 4–25°; 2. Ac_2O, Py, 4°; 3. AcOH, MeOH, –20°. *c*) 1. Ac_2O, Py, DMF, reflux; 2. NaOH, THF, MeOH, H_2O, 25°. *d*) $(MeO)_2TrCl$, Py, 25°. *e*) See *Scheme 2*. *f*) 2-Cyanoethyl diisopropylphosphoramidochloridite, $^iPr_2NEt$, CH_2Cl_2, 25°. *g*) Bis(4-nitrophenyl) heptanedioate, Py, *N*,*N*-dimethylpyridin-4-amine (DMAP), 25°. *h*) 1. Long-chain-alkylamino CPG, $^iPr_2NEt$, DMF, 25°; 2. Ac_2O, Py, 25°.

The synthesis of N^4-acetylcytidine (**1**) was carried out by selective *N*-acetylation of cytidine with Ac_2O in DMF as described in [22], but in much less solvent. After partial removal of the DMF, the crude product was directly transformed into its 5′-*O*-dimethoxytritylated derivative **4** with $(MeO)_2TrCl$/pyridine. N^6-Acetyladenosine (**2**) was synthesized by modification of an established procedure [23], involving first trimethylsilylation of the OH groups with Me_3SiCl/pyridine, followed by acetylation of the NH_2 group with Ac_2O, extractive workup, and cleavage of the trimethylsilyl ethers with AcOH in MeOH. A convenient synthesis of N^2-acetylguanosine (**3**) was achieved by peracetylation of guanosine with Ac_2O in DMF/pyridine, followed by cleavage of the *O*-bound Ac groups with NaOH in THF/MeOH/H_2O. The introduction of the 5′-*O*-$(MeO)_2Tr$ group into the nucleobase-protected nucleosides **1**–**3** and into uridine was carried out under standard conditions with $(MeO)_2TrCl$ pyridine and gave the 5′-*O*-protected nucleosides **4**–**7** in good yields. DMF was used as a cosolvent in the reactions with the cytidine and guanosine derivatives **1** and **3** (*Scheme 1*).

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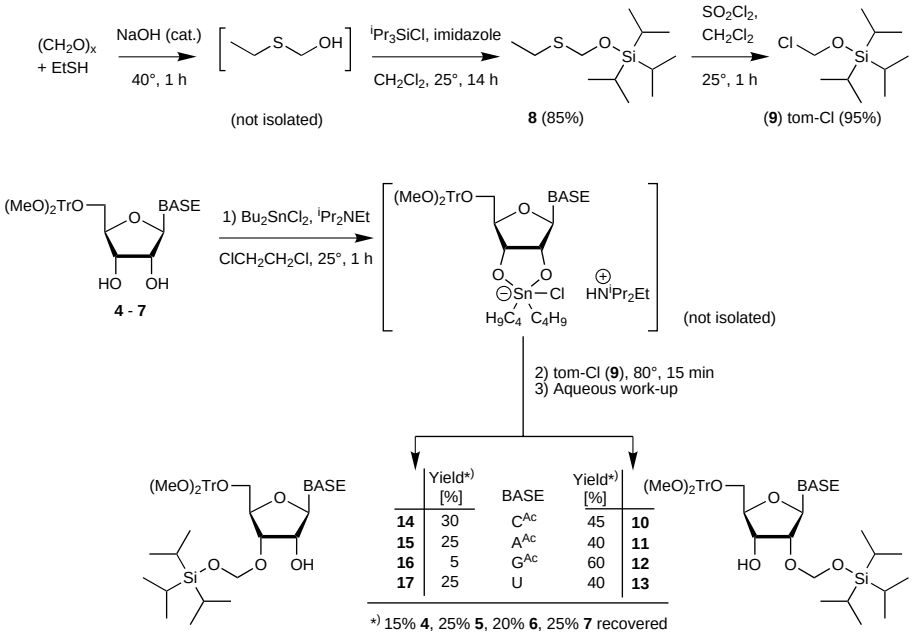
The tom group was introduced into the protected ribonucleosides **4**–**7** under the conditions we had earlier developed for the introduction of the nbm group [12] (*Scheme 2*). The reagent [(triisopropylsilyl)oxy]methyl chloride (tom-Cl; **9**) was synthesized by modifying the general procedure for the synthesis of [(trialkylsilyl)-oxy]methyl chlorides by *Benneche* and co-workers: silylation of (ethylthio)methanol [24][25] with triisopropylsilyl chloride/1*H*-imidazole in CH_2Cl_2 gave, after extraction and distillation, the silylated derivative **8** in good yield. This intermediate was transformed with SO_2Cl_2 in CH_2Cl_2 into tom-Cl (**9**) according to [26]. By distillation below 70°, **9** was isolated in an excellent yield.

Scheme 2. *Preparation of tom-Cl* (**9**) *and Introduction of the tom Group into Protected Ribonucleosides*



Alkylation of the diol moieties of the ribonucleosides **4**–**7** was achieved by first forming their cyclic 2′,3′-*O*-dibutylstannylidene derivatives under basic conditions with Bu_2SnCl_2/$^iPr_2NEt$ in 1,2-dichloroethane for 1 h at 25°. These activated intermediates were subsequently treated with 1.1–1.3 equiv. of tom-Cl (**9**) at 80° for 20 min. After aqueous workup, the 2′-*O*- and 3′-*O*-alkylated nucleosides **10**–**13** and **14**–**17**, respectively, were isolated both in pure form by chromatography on silica gel (unreacted starting materials **4**–**7** could also be recovered). Under these optimized reaction conditions, side reactions (alkylation of the base moieties) were observed only to a very small extent (< 5%). Fair to good yields of the desired 2′-*O*-alkylated products **10**–**13** besides various amounts of the 3′-*O*-alkylated isomers **14**–**17** were obtained. In all cases, the predominant, first eluting isomer was the 2′-*O*-alkylated compound. The unambiguous identification of the products was carried out by ^{1}H-NMR spectroscopy and irradiation experiments according to [12].

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Alkylation of the dialkylstannylated derivatives of nucleosides **4**–**7** with tom-Cl (**9**) occurred under a variety of reaction conditions. Performing the reaction at 80° in 1,2-dichloroethane as solvent favored the formation of the 2′-*O*-alkylated products **10**–**13**, whereas, at lower temperatures and with other solvents (THF, MeCN, C_6H_6), formation of the 3′-*O*-alkylated products **14**–**17** predominated. Slightly higher yields (5–10%) of the nucleosides **10**–**13** were obtained by forming the stannylated nucleosides with $^tBu_2SnCl_2$ instead of Bu_2SnCl_2. However, since $^tBu_2SnCl_2$ is very expensive, we used it only for the alkylation of precious nucleosides. Under our preferred reaction conditions, with only 1.1–1.3 equiv. of tom-Cl (**9**), a significant portion of starting material was recovered. Still, employing a larger excess of **9** did not result in better product yields, but only in the formation of more nucleobase-alkylated by-products.

The 2′-*O*-tom-protected intermediates **10**–**13** were converted with 2-cyanoethyl diisopropylphosphoramidochloridite/$^iPr_2NEt$ into the corresponding phosphoramidite building blocks **18**–**21** (*Scheme 1*). In *Fig. 1*, the ^{31}P-NMR spectrum of the regular

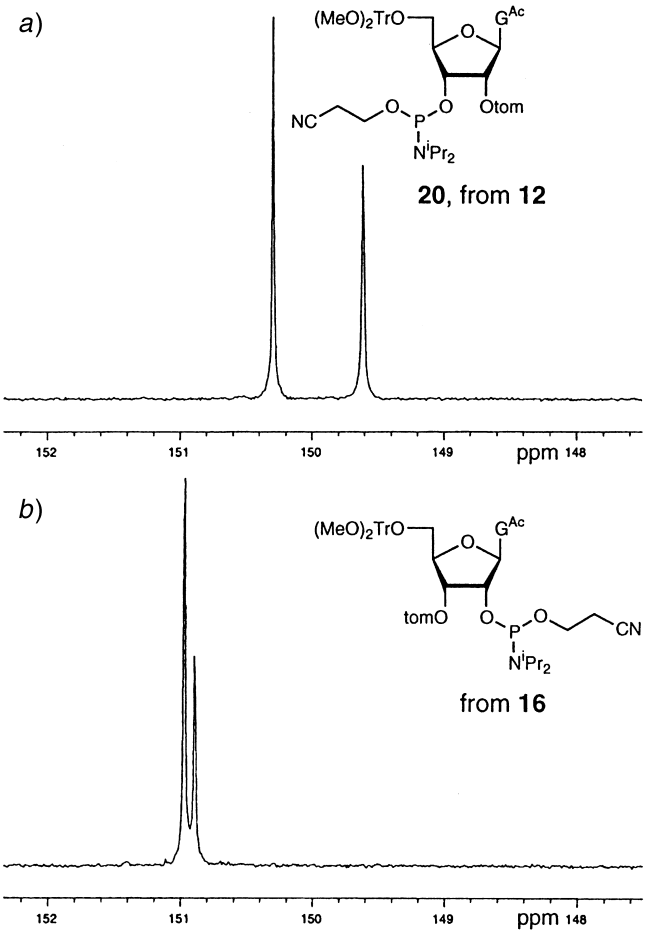

Fig. 1. ^{31}*P-NMR Spectrum* a) *of the regular phosphoramidite* **20** (prepared from the 2′-*O*-tom-protected nucleoside **12**) *and* b) *of its regioisomer* (prepared from the 3′-*O*-tom-protected nucleoside **16**), *demonstrating the purity of the RNA building block* **20**

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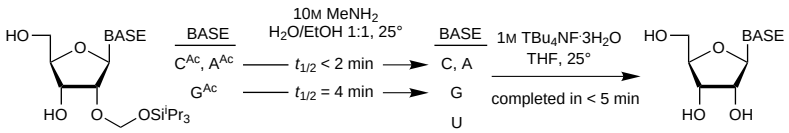
guanosine 3′-phosphoramidite **20** is shown together with that of its 2′-regioisomer (prepared from the 3′-*O*-tom-substituted guanine nucleoside **16** for comparison). Analogous analyses were also carried out with the three other phosphoramidites **18**, **19**, and **21**. They all established the absence of regioisomeric phosphoramidites that, upon incorporation into oligonucleotides, would lead to the formation of undesired 2′ → 5′ phosphodiester linkages.

The solid supports were synthesized by first preparing the 2′-(nitrophenyl heptanedioates) **22**–**24** from the 3′-*O*-alkylated nucleosides **14**, **15**, and **17**, respectively, and the 3′-(nitrophenyl heptanedioate) **25** from the 2′-*O*-alkylated guanosine derivative **12**, and then immobilizing these activated esters on (aminoalkyl)-functionalized controlled-pore glass (CPG) (*Scheme 1*). With 500-Å and 1000-Å CPG materials, typical loadings of 30 and 20 μmol/g, respectively, were obtained.

The compounds **2**–**25** were fully characterized by TLC, $[\alpha]_D$, IR, UV, ^{1}H-, ^{13}C-, and ^{31}P-NMR, and FAB-MS (see *Exper. Part*).

For protection of the exocyclic NH_2 functions of the nucleobases, we have chosen the Ac group, which, as a protecting group for cytosine nucleosides, is already used in combination with a convenient deprotection scheme involving a mixture of aqueous NH_3 solution and $MeNH_2$ [22]. Prior to the synthesis of oligonucleotides, we established the conditions required for the removal of the base-protecting Ac groups with $MeNH_2$ (10M in H_2O/EtOH 1:1, 25°). By UV measurements and reversed-phase HPLC, we determined half-lives of < 2 min for the 2′-*O*-tom-protected N^4-acetylcytidine and N^6-acetyladenosine derivatives, and a half-life of 4 min for the 2′-*O*-tom-protected N^2-acetylguanosine, respectively (*Scheme 3*). These values indicated that complete base-deprotection of oligonucleotide sequences can be achieved within 1 h (equal to 15 half-lives for the acetylated guanosine residues) and demonstrated that the Ac group, in combination with $MeNH_2$, is indeed suited for a labile universal nucleobase protection. Under the deprotection conditions with $MeNH_2$, the 2′-*O*-tom group was completely stable for a period of at least 48 h. Analogous model experiments for the removal of the 2′-*O*-tom protecting group were then carried out and the products analyzed by TLC, reversed-phase HPLC, and ^{1}H-NMR. With an excess of 1M $Bu_4NF \cdot 3\ H_2O$ in THF at 25°, the four deacetylated 2′-*O*-tom-protected ribonucleosides were deprotected completely and cleanly within < 5 min (*Scheme 3*).

Scheme 3. *Stepwise Deprotection of Nucleobase- and 2′-*O*-tom-Protected Ribonucleosides.* The reactions were followed by TLC, reversed-phase HPLC, and ^{1}H-NMR.



2.2. *Oligoribonucleotides.* Fortunately, already our first RNA syntheses with 2′-*O*-tom-protected phosphoramidites showed very good coupling yields and indicated a very clean and reliable deprotection process. Subsequently, we optimized all feasible reaction and deprotection parameters, to identify the scope of our new building blocks for RNA synthesis. First, we tested a variety of different known activators for the phosphoramidite coupling reaction; we found (by detritylation assay) the following

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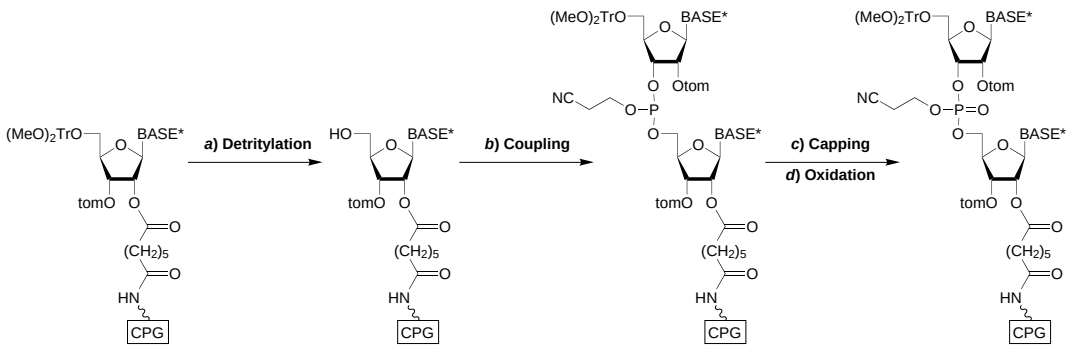
coupling-efficiency order: 5-(ethylthio)-1*H*-tetrazole ≈ 5-(methylthio)-1*H*-tetrazole > 5-(4-nitrophenyl)-1*H*-tetrazole > DCI (1*H*-imidazole-4,5-dicarbonitrile) > 1*H*-tetrazole. The two (alkylthio)tetrazoles clearly showed the best results (coupling yields ≈ 99%). Even better results were obtained with 5-(benzylthio)-1*H*-tetrazole (BTT) (synthesized according to [27]) as activator. This compound is slightly more acidic ($pK_a = 4.1$ [27]) than 5-(ethylthio)- and 5-(methylthio)-1*H*-tetrazole ($pK_a = 4.3$ [27]), crystallizes readily from MeCN/toluene, and allows the preparation of up to 0.4M solutions in MeCN.

Next, the amounts of phosphoramidites and the coupling times were optimized. We found that a minimal amount of 6 equiv. of phosphoramidites/1.5 min coupling time or of 2.5 equiv. of phosphoramidites/7 min coupling time on a 1.5-μmol or 10-μmol scale, respectively, were required to obtain reproducible coupling yields of > 99%; fewer equivalents or shorter coupling times resulted in lower yields, whereas more equivalents and/or longer coupling times did not enhance the coupling yields significantly. Finally, we established our coupling conditions for the routine assembly of RNA sequences with 2′-*O*-tom phosphoramidites, which are presented in *Scheme 4*. Additionally, we found that, with conventional CPG supports (containing succinate-linked nucleosides), the coupling yields within the first ten coupling reactions increased steadily from ≈ 95% to the average yields of > 99%. With our supports (containing heptanedioate-linked nucleosides, see *Scheme 1*), however, coupling yields > 99% were obtained from the beginning and independent of the sequence length.

Scheme 4. *Reagents and Conditions for the Assembly of RNA Sequences on a DNA Synthesizer*



a) Detritylation with 4% dichloroacetic acid in $ClCH_2CH_2Cl$ (1.5-μmol scale: 1.5 min; 10-μmol scale: 4 min). *b*) Coupling with 0.1M phosphoramidite in MeCN, promoted by 0.25M BTT in MeCN (1.5-μmol scale: 0.1 ml + 0.4 ml, 2.5 min; 10-μmol scale: 0.4 ml + 0.6 ml, 7 min). *c*) Capping with Ac_2O/2,6-lutidine (= 2,6-dimethylpyridine)/THF 1:1:8 and 16% (*v*/*v*) 1-methyl-1*H*-imidazole/THF in a 1:1 ratio (1.5-μmol scale: 1 min; 10-μmol scale: 3 min). *d*) Oxidation with I_2/H_2O/Py/THF 3:2:20:75 (1.5-μmol scale: 0.7 min; 10-μmol scale: 2.5 min).

Under the conditions shown in *Scheme 4*, stable and reproducible individual coupling yields between 99.0 and 99.9% (1.5- and 10-μmol scale, resp.) were obtained; the average value (determined from a number of meanwhile > 50000 couplings) was 99.4%. Under identical conditions, commercially available 2′-*O*-methyl-RNA and DNA phosphoramidites exhibited similar average coupling yields of ≈ 99.5 and ≈ 99.7%, respectively. In *Fig. 2*, the anion-exchange (= AE) HPLC of a crude 47mer

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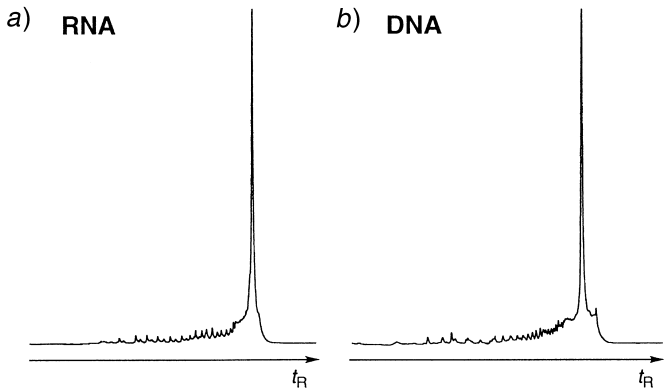

Fig. 2. *AE-HPLC Traces* a) *of the oligoribonucleotide r(CAGXAGXXCCAAXCGACCXGXCGACGGX-CGGXCGCCGAGGXGCGXAA) and* b) *of the corresponding oligodeoxyribonucleotide* (47mers), *assembled under identical conditions* (see *Scheme 4*). The DNA sequence (X = T) was prepared from commercial DNA phosphoramidites, and the RNA sequence (X = U) was prepared from the 2′-*O*-tom-protected phosphoramidites **18**–**21**. For AE-HPLC conditions, see *Exper. Part* (*A* → *A*/*B* 1:4 within 60 min); UV detection at 260 nm.

RNA oligonucleotide (prepared under the conditions of *Scheme 4*) is shown, together with the trace of a crude 47mer DNA oligonucleotide of the same sequence and prepared under identical coupling conditions. The similarity of the two traces indicates a similar reaction course and, specifically, comparable coupling efficiencies achieved with DNA and 2′-*O*-tom-protected RNA phosphoramidites.

To establish the deprotection conditions, model experiments similar to those described for the nucleosides in *Scheme 3* were carried out with oligoribonucleotides (see *Scheme 5*). In *Fig. 3*, reversed-phase HPLC traces from a crude 18mer RNA sequence, obtained under different deprotection conditions are presented. The synthesis of this oligonucleotide was carried out on a 10-μmol scale according to *Scheme 4*. Aliquots of the solid support (≈1 μmol each) were then treated with 10M $MeNH_2$ in H_2O/EtOH 1:1 (0.5 ml) at 25°, the supernatants evaporated, and the residues treated with 1M $Bu_4NF \cdot 3\ H_2O$ in THF (0.5 ml) at 25°. After quenching with 1M *Tris* · HCl buffer (pH 7.4; 0.5 ml) and desalting (see *Exper. Part*), the crude samples were analyzed by reversed-phase HPLC. Already under short-time deprotection conditions, *i.e.* 1 h $MeNH_2$/1 h Bu_4NF, quantitative removal of all protecting groups and formation of a uniform product was observed (*Fig. 3*, center); importantly, identical results were obtained after prolonged treatment with $MeNH_2$ for 24 h (*Fig. 3*, left) or with Bu_4NF for 50 h (*Fig. 3*, right), respectively.

The proposed mechanism of the fluoride-induced removal of a tom protecting group is shown in *Scheme 5*. Attack of F^- at the Si-atom results in cleavage of the Si–O bond and formation of a formaldehyde hemiacetal anion. During the deprotection reaction or upon addition of aqueous buffer solution, this intermediate then undergoes fragmentation to the ribonucleoside moiety and formaldehyde.

We later found that some 2′-*O*-tom-protected oligonucleotides, specifically adenosine-rich sequences and DNA/RNA hybrid sequences, were only slightly soluble in THF. Still, they could be deprotected with 1M $Bu_4NF \cdot 3\ H_2O$ or $Et_4NF \cdot 2\ H_2O$ in

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Scheme 5. *Stepwise Deprotection of the RNA Sequence r(GUAUCGAGCCUACG) with Variation of the Reaction Times.* The reactions were followed by reversed-phase HPLC (see *Fig. 3*)

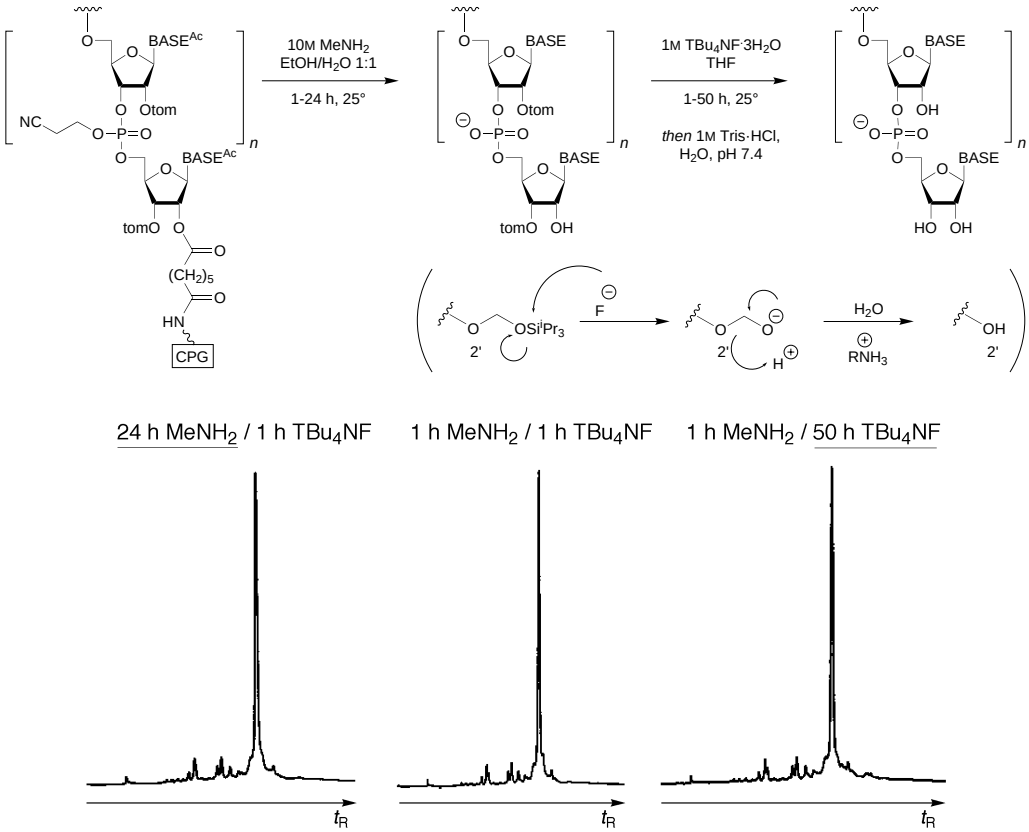

Fig. 3. *Reversed-phase HPLC traces of the crude RNA sequence r(GUAUCGAGCCUACG)* (18mer), *assembled under standard conditions* (see *Scheme 4*; 10-µmol scale) *and deprotected according to the conditions given in the* Table, *but with different deprotection times.* For reversed-phase HPLC conditions, see *Exper. Part* ($A \rightarrow A/B$ 7:3 within 30 min); UV detection at 260 nm.

DMSO, DMF, and NMP (1-methylpyrrolidin-2-one), or mixtures of these solvents with THF (often the addition of a few drops of one of the above-mentioned polar solvents to the THF deprotection solution was sufficient to solubilize the oligonucleotide). Even the addition of up to 20% H_2O to the Bu_4NF/THF deprotection solution still led to a quantitative removal of the 2′-*O*-tom groups within a few hours. As an illustration, the almost identical capillary-electrophoresis (CE) traces of a crude 69mer RNA sequence (containing one DNA nucleoside), deprotected under standard conditions (1M $Bu_4NF \cdot 3\ H_2O$, 25°, 14 h) in THF or THF/H_2O 4:1 are presented in *Fig. 4*.

Meanwhile, we have developed robust deprotection conditions, which are listed in the *Table*. Depending on the synthesis scale and the length of the product sequence, the minimal amount of deprotection solution, the reaction temperature, and the reaction time were varied. Following these empirically determined conditions, we never

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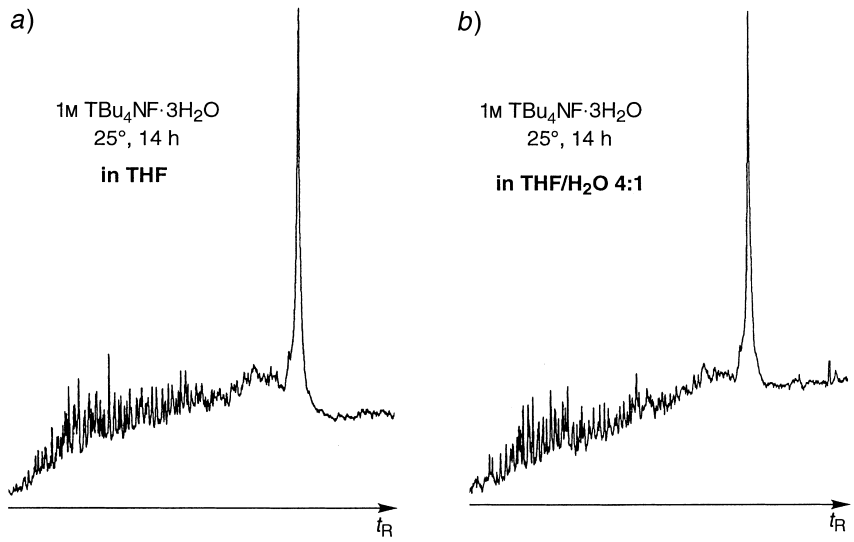

Fig. 4. *Capillary electrophoresis of the crude sequence r(GCGC)dAr(GUACUGUUUCUCAAACAGC-GAAGCGCGCCAGGGAAACACACCAUGUGUGGUAUAUUAUCUGGCA)* (69mer), *assembled under standard conditions* (see *Scheme 4) and deprotected first with 10*M *$MeNH_2$* (H_2O/EtOH 1:1, 25°, 6 h), *followed by* 1M *$Bu_4NF \cdot 3\ H_2O$* (25°, 14 h) a) *in THF and* b) *in THF/H_2O 4:1*. For CE conditions, see *Exper. Part*; UV detection at 260 nm.

Table. *Minimal Deprotection Conditions Depending on the Synthesis Scale and on the Sequence Length, for RNA Sequences Prepared from* N-*Acetylated, 2′*-O-*tom-Protected Building Blocks.* For further details, see *Exper. Part*.

Deprotection conditions	1.5-µmol Scale			10-µmol Scale		
	< 20mer	< 50mer	> 50mer	< 20mer	< 50mer	> 50mer
1) 10M $MeNH_2$ ($EtOH/H_2O$ 1:1)	0.5 ml, 25°, 3 h	1 ml, 35°, 4 h	1.5 ml, 35°, 6 h	4 ml, 25°, 4 h	4 ml, 35°, 5 h	5 ml, 35°, 6 h
2) 1M $Bu_4NF \cdot 3\ H_2O$ (THF)	0.5 ml, 25°, 5 h	1 ml, 25°, 10 h	1.5 ml, 30°, 14 h	3 ml, 25°, 5 h	4 ml, 25°, 10 h	5 ml, 30°, 14 h

observed indications of incomplete deprotection and/or for concomitant strand scission.

After the second deprotection process with Bu_4NF, the reactions were quenched by addition of an equal volume of 1M *Tris* · HCl buffer (pH 7.4). Subsequently, the excess Bu_4NF was removed by chromatography on *Sephadex G-10* or on *NAP* cartridges (*Pharmacia*). The thus obtained crude products were isolated either by evaporation, by precipitation with aqueous NaOAc solution/EtOH or by purification by HPLC polyacrylamide-gel electrophoresis (PAGE). In *Fig. 5*, the CE trace of a crude 84mer sequence and, in *Fig. 6*, the PAGE analysis of five crude products with sequence lengths between 81 and 40 are presented (all products were synthesized on a 1.5-µmol scale). Depending on the sequence length, overall coupling yields of 60–80% were obtained in these syntheses (trityl assay). The amounts of crude products ranged from 250 to 600 OD_{260} (8–19 mg). In all examples, the main product corresponded to the

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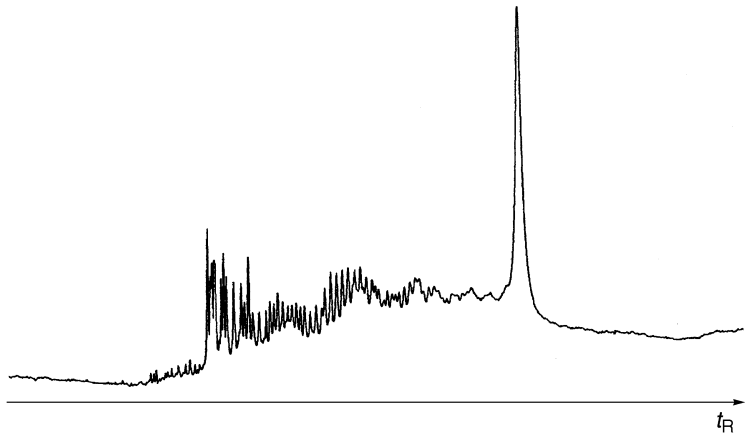

Fig. 5. *Capillary electrophoresis of the crude RNA sequence r(GGCCGGCAUGGUCCCAGCCUCCUCG-CUGGCGCCGGCUGGGCAACAUUCCGAGGGGACCGUCCCCUCGGUAAUGGGCGAAUGGAC)* (84mer), *assembled and deprotected under standard conditions* (see *Scheme 4* and *Table*). For CE conditions, see *Exper. Part*; UV detection at 260 nm.

completely deprotected full-length sequence. After purification of these crude products by prep. gel electrophoresis or anion-exchange HPLC, 50–120 OD_{260} (2–4 mg) of purified product sequences were obtained, which is equivalent to an overall yield of 20–40%.

The identity of the product RNA sequences was determined by various methods. Purified products (up to a sequence length of 40) were routinely characterized by MALDI-TOF mass spectrometry. In all such analyses, the products exhibited the correct mass, indicating the correct sequence composition and the absence of protecting groups. Several MS data of RNA sequences prepared from 2′-*O*-tom phosphoramidites are published [13][18–21][28]. In *Fig. 7*, the MALDI-TOF mass spectrum (positive mode) of a 35mer RNA sequence is presented. The sharp signals at *m/z* 11254 and 5628 amu agree very well with the calculated mass of 11255 amu for $[M+H]^+$ and 5627 amu for $[M+2\,H]^{2+}$, respectively.

We routinely carried out enzymatic digestion experiments of crude and purified RNA sequences according to [30]. The phosphodiesters were cleaved with nuclease P1 (which cleaves only natural 3′ → 5′ phosphodiester bonds [30][31]), and the resulting nucleotides were subsequently dephosphorylated with alkaline phosphatase. In *Fig. 8*, the reversed-phase HPLC traces of the products of such experiments, performed with the five crude RNA sequences from *Fig. 6*, are presented. In all examples, only the peaks corresponding to the four ribonucleosides are visible. This result indicates first the complete absence of any unnatural connections, such as 2′ → 5′ phosphodiester linkages and branched sequences, second the complete removal of all sugar and nucleobase protecting groups, and third the full integrity of the nucleosides during the phosphoramidite synthesis, the sequence assembly, and the two deprotection reactions.

Additionally, some functional and biologically active RNA sequences prepared from 2′-*O*-tom-protected phosphoramidites have been reported in the literature. The synthesis and pairing properties of RNA analogues containing flexible linkers was

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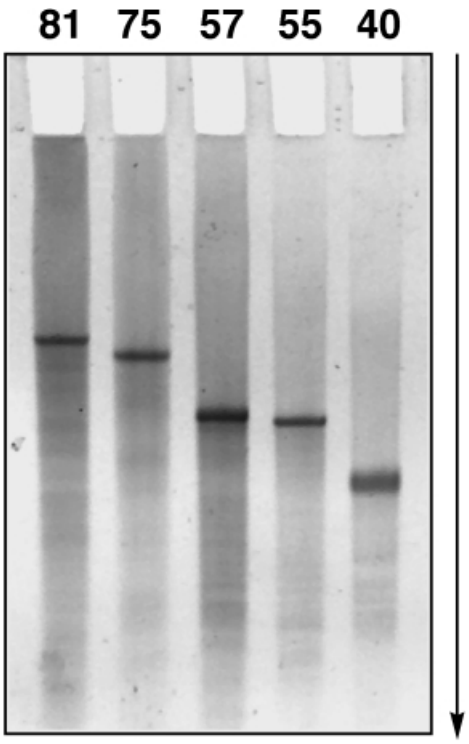

Fig. 6. *PAGE* (ethidium-bromide staining) *of five crude products, assembled and deproteced under standard conditions* (see *Scheme 4* and *Table*): *r(CCAAUCAACCUAAAACUUACACACUACAGACAUUG-CACGGCGCUGUCUGGGACGUACUAAAACAGCAUCAACGUGACACUU)* (81mer), *r(GCUCCUAUA-GUGUAGCGGUUAUCACCUCGGACUCUGAAUCCGAAACCCUGGUUCGAGUCCAGGUAGGAGUG-CCA)* (75mer), *r(CCAAAAUCAACCUAAAACUUACACACCGUACAGACAUUGCACCUGGCGCUGU-CUGGG)* (57mer), *r(CUACUUAGUGGUUCAGCUACAAUUCUUCUCCUUAACUACACCACAAUGC-AAGCCC)* (55mer), *and r(GUAAUGAUGGCAGGGCGUACCGAUAUACGAACAACAUUAA)* (40mer). PAGE conditions: see *Exper. Part*.

reported by *Micura* and co-workers [32–35], ribozyme activity was demonstrated by us [13], binding studies and NMR experiments of a RNA-protein complex were carried out by *Huenges et al.* [36], RNase P mediated cleavage of a hairpin-loop structure was investigated by *Brännvall et al.* [37], and investigations concerning the aminoacylation activity and specificity were carried out by *Nagan et al.* [38].

3. Discussion. – The tom group displays some unique properties, which render it a very valuable 2′-*O*-protecting group for the chemical synthesis of oligoribonucleotides. It is completely stable under all reaction conditions required for the assembly and the deprotection of RNA sequences. The excellent stability of the tom group, towards both strongly acidic conditions (employed during the detritylation reactions) and strongly basic conditions (employed for the deprotection of the nucleobase-protecting groups) is a consequence of the sterically very hindered triisopropylsilyl moiety. To the contrary,

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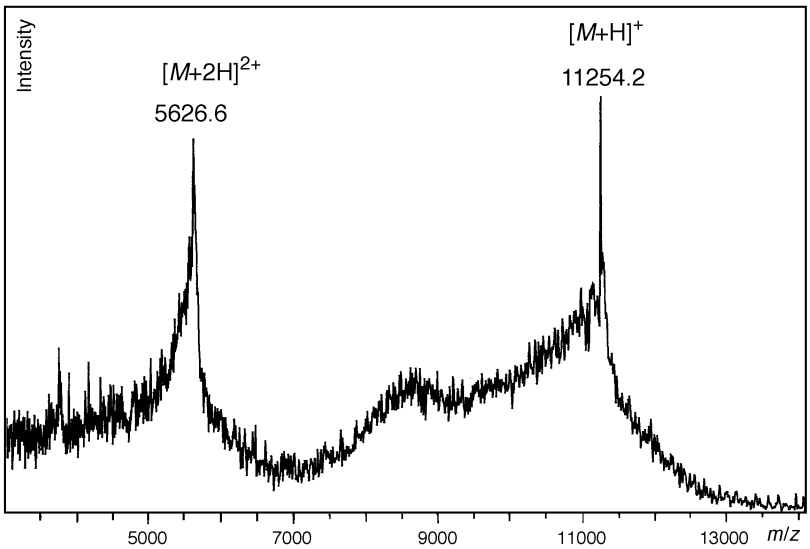

Fig. 7. *MALDI-TOF-MS* (positive mode) *of the sequence r(GGUCGCCGAGGUGCGUAAAUGCUAUG-GUAUCCGUU)* (35mer), *after purification by AE-HPLC.* $[M+H]^+$, calc. at *m/z* 11255 amu; $[M+2H]^{2+}$, calc. at *m/z* 5628 amu. MS measured according to [29].

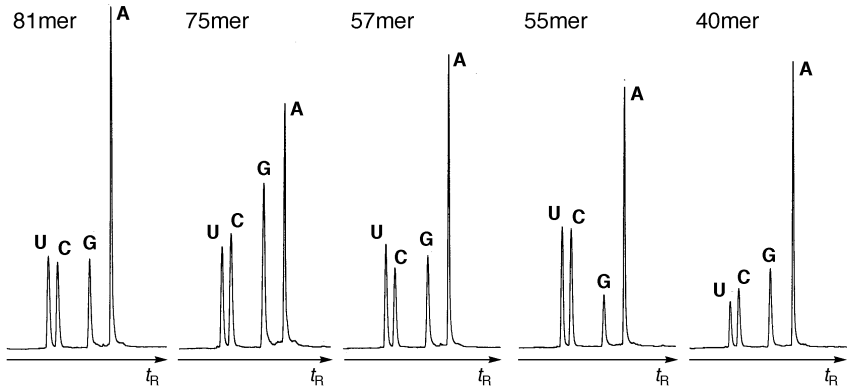

Fig. 8. *Reversed-phase HPLC of the products obtained by digestion of the five crude RNA sequences from* Fig. 6 *with nuclease P1/bacterial alkaline phosphatase* (according to [30]). A = adenosine, C = cytidine, G = guanosine, U = uridine. For reversed-phase HPLC conditions, see *Exper. Part* (*A*/*C* 96 : 4 → *A*/*C* 93 : 7 within 6 min, then → *A*/*B*/*C* 87 : 6 : 7 within 4 min, then → *A*/*B*/*C* 81 : 12 : 7 within 20 min)

the tom group is very labile towards Bu_4NF and is completely removed, even in the presence of up to 20% H_2O. Complete removal of the tbdms group, in contrast, occurs only in the absence of H_2O, and requires drying of the Bu_4NF solution with molecular sieves [39]. This difference in the reactivity of the two protecting groups can be attributed to the nature of the leaving groups. The deprotonated formaldehyde hemiacetals, which are formed upon cleaving the tom groups (see *Scheme 5*), have pK_a

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values of *ca*. 10.5 and are, therefore, much better leaving groups then the alcoholates, formed upon cleaving the tbdms groups. We have indications that, during the deprotection of the 2′-*O*-tom groups with Bu_4NF, some formaldehyde hemiacetal groups remain attached to the 2′-*O* positions; during the aqueous workup with *Tris* buffer, however, they are removed immediately and completely.

Many interesting RNA sequences, such as t-RNAs, ribozymes, and aptamers consist of 60 to 80 nucleotides. To obtain a reasonable overall coupling yield of $\approx 50\%$, an individual coupling yield of $\approx 99.3\%$ must be achieved during their assembly. Lower yields result not only in small product quantities, but also in complex crude products, which are often very difficult to purify to homogeneity. The average coupling yields obtained with 2′-*O*-tom phosphoramidites are in the range of $\approx 99.5\%$, and, therefore, even relatively long RNA sequences can be prepared routinely. These excellent coupling yields are most probably the consequence of the minimal steric demand of the tom protecting group, obtained by connecting the fluoride-labile triisopropylsilyl group and the nucleoside by a small formaldehyde acetal linker.

In addition to hgh coupling yields, a reliable deprotection scheme is crucial for the efficient synthesis of longer sequences. Our combination of protecting groups allows, for both required deprotection processes, reaction times that correspond to more than 100 individual half-lives (with respect to the removal of one protecting group) without destruction of the oligonucleotide product. Therefore, it is possible to carry out the deprotection reactions even for extremely prolonged periods of time, assuring complete deprotection without risking concomitant destruction of the product.

The separation of the two regioisomeric tom-substituted ribonucleosides, formed during the dibutyltin-mediated alkylation reaction, is straightforward and leads to uniform products. In contrast to the tbdms protecting group, the tom group does not migrate from the 2′-*O* to the 3′-*O* position, even under strongly basic conditions. These factors allow the routine preparation of very pure phosphoramidites, which hence result in the exclusive formation of $3' \rightarrow 5'$ phosphodiester moieties. Furthermore, due to the stability of the 2′-*O*-tom group, a variety of nucleobase and sugar manipulations can be carried out after its introduction. Therefore, we were able to carry out short and convergent syntheses of ribonucleoside analogues [18][19] and of building blocks with different nucleobase-protecting groups [21].

The chemical synthesis of oligonucleotides allows the more or less unrestricted incorporation of nucleobase, sugar, and backbone modifications, the preparation of hybrid sequences, and the labeling with specific reporter groups. By having adapted our RNA chemistry to the established DNA chemistry, the 2′-*O*-tom-protected building blocks can be combined with all of these modifications and even with 2′-*O*-tbdms-protected RNA phosphoramidites [28].

This work was supported by the ETH Zürich.

Experimental Part

General. Reagents and solvents (Py = pyridine) from *Fluka*, enzymes from *Sigma*, unless otherwise stated; DNA phosphoramidites and supports from *Glen Research*; 5-(benzylthio)-1*H*-tetrazole (BTT) was synthesized according to [27]. Workup implies partitioning 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

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by dipping into a soln. of anisaldehyde (10 ml), conc. H_2SO_4 soln. (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*. Reversed-phase HPLC: *LiChrosphere 100 RP-18* (4.0 × 250 mm; *Merck*), flow 1 ml/min, elution at 40°; eluent *A* 0.1M (Et_3NH)OAc in H_2O (pH 7), eluent *B* MeCN, eluent *C* MeOH; detection at 260 nm. Anion-exchange (AE) HPLC: *DNAPAC PA-100* (4 × 250 nm; *Dionex*), flow 0.75 ml/min; eluent *A:* 2 mM *Tris* · HCl (pH 7.4), 10 mM $NaClO_4$, 6M urea; eluent *B:* 2 mM *Tris* · HCl (pH 7.4), 0.55M $NaClO_4$, 6M urea; detection at 260 nm, elution at 80°. Capillary electrophoresis (CE): *BioFocus 3000* (*Biorad*) with coated *BioCap-XL* column (75 μm × 40 cm), 'run buffer' (No. 184-5026) + 6M urea, injection with pressure, elution with 15 kV at 40°, detection at 260 nm. Polyacrylamide-gel electrophoresis (PAGE): 15% acrylamide (acrylamide/bisacrylamide 29:1), 0.75 × 100 mm, 90 mM *Tris* · borate (pH 8.3), 7M urea, 2 mM EDTA, elution at 200 V, stained with ethidium bromide. Optical rotation ($[\alpha]^{25}_{D}$): *Jasco DIP-370*, *c* in g/100 ml. UV Spectra: *Uvikon 931*; λ_{max} in nm, ε in dm^3/mol/cm; all measurements at 25°. IR Spectra: $\tilde{\nu}$ in cm^{-1}. NMR: chemical shift δ in ppm, coupling constants *J* in Hz. MS: *VG-ZAB2-SEQ*; FAB pos. mode; 3-nitrobenzyl alcohol as matrix; m/z (rel. %).

Oligonucleotide Synthesis and Deprotection. The oligonucleotides were assembled on a *Pharmacia Gene Assembler Plus* under the conditions given in *Scheme 4*. HPLC-Grade MeCN was dried by refluxing in the presence of CaH_2 (24 h). Prior to the assembly, the phosphoramidite solns. (0.1M in MeCN), the BTT soln. (0.25M in MeCN), and the MeCN were stored over 4-Å molecular sieves for 14 h. CPG Supports with a pore size of 500 and 1000 Å were used for the synthesis of ≤47mers and >47mers, respectively. All syntheses were carried out in the 'trityl-off' mode.

After the assembly, the solid supports were removed from the cartridges and treated with an equal volume of 12M $MeNH_2$ in H_2O and 8M $MeNH_2$ in EtOH according to the conditions given in the *Table*. By centrifugation, the supernatant solns. were separated from the solid supports, evaporated and treated with 1M $Bu_4NF \cdot 3\,H_2O$ in THF according to the conditions given in the *Table*. Upon vortexing, the 2′-*O*-tom-protected oligonucleotide intermediates dissolved immediately. The reactions were quenched by addition of an equal volume of 1M *Tris* · HCl buffer (pH 7.4), and the products were desalted by chromatography (*Sephadex G-10*, 1.5 × 25 cm, H_2O, 1 ml/min) or on *NAP* columns (*Pharmacia*) according to the manufacturer's instructions. The UV-absorbing fractions were pooled, evaporated, and precipitated with 0.3M aq. NaOAc/EtOH 1:8.

N^6-*Acetyladenosine* (**2**). At 4°, Me_3SiCl (473 ml, 3.7 mol) was added dropwise to a suspension of adenosine (100 g, 0.37 mol) in Py (750 ml). The suspension was stirred for 14 h at r.t., diluted with MeCN (1.1 l), cooled to 4°, and treated with AcCl (40 ml, 0.56 mol). After 1 h, the mixture was diluted with CH_2Cl_2 (2.2 l) and added under stirring to H_2O (1.5 l, 4°). The org. phase was washed with H_2O (2 l, 4°), dried ($MgSO_4$), evaporated, and co-evaporated with toluene until all Py was removed. The residue was dissolved in MeOH (750 ml) and treated with AcOH (225 ml). Filtration after 2 d at −20° gave **2** (91 g, 80%). Off-white powder. TLC (CH_2Cl_2/MeOH 7:3): R_f 0.50. M.p. 173–175°. $[\alpha]^{25}_{D} = -53.7$ ($c = 0.98$, H_2O/MeOH 1:1). UV (H_2O): 273 (16700), 232 (3600), 209 (21700). IR (KBr): 3270*m*, 2935*w*, 1720*m*, 1685*s*, 1620*s*, 1590*m*, 1535*w*, 1465*s*, 1415*w*, 1375*m*, 1360*m*, 1310*s*, 1250*m*, 1225*m*, 1190*w*, 1130*w*, 1100*m*, 1085*m*, 1040*m*, 1010*w*, 990*w*, 895*w*, 820*w*. ^{1}H-NMR (300 MHz, CD_3OD/D_2O 1:1): 2.36 (*s*, MeCO); 3.80 (*dd*, *J* = 3.3, 12.6, H−C(5′)); 3.92 (*dd*, *J* = 2.8, 12.6, H′−C(5′)); 4.23 (br. *q*, *J* ≈ 3, H−C(4′)); 4.39 (*dd*, *J* = 3.4, 5.5, H−C(3′)); 4.77 (*t*, *J* = 5.6, H−C(2′)); 6.10 (*d*, *J* = 5.9, H−C(1′)); 8.58, 8.65 (2*s*, H−C(2), H−C(8)). ^{13}C-NMR (75 MHz, CD_3OD/D_2O 1:1): 24.9 (*q*, *Me*CO); 63.2 (*t*, C(5′)); 72.4, 75.7, 87.8 (3*d*, C(2′), C(3′), C(4′)); 90.8 (*d*, C(1′)); 125.1 (*s*, C(5)); 145.3 (*d*, C(8)); 150.9 (*s*, C(4)); 153.0 (*s*, C(6)); 153.5 (*d*, C(2)); 173.4 (*s*, CO). FAB-MS: 310 (100, $[M+H]^+$), 178 (55), 120 (24), 107 (40). Anal. calc. for $C_{12}H_{15}N_5O_5 \cdot 0.25\,H_2O$: C 45.93, H 4.98, N 22.31; found: C 46.00, H 4.87, N 22.23.

N^2-*Acetylguanosine* (**3**). A mixture of guanosine (56.6 g, 0.2 mol) in DMF/Py/Ac_2O (250 ml each) was heated under reflux for 3 h. All liquids were removed by distillation (100°/30 mbar), and the residue was dissolved in CH_2Cl_2 (400 ml). After extraction (1M HCl, then sat. $NaHCO_3$ soln.) and drying ($MgSO_4$), the intermediate (N^2, 2′-*O*,3′-*O*,5′-*O*-tetraacetylguanosine) was adsorbed on SiO_2 (150 g). After CC (150 g of SiO_2, $CH_2Cl_2 \rightarrow CH_2Cl_2$/MeOH 9:1), the intermediate was dissolved in THF/MeOH/H_2O 10:8:7 and treated with 10M aq. NaOH (20 ml). After 20 min at r.t., AcOH (25 ml) was added and the soln. concentrated to 400 ml and cooled to 4°. Filtration gave **3** (50.5 g, 77%). Off-white powder. TLC (CH_2Cl_2/MeOH 7:3): R_f 0.30. M.p. 224–226°. $[\alpha]^{25}_{D} = -19.0$ ($c = 0.94$, H_2O). UV (H_2O): 280 (sh, 11200), 259 (16300), 228 (3800). IR (KBr): 3465*m*, 3190*m*, 2120*m*, 2930*m*, 1690*s*, 1610*s*, 1565*s*, 1480*m*, 1445*w*, 1405*s*, 1370*m*, 1315*w*, 1265*m*, 1235*s*, 1205*w*, 1155*w*, 1120*w*, 1100*s*, 1065*m*, 1050*m*, 1035*m*, 1025*m*, 1000*w*, 945*w*, 890*w*, 875*w*, 800*m*. ^{1}H-NMR (300 MHz, D_2O): 2.28 (*s*, MeCO); 3.83 (*dd*, *J* = 4.0, 12.6, H−C(5′)); 3.92 (*dd*, *J* = 1.0, 12.6, H′−C(5′)); 4.21 (*m*, H−C(4′)); 4.45 (*t*, *J* = 4.7, H−C(3′)); 4.72 (*t*, *J* = 4.6, H−C(2′)); 5.97 (*d*, *J* = 4.4, H−C(1′)); 8.18 (*s*, H−C(8)). ^{13}C-NMR (75 MHz, D_2O): 26.2 (*q*, *Me*CO); 64.0 (*t*, C(5′)); 72.9, 76.8, 87.8 (3*d*, C(2′), C(3′), C(4′)); 90.9 (*d*, C(1′)); 122.9 (*s*, C(5));

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142.5 (*d*, C(8)); 150.6 (*s*, C(4)); 152.1 (*s*, C(2)); 160.2 (*s*, C(6)); 178.3 (*s*, CO). FAB-MS: 326 (100, $[M+H]^+$). Anal. calc. for $C_{12}H_{15}N_5O_6 \cdot 0.33\ H_2O$: C 43.51, H 4.77, N 21.14; found: C 43.65, H 5.14, N 21.16.

N^4-*Acetyl-5′-O-(4,4′-dimethoxytrityl)cytidine* (**4**). A suspension of cytidine (121.6 g, 0.5 mol) in DMF (1 l) was treated with Ac_2O (52 ml, 0.55 mol) and stirred for 14 h at r.t. The soln. was concentrated (45°/1 mbar) to 100 ml, diluted with Py (900 ml), treated in 4 portions with $(MeO)_2TrCl$ (187 g, 0.55 mol), and stirred for 1 h at r.t. After workup and evaporation (first at 30 mbar, then at 1 mbar), the residue was crystallized from MeOH: **4** (205 g, 70%). White powder. TLC (CH_2Cl_2/MeOH 9:1): R_f 0.50. $[\alpha]^{25}_D = +26.1$ ($c = 0.88$, $CHCl_3$). UV (MeOH): 299 (14800), 272 (8000), 247 (30200), 227 (12000), 212 (42600). IR ($CHCl_3$): 3510*w*, 3390*m*, 3000*m*, 2940*w*, 2820*w*, 1740*m*, 1640*s*, 1600*s*, 1550*m*, 1500*s*, 1480*s*, 1180*m*, 1100*m*, 1100*s*, 1030*s*. ^{1}H-NMR (300 MHz, $CDCl_3$): 2.24 (*s*, MeCO); 3.42 (*dd*, $J = 2.8$, 10.9, H–C(5′)); 3.48 (*dd*, $J = 2.5$, 10.9, H′–C(5′)); 3.54 (br. *s*, OH); 3.79 (*s*, 2 MeO); 4.39–4.43 (*m*, H–C(2′), H–C(3′), H–C(4′)); 5.67 (br. *s*, OH); 5.87 (*d*, $J = 3.7$, H–C(1′)); 6.80–6.86 (*m*, 4 arom. H); 7.20–7.62 (*m*, 10 H, arom. H, H–C(5)); 8.18 (*d*, $J = 7.5$, H–C(6)); 8.80 (br. *s*, NH–C(4)). ^{13}C-NMR (75 MHz, $CDCl_3$): 25.0 (*q*, *Me*CO); 55.3 (*q*, MeO); 62.6 (*t*, C(5′)); 71.1, 76.9, 85.3 (3*d*, C(2′), C(3′), C(4′)); 87.2 (*s*, $(MeO)_2$*Tr*); 93.0 (*d*, C(1′)); 97.0 (*d*, C(5)); 113.5 (*d*, arom. C); 127.3, 128.2, 128.3, 130.2, 130.3 (5*d*, arom. C); 135.4 (*d*, arom. C); 135.7 (*s*, arom. C); 145.0 (*d*, C(6)); 156.9 (*s*, C(2)); 159.0 (*s*, arom. C); 162.9 (*s*, C(4)); 170.7 (*s*, CO). FAB-MS: 588 (25, $[M+H]^+$), 303 (100, $[(MeO)_2Tr]^+$).

N^6-*Acetyl-5′-O-(4,4′-dimethoxytrityl)adenosine* (**5**). A soln. of **2** (77 g, 0.25 mol) in Py (800 ml) was treated in 4 portions with $(MeO)_2TrCl$ (102 g, 0.30 mol) and kept for 1 h at r.t. Workup and CC (400 g of SiO_2, $CH_2Cl_2 \rightarrow CH_2Cl_2$/MeOH 9:1 (+1% Et_3N)) gave **5** (114 g, 75%). Yellow, solid foam. TLC (CH_2Cl_2/MeOH 9:1): R_f 0.50. $[\alpha]^{25}_D = -7.4$ ($c = 0.87$, $CHCl_3$). UV (MeOH): 272 (18700), 252 (13000), 234 (22700), 228 (22100). IR ($CHCl_3$): 3700*w*, 3540*w*, 3405*w*, 3380*w*, 3000*m*, 2940*w*, 2840*w*, 1740*w*, 1710*m*, 1620*s*, 1590*m*, 1520*s*, 1470*m*, 1460*m*, 1410*w*, 1380*m*, 1300*m*, 1130*m*, 1040*m*, 910*m*. ^{1}H-NMR (300 MHz, $CDCl_3$): 2.58 (*s*, MeCO); 3.31 (*dd*, $J =$ 3.4, 10.6, H–C(5′)); 3.44 (*dd*, $J = 3.1$, 10.6, H′–C(5′)); 3.53 (br. *s*, OH); 3.75 (*s*, 2 MeO); 4.43 (br. *q*, $J \approx 2.5$, H–C(4′)); 4.49 (*dd*, $J = 2.5$, 5.3, H–C(3′)); 4.88 (*t*, $J = 5.3$, H–C(2′)); 5.74 (br. *s*, OH); 6.05 (*d*, $J = 5.6$, H–C(1′)); 6.72–6.75 (*m*, 4 arom. H); 7.16–7.29 (*m*, 9 arom. H); 8.23 (*s*, H–C(2)); 8.60 (*s*, H–C(8)); 8.94 (br. *s*, NH–C(6)). ^{13}C-NMR (75 MHz, $CDCl_3$): 25.7 (*q*, *Me*CO); 55.3 (*q*, MeO); 63.7 (*t*, C(5′)); 72.7, 76.0, 86.1 (3*d*, C(2′), C(3′), C(4′)); 86.8 (*s*, arom. C); 90.7 (*d*, C(1′)); 113.4 (*d*, arom. C); 122.2 (*s*, C(5)); 127.2, 128.1, 128.2, 130.1, 130.2 (5 arom. C); 135.7 (*s*, arom. C); 141.7 (*d*, C(8)); 144.6 (*s*, arom. C); 149.6 (*s*, C(4)); 150.8 (*s*, C(6)); 152.2 (*d*, C(2)); 158.9 (*s*, arom. C); 170.7 (*s*, CO). FAB-MS: 612 (17, $[M+H]^+$), 303 (100, $[(MeO)_2Tr]^+$), 136 (26), 102 (83).

N^2-*Acetyl-5′-O-(4,4′-dimethoxytrityl)guanosine* (**6**). A suspension of **3** (42.3 g, 0.13 mol) in Py (390 ml), DMF (130 ml), and 4-Å molecular sieves (50 g) was stirred for 1 h at r.t. Then $(MeO)_2TrCl$ (49 g, 0.15 mol) was added in 4 portions, and the mixture was stirred for 1 h at r.t. After workup and evaporation (first at 30 mbar, then at 1 mbar), the residue was crystallized from CH_2Cl_2: **6** (65.7 g, 81%). Yellow powder. TLC (CH_2Cl_2/MeOH 9:1): R_f 0.35. M.p. 170–174° (dec.). $[\alpha]^{25}_D = +15.5$ ($c = 0.93$, MeOH). UV (MeOH): 282 (sh, 13700), 274 (14600), 271 (14300), 260 (sh, 18300), 251 (sh, 19600), 235 (25800), 226 (22400). IR (KBr): 3430*m*, 3155*m*, 3060*m*, 2935*m*, 2835*w*, 1675*s*, 1610*s*, 1560*s*, 1510*s*, 1475*m*, 1445*m*, 1405*w*, 1375*m*, 1300*w*, 1250*s*, 1175*m*, 1155*m*, 1120*m*, 1085*m*, 1035*m*, 905*w*, 830*m*. ^{1}H-NMR (300 MHz, $CDCl_3/CD_3OD$ 3:1): 2.02 (*s*, MeCO); 3.20 (*dd*, $J = 4.3$, 10.5, H–C(5′)); 3.26 (*dd*, $J = 2.8$, 10.5, H–C(5′)); 3.62 (*s*, 2 MeO); 3.69 (br. *s*, H–C(4′)); 4.22 (*t*, $J = 4.9$, H–C(3′)); 4.45 (*t*, $J = 4.9$, H–C(2′)); 5.76 (*d*, $J = 4.8$, H–C(1′)); 6.64 (*m*, 4 arom. H); 7.03–7.26 (*m*, 9 arom. H); 7.74 (*s*, H–C(8)). ^{13}C-NMR (75 MHz, $CDCl_3/CD_3OD$ 3:1): 23.4 (*q*, *Me*CO); 55.1 (*q*, MeO); 63.6 (*t*, C(5′)); 71.0, 74.8, 84.2 (3*d*, C(2′), C(3′), C(4′)); 86.6 (*d*, C(1′)); 88.6 (*s*, arom. C); 113.2 (*d*, arom. C); 120.8 (*s*, C(5)); 127.0, 127.9, 128.2, 130.1 (4 arom. C); 135.8 (*s*, arom. C); 137.8 (*d*, C(8)); 144.6 (*s*, arom. C); 147.9 (*s*, C(4)); 148.8 (*s*, C(2)); 156.2 (*s*, C(6)); 158.7 (*s*, arom. C); 173.3 (*s*, CO). FAB-MS: 628 (74, $[M+H]^+$), 303 (100, $[(MeO)_2Tr]^+$), 242 (20). Anal. calc. for $C_{32}H_{35}N_4O_8$: C 63.15, H 5.30, N 11.16; found: C 63.08, H 5.35, N 10.91.

5′-O-*(4,4′-Dimethoxytrityl)uridine* (**7**). A soln. of uridine (61 g, 0.25 mol) in Py (800 ml) was treated in 4 portions with $(MeO)_2TrCl$ (102 g, 0.3 mol) and kept for 1 h at r.t. Workup and CC (400 g of SiO_2, $CH_2Cl_2 \rightarrow CH_2Cl_2$/MeOH 9:1 (+1% Et_3N)) gave **7** (109 g, 80%). Yellow, solid foam. TLC (CH_2Cl_2/MeOH 9:1): R_f 0.50. $[\alpha]^{25}_D = +2.4$ ($c = 0.92$, $CHCl_3$). UV (MeOH): 265 (11900), 255 (11400), 233 (23100), 227 (12800). IR ($CHCl_3$): 3700*w*, 3610*w*, 3540*w*, 3400*m*, 3320*w*, 3040*w*, 2930*w*, 2340*w*, 1705*s*, 1690*s*, 1610*m*, 1510*m*, 1470*m*, 1400*w*, 1300*w*, 1110*m*, 1070*w*, 1040*m*, 910*m*. ^{1}H-NMR (300 MHz, $CDCl_3$): 3.48 (*dd*, $J = 2.5$, 10.9, H–C(5′)); 3.53 (*dd*, $J = 2.2$, 10.9, H′–C(5′)); 3.78 (*s*, 2 MeO); 4.19 (*td*, $J = 2.3$, 5.5, H–C(4′)); 4.33 (*dd*, $J = 2.8$, 5.3, H–C(2′)); 4.42 (*t*, $J = 5.5$, H–C(3′)); 5.37 (*d*, $J = 8.1$, H–C(5)); 5.88 (*d*, $J = 2.5$, H–C(1′)); 6.82–6.87 (*m*, 4 arom. H); 7.23–7.40 (*m*, 9 arom. H); 8.00 (*d*, $J = 8.1$, H–C(6)). ^{13}C-NMR (75 MHz, $CDCl_3$): 55.3 (*q*, MeO); 62.2 (*t*, C(5′)); 70.1, 75.7, 84.0 (3*d*, C(2′), C(3′), C(4′)); 87.2 (*s*, arom. C); 90.5 (*d*, C(1′)); 102.4 (*d*, C(5)); 113.5 (*d*, arom. C); 128.2, 128.4, 128.6, 130.3, 130.4 (5*d*, arom. C); 135.5, 135.6 (2*s*, arom. C); 140.7 (*d*, C(6)); 144.7 (*s*, arom. C); 151.4

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(*s*, C(2)); 159.0 (*s*, arom. C); 164.0 (*s*, C(4)). FAB-MS: 547 (25, $[M+H]^+$), 546 (24, M^+), 303 (100, $[(MeO)_2Tr]^+$).

Triisopropylsilyl (Ethylthio)methyl Ether (= *[(Ethylthio)methoxy]tris(1-methylethyl)silane*; **8**). At 4°, a suspension of paraformaldehyde (30.6 g, 1.02 mol) in EtSH (75 ml, 1.02 mol) was treated with 10M aq. NaOH (1 drop) and slowly allowed to warm up. At ca. 15°, a violent exothermic reaction occurred. The resulting soln. was stirred for 1 h at 40°, cooled to r.t., and treated with CH_2Cl_2 (1 l), 1*H*-imidazole (138.6 g, 2.04 mol), and $^iPr_3SiCl$ (205 ml, 0.97 mol). The suspension was stirred for 14 h at r.t., diluted with hexane (2 l), and extracted with 10% aq. NaH_2PO_4 soln. (1 l). The org. layer was dried ($MgSO_4$) and evaporated. Distillation at 0.05 Torr gave **8** (206 g, 85%). Colorless liquid. B.p. 72°/0.05 Torr. ^{1}H-NMR (300 MHz, $CDCl_3$): 1.08–1.18 (*m*, $^iPr_3Si$); 1.31 (*t*, *J* = 7.4, *Me*CH_2); 2.71 (*q*, *J* = 7.4, MeCH_2); 4.88 (*s*, SCH_2O). ^{13}C-NMR (75 MHz, $CDCl_3$): 12.0 (*d*, Me_2CH); 15.0 (*q*, Me_2CH); 17.9 (*q*, *Me*CH_2); 24.7 (*t*, MeCH_2); 66.1 (*t*, SCH_2O).

[(Triisopropylsilyl)oxy]methyl Chloride (= *(Chloromethoxy)tris(1-methylethyl)silane*; tom-Cl; **9**). At 4°, a soln. of **8** (176 g, 0.7 mol) in CH_2Cl_2 (500 ml) was treated slowly with SO_2Cl_2 (57.4 ml, 0.7 mol). After the addition, the yellow soln. was stirred for 1 h at r.t. and then evaporated. Distillation at 0.01 Torr gave **9** (148 g, 95%). Colorless liquid. B.p. 40°/0.01 Torr. ^{1}H-NMR (300 MHz, $CDCl_3$): 1.08–1.10 (*m*, $^iPr_3Si$); 5.66 (*s*, SCH_2O). ^{13}C-NMR (75 MHz, $CDCl_3$): 11.8 (*d*, Me_2CH); 17.7 (*q*, Me_2CH); 76.6 (*t*, SCH_2O).

N^4*-Acetyl-5′-*O*-(4,4′-dimethoxytrityl)-2′-*O*- and* N^4*-Acetyl-5′-*O*-(4,4′-dimethoxytrityl-3′-*O*-{[(triisopropylsilyl)oxy]methyl}cytidine* (**10** and **14**, resp.). A soln. of **4** (147 g, 0.25 mol) and $^iPr_2NEt$ (146 ml, 0.88 mol) in $ClCH_2CH_2Cl$ (1 l) was treated with Bu_2SnCl_2 (94 g, 0.28 mmol), stirred for 1 h at r.t., heated to 80°, treated with **9** (72 g, 0.33 mol), and stirred for 20 min at 80°. Workup and CC (2 kg of SiO_2, hexane/AcOEt 7:3 → 3:7 (+1% Et_3N)) afforded **10** (87 g, 45%) and **14** (58 g, 30%) as pale yellow, solid foams. By continuing the CC with CH_2Cl_2 → CH_2Cl_2/MeOH 9:1 (+1% Et_3N), **4** (22 g, 15%) was recovered.

Data of **10**: TLC (hexane/AcOEt 3:7): R_f 0.50. $[\alpha]^{25}_D = +46.8$ (*c* = 0.94, $CHCl_3$). UV (MeOH): 300 (6800), 290 (6400), 283 (6800), 278 (6400), 236 (27200), 225 (24500). IR ($CHCl_3$): 3520*w*, 3400*w*, 3000*m*, 2940*m*, 2860*m*, 1740*m*, 1660*s*, 1610*m*, 1550*m*, 1510*s*, 1480*s*, 1380*m*, 1300*m*, 1100*m*, 1040*m*, 990*m*, 910*w*, 880*m*. ^{1}H-NMR (300 MHz, $CDCl_3$): 1.04–1.15 (*m*, $^iPr_3Si$); 2.21 (*s*, MeCO); 3.34 (*d*, *J* = 8.4, OH–C(3′)); 3.53 (*dd*, *J* = 2.5, 10.9, H–C(5′)); 3.62 (*dd*, *J* = 1.8, 10.9, H′–C(5′)); 3.81, 3.82 (2*s*, 2 MeO); 4.09 (br. *dt*, *J* ≈ 9, 2, H–C(4′)); 4.23 (*d*, *J* = 5.0, H–C(2′)); 4.37 (*m*, H–C(3′)); 5.15, 5.28 (2*d*, *J* = 4.6, OCH_2O); 5.97 (*s*, H–C(1′)); 6.84–6.88 (*m*, 4 arom. H); 7.04 (*d*, *J* = 7.1, H–C(5)); 7.24–7.44 (*m*, 9 arom. H); 8.48 (*d*, *J* = 7.4, H–C(6)); 8.62 (br. *s*, NH–C(4)). ^{13}C-NMR (75 MHz, $CDCl_3$): 11.9 (*d*, Me_2CH); 17.8 (*q*, Me_2CH); 24.9 (*q*, *Me*CO); 55.3 (*q*, MeO); 61.4 (*t*, C(5′)); 67.9, 83.50, 83.55 (3*d*, C(2′), C(3′), C(4′)); 87.2 (*s*, arom. C); 90.2 (*d*, C(1′)); 90.9 (*t*, OCH_2O); 96.8 (*d*, C(5)); 113.5 (*d*, arom. C); 127.4, 128.3, 128.4, 128.5, 130.4 (5*d*, arom. C); 135.6, 135.8, 144.6 (3*s*, arom. C); 145.1 (*d*, C(6)); 155.3 (*s*, C(2)); 159.0 (*s*, arom. C); 163.1 (*s*, C(4)); 170.6 (*s*, CO). FAB-MS: 774 (23, $[M+H]^+$), 730 (27), 303 (100, $[(MeO)_2Tr]^+$). Anal. calc. for $C_{42}H_{55}N_3O_9Si$: C 65.18, H 7.16, N 5.43; found: C 64.89, H 7.18, N 5.42.

Data of **14**: TLC (AcOEt): R_f 0.40. $[\alpha]^{25}_D = +16.7$ (*c* = 0.87, $CHCl_3$). UV (MeOH): 299 (7500), 290 (6700), 283 (7500), 272 (6700), 235 (29000), 25 (24800). IR ($CHCl_3$): 3540*w*, 3400*m*, 3000*m*, 2940*m*, 2860*m*, 1740*m*, 1670*s*, 1620*m*, 1550*m*, 1510*s*, 1480*s*, 1370*m*, 1300*m*, 1110*m*, 1040*s*, 880*w*. ^{1}H-NMR (300 MHz, $CDCl_3$): 1.02–1.06 (*m*, $^iPr_3Si$); 2.25 (*s*, MeCO); 3.39 (*dd*, *J* = 3.1, 10.9, H–C(5′)); 3.58 (br. *s*, OH–C(2′)); 3.60 (*dd*, *J* = 2.2, 10.9, H′–C(5′)); 3.81 (*s*, MeO); 4.27–4.38 (*m*, H–C(2′), H–C(3′), H–C(4′)); 4.90, 5.02 (2*d*, *J* = 5.0, OCH_2O); 5.99 (*d*, *J* = 2.5, H–C(1′)); 6.83–6.87 (*m*, 4 arom. H); 7.18 (*d*, *J* = 7.5, H–C(5)); 7.5–7.40 (*m*, 9 arom. H); 8.29 (*d*, *J* = 7.5, H–C(6)); 9.67 (br. *s*, NH–C(4)). ^{13}C-NMR (75 MHz, $CDCl_3$): 11.9 (*d*, Me_2CH); 17.8 (*q*, Me_2CH); 24.9 (*q*, *Me*CO); 55.3 (*q*, MeO); 62.1 (*t*, C(5′)); 75.8, 76.9, 82.5 (3*d*, C(2′), C(3′), C(4′)); 87.2 (*s*, arom. C); 92.6 (*d*, C(1′)); 90.4 (*t*, OCH_2O); 96.9 (*d*, C(5)); 113.5 (*d*, arom. C); 127.4, 128.2, 128.4, 130.3 (4*d*, arom. C); 135.6, 135.7 (2*s*, arom. C); 144.5 (*d*, C(6)); 145.0 (*s*, arom. C); 155.8 (*s*, C(2)); 159.0 (*s*, arom. C); 163.3 (*s*, C(4)); 171.1 (*s*, CO). FAB-MS: 774 (47, $[M+H]^+$), 303 (100, $[(MeO)_2Tr]^+$). Anal. calc. for $C_{42}H_{55}N_3O_9Si$: C 65.18, H 7.16, N 5.43; found: C 64.98, H 7.29, N 5.45.

N^6*-Acetyl-5′-*O*-(4,4′-dimethoxytrityl)-2′-*O*- and* N^6*-Acetyl-5′-*O*-(4,4′-dimethoxytrityl)-3′-*O*-{[(triisopropylsilyl)oxy]methyl}adenosine* (**11** and **15**, resp.). As described for **10** and **14**, with **5** (122 g, 0.2 mol), $^iPr_2NEt$ (117 ml, 0.7 mol), $ClCH_2CH_2Cl$ (800 ml), Bu_2SnCl_2 (67 g, 0.22 mol), and **9** (49 g, 0.22 mol). Workup and CC (2 kg of SiO_2, hexane/AcOEt 4:1 → 1:4 (+1% Et_3N)] afforded **11** (64 g, 40%) and **15** (40 g, 25%) as colorless, solid foams. With CH_2Cl_2 → CH_2Cl_2/MeOH 9:1 (+1% Et_3N), **5** (30 g, 25%) was recovered.

Data of **11**: TLC (hexane/AcOEt 3:7): R_f 0.45. $[\alpha]^{25}_D = +11.5$ (*c* = 1.36, $CHCl_3$). UV (MeOH): 280 (sh, 15500), 272 (20500), 253 (14200), 234 (24700), 220 (23800). IR ($CHCl_3$): 3540*w*, 3400*w*, 3380*w*, 3000*m*, 2940*m*, 2880*m*, 1730*w*, 1700*m*, 1610*s*, 1590*m*, 1510*m*, 1470*m*, 1380*m*, 1300*m*, 1040*s*, 1010*w*, 1000*w*, 910*w*, 890*w*, 830*w*. ^{1}H-NMR (300 MHz, $CDCl_3$): 0.99–1.08 (*m*, $^iPr_3Si$); 2.61 (*s*, MeCO); 3.07 (br. *d*, *J* ≈ 3, OH–C(3′)); 3.40

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(*dd*, $J = 3.9$, 10.6, H–C(5′)); 3.53 (*dd*, $J = 3.5$, 10.6, H′–C(5′)); 3.78 (*s*, 2 MeO); 4.30 (*q*, $J = 3.7$, H–C(4′)); 4.57 (br. *q*, $J \approx 3$, H–C(3′)); 4.96 (*t*, $J = 5.3$, H–C(2′)); 4.98, 5.14 (2*d*, $J = 5.0$, OCH_2O); 6.21 (*d*, $J = 5.3$, H–C(1′)); 6.78–6.81 (*m*, 4 arom. H); 7.23–7.44 (*m*, 9 arom. H); 8.16 (*s*, H–C(2)); 8.60 (*s*, H–C(8)); 8.65 (br. *s*, NH–C(6)). ^{13}C-NMR (75 MHz, $CDCl_3$): 11.8 (*d*, Me_2CH); 17.8 (*q*, Me_2CH); 25.9 (*q*, *Me*CO); 55.3 (*q*, MeO); 63.4 (*t*, C(5′)); 71.0, 82.2, 84.6 (3*d*, C(2′), C(3′), C(4′)); 86.8 (*s*, arom. C); 87.4 (*d*, C(1′)); 91.0 (*t*, OCH_2O); 113.4 (*d*, arom. C); 122.5 (*s*, C(5)); 127.2, 128.1, 128.4, 130.3 (4*d*, arom. C); 135.9 (*s*, arom. C); 142.1 (*d*, C(8)); 144.8 (*s*, arom. C); 149.4 (*s*, C(4)); 151.4 (*s*, C(6)); 152.7 (*d*, C(2)); 158.9 (*s*, arom. C); 170.7 (*s*, CO). FAB-MS: 798 (29, $[M+H]^+$), 303 (100, $[(MeO)_2Tr]^+$). Anal. calc. for $C_{43}H_{55}N_5O_8Si$: C 64.72, H 6.95, N 8.78; found: C 64.64, H 7.06, N 8.74.

Data of **15**: TLC (hexane/AcOEt 1:9): R_f 0.45. $[\alpha]^{25}_D = -19.0$ ($c = 0.78$, $CHCl_3$). UV (MeOH): 282 (sh, 13600), 272 (19500), 253 (14300), 234 (25700), 220 (25000). IR ($CHCl_3$): 3520*w*, 3400*w*, 3380*w*, 3000*m*, 2950*m*, 2860*m*, 1730*w*, 1700*m*, 1610*s*, 1590*m*, 1510*m*, 1470*m*, 1380*m*, 1300*m*, 1040*s*, 880*w*. ^{1}H-NMR (300 MHz, $CDCl_3$): 1.04–1.07 (*m*, $^iPr_3Si$); 2.62 (*s*, MeCO); 3.34 (*dd*, $J = 4.0$, 10.2, H–C(5′)); 3.49 (*dd*, $J = 3.4$, 10.2, H′–C(5′)); 3.78 (*s*, 2 MeO); 3.78 (br. *s*, OH–C(2′)); 4.38 (br. *q*, $J \approx 4$, H–C(4′)); 4.52 (*t*, $J = 4.8$, H–C(3′)); 4.91 (br. *t*, $J \approx 5$, H–C(2′)); 4.96, 5.11 (2*d*, $J = 4.7$, OCH_2O); 6.04 (*d*, $J = 5.0$, H–C(1′)); 6.77 (*d*, $J = 8.7$, 4 arom. H); 7.19–7.39 (*m*, 9 arom. H); 8.15 (*s*, H–C(2)); 8.51 (br. *s*, NH–C(6)); 8.62 (*s*, H–C(8)). ^{13}C-NMR (75 MHz, $CDCl_3$): 11.9 (*d*, Me_2CH); 17.8 (*q*, Me_2CH); 25.7 (*q*, *Me*CO); 55.3 (*q*, MeO); 63.1 (*t*, C(5′)); 74.3, 79.4, 83.1 (3*d*, C(2′), C(3′), C(4′)); 86.7 (*s*, arom. C); 89.5 (*d*, C(1′)); 90.9 (*t*, OCH_2O); 113.3 (*d*, arom. C); 122.5 (*s*, C(5)); 127.1, 128.1, 128.3, 130.3 (4*d*, arom. C); 135.9 (*s*, arom. C); 142.1 (*d*, C(8)); 144.8 (*s*, arom. C); 149.5 (*s*, C(4)); 151.3 (*s*, C(6)); 152.6 (*d*, C(2)); 158.9 (*s*, arom. C); 170.7 (*s*, CO). FAB-MS: 798 (66, $[M+H]^+$), 303 (100, $[(MeO)_2Tr]^+$). Anal. calc. for $C_{43}H_{55}N_5O_8Si \cdot 0.5\ H_2O$: C 64.00, H 7.00, N 8.68; found: C 64.05, H 6.95, N 8.49.

N^2*-Acetyl-5′-*O*-(4,4′-dimethoxytrityl)-2′-*O*- and N*^{2}*-Acetyl-5′-*O*-(4,4′-dimethoxytrityl)-3′-*O*-{[(triisopropylsilyl)oxy]methyl}guanosine* (**12** and **16**, resp.). As described for **10** and **14**, with **6** (100 g, 0.16 mol), $^iPr_2NEt$ (96 ml, 0.56 mol), $ClCH_2CH_2Cl$ (640 ml), Bu_2SnCl_2 (54 g, 0.17 mol), and **9** (36 g, 0.16 mol). Workup and CC (1.5 kg of SiO_2, $CH_2Cl_2 \rightarrow CH_2Cl_2$/MeOH 97:3 (+5% Et_3N)) afforded **12** (78 g, 60%) and impure **16** (7 g, ≈5%) as yellow, solid foams. With CH_2Cl_2/MeOH 19:1 → 9:1 (+1% Et_3N), **6** (20 g, 20%) was recovered.

Data of **12**: TLC (CH_2Cl_2/MeOH 19:1): R_f 0.55. $[\alpha]^{25}_D = +7.7$ ($c = 0.85$, $CHCl_3$). UV (MeOH): 282 (sh, 13500), 275 (14300), 271 (13900), 260 (sh, 17500), 251 (19200), 235 (26500), 223 (22800). IR ($CHCl_3$): 3370*w*, 3225*w*, 3010*w*, 2945*m*, 2870*w*, 1700*s*, 1605*s*, 1560*m*, 1510*m*, 1465*w*, 1410*m*, 1300*w*, 1255*m*, 1090*m*, 1040*m*, 995*m*, 920*w*, 880*w*, 830*w*. ^{1}H-NMR (300 MHz, $CDCl_3$): 0.94–1.12 (*m*, $^iPr_3Si$); 1.46 (*s*, MeCO); 3.03 (br. *d*, $J \approx 3$, OH–C(3′)); 3.13 (*dd*, $J = 2.8$, 10.6, H–C(5′)); 3.53 (*dd*, $J = 2.0$, 10.6, H′–C(5′)); 3.76, 3.78 (2*s*, 2 MeO); 4.24 (*q*, $J = 2.0$, H–C(4′)); 4.57 (*ddd*, $J = 1.5$, 5.0, 3.0, H–C(3′)); 4.95, 5.14 (2*d*, $J = 4.7$, OCH_2O); 5.09 (*dd*, $J = 5.3$, 7.5, H–C(2′)); 5.90 (*d*, $J = 7.5$, H–C(1′)); 6.78–6.83 (*m*, 4 arom. H); 7.18–7.57 (*m*, 9 arom. H); 7.75 (br. *s*, NH–C(2)); 7.81 (*s*, H–C(8)); 11.82 (br. *s*, H–N(1)). ^{13}C-NMR (75 MHz, $CDCl_3$): 11.8 (*d*, Me_2CH); 17.8 (*q*, Me_2CH); 23.7 (*q*, *Me*CO); 55.4 (*q*, MeO); 63.9 (*t*, C(5′)); 70.8, 81.5, 84.5 (3*d*, C(2′), C(3′), C(4′)); 86.5 (*d*, C(1′)); 86.9 (*s*, arom. C); 91.1 (*t*, OCH_2O); 113.5 (*d*, arom. C); 122.4 (*s*, C(5)); 127.3, 128.2, 128.3, 130.3 (4*d*, arom. C); 135.9, 136.3 (2*s*, arom. C); 139.1 (*d*, C(8)); 145.3 (*s*, arom. C); 147.4 (*s*, C(4)); 148.7 (*s*, C(2)); 156.3 (*s*, C(6)); 159.0 (*s*, arom. C); 171.8 (*s*, CO). FAB-MS: 814 (17, $[M+H]^+$), 303 (100, $[(MeO)_2Tr]^+$). Anal. calc. for $C_{43}H_{55}N_5O_9Si$: C 63.45, H 6.81, N 8.60; found: C 63.34, H 6.84, N 8.35.

Data of **16**: Additional purification by prep. TLC. TLC (CH_2Cl_2/MeOH 19:1): R_f 0.50. $[\alpha]^{25}_D = -23.6$ ($c = 1.00$, $CHCl_3$). UV (MeOH): 282 (sh, 13000), 275 (14500), 271 (14000), 260 (sh, 17500), 251 (19500), 235 (26500), 223 (23000). IR ($CHCl_3$): 3370*w*, 3220*w*, 3010*w*, 2945*m*, 2870*w*, 1700*s*, 1610*s*, 1560*m*, 1510*m*, 1465*w*, 1410*m*, 1300*w*, 1250*m*, 1040*m*, 995*m*, 915*w*, 880*w*, 830*w*. ^{1}H-NMR (300 MHz, $CDCl_3$): 0.99–1.13 (*m*, $^iPr_3Si$); 1.47 (*s*, MeCO); 3.13 (*dd*, $J = 3.1$, 10.6, H–C(5′)); 3.53 (*dd*, $J = 2.0$, 10.6, H′–C(5′)); 3.76–3.78 (*m*, 2 MeO, OH–C(2′)); 4.32 (br. *q*, $J \approx 2$, H–C(4′)); 4.45 (*dd*, $J = 2.8$, 5.6, H–C(3′)); 4.99, 5.10 (2*d*, $J = 5.0$, OCH_2O); 5.17 (br. *t*, $J \approx 6$, H–C(2′)); 5.73 (*d*, $J = 6.8$, H–C(1′)); 6.78–6.83 (*m*, 4 arom. H); 7.18–7.55 (*m*, 9 arom. H); 7.60 (br. *s*, NH–C(2)); 7.77 (*s*, H–C(8)); 11.85 (br. *s*, H–N(1)). ^{13}C-NMR (75 MHz, $CDCl_3$): 11.8 (*d*, Me_2CH); 17.8 (*q*, Me_2CH); 23.4 (*q*, *Me*CO); 55.4 (*q*, MeO); 63.4 (*t*, C(5′)); 72.8, 78.0, 83.9 (3*d*, C(2′), C(3′), C(4′)); 86.3 (*d*, C(1′)); 89.8 (*s*, arom. C); 90.8 (*t*, OCH_2O); 113.4, 113.5 (2*d*, arom. C); 122.3 (*s*, C(5)); 127.3, 128.3, 130.2, 130.4 (4*d*, arom. C); 136.0, 136.7 (2*s*, arom. C); 139.6 (*d*, C(8)); 145.3 (*s*, arom. C); 146.7 (*s*, C(4)); 148.2 (*s*, C(2)); 155.6 (*s*, C(6)); 159.0 (*s*, arom. C); 171.9 (*s*, CO). FAB-MS: 814 (27, $[M+H]^+$), 303 (100, $[(MeO)_2Tr]^+$).

*5′-*O*-(4,4′-Dimethoxytrityl)-2′-*O*- and 5′-*O*-(4,4′-Dimethoxytrityl)-3′-*O*-{[(triisopropylsilyl)oxy]methyl}uridine* (**13** and **17**, resp.). As described for **10** and **14**, with **7** (109 g, 0.2 mol), $^iPr_2NEt$ (117 ml, 0.7 mol), $ClCH_2CH_2Cl$ (800 ml), Bu_2SnCl_2 (67 g, 0.22 mol), and **9** (53 g, 0.24 mol). Workup and CC (2 kg of SiO_2; hexane/

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AcOEt 4:1 → 1:4 (+1% Et_3N)) afforded **13** (59 g, 40%) and **17** (37 g, 25%) as colorless, solid foams. With CH_2Cl_2 → CH_2Cl_2/MeOH 9:1 (+1% Et_3N), **7** (27 g, 25%) was recovered.

Data of **13**: TLC (hexane/AcOEt 3:7): R_f 0.55. $[\alpha]^{25}_D = +42.9$ ($c = 1.00$, $CHCl_3$). UV (MeOH): 267 (13100), 233 (25900), 226 (28600). IR ($CHCl_3$): 3680*w*, 3520*w*, 3400*w*, 3000*m*, 2940*m*, 2880*m*, 1710*m*, 1690*s*, 1610*m*, 1510*m*, 1460*m*, 1390*w*, 1300*w*, 1100*m*, 1040*m*, 910*w*, 880*w*. ^{1}H-NMR (300 MHz, $CDCl_3$): 1.05–1.15 (*m*, $^iPr_3Si$); 3.17 (br. *d*, $J \approx 3$, OH–C(3′)); 3.52 (br. *d*, $J \approx 11$, H–C(5′)); 3.55 (br. *d*, $J \approx 11$, H′–C(5′)); 3.80 (*s*, 2 MeO); 4.11 (*m*, H–C(4′)); 4.26 (*dd*, $J = 3.5$, 5.0, H–C(2′)); 4.55 (br. *t*, $J \approx 3$, H–C(3′)); 5.03, 5.23 (2*d*, $J = 5.0$, OCH_2O); 5.29 (*d*, $J = 8.1$, H–C(5)); 6.03 (*d*, $J = 3.1$, H–C(1′)); 6.83–6.87 (*m*, 4 arom. H); 7.24–7.40 (*m*, 9 arom. H); 7.94 (*d*, $J = 8.1$, H–C(6)); 8.38 (br. *s*, H–N(3)). ^{13}C-NMR (75 MHz, $CDCl_3$): 11.9 (*d*, Me_2CH); 17.8 (*q*, Me_2CH); 55.3 (*q*, MeO); 62.3 (*t*, C(5′)); 69.5, 83.0, 83.8 (3*d*, C(2′), C(3′), C(4′)); 87.3 (*s*, arom. C); 88.0 (*d*, C(1′)); 90.8 (*t*, OCH_2O); 102.4 (*d*, C(5)); 113.5 (*d*, arom. C); 127.4, 128.2, 128.4, 130.4 (4*d*, arom. C); 135.4, 135.6 (2*s*, arom. C); 140.4 (*d*, C(6)); 144.6 (*s*, arom. C); 150.4 (*s*, C(2)); 159.0, 159.1 (2*s*, arom. C); 163.3 (*s*, C(4)). FAB-MS: 732 (21, M^+), 303 (100, $[(MeO)_2Tr]^+$). Anal. calc. for $C_{40}H_{52}N_2O_9Si$: C 65.55, H 7.15, N 3.82; found: C 65.29, H 7.08, N 4.02.

Data of **17**: TLC (hexane/AcOEt 1:4): R_f 0.60. $[\alpha]^{25}_D = -9.5$ ($c = 0.98$, $CHCl_3$). UV (MeOH): 263 (11500), 234 (25300), 224 (21600). IR ($CHCl_3$): 3540*w*, 3400*m*, 2950*m*, 2880*m*, 1710*s*, 1700*s*, 1610*w*, 1510*m*, 1460*m*, 1390*w*, 1300*w*, 1120*m*, 1040*s*, 1000*w*, 890*w*. ^{1}H-NMR (300 MHz, $CDCl_3$): 1.02–1.15 (*m*, $^iPr_3Si$); 3.39 (*dd*, $J = 2.5$, 10.9, H–C(5′)); 3.43 (br. *s*, OH–C(2′)); 3.55 (*dd*, $J = 2.5$, 10.9, H′–C(5′)); 3.80 (*s*, 2 MeO); 4.26–4.34 (*m*, H–C(2′), H–C(3′), H–C(4′)); 4.90, 5.06 (2*d*, $J = 5.0$, OCH_2O); 5.39 (*d*, $J = 8.1$, H–C(5)); 5.97 (*d*, $J = 4.0$, H–C(1′)); 6.83 (*d*, $J = 8.4$, 4 arom. H); 7.24–7.39 (*m*, 9 arom. H); 7.77 (*d*, $J = 8.1$, H–C(6)); 8.42 (br. *s*, H–N(3)). ^{13}C-NMR (75 MHz, $CDCl_3$): 11.9 (*d*, Me_2CH); 17.8 (*q*, Me_2CH); 55.3 (*q*, MeO); 62.6 (*t*, C(5′)); 74.7, 78.3, 82.3 (3*d*, C(2′),C(3′),C(4′)); 87.2 (*s*, arom. C); 89.5 (*d*, C(1′)); 90.7 (*t*, OCH_2O); 102.5 (*d*, C(5)); 113.5 (*d*, arom. C); 127.4, 128.2, 128.4, 130.4 (4*d*, arom. C); 135.5, 135.6 (2*s*, arom. C); 140.6 (*d*, C(6)); 144.6 (*s*, arom. C); 150.6 (*s*, C(2)); 159.0 (*s*, arom. C); 163.2 (*s*, C(4)). FAB-MS: 732 (39, $[M + H]^+$), 303 (100, $[(MeO)_2Tr]^+$). Anal. calc. for $C_{40}H_{52}N_2O_9Si$: C 65.55, H 7.15, N 3.82; found: C 65.47, H 7.06, N 3.96.

N^4*-Acetyl-5′-*O*-(4,4′-dimethoxytrityl)-2′-*O*-{[(triisopropyl)silyloxy]methyl}cytidine 3′-(2-Cyanoethyl Diisopropylphosphoramidite)* (**18**). A soln. of **10** (77 g, 0.1 mol) in CH_2Cl_2 (220 ml) was treated consecutively with $^iPr_2NEt$ (43 ml, 0.25 mol) and 2-cyanoethyl diisopropylphosphoramidochloridite (28 g, 0.12 mol). After stirring for 14 h at r.t., the mixture was subjected to CC (1.5 kg of SiO_2, hexane/AcOEt 9:1 → 3:7 (+3% Et_3N)): **18** (91 g, 94%; 1:1 mixture of diastereoisomers). Colorless foam. TLC (hexane/AcOEt 3:7): R_f 0.75. UV (MeCN): 305 (6700), 290 (5700), 279 (6200), 237 (29000), 226 (23800). IR ($CHCl_3$): 3400*w*, 3000*m*, 2980*m*, 2860*m*, 1720*m*, 1670*s*, 1610*s*, 1560*m*, 1500*s*, 1480*s*, 1460*m*, 1380*m*, 1360*m*, 1300*m*, 1100*m*, 1040*s*, 990*m*, 880*w*. ^{1}H-NMR (500 MHz, $CDCl_3$): 0.87–1.11 (*m*, $^iPr_3Si$); 1.13, 1.16 (2*d*, $J = 6.8$, $(Me_2CH)_2N$); 2.22, 2.33 (2*s*, MeCO); 2.38, 2.59 (2*t*, $J = 6.5$, CH_2CN); 3.42–3.68 (*m*, 1 H of $POCH_2$, $(Me_2CH)_2N$, H–C(5′)); 3.806, 3.807, 3.814, 3.817 (4*s*, 2 MeO); 3.91 (*m*, 1 H, $POCH_2$); 4.28–4.41 (*m*, H–C(2′), H–C(4′)); 4.50 (*ddd*, $J = 4.8$, 8.0, 9.9, H–C(3′)); 5.15–5.22 (*m*, OCH_2O); 6.15 (*d*, $J = 1.8$, 0.5 H, H–C(1′)); 6.16 (*d*, $J = 2.4$, 0.5 H, H–C(1′)); 6.82–6.88 (*m*, 4 arom. H); 6.96, 7.03 (2*d*, $J = 7.4$, H–C(5)); 7.24–7.47 (*m*, 9 arom. H); 8.36, 8.45 (2*d*, $J = 7.4$, H–C(6)); 9.52, 9.60 (2 br. *s*, NH–C(4)). ^{13}C-NMR (125 MHz, $CDCl_3$): 12.0 (*d*, $(Me_2CH)_3Si$); 17.8, 17.9 (2*q*, $(Me_2CH)_3Si$); 20.2, 20.3 (2*t*, J(C,P) = 7, CH_2CN); 24.48, 24.54, 24.58, 24.61, 24.64 (5*q*, $(Me_2CH)_2N$); 24.7, 24.9 (2*q*, *Me*CO); 43.2, 43.3 (2*d*, J(C,P) = 7, $(Me_2CH)_2N$); 55.2, 55.3 (2*q*, MeO); 58.2, 58.7 (2*t*, J(C,P) = 20, $POCH_2$); 60.9, 61.6 (2*t*, C(5′)); 69.4, 69.7 (2*d*, J(C,P) = 14), 78.7 (*d*, J(C,P) = 3), 78.9 (*d*), 82.3 (*d*, J(C,P) = 3), 82.5 (*d*, J(C,P) = 5), (C(2′),C(3′),C(4′)); 87.0, 87.2 (2*s*, arom. C); 89.6, 89.7 (2*d*, C(1′)); 89.8 (*t*, OCH_2O); 96.5 (br. *d*, C(5)); 113.2, 113.3 (2*d*, arom. C); 117.4, 117.7 (2*s*, CN); 127.2, 128.0, 128.2, 128.4, 130.1, 130.2, 130.3, 130.4 (8*d*, arom. C); 135.2, 135.3, 135.4, 135.5, 144.1, 144.3 (6*s*, arom. C); 145.0, 145.1 (2*d*, C(6)); 154.9 (*s*, C(2)); 158.7 (*s*, arom. C); 162.6, 162.7 (2*s*, C(4)); 170.5 (*s*, CO). ^{31}P-NMR (150 MHz, $CDCl_3$): 150.6, 150.9. FAB-MS: 974 (21, $[M + H]^+$), 303 (100, $[(MeO)_2Tr]^+$). Anal. calc. for $C_{51}H_{72}N_5O_{10}PSi$: C 62.88, H 7.45, N 7.19; found: C 62.74, H 7.56, N 7.00.

N^6*-Acetyl-5′-*O*-(4,4′-dimethoxytrityl)-2′-*O*-{[(triisopropylsilyl)oxy]methyl}adenosine 3′-(2-Cyanoethyl Diisopropylphosphoramidite)* (**19**). As described for **18**, with **11** (80 g, 0.1 mol), CH_2Cl_2 (220 ml), $^iPr_2NEt$ (43 ml, 0.25 mol), and 2-cyanoethyl diisopropylphosphoramidochloridite (28 g, 0.12 mmol). CC (1.5 kg of SiO_2, hexane/AcOEt 9:1 → 3:7 (+3% Et_3N)): **19** (93 g, 93%; 1:1 mixture of diastereoisomers). Colorless foam. TLC (hexane/AcOEt 3:7): R_f 0.75. UV (MeCN): 278 (sh, 17100), 270 (21800), 253 (16600), 235 (25400), 228 (23800). IR ($CHCl_3$): 3400*w*, 3390*w*, 3000*m*, 2980*m*, 2860*m*, 1740*m*, 1700*m*, 1610*s*, 1590*m*, 1510*s*, 1460*m*, 1380*m*, 1300*m*, 1080*m*, 1050*s*, 1000*m*, 900*w*. ^{1}H-NMR (500 MHz, $CDCl_3$): 0.88–0.90 (*m*, $^iPr_3Si$); 1.09, 1.18, 1.19 (3*d*, $J = 6.8$, $(Me_2CH)_2N$); 2.38 (*t*, $J = 6.4$, 1 H, CH_2CN); 2.62 (*s*, MeCO); 2.65 (*dt*, $J = 3.3$, 6.6, 1 H, CH_2CN); 3.35 (*ddd*, $J = 4.6$, 7.5, 11.7, 1 H, $POCH_2$); 3.51–3.75 (*m*, 1 H of $POCH_2$, $(Me_2CH)_2N$, H–C(5′)); 3.77, 3.78 (2*s*, 2 MeO); 3.85–3.97 (*m*, 1 H, $POCH_2$); 4.36, 4.41 (2*q*, $J = 4.0$, H–C(4′)); 4.68 (*m*, H–C(3′)); 4.92, 4.95, 4.99

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(3*d*, *J* = 5.0, OCH_2O); 5.17, 5.20 (2*t*, *J* = 5.5, H–C(2′)); 6.17, 6.20 (2*d*, *J* = 5.8, H–C(1′)); 6.76–6.80 (*m*, 4 arom. H); 7.18–7.42 (*m*, 9 arom. H); 8.12, 8.15 (2*s*, H–C(2)); 8.53 (br. *s*, NH–C(6)); 8.54, 8.56 (2*s*, H–C(8)). ^{13}C-NMR (125 MHz, $CDCl_3$): 11.8 (*d*, $(Me_2CH)_3Si$); 17.5, 17.6, 17.7 (3*q*, $(Me_2CH)_3Si$); 20.1, 20.3 (2*t*, *J*(C,P) = 7, CH_2CN); 24.52, 24.56, 24.58, 24.62 (4*q*, $(Me_2CH)_2N$); 25.6 (*q*, *Me*CO); 43.2, 43.4 (2*d*, *J*(C,P) = 13, $(Me_2CH)_2N$); 55.1, 55.2 (2*q*, MeO); 58.0, 58.9 (2*t*, *J*(C,P) = 18, $POCH_2$); 62.8, 63.2 (2*t*, C(5′)); 71.2, 71.8 (2*d*, *J*(C,P) = 16), 77.0, 77.6 (2*d*, *J*(C,P) = 4), 84.1, 84.2 (2*d*) (C(2′), C(3′), C(4′)); 86.5, 86.6 (2*s*, arom. C); 87.4, 87.5 (2*d*, C(1′)); 89.2, 89.5 (2*t*, OCH_2O); 113.0, 113.1 (2*d*, arom. C); 117.3, 117.6 (2*s*, CN); 122.2 (*s*, C(5)); 126.8, 126.9, 127.8, 128.1, 128.2, 128.3, 130.0, 130.1, 130.2 (9*d*, arom. C); 135.6, 135.7, 135.8 (3*s*, arom. C); 142.1, 142.2 (2*d*, C(8)); 144.4, 144.5 (2*s*, arom. C); 149.0, 149.1 (2*s*, C(4)); 151.0, 151.1 (2*s*, C(6)); 152.2 (*d*, C(2)); 158.5, 158.6 (2*s*, arom. C); 170.3 (*s*, CO). ^{31}P-NMR (150 MHz, $CDCl_3$): 150.8, 151.5. FAB-MS: 998 (30, $[M+H]^+$), 821 (63), 303 (100, $[(MeO)_2Tr]^+$). Anal. calc. for $C_{52}H_{72}N_7O_9PSi$: C 62.57, H 7.27, N 9.82; found: C 62.62, H 7.25, N 9.72.

N^2-*Acetyl-5′-*O*-(4,4′-dimethoxytrityl)-2′-*O*-{[(triisopropylsilyl)oxy]methyl}guanosine 3′-(2-Cyanoethyl Diisopropylphosphoramidite)* (**20**). As described for **18**, with **12** (81 g, 0.1 mol), CH_2Cl_2 (220 ml), $^iPr_2NEt$ (43 ml, 0.25 mol) and 2-cyanoethyl diisopropylphosphoramidochloridite (28 g, 0.12 mmol). CC (1.5 kg of SiO_2, hexane/AcOEt 1:1 → AcOEt, then AcOEt → AcOEt/MeOH 9:1 (+3% Et_3N)): **20** (91 g, 90%; 1:1 mixture of diastereoisomers). Colorless foam. TLC (hexane/AcOEt 3:7): R_f 0.55. UV (MeCN): 276 (13500), 269 (12800), 259 (sh, 17700), 250 (sh, 19700), 237 (24600), 225 (21200). IR ($CHCl_3$): 3360*w*, 3230*w*, 3220*w*, 3000*w*, 2960*m*, 2860*m*, 1700*s*, 1600*s*, 1570*m*, 1510*m*, 1980*m*, 1910*w*, 1860*w*, 1800*w*, 1140*w*, 1100*m*, 1050*m*, 1000*m*, 910*w*, 880*w*. ^{1}H-NMR (500 MHz, $CDCl_3$): 0.91–0.94 (*m*, $^iPr_3Si$); 1.02–1.19 (5*d*, *J* = 7, $(Me_2CH)_2N$); 1.73, 1.82 (2*s*, MeCO); 2.25, 2.75 (2*m*, CH_2CN); 3.22 (*dd*, *J* = 3.7, 10.7, 0.5 H, H–C(5′)); 3.28 (*dd*, *J* = 5.3, 10.6, 0.5 H, H–C(5′)); 3.47–3.62 (*m*, 3.5 H, $(Me_2CH)_2N$, H–C(5′), $POCH_2$); 3.68 (*m*, 0.5 H, $POCH_2$); 3.757, 3.760, 3.768, 3.776 (4*s*, 2 MeO); 3.92, 4.04 (2*m*, 1.5 H, $POCH_2$); 4.23 (br. *q*, *J* ≈ 2.5, 0.5 H, H–C(4′)); 4.32 (br. *dt*, *J* ≈ 5, 2, 0.5 H, H–C(4′)); 4.52 (*ddd*, *J* = 2.0, 4.8, 12.1, 0.5 H, H–C(3′)); 4.62 (*ddd*, *J* = 4.8, 5.8, 10.6, 0.5 H, H–C(3′)); 4.91 (*s*, 1 H, OCH_2O); 4.90, 5.00 (2*d*, *J* = 5.2, 1 H, OCH_2O); 5.02 (*dd*, *J* = 4.8, 7.4, 0.5 H, H–C(2′)); 5.05 (*t*, *J* = 5.8, 0.5 H, H–C(2′)); 5.87 (*d*, *J* = 5.7, 0.5 H, H–C(1′)); 5.97 (*d*, *J* = 7.4, 0.5 H, H–C(1′)); 6.76–6.82 (*m*, 4 arom. C); 7.14–7.52 (*m*, 9 arom. H); 7.74, 7.80 (2*s*, H–C(8)); 8.29, 8.57 (2 br. *s*, NH–C(2)); 11.89 (br. *s*, H–N(1)). ^{13}C-NMR (125 MHz, $CDCl_3$): 11.8 (*d*, $(Me_2CH)_3Si$); 17.6, 17.7, 17.8 (3*q*, $(Me_2CH)_3Si$); 20.1 (*t*, *J*(C,P) = 3, CH_2CN); 20.2 (*t*, CH_2CN); 23.5, 23.6 (2*q*, *Me*CO); 24.5, 24.6, 24.7 (3*q*, $(Me_2CH)_2N$); 43.1, 43.3 (2*d*, *J*(C,P) = 13, $(Me_2CH)_2N$); 55.2, 55.3 (2*q*, MeO); 56.9 (*t*, *J*(C,P) = 19, $POCH_2$); 58.8 (*t*, *J*(C,P) = 13, $POCH_2$); 63.5, 63.9 (2*t*, C(5′)); 70.7 (*d*, *J*(C,P) = 17), 71.7 (*d*, *J*(C,P) = 14), 76.9 (*d*), 78.3 (*d*), 84.2 (*d*), 84.3 (*d*, *J*(C,P) = 4), (C(2′), C(3′), C(4′)); 86.2 (*d*, C(1′)); 86.3, 86.7 (2*s*, arom. C); 88.9 (*d*, C(1′)); 89.4, 89.5 (2*t*, OCH_2O); 113.1, 113.2 (2*d*, arom. C); 117.5, 118.1 (2*s*, CN); 122.0, 122.7 (2*s*, C(5)); 127.0, 127.1, 127.9, 128.0, 128.1, 130.0, 130.1, 130.2 (8*d*, arom. C); 135.6, 135.8, 136.0, 136.3 (4*s*, arom. C); 137.7, 139.1 (2*d*, C(8)); 144.6, 145.0 (2*s*, arom. C); 146.8, 147.1 (2*s*, C(4)); 148.0, 148.5 (2*s*, C(2)); 155.6 (*s*, C(6)); 158.6, 158.7 (2*s*, arom. C); 171.5, 171.6 (2*s*, CO). ^{31}P-NMR (150 MHz, $CDCl_3$): 149.9, 150.5. FAB-MS: 1014 (62, $[M+H]^+$), 303 (100, $[(MeO)_2Tr]^+$). Anal. calc. for $C_{52}H_{72}N_7O_{10}PSi$: C 61.58, H 7.15, N 9.67; found: C 61.22, H 7.19, N 9.55.

*5′-*O*-(4,4′-Dimethoxytrityl)-2′-*O*-{[(triisopropylsilyl)oxy]methyl}uridine 3′-(2-Cyanoethyl Diisopropylphosphoramidite)* (**21**). As described for **18**, with **13** (73 g, 0.1 mol), CH_2Cl_2 (220 ml), $^iPr_2NEt$ (43 ml, 0.25 mol), and 2-cyanoethyl diisopropylphosphoramidochloridite (28 g, 0.12 mmol). CC (1.5 kg of SiO_2, hexane/AcOEt 9:1 → 3:7 (+3% Et_3N)): **21** (86 g, 92%); 1:1 mixture of diastereoisomers). Colorless foam. TLC (hexane/AcOEt 1:1): R_f 0.65. UV (MeCN): 264 (12100), 236 (23700), 225 (20500). IR ($CHCl_3$): 3380*w*, 3000*w*, 2960*m*, 2860*m*, 1730*s*, 1700*s*, 1600*w*, 1500*m*, 1450*m*, 1350*w*, 1100*w*, 1050*m*, 980*w*, 890*w*. ^{1}H-NMR (500 MHz, $CDCl_3$): 1.02–1.05 (*m*, $^iPr_3Si$); 1.06, 1.17 (*m*, $(Me_2CH)_2N$); 2.39, 2.63 (2*t*, *J* = 6.7, CH_2CN); 3.39 (*m*, H–C(5′)); 3.53–3.70 (*m*, 4 H, $(Me_2CH)_2N$, H′–C(5′), $POCH_2$); 3.79, 3.80 (2*s*, 2 MeO); 3.81–3.96 (*m*, 1 H, $POCH_2$); 4.19, 4.27 (2br. *dt*, *J* ≈ 5, 2, H–C(4′)); 4.40–4.55 (*m*, H–C(2′), H–C(3′)); 4.96–5.06 (*m*, OCH_2O); 5.32, 5.36 (2*d*, *J* = 8.1, H–C(5)); 6.12 (*d*, *J* = 5.1, 0.5 H, H–C(1′)); 6.14 (*d*, *J* = 4.9, 0.5 H, H–C(1′)); 6.82–6.85 (*m*, 4 arom. H); 7.05–7.47 (*m*, 9 arom. H); 7.80, 7.86 (2*d*, *J* = 8.1, H–C(6)); 8.33 (br. *s*, H–N(3)). ^{13}C-NMR (125 MHz, $CDCl_3$): 11.9, 12.0 (2*d*, $(Me_2CH)_3Si$); 17.7, 17.8, 17.9 (3*q*, $(Me_2CH)_3Si$); 20.2, 20.4 (2*t*, *J*(C,P) = 7, CH_2CN); 24.52, 24.55, 24.58, 24.62 (4*q*, $(Me_2CH)_2N$); 43.2, 43.4 (2*d*, *J*(C,P) = 12, $(Me_2CH)_2N$); 55.2, 55.3 (2*q*, MeO); 57.8, 58.9 (2*t*, *J*(C,P) = 18, $POCH_2$); 62.1, 62.5 (2*t*, C(5′)); 70.4, 70.9 (2*d*, *J*(C,P) = 15), 77.4, 78.1 (2*d*, *J*(C,P) = 4), 83.3 (*d*, *J*(C,P) = 4, 83.5 (*d*) (C(2′), C(3′), C(4′)); 87.1, 87.2 (2*s*, arom. C); 87.4, 89.0 (2*d*, C(1′)); 89.3, 89.4 (2*t*, OCH_2O); 102.3, 102.4 (2*d*, C(5)); 113.1, 113.2, 113.3 (3*d*, arom. C); 117.3, 117.7 (2*s*, CN); 127.2, 127.9, 128.0, 128.2, 128.3, 130.2, 130.3, 130.4 (8*d*, arom. C); 135.1, 135.3, 135.4, 135.5 (4*s*, arom. C); 140.3, 140.4 (2*d*, C(6)); 144.2, 144.3 (2*s*, arom. C); 150.1 (*s*, C(2)); 158.7 (*s*, arom. C); 162.8, 162.9 (2*s*, C(4)). ^{31}P-NMR (150 MHz, $CDCl_3$): 150.9, 151.3. FAB-MS: 933 (57, $[M+H]^+$), 303 (100, $[(MeO)_2Tr]^+$). Anal. calc. for $C_{49}H_{69}N_4O_{10}PSi$: C 63.07, H 7.45, N 6.00; found: C 62.84, H 7.49, N 5.98.

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N^4-*Acetyl-5′-*O*-(4,4′-dimethoxytrityl)-3′-*O*-{[(triisopropylsilyl)oxy]methyl}cytidine 2′-(4-Nitrophenyl Heptanedioate)* (**22**). A soln. of **14** (7.7 g, 10 mmol) in Py (50 ml) was added during 3 h to a soln. of bis(4-nitrophenyl) heptanedioate (20 g, 50 mmol) and DMAP (1.6 g, 10 mmol) in Py (100 ml). After stirring for 14 h at r.t., the mixture was evaporated and co-evaporated twice with toluene (200 ml). CC (150 g of SiO_2, hexane/AcOEt 1:1 → 1:4) gave **22** (6.7 g, 65%). Colorless foam. TLC (hexane/AcOEt 3:7): R_f 0.50. IR ($CHCl_3$): 3440*w*, 3000*m*, 2990*m*, 2880*m*, 1740*s*, 1670*s*, 1610*s*, 1600*m*, 1550*m*, 1520*s*, 1510*s*, 1490*s*, 1350*s*, 1300*m*, 1190*s*, 1080*m*, 1040*m*, 1000*m*, 920*w*, 900*w*, 880*w*. ^{1}H-NMR (300 MHz, $CDCl_3$): 0.96 – 1.01 (*m*, $^iPr_3Si$); 1.47 (*m*, CH_2); 1.66 – 1.80 (*m*, 2 CH_2); 2.21 (*s*, MeCO); 2.44 (*td*, *J* = 7.8, 1.8, 2 CH_2); 2.60 (*t*, *J* = 7.4, CH_2); 3.47 (*dd*, *J* = 3.4, 10.9, H – C(5′)); 3.67 (*dd*, *J* = 2.2, 10.9, H′ – C(5′)); 3.80 (*s*, 2 MeO); 4.33 (br. *dt*, *J* ≈ 6, 3, H – C(4′)); 4.49 (*dd*, *J* = 4.7, 6.5, H – C(3′)); 4.80, 4.86 (2*d*, *J* = 5.0, OCH_2O); 5.45 (*dd*, *J* = 3.1, 4.7, H – C(2′)); 6.16 (*d*, *J* = 3.1, H – C(1′)); 6.85 (*d*, *J* = 8.1, 4 arom. H); 7.03 (*d*, *J* = 7.6, H – C(5)); 7.26 – 7.42 (*m*, 11 arom. H); 8.20 (*d*, *J* = 7.8, H – C(6)); 8.23 – 8.28 (*m*, 2 arom. H); 9.09 (br. *s*, NH – C(4)). ^{13}C-NMR (75 MHz, $CDCl_3$): 11.9 (*d*, Me_2*C*H); 17.8 (*q*, *Me*$_2$CH), 24.3, 24.4, 28.4, 33.8, 34.1 (5*t*, CH_2); 25.0 (*q*, *Me*CO); 55.3 (*q*, MeO); 62.2 (*t*, C(5′)); 72.7, 74.6, 82.4 (3*d*, C(2′), C(3′), C(4′)); 87.3 (*s*, arom. C); 89.0 (*d*, C(1′)); 89.5 (*t*, OCH_2O); 96.8 (*d*, C(5)); 113.5, 116.0 (2*d*, arom. C); 122.7, 125.4, 126.3, 127.5, 128.2, 128.6, 130.5 (7*d*, arom. C); 135.4 (*s*, arom. C); 135.5 (*d*, C(6)); 144.4, 144.8, 145.5 (3*s*, arom. C); 155.3 (*s*, C(2)); 155.8 (*s*, arom. C); 159.0 (*s*, arom. C); 162.8 (*s*, C(4)); 170.4, 172.2 (2*s*, CO). FAB-MS: 1038 (25, $[M + H]^+$), 303 (100, $[(MeO)_2Tr]^+$).

N^6-*Acetyl-5′-*O*-(4,4′-dimethoxytrityl)-3′-*O*-{[(triisopropylsilyl)oxy]methyl}adenosine 2′-(4-Nitrophenyl Heptanedioate)* (**23**). As described for **22**, with **15** (8.0 g, 10 mmol), Py (50 ml), bis(4-nitrophenyl) heptanedioate (20 g, 50 mmol), and DMAP (1.6 g, 10 mmol) in Py (100 ml). CC (150 g of SiO_2, hexane/AcOEt 1:1 → 1:5) gave **23** (6.4 g, 60%). Colorless foam. TLC (hexane/AcOEt 3:7): R_f 0.50. IR ($CHCl_3$): 3390*w*, 3380*w*, 3000*m*, 2930*m*, 2900*w*, 1750*m*, 1700*m*, 1610*s*, 1600*s*, 1510*m*, 1500*m*, 1490*w*, 1450*m*, 1400*s*, 1300*m*, 1130*m*, 1040*m*, 1040*w*, 990*w*, 900*w*, 890*w*, 880*w*. ^{1}H-NMR (300 MHz, $CDCl_3$): 0.98 – 1.05 (*m*, $^iPr_3Si$); 1.41 (*m*, CH_2); 1.61 – 1.76 (*m*, 2 CH_2); 2.41 (*t*, *J* = 7.5, CH_2); 2.57 (*t*, *J* = 7.5, CH_2); 2.21 (*s*, MeCO); 3.42 (*dd*, *J* = 4.3, 10.2, H – C(5′)); 3.53 (*dd*, *J* = 2.8, 10.2, H′ – C(5′)); 3.77 (*s*, 2 MeO); 4.46 (br. *q*, *J* ≈ 4, H – C(4′)); 4.80 (*t*, *J* = 4.9, H – C(3′)); 4.84, 4.97 (2*dd*, *J* = 5.0, OCH_2O); 5.91 (*t*, *J* = 5.2, H – C(2′)); 6.27 (*d*, *J* = 5.2, H – C(1′)); 6.77 (*d*, *J* = 8.4, 4 arom. H); 7.22 – 7.42 (*m*, 11 arom. H); 8.11 (*s*, H – C(2)); 8.23 – 8.26 (*m*, 2 arom. H); 8.60 (*s*, H – C(8)); 8.65 (br. *s*, NH – C(6)). ^{13}C-NMR (75 MHz, $CDCl_3$): 11.9 (*d*, Me_2*C*H); 17.8 (*q*, *Me*$_2$CH); 24.3, 24.4, 28.4, 33.6, 34.0 (5*t*, CH_2); 25.6 (*q*, *Me*CO); 55.3 (*q*, MeO); 63.4 (*t*, C(5′)); 74.7, 74.8, 83.7 (3*d*, C(2′), C(3′), C(4′)); 86.5 (*s*, arom. C); 86.9 (*d*, C(1′)); 89.9 (*t*, OCH_2O); 113.4 (*d*, arom. C); 122.6 (*s*, C(5)); 125.4, 126.4, 127.2, 128.1, 128.5, 130.3 (6*d*, arom. C); 135.8 (*s*, arom. C); 141.7 (*d*, C(8)); 144.6, 145.6 (2*s*, arom. C); 149.4 (*s*, C(4)); 151.4 (*s*, C(6)); 152.8 (*d*, C(2)); 155.7 (*s*, arom. C); 158.9 (*s*, arom. C); 170.6, 171.3, 172.6 (3*s*, CO). FAB-MS: 1061 (10, $[M + H]^+$), 303 (100, $[(MeO)_2Tr]^+$).

*5′-*O*-(4,4′-Dimethoxytrityl)-3′-*O*-{[(triisopropylsilyl)oxy]methyl}uridine 2′-(4-Nitrophenyl Heptanedioate)* (**24**). As described for **22**, with **17** (7.3 g, 10 mmol), Py (50 ml), bis(4-nitrophenyl) heptanedioate (20 g, 50 mmol), and DMAP (1.6 g, 10 mmol) in Py (100 ml). CC (150 g of SiO_2, hexane/AcOEt 4:1 → 2:3) gave **24** (6.9 g, 70%). Colorless foam. TLC (hexane/AcOEt 1:1): R_f 0.45. IR ($CHCl_3$): 3380*w*, 3000*w*, 2960*w*, 2880*w*, 1750*m*, 1720*s*, 1690*s*, 1610*w*, 1600*w*, 1460*m*, 1350*m*, 1120*m*, 1080*w*, 1040*m*, 1000*w*, 930*w*, 1390*w*, 1360*w*. ^{1}H-NMR (300 MHz, $CDCl_3$): 1.00 (*m*, $^iPr_3Si$); 1.46 (*m*, CH_2); 1.66 – 1.83 (*m*, 2 CH_2); 2.44 (*t*, *J* = 7.8, CH_2); 2.61 (*t*, *J* = 7.5, CH_2); 3.46 (*dd*, *J* = 2.8, 10.9, H – C(5′)); 3.56 (*dd*, *J* = 2.1, 10.9, H′ – C(5′)); 3.79 (*s*, 2 MeO); 4.32 (br. *td*, *J* ≈ 2, 5, H – C(4′)); 4.58 (*t*, *J* = 4.7, H – C(3′)); 4.83, 4.95 (2*d*, *J* = 5.0, OCH_2O); 5.30 (*d*, *J* = 8.1, H – C(5)); 5.42 (*t*, *J* = 5.3, H – C(2′)); 6.19 (*d*, *J* = 5.6, H – C(1′)); 6.81 – 6.85 (*m*, 4 arom. H); 7.26 – 7.40 (*m*, 11 arom. H); 7.75 (*d*, *J* = 8.1, H – C(6)); 8.24 – 8.27 (*m*, 2 arom. H); 8.50 (br. *s*, H – N(3)). ^{13}C-NMR (75 MHz, $CDCl_3$): 11.9 (*d*, Me_2*C*H); 17.8 (*q*, *Me*$_2$CH); 24.3, 24.4, 28.4, 33.7, 34.1 (5*t*, CH_2); 55.3 (*q*, MeO); 62.8 (*t*, C(5′)); 74.1, 74.5, 83.3 (3*d*, C(2′), C(3′), C(4′)); 86.6 (*s*, arom. C); 87.5 (*d*, C(1′)); 89.7 (*t*, OCH_2O); 102.8 (*d*, C(5)); 113.5 (*d*, arom. C); 122.6, 125.4, 127.5, 128.3, 128.5, 130.4, 130.5 (7*d*, arom. C); 135.2, 135.4, (2*s*, arom. C); 140.2 (*d*, C(6)); 144.3, 145.6 (2*s*, arom. C); 150.4 (*s*, C(2)); 155.7 (*s*, arom. C); 159.1 (*s*, arom. C); 163.0 (*s*, C(4)); 171.3, 172.6 (2*s*, CO). FAB-MS: 995 (18, $[M + H]^+$), 303 (100, $[(MeO)_2Tr]^+$).

N^2-*Acetyl-5′-*O*-(4,4′-dimethoxytrityl)-2′-*O*-{[(triisopropylsilyl)oxy]methyl}guanosine 3′-(4-Nitrophenyl Heptanedioate)* (**25**). A soln. of **13** (8.1 g, 10 mmol) in Py (50 ml) was added during 3 h to a soln. of bis(4-nitrophenyl) heptanedioate (20 g, 50 mmol) and DMAP (1.6 g, 10 mmol) in Py (100 ml). After stirring for 72 h at 35°, the mixture was evaporated and co-evaporated twice with toluene (200 ml). CC (150 g of SiO_2, hexane/AcOEt 1:1 → AcOEt, then AcOEt → AcOEt/EtOH 9:1) gave **25** (6.5 g, 65%). Colorless foam. TLC (hexane/AcOEt 1:9): R_f 25. IR ($CHCl_3$): 3370*w*, 3005*w*, 2945*w*, 2870*w*, 1700*s*, 1680*s*, 1560*m*, 1525*m*, 1510*m*, 1490*w*, 1465*w*, 1415*w*, 1350*m*, 1300*m*, 1120*m*, 1035*m*, 885*w*, 830*w*. ^{1}H-NMR (300 MHz, $CDCl_3$): 0.91 – 1.00 (*m*, $^iPr_3Si$); 1.41 (*s*, MeCO); 1.48 (*m*, CH_2); 1.71 (*m*, CH_2); 1.79 (*m*, CH_2); 2.42 (*m*, CH_2); 2.62 (*t*, *J* = 7.5, CH_2); 3.17 (*dd*, *J* = 2.8,

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10.6, H–C(5′)); 3.55 (*dd*, $J = 2.2$, 10.6, H′–C(5′)); 3.78, 3.75 (2*s*, 2 MeO); 4.22 (br. *q*, $J \approx 2$, H–C(4′)); 4.85, 4.89 (2*d*, $J = 5.3$, OCH_2O); 5.47 (*dd*, $J = 5.3$, 7.8, H–C(2′)); 5.61 (*dd*, $J = 1.8$, 5.3, H–C(3′)); 5.85 (*d*, $J = 7.8$, H–C(1′)); 6.76–6.80 (*m*, 4 arom. H); 7.24–7.57 (*m*, 11 arom. H, NH–C(2)); 7.78 (*s*, H–C(8)); 8.23–8.28 (*m*, 2 arom. H); 11.77 (br. *s*, H–N(1)). ^{13}C-NMR (75 MHz, $CDCl_3$): 11.8 (*d*, Me_2CH); 17.6 (*q*, Me_2CH); 23.4 (*q*, *Me*CO), 24.3, 24.4, 28.4, 33.9, 34.1 (5*t*, CH_2); 55.4 (*q*, arom. H); 63.6 (*t*, C(5′)); 71.8, 76.3, 82.8 (3*d*, C(2′), C(3′), C(4′)); 86.6 (*d*, C(1′)); 86.9 (*s*, arom. C); 90.0 (*t*, OCH_2O); 113.5, 113.6 (2*d*, arom. C); 122.8 (*s*, C(5)); 122.6, 125.5, 126.3, 127.5, 128.3, 128.4, 128.6, 130.2, 130.3 (9*d*, arom. C); 135.7, 136.3 (2*s*, arom. C); 139.5 (*d*, C(8)); 145.2, 145.6 (2*s*, arom. C); 146.9 (*s*, C(4)); 148.5 (*s*, C(2)); 155.6 (*s*, C(6)); 155.7 (*s*, arom. C); 159.1 (*s*, arom. C); 171.4, 171.5, 172.9 (3*s*, CO). FAB-MS: 1077 (20, $[M+H]^+$), 303 (100, $[(MeO)_2Tr]^+$).

Preparation of Solid Supports. To a soln. of the active esters **22**–**25** (0.25 mmol) in DMF (8 ml) was added long-chain-alkylamino CPG (2 g) and then $^iPr_2NEt$ (0.8 ml). The mixtures were shaken for 20 h at r.t. After filtration, the solids were washed with DMF and CH_2Cl_2, dried, suspended in Py (5 ml) and Ac_2O (3 ml), and shaken for 2 h at r.t. After filtration, the solids were washed with DMF and CH_2Cl_2 and dried under high vacuum. Typical loadings of the solid supports were 30 and 20 µmol/g with 500-Å and 1000-Å CPG, respectively.

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Received June 26, 2001

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