Nucleotides

Part LXXII[1])

Synthesis of *N*-Methylated Ribonucleosides for RNA Synthesis

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The synthesis of various *N*-methylated nucleosides (m^6A, m^3C, m^4C, m^3U) is described. These minor nucleosides can be obtained by simple methylation with diazomethane of [2-(4-nitrophenyl)ethoxy]carbonyl(npeoc)-protected nucleosides. These methylated compounds are easily further derivatized to fit into the scheme of the [2-(dansyl)ethoxy]carbonyl (dnseoc) approach for RNA synthesis (dansyl = [5-(dimethylamino)naphthalen-1-yl]sulfonyl). Various oligoribonucleotides containing N^6-methyladenosine were synthesized, underlining the usefulness of the dnseoc approach, especially for the synthesis of natural tRNA-derived oligoribonucleotide sequences.

1. Introduction. – In addition to the standard ribonucleosides adenosine, guanosine, cytidine, and uridine, more than 100 so-called minor nucleosides have, so far, been found in nature, especially as components of tRNAs. These represent modified derivatives of the usual purine and pyridmidine bases and of 2′-*O*-methylribose. Despite their low abundance, most of them play important roles in various biological mechanisms. Among those minor nucleosides, the ones that show one or more *N*-methylations at the heterocyclic nucleobase can be grouped together in comparison to the standard nucleosides. Isolation from natural RNA led to the characterization of, *e.g.*, 1-methyl- (m^1A), 2-methyl- (m^2A), and N^6-methyladenosine (m^6A), N^3-methyl- (m^3C), N^4-methyl (m^4C) [2], and 5-methylcytidine (m^5C), N^1-methyl- (m^1G), N^2-methyl- (m^2G), N^2,N^2-dimethyl- ($m^2{}_2G$), and N^7-methylguanosine (m^7G), as well as N^3-methyluridine (m^3U) [3–5] (*Fig. 1*).

These *N*-methylated nucleosides play an important role, *e.g.*, in the investigation of certain receptors (m^6A) [6], mechanisms of splicing ($m^2{}_2G$) [7], regulation of genes (m^5C) [8], or in studies of the HIV problem (m^4C) [9]. Therefore, it would be beneficial to have easy synthetic access to these minor nucleosides that would allow their incorporation in oligoribonucleotides *via* commonly used phosphoramidite chemistry.

Performing RNA synthesis *via* phosphoramidite chemistry applying *N*-methylated ribonucleosides usually implies the introduction of the Me group in an early stage of the synthetic scheme. Therefore, a large number of extra synthetic steps have to be undertaken to finally reach the *N*-methylated phosphoramidites. Because of that, it would be desirable to introduce the Me groups at a later stage to avoid additional

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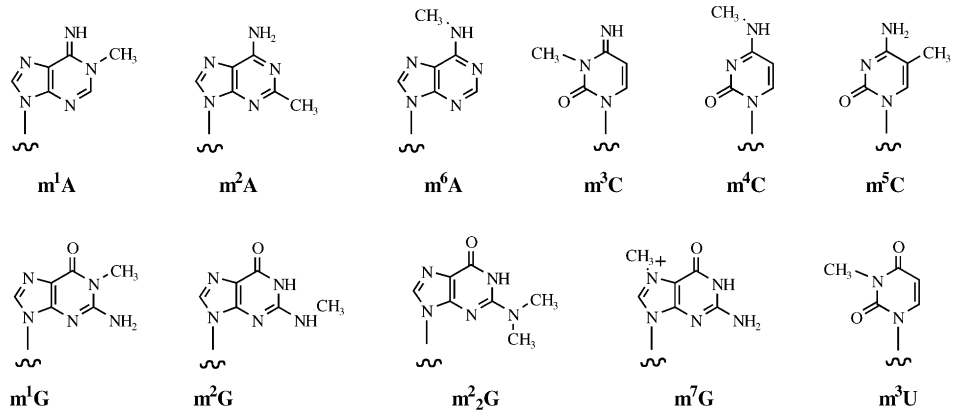


Fig. 1. *Methylated minor nucleosides isolated from natural RNA*

synthetic steps. For RNA synthesis, we followed the successful dnseoc strategy described by *Bergmann* and *Pfleiderer* [10] (*Fig. 2*). Within this strategy, the NH_2 functions of the heterocyclic bases are protected by the [2-(4-nitrophenyl)ethoxy]carbonyl group (npeoc) [11], which is cleavable by β-elimination with a non-nucleophilic base, *e.g.*, DBU (=1,8-diazabicyclo[5.4.0]undec-7-ene), in an aprotic solvent. Furthermore it was shown that it is of great advantage to protect also the lactam function of guanosine by applying the 2-(4-nitrophenyl)ethyl group (npe), which follows the same cleavage pattern. Compared to other strategies for RNA synthesis, the 5′-position is protected within the dnseoc approach by the highly base-labile (2-dansylethoxy)carbonyl group (dnseoc) (dansyl = [5-(dimethylamino)naphthalen-1-yl]sulfonyl). The 2′-position is blocked by the tetrahydro-4-methoxy-2*H*-pyran-4-yl group (mthp) [12] and the phosphate also by the 2-(4-nitrophenyl)ethyl group (npe). Since the temporary base-labile 5′-dnseoc and the permanent 2′-mthp protecting groups are strictly orthogonal, no loss of the 2′-protection has to be considered during machine-aided synthesis, compared to other strategies. Furthermore, combining this protection scheme with a DBU-stable linker system (LCAMA-CPG) [13], the removal of all protecting groups – except the 2′-*O*-mthp group – can be performed in one step with the oligomer still attached to the solid support. Therefore, all protecting-group residues can simply be washed away, making a time-consuming HPLC purification unnecessary. Ammonia treatment cleaves the oligomer from the support, and, in the final deprotection step, the removal of the 2′-*O*-mthp acetal function can be achieved by mildly acidic treatment [14], leading to RNA oligomers of high purity without any significant tendency to undergo strand scission.

2. Synthesis. – The purpose of these investigations was to make some of the naturally occurring *N*-methylated minor nucleosides easily available for phosphoramidite chemistry, starting from appropriately protected monomeric building blocks applied already in the dnseoc strategy for RNA synthesis. Generally, heterocyclic bases are known to be *N*-methylated either by direct alkylation with diazomethane [15],

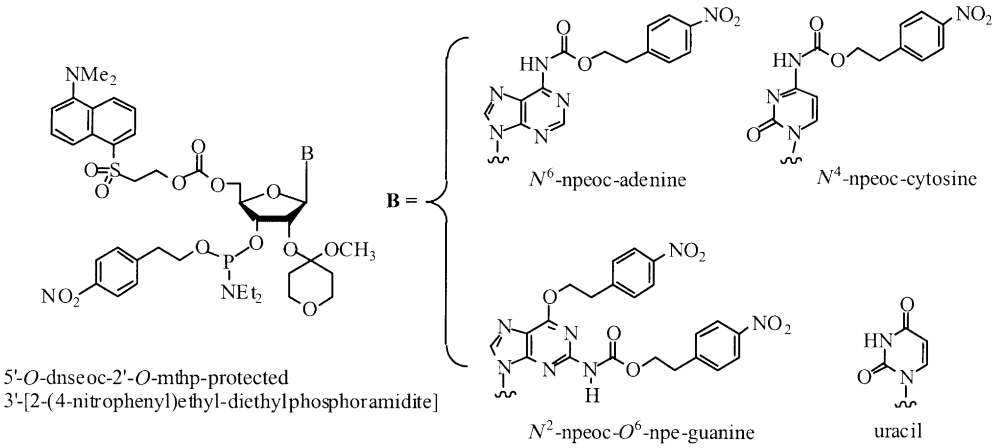


Fig. 2. *Protection scheme within the dnseoc approach*

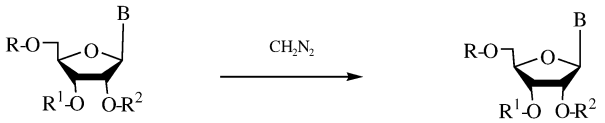
iodomethane [16][17], or dimethylsulfate [18], or by nucleophilic displacement of a chloro [19], fluoro [9], or triazolyl group [20].

Using npe/npeoc-protected nucleosides, *Sigmund* [21] obtained N^6-methyladenosine by a *Dimroth* rearrangement from N^1-methyl adenosine during deprotection of the sugar moiety. Based on the findings of *Cramer* and *Pfleiderer* [22], who isolated *N*-methylated nucleosides as by-products during the methylation of the 2′-*O*- and 3′-*O* position of ribonucleosides with diazomethane omitting a *Lewis* catalyst, we concentrated on the introduction of the Me groups by this procedure. Various *N*-methylated ribonucleosides could be obtained by attaching the Me group to the appropriately protected ribonucleosides on the latest stage of the synthesis to decrease the overall number of steps during the conventional approach.

Initially employing diazomethane as a mild methylation agent for the heterocyclic-moieties-protected ribonucleosides, 3′,5′-*O*-tipds-N^4-npeoc-cytidine (**1**; tips = 1,1,3,3-tetraisopropyldisiloxane-1,3-diyl), 2′-*O*-mthp-uridine (**2**), and 2′-*O*-mthp-N^2-npeoc-O^6-npe-guanosine (**3**) were subjected to diazomethane treatment (*Scheme 1*). Typically, 3–6 equiv. of diazomethane were employed in these reactions at 0°. The uridine and the cytidine derivatives showed relatively clean alkylations, giving only the expected N^3- and N^4-methylated products. From 3′,5′-*O*-tipds-N^4-npeoc-cytidine (**1**), two methylated products were obtained, the N^3-methyl derivative **4** and the N^4-methyl derivative **5**. Alkylation at the sterically less-hindered N^3-position was clearly favored over methylation at the N^4-position. As expected, 2′-*O*-mthp-uridine (**2**) was alkylated only at the N^3-position. Although up to 14 equiv. of diazomethane were used in the reaction of 2′-*O*-mthp-N^2-npeoc-O^6-npe-guanosine (**3**), no complete turnover was observed. Instead a complex mixture that could hardly be separated by chromatography was obtained. This mixture contained two methylated products and the educt. The major methylated product was obtained almost pure by several chromatographic purification steps. However, no clearcut assignment of the location of methylation was possible by NMR. Elemental analysis established that only a single methylation had taken place. From the similarity of the UV spectra of the isolated compound and the

educt, we suggest that methylation had taken place at the N^2-position. Alkylation at the N^1- or at the N^7-position would have altered the chromophore of the aromatic moiety more strongly, essentially resulting in a more-severe change of the UV spectrum of the compound.

Scheme 1. *Methylation of Cytidine and Uridine Nucleosides with Diazomethane*



	B	R	R1	R2
1	C^{npeoc}	tipds		mthp
2	U	H	H	mthp
3	G^{npe}_{npeoc}	H	H	mthp

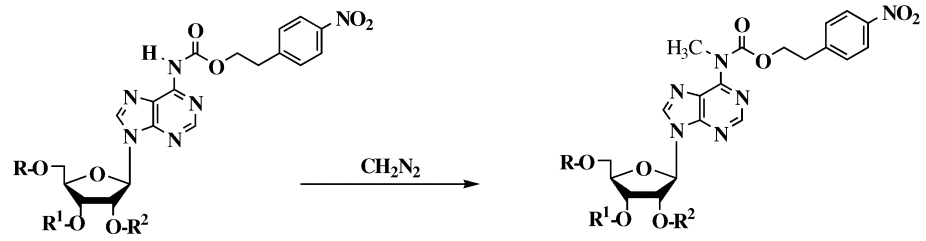
	B	R	R1	R2	%
4	m^3C^{npeoc}	tipds		mthp	55
5	m^4C^{npeoc}	tipds		mthp	30
6	m^3U	H	H	mthp	72
7	$m^2G^{npe}_{npeoc}$	H	H	mthp	13

To explore in more detail at which stage the Me groups should be introduced, we subjected various npe/npeoc-protected adenosine compounds **8**–**12** with different substitution patterns to the diazomethane reaction (*Scheme 2*). Besides the common mthp acetal for 2′-protection, the very acid-labile 2-methoxypropan-2-yl (=1-methoxy-1-methylethyl) group (mep) was tested. All reactions performed well, giving exclusively the N^6-methylated products **13**–**17** in good yields. This underlined the finding of *Cramer* and *Pfleiderer* [22] that, omitting the *Lewis* catalyst, methylation with diazomethane takes place only at the moderately nucleophilic amino position of the aglycone, no matter whether or not the sugar OH groups are protected. Even the 2′-*O*-mep protection proved to be orthogonal to the diazomethane reaction. However, in the methylation of the 5′-*O*-dnseoc-protected derivative **12** with diazomethane, unexpectedly, loss of the 5′-*O*-dnseoc protecting group was observed simulteanously with N^6-methylation. It seems that the basicity of diazomethane is strong enough to promote β-elimination of the dnseoc group. Therefore, the introduction of the Me group into the heterocylic bases with diazomethane has to be performed one step prior to the introduction of the 5′-*O*-dnseoc group. Nevertheless, only one extra synthetic step is necessary to make the minor *N*-methylated nucleosides available for RNA synthesis, since all protecting groups (npeoc, npe, mthp, mep) employed in the dnseoc strategy are stable during diazomethane methylation.

Interesting to note is that less selectivity was observed when the N^6-benzoylated adenosine compound **18** was submitted to the diazomethane reaction as compared to the npeoc-protected analogues **8**–**12**. Additional methylation was observed at the N^1-position and at the carbonyl function of the benzoyl moiety (*Scheme 3*).

To make the *N*-methylated nucleosides of uridine, cytidine, and adenosine available for machine-aided RNA synthesis *via* the dnseoc strategy, the *N*-methylated compounds were processed according to the usual protection/deprotection scheme (*Scheme 4*). Starting from the 3′,5′-*O*-tipds derivative **14**, the 2′-*O*-mthp group was introduced by means of 5,6-dihydro-4-methoxy-2*H*-pyran under acidic catalysis to give **15**. Then, the cyclic silyl group was removed from **4**, **5**, and **15** in the usual manner by

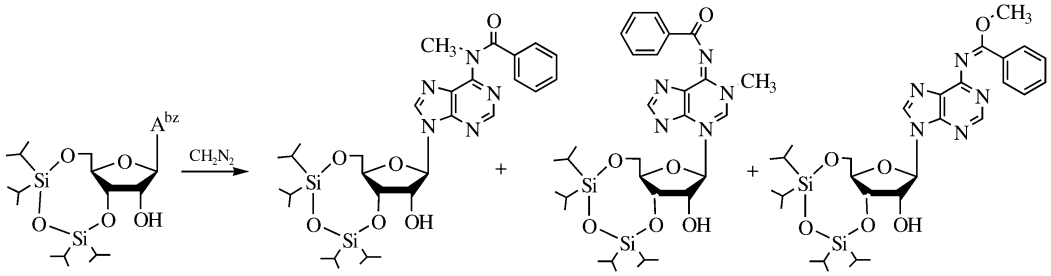
Scheme 2. N-*Methylation of* N^6*-npeoc-Protected Adenosine Derivatives*



	R	R^1	R^2
8	H	H	H
9	tipds		H
10	tipds		mthp
11	tipds		mep
12	dnseco	H	mthp

	R	R^1	R^2	%
13	H	H	H	65
14	tipds		H	54
15	tipds		mthp	75
16	tipds		mep	85
17	dnseco	H	mthp	–

Scheme 3. *Reaction of 3′,5′-*O*-tipds-*N^6*-benzoyladenosine* (**18**) *with Diazomethane*



18 **19** (33 %) **20** (11 %) **21** (22 %)

Bu_4NF, resulting in formation of the 2′-*O*-mthp protected derivatives **22**–**24**. In the following step, the dnseoc group was selectively introduced at the 5′-*O*-position of **22**–**24** and **6** by means of the hydrochloride salt of 2-(dansyl)ethyl carbonochloridate leading to **17** and **25**–**27**. The intermediary adenosine derivative **17** was then converted to the highly reactive diethylphosphoramidite **28** as well as the moderately reactive diisopropylphosphoramidites **29** and **30** by standard procedures. For attachment of the starting nucleoside to the solid support, the 5′-*O*-dnseoc-2′-*O*-mthp-*N*^{6}-npeoc-adenosine (**17**) was reacted with succinic anhydride to give the succinate **31**, which was used to derivatize the solid support 1400-Å LCAMA-CPG according to the reported procedure [23].

Having synthesized all the necessary building blocks for machine-aided oligoribonucleotide synthesis, *N*^{6}-methylated adenosine was incorporated first in homomeric (see **P1** and **P2**) and then in mixed oligoribo sequences (see **P3**–**P8**; *Table*). The *N*^{6}-methyladenosine-containing ribonucleotides represent parts of the anticodon loop of natural t-RNAs (RV 1460, RV 1180, RV 1662, RY 1440, RG 1140, RV 1180).

Scheme 4. *Synthesis of* N-*Methylated Ribonucleosides*

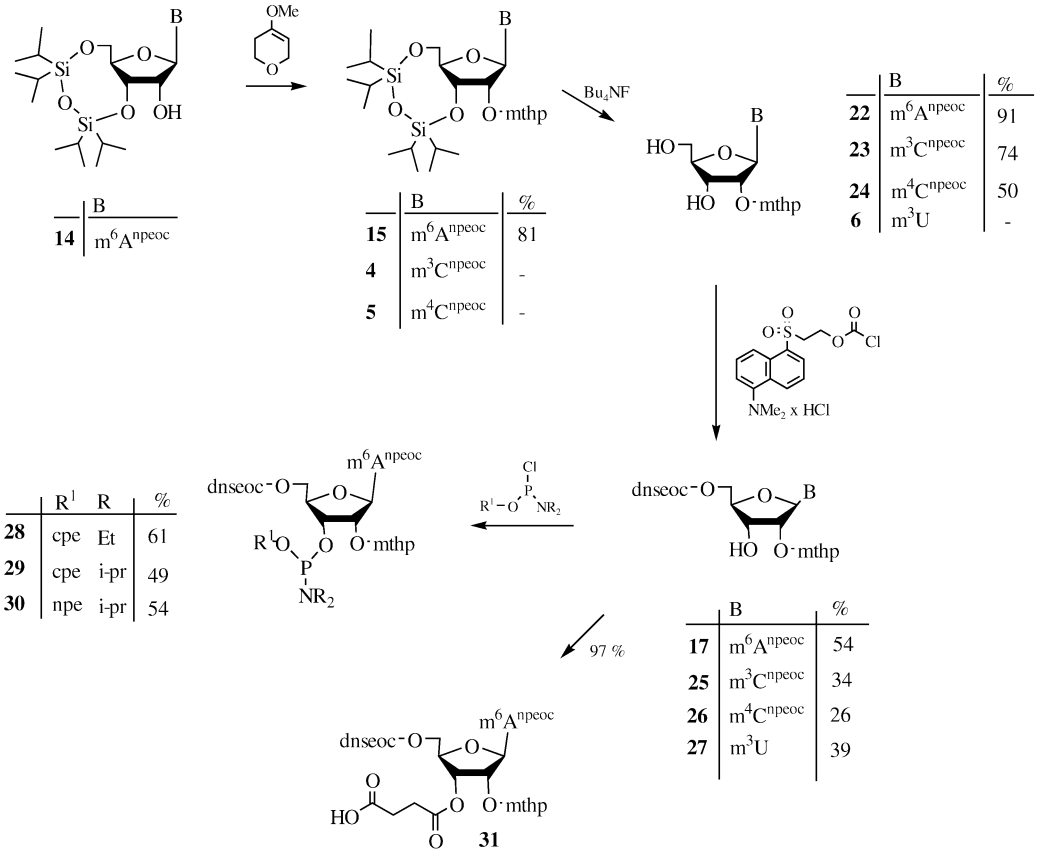


Table. *Oligoribonucleotide Sequences*

	RNA sequences
P1	$m^6Am^6Am^6Am^6Am^6Am^6A$
P2	$m^6Am^6Am^6Am^6Am^6Am^6Am^6Am^6Am^6A$
P3	CAC CUU GAC **m^6A**
P4	AC**m^6A** AGC AGG CG
P5	AC**m^6A** AGG AGG GG
P6	CUG UA**m^6A** CCCACUU
P7	UUCC**m^6A** AGC UGA UU
P8	AGA GCU CCU GCC UUA C**m^6A**

RNA Syntheses were performed on a *ABI-392-DNA* synthesizer according to a standard protocol for RNA synthesis employing 5′-*O*-dnseoc-protected phosphoramidites. Instead of 1*H*-tetrazole, 0.5M pyridine hydrochloride in MeCN was used as the activator of choice [24], resulting in a shortened coupling step of 280 s without loss of performance. Post-synthesis manipulations included overnight treatment with 1M DBU

at room temperature for the removal of the npe and npeoc groups and treatment with conc. NH_3 solution (55°) to cleave off the 2′-*O*-mthp-oligoribonucleotide from the solid support, followed by analysis by reversed-phase and ion-exchange HPLC (*Fig. 3*). Final removal of the 2′-*O*-mthp groups was accomplished by an overnight treatment with 0.5M AcOH/NaOAc buffer pH 3.25. After neutralization, the free oligoribonucleotides **P1** – **P8** were isolated by precipitation with EtOH.

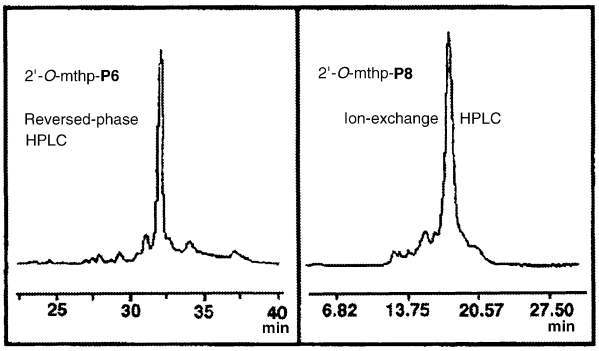


Fig. 3. *HPLC of 2′-*O*-mthp-protected oligoribonucleotides*

HPLC Analysis of all oligoribonucleotides showed high purity of the crude products (*Fig. 4*). Enzymatic digestion of the free oligoribonucleotides with alkaline phosphatase and snake-venom phosphodiesterase gave the expected ratios of the five mononucleosides when analyzed by reversed-phase HPLC (*Fig. 5*).

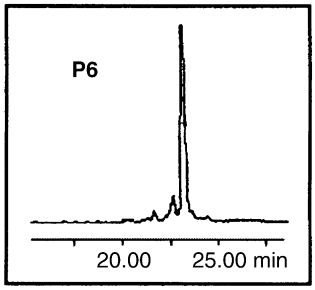


Fig. 4. *Reversed-phase HPLC of ribonucleotide* **P6**

3. Conclusions. – We were able to demonstrate an easy approach to the preparation of the appropriately protected monomeric units of N^6-methyladenosine, N^3-methyl- and N^4-methylcytidine, and N^3-methyluridine prone to RNA synthesis *via* the dnseoc strategy. Various oligoribonucleotide sequences containing N^6-methyl adenosine were synthesized successfully. The applied blocking-group strategy allows the isolation of the 2′-*O*-mthp-oligoribonucleotides as stable storage forms that can easily be converted to the free oligoribonucleotides by mild acid treatment. The dnseoc strategy for RNA synthesis can be regarded as a powerful tool, especially for chemical synthesis of tRNA sequences of biological interest containing minor nucleotides.

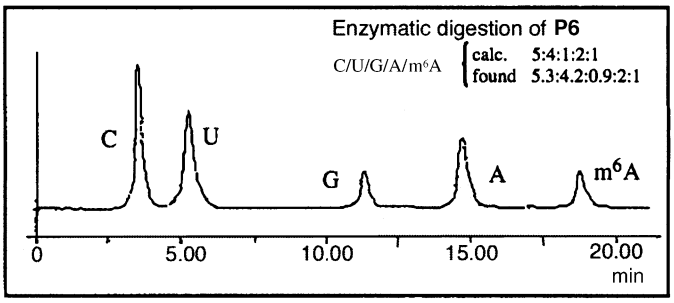


Fig. 5. *HPLC Analysis of enzymatic digestion of* **P6**

Experimental Part

General. Products were dried under high vacuum. All solvents used were anh. grade. TLC: precoated silica-gel thin-layer sheets *60 F254* from *Merck*. Flash chromatography (FC): silica gel (*Baker*, 30–60 μm); 0.2–0.3 bar. M.p.: *Gallenkamp* melting-point apparatus; no corrections. UV/VIS: *Perkin-Elmer Lambda 5*; λ_{max} in nm (log ε). ^{1}H-NMR: *Bruker AC-250*; δ in ppm rel. to $SiMe_4$ or $CDCl_3$ ((D_6)DMSO) as internal standard. ^{31}P-NMR: *Jeol JMN-GX400*.

1. *Diazomethane*. A 2-layer mixture of 1,2-dimethoxyethane (50 ml) and 40% KOH soln. (33 ml) was cooled to 0°, and then *N*-methyl-*N*-nitrosourea (10 g) was added carefully in small portions within 30 min keeping the temp. of the mixture below +1°. After additional stirring for 20 min at this temp., the org. phase was separated and the aq. layer washed with 1,2-dimethoxyethane. The combined org. phase was dried for 4 h at 0° over KOH pellets. The solid was separated and the *ca.* 1M diazomethane soln. in 1,2-dimethoxyethane directly used for the methylation reactions.

2. N^3-*Methyl*-N^4-*{[2-(4-nitrophenyl)ethoxy]carbonyl}-2′*-O-*(tetrahydro-4-methoxy-2*H*-pyran-4-yl)-3′,5′*-O-*(1,1,3,3-tetraisopropyldisiloxane-1,3-diyl)cytidine* (**4**) *and* N^4-*Methyl*-N^4-*{[2-(4-nitrophenyl)ethoxy]carbonyl}-2′*-O-*(tetrahydro-4-methoxy-*2H*-pyran-4-yl)-3′,5′*-O-*(1,1,3,3-tetraisopropyldisiloxane-1,3-diyl)cytidine* (**5**). A soln. of *N*^{4}-{[2-(4-nitrophenyl)ethoxy]carbonyl}-2′-*O*-(tetrahydro-4-methoxy-2*H*-pyran-4-yl)-3′,5′-*O*-(1,1,3,3-tetraisopropyldisiloxane-1,3-diyl)cytidine (**1**; 3.97 g, 5 mmol) in toluene (30 ml) was cooled to 0°, and then 1M CH_2N_2 in 1,2-dimethoxyethane (20 ml) was added dropwise within 20 min. The mixture was stirred for 9 h at 0°, the ice-bath removed, and stirring at r.t. continued overnight. The mixture was evaporated and purification achieved by CC (14–17% acetone/hexane): 2.2 g (55%) of **4** and 1.22 (30%) of **5**.

Data of **4**: R_f (hexane/acetone 3:2) 0.69. UV (MeOH): 282 (4.38), 213 (sh, 4.25). ^{1}H-NMR ((D_6)DMSO): 8.15 (*d*, 2 H_o to NO_2); 7.67 (*d*, H–C(6)); 7.54 (*d*, 2 H_m to NO_2); 6.18 (*d*, H–C(5)); 5.64 (*s*, H–C(1′)); 4.48 (*d*, H–C(2′)); 4.32 (*t*, CH_2CH_2O); 4.18 (*m*, H–C(3′)); 4.16 (*m*, 1 H–C(5′)); 4.03 (*m*, H–C(4′)); 3.92 (*m*, 1 H–C(5)); 3.66–3.47 (2*m*, CH_2OCH_2); 3.22 (2*s*, MeN, MeO); 3.08 (*t*, CH_2CH_2O); 1.75–1.83 (2*m*, CH_2CCH_2); 0.92–1.05 (*m*, 4 Me_2CH). Anal. calc. for $C_{37}H_{58}N_4O_{12}Si_2$ (807.1): C 55.06, H 7.24, N 6.94; found: C 54.92, H 7.22, N 6.84.

Data of **5**: R_f (hexane/acetone 3:2) 0.59. UV (MeOH): 284 (sh, 4.21), 251 (4.31), 203 (sh, 4.51). ^{1}H-NMR ((D_6)DMSO): 8.17 (*d*, 2 H_o to NO_2); 8.10 (*d*, H–C(6)); 7.58 (*d*, 2 H_m, to NO_2); 7.07 (*d*, H–C(5)); 5.67 (*s*, H–C(1′)); 4.46 (*t*, CH_2CH_2O); 4.41 (*m*, H–C(2′)); 4.19 (*m*, H–C(3′), 1 H–C(5′)); 4.09 (*m*, H–C(4′)); 3.93 (*m*, 1 H–C(5′)); 3.48, 3.67 (2*m*, CH_2OCH_2); 3.23 (*s*, MeN); 3.22 (*s*, MeO); 3.14 (*t*, CH_2CH_2O); 1.94, 1.77 (2*m*, CH_2CCH_2); 1.91–1.05 (*m*, 4 Me_2CH). Anal. calc. for $C_{37}H_{58}N_4O_{12}Si_2$ (807.1): C 55.06, H 7.24, N 6.94; found: C 54.91, H 7.22, N 6.98.

3. N^3-*Methyl-2′*-O-*(tetrahydro-4-methoxy-2*H*-pyran-4-yl)-uridine* (**6**). According to *Exper. 2*, 2′-*O*-(tetrahydro-4-methoxy-2*H*-pyran-4-yl)uridine (**2**; 1.07 g, 3 mmol) in CH_2Cl_2 (30 ml) and AcOEt (15 ml) was treated with 1M CH_2N_2 (20 ml). After evaporation of the mixture, the residue was suspended in AcOEt, whereby on stirring, 0.6 g (54%) of crystalline **6** separated out and was collected by filtration. Purification of the filtrate by CC (silica gel, toluene/AcOEt 5:4 with 0–3.5% MeOH) gave additional 0.21 g (18%) of **6**. R_f ($CHCl_3$/MeOH 9:1) 0.30. UV (MeOH): 260 (3.89), 207 (3.92). ^{1}H-NMR ((D_6)DMSO): 7.99 (*d*, H–C(6)); 6.05 (*d*, H–C(5)); 5.86 (*d*, H–C(1′)); 5.21 (*t*, OH–C(5′)); 5.17 (*d*, OH–C(3′)); 4.33 (*m*, H–C(2′)); 3.97 (*m*, H–C(3′)); 3.91

(*m*, H–C(4′)); 3.30–3.80 (*m*, CH_2OCH_2, 2 H–C(5′)); 3.15 (*s*, MeN); 2.91 (*s*, MeO); 1.55–1.80 (*m*, CH_2CCH_2). Anal. calc. for $C_{16}H_{24}N_2O_8$ (372.4): C 51.61, H 6.50, N 7.52; found: C 51.28, H 6.63, N 7.30.

4. N^2-*Methyl*-N^2-*{[2-(4-nitrophenyl)ethoxy]carbonyl}*-O^6-*[2-(4-nitrophenyl)ethyl](tetrahydro-4-methoxy-2*H*-pyran-4-yl)guanosine* (**7**). A soln. of **3** (1.7 g, 1.73 mmol) in a mixture of toluene (15 ml) and CH_2Cl_2 was cooled to 0° and then, under stirring, 1M diazomethane in 1,2-dimethoxyethane (25 ml) was dropwise slowly added within 20 min. After stirring for 9 h at 0°, the mixture was evaporated and the residue purified by CC (silica gel (50 g), toluene/AcOEt 5:4 with 0–4% MeOH) to give, in the first fraction, 0.165 g (13%) of **7** as a solid foam. The following fractions consisted of substance mixtures. **7**: R_f (toluene/AcOEt/MeOH 5:4:1) 0.33. UV (MeOH): 216 (4.49), 267 (5.00). ^{1}H-NMR ((D$_6$)DMSO): 8.60 (*s*, H–C(8)); 8.17 (*d*, 2 H_o to NO_2); 8.05 (*d*, 2 H_o to NO_2); 7.58 (*d*, 2 H_m to NO_2); 7.44 (*d*, 2 H_m to NO_2); 6.02 (*d*, H–C(1′)); 5.26 (*d*, OH–C(3′)); 5.09 (*t*, OH–C(5′)); 4.88 (*m*, H–C(2′)); 4.72 (*t*, CH_2CH_2O); 4.36 (*t*, CH_2CH_2O); 4.15 (*m*, H–C(3′)); 3.99 (*m*, H–C(4′)); 3.68–3.55 (*m*, 2 H–C(5′), 1 H of CH_2OCH_2); 3.38 (*m*, 2 H of CH_2OCH_2); 3.27 (*m*, 1 H of CH_2OCH_2, CH_2CH_2O, MeN); 3.18 (*m*, CH_2CH_2O); 2.56 (*s*, MeO); 1.76–1.43 (*m*, CH_2CCH_2). Anal. calc. for $C_{34}H_{39}N_7O_{13}$ (753.7): C 54.18, H 5.21, N 13.01; found: C 53.95, H 5.33, N 12.17.

5. N^6-*Methyl*-N^6-*{[2-(4-nitrophenyl)ethoxy]carbonyl}adenosine* (**13**) [22]. Analogously to *Exper. 2*, with N^6-{[2-(4-nitrophenyl)ethoxy]carbonyl}adenosine (**8**; 2.31 g, 5 mmol) in anh. DMF (50 ml) and 1M CH_2N_2 (20 ml). Purification by CC (0–50% acetone/petroleum ether) gave 1.54 g (65%) of **13** ([22]: 72%).

6. N^6-*Methyl*-N^6-*{[2-(4-nitrophenyl)ethoxy]carbonyl}-3′,5′*-O-*(1,1,3,3-tetraisopropyldisiloxane-1,3-diyl)adenosine* (**14**). 6.1. Analogously to *Exper. 2*, with N^6-{[2-(4-nitrophenyl)ethoxy]carbonyl}-3′,5′-*O*-(1,1,3,3-tetraisopropyldisiloxane-1,3-diyl)adenosine (**9**; 4.64 g, 6.61 mmol) in DMF (50 ml) and 1M CH_2N_2 (20 ml). Purification by CC (toluene/AcOEt 5:4 with 0–1.5% MeOH) gave 2.56 g (54%) of **14**.

6.2. After co-evaporation with anh. pyridine, **13** (1.46 g, 3.08 mmol) was dissolved in anh. pyridine (30 ml), and 1,3-dichloro-1,1,3,3-tetraisopropyldisiloxane (1.1 ml, 3.39 mmol) was added. After 3 d, the reaction was quenched with MeOH, the mixture evaporated, and the residue dissolved in CH_2Cl_2 (50 ml) and extracted with $NaHCO_3$ soln. (100 ml). The org. layer was dried ($MgSO_4$) and purified by CC (toluene/AcOEt 5:4 with 0–1.5% MeOH): 1.66 g (75%) of **14**. R_f (toluene/AcOEt 1:1) 0.35. UV (MeOH): 272 (4.36), 212 (4.39). ^{1}H-NMR ((D$_6$)DMSO): 8.65 (*s*, H–C(2)); 8.52 (*s*, H–C(8)); 8.02 (*d*, 2 H_o to NO_2); 7.26 (*d*, 2 H_m to NO_2); 5.96 (*s*, H–C(1′)); 5.69 (*d*, OH–C(2′)); 4.75 (*m*, H–C(3′)); 4.60 (*t*, H–C(2′)); 4.35 (*m*, CH_2CH_2CO); 4.04 (*m*, H–C(4′), 2 H–C(5′)); 3.35 (*s*, MeN); 2.96 (*t*, CH_2CH_2O); 1.02 (*m*, 4 Me_2CH). Anal. calc. for $C_{32}H_{48}N_6O_9Si_2$ (716.95): C 53.61, H 6.75, N 11.72; found: C 53.66, H 6.76, N 11.73.

7. N^6-*Methyl*-N^6-*{[2-(4-nitrophenyl)ethoxy]carbonyl}-2′*-O-*(tetrahydro-4-methoxy-2*H*-pyran-4-yl)-3′,5′*-O-*(1,1,3,3-tetraisopropyldisiloxane-1,3-diyl)adenosine* (**15**). 7.1. Analogously to *Exper. 2*, with 2′-*O*-mthp-N^6-{[2-(4-nitrophenyl)ethoxy]carbonyl}-3′,5′-*O*-(1,1,3,3-tetraisopropyldisiloxane-1,3-diyl)adenosine (**10**; 2.07 g, 2.53 mmol) in CH_2Cl_2 (40 ml) and MeOH (2 ml) and 1M CH_2N_2 (24 ml). Purification by CC (0–20% acetone/petroleum ether) gave 1.58 g (75%) of **15**.

7.2. To **14** (2.0 g, 2.78 mmol) and $TsOH \cdot H_2O$ (53 mg, 0.278 mmol) in anh. CH_2Cl_2 (20 ml) was added 5,6-dihydro-4-methoxy-2*H*-pyran (1.80 g, 65%, 10.2 mmol) and the mixture was stirred for 7 h. The reaction was quenched with sodium methoxide and the mixture extracted with CH_2Cl_2 (70 ml) and sat. $NaHCO_3$ soln. (100 ml). The org. layer was dried ($MgSO_4$) and evaporated and the residue purified by CC (0–20% acetone/petroleum ether): 1.87 g (81%) of **15**. R_f (toluene/AcOEt/MeOH 5:4:1) 0.80. UV (MeOH): 272 (4.32), 212 (4.35). ^{1}H-NMR ((D$_6$)DMSO): 8.64 (*s*, H–C(2)); 8.56 (*s*, H–C(8)); 8.02 (*d*, 2 H_o to NO_2); 7.26 (*d*, 2 H_m to NO_2); 6.10 (*s*, H–C(1′)); 4.90 (*m*, H–C(3′), H–C(2′)); 4.35 (*m*, CH_2CH_2O); 4.05 (*m*, H–C(4′), 2 H–C(5′)); 3.80–3.30 (2*m*, CH_2OCH_2); 3.35 (*s*, MeN); 3.16 (*s*, MeO); 2.96 (*t*, CH_2CH_2O); 1.78 (*m*, CH_2CCH_2); 1.02 (*m*, 4 Me_2CH). Anal. calc. for $C_{38}H_{58}N_6O_{11}Si_2$ (831.1): C 54.92, H 7.03, N 10.11; found: C 55.13, H 7.17, N 10.50.

8. 2′-O-*1-Methoxy-1-methylethyl)*-N^6-*methyl*-N^6-*{[2-(4-nitrophenyl)ethoxy]carbonyl}-3′,5′*-O-*(1,1,3,3-tetraisopropyldisiloxane-1,3-diyl)adenosine* (**16**). Analogously to *Exper. 2*, with 2′-*O*-1-methoxy-1-methylethyl)-N^6-{[2-(4-nitrophenyl)ethoxy]carbonyl}-3′,5′-*O*-(1,1,3,3-tetraisopropyldisiloxane-1,3-diyl)adenosine (**11**; 2.63 g, 3.39 mmol) in DMF (50 ml) and 1M CH_2N_2 (14 ml). Purification by CC (0–50% AcOEt/toluene) gave 2.27 g (85%) of **16**. R_f (toluene/AcOEt 1:1) 0.43. UV (MeOH): 326 (sh, 3.34), 272 (4.33), 212 (4.37). ^{1}H-NMR ((D$_6$)DMSO): 8.64 (*s*, H–C(2)); 8.57 (*s*, H–C(8)); 8.01 (*d*, 2 H_o to NO_2); 7.25 (*d*, 2 H_m to NO_2); 6.05 (*s*, H–C(1′)); 4.85 (*m*, H–C(2′), H–C(3′)); 4.36 (*m*, CH_2CH_2O); 3.96 (*m*, H–C(4′), 2 H–C(5′)); 3.35 (*s*, MeN); 3.12 (*s*, MeO); 2.95 (*t*, CH_2CH_2O); 1.32 (2*s*, 2 Me); 1.02 (*m*, 4 Me_2CH). Anal. calc. for $C_{36}H_{56}N_6O_{10}Si_2 \cdot 0.25$ toluene (812.1): C 55.83, H 7.20, N 10.35; found: C 56.20, H 7.41, N 10.69.

9. *5′*-O-*{{2-{[5-(Dimethylamino)naphthalen-1-yl]sulfonyl}ethoxy}carbonyl}*-N^6-*methyl*-N^6-*{[2-(4-nitrophenyl)ethoxy]carbonyl}2′*-O-*(tetrahydro-4-methoxy-2*H*-pyran-4-yl)adenosine* (**17**). After co-evaporation with anh. pyridine, N^6-methyl-N^6-{[2-(4-nitrophenyl)ethoxy]carbonyl}-2′-*O*-(tetrahydro-4-methoxy-2*H*-pyran-4-

yl)adenosine (**22**; 1.05 g, 1.78 mmol) was dissolved in anh. pyridine (20 ml) and cooled to 0°. Then 2-{[5-(dimethylamino)naphthalen-1-yl]sulfonyl}ethyl carbonochloridate hydrochloride (0.742 g) was added. The reaction was quenched after 75 min with MeOH and the mixture evaporated. The residue was extracted with CH_2Cl_2, the org. phase washed with sat. $NaHCO_3$ soln. (100 ml), dried ($MgSO_4$), and evaporated, and the residue purified by CC (toluene/AcOEt 5:4 with 0–4% MeOH): 0.86 g (54%) of **17**. R_f (toluene/AcOEt/MeOH 5:4:1) 0.51. UV (MeOH): 343 (3.67), 261 (4.48), 213 (4.82). ^{1}H-NMR ((D$_6$)DMSO): 8.73 (2*s*, H–C(2), H–C(8)); 8.51 (*d*, H–C(2)(npht)); 8.15 (*m*, H–C(4)(npht), H–C(8)(npht); 8.04 (*d*, 2 H$_o$ to NO$_2$); 7.64 (*m*, H–C(3)(npht), H–C(7)(npht)); 7.34 (*d*, 2 H$_m$ to NO$_2$); 7.24 (*d*, H–C(6)(npht)); 6.13 (*d*, H–C(1′)); 5.52 (*m*, OH–C(3′)); 5.05 (*m*, H–C(2′)); 4.35 (2*t*, 2 CH$_2$C*H*$_2$O); 4.20 (*m*, H–C(3′), H–C(4′)); 3.83 (*t*, CH$_2$SO$_2$); 3.20–3.70 (*m*, 2 H–C(5′), CH$_2$OCH$_2$); 3.37 (*s*, MeN); 2.95 (*t*, C*H*$_2$CH$_2$O); 2.79 (*s*, Me$_2$N); 2.48 (*s*, MeO); 1.60 (2*m*, CH$_2$CCH$_2$). Anal. calc. for $C_{41}H_{47}N_7O_{14}S$ (893.9): C 55.09, H 5.30, N 10.97; found: C 55.48, H 5.54, N 10.21.

10. N^6-*Benzoyl*-N^6-*methyl*-3′,5′-O-*(1,1,3,3-tetraisopropyldisiloxane-1,3-diyl)adenosine* (**19**), N^6-*Benzoyl*-N^1-*methyl*-3′,5′-O-*(1,1,3,3-tetraisopropyldisiloxane-1,3-diyl)adenosine* (**20**), *and* N^6-*[Methoxy(phenyl)methylene]*-3′,5′-O-*(1,1,3,3-tetraisopropyldisiloxane-1,3-diyl)purine* (**21**). Analogously to *Exper. 2*, with *N*^{6}-benzoyl-3′,5′-*O*-(1,1,3,3-tetraisopropyldisiloxane-1,3-diyl)adenosine (**18**; 4.0 g, 6.51 mmol) in CH_2Cl_2 (40 ml) with 1M CH_2N_2 (20 ml). Purification by CC (14–50% acetone/petroleum ether) gave 1.37 g (33%) of **19**, 0.44 g (11%) of **20**, and 0.91 g (22%) of **21**.

Data of **19**: R_f (petroleum ether/AcOEt 3:2) 0.63. UV (MeOH): 280 (4.10), 206 (4.40). ^{1}H-NMR ((D$_6$)DMSO): 8.50 (2*s*, H–C(2), H–C(8)); 7.31 (*m*, 3 H, bz); 7.20 (*m*, 2 H, bz); 5.92 (*s*, H–C(1′)); 5.66 (*d*, OH–C(2′)); 4.67 (*m*, H–C(3′)); 4.53 (*m*, H–C(2′)); 3.99–4.04 (*m*, 1 H–C(5′), H–C(4′)); 3.91 (*m*, 1 H–C(5′)); 3.65 (*s*, MeN); 0.94–1.02 (*m*, 4 Me$_2$CH). Anal. calc. for $C_{30}H_{45}N_5O_6Si_2$ (627.9): C 57.39, H 7.22, N 11.15; found: C 57.36, H 7.13, N 11.05.

Data of **20**: R_f (petroleum ether/AcOEt 3:2) 0.42. UV (MeOH): 312 (4.08), 286 (3.99), 236 (4.23), 204 (4.48). ^{1}H-NMR ((D$_6$)DMSO): 8.37 (*s*, H–C(2)); 7.97 (*s*, H–C(8)); 7.92 (*d*, 2 H, bz); 7.49 (*m*, 1 H, bz); 7.41 (*m*, 2 H, bz); 5.77 (*s*, H–C(1′)); 5.60 (*d*, OH–C(2′)); 4.59 (*m*, H–C(3′)); 4.46 (*m*, H–C(2′)); 3.90–4.01 (*m*, H–C(4′), 2 H–C(5′)); 3.66 (*s*, MeN); 0.95–1.03 (*m*, Me$_2$CH). Anal. calc. for $C_{30}H_{45}N_5O_6Si_2$ (627.9): C 57.39, H 7.22, N 11.15; found: C 57.21, H 7.25, N 10.93.

Data of **21**: R_f (petroleum ether/AcOEt 3:2) 0.57. UV (MeOH): 266 (4.02), 239 (4.10), 207 (4.41). ^{1}H-NMR ((D$_6$)DMSO): 8.49 (*s*, H–C(2)); 8.35 (*s*, H–C(8)); 7.23–7.36 (*m*, 5 H, bz); 5.90 (*s*, H–C(1′)); 5.66 (*d*, OH–C(2′)); 4.69 (*m*, H–C(3′)); 4.56 (*m*, H–C(2′)); 4.04 (*s*, MeO); 4.01 (*m*, 1 H–C(5′), H–C(4′)); 3.90 (*m*, 1 H–C(5′)); 0.98 (*m*, 4 Me$_2$CH). Anal. calc. for $C_{30}H_{45}N_5O_6Si_2$ (627.9): C 57.39, H 7.22, N 11.15; found: C 57.63, H 7.42, N 10.41.

11. N^6-*Methyl*-N^6-*{[2-(4-nitrophenyl)ethoxy]carbonyl}*-2′-O-*(tetrahydro-4-methoxy-2*H*-pyran-4-yl)adenosine* (**22**). To *N*^{6}-methyl-*N*^{6}-{[2-(4-nitrophenyl)ethoxy]carbonyl}-3′,5′-*O*-(1,1,3,3-tetraisopropyldisiloxane-1,3-diyl)adenosine (**15**; 1.82 g, 2.19 mmol) in anh. THF (25 ml) was added Bu_4NF (2.0 g, 6.34 mmol). After 10 min, the solvent was evaporated, the residue dissolved in CH_2Cl_2 (80 ml), and the org. soln. washed with sat. $NaHCO_3$ soln. (100 ml), dried ($MgSO_4$), and adsorbed on silica gel. Purification by CC (toluene/AcOEt 5:4 with 0–4% MeOH) gave 1.17 g (91%) of **22**. R_f (toluene/AcOEt/MeOH 5:4:1) 0.22. UV (MeOH): 272 (4.37), 211 (4.39). ^{1}H-NMR ((D$_6$)DMSO): 8.77 (2*s*, H–C(2), H–C(8)); 8.05 (*d*, 2 H$_o$ to NO$_2$); 7.35 (*d*, 2 H$_m$ to NO$_2$); 6.13 (*d*, H–C(1′)); 5.32 (*d*, OH–C(3′)); 5.23 (*t*, OH–C(5′)); 4.90 (*m*, H–C(2′)); 4.36 (*t*, CH$_2$C*H*$_2$O); 4.16 (*m*, H–C(3′)); 4.02 (*m*, H–C(4′)); 3.75–3.15 (3*m*, 2 H–C(5′), CH$_2$OCH$_2$); 3.38 (*s*, MeN); 2.97 (*t*, C*H*$_2$CH$_2$O); 2.44 (*s*, MeO); 1.60 (*m*, CH$_2$CCH$_2$). Anal. calc. for $C_{26}H_{32}N_6O_{10}\cdot 0.5\ H_2O$ (597.6): C 52.26, H 5.57, N 14.06; found: C 52.20, H 5.67, N 13.40.

12. N^3-*Methyl*-N^4-*{[2-(4-nitrophenyl)ethoxy]carbonyl}*-2′-O-*(tetrahydro-4-methoxy-2*H*-pyran-4-yl)cytidine* (**23**). A soln. of *N*^{3}-methyl-*N*^{4}-{[2-(4-nitrophenyl)ethoxy]carbonyl}-2′-*O*-(tetrahydro-4-methoxy-2*H*-pyran-4-yl)-3′,5′-*O*-(1,1,3,3-tetraisopropyldisiloxane-1,3-diyl)cytidine (**4**; 2 g, 2.48 mmol) in anh. THF (30 ml) and Bu_4NF (1.95 g, 6.19 mmol) was stirred for 10 min. Then the solvent was evaporated, the residue dissolved in CH_2Cl_2 (100 ml), the org. soln. washed with sat. $NaHCO_3$ soln. (100 ml), dried ($MgSO_4$), and evaporated, and the residue purified by CC (toluene/AcOEt 5:4) with 0–4% MeOH): 1.03 g (74%) of **23**. R_f (toluene/AcOEt/MeOH 5:4:1) 0.35. UV (MeOH): 280 (4.36). ^{1}H-NMR ((D$_6$)DMSO): 8.16 (*d*, 2 H$_o$ to NO$_2$); 7.85 (*d*, H–C(6)); 7.56 (*d*, 2 H$_m$ to NO$_2$): 6.26 (*d*, H–C(5)); 6.01 (*s*, H–C(1′)); 5.19 (*m*, OH–C(5′), OH–C(3′)); 4.33 (*m*, CH$_2$C*H*$_2$O, H–C(2′)): 3.95 (*m*, H–C(3′)); 3.90 (*m*, 1 H–C(5′)); 3.70–3.30 (*m*, CH$_2$OCH$_2$, H–C(4′), 1 H–C(5′)); 3.22 (*s*, MeN); 3.08 (*t*, C*H*$_2$CH$_2$O); 2.89 (*s*, MeO); 1.80–1.60 (*m*, CH$_2$CCH$_2$). Anal. calc. for $C_{25}H_{32}N_4O_{11}\cdot 0.5\ H_2O$ (573.6): C 52.35, H 5.80, N 9.77; found: C 52.31, H 5.81, N 9.70.

13. N^4-*Methyl*-N^4-*{[2-(4-nitrophenyl)ethoxy]carbonyl}*-2′-O-*(tetrahydro-4-methoxy-2*H*-pyran-4-yl)cytidine* (**24**). Analogously to *Exper. 11* with *N*^{4}-methyl-*N*^{4}-{[2-(4-nitrophenyl)ethoxy]carbonyl}-2′-*O*-(tetrahydro-4-

methoxy-2*H*-pyran-4-yl)-3′,5′-*O*-(1,1,3,3-tetraisopropyldisiloxane-1,3-diyl)cytidine (**5**; 1 g, 1.24 mmol) and Bu_4NF (0.98 g, 3.1 mmol) in anh. THF (20 ml). Purification by CC (toluene/AcOEt 5:4) with 0–4% MeOH) gave 0.35 g (50%) of **24**. R_f (toluene/AcOEt/MeOH 5:4:1) 0.25. UV (MeOH): 279 (4.16), 254 (4.29), 204 (4.40). ^{1}H-NMR ((D_6)DMSO): 8.23 (*d*, H–C(6)); 8.18 (*d*, 2 H_o to NO_2); 7.60 (*d*, 2 H_m to NO_2): 7.09 (*d*, H–C(5)); 6.09 (*s*, H–C(1′)); 5.20 (*t*, OH–C(5′)); 5.16 (*d*, OH–C(3′)); 4.47 (*t*, CH_2CH_2O); 4.32 (*m*, H–C(2′)); 3.98 (*m*, H–C(3′)); 3.92 (*m*, 1 H–C(5′)); 3.70–3.25 (*m*, CH_2OCH_2, H–C(4′), 1 H–C(5′)); 3.21 (*s*, MeN); 3.14 (*t*, CH_2CH_2O); 2.81 (*s*, MeO); 1.85–1.60 (*m*, CH_2CCH_2). Anal. calc. for $C_{25}H_{32}N_4O_{11}\cdot 0.5\ H_2O$ (573.6): C 52.35, H 5.80, N 9.77; found: C 52.50, H 5.80, N 9.80.

14. *5′-O-{[2-[[5-(Dimethylamino)naphthalen-1-yl]sulfonyl}ethoxy}carbonyl]-*N^3*-methyl-*N^4*-{[2-(4-nitrophenyl)ethoxy]carbonyl}-2′-O-(tetrahydro-4-methoxy-2*H*-pyran-4-yl)cytidine* (**25**). Analogously to *Exper. 9*, with **23** (0.94 g, 1.41 mmol) and 2-{[5-(dimethylamino)naphthalen-1-yl]sulfonyl}ethyl carbonochloridate hydrochloride (0.756 g, 1.2 mmol) in anh. pyridine (20 ml) and anh. MeCN (10 ml) for 1 h at 0°. Purification by CC (toluene/AcOEt 5:4 with 0–1% MeOH) gave 0.48 g (34%) of **25**. R_f (toluene/AcOEt/MeOH 5:4:1) 0.56. UV (MeOH): 344 (3.64), 282 (sh, 4.35), 262 (4.45), 214 (4.74). ^{1}H-NMR ((D_6)DMSO): 8.52 (*d*, H–C(2)(npht)); 8.21–8.09 (*m*, H–C(4)(npht), H–C(8)(npht), 2 H_o to NO_2); 7.70–7.51 (*m*, H–C(3)(npht), H–C(7)(npht), H–C(6), 2 H_m to NO_2); 7.26 (*d*, H–C(6)(npht)); 6.25 (*d*, H–C(5)); 5.94 (*d*, H–C(1′)); 5.38 (*d*, OH–C(3′)); 4.40–4.27 (*m*, 2 CH_2CH_2O, H–C(2′)); 4.15 (*m*, H–C(3′), H–C(4′)); 3.95–3.83 (*m*, CH_2SO_2, 2 H–C(5′)); 3.80–3.30 (*m*, CH_2OCH_2); 3.24 (*s*, MeN); 3.04 (*t*, CH_2CH_2O); 2.93 (*s*, MeO); 2.80 (*s*, Me_2N); 1.80–1.60 (*m*, CH_2CCH_2). Anal. calc. for $C_{40}H_{47}N_5O_{15}S$ (869.9): C 55.23, H 5.44, N 8.05; found: C 55.38, H 5.61, N 7.70.

15. *5′-O-{[2-[[5-(Dimethylamino)naphthalen-1-yl]sulfonyl}ethoxy}carbonyl]-*N^4*-methyl-*N^4*-{[2-(4-nitrophenyl)ethoxy]carbonyl}-2′-O-(tetrahydro-4-methoxy-2*H*-pyran-4-yl)cytidine* (**26**). Analogously to *Exper. 9*, with **24** (0.5 g, 1.34 mmol) and 2-{[5-(dimethylamino)naphthalen-1-yl]sulfonyl}ethyl carbonochloridate hydrochloride (0.265 g, 0.70 mmol) in anh. pyridine (10 ml) and anh. MeCN (5 ml) for 3 h at 0°. Purification by CC (toluene/AcOEt 5:4 with 0–3.5% MeOH) gave 0.13 g (26%) of **24**. R_f (toluene/AcOEt/MeOH 5:4:1) 0.34. UV (MeOH): 342 (3.67), 288 (sh, 4.17), 253 (4.54), 213 (4.83). ^{1}H-NMR ((D_6)DMSO): 8.55 (*d*, H–C(2)(npht)); 8.15 (*m*, H–C(4)(npht), H–C(8)(npht), 2 H_o to NO_2); 7.92 (*d*, H–C(6)); 7.67–7.55 (*m*, H–C(3)(npht), H–C(7)(npht), 2 H_m to NO_2); 7.25 (*d*, H–C(6)(npht)); 7.07 (*d*, H–C(5)); 6.00 (*d*, H–C(1′)); 5.36 (*d*, OH–C(3′)); 4.50–4.30 (*m*, 2 CH_2CH_2O, H–C(2′)); 4.18 (*m*, H–C(3′), H–C(4′)); 4.00–3.70 (*m*, CH_2SO_2, 2 H–C(5′)); 3.70–3.30 (*m*, CH_2OCH_2), 3.24 (*s*, MeN); 3.13 (*t*, CH_2CH_2O); 2.86 (*s*, MeO); 2.80 (*s*, Me_2N); 1.80–1.60 (*m*, CH_2CCH_2). Anal. calc. for $C_{40}H_{47}N_5O_{15}S$ (869.9): C 55.23, H 5.44, N 8.05; found: C 54.98, H 5.55, N 7.72.

16. *5′-O-{[2-[[5-(Dimethylamino)naphthalen-1-yl]sulfonyl}ethoxy}carbonyl]-*N^3*-methyl-2′-O-(tetrahydro-4-methoxy-2*H*-pyran-4-yl)uridine* (**27**). Analogously to *Exper. 9*, with **6** (0.5 g, 1.34 mmol) and 2-{[5-(dimethylamino)naphthalen-1-yl]sulfonyl}ethyl carbonochloridate hydrochloride (0.584 g, 1.54 mmol) in anh. pyridine (10 ml) for 1 h at 0°. Purification by CC (toluene/AcOEt 5:4 with 0–2.5% MeOH) gave 0.35 g (39%) of **27**. R_f (toluene/AcOEt/MeOH 5:4:1) 0.44. UV (MeOH): 343 (3.68), 255 (4.36), 212 (4.74). ^{1}H-NMR ((D_6)DMSO): 8.54 (*d*, H–C(2)(npht)); 8.19 (*m*, H–C(4)(npht), H–C(8)(npht)); 7.69 (*m*, H–C(3)(npht), H–C(7)(npht), H–C(6)); 7.28 (*d*, H–C(6)(npht)); 5.96 (*d*, H–C(5)); 5.84 (*d*, H–C(1′)); 5.37 (*d*, OH–C(3′)); 4.38 (*m*, CH_2CH_2O, H–C(2′)); 4.15 (*m*, H–C(3′)); 4.00–3.82 (*m*, CH_2SO_2, H–C(4′)); 3.80–3.30 (*m*, CH_2OCH_2, 2 H–C(5′)); 3.18 (*s*, MeN); 2.94 (*s*, MeO); 2.82 (*s*, Me_2N); 1.82–1.55 (*m*, CH_2CCH_2). Anal. calc. for $C_{31}H_{39}N_3O_{12}S\cdot 1/4$ toluene (700.8): C 56.13, H 5.90, N 6.00; found: C 56.16, H 5.90, N 6.01.

17. *5′-O-{[2-[[5-(Dimethylamino)naphthalen-1-yl]sulfonyl}ethoxy}carbonyl]-*N^6*-methyl-*N^6*-{[2-(4-nitrophenyl)ethoxy]carbonyl}-2′-O-(tetrahydro-4-methoxy-2*H*-pyran-4-yl)adenosine 3′-[2-(4-Cyanophenyl)ethyl Diethylphosphoramidite]* (**28**). Under exclusion of humidity, **17** (0.45 g, 0.5 mmol) was dissolved in anh. THF (8 ml). Then, ethyldiisopropylamine (0.5 ml, 3 mmol) and 2-(4-cyanophenyl)ethyl diethylphosphoramidochloridite (0.285 g, 1 mmol) were added, and the mixture was stirred for 1 h. The reaction was quenched with ^{i}PrOH (0.2 ml), and after extraction with CH_2Cl_2 (30 ml) and $NaHCO_3$ soln. (30 ml), the org. layer was dried ($MgSO_4$), and evaporated. Purification by CC (40–50% AcOEt/toluene) gave 0.35 g (61%) of **28**. R_f (petroleum ether/AcOEt/Et_3N 1:9:1) 0.73. ^{1}H-NMR ((D_6)DMSO): 8.75 (*m*, H–C(2), H–C(8)); 8.50 (*d*, H–C(2)(npht)); 8.20 (*m*, H–C(4)(npht), H–C(8)(npht)); 8.03 (*m*, 2 H_o to NO_2); 7.75 (*m*, 2 H_o to CN); 7.70 (*m*, 2 H_m to NO_2); 7.46 (*m*, H–C(3)(npht), H–C(7)(npht)); 7.36 (*d*, 2 H_m to CN); 7.26 (*m*, H–C(6)(npht)); 6.10 (2*d*, H–C(1′)); 5.25 (2*m*, H–C(2′)); 4.35 (*m*, 3 · CH_2CH_2O, H–C(3′), H–C(4′), 2 H–C(5′)); 3.82 (*t*, SO_2CH_2); 3.55 (*m*, CH_2OCH_2); 3.37 (*s*, MeN); 2.96 (*m*, 2 CH_2CH_2O, 2 CH_2N); 2.79 (*s*, Me_2N); 2.31 (2*s*, MeO); 1.66 (*m*, CH_2CCH_2); 1.02 (*m*, 2 *Me*CH_2). ^{31}P-NMR ((D_6)DMSO): 149.91, 149.03 (2*s*). Anal. calc. for $C_{54}H_{64}N_9O_{15}PS$ (1142.2): C 56.78, H 5.65, N 11.04; found: C 57.35, H 6.04, N 11.32.

18. *5′*-O-*{[2-[[5-(Dimethylamino)naphthalen-1-yl]sulfonyl}ethoxy}carbonyl}*-N^6-*methyl*-N^6-*{[2-(4-nitrophenyl)ethoxy]carbonyl}-2′*-O-*(tetrahydro-4-methoxy-2*H*-pyran-4-yl)-adenosine 3′-[2-(4-Cyanophenyl)ethyl Diisopropylphosphoramidite]* (**29**). Analogously to *Exper. 17*, with **17** (0.447 g, 0.5 mmol), ethyldiisopropylamine (0.5 ml, 3 mmol), and 2-(4-cyanophenyl)ethyl diisopropylphosphoramidochloridite (0.313 g, 1 mmol). Purification by CC (20–50% AcOEt/toluene) gave 0.29 g (49%) of **29**. R_f (petroleum ether/AcOEt/Et_3N 1:9:1) 0.76. ^{1}H-NMR ((D_6)DMSO): 8.75 (2*s*, H–C(2), H–C(8)); 8.51 (*m*, H–C(2)(npht)); 8.16 (*m*, H–C(4)(npht), H–C(8)(npht)); 8.04 (*d*, 2 H_o to NO_2); 7.77 (*m*, 2 H_o to CN); 7.62 (*m*, H–C(3)(npht), H–C(7)(npht)); 7.46 (*m*, 2 H_m to NO_2); 7.33 (*d*, 2 H_m to CN); 7.22 (*m*, H–C(6)(npht)); 6.07 (2*d*, H–C(1′)); 5.25 (2*m*, H–C(2′)); 4.30 (*m*, 3 CH_2CH_2O, H–C(3′), H–C(4′), 2 H–C(5′)); 3.80 (*m*, SO_2CH_2); 3.40 (*m*, CH_2OCH_2); 3.38 (*s*, MeN); 2.97 (*m*, 2 CH_2CH_2O, 2 Me_2CH); 2.79 (*s*, Me_2N); 2.49 (*s*, MeO); 1.66 (*m*, CH_2CCH_2); 1.08 (*m*, 2 Me_2CH). ^{31}P-NMR ((D_6)DMSO): 150.29, 148.65 (2*s*). Anal. calc. for $C_{56}H_{68}N_9O_{15}PS$ (1170.25): C 57.48, H 5.86, N 10.77; found: C 58.18, H 6.31, N 10.56.

19. *5′*-O-*{[2-[[5-(Dimethylamino)naphthalen-1-yl]sulfonyl}ethoxy}carbonyl}*-N^6-*methyl*-N^6-*{[2-(4-nitrophenyl)ethoxy]carbonyl}-2′*-O-*(tetrahydro-4-methoxy-2*H*-pyran-4-yl)adenosine 3′-[2-(4-Nitrophenyl)ethyl Diisopropylphosphoramidite]* (**30**). Analogously to *Exper. 17*, with **17** (0.894 g, 1 mmol), ethyldiisopropylamine (1 ml, 6 mmol), and [2-(4-nitrophenyl)ethyl diisopropylphosphoramidochloridite (0.7 g, 2.5 mmol). Purification by CC 25–50% acetone/petroleum ether) gave 0.64 g (54%) of **30**. R_f (petroleum ether/AcOEt/Et_3N 1:9:1) 0.74. ^{1}H-NMR ((D_6)DMSO): 8.76 (2*s*, H–C(2), H–C(8)); 8.49 (*m*, H–C(2)(npht)); 8.19–8.02 (*m*, H–C(4)(npht), H–C(8)(npht), 4 H_o to NO_2); 7.65–7.49 (*m*, H–C(3)(npht), H–C(7)(npht), 2 H_m to NO_2); 7.35 (*m*, 2 H_m to NO_2); 7.24 (*m*, H–C(6)(npht)); 6.07 (2*d*, H–C(1′)); 5.28 (*m*, H–C(2′)); 4.35–4.12 (*m*, 3 CH_2CH_2O, H–C(3′), H–C(4′)); 4.06–3.69 (*m*, 2 H–C(5′), SO_2CH_2); 3.65–3.25 (*m*, 2 Me_2CH, MeN, CH_2OCH_2); 3.20–2.90 (*m*, 2 CH_2CH_2O); 2.78 (*s*, Me_2N); 2.31 (*s*, MeO); 1.75–1.25 (*m*, CH_2CCH_2); 1.19–1.06 (*m*, 12 H, 2 Me_2CH). ^{31}P-NMR ((D_6)DMSO): 153.30, 151.64 (2*s*). Anal. calc. for $C_{55}H_{68}N_9O_{17}PS$ (1190.2): C 55.50, H 5.76, N 10.59; found: C 54.96, H 5.73, N 10.22.

20. *5′*-O-*{[2-[[5-(Dimethylamino)naphthalen-1-yl]sulfonyl}ethoxy}carbonyl}*-N^6-*methyl*-N^6-*{[2-(4-nitrophenyl)ethoxy]carbonyl}-2′*-O-*(tetrahydro-4-methoxy-2*H*-pyran-4-yl)adenosine 3′-(Hydrogen Butanedioate)* (**31**). A soln. of **17** (0.268 g, 0.3 mmol), *N,N*-dimethylpyridin-4-amine (DMAP; 48 mg, 0.39 mmol), and succinic anhydride (60 mg, 0.6 mmol) was stirred for 9.5 h in anh. CH_2Cl_2 (30 ml). The mixture was extracted with CH_2Cl_2 (20 ml) and sat. $NaHCO_3$ soln. (25 ml) and the org. layer washed with 10% citric acid soln., dried ($MgSO_4$), and evaporated: 0.29 g (97%) of **31**. Anal. pure material was obtained by CC (33–66% acetone/petroleum ether). R_f (toluene/AcOEt/MeOH 5:4:1) 0.27. UV (MeOH): 343 (3.63), 262 (4.47), 213 (4.80). ^{1}H-NMR ((D_6)DMSO): 12.32 (*s*, COOH); 8.76 (2*s*, H–C(2), H–C(8)); 8.52 (*d*, H–C(2)(npht)); 8.18 (*m*, H–C(4)(npht), H–C(8)(npht)); 8.04 (*d*, 2 H_o to NO_2); 7.62 (*m*, H–C(3)(npht), H–C(7)(npht))); 7.35 (*d*, 2 H_m to NO_2); 7.25 (*d*, H–C(6)(npht)); 6.17 (*d*, H–C(1′)); 5.34 (*m*, H–C(2′), H–C(3′)); 4.38–4.23 (*m*, 2 CH_2CH_2O, 2 H–C(5′)); 3.83 (*t*, CH_2SO_2); 3.50–3.10 (2*m*, CH_2OCH_2); 3.38 (*s*, MeN); 2.96 (*t*, CH_2CH_2O); 2.79 (*s*, Me_2N); 2.56 (2*m*, CH_2CH_2); 2.35 (*s*, MeO); 1.75–1.30 (*m*, CH_2CCH_2). Anal. calc. for $C_{45}H_{51}N_7O_{17}S$ (994.0): C 54.38, H 5.17, N 9.86; found: C 54.31, H 5.36, N 9.50.

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