Nucleotides

Part LXXIII[1])

Oligoribonucleotide Synthesis with the (2-Cyano-1-phenylethoxy)carbonyl (2c1peoc) Group for the 5′-Hydroxy Protection

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The (2-cyano-1-phenylethoxy)carbonyl (2c1peoc) group was developed as a new base-labile protecting group for the 5′-OH function in solid-phase synthesis of oligoribonucleotides *via* the phosphoramidite approach. The half-lives of its β-elimination process by 0.1M DBU (1,8-diazabicyclo[5.4.0]undec-7-ene) were determined to be 7–14 s by HPLC investigations. The 2′-OH function was protected with the acid-labile tetrahydro-4-methoxy-2*H*-pyran-4-yl (thmp) group, while the 2-(4-nitrophenyl)ethyl (npe) and 2-(4-nitrophenyl)ethoxycarbonyl (npeoc) groups were used for the protection of the base and phosphate moieties. The syntheses of the monomeric building blocks, both phosphoramidites and nucleoside-functionalized supports, as well as the build-up of oligoribonucleotides by means of this approach are described.

1. Introduction. – Whereas the machine-aided assembly of oligodeoxyribonucleotides has been established for many years, the solid-phase synthesis of RNA is still less efficient. The crucial problem in RNA synthesis is to find an adequate 2′-OH protecting group, which does not sterically interfere the coupling reaction and remains intact during all steps of RNA assembly as well as during the workup process. At the end, however, the 2′-OH protecting group has to be removed under mild conditions to avoid internucleotide cleavage or intramolecular 2′-*O* → 3′-*O* migration.

The most common 2′-OH protecting group is the (*tert*-butyl)dimethylsilyl (tbdms) group [2][3], which is removed by F^- ions and is, thus, completely compatible with the conventional 5′-*O*-(dimethoxytrityl) group. But the (*tert*-butyl)dimethylsilyl group involves other problems, *e.g.*, partial loss during deprotection of the base functions, tendency to 2′-*O* → 3′-*O* phosphodiester migration, or insufficient deprotection of longer sequences. The recently developed 2′-*O*-[(triisopropylsilyl)oxy]methyl (tom) group [4][5], which is structurally related to the tbdms group, presents better features like less steric hindrance, reliable deprotection, and lack of phosphodiester isomerization. However, the cleavage of those groups with F^- ions leads to salt by-products that have to be removed in an additional cleaning step.

An alternative strategy is represented by the acid-labile acetal functions like the tetrahydro-2*H*-pyranyl (thp) [6] or the achiral tetrahydro-4-methoxy-2*H*-pyran-4-yl (thmp) group [7], which are removed under mild conditions without formation of by-products. Unfortunately, these groups are insufficiently compatible with the 5′-*O*-trityl groups, especially for the synthesis of longer oligonucleotide chains. One solution to

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this problem is the replacement of the acid-labile 5′-OH protecting groups with base-labile functions. For example, the (9*H*-fluoren-9-ylmethoxy)carbonyl (fmoc) function [8] and the (2-dansylethoxy)carbonyl (danseoc) group [9] (dansyl = [5-(dimethylamino)-naphthalen-1-yl]sulfonyl), both cleaved in a β-elimination process, have been earlier used in combination with the 2′-*O*-(tetrahydro-4-methoxy-2*H*-pyran-4-yl) group.

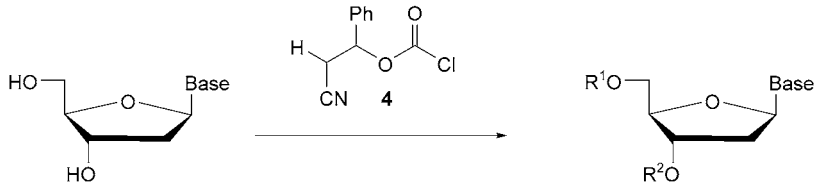
In this context, we suggested recently the β-eliminating (2-cyano-1-phenylethoxy)-carbonyl (2c1peoc) group as a new 5′-*O*-protecting group [10]. The good features of this group are its fairly easy chemical approach in good yields and its short half-life of 12 s, determined with the 5′-*O*-2c1peoc-protected thymidine derivative **12** with 0.1M DBU (see below). Due to this short deprotection time, the 2c1peoc group should be compatible with the 2-(4-nitrophenyl)ethyl (npe) and [2-(4-nitrophenyl)ethoxy]carbonyl (npeoc) groups, which we use in our npe/npeoc strategy for the blocking of the base and phosphate functions [11–14]. In this approach, all permanent npe/npeoc protecting groups are cleaved after oligonucleotide synthesis *via* β-elimination with DBU in an aprotic solvent and washed out, while the oligonucleotide is still resin-bound. In this way, it is possible to synthesize very pure oligonucleotides in a direct manner without further purification.

To reveal a possible impact of the npe/npeoc-protected bases adenine, cytosine, and guanine on the deprotection rate of the 5′-*O*-2c1peoc group, we also evaluated the half-lives of the corresponding nucleosides. Then we synthesized the fully protected monomeric building blocks used during the phosphoramidite approach, and finally, we employed these new monomers in oligoribonucleotide synthesis. The usefulness of this protecting-group combination is discussed in detail.

2. Kinetics Studies. – As model substances for the kinetics studies, the 5′-*O*-protected nucleosides **5**, **7**, and **10** had to be synthesized (*Scheme 1*). Therefore, 2′-deoxy-N^6-{[2-(4-nitrophenyl)ethoxy]carbonyl}adenosine (**1**) [11][15], 2′-deoxy-N^4-{[2-(4-nitrophenyl)ethoxy]carbonyl}cytidine (**2**) [11][15], or 2′-deoxy-N^2-{[2-(4-nitrophenyl)ethoxy]carbonyl}-O^6-[2-(4-nitrophenyl)ethyl]guanosine (**3**) [11][15] was reacted with a slight excess (1.3 equiv.) of 2-cyano-1-phenylethyl carbonochloridate (**4**) [10] at low temperature in CH_2Cl_2/pyridine. After workup and purification, the desired 5′-*O*-substituted products **5**, **7**, and **10**, respectively, were obtained in yields between 58 and 73%. As by-products, the 3′,5′-bis-*O*-substituted compounds **6**, **9**, and **11** were formed in 14–18% yield and, in the case of the cytidine derivative, the 3′-*O*-substituted compound **8** could also be isolated in 8% yield.

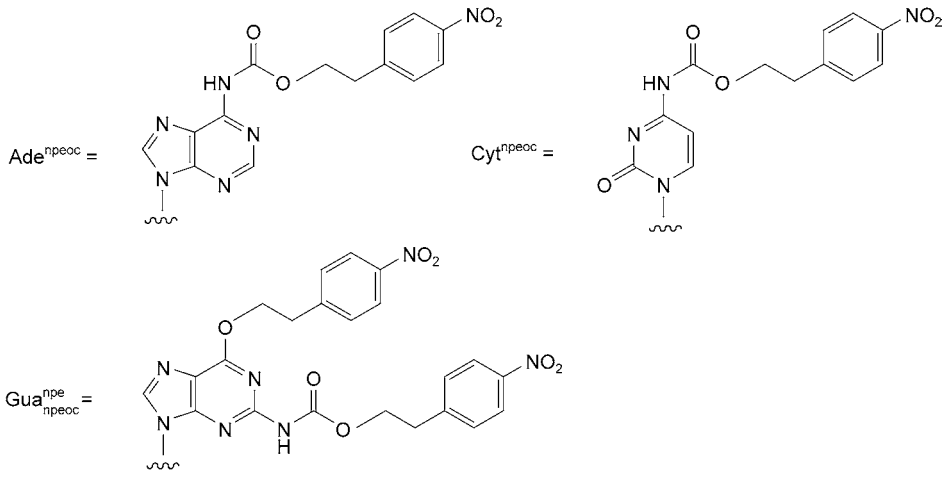
To study the cleavage kinetics of the 5′-*O*-2c1peoc-protected nucleosides **5**, **7**, and **10**, a 10-fold excess of 0.1M DBU in MeCN was used as the standard. After defined time periods, aliquots were taken, the reaction was quenched with 0.5M AcOH in MeCN, and the mixture was analyzed by reversed-phase HPLC. From these data, we calculated the half-lives for the pseudo-first-order reactions (*Table 1*). The half-life of the 5′-*O*-[(2-cyano-1-phenylethoxy)carbonyl]thymidine (**12**), which was determined earlier [10], is also given in *Table 1*. According to the results, the cleavage of the 2c1peoc group from the nucleosides **5** and **7** with the npeoc-protected bases adenine and cytosine, respectively, as well as from **12** with thymine, is approximately the same, whereby half-lives of 12–14 s were calculated. In contrast to this and with a half-live of *ca.* 7 s, the

Scheme 1



	Base
1	Ade^{npeoc}
2	Cyt^{npeoc}
3	Gua^{npe}_{npeoc}

	Base	R^1	R^2	%
5	Ade^{npeoc}	2c1peoc	H	67
6		2c1peoc	2c1peoc	18
7	Cyt^{npeoc}	2c1peoc	H	58
8		H	2c1peoc	8
9		2c1peoc	2c1peoc	16
10	Gua^{npe}_{npeoc}	2c1peoc	H	73
11		2c1peoc	2c1peoc	14

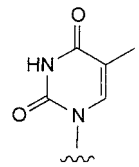


2c1peoc function seems to be removed faster from the npe/npeoc-protected guanosine derivative **10**.

3. Syntheses. – The synthesis of the monomeric building blocks started with the protection of the functional groups of the nucleobases with the 2-(4-nitrophenyl)ethyl (npe) and [2-(4-nitrophenyl)ethoxy]carbonyl (npeoc) group according to known literature procedures (**13** → **15**; see *Scheme 2*) [11][15][16]. While the protection of the amino functions of adenine, cytosine, and guanine is crucial, the protection of the lactam function of guanine is not absolutely necessary but highly recommended. The

Table 1. *Half-Lives of 5′-*O*-2c1peoc-2′-Deoxynucleosides with 0.1*M *DBU*

	Base	Half-life $t_{1/2}$
5	Ade^{npeoc}	14 s
7	Cyt^{npeoc}	12 s
10	Gua^{npe}_{npeoc}	7 s
12 [10]	Thy	12 s

Thy = 

lactam group in uridine (**16**) remains normally free, but, for comparative studies, we also synthesized the O^4-npe-protected uridine derivative **17** [17].

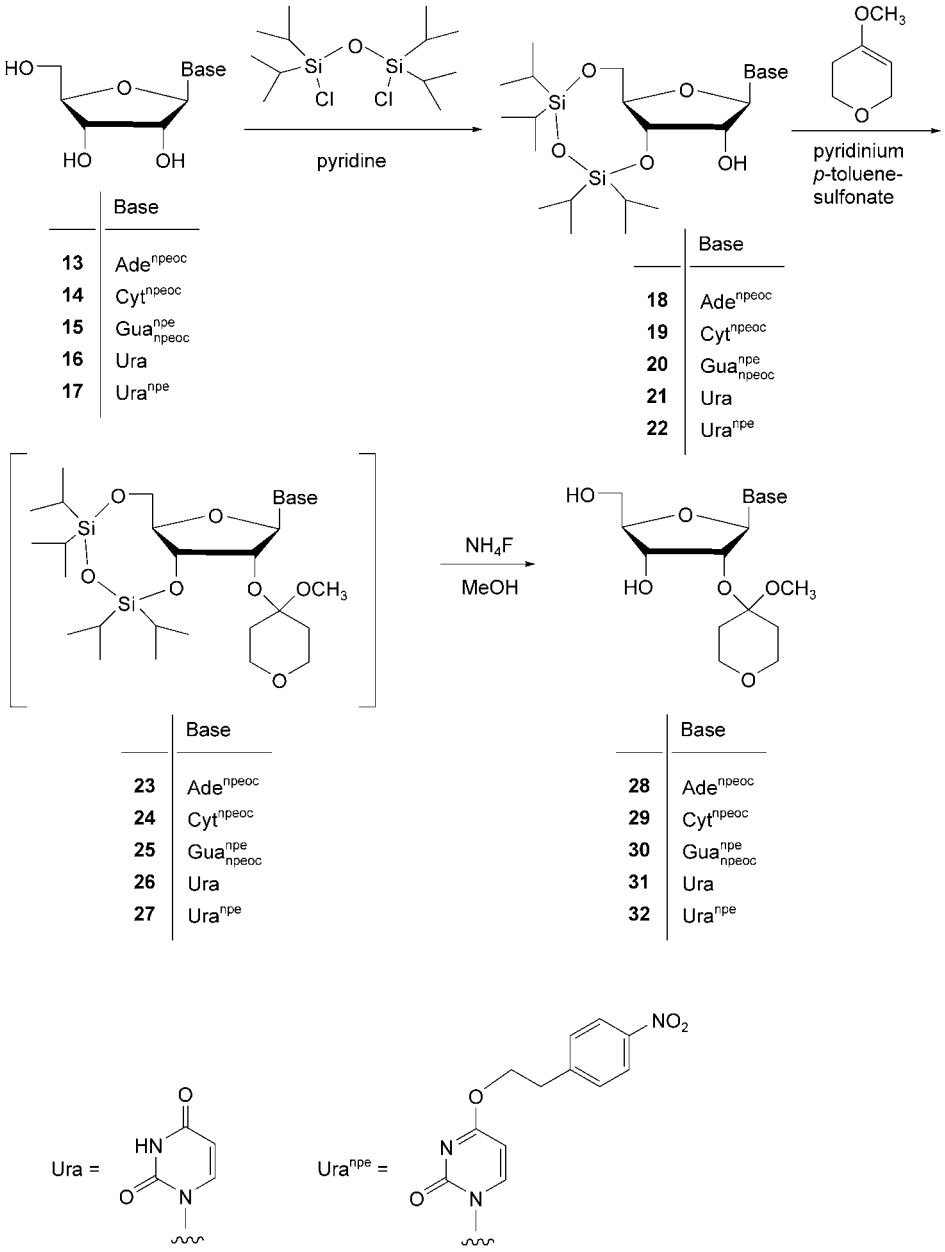
The next step in the reaction scheme was the protection of the 2′-OH function by the tetrahydro-4-methoxy-2*H*-pyran-4-yl (thmp) group [7]. The advantage of the thmp group lies in its achirality, which simplifies immensely the synthesis and characterization of the monomers by spectroscopic means as well as the analysis of the oligonucleotides. To achieve selective introduction of the 2′-*O*-protecting group, the 3′- and 5′-OH functions were intermediately blocked with the bifunctional 1,1,3,3-tetraisopropyldisiloxane-1,3-diyl (tipds) group developed by *Markiewicz* [18]. Reaction of nucleosides **13**–**17** with a slight excess (1.1 equiv.) of 1,3-dichloro-1,1,3,3-tetraisopropyldisiloxane in abs. pyridine resulted in the 3′,5′-*O*-tipds-nucleosides **18**–**22** [17–20] (*Scheme 2*). Subsequent treatment with an excess (3.9–5.6 equiv.) of 3,6-dihydro-4-methoxy-2*H*-pyran [21–23] in the presence of a catalytic amount (0.20–0.35 equiv.) of pyridinium *p*-toluenesulfonate in CH_2Cl_2 led to the intermediates **23**–**27**, which were, without isolation, desilylated with NH_4F in MeOH to give the 2′-*O*-thmp-nucleosides **28**–**32** [9][17][23] in yields of 83–98%.

The subsequent introduction of the 2c1peoc group was carried out with a slight excess (1.3–1.5 equiv.) of 2-cyano-1-phenylethyl carbonochloridate (**4**) at low temperature (^{i}PrOH/N_2 bath) in CH_2Cl_2/pyridine (*Scheme 3*). Separation of the product mixture by flash chromatography afforded the desired 5′-*O*-isomers **33**, **35**, **38**, **40**, and **42** in 59–83% yield and the 3′,5′-bis-*O*-substituted by-products **34**, **37**, **39**, **41**, and **44** in 10–22% yield. The 3′-*O*-substituted derivatives could be isolated only in the cases of cytidine and the O^4-npe protected uridine in a yield of 3% (**36**) and 2% (**43**), respectively.

The preparation of the 3′-phosphoramidites **46**–**50** was achieved by a standard phosphitylation method of the 5′-*O*-2c1peoc-substituted nucleosides **33**, **35**, **38**, **40**, and **42** with 2-(4-nitrophenyl)ethyl tetraisopropylphosphordiamidite (**45**) [24] (*Scheme 4*). The yields after workup and flash chromatography (petroleum ether/acetone gradient) ranged between 68 and 81%.

As starting nucleosides for the solid-phase synthesis of oligoribonucleotides, the 3′-succinates **51**–**55** were required; these resulted from the reaction of the appropriately protected nucleosides **33**, **35**, **38**, **40**, and **42** with succinic anhydride and 1-methyl-1*H*-imidazole in CH_2Cl_2 in 86–99% yield. In a normal workup procedure of this reaction, the organic phase was washed with 10% citric acid and, subsequently, with sat. $NaHCO_3$ solution to get rid of the excess of 1-methyl-1*H*-imidazole and succinic anhydride, respectively. However, in the case of the uridine derivatives **54** and **55**, the washing step with sat. $NaHCO_3$ solution had to be skipped, since it caused loss of the 5′-

Scheme 2



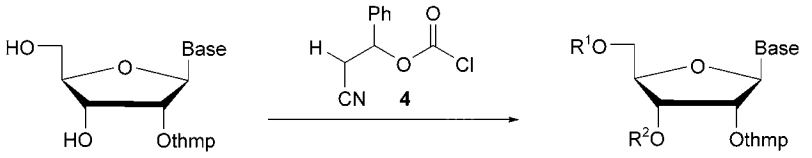
	Base
13	Ade^{npeoc}
14	Cyt^{npeoc}
15	Gua$^{npe}_{npeoc}$
16	Ura
17	Ura^{npe}

	Base
18	Ade^{npeoc}
19	Cyt^{npeoc}
20	Gua$^{npe}_{npeoc}$
21	Ura
22	Ura^{npe}

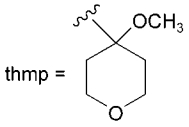
	Base
23	Ade^{npeoc}
24	Cyt^{npeoc}
25	Gua$^{npe}_{npeoc}$
26	Ura
27	Ura^{npe}

	Base
28	Ade^{npeoc}
29	Cyt^{npeoc}
30	Gua$^{npe}_{npeoc}$
31	Ura
32	Ura^{npe}

Scheme 3



	Base
28	Ade^{npeoc}
29	Cyt^{npeoc}
30	Gua^{npe}_{npeoc}
31	Ura
32	Ura^{npe}



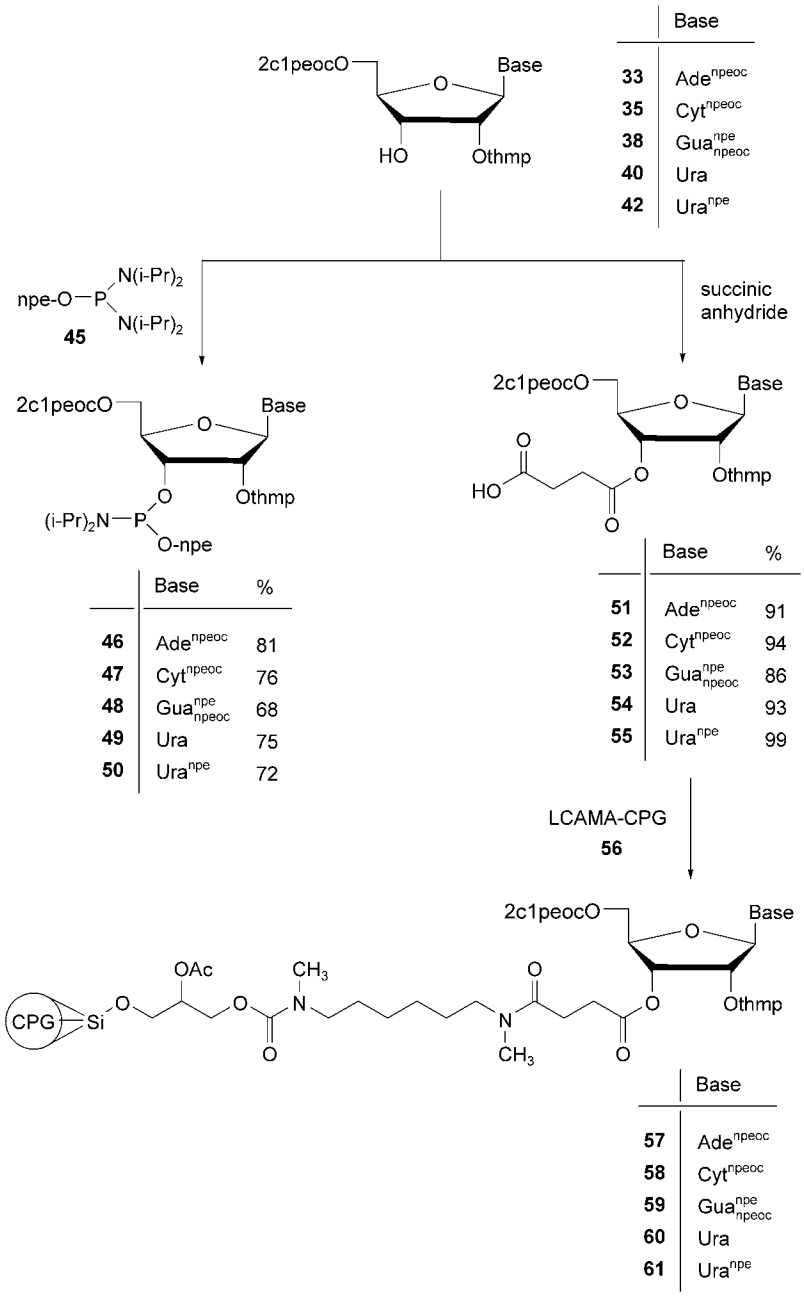
	Base	R^1	R^2	%
33	Ade^{npeoc}	2c1peoc	H	72
34		2c1peoc	2c1peoc	17
35	Cyt^{npeoc}	2c1peoc	H	58
36		H	2c1peoc	3
37		2c1peoc	2c1peoc	20
38	Gua^{npe}_{npeoc}	2c1peoc	H	83
39		2c1peoc	2c1peoc	10
40	U	2c1peoc	H	65
41		2c1peoc	2c1peoc	23
42	U^{npe}	2c1peoc	H	59
43		H	2c1peoc	2
44		2c1peoc	2c1peoc	19

O-2c1peoc group leading to lower yields of the succinates **54** and **55**. Instead, compounds **54** and **55** were purified by flash chromatography.

The condensation of the succinates **51**–**55** with LCAMA-CPG (=(long-chain-alkyl)methylamine controlled-pore glass; **56**) [12][25][26] was achieved in the presence of the coupling reagent *O*-{[cyano(ethoxycarbonyl)methylene]amino}-*N*,*N*,*N*′,*N*′-tetramethyluronium tetrafluoroborate (TOTU) and *N*-methylmorpholine in MeCN. After capping with Ac_2O and 1-methyl-1*H*-imidazole, the functionalized solid supports **57**–**61** were obtained with loadings of 13–15 μmol/g.

4. Oligoribonucleotide Syntheses. – Solid-phase synthesis of the oligoribonucleotides was performed according to the phosphoramidite approach by *Caruthers* and coworkers [27–30] with an *Applied Biosystems 380B* synthesizer. After attachment of a small column filled with the desired starting nucleoside **57**–**61** (0.15 to 0.2-μmol scale), the assembly was performed by a programmed repetitive cycle of four chemical steps with intermediate washing steps. First the terminal 5′-*O*-2c1peoc group was removed with 0.1M DBU in MeCN within 120 s. Then the coupling was carried out with different activators and various condensation times applying the phosphoramidites **51**–**54** normally as 0.1M solutions in MeCN. Only the O^4-npe-protected uridine phosphoramidite **50** was employed as a 0.1M solution in CH_2Cl_2 for solubility reasons. After condensation, unreacted OH functions were capped by acetylation with Ac_2O/2,6-dimethylpyridine/1-methyl-1*H*-imidazole in THF for 25 s, and finally the phosphite triester links were oxidized with 0.05M I_2 in THF/H_2O/pyridine.

Scheme 4


2c1peocO
Base
HO
Othmp
Base
33 Ade^npeoc
35 Cyt^npeoc
38 Gua^npe_npeoc
40 Ura
42 Ura^npe
npe-O—P
N(i-Pr)2
N(i-Pr)2
45
succinic anhydride
(i-Pr)2N—P
O-npe
Base %
46 Ade^npeoc 81
47 Cyt^npeoc 76
48 Gua^npe_npeoc 68
49 Ura 75
50 Ura^npe 72
HO
Base %
51 Ade^npeoc 91
52 Cyt^npeoc 94
53 Gua^npe_npeoc 86
54 Ura 93
55 Ura^npe 99
LCAMA-CPG
56
CPG
Si
OAc
CH3
N
CH3
Base
57 Ade^npeoc
58 Cyt^npeoc
59 Gua^npe_npeoc
60 Ura
61 Ura^npe

After the RNA assembly was completed, the base- and phosphate-protecting groups were removed with either 1M DBU (12.5 h) or 2M DBU (10 h) in MeCN. The 2M DBU instead of 1M DBU was used comparatively to ensure that, indeed, all of the npe/npeoc protecting groups had been removed; however, no differences could be observed between 1M or 2M DBU. After washing out the cleaved npe/npeoc blocking groups, the 2′-*O*-thmp-protected oligonucleotide was released from the solid support by treatment with concentrated NH_3 solution for 2 h. Finally, the ammonia solutions were lyophilized in a *Speed-vac* concentrator. The quality of the synthesized crude oligonucleotides was analyzed by reversed-phase and anion-exchange HPLC without further purification.

First, the homologous tetra- and octamers **62**–**69** and pentadecamer **70** were synthesized under different condensation conditions (*Table 2*). As condensation agents, 1*H*-tetrazole (0.5M), pyridinium chloride (0.5M) [31][32], and 5-(ethylthio)-1*H*-tetrazole (0.6M) [33] were tested. In general, for the synthesis of the adenylate and guanosylate sequences, no differences in the purity of the products due to the coupling

Table 2. *Formation of 2′-*O*-Thmp-Protected Homooligomers in the Presence of Different Activators after Different Condensation Times*

5′-Sequence-3′	Activator	Condensation time [s]
$[A(thmp)]_4$ (**62**)	0.5M 1*H*-tetrazole	700
	0.5M 1*H*-tetrazole	140
	0.5M pyridinium chloride	140
	0.5M pyridinium chloride	10
	0.6M 5-(ethylthio)-1*H*-tetrazole	300
$[A(thmp)]_8$ (**63**)	0.5M 1*H*-tetrazole	700
	0.5M pyridinium chloride	70
	0.6M 5-(ethylthio)-1*H*-tetrazole	300
$[C(thmp)]_4$ (**64**)	0.5M 1*H*-tetrazole	700
	0.5M pyridinium chloride	140
	0.6M 5-(ethylthio)-1*H*-tetrazole	300
$[C(thmp)]_8$ (**65**)	0.5M pyridinium chloride	70
	0.6M 5-(ethylthio)-1*H*-tetrazole	300
$[G(thmp)]_4$ (**66**)	0.5M 1*H*-tetrazole	700
	0.5M pyridinium chloride	140
	0.6M 5-(ethylthio)-1*H*-tetrazole	300
$[G(thmp)]_8$ (**67**)	0.5M pyridinium chloride	70
	0.6M 5-(ethylthio)-1*H*-tetrazole	300
$[U(thmp)]_4$ (**68**)[a])	0.5M 1*H*-tetrazole	700
	0.5M pyridinium chloride	140
	0.6M 5-(ethylthio)-1*H*-tetrazole	300
$[U(thmp)]_8$ (**69**)[a])	0.5M pyridinium chloride	140
	0.6M 5-(ethylthio)-1*H*-tetrazole	300
$[U(thmp)]_4$ (**68**)[b])	0.5M 1*H*-tetrazole	700
	0.5M pyridinium chloride	140
	0.6M 5-(ethylthio)-1*H*-tetrazole	300
$[U(thmp)]_8$ (**69**)[b])	0.5M pyridinium chloride	140
$[U(thmp)]_{15}$ (**70**)[b])	0.5M pyridinium chloride	140
	0.5M pyridinium chloride	280

[a]) Syntheses with unprotected uridine phosphoramidite **49**. [b]) Syntheses with O^4-npe-protected uridine phosphoramidite **50**.

reagent and time could be observed. In contrast to this, the activation of the phosphoramidites with the pyrimidine bases cytosine, *i.e.*, **47**, and uracil, *i.e.*, **49** and **50**, were less efficient with 1*H*-tetrazole. In the case of the O^4-npe-protected uridine phosphoramidite **50**, the condensation property seemed to be best with pyridinium chloride.

The synthesis of the adenylate sequences came always along with impurities that had longer retention times in the reversed-phase HPLC. These by-products, which are especially visible for the 2′-*O*-mthp-protected tetramer **62** (*Fig. 1,a*), are probably formed by undesired *N*-phosphitylation at the N^6-npeoc-protected moiety of adenosine [17][34]. *Beier* and *Pfleiderer* [34] could prevent the formation of such by-products by using pyridinium chloride as activator, since the latter allows to reduce the condensation time drastically because of its high activity. In this work, however, it was not possible to prevent formation of these by-products. Even reduction of the condensation times from 700 to 140 s for 1*H*-tetrazole and from 140 to 10 s for pyridinium chloride did not lead to a more-homogeneous product, instead the oligomers were more contaminated by failure sequences due to poorer step-wise condensation yields.

The HPLC profiles of the uridine sequences, which were synthesized with the unprotected uridine phosphoramidite **49**, showed a shoulder towards longer retention times (*Fig. 1,b*). This result is in accord with observations made earlier by *Bergmann* and *Pfleiderer* on uridine-rich sequences [35]. With the O^4-npe-protected uridine phosphoramidite **50**, the formation of these by-products could be avoided to a great extent (*Fig. 1,c*), suggesting an undesired condensation at the unprotected O^4-lactam function in the case of **49**. Unfortunately, however, the HPLC traces showed earlier eluted failure sequences attributed obviously to deficient condensation properties of the phosphoramidite **50**. Attempts to solve this problem by using a more-concentrated phosphoramidite solution (0.12M) or by extending the condensation time failed.

Subsequently, the synthesis of the mixed sequences **71**–**82** were carried out with pyridinium chloride as activator and a condensation time between 140 and 300 s (*Table 3*). Moreover, for incorporation of a uridine building block, we used only the O^4-npe-protected phosphoramidite **50**.

Table 3. *Formation of Mixed 2′-*O*-Thmp-Protected Oligonucleotide Sequences*

5′-Sequence-3′	Length	Condensation time [s]
UAAGGAU[a]) (**71**)	7	140
GGGGUAAU[a]) (**72**)	9	280
ACUGCAAUAA[a]) (**73**)	10	280
UAUUUCUACCA[a]) (**74**)	11	280
GGAGGUUAGAUC[a]) (**75**)	12	280
GGGAUGGGUCUUGA[a]) (**76**)	14	280
CUCUUAUUUCUACCA[a]) (**77**)	15	280
CCAAUAGCUCAGUCAGGU[a]) (**78**)	18	280
AAUCCCUCAUCUUCCGCCA[a]) (**79**)	19	280
CGGAGACUUGGAGAAGUCGU[a]) (**80**)	20	350
CACACCUUUUAGAGAUCGUCA[a]) (**81**)	21	300
UGACGAUCUCUAAAAGGUGUG[a]) (**82**)	21	280

[a]) Short-form representation of the corresponding 2′-*O*-thmp-protected oligonucleotides.

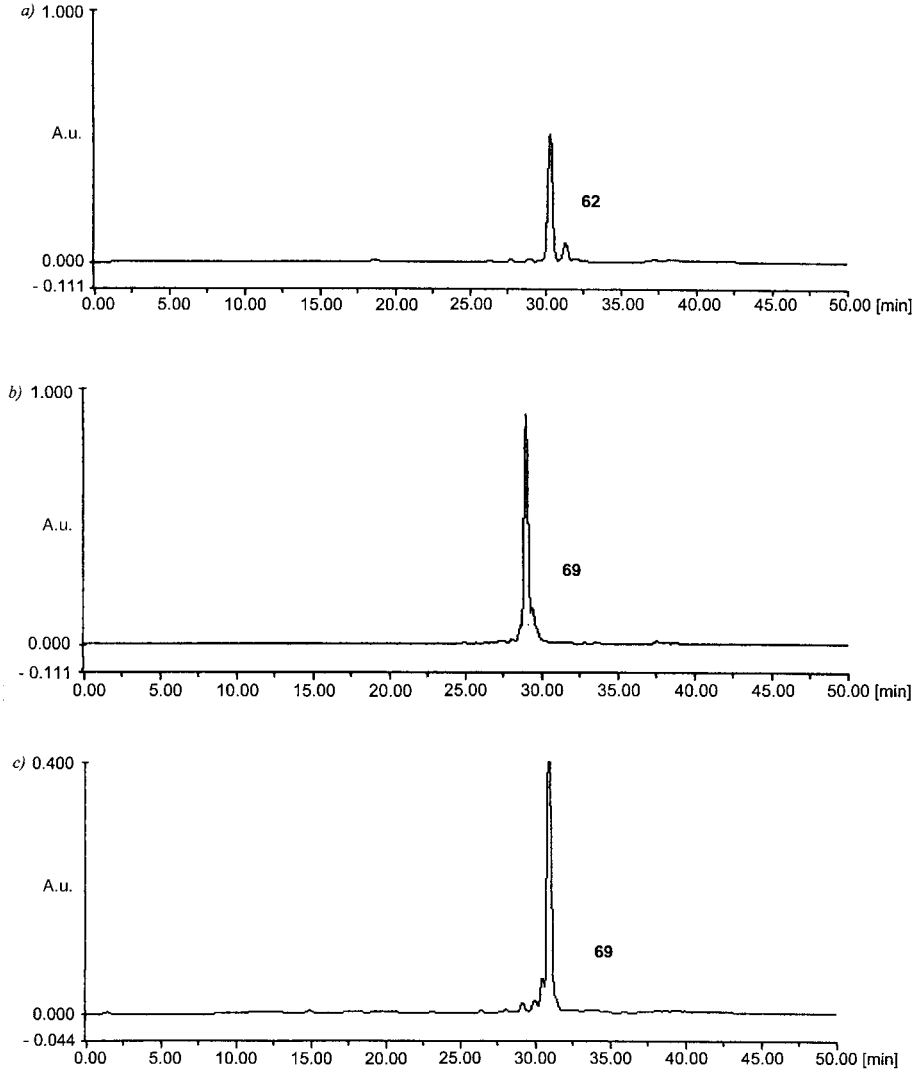


Fig. 1. *Reversed-phase HPLC traces of crude homomer sequences:* a) $[A(thmp)]_4$ (**62**), b) $[U(thmp)]_8$ (**69**), *synthesized with unprotected phosphoramidite* **49**, *and* c) $[U(thmp)]_8$ (**69**), *synthesized with* O^4*-npe-protected phosphoramidite* **50**

In *Fig. 2*, some HPLC traces of crude 2′-*O*-mthp-oligomers are shown. While products with a length of up to 15 bases look fairly pure, longer sequences show more impurities and required further purification before use. The anion-exchange HPLC profiles of oligomers **78** and **82** show impurities eluted after the product peak, probably due to the relatively high content of adenosine compared to sequence **79**.

The cleavage of the 2′-O-mthp group was performed with NaOAc according to *Rao* and *Macfarlane* [36], who used this reagent earlier for the cleavage of the 2′-*O*-[1-(2-

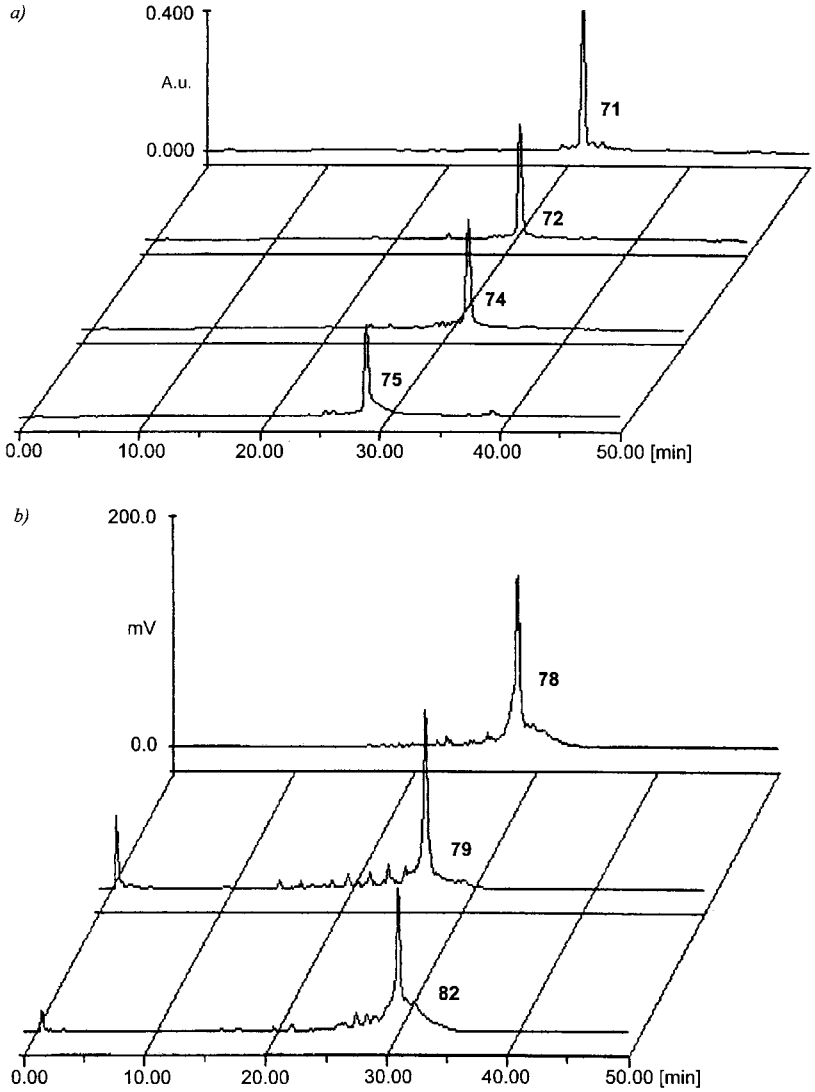


Fig. 2. *HPLC Traces of crude mixed 2′-*O*-thmp-protected oligoribonuceotide sequences:* a) *reversed-phase HPLC of* **71**, **72**, **74**, *and* **75**; b) *anion-exchange HPLC of* **78**, **79**, *and* **82**

fluorophenyl)-4-methoxypiperidin-4-yl] (fpmp) group. The quality of the free oligoribonucleotides was analyzed by reversed-phase and anion-exchange HPLC; by using this mild deprotection method, no impurities resulting from the deprotection step could be observed.

Experimental Part

General. Products were dried under high vacuum. TLC: precoated silica-gel thin-layer sheets *60 F_{254}* from *Merck*. Flash chromatography (FC): silica gel (*Baker*, 30–60 μm); 0.2–0.3 bar. Reversed-phase HPLC: pump *L 6000*, autosampler *AS 4000*, *Merck-Hitachi*, UV detector *Uvikon 730 SLC*, *Kontron*; column *RP 18*, *LiChrospher*, 125 × 4 mm, 5 μm, *Merck*; elution: *A* = 0.1M (Et_3NH)OAc buffer (pH 7), *B* = MeCN; flow rate 1 ml/min, t_R in min. Anion-exchange HPLC: column *NucleoPak PA-100*, 250 × 4 mm, *Dionex*; elution: *C* = 0.02M NaOH and 0.02M NaCl in H_2O (pH 12), *D* = 0.02M NaOH and 1.5M NaCl in H_2O (pH 12), *E* = 0.025M *Tris* in 0.5% MeCN/H_2O (pH 8), *F* = 0.025M *Tris* and 0.8M NH_4Cl in 0.5% MeCN/H_2O (pH 8); flow rate 1 ml/min; t_R in min. 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: *Joel 400 MHz*; δ in ppm rel. to H_3PO_4.

1. *5′-O-[(2-Cyano-1-phenylethoxy)carbonyl]-2′-deoxy-*N^6*-[[2-(4-nitrophenyl)ethoxy]carbonyl]adenosine* (**5**) *and 3′,5′-Bis-*O*-[(2-cyano-1-phenylethoxy)carbonyl]-2′-deoxy-*N^6*-[[2-(4-nitrophenyl)ethoxy]carbonyl]adenosine* (**6**). After co-evaporation with dry pyridine (3 × 5 ml), **1** [11][15] (402 mg, 0.91 mmol) was dissolved in dry pyridine (5 ml) and cooled under N_2 to −50° (^{i}PrOH/N_2 bath). A soln. of an oil, containing *ca.* 80% of **4** [10] (250 mg, 1.19 mmol), in dry CH_2Cl_2 (4 ml) was added dropwise within 45 min, and stirring was continued for 4 h at −50 to −20°. Then the mixture was diluted with CH_2Cl_2 (20 ml) and washed with H_2O (20 ml). The aq. layer was extracted with CH_2Cl_2 (2 × 20 ml), the combined org. phase dried (Na_2SO_4), evaporated, and co-evaporated with toluene (4 × 10 ml), and the residue separated by FC (silica gel (20 g), 14.5 × 2.1 cm, CH_2Cl_2 (100 ml), CH_2Cl_2/MeOH 100:1 (101 ml), 100:2 (102 ml), 100:3 (103 ml), 100:4 (104 ml), 100:5 (210 ml)): 0.38 g (67%) of **5**. Combined fractions containing **6** were still contaminated, and were purified by another FC (silica gel (10 g), 14.5 × 1.4 cm, CH_2Cl_2 (100 ml), CH_2Cl_2/MeOH 100:1 (101 ml), 100:2 (102 ml), 100:3 (51.5 ml)): 0.13 g (18%) of **6**.

Data of **5**: Colorless foam. TLC (toluene/AcOEt/MeOH 5:4:1): R_f 0.34. UV (MeOH): 273 (sh, 4.39), 267 (4.45), 213 (sh, 4.55). ^{1}H-NMR ($CDCl_3$): 9.11, 9.03 (2 br. *s*, NH); 8.63 (*s*, H–C(2)); 8.18, 8.16 (2 *s*, H–C(8)); 8.08 (*d*, 2 H_o to NO_2); 7.38–7.30 (*m*, 2 H_m to NO_2, Ph (2c1peoc)); 6.44 (*t*, H–C(1′)); 5.78 (*m*, CH (2c1peoc)); 4.69 (*m*, H–C(3′)); 4.50 (*t*, CH_2CH_2O); 4.42 (*m*, 1 H–C(5′)); 4.36–4.21 (*m*, H–C(5′), H–C(4′)); 3.95 (2 br. *s*, OH–C(3′)); 3.10 (*t*, CH_2CH_2O); 2.91 (*m*, CH_2 (2c1peoc), 1 H–C(2′)); 2.55 (*m*, 1 H–C(2′)). Anal. calc. for $C_{29}H_{27}N_7O_9 \cdot 0.5\ H_2O$ (626.6): C 55.59, H 4.50, N 15.65; found: C 55.43, H 4.37, N 15.36.

Data of **6**: Colorless foam. TLC (toluene/AcOEt/MeOH 5:4:1): R_f 0.58. UV (MeOH): 272 (sh, 4.41), 266 (4.46), 212 (sh, 4.72). ^{1}H-NMR ($CDCl_3$): 8.69 (*s*, H–C(2)); 8.13, 8.11 (2 *s*, H–C(8)); 8.38, 8.35 (2 br. *s*, NH); 8.13 (*d*, 2 H_o to NO_2); 7.43–7.33 (*m*, 2 H_m to NO_2, 2 Ph (2c1peoc)); 6.46 (*m*, H–C(1′)); 5.87–5.75 (*m*, 2 CH (2c1peoc)); 5.38–5.27 (*m*, H–C(3′)); 4.54–4.33 (*m*, CH_2CH_2O, 2 H–C(5′), H–C(4′)); 3.13 (*t*, CH_2CH_2O); 2.96–2.71 (*m*, 2 CH_2 (2c1peoc), 1 H–C(2′)); 2.55 (*m*, 1 H–C(2′)). Anal. calc. for $C_{39}H_{34}N_8O_{11}$ (790.75): C 59.24, H 4.33, N 14.17; found: C 58.85, H 4.48, N 13.89.

2. *5′-O-[(2-Cyano-1-phenylethoxy)carbonyl]-2′-deoxy-*N^4*-[[2-(4-nitrophenyl)ethoxy]carbonyl]cytidine* (**7**), *3′-O-[(2-Cyano-1-phenylethoxy)carbonyl]-2′-deoxy-*N^4*-[[2-(4-nitrophenyl)ethoxy]carbonyl]cytidine* (**8**), *and 3′,5′-Bis-*O*-[(2-cyano-1-phenylethoxy)carbonyl]-2′-deoxy-*N^4*-[[2-(4-nitrophenyl)ethoxy]carbonyl]cytidine* (**9**). As described in *Exper. 1*, with **2** [11][15] (405 mg, 0.96 mmol), pyridine (6 ml), **4** (265 mg, 1.25 mmol; oil, *ca.* 80% pure), and CH_2Cl_2 (4 ml); addition within 10 min at −55°, stirring for 6 h at −55 to −20°. FC (silica gel (20 g), 14.5 × 2.1 cm, CH_2Cl_2 (100 ml), CH_2Cl_2/MeOH 100:1 (101 ml), 100:2 (102 ml), 100:3 (103 ml), 100:4 (104 ml), 100:5 (210 ml)) gave 334 mg (58%) of **7** and 44 mg (8%) of **8**. Combined fractions containing **9** were still contaminated, and were purified by another FC (silica gel (10 g), 14.5 × 1.4 cm, CH_2Cl_2 (100 ml), CH_2Cl_2/MeOH 100:1 (101 ml), 100:2 (102 ml), 100:3 (51.5 ml)): 114 mg (16%) of **9**.

Data of **7**: Colorless foam. TLC (toluene/AcOEt/MeOH 5:4:1): R_f 0.28. UV (MeOH): 280 (4.16), 242 (4.24), 213 (sh, 4.44). ^{1}H-NMR ($CDCl_3$): 8.31 (br. *s*, NH); 8.13 (*d*, 2 H_o to NO_2); 7.97 (*d*, H–C(6)); 7.37 (*m*, 2 H_m to NO_2, Ph (2c1peoc)); 7.22, 7.19 (2 *d*, H–C(5)); 6.23, 6.21 (2 *t*, H–C(1′)); 5.86–5.72 (*m*, CH (2c1peoc)); 4.47–4.28 (*m*, CH_2CH_2O, H–C(3′), OH–C(3′), 2 H–C(5′)); 4.22 (*m*, H–C(4′)); 3.08 (*t*, CH_2CH_2O); 2.98–2.90 (*m*, CH_2 (2c1peoc)); 2.68 (*m*, H–C(2′)); 2.14–2.02 (*m*, 1 H–C(2′)). Anal. calc. for $C_{28}H_{27}N_5O_{10} \cdot 0.5\ H_2O$ (602.6): C 55.81, H 4.68, N 11.62; found: C 55.78, H 4.48, N 11.51.

Data of **8**: Colorless foam. TLC (toluene/AcOEt/MeOH 5:4:1): R_f 0.39. UV (MeOH): 280 (4.14), 242 (4.24), 213 (sh, 4.46). ^{1}H-NMR ($CDCl_3$): 8.30 (br. *s*, NH); 8.13 (*d*, 2 H_o to NO_2); 8.13 (*d*, H–C(6)); 7.42–7.34 (*m*, 2 H_m to NO_2, Ph (2c1peoc)); 7.14 (*d*, H–C(5)); 6.13 (*t*, H–C(1′)); 5.81 (*m*, CH (2c1peoc)); 5.28 (*m*, H–C(3′)); 4.39 (*t*, CH_2CH_2O); 4.22 (*m*, H–C(4′)); 3.90–3.76 (*m*, 2 H–C(5′)); 3.54 (br. *s*, OH–C(5′)); 3.06 (*t*, CH_2CH_2O); 2.99–2.91 (*m*, CH_2 (2c1peoc)); 2.68–2.56 (*m*, 1 H–C(2′)); 2.48–2.39 (*m*, 1 H–C(2′)). Anal. calc. for $C_{28}H_{27}N_5O_{10} \cdot H_2O$ (611.6): C 54.99, H 4.78, N 11.45; found: C 55.28, H 4.44, N 10.93.

Data of **9**: Colorless foam. TLC (toluene/AcOEt/MeOH 5:4:1): R_f 0.56. UV (MeOH): 278 (4.19), 243 (4.29), 213 (sh, 4.73). ^{1}H-NMR ($CDCl_3$): 8.20 (br. *s*, NH); 8.16 (*d*, 2 H_o to NO_2); 7.84, 7.80 (2 *d*, H–C(6)); 7.36 (*m*, 2 H_m to NO_2, 2 Ph (2c1peoc)); 7.20, 7.08 (2 *d*, H–C(5)); 6.21 (*m*, H–C(1′)); 5.81 (*m*, 2 CH (2c1peoc)); 5.16 (*m*, H–C(3′)); 4.44–4.28 (*m*, CH_2CH_2O, 2 H–C(5′), H–C(4′)); 3.10 (*m*, CH_2CH_2O); 2.90 (*m*, 2 CH_2 (2c1peoc)); 2.77 (*m*, 1 H–C(2′)); 2.13 (*m*, 1 H–C(2′)). Anal. calc. for $C_{38}H_{34}N_6O_{12}$ (766.7): C 59.53, H 4.47, N 10.96; found: C 59.50, H 4.73, N 10.51.

3. *5′-*O*-[(2-Cyano-1-phenylethoxy)carbonyl]-2′-deoxy-*N^2*-[[2-(4-nitrophenyl)ethoxy)carbonyl]-*O^6*-[2-(4-nitrophenyl)ethyl]guanosine* (**10**) *and 3′,5′-Bis-*O*-[(2-cyano-1-phenylethoxy)carbonyl]-2′-deoxy-*N^2*-[[2-(4-nitrophenyl)ethoxy]carbonyl]-*O^6*-[2-(4-nitrophenyl)ethyl]guanosine* (**11**). As described in *Exper. 1*, with **3** [11][15] (403 mg, 0.66 mmol), pyridine (5 ml), **4** (180 mg, 0.86 mmol; oil of *ca.* 80% purity), and CH_2Cl_2 (4 ml); addition within 45 min at $-55°$, stirring for 6 h at -55 to $-20°$. FC (silica gel (20 g), 14.5 × 2.1 cm, CH_2Cl_2 (100 ml), CH_2Cl_2/MeOH 100:1 (101 ml), 100:2 (102 ml), 100:3 (103 ml), 100:4 (104 ml), 100:5 (105 ml)) gave 376 mg (73%) of **10**. Combined fractions containing **11** were still contaminated and purified by another FC (silica gel (10 g), 14.5 × 1.4 cm, CH_2Cl_2 (100 ml), CH_2Cl_2/MeOH 100:1 (101 ml), 100:2 (102 ml)): 88 mg (14%) of **11**.

Data of **10**: Colorless foam. TLC (toluene/AcOEt/MeOH 5:4:1): R_f 0.32. UV (MeOH): 279 (sh, 4.46), 268 (4.54), 213 (sh, 4.68). ^{1}H-NMR ($CDCl_3$): 8.11 (*d*, 2 H_o to NO_2); 8.10 (*d*, 2 H_o to NO_2); 8.03, 7.98 (2 *s*, H–C(8)); 7.54 (br. *s*, NH); 7.45 (*d*, 2 H_m to NO_2); 7.41 (*d*, 2 H_m to NO_2); 7.32 (*m*, Ph (2c1peoc)); 6.54, 6.50 (2 *t*, H–C(1′)); 5.88 (*m*, CH (2c1peoc)); 4.80 (*m*, H–C(3′)); 4.73 (*t*, CH_2CH_2O (*O*^{6}-npe)); 4.51–4.32 (*m*, CH_2CH_2O (*N*^{2}-npeoc), 2 H–C(5′)); 4.27 (*m*, H–C(4′)); 3.88, 3.81 (2 br. *s*, OH–C(3′)); 3.24 (*t*, CH_2CH_2O (*O*^{6}-npe)); 3.06 (*t*, CH_2CH_2 (*N*^{2}-npeoc)); 2.88 (*m*, CH_2 (2c1peoc)); 2.77 (*m*, 1 H–C(2′)); 2.50 (*m*, 1 H–C(2′)). Anal. calc. for $C_{37}H_{34}N_8O_{12}$ (782.7): C 56.78, H 4.38, N 14.32; found: C 56.47, H 4.41, N 14.13.

Data of **11**: Colorless foam. TLC (toluene/AcOEt/MeOH 5:4:1): R_f 0.56. UV (MeOH): 277 (sh, 4.51), 268 (4.57), 214 (sh, 4.83). ^{1}H-NMR ($CDCl_3$): 8.13 (*d*, 2 H_o to NO_2); 8.10 (*d*, 2 H_o to NO_2); 7.85 (*s*, H–C(8)); 7.49–7.30 (*m*, 4 H_m to NO_2, NH, 2 Ph (2c1peoc)); 6.31 (*m*, H–C(1′)); 5.86–5.73 (*m*, 2 CH (2c1peoc)); 5.48 (*m*, H–C(3′)); 4.72 (*t*, CH_2CH_2O (*O*^{6}-npe)); 4.48–4.33 (*m*, CH_2CH_2O (*N*^{2}-npeoc), 2 H–C(5′), H–C(4′)); 3.23 (*t*, CH_2CH_2 (*O*^{6}-npe)); 3.17–3.02 (*m*, CH_2CH_2O (*N*^{2}-npeoc)); 3.06–2.81 (*m*, 2 CH_2 (2c1peoc)); 2.72 (*m*, 1 H–C(2′)); 2.59 (*m*, 1 H–C(2′)). Anal. calc. for $C_{47}H_{41}N_9O_{14}$ (955.9): C 59.06, H 4.32, N 13.19; found: C 58.85, H 4.48, N 12.83.

4. *Kinetic Studies.* Nucleoside **5**, **7**, or **10** was dissolved in MeCN (concentration of nucleoside, 0.02M) in an attached cap *Eppendorf* tube. Then 2–3 aliquots (50 µl) were taken, quenched in AcOH/MeCN (0.05M, 450 µl), and analyzed by HPLC for the determination of the area under the peak of starting material at time t_0 (A_0). Then the cleavage reaction was started by addition of 10 equiv. of DBU/MeCN (0.2M, end concentration of the reaction soln. 0.1M) and stirred at r.t. After defined time intervals, aliquots (50 µl) were removed, quenched in AcOH/MeCN (0.05M, 450 µl), and analyzed by reversed-phase HPLC. The decrease of starting material was detected at 268 nm. For each experiment, 5–10 aliquots were taken. The half-lives ($t_{1/2}$) for the pseudo-first-order reactions were obtained from the graphical plots of $\ln(A_t/A_0)$ *vs.* time, where A_t is the area under the peak of starting material at time *t*.

HPLC for **5**: gradient: 0% *B* (0–3 min), 0–50% *B* (3–40 min), 50–100% *B* (40–45 min), 100–50% *B* (45–50 min), 50–0% *B* (50–53 min), 0% *B* (53–55 min); **1** t_R 26.79; **5** t_R 38.08; 3-phenyl-prop-2-enenitrile, t_R 34.19 and 35.04.

HPLC for **7** and **10**: gradient: 0% *B* (0–3 min), 0–50% *B* (3–30 min), 50–100% *B* (30–35 min), 100–50% *B* (35–40 min), 50–0% *B* (40–43 min), 0% *B* (43–45 min); **2**, t_R 23.04; **3**, t_R 32.13; **7**, t_R 31.41; **10**: t_R 35.20; 3-phenyl-prop-2-enenitrile, t_R 28.72 and 29.31 min.

5. *N^6-{[2-(4-Nitrophenyl)ethoxy]carbonyl}-2′-*O*-(tetrahydro-4-methoxy-2*H*-pyran-4-yl)adenosine* (**28**) [9]. To a soln. of **18** [19] (5.01 g, 7.12 mmol) and pyridinium *p*-toluenesulfonate (500 mg, 1.99 mmol) in dry CH_2Cl_2 (50 ml), 70% pure 5,6-dihydro-4-methoxy-2*H*-pyran [21–23] (5 ml, 30.7 mmol) was added and the soln. stirred at r.t. After 3 d, the mixture was neutralized by adding sodium methoxide (*ca.* 10% in MeOH), whereby the color turned from orange-red to light orange, and the mixture was evaporated. The residual yellow oil was dissolved in MeOH (50 ml), NH_4F (2.64 g, 71.3 mmol) was added, and the mixture was stirred for additional 2 d at r.t. Then the mixture was diluted with CH_2Cl_2 (100 ml) and washed with H_2O (100 ml). The aq. layer was extracted with CH_2Cl_2 (2 × 100 ml), the combined org. phase dried (Na_2SO_4), and the residue purified by FC (silica gel (100 g), 14 × 4.3 cm, CH_2Cl_2/MeOH 100:1 (252.5 ml), 100:2 (255 ml), 100:3 (275.5 ml), 100:4 (260 ml), 100:5 (525 ml), 100:6 (106 ml)): 3.67 g (90%) of **28**. Colorless foam. TLC (toluene/AcOEt/MeOH 5:4:1): R_f 0.18. UV (MeOH): 275 (sh, 4.37), 267 (4.45). ^{1}H-NMR ($CDCl_3$): 8.74 (*s*, H–C(2)); 8.41 (br. *s*, NH); 8.13 (*d*, 2 H_o to NO_2); 8.01 (*s*, H–C(8)); 7.42 (*d*, 2 H_m to NO_2); 5.90 (*d*, OH–C(5′)); 5.88 (*d*, H–C(1′)); 5.28 (*dd*, H–C(2′)); 4.53 (*t*, CH_2CH_2O); 4.36 (*m*, H–C(3′), H–C(4′)); 3.93 (*m*, 1 H–C(5′)); 3.75 (*m*, 1 H–C(5′));

3.71 – 3.26 (*m*, CH_2OCH_2); 3.14 (*t*, CH_2CH_2O); 2.87 (br. *s*, OH–C(3′)); 2.59 (*s*, MeO); 1.83 – 1.31 (*m*, CH_2CCH_2).

6. N^4-*[[2-(4-Nitrophenyl)ethoxy]carbonyl]-2′*-O-*(tetrahydro-4-methoxy-2*H*-pyran-4-yl)cytosine* (**29**) [9]. As described in *Exper. 5*, with **19** [19] (5.01 g, 7.38 mmol), pyridinium *p*-toluenesulfonate (507 mg, 2.02 mmol), CH_2Cl_2 (50 ml), and 60% pure 5,6-dihydro-4-methoxy-2*H*-pyran (5.5 ml, 28.9 mmol); stirring for 2 d. After desilylation of the residual yellow oil in MeOH (50 ml) with NH_4F (2.73 g, 73.7 mmol) for 3 d at r.t., the mixture was treated with H_2O (150 ml), shaken, and let stand. After *ca.* 10 min, the precipitated product was filtered by suction and washed with Et_2O: 3.45 g (85%) of **29**. Colorless solid. TLC (toluene/AcOEt/MeOH 5 : 4 : 1): R_f 0.19. UV (MeOH): 276 (sh, 4.13), 244 (4.25), 210 (sh, 4.39). ^{1}H-NMR ((D_6)DMSO): 10.60 (br. *s*, NH); 8.32 (*d*, H–C(6)); 8.18 (*d*, 2 H_o to NO_2); 7.60 (*d*, 2 H_m to NO_2); 7.01 (*d*, H–C(5)); 6.08 (*d*, H–C(1′)); 5.19 (*t*, OH–C(5′)); 5.16 (*d*, OH–C(3′)); 4.38 – 3.92 (*m*, H–C(2′), CH_2CH_2O); 3.98 (*m*, H–C(3′)); 3.92 (*m*, H–C(4′)); 3.68 – 3.36 (*m*, 2 H–C(5′), CH_2OCH_2); 3.07 (*t*, CH_2CH_2O); 2.86 (*s*, MeO); 1.74 – 1.65 (*m*, CH_2CCH_2).

7. N^2-*[[2-(4-Nitrophenyl)ethoxy]carbonyl]*-O^6-*[2-(4-nitrophenyl)ethyl]-2′*-O-*(tetrahydro-4-methoxy-2*H*-pyran-4-yl)guanosine* (**30**) [9]. As described in *Exper. 5*, with **20** [20] (5.00 g, 5.76 mmol), pyridinium *p*-toluenesulfonate (500 mg, 1.99 mmol), CH_2Cl_2 (50 ml), and 73% pure 5,6-dihydro-4-methoxy-2*H*-pyran (5.0 ml, 32.0 mmol); stirring for 3 d. After desilylation of the residual yellow oil in MeOH (50 ml) with NH_4F (2.14 g, 57.6 mmol) for 3 d at r.t. and usual workup, purification by FC (silica gel (100 g), 14 × 4.3 cm, CH_2Cl_2/MeOH 100 : 1 (252.5 ml), 100 : 2 (255 ml), 100 : 3 (275.5 ml), 100 : 4 (520 ml), 100 : 5 (262.5 ml)) gave 3.54 g (83%) of **30**. Slightly yellow foam. TLC (toluene/AcOEt/MeOH 5 : 4 : 1): R_f 0.25. UV (MeOH): 276 (sh, 4.46), 268 (4.51), 215 (4.58). ^{1}H-NMR ($CDCl_3$): 8.17 (*d*, 2 H_o to NO_2); 8.14 (*d*, 2 H_o to NO_2); 7.84 (*s*, H–C(8)); 7.49 (*d*, 2 H_m to NO_2); 7.41 (*d*, 2 H_m to NO_2); 7.29 (*s*, NH); 5.83 (*d*, H–C(1′)); 5.28 – 5.13 (*m*, H–C(2′), OH–C(5′)); 4.86 – 4.72 (*m*, CH_2O (O^6-npe)); 4.46 (*t*, CH_2CH_2O (N^2-npeoc)); 4.38 (*d*, H–C(3′)); 4.30 (*m*, H–C(4′)); 3.92 (*m*, 1 H–C(5′)); 3.79 (*m*, 1 H–C(5′)); 3.74 – 3.37 (*m*, CH_2OCH_2); 3.29 (*t*, CH_2CH_2 (O^6-npe)); 3.10 (*t*, CH_2CH_2 (N^2-npeoc)); 2.71 (*s*, OH–C(3′)); 2.63 (*s*, MeO); 1.85 – 1.41 (*m*, CH_2CCH_2).

8. *2′*-O-*(Tetrahydro-4-methoxy-2*H*-pyran-4-yl)uridine* (**31**) [23]. As described in *Exper. 5*, with **21** [18] (5.07 g, 10.4 mmol), pyridinium *p*-toluenesulfonate (509 mg, 2.03 mmol), CH_2Cl_2 (50 ml), and 66% pure 5,6-dihydro-4-methoxy-2*H*-pyran (8.5 ml, 49.2 mmol); stirring for 2 d. After desilylation of the residual brown oil in MeOH (50 ml) with NH_4F (3.86 g, 104 mmol) for 3 d at r.t. and usual workup, purification by FC (silica gel (100 g), 14 × 4.3 cm, CH_2Cl_2/MeOH 100 : 1 (252.5 ml), 100 : 2 (255 ml), 100 : 3 (275.5 ml), 100 : 4 (520 ml), 100 : 5 (262.5 ml), 100 : 6 (265 ml), 100 : 7 (535 ml), 100 : 8 (270 ml)) gave 3.65 g (98%) of **31**. Colorless foam. TLC (toluene/AcOEt/MeOH 5 : 4 : 1): R_f 0.15. UV (MeOH): 260 (3.92). ^{1}H-NMR ((D_6)DMSO): 11.36 (*s*, NH); 7.92 (*s*, H–C(6)); 6.00 (*d*, H–C(1′)); 5.72 (*d*, H–C(5)); 5.21 – 5.14 (*m*, OH–C(3′), OH–C(5′)); 4.31 (*dd*, H–C(2′)); 3.96 (*t*, H–C(3′)); 3.89 (*m*, H–C(4′)); 3.69 – 3.32 (*m*, 2 H–C(5′), CH_2OCH_2); 2.95 (*s*, MeO); 1.81 – 1.56 (*m*, CH_2CCH_2).

9. O^4-*[2-(4-Nitrophenyl)ethyl]-2′*-O-*(tetrahydro-4-methoxy-2*H*-pyran-4-yl)uridine* (**32**) [17]. As described in *Exper. 5*, with **22** [17] (3.00 g, 4.72 mmol), pyridinium *p*-toluenesulfonate (300 mg, 1.19 mmol), CH_2Cl_2 (30 ml), and 70% pure 5,6-dihydro-4-methoxy-2*H*-pyran (8.5 ml, 49.2 mmol); stirring for 3 d. After desilylation of the residual brown oil in MeOH (30 ml) with NH_4F (1.75 g, 47.3 mmol) for 4 d at r.t., the mixture was treated with H_2O (130 ml) and the precipitated product filtered by suction and washed with Et_2O: 1.42 g (59%) of **32**. The filtrate was diluted with CH_2Cl_2 (50 ml), the aq. layer extracted with CH_2Cl_2 (2 × 20 ml), the combined org. phase dried (Na_2SO_4) and evaporated; and the residue purified by FC (silica gel (40 g), 15 × 2.6 cm, CH_2Cl_2/MeOH 100 : 1 (202 ml), 100 : 2 (204 ml), 100 : 3 (206 ml), 100 : 4 (208 ml), 100 : 5 (210 ml)): 679 mg (28%) of **32**. Total yield: 2.09 g (87%). Colorless solid. TLC (toluene/AcOEt/MeOH 5 : 4 : 1): R_f 0.22. UV (MeOH): 275 (4.21). ^{1}H-NMR ((D_6)DMSO): 8.23 (*d*, H–C(6)); 8.17 (*d*, 2 H_o to NO_2); 7.57 (*d*, 2 H_m to NO_2); 6.10 (*d*, H–C(5)); 6.07 (*d*, H–C(1′)); 5.21 (*t*, OH–C(5′)); 5.15 (*d*, OH–C(3′)); 4.54 (*t*, CH_2CH_2O); 4.30 (*dd*, H–C(2′)); 3.96 (*t*, H–C(3′)); 3.91 (*m*, H–C(4′)); 3.63 – 3.33 (*m*, 2 H–C(5′), CH_2OCH_2); 3.15 (*t*, CH_2CH_2O); 2.82 (*s*, MeO); 1.82 – 1.63 (*m*, CH_2CCH_2).

10. *5′*-O-*[(2-Cyano-1-phenylethoxy)carbonyl]*-N^6-*[[2-(4-nitrophenyl)ethoxy]carbonyl]-2′*-O-*(tetrahydro-4-methoxy-2*H*-pyran-4-yl)adenosine* (**33**) *and 3′,5′-Bis*-O-*[(2-cyano-1-phenylethoxy)carbonyl]*-N^6-*[[2-(4-nitrophenyl)ethoxy]carbonyl]-2′*-O-*(tetrahydro-4-methoxy-2*H*-pyran-4-yl)adenosine* (**34**). As described in *Exper. 1*, with **28** (1.01 g, 1.76 mmol; co-evaporated with pyridine (3 × 10 ml)), pyridine (35 ml), **4** (479 mg, 2.29 mmol; oil of *ca.* 80% purity), and CH_2Cl_2 (25 ml); addition within 2 h 30 min at −55 to −45°, stirring for 3 h at −50 to −10°. Workup with CH_2Cl_2 (80 ml), H_2O (100 ml), and CH_2Cl_2 (2 × 100 ml) and co-evaporation with toluene (4 × 20 ml). FC (silica gel (50 g), 20 × 2.9 cm, CH_2Cl_2 (250 ml), CH_2Cl_2/MeOH 100 : 1 (252.5 ml), 100 : 2 (255 ml), 100 : 3 (257.5 ml), 100 : 4 (260 ml)) gave 940 mg (72%) of **33**. Combined fractions containing **34** were

still contaminated, and were purified by another FC (silica gel (10 g), 14.5 × 1.4 cm, CH_2Cl_2 (100 ml), CH_2Cl_2/MeOH 100 : 1 (101 ml), 100 : 2 (102 ml), 100 : 3 (51.5 ml)): 270 mg (17%) of **34**.

Data of **33**: Colorless foam. TLC (toluene/AcOEt/MeOH 5 : 4 : 1): R_f 0.34. UV (MeOH): 273 (sh, 4.39), 266 (4.44). ^{1}H-NMR ($CDCl_3$): 8.71 (*s*, H–C(2)); 8.15 (*s*, H–C(8)); 8.29, 8.25 (2 *s*, NH); 8.12 (*d*, 2 H_o to NO_2); 7.41–7.33 (*m*, 2 H_m to NO_2, Ph (2c1peoc)); 6.13, 6.12 (2 *d*, H–C(1′)); 5.82 (*m*, CH (2c1peoc)); 5.14 (*m*, H–C(2′)); 4.52 (*t*, CH_2CH_2O); 4.50–4.33 (*m*, H–C(3′), H–C(4′), 2 H–C(5′)); 3.74–3.35 (*m*, CH_2OCH_2); 3.14 (*t*, CH_2CH_2O); 2.96–2.87 (*m*, CH_2 (2c1peoc), OH–C(3′)); 2.84 (*s*, MeO); 1.78–1.56 (*m*, CH_2CCH_2). Anal. calc. for $C_{35}H_{37}N_7O_{12} \cdot 0.5\ H_2O$ (756.7): C 55.55, H 5.06, N 12.96; found: C 55.48, H 5.02, N 12.54.

Data of **34**: Colorless foam. TLC (toluene/AcOEt/MeOH 5 : 4 : 1): R_f 0.54. UV (MeOH): 274 (sh, 4.42), 266 (4.48), 213 (sh, 4.66). ^{1}H-NMR ($CDCl_3$): 8.72, 8.71 (2 *s*, H–C(2)); 8.11, 8.06 (2 *s*, H–C(8)); 8.00, 7.98 (2 br. *s*, NH); 8.18 (*d*, 2 H_o to NO_2); 7.44–7.27 (*m*, 2 H_m to NO_2, 2 Ph (2c1peoc)); 6.10 (*m*, H–C(1′)); 5.86–5.71 (*m*, 2 CH (2c1peoc)); 5.54–5.32 (*m*, H–C(2′)); 5.22 (*m*, H–C(3′)); 4.45 (*m*, CH_2CH_2O, H–C(4′), 2 H–C(5′)); 3.74–3.26 (*m*, CH_2OCH_2); 3.10 (*t*, CH_2CH_2O); 3.00–2.83 (*m*, 2 CH_2 (2c1peoc)); 2.71, 2.48 (2 *s*, MeO); 1.81–1.51 (*m*, CH_2CCH_2). Anal. calc. for $C_{45}H_{44}N_8O_{14}$ (920.9): C 58.69, H 4.82, N 12.17; found: C 58.77, H 4.92, N 11.92.

11. *5′-*O*-[(2-Cyano-1-phenylethoxy)carbonyl]-*N^4*-{[2-(4-nitrophenyl)ethoxy]carbonyl}-2′-*O*-(tetrahydro-4-methoxy-2*H*-pyran-4-yl)cytidine* (**35**), *3′-*O*-[(2-Cyano-1-phenylethoxy)carbonyl]-*N^4*-{[2-(4-nitrophenyl)ethoxy]carbonyl}-2′-*O*-(tetrahydro-4-methoxy-2*H*-pyran-4-yl)cytidine* (**36**), *and 3′,5′-Bis-*O*-[(2-cyano-1-phenylethoxy)carbonyl]-*N^4*-{[2-(4-nitrophenyl)ethoxy]carbonyl}-2′-*O*-(tetrahydro-4-methoxy-2*H*-pyran-4-yl)cytidine* (**37**). As described in *Exper. 1*, with **29** (1.00 g, 1.82 mmol; co-evaporated with dry pyridine (3 × 10 ml)), pyridine (20 ml), **4** (575 mg, 2.74 mmol; oil of *ca.* 80% purity), and CH_2Cl_2 (16 ml); addition within 2 h at −60 to −50°, stirring for 4 h 35 min at −55 to −20°. Workup with CH_2Cl_2 (80 ml), H_2O (100 ml), and CH_2Cl_2 (2 × 100 ml) and co-evaporation with toluene (5 × 20 ml). FC (silica gel (40 g), 15 × 2.6 cm, CH_2Cl_2 (200 ml), CH_2Cl_2/MeOH 100 : 1 (202 ml), 100 : 2 (204 ml), 100 : 3 (206 ml), 100 : 4 (208 ml), 100 : 5 (105 ml)) gave 763 mg (58%) of **35** and 37 mg (3%) of **36**. Combined fractions containing **37** were still contaminated and purified by another FC (silica gel (10 g), 14.5 × 1.4 cm, CH_2Cl_2 (100 ml), CH_2Cl_2/MeOH 100 : 0.5 (100.5 ml), 100 : 1 (101 ml), 100 : 2 (102 ml)): 322 mg (20%) of **37**.

Data of **35**: Colorless foam. TLC (toluene/AcOEt/MeOH 5 : 4 : 1): R_f 0.30. UV (MeOH): 277 (sh, 4.15), 245 (4.28). ^{1}H-NMR ($CDCl_3$): 8.40 (br. *s*, NH); 8.14 (*d*, 2 H_o to NO_2); 7.90, 7.88 (2 *d*, H–C(6)); 7.42–7.36 (*m*, 2 H_m to NO_2, Ph (2c1peoc)); 7.26, 7.18 (2 *d*, H–C(5)); 6.03, 6.02 (2 *d*, H–C(1′)); 5.84 (*m*, CH (2c1peoc)); 4.52–4.37 (*m*, H–C(2′), CH_2CH_2O, 2 H–C(5′)); 4.22 (*m*, H–C(4′)); 4.08 (*m*, H–C(3′)); 3.74–3.53 (*m*, CH_2OCH_2); 3.35 (br. *s*, OH–C(3′)); 3.10 (*m*, CH_2CH_2O, MeO); 2.95 (*m*, CH_2 (2c1peoc)); 1.85–1.70 (*m*, CH_2CCH_2). Anal. calc. for $C_{34}H_{37}N_5O_{13}$ (723.7): C 56.43, H 5.15, N 9.68; found: C 56.03, H 5.25, N 9.36.

Data of **36**: Colorless foam. TLC (toluene/AcOEt/MeOH 5 : 4 : 1): R_f 0.39. UV (MeOH): 274 (sh, 4.14), 245 (4.28). ^{1}H-NMR ($CDCl_3$): 8.47 (br. *s*, NH); 8.14 (*d*, 2 H_o to NO_2); 7.81, 7.79 (2 *d*, H–C(6)); 7.38 (*m*, 2 H_m to NO_2, Ph (2c1peoc)); 7.20 (*m*, H–C(5)); 5.83 (*m*, CH (2c1peoc)); 5.58 (*m*, H–C(1′)); 5.32, 5.25 (2 *m*, H–C(2′)); 5.22 (*m*, H–C(3′)); 4.41 (*m*, CH_2CH_2O, OH–C(5′)); 4.29, 4.18 (2 *m*, H–C(4′)); 3.84–3.26 (*m*, 2 H–C(5′), CH_2OCH_2); 3.09 (*t*, CH_2CH_2O); 2.97, 2.79 (2 *s*, MeO); 2.94 (*m*, CH_2 (2c1peoc)); 1.83–1.75 (*m*, CH_2CCH_2). Anal. calc. for $C_{34}H_{37}N_5O_{13}$ (723.7): C 56.43, H 5.15, N 9.68; found: C 55.97, H 5.35, N 9.23.

Data of **37**: Colorless foam. TLC (toluene/AcOEt/MeOH 5 : 4 : 1): R_f 0.49. UV (MeOH): 280 (sh, 4.07), 246 (4.22), 214 (sh, 4.09). ^{1}H-NMR ($CDCl_3$): 8.16 (*d*, 2 H_o to NO_2); 7.75 (*m*, H–C(6)); 7.55 (br. *s*, NH); 7.40 (*m*, 2 H_m to NO_2, 2 Ph (2c1peoc)); 7.24–7.12 (*m*, H–C(5)); 6.12 (*m*, H–C(1′)); 5.81 (*m*, 2 CH (2c1peoc)); 5.00 (*m*, H–C(3′)); 4.72, 4.60 (2 *m*, H–C(2′)); 4.46–4.33 (*m*, CH_2CH_2O, H–C(4′), 2 H–C(5′)); 3.75–3.38 (*m*, CH_2OCH_2); 3.13–2.84 (*m*, CH_2CH_2O, 2 CH_2 (2c1peoc), MeO); 1.82–1.59 (*m*, CH_2CCH_2). Anal. calc. for $C_{44}H_{44}N_6O_{15}$ (896.9): C 58.93, H 4.95, N 9.37; found: C 58.54, H 5.05, N 9.53.

12. *5′-*O*-[(2-Cyano-1-phenylethoxy)carbonyl]-*N^2*-{[2-(4-nitrophenyl)ethoxy]carbonyl}-*O^6*-[2-(4-nitrophenyl)ethyl]-2′-*O*-(tetrahydro-4-methoxy-2*H*-pyran-4-yl)guanosine* (**38**) *and 3′,5′-Bis-*O*-[(2-cyano-1-phenylethoxy)carbonyl]-*N^2*-{[2-(4-nitrophenyl)ethoxy]carbonyl}-*O^6*-[2-(4-nitrophenyl)ethyl]-2′-*O*-(tetrahydro-4-methoxy-2*H*-pyran-4-yl)guanosine* (**39**). As described in *Exper. 1*, with **30** (1.00 g, 1.35 mmol), co-evaporated with dry pyridine (3 × 10 ml)), pyridine (20 ml), **4** (395 mg, 1.88 mmol; oil of *ca.* 80% purity), and CH_2Cl_2 (16 ml); addition within 1 h 15 min at −60 to −45°, stirring for 4 h 30 min at −50 to −20°. Workup with CH_2Cl_2 (100 ml), H_2O (100 ml), and CH_2Cl_2 (2 × 100 ml) and co-evaporation with toluene (5 × 20 ml). FC (silica gel (40 g), 15 × 2.6 cm, CH_2Cl_2/MeOH 100 : 1 (202 ml), 100 : 2 (204 ml), 100 : 3 (206 ml), 100 : 4 (208 ml)) gave 1.03 g (83%) of **38**. Combined fractions containing **39** were still contaminated, and were purified by another FC (silica gel (10 g), 14.5 × 1.4 cm, CH_2Cl_2 (100 ml), CH_2Cl_2/MeOH 100 : 1 (101 ml), 100 : 2 (102 ml)): 139 mg (10%) of **39**.

Data of **38**: Colorless foam. TLC (toluene/AcOEt/MeOH 5:4:1): R_f 0.36. UV (MeOH): 279 (sh, 4.33), 268 (4.43), 213 (4.51). ^{1}H-NMR ($CDCl_3$): 8.16–8.11 (2 *d*, 4 H_o to NO_2); 7.89, 7.88 (2 *s*, H–C(8)); 7.48 (*d*, 2 H_m to NO_2); 7.42 (*d*, 2 H_m to NO_2); 7.35 (*m*, Ph (2c1peoc), NH); 5.96, 5.94 (2 *d*, H–C(1′)); 5.78 (*t*, CH (2c1peoc)); 5.18 (*m*, H–C(2′)); 4.76 (*t*, CH_2CH_2O (O^6-npe)); 4.55–4.36 (*m*, CH_2CH_2O (N^2-npeoc), 2 H–C(5′), H–C(3′)); 4.30 (*m*, H–C(4′)); 3.72–3.40 (*m*, CH_2OCH_2); 3.27 (*t*, CH_2CH_2O (O^6-npe)); 3.09 (*t*, CH_2CH_2O (N^2-npeoc)); 2.89 (*m*, CH_2 (2c1peoc), MeO); 2.82 (br. *s*, OH–C(3′)); 1.74–1.57 (*m*, CH_2CCH_2). Anal. calc. for $C_{43}H_{44}N_8O_{15}\cdot 0.5\ H_2O$ (921.9): C 56.02, H 4.92, N 12.15; found: C 55.88, H 4.84, N 12.35.

Data of **39**: Colorless foam. TLC (toluene/AcOEt/MeOH 5:4:1): R_f 0.58. UV (MeOH): 277 (sh, 4.37), 268 (4.44), 211 (4.64). ^{1}H-NMR ($CDCl_3$): 8.15 (*m*, 4 H_o to NO_2); 7.86, 7.85 (2 *s*, H–C(8)); 7.49–7.28 (*m*, 4 H_m to NO_2, 2 Ph (2c1peoc), NH); 5.98–5.66 (*m*, H–C(1′), 2 CH (2c1peoc), H–C(2′)); 5.30 (*m*, H–C(3′)); 4.76 (*t*, CH_2CH_2O (O^6-npe)); 4.57–4.35 (*m*, CH_2CH_2O (N^2-npeoc), 2 H–C(5′), H–C(4′)); 3.70–3.26 (*m*, CH_2OCH_2, CH_2CH_2O (O^6-npe)); 3.14–2.82 (*m*, CH_2CH_2O (N^2-npeoc), 2 CH_2 (2c1peoc)); 2.72, 2.51 (2 *s*, MeO); 1.76–1.48 (*m*, CH_2CCH_2). Anal. calc. for $C_{53}H_{51}N_9O_{17}$ (1086.0): C 58.62, H 4.73, N 11.61; found: C 58.36, H 4.78, N 11.44.

13. *5′*-O-*[(2-Cyano-1-phenylethoxy)carbonyl]-2′*-O-*(tetrahydro-4-methoxy-2*H-*pyran-4-yl)uridine* (**40**) and *3′,5′-Bis*-O-*[(2-cyano-1-phenylethoxy)carbonyl]-2′*-O-*(tetrahydro-4-methoxy-2*H-*pyran-4-yl)uridine* (**41**). As described in *Exper. 1*, with **31** (1.00 g, 2.79 mmol; co-evaporated with dry pyridine (3 × 10 ml)), pyridine (20 ml), **4** (800 mg, 3.82 mmol; oil of *ca.* 80% purity), and CH_2Cl_2 (16 ml); addition within 1 h 40 min at −60 to −45°, stirring for 3 h 40 min at −55 to −20°. Workup with CH_2Cl_2 (100 ml), H_2O (100 ml), and CH_2Cl_2 (2 × 100 ml) and co-evaporation with toluene (5 × 20 ml). FC (silica gel (50 g), 19 × 3 cm, CH_2Cl_2 (250 ml), CH_2Cl_2/MeOH 100:1 (252.5 ml), 100:2 (255 ml), 100:3 (257.5 ml), 100:4 (208 ml), 100:5 (157.5 ml)) gave 957 mg (65%) of **40** and 443 mg (23%) of **41**.

Data of **40**: Colorless foam. TLC (toluene/AcOEt/MeOH 5:4:1): R_f 0.31. UV (MeOH): 259 (3.96). ^{1}H-NMR ($CDCl_3$): 9.27 (br. *s*, NH); 7.44–7.33 (*m*, Ph (2c1peoc), H–C(6)); 5.99 (2 *d*, H–C(1′)); 5.84 (*m*, CH (2c1peoc)); 5.84, 5.58 (2 *d*, H–C(5)); 4.44 (*m*, H–C(2′), 2 H–C(5′)); 4.36–4.21 (*m*, H–C(4′)); 4.20–4.06 (*m*, H–C(3′)); 3.80–3.52 (*m*, CH_2OCH_2); 3.15, 3.13 (2 *s*, MeO); 3.14–2.92 (*m*, CH_2 (2c1peoc), OH–C(3′)); 1.90–1.59 (*m*, CH_2CCH_2). Anal. calc. for $C_{25}H_{29}N_3O_{10}$ (531.5): C 56.49, H 5.50, N 7.91; found: C 56.27, H 5.57, N 8.07.

Data of **41**: Colorless foam. TLC (toluene/AcOEt/MeOH 5:4:1): R_f 0.48. UV (MeOH): 257 (3.99), 214 (sh, 4.22). ^{1}H-NMR ($CDCl_3$): 9.54 (br. *s*, NH); 7.44–7.27 (*m*, 2 Ph (2c1peoc), H–C(6)); 6.05 (*m*, H–C(1′)); 5.83 (*m*, 2 CH (2c1peoc)); 5.55 (2 *m*, H–C(5)); 5.02 (*m*, H–C(3′)); 4.64, 4.55 (2 *m*, H–C(2′)); 4.32 (*m*, 2 H–C(5′), H–C(4′)); 3.78–3.27 (*m*, CH_2OCH_2); 3.11, 3.09 (2 *s*, MeO); 2.93 (*m*, 2 CH_2 (2c1peoc)); 1.85–1.55 (*m*, CH_2CCH_2). Anal. calc. for $C_{35}H_{36}N_4O_{12}$ (704.7): C 56.66, H 5.15, N 7.95; found: C 56.44, H 5.24, N 8.02.

14. *5′*-O-*[(2-Cyano-1-phenylethoxy)carbonyl]*-O^4-*[2-(4-nitrophenyl)ethyl]-2′*-O-*(tetrahydro-4-methoxy-2*H-*pyran-4-yl)uridine* (**42**), *3′*-O-*[(2-Cyano-1-phenylethoxy)carbonyl]*-O^4-*[2-(4-nitrophenyl)ethyl]-2′*-O-*(tetrahydro-4-methoxy-2*H-*pyran-4-yl)uridine* (**43**), and *3′,5′-Bis*-O-*[(2-cyano-1-phenylethoxy)carbonyl]*-O^4-*[2-(4-nitrophenyl)ethyl]-2′*-O-*(tetrahydro-4-methoxy-2*H-*pyran-4-yl)uridine* (**44**). As described in *Exper. 1*, with **32** (1.83 g, 3.60 mmol), co-evaporated with dry pyridine (3 × 10 ml)), pyridine (35 ml), **4** (1.10 g, 5.25 mmol; oil of *ca.* 80% purity), and CH_2Cl_2 (25 ml); addition within 1 h 45 min at −70 to −50°, stirring for 4 h 15 min at −55 to −20°. Workup with CH_2Cl_2 (80 ml), H_2O (100 ml), and CH_2Cl_2 (2 × 80 ml) and co-evaporation with toluene (5 × 20 ml). FC (silica gel (50 g), 19 × 3 cm, CH_2Cl_2 (250 ml), CH_2Cl_2/MeOH 100:1 (252.5 ml), 100:2 (255 ml), 100:3 (257.5 ml), 100:4 (260 ml)) gave 1.45 g (59%) of **42**. Combined fractions containing **43** and **44** were still contaminated and purified by another FC (silica gel (20 g), 14 × 2.1 cm, toluene/AcOEt 5:4 (100 ml), toluene/AcOEt/MeOH 5:4:0.025 (100.25 ml), 5:4:0.05 (100.5 ml), 5:4:0.1 (101 ml)): 44 mg (2%) of **43** and 0.59 g (19%) of **44**.

Data of **42**: Colorless foam. TLC (toluene/AcOEt/MeOH 5:4:1): R_f 0.30. UV (MeOH): 274 (4.19), 211 (sh, 4.44). ^{1}H-NMR ($CDCl_3$): 8.16 (2 *d*, 2 H_o to NO_2); 7.76, 7.71 (2 *d*, H–C(6)); 7.41–7.37 (*m*, 2 H_m to NO_2, Ph (2c1peoc)); 6.05, 6.03 (2 *d*, H–C(1′)); 5.99, 5.77 (2 *d*, H–C(5)); 5.84 (*m*, CH (2c1peoc)); 4.65–4.31 (*m*, H–C(2′), CH_2CH_2O, 2 H–C(5′)); 4.20 (*m*, H–C(4′)); 4.07 (*m*, H–C(3′)); 3.74–3.57 (*m*, CH_2OCH_2); 3.16–3.08 (*m*, CH_2CH_2O, OH–C(3′), MeO); 2.96 (*d*, CH_2 (2c1peoc)); 1.90–1.73 (*m*, CH_2CCH_2). Anal. calc. for $C_{33}H_{36}N_4O_{12}$ (680.7): C 58.23, H 5.33, N 8.23; found: C 58.28, H 5.44, N 8.02.

Data of **43**: Colorless foam. TLC (toluene/AcOEt/MeOH 5:4:1): R_f 0.42. UV (MeOH): 274 (4.17), 211 (sh, 4.46). ^{1}H-NMR ($CDCl_3$): 8.15 (*d*, 2 H_o to NO_2); 7.62, 7.59 (2 *d*, H–C(6)); 7.38 (*m*, 2 H_m to NO_2, Ph (2c1peoc)); 5.91, 5.89 (2 *d*, H–C(1′)); 5.82 (*m*, CH (2c1peoc)); 5.55, 5.52 (2 *d*, H–C(5)); 5.35, 5.23 (2 *m*, H–C(2′), H–C(3′)); 4.64–4.60 (*m*, CH_2CH_2O); 4.37–4.17 (2 *m*, OH–C(5′), H–C(4′)); 3.85–3.35

(*m*, 2 H–C(5′), CH_2OCH_2); 3.15 (*t*, CH_2CH_2O); 3.08–2.78 (*m*, CH_2 (2c1peoc), MeO); 1.82–1.54 (*m*, CH_2CCH_2). Anal. calc. for $C_{33}H_{36}N_4O_{12}$ (680.7): C 58.23, H 5.33, N 8.23; found: C 58.95, H 5.58, N 7.71.

Data of **44**: Colorless foam. TLC (toluene/AcOEt/MeOH 5:4:1): R_f 0.61. UV (MeOH): 274 (4.24), 211 (sh, 4.56). ^{1}H-NMR ($CDCl_3$): 8.16 (*d*, 2 H_o to NO_2); 7.65, 7.59, 7.58 (3 *d*, H–C(6)); 7.38 (*m*, 2 H_m to NO_2, 2 Ph (2c1peoc)); 6.20, 6.17 (2 *d*, H–C(1′)); 5.99, 5.76, 5.74 (3 *d*, H–C(5)); 5.80 (*m*, 2 CH (2c1peoc)); 4.98 (*m*, H–C(3′)); 4.71–4.59 (*m*, H–C(2′)); 4.35 (*m*, CH_2CH_2O, H–C(4′), 2 H–C(5′)); 3.72–3.35 (*m*, CH_2OCH_2); 3.14 (*t*, CH_2CH_2O); 3.08, 3.05, 2.85, 2.83 (4 *s*, MeO); 2.93 (*m*, 2 CH_2 (2c1peoc)); 1.83–1.65 (*m*, CH_2CCH_2). Anal. calc. for $C_{43}H_{43}N_5O_{14}$ (853.8): C 60.49, H 5.08, N 8.20; found: C 60.02, H 5.29, N 8.17.

15. *5′-*O*-[(2-Cyano-1-phenylethoxy)carbonyl]-*N^6*-{[2-(4-nitrophenyl)ethoxy]carbonyl}-2′-*O*-(tetrahydro-4-methoxy-2*H*-pyran-4-yl)adenosine 3′-[2-(4-Nitrophenyl)ethyl Diisopropylphosphoramidite]* (**46**). A soln. of **33** (0.19 g, 0.25 mmol), 2-(4-nitrophenyl)ethyl tetraisopropylphosphordiamidite [24] (**45**; 0.2 g, 0.51 mmol), and 1*H*-tetrazole (9 mg, 0.13 mmol) in dry CH_2Cl_2 (1.6 ml) was stirred overnight under N_2 for 15 h. After dilution with CH_2Cl_2 (20 ml), the soln. was washed with 5% $NaHCO_3$/sat. NaCl soln. 2:1 (30 ml), the aq. layer extracted with CH_2Cl_2 (2 × 30 ml), the combined org. phase dried (Na_2SO_4) and evaporated, and the residue purified by FC (silica gel (10 g), 14.5 × 1.7 cm, petroleum ether/acetone 3:1 (80 ml), 3:2 (100 ml), 1:1 (100 ml), 2:3 (100 ml)): 215 mg (81%) of **46**. Colorless foam. TLC (petroleum ether/acetone 3:2): R_f 0.22. UV (MeOH): 275 (sh, 4.50), 267 (4.55), 211 (sh, 4.71). ^{1}H-NMR ($CDCl_3$): 8.73, 8.72, 8.70 (3 *s*, H–C(2)); 8.19–8.10 (*m*, 4 H_o to NO_2, NH, H–C(8)); 7.45–7.31 (*m*, 4 H_m to NO_2, Ph (2c1peoc)); 6.13, 6.05 (2 *d*, H–C(1′)); 5.84 (*m*, CH (2c1peoc)); 5.28, 5.17–5.10 (2 *m*, H–C(2′)); 4.52 (*t*, CH_2CH_2O (*N*^{6}-npeoc)); 4.43–3.84 (*m*, CH_2OP, H–C(3′), H–C(4′), 2 H–C(5′)); 3.78–3.25 (*m*, CH_2OCH_2, 2 Me_2CH); 3.15 (*t*, CH_2CH_2O (*N*^{6}-npeoc)); 3.05–2.91 (*m*, C–CH_2, CH_2 (2c1peoc)); 2.65, 2.62, 2.58 (3 *s*, MeO); 1.86–1.39 (*m*, CH_2CCH_2); 1.26–1.05 (*m*, 2 Me_2CH). ^{31}P-NMR ($CDCl_3$): 151.14; 151.20; 149.42; 149.13. Anal. calc. for $C_{49}H_{58}N_9O_{15}P$ (1044.0): C 56.37, H 5.60, N 12.07; found: C 56.08, H 5.87, N 11.59.

16. *5′-*O*-[(2-Cyano-1-phenylethoxy)carbonyl]-*N^4*-{[2-(4-nitrophenyl)ethoxy]carbonyl}-2′-*O*-(tetrahydro-4-methoxy-2*H*-pyran-4-yl)cytidine 3′-[2-(4-Nitrophenyl)ethyl Diisopropylphosphoramidite]* (**47**). As described in *Exper. 15*, with **35** (962 mg, 1.33 mmol), **45** (1.06 g, 2.66 mmol), 1*H*-tetrazole (47 mg, 0.67 mmol), and dry CH_2Cl_2 (12 ml); stirring for 17 h. Workup with CH_2Cl_2 (80 ml), 5% $NaHCO_3$/sat. NaCl 4:1 soln. (100 ml), and CH_2Cl_2 (2 × 100 ml). FC (silica gel (50 g), 19 × 3 cm, petroleum ether/acetone 3:1 (200 ml), 2:1 (225 ml), 3:2 (250 ml), 1:1 (300 ml)) gave 1.03 g (76%) of **47**. Colorless foam. TLC (petroleum ether/acetone 3:2): R_f 0.23. UV (MeOH): 269 (4.50), 252 (sh, 4.48), 212 (sh, 4.70). ^{1}H-NMR ($CDCl_3$): 8.14 (*m*, 4 H_o to NO_2); 7.82 (*m*, H–C(6)); 7.54 (br. *s*, NH); 7.38 (*m*, 4 H_m to NO_2, Ph (2c1peoc)); 7.29–7.09 (*m*, H–C(5)); 6.21, 6.09, 6.07 (3 *d*, H–C(1′)); 5.85 (*m*, CH (2c1peoc)); 4.44–4.31 (*m*, H–C(2′), 2 CH_2O); 4.11–3.46 (*m*, H–C(3′), H–C(4′), 2 H–C(5′), CH_2OCH_2, 2 Me_2CHN); 3.13–2.86 (*m*, 2 CH_2CH_2O, CH_2 (2c1peoc), MeO); 1.83–1.47 (*m*, CH_2CCH_2); 1.18–1.00 (*m*, 2 Me_2CH). ^{31}P-NMR ($CDCl_3$): 150.67; 150.61; 149.41; 148.88. Anal. calc. for $C_{48}H_{58}N_7O_{16}P$ (1020.0): C 56.52, H 5.73, N 9.61; found: C 56.36, H 5.92, N 9.47.

17. *5′-*O*-[(2-Cyano-1-phenylethoxy)carbonyl]-*N^2*-{[2-(4-nitrophenyl)ethoxy]carbonyl}-*O^6*-[2-(4-nitrophenyl)ethyl]-2′-*O*-(tetrahydro-4-methoxy-2*H*-pyran-4-yl)guanosine 3′-[2-(4-Nitrophenyl)ethyl Diisopropylphosphoramidite]* (**48**). As described in *Exper. 15*, with **38** (976 mg, 1.07 mmol), **45** (850 mg, 2.14 mmol), 1*H*-tetrazole (37 mg, 0.53 mmol), and CH_2Cl_2 (10 ml), stirring for 19 h. Workup with CH_2Cl_2 (80 ml), 5% $NaHCO_3$/sat. NaCl 4:1 soln. (100 ml), and CH_2Cl_2 (2 × 100 ml). FC (silica gel (50 g), 19 × 3 cm, petroleum ether/acetone 3:1 (200 ml), 2:1 (225 ml), 3:2 (250 ml), 1:1 (250 ml)) gave 880 mg (68%) of **48**. Colorless foam. TLC (petroleum ether/acetone 3:2): R_f 0.24. UV (MeOH): 279 (sh, 4.54), 269 (4.61), 212 (sh, 4.73). ^{1}H-NMR ($CDCl_3$): 8.21–8.10 (*m*, 6 H_o to NO_2); 7.91, 7.89, 7.86 (3 *s*, H–C(8)); 7.51–7.22 (*m*, 6 H_m to NO_2, Ph (2c1peoc), NH); 6.00–5.83 (4 *d*, H–C(1′)); 5.76 (*m*, CH (2c1peoc)); 5.40, 5.28 (2 *m*, H–C(2′)); 4.83–4.75 (*m*, CH_2CH_2O (*O*^{6}-npe)); 4.68–3.40 (*m*, 2 CH_2O, H–C(3′), H–C(4′), 2 H–C(5′), CH_2OCH_2, 2 Me_2CH); 3.29 (*t*, CH_2CH_2O (*O*^{6}-npe)); 3.12–2.96 (*m*, 2 C–CH_2); 2.87 (*m*, CH_2 (2c1peoc)); 2.56, 2.55, 2.54, 2.52 (4 *s*, MeO); 1.75–1.48 (*m*, CH_2CCH_2); 1.23–1.07 (*m*, 2 Me_2CH). ^{31}P-NMR ($CDCl_3$): 151.37, 151.31, 149.47, 149.44. Anal. calc. for $C_{57}H_{65}N_{10}O_{18}P$ (1209.2): C 56.62, H 5.42, N 11.58; found: C 56.64, H 5.59, N 11.18.

18. *5′-*O*-[(2-Cyano-1-phenylethoxy)carbonyl]-2′-*O*-(tetrahydro-4-methoxy-2*H*-pyran-4-yl)uridine 3′-[2-(4-Nitrophenyl)ethyl Diisopropylphosphoramidite]* (**49**). As described in *Exper. 15*, with **40** (1.60 g, 3.01 mmol), **45** (2.40 g, 6.03 mmol), 1*H*-tetrazole (106 mg, 1.51 mmol), and CH_2Cl_2 (25 ml), stirring for 17 h. Workup with CH_2Cl_2 (80 ml), 5% $NaHCO_3$/sat. NaCl 4:1 soln. (100 ml), and CH_2Cl_2 (2 × 100 ml). FC (silica gel (40 g), 15 × 3 cm, petroleum ether/acetone 3:1 (200 ml), 2:1 (225 ml), 3:2 (250 ml), 1:1 (300 ml)) gave 1.86 g (75%) of **49**. Colorless foam. TLC (petroleum ether/acetone 3:2): R_f 0.22. UV (MeOH): 263 (4.25), 213 (sh, 4.41). ^{1}H-NMR ($CDCl_3$): 8.14 (*m*, 2 H_o to NO_2, NH); 7.42–7.29 (*m*, 2 H_m to NO_2, Ph (2c1peoc), H–C(6)); 6.12, 6.02 (2 *d*, H–C(1′)); 5.83 (*m*, CH (2c1peoc)); 5.52, 5.51 (2 *d*, H–C(5)); 4.45 (*m*, CH_2CH_2O, H–C(2′), H–C(3′),

H–C(4′), 2 H–C(5′), CH_2OCH_2, 2 Me_2CH); 3.05–2.93 (*m*, CH_2CH_2O, MeO, CH_2 (2c1peoc)); 1.86–1.59 (*m*, CH_2CCH_2); 1.21–1.07 (*m*, 2 Me_2CH). ^{31}P-NMR ($CDCl_3$): 151.31; 149.16; 149.07. Anal. calc. for $C_{39}H_{50}N_5O_{13}P$ (827.8): C 56.59, H 6.09, N 8.46; found: C 56.23, H 6.20, N 8.31.

19. *5′-O-[(2-Cyano-1-phenylethoxy)carbonyl]-*O^4*-[2-(4-nitrophenyl)ethyl]-2′-*O*-(tetrahydro-4-methoxy-2*H*-pyran-4-yl)uridine 3′-[2-(4-Nitrophenyl)ethyl Diisopropylphosphoramidite]* (**50**). As described in *Exper. 15*, with **42** (913 mg, 1.34 mmol), **45** (1.07 g, 2.68 mmol), 1*H*-tetrazole (47 mg, 0.67 mmol), and CH_2Cl_2 (12 ml); stirring for 18 h. Workup with CH_2Cl_2 (80 ml), 5% $NaHCO_3$/sat. NaCl 4:1 soln. (100 ml), and CH_2Cl_2 (2 × 80 ml). FC (silica gel (50 g), 19 × 3 cm, petroleum ether/acetone 3:1 (200 ml), 2:1 (225 ml), 3:2 (250 ml), 1:1 (300 ml)) gave 945 mg (72%) of **50**. Colorless foam. TLC (petroleum ether/acetone 3:2): R_f 0.22. UV (MeOH): 273 (4.41), 211 (sh, 4.64). ^{1}H-NMR ($CDCl_3$): 8.13 (*m*, 4 H_o to NO_2); 7.72, 7.71, 7.12 (3 *d*, H–C(6)); 7.41–7.25 (*m*, 4 H_m to NO_2, Ph (2c1peoc)); 6.23, 6.20, 6.10, 6.08 (4 *d*, H–C(1′)); 5.97, 5.95, 5.70, 5.68 (4 *d*, H–C(5)); 5.84 (*m*, CH (2c1peoc)); 4.61 (*m*, CH_2CH_2O); 4.50–3.40 (*m*, H–C(2′), CH_2CH_2O, H–C(3′), H–C(4′), 2 H–C(5′), CH_2OCH_2, 2 Me_2CH); 3.14–2.98 (*m*, 2 CH_2CH_2O, CH_2 (2c1peoc), MeO); 1.90–1.65 (*m*, CH_2CCH_2); 1.23–1.04 (*m*, 2 Me_2CH). ^{31}P-NMR ($CDCl_3$): 150.66; 150.63; 149.42; 149.34. Anal. calc. for $C_{47}H_{57}N_6O_{15}P$ (977.0): C 57.78, H 5.88, N 8.60; found: C 57.76, H 6.06, N 8.53.

20. *5′-O-[(2-Cyano-1-phenylethoxy)carbonyl]-*N^6*-[[2-(4-nitrophenyl)ethoxy]carbonyl]-2′-*O*-(tetrahydro-4-methoxy-2*H*-pyran-4-yl)adenosine 3′-(Hydrogen Butanedioate)* (**51**). In dry CH_2Cl_2 (10 ml), succinic anhydride (643 mg, 6.43 mmol) and 1-methyl-1*H*-imidazole (0.255 ml, 263 mg, 3.21 mmol) were stirred at r.t. for 0.5 h. After addition of **33** (0.2 g, 0.27 mmol), the mixture was stirred overnight for 18 h, diluted with CH_2Cl_2 (30 ml), and washed with 10% citric acid (30 ml). The aq. layer was extracted with CH_2Cl_2 (2 × 30 ml) and the combined org. phase washed with sat. $NaHCO_3$ soln. (50 ml). The aq. phase was re-extracted with CH_2Cl_2 (2 × 30 ml), the combined org. layer washed with H_2O (100 ml), and the aq. phase re-extracted with CH_2Cl_2 (2 × 50 ml). The combined org. phase was dried (Na_2SO_4) and evaporated to a colorless solid, which was dissolved in CH_2Cl_2 (4 ml) and added dropwise under stirring into hexane (50 ml): 209 mg (91%) of **51** after filtration and drying under high vacuum at 40°. Colorless powder. TLC (CH_2Cl_2/MeOH 9:1): R_f 0.60. UV (MeOH): 272 (sh, 4.36), 266 (4.40). ^{1}H-NMR ((D$_6$)DMSO): 12.30 (br. *s*, COOH); 10.65 (br. *s*, NH); 8.74, 8.73 (2 *s*, H–C(2)); 8.61 (*s*, H–C(8)); 8.16 (*d*, 2 H_o to NO_2); 7.62 (*d*, 2 H_m to NO_2); 7.36 (*m*, Ph (2c1peoc)); 6.19 (*d*, H–C(1′)); 5.86 (*t*, CH (2c1peoc)); 5.49–5.36 (*m*, H–C(2′), H–C(3′)); 4.51 (*m*, CH_2CH_2O); 4.39 (*m*, H–C(4′), 2 H–C(5′)); 3.63–3.32 (*m*, CH_2OCH_2); 3.30 (*m*, CH_2 (2c1peoc)); 3.11 (*t*, CH_2CH_2O); 2.65, 2.56 (2 *m*, $C(O)CH_2CH_2C(O)$); 2.40 (*s*, MeO); 1.69–1.30 (*m*, CH_2CCH_2). Anal. calc. for $C_{39}H_{41}N_7O_{15}\cdot 0.5\ C_6H_{14}$ (904.8): C 55.75, H 5.35, N 10.84; found: C 55.49, H 5.38, N 11.30.

21. *5′-O-[(2-Cyano-1-phenylethoxy)carbonyl]-*N^4*-[[2-(4-nitrophenyl)ethoxy]carbonyl]-2′-*O*-(tetrahydro-4-methoxy-2*H*-pyran-4-yl)cytidine 3′-(Hydrogen Butanedioate)* (**52**). As described in *Exper. 20*, with succinic anhydride (667 mg, 6.67 mmol), 1-methyl-1*H*-imidazole (0.264 ml, 273 mg, 3.32 mmol), **35** (200 mg, 0.28 mmol), and CH_2Cl_2 (10 ml) for 17 h: 215 mg (94%) of **52**. Colorless powder. TLC (CH_2Cl_2/MeOH 9:1): R_f 0.61. UV (MeOH): 275 (sh, 4.10), 247 (4.24), 211 (sh, 4.46). ^{1}H-NMR ((D$_6$)DMSO): 12.28 (br. *s*, COOH); 10.90 (br. *s*, NH); 8.16 (*d*, 2 H_o to NO_2); 8.09, 8.06 (2 *d*, H–C(6)); 7.60 (*d*, 2 H_m to NO_2); 7.38 (*m*, Ph (2c1peoc)); 7.04, 7.01 (2 *d*, H–C(5)); 6.07, 6.04 (2 *d*, H–C(1′)); 5.85 (*m*, CH (2c1peoc)); 5.17 (*m*, H–C(3′)); 4.78 (*m*, H–C(2′)); 4.36 (*m*, CH_2CH_2O, 2 H–C(5′)); 4.24 (*m*, H–C(4′)); 3.53–3.40 (*m*, CH_2OCH_2); 3.25 (*m*, CH_2 (2c1peoc)); 3.08 (*t*, CH_2CH_2O); 2.79 (*s*, MeO); 2.61, 2.52 (2 *m*, $C(O)CH_2CH_2C(O)$); 1.69–1.42 (*m*, CH_2CCH_2). Anal. calc. for $C_{38}H_{41}N_5O_{16}\cdot 0.25\ C_6H_{14}$ (845.3): C 56.13, H 5.31, N 8.29; found: C 55.91, H 5.63, N 8.05.

22. *5′-O-[(2-Cyano-1-phenylethoxy)carbonyl]-*N^2*-[[2-(4-nitrophenyl)ethoxy]carbonyl]-*O^6*-[2-(4-nitrophenyl)ethyl]-2′-*O*-(tetrahydro-4-methoxy-2*H*-pyran-4-yl)guanosine 3′-(Hydrogen Butanedioate)* (**53**). As described in *Exper. 20*, with succinic anhydride (528 mg, 5.28 mmol), 1-methyl-1*H*-imidazole (0.209 ml, 216 mg, 2.63 mmol), **38** (198 mg, 0.22 mmol), and CH_2Cl_2 (10 ml) for 19 h: 188 mg (86%) of **53**. Colorless powder. TLC (CH_2Cl_2/MeOH 9:1): R_f 0.62. UV (MeOH): 277 (sh, 4.32), 269 (4.38), 213 (4.48). ^{1}H-NMR ((D$_6$)DMSO): 12.30 (br. *s*, COOH); 10.40, 10.38 (2 *s*, NH); 8.45 (*s*, H–C(8)); 8.16 (*d*, 4 H_o to NO_2); 7.65–7.56 (*m*, 4 H_m to NO_2); 7.35 (*m*, Ph (2c1peoc)); 6.07 (*d*, H–C(1′)); 5.83 (*t*, CH (2c1peoc)); 5.64 (*m*, H–C(3′)); 5.32 (*m*, H–C(2′)); 4.75 (*t*, CH_2CH_2O (*O*^{6}-npe)); 4.62–4.28 (*m*, CH_2O (*N*^{2}-npeoc), 2 H–C(5′), H–C(4′)); 3.63–3.12 (*m*, 2 CH_2CH_2O, CH_2OCH_2); 3.06 (*m*, CH_2 (2c1peoc)); 2.64, 2.54 (2 *m*, $C(O)CH_2CH_2C(O)$); 2.39 (*s*, MeO); 1.68–1.35 (*m*, CH_2CCH_2). Anal. calc. for $C_{47}H_{48}N_8O_{18}\cdot 0.5\ C_6H_{14}$ (1056.0): C 56.87, H 5.25, N 10.61; found: C 57.22, H 5.46, N 10.11.

23. *5′-O-[(2-Cyano-1-phenylethoxy)carbonyl]-2′-*O*-(tetrahydro-4-methoxy-2*H*-pyran-4-yl)uridine 3′-(Hydrogen Butanedioate)* (**54**). As described in *Exper. 20*, with succinic anhydride (904 mg, 9.03 mmol), 1-methyl-1*H*-imidazole (0.359 ml, 371 mg, 4.52 mmol), **40** (200 mg, 0.38 mmol), and CH_2Cl_2 (10 ml) for 17 h: 74 mg (31%) of **54**.

An alternative workup by dilution with CH_2Cl_2 (30 ml), washing with 10% citric acid (30 ml), extraction of the aq. layer with CH_2Cl_2 (2 × 30 ml), washing of the combined org. phase with H_2O (100 ml), re-extraction of the aq. phase with CH_2Cl_2 (2 × 50 ml), and purification by FC (silica gel (10 g), 14.5 × 1.4 cm, CH_2Cl_2/MeOH 100:1 (101 ml), 100:2 (102 ml), 100:3 (103 ml), 100:4 (104 ml)) gave 220 mg (93%) of **54**. Colorless powder. TLC (CH_2Cl_2/MeOH 9:1): R_f 0.59. UV (MeOH): 258 (3.95). ^{1}H-NMR ((D$_6$)DMSO): 12.25 (br. *s*, COOH); 11.51 (br. *s*, NH); 7.72 (*d*, H–C(6)); 7.40 (*m*, Ph (2c1peoc)); 5.98, 5.96 (2 *d*, H–C(1′)); 5.88 (*t*, CH (2c1peoc)); 5.72, 5.69 (2 *d*, H–C(5)); 5.13 (*m*, H–C(3′)); 4.71 (*m*, H–C(2′)); 4.38 (*m*, 2 H–C(5′)); 4.22 (*m*, H–C(4′)); 3.54–3.26 (*m*, CH_2OCH_2, CH_2 (2c1peoc)); 2.91 (*s*, MeO); 2.62–2.60, 2.53–2.49 (2 *m*, C(O)CH_2CH_2C(O)); 1.70–1.50 (*m*, CH_2CCH_2). Anal. calc. for $C_{29}H_{33}N_3O_{13}\cdot 0.5\ C_6H_{14}$ (674.7): C 56.97, H 5.98, N 6.23; found: C 56.69, H 6.12, N 6.10.

24. *5′*-O-*[(2-Cyano-1-phenylethoxy)carbonyl]*-O^4-*[2-(4-nitrophenyl)ethyl]-2′*-O-*(tetrahydro-4-methoxy-2*H*-pyran-4-yl)uridine 3′-(Hydrogen Butanedioate)* (**55**). As described in *Exper. 20*, with succinic anhydride (541 mg, 5.41 mmol), 1-methyl-1*H*-imidazole (0.215 ml, 222 mg, 2.71 mmol), **42** (0.15 g, 0.23 mmol), and CH_2Cl_2 (12 ml) for 18 h. Workup by dilution with CH_2Cl_2 (30 ml), washing with 10% citric acid (30 ml), extraction of the aq. layer with CH_2Cl_2 (2 × 80 ml), washing of the combined org. phase with H_2O (80 ml), re-extraction of the aq. phase with CH_2Cl_2 (2 × 80 ml), and purification by FC (silica gel (10 g), 14.5 × 1.4 cm, CH_2Cl_2/MeOH 100:1 (101 ml), 100:2 (102 ml), 100:3 (103 ml)) gave 170 mg (99%) of **55**. Colorless foam. TLC (CH_2Cl_2/MeOH 9:1): R_f 0.60. UV (MeOH): 274 (4.22), 213 (sh, 4.44). ^{1}H-NMR ((D$_6$)DMSO): 12.30 (br. *s*, COOH); 8.17 (*d*, 2 H$_o$ to NO_2); 8.02, 7.99 (2 *d*, H–C(6)); 7.58 (*d*, 2 H$_m$ to NO_2); 7.37 (*m*, Ph (2c1peoc)); 6.06 (*m*, H–C(1′), H–C(5)); 5.87 (*t*, CH (2c1peoc)); 5.16 (*m*, H–C(3′)); 4.75 (*m*, H–C(2′)); 4.60 (*t*, CH_2CH_2O); 4.38 (*m*, 2 H–C(5′)); 4.24 (*m*, H–C(4′)); 3.57–3.40 (*m*, CH_2OCH_2); 3.26 (*m*, CH_2 (2c1peoc)); 3.16 (*t*, CH_2CH_2O); 2.77 (*s*, MeO); 2.61, 2.51 (2 *m*, C(O)CH_2CH_2C(O)); 1.68 (*m*, CH_2CCH_2). Anal. calc. for $C_{37}H_{40}N_4O_{15}$ (780.7): C 56.92, H 5.16, N 7.18; found: C 56.57, H 5.50, N 7.03.

25. *Solid-Support Material* **57**–**61** *from LCAMA-CPG 500 Å* (**56**) *and 5′*-O-*[(2-Cyano-1-phenylethoxy)-carbonyl]-nucleoside 3′-(Hydrogen Butanedioate)* **51**–**55**. To a mixture of LCAMA-CPG **56** [12][25][26] (0.1 g), one of the 5′-O-[(2-cyano-1-phenylethoxy)carbonyl]-nucleoside 3′-(hydrogen butanedioates) **51**–**55** (16–22 μmol), and TOTU (18–20 μmol), MeCN (3–5 ml) and *N*-methylmorpholine (5 μl, 45.4 μmol) were added. After shaking for 2–4 h, the CPG material was collected in a sintered glass funnel and washed with pyridine, DMF, MeOH, and acetone. Capping procedure: The nucleoside-functionalized CPG was suspended in pyridine (5 ml), treated with 1-methyl-1*H*-imidazole (25 μl, 315 μmol) and Ac_2O (1 ml, 10.6 mmol), and shaken at r.t. for 1 h. Then, the CPG material **57**–**61** was filtered through a sintered glass funnel and washed with pyridine, DMF, MeOH, and acetone. Determination of loading: A defined amount of **57**–**61** (2–5 mg) was treated in a 1-ml microcuvette (d = 1 cm) with 1 ml of 0.1M DBU in MeCN. After shaking for 2 min, the absorbance at the UV maximum (λ_{max} 272–282 nm) was measured against 0.1M DBU/MeCN. The loading was calculated by the formula L [μmol/g] = 51.7 · A/m (L = loading, A = absorbance at λ_{max}, m = weighed CPG **57**–**61** in mg): L = 13–15 μmol/g. This calculation considers ε = 19336 (λ_{max} 273) of the formed 3-phenyl-prop-2-enenitrile, determined for its (2*Z*)/(2*E*) mixture 3:7 [10].

26. *Assembly of Oligonucleotides* **62**–**82**. The syntheses were carried out with an *Applied Biosystems 380B* DNA synthesizer. Nucleoside-functionalized CPG material **57**–**61** (0.1 μmol) was packed into a small *ABI* column. Cycles of chain elongation were carried out by a programmed series of reagent and solvent washes based on recommended procedures. The following main steps were performed: *1*) 5′-*O*-2c1peoc Deprotection: 0.1 DBU in MeCN delivered in 2 × 30 s and 6 × 10 s with intermediate 1-s reverse flushes. *2*) Coupling: 0.1–0.12M phosphoramidite and activator in dry MeCN (0.5M 1*H*-tetrazole, or 0.5M pyridinium chloride, or 0.6M 5-(ethylthio)-1*H*-tetrazole), delivered in alternating reagent pulses with a subsequent waiting time of 700 s (1*H*-tetrazole), 70–350 s (pyridinium chloride), and 300 s (5-(ethylthio)-1*H*-tetrazole), respectively. *3*) Capping: Ac_2O/2,6-dimethylpyridine/THF 1:1:8 and 1-methyl-1*H*-imidazole/THF 16:84, delivered in one 10-s burst with a subsequent waiting time of 15 s. *4*) Oxidation: 0.05M I_2 in THF/H_2O/pyridine 7:2:1, delivered in one 15-s burst with a subsequent waiting time of 15 s.

Deprotection and cleavage program: *1*) npe/npeoc Deprotection: 1M DBU in MeCN delivered in 12 × one 45-s push with a subsequent waiting time of 4 × 1800 s, 3 × 3600 s, and 5 × 5400 s (total waiting time of 12.5 h) or 2M DBU in MeCN delivered in 12 × one 45-s push with a subsequent waiting time of 4 × 1800 s and 8 × 3600 s (total waiting time of 10 h). *2*) Cleavage from the support: conc. NH_3 soln. delivered in a 18-s burst with a consecutive waiting time of 1800 s, repeated 3 times (total waiting time 2 h).

The NH_3 soln. containing the oligonucleotide was collected and, after determination of the isolated amount of oligonucleotide by measurement of the absorbance at 260 nm, lyophilized in a *Speed-vac* concentrator under high vacuum.

Reversed-phase HPLC: gradient: 2.5% *B* (0–2 min), 2.5–20% *B* (2–32 min), 20–50% *B* (32–45 min), 50% *B* (45–50 min), 50–2.5% *B* (50–55 min), and 2.5% *B* (55–60 min).

Anion-exchange HPLC: gradient: 0% *D* (0–5 min), 0–70% *D* (5–45 min), 70–100% *D* (45–47 min), 100% *D* (47–53 min), 100–0% *D* (53–58 min), and 0% *D* (58–65 min).

*27. Deprotection of 2′-*O*-Thmp Groups* [17][36]. A soln. of 0.4 *OD* of 2′-*O*-thmp-oligoribonucleotide in 40 µl of 0.5M NaOAc (pH 3.25) was shaken for 1 h and kept at r.t. for 12–36 h. Then the soln. was neutralized with 8 µl of 3M buffer *Tris* (pH 8) and the oligonucleotide precipitated by adding 500 µl of EtOH. After centrifugation, the supernatant solvent was removed with a pipette and the fully deprotected oligoribonucleotide lyophilized briefly in a *Speed-vac* concentrator under high vacuum.

Reversed-phase HPLC: gradient: 2.5% *B* (0–2 min), 2.5–20% *B* (2–32 min), 20–50% *B* (32–45 min), 50% *B* (45–50 min), 50–2.5% *B* (50–55 min), and 2.5% *B* (55–60 min).

Anion-exchange HPLC: gradient: 0% *F* (0–2 min), 0–50% *F* (2–20 min), 50–100% *F* (20–30 min), 100% *F* (30–35 min), 100–0% *F* (35–38 min), and 0% *F* (38–43 min).

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