Nucleotides

Part LXV[1])

Synthesis of 2′-Deoxyribonucleoside 5′-Phosphoramidites: New Building Blocks for the Inverse (5′-3′)-Oligonucleotide Approach

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With best personal wishes dedicated to Prof. Dr. *Albert Eschenmoser* on the occassion of his 75th birthday

The syntheses of the 3′-*O*-(4,4′-dimethoxytrityl)-protected 5′-phosphoramidites **25**–**28** and 5′-(hydrogen succinates) **29**–**32**, which can be used as monomeric building blocks for the inverse (5′-3′)-oligodeoxyribonucleotide synthesis are described (*Scheme*). These activated nucleosides and nucleotides were obtained by two slightly different four-step syntheses starting with the base-protected nucleosides **13**–**20**. For the protection of the aglycon residues, the well-established 2-(4-nitrophenyl)ethyl (npe) and [2-(4-nitrophenyl)ethoxy]carbonyl (npeoc) groups were used. The assembly of the oligonucleotides required a slightly increased coupling time of 3 min in application of the common protocol (see *Table 1*). The use of pyridinium hydrochloride as an activator (instead of 1*H*-tetrazole) resulted in an extremely shorter activation time of 30 seconds. We established the efficiency of this inverse strategy by the synthesis of the oligonucleotide 3′-conjugates **33** and **34** which carry lipophilic caps derived from cholesterol and vitamin E, respectively, as well as by the formation of (3′-3′)- and (5′-5′)-internucleotide linkages (see *Table 2*).

1. Introduction. – In nature, oligonucleotides are synthesized in the 5′ → 3′ direction, while the chemical build-up of these biopolymers, due to the easy accessibility of the corresponding monomeric building blocks, are carried out in DNA synthesizers by the opposite 3′ → 5′ approach. Even though primary phosphoramidites are often used for the synthesis of (3′-3′)- and (5′-5′)-internucleotide linkages [2][3], very little is known in literature [4] about the detailed specification of the synthesis of the appropriate 5′-phosphoramidites suitable for the built-up of oligonucleotides in the 5′ → 3′ direction.

Beside the use of oligonucleotides in medicinal chemistry as potential antisense and antigene drugs, there is increasing interest in their use in oligonucleotide-chip technology [5–10], diagnostic tools, biological applications, and nanotechnology [11]. For these purposes, efficient and diverse oligonucleotide syntheses are essential. Application of the npe/npeoc strategy (npe = 2-(4-nitrophenyl)ethyl, npeoc = [2-(4-nitrophenyl)ethoxy]carbonyl) [12] allows also the inverse (5′ → 3′)-directed built-up of oligonucleotides of high purity starting with the appropriately protected 5′-phosphoramidites. By this strategy, totally unprotected oligonucleotides still attached to the solid support are available, and final cleavage with ammonia affords the oligonucleotides for direct use without further purification.

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In this paper, we describe the syntheses of new 5′-phosphoramidites and 5′-(hydrogen succinates) and show their efficiency in inverse (5′-3′)-oligodeoxyribonucleotide syntheses [13]. Furthermore, an improved coupling step, brought about by the use of pyridinium chloride [14] instead of the commonly applied 1*H*-tetrazole, is recommended.

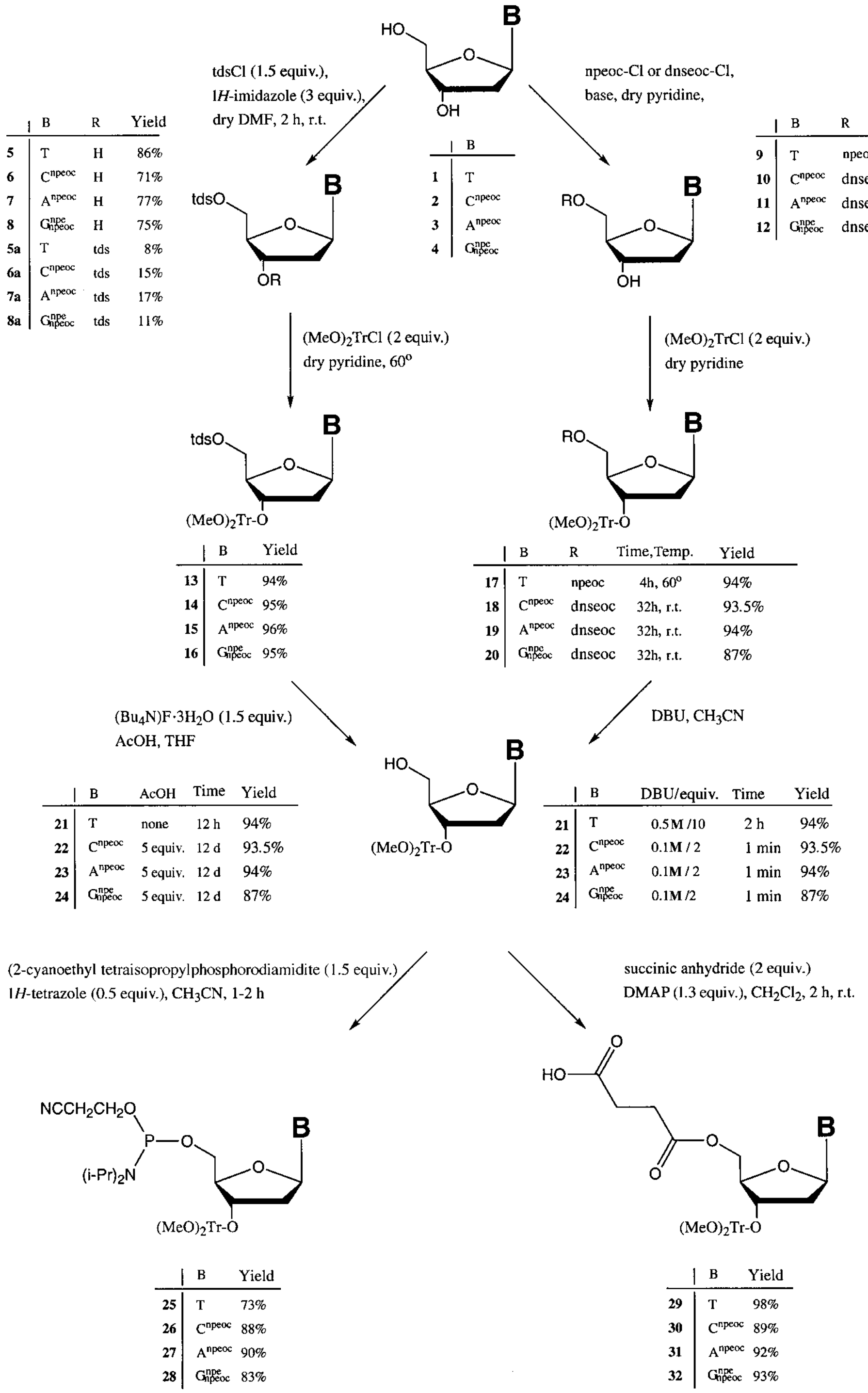
2- Syntheses. – At first, the aglycone functions of the common nucleosides were protected in the usual manner by the 2-(4-nitrophenyl)ethyl (npe) and the [2-(4-nitrophenyl)ethoxy]carbonyl (npeoc) group (→**1**–**4**) [15], which have already been shown to be very efficient for the synthesis of oligodeoxynucleotides [16–24]. The modification of the sugar moiety was then undertaken by the introduction at the 5′-position of a protecting group compatible with the dimethoxytrityl residue. In our first route, the primary OH function of **1**–**4** was blocked by the bulky dimethyl(thexyl)silyl (tds) group after treatment with [dimethyl(1,1,2-trimethylpropyl)silyl] chloride (tdsCl) and 1*H*-imidazole in DMF to furnish the singly protected nucleosides **5**–**8** in 78–86% besides 8–17% of the doubly silylated derivatives **5a**–**8a**, which could be seperated easily by silica-gel column chromatography (*Scheme*). Subsequent dimethoxytritylation with $(MeO)_2TrCl$ in pyridine at 60° led to the fully protected derivatives **13**–**16** in almost quantitative yields. Due to the steric hindrance of the secondary alcohol group, the higher temperature was necessary to obtain complete conversion in 2–4 h. Desilylation was then achieved by fluoride ion in the presence of small amounts of AcOH to avoid β-elimination of the aglycone protecting groups. Although an extended reaction time had to be tolerated, the 3′-*O*-(dimethoxytrityl)nucleosides **21**–**24** were obtained in high yields (87–94%).

In an alternative approach, the primary-alcohol function was protected with the base-labile (2-dansylethoxy)carbonyl (=(2-{[5-(dimethylamino)naphthalen-1-yl]sulfonyl}ethoxy)carbonyl; dnseoc) [25] or npeoc group to give compounds **9**–**12**. Subsequent dimethoxytritylation of **9** at 60° and of **10**–**12** at room temperature due to the sensitivity of the dnseoc group led to the fully protected nucleosides **17**–**20**. The precursors **21**–**24** of the monomeric building blocks were then obtained in high yields by a mild β-elimination process with 1,8-diazabicyclo[5.4.0]undec-7-ene (DBU) in MeCN. The phosphitylations to **25**–**28** and succinoylations to **29**–**32** were carried out following published procedures [26].

The loading of the LCAMA-CPG support ((long-chain-alkyl)methylamine controlled-pore glass [12]) with **29**–**32** was performed in the presence of 2-{[cyano-(ethoxycarbonyl)-methylidene]amino}-1,1,3,3-tetramethyluronium tetrafluoroborate (TOTU) and *N*-methylmorpholine in MeCN followed by a capping process with Ac_2O and *N*,*N*-dimethylpyridin-4-amine (DMAP) in pyridine leading to supports loaded with 29–35 μmol/g.

Standard solid-phase phosphoramidite chemistry [27–30] was applied for the assembly of the oligonucleotides in a commercial DNA synthesizer (*ABI 392*), changing only the coupling time to 3 min for the phosphoramidites **25**–**28** to achieve high coupling efficiencies. Since the stability and handling of the 5′-phosphoramidites are comparatively similar to that of their 3′-isomers, no special precautions were necessary. The following slightly modified protocol was used (washing steps not shown): 1) Detritylation of the terminal dimethoxytrityl group with 3% CCl_3COOH in CH_2Cl_2 for

Scheme



	B
1	T
2	C^{npeoc}
3	A^{npeoc}
4	G^{npe}_{npeoc}

	B	R	Yield
5	T	H	86%
6	C^{npeoc}	H	71%
7	A^{npeoc}	H	77%
8	G^{npe}_{npeoc}	H	75%
5a	T	tds	8%
6a	C^{npeoc}	tds	15%
7a	A^{npeoc}	tds	17%
8a	G^{npe}_{npeoc}	tds	11%

	B	R	Yield
9	T	npeoc	86%
10	C^{npeoc}	dnseoc	71%
11	A^{npeoc}	dnseoc	77%
12	G^{npe}_{npeoc}	dnseoc	75%

	B	Yield
13	T	94%
14	C^{npeoc}	95%
15	A^{npeoc}	96%
16	G^{npe}_{npeoc}	95%

	B	R	Time,Temp.	Yield
17	T	npeoc	4h, 60°	94%
18	C^{npeoc}	dnseoc	32h, r.t.	93.5%
19	A^{npeoc}	dnseoc	32h, r.t.	94%
20	G^{npe}_{npeoc}	dnseoc	32h, r.t.	87%

	B	AcOH	Time	Yield
21	T	none	12 h	94%
22	C^{npeoc}	5 equiv.	12 d	93.5%
23	A^{npeoc}	5 equiv.	12 d	94%
24	G^{npe}_{npeoc}	5 equiv.	12 d	87%

	B	DBU/equiv.	Time	Yield
21	T	0.5M /10	2 h	94%
22	C^{npeoc}	0.1M / 2	1 min	93.5%
23	A^{npeoc}	0.1M / 2	1 min	94%
24	G^{npe}_{npeoc}	0.1M /2	1 min	87%

	B	Yield
25	T	73%
26	C^{npeoc}	88%
27	A^{npeoc}	90%
28	G^{npe}_{npeoc}	83%

	B	Yield
29	T	98%
30	C^{npeoc}	89%
31	A^{npeoc}	92%
32	G^{npe}_{npeoc}	93%

135 s; 2) coupling with 0.1M phosphoramidite **25**–**28** and 0.5M 1*H*-tetrazole in MeCN for 180 s; 3) capping with Ac_2O/2,6-dimethylpyridine/1-methyl-1*H*-imidazole in THF for 15 s; 4) Oxidation with 0.05M I_2 in THF/pyridine/H_2O for 32 s. After the synthesis, the cyanoethyl, npe, and npeoc groups were removed with 1M DBU and washed away, and then the deprotected oligonucleotide was detached from the solid support by common ammonia treatment. The oligonucleotides **A**–**J** obtained were checked by HPLC (*Fig. 1*) and demonstrated high purity so that they did not require further purification (*Table 1*).

In an analogous manner, we also synthesized the oligomers **K**–**M** (see *Table 2*) in the presence of the coupling catalyst pyridinium chloride instead of 1*H*-tetrazole and found the same high quality of the oligonucleotide with a reduced coupling time of 30 s (*Fig. 1*).

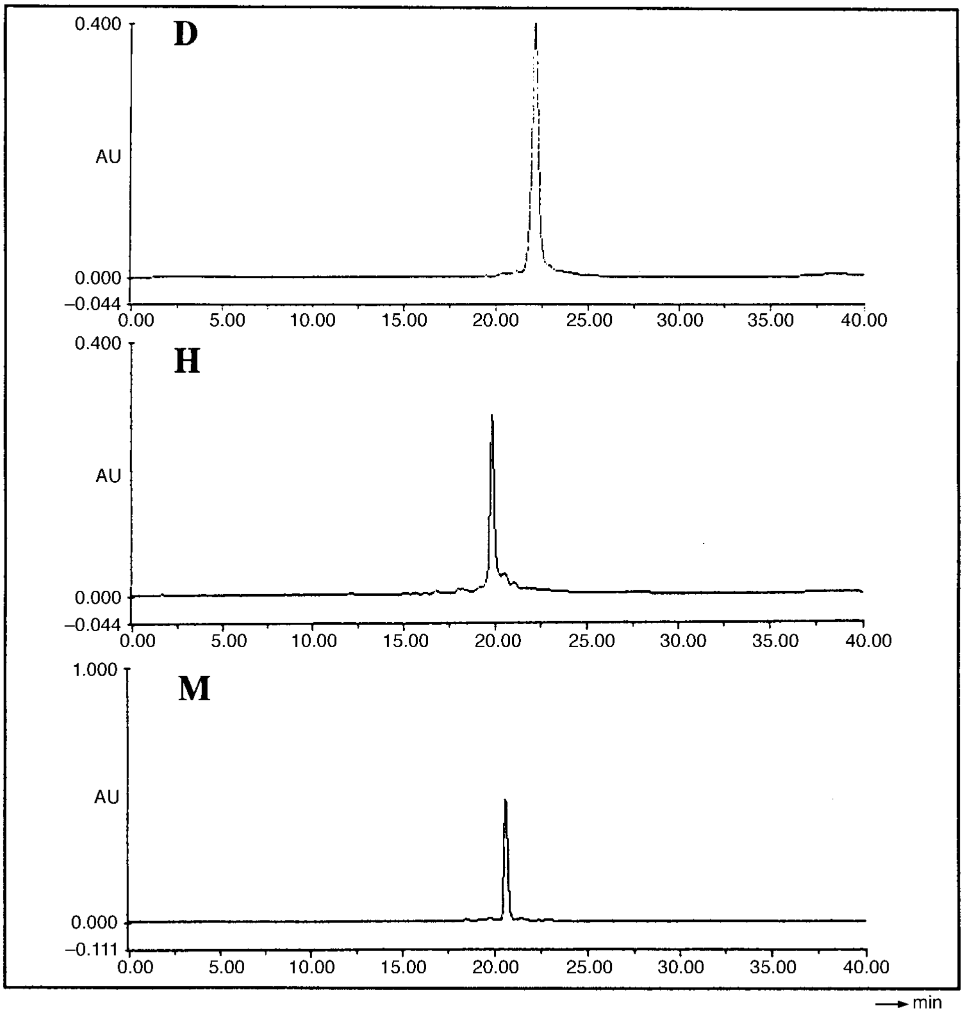


Fig. 1. *HPLC Traces of the crude oligo-2′-deoxyribonucleotides* **D**, **H**, *and* **M**. AU = arbitrary unit.

Table 1. *Oligo-2′-deoxyribonucleotide Syntheses by the Inverse 5′ → 3′ Approach*

Phosphor-amidite	Oligonucleotide sequence		Coupling time [s]	Average condensation yield [%]	Overall yield [%]	Size [µmol]	Yield in *OD* units	HPLC quality
25	5′-TTT-TT-3′	(**A**)	40	96	88	0.24	8.0	1–2
25	5′-TTT-TTT-T-3′	(**B**)	120	99.9	89	0.16	5.8	1
26	5′-d(CCC-CCC)-3′	(**C**)	120	95.6	86.7	0.21	12	2
26	5′-d(CCC-CCC-CCC)-3′	(**D**)	180	100	100	0.17	6.9	1
27	5′-d(AAA-AAA)-3′	(**E**)	180	95.4	84	0.17	6.2	1
27	5′-d(AAA-AAA-AAA)-3′	(**F**)	180	100	100	0.16	10.4	1
26, **27**, **28**	5′-d(CAC-AGC-GGC-CGC)-3′	(**G**)	180	100	86	0.18	11.2	1
25, **26**, **27**, **28**	5′-d(TGA-GCA-GA)-3′	(**H**)	180	99	93	0.23	11.1	1
25, **28**	5′-d(TTG-TGT-GTG-TGT-G)-3′	(**I**)	180	98.6	83.7	0.19	9.9	1
25, **26**	5′d(TCC-CCC-CCC-CC)-3′	(**J**)	180	98.5	95.1	0.22	18	1–2

Furthermore, the monomeric building blocks **25**–**32** could be used for the introduction of so-called (3′-3′)- and/or (5′-5′)-end terminal caps (*Tab. 2*). Loading of the LCAMA-CPG support in the usual manner with one of the 5′-succinates **29**–**32** and subsequent elongation of the oligonucleotides with the common 3′-phosphoramidites led to (3′-3′)-phosphodiester bonds. The opposite (5′-5′)-phosphodiester linkages were achieved by synthesizing the oligonucleotide in the common 3′ → 5′ direction with subsequent condensation of one of the 5′-phosphoramidites **25**–**28**. The combination of both approaches led to oligonucleotides carrying a (3′-3′)- as well as a (5′-5′)-terminal cap structure (see *Table 2*).

Table 2. *Modified Oligo-2′-deoxyribonucleotide Sequences*

Sequence	Sequence
5′-d(TCC-CCC-CC)-3′ (**K**)	T-**5′-5′**-d(CAC-CGA-CGG-CGC)
5′-d(AAA-AA)-3′ (**L**)	dC-**5′-5′**-d(CAC-CGA-CGG-CGC)
5′-d(TTG-TGT-GT)-3′ (**M**)	dC-**5′-5′**-d(CAC-CGA-CGG-CGC)
	dC-**5′-5′**-d(CAC-CGA-CGG-CGC)
5′-d(CAC-CGA-CGG-CGC)-**3′-3′**-T	T-**5′-5′**-d(CAC-CGA-CGG-CGC)-**3′-3′**-T
5′-d(CAC-CGA-CGG-CGC)-**3′-3′**-dC	dC-**5′-5′**-d(CAC-CGA-CGG-CGC)-**3′-3′**-dC
5′-d(CAC-CGA-CGG-CGC)-**3′-3′**-dA	dA-**5′-5′**-d(CAC-CGA-CGG-CGC)-**3′-3′**-dA
5′-d(CAC-CGA-CGG-CGC)-**3′-3′**-dG	dG-**5′-5′**-d(CAC-CGA-CGG-CGC)-**3′-3′**-dG

The described inverse strategy allowed also for easy formation of 3′-*O*-conjugates, as demonstrated by the reaction of cholesteryl [31] or α-tocopheryl 2-cyanoethyl diisopropylphosphoramidite [32], in the last step by prolonged condensation time and the use of pyridinium chloride as activator to form the modified oligonucleotides. Deprotection and cleavage from the solid support in the usual manner led to the 3′-*O*-conjugates **33** and **34** with a lipophilic cap at the 3′-terminal end in 38 and 55% yield, respectively. The HPLC traces of the crude oligonucleotide 3′-*O*-conjugates (*Fig. 2*) indicated that the attachment of cholesterol and vitamin E is not complete due to steric effects, but purification of the conjugates could be easily achieved since the retention times of the unmodified oligonucleotides and their functionalized derivatives are quite different.

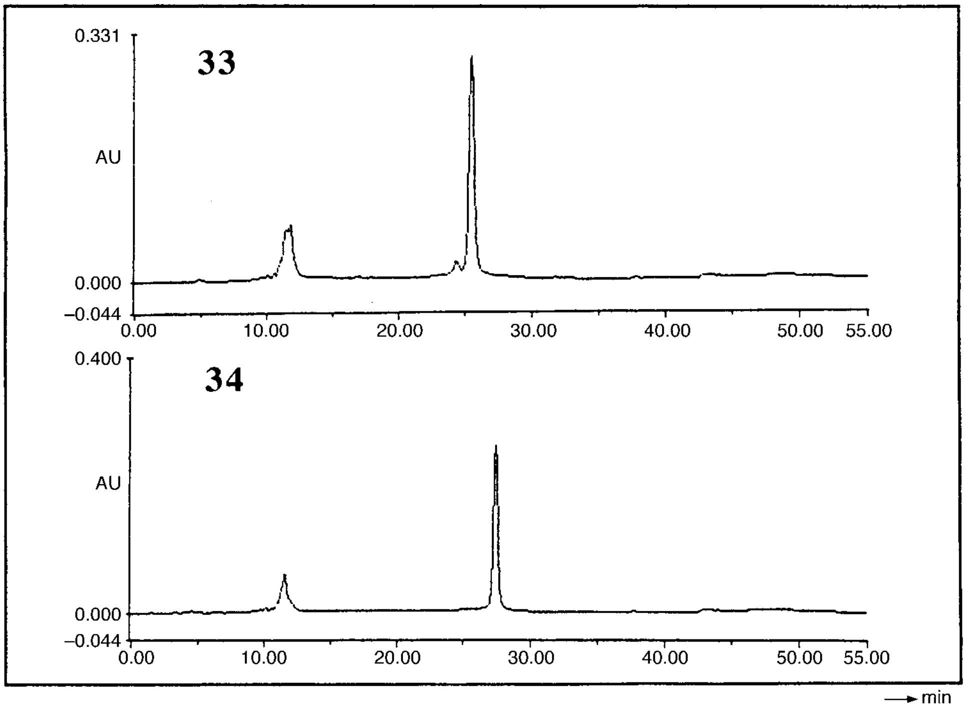
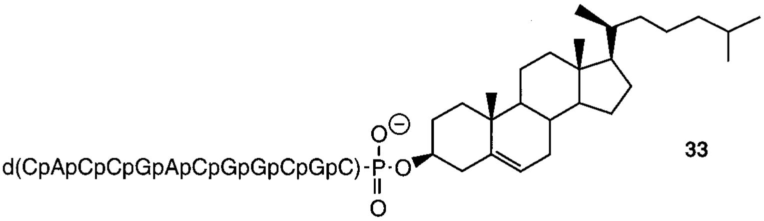
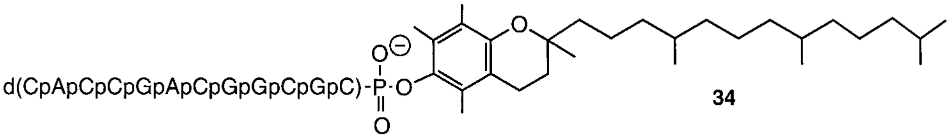


Fig. 2. *HPLC Traces of the crude oligonucleotide 3′-*O*-conjugates* **33** *and* **34**. AU = arbitrary unit.



33



34

Experimental Part

General. Products were dried under high vacuum. Oligonucleotide syntheses: *Applied-Biosystems-392* DNA/RNA synthesizer. TLC: precoated silica-gel thin-layer sheets *F1500 LS 254* from *Schleicher & Schuell.* 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 15*; 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-[Dimethyl(1,1,2-trimethylpropyl)silyl]thymidine* (**5**) *and 3′,5′-Bis-O-[dimethyl(1,1,2-trimethylpropyl)silyl]thymidine* (**5a**). Thymidine (**1**; 2.42 g, 10 mmol) was co-evaporated with dry pyridine (2 × 10 ml) and then taken up in abs. DMF (60 ml). Then, 1*H*-imidazole (2 g, 30 mmol) and dimethyl(1,1,2-trimethylpropyl)silyl chloride (= dimethyl(thexyl)silyl chloride; 3 ml, 15 mmol) were added and stirred for 2 h at r.t. After dilution with AcOEt (200 ml), the soln. was extracted with H_2O (3 × 150 ml), the org. layer dried (Na_2SO_4) and evaporated, and the residue purified by FC (16 × 3.5 cm, toluene/AcOEt/MeOH 4 : 1 : 0 (0.5 l) and 10 : 10 : 1 (0.5 l)): 3.4 g (86%) of **5** and 430 mg (8%) of **5a**.

Data of **5**: M.p. 178–180°. UV (MeOH): 266 (4.04). ^{1}H-NMR ((D_6)DMSO): 11.3 (br. *s*, NH); 7.4 (*s*, H−C(6)); 6.12 (*t*, H−C(1′)); 5.25 (*d*, OH−C(3′)); 4.17 (*m*, H−C(3′)); 3.77–3.69 (*m*, H−C(4′), 2 H−C(5′)); 2.07–2.02 (*m*, 2 H−C(2′)); 1.76 (*s*, Me−C(5)); 1.65–1.55 (*m*, Me_2CH); 0.85–0.81 (*m*, 2 Me_2C); 0.094 (*s*, Me_2Si). Anal. calc. for $C_{18}H_{32}N_2O_5Si$ (384.6): C 56.32, H 8.39, N 7.28; found: C 56.32, H 8.33, N 7.38.

Data of **5a**: M.p. 115–118°. UV (MeOH): 266 (4.06). ^{1}H-NMR ($CDCl_3$): 8.42 (br. *s*, NH); 7.42 (*s*, H−C(6)); 6.34–6.28 (*t*, H−C(1′)); 4.41–4.39 (*m*, H−C(3′)); 3.94–3.72 (*m*, H−C(4′), 2 H−C(5′)); 2.23–2.18 (*m*, 1 H−C(2′)); 2.05–1.90 (*m*, 1 H−C(2′), Me−C(5)); 1.68–1.54 (*m*, 2 Me_2CH); 0.91–0.82 (*m*, 4 Me_2C); 0.15–0.07 (*s*, 2 Me_2Si). Anal. calc. for $C_{26}H_{50}N_2O_5Si_2$ (526.8): C 59.27, H 9.57, N 5.32; found: C 58.93, H 9.47, N 5.29.

2. *2′-Deoxy-5′-O-[dimethyl(1,1,2-trimethylpropyl)silyl]-N^4-{[2-(4-nitrophenyl)ethoxy]carbonyl}cytidine* (**6**) and *2′-Deoxy-3′,5′-bis-O-[dimethyl(1,1,2-trimethylpropyl)silyl]-N^4-{[2-(4-nitrophenyl)ethoxy]carbonyl}cytidine* (**6a**). As described in *Exper. 1*, with **2** (1.26 g, 3 mmol), dry DMF (30 ml), 1*H*-imidazole (600 mg, 9 mmol), and dimethyl(thexyl)silyl chloride (1.2 ml, 6 mmol; 2 h, r.t.). Purification by FC (12 × 3.5 cm, toluene/AcOEt 1 : 1 (0.5 l) and 1 : 10 (0.5 l)) gave 1.19 g (71%) of **6** and 320 mg (15%) of **6a**.

Data of **6**: Crystals. M.p. 148°. UV (MeOH): 211 (4.49), 241 (4.27), 280 (4.20). ^{1}H-NMR ((D_6)DMSO): 10.8 (br. *s*, NH); 8.18–8.13 (*m*, H−C(6), 2 H *o* to NO_2); 7.59 (*d*, 2 H *m* to NO_2); 6.97 (*d*, H−C(5)); 6.06 (*t*, H−C(1′)); 5.29 (*d*, OH−C(3′)); 4.34 (*t*, CH_2CH_2OCO); 4.20–4.12 (*m*, H−C(3′)); 3.90–3.73 (*m*, H−C(4′), 2 H−C(5′)); 3.06 (*t*, CH_2CH_2OCO); 2.34–2.24 (*m*, 1 H−C(2′)); 2.07–1.92 (*m*, 1 H−C(2′)); 1.58–1.53 (*m*, Me_2CH); 0.83–0.78 (*m*, 2 Me_2C); 0.09 (*s*, Me_2Si). Anal. calc. for $C_{26}H_{38}N_4O_8Si$ (562.7): C 55.50, H 6.81, N 9.96; found: C 55.55, H 6.87, N 9.95.

Data of **6a**: Oil. UV (MeOH): 211 (4.47), 240 (4.27), 280 (4.18). ^{1}H-NMR ((D_6)DMSO): 10.81 (br. *s*, NH); 8.16–8.10 (*m*, H−C(6), 2 H *o* to NO_2); 7.58 (*d*, 2 H *m* to NO_2); 6.98 (*d*, H−C(5)); 6.06 (*t*, H−C(1′)); 4.37–4.32 (*m*, CH_2CH_2OCO, H−C(3′)); 3.86–3.66 (*m*, H−C(4′), 2 H−C(5′)); 3.06 (*t*, CH_2CH_2OCO); 2.30–2.22 (*m*, 1 H−C(2′)); 2.14–2.07 (*m*, 1 H−C(2′)); 1.62–1.51 (*m*, 2 Me_2CH); 0.85–0.77 (*m*, 4 Me_2C); 0.11–0.01 (*m*, 2 Me_2Si). Anal. calc. for $C_{34}H_{56}N_4O_8Si_2$ (705.0): C 57.92, H 8.00, N 7.95; found: C 57.59, H 8.11, N 7.56.

3. *2′-Deoxy-5′-O-[dimethyl(1,1,2-trimethylpropyl)silyl]-N^t-{[2-(4-nitrophenyl)ethoxy]carbonyl}adenosine* (**7**) *and 2′-Deoxy-3′,5′-bis-O-[dimethyl(1,1,2-trimethylpropyl)silyl]-N^6-{[2-(4-nitrophenyl)ethoxy]carbonyl}adenosine* (**7a**). As described in *Exper. 1*, with **3** (1.33 g, 3 mmol), dry DMF (30 ml), 1*H*-imidazole (0.6 g, 9 mmol), and dimethyl(thexyl)silyl chloride (1.2 ml, 6 mmol; 2 h, r.t.). Purification by FC (12 × 3.5 cm, toluene/AcOEt 1 : 1 (0.5 l) and 1 : 10 (0.5 l)) gave 1.36 g (77%) of **7** and 380 mg (17%) of **7a**.

Data of **7**: M.p. 110–114°. UV (MeOH): 206 (4.58), 266 (4.46). ^{1}H-NMR ((D_6)DMSO): 10.57 (br. *s*, NH); 8.60, 8.54 (2*s*, H−C(2), H−C(8)); 8.14 (*d*, 2 H *o* to NO_2); 7.59 (*d*, 2 H *m* to NO_2); 6.41 (*t*, H−C(1′)); 5.38 (*d*, OH−C(3′)); 4.40–4.35 (*m*, H−C(3′), CH_2CH_2OCO); 3.88–3.65 (*m*, H−C(4′), 2 H−C(5′)); 3.09 (*t*, CH_2CH_2OCO); 2.82–2.76 (*m*, 1 H−C(2′)); 2.38–2.32 (*m*, 1 H−C(2′)); 1.55–1.49 (*m*, Me_2CH); 0.81–0.76 (*m*, 2 Me_2C); 0.02 (*s*, Me_2Si). Anal. calc. for $C_{27}H_{38}N_6O_7Si$ (586.7): C 55.27, H 6.52, N 14.32; found: C 55.28, H 6.60, N 14.21.

Data of **7a**: M.p. 141–142°. UV (MeOH): 208 (4.56), 266 (4.47). ^{1}H-NMR ($CDCl_3$): 8.72 (*s*, H−C(2)); 8.26–8.13 (*m*, H−C(8), NH, 2 H *o* to NO_2); 7.45–7.41 (*m*, 2 H *m* to NO_2); 6.45 (*t*, H−C(1′)); 4.58–4.49 (*m*, H−C(3′), CH_2CH_2OCO); 4.02–3.98 (*m*, H−C(4′)); 3.86–3.69 (*m*, 2 H−C(5′)); 3.13 (*t*, CH_2CH_2OCO); 2.64–2.58 (*m*, 1 H−C(2′)); 2.46–2.34 (*m*, 1 H−C(2′)); 1.64–1.57 (*m*, 2 Me_2CH); 0.89–0.83 (*m*, 4 Me_2C); 0.11–0.09 (*m*, 2 Me_2Si). Anal. calc. for $C_{35}H_{56}N_6O_7Si_2$ (729.1): C 57.66, H 7.74, N 11.53; found: C 57.28, H 7.67, N 11.27.

4. *2′-Deoxy-5′-O-[dimethyl(1,1,2-trimethylpropyl)silyl]-N^2-{[2-(4-nitrophenyl)ethoxy]carbonyl}-O^6-[2-(4-nitrophenyl)ethyl]guanosine* (**8**) *and 2′-Deoxy-3′,5′-bis-O-[dimethyl(1,1,2-trimethylpropyl)silyl]-N^2-{[2-(4-nitrophenyl)ethoxy]carbonyl}-O^6-[2-(4-nitrophenyl)ethyl]guanosine* (**8a**). As described in *Exper. 1*, with **4** (1.22 g, 2 mmol), dry DMF (20 ml), 1*H*-imidazole (0.4 g, 6 mmol), and dimethyl(thexyl)silyl chloride (0.8 ml, 4 mmol; 2 h, r.t.). Purification by FC (10 × 3.5 cm, toluene/AcOEt 1 : 1 (0.5 l) and 1 : 10 (0.5 l)) gave 1.14 g (75%) of **8** and 200 mg (11%) of **8a**.

Data of **8**: M.p. 184–185°. UV (MeOH): 214 (4.66), 268 (4.56). ^{1}H-NMR ((D_6)DMSO): 10.3 (br. *s*, NH); 8.30 (1*s*, H−C(8)); 8.17–8.14 (*m*, 4 H *o* to NO_2); 7.64–7.59 (*m*, 4 H *m* to NO_2); 6.37 (*t*, H−C(1′)); 5.31 (*d*, OH−C(3′)); 4.74 (*t*, $\mathrm{CH_2C}\mathit{H}_2\mathrm{OCO}$); 4.38–4.33 (*m*, H−C(3′), $\mathrm{CH_2C}\mathit{H}_2\mathrm{O}$ of *O*^{6}-npe); 3.85–3.63 (*m*, H−C(4′), 2 H−C(5′)); 3.31 (*t*, $\mathrm{C}\mathit{H}_2\mathrm{CH_2OCO}$); 3.09 (*t*, $\mathrm{C}\mathit{H}_2\mathrm{CH_2O}$ of *O*^{6}-npe); 2.86–2.75 (*m*, 1 H−C(2′)); 2.27–2.23 (*m*, 1 H−C(2′)); 1.53–1.45 (*m*, $\mathrm{Me_2C}\mathit{H}$); 0.79–0.74 (*m*, 2 Me_2C); 0.04 (*s*, Me_2Si). Anal. calc. for $C_{35}H_{45}N_7O_{10}Si$ (751.9): C 55.91, H 6.03, N 13.06; found: C 55.90, H 6.12, N 12.86.

Data of **8a**: UV (MeOH): 214 (4.68), 268 (4.57). ^{1}H-NMR ($CDCl_3$): 8.18–8.06 (*m*, H−C(8), 4 H *o* to NO_2); 7.49/7.40 (*m*, 4 H *m* to NO_2); 7.30 (br. *s*, NH); 6.33 (*t*, H−C(1′)); 4.79 (*t*, $\mathrm{CH_2C}\mathit{H}_2\mathrm{OCO}$); 4.58–4.45 (*m*, H−C(1′), $\mathrm{C}\mathit{H}_2\mathrm{O}$ of *O*^{6}-npe); 3.96–3.94 (*m*, H−C(4′)); 3.82–3.68 (*m*, 2 H−C(5′)); 3.29 (*t*, $\mathrm{C}\mathit{H}_2\mathrm{CH_2OCO}$); 3.10 (*t*, $\mathrm{C}\mathit{H}_2\mathrm{CH_2O}$ of *O*^{6}-npe); 2.64–2.57 (*m*, 1 H−C(2′)); 2.39–2.30 (*m*, 1 H−C(2′)); 1.64–1.54 (*m*, 2 $\mathrm{Me_2C}\mathit{H}$); 0.89–0.83 (*m*, 4 Me_2C), 0.11–0.06 (*m*, 2 Me_2Si). Anal. calc. for $C_{48}H_{63}N_7O_{10}Si_2$ (894.2): C 57.76, H 7.10, N 10.97; found: C 57.65, H 7.14, N 10.75.

5. *3′-*O*-(4,4′-Dimethoxytrityl)-5′-*O*-[dimethyl(1,1,2-trimethylpropyl)silyl]thymidine* (**13**). In dry pyridine (10 ml), **5** (812 mg, 2.1 mmol) was co-evaporated, taken up in dry pyridine (20 ml), and treated with $(MeO)_2TrCl$ (1.43 g, 4.2 mmol). After stirring for 2 h at 60°, the soln. was diluted with CH_2Cl_2 (60 ml) and extracted with sat. $NaHCO_3$ soln. (3 × 20 ml). The aq. phase was extracted with CH_2Cl_2 (20 ml), the combined org. layer dried (Na_2SO_4), evaporated, and co-evaporated with toluene (2 × 20 ml), and the residue purified by FC (15 × 2.5 cm, toluene/AcOEt 10:1 (0.4 l and 4:1 (0.5 l)): 1.35 g (94%) of **13**. Slightly yellowish foam. UV (MeOH): 234 (4.38), 275 (4.27). ^{1}H-NMR ($CDCl_3$): 8.37 (*s*, NH); 7.50–7.13 (*m*, H−C(6), 9 H of $(\mathrm{MeO})_2\mathit{Tr}$); 6.87 (*d*, 4 H *o* to MeO); 6.45–6.38 (*m*, H−C(1′)); 4.34–4.30 (*m*, H−C(3′)); 4.08–4.06 (*m*, H−C(4′)); 3.84 (*s*, 2 MeO); 3.71, 3.69 (*dd*, 1 H−C(5′)); 3.41, 3.39 (*dd*, 1 H−C(5′)); 1.91 (*s*, Me−C(5)); 1.73–1.53 (*m*, 2 H−C(2′), $\mathrm{Me_2C}\mathit{H}$); 0.84–0.80 (*m*, 2 Me_2C); 0.04–0.01 (*m*, Me_2Si). Anal. calc. for $C_{39}H_{51}N_2O_7Si$ (687.9): C 68.09, H 7.47, N 4.07; found: C 68.32, H 7.43, N 4.33.

6. *2′-Deoxy-3′-*O*-(4,4′-dimethoxytrityl)-5′-*O*-[dimethyl(1,1,2-trimethylpropyl)silyl]-*N^4*{[2-(4-nitrophenyl)-ethoxy]carbonyl}cytidine* (**14**). As described in *Exper. 5*, with **6** (670 mg, 1.21 mmol), dry pyridine (20 ml), and $(MeO)_2TrCl$ (820 mg, 2.42 mmol; 2 h, 60°). Purification by FC (15 × 2.5 cm, toluene/AcOEt 1:1 (0.7 l) and 1:4 (0.2 l)) gave 950 mg (95%) of **14**. Slightly yellowish foam. UV (MeOH): 203 (4.96), 236 (4.59), 275 (4.27), 282 (sh, 4.27). ^{1}H-NMR ($CDCl_3$): 8.17–8.08 (*m*, 2 H *o* to NO_2, H−C(6)); 7.72 (br. *s*, NH); 7.72–7.16 (*m*, H−C(5), 9 H of $(\mathrm{MeO})_2\mathit{Tr}$, 2 H *m* to NO_2); 7.05 (*d*, H−C(5)); 6.79 (*d*, 4 H *o* to MeO); 6.43–6.35 (*m*, H−C(1′)); 4.39 (*t*, $\mathrm{CH_2C}\mathit{H}_2\mathrm{OCO}$); 4.23–4.20 (*m*, H−C(3′)); 3.93–3.90 (*m*, H−C(4′)); 3.76 (*s*, 2 MeO); 3.58, 3.54 (*dd*, 1 H−C(5′)); 3.22, 3.18 (*dd*, 1 H−C(5′)); 3.08 (*t*, $\mathrm{C}\mathit{H}_2\mathrm{CH_2OCO}$); 2.29–2.17 (*m*, 1 H−C(2′)); 1.68–1.42 (*m*, 1 H−C(2′), $\mathrm{Me_2C}\mathit{H}$); 0.85–0.70 (*m*, 2 Me_2C); 0.02 (*d*, Me_2Si). Anal. calc. for $C_{47}H_{54}N_4O_{10}Si$ (863.1): C 65.40, H 6.31, N 6.49; found: C 65.68, H 6.62, N 6.64.

7. *2′-Deoxy-3′-*O*-(4,4′-dimethoxytrityl)-5′-*O*-[dimethyl(1,1,2-trimethylpropyl)silyl]-*N^6*-{[2-(4-nitrophenyl)-ethoxy]carbonyl}adenosine* (**15**). As described in *Exper. 5*, with **7** (1.04 g, 1.76 mmol), dry pyridine (20 ml), and $(MeO)_2TrCl$ (1.18 g, 3.52 mmol; 2 h, 60°). Purification by FC (silica gel, 15 × 2.5 cm, toluene/AcOEt 10:1 (0.5 l) and 1:4 (0.2 l)) gave 5 g (96%) of **15**. Slightly yellowish foam. UV (MeOH): 203 (4.98), 236 (4.46), 267 (4.50). ^{1}H-NMR ($CDCl_3$): 8.72 (*s*, NH); 8.33 (*s*, H−C(2)); 8.16–8.13 (*m*, 2 H *o* to NO_2, H−C(8)); 7.47–7.16 (*m*, 9 H of $(\mathrm{MeO})_2\mathit{Tr}$, 2 H *m* to NO_2); 7.05 (*d*, H−C(5)); 6.83, 6.80 (*dd*, 4 H *o* to MeO); 6.56–6.50 (*m*, H−C(1′)); 4.50 (*t*, $\mathrm{CH_2C}\mathit{H}_2\mathrm{OCO}$); 4.40–4.38 (*m*, H−C(3′)); 4.09–4.07 (*m*, H−C(4′)); 3.76 (*s*, 2 MeO); 3.61, 3.60 (*dd*, 1 H−C(5′)); 3.39, 3.36 (*dd*, 1 H−C(5′)); 3.11 (*t*, $\mathrm{C}\mathit{H}_2\mathrm{CH_2OCO}$); 2.12–1.93 (*m*, 2 H−C(2′)); 1.58–1.42 (*m*, $\mathrm{Me_2C}\mathit{H}$); 0.76–0.72 (*m*, 2 Me_2C); 0.01 (*d*, Me_2Si). Anal. calc. for $C_{48}H_{56}N_6O_9Si$ (889.1): C 64.84, H 6.35, N 9.45; found: C 64.73, H 6.33, N 9.25.

8. *2′-Deoxy-3′-*O*-(4,4′-dimethoxytrityl)-5′-*O*-[dimethyl(1,1,2-trimethylpropyl)silyl]-*N^2*-{[2-(4-nitrophenyl)-ethoxy]carbonyl}-*O^6*-[2-(4-nitrophenyl)ethyl]guanosine* (**16**). As described in *Exper. 5*, with **8** (570 mg, 0.76 mmol), dry pyridine (10 ml), and $(MeO)_2TrCl$ (500 mg, 1.44 mmol; 2 h, 60°). Purification by FC (11 × 2.5 cm, toluene/AcOEt 20:1 (0.5 l), 10:1 (0.4 l), and 1:4 (0.2 l)) gave 730 mg (95%) of **16**. Slightly yellowish foam. UV (MeOH): 203 (5.02), 237 (4.54), 268 (4.60). ^{1}H-NMR ($CDCl_3$): 8.21–8.12 (*m*, 4 H *o* to NO_2); 8.00 (*s*, H−C(8)); 7.51–7.13 (*m*, 9 H of $(\mathrm{MeO})_2\mathit{Tr}$, 4 H *m* to NO_2); 6.85–6.82 (*m*, 4 H *o* to MeO); 6.44–6.39 (*m*, H−C(1′)); 4.80 (*t*, $\mathrm{CH_2C}\mathit{H}_2\mathrm{OCO}$); 4.46 (*t*, $\mathrm{CH_2C}\mathit{H}_2\mathrm{O}$ of *O*^{6}-npe); 4.43–4.39 (*m*, H−C(3′)); 4.09–4.07 (*m*, H−C(4′)); 3.79 (*s*, 2 MeO); 3.65, 3.61 (*dd*, 1 H−C(5′)); 3.39–3.32 (*m*, 1 H−C(5′), $\mathrm{C}\mathit{H}_2\mathrm{CH_2OCO}$); 3.14 (*t*, $\mathrm{C}\mathit{H}_2\mathrm{CH_2O}$ of *O*^{6}-npe); 2.07–2.00 (*m*, 1 H−C(2′)); 1.98–1.83 (*m*, 1 H−C(2′)); 1.56–1.48 (*m*, $\mathrm{Me_2C}\mathit{H}$); 0.78–0.74 (*m*, 2 Me_2C); 0.09–0.00 (*m*, Me_2Si). Anal. calc. for $C_{56}H_{63}N_7O_{12}Si$ (1054.1): C 63.80, H 6.02, N 9.30; found: C 63.91, H 6.08, N 9.06.

9. *3′-*O*-(4,4′-Dimethoxytrityl)-5′-*O*-{[2-(4-nitrophenyl)ethoxy]carbonyl}thymidine* (**17**). As described in *Exper. 5*, with **9** (400 mg, 0.9 mmol), dry pyridine/CH_2Cl_2 1:1 (10 ml), and $(MeO)_2TrCl$ (610 mg, 1.8 mmol; 2 h,

60°). Purification by FC (14 × 2 cm, toluene/AcOEt/MeOH 10 : 1 : 0 (0.4 l) and 5 : 5 : 1 (0.2 l)) gave 640 mg (94%) of **7**. Colorless foam. UV (MeOH): 203 (4.85), 236 (4.40), 266 (4.33), 282 (sh, 4.14). ^{1}H-NMR ($CDCl_3$): 8.18 (*d*, 2 H *o* to NO_2); 7.46 – 7.17 (*m*, 2 H *m* to NO_2, 9 H of $(MeO)_2$*Tr*, H–C(6)); 6.84 (*d*, 4 H *o* to MeO); 6.38 – 6.37 (*m*, H–C(1′)); 4.39 – 4.31 (*m*, CH_2*CH_2*OCO); 4.25 – 4.21 (*m*, H–C(3′)); 4.02 – 3.84 (*m*, H–C(4′), 1 H–C(5′)); 3.80 (*s*, 2 MeO); 3.73 – 3.68 (*m*, 1 H–C(5′)); 3.06 (*t*, *CH_2*CH_2OCO); 2.12 – 1.98 (*m*, 1 H–C(2′)); 1.82 (*s*, Me–C(5)); 1.78 – 1.64 (*m*, 1 H–C(2′)). Anal. calc. for $C_{40}H_{39}N_3O_{11}$ (737.8): C 65.12, H 5.33, N 5.69; found: C 65.35, H 5.42, N 5.64.

10. *2′-Deoxy-3′-*O*-(4,4′-dimethoxytrityl)-5′-*O*-{[5-(dimethylamino)naphthalen-1-yl]sulfonyl}-*N^4*-{[2-(4-nitrophenyl)ethoxy]carbonyl}cytidine* (**18**). As described in *Exper. 5*, with **10** (1.01 g, 1.39 mmol), dry pyridine/CH_2Cl_2 1 : 1 (10 ml), and $(MeO)_2TrCl$ (940 mg, 2.79 mmol; 32 h, r.t.). Purification by FC (silica gel, 14 × 2.5 cm, toluene/AcOEt 1 : 1 (0.5 l) and 1 : 4 (0.4 l)) gave 1.33 g (93%) of **18**. Yellow foam. UV (MeOH): 204 (5.05), 235 (4.57), 278 (sh, 4.56), 346 (sh, 3.58). ^{1}H-NMR ((D_6)DMSO): 10.8 (br. *s*, NH); 8.45 (*d*, H–C(2)(dns)); 8.19 – 8.12 (*m*, 2 H *o* to NO_2, H–C(4)(dns), H–C(8)(dns)); 7.72 (*d*, H–C(6)); 7.62 – 7.56 (*m*, 2 H *m* to NO_2, H–C(3)(dns), H–C(7)(dns)); 7.41 – 7.12 (*m*, 9 H of $(MeO)_2$*Tr*, H–C(6)(dns)); 6.91 – 6.86 (*m*, 4 H *o* to MeO, H–C(5)); 6.12 (*t*, H–C(1′)); 4.33 – 4.31 (*m*, 2 CH_2*CH_2*OCO); 4.12 – 4.10 (*m*, H–C(3′)); 3.85 – 3.65 (*m*, H–C(4′), 2 H–C(5′), 2 MeO, SO_2CH_2); 3.06 (*t*, *CH_2*CH_2OCO); 2.77 (*s*, Me_2N); 1.98 – 1.90 (*m*, 1 H–C(2′)); 1.64 – 1.56 (*m*, 1 H–C(2′)). Anal. calc. for $C_{54}H_{53}N_5O_{14}S$ (1028.1): C 63.09, H 5.20, N 6.81; found: C 63.01, H 5.30, N 6.89.

11. *2′-Deoxy-5′-*O*-{[5-(dimethylamino)naphthalen-1-yl]sulfonyl}-3′-*O*-(4,4′-dimethoxytrityl)-*N^6*-{[2-(4-nitrophenyl)ethoxy]carbonyl}adenosine* (**19**). As described in *Exper. 5*, with **11** (680 mg, 0.91 mmol), dry pyridine/CH_2Cl_2 1 : 1 (10 ml); and $(MeO)_2TrCl$ (610 mg, 1.82 mmol; 32 h, r.t.). Purification by FC (14 × 2.5 cm, toluene/AcOEt 4 : 1 (0.5 l), 1 : 1 (0.4 l), and 1 : 4 (0.4 l)) gave 900 mg (94%) of **19**. Yellow foam. UV (MeOH): 204 (5.08), 238 (4.57), 264 (4.60), 346 (sh, 3.59). ^{1}H-NMR ((D_6)DMSO): 10.6 (br. *s*, NH); 8.57 – 8.43 (*m*, H–C(2), H–C(8), H–C(2)(dns)); 8.19 – 8.11 (*m*, 2 H *o* to NO_2, H–C(4)(dns), H–C(8)(dns)); 7.63 – 7.10 (*m*, 2 H *m* to NO_2, H–C(3)(dns), H–C(7)(dns), 9 H of $(MeO)_2$*Tr*, H–C(6)(dns)); 6.89 (*d*, 4 H *o* to MeO); 6.44 (*t*, H–C(1′)); 4.39 – 4.22 (*m*, 2 CH_2*CH_2*OCO, H–C(3′)); 3.81 – 3.71 (*m*, H–C(4′), 2 H–C(5′), 2 MeO, SO_2CH_2); 3.09 (*t*, *CH_2*CH_2OCO); 2.74 (*s*, Me_2N); 2.58 – 2.47 (*m*, 1 H–C(2′)); 2.05 – 1.97 (*m*, 1 H–C(2′)). Anal. calc. for $C_{55}H_{53}N_7O_{13}S$ (1052.1): C 62.77, H 5.08, N 9.32; found: C 62.68, H 5.17, N 9.20.

12. *2′-Deoxy-5′-*O*-{[5-(dimethylamino)naphthalen-1-yl]sulfonyl}-3′-*O*-(4,4′-dimethoxytrityl)-*N^2*-{[2-(4-nitrophenyl)ethoxy]carbonyl}-*O^6*-[2-(4-nitrophenyl)ethyl]guanosine* (**20**). As described in *Exper. 5*, with **12** (880 mg, 0.96 mmol), dry pyridine/CH_2Cl_2 1 : 1 (10 ml), and $(MeO)_2TrCl$ (650 mg, 1.92 mmol; 32 h, r.t.). Purification by FC (14 × 2.5 cm, toluene/AcOEt 4 : 1 (0.4 l) and 1 : 1 (0.3 l)) gave 1.03 g (87%) of **20**. Yellow foam. UV (MeOH): 203 (5.12), 238 (4.63), 264 (4.68), 346 (sh, 3.59). ^{1}H-NMR ((D_6)DMSO): 10.31 (br. *s*, NH); 8.43 (*d*, H–C(2)(dns)); 8.19 – 8.10 (*m*, 4 H *o* to NO_2, H–C(4)(dns), H–C(8)(dns), H–C(8)); 7.63 – 7.53 (*m*, 4 H *m* to NO_2, H–C(3)(dns), H–C(7)(dns)); 7.44 – 7.14 (*m*, 9 H of $(MeO)_2$*Tr*, H–C(6)(dns)); 6.87 (*d*, 4 H *o* to MeO); 6.31 (*t*, H–C(1′)); 4.73 (*t*, CH_2*CH_2*OCO); 4.31 – 4.17 (*m*, CH_2*CH_2*OCO, CH_2*CH_2*O of *O*^{6}-npe, H–C(3′)); 3.87 – 3.70 (*m*, H–C(4′), 2 H–C(5′), 2 MeO, SO_2CH_2); 3.27 (*t*, *CH_2*CH_2OCO); 3.06 (*t*, *CH_2*CH_2O of *O*^{6}-npe); 2.73 (*s*, 2 Me_2N); 2.53 – 2.48 (*m*, 1 H–C(2′)); 1.93 – 1.87 (*m*, 1 H–C(2′)). Anal. calc. for $C_{63}H_{61}N_8O_{16}S$ (1217.3): C 62.16, H 4.97, N 9.20; found: C 62.30, H 5.09, N 8.68.

13. *3′-*O*-(4,4′-Dimethoxytrityl)thymidine* (**21**). 13.1. To a soln. of $(Bu_4N)F \cdot H_2O$ in THF (100 ml), **13** (10.2 g, 14.80 mmol) was added. After stirring overnight at r.t., the soln. was diluted with AcOEt (250 ml) and washed with H_2O (3 × 100 ml) and the org. layer dried (Na_2SO_4) and evaporated. The crude product was dissolved in CH_2Cl_2 (50 ml) and added dropwise to pentane (600 ml). The resulting precipitate was collected and dried at 60° *in vacuo:* 7.7 g (96%) of **21**.

13.2. Compound **17** (1.3 g, 1.8 mmol) was dissolved in 0.5M DBU in MeCN (30 ml) and stirred for 2 h at r.t. The reaction was stopped by addition of 0.5M aq. AcOH (30 ml), the mixture diluted with H_2O (10 ml) and extracted with CH_2Cl_2 (3 × 10 ml), the org. layer washed with sat. $NaHCO_3$ soln. (2 × 20 ml), the aq. layer extracted back with CH_2Cl_2 (10 ml), the combined org. layer dried (Na_2SO_4) and evaporated, and the residue purified by FC (10 × 2.5 cm, toluene/AcOEt 10 : 1 (0.3 l) and 1 : 1 (0.2 l)): 850 mg (90%) of **21**. Colorless foam. UV (MeOH): 236 (4.37), 265 (4.12). ^{1}H-NMR ((D_6)DMSO): 11.30 (br. *s*, NH); 7.62 (*s*, H–C(6)); 7.43 – 7.19 (*m*, 9 H of $(MeO)_2$*Tr*); 6.94 – 6.90 (*d*, 4 H *o* to MeO); 6.20 – 6.19 (*m*, H–C(1′)); 4.98 (*t*, OH–C(5′)); 4.25 – 4.23 (*m*, H–C(3′)); 3.78 – 3.74 (*m*, H–C(4′), 2 MeO); 3.49 – 3.15 (*m*, 2 H–C(5′)); 1.80 – 1.40 (*m*, 2 H–C(2′), Me–C(5)). Anal. calc. for $C_{31}H_{33}N_2O_7$ (544.6): C 68.24, H 6.10, N 5.13; found: C 68.28, H 6.07, N 4.87.

14. *2′-Deoxy-3′-*O*-(4,4′-dimethoxytrityl)-*N^4*-{[2-(4-nitrophenyl)ethoxy]carbonyl}cytidine* (**22**). 14.1. As described in *Exper. 13.1*, with $(Bu_4N)F \cdot H_2O$ (4.6 g, 14.63 mmol), AcOH (2.8 ml, 48.8 mmol), THF (100 ml),

and **14** (8.42 g, 9.76 mmol; 12 d, r.t.). The crude product was purified by FC (18 × 5.5 cm, toluene/AcOEt 1 : 1 (1.0 l), 1 : 4 (1.0 l), and 1 : 8 (1.0 l)): 6.35 g (90%) of **22**. Colorless foam.

14.2. As described in *Exper. 13.2*, with 0.1M DBU (22 ml) in MeCN, **18** (1.13 g, 1.1 mmol; 1 min, r.t.), and 0.1M aq. AcOH (22 ml). Purification by FC (14 × 2.5 cm, toluene/AcOEt/MeOH 4 : 1 : 0 (0.3 l) and 5 : 4 : 1 (0.2 l)) gave 760 mg (90%) of **22**. Colorless foam. UV (MeOH): 238 (4.63), 275 (4.25), 280 (sh, 4.25) ^{1}H-NMR ((D_6)DMSO): 10.74 (br. *s*, NH); 8.17 – 8.14 (*m*, H−C(6), 2 H *o* to NO_2); 7.57 (*d*, 2 H *m* to NO_2); 7.42 – 7.12 (*m*, 9 H of $(MeO)_2$*Tr*); 6.95 – 6.88 (*m*, H−C(5), 4 H *o* to MeO); 6.19 – 6.14 (*m*, H−C(1′)); 4.89 (*t*, OH−C(5′)); 4.33 (*t*, CH_2CH_2OCO); 4.23 – 4.21 (*m*, H−C(3′)); 3.74 – 3.72 (*m*, H−C(4′), 2 MeO); 3.32 – 3.30 (*m*, 1 H−C(5′)); 3.16 – 3.03 (*m*, 1 H−C(5′), CH_2CH_2OCO); 1.92 – 1.80 (*m*, 1 H−C(2′)); 1.70 – 1.58 (*m*, 1 H−C(2′)). Anal. calc. for $C_{39}H_{36}N_4O_{10}$ (720.8): C 64.99, H 5.03, N 7.77; found: C 64.94, H 5.50, N 7.69.

15. *2′-Deoxy-3′-*O*-(4,4′-dimethoxytrityl)-*N^6*-{[2-(4-nitrophenyl)ethoxy]carbonyl}adenosine* (**23**). 15.1. As described in *Exper. 13.1*, with $(Bu_4N)F \cdot H_2O$ (2.22 g, 7.05 mmol), AcOH (1.34 ml, 23.5 mmol), THF (50 ml), and **15** (4.18 g, 4.7 mmol; 12 d, r.t.). The crude product was dissolved in toluene/CH_2Cl_2 3 : 1 (50 ml) and the soln. added dropwise to pentane (500 ml). The resulting residue was collected and dried at 60°: 3.37 g (96%) of **23**.

15.2. As described in *Exper. 13.2*, with 0.1M DBU (14.5 ml) in MeCN, **19** (770 mg, 0.73 mmol; 1 min, r.t.), and 0.1M aq. AcOH (14.5 ml). Purification by FC (8 × 2.5 cm, toluene/AcOEt/MeOH 4 : 1 : 0 (0.3 l) and 5 : 4 : 1 (0.2 l)) gave 520 mg (95%) of **23**. Colorless foam. UV (MeOH): 203 (4.96), 236 (4.46), 268 (4.50). ^{1}H-NMR ((D_6)DMSO): 10.60 (br. *s*, NH); 8.58, 8.54 (2*s*, H−C(2), H−C(8)); 8.13 (*d*, 2 H *o* to NO_2); 7.59 (*d*, 2 H *m* to NO_2); 7.47 – 7.12 (*m*, 9 H of $(MeO)_2$*Tr*); 6.91 (*d*, 4 H *o* to MeO); 6.48 – 6.42 (*m*, H−C(1′)); 5.00 (*t*, OH−C(5′)); 4.37 – 4.34 (*m*, CH_2CH_2OCO, H−C(3′)); 3.83 – 3.72 (*m*, H−C(4′), 2 MeO); 3.38 – 3.35 (*m*, 1 H−C(5′)); 3.21 – 3.18 (*m*, 1 H−C(5′)); 3.08 (*t*, CH_2CH_2OCO); 2.45 – 2.36 (*m*, 1 H−C(2′)); 1.90 – 1.83 (*m*, 1 H−C(2′)). Anal. calc. for $C_{40}H_{38}N_6O_9$ (746.8): C 64.33, H 5.13, N 11.25; found: C 64.12, H 5.26, N 10.65.

16. *2′-Deoxy-3′-*O*-(4,4′-dimethoxytrityl)-*N^2*-{[2-(4-nitrophenyl)ethoxy]carbonyl}-*O^6*-[2-(4-nitrophenyl)ethyl]guanosine* (**24**). 16.1. As described in *Exper. 13.1*, with $(Bu_4N)F \cdot H_2O$ (2.78 g, 9.84 mmol), AcOH (1.69 ml, 29.5 mmol), THF (70 ml), and **16** (6.22 g, 5.9 mmol; 12 d, r.t.). The crude product was dissolved in CH_2Cl_2 (15 ml) and the soln. added dropwise to Et_2O/pentane (500 ml). The resulting precipitate was collected and co-evaporated with CH_2Cl_2: 5.10 g (95%) of **24**.

16.2. As described in *Exper. 13.2*, with 0.1M DBU (15.2 ml) in MeCN, **20** (930 mg, 0.76 mmol; 1 min, r.t.), and 0.1M aq. AcOH (15.2 ml). Purification by FC (9 × 2.5 cm, toluene/AcOEt/MeOH 10 : 1 : 0 (0.3 l) and 5 : 4 : 1 (0.3 l)) gave 680 mg (95%) of **24**. Colorless foam. UV (MeOH): 203 (5.01), 236 (4.53), 268 (4.57). ^{1}H-NMR ((D_6)DMSO): 10.29 (br. *s*, NH); 8.47 (*s*, H−C(8)); 8.14 (*d*, 4 H *o* to NO_2); 7.63 – 7.58 (*m*, 4 H *m* to NO_2); 7.46 – 7.20 (*m*, 9 H of $(MeO)_2$*Tr*); 6.90 (*d*, 4 H *o* to MeO); 6.35 – 6.29 (*m*, H−C(1′)); 4.81 – 4.71 (*m*, CH_2CH_2OCO, OH−C(5′)); 4.36 – 4.32 (*m*, CH_2CH_2O of *O*^{6}-npe, H−C(3′)); 3.83 – 3.72 (*m*, H−C(4′), 2 MeO); 3.37 – 3.22 (*m*, 2 H−C(5′), CH_2CH_2OCO); 3.08 (*t*, CH_2CH_2O of *O*^{6}-npe); 2.40 – 2.32 (*m*, 1 H−C(2′)); 1.79 – 1.72 (*m*, 1 H−C(2′)). Anal. calc. for $C_{48}H_{45}N_7O_{12}$ (911.9): C 63.15, H 5.08, N 10.74; found: C 63.41, H 5.21, N 10.06.

17. *3′-*O*-(4,4′-Dimethoxytrityl)thymidine 5′-(2-Cyanoethyl Diisopropylphosphoramidite)* (**25**). A mixture of **21** (1.63 g, 3 mmol), 2-cyanoethyl tetraisopropylphosphorodiamidite (1.35 g, 4.5 mmol) and 1*H*-tetrazole (110 mg, 1.5 mmol) was stirred in abs. MeCN (3 ml) under N_2 for 1 h. After dilution with CH_2Cl_2 (20 ml), the soln. was washed with phosphate buffer (pH 7; 2 × 10 ml), the aq. phase extracted with CH_2Cl_2 (2 × 10 ml), the combined org. layer dried (Na_2SO_4) and evaporated, and the crude foam purified by FC (20 × 2.5 cm, toluene/AcOEt (1% Et_3N) 4 : 1 (0.3 l) and 2 : 1 (0.3 l). The product fractions were co-evaporated with CH_2Cl_2: 1.63 g (73%) of **25**. Colorless foam. UV (MeOH): 203 (4.85), 235 (4.35), 268 (4.09). ^{1}H-NMR ((D_6)DMSO): 11.30 (br. *s*, NH); 7.42 – 7.12 (*m*, 9 H of $(MeO)_2$*Tr*, H−C(6)); 6.89 (*d*, 4 H *o* to MeO); 6.21 – 6.14 (*m*, H−C(1′)); 4.21 – 4.17 (*m*, H−C(3′)); 3.96 – 3.89 (*m*, H−C(4′)); 3.72 – 3.33 (*m*, 2 MeO, 2 Me_2CH, CH_2CH_2OP, 2 H−C(5′)); 2.72 – 2.60 (*m*, CH_2CH_2OP); 1.71 – 1.62 (*m*, Me−C(5), 2 H−C(2′)); 1.10 – 0.93 (*m*, 2 Me_2CH)). ^{31}P-NMR ((D_6)DMSO): 148.86, 148.26. Anal. calc. for $C_{40}H_{49}N_4O_8P$ (744.8): C 64.50, H 6.63, N 7.52; found: C 64.25, H 6.52, N 7.20.

18.2. *2′-Deoxy-3′-*O*-(4,4′-dimethoxytrityl)-*N^4*-{[2-(4-nitrophenyl)ethoxy]carbonyl}cytidine 5′-(2-Cyanoethyl Diisopropylphosphoramidite)* (**26**). As described in *Exper. 17*, with **22** (720 mg, 1 mmol), 2-cyanoethyl tetraisopropylphosphorodiamidite (450 mg, 1.5 mmol), 1*H*-tetrazole (33 mg, 0.5 mmol), and MeCN (3 ml). Purification by FC (14 × 2.5 cm, AcOEt (1% Et_3N) (0.1 l)) gave 810 mg (88%) of **26**. Colorless foam. UV (MeOH): 202 (4.99), 236 (4.58), 280 (4.25). ^{1}H-NMR ((D_6)DMSO): 10.73 (br. *s*, NH); 8.13 (*d*, 2 H *o* to NO_2); 7.98 – 7.94 (*m*, H−C(6)); 7.57 (*d*, 2 H *m* to NO_2); 7.42 – 7.14 (*m*, 9 H of $(MeO)_2$*Tr*); 6.96 – 6.88 (*m*, 4 H *o* to MeO, H−C(5)); 6.14 – 6.09 (*m*, H−C(1′)); 4.33 (*t*, CH_2CH_2OCO); 4.18 – 4.16 (*m*, H−C(3′)); 3.97 – 3.87 (*m*, H−C(4′)); 3.72 – 3.39 (*m*, 2 MeO, 2 Me_2CH, CH_2CH_2OP, 2 H−C(5′)); 3.05 (*t*, CH_2CH_2OCO); 2.71 – 2.60

(*m*, C*H*$_2$CH$_2$OP); 2.12 – 1.91 (*m*, 1 H–C(2′)); 1.64 – 1.48 (*m*, 1 H–C(2′)); 1.19 – 0.92 (*m*, 2 *Me*$_2$CH). ^{31}P-NMR ((D$_6$)DMSO): 148.74, 148.29. Anal. calc. for $C_{48}H_{55}N_6O_{11}P$ (923.0): C 62.46, H 6.00, N 9.11; found: C 61.86, H 6.05, N 8.81.

19. *2′-Deoxy-3′-*O*-(4,4′-dimethoxytrityl)-*N^6*-{[2-(4-nitrophenyl)ethoxy]carbonyl}adenosine 5′-(2-Cyanoethyl Diisopropylphosphoramidite)* (**27**). As described in *Exper. 17*, **23** (700 mg, 0.95 mmol), 2-cyanethyl tetraisopropylphosphorodiamidite (430 mg, 1.43 mmol), 1*H*-tetrazole (32 mg, 0.48 mmol), and MeCN (3 ml). Purification by FC (14 × 2.5 cm, AcOEt (1% Et$_3$N) (0.1 l)) gave 800 mg (90%) of **27**. Colorless foam. UV (MeOH): 204 (4.96), 237 (4.44), 267 (4.48). ^{1}H-NMR ((D$_6$)DMSO): 10.56 (br. *s*, NH); 8.57 (*d*, H–C(2)); 8.42 (*d*, H–C(8)); 8.13 (*d*, 2 H *o* to NO$_2$); 7.46 (*d*, 2 H *m* to NO$_2$); 7.35 – 7.16 (*m*, 9 H of (MeO)$_2$*Tr*); 6.91 (*m*, 4 H *o* to MeO); 6.50 – 6.41 (*m*, H–C(1′)); 4.39 – 4.35 (*m*, CH$_2$C*H*$_2$OCO, H–C(3′)); 4.05 – 3.96 (*m*, H–C(4′)); 3.72 – 3.38 (*m*, 2 MeO, 2 Me$_2$C*H*, CH$_2$C*H*$_2$OP, 2 H–C(5′)); 3.08 (*t*, C*H*$_2$CH$_2$OCO); 2.71 – 2.62 (*m*, C*H*$_2$CH$_2$OP); 2.38 – 2.28 (*m*, 1 H–C(2′)); 2.11 – 1.94 (*m*, 1 H–C(2′)); 1.21 – 0.84 (*m*, 2 *Me*$_2$CH). ^{31}P-NMR ((D$_6$)DMSO): 148.29, 148.12. Anal. calc. for $C_{48}H_{55}N_6O_{11}P$ (947.0): C 62.15, H 5.85, N 11.83; found: C 61.78, H 5.95, N 11.60.

20. *2′-Deoxy-3′-*O*-(4,4′-dimethoxytrityl)-*N^2*-{[2-(4-nitrophenyl)ethoxy]carbonyl}-*O^6*-[2-(4-nitrophenyl)ethyl]guanosine 5′-(2-Cyanoethyl Diisopropylphosphoramidite)* (**28**). As described in *Exper. 17*, with **24** (810 mg, 0.96 mmol), 2-cyanethyl tetraisopropylphosphorodiamidite (430 mg, 1.43 mmol), 1*H*-tetrazole (32 mg, 0.48 mmol), and MeCN (3 ml). Purification by FC (14 × 2.5 cm, AcOEt (1% Et$_3$N) (0.1 l)) gave 810 mg (83%) of **28**. Colorless foam. UV (MeOH): 202 (5.02), 237 (4.51), 269 (4.56). ^{1}H-NMR ((D$_6$)DMSO): 10.29 (br. *s*, NH); 8.21 – 8.13 (*m*, H–C(8), 4 H *o* to NO$_2$); 7.63 – 7.53 (*m*, 4 H *m* to NO$_2$); 7.46 – 7.18 (*m*, 9 H of (MeO)$_2$*Tr*); 6.89 (*m*, 4 H *o* to MeO); 6.39 – 6.29 (*m*, H–C(1′)); 4.73 (*t*, CH$_2$C*H*$_2$OCO); 4.34 – 4.29 (*m*, CH$_2$C*H*$_2$O of *O*^{6}-npe, H–C(3′)); 3.98 – 3.80 (*m*, H–C(4′)); 3.71 – 3.24 (*m*, 2 MeO, 2 Me$_2$C*H*, CH$_2$C*H*$_2$OP, 2 H–C(5′), C*H*$_2$CH$_2$OCO); 3.07 (*t*, C*H*$_2$CH$_2$ of *O*^{6}-npe); 2.71 – 2.60 (*m*, C*H*$_2$CH$_2$OP); 2.36 – 2.28 (*m*, 1 H–C(2′)); 2.05 – 1.86 (*m*, 1 H–C(2′)); 1.21 – 0.82 (*m*, 2 *Me*$_2$CH). ^{31}P-NMR ((D$_6$)DMSO): 148.35. Anal. calc. for $C_{57}H_{62}N_9O_{13}P$ (1112.2): C 61.56, H 5.62, N 11.33; found: C 61.31, H 5.75, N 10.94.

21. *3′-*O*-(4,4′-Dimethoxytrityl)thymidine 5′-(Hydrogen Butanedioate)* (**29**). In CH$_2$Cl$_2$ (5 ml), **21** (250 mg, 0.46 mmol), succinic anhydride (92 mg, 0.92 mmol), and *N,N*-dimethylpyridin-4-amine (DMAP; 74 mg, 0.6 mmol) were stirred at r.t. for 2 h. Then the mixture was diluted with CH$_2$Cl$_2$ (20 ml) and washed with 10% citric acid (2 × 20 ml) and NaCl soln. (2 × 20 ml). The aq. phase was extracted with CH$_2$Cl$_2$ (2 × 10 ml), the combined org. layer dried (Na$_2$SO$_4$) and evaporated, the resulting foam dissolved in CH$_2$Cl$_2$ (5 ml), and the soln. added dropwise to pentane (70 ml). The resulting residue was collected 290 mg (98%) of **29**. Colorless powder. UV (MeOH): 203 (4.83), 234 (4.37), 266 (4.37). ^{1}H-NMR ((D$_6$)DMSO): 12.23 (br. *s*, COOH); 11.32 (br. *s*, NH); 7.43 – 7.20 (*m*, 9 H of (MeO)$_2$*Tr*, H–C(6)); 6.90 (*d*, 4 H *o* to MeO); 6.18 – 6.12 (*m*, H–C(1′)); 4.17 – 4.15 (*m*, H–C(3′)); 3.95 – 3.73 (*m*, H–C(4′), 2 H–C(5′), 2 MeO); 2.49 – 2.41 (*m*, CH$_2$CH$_2$); 1.78 – 1.62 (*m*, 2 H–C(2′), Me–C(5)). Anal. calc. for $C_{35}H_{31}N_2O_{10} \cdot H_2O$ (657.7): C 63.92, H 5.06, N 4.26; found: C 64.08, H 5.58, N 4.37.

22. *2′-Deoxy-3′-*O*-(4,4′-dimethoxytrityl)-*N^4*-{[2-(4-nitrophenyl)ethoxy]carbonyl}cytidine 5′-(Hydrogen Butanedioate)* (**30**). As described in *Exper. 21*, with **22** (320 mg, 0.44 mmol): 320 mg (89%) of **30**. Colorless powder. UV (MeOH): 202 (4.98), 236 (4.63), 275 (sh, 4.25), 280 (4.25). ^{1}H-NMR ((D$_6$)DMSO): 10.8 (br. *s*, NH); 8.15 (*d*, 2 H *o* to NO$_2$); 7.89 (*d*, H–C(6)); 7.58 (*d*, 2 H *m* to NO$_2$); 7.41 – 7.21 (*m*, 9 H of (MeO)$_2$*Tr*); 6.99 – 6.88 (*m*, H–C(5), 4 H *o* to MeO); 6.13 (*t*, H–C(1′)); 4.33 (*t*, CH$_2$C*H*$_2$OCO); 4.20 – 4.13 (*m*, H–C(3′)); 3.94 – 3.73 (*m*, H–C(4′), 2 H–C(5′), 2 MeO); 3.06 (*t*, C*H*$_2$CH$_2$OCO); 2.41 – 2.39 (*m*, CH$_2$CH$_2$); 1.99 – 1.94 (*m*, 1 H–C(2′)); 1.72 – 1.67 (*m*, 1 H–C(2′)). Anal. calc. for $C_{43}H_{40}N_4O_{13}$ (820.8): C 62.92, H 4.91, N 6.83; found: C 62.54, H 5.15, N 6.81.

23. *2′-Deoxy-3′-*O*-(4,4′-dimethoxytrityl)-*N^6*-{[2-(4-nitrophenyl)ethoxy]carbonyl}adenosine 5′-(Hydrogen Butanedioate)* (**31**). As described in *Exper. 21*, with **23** (260 mg, 0.44 mmol): 320 mg (89%) of **31**. Colorless powder. UV (MeOH): 203 (5.02), 236 (4.51), 266 (4.54). ^{1}H-NMR ((D$_6$)DMSO): 10.50 (br. *s*, NH); 8.57, 8.49 (2*s*, H–C(2), H–C(8)); 8.14 (*d*, 2 H *o* to NO$_2$); 7.59 (*d*, 2 H *m* to NO$_2$); 7.48 – 7.21 (*m*, 9 H of (MeO)$_2$*Tr*); 6.90 (*d*, 4 H *o* to MeO); 6.44 (*m*, H–C(1′)); 4.40 – 4.35 (*m*, CH$_2$C*H*$_2$OCO, H–C(3′)); 4.02 – 3.72 (*m*, H–C(4′), 2 H–C(5′), 2 MeO); 3.09 (*t*, C*H*$_2$CH$_2$OCO); 2.57 – 2.36 (*m*, 1 H–C(2′), CH$_2$CH$_2$); 1.98 – 1.91 (*m*, 1 H–C(2′)). Anal. calc. for $C_{44}H_{42}N_6O_{12}$ (846.9): C 62.40, H 5.00, N 9.92; found: C 62.20, H 5.28, N 9.46.

24. *2′-Deoxy-3′-*O*-(4,4′-dimethoxytrityl)-*N^2*-{[2-(4-nitrophenyl)ethoxy]carbonyl}-*O^6*-[2-(4-nitrophenyl)ethyl]guanosine 5′-(Hydrogen Butanedioate)* (**32**). As described in *Exper. 21*, with **24** (320 mg, 0.35 mmol): 330 mg (93%) of **32**. Colorless powder. UV (MeOH): 202 (5.00), 236 (4.51), 269 (4.55). ^{1}H-NMR ((D$_6$)DMSO): 12.26 (br. *s*, COOH); 10.30 (br. *s*, NH); 8.24 (*s*, H–C(8)); 8.15 (*d*, 4 H *o* to NO$_2$); 7.63 – 7.58 (*m*, 4 H *m* to NO$_2$); 7.46 – 7.20 (*m*, 9 H of (MeO)$_2$*Tr*); 6.89 (*d*, 4 H *o* to MeO); 6.30 (*t*, H–C(1′)); 4.72 (*m*, CH$_2$C*H*$_2$OCO); 4.39 – 4.28 (*m*, CH$_2$C*H*$_2$O of *O*^{6}-npe, H–C(3′)); 4.02 – 3.71 (*m*, 2 H–C(5′), H–C(4′),

2 MeO); 3.26 (*t*, CH_2CH_2OCO); 3.07 (*t*, CH_2CH_2O of O^6-npe); 2.51 – 2.39 (*m*, 1 H–C(2′), CH_2CH_2); 1.86 – 1.78 (*m*, 1 H–C(2′)). Anal. calc. for $C_{52}H_{50}N_7O_{15}$ (1013.2): C 61.65, H 4.98, N 9.68; found: C 61.58, H 4.92, N 9.50.

25. *Solid-Support Material from 500-Å LCAMA-CPG and* **29**–**32**. A mixture of LCAMA-CPG (250–300 mg), 5′-(hydrogen butanedioate) **29** (29 mg, 45 µmol) (or **30** (37 mg, 45 µmol), **31** (38 mg, 45 µmol), or **32** (46 mg, 45 µmol)), TOTU (22 mg, 67 µmol), abs. MeCN (5 ml), and *N*-methylmorpholine (15 ml, 145 µmol) was gently shaken for 2 h. The CPG material was collected in a glass funnel and washed with MeOH, DMF, pyridine, MeOH, acetone, and Et_2O. *Capping procedure:* The nucleoside-functionalized CPG was treated with a mixture of DMAP (50 mg, 0.41 mmol), abs. pyridine (10 ml), and Ac_2O (1 ml, 10.6 mmol) for 45 min at r.t. by gently shaking. Then the nucleoside-bearing, capped solid support was collected, washed with MeOH, DMF, MeOH, acetone, and Et_2O, and dried in a desiccator. *Determination of loading:* A defined amount of the nucleoside-bearing support (*ca.* 5 mg) was treated in a 10-ml calibrated flask (10 ml) with 0.2M TsOH in MeCN (10 ml). After 1 min, the absorbance at 498 nm was measured against 0.2M TsOH in MeCN. The loading L [µmol/g] was calculated by the formula $L = A \cdot 10 \cdot 14.4/m$ (A = absorbance at 498 nm; m = weight of loaded CPG material in mg): $L = 35$ (loaded with **29**), 33 (loaded with **30**), 34 (loaded with **31**) and 29 µmol/g (loaded with **32**).

26. *Assembly of Oligonucleotides.* The syntheses were carried out in an *Applied-Biosystems-392-DNA/RNA* synthesizer. LCAMA-CPG Material loaded with **29**, **30**, **31**, or **32** (see *Exper. 25*) was packed into a small *ABI* column, and cycles of nucleotide addition were carried out by programmed series of reagent and solvent washes based on recommended procedures with the following main steps: *1*) 3′-*O*-$(MeO)_2Tr$ deprotection with 3% CCl_3COOH in CH_2Cl_2 for 135 s; the eluate from this step was collected and the absorbance at 498 nm measured to determine the condensation yields. *2*) Coupling: 0.1M phosphoramidite and 0.5M 1*H*-tetrazole in dry MeCN (or 0.5M pyridinium chloride in dry MeCN), delivered in alternating reagent pushes with a subsequent wait time of 180 s. *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 push with a subsequent wait time of 5 s. *4*) Oxidation: 0.05M I_2 in THF/H_2O/pyridine 7:2:1, delivered in one 10 s push with a subsequent wait time of 15 s.

Then a cleavage program was carried out: *1*) Cleavage of the base-protecting groups; 1M DBU in MeCN delivered in several pushes and following wait steps (total wait time: 11 h). 2) Cleavage from the solid support: conc. NH_3 soln. delivered in one push with a consecutive wait time of 4 × 900 s (total wait time 2 h). The reaction soln. containing only the oligonucleotide and NH_3 was collected and, after determination of the isolated amount of oligonucleotide by measurement of the absorbance at 260 nm, lyophilized in a *Speedvac* concentrator under high vacuum.

27. *Oligonucleotide 3′-Conjugates* **33** *and* **34**. After the oligonucleotide synthesis, the solid support was dried and transferred into a 25-ml flask. Cholesteryl phosphoramidite [31] (52 mg, 88 µmol) or phosphoramidite ester with vitamin E [32] (52 mg, 82 µmol), resp., was dissolved in dry MeCN/CH_2Cl_2 (1.6 ml), and pyridinium chloride (23.8 mg (176 µmol) and 22.7 mg (164 µmol), resp.) in dry MeCN/CH_2Cl_2 (1 ml) was added. The heterogenous mixture was gently shaken for 18 h at r.t. After oxidation with 0.05M I_2 in THF/H_2O/pyridine 7:2:1 (dropwise addition until the soln. remained brownish), the solid support was washed carefully and then transferred back into a synthesis column for deprotection and cleavage from the support as described above.

REFERENCES

[1] H. Cramer, W. Pfleiderer, *Nucleosides Nucleotides Nucleic Acids* **2000**, *19*, in press.

[2] C. Chaix, R. P. Iyer, S. Agrawal, *Bioorg. Med. Chem. Lett.* **1996**, *6*, 827.

[3] M. Koga, A. Wilk, M. F. Moore, C. L. Scremin, L. Zhou, S. L. Beaucage, *J. Org. Chem.* **1995**, *60*, 1520.

[4] J. Bogan, L. Ignatovich, E. Stankevich, *Nucleosides Nucleotides* **1999**, *18*, 1183.

[5] S. P. A. Fodor, J. L. Read, M. C. Pirrung, L. Stryer, A. T. Lu, D. Solas, *Science* (*Washington*, *D.C.*) **1991**, *251*, 767.

[6] A. C. Pease, D. Solas, E. J. Sullivan, M. T. Cronin, C. P. Holmes, S. P. A. Fodor, *Proc. Natl. Acad. Sci. U.S.A.* **1994**, *91*, 5022.

[7] J. D. Hoheisel, *Tibtech* **1997**, *15*, 465.

[8] R. J. Lipshutz, S. P. A. Fodor, T. R. Gingeras, D. J. Lockhart, *Nat. Genet.* **1999**, *21*, 20.

[9] A. Marshall, J. Hodgson, *Nat. Biotechnol.* **1998**, *27.*

[10] S. Singh-Gasson, R. D. Green, Y. Yue, C. Nelson, F. Blattner, M. S. Sussman, F. Cerrina, *Nat. Biotechnol.* **1999**, *17*, 974.

[11] G. Ramsay, *Nat. Biotechnol.* **1998**, *40.*

[12] K. P. Stengele, W. Pfleiderer, *Nucleic Acids Symp. Ser*. **1989**, *21*, 101; K. P. Stengele, W. Pfleiderer, *Tetrahedron Lett*. **1990**, *31*, 2549.

[13] T. Wagner, W. Pfleiderer, *Nucleosides Nucleotides* **1997**, *16*, 1657.

[14] M. Beier, W. Pfleiderer, *Helv. Chim. Acta* **1999**, *82*, 879.

[15] F. Himmelsbach, B. S. Schulz, T. Trichtinger, R. Charubala, W. Pfleiderer, *Tetrahedron* **1984**, *40*, 59.

[16] R. Charubala, W. Pfleiderer, in 'Progress in Molecular and Submolecular Biology', Eds. W. E. G. Müller and H. C. Schröder, Springer-Verlag, Berlin, 1994, Vol. 14, p. 114.

[17] M. Resmini, W. Pfleiderer, *Bioorg. Med. Chem. Lett*. **1994**, *16*, 1909.

[18] H. Schirmeister, W. Pfleiderer, *Helv. Chim. Acta* **1994**, *77*, 10.

[19] M. Wasner, E. E. Henderson, R. J. Suhadolnik, W. Pfleiderer, *Helv. Chim. Acta* **1994**, *77*, 1757.

[20] W. Pfleiderer, S. Matysiak, F. Bergmann, R. Schnell, *Acta Biochim. Polon.* **1996**, *43*, 37.

[21] C. Hörndler, R. J. Suhadolnik, N. F. Muto, E. E. Henderson, M. X. Guan, W. Pfleiderer, *Helv. Chim. Acta* **1997**, *80*, 767.

[22] B. Greiner, W. Pfleiderer, *Helv. Chim. Acta* **1998**, *81*, 1528.

[23] H. Cramer, W. Pfleiderer, *Helv. Chim. Acta* **1999**, *82*, 614.

[24] H. Lang, M. Gottlieb, M. Schwarz, S. Farkas, B. S. Schulz, F. Himmelsbach, R. Charubala, W. Pfleiderer, *Helv. Chim. Acta* **1999**, *82*, 2172.

[25] F. Bergmann, W. Pfleiderer, *Helv. Chim. Acta* **1994**, *77*, 203, 481, 988.

[26] T. Wagner, W. Pfleiderer, *Helv. Chim. Acta* **1997**, *80*, 200.

[27] M. D. Matteucci, M. H. Caruthers, *Tetrahedron Lett*. **1980**, *21*, 719.

[28] S. L. Beaucage, M. H. Caruthers, *Tetrahedron Lett*. **1981**, *22*, 1859.

[29] M. D. Matteucci, M. H. Caruthers, *J. Am. Chem. Soc*. **1981**, *103*, 3185.

[30] T. Brown, D. J. S. Brown, in 'Oligonucleotides and Analogues – A Practical Approach', Ed. F. Eckstein, IRL Press, Oxford, 1991, p. 1.

[31] C. MacKellar, D. Graham, D. W. Will, S. Burgess, T. Brown, *Nucleic Acids Res.* **1992**, *20*, 3411.

[32] D. W. Will, T. Brown, *Tetrahedron Lett*. **1992**, *33*, 2729.

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