

Nucleotides

Part LXV¹⁾

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 occasion 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.

¹⁾ Part LXIV: [1].

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.

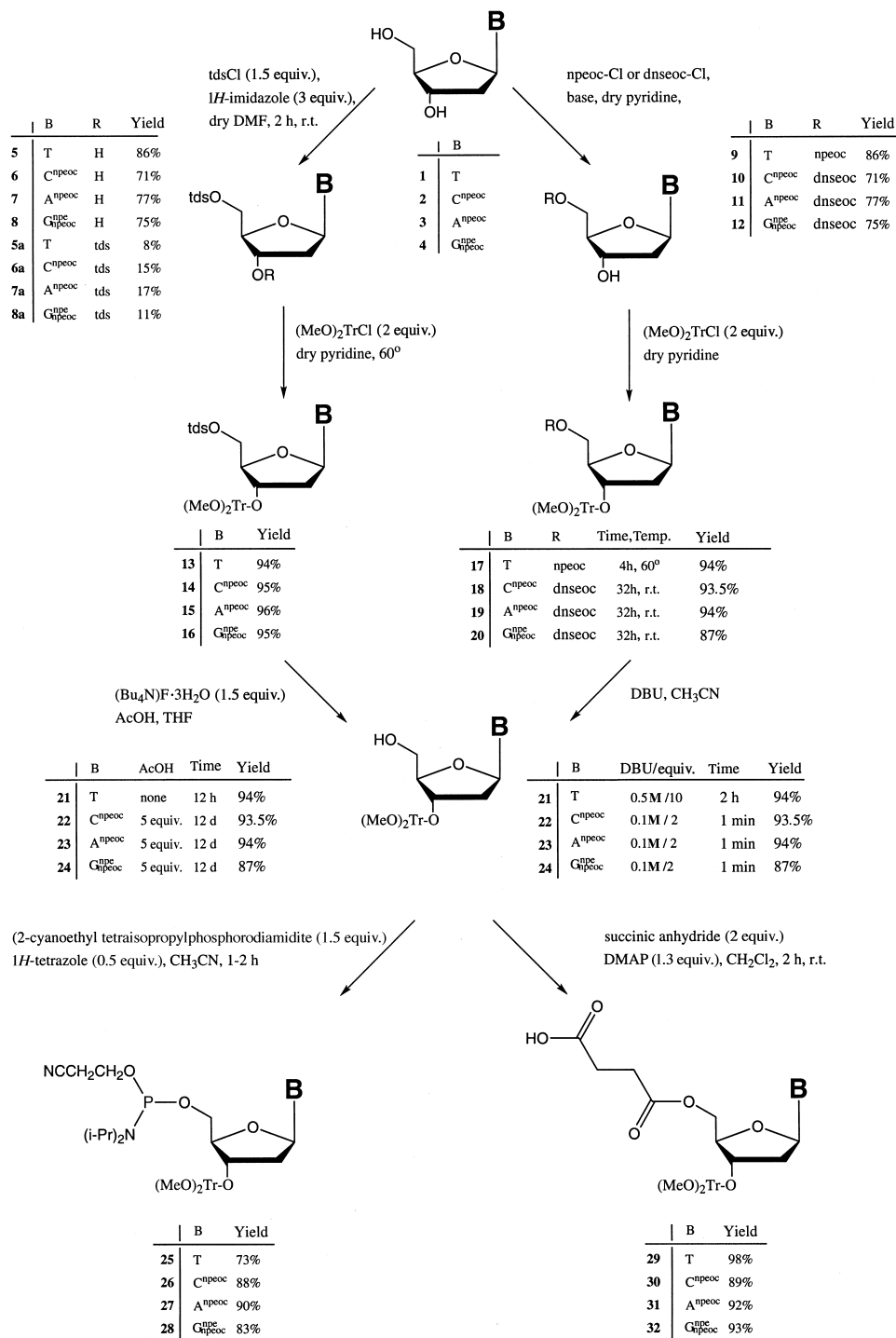
2- Syntheses. – At first, the aglycone functions of the common nucleosides were protected in the usual manner by the 2-(4-nitrophenyl)ethyl (npe) and the [2-(4-nitrophenyl)ethoxy]carbonyl (npeoc) group ($\rightarrow$ **1–4**) [15], which have already been shown to be very efficient for the synthesis 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 separated 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 Ac₂O and *N,N*-dimethylpyridin-4-amine (DMAP) in pyridine leading to supports loaded with 29–35 μ mol/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% CCl₃COOH in CH₂Cl₂ for

Scheme



135 s; 2) coupling with 0.1M phosphoramidite **25–28** and 0.5M 1*H*-tetrazole in MeCN for 180 s; 3) capping with Ac₂O/2,6-dimethylpyridine/1-methyl-1*H*-imidazole in THF for 15 s; 4) Oxidation with 0.05M I₂ in THF/pyridine/H₂O 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*).

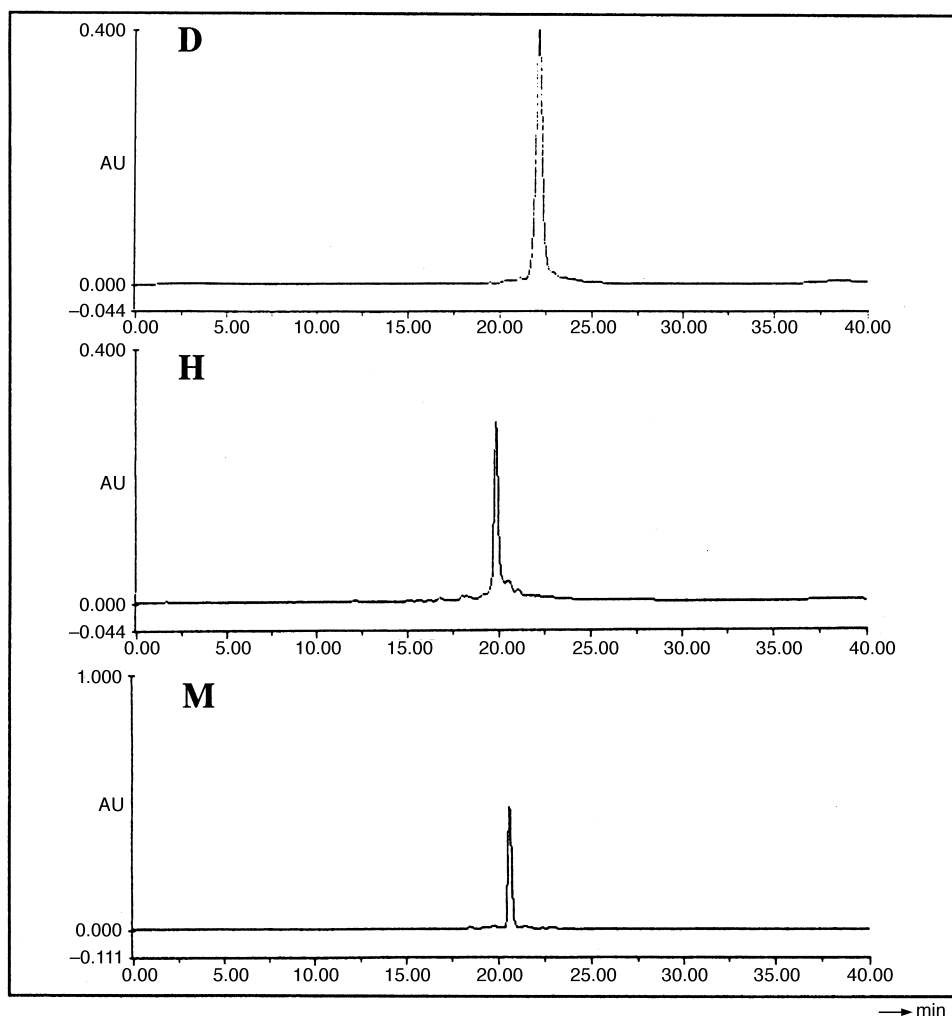


Fig. 1. HPLC Traces of the crude oligo-2'-deoxyribonucleotides **D**, **H**, and **M**. AU = arbitrary unit.

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

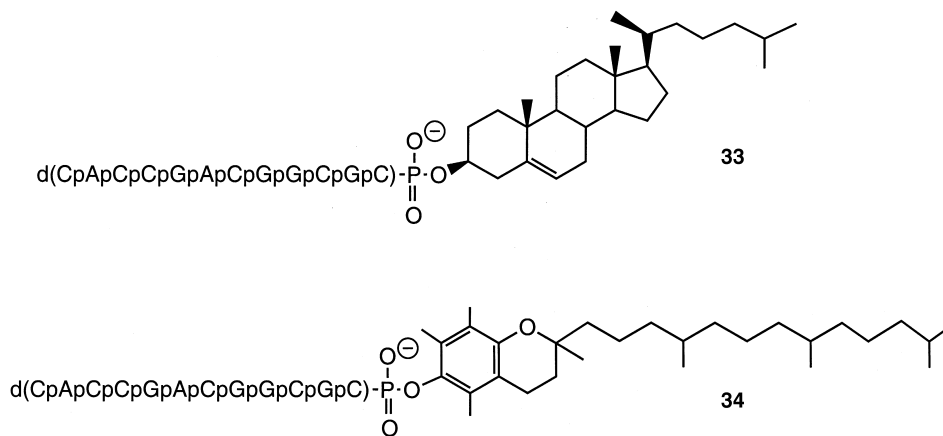
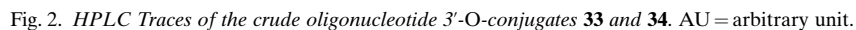
Phosphor- amidite	Oligonucleotide sequence		Coupling time [s]	Average condensation yield [%]	Overall yield [%]	Size [μmol]	Yield in OD units	HPLC quality
25	5'-TTT-TT-3'	(A)	40	96	88	0.24	8.0	1–2
25	5'-TTT-TTT-T-3'	(B)	120	99.9	89	0.16	5.8	1
26	5'-d(CCC-CCC)-3'	(C)	120	95.6	86.7	0.21	12	2
26	5'-d(CCC-CCC-CCC)-3'	(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.



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). $^1\text{H-NMR}$: *Bruker AC 250*; δ in ppm rel. to SiMe_4 or CDCl_3 ($(\text{D}_6)\text{DMSO}$) as internal standard. $^{31}\text{P-NMR}$: *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 H₂O (3 × 150 ml), the org. layer dried (Na₂SO₄) and evaporated, and the residue purified by FC (16 × 3.5 cm, toluene/AcOEt/MeOH 4:1:0 (0.5:1) and 10:10:1 (0.5:1)); 3.4 g (86%) of **5** and 430 mg (8%) of **5a**.

Data of **5**: M.p. 178–180°. UV (MeOH): 266 (4.04). ¹H-NMR ((D₆)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, Me₂CH); 0.85–0.81 (m, 2 Me₂C); 0.094 (s, Me₂Si). Anal. calc. for C₁₈H₃₂N₂O₃Si (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). ¹H-NMR (CDCl₃): 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 Me₂CH); 0.91–0.82 (m, 4 Me₂C); 0.15–0.07 (s, 2 Me₂Si). Anal. calc. for C₂₆H₃₀N₂O₃Si₂ (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]-N⁴-[2-(4-nitrophenyl)ethoxy]carbonyl]cytidine (**6**) and 2'-Deoxy-3',5'-bis-O-[dimethyl(1,1,2-trimethylpropyl)silyl]-N⁴-[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 (0.5:1) and 1:10 (0.5:1)) 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). ¹H-NMR ((D₆)DMSO): 10.8 (br. s, NH); 8.18–8.13 (m, H–C(6), 2 H *o* to NO₂); 7.59 (d, 2 H *m* to NO₂); 6.97 (d, H–C(5)); 6.06 (t, H–C(1')); 5.29 (d, OH–C(3')); 4.34 (t, CH₂CH₂OCO); 4.20–4.12 (m, H–C(3')); 3.90–3.73 (m, H–C(4'), 2 H–C(5')); 3.06 (t, CH₂CH₂OCO); 2.34–2.24 (m, 1 H–C(2')); 2.07–1.92 (m, 1 H–C(2')); 1.58–1.53 (m, Me₂CH); 0.83–0.78 (m, 2 Me₂C); 0.09 (s, Me₂Si). Anal. calc. for C₂₆H₃₈N₄O₈Si (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). ¹H-NMR ((D₆)DMSO): 10.81 (br. s, NH); 8.16–8.10 (m, H–C(6), 2 H *o* to NO₂); 7.58 (d, 2 H *m* to NO₂); 6.98 (d, H–C(5)); 6.06 (t, H–C(1')); 4.37–4.32 (m, CH₂CH₂OCO, H–C(3')); 3.86–3.66 (m, H–C(4'), 2 H–C(5')); 3.06 (t, CH₂CH₂OCO); 2.30–2.22 (m, 1 H–C(2')); 2.14–2.07 (m, 1 H–C(2')); 1.62–1.51 (m, 2 Me₂CH); 0.85–0.77 (m, 4 Me₂C); 0.11–0.01 (m, 2 Me₂Si). Anal. calc. for C₃₄H₅₆N₄O₈Si₂ (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]-N⁶-[2-(4-nitrophenyl)ethoxy]carbonyl]adenosine (**7**) and 2'-Deoxy-3',5'-bis-O-[dimethyl(1,1,2-trimethylpropyl)silyl]-N⁶-[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 (0.5:1) and 1:10 (0.5:1)) 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). ¹H-NMR ((D₆)DMSO): 10.57 (br. s, NH); 8.60, 8.54 (2s, H–C(2), H–C(8)); 8.14 (d, 2 H *o* to NO₂); 7.59 (d, 2 H *m* to NO₂); 6.41 (t, H–C(1')); 5.38 (d, OH–C(3')); 4.40–4.35 (m, H–C(3'), CH₂CH₂OCO); 3.88–3.65 (m, H–C(4'), 2 H–C(5')); 3.09 (t, CH₂CH₂OCO); 2.82–2.76 (m, 1 H–C(2')); 2.38–2.32 (m, 1 H–C(2')); 1.55–1.49 (m, Me₂CH); 0.81–0.76 (m, 2 Me₂C); 0.02 (s, Me₂Si). Anal. calc. for C₂₇H₃₈N₆O₇Si (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). ¹H-NMR (CDCl₃): 8.72 (s, H–C(2)); 8.26–8.13 (m, H–C(8), NH, 2 H *o* to NO₂); 7.45–7.41 (m, 2 H *m* to NO₂); 6.45 (t, H–C(1')); 4.58–4.49 (m, H–C(3'), CH₂CH₂OCO); 4.02–3.98 (m, H–C(4')); 3.86–3.69 (m, 2 H–C(5')); 3.13 (t, CH₂CH₂OCO); 2.64–2.58 (m, 1 H–C(2')); 2.46–2.34 (m, 1 H–C(2')); 1.64–1.57 (m, 2 Me₂CH); 0.89–0.83 (m, 4 Me₂C); 0.11–0.09 (m, 2 Me₂Si). Anal. calc. for C₃₅H₅₆N₆O₇Si₂ (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]-N²-[2-(4-nitrophenyl)ethoxy]carbonyl]-O⁶-[2-(4-nitrophenyl)ethyl]guanosine (**8**) and 2'-Deoxy-3',5'-bis-O-[dimethyl(1,1,2-trimethylpropyl)silyl]-N²-[2-(4-nitrophenyl)ethoxy]carbonyl]-O⁶-[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 (0.5:1) and 1:10 (0.5:1)) 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). ¹H-NMR ((D₆)DMSO): 10.3 (br. s, NH); 8.30 (1s, H–C(8)); 8.17–8.14 (m, 4 H *o* to NO₂); 7.64–7.59 (m, 4 H *m* to NO₂); 6.37 (t, H–C(1')); 5.31 (d, OH–C(3')); 4.74 (t, CH₂CH₂OCO); 4.38–4.33 (m, H–C(3'), CH₂CH₂O of *O*⁶-npe); 3.85–3.63 (m, H–C(4'), 2 H–C(5')); 3.31 (t, CH₂CH₂OCO); 3.09 (t, CH₂CH₂O 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, Me₂CH); 0.79–0.74 (m, 2 Me₂C); 0.04 (s, Me₂Si). Anal. calc. for C₃₅H₄₅N₇O₁₀Si (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). ¹H-NMR (CDCl₃): 8.18–8.06 (m, H–C(8), 4 H *o* to NO₂); 7.49/7.40 (m, 4 H *m* to NO₂); 7.30 (br. s, NH); 6.33 (t, H–C(1')); 4.79 (t, CH₂CH₂OCO); 4.58–4.45 (m, H–C(1'), CH₂O of *O*⁶-npe); 3.96–3.94 (m, H–C(4')); 3.82–3.68 (m, 2 H–C(5')); 3.29 (t, CH₂CH₂OCO); 3.10 (t, CH₂CH₂O 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 Me₂CH); 0.89–0.83 (m, 4 Me₂C), 0.11–0.06 (m, 2 Me₂Si). Anal. calc. for C₄₈H₆₃N₇O₁₀Si₂ (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 CH₂Cl₂ (60 ml) and extracted with sat. NaHCO₃ soln. (3 × 20 ml). The aq. phase was extracted with CH₂Cl₂ (20 ml), the combined org. layer dried (Na₂SO₄), 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). ¹H-NMR (CDCl₃): 8.37 (s, NH); 7.50–7.13 (m, H–C(6), 9 H of (MeO)₂Tr); 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'), Me₂CH); 0.84–0.80 (m, 2 Me₂C); 0.04–0.01 (m, Me₂Si). Anal. calc. for C₃₉H₅₁N₂O₇Si (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]-N⁴[[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 (1.18 g, 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). ¹H-NMR (CDCl₃): 8.17–8.08 (m, 2 H *o* to NO₂, H–C(6)); 7.72 (br. s, NH); 7.72–7.16 (m, H–C(5), 9 H of (MeO)₂Tr, 2 H *m* to NO₂); 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, CH₂CH₂OCO); 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, CH₂CH₂OCO); 2.29–2.17 (m, 1 H–C(2')); 1.68–1.42 (m, 1 H–C(2'), Me₂CH); 0.85–0.70 (m, 2 Me₂C); 0.02 (d, Me₂Si). Anal. calc. for C₄₇H₅₄N₄O₁₀Si (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]-N⁶[[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). ¹H-NMR (CDCl₃): 8.72 (s, NH); 8.33 (s, H–C(2)); 8.16–8.13 (m, 2 H *o* to NO₂, H–C(8)); 7.47–7.16 (m, 9 H of (MeO)₂Tr, 2 H *m* to NO₂); 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, CH₂CH₂OCO); 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, CH₂CH₂OCO); 2.12–1.93 (m, 2 H–C(2')); 1.58–1.42 (m, Me₂CH); 0.76–0.72 (m, 2 Me₂C); 0.01 (d, Me₂Si). Anal. calc. for C₄₈H₅₆N₆O₉Si (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]-N²[[2-(4-nitrophenyl)ethoxy]carbonyl]-O⁶[[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). ¹H-NMR (CDCl₃): 8.21–8.12 (m, 4 H *o* to NO₂); 8.00 (s, H–C(8)); 7.51–7.13 (m, 9 H of (MeO)₂Tr, 4 H *m* to NO₂); 6.85–6.82 (m, 4 H *o* to MeO); 6.44–6.39 (m, H–C(1')); 4.80 (t, CH₂CH₂OCO); 4.46 (t, CH₂CH₂O 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'), CH₂CH₂OCO); 3.14 (t, CH₂CH₂O 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, Me₂CH); 0.78–0.74 (m, 2 Me₂C); 0.09–0.00 (m, Me₂Si). Anal. calc. for C₅₆H₆₃N₇O₁₂Si (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/CH₂Cl₂ 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). ¹H-NMR (CDCl₃): 8.18 (*d*, 2 H *o* to NO₂); 7.46–7.17 (*m*, 2 H *m* to NO₂, 9 H of (MeO)₂Tr, H–C(6)); 6.84 (*d*, 4 H *o* to MeO); 6.38–6.37 (*m*, H–C(1')); 4.39–4.31 (*m*, CH₂CH₂OCO); 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*, CH₂CH₂OCO); 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 C₄₀H₃₉N₃O₁₁ (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]-N⁴-[[2-(4-nitrophenyl)ethoxy]carbonyl]cytidine (**18**). As described in *Exper. 5*, with **10** (1.01 g, 1.39 mmol), dry pyridine/CH₂Cl₂ 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). ¹H-NMR ((D₆)DMSO): 10.8 (br. *s*, NH); 8.45 (*d*, H–C(2)(dns)); 8.19–8.12 (*m*, 2 H *o* to NO₂, H–C(4)(dns), H–C(8)(dns)); 7.72 (*d*, H–C(6)); 7.62–7.56 (*m*, 2 H *m* to NO₂, H–C(3)(dns), H–C(7)(dns)); 7.41–7.12 (*m*, 9 H of (MeO)₂Tr, 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 CH₂CH₂OCO); 4.12–4.10 (*m*, H–C(3')); 3.85–3.65 (*m*, H–C(4'), 2 H–C(5'), 2 MeO, SO₂CH₂); 3.06 (*t*, CH₂CH₂OCO); 2.77 (*s*, Me₂N); 1.98–1.90 (*m*, 1 H–C(2')); 1.64–1.56 (*m*, 1 H–C(2')). Anal. calc. for C₅₄H₅₃N₃O₁₄S (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)-N⁶-[[2-(4-nitrophenyl)ethoxy]carbonyl]adenosine (**19**). As described in *Exper. 5*, with **11** (680 mg, 0.91 mmol), dry pyridine/CH₂Cl₂ 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). ¹H-NMR ((D₆)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 NO₂, H–C(4)(dns), H–C(8)(dns)); 7.63–7.10 (*m*, 2 H *m* to NO₂, H–C(3)(dns), H–C(7)(dns), 9 H of (MeO)₂Tr, H–C(6)(dns)); 6.89 (*d*, 4 H *o* to MeO); 6.44 (*t*, H–C(1')); 4.39–4.22 (*m*, 2 CH₂CH₂OCO, H–C(3')); 3.81–3.71 (*m*, H–C(4'), 2 H–C(5'), 2 MeO, SO₂CH₂); 3.09 (*t*, CH₂CH₂OCO); 2.74 (*s*, Me₂N); 2.58–2.47 (*m*, 1 H–C(2')); 2.05–1.97 (*m*, 1 H–C(2')). Anal. calc. for C₅₅H₅₃N₇O₁₃S (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)-N²-[[2-(4-nitrophenyl)ethoxy]carbonyl]-O⁶-[2-(4-nitrophenyl)ethyl]guanosine (**20**). As described in *Exper. 5*, with **12** (880 mg, 0.96 mmol), dry pyridine/CH₂Cl₂ 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). ¹H-NMR ((D₆)DMSO): 10.31 (br. *s*, NH); 8.43 (*d*, H–C(2)(dns)); 8.19–8.10 (*m*, 4 H *o* to NO₂, H–C(4)(dns), H–C(8)(dns), H–C(8)); 7.63–7.53 (*m*, 4 H *m* to NO₂, H–C(3)(dns), H–C(7)(dns)); 7.44–7.14 (*m*, 9 H of (MeO)₂Tr, H–C(6)(dns)); 6.87 (*d*, 4 H *o* to MeO); 6.31 (*t*, H–C(1')); 4.73 (*t*, CH₂CH₂OCO); 4.31–4.17 (*m*, CH₂CH₂OCO, CH₂CH₂O of O⁶-npe, H–C(3')); 3.87–3.70 (*m*, H–C(4'), 2 H–C(5'), 2 MeO, SO₂CH₂); 3.27 (*t*, CH₂CH₂OCO); 3.06 (*t*, CH₂CH₂O of O⁶-npe); 2.73 (*s*, 2 Me₂N); 2.53–2.48 (*m*, 1 H–C(2')); 1.93–1.87 (*m*, 1 H–C(2')). Anal. calc. for C₆₃H₆₁N₈O₁₆S (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 (Bu₄N)F·H₂O 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 H₂O (3 × 100 ml) and the org. layer dried (Na₂SO₄) and evaporated. The crude product was dissolved in CH₂Cl₂ (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 H₂O (10 ml) and extracted with CH₂Cl₂ (3 × 10 ml), the org. layer washed with sat. NaHCO₃ soln. (2 × 20 ml), the aq. layer extracted back with CH₂Cl₂ (10 ml), the combined org. layer dried (Na₂SO₄) 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). ¹H-NMR ((D₆)DMSO): 11.30 (br. *s*, NH); 7.62 (*s*, H–C(6)); 7.43–7.19 (*m*, 9 H of (MeO)₂Tr); 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 C₃₁H₃₃N₂O₇ (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)-N⁴-[[2-(4-nitrophenyl)ethoxy]carbonyl]cytidine (**22**). 14.1. As described in *Exper. 13.1*, with (Bu₄N)F·H₂O (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). ¹H-NMR ((D₆)DMSO): 10.74 (br. s, NH); 8.17–8.14 (*m*, H–C(6), 2 H *o* to NO₂); 7.57 (*d*, 2 H *m* to NO₂); 7.42–7.12 (*m*, 9 H of (MeO)₂Tr); 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*, CH₂CH₂OCO); 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'), CH₂CH₂OCO); 1.92–1.80 (*m*, 1 H–C(2')); 1.70–1.58 (*m*, 1 H–C(2')). Anal. calc. for C₃₉H₃₆N₄O₁₀ (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)-N⁶-[2-(4-nitrophenyl)ethoxy]carbonyl]adenosine (**23**). 15.1. As described in *Exper. 13.1*, with (Bu₄N)F·H₂O (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/CH₂Cl₂ 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). ¹H-NMR ((D₆)DMSO): 10.60 (br. s, NH); 8.58, 8.54 (2s, H–C(2), H–C(8)); 8.13 (*d*, 2 H *o* to NO₂); 7.59 (*d*, 2 H *m* to NO₂); 7.47–7.12 (*m*, 9 H of (MeO)₂Tr); 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*, CH₂CH₂OCO, 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*, CH₂CH₂OCO); 2.45–2.36 (*m*, 1 H–C(2')); 1.90–1.83 (*m*, 1 H–C(2')). Anal. calc. for C₄₀H₃₈N₆O₉ (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)-N²-[2-(4-nitrophenyl)ethoxy]carbonyl]-O⁶-[2-(4-nitrophenyl)-ethyl]guanosine (**24**). 16.1. As described in *Exper. 13.1*, with (Bu₄N)F·H₂O (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 CH₂Cl₂ (15 ml) and the soln. added dropwise to Et₂O/pentane (500 ml). The resulting precipitate was collected and co-evaporated with CH₂Cl₂: 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). ¹H-NMR ((D₆)DMSO): 10.29 (br. s, NH); 8.47 (s, H–C(8)); 8.14 (*d*, 4 H *o* to NO₂); 7.63–7.58 (*m*, 4 H *m* to NO₂); 7.46–7.20 (*m*, 9 H of (MeO)₂Tr); 6.90 (*d*, 4 H *o* to MeO); 6.35–6.29 (*m*, H–C(1')); 4.81–4.71 (*m*, CH₂CH₂OCO, OH–C(5')); 4.36–4.32 (*m*, CH₂CH₂O of O⁶-npe, H–C(3')); 3.83–3.72 (*m*, H–C(4'), 2 MeO); 3.37–3.22 (*m*, 2 H–C(5'), CH₂CH₂OCO); 3.08 (*t*, CH₂CH₂O of O⁶-npe); 2.40–2.32 (*m*, 1 H–C(2')); 1.79–1.72 (*m*, 1 H–C(2')). Anal. calc. for C₄₈H₄₅N₇O₁₂ (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 1H-tetrazole (110 mg, 1.5 mmol) was stirred in abs. MeCN (3 ml) under N₂ for 1 h. After dilution with CH₂Cl₂ (20 ml), the soln. was washed with phosphate buffer (pH 7; 2 × 10 ml), the aq. phase extracted with CH₂Cl₂ (2 × 10 ml), the combined org. layer dried (Na₂SO₄) and evaporated, and the crude foam purified by FC (20 × 2.5 cm, toluene/AcOEt (1% Et₃N) 4:1 (0.3 l) and 2:1 (0.3 l)). The product fractions were co-evaporated with CH₂Cl₂: 1.63 g (73%) of **25**. Colorless foam. UV (MeOH): 203 (4.85), 235 (4.35), 268 (4.09). ¹H-NMR ((D₆)DMSO): 11.30 (br. s, NH); 7.42–7.12 (*m*, 9 H of (MeO)₂Tr, 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 Me₂CH, CH₂CH₂OP, 2 H–C(5')); 2.72–2.60 (*m*, CH₂CH₂OP); 1.71–1.62 (*m*, Me–C(5), 2 H–C(2')); 1.10–0.93 (*m*, 2 Me₂CH). ³¹P-NMR ((D₆)DMSO): 148.86, 148.26. Anal. calc. for C₄₀H₄₉N₄O₈P (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)-N⁴-[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), 1H-tetrazole (33 mg, 0.5 mmol), and MeCN (3 ml). Purification by FC (14 × 2.5 cm, AcOEt (1% Et₃N) (0.1 l)) gave 810 mg (88%) of **26**. Colorless foam. UV (MeOH): 202 (4.99), 236 (4.58), 280 (4.25). ¹H-NMR ((D₆)DMSO): 10.73 (br. s, NH); 8.13 (*d*, 2 H *o* to NO₂); 7.98–7.94 (*m*, H–C(6)); 7.57 (*d*, 2 H *m* to NO₂); 7.42–7.14 (*m*, 9 H of (MeO)₂Tr); 6.96–6.88 (*m*, 4 H *o* to MeO, H–C(5)); 6.14–6.09 (*m*, H–C(1')); 4.33 (*t*, CH₂CH₂OCO); 4.18–4.16 (*m*, H–C(3')); 3.97–3.87 (*m*, H–C(4')); 3.72–3.39 (*m*, 2 MeO, 2 Me₂CH, CH₂CH₂OP, 2 H–C(5')); 3.05 (*t*, CH₂CH₂OCO); 2.71–2.60

(*m*, CH₂CH₂OP); 2.12–1.91 (*m*, 1H–C(2')); 1.64–1.48 (*m*, 1H–C(2')); 1.19–0.92 (*m*, 2 Me₂CH). ³¹P-NMR ((D₆)DMSO): 148.74, 148.29. Anal. calc. for C₄₈H₅₅N₆O₁₁P (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)-N⁶-[[2-(4-nitrophenyl)ethoxy]carbonyl]adenosine 5'-(2-Cyanoethyl Diisopropylphosphoramidite) (**27**). As described in *Exper. 17*, **23** (700 mg, 0.95 mmol), 2-cyanoethyl tetraisopropylphosphorodiamidite (430 mg, 1.43 mmol), 1H-tetrazole (32 mg, 0.48 mmol), and MeCN (3 ml). Purification by FC (14 × 2.5 cm, AcOEt (1% Et₃N) (0.1 l)) gave 800 mg (90%) of **27**. Colorless foam. UV (MeOH): 204 (4.96), 237 (4.44), 267 (4.48). ¹H-NMR ((D₆)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 NO₂); 7.46 (*d*, 2 H *m* to NO₂); 7.35–7.16 (*m*, 9 H of (MeO)₂Tr); 6.91 (*m*, 4 H *o* to MeO); 6.50–6.41 (*m*, H–C(1')); 4.39–4.35 (*m*, CH₂CH₂OCO, H–C(3')); 4.05–3.96 (*m*, H–C(4')); 3.72–3.38 (*m*, 2 MeO, 2 Me₂CH, CH₂CH₂OP, 2 H–C(5')); 3.08 (*t*, CH₂CH₂OCO); 2.71–2.62 (*m*, CH₂CH₂OP); 2.38–2.28 (*m*, 1 H–C(2')); 2.11–1.94 (*m*, 1 H–C(2')); 1.21–0.84 (*m*, 2 Me₂CH). ³¹P-NMR ((D₆)DMSO): 148.29, 148.12. Anal. calc. for C₄₈H₅₅N₆O₁₁P (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)-N²-[[2-(4-nitrophenyl)ethoxy]carbonyl]-O⁶-[2-(4-nitrophenyl)-ethyl]guanosine 5'-(2-Cyanoethyl Diisopropylphosphoramidite) (**28**). As described in *Exper. 17*, with **24** (810 mg, 0.96 mmol), 2-cyanoethyl tetraisopropylphosphorodiamidite (430 mg, 1.43 mmol), 1H-tetrazole (32 mg, 0.48 mmol), and MeCN (3 ml). Purification by FC (14 × 2.5 cm, AcOEt (1% Et₃N) (0.1 l)) gave 810 mg (83%) of **28**. Colorless foam. UV (MeOH): 202 (5.02), 237 (4.51), 269 (4.56). ¹H-NMR ((D₆)DMSO): 10.29 (br. s, NH); 8.21–8.13 (*m*, H–C(8)), 4 H *o* to NO₂; 7.63–7.53 (*m*, 4 H *m* to NO₂); 7.46–7.18 (*m*, 9 H of (MeO)₂Tr); 6.89 (*m*, 4 H *o* to MeO); 6.39–6.29 (*m*, H–C(1')); 4.73 (*t*, CH₂CH₂OCO); 4.34–4.29 (*m*, CH₂CH₂O of O⁶-npe, H–C(3')); 3.98–3.80 (*m*, H–C(4')); 3.71–3.24 (*m*, 2 MeO, 2 Me₂CH, CH₂CH₂OP, 2 H–C(5'), CH₂CH₂OCO); 3.07 (*t*, CH₂CH₂ of O⁶-npe); 2.71–2.60 (*m*, CH₂CH₂OP); 2.36–2.28 (*m*, 1 H–C(2')); 2.05–1.86 (*m*, 1 H–C(2')); 1.21–0.82 (*m*, 2 Me₂CH). ³¹P-NMR ((D₆)DMSO): 148.35. Anal. calc. for C₅₇H₆₂N₆O₁₃P (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 CH₂Cl₂ (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 CH₂Cl₂ (20 ml) and washed with 10% citric acid (2 × 20 ml) and NaCl soln. (2 × 20 ml). The aq. phase was extracted with CH₂Cl₂ (2 × 10 ml), the combined org. layer dried (Na₂SO₄) and evaporated, the resulting foam dissolved in CH₂Cl₂ (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). ¹H-NMR ((D₆)DMSO): 12.23 (br. s, COOH); 11.32 (br. s, NH); 7.43–7.20 (*m*, 9 H of (MeO)₂Tr, 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*, CH₂CH₂); 1.78–1.62 (*m*, 2 H–C(2'), Me–C(5')). Anal. calc. for C₃₅H₃₁N₂O₁₀ · H₂O (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)-N⁴-[[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). ¹H-NMR ((D₆)DMSO): 10.8 (br. s, NH); 8.15 (*d*, 2 H *o* to NO₂); 7.89 (*d*, H–C(6)); 7.58 (*d*, 2 H *m* to NO₂); 7.41–7.21 (*m*, 9 H of (MeO)₂Tr); 6.99–6.88 (*m*, H–C(5), 4 H *o* to MeO); 6.13 (*t*, H–C(1')); 4.33 (*t*, CH₂CH₂OCO); 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*, CH₂CH₂OCO); 2.41–2.39 (*m*, CH₂CH₂); 1.99–1.94 (*m*, 1 H–C(2')); 1.72–1.67 (*m*, 1 H–C(2')). Anal. calc. for C₄₃H₄₀N₄O₁₃ (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)-N⁶-[[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). ¹H-NMR ((D₆)DMSO): 10.50 (br. s, NH); 8.57, 8.49 (2s, H–C(2), H–C(8)); 8.14 (*d*, 2 H *o* to NO₂); 7.59 (*d*, 2 H *m* to NO₂); 7.48–7.21 (*m*, 9 H of (MeO)₂Tr); 6.90 (*d*, 4 H *o* to MeO); 6.44 (*m*, H–C(1')); 4.40–4.35 (*m*, CH₂CH₂OCO, H–C(3')); 4.02–3.72 (*m*, H–C(4'), 2 H–C(5'), 2 MeO); 3.09 (*t*, CH₂CH₂OCO); 2.57–2.36 (*m*, 1 H–C(2'), CH₂CH₂); 1.98–1.91 (*m*, 1 H–C(2')). Anal. calc. for C₄₄H₄₂N₆O₁₂ (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)-N²-[[2-(4-nitrophenyl)ethoxy]carbonyl]-O⁶-[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). ¹H-NMR ((D₆)DMSO): 12.26 (br. s, COOH); 10.30 (br. s, NH); 8.24 (s, H–C(8)); 8.15 (*d*, 4 H *o* to NO₂); 7.63–7.58 (*m*, 4 H *m* to NO₂); 7.46–7.20 (*m*, 9 H of (MeO)₂Tr); 6.89 (*d*, 4 H *o* to MeO); 6.30 (*t*, H–C(1')); 4.72 (*m*, CH₂CH₂OCO); 4.39–4.28 (*m*, CH₂CH₂O of O⁶-npe, H–C(3')); 4.02–3.71 (*m*, 2 H–C(5'), H–C(4'),

2 MeO); 3.26 (*t*, CH₂CH₂OCO); 3.07 (*t*, CH₂CH₂O of *O*⁶-npe); 2.51–2.39 (*m*, 1 H–C(2'), CH₂CH₂); 1.86–1.78 (*m*, 1 H–C(2')). Anal. calc. for C₅₂H₅₀N₇O₁₅ (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 Et₂O. *Capping procedure*: The nucleoside-functionalized CPG was treated with a mixture of DMAP (50 mg, 0.41 mmol), abs. pyridine (10 ml), and Ac₂O (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 Et₂O, 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% CCl₃COOH in CH₂Cl₂ 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: Ac₂O/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 I₂ in THF/H₂O/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. NH₃ 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 NH₃ 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/CH₂Cl₂ (1.6 ml), and pyridinium chloride (23.8 mg (176 μmol) and 22.7 mg (164 μmol), resp.) in dry MeCN/CH₂Cl₂ (1 ml) was added. The heterogenous mixture was gently shaken for 18 h at r.t. After oxidation with 0.05M I₂ in THF/H₂O/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.

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