Nucleotides

Part LXXV[1])

New Types of Fluorescence Labeling of 2′-Deoxycytidine

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The reactivity of the 2′-deoxy-N^4-(phenoxycarbonyl)cytidine derivatives **3** and **4** with aromatic amines was studied to form new types of urea derivatives (see **5**–**10**). On the same basis, labeling of **3** and **4** with 5-aminofluorescein (**14**) was achieved to give the conjugates **15** and **17**, respectively (*Scheme 1*). Treatment of **17** with 2-(4-nitrophenyl)ethanol in a *Mitsunobu* reaction led to double protection of the fluorescein moiety (→**18**) and desilylation yielded **19**. Dimethoxytritylation (→**20**) and subsequent phosphitylations afforded the new building blocks **21** and **22**. Synthesis of the fully protected trimer **28** was achieved by condensation of **21** with **23** to **26** which after detritylation (→**27**) was coupled with **25** to give **28** (*Scheme 2*). Deprotection of all blocking groups was performed with 1,8-diazabicyclo[5.4.0]undec-7-ene (DBU) in one step to give **29**. The synthesis of the decamer 5′-d(C^{Flu}CCG GCC CGC)-3′ (**33**) started from **30** which was attached to the solid support and then elongated with **31**, **32**, and **22** at the 5′-terminal end (C^{Flu} = deprotected phosphate derivative of **22**). Hybridization with the complementary oligomer 5′-d(G GGC CGG GCG)-3′ (**34**) showed the influence of the fluorescein label on the stability of the duplex.

1. Introduction. – Radioactive labeling of nucleotides and oligonucleotides [2][3] has recently been more and more replaced by fluorescent or biochemical markers [4–6], especially in research areas where a high degree of automatization is applied. In these cases, the chromophore is attached at the oligonucleotide chain by a flexible hydrocarbon linker of several atoms length at the 3′- or 5′-end of an oligonucledotide [7–10], at the phosphate-ribose backbone [11–15], or at the nucleobase [16–18]. Modified nucleosides are able to bind covalently a fluorophore after completion of the oligonucleotide synthesis. Examples are N^4-(aminoalkyl)-2′-deoxycytidine [19][20], 2′-deoxy-5-{(1*E*)-3-oxo-3-{[6-(trifluoroacetyl)amino]hexyl}amino}prop-1-en-1-yl}uridine [21][22], 5-(3-aminopropyl)-2′-deoxyuridine [23] and 8-[(aminoalkyl)amino]-2′-deoxyadenosine [24–26]. The N^6-position of 2′-deoxyadenosine was also modified to incorporate a (6-trifluoroacetylaminohexyl)amino spacer [27], and the ribose moiety was converted into a 5′-amino- [14][23] and 5′-mercapto derivative [28], respectively. Our intention was the direct coupling of the highly efficient fluorophor fluorescein onto the amino functions of all nucleobase moieties by a carbamoyl linker [29], and here we describe our efforts with 2′-deoxycytidine. The activated phenyl carbamate intermediates react easily with 5-aminofluorescein (= 5-amino-3′,6′-dihydroxyspiro[isobenzofuran-1(3*H*),9′-[9*H*]xanthen-3-one) to the corresponding dye conjugate. Applications in

[1]) Part LXXIV: [1].

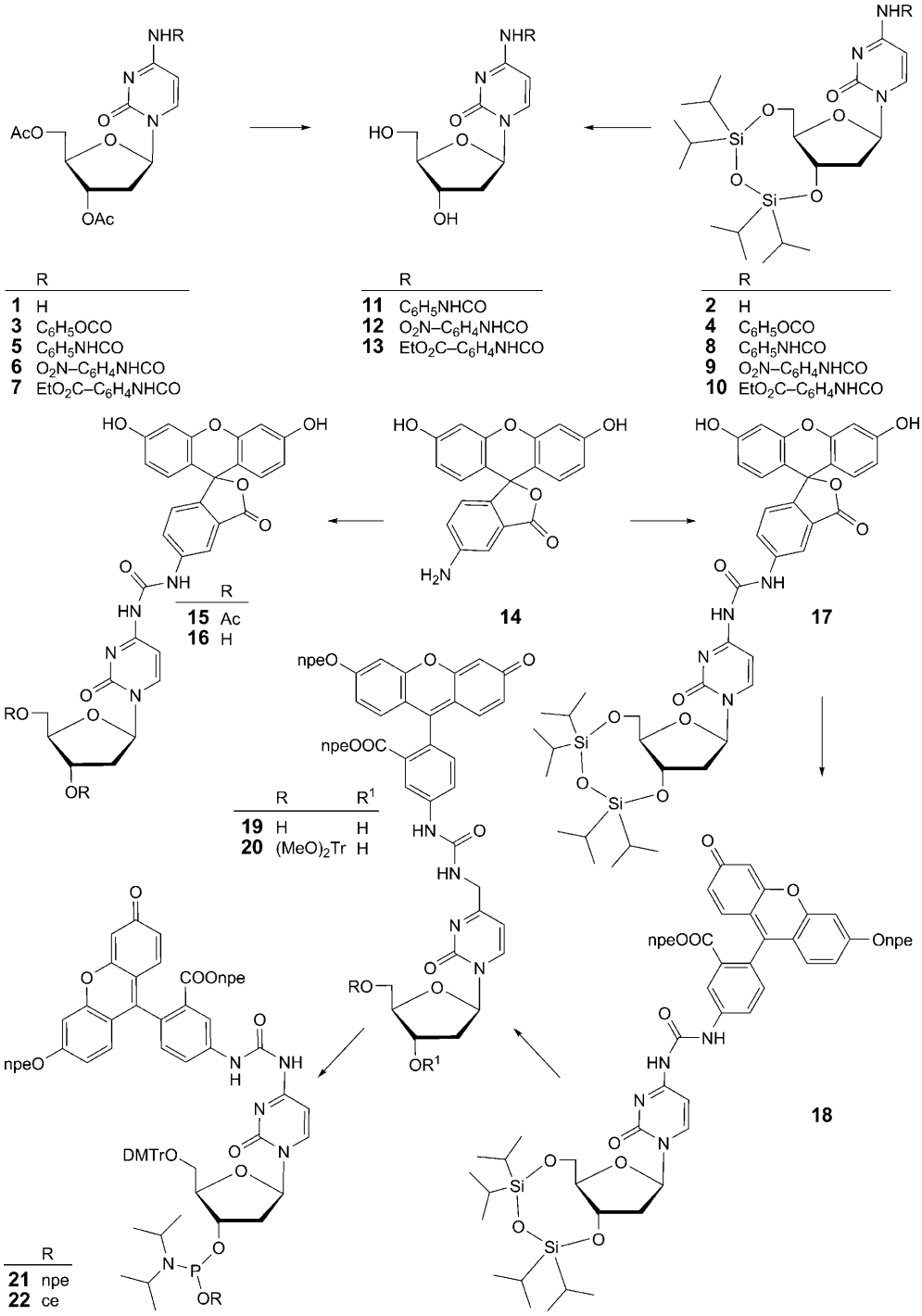
oligonucleotide syntheses, however, needs further protection of the two acidic functionalities of the fluorescein moiety which could be achieved by use of the 2-(4-nitrophenyl)ethyl (npe) and 2-(4-nitrophenylethoxy)carbonyl (npeoc) blocking group strategy [30].

2. Syntheses. – Analogously to the adenosine strategy [16], 3′,5′-di-*O*-acetylcytidine (**1**) [31] and 2′-deoxy-3′,5′-*O*-(tetraisopropyldisiloxane-1,3-diyl)-cytidine (**2**) [32] were converted into the corresponding N^4-(phenoxycarbonyl) derivatives **3** and **4** by treatment with phenyl 1*H*-tetrazole-1-carboxylate (*Scheme 1*). Model reactions of **3** and **4** with aromatic amines such as aniline, 4-nitroaniline, and ethyl 4-aminobenzoate proceeded well at 70° to give **5**–**10** in good yields. Treatment of **5**–**7** or **8**–**10** with ammonia or tetrabutylammonium fluoride (Bu_4NF), respectively, afforded deprotection at the sugar moiety yielding **11**–**13**. Reaction of **1** and **2** with 5-aminofluorescein (**14**) [2] led in excellent yields to the 2′-deoxy-N^4-[(fluorescein-5-ylamino)carbonyl]-cytidines **15** and **17**, respectively. Deacetylation of **15** by ammonia and K_2CO_3 gave **16** in 68% yield. To protect the fluorescein moiety for further reactions, the *Mitsunobu* reaction with 2-(4-nitrophenyl)ethanol, triphenylphosphine, and diethyl azodicarboxylate (=diethyl diazene-1,2-dicarboxylate) was performed and led under twofold substitution to **18** in excellent yield. Cleavage of the silyl protecting group with Bu_4NF in THF/AcOH afforded **19** which was converted into the oligonucleotide building block **22** first by reaction with dimethoxytrityl chloride to **20** and then by phosphitylation with 2-(4-nitrophenyl)ethyl *N*,*N*,*N*′,*N*′-tetraisopropylphosphorodiamidite giving **21** and 2-cyanoethyl *N*,*N*,*N*′,*N*′-tetraisopropylphosphorodiamidite to yield **22**.

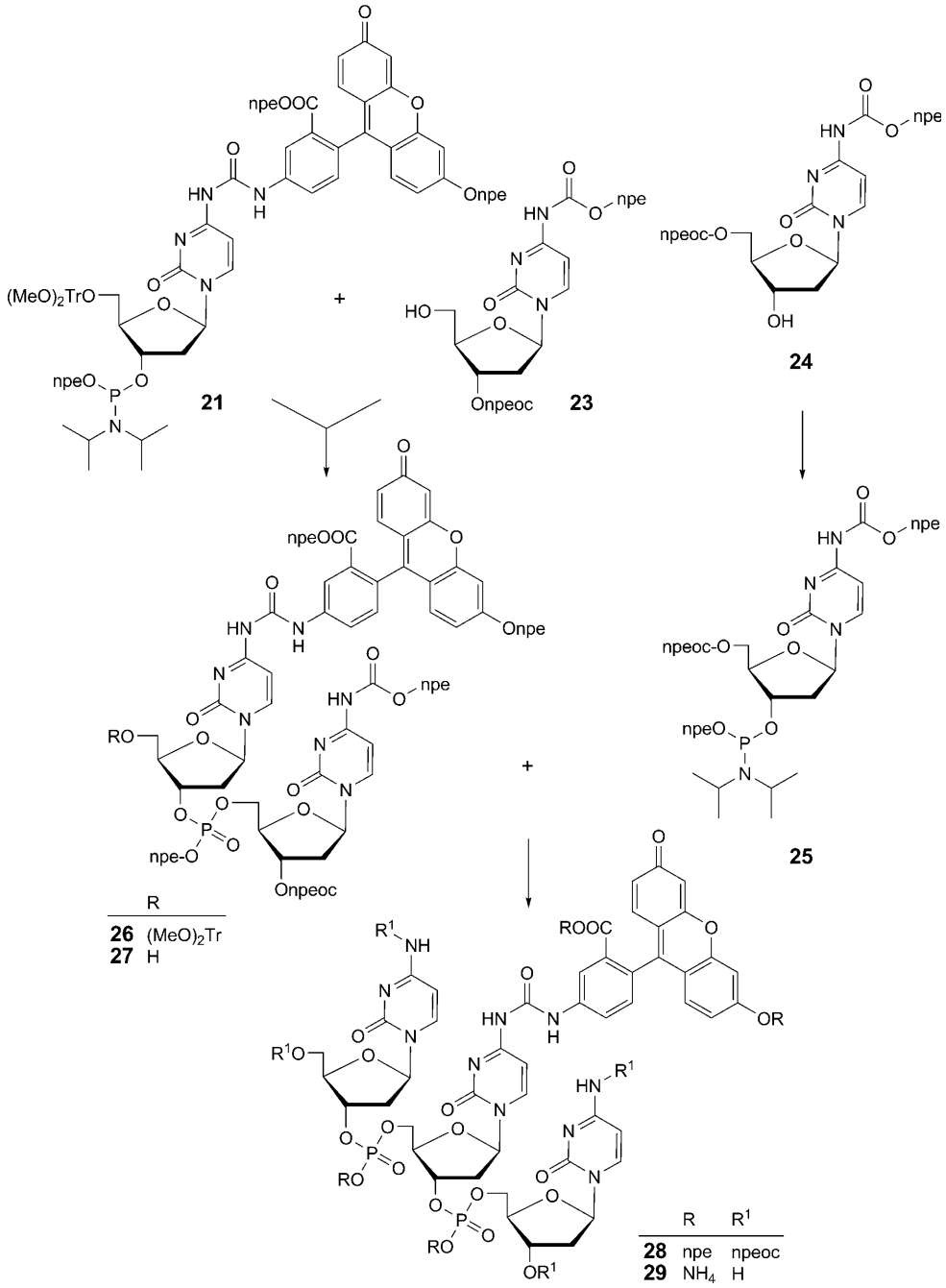
For the synthesis of the fully protected 2′-deoxycytidine trimer **28** (*Scheme 2*) carrying as a universal pattern the npe and npeoc blocking groups at all functionalities, 2′-deoxy-N^4,3′-*O*-bis{[2-(4-nitrophenyl)ethoxy]carbonyl}cytidine (**23**) [33] and 2′-deoxy-N^4,5′-*O*-bis{[2-(4-nitrophenyl)ethoxy]carbonyl}cytidine 3′-[2-(4-nitrophenyl)ethyl *N*,*N*-diisopropylphosphoramidite] (**25**) derived from **24** [33] were prepared. In the first step of the oligonucleotide synthesis, the phosphoramidite **21** was treated with **23** under tetrazole activation and subsequent oxidation to give the dimer **26**. Detritylation of **26** led to **27** which was then coupled with **25** to achieve the fully protected trimer **28** in 83% yield. The total deprotection of **28** was achieved by 1,8-diazabicyclo[5.4.0]undec-7-ene (DBU) in pyridine in one step, and workup proceeded by DEAE-*Sephadex* chromatography with triethylammonium hydrogen carbonate buffer followed by conversion of the resulting triethylammonium salt into the ammonium salt **29**. Its purity was established by reversed-phase HPLC (buffer pH 9/MeOH/H_2O 1:1:2) yielding one peak with a retention time of 16.8 min and detection either at 260 nm or 480 nm. Cleavage of **29** by snake-venom phosphodiesterase (SVP) furnished a mixture which showed in the HPLC, expectedly, only two peaks of 2′-deoxycytidine and **16**.

The labeled 5′-d(C^{Flu}CCG GCC CGC)-3′ (**33**) decamer was prepared by automated solid-support synthesis on glyceryl-CPG (500 Å) which was activated with phenyl 1*H*-tetrazole-1-carboxylate and subsequently loaded with the N^1,N^6-dimethylhexane-1,6-diamine spacer [34], followed by reaction with 2′-deoxy-5′-*O*-(4,4′-dimethoxytrityl)-N^4-{[2-(4-nitrophenyl)ethoxy]carbonyl}cytidine 3′-succinate (**30**). The next cycles for the built-up of the oligonucleotide chain were performed with 2′-deoxy-5′-*O*-(4,4′-dimethoxytrityl)-N^2-{[2-(4-nitrophenyl)ethoxy]carbonyl}-O^6-[2-(4-nitrophenyl)ethyl]-

Scheme 1


NHR
AcO
OAc
HO
OH
Si
R
1 H
3 C6H5OCO
5 C6H5NHCO
6 O2N–C6H4NHCO
7 EtO2C–C6H4NHCO
11 C6H5NHCO
12 O2N–C6H4NHCO
13 EtO2C–C6H4NHCO
2 H
4 C6H5OCO
8 C6H5NHCO
9 O2N–C6H4NHCO
10 EtO2C–C6H4NHCO
NH
HN
RO
OR
15 Ac
16 H
14
17
npeO
npeOOC
R R1
19 H H
20 (MeO)2Tr H
OR1
COOnpe
Onpe
18
DMTrO
N P O
21 npe
22 ce

Scheme 2



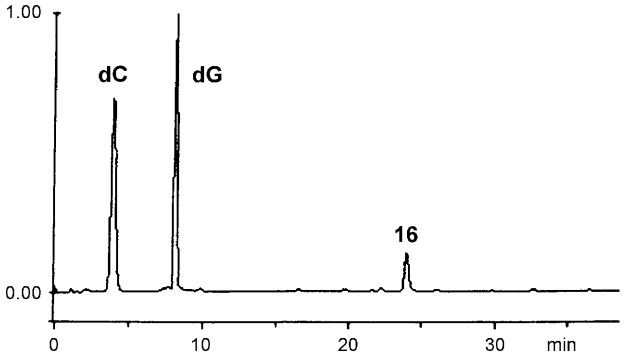
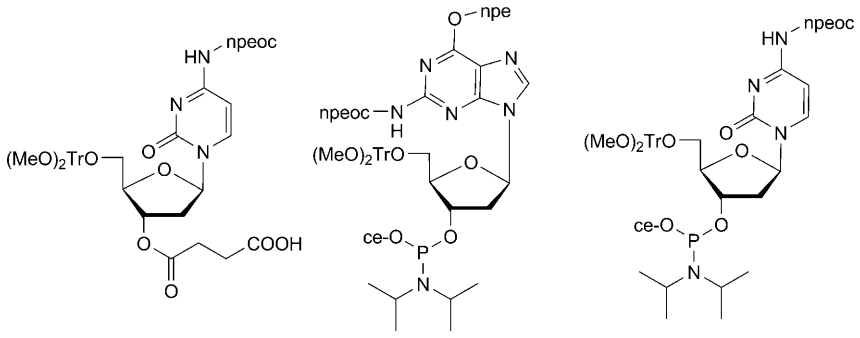


Fig. 1. *HPLC of the mixture obtained after enzymatic-hydrolysis of* **33** *by SVP*

guanosine 3′-(2-cyanoethyl *N*,*N*-diisopropylphosphoramidite) (**31**) [35], 2′-deoxy-5′-*O*-(4,4′-dimethoxytrityl)-N^4-{[2-(4-nitrophenyl)ethoxy]carbonyl}cytidine 3′-(2-cyanoethyl *N*,*N*-diisopropylphosphoramidite) (**32**) [35] and with **22** as the last building block. Deprotection was achieved first with $CHCl_2COOH$ to remove the dimethoxytrityl group, then with DBU to cleave the ce, npe, and npeoc groups, and finally by washing with ammonia to detach the oligomer from the solid support. Enzymatic degradation by snake venom diesterase (SVP) showed in HPLC the three expected peaks of dC, dG, and **16** (*Fig. 1*). The complementary 5′-d(G GGC CGG GCG)-3′ (**34**) and unmasked 5′-d(C CCG GCC CGC)-3′ (**35**) decamers were analogously assembled and obtained after deprotection. The HPLC trace of **33** and **35** showed only one peak, thus establishing these very efficient and clean syntheses (*Fig. 2*).



Hybridization experiments in sodium phosphate buffer solution (pH 7.4) between **33** and **34** showed a T_m of 57–58°, whereas the T_m of the combination **34**·**35** was slightly higher (60–61°; *Table*).

3. Structures. – The newly synthesized 2′-deoxycytidine derivatives were characterized and their structures established by elemental analyses, ^{1}H-NMR and UV spectra.

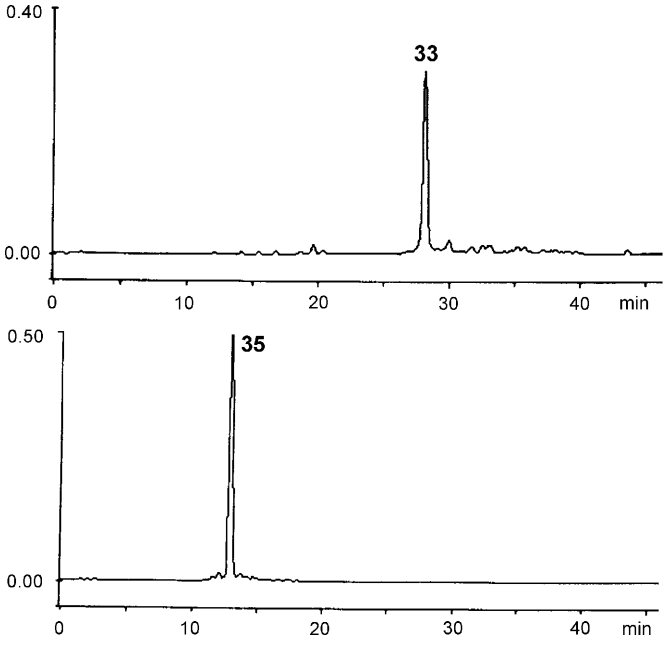


Fig. 2. *HPLC of 5′-d(C^{Flu}CCG GCC CGC)-3′* (**33**) *and 5′-d(G GGC CGG GCG)-3′* (**35**)

Table. *Oligodeoxynucleotide Sequences and* T_m *of Hybridizations*[a])

Sequence	Hybrid	T_m [°]
5′-d(C^{Flu} CCG GCC CGC)-3′ (**33**)		
	33·**34**	56–57
5′-d(G GGC CGG GCG)-3′ (**34**)		
	34·**35**	60–61
5′-d(C CCG GCC CGC)-3′ (**35**)		

[a]) Salt concentration: 0.12M (Na^+).

Experimental Part

General. Products were dried under high vacuum. All solvents used were of anh. grade. DNA Synthesizer from *Applied Biosystems*, model 392. TLC: precoated silica gel thin-layer sheets *60 F254* from *Merck*. Flash Chromatography (FC): silica gel (SiO_2, 30–60 μm; *Baker*); 0.2–0.3 bar. CC = Column chromatography. HPLC: pump *L 6200*, autosampler *AS 4000*, UV detector *L 4000*, *Merck-Hitachi*; column *Lichrosorb RP 18*, (125 × 4 mm, 5 μm; *Merck*); elution: *A* = 0.1M buffer (pH 9) (2 min); *B* = 0.1M buffer (pH 9)/MeOH/H_2O 1:1:2 (20 min); *C* = MeOH (20 min); flow rate 1 ml/min. Ion-exchange CC: *DEAE-Sephadex A25*, from *Pharmacia Fine Chemicals*. M.p.: *Gallenkamp* melting-point apparatus; no corrections. UV/VIS Spectra: *Perkin-Elmer Lambda 5*; λ_{max} in nm (log ε). ^{1}H-NMR Spectra: *Bruker AC 250*; δ in ppm rel. to Me_4Si or $CDCl_3$ ((D_6)DMSO) as internal standard. ^{31}P-NMR Spectra: *Jeol JMN-GX400*.

3′,5′-Di-O-*acetyl-2′-deoxy*-N^4-*(phenoxycarbonyl)cytidine* (= *Phenyl {1-{4-(Acetyloxy)-5-[(acetyloxy)methyl]tetrahydrofuran-2-yl}-1,2-dihydro-2-oxopyrimidin-4-yl}carbamate*; **3**). To a soln. of 3′,5′-di-

O-acetyl-2′-deoxycytidine (**1**) [31] (6.0 g, 20 mmol) in dioxane (100 ml) was added phenyl 1*H*-tetrazole-1-carboxylate [36] (5.0 g. 26 mmol), and the mixture was stirred at 40° for 1 h. The soln. was concentrated, the residue suspended in cold abs. $CHCl_3$ (50 ml) and kept at 0° for 2 h. The tetrazole was filtered off and washed with little cold $CHCl_3$, and then the filtrate again concentrated. The residue was purified by CC (SiO_2 (3 × 25 cm), toluene (200 ml), toluene/acetone 9:1 (200 ml), toluene/acetone 7:3 (200 ml), and toluene/acetone 1:1 (200 ml)). The residue of the main fraction was dissolved in CH_2Cl_2 and the soln. concentrated: 8.0 g (92%) of **3**. Solid foam. R_f 0.48 (toluene/acetone 1:1). UV (CH_2Cl_2): 240 (4.19), 300 (3.87), 310 (sh, 3.76). ^{1}H-NMR ((D$_6$)DMSO): 11.30 (br. *s*, NH); 8.11 (*d*, H–C(6)); 7.44 ('*t*', 2 arom. H); 7.30–7.15 (*m*, 3 arom. H); 6.98 (*d*, H–C(5)); 6.11 (*dd*, H–C(1′)); 5.18 (*m*, H–C(3′)); 4.28–4.20 (*m*, H–C(4′), CH_2(5′)); 2.54–2.46 (*m*, 1 H–C(2′)); 2.40–2.26 (*m*, 1 H–C(2′)); 2.05 (*s*, 2 AcO). Anal. calc. for $C_{20}H_{21}N_3O_8$ (431.4): C 55.68, H 4.91, N 9.73; found: C 55.11, H 4.96, N 9.54.

*2′-Deoxy-3′,5′-*O*-(1,1,3,3-tetraisopropyldisiloxane-1,3-diyl)cytidine* (= *Phenyl* N*-[1,2-Dihydro-2-oxo-1-(tetrahydro-2,2,4,4-tetraisopropyl-6*H*-furo[3,2-f]-1,3,5,2,4-trioxadisilocin-8-yl)pyrimidin-4-yl]carbamate*; **4**). As described for **3**, with 2′-deoxy-3′,5′-di-*O*-1,1,3,3-(tetraisopropyldisiloxane-1,3-diyl)cytidine (**2**) [32] (4.7 g, 10 mmol) and phenyl 1*H*-tetrazole-1-carboxylate (2.5 g, 13 mmol) in dry dioxane (100 ml). After purification by CC (SiO_2 (4.7 × 13 cm), toluene (400 ml), toluene/acetone 95:5 (200 ml), toluene/acetone 9:1 (400 ml), and toluene/acetone 7:3 (400 ml)), the residue of the main fraction was dissolved in CH_2Cl_2 and the soln. concentrated: 5.52 g (93%) of **4**. Solid foam. UV (CH_2Cl_2): 238 (4.18), 301 (3.90), 309 (sh, 3.81). ^{1}H-NMR ((D$_6$)DMSO): 11.29 (br. *s*, NH); 8.07 (*d*, H–C(6)); 7.46 ('*t*', 2 arom. H); 7.30–7.10 (*m*, 3 arom. H); 6.97 (*d*, H–C(5)); 5.99 (*dd*, H–C(1′)); 4.41 (*m*, H–C(3′)); 4.10 (*dd*, 1 H–C(5′)); 3.01 (*dd*, 1 H–C(5′)); 3.80 (*m*, H–C(4′)); 2.50–2.28 (*m*, CH_2(2′)); 1.05–0.90 (*m*, 4 Me_2CH). Anal. calc. for $C_{28}H_{43}N_3O_7Si_2$ (589.8): C 57.02, H 7.34, N 7.12; found: C 56.57, H 7.30, N 7.19.

*3′,5′-Di-*O*-acetyl-2′-deoxy-*N^4*-[(phenylamino)carbonyl]cytidine* (= N*-{1-{4-(Acetyloxy)-5-[(acetyloxy)methyl]tetrahydrofuran-2-yl}-1,2-dihydro-2-oxo-pyrimidin-4-yl}-*N′*-phenylurea*; **5**). A soln. of **3** (1.0 g, 2.3 mmol) in abs. pyridine (20 ml) was treated with aniline (0.34 g, 3.6 mmol) at 70° for 3 h. The mixture was concentrated, 3 × the residue dissolved in toluene and the soln. concentrated, and the residue recrystallized from EtOH: 0.7 g (71%) of **5**. Colorless crystals. M.p. 176–177°. UV (MeOH): 214 (sh, 4.19), 282 (4.17), 293 (4.25). ^{1}H-NMR ((D$_6$)DMSO): 11.20 (br. *s*, NH); 11.02 (br. *s*, NH); 7.95 (*d*, H–C(6)); 7.75 (*m*, 3 arom. H); 7.37 (*m*, 2 arom. H); 7.10 (*d*, H–C(5)); 6.30 (*dd*, H–C(1′)); 5.22 (*m*, H–C(3′)); 4.37 (*m*, H–C(4′), CH_2(5′)); 2.79–2.70 (*m*, 1 H–C(2′)); 2.20–2.11 (*m*, 1 H–C(2′)); 2.09 (*s*, 2 AcO). Anal. calc. for $C_{20}H_{22}N_4O_7$ (430.4): C 55.81, H 5.15, N 13.01; found: C 55.39, H 5.13, N 12.85.

*3′,5′-Di-*O*-acetyl-2′-deoxy-*N^4*-{[(4-nitrophenyl)amino]carbonyl}cytidine* (= N*-{1-{4-(Acetyloxy)-5-[(acetyloxy)methyl]tetrahydrofuran-2-yl]-1,2-dihydro-2-oxopyrimidin-4-yl}-*N′*-(4-nitrophenyl)urea*; **6**). As described for **5**, with **3** (0.6 g, 1.4 mmol) and 4-nitroaniline (0.23 g, 1.67 mmol). The residue was washed with EtOH, filtered, and recrystallized from DMF (2 ml): 0.2 g (35%) of **6**. Yellowish crystals. M.p. 249° (dec.). UV (MeOH): 214 (sh, 4.29), 236 (4.07), 315 (4.39). ^{1}H-NMR ((D$_6$)DMSO): 11.80 (br. *s*, NH); 11.42 (br. *s*, NH); 8.24 (*d*, 2 arom. H); 8.08 (*d*, H–C(6)); 7.71 (*d*, 2 arom. H); 6.48 (*d*, H–C(5)); 6.11 (*dd*, H–C(1′)); 5.19 (*m*, H–C(3′)); 4.25 (*m*, H–C(4′), CH_2(5′)); 2.50–2.30 (*m*, CH_2(2′)); 2.04 (*s*, 2 AcO). Anal. calc. for $C_{20}H_{21}N_5O_9$ (475.4): C 50.57, H 4.45, N 14.73; found: C 50.16, H 4.51, N 14.73.

*3′,5′-Di-*O*-acetyl-2′-deoxy-*N^4*-{[[4-(ethoxycarbonyl)phenyl]amino}carbonyl}cytidine* (= *Ethyl 4-{3-{1-{4-(Acetyloxy)-5-[(acetyloxy)methyl]tetrahydrofuran-2-yl}-1,2-dihydro-2-oxopyrimidin-4-yl}ureido}-benzoate*; **7**). To a soln. of **3** (1.0 g, 2.3 mmol) in abs. pyridine (20 ml) at 70° was added dropwise a soln. of ethyl 4-benzoate (0.38 g, 2,3 mmol) in pyridine (15 ml). After heating for 2 h, the mixture was concentrated, 3 × the residue dissolved in toluene and the soln. concentrated, and the residue recrystallized from EtOH: 0.8 g (70%) of **7**. Colorless crystals. M.p. 216°. UV (MeOH): 210 (sh, 4.36), 244 (4.13), 297 (4.50). ^{1}H-NMR ((D$_6$)DMSO): 11.31 (br. *s*, NH); 11.23 (br. *s*, NH); 8.00 (*m*, H–C(6), 2 arom. H); 7.79–7.67 (*m*, H–C(5), 2 arom. H); 6.28 (*dd*, H–C(1′)); 5.22 (*m*, H–C(3′)); 4.42–4.30 (*m*, H–C(4′), CH_2(5′), CH_2); 2.47–2.30 (*m*, 1 H–C(2′)); 2.21–2.11 (*m*, 1 H–C(2′)); 2.09 (*s*, 2 AcO); 1.38 (*t*, Me). Anal. calc. for $C_{23}H_{26}N_4O_7$ (502.5): C 54.98, H 5.22, N 11.15; found: C 55.07, H 5.25, N 11.20.

*2′-Deoxy-*N^4*-[(phenylamino)carbonyl]-3′,5′-*O*-(1,1,3,3-tetraisopropyldisiloxane-1,3-diyl)cytidine* (= N*-[1,2-Dihydro-2-oxo-1-(tetrahydro-2,2,4,4-tetraisopropyl-6*H*-furo[3,2-*f*]-1,3,5,2,4-trioxadisilocin-8-yl)pyrimidin-4-yl]-*N′*-phenylurea*; **8**). As described for **5**, with **4** (1.0 g, 2.3 mmol), aniline (0.34 g, 3.6 mmol), and abs. pyridine (20 ml): 0.7 g (71%) of **8**. Colorless crystals. M.p. 200–201°. UV (MeOH):

214 (sh, 4.27), 228 (sh, 4,21), 294 (4.26). ^{1}H-NMR ($CDCl_3$): 11.5 (br. *s*, NH); 11.3 (br. *s*, NH); 8.18 (*d*, H–C(6)); 7.78 (*m*, 3 arom. H); 7.31 (*m*, 2 arom. H); 7.08 (*d*, H–C(5)); 6.14 (*dd*, H–C(1′)); 4.44 (*m*, H–C(3′)); 4.21 (*m*, 1 H–C(5′)); 4.06 (*m*, 1 H–C(5′)); 3.83 (*m*, H–C(4′)); 2.50–2.28 (*m*, CH_2(2′)); 0.96 (*m*, 4 Me_2CH). Anal. calc. for $C_{28}H_{44}N_4O_6Si_2 \cdot 0.5\ H_2O$ (593.3): C 56.67, H 7.55, N 9.43; found: C 56.49, H 7.47, N 9.36.

*2′-Deoxy-*N^4*-{[(4-nitrophenyl)amino]carbonyl}-3′,5′-*O*-(1,1,3,3-tetraisopropyldisiloxane-1,3-diyl)cytidine* (= N-*[1,2-Dihydro-2-oxo-1-(tetrahydro-2,2,4,4-tetraisopropyl-6*H*-furo[3,2-*f*]-1,3,5,2,4-trioxadisilocin-8-yl)pyrimidin-4-yl]-*N′*-(4-nitrophenyl)urea*; **9**). As described for **5**, with **4** (0.2 g, 0.34 mmol), 4-nitroaniline (30 mg, 0.2 mmol), and abs. pyridine (10 ml): 55 mg (88%) of **9**. Colorless crystals. M.p. 235° (dec.). UV (MeOH): 214 (4.23), 236 (sh, 4.06), 314 (4.40). ^{1}H-NMR (($CDCl_3$): 11.65 (br. *s*, NH); 10.35 (br. *s*, NH); 8.15 (*d*, 2 arom. H); 7.90 (*d*, H–C(6)); 7.56 (*d*, 2 arom. H); 7.32 (*d*, H–C(5)); 5.93 (*dd*, H–C(1′)); 4.31 (*m*, H–C(3′)); 3.96 (*m*, 1 H–C(5′)); 3.83 (*m*, 1 H–C(5′)); 3.67 (*m*, H–C(4′)); 2.48–2.28 (*m*, CH_2(2′)); 1.00–0.83 (*m*, 4 Me_2CH). Anal. calc. for $C_{28}H_{43}N_5O_8Si_2$ (633.9): C 53.06, H 6.84, N 11.05; found: C 52.55, H 6.82, N 10.93.

*2′-Deoxy-*N^4*-{{[4-(ethoxycarbonyl)phenyl]amino}carbonyl}-3′,5′-*O*-(1,1,3,3-tetraisopropyldisiloxane-1,3-diyl)cytidine* (= *Ethyl 4-{3-[1,2-Dihydro-2-oxo-1-(tetrahydro-2,2,4,4-tetraisopropyl-6*H*-furo[3,2-*f*]-1,3,5,2,4-trioxadisilocin-8-yl]pyrimidin-4-yl]ureido}benzoate*; **10**). As described for **5**, with **4** (1.0 g, 2.3 mmol), ethyl 4-aminobenzoate (0.38 g, 2.3 mmol), and pyridine (20 ml): 0.8 g (70%) of **10**. Colorless crystals. M.p. 243° (dec.). UV (MeOH): 203 (4.37), 211 (4.37), 244 (4.12), 297 (4.51). ^{1}H-NMR ($CDCl_3$): 11.4 (br. *s*, NH); 11.3 (br. *s*, NH); 8.23 (*d*, H–C(6)); 8.00 (*d*, 2 arom. H); 7.78 (*d*, 2 arom. H); 7.61 (*d*, H–C(5)); 6.15 (*dd*, H–C(1′)); 4.35 (*m*, H–C(3′), CH_2); 4.23 (*m*, 1 H–C(5′)); 4.05 (*m*, 1 H–C(5′)); 3.86 (*m*, H–C(4′)); 2.60–2.42 (*m*, 1 H–C(2′)); 2.40–2.25 (*m*, 1 H–C(2′)); 1.39 (*t*, Me); 1.10–0.90 (*m*, 4 Me_2CH). Anal. calc. for $C_{31}H_{48}N_4O_8Si_2 \cdot 0.5\ H_2O$ (669.9): C 55.58, H 7.37, N 8.37; found: C 55.55, H 7.33, N 8.59.

*2′-Deoxy-*N^4*-[(phenylamino)carbonyl]cytidine* (= N-*{1,2-Dihydro-2-oxo-1-[tetrahydro-4-hydroxy-5-(hydroxymethyl)furan-2-yl]pyrimidin-4-yl}-*N′*-phenylurea*; **11**). *a*) A soln. of **5** (0.55 g, 1.2 mmol) in sat. methanolic ammonia (15 ml) was stirred overnight. After evaporation, the residue was recrystallized from EtOH: 0.3 g (73%) of **11**. Colorless crystals. M.p. 175°.

b) A soln. of **8** (0.9 g, 1.5 mmol) and $Bu_4NF \cdot 3\ H_2O$ (1.2 g, 3.8 mmol) in THF (20 ml) was stirred at r.t. for 15 min. The solvent was evaporated, the residue treated with little H_2O, and then the precipitate collected. Recrystallization from EtOH gave 0.34 g (65%) of **11**. Colorless crystals. M.p. 175°. UV (MeOH): 214 (sh, 4.26), 227 (sh, 4.19), 294 (4.24). ^{1}H-NMR ((D_6)DMSO): 11.30 (br. *s*, NH); 10.20 (br. *s*, NH); 8.23 (*d*, H–C(6)); 7.46 (*m*, 2 arom. H); 7.34 (*m*, 1 arom. H); 7.05 (*t*, 1 arom. H); 6.41 (*d*, H–C(5)); 6.13 (*dd*, H–C(1′)); 5.28 (*d*, OH–C(3′)); 5.05 (*t*, OH–C(5′)); 4.22 (*m*, H–C(3′)); 3.86 (*m*, H–C(4′)); 3.69–3.51 (*m*, CH_2(5′)); 2.30–2.20 (*m*, 1 H–C(2′)); 2.09–1.98 (*m*, 1 H–C(2′)). Anal. calc. for $C_{16}H_{18}N_4O_5$ (346.3): C 55.49, H 5.24, N 16.18; found: C 55.62, H 5.34, N 16.12.

*2′-Deoxy-*N^4*-{[(4-nitrophenyl)amino]carbonyl}cytidine* (= N-*{1,2-Dihydro-2-oxo-1-[tetrahydro-4-hydroxy-5-(hydroxymethyl)furan-2-yl]pyrimidin-4-yl}-*N′*-(4-nitrophenyl)urea*; **12**). *a*) A suspension of **6** (0.08 g, 0.17 mmol) in sat. methanolic ammonia (10 ml) was stirred overnight. The solvent was evaporated and the residue treated with EtOH to give, after drying 0.05 g (73%) of **12**. Yellowish solid. M.p. 205–206° (dec.).

b) As described for **11** (*Exper. b*), with **9** (0.4 g, 0.6 mmol), $Bu_4NF \cdot 3\ H_2O$ (0.5 g, 1.5 mmol), and THF (20 ml) for 20 min: 0.13 g (55%) of **12**. Yellowish crystals. M.p. 205–206° (dec.). UV (MeOH): 214 (sh, 4.29), 236 (sh, 4.07), 316 (4.39). ^{1}H-NMR ((D_6)DMSO): 11.87 (br. *s*, NH); 10.34 (br. *s*, NH); 8.27 (*m*, 2 arom. H, H–C(6)); 7.73 (*d*, 2 arom. H); 6.44 (*d*, H–C(5)); 6.11 (*dd*, H–C(1′)); 5.31 (*d*, OH–C(3′)); 5.09 (*dd*, OH–C(5′)); 4.23 (*m*, H–C(3′)); 3.88 (*m*, H–C(4′)); 3.66–3.55 (*m*, CH_2(5′)); 2.31–2.24 (*m*, 1 H–C(2′)); 2.05–1.98 (*m*, 1 H–C(2′)). Anal. calc. for $C_{16}H_{17}N_5O_7 \cdot 0.5\ H_2O$ (400.3): C 48.05, H 4.54, N 17.48; found: C 48.19, H 4.53, N 17.20.

*2′-Deoxy-*N^4*-{{[4-(ethoxycarbonyl)phenyl]amino}carbonyl}cytidine* (= *Ethyl 4-{3-{1,2-Dihydro-2-oxo-1-[tetrahydro-4-hydroxy-5-(hydroxymethyl)furan-2-yl]pyrimidin-4-yl}ureido}benzoate*; **13**). *a*) As described for **12** (*Exper. a*), with **7** (0.7 g, 1.4 mmol) in sat. methanolic ammonia (10 ml): 0.45 g (78%) of **13**. Colorless solid. M.p. 169° (dec.).

b) As described for **11** (*Exper. b*), with **10** (2.0 g, 3 mmol), $Bu_4NF \cdot 3\,H_2O$ (2.37 g, 7.5 mmol), and THF (20 ml) for 30 min (treatment with little H_2O and EtOH): 0.95 g (76%) of **13**. Colorless crystals. M.p. 169° (dec.). UV (MeOH): 203 (4.36), 212 (4.37), 244 (4.11), 297 (4.50). ^{1}H-NMR ((D_6)DMSO): 11.7 (br. *s*, NH); 10.3 (br. *s*, NH); 8.28 (*d*, H–C(6)); 7.93 (*d*, 2 arom. H); 7.59 (*d*, 2 arom. H); 6.43 (*d*, H–C(5)); 6.12 (*dd*, H–C(1′)); 5.30 (*d*, OH–C(3′)); 5.06 (*dd*, OH–C(5′)); 4.35–4.21 (*m*, H–C(3′), CH_2); 3.88 (*m*, H–C(4′)); 3.31–3.18 (*m*, CH_2(5′)); 2.31–2.18 (*m*, 1 H–C(2′)); 2.10–1.99 (*m*, 1 H–C(2′)); 1.28 (*t*, Me). Anal. calc. for $C_{19}H_{22}N_4O_7$ (418.4): C 54.54, H 5.30, N 13.39; found: C 53.95, H 5.30, N 13.29.

3′,5′-Di-O*-acetyl-2′-deoxy*-N^4*-[(fluorescein-5-ylamino)carbonyl]cytidine* (= *3′,5′-Di*-O*-acetyl-2′-deoxy*-N^4*-{[(3′,6′-dihydroxy-3-oxospiro[isobenzofuran-1(3*H*),9′-[9*H*]xanthen]-5-yl)amino]carbonyl}cytidine*; **15**). As described for **5**, with 5-aminofluorescein (**14**; 0.33 g, 0.9 mmol), **3** (0.6 g, 1.5 mmol), and pyridine (50 ml). Workup with toluene (3 × 50 ml) and purification by CC (SiO_2 (5 × 20 cm), CH_2Cl_2/MeOH 9.5 : 0.5 (150 ml), CH_2Cl_2/MeOH 9 : 1 (150 ml), and CH_2Cl_2/MeOH 7 : 3 (1 l)). The main fraction was concentrated, and the residue treated with EtOH, washed with Et_2O, and dried in a vacuum desiccator: 0.6 g (93%) of **15**. Orange solid. UV (MeOH): 230 (4.64), 291 (4.36), 358 (sh, 3.23), 458 (sh, 3.82), 489 (3.92). ^{1}H-NMR ((D_6)DMSO): 11.52 (br. *s*, NH); 10.15 (br. *s*, NH, 2 OH); 8.18 (*d*, H–C(4_{iso})); 8.00 (*d*, H–C(6)); 7.70 (*dd*, H–C(6_{iso})); 7.21 (*d*, H–C(7_{iso})); 6.65–6.45 (*m*, H–C(5), 6 arom. H (xan)); 6.18 (*dd*, H–C(1′)); 5.22 (*m*, H–C(3′)); 4.27 (*m*, H–C(4′), CH_2(5′)); 2.50–2.30 (*m*, CH_2(2′)); 2.10 (*s*, 2 AcO). Anal. calc. for $C_{34}H_{28}N_4O_{12} \cdot H_2O$ (702.6): C 58.12, H 4.30, N 7.97; found: C 58.34, H 4.34, N 7.95.

2′-Deoxy-N^4*-[(fluorescein-5-ylamino)carbonyl]cytidine* (= *2′-Deoxy*-N^4*-{[(3′,6′-dihydroxy-3-oxospiro[isobenzofuran-1(3*H*),9′-[9*H*]xanthen]-5-yl)amino]carbonyl}cytidine*; **16**). A soln. of **15** (0.45 g, 0.64 mmol) in sat. methanolic ammonia was stirred at r.t. for 15 h. Then K_2CO_3 (0.1 mmol) was added, and stirring continued for another 24 h. The solvent was evaporated, the residue dissolved in phosphate buffer (pH 7; 20 ml) followed by AcOH, and the resulting precipitate washed with little H_2O, EtOH, and Et_2O, and dried: 0.25 g (68%) of **16**. Orange solid. UV (MeOH): 210 (4.70), 238 (4.73), 289 (4.49), 347 (sh, 3.87), 467 (sh, 4.51), 4.92 (4.91). ^{1}H-NMR ((D_6)DMSO): 11.73 (br. *s*, NH); 10.35 (br. *s*, NH, 2 OH); 8.26 (*d*, H–C(4_{iso})); 8.18 (*d*, H–C(6)); 7.65 (*dd*, H–C(6_{iso})); 7.21 (*d*, H–C(7_{iso})); 6.65–6.45 (*m*, H–C(5), 6 arom. H (xan)); 6.13 (*dd*, H–C(1′)); 5.31 (*d*, OH–C(3′)); 5.15 (*t*, OH–C(5′)); 4.22 (*m*, H–C(3′)); 3.85 (*m*, H–C(4′)); 3.59 (*m*, CH_2(5′)); 2.30 (*m*, 1 H–C(2′)); 2.08 (*m*, 1 H–C(2′)). Anal. calc. for $C_{30}H_{24}N_4O_{10} \cdot H_2O$ (618.6): C 58.25, H 4.23, N 9.05; found: C 57.82, H 4.44, N 8.87.

2′-Deoxy-N^4*-[(fluorescein-5-ylamino)carbonyl]-3′,5′*-O*-(1,1,3,3-tetraisopropyldisiloxane-1,3-diyl)cytidine* (= *2′-Deoxy*-N^4*-{[(3′,6′-dihydroxy-3-oxospiro[isobenzofuran-1(3*H*),9′-[9*H*]xanthen]-5-yl)amino]carbonyl}-3′,5′*-O*-(1,1,3,3-tetraisopropyldisiloxane-1,3-diyl)cytidine*; **17**). As described for **5**, with **4** (7.0 g, 12 mmol), **14** (2.6 g, 7.5 mmol), and pyridine (100 ml). Workup with toluene (3 × 80 ml) and purification of the oil by CC (SiO_2 (3 × 30 cm), toluene/AcOEt 1 : 1 (300 ml), toluene/AcOEt/MeOH 4.75 : 4.75 : 1 (600 ml), and toluene/AcOEt/MeOH 4.5 : 4.5 : 1 (600 ml)). The main fraction was concentrated to 50 ml and the suspension kept overnight. The precipitate was dried under high vacuum: 5.8 g (92%) of **17**. Orange solid. UV (MeOH/CH_2Cl_2 1 : 1): 219 (4.42), 231 (4.65), 291 (4.39), 371 (sh, 3.19), 455 (sh, 3.86), 485 (4.93). ^{1}H-NMR ((D_6)DMSO): 11.69 (br. *s*, NH); 10.08 (br. *s*, NH, 2 OH); 8.21 (*d*, H–C(4_{iso})); 8.03 (*d*, H–C(6)); 7.72 (*dd*, H–C(6_{iso}))); 7.22 (*d*, H–C(7_{iso})); 6.65–6.40 (*m*, H–C(5), 6 arom. H (xan)); 6.03 (*dd*, H–C(1′)); 4.51 (*m*, H–C(3′)); 4.12–3.98 (*m*, CH_2(5′)); 3.83 (*m*, H–C(4′)); 2.39 (*m*, CH_2(2′)); 1.10–0.95 (*m*, 4 Me_2CH). Anal. calc. for $C_{42}H_{50}N_4O_{11}Si_2 \cdot H_2O$ (861.1): C 58.58, H 6.09, N 6.50; found: C 58.74, H 6.08, N 6.62.

2′-Deoxy-N^4*-{{{3-{[2-(4-nitrophenyl)ethoxy]carbonyl}-4-{6-[2-(4-nitrophenyl)ethoxy]-3-oxo-3*H*-xanthen-9-yl}-phenyl}amino}carbonyl}-3′,5′*-O*-(1,1,3,3-tetraisopropyldisiloxane-1,3-diyl)cytidine* (**18**). To a mixture of **17** (4.7 g, 5.5 mmol), 2-(4-nitrophenyl)ethanol (4.6 g, 28 mmol), and diethyl azodicarboxylate (1.93 g, 11 mmol) in abs. dioxane (100 ml) was added dropwise at 60° PPh_3 in abs. dioxane (5 ml), and the mixture was stirred for 1 h. The mixture was concentrated, the residue dissolved in CH_2Cl_2, the soln. washed with 1% KH_2PO_4 soln. (2 × 50 ml) and H_2O (2 × 50 ml), dried (Na_2SO_4), and concentrated, and the residue purified by CC (SiO_2 (6 × 30 cm), CH_2Cl_2 (100 ml), CH_2Cl_2/MeOH 195 : 5 (600 ml), CH_2Cl_2/MeOH 95 : 5 (400 ml), CH_2Cl_2/MeOH 94 : 6 (800 ml), and CH_2Cl_2/MeOH 93 : 7 (400 ml)). The residue of the product fraction was treated with MeOH (80 ml) and stirred for 1 h, and the precipitate collected: 5.2 g (84%) of **18**. Orange-red solid. UV(MeOH/CH_2Cl_2 1 : 1): 225 (4.75), 232 (4.76), 270 (sh,

4.68), 276 (4.69), 294 (sh, 4.60), 308 (sh, 4.45), 355 (4.03), 406 (sh, 3.98), 434 (sh, 4.29), 458 (4.44), 487 (4.32). ^{1}H-NMR ((D_6)DMSO): 11.63 (br. *s*, NH); 10.22 (br. *s*, NH); 8.24 (*d*, H–C($2_{arom.}$)); 8.16 (*d*, 2 arom. H *o* to NO_2); 8.03 (*d*, 2 arom. H *o* to NO_2, H–C(6)); 7.89 (*dd*, H–C($6_{arom.}$)); 7.63 (*d*, 2 arom. H *m* to NO_2); 7.36 (*d*, H–C($5_{arom.}$), 2 arom. H *m* to NO_2); 7.15 (*d*, H–C(xan); 6.88–6.79 (*m*, 3 H–C(xan)); 6.48 (*d*, H–C(5)): 6.34–6.14 (*m*, 2 H–C(xan)); 6.03 (*m*, H–C(1′)); 4.59–4.40 (*m*, H–C(3′), $CH_2(\alpha)$); 4.28 (*t*, $CH_2(\alpha)$); 4.05 (*m*, CH_2(5′)); 3.80 (*m*, H–C(4′)); 3.21 (*t*, $CH_2(\beta)$); 2.81 (*t*, $CH_2(\beta)$); 2.40 (*m*, CH_2(2′)); 1.10–0.95 (*m*, 4 Me_2CH). Anal. calc. for $C_{58}H_{63}N_6O_{15}Si_2$ (1140.3): C 61.09, H 5.57, N 7.37; found: C 61.03, H 5.74, N 7.55.

*2′-Deoxy-*N^4*-{{{3-[[2-(4-nitrophenyl)ethoxy]carbonyl}-4-{6-[2-(4-nitrophenyl)ethoxy]-3-oxo-3*H*-xanthen-9-yl}phenyl}amino}carbonyl}cytidine* (**19**). A soln. of **18** (2.5 g, 3.13 mmol) in THF (30 ml) was stirred for 15 h in AcOH (3.1 ml) in the presence of $Bu_4NF \cdot 3\ H_2O$ (1.68 g, 5.33 mmol). The soln. was diluted with CH_2Cl_2 (200 ml) and washed with H_2O (100 ml) whereby a part of the product separated out. The solid was filtered off, and the org. phase dried (Na_2SO_4) and concentrated. Both solid fractions were combined and treated with EtOH (30 ml) under stirring for 5 h. The solid was collected and dried: 1.8 g (92%) of **19**. Orange solid. UV(MeOH/CH_2Cl_2 9:1): 227 (sh, 4.74), 232 (4.75), 271 (sh, 4.67), 276 (4.68), 294 (sh, 4.58), 306 (sh, 4.48), 354 (4.02), 406 (sh, 3.97), 433 (sh, 4.28), 459 (4.44), 487 (4.33). ^{1}H-NMR ((D_6)DMSO): 11.80 (br. *s*, NH); 10.38 (br. *s*, NH); 8.30 (*d*, H–C($2_{arom.}$)); 8.16 (*d*, 2 arom. H *o* to NO_2); 8.03 (*d*, 2 arom. H *o* to NO_2, H–C(6)); 7.89 (*d*, H–C($6_{arom.}$)); 7.61 (*d*, 2 arom. H *m* to NO_2); 7.37 (*d*, H–C($5_{arom.}$), 2 arom. H *m* to NO_2); 7.16 (*d*, 1 H–C(xan); 6.81 (*m*, 3 H–C(xan)); 6.46 (*d*, H–C(5)): 6.33 (*d*, 1 H–C(xan); 6.17 (*m*, H–C(1′), 1 H–C(xan)); 5.30 (*d*, OH–C(3′)); 4.45 (*t*, $CH_2(\alpha)$); 4.23 (*t*, H–C(3′), $CH_2(\alpha)$); 3.88 (*m*, H–C(4′)); 3.60 (*m*, CH_2(5′)); 3.21 (*t*, $CH_2(\beta)$); 2.83 (*t*, $CH_2(\beta)$); 2.28 (*m*, 1 H–C(2′)); 2.10 (*m*, 1 H–C(2′)). Anal. calc. for $C_{46}H_{38}N_6O_{14}$ (898.8): C 61.47, H 4.26, N 9.34; found: C 61.24, H 4.49, N 9.20.

*2′-Deoxy-5′-*O*-(4,4′-dimethoxytrityl)-*N^4*-{{{3-[[2-(4-nitrophenyl)ethoxy]carbonyl}-4-{6-[2-(4-nitrophenyl)ethoxy]-3-oxo-3*H*-xanthen-9-yl}phenyl}amino}carbonyl}cytidine* (**20**). A soln. of **19** (6.3 g, 7 mmol) in dry pyridine was concentrated, and 2× the residue dissolved in pyridine, and the soln. concentrated. The residue was dissolved in dry pyridine (200 ml), and then 4,4′-dimethoxytrityl chloride (2.86 g, 8.4 mmol) was added, and the mixture stirred at r.t. overnight. The soln. was diluted with CH_2Cl_2 (400 ml) and extracted with sat. $NaHCO_3$ soln. (3 × 100 ml). The org. phase was dried (Na_2SO_4) and concentrated and the residue subject to FC (SiO_2, 150 g), CH_2Cl_2/MeOH 99:1 (300 ml), CH_2Cl_2/MeOH 98:2 (300 ml), CH_2Cl_2/MeOH 97:3 (300 ml), CH_2Cl_2/MeOH 95:5 (600 ml), and CH_2Cl_2/MeOH 96:4 (400 ml)). The residue of the product fractions was dried: 7.0 g (83%) of **20**. Orange-red solid. UV (MeOH/CH_2Cl_2 1:1): 210 (4.68), 227 (sh, 4.87), 232 (4.88), 270 (sh, 4.70), 275 (4.71), 294 (sh, 4.60), 306 (sh, 4.50), 354 (4.04), 406 (sh, 3.98), 434 (sh, 4.29), 459 (4.44), 487 (4.24). ^{1}H-NMR ((D_6)DMSO): 11.80 (br. *s*, NH); 10.45 (br. *s*, NH); 8.35 (*d*, H–C($2_{arom.}$)); 8.13 (*d*, 2 arom. H *o* to NO_2, H–C(6)); 8.06 (*d*, 2 arom. H *o* to NO_2); 7.89 (*d*, H–C($6_{arom.}$)); 7.60 (*d*, 2 arom. H *m* to NO_2); 7.41–7.20 (*m*, 12 H, H–C($5_{arom.}$), 2 arom. H *m* to NO_2, 4 arom. H *m* to MeO, 5 arom. H); 7.13 (*d*, H–C(xan)); 6.96–6.78 (*m*, 3 H–C(xan), 4 arom. H *o* to MeO); 6.37 (*d*, H–C(5), H–C(xan)); 6.15 (*m*, H–C(1′), H–C(flu)); 5.31 (*d*, OH–C(3′)); 4.41–4.25 (*m*, H–C(3′), 2 $CH_2(\alpha)$); 3.99 (*m*, H–C(4′)); 3.71 (*s*, 2 MeO); 3.31–3.15 (*m*, CH_2(5′), $CH_2(\beta)$); 2.82 (*t*, $CH_2(\beta)$); 2.50–2.10 (*m*, CH_2(2′)). ^{31}P-NMR ((D_6)DMSO): 147.72; 147.63. Anal. calc. for $C_{67}H_{56}N_6O_{16}$ (1201.2): C 66.99, H 4.70, N 6.99; found: C 66.87, H 4.91, N 6.93.

*2′-Deoxy-5′-*O*-(4,4′-dimethoxytrityl)-*N^4*-{{{3-[[2-(4-nitrophenyl)ethoxy]carbonyl}-4-{6-[2-(4-nitrophenyl)ethoxy]-3-oxo-3*H*-xanthen-9-yl}phenyl}amino}carbonyl}cytidine 3′-[2-(4-Nitrophenyl)ethyl* N,N*-Diisopropylphosphoramidite]* (**21**). To a soln. of **20** (0.8 g 0.67 mmol) and 1*H*-tetrazole (38 mg, 0.54 mmol) in CH_2Cl_2 (10 ml) under N_2 was added MeCN (10 ml) and then 2-(4-nitrophenyl)ethyl *N,N,N′,N′*-tetraisopropylphosphorodiamidite [37] (0.8 g, 2 mmol). The mixture was stirred at r.t. for 3 h, then diluted with CH_2Cl_2 (100 ml), and washed with sat. $NaHCO_3$ soln. The org. layer was dried (Na_2SO_4) and concentrated, and the residue purified by CC (SiO_2 (3 × 10 cm), hexane/acetone 1:1 (200 ml), hexane/acetone 1:3 (200 ml), and hexane/acetone 1:4 (400 ml)). The residue of the product fractions was dissolved in little CH_2Cl_2 and slowly added dropwise into abs. Et_2O. The resulting precipitate was collected under N_2 and dried under high vacuum: 0.818 g (82%) of **21**. Orange-red powder. UV (MeOH/CH_2Cl_2 1:1): 216 (4.68), 235 (4.82), 275 (4.77), 293 (sh, 4.65), 355 (sh, 4.04), 415

(4.05), 436 (sh, 4.29), 459 (4.44), 487 (4.33). ^{1}H-NMR ((D_6)DMSO): 11.79 (br. *s*, NH); 10.41 (br. *s*, NH); 8.30 (*d*, H–C($2_{arom.}$)); 8.20–8.00 (*d*, 4 arom. H *o* to NO_2, H–C(6)); 7.90 (*d*, H–C($6_{arom.}$)); 7.62 (*d*, 2 arom. H *m* to NO_2); 7.50–7.19 (*m*, H–C($5_{arom.}$), 2 arom. H *m* to NO_2, 4 arom. H *m* to MeO, 5 arom. H); 7.14 (*s*, 1 H–C(xan); 6.90–6.78 (*m*, 3 H–C(xan), 4 arom. H *o* to MeO); 6.33 (2*d*, H–C(5), H–C(xan)); 6.15 (*m*, H–C(1′), H–C(xan)); 4.41 (*t*, H–C(3′), $CH_2(\alpha)$); 4.27 (*t*, $CH_2(\alpha)$); 4.03 (*m*, H–C(4′)); 3.72 (*m*, CH_2O–P); 3.69 (*s*, 2 MeO); 3.41 (*m*, 2 Me_2CH); 3.21 (*m*, CH_2(5′), $CH_2(\beta)$); 3.00–2.79 (*m*, 2 $CH_2(\beta)$); 2.49–2.10 (*m*, CH_2(2′)); 1.10–0.90 (*m*, 2 Me_2CH). Anal. calc. for $C_{81}H_{77}N_8O_{19}P$ (1497.5): C 64.97, H 5.18, N 7.48; found: C 63.82, H 5.51, N 7.18.

2′-Deoxy-5′-O-*(4,4′-dimethoxytrityl)*-N^4*-{{{3-{[2-(4-nitrophenyl)ethoxy]carbonyl}-4-{6-[2-(4-nitrophenyl)ethoxy]-3-oxo-3*H*-xanthen-9-yl}phenyl}amino}carbonyl}cytidine 3′-[2-Cyanoethyl* N,N-*Diisopropylphosphoramidite]* (**22**). As described for **21**, with **20** (0.8 g 0.67 mmol), 1*H*-tetrazole (38 mg, 0.54 mmol), CH_2Cl_2 and MeCN under N_2, and 2-cyanoethyl *N,N,N′,N′*-tetraisopropylphosphorodiamidite [38][39] (0.6 g, 2 mmol): 0.75 g (80%) of **22**. Orange-red solid. UV (MeOH/CH_2Cl_2 1:1): 217 (4.65), 234 (4.81), 276 (4.69), 294 (sh, 4.57), 356 (sh, 3.98), 409 (sh, 3.95), 436 (sh, 4.28), 459 (4.44), 487 (4.34). ^{1}H-NMR ((D_6)DMSO): 11.75 (br. *s*, NH); 10.40 (br. *s*, NH); 8.31 (*d*, H–C($2_{arom.}$)); 8.18 (*d*, 2 arom. H *o* to NO_2, H–C(6)); 8.04 (*d*, 2 arom. H *o* to NO_2); 7.88 (*d*, H–C($6_{arom.}$)); 7.62 (*d*, 2 arom. H *m* to NO_2); 7.41–7.19 (*m*, H–C($5_{arom.}$), 2 arom. H *m* to NO_2, 4 arom. H *m* to MeO, 3 arom. H); 7.16 (*s*, 1 H–C(xan); 6.92–6.80 (*m*, 3 H–C(xan), 4 arom. H *o* to MeO); 6.31 (*d*, H–C(5), 1 H–C(xan)); 6.13 (*m*, H–C(1′), 1 H–C(xan)); 4.55 (*t*, H–C(3′)); 4.40 (*t*, $CH_2(\alpha)$); 4.24 (*t*, $CH_2(\alpha)$); 4.12 (*m*, H–C(4′)); 3.72 (*s*, 2 MeO); 3.70–3.40 (*m*, CH_2O–P, 2 Me_2CH, CH_2(5′)); 3.22 (*m*, $CH_2(\beta)$); 2.84–2.60 (*m*, CH_2CN, $CH_2(\beta)$); 2.37 (*m*, CH_2(2′)); 1.16–0.95 (*m*, 2 Me_2CH). ^{31}P-NMR ((D_6)DMSO): 148.62; 148.31. Anal. calc. for $C_{76}H_{73}N_8O_{17}P$ (1401.4): C 65.14, H 5.25, N 7.99; found: C 64.90, H 5.71, N 7.34.

*2′-Deoxy-*N^4*,3′*-O-*bis{[2-(4-nitrophenyl)ethoxy]carbonyl}cytidine* (**23**). A soln. of 2′-deoxy-5′-*O*-(monomethoxytrityl)-*N*^{4},3′-*O*-bis{[2-(4-nitrophenyl)ethoxy]carbonyl}cytidine (0.885 g, 1 mmol) in 1% TsOH in CH_2Cl_2/MeOH 4:1 (40 ml) was stirred at r.t. for 30 min. The mixture was diluted with $CHCl_3$ (60 ml) and washed with phosphate buffer (pH 7; 3 × 100 ml), the org. phase dried (Na_2SO_4) and concentrated, and the residue purified by CC (SiO_2 (2.5 × 25 cm), CH_2Cl_2, $CHCl_3$/MeOH 100:1, and $CHCl_3$/MeOH 100:2). The residue of the product fractions was dried under high vacuum: 0.57 g (93%) of **23**. Colorless solid foam. Recrystallization from AcOEt gave crystals with m.p. 143–145°. UV (MeOH): 247 (4.36), 273 (4.39). ^{1}H-NMR ($(CDCl_3)$: 8.40 (br. *s*, NH); 8.20 (*d*, H–C(6)); 8.12 (*d*, 2 arom. H *o* to NO_2); 8.10 (*d*, 2 arom. H *o* to NO_2); 7.38 (*d*, 2 arom. H *m* to NO_2); 7.34 (*d*, 2 arom. H *m* to NO_2); 7.15 (*d*, H–C(5)); 6.16 (*m*, H–C(1′)); 5.27 (*d*, H–C(3′)); 4.37 (*t*, 2 $CH_2(\alpha)$); 4.20 (br. *s*, H–C(4′)); 3.8 (*m*, CH_2(5′)); 3.75 (br. *s*, OH–C(5′)); 3.05 (*t*, 2 $CH_2(\beta)$); 2.64 (*m*, 1 H–C(2′)); 2.37 (*m*, 1 H–C(2′)). Anal. calc. for $C_{27}H_{27}N_5O_{12}$ (613.5): C 52.86, H 4.44, N 11.41; found: C 52.86, H 4.50, N 11.32.

*2′-Deoxy-*N^4*,5′*-O-*bis{[2-(4-nitrophenyl)ethoxy]carbonyl}cytidine* (**24**). A soln. of 2′-deoxy-*N*^{4}-{[2-(4-nitrophenyl)ethoxy]carbonyl}cytidine [40] (4.1 g, 9.7 mmol) in abs. pyridine (40 ml) was cooled to −25°, and then 2-(4-nitrophenyl)ethyl carbonochloridate (3.36 g, 14.6 mmol) in abs. CH_2Cl_2 (40 ml) was added dropwise. After stirring for 3 h at −20°, the mixture was diluted with CH_2Cl_2 (300 ml) and washed with H_2O, the org. layer dried (Na_2SO_4) and concentrated, and the residue purified by CC (SiO_2 (4.5 × 20 cm), CH_2Cl_2 (300 ml), CH_2Cl_2/MeOH 98:2 (600 ml), and CH_2Cl_2/MeOH 96:4 (600 ml)). The residue of the product fractions was dried under high vacuum: 3.6 g (61%) of **24**. Colorless solid. UV (MeOH): 213 (4.55), 246 (sh, 4.39), 273 (4.32). ^{1}H-NMR ($(CDCl_3)$: 8.42 (br. *s*, NH); 8.15 (*dd*, 4 arom. H *o* to NO_2); 7.99 (*d*, H–C(6)); 7.37 (*d*, 4 arom. H *m* to NO_2); 7.11 (*d*, H–C(5)); 6.25 (*m*, H–C(1′)); 4.70 (br. *s*, OH–C(3′)); 4.41–4.20 (*m*, H–C(3′), H–C(4′), CH_2(5′), 2 $CH_2(\alpha)$); 3.09 (*t*, 2 $CH_2(\beta)$); 2.70 (*m*, 1 H–C(2′)); 2.08 (*m*, 1 H–C(2′)). Anal. calc. for $C_{27}H_{27}N_5O_{12}$ (613.5): C 52.86, H 4.44, N 11.41; found: C 53.03, H 4.57, N 10.94.

*2′-Deoxy-*N^4*,5′*-O-*bis{[2-(4-nitrophenyl)ethoxy]carbonyl}cytidine 3′-[2-(4-Nitrophenyl)ethyl* N,N-*Diisopropylphosphoramidite]* (**25**). As described for **21**, with **24** (1.0 g 1.6 mmol), 1*H*-tetrazole (70 mg, 1 mmol), CH_2Cl_2 (20 ml), MeCN (10 ml) and 2-(4-nitrophenyl)ethyl *N,N,N′,N′*-tetraisopropylphosphorodiamidite [37] (1.4 g, 3.6 mmol), for 6 h. After CC (SiO_2 (3 × 15 cm), hexane/AcOEt 1:1 (200 ml), hexane/AcOEt 1:2 (100 ml), and hexane/AcOEt 1:4 (400 ml)), the residue of the product fractions was dried under high vacuum: 1.2 g (81%) of **25**. Colorless powder. UV (MeOH): 203 (4.54), 211 (sh, 4.46), 250 (sh, 4.26), 272 (4.34). ^{1}H-NMR ($CDCl_3$): 8.20–8.15 (*m*, 6 arom. H *o* to NO_2); 7.43 (*d*, H–C(6));

7.44–7.36 (*m*, 6 arom. H *m* to NO_2); 7.13 (br. *s*, NH); 7.08 (*d*, H–C(5)); 6.19 (*m*, H–C(1′)); 4.49–4.19 (*m*, H–C(3′), H–C(4′), CH_2(5′), 2 CH_2(α)); 3.90–3.71 (*m*, CH_2O–P); 3.60–3.48 (*m*, 2 Me_2CH); 3.16–2.99 (*m*, 3 CH_2(β)); 2.65 (*m*, 1 H–C(2′)); 2.03 (*m*, 1 H–C(2′)); 1.13–1.04 (*m*, 2 Me_2CH). ^{31}P-NMR: 148.79; 148.66. Anal. calc. for $C_{41}H_{48}N_7O_{15}P$ (909.8): C 54.12, H 5.32, N 10.77; found: C 53.60, H 5.33, N 10.45.

2′-Deoxy-5′-O-*(4,4′-dimethoxytrityl)*-N^4-*{{{3-{[2-(4-nitrophenyl)ethoxy]carbonyl}-4-{6-[2-(4-nitrophenyl)ethoxy]-3-oxo-3*H*-xanthen-9-yl}phenyl}amino}carbonyl}*-O^P-*[2-(4-nitrophenyl)ethyl]cytidylyl-3′ → 5′)-2′-deoxy*-N^4,*3′*-O-*bis{[2-(4-nitrophenyl)ethoxy]carbonyl}cytidine* (**26**). A mixture of **23** (0.35 g, 0.57 mmol) and 1*H*-tetrazole (0.1 g, 1.5 mmol) was dissolved in CH_2Cl_2 (20 ml) and MeCN (10 ml) under N_2. Then **21** (0.57 g, 0.38 mmol) was added, and the mixture was stirred at r.t. for 3 h, followed by treatment with I_2 (0.5 g) in CH_2Cl_2/pyridine/H_2O 1:3:1 (4 ml) for 30 min. The mixture was diluted with CH_2Cl_2 (150 ml) and the soln. extracted with a sat. soln. of $Na_2S_2O_3$/NaCl (3 × 100 ml). The aq. phases were reextracted with CH_2Cl_2 (50 ml). The combined org. extract was dried (Na_2SO_4) and concentrated, 3 × the residue dissolved in toluene (100 ml), and the soln. concentrated, and the residue purified by CC (SiO_2 (1 × 10 cm), CH_2Cl_2 (100 ml), CH_2Cl_2/MeOH 98:2 (100 ml), CH_2Cl_2/MeOH 96:4 (200 ml), and CH_2Cl_2/MeOH 95:5 (100 ml)). The residue of the product fractions was dissolved in little CH_2Cl_2/MeOH 1:1, and this soln. slowly added dropwise to cold (0°) MeOH (25 ml). The precipitate was washed with MeOH and Et_2O and dried under high vacuum: 0.585 g (76%) of **26**. Orange-red solid. UV (MeOH): 224 (4.97), 275 (4.91), 348 (sh, 3.99), 409 (sh, 3.96), 439 (sh, 4.28), 460 (4.44), 488 (4.33). ^{1}H-NMR ((D$_6$)DMSO): 11.71 (*s*, NH); 10.80 (*s*, NH); 10.40 (*s*, NH); 8.29 (s, H–C(2$_{arom.}$)); 8.19–7.95 (*m*, 2 H–C(6), 10 arom. H *o* to NO_2); 7.89 (*d*, H–C(6$_{arom.}$)); 7.65–7.18 (*m*, 21 H, 10 arom. H *m* to NO_2, 9 arom. H, H–C(5$_{arom.}$), H–C(5′$_{xan}$)); 6.96 (*d*, H–C(5)); 6.90–6.80 (*m*, H–C(1′$_{xan}$), H–C(7′$_{xan}$), H–C(8′$_{xan}$)), 4 arom. H *o* to MeO); 6.18–6.01 (*m*, 2 H–C(1′), H–C(3′$_{xan}$), H–C(4′$_{xan}$)); 5.83 (*m*, H–C(5), H–C(5′$_{xan}$)); 5.03 (*m*, H–C(3′)); 4.91 (*m*, H–C(3′)); 4.50–4.10 (*m*, 14 H, 5 CH_2(α), 2 H–C(4′), CH_2(5′)); 3.70 (*s*, 2 MeO); 3.30–2.83 (*m*, 5 CH_2(β), CH_2(5′)); 2.50–2.20 (*m*, 2 CH_2(2′)). ^{31}P-NMR ((D$_6$)DMSO): −1.51. Anal. calc. for $C_{102}H_{89}N_{12}O_{32}P$ (2025.9): C 60.47, H 4.43, N 8.30; found: C 60.26, H 4.58, N 8.23.

2′-Deoxy-N^4-*{{{3-{[2-(4-nitrophenyl)ethoxy]carbonyl}-4-{6-[2-(4-nitrophenyl)ethoxy]-3-oxo-3*H*-xanthen-9-yl}phenyl}amino}carbonyl}*-O^P-*[2-(4-nitrophenyl)ethyl]cytidylyl-(3′ → 5′)-2′-deoxy*-N^4,*3′*-O-*bis{[2-(4-nitrophenyl)ethoxy]carbonyl}cytidine* (**27**). To 1% TsOH in CH_2Cl_2/MeOH 4:1 (20 ml) was added **26** (0.48 g, 0.24 mmol) and stirred at r.t. for 1 h. The mixture was diluted with CH_2Cl_2 (150 ml), and the soln. extracted with sat. $NaHCO_3$ soln. (3 × 80 ml). The aq. extracts were reextracted with CH_2Cl_2 (50 ml). Then the combined org. phase was dried (Na_2SO_4) and concentrated and the resulting solid purified by CC (SiO_2 (1.5 × 8 cm), CH_2Cl_2 (50 ml), CH_2Cl_2/MeOH 98.5:1:5 (50 ml), CH_2Cl_2/MeOH 96:4 (50 ml), and CH_2Cl_2/MeOH 94:6 (100 ml)). The residue of the product fractions was dissolved in little CH_2Cl_2/MeOH 1:1, and the soln. slowly and dropwise added to cold (0°) MeOH (25 ml). The precipitate was washed with Et_2O and dried under high vacuum: 0.35 g (85%) of **27**. Orange-red powder. UV (MeOH): 224 (4.85), 275 (4.88), 352 (4.01), 407 (sh, 3.94), 438 (sh, 4.28), 459 (4.44), 487 (4.33). ^{1}H-NMR ((D$_6$)DMSO): 11.75 (*s*, NH); 10.80 (*s*, NH); 10.49 (*s*, NH); 8.28 (*s*, H–C(2$_{arom.}$)); 8.23–7.99 (*m*, 2 H–C(6), 10 arom. H *o* to NO_2); 7.88 (*d*, H–C(6$_{arom.}$)); 7.65–7.48 (*m*, 8 arom. H *m* to NO_2); 7.40–7.30 (*m*, 2 arom. H *m* to NO_2, H–C(5$_{arom.}$)); 7.17 (*s*, H–C(5′$_{xan}$)); 6.98 (*d*, H–C(5)); 6.83 (*m*, H–C(1′$_{xan}$), H–C(7′$_{xan}$), H–C(8′$_{xan}$)); 6.41 (*d*, H–C(5)); 6.36 (*d*, H–C(2′$_{xan}$)); 6.19–6.00 (*m*, 2 H–C(1′), H–C(4′$_{xan}$)); 5.19 (*t*, OH–C(5′)); 5.10 (*m*, H–C(3′)); 4.88 (*m*, H–C(3′)); 4.50–4.10 (*m*, 5 CH_2(α), 2 H–C(4′), CH_2(5′)); 3.58 (*m*, CH_2(5′)); 3.30–2.80 (*m*, 5 CH_2(β)); 2.43–2.21 (*m*, 2 CH_2(2′)). ^{31}P-NMR ((D$_6$)DMSO): −1.53; −1.42. Anal. calc. for $C_{81}H_{71}N_{12}O_{30}P$ (1723.5): C 56.45, H 4.15, N 9.75; found: C 56.02, H 4.31, N 9.69.

2′-Deoxy-N^4,*5′*-O-*bis{[2-(4-nitrophenyl)ethoxy]carbonyl}*-O^P-*[2-(4-nitrophenyl)ethyl]cytidylyl-(3′ → 5′)-2′-deoxy*-N^4-*{{{3-{[2-(4-nitrophenyl)ethoxy]carbonyl}-4-{6-[2-(4-nitrophenyl)ethoxy]-3-oxo-3*H*-xanthen-9-yl}phenyl}amino}carbonyl}*-O^P-*[2-(4-nitrophenyl)ethyl]cytidylyl-(3′ → 5′)-2′-deoxy*-N^4,*3′*-O-*bis{[2-(4-nitrophenyl)ethoxy]carbonyl}cytidine* (**28**). A mixture of **27** (0.22 g, 0.13 mmol) and 1*H*-tetrazole (95 mg, 1.35 mmol) in abs. CH_2Cl_2 (10 ml) was concentrated, and 2 × the residue dissolved in abs. toluene (10 ml), and the soln. concentrated. The residue was dissolved in CH_2Cl_2 (15 ml) and MeCN (8 ml), and under N_2, **25** (0.45 g, 0.5 mmol) was added and the mixture stirred overnight. After treatment with I_2 (0.5 g) in CH_2Cl_2/pyridine/H_2O 1:3:1 (4 ml), the mixture was stirred for 30 min, diluted with

CH_2Cl_2 (150 ml), and extracted with a sat. soln. of $Na_2S_2O_3$/NaCl (3×100 ml). The aq. phases were reextracted with CH_2Cl_2 (50 ml). The combined org. extract was dried (Na_2SO_4) and concentrated, and the residue $3\times$ dissolved in toluene (50 ml), and the soln. concentrated. The residue was dissolved in CH_2Cl_2 (8 ml) and MeOH (2 drops) and purified by CC (SiO_2 (1×20 cm), CH_2Cl_2 (100 ml), CH_2Cl_2/MeOH 98 : 2 (100 ml), CH_2Cl_2/MeOH 96 : 4 (200 ml), CH_2Cl_2/MeOH 95 : 5 (300 ml), and CH_2Cl_2/MeOH 93 : 7)). The residue of the product fractions was dissolved in little CH_2Cl_2/MeOH and then dropwise added to MeOH (25 ml) with stirring. The precipitate was washed with little Et_2O and dried under high vacuum: 0.27 g (83%) of **28**. Orange-red solid. UV (MeOH): 216 (4.86), 274 (5.02), 350 (sh, 4.01), 408 (sh, 3.93), 436 (sh, 4.26), 459 (4.43), 484 (4.32). ^{1}H-NMR ((D$_6$)DMSO): 11.70 (*s*, NH); 10.80 (*s*, NH); 10.38 (*s*, NH); 8.27 (*s*, H–C(2$_{arom.}$)); 8.22–7.90 (*m*, 3 H–C(6), 16 arom. H *o* to NO_2); 7.86 (*d*, H–C(6$_{arom.}$)); 7.70–7.30 (*m*, 16 arom. H *m* to NO_2, H–C(5$_{arom.}$)); 7.19 (*s*, H–C(5′$_{xan}$)); 6.98 (*m*, 2 H–C(5)); 6.83 (*m*, H–C(1′$_{xan}$), H–C(7′$_{xan}$), H–C(8′$_{xan}$)); 6.40 (*m*, H–C(5), H–C(2′$_{xan}$)); 6.12 (*m*, 3 H–C(1′), H–C(4′)); 5.10 (*m*, H–C(3′)); 4.92 (*m*, 2 H–C(3′)); 4.45–4.20 (*m*, 8 $CH_2(\alpha)$, 3 H–C(4′), 3 CH_2(5′)); 3.26 (*t*, $CH_2(\beta)$); 3.12 (*m*, 6 $CH_2(\beta)$); 2.81 (*t*, $CH_2(\beta)$); 2.45–2.20 (*m*, 3 CH_2(2′)). ^{31}P-NMR((D$_6$)DMSO): −1.48; −1.45; −1.36. Anal. calc. for $C_{113}H_{104}N_{18}O_{46}P_2$ (2512.0): C 54.03, H 4.17, N 10.04; found: C 53.81, H 4.19, N 9.83.

*2′-Deoxycytidylyl-(3′ → 5′)-2′-deoxy-*N^4*-[(fluorescein-5-ylamino)carbonyl]cytidylyl-(3′ → 5′)-2′-deoxycytidine Ammonium Salt (1 : 2)* (**29**). A soln. of **28** (0.1 g, 0.039 mmol) in abs. pyridine (20 ml) was concentrated, the residue again dissolved in abs. pyridine (20 ml), DBU (1.4 ml, 9.4 mmol) added, and then the mixture stirred at r.t. for 3 d. The mixture was neutralized with AcOH and concentrated. The residue was dissolved in H_2O (10 ml), the soln. extracted with CH_2Cl_2 (2×5 ml), and the aq. layer subjected to ion-exchange CC (*DEAE-Sephadex A-25* (HCO_3^- form, 1.5×60 cm), H_2O (200 ml), then linear gradient of 0–0.3M triethylammonium hydrogencarbonate buffer (pH 7.5, 5 l)). The product fractions eluted with 0.24–0.26M buffer, and their residue was several times dissolved in MeOH and the soln. evaporated. The conversion into the ammonium salt was achieved by dissolving the residue in little ammonia and purifying by paper chromatography (six large paper sheets (30×52 cm; *Schleicher & Schuell*), ^{i}PrOH/25% ammonia/H_2O 1 : 3 : 1). The product bands were cut out and extracted with dilute ammonia. The combined extract was concentrated: 2200 OD_{490} (65%, 0.025 mmol) of **29**. Orange-red solid. HPLC (*RP-18*, system *A* followed by *B* and *C*): t_R 16.8 min (detection at 260 and 480 nm). ^{1}H-NMR (D_2O): 8.04 (*d*, H–C(6)); 7.90 (*s*, H–C(2$_{arom.}$)); 7.73 (*d*, H–C(6)); 7.61 (*d*, H–C(6)); 7.43 (*d*, H–C(6$_{arom.}$)); 7.21 (*d*, H–C(5$_{arom.}$)); 7.09 (*d*, H–C(5)); 7.00 (*d*, H–C(5)); 6.53 (*m*, H–C(2′), H–C(7′$_{xan}$)); 6.42 (*s*, H–C(4′$_{xan}$), H–C(6′$_{xan}$)); 6.22 (*d*, H–C(5)); 6.15 (*dd*, H–C(1′$_{xan}$)); 5.98 (*dd*, H–C(1′)); 5.89 (*m*, H–C(1′$_{xan}$), H–C(5′$_{xan}$)); 5.71 (*d*, H–C(5)); 4.39 (*m*, H–C(3′)); 4.21 (*m*, 2 H–C(3′)); 4.05–3.90 (*m*, 3 CH_2(5′)); 3.63 (*m*, 3 H–C(4′)); 2.60–2.00 (*m*, 3 CH_2(2′)).

*2′-Deoxy-5′-*O*-(4,4′-dimethoxytrityl)-*N^4*-{[2-(4-nitrophenyl)ethoxy]carbonyl}cytidine 3′-(Hydrogen Butanedioate)* (*=1-{Tetrahydro-5-{4-{{[2-(4-nitrophenyl)ethoxy]carbonyl}amino}-2-oxopyrimidin-1(2*H*)-yl}-2-{[bis(4-methoxyphenyl)phenylmethoxy]methyl}furan-3-yl} Hydrogen Butanoedioate*; **30**). A soln. of 2′-deoxy-5′-*O*-(4,4′-dimethoxytrityl)-*N*^{4}-{[2-(4-nitrophenyl)ethoxy]carbonyl}cytidine [41] (0.723 g, 1 mmol), succinic anhydride (0.2 g, 2 mmol), and *N*,*N*-dimethylpyridin-4-amine (0.16 g, 1.3 mmol) in abs. CH_2Cl_2 (5 ml) was stirred until the starting material had disappeared. The mixture was diluted with CH_2Cl_2 (50 ml), the soln. extracted subsequently with cold 10% aq. citric acid (30 ml), followed by sat. $NaHCO_3$ soln. (30 ml) and H_2O (30 ml). The aq. phases were reextracted with CH_2Cl_2. The combined org. phase was dried (Na_2SO_4) and concentrated, and the residue dried under high vacuum: 0.658 g (80%) of **30**. UV (MeOH): 214 (4.48), 236 (4.45), 275 (4.17), 282 (sh, 4.17). ^{1}H-NMR ($CDCl_3$): 8.25–8.15 (*m*, 2 arom. H *o* to NO_2, NH, H–C(6)); 7.45–7.12 (*m*, 11 arom. H), 6.95 (*d*, H–C(5)); 6.85 (*m*, 4 arom. H *o* to MeO); 6.24 (*dd*, H–C(1′)); 4.52 (*m*, H–C(3′)); 4.40 (*t*, OCH_2CH_2); 4.26 (*m*, H–C(4′)); 3.78 (*s*, MeO); 3.45–3.35 (*m*, CH_2(5′)); 3.09 (*t*, OCH_2CH_2); 2.85 (*m*, 1 H–C(2′)); 2.70–2.50 (*m*, $OCCH_2CH_2CO$); 2.35–2.20 (*m*, 1 H–C(2′)). Anal. calc. for $C_{43}H_{42}N_4O_{13}$ (822.8): C 62.77, H 5.15, N 6.81; found: C 62.62, H 4.95, N 6.60.

5′-d(C^{Flu}CCG GCC CGC)-3′ DNA (**33**). The automated solid-phase syntheses of the various oligodeoxyribonucleotides were performed in a DNA synthesizer. The solid phase was glyceryl-CPG (500 Å) from *Fluka* which was activated by phenyl 1*H*-tetrazole-1-carboxylate and coupled with *N*^{1},*N*^{6}-dimethylhexane-1,6-diamine [42]. Then **30** was attached and led to a loading of 14 μmol/g. This solid

support (43 mg, 0.6 μmol) was applied in the synthesizer for chain elongation with **31** [35], **32** [35], and **22** as 0.095M soln. in CH_2Cl_2 in the usual manner. The overall yield was 89.3% which corresponds to a stepwise yield of 98.4%. The isolation of the oligomer was achieved by treatment of the solid-support material first with DBU to deprotect the ce, npe, and npeoc groups, followed by washing and finally treatment with ammonia to cleave **33** from the support. HPLC ($A = 0.1$M $Et_4N(OAc)$, $B = Et_4N(OAc)$/MeCN 1:1; elution: 0–2 min 95% A + 5% B; 2–32 min 60% A + 40% B; 32–52 min 100% B): t_R 28.2 min.

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