

Nucleotides

Part LXXV¹⁾

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 (\rightarrow **18**) and desilylation yielded **19**. Dimethoxytritylation (\rightarrow **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 (\rightarrow **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 oligonucleotide [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 fluorophore 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

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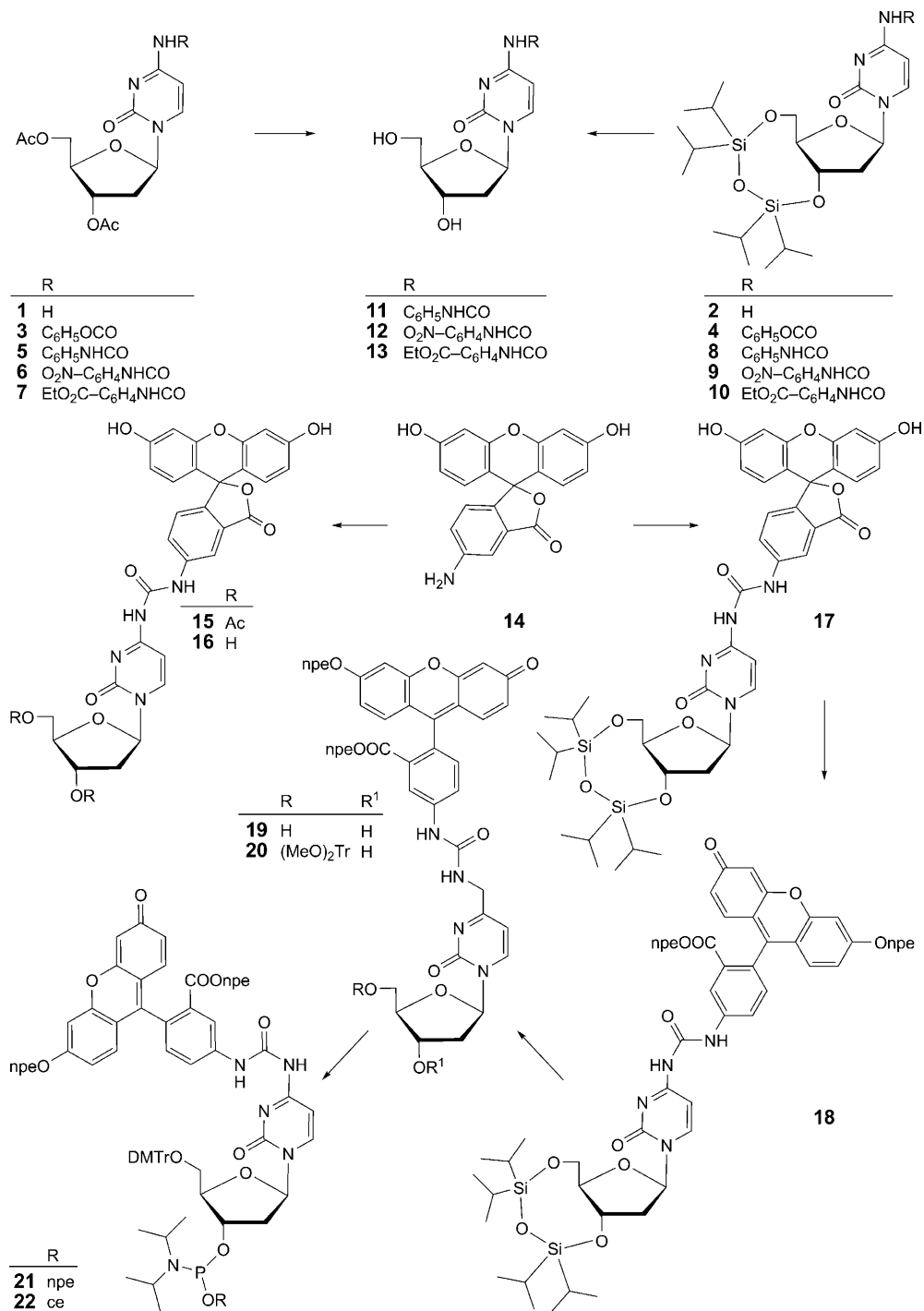
oligonucleotide syntheses, however, needs further protection of the two acidic functionalities of the fluorescein moiety which could be achieved by use of the 2-(4-nitrophenyl)ethyl (npe) and 2-(4-nitrophenylethoxy)carbonyl (npeoc) blocking group strategy [30].

2. Syntheses. – Analogously to the adenosine strategy [16], 3',5'-di-*O*-acetylcytidine (**1**) [31] and 2'-deoxy-3',5'-*O*-(tetraisopropylidisiloxane-1,3-diyl)-cytidine (**2**) [32] were converted into the corresponding *N*⁴-(phenoxy-carbonyl) 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 (Bu₄NF), 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 K₂CO₃ 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 Bu₄NF 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**.

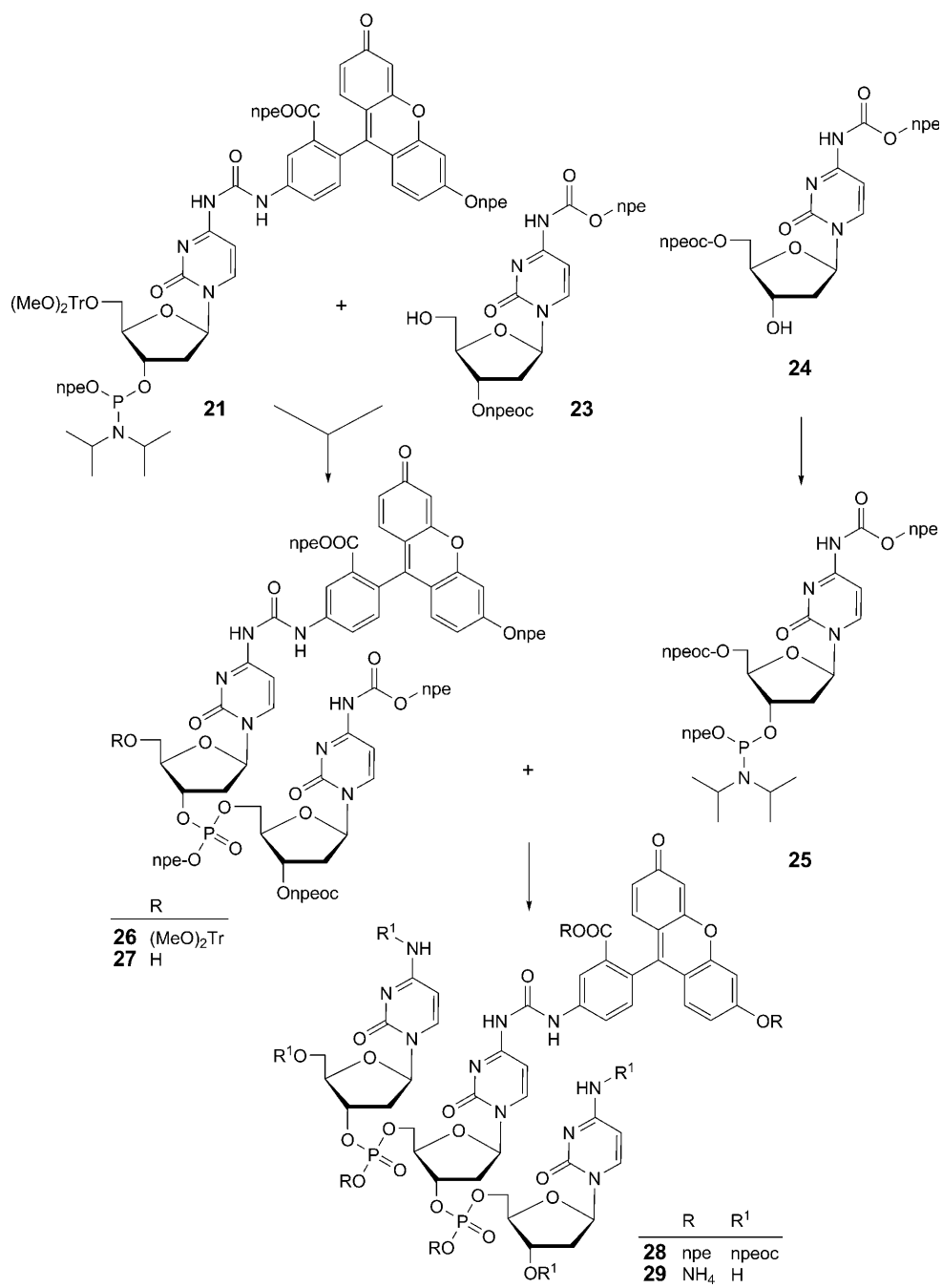
For the synthesis of the fully protected 2'-deoxycytidine trimer **28** (*Scheme 2*) carrying as a universal pattern the npe and npeoc blocking groups at all functionalities, 2'-deoxy-*N*⁴,3'-*O*-bis[[2-(4-nitrophenyl)ethoxy]carbonyl]cytidine (**23**) [33] and 2'-deoxy-*N*⁴,5'-*O*-bis[[2-(4-nitrophenyl)ethoxy]carbonyl]cytidine 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/H₂O 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



Scheme 2



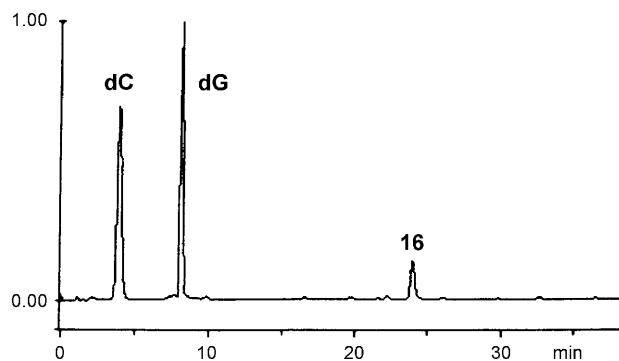
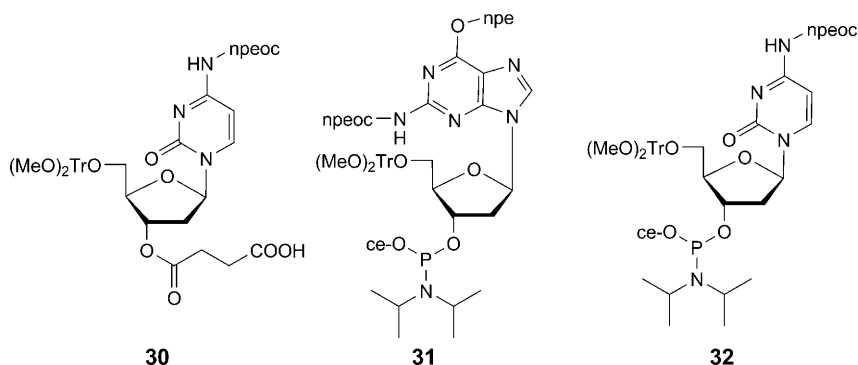


Fig. 1. HPLC of the mixture obtained after enzymatic-hydrolysis of **33** by SVP

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, ^1H -NMR and UV spectra.

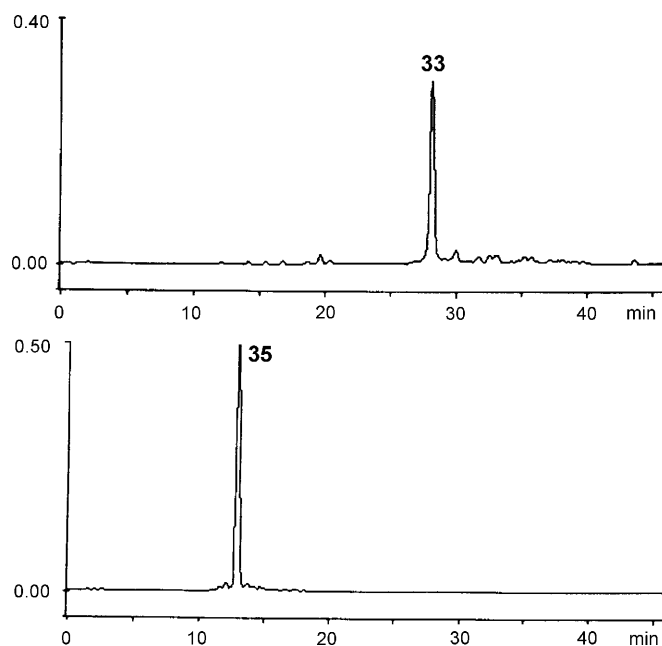


Fig. 2. HPLC of 5'-d(C^{Flu} CCG GCC CGC)-3' (**33**) and 5'-d(G GGC CGG GCG)-3' (**35**)

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)		
5'-d(C CCG GCC CGC)-3' (35)	34 · 35	60–61

^{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 (SiO₂, 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/H₂O 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). ¹H-NMR Spectra: *Bruker AC 250*; δ in ppm rel. to Me₄Si or CDCl₃ ((D₆)DMSO) as internal standard. ³¹P-NMR Spectra: *Jeol JMN-GX400*.

3',5'-Di-O-acetyl-2'-deoxy-N⁴-(phenoxycarbonyl)cytidine (= Phenyl {1-[4-(Acetyloxy)-5-[(acetyloxy)methyl]tetrahydrofuran-2-yl]-1,2-dihydro-2-oxypyrimidin-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. CHCl₃ (50 ml) and kept at 0° for 2 h. The tetrazole was filtered off and washed with little cold CHCl₃, and then the filtrate again concentrated. The residue was purified by CC (SiO₂ (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 CH₂Cl₂ and the soln. concentrated: 8.0 g (92%) of **3**. Solid foam. *R*_f 0.48 (toluene/acetone 1:1). UV (CH₂Cl₂): 240 (4.19), 300 (3.87), 310 (sh, 3.76). ¹H-NMR ((D₆)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'), CH₂(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 C₂₀H₂₁N₃O₈ (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-tetraisopropylidisiloxane-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-(tetraisopropylidisiloxane-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 (SiO₂ (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 CH₂Cl₂ and the soln. concentrated: 5.52 g (93%) of **4**. Solid foam. UV (CH₂Cl₂): 238 (4.18), 301 (3.90), 309 (sh, 3.81). ¹H-NMR ((D₆)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*, CH₂(2')); 1.05–0.90 (*m*, 4 Me₂CH). Anal. calc. for C₂₈H₄₃N₃O₇Si₂ (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-N⁴-[(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). ¹H-NMR ((D₆)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'), CH₂(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 C₂₀H₂₂N₄O₇ (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-N⁴-[[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). ¹H-NMR ((D₆)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'), CH₂(5')); 2.50–2.30 (*m*, CH₂(2')); 2.04 (*s*, 2 AcO). Anal. calc. for C₂₀H₂₁N₅O₉ (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-N⁴-[[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). ¹H-NMR ((D₆)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'), CH₂(5'), CH₂); 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 C₂₃H₂₆N₄O₇ (502.5): C 54.98, H 5.22, N 11.15; found: C 55.07, H 5.25, N 11.20.

2'-Deoxy-N⁴-[(phenylamino)carbonyl]-3',5'-O-(1,1,3,3-tetraisopropylidisiloxane-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). $^1\text{H-NMR}$ (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, $\text{CH}_2(2')$); 0.96 (m, 4 Me_2CH). Anal. calc. for $\text{C}_{28}\text{H}_{44}\text{N}_4\text{O}_6\text{Si}_2 \cdot 0.5 \text{H}_2\text{O}$ (593.3): C 56.67, H 7.55, N 9.43; found: C 56.49, H 7.47, N 9.36.

2'-Deoxy-N⁴-[[4-(4-nitrophenyl)amino]carbonyl]-3',5'-O-(1,1,3,3-tetraisopropylidisiloxane-1,3-diyl)cytidine (= N-[1,2-Dihydro-2-oxo-1-(tetrahydro-2,2,4,4-tetraisopropyl-6H-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). $^1\text{H-NMR}$ (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, $\text{CH}_2(2')$); 1.00–0.83 (m, 4 Me_2CH). Anal. calc. for $\text{C}_{28}\text{H}_{43}\text{N}_5\text{O}_8\text{Si}_2$ (633.9): C 53.06, H 6.84, N 11.05; found: C 52.55, H 6.82, N 10.93.

2'-Deoxy-N⁴-[[4-(ethoxycarbonyl)phenyl]amino]carbonyl]-3',5'-O-(1,1,3,3-tetraisopropylidisiloxane-1,3-diyl)cytidine (= Ethyl 4-[3-[1,2-Dihydro-2-oxo-1-(tetrahydro-2,2,4,4-tetraisopropyl-6H-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). $^1\text{H-NMR}$ (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 $\text{C}_{31}\text{H}_{48}\text{N}_4\text{O}_8\text{Si}_2 \cdot 0.5 \text{H}_2\text{O}$ (669.9): C 55.58, H 7.37, N 8.37; found: C 55.55, H 7.33, N 8.59.

2'-Deoxy-N⁴-[(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 $\text{Bu}_4\text{NF} \cdot 3 \text{H}_2\text{O}$ (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). $^1\text{H-NMR}$ ($(\text{D}_6)\text{DMSO}$): 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, $\text{CH}_2(5')$); 2.30–2.20 (m, 1 H–C(2')); 2.09–1.98 (m, 1 H–C(2')). Anal. calc. for $\text{C}_{16}\text{H}_{18}\text{N}_4\text{O}_5$ (346.3): C 55.49, H 5.24, N 16.18; found: C 55.62, H 5.34, N 16.12.

2'-Deoxy-N⁴-[[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), $\text{Bu}_4\text{NF} \cdot 3 \text{H}_2\text{O}$ (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). $^1\text{H-NMR}$ ($(\text{D}_6)\text{DMSO}$): 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, $\text{CH}_2(5')$); 2.31–2.24 (m, 1 H–C(2')); 2.05–1.98 (m, 1 H–C(2')). Anal. calc. for $\text{C}_{16}\text{H}_{17}\text{N}_5\text{O}_7 \cdot 0.5 \text{H}_2\text{O}$ (400.3): C 48.05, H 4.54, N 17.48; found: C 48.19, H 4.53, N 17.20.

2'-Deoxy-N⁴-[[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), Bu₄NF · 3 H₂O (2.37 g, 7.5 mmol), and THF (20 ml) for 30 min (treatment with little H₂O 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). ¹H-NMR ((D₆)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'), CH₂); 3.88 (m, H–C(4')); 3.31–3.18 (m, CH₂(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 C₁₉H₂₂N₄O₇ (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-N⁴-[(fluorescein-5-ylamino)carbonyl]cytidine (= 3',5'-Di-O-acetyl-2'-deoxy-N⁴-[(3',6'-dihydroxy-3-oxospiro[isobenzofuran-1(3H),9'-[9H]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 (SiO₂ (5 × 20 cm), CH₂Cl₂/MeOH 9.5:0.5 (150 ml), CH₂Cl₂/MeOH 9:1 (150 ml), and CH₂Cl₂/MeOH 7:3 (1 l)). The main fraction was concentrated, and the residue treated with EtOH, washed with Et₂O, 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). ¹H-NMR ((D₆)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'), CH₂(5')); 2.50–2.30 (m, CH₂(2')); 2.10 (s, 2 AcO). Anal. calc. for C₃₄H₂₈N₄O₁₂ · H₂O (702.6): C 58.12, H 4.30, N 7.97; found: C 58.34, H 4.34, N 7.95.

2'-Deoxy-N⁴-[(fluorescein-5-ylamino)carbonyl]cytidine (= 2'-Deoxy-N⁴-[(3',6'-dihydroxy-3-oxospiro[isobenzofuran-1(3H),9'-[9H]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 K₂CO₃ (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 H₂O, EtOH, and Et₂O, 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). ¹H-NMR ((D₆)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, CH₂(5')); 2.30 (m, 1 H–C(2')); 2.08 (m, 1 H–C(2')). Anal. calc. for C₃₀H₂₄N₄O₁₀ · H₂O (618.6): C 58.25, H 4.23, N 9.05; found: C 57.82, H 4.44, N 8.87.

2'-Deoxy-N⁴-[(fluorescein-5-ylamino)carbonyl]-3',5'-O-(1,1,3,3-tetraisopropylidisiloxane-1,3-diyl)cytidine (= 2'-Deoxy-N⁴-[(3',6'-dihydroxy-3-oxospiro[isobenzofuran-1(3H),9'-[9H]xanthen]-5-yl)amino]carbonyl]-3',5'-O-(1,1,3,3-tetraisopropylidisiloxane-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 (SiO₂ (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/CH₂Cl₂ 1:1): 219 (4.42), 231 (4.65), 291 (4.39), 371 (sh, 3.19), 455 (sh, 3.86), 485 (4.93). ¹H-NMR ((D₆)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, CH₂(5')); 3.83 (m, H–C(4')); 2.39 (m, CH₂(2')); 1.10–0.95 (m, 4 Me₂CH). Anal. calc. for C₄₂H₅₀N₄O₁₁Si₂ · H₂O (861.1): C 58.58, H 6.09, N 6.50; found: C 58.74, H 6.08, N 6.62.

2'-Deoxy-N⁴-[[3-{[2-(4-nitrophenyl)ethoxy]carbonyl}-4-{6-[2-(4-nitrophenyl)ethoxy]-3-oxo-3H-xanthen-9-yl]-phenyl}amino]carbonyl]-3',5'-O-(1,1,3,3-tetraisopropylidisiloxane-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° PPh₃ in abs. dioxane (5 ml), and the mixture was stirred for 1 h. The mixture was concentrated, the residue dissolved in CH₂Cl₂, the soln. washed with 1% KH₂PO₄ soln. (2 × 50 ml) and H₂O (2 × 50 ml), dried (Na₂SO₄), and concentrated, and the residue purified by CC (SiO₂ (6 × 30 cm), CH₂Cl₂ (100 ml), CH₂Cl₂/MeOH 195:5 (600 ml), CH₂Cl₂/MeOH 95:5 (400 ml), CH₂Cl₂/MeOH 94:6 (800 ml), and CH₂Cl₂/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/CH₂Cl₂ 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). ¹H-NMR ((D₆)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 NO₂); 8.03 (d, 2 arom. H *o* to NO₂, H–C(6)); 7.89 (dd, H–C(6_{arom.})); 7.63 (d, 2 arom. H *m* to NO₂); 7.36 (d, H–C(5_{arom.}), 2 arom. H *m* to NO₂); 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'), CH₂(α)); 4.28 (t, CH₂(α)); 4.05 (m, CH₂(5')); 3.80 (m, H–C(4')); 3.21 (t, CH₂(β)); 2.81 (t, CH₂(β)); 2.40 (m, CH₂(2')); 1.10–0.95 (m, 4 Me₂CH). Anal. calc. for C₃₈H₆₃N₆O₁₅Si₂ (1140.3): C 61.09, H 5.57, N 7.37; found: C 61.03, H 5.74, N 7.55.

2'-Deoxy-N⁴-{[3-{[2-(4-nitrophenyl)ethoxy]carbonyl}-4-{6-[2-(4-nitrophenyl)ethoxy]-3-oxo-3H-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 Bu₄NF·3 H₂O (1.68 g, 5.33 mmol). The soln. was diluted with CH₂Cl₂ (200 ml) and washed with H₂O (100 ml) whereby a part of the product separated out. The solid was filtered off, and the org. phase dried (Na₂SO₄) 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/CH₂Cl₂ 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). ¹H-NMR ((D₆)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 NO₂); 8.03 (d, 2 arom. H *o* to NO₂, H–C(6)); 7.89 (d, H–C(6_{arom.})); 7.61 (d, 2 arom. H *m* to NO₂); 7.37 (d, H–C(5_{arom.}), 2 arom. H *m* to NO₂); 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, CH₂(α)); 4.23 (t, H–C(3'), CH₂(α)); 3.88 (m, H–C(4')); 3.60 (m, CH₂(5')); 3.21 (t, CH₂(β)); 2.83 (t, CH₂(β)); 2.28 (m, 1 H–C(2')); 2.10 (m, 1 H–C(2')). Anal. calc. for C₄₆H₅₈N₆O₁₄ (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)-N⁴-{[3-{[2-(4-nitrophenyl)ethoxy]carbonyl}-4-{6-[2-(4-nitrophenyl)ethoxy]-3-oxo-3H-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 CH₂Cl₂ (400 ml) and extracted with sat. NaHCO₃ soln. (3 × 100 ml). The org. phase was dried (Na₂SO₄) and concentrated and the residue subject to FC (SiO₂, 150 g), CH₂Cl₂/MeOH 99:1 (300 ml), CH₂Cl₂/MeOH 98:2 (300 ml), CH₂Cl₂/MeOH 97:3 (300 ml), CH₂Cl₂/MeOH 95:5 (600 ml), and CH₂Cl₂/MeOH 96:4 (400 ml). The residue of the product fractions was dried: 7.0 g (83%) of **20**. Orange-red solid. UV (MeOH/CH₂Cl₂ 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). ¹H-NMR ((D₆)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 NO₂, H–C(6)); 8.06 (d, 2 arom. H *o* to NO₂); 7.89 (d, H–C(6_{arom.})); 7.60 (d, 2 arom. H *m* to NO₂); 7.41–7.20 (m, 12 H, H–C(5_{arom.}), 2 arom. H *m* to NO₂, 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 CH₂(α)); 3.99 (m, H–C(4')); 3.71 (s, 2 MeO); 3.31–3.15 (m, CH₂(5'), CH₂(β)); 2.82 (t, CH₂(β)); 2.50–2.10 (m, CH₂(2')). ³¹P-NMR ((D₆)DMSO): 147.72; 147.63. Anal. calc. for C₆₇H₅₆N₆O₁₆ (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)-N⁴-{[3-{[2-(4-nitrophenyl)ethoxy]carbonyl}-4-{6-[2-(4-nitrophenyl)ethoxy]-3-oxo-3H-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 1H-tetrazole (38 mg, 0.54 mmol) in CH₂Cl₂ (10 ml) under N₂ 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 CH₂Cl₂ (100 ml), and washed with sat. NaHCO₃ soln. The org. layer was dried (Na₂SO₄) and concentrated, and the residue purified by CC (SiO₂ (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 CH₂Cl₂ and slowly added dropwise into abs. Et₂O. The resulting precipitate was collected under N₂ and dried under high vacuum: 0.818 g (82%) of **21**. Orange-red powder. UV (MeOH/CH₂Cl₂ 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). $^1\text{H-NMR}$ ((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 NO₂, H–C(6)); 7.90 (*d*, H–C(6_{arom.})); 7.62 (*d*, 2 arom. H *m* to NO₂); 7.50–7.19 (*m*, H–C(5_{arom.}), 2 arom. H *m* to NO₂, 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 (*2d*, H–C(5), H–C(xan)); 6.15 (*m*, H–C(1'), H–C(xan)); 4.41 (*t*, H–C(3'), CH₂(α)); 4.27 (*t*, CH₂(α)); 4.03 (*m*, H–C(4')); 3.72 (*m*, CH₂O–P); 3.69 (*s*, 2 MeO); 3.41 (*m*, 2 Me₂CH); 3.21 (*m*, CH₂(5'), CH₂(β)); 3.00–2.79 (*m*, 2 CH₂(β)); 2.49–2.10 (*m*, CH₂(2')); 1.10–0.90 (*m*, 2 Me₂CH). Anal. calc. for C₈₁H₇₇N₈O₁₉P (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)-N⁴-{[3-{[2-(4-nitrophenyl)ethoxy]carbonyl]-4-[6-[2-(4-nitrophenyl)ethoxy]-3-oxo-3H-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), 1H-tetrazole (38 mg, 0.54 mmol), CH₂Cl₂ and MeCN under N₂, 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/CH₂Cl₂ 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). $^1\text{H-NMR}$ ((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 NO₂, H–C(6)); 8.04 (*d*, 2 arom. H *o* to NO₂); 7.88 (*d*, H–C(6_{arom.})); 7.62 (*d*, 2 arom. H *m* to NO₂); 7.41–7.19 (*m*, H–C(5_{arom.}), 2 arom. H *m* to NO₂, 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*, CH₂(α)); 4.24 (*t*, CH₂(α)); 4.12 (*m*, H–C(4')); 3.72 (*s*, 2 MeO); 3.70–3.40 (*m*, CH₂O–P, 2 Me₂CH, CH₂(5')); 3.22 (*m*, CH₂(β)); 2.84–2.60 (*m*, CH₂CN, CH₂(β)); 2.37 (*m*, CH₂(2')); 1.16–0.95 (*m*, 2 Me₂CH). $^{31}\text{P-NMR}$ ((D_6) DMSO): 148.62; 148.31. Anal. calc. for C₇₆H₇₃N₈O₁₇P (1401.4): C 65.14, H 5.25, N 7.99; found: C 64.90, H 5.71, N 7.34.

2'-Deoxy-N⁴,3'-O-bis[2-(4-nitrophenyl)ethoxy]carbonyl]cytidine (**23**). A soln. of 2'-deoxy-5'-O-(monomethoxytrityl)-N⁴,3'-O-bis[2-(4-nitrophenyl)ethoxy]carbonyl]cytidine (0.885 g, 1 mmol) in 1% TsOH in CH₂Cl₂/MeOH 4:1 (40 ml) was stirred at r.t. for 30 min. The mixture was diluted with CHCl₃ (60 ml) and washed with phosphate buffer (pH 7; 3 \times 100 ml), the org. phase dried (Na₂SO₄) and concentrated, and the residue purified by CC (SiO₂ 2.5 \times 25 cm), CH₂Cl₂, CHCl₃/MeOH 100:1, and CHCl₃/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). $^1\text{H-NMR}$ ((CDCl₃): 8.40 (br. s, NH); 8.20 (*d*, H–C(6)); 8.12 (*d*, 2 arom. H *o* to NO₂); 8.10 (*d*, 2 arom. H *o* to NO₂); 7.38 (*d*, 2 arom. H *m* to NO₂); 7.34 (*d*, 2 arom. H *m* to NO₂); 7.15 (*d*, H–C(5)); 6.16 (*m*, H–C(1')); 5.27 (*d*, H–C(3')); 4.37 (*t*, 2 CH₂(α)); 4.20 (br. s, H–C(4')); 3.8 (*m*, CH₂(5')); 3.75 (br. s, OH–C(5')); 3.05 (*t*, 2 CH₂(β)); 2.64 (*m*, 1 H–C(2')); 2.37 (*m*, 1 H–C(2')). Anal. calc. for C₂₇H₂₇N₅O₁₂ (613.5): C 52.86, H 4.44, N 11.41; found: C 52.86, H 4.50, N 11.32.

2'-Deoxy-N⁴,5'-O-bis[2-(4-nitrophenyl)ethoxy]carbonyl]cytidine (**24**). A soln. of 2'-deoxy-N⁴-[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. CH₂Cl₂ (40 ml) was added dropwise. After stirring for 3 h at –20°, the mixture was diluted with CH₂Cl₂ (300 ml) and washed with H₂O, the org. layer dried (Na₂SO₄) and concentrated, and the residue purified by CC (SiO₂ 4.5 \times 20 cm), CH₂Cl₂ (300 ml), CH₂Cl₂/MeOH 98:2 (600 ml), and CH₂Cl₂/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). $^1\text{H-NMR}$ ((CDCl₃): 8.42 (br. s, NH); 8.15 (*dd*, 4 arom. H *o* to NO₂); 7.99 (*d*, H–C(6)); 7.37 (*d*, 4 arom. H *m* to NO₂); 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'), CH₂(5'), 2 CH₂(α)); 3.09 (*t*, 2 CH₂(β)); 2.70 (*m*, 1 H–C(2')); 2.08 (*m*, 1 H–C(2')). Anal. calc. for C₂₇H₂₇N₅O₁₂ (613.5): C 52.86, H 4.44, N 11.41; found: C 53.03, H 4.57, N 10.94.

2'-Deoxy-N⁴,5'-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), 1H-tetrazole (70 mg, 1 mmol), CH₂Cl₂ (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 (SiO₂ 3 \times 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). $^1\text{H-NMR}$ (CDCl₃): 8.20–8.15 (*m*, 6 arom. H *o* to NO₂); 7.43 (*d*, H–C(6));

7.44–7.36 (*m*, 6 arom. H *m* to NO₂); 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'), CH₂(5')), 2 CH₂(*α*)); 3.90–3.71 (*m*, CH₂O–P); 3.60–3.48 (*m*, 2 Me₂CH); 3.16–2.99 (*m*, 3 CH₂(β)); 2.65 (*m*, 1 H–C(2')); 2.03 (*m*, 1 H–C(2')); 1.13–1.04 (*m*, 2 Me₂CH). ³¹P-NMR: 148.79; 148.66. Anal. calc. for C₄₁H₄₈N₇O₁₅P (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)-N⁴-{[3-{[2-(4-nitrophenyl)ethoxy]carbonyl}-4-{6-[2-(4-nitrophenyl)ethoxy]-3-oxo-3H-xanthen-9-yl]phenyl}amino]carbonyl}-O^P-[2-(4-nitrophenyl)ethyl]cytidyl-3' → 5'-2'-deoxy-N⁴,3'-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 CH₂Cl₂ (20 ml) and MeCN (10 ml) under N₂. 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 I₂ (0.5 g) in CH₂Cl₂/pyridine/H₂O 1:3:1 (4 ml) for 30 min. The mixture was diluted with CH₂Cl₂ (150 ml) and the soln. extracted with a sat. soln. of Na₂S₂O₃/NaCl (3 × 100 ml). The aq. phases were reextracted with CH₂Cl₂ (50 ml). The combined org. extract was dried (Na₂SO₄) and concentrated, 3 × the residue dissolved in toluene (100 ml), and the soln. concentrated, and the residue purified by CC (SiO₂ (1 × 10 cm), CH₂Cl₂ (100 ml), CH₂Cl₂/MeOH 98:2 (100 ml), CH₂Cl₂/MeOH 96:4 (200 ml), and CH₂Cl₂/MeOH 95:5 (100 ml)). The residue of the product fractions was dissolved in little CH₂Cl₂/MeOH 1:1, and this soln. slowly added dropwise to cold (0°) MeOH (25 ml). The precipitate was washed with MeOH and Et₂O 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). ¹H-NMR ((D₆)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 NO₂); 7.89 (*d*, H–C(6_{arom.})); 7.65–7.18 (*m*, 21 H, 10 arom. H *m* to NO₂, 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 CH₂(*α*), 2 H–C(4'), CH₂(5')); 3.70 (*s*, 2 MeO); 3.30–2.83 (*m*, 5 CH₂(β), CH₂(5')); 2.50–2.20 (*m*, 2 CH₂(2')). ³¹P-NMR ((D₆)DMSO): –1.51. Anal. calc. for C₁₀₂H₈₉N₁₂O₃₂P (2025.9): C 60.47, H 4.43, N 8.30; found: C 60.26, H 4.58, N 8.23.

2'-Deoxy-N⁴-{[3-{[2-(4-nitrophenyl)ethoxy]carbonyl}-4-{6-[2-(4-nitrophenyl)ethoxy]-3-oxo-3H-xanthen-9-yl]phenyl}amino]carbonyl}-O^P-[2-(4-nitrophenyl)ethyl]cytidyl-3' → 5'-2'-deoxy-N⁴,3'-O-bis{[2-(4-nitrophenyl)ethoxy]carbonyl}cytidine (**27**). To 1% TsOH in CH₂Cl₂/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 CH₂Cl₂ (150 ml), and the soln. extracted with sat. NaHCO₃ soln. (3 × 80 ml). The aq. extracts were reextracted with CH₂Cl₂ (50 ml). Then the combined org. phase was dried (Na₂SO₄) and concentrated and the resulting solid purified by CC (SiO₂ (1.5 × 8 cm), CH₂Cl₂ (50 ml), CH₂Cl₂/MeOH 98.5:1:5 (50 ml), CH₂Cl₂/MeOH 96:4 (50 ml), and CH₂Cl₂/MeOH 94:6 (100 ml)). The residue of the product fractions was dissolved in little CH₂Cl₂/MeOH 1:1, and the soln. slowly and dropwise added to cold (0°) MeOH (25 ml). The precipitate was washed with Et₂O 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). ¹H-NMR ((D₆)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 NO₂); 7.88 (*d*, H–C(6_{arom.})); 7.65–7.48 (*m*, 8 arom. H *m* to NO₂); 7.40–7.30 (*m*, 2 arom. H *m* to NO₂, 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 CH₂(*α*), 2 H–C(4'), CH₂(5')); 3.58 (*m*, CH₂(5')); 3.30–2.80 (*m*, 5 CH₂(β)); 2.43–2.21 (*m*, 2 CH₂(2')). ³¹P-NMR ((D₆)DMSO): –1.53; –1.42. Anal. calc. for C₈₁H₇₁N₁₂O₃₀P (1723.5): C 56.45, H 4.15, N 9.75; found: C 56.02, H 4.31, N 9.69.

2'-Deoxy-N⁴,5'-O-bis{[2-(4-nitrophenyl)ethoxy]carbonyl}-O^P-[2-(4-nitrophenyl)ethyl]cytidyl-3' → 5'-2'-deoxy-N⁴,3'-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. CH₂Cl₂ (10 ml) was concentrated, and 2 × the residue dissolved in abs. toluene (10 ml), and the soln. concentrated. The residue was dissolved in CH₂Cl₂ (15 ml) and MeCN (8 ml), and under N₂, **25** (0.45 g, 0.5 mmol) was added and the mixture stirred overnight. After treatment with I₂ (0.5 g) in CH₂Cl₂/pyridine/H₂O 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 $\text{Na}_2\text{S}_2\text{O}_3/\text{NaCl}$ (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), $\text{CH}_2\text{Cl}_2/\text{MeOH}$ 98:2 (100 ml), $\text{CH}_2\text{Cl}_2/\text{MeOH}$ 96:4 (200 ml), $\text{CH}_2\text{Cl}_2/\text{MeOH}$ 95:5 (300 ml), and $\text{CH}_2\text{Cl}_2/\text{MeOH}$ 93:7)). The residue of the product fractions was dissolved in little $\text{CH}_2\text{Cl}_2/\text{MeOH}$ 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). $^1\text{H-NMR}$ ((D_6) DMSO): 11.70 (s, NH); 10.80 (s, NH); 10.38 (s, NH); 8.27 (s, $\text{H-C}(2_{\text{arom.}})$); 8.22–7.90 (m, 3 $\text{H-C}(6)$, 16 arom. H *o* to NO_2); 7.86 (d, $\text{H-C}(6_{\text{arom.}})$); 7.70–7.30 (m, 16 arom. H *m* to NO_2 , $\text{H-C}(5_{\text{arom.}})$); 7.19 (s, $\text{H-C}(5'_{\text{xan}})$); 6.98 (m, 2 $\text{H-C}(5)$); 6.83 (m, $\text{H-C}(1'_{\text{xan}})$, $\text{H-C}(7'_{\text{xan}})$, $\text{H-C}(8'_{\text{xan}})$); 6.40 (m, $\text{H-C}(5)$, $\text{H-C}(2'_{\text{xan}})$); 6.12 (m, 3 $\text{H-C}(1')$, $\text{H-C}(4')$); 5.10 (m, $\text{H-C}(3')$); 4.92 (m, 2 $\text{H-C}(3')$); 4.45–4.20 (m, 8 $\text{CH}_2(\alpha)$, 3 $\text{H-C}(4')$, 3 $\text{CH}_2(5')$); 3.26 (t, $\text{CH}_2(\beta)$); 3.12 (m, 6 $\text{CH}_2(\beta)$); 2.81 (t, $\text{CH}_2(\beta)$); 2.45–2.20 (m, 3 $\text{CH}_2(2')$). $^{31}\text{P-NMR}$ ((D_6) DMSO): –1.48; –1.45; –1.36. Anal. calc. for $\text{C}_{113}\text{H}_{104}\text{N}_{18}\text{O}_{46}\text{P}_2$ (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-N⁴-[(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*), $^i\text{PrOH}/25\%$ 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). $^1\text{H-NMR}$ (D_2O): 8.04 (d, $\text{H-C}(6)$); 7.90 (s, $\text{H-C}(2_{\text{arom.}})$); 7.73 (d, $\text{H-C}(6)$); 7.61 (d, $\text{H-C}(6)$); 7.43 (d, $\text{H-C}(6_{\text{arom.}})$); 7.21 (d, $\text{H-C}(5_{\text{arom.}})$); 7.09 (d, $\text{H-C}(5)$); 7.00 (d, $\text{H-C}(5)$); 6.53 (m, $\text{H-C}(2')$, $\text{H-C}(7'_{\text{xan}})$); 6.42 (s, $\text{H-C}(4'_{\text{xan}})$, $\text{H-C}(6'_{\text{xan}})$); 6.22 (d, $\text{H-C}(5)$); 6.15 (dd, $\text{H-C}(1'_{\text{xan}})$); 5.98 (dd, $\text{H-C}(1')$); 5.89 (m, $\text{H-C}(1'_{\text{xan}})$, $\text{H-C}(5'_{\text{xan}})$); 5.71 (d, $\text{H-C}(5)$); 4.39 (m, $\text{H-C}(3')$); 4.21 (m, 2 $\text{H-C}(3')$); 4.05–3.90 (m, 3 $\text{CH}_2(5')$); 3.63 (m, 3 $\text{H-C}(4')$); 2.60–2.00 (m, 3 $\text{CH}_2(2')$).

2'-Deoxy-5'-O-(4,4'-dimethoxytrityl)-N⁴-[[2-(4-nitrophenyl)ethoxy]carbonyl]cytidine 3'-(Hydrogen Butanedioate) (=1-[Tetrahydro-5-[4-[[2-(4-nitrophenyl)ethoxy]carbonyl]amino]-2-oxopyrimidin-1(2H)-yl]-2-[[bis(4-methoxyphenyl)phenylmethoxy]methyl]furan-3-yl] Hydrogen Butanedioate; 30). A soln. of 2'-deoxy-5'-O-(4,4'-dimethoxytrityl)-N⁴-[[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). $^1\text{H-NMR}$ (CDCl_3): 8.25–8.15 (m, 2 arom. H *o* to NO_2 , NH, $\text{H-C}(6)$); 7.45–7.12 (m, 11 arom. H), 6.95 (d, $\text{H-C}(5)$); 6.85 (m, 4 arom. H *o* to MeO); 6.24 (dd, $\text{H-C}(1')$); 4.52 (m, $\text{H-C}(3')$); 4.40 (t, OCH_2CH_2); 4.26 (m, $\text{H-C}(4')$); 3.78 (s, MeO); 3.45–3.35 (m, $\text{CH}_2(5')$); 3.09 (t, OCH_2CH_2); 2.85 (m, 1 $\text{H-C}(2')$); 2.70–2.50 (m, $\text{OCCH}_2\text{CH}_2\text{CO}$); 2.35–2.20 (m, 1 $\text{H-C}(2')$). Anal. calc. for $\text{C}_{43}\text{H}_{42}\text{N}_4\text{O}_{13}$ (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 ($A = 0.1\text{M Et}_4\text{N}(\text{OAc})$, $B = \text{Et}_4\text{N}(\text{OAc})/\text{MeCN } 1:1$; 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.

REFERENCES

- [1] C. Henke, W. Pfeleiderer, *Nucleosides, Nucleotides Nucleic Acids* **2005**, 24, 1665.
- [2] F. Sanger, S. Nicklen, A. R. Coulson, *Proc. Natl. Acad. Sci. U.S.A.* **1977**, 74, 5463.
- [3] E. M. Southern, *J. Mol. Biol.* **1975**, 98, 503.
- [4] L. M. Smith, J. Z. Sanders, R. J. Kaiser, P. Hughes, C. Dodd, C. R. Connell, C. Heiner, S. B. H. Kent, L. E. Hood, *Nature (London, U.K.)* **1986**, 321, 674.
- [5] W. Ansorge, B. Sproat, J. Stegemann, C. Schwager, M. Zenke, *Nucleic Acids Res.* **1987**, 15, 4593.
- [6] A. E. Karger, J. M. Harris, R. F. Gesteland, *Nucleic Acids Res.* **1991**, 19, 4955.
- [7] S. Agrawal, C. Christodoulou, M. Gait, *Nucleic Acids Res.* **1986**, 14, 6227.
- [8] N. T. Thuong, M. Chassignol, *Tetrahedron Lett.* **1988**, 29, 5905.
- [9] T. Tanaka, Y. Yamada, M. Ikehara, *Chem. Pharm. Bull.* **1988**, 36, 1386.
- [10] B. A. Connolly, *Nucleic Acids Res.* **1997**, 15, 3131.
- [11] S. Agrawal, P. C. Zamecnik, *Nucleic Acids Res.* **1990**, 18, 5419.
- [12] J. Y. Tang, S. Agrawal, *Nucleic Acids Res.* **1990**, 18, 6461.
- [13] U. Asseline, N. T. Thuong, *Nucleosides, Nucleotides Nucleic Acids* **1991**, 10, 359.
- [14] L. M. Smith, S. Fung, M. W. Hunkapiller, T. J. Hunkapiller, L. E. Hood, *Nucleic Acids Res.* **1985**, 13, 2399.
- [15] K. Yamana, T. Gokota, H. Ozaki, H. Nakano, O. Sangen, T. Shimidzu, *Nucleosides, Nucleotides Nucleic Acids* **1992**, 11, 383.
- [16] H. Sigmund, W. Pfeleiderer, *Helv. Chim. Acta* **2003**, 86, 2299.
- [17] P. R. Langer, D. C. Waldrop, D. C. Ward, *Proc. Natl. Acad. Sci. U.S.A.* **1981**, 78, 6633.
- [18] A. Roget, H. Bazin, R. Teoule, *Nucleic Acids Res.* **1989**, 17, 7643.
- [19] R. Kierzek, W. T. Markiewicz, *Nucleosides, Nucleotides Nucleic Acids* **1991**, 10, 1257.
- [20] J. Telser, K. A. Cruickshank, L. E. Morrison, J. Netzel, *J. Am. Chem. Soc.* **1989**, 111, 6966.
- [21] J. L. Ruth, *DNA* **1984**, 3, 123.
- [22] J. L. Ruth, C. Morgan, A. Pasko, *DNA* **1985**, 4, 93.
- [23] B. S. Sproat, B. Beijer, P. Rider, P. Neuner, *Nucleic Acids Res.* **1987**, 15, 4837.
- [24] D. Singh, V. Kumar, K. N. Ganesh, *Nucleic Acids Res.* **1990**, 18, 3339.
- [25] S. R. Sarfati, A. Namane, *Tetrahedron Lett.* **1990**, 31, 2581.
- [26] S. R. Sarfati, S. Pochet, C. Guerreiro, A. Namane, T. Huynh-Dinh, J. Igolen, *Tetrahedron* **1987**, 43, 3491.
- [27] E. Krzymańska-Olejnik, R. W. Adamiak, *Nucleosides Nucleotides* **1991**, 10, 595.
- [28] B. S. Sproat, B. Beijer, P. Rider, *Nucleic Acids Res.* **1987**, 15, 6181.
- [29] H. Sigmund, T. Maier, W. Pfeleiderer, *Nucleosides, Nucleotides Nucleic Acids* **1997**, 16, 685.
- [30] H. Lang, M. Gottlieb, M. Schwarz, S. Farkas, B. S. Schulz, F. Himmelsbach, R. Charubala, W. Pfeleiderer, in 'Perspectives in Nucleoside and Nucleic Acid Chemistry', Eds. M. V. Kisakürek and H. Rosemeyer, Verlag Helvetica Chimica Acta, Zürich, Wiley–VCH, Weinheim, 2000, p. 61.
- [31] R. Kierzek, H. Ito, R. Bhatt, K. Itakura, *Tetrahedron Lett.* **1981**, 22, 3761.
- [32] W. T. Markiewicz, *J. Chem. Res. Synop.* **1979**, 24.
- [33] H. Schirmeister, F. Himmelsbach, W. Pfeleiderer, *Helv. Chim. Acta* **1993**, 76, 385.
- [34] K.-P. Stengele, W. Pfeleiderer, *Tetrahedron Lett.* **1990**, 31, 2549.
- [35] H. Lang, M. Gottlieb, M. Schwarz, S. Farkas, B. S. Schulz, F. Himmelsbach, R. Charubala, W. Pfeleiderer, *Helv. Chim. Acta* **1999**, 82, 2172.
- [36] R. W. Adamiak, J. Stawiński, *Tetrahedron Lett.* **1977**, 18, 1935.

- [37] R. W. Sobol, E. E. Hendersen, N. Kon, J. Shao, P. Hitzges, C. Mordechai, N. L. Reichenbach, R. Charubala, H. Schirmeister, W. Pfeleiderer, R. J. Suhadolnik, *J. Biol. Chem.* **1995**, 270, 5963.
- [38] N. D. Sinha, J. Biernat, J. McManus, H. Köster, *Nucleic Acids Res.* **1984**, 12, 4539.
- [39] A. Kraszewski, K. E. Norris, *Nucleic Acids Res. Symp. Ser.* **1987**, 18, 177.
- [40] F. Himmelsbach, B. S. Schulz, T. Trichtinger, R. Charubala, W. Pfeleiderer, *Tetrahedron* **1984**, 40, 59.
- [41] S. R. Waldvogel, W. Pfeleiderer, *Helv. Chim. Acta* **1998**, 81, 46.
- [42] K.-P. Stengele, W. Pfeleiderer, *Nucleic Acids Res. Symp. Ser.* **1989**, 21, 101.

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