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NUCLEOTIDES PART: LXXVII¹ NEW TYPES OF FLUORESCENCE LABELING OF 2'-DEOXYGUANOSINE

Thomas Maier and Wolfgang Pfeleiderer*

Fachbereich Chemie, Universität Konstanz, 78457 Konstanz, Germany
Wolfgang.Pfeleiderer@uni-konstanz.de

Abstract - Protected 3',5'-diacetyl- (**5**) and 3',5'-*O*-(tetraisopropylidisiloxan-1,3-diyl)-2'-deoxyguanosine (**6**) have been labeled with 5-aminofluorescein (**9**) to form **10** and **11** which reacted under Mitsunobu conditions with 2-(4-nitrophenyl)ethanol to **13** and **14**, respectively. **14** could also be synthesized directly from **6** with **15** in one step. Selective desilylation with fluoride ion led from **1** to **12** and from **14** to **16**. Dimethoxytritylation of **16** gave **18** which was further converted into the building blocks **19-21**. The synthesis of the 2'-deoxyguanosyl[3'→5']-2'-deoxy-*N*²-{[(fluorescein-5-yl)amino]carbonyl}guanosyl[3'→5']-2'-deoxyguanosine trimer (**28**) was achieved by step-wise built-up from **20** with **22** to **23** and followed by coupling of **24** with **26** yielding **27**. Due to the unified blocking group pattern in **27** fully deprotection afforded only one step by treatment with DBU to give **28**. Several oligo-2'-deoxynucleotides containing fluorescein labeled 2'-deoxyguanosines at various positions of the chain were formed in a DNA-synthesizer and their fluorescence properties and the T_m s of the corresponding duplexes measured.

Dedicated to Prof. Albert Eschenmoser on the occasion of his 85th birthday and in admiration of his outstanding scientific achievements.

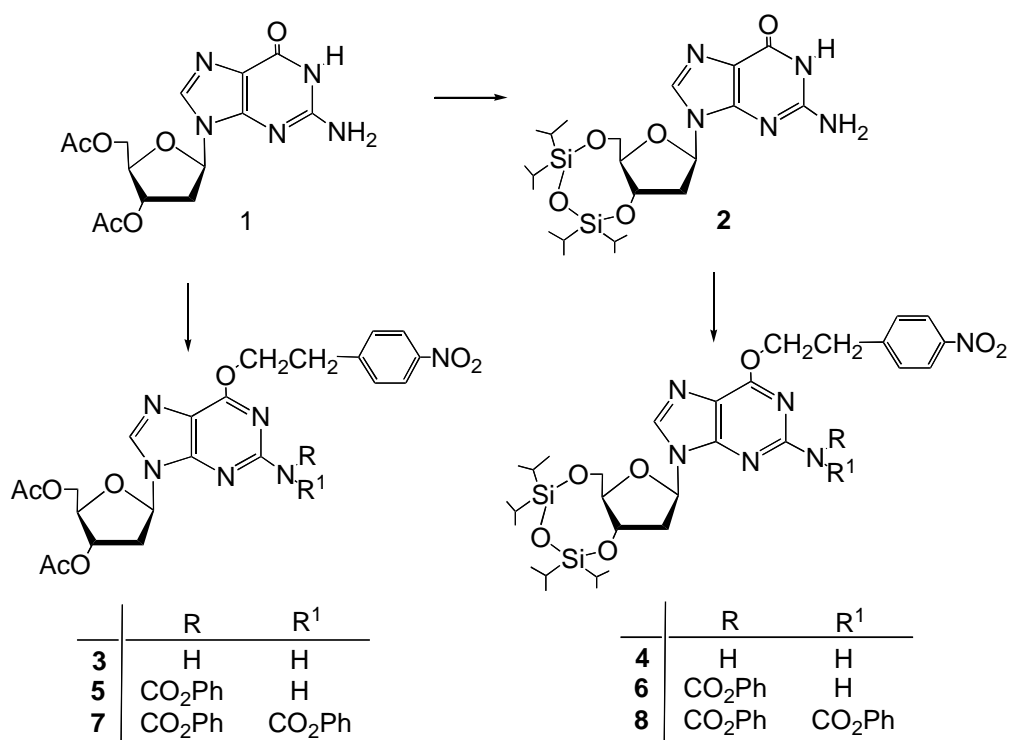
1. INTRODUCTION

In recent years the trend of labeling nucleotides and oligonucleotides has been switched from radioactive labeling² towards the use of fluorescent and biochemical markers³ to eliminate hazardous irradiation. All open positions in oligonucleotides have been subject to modifications by attachment of various types of

chromophores usually via flexible hydrocarbon linkers. The 3'- or 5'-end⁴ of the oligonucleotide, its phosphate bridge,⁵ the sugar moiety⁶ or the nucleobase⁷ have selectively been chosen from special reasons. In continuation of our efforts to label the nucleobases with fluorescein we investigated now after 2'-deoxy-adenosine⁸ and 2'-deoxycytidine⁹ the 2'-deoxyguanosine series.

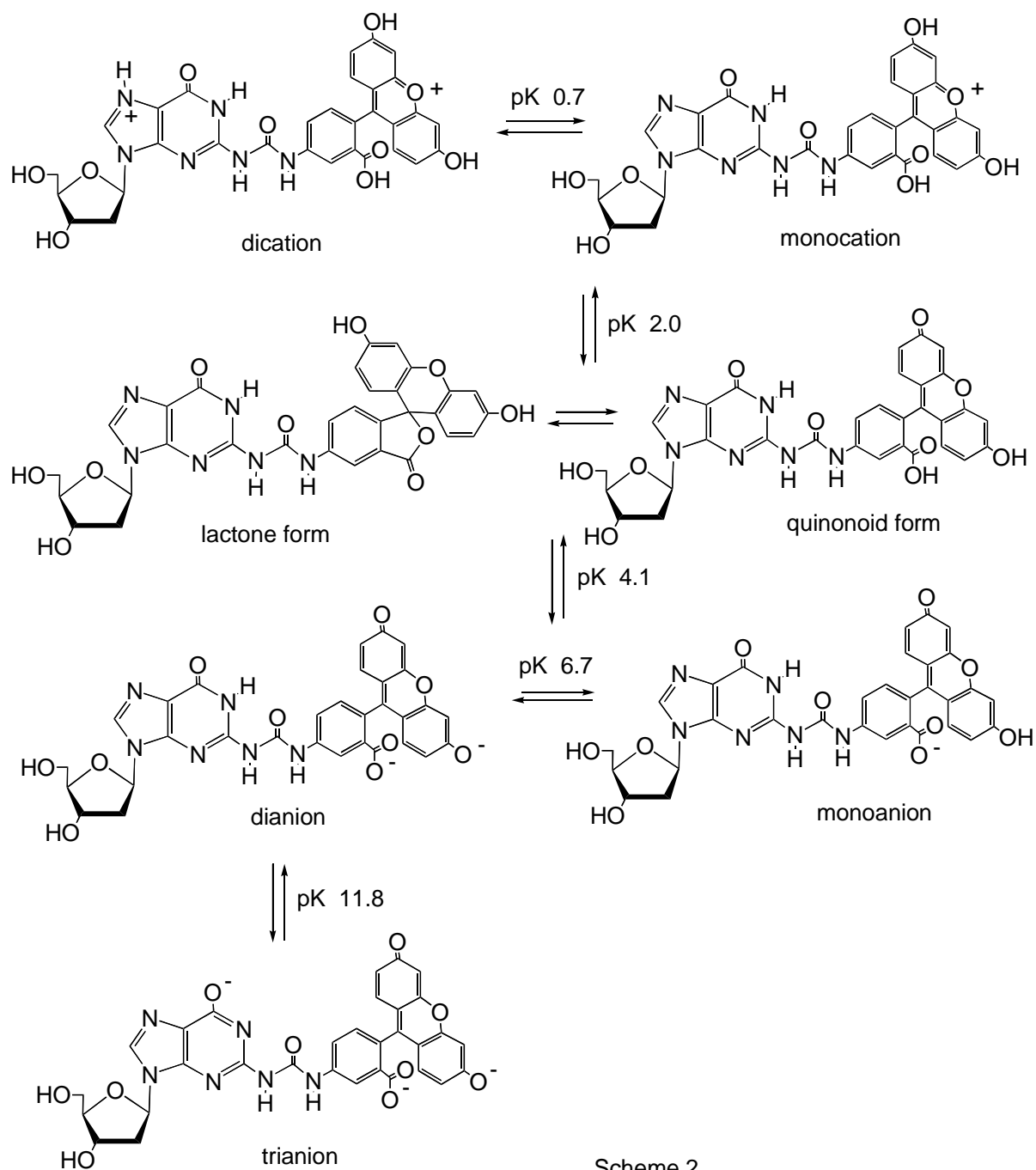
2. SYNTHESSES

The chemical modifications of 2'-deoxyguanosine was achieved in two parallel series of reactions starting from 3',5'-di-*O*-acetyl-2'-deoxy- (1)¹⁰ and 2'-deoxy-3',5'-*O*-(tetraisopropylidisiloxan-1,3-diyl)guanosine (2).¹¹ In the first step the 2-(4-nitrophenyl)ethyl- (NPE) group was introduced onto *O*⁶ by a Mitsunobu reaction yielding 3⁸ and 4,¹¹ respectively. The amino group was then protected by the phenoxycarbonyl group to give 5 and 6 and by prolonged heating a double protection could be achieved forming the *N*²-diphenoxycarbonyl derivatives 7 and 8 (Scheme 1).

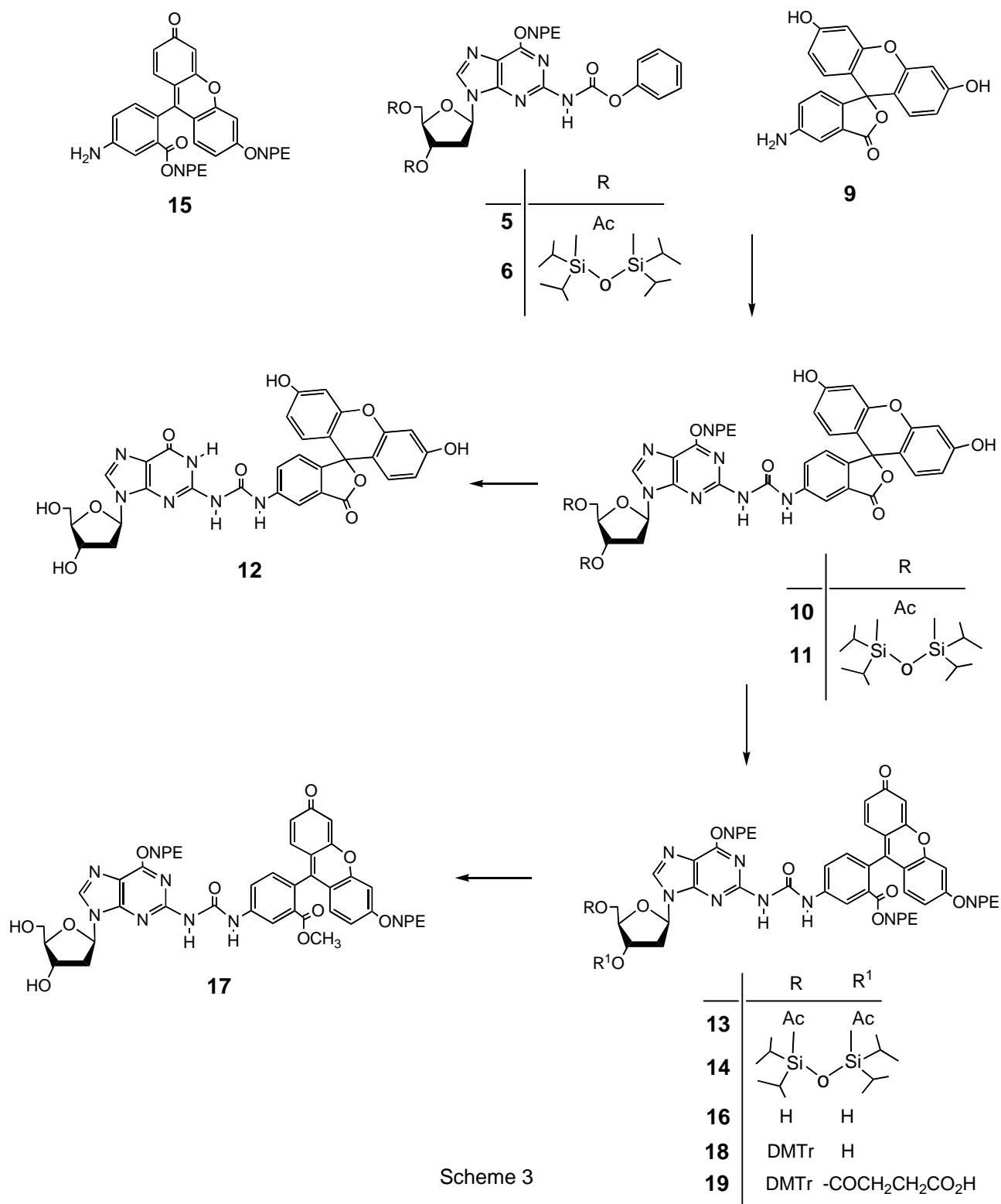


Scheme 1

The reaction of 5 and 6 respectively, with 5-aminofluorescein (9) proceeded in pyridine very well and formed the corresponding 2'-deoxy-*N*²-{[(fluorescein-5-yl)amino]carbonyl}-*O*⁶-[2-(4-nitrophenyl)ethyl]guanosine derivatives 10⁸ and 11 in almost quantitative yields (Scheme 3). Deblocking of 10 by treatment with DBU and followed by ammonia led to 2'-deoxy-*N*²-{[(fluorescein-5-yl)amino]carbonyl}guanosine (12) which was used to determine the pK_a values of the equilibria of the various molecular forms in the range of dication till trianion (Scheme 2).



The protection of the fluorescein moiety in **10** and **11**, respectively, by the NPE group could be achieved by a Mitsunobu reaction applying 2-(4-nitrophenyl)ethanol, triphenylphosphane and ethyl azodicarboxylate in dry dioxane to form **13** in 73% and **14** in 75% yield. Compound **14** was also obtained directly from **6** with 5-amino-2-{6-[2-(4-nitrophenyl)ethoxy]-3-oxo-3*H*-xanthen-9-yl}benzoic acid 2-(4-nitrophenyl)ethyl ester (**15**) (Scheme 3).



Deprotection of the sugar moiety worked best with **14** on treatment with tetrabutylammonium fluoride to yield **16** whereas deacetylation of **13** with dry K₂CO₃ in abs. MeOH resulted in a simultaneous transesterification to the corresponding benzoic acid methyl ester **17**. Compound **16** was further modified by dimethoxytritylation at the 5'-OH group yielding **18** and followed by reaction with succinic acid

anhydride to get the fully protected building block **19**.

Compound **18** was also the starting material for the syntheses of the the 3'-(4-nitrophenylethyl)- (**20**) and 3'-(2-cyanoethyl)phosphoramidite (**21**), respectively (Scheme 4). The strategy for the synthesis of a 2'-deoxyguanosine trimer (**28**) carrying the fluorescent label at the middle moiety was based on the idea to protect all functionalities by the same type of blocking groups consisting of the 2-(4-nitrophenyl)ethyl (NPE) and the 2-(4-nitrophenyl)ethyloxycarbonyl (NPEOC) group. In the first step the phosphoramidite **20** was coupled with 2'-{*O*⁶-[2-(4-nitrophenyl)ethyl]-2,3'-bis-[2-(4-nitrophenyl)ethyloxycarbonyl]}-guanosine (**22**)¹² to form **23** in 86% yield. Detritylation led to **24** which was condensed with **26** resulting from phosphorylation of 2'-{*O*⁶-[2-(4-nitrophenyl)ethyl]-2,5'-bis-[2-(4-nitrophenyl)ethyloxycarbonyl]}-guanosine (**25**)¹² to the fully protected 2'-deoxyguanosine trimer **27** in 85% yield. Due to the fact that all blocking groups could be cleaved by β -elimination with DBU the interconversion of **28** into the unprotected trimer **28** afforded only one step and was isolated after chromatography in 60% yield as ammonium salt. Trimer **28** was also synthesized in a DNA-synthesizer on solid support applying commercially available building blocks and **21** showing the same purity in HPLC-analysis. The synthesis of TTTTdG^{Flu} started with **19** attached via its succinate function to the LCMAA-CPG-support¹³ and followed by conventional chain-elongation with 5'-dimethoxytrityl-thymidine-3'-phosphoramidite. Furthermore three 21-mer oligomers carrying the fluorescence label at various positions were prepared in a DNA-synthesizer to study the fluorescence quantum yields in the single strand and duplex form (Table 1) and to determine the melting point of the duplexes in comparison to the non-modified standard (Table 2). The bulky label causes a substantial decrease in the melting point of the duplex and the fluorescence quantum yield is highly quenched if 2 or more fluorescence labels are adjacent in the oligonucleotide chain.

Table 1. Fluorescence Quantum Yields of Fluorescein-labeled 2'-Deoxyguanosines

Sequence	Quantum yield	
	Single strand	Duplex
Fluorescein	1.00	
dG ^{Flu} (12)	0.92	
d(G G ^{Flu} G) (29)	0.80	
T T T T dG ^{Flu}	0.87	
d(AAA GGG AAC AAA AG ^{Flu} C TGG GTA)	0.89	0.87
d(AAA G ^{Flu} G ^{Flu} G ^{Flu} AAC AAA AGC TGG GTA)	0.05	0.06
d(AAA G ^{Flu} GG ^{Flu} AAC AAA AG ^{Flu} C TGG GTA)	0.35	0.52

Measured in phosphate buffer pH 8 at 25°C; c (Na⁺) = 0.12 M; λ = 493 nm.

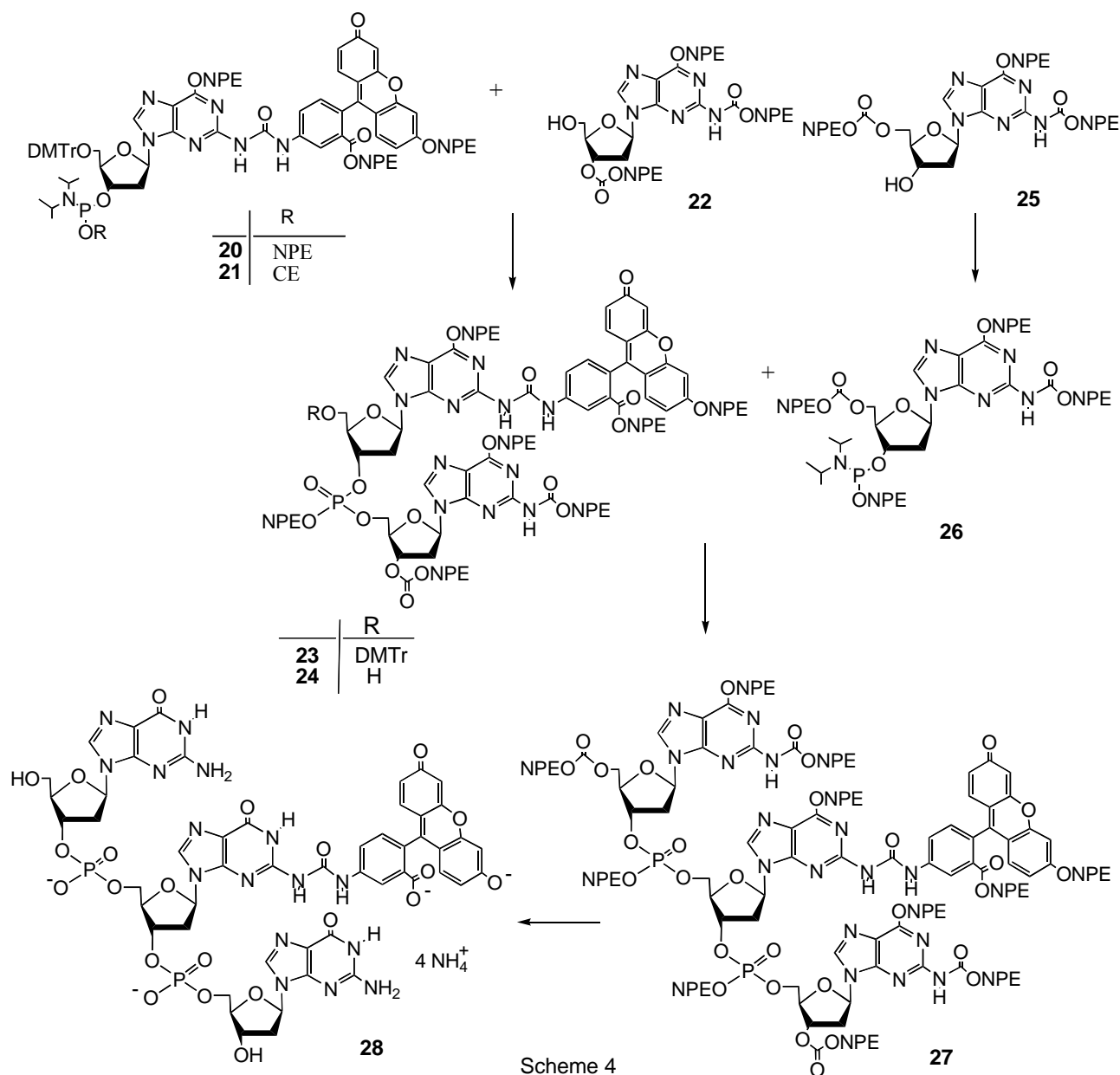


Table 2. Melting Points of Modified Oligonucleotides

Sequence	T_m at pH 8 °C
d(AAA GGG AAC AAA AGC TGG GTA)	60.5
d(TTT CCC TTG TTT TCG ACC CAT)	
d(AAA GGG AAC AAA AG ^{Flu} C TGG GTA)	55.7
d(TTT CCC TTG TTT T C G ACC CAT)	
d(AAA G ^{Flu} G ^{Flu} G ^{Flu} AAC AAA AGC TGG GTA)	54.9
d(TTT C C C TTG TTT TCG ACC CAT)	
d(AAA G ^{Flu} GG ^{Flu} AAC AAA AG ^{Flu} C TGG GTA)	50.6
d(TTT C C C TTG TTT T C G ACC CAT)	

Measured in phosphate buffer pH 8; $c(\text{Na}^+) = 0.12 \text{ M}$.

3. STRUCTURES

The newly synthesized 2'-deoxyguanosine derivatives have been characterized and proven in their structures by elemental analyses, ¹H-NMR- and UV-spectra.

Table 3. UV-Data of Fluorescein-labeled 2'-Deoxyguanosine Derivatives

Substance	λ_{\max} nm						in MeOH/CH ₂ Cl ₂ 1:1			log ϵ		
10	227	262	274	360	(429)	455 (482)	4.86	4.67	4.66	3.28 (3.52)	3.75 (3.76)	
11	228	261	275	362	(432)	456 482	4.85	4.66	4.65	3.33 (3.58)	3.80 3.81	
13	231	270	(304)	356	(437)	460 (489)	4.82	4.86	(4.28)	4.06 (4.31)	4.46 (4.35)	
14	230	268	(305)	353	(438)	459 (488)	4.84	4.90	(4.26)	4.08 (4.33)	4.45 (4.33)	
16	232	274	(306)	359	(438)	460 (488)	4.80	4.85	(4.25)	4.05 (4.30)	4.45 (4.34)	
17	231	274	(303)	356	(435)	459 (487)	4.79	4.79	(4.24)	4.04 (4.28)	4.43 (4.32)	
18	232	274	(302)	359	(438)	460 (489)	4.92	4.87	(4.31)	4.06 (4.31)	4.46 (4.36)	
19	231	274	(305)	357	(438)	460 (488)	4.94	4.87	(4.30)	4.09 (4.32)	4.45 (4.34)	
20	232	273	(305)	354	(439)	460 (487)	4.91	4.91	(4.33)	4.07 (4.30)	4.44 (4.33)	
21	232	267	(300)	357	(439)	460 (487)	4.90	4.88	(4.33)	3.98 (4.28)	4.44 (4.32)	
23-Dimer	233	270		354	(436)	460 (490)	4.98	5.08		3.98 (4.26)	4.43 (4.31)	
24-Dimer	232	271		352	(437)	460 (488)	4.93	5.10		4.05 (4.30)	4.45 (4.35)	
27-Trimer	222	270		352	(437)	460 (487)	5.02	5.27		4.12 (4.29)	4.45 (4.33)	

() = shoulder.

EXPERIMENTAL

Products were dried under high vacuum. All solvents used were of anhydrous grade. TLC: precoated silica gel thin-layer sheets 60 F254 from Merck. Flash Chromatography (FC): silica gel (Baker, 30-60 μm); 0.2-0.3 bar. Column Chromatography (CC). HPLC: pump L 6200, autosampler AS 4000, UV-detector L 4000, Merck-Hitachi; column RP 18, Lichrosorb, 125 \times 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 chromatography: DEAE-Sephadex A25, from Pharmacia Fine Chemicals. M. p.: Büchi 510 melting-point apparatus; no corrections. DNA-Synthesizer from Applied Biosystems Model 392. UV/VIS: Perkin-Elmer Lambda 5; λ_{\max} in nm (log ϵ). Fluorescence Spectra: Perkin-Elmer, model LS 50. Temperature dependent absorption spectra: Perkin-Elmer Lambda 2 with Peltier temperature

programmer. $^1\text{H-NMR}$: Bruker AC 250; δ in ppm rel. to SiMe_4 or CDCl_3 and $(\text{DMSO}d_6)$ as internal standard. $^{31}\text{P-NMR}$: Jeol JMN-GX400.

2'-Deoxy- O^6 -[2-(4-nitrophenyl)ethyl]-3',5'- O -(tetraisopropylidisiloxan-1,3-diyl)guanosine (4). A mixture of 2'-deoxy-3',5'- O -(tetraisopropylidisiloxan-1,3-diyl)guanosine (**2**)¹¹ (12.5 g, 24.5 mmol) 2-(4-nitrophenyl)ethanol (6.2 g, 36.7 mmol) and triphenylphosphane (9.65 g, 36.7 mmol) in dry dioxane (180 mL) was stirred at 80 °C for 1 h. Evaporation and twice coevaporation with dry dioxane. The residue was suspended in dry dioxane (100 mL) and then at 60 °C diethyl azodicarboxylate (5.7 mL, 36.7 mmol) dropwise added and stirred for 2 h. The solution was evaporated then aqueous potassium dihydrogenphosphate (1%, 100 mL) added and extracted with CH_2Cl_2 (4 x 100 mL). The organic layers were united, dried over Na_2SO_4 and evaporated to an oil, which was dissolved in CH_2Cl_2 (20 mL) and stored over night in the icebox. The resulting crystals of diethyl sym-hydrazinecarboxylate filtered off and washed with CH_2Cl_2 . The filtrate was evaporated, the residue dissolved in a mixture of CH_2Cl_2 (20 mL), Et_2O (30 mL) and *n*-hexane (20 mL) and put on a silica gel column (8 cm, 700 g) for FC with $\text{Et}_2\text{O}/n$ -hexane (7:3; 1 L) to remove all diethyl sym-hydrazinecarboxylate. The column was reconditioned with toluene/ EtOAc (3:1; 1 L) and the product eluted with toluene/EtOAc (2:1; 600 mL). Evaporation of the product fraction and coevaporation several times with CH_2Cl_2 yielded 13.5 g (85%) of a colorless solid foam. UV (MeOH): 210 (4.50), 252 (4.20), 280 (4.28). $^1\text{H-NMR}$ ($\text{DMSO}d_6$): 8.18 (d, 2H, *o* to NO_2); 7.98 (s, H-C(8)); 7.60 (d, 2H, *m* to NO_2); 6.42 (bs, NH_2); 6.13 (dd, H-C(1')); 4.93-4.72 (m, 3H, H-C(4'). H-C(5')); 4.71 (t, H-C(3')); 4.68 (t, OCH_2CH_2); 3.22 (t, OCH_2CH_2); 2.80-2.65 (m, H-C(2')); 2.50 (m, H-C(2'')); 1.18-0.95 (m, 28 H, isopropyl). Anal. Calcd for $\text{C}_{30}\text{H}_{46}\text{N}_6\text{O}_7\text{Si}_2$ (659.9): C 54.69, H 7.04, N 12.75. Found: C 54.43, H 7.09, N 12.59.

3',5'-Diacetyl-2'-deoxy- O^6 -[2-(4-nitrophenyl)ethyl]- N^2 -phenoxy carbonylguanosine (5). A solution of 3',5'-di-*O*-acetyl-2'-deoxy- O^6 -[2-(4-nitrophenyl)ethyl]guanosine (**3**)⁸ (1.5 g, 3 mmol) in dry pyridine was evaporated and then dissolved in dry pyridine (10 mL). The solution was cooled by ice and then phenyl chloroformate (0.53 ml, 4.2 mmol) slowly added and stirred for 1 h at 0 °C and another 1 h at rt. It was evaporated to half of the volume and a saturated aqueous NaHCO_3 solution was added and stirred for 10 min. Extraction by CH_2Cl_2 (3 x 50 mL), drying over Na_2SO_4 , evaporation and coevaporation by toluene. The residue was dissolved in toluene/ CH_2Cl_2 (1:1) and put on a silica gel column (20 g, 4 cm) for FC with toluene (200 mL), toluene/EtOAc (1:1, 400 mL), toluene/EtOAc (3:7, 200 mL) and EtOAc (100 mL). The main fractions were collected, evaporated and coevaporated with CH_2Cl_2 to give 1.5 g (80%) as a solid foam. UV (MeOH): 215 (4.58), 267 (4.44), 300 sh 3.69). $^1\text{H-NMR}$ ($\text{DMSO}d_6$): 10.64 (s, NH); 8.40 (s, H-C(8)); 8.13 (d, 2H, *o* to NO_2); 7.62 (d, 2H, *m* to NO_2); 7.42 (m, 2H, arom.H); 7.31-7.20 (m, 3H, arom.H); 6.31 (pt, H-C(1')); 5.41 (m, H.C(3')); 4.81 (t, OCH_2CH_2); 4.32-4.22(m, 3H, H-C(4'). H-C(5'));

3.28 (t, OCH_2CH_2); 3.25-3.18 (m, H-C(2')); 2.55 (m, H-C(2'')); 2.07 (s, 3H, Ac); 2.00 (s, 3H, Ac). Anal. Calcd for $\text{C}_{29}\text{H}_{28}\text{N}_6\text{O}_{10} \times 0.5 \text{H}_2\text{O}$ (629.5): C 55.33, H 4.64, N 13.34. Found: C 55.45, H 4.59, N 13.19.

2'-Deoxy- O^6 -[2-(4-nitrophenyl)ethyl]- N^2 -phenoxy carbonyl-3',5'- O -(tetraisopropylidisiloxan-1,3-diyl)guanosine (6). Analogous to the preceding procedure with **4** (5.0 g, 7.6 mmol) and phenyl chloroformate (1.2 ml, 10 mmol). After stirring 30 min at 0 °C and 3 h at rt the crude extract was dissolved in a little toluene and purified by FC (5 cm, 140 g flash silica gel) with toluene (200 mL), toluene/EtOAc (9:1, 200 mL), toluene/AcOEt (7:3, 200 mL), toluene/AcOEt (6:4, 300 mL). The first elution product (0.3 g, 4%) was identified as **8**. The main fraction was evaporated and coevaporated several times with CH_2Cl_2 to give 5.0 g (84%) of a solid foam. UV (CH_2Cl_2): 256 (4.34), 268 (4.44), 298 (sh 3.65). $^1\text{H-NMR}$ ($\text{DMSO-}d_6$): 10.98 (s, NH); 8.31 (s, H-C(8)); 8.14 (d, 2H, *o* to NO_2); 7.63 (d, 2H, *m* to NO_2); 7.44 (m, 2H, arom.H); 7.30-7.15 (m, 3H, arom.H); 6.24 (dd, H-C(1')); 4.82 (m, H-C(3')); 4.78 (t, OCH_2CH_2); 3.88 (m, 2H, H-C(5')); 3.82 (m, H-C(4')); 3.29 (t, OCH_2CH_2); 3.02-2.90 (m, H-C(2')); 2.49 (m, H-C(2'')); 1.10-0.95 (m, 28H, isopropyl). Anal. Calcd for $\text{C}_{37}\text{H}_{50}\text{N}_6\text{O}_9\text{Si}_2$ (779.0): C 57.05, H 6.47, N 10.78. Found: C 56.73, H 6.42, N 10.64.

3',5'-Diacetyl-2'-deoxy- O^6 -[2-(4-nitrophenyl)ethyl]- N^2,N^2 -bis(phenoxy carbonyl)guanosine (7). To a cold solution of **3**⁸ (0.3 g, 0.6 mmol) in dry pyridine (5 mL) was added dropwise at 0 °C phenyl chloroformate (0.3 mL, 2.3 mmol). Stirring for 30 min at 0 °C was continued at rt for 12 h. It was diluted by CH_2Cl_2 (80 mL) and treated twice with saturated aqueous NaHCO_3 solution. The organic phase was dried with Na_2SO_4 , evaporated and the residue dissolved in a little toluene for FC (d = 3 cm, 20 g flash silica gel) with toluene (200 mL), toluene/AcOEt (4:1, 200 mL), toluene/AcOEt (7:3, 200 mL), toluene/AcOEt (3:2, 200 mL) and toluene/AcOEt (1:1, 200 mL). The product fractions were collected, evaporated and coevaporated with CH_2Cl_2 to give 0.346 g (78%) of a solid foam. UV (CH_2Cl_2): 223 (4.07), 259 (4.31), 289 (sh 3.85). $^1\text{H-NMR}$ ($\text{DMSO-}d_6$): 8.71 (s, H-C(8)); 8.12 (d, 2H, *o* to NO_2); 7.60 (d, 2H, *m* to NO_2); 7.42 (m, 4H, arom.H); 7.28 (m, 2H, arom.H); 7.21 (2d, 4H, arom.H); 6.47 (d, H-C(1')); 5.44 (m, H-C(3')); 4.97 (t, OCH_2CH_2); 4.38-4.20 (m, 3H, H-C(4'), H-C(5')); 3.41 (t, OCH_2CH_2); 3.25-3.19 (m, H-C(2')); 2.69-2.58 (m, H-C(2'')); 2.10 (s, 3H, Ac); 1.94 (s, 3H, Ac). Anal. Calcd for $\text{C}_{36}\text{H}_{32}\text{N}_6\text{O}_{12}$ (740.7): C 58.37, H 4.35, N 11.35. Found: C 58.21, H 4.18, N 11.43.

2'-Deoxy- O^6 -[2-(4-nitrophenyl)ethyl]- N^2,N^2 -bisphenoxy carbonyl-3',5'- O -(tetraisopropylidisiloxan-1,3-diyl)guanosine (8). Analogous to the preceding procedure with **4** (1.0 g, 1.5 mmol) and phenyl chloroformate (0.6 mL, 4.5 mmol) in dry pyridine (20 mL) at 0 °C. After 30 min at 0 °C stirring was continue at rt overnight. Column chromatography was done by FC (d = 3 cm, 50 g) with toluene (200

mL), toluene/AcOEt (9:1, 200 mL), toluene/AcOEt (4:1, 200 mL) and toluene/AcOEt (7:3, 200 mL). The product fractions were evaporated and the residue coevaporated with CH_2Cl_2 to give 1.0 g (75%) of a solid foam. UV (CH_2Cl_2): 224 (4.06), 260 (4.30, 287 (sh 3.89). $^1\text{H-NMR}$ (DMSO-d_6): 8.59 (s, H-C(8)); 8.12 (d, 2H, *o* to NO_2); 7.60 (d, 2H, *m* to NO_2); 7.44 (2t, 4H, arom.H); 7.31 (2t, 2H, arom.H); 7.16 (2d, 4H, arom.H); 6.42 (d, H-C(1')); 4.82 (m, H.C(3')); 4.90 (m, OCH_2CH_2 , H-C(3')); 3.91-3.82 (m, 3H, H-C(4'), H-C(5')); 3.32 (t, OCH_2CH_2); 3.05-2.90 (m. H-C(2')); 2.69-2.52 (m, H-C(2'')); 1.10-0.93 (m, 28H, isopropyl). Anal. Calcd for $\text{C}_{44}\text{H}_{54}\text{N}_6\text{O}_{11}\text{Si}_2$ (900.9): C 58.66, H 6.04, N 9.33. Found: C 59.19, H 6.08, N 9.11.

3',5'-Di-*O*-acetyl-2'-deoxy- N^2 -[(fluorescein-5-yl)aminocarbonyl]- O^6 -[2-(4-nitrophenyl)ethyl]-guanosine (10). A mixture of **5** (0.5 g, 0.8 mmol) and 5-aminofluorescein (**9**) (0.35 g, 1 mmol) was coevaporated three times with dry pyridine (10 mL) and then the residue dissolved in dry pyridine (10 mL). After heating over night at 70 °C was evaporated and coevaporated with toluene and then the red sirup dissolved in a little $\text{CH}_2\text{Cl}_2/\text{MeOH}$ (97:3) for colum chromatography ($d = 3$ cm, 25 g flash silica gel) with CH_2Cl_2 (200 mL), $\text{CH}_2\text{Cl}_2/\text{MeOH}$ (98:2, 100 mL), $\text{CH}_2\text{Cl}_2/\text{MeOH}$ (95:5, 300 mL), $\text{CH}_2\text{Cl}_2/\text{MeOH}$ (93:7, 100 mL) and $\text{CH}_2\text{Cl}_2/\text{MeOH}$ (9:1, 200 mL). The product fractions were collected, evaporated and the residue treated with hot isopropanol (50 mL). After cooling the precipitate was filtered off, dried in a vacuum desiccator to give 0.65 g (94%) of an orange solid. Mp 181° (decomp.). $^1\text{H-NMR}$ (DMSO-d_6): 11.42 (s, NH); 10.33 (s, NH); 10.14 (bs, 2 OH); 8.43 (s, H-C(8)); 8.32 (d, Flu-H(6)); 8.19 (d, 2H, *o* to NO_2); 7.81 (dd, Flu-H-C(4)); 7.68 (d, 2H, *m* to NO_2); 7.20 (d, Flu-H-C(3)); 6.70-6.51 (m, 6H, Flu-H-C(1'), H-C(2'), H-C(4'), H-C(5'), H-C(7'), H-C(8')); 6.42 (pt, H-C(1')); 5.40 (m, H-C(3')); 4.89 (t, OCH_2CH_2); 4.39-4.15 (m, 3H, H-C(4'), H-C(5')); 4.35 (t, OCH_2CH_2); 3.18-3.10 (m. H-C(2')); 2.64 (m, H-C(2'')); 1.96 (2s, 6H, 2 Ac). Anal. Calcd for $\text{C}_{43}\text{H}_{35}\text{N}_7\text{O}_{14} \times 2 \text{H}_2\text{O}$ (909.8): C 56.77, H 4.32, N 10.77. Found: C 56.73, H 4.09, N 10.71.

2'-Deoxy- N^2 -[(fluorescein-5-yl)aminocarbonyl]- O^6 -[2-(4-nitrophenyl)ethyl]-3',5'-*O*-(tetraisopropyl-disiloxan-1,3-diyl)guanosine (11). Analogous to the preceding procedure with **6** (4.0 g, 5.1 mmol) and **9** (1.3 g, 3.7 mmol) in dry pyridine (80 mL) at 70 °C for 3 h. Workup by FC ($d = 3$ cm, 120 g) with CH_2Cl_2 (200 mL), $\text{CH}_2\text{Cl}_2/\text{MeOH}$ (97:3, 200 mL), $\text{CH}_2\text{Cl}_2/\text{MeOH}$ (95:5, 400 mL), $\text{CH}_2\text{Cl}_2/\text{MeOH}$ (94:6, 200 mL) and $\text{CH}_2\text{Cl}_2/\text{MeOH}$ (93:7, 200 mL). The residue after evaporation was dissolved in little $\text{CH}_2\text{Cl}_2/\text{MeOH}$ (95:5) and then dropwise added to hot MeOH. The precipitate was collected, washed with Et_2O to give 3.8 g (97%) of an orange solid. $^1\text{H-NMR}$ (DMSO-d_6): 11.49 (s, NH); 10.26 (s, NH); 10.11 (s, 2 OH); 8.38 (d, Flu-H(6)); 8.37 (s, H-C(8)); 8.14 (d, 2H, *o* to NO_2); 7.75 (dd, Flu-H-C(4)); 7.71 (d, 2H, *m* to NO_2); 7.17 (d, Flu-H-C(3)); 6.67-6.51 (m, 6H, Flu-H-C(1'), H-C(2'), H-C(4'), H-C(5'), H-C(7'), H-C(8')); 6.38 (pt, H-C(1')); 4.83 (t, OCH_2CH_2); 4.68 (m, H-C(3')); 4.35 (t, OCH_2CH_2); 3.88 (m, 3H, H-

C(4'). H-C(5'')); 3.00-2.90 (m, H-C(2'')); 2.63-2.50 (m, H-C(2'')); 1.06-0.91 (m, 28H, 4 i-Pr). Anal. Calcd for $C_{51}H_{57}N_7O_{13}Si_2 \times 2 H_2O$ (1068.2): C 58.33, H 5.66, N 9.33. Found: C 58.31, H 5.60, N 9.35.

2'-Deoxy- N^2 -[(fluorescein-5-yl)aminocarbonyl]guanosine (12). A solution of **10** (0.35 g, 0.44 mmol) in dry pyridine was twice coevaporated and then dissolved in dry pyridine (7 mL). DBU (0.52 mL, 3.5 mmol) was added, stirred at rt for 36 h and then treated with methanolic ammonia overnight. The red solution was evaporated, twice coevaporated with toluene, the residue treated with H_2O (20 mL) and dilute ammonia added till pH 8. The solution was extracted with CH_2Cl_2 (3 x 20 mL), the extract washed with dilute ammonia and then the aqueous phases acidified with AcOH to pH 4 forming a red precipitate. The solid was collected, washed with a little H_2O and dried in a vacuum desiccator to give 0.22 g (78%). pK_a : 0.7, 2.0, 4.1, 6.7, 11.8. UV ($H_0 -1$): 225 (4.81), 248 (4.62), 312 (4.62), 438 (4.77); (pH 1.6): 220 (4.83), 247 (sh 4.61), 296 (4.51), 438 (4.68), 478 (sh 3.88); (pH 3.0): 221 (4.83), 254 (4.62), 301 (4.49), 438 (4.39), 475 (sh 3.88); (pH 5.0): 208 (4.80), 238 (4.77), 292 (4.53), 422 (sh 4.26), 454 (4.36), 475 (4.36); (pH 9.0): 222 (4.79), 260 (sh 4.47), 291 (4.48), 319 (sh 3.98), 461 (sh 4.59), 492 (4.93); (pH 14): 221 (4.77), 264 (sh 4.44), 308 (4.61), 377 (sh 4.00), 460 (sh 4.58), 493 (4.92). 1H -NMR ($DMSO-d_6$): 11.60 (s, NH); 10.17 (s, 3H, NH, 2 OH); 8.21 (2s, H-C(8), Flu-H(6)); 7.71 (d, Flu-H-C(4)); 7.20 (d, Flu-H-C(3)); 6.76-6.60 (m, 6H, Flu-H-C(1'), H-C(2'), H-C(4'), H-C(5'), H-C(7'), H-C(8')); 6.23 (pt, H-C(1')); 5.33 (d, HO-C(3')); 4.93 (t, HO-C(5')); 4.38 (m, H-C(4')); 3.52 (m, 2H, H-C(5')); 2.65-2.55 (m, H-C(2')); 2.31-2.21(m, H-C(2'')). Anal. Calcd for $C_{31}H_{24}N_6O_{10} \times 3.5 H_2O$ (703.5): C 52.92, H 4.44, N 11.94. Found: C 52.96, H 4.37, N 11.89.

3',5'-Di-*O*-acetyl-2'-deoxy- N^2 -{[[3-[[2-(4-nitrophenyl)ethoxycarbonyl]-4-[6-[2-(4-nitrophenyl)ethoxy]-3-oxo-3*H*-xanthen-9-yl]phenylaminocarbonyl]- O^6 -[2-(4-nitrophenyl)ethyl]guanosine (13). A mixture of **10** (0.7 g, 0.8 mmol) and 2-(4-nitrophenyl)ethanol (1.0 g, 6 mmol) was three times coevaporated with dry dioxane and then dissolved in dry dioxane (10 mL). Diethyl azodicarboxylate (0.25 mL, 1.6 mmol) was added, heated to 60 °C and then a solution of triphenylphosphane (0.42 g, 1.6 mmol) in dry dioxane (2 mL) dropwise added. After heating to 60 °C for 1 h and cooling, the mixture was evaporated. The residue was dissolved in CH_2Cl_2 (50 mL), extracted with 1% potassium dihydrogenphosphate solution (3 x 20 mL), the organic phase separated, dried over Na_2SO_4 and then evaporated. The red sirup was dissolved in a little $CH_2Cl_2/MeOH$ (95:5) and purified by FC (3 cm, 60 g flash silica gel) with CH_2Cl_2 (200 mL), $CH_2Cl_2/MeOH$ (98:2, 200 mL), $CH_2Cl_2/MeOH$ (96:4, 200 mL), $CH_2Cl_2/MeOH$ (95:5, 200 mL), $CH_2Cl_2/MeOH$ (94:6, 200 mL) and $CH_2Cl_2/MeOH$ (9:1, 200 mL). The product fractions were evaporated and gave 0.68 g (73%) of a red solid. 1H -NMR ($DMSO-d_6$): 11.33 (s, NH); 10.31 (bs NH); 8.61 (d, phe-H-C(2)); 8.42 (s, H-C(8)); 8.12 (2d, 4H *o* to NO_2); 8.08 (d, 2H, *o* to

NO₂); 7.86 (dd, phe-H-C(6)); 7.65 (2d, 4H, m to NO₂); 7.31 (m, 5H, m to NO₂, phe-H-C(5)); 7.17 (d, xan-H-C(5)); 6.88-6.80 (m, xan-H-C(8), xan-H-C(7), xan-H-C(1)); 6.44 (dd, H-C(1')); 6.36 (dd, xan-H-C(2)); 6.15 (d, xan-H-C(4)); 5.40 (m, H-C(3')); 4.90 (t, OCH₂CH₂); 4.46 (t, OCH₂CH₂); 4.32-4.20 (m, OCH₂CH₂, H-C(4'), H-C(5',5'')); 3.38-3.20 (2t, OCH₂CH₂); 3.20-3.12 (m, H-C(2')); 2.79 (t, OCH₂CH₂); 2.68-2.59 (m, H-C(2''), 2.08 (s, 3H, Ac); 1.96 (s, 3H, Ac). Anal. Calcd for C₅₉H₄₉N₉O₁₈ (1172.0): C 60.46, H 4.21, N 10.75. Found: C 60.36, H 4.28, N 10.69.

2'-Deoxy-N²-{[[[3-[[2-(4-nitrophenyl)ethoxycarbonyl]-4-[6-[2-(4-nitrophenyl)ethoxy]-3-oxo-3H-xanthen-9-yl]phenylaminocarbonyl]-O⁶-[2-(4-nitrophenyl)ethyl]-3',5'-O-(tetraisopropylidisiloxan-1,3-diyl)guanosine (14). a) A mixture of **6** (2.3 g, 3.3 mmol) and **15** (1.8 g, 2.8 mmol) was coevaporated in dry pyridine, the residue dissolved in dry pyridine (40 mL) and then the red solution stirred at 80 °C for 6 h. It was evaporated, three times coevaporated with toluene and then the residue dissolved in a little CH₂Cl₂ for flash chromatography (column d = 3 cm, 80 g flash silica gel) with toluene/AcOEt (1:1, 200 mL), toluene/EtOAc/MeOH (49:49:2, 100 mL), toluene/EtOAc/MeOH (48.5:48.5:3, 100 mL), toluene/EtOAc/MeOH (48:48:4, 100 mL), toluene/EtOAc/MeOH (47.5:47.5:5, 300 mL) and toluene/EtOAc/MeOH (47:47:6, 300 mL). The product fractions were evaporated and the residue coevaporated with CH₂Cl₂ to give 3.4 g (91%) of a solid foam. For analysis 0.5 g were dissolved in CH₂Cl₂ filtered through cellulose and then dropwise added under stirring into cold MeOH at 0 °C. The precipitate was collected and dried in a vacuum desiccator to give 0.4 g of an analytically pure, orange solid. b). A mixture of **11** (2.5 g, 2.4 mmol), 2-(4-nitrophenyl)ethanol (3.1 g, 18.4 mmol) and triphenylphosphane (1.57 g, 6 mmol) was coevaporated in dry dioxane and then the residue dissolved in the same solvent (80 mL). The solution was heated to 70 °C and the diethyl azodicarboxylate (0.93 ml, 6 mmol) added. After 3 h the mixture was evaporated and the residue was dissolved in CH₂Cl₂ (100 mL) followed by two extractions with 1% potassium dihydrogenphosphate solution and washing with H₂O. The organic phase was dried over Na₂SO₄, evaporated and the resulting red sirup dissolved in a little CH₂Cl₂ for purification by flash column chromatography (d = 4 cm, 160 g) with toluene/AcOEt (1:1, 200 mL), toluene/EtOAc/MeOH (49:49:2, 200 mL), toluene/EtOAc/MeOH (48.5:48.5:3, 200 mL), toluene/EtOAc/MeOH (48:48:4, 200 mL), toluene/EtOAc/MeOH (47.5:47.5:5, 400 mL) and toluene/EtOAc/MeOH (47:47:6, 400 mL). The product fractions were collected, evaporated and coevaporated with CH₂Cl₂ to give 2.7 g (85%) of an orange solid foam. Further purification was done by solution in CH₂Cl₂, filtration through cellulose and dropwise addition to cold MeOH. The orange precipitate was collected and dried in a vacuum desiccator to give 2.4 g (75%). ¹H-NMR (DMSO-d₆): 11.40 (s, NH); 10.21 (bs NH); 8.58 (d, phe-H-C(2)); 8.34 (s, H-C(8)); 8.12 (2d, 4H *o* to NO₂); 8.00 (d, 2H, *o* to NO₂); 7.85 (dd, phe-H-C(6)); 7.70-7.60 (2d, 4H, m to NO₂); 7.29 (m, 5H, m to NO₂, phe-H-C(5));

7.15 (d, xan-H-C(5)); 6.81 (m, 3H, xan-H-C(8), xan-H-C(7), xan-H-C(1)); 6.41 (dd, H-C(1')); 6.35 (dd, xan-H-C(2)); 6.11 (d, xan-H-C(4)); 4.86 (t, OCH_2CH_2); 4.76 (m, H-C(3')); 4.44 (t, OCH_2CH_2); 4.27 (m, OCH_2CH_2); 3.89 (m, H-C(4'), H-C(5',5'')); 3.35 (t, OCH_2CH_2); 3.22 (t, OCH_2CH_2); 3.01-2.90 (m, H-C(2')); 2.86 (t, OCH_2CH_2); 2.68 (m, H-C(2'')), 1.10-0.80 (m, 28H, 4 *i*-Pr). Anal. Calcd for $C_{67}H_{71}N_9O_{17}Si_2$ (1330.5): C 60.48, H 5.58, N 9.47. Found: C 60.24, H 5.41, N 9.57.

2-(4-Nitrophenyl)ethyl 5-Amino-2-{6-[2-(4-nitrophenyl)ethoxy]-3-oxo-3*H*-xanthen-9-yl]benzoate

(15). A mixture of 5-aminofluorescein⁸ (0.5 g, 1.44 mmol), triphenylphosphane (0.9 g, 3.46 mmol) and 2-(4-nitrophenyl)ethanol (1.4 g, 8.64 mmol) was coevaporated in dry dioxane, the residue dissolved in the same solvent (50 mL) and then refluxed for 2 h. The temperature was reduced to 60 °C and then a solution of diethyl azodicarboxylate (0.52 mL, 3.46 mmol) in dry dioxane (8 mL) very slowly dropwise added within 1 h. After another 4 h the reaction was stopped, evaporated and the red, oily residue dissolved in a little CH_2Cl_2 for CC (d = 2.5 cm, 70 g flash silica gel) with toluene/EtOAc/ NEt_3 (49:49:2, 200 mL), toluene/EtOAc/ NEt_3 (48.5:48.5:3, 200 mL), toluene/EtOAc/ NEt_3 (48:48:4, 200 mL), toluene/EtOAc/ NEt_3 (47.5:47.5:5, 200 mL) and toluene/EtOAc/ NEt_3 (47:47:6, 200 mL). The product fractions were collected, evaporated and dissolved in a little CH_2Cl_2 . The solution was added dropwise into cold EtOH (0 °C, 20 mL), cooled in the icebox for 2 days, the precipitate collected, washed with Et_2O and dried to give 0.88 g (88%) of a red solid. UV (MeOH/ CH_2Cl_2 1:1): 230 (4.73), 267 (4.63), 360 (4.02), 436 (sh 4.35), 459 (4.48), 486 (4.38). ¹H-NMR ($DMSO-d_6$): 8.12 (2d, 4H *o* to NO_2); 8.02 (d, 4H, *o* to NO_2); 7.63 (d, 4H, *m* to NO_2); 7.29 (s, H-C(6)); 7.24 (d, 4H, *m* to NO_2); 7.13 (d, xan-H-C(5)); 7.01 (d, H-C(3)); 6.96-6.88 (m, 3H, xan-H.C(1), xan-H-C(7), xan-H-C(8)); 6.80 (d, H-C(4)); 6.35 (d, xan-H-C(2)); 6.10 (d, xan-H-C(4)); 5.90 (bs, NH_2); 4.42 (t, OCH_2CH_2); 4.18 (t, OCH_2CH_2); 3.21 (t, OCH_2CH_2); 2.73 (t, OCH_2CH_2). Anal. Calcd for $C_{36}H_{27}N_3O_9$ (645.6): C 66.98, H 4.22, N 6.51. Found: C 66.64, H 4.28, N 6.33.

2'-Deoxy-*N*²-{[[3-[[2-(4-nitrophenyl)ethoxycarbonyl]-4-[6-[2-(4-nitrophenyl)ethoxy]-3-oxo-3*H*-xanthen-9-yl]phenylaminocarbonyl]-*O*⁶-[2-(4-nitrophenyl)ethyl]guanosine (16). To a solution of 14 (2.2 g, 1.6 mmol) in THF (30 ml) and AcOH (2.3 ml, 40 mmol) was added tetrabutylammonium fluoride trihydrate (1.3 g, 4 mmol) and stirred at rt for 12 h. It was diluted with CH_2Cl_2 (150 ml) and MeOH (8 mL) and three times extracted with a saturated aqueous $NaHCO_3$ solution. The organic phase was dried over Na_2SO_4 , evaporated and then the residue dissolved in little CH_2Cl_2 /MeOH (95:5) for CC (d = 3 cm, 50 g flash silica gel) with CH_2Cl_2 /MeOH (93:7, 200 mL), CH_2Cl_2 /MeOH (92:8, 200 mL), CH_2Cl_2 /MeOH (91:9, 200 mL) and CH_2Cl_2 /MeOH (9:1, 200 mL). The product fractions were evaporated, the residue dissolved in a little CH_2Cl_2 /MeOH (95:5) and then dropwise added under stirring into MeOH (50 mL). The red precipitate was collected, washed with Et_2O and dried in a vacuum

desiccator to give 1.6 g (94%) of a red solid. $^1\text{H-NMR}$ ($\text{DMSO-}d_6$): 11.49 (s, NH); 10.30 (bs NH); 8.62 (d, phe-H-C(2)); 8.46 (s, H-C(8)); 8.17 (2d, 4H *o* to NO_2); 8.03 (d, 2H, *o* to NO_2); 7.79 (dd, phe-H-C(6)); 7.68-7.60 (2d, 4H, *m* to NO_2); 7.33 (m, 5H, *m* to NO_2 , phe-H-C(5)); 7.17 (d, xan-H-C(5)); 6.88-6.78 (m, 3H, xan-H-C(8), xan-H-C(7), xan-H-C(1)); 6.42 (dd, H-C(1')); 6.36 (dd, xan-H-C(2)); 6.16 (d, xan-H-C(4)); 5.38 (d, HO-C(3')); 4.97 (t, HO-C(5')); 4.89 (t, OCH_2CH_2); 4.45 (m, OCH_2CH_2 , H-C(3')); 4.23 (m, OCH_2CH_2); 3.89 (m, H-C(4')); 3.57 (m, H-C(5',5'')); 3.36 (t, OCH_2CH_2); 3.27 (t, OCH_2CH_2); 2.84 (t, OCH_2CH_2); 2.72 (m, H-C(2')); 2.29 (m, H-C(2'')). Anal. Calcd for $\text{C}_{55}\text{H}_{45}\text{N}_9\text{O}_{16} \times \text{H}_2\text{O}$ (1106.0): C 59.72, H 4.28, N 11.36. Found: C 59.66, H 4.38, N 11.57.

2'-Deoxy- N^2 -{[[3-[(methoxy)carbonyl]-4-[6-[2-(4-nitrophenyl)ethoxy]-3-oxo-3*H*-xanthen-9-yl]-phenylaminocarbonyl]- O^6 -[2-(4-nitrophenyl)ethyl]guanosine (17). A solution of **13** (0.25 g, 0.21 mmol) in dry MeOH (5 mL) and CH_2Cl_2 (2 mL) was treated with dry K_2CO_3 (50 mg, 0.36 mmol) at rt for 2 h with stirring. It was diluted with CH_2Cl_2 (30 mL), extracted twice with 1% aqueous KH_2PO_4 solution, the organic phase dried over Na_2SO_4 and then evaporated. The resulting red sirup was dissolved in a little $\text{CH}_2\text{Cl}_2/\text{MeOH}$ (95:5) and put on a flash silica gel column ($d = 1$ cm, 5 g) for purification with $\text{CH}_2\text{Cl}_2/\text{MeOH}$ (94:6, 100 mL), $\text{CH}_2\text{Cl}_2/\text{MeOH}$ (93:7, 100 mL), $\text{CH}_2\text{Cl}_2/\text{MeOH}$ (92:8, 100 mL) and $\text{CH}_2\text{Cl}_2/\text{MeOH}$ (9:1, 100 mL). The product fractions were evaporated, the residue dissolved in little $\text{CH}_2\text{Cl}_2/i\text{-PrOH}$ (1:1) and then added dropwise to hot *i*-PrOH. After cooling the precipitate collected and dried to give 0.19 g (95%) of a red solid. Mp 170 °C (decomp.). $^1\text{H-NMR}$ ($\text{DMSO-}d_6$): 11.52 (s, NH); 10.29 (bs NH); 8.54 (d, phe-H-C(2)); 8.49 (s, H-C(8)); 8.19 (2d, 4H *o* to NO_2); 7.94 (dd, phe-H-C(6)); 7.68-7.63 (2d, 4H, *m* to NO_2); 7.33 (m, phe-H-C(5)); 7.29 (d, xan-H-C(5)); 6.91-6.85 (m, 3H, xan-H-C(8), xan-H-C(7), xan-H-C(1)); 6.40 (m, 2H, H-C(1'), xan-H-C(2)); 6.21 (d, xan-H-C(4)); 5.37 (d, HO-C(3')); 4.95 (t, HO-C(5')); 4.89 (t, OCH_2CH_2); 4.45 (m, OCH_2CH_2 , H-C(3')); 3.94 (m, H-C(4')); 3.61 (s, OCH_3); 3.56 (m, H-C(5',5'')); 3.31 (t, OCH_2CH_2); 3.24 (t, OCH_2CH_2); 2.79-2.68 (m, H-C(2')); 2.41-2.32 (m, H-C(2'')). Anal. Calcd for $\text{C}_{48}\text{H}_{40}\text{N}_8\text{O}_{14}$ (952.8): C 60.50, H 4.23, N 11.75. Found: C 60.16, H 4.30, N 11.58.

2'-Deoxy-5'-*O*-dimethoxytrityl- N^2 -{[[3-[[2-(4-nitrophenyl)ethoxycarbonyl]-4-[6-[2-(4-nitrophenyl)ethoxy]-3-oxo-3*H*-xanthen-9-yl]phenylaminocarbonyl]- O^6 -[2-(4-nitrophenyl)ethyl]guanosine (18). A solution of **16** (1.3 g, 1.2 mmol) in dry pyridine was coevaporated and then the residue dissolved in dry pyridine. Dimethoxytrityl chloride (0.5 g, 1.4 mmol) was added and the mixture stirred at rt overnight. It was evaporated, coevaporated twice with toluene, the residue dissolved in CH_2Cl_2 (100 mL) and three times extracted with a saturated aqueous NaHCO_3 solution. The organic phase was dried over Na_2SO_4 , then evaporated and the residue dissolved in a little CH_2Cl_2 for CC ($d = 2$ cm, flash silica gel 40 g) with toluene/EtOAc (1:1, 100 mL), toluene/EtOAc/MeOH (46:46:8, 100 mL), toluene/EtOAc/MeOH

(45.5:45,5:9, 100 mL), toluene/EtOAc/MeOH (45:45:10, 100 mL), toluene/EtOAc/MeOH (44:44:12, 100 mL) and toluene/EtOAc/MeOH (43:43:14, 100 mL). The product fraction was evaporated, the residue dissolved in a little CH₂Cl₂ and the solution dropwise added under stirring in cold MeOH (0 °C). The resulting red precipitate was collected after 1 h, washed with a little MeOH and dried in a vacuum desiccator to give 1.4 g (83%) of a red solid. ¹H-NMR (DMSO-d₆): 11.90 (s, NH); 10.33 (bs NH); 8.62 (d, phe-H-C(2)); 8.41 (s, H-C(8)); 8.17 (2d, 4H *o* to NO₂); 8.09 (2d, 3H *o* to NO₂), phe-H-C(6)); 7.62 (2d, 4H, *m* to NO₂); 7.39 (m, phe-H-C(5)); 7.30 (d, 2H, *m* to NO₂); 7.17-7.09 (m, 4H, *m* to OCH₃, 7 phenyl-H, xan-H-C(5)); 6.80 (m, 3H, xan-H-C(8), xan-H-C(7), xan-H-C(1)); 6.41 (m, 2H, H-C(1')); 6.32 (t, xan-H-C(2)); 6.13 (d, xan-H-C(4)); 5.41 (d, HO-C(3')); 4.82 (t, OCH₂CH₂); 4.47 (m, H-C(3')); 4.45 (t, OCH₂CH₂); 4.21 (t, OCH₂CH₂); 4.08 (m, H-C(4')); 3.65 (2s, 2 OCH₃); 3.34 (t, OCH₂CH₂); 3.23 (t, OCH₂CH₂); 3.08 (m, H-C(5',5'')); 3.00 (m, H-C(2')); 2.77 (t, OCH₂CH₂); 2.50 (m, H-C(2'')). Anal. Calcd for C₇₆H₆₃N₉O₁₈ (1390.5): C 65.65, H 4.57, N 9.06. Found: C 65.16, H 4.77, N 9.22.

3'-O-(3-Carboxypropanoyl)-2'-deoxy-5'-O-dimethoxytrityl-N²-{[[3-[[2-(4-nitrophenyl)ethoxycarbonyl]-4-[6-[2-(4-nitrophenyl)ethoxy]-3-oxo-3*H*-xanthen-9-yl]phenylaminocarbonyl]-O⁶-[2-(4-nitrophenyl)ethyl]guanosine (19). A mixture of **18** (0.35 g, 0.25 mmol) and DMAP (62 mg, 0.5 mmol) in dry CH₂Cl₂ (5 mL) was treated with succinic anhydride (40 mg, 0.4 mmol) for 24 h at rt. It was diluted with CH₂Cl₂, extracted three times with a cold solution of 10% aqueous citric acid (50 mL) and then twice with H₂O (50 mL). The aqueous phase was reextracted with CH₂Cl₂, then the organic layers united, dried over Na₂SO₄, filtered and evaporated. The oily residue was coevaporated with CH₂Cl₂ to give 0.365 g (98%) of a red solid foam. ¹H-NMR (DMSO-d₆): 12.21 (bs, COOH); 11.54 (s, NH); 10.28 (bs NH); 8.62 (d, phe-H-C(2)); 8.38 (s, H-C(8)); 8.16 (2d, 4H *o* to NO₂); 8.00 (2d, 3H *o* to NO₂); 7.87 (dd, phe-H-C(6)); 7.64 (2d, 4H, *m* to NO₂); 7.35 (m, phe-H-C(5)); 7.26 (d, 2H, *m* to NO₂); 7.20-7.08 (m, 4H, *m* to OCH₃, 7 phenyl-H, xan-H-C(5)); 6.80 (m, 3H, xan-H-C(8), xan-H-C(7), xan-H-C(1)); 6.48 (t, xan-H-C(2)); 6.32 (m, 2H, H-C(1')); 6.12 (d, xan-H-C(4)); 5.36 (m, H-C(3')); 4.86 (t, OCH₂CH₂); 4.42 (t, OCH₂CH₂); 4.25 (m, OCH₂CH₂, H-C(4')); 3.75 (t, OCH₂CH₂); 3.65 (2s, 2 OCH₃); 3.39-3.18 (m, 6H, 2 OCH₂CH₂, H-C(5',5'')); 2.68-2.40 (m, H-C(2',2''), 4H, succ). Anal. Calcd for C₈₀H₆₆N₉O₂₁ (1489.4): C 64.45, H 4.47, N 8.46. Found: C 64.55, H 4.64, N 8.30.

2'-Deoxy-5'-O-dimethoxytrityl-N²-{[[3-[[2-(4-nitrophenyl)ethoxycarbonyl]-4-[6-[2-(4-nitrophenyl)ethoxy]-3-oxo-3*H*-xanthen-9-yl]phenylaminocarbonyl]-O⁶-[2-(4-nitrophenyl)ethyl]guanosine 3'-O-[2-(4-Nitrophenyl)ethyl *N,N*-Diisopropylphosphoramidite] (20). In a dry two-necked flask (50 ml) **20** (1.39 g, 1 mmol) was dissolved in dry CH₂Cl₂ (10 mL) and then dry acetonitrile (10 mL) added. The flask was treated with a slow stream of dry N₂ for 10 min, then bis(diisopropylamino)-[2-(4-

nitrophenyl)ethyl]phosphane (0.733 g, 2 mmol) and sublimed 1*H*-tetrazole (35 mg, 0.5 mmol) added and stirred at rt for 3 h. It was diluted with CH₂Cl₂ (50 mL) and extracted with 1 N NaHCO₃ solution saturated with NaCl (2 x 100 mL). The organic phase was dried over Na₂SO₄, filtered and evaporated. The red oily residue was dissolved in a little CH₂Cl₂ for fast flash column chromatography (d = 3 cm, 40 g, 15 min) with acetone/*n*-hexane (1:1, 600 mL), acetone/*n*-hexane (9:1, 700 mL) and acetone (300 mL). The product fractions were collected, evaporated, the resulting red sirup dissolved in CH₂Cl₂ again and then dropwise added under stirring into cold dry Et₂O (0 °C) to give 1.4 g (83%) of a red solid. ¹H-NMR (DMSO-d₆): 11.80 (s, NH); 10.38 (bs NH); 8.67 (s, H-C(8)); 8.33 (d, phe-H-C(2)); 8.17 (2d, 4H *o* to NO₂); 8.04-7.93 (m, 3H *o* to NO₂), phe-H-C(6)); 7.68 (2d, 4H, *m* to NO₂); 7.43-7.21 (m, 2H, *m* to NO₂), phe-H-C(5)); 7.17-7.05 (m, 4H, *m* to OCH₃, 5 phenyl-H, xan-H-C(5)); 6.78 (m, 3H, xan-H-C(8), xan-H-C(7), xan-H-C(1)); 6.72 (m, *m* to OCH₃); 6.48 (m, 2H, H-C(1')); 6.32 (t, xan-H-C(2)); 6.16 (d, xan-H-C(4)); 4.90 (t, OCH₂CH₂); 4.69 (m, H-C(3')); 4.47 (t, OCH₂CH₂); 4.22 (t, OCH₂CH₂), H-C(4')); 3.69 (2s, 2 OCH₃); 3.40-2.63 (m, 4 x OCH₂CH₂), H-C(5',5''), H-C(2'), 2 Me₂CH); 2.50 (m, H-C(2'')); 1.09-0.89 (m, 2 Me₂CH). ³¹P-NMR (DMSO-d₆): 147.9, 147.6. Anal. Calcd for C₉₀H₈₄N₁₁O₂₁P (1686.7): C 64.09, H 5.02, N 9.13. Found: C 63.32, H 5.11, N 9.18.

2'-Deoxy-5'-*O*-dimethoxytrityl-*N*²-{[[3-[[2-(4-nitrophenyl)ethoxycarbonyl]-4-[6-[2-(4-nitrophenyl)ethoxy]-3-oxo-3*H*-xanthen-9-yl]phenylaminocarbonyl]-*O*⁶-[2-(4-nitrophenyl)ethyl]guanosine 3'-*O*-[2-Cyanoethyl *N,N*-Diisopropylphosphoramidite] (21). Analogous to the preceding procedure with **20** (1.39 g, 1 mmol) and bis(diisopropylamino)(2-cyanoethoxy)phosphane (0.58 g, 2 mmol) yielding 1.3 g (82%) of a red solid. ¹H-NMR (DMSO-d₆): 11.81 (s, NH); 10.32 (bs NH); 8.62 (s, H-C(8)); 8.39 (d, phe-H-C(2)); 8.11 (2d, 4H *o* to NO₂); 7.99 (m, 3H *o* to NO₂), phe-H-C(6)); 7.61 (2d, 4H, *m* to NO₂); 7.33 (d, phe-H-C(5)); 7.24 (m, 2H, *m* to NO₂); 7.18-7.01 (m, 4H, *m* to OCH₃, 5 phenyl-H, xan-H-C(5)); 6.85-6.63 (m, 3H, xan-H-C(8), xan-H-C(7), xan-H-C(1), *o* to OCH₃); 6.50 (m, 2H, H-C(1')); 6.26 (dd, xan-H-C(2)); 6.13 (d, xan-H-C(4)); 4.81 (t, OCH₂CH₂); 4.67 (m, H-C(3')); 4.39 (t, OCH₂CH₂); 4.19 (t, OCH₂CH₂); 3.63 (2s, 2 OCH₃), H-C(4')); 3.53-3.00 (m, 3 x OCH₂CH₂), H-C(5',5''), 2 Me₂CH); 2.88 (t, OCH₂CH₂CN); 2.58-2.50 (m, H-C(2',2'')); 1.07-0.89 (m, 2 Me₂CH). ³¹P-NMR (DMSO-d₆): 148.99, 148.14. Anal. Calcd for C₈₅H₈₀N₁₁O₁₉P (1590.5): C 64.18, H 5.07, N 9.68. Found: C 63.57, H 5.21, N 9.45.

2'-Deoxy-5'-*O*-dimethoxytrityl-*N*²-{[[3-[[2-(4-nitrophenyl)ethoxycarbonyl]-4-[6-[2-(4-nitrophenyl)ethoxy]-3-oxo-3*H*-xanthen-9-yl]phenylaminocarbonyl]-*O*⁶-[2-(4-nitrophenyl)ethyl]guanosyl]-[3'-{*O*^P-[2-(4-nitrophenyl)ethyl]}→5']-2'-deoxy-*O*⁶-[2-(4-nitrophenyl)ethyl]-*N*²,3'-*O*-bis[2-(4-nitrophenyl)ethoxycarbonyl]guanosine (23). In a dry two-necked flask **20** (0.3 g, 0.18 mmol) and **22**¹² (0.361 g, 0.44 mmol) were dissolved under N₂-atmosphere in dry CH₂Cl₂ (3 mL) and dry MeCN (5 mL),

then sublimed 1*H*-tetrazole (60 mg, 0.9 mmol) added and stirred for 1 h. The intermediary phosphite was oxydized with a solution (3 mL) of iodine (0.5 g) in CH₂Cl₂/H₂O/pyridine (1:1:1, 3 ml) by stirring for 30 min. It was dilute with CH₂Cl₂ (50 mL), extracted three times with a saturated aqueous solution of Na₂S₂O₃/NaCl and followed by a saturated aqueous NaHCO₃ solution. The organic phase was dried over Na₂SO₄, filtered and evaporated. The residue was dissolved in a little CH₂Cl₂/MeOH (95:5) for CC (d = 2 cm, flash silica gel 20 g) with CH₂Cl₂ (150 ml), CH₂Cl₂/MeOH (96:4, 100 ml) and CH₂Cl₂/MeOH (93:7, 200 ml). The product fractions were evaporated, the residue dissolved in a little CH₂Cl₂/MeOH (95:5) and then dropwise added into cold MeOH (0 °C, 20 mL) under stirring. The resulting suspension was kept in the icebox overnight, the precipitate collected and dried in a vacuum desiccator to give 0.37 g (86%) of a red solid. ¹H-NMR (DMSO_d₆): 11.59 (s, NH); 10.28 (bs NH); 8.65 (s, H-C(8)); 8.32 (m, H-C(8), phe-H-C(2)); 8.80-7.90 (m, 14H *o* to NO₂, phe-H-C(6)); 7.70-7.00 (m, 14H *m* to NO₂, phe-H-C(5), 4H *m* to OCH₃, 5 phenyl-H, phen-H-C(5)); 6.81-6.60 (m, 3H, xan-H-C(8), xan-H-C(7), xan-H-C(1), 4H *m* to OCH₃); 6.42-6.23 (m, 2H, H-C(1'), xan-H-C(2)); 6.13 (m, xan-H-C(4)); 5.30-5.21 (m, H-C(3')); 5.08 (m, H-C(3')); 4.88 (m, H-C(5',5'')); 4.65 (m, H-C(4')); 4.40-4.07 (m, 14H OCH₂CH₂), H-C(4'), H-C(5',5'')); 3.61 (2s, 2 OCH₃); 3.30-2.45 (m, 14H OCH₂CH₂), H-C(2')). ³¹P-NMR (DMSO_d₆): -1.82, -1.78. Anal. Calcd for C₁₂₀H₁₀₃N₁₈O₃₆P (2404.1): C 59.95, H 4.32, N 10.48. Found: C 59.94, H 4.31, N 10.37.

2'-Deoxy-N²-{[[3-[[2-(4-nitrophenyl)ethoxycarbonyl]-4-[6-[2-(4-nitrophenyl)ethoxy]-3-oxo-3*H*-xanthen-9-yl]phenylaminocarbonyl]-O⁶-[2-(4-nitrophenyl)ethyl]guanosyl]-[3'-{O^P-[2-(4-nitrophenyl)ethyl]}→5']-2'-deoxy-O⁶-[2-(4-nitrophenyl)ethyl]-N²,3'-O-bis[2-(4-nitrophenyl)ethoxycarbonyl]guanosine (24). A solution of **23** (0.25 g, 0.104 mmol) in CH₂Cl₂/MeOH (4:1, 10 ml) was treated with *p*-toluenesulfonic acid (0.1 g) and stirred for 10 min at rt. It was extracted with a saturated aqueous NaHCO₃ solution (3 x 30 mL), the organic phase dried over Na₂SO₄, filtered and again evaporated. The residue was dissolved in CH₂Cl₂/MeOH (95:5) for CC (d = 1 cm, flash silica gel 20 g) with CH₂Cl₂ (100 mL), CH₂Cl₂/MeOH (98:2, 100 mL), CH₂Cl₂/MeOH (95:5, 100 mL), CH₂Cl₂/MeOH (93:7, 100 mL) and CH₂Cl₂/MeOH (9:1, 100 mL). The product fraction were evaporated, the residue dissolved in a little CH₂Cl₂/MeOH (95:5) and then added under stirring to cold MeOH (0 °C, 20 ml). The suspension was kept over night in the icebox, the precipitate collected and dried in a vacuum desiccator to give 0.18 g (82%) of a red solid. ¹H-NMR (DMSO_d₆): 11.40 (s, NH); 10.28 (bs NH); 8.70 (s, H-C(8)); 8.43 (s, H-C(8)); 8.25-7.94 (m, 14H *o* to NO₂, phe-H-C(2)); 7.72-7.29 (m, 14H *m* to NO₂, phe-H-C(5), phe-H-C(6)); 4H *m* to OCH₃, 5 phenyl-H, phen-H-C(5)); 6.81-6.60 (m, 3H, xan-H-C(8), xan-H-C(7), xan-H-C(1), 4H *m* to OCH₃); 6.42-6.23 (m, 2H, H-C(1'), xan-H-C(2)); 6.12 (m, xan-H-C(4)); 5.30 (m, H-C(3')); 5.11 (m, HO-C(5')); 4.92 (m, H-C(5',5''), H-C(3')); 4.61 (m, H-C(4')); 4.46-4.10 (m, 14H OCH₂CH₂), H-C(4'), H-C(5',5'')); 3.50-2.49 (m, 14H OCH₂CH₂), 4 H-C(2')). ³¹P-NMR ((D₆)DMSO): -

1.86, -1.65. Anal. Calcd for C₉₉H₈₅N₁₈O₃₄P (2101.7): C 56.57, H 4.09, N 11.99. Found: C 56.76, H 4.11, N 11.19.

2'-Deoxy-*N*²,5'-*O*-bis[2-(4-nitrophenyl)ethoxycarbonyl]-*O*⁶-[2-(4-nitrophenyl)ethyl]guanosine 3'-*O*-[2-(4-Nitrophenyl)ethyl *N,N*-Diisopropylphosphoramidite] (26). A solution of **25**¹² (0.8 g, 1 mmol) in dry CH₂Cl₂ (5 mL) and dry acetonitrile (5 mL) was treated under N₂-atmosphere with bis(isopropylamino)[2-(4-nitrophenyl)ethoxy]phosphane (1.0 g, 2.5 mmol) and sublimed 1*H*-tetrazole (35 mg, 0.5 mmol). After stirring overnight was diluted with CH₂Cl₂ (100 mL), extracted twice with a saturated aqueous NaHCO₃ solution, the organic phase dried over Na₂SO₄, filtered and again evaporated. The residue was dissolved in a little CH₂Cl₂ for CC (d = 2.5 cm, flash silica gel 60 g) with toluene (150 ml), toluene/AcOEt (4:1, 150 mL), toluene/AcOEt (7:3, 150 mL), toluene/AcOEt (3:2, 150 mL) and toluene/AcOEt (1:1, 150 mL). The product fractions were evaporated and the residue coevaporated several times with CH₂Cl₂ to give 0.82 g (75%) of a solid foam. UV (MeOH): 213 (4.65), 269 (4.57). ¹H-NMR-(CDCl₃): 8.18-8.12 (m, 8H *o* to NO₂); 7.96 (s, H-C(8)); 7.57-7.36 (m, 8H *m* to NO₂), H-N); 6.33 (dd, H-C(1')); 4.81 (t, OCH₂CH₂); 4.60 (m, H-C(3')); 4.47 (t, OCH₂CH₂); 4.39 (t, OCH₂CH₂); 4.30-4.21 (m, H-C(4'), H-C(5',5'')); 3.92-3.80 (m, POCH₂CH₂); 3.60 -3.49 (m, 2 Me₂CH); 3.31 (t, OCH₂CH₂); 3.16-3.00 (m, 3 x OCH₂CH₂); 2.90-2.70 (m, H-C(2')); 2.53-2.45 (m, H-C(2'')); 1.18-1.05 (m, 2 (H₃C)₂CH). ³¹P-NMR ((CDCl₃):149.3, 148.8. Anal. Calcd for C₅₀H₅₅N₁₀O₁₇P (1099.0): C 54.64, H 5.04, N 12.74. Found: C 54.75, H 5.19, N 12.46.

2'-Deoxy-*O*⁶-[2-(4-nitrophenyl)ethyl]-*N*²,5'-*O*-bis[4-nitrophenyl)ethoxycarbonyl]guanosyl-yl-{3'-[*O*^P-[2-(4-nitrophenyl)ethyl]]→5'}-2'-deoxy-*N*²-{[[3-[[2-(4-nitrophenyl)ethoxycarbonyl]-4-[6-[2-(4-nitrophenyl)ethoxy]-3-oxo-3*H*-xanthen-9-yl]]phenylaminocarbonyl]-*O*⁶-[2-(4-nitrophenyl)ethyl]-guanosyl-yl{3'-{*O*^P-[2-(4-nitrophenyl)ethyl]]→5'}-2'-deoxy-*O*⁶-[2-(4-nitrophenyl)ethyl]-*N*²,3'-*O*-bis[2-(4-nitrophenyl)ethoxycarbonyl]guanosine (27). In a dry two-necked flask the dimer **24** (0.2 g, 0.095 mmol) and **26** (0.313 g, 0.28 mmol) were dissolved under N₂-atmosphere in dry CH₂Cl₂/MeCN (1:1, 5 mL), then sublimed 1*H*-tetrazole (100 mg, 1.4 mmol) added and stirred for 3 h. The intermediary phosphite was oxydized with a solution (3 mL) of iodine (0.5 g) in CH₂Cl₂/H₂O/pyridine (1:1:1, 3 mL) by stirring for 30 min. It was dilute with CH₂Cl₂ (50 mL), extracted three times with a saturated aqueous solution of Na₂S₂O₃/NaCl and followed by a saturated aqueous NaHCO₃ solution. The organic phase was dried over Na₂SO₄, filtered and evaporated. The residue was dissolved in little CH₂Cl₂/MeOH (95:5) for CC (d = 2 cm, flash silica gel 30 g) with CH₂Cl₂ (150 mL), CH₂Cl₂/MeOH (98:2, 100 mL), CH₂Cl₂/MeOH (97:3, 200 mL) and CH₂Cl₂/MeOH (93:7, 200 mL). The product fractions were evaporated, the residue dissolved in a little CH₂Cl₂/MeOH (95:5) and then dropwise added into cold

MeOH (0 °C, 10 mL) under stirring. The resulting suspension was kept in the icebox overnight, the precipitate collected and dried in a vacuum desiccator to give 0.25 g (85%) of a red solid. ¹H-NMR (DMSO-*d*₆): 11.16 (s, NH); 10.21-10.08 (3s, 3 NH); 8.59 (s, H-C(8)); 8.32 (m, H-C(8)); 8.32 (2s, 2 H-C(8)); 8.20-7.95 (m, phe-H-C(2), phe-H-C(6), 22H *o* to NO₂); 7.70-7.30 (m, 22H *m* to NO₂), phen-H-C(5)); 7.11 (m, xan-H-C(5)); 6.82 (m, 3H, xan-H-C(8), xan-H-C(7), xan-H-C(1)); 6.30 (m, 2H, H-C(1'), xan-H-C(2)); 6.13 (s, xan-H-C(4)); 6.11-5.40 (m, 3H, H-C(3'')); 4.88-4.72 (m, 6H, OCH₂CH₂); 4.50-4.20 (m, 16H, OCH₂CH₂), 6H, H-C(5',5''), 3H, H-C(4'')); 3.30-2.49 (m, 22H, OCH₂CH₂), 6H, H-C(2'')). ³¹P-NMR (DMSO-*d*₆): -1.82. Anal. Calcd for C₁₄₃H₁₂₅N₂₇O₅₂P₂ (3115.5): C 55.13, H 4.04, N 12.13. Found: C 55.02, H 4.11, N 12.05.

2'-Deoxyguanosyl[3'→5']-2'-deoxy-*N*²-{[(fluorescein-5-yl)amino]carbonyl}guanosyl[3'→5']-2'-deoxyguanosine Tetraammonium Salt (28). The trimer **27** (90 mg, 0.028 mmol) was coevaporated twice with dry pyridine (20 mL), then dissolved in dry pyridine (20 mL), DBU (1.6 g, 9.45 mmol) added and stirred for 18 h at rt. It was evaporated, neutralized with AcOH, again neevaporated and three times coevaporated with toluene. The red sirup was dissolved in dilute ammonia and then extracted with CHCl₃ (5 x 30 mL). The aqueous layer was concentrated to 3-5 mL and put onto a DEAE Sephadex column (A25, 60 x 1.5 cm). A gradient of 100% H₂O till 1 M triethylammonium bicarbonate buffer (pH 7.5) was applied. The product eluted at highest salt concentrations, the fractions were collected and evaporated. The residue was dissolved in a little dilute ammonia and put on 6 cellulose sheets (52 x 30 cm, Schleicher & Schüll) for chromatography with *i*PrOH/conc. NH₃/H₂O (6:1:3). The red bands were cut out, eluted with dilute ammonia, filtered and then lyophilized to give 1400 OD unit (490 nm) of a red solid foam. The yield of 60% was calculated on an extinction of 85000 (490 nm). ¹H-NMR (D₂O): 8.48 (m, H-C(8), flu-H-C(6)); 8.23 (s, H-C(8)); 8.02 (s, H-C(8)); 7.78 (dd, flu-H-C(4)); 7.63 (m, flu-H-C(1'), flu-H-C(8'), flu-H-C(3)); 7.12 (m, flu-H-C(4'), flu-H-C(5'), flu-H-C(2'), flu-H-C(7'')); 6.42-6.30 (m, 3 H-C(1'')); 5.37 (m, H-C(3'')); 5.09 (m, H-C(3'')); 4.58-4.00 (m, H-C(3'), 3 H-C(4'), 6 H-C(5',5'')); 3.20-2.42 (m, 6 H-C(2',2'')).

Oligonucleotide synthesis: Solid-support material 500Å LCAMA-CPG¹³ (200 mg) was loaded with **19** (30 mmol) by TOTU (10 mg, 30 mmol) in dry MeCN (3 mL) and *N*-methylmorpholine (8 μL) and gentle shaking for 1.5 h. The CPG-material was collected in a glass funnel and washed with MeOH, DMF, pyridine, MeOH, acetone and Et₂O. Capping procedure: The nucleoside-functionalized CPG was treated with a mixture of DMAP (50 mg, 0.41 mmol), abs. pyridine (10 ml) and Ac₂O (1 ml, 10.6 mmol) for 1 h at rt by gently shaking. The material was collected, washed with MeOH, DMF, MeOH, acetone and Et₂O and dried in a vacuum desiccator. Assembly of oligodeoxynucleotides: The syntheses were carried out in

an *Applied Biosystems 392 DNA synthesizer* applying the functionalized CPG material packed into a small ABI column and followed by cycles of nucleotide addition according to programmed series of reagents and solvent washes based on recommended procedures with the following main steps: 1) 5'-*O*-(MeO)₂Tr-deprotection in 135 sec. 2) Coupling: 0.1 M phosphoramidite and 0.5 M 1*H*-tetrazole in dry MeCN, delivered in alternating reagent pushes with a subsequent wait time of 60 sec. 3) Capping: Ac₂O/2,6-dimethylpyridine/THF 1:1:8 and 1-methylimidazole/THF 16:84, delivered in one 10 sec push with a subsequent wait time of 5 sec. 4). Oxidation: 0.05 M I₂ in THF/H₂O/pyridine 7:2:1, delivered in one 10 sec push with a subsequent wait time of 15 sec. Then a cleavage programme was carried out: 1) Cleavage of NPE groups by 1 M DBU in MeCN delivered in several pushes and following wait steps (total wait time 20 min). 2) Cleavage of the base-labile groups and from the support: conc. NH₃ solution delivered in one push with consecutive wait time of 1 h. The reaction solution was collected and lyophilized in a *Speed-vac* concentrator under high vacuum.

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