Nucleotides

Part LXXVIII[1])

Double Labeling of Nucleosides and Nucleotides

by **Thomas Maier** and **Wolfgang Pfleiderer***

Fachbereich Chemie, Universität Konstanz, Postfach 5560, D-78457 Konstanz

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

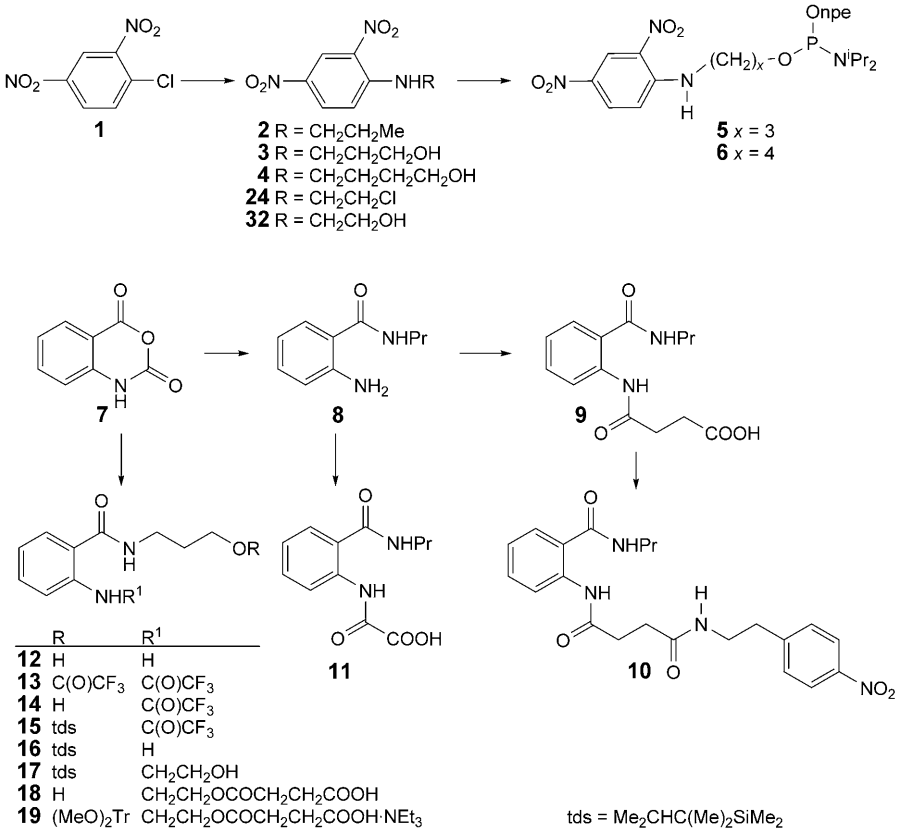
Several *N*(-hydroxyalkyl)-2,4-dinitroanilines were transformed into their phosphoramidites (see **5** and **6** in *Scheme 1*) in view of their use as fluorescence quenchers, and modified 2-aminobenzamides (see **9**, **10**, **18**, and **19** in *Scheme 1*) were applied in model reactions as fluorophors to determine the relative fluorescence quantum yields of the 3′-Aba and 5′-Dnp-3′-Aba conjugates (Aba = aminobenzamide, Dnp = dinitroaniline). Thymidine was alkylated with *N*-(2-chloroethyl)-2,4-dinitroaniline (**24**) to give **25** which was further modified to the building blocks **27** and **28** (*Scheme 3*). The 2-amino group in **29** was transformed by diazotation into the 2-fluoroinosine derivative **30** used as starting material for several reactions at the pyrimidine nucleus (→ **31**, **33**, and **35**; *Scheme 4*). The 3′,5′-di-*O*-acetyl-2′-deoxy-N^2-[(dimethylamino)methylene]guanosine (**37**) was alkylated with methyl and ethyl iodide preferentially at N(1) to **43** and **44**, and similarly reacted *N*-(2-chloroethyl)-2,4-dinitroaniline (**24**) to **38** and the *N*-(2-iodoethyl)-*N*-methyl analog **50** to **53** (*Scheme 5*). The 2′-deoxyguanosine derivative **53** was transformed into 3′,5′-di-*O*-acetyl-2-fluoro-1-{2-[(2,4-dinitrophenyl)methylamino]ethyl}inosine (**54**; *Scheme 5*) which reacted with 2,2′-[ethane-1,2-diylbis(oxy)]bis[ethanamine] to modify the 2-position with an amino spacer resulting in **56** (*Scheme 6*). Attachment of the fluorescein moiety **55** at **56** *via* a urea linkage led to the doubly labeled 2′-deoxyguanosine derivative **57** (*Scheme 6*). Dimethoxytritylation to **58** and further reaction to the 3′-succinate **59** and 3′-phosphoramidite **60** afforded the common building blocks for the oligonucleotide synthesis (*Scheme 6*). Similarly, **30** reacted with *N*-(2-aminoethyl)-2,4-dinitroaniline (**61**) thus attaching the quencher at the 2-position to yield **62** (*Scheme 7*). The amino spacer was again attached at the same site *via* a urea bridge to form **64**. The labeling of **64** with the fluorescein derivative **55** was straigthforward giving **65**. and dimethoxytritylation to **66** and further phosphitylation to **67** followed known procedures (*Scheme 7*). Several oligo-2′-deoxynucleotides containing the doubly labeled 2′-deoxyguanosines at various positions of the chain were formed in a DNA synthesizer, and their fluorescence properties and the T_ms in comparison to their parent duplexes were measured (*Tables 1–5*).

Introduction. – Since 1992 [2], it is known from peptide chemistry that the fluorescence of 2-aminobenzamides can intramolecularly be quenched by 2,4-dinitroaniline residues. This fact was applied to a nucleotide dimer [3] carrying at the 5′-end a dinitroaniline and at the 3′-end a 2-aminobenzamide residue. Fluorescence could only be observed after splitting of the nucleotide linkage. To evaluate the capacity of 2-aminobenzamides as fluorescence markers for oligonucleotides and to study the interaction with 2,4-dinitroaniline derivatives, a series of labeled oligo-2′-deoxynucleotides were prepared by solid-phase synthesis.

[1]) Part LXXVII: [1].

Synthesis. – *N*-Substituted 2,4-dinitroanilines **2**–**4** were easily available from 1-chloro-2,4-dinitrobenzene (**1**) and the corresponding amines (*Scheme 1*). The hydroxy functions of **3** and **4** were coupled with 2-(4-nitrophenyl)ethyl tetraisopropylphosphorodiamidite to the phosphoramidites **5** and **6** which were obtained as yellow oils. The succinic acid derivative **9** for solid-support attachment was synthesized from isatoic anhydride (=2*H*-3,1-benzoxazine-2,4(1*H*)-dione; **7**) with propylamine *via* 2-amino-*N*-propylbenzamide (**8**) and subsequent reaction with succinic anhydride. Coupling of **9** with 2-(4-nitrophenyl)ethylamine and TOTU (=*N*-{{[(1-cyano-2-ethoxy-2-oxoethylidene)amino]oxy}(dimethylamino)methylene}-*N*-methylmethanaminium tetrafluoroborate(1−)) yielded **10** which was used as model substance for cleavage studies from solid-support material. Unfortunately, the amide bond turned out to be too stable for further use. Also the oxalic acid derived linker **11** showed negative results.

Scheme 1

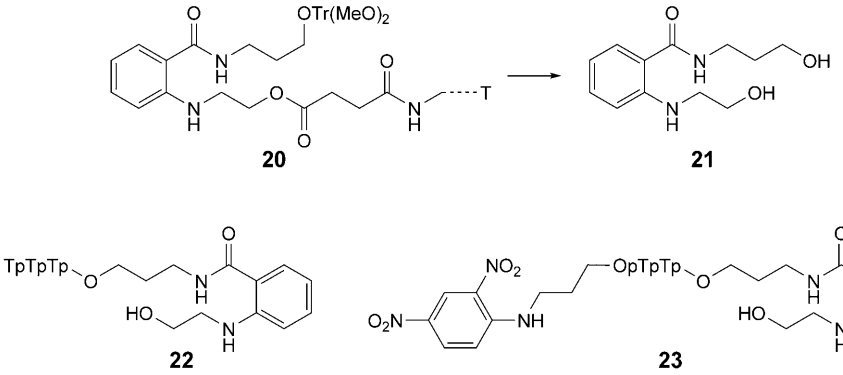


Next, we converted **7** with 3-aminopropan-1-ol into **12** which reacted with trifluoroacetic anhydride to the bistrifluoroacetyl derivative **13** which led, under selective cleavage of the ester bond, to *N*-(3-hydroxypropyl)-2-(2,2,2-trifluoroacetamido)benzamide (**14**) (*Scheme 1*). Silylation of **14** with dimethylthexylsilyl chloride

(=chlorodimethyl(1,1,2-trimethylpropyl)silane = tds-Cl) gave **15** which was subsequently hydrolyzed with K_2CO_3 in a one-pot reaction to 2-amino-*N*-{3-[(dimethylthexylsilyl)oxy]propyl}benzamide (**16**). Treatment of **16** with oxirane led to **17** which was acylated with succinic anhydride and then desilylated with fluoride ion yielding **18**. Dimethoxytritylation gave the linker in form of its triethylammonium salt **19** for further attachment to the solid-support material.

The modified LCAMA-CPG (=(long-chain-alkyl)methylamine controlled-pore glass), LCAA-CPG 500Å [4], was used and loaded with **19** in the usual manner up to 15 μm/g (→**20**; *Scheme 2*). Stability tests showed that this material **20** was well suited for oligonucleotide syntheses since it was stable against the applied reagents and gave, after detritylation and subsequent cleavage with ammonia, chromatographically pure 2-[(2-hydroxyethyl)amino]-*N*-(3-hydroxypropyl)benzamide (**21**).

Scheme 2



After deprotection, short oligo-T-nucleotides with an aminobenzamide (Aba) cap at the 3′-end (see **22**) and an additional dinitroaniline (Dnp) cap at the 5′-end (see **23**) were obtained as products of high purity (*Scheme 2* and *Fig. 1*).

A series of aminobenzamide conjugates with nucleic acid building blocks were synthesized to determine their influence on the fluorescence quantum yield of **21** as the reference (*Table 1*). The purine nucleosides dG and dA showed a small quenching effect by the *Förster* or short-range mechanism [5][6], whereas the pyrimidine nucleosides dC and T quenched by *ca.* 80%. This effect was less severe if the chain started with dG and did not alter on elongation.

Additional attachment of a 2,4-dinitroaniline spacer to the 5′-end, besides the aminobenzamide cap at the 3′-end, caused a strong quenching effect specially in short oligonucleotide chains (*Table 2*). Since 2,4-dinitroaniline shows a transition at 348 nm and a shoulder at 406 nm, there is an overlap with the fluorescence maximum of the aminobenzamide explaining the quench effect by a short-range and/or long-range mechanism.

Since the 2,4-dinitroaniline-derived phosphoramidites **5** and **6** can only be attached to the 5′-end of an oligodeoxynucleotide, we also modified thymidine at the N(3) position by alkylation with *N*-(2-chloroethyl)-2,4-dinitroaniline (**24**) to give **25** (*Scheme 3*). The latter was dimethoxytritylated to **26** followed by phosphitylation to

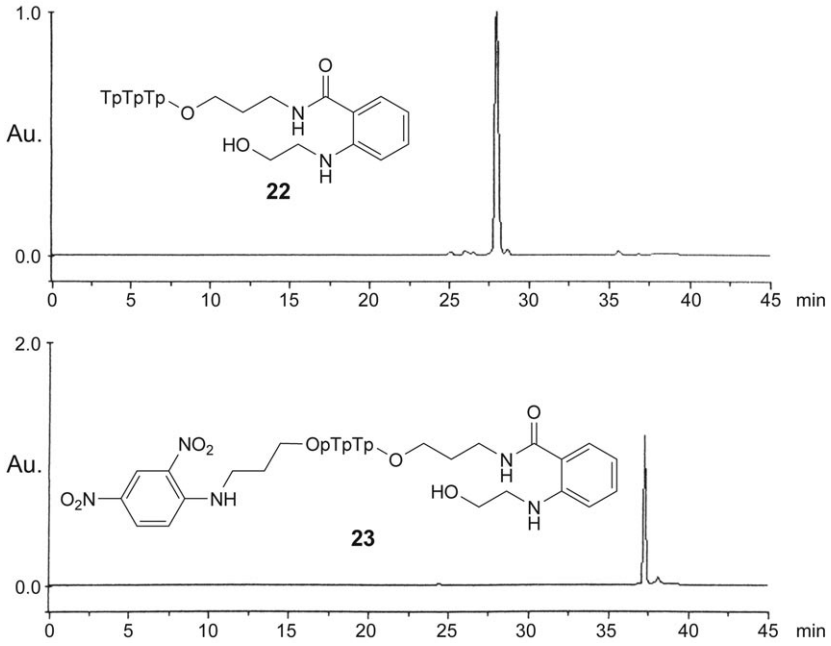


Fig. 1. *HPLC* (*RP 18*) *of* **22** *and* **23**

Table 1. *Relative Fluorescence Quantum Yields of 3′-Aba Conjugates.* Aba = Aminobenzamide, *i.e.*, 2-[(2-hydroxyethyl)amino]-*N*-(3-hydroxypropyl)benzamide (**21**).

Conjugate	Relative quantum yield
Aba	1.0
dG-Aba	0.97
dA-Aba	0.84
dC-Aba	0.18
T-Aba	0.14
TT-Aba	0.14
TTT-Aba	0.14
TTTT-Aba	0.14
d(GG)-Aba	0.62
d(AG)-Aba	0.68
d(CG)-Aba	0.68
d(TG)-Aba	0.67
d(AAA GGG AAC AAA AGC TGG GTA G)-Aba	0.60

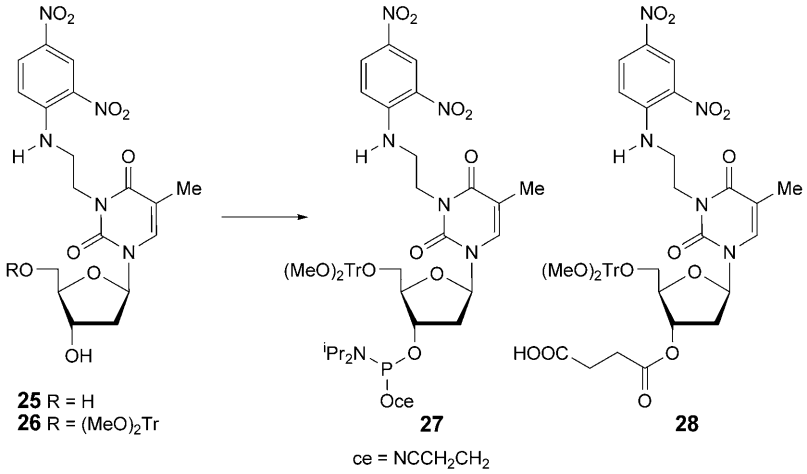
the phosphoramidite **27**. The reaction of **26** with succinic anhydride gave the building block **28**.

A few oligodeoxynucleotides carrying the base-modified thymidine moiety dT^{Dnp} (see **25**) at the 3′- and the 5′-end as well as in the middle of the chain were synthesized,

Table 2. *Relative Fluorescence Quantum Yields of 5′-Dnp-3′-Aba Conjugates.* For Aba, see *Table 1.* Dnp = *N*-Substituted 2,4-dinitroaniline.

Conjugate	Relative quantum yield	Rel. to **21**
TT-Aba	1.00	(0.14)
Dnp-TT-Aba	0.05	
d(TG)-Aba	1.00	(0.69)
d(Dnp-TG)-Aba	0.03	
TTTT-Aba	1.00	(0.14)
Dnp-TTTT-Aba	0.26	
d(AAA GGG AAC AAA AGC TGG GTA G)-Aba	1.00	(0.61)
d(Dnp-AAA GGG AAC AAA AGC TGG GTA G)-Aba	0.76	

Scheme 3



and the melting points of the duplexes with the complementary sequence of the 21-mers were determined (*Table 3*). Attachments at the modified thymidine base at the ends of the oligodeoxynucleotides harmed the duplex formation very little, but attachment in the middle produced a strong hyperchromicity effect of 20%.

Furthermore, we found that the thymidine quencher dT^{Dnp} (see **25**) reduced the fluorescence quantum yields of fluorescein-labeled nucleosides in dimers tremendously, but separation of the two modifiers by increasing chain lengths reduced the interaction as expected (*Table 4*). If adjoined modified dimers were part of an oligodeoxynucleotide chain, the quenching effect could partly be reversed by duplex formation (*Table 4*).

If such modified oligodeoxynucleotide systems are used in DNA diagnostics, complications may arise due to the fact that the neighborhood of the modified units will play an important role, and special sequences have to be chosen to get reliable results.

Table 3. *Melting Points of Duplexes with Modified Oligodeoxynucleotides.* See **25** for dT^{Dnp}. Phosphate buffer pH 7.0: $c(Na^+) = 0.12$M.

Duplex	T_m [°]
d(TCA CCA GCT TTT GTT CCC TTT) d(ATG GGT CGA AAA CAA GGG AAA)	61.2
d(TCA CCA GCT TTT GTT CCC TTT^{Dpn}) d(ATG GGT CGA AAA CAA GGG AAA)	60.6
d(T^{Dnp}CA CCA GCT TTT GTT CCC TTT) d(A TG GGT CGA AAA CAA GGG AAA)	61.1
d(TCA CCA GCT^{Dnp} TTT GTT CCC TTT) d(ATG GGT CGA AAA CAA GGG AAA)	56.4

Table 4. *Relative Fluorescence Quantum Yields in Comparison to Fluorescein.* Flu = Fluorescein; Dnp = *N*-substituted 2,4-dinitroaniline. See **25** for dT^{Dnp}.

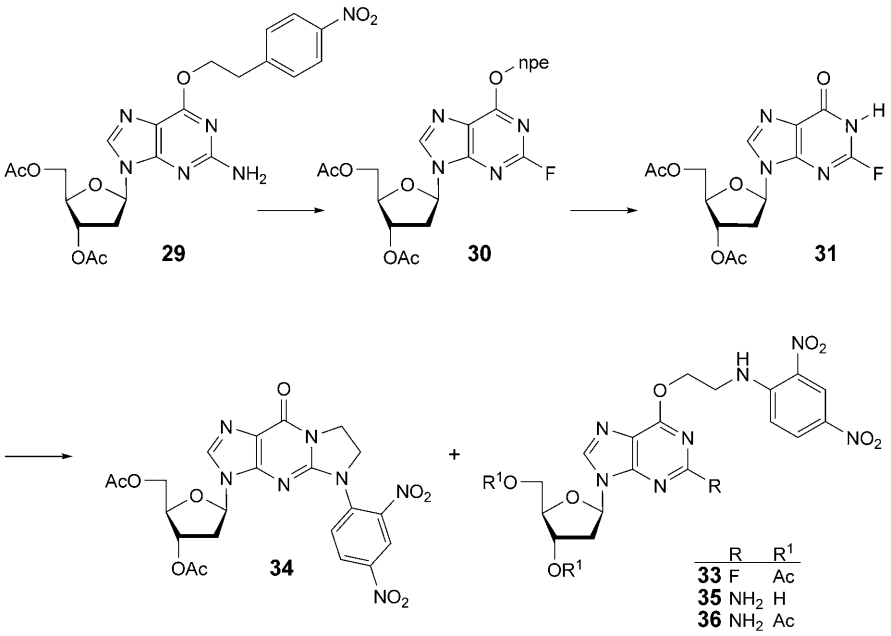
Oligonucleotide conjugates	Fluorescence quantum yields	Oligonucleotide conjugates	Fluorescence quantum yields
Fluorescein	1.00	d(AAA GGG AAC AAA $AGC^{Flu}T^{Dnp}$ GGG TA)	0.16
dC^{Flu}	0.99		
dG^{Flu}	1.01	d(AAA GGG AAC AAA $AGC^{Flu}T^{Dnp}$ GGG TA)	0.27
dA^{Flu}	1.03	d(TTT CCC TTG TTT TCG A CCC AT)	
Dnp-dT-dC^{Flu}	0.05		
Dnp-dT-dG^{Flu}	0.06	d(ACT GCT $GT^{Dnp}C^{Flu}$ GAT TTC CCA C)	0.19
Dnp-dT-dA^{Flu}	0.05		
Dnp-$(dT)_2$-dC^{Flu}	0.11	d(ACT GCT $GT^{Dnp}C^{Flu}$ GAT TTC CCA C)	0.43
Dnp-$(dT)_4$-dC^{Flu}	0.25	d(TGA CGA CA G CTA AAG GGT G)	
Dnp-$(dT)_4$-dG^{Flu}	0.23		
Dnp-$(dT)_4$-dA^{Flu}	0.30	d(ACT GCT $GT^{Dnp}C^{Flu}$ GAT TTC CCA C)	0.31
Dnp-$(dT)_6$-dC^{Flu}	0.38		
Dnp-$(dT)_{10}$-dC^{Flu}	0.77	d(ACT GCT $GT^{Dnp}C^{Flu}$ GAT TTC CCA C)	0.53
Dnp-$(dT)_{15}$-dC^{Flu}	0.85	d(TGA CGA CA G CTA AAG GGT G)	
Dnp-$(dT)_{20}$-dC^{Flu}	0.85		
Dnp-$(dT)_{30}$-dC^{Flu}	0.83		

To overcome these potential difficulties, we started to develop new modified monomeric building blocks carrying both functions – the fluorophor and the quencher – at the same molecule.

Various strategies involving modifications at N(1) and NH_2–C(2) of 2′-deoxyguanosine were followed. Starting with 3′,5′-di-*O*-acetyl-2′-deoxy-O^6-[2-(4-nitrophenyl)ethyl]guanosine (**29**) [7], the amino group was converted by diazotation with *tert*-butyl nitrite in HF [8] into 3′,5′-di-*O*-acetyl-2′-deoxy-2-fluoro-O^6-[2-(4-nitrophenyl)-ethyl]inosine (**30**) (*Scheme 4*). Treatment with DBU (=1,8-diazabicyclo[5.4.0]undec-7-ene) eliminated the 2-(4-nitrophenyl)ethyl (npe) group to give **31** which was alkylated with *N*-(2-iodoethyl)-2,4-dinitroaniline/K_2CO_3 in DMF forming a mixture of 3′,5′-di-*O*-acetyl-2′-deoxy-O^6-{2-[(2,4-dinitrophenyl)amino]ethyl}-2-fluoroinosine (**33**) and 3′,5′-di-*O*-acetyl-2′-deoxy-N^2-(2,4-dinitrophenyl)-1,N^2-ethanoguanosine (**34**), and

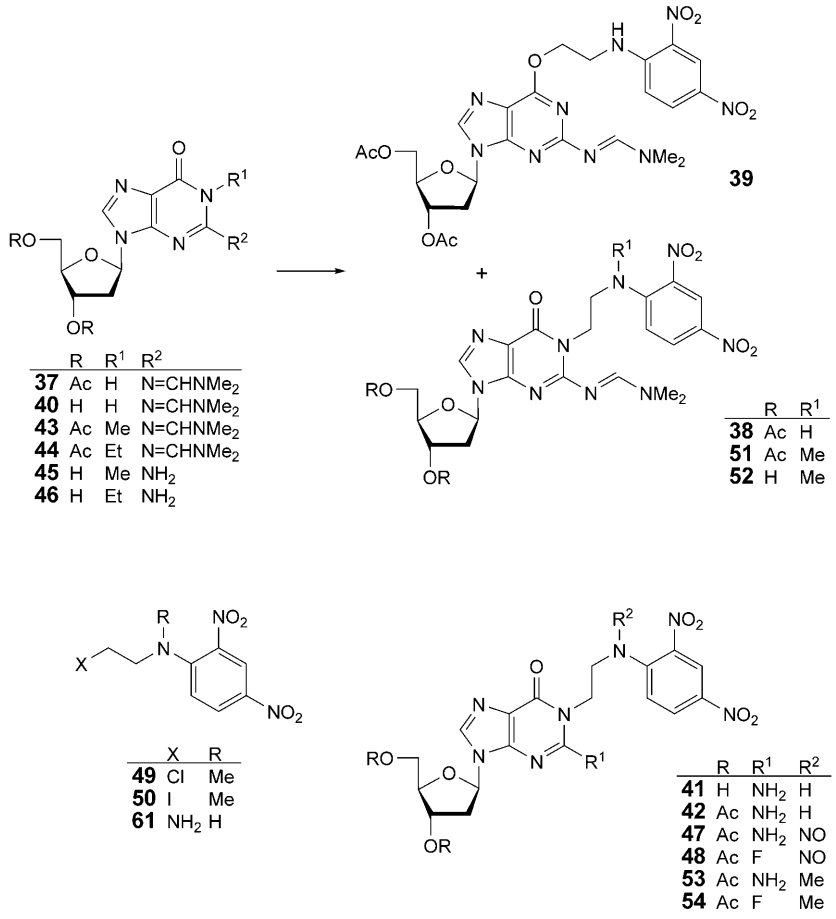
ammonia converted **33** into 2′-deoxy-O^6-{2-[(2,4-dinitrophenyl)amino]ethyl}guanosine (**35**). A *Mitsunobu* reaction of 3′,5′-di-*O*-acetyl-2′-deoxyguanosine with 2-[(2,4-dinitrophenyl)amino]ethanol (**32**; see *Scheme 1*) [9] led directly in high yield to 3′,5′-di-*O*-acetyl-2′-deoxy-O^6-{2-[(2,4-dinitrophenyl)amino]ethyl}guanosine (**36**) which could be deacetylated by ammonia to give **35** again.

Scheme 4



Based upon former results in the pterin series [10], we applied the *Mitsunobu* reaction also to 3′,5′-di-*O*-acetyl-2′-deoxy-N^2-[(dimethylamino)methylene]guanosine [11] (**37**) and obtained with 2-[(2,4-dinitrophenyl)amino]ethanol (**32**; see *Scheme 1*) a mixture of the 1- and O^6-{2-[(2,4-dinitrophenyl)amino]ethyl}-substituted derivatives **38** and **39**) in 60 and 17% yield, respectively (*Scheme 5*). Furthermore, 2′-deoxy-N^2-[(dimethylamino)methylene]guanosine (**40**) reacted with *N*-(2-chloroethyl)-2,4-dinitroaniline in DMF, in the presence of K_2CO_3 under N(1) substitution which gave, after base treatment, 2′-deoxy-1-{2-[(2,4-dinitrophenyl)amino]ethyl}guanosine (**41**). Mild acetylation of **41** led to the protection of the sugar moiety yielding **42** in 84% yield. Compound **37** was also subjected to alkylations with methyl and ethyl iodide, respectively, leading in high yields to N(1) substitution (see **43** and **44**), and after cleavage of the blocking groups to 1-methyl- (**45**) and 1-ethyl-2′-deoxyguanosine (**46**) [12]. Structural assignments of N(1)- and O^6-alkylated 2′-deoxyguanosines can be performed by ^{1}H-NMR and UV spectra. Thus, the chemical shift of the α-protons of the alkyl substituents is characteristically at δ 4.21 for **41** and at δ 4.65 for **35**. The UV spectrum of **35** shows a maximum at 248 and a shoulder at 264 nm, whereas **41** exhibits an absorption maximum at 257 nm.

Scheme 5



	R	R^1	R^2
37	Ac	H	$N{=}CHNMe_2$
40	H	H	$N{=}CHNMe_2$
43	Ac	Me	$N{=}CHNMe_2$
44	Ac	Et	$N{=}CHNMe_2$
45	H	Me	NH_2
46	H	Et	NH_2

	R	R^1
38	Ac	H
51	Ac	Me
52	H	Me

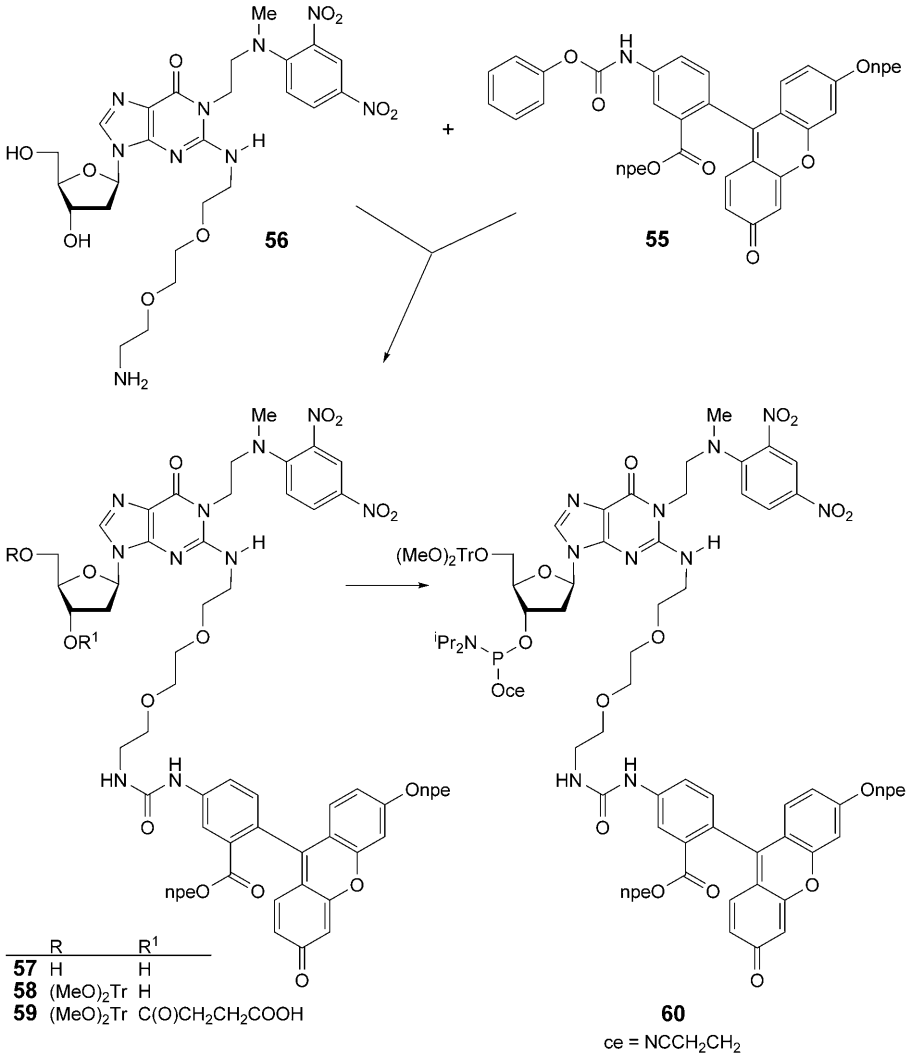
	X	R
49	Cl	Me
50	I	Me
61	NH_2	H

	R	R^1	R^2
41	H	NH_2	H
42	Ac	NH_2	H
47	Ac	NH_2	NO
48	Ac	F	NO
53	Ac	NH_2	Me
54	Ac	F	Me

Diazotation of **42** in 60% HF solution with 1 equiv. of *tert*-butyl nitrite led in low yield to 3′,5′-di-*O*-acetyl-2′-deoxy-1-{2-[(2,4-dinitrophenyl)nitrosoamino]ethyl}guanosine (**47**), and with 5 equiv. of *tert*-butyl nitrite to the 2-fluoroinosine analog **48** (*Scheme 5*). Both compounds were not isolated in pure form but the proposed structures were derived from NMR studies. These experiments showed that the amino function of the side-chain has to be protected to achieve good yields on diazotation. For this purpose, *N*-methyl-2,4-dinitro-*N*-(2-iodoethyl)aniline (**50**) was synthesized from 1-chloro-2,4-dinitrobenzene with 2-(methylamino)ethanol and *via* the corresponding chloro derivative **49**. Alkylation of **37** with **50** in DMF/K_2CO_3 led in 76% yield to 3′,5′-di-*O*-acetyl-2′-deoxy-N^2-[(dimethylamino)methylene]-1-{2-[(2,4-dinitrophenyl)methylamino]ethyl}guanosine (**51**) which was first treated with ammonia to form **52**, then acetylated mildly with Ac_2O to give N^2-deprotected **53**, and finally converted with *tert*-butyl nitrite in 60% HF solution into 3′,5′-di-*O*-acetyl-2′-deoxy-2-fluoro-1-{2-[(2,4-dinitrophenyl)methylamino]ethyl)inosine (**54**) in 78% yield.

Compound **54** was then treated with 2,2′-[ethane-1,2-diylbis(oxy)]bis[ethanamine] to introduce the spacer into position 2, followed by deprotection of the sugar moiety to give N^2-{[2-[2-(2-aminoethoxy)ethoxy]ethyl}amino}-2′-deoxy-1-{2-[(2,4-dinitrophenyl)-methylamino]ethyl}guanosine (**56**) in 77% yield (*Scheme 6*). In two subsequent steps, **56** was first treated with 2-(4-nitrophenyl)ethyl 5-[(phenoxycarbonyl)amino]-2-{6-[2-(4-nitrophenyl)ethoxy]-3-oxo-3*H*-xanthen-9-yl}benzoate (**55**) in presence of *N*,*N*-dimethylpyridin-4-amine (DMAP) to introduce the fluorescent label yielding **57**, then dimethoxytritylation proceeded in the usual manner to give **58** in high (88%) yield. In TLC (thin-layer chromatography) experiments, we established that **58** reacted with the

Scheme 6



	R	R^1
57	H	H
58	$(MeO)_2Tr$	H
59	$(MeO)_2Tr$	$C(O)CH_2CH_2COOH$

capping mixture (Ac_2O/1-methyl-1*H*-imidazole/lutidine/THF; used subsequently in the oligonucleotide synthesis) only at the sugar moiety, whereas the NH–C(2) function was fortunately not protected. Acylation at NH–C(2) during capping would have created problems in oligonucleotide synthesis since N^2-deacetylation with ammonia would have required too long reaction times and higher temperatures. Compound **58** was, therefore, a perfect starting material for the syntheses of the two building blocks, the 3′-succinate **59** and the 3′-phosphoramidite **60**.

A second strategy of double labeling of 2′-deoxyguanosine consisted in the introduction of both the quencher and fluorophor at the 2-amino group. A corresponding synthesis started from **30** (see *Scheme 4*) which was treated with *N*-(2-aminoethyl)-2,4-dinitroaniline (= *N*-(2,4-dinitrophenyl)ethane-1,2-diamine; **61**) yielding **62** (*Scheme 7*). Activation of the secondary amino group by phenyl carbonochloridate gave 3′,5′-di-*O*-acetyl-2′-deoxy-N^2-{2-[(2,4-dinitrophenyl)amino]ethyl}-O^6-[2-(4-nitrophenyl)ethyl]-N^2-(phenoxycarbonyl)guanosine (**63**) which reacted with the 2,2′-[ethane-1,2-diylbis(oxy)]bis[ethanamine] spacer to the urethane **64**. Deacetylation by ammonia in MeOH/dioxane and subsequent treatment with **55** under DMAP catalysis led in 78% yield to **65**. Finally, the building block **67** was obtained in two more steps by dimethoxytritylation to **66** and its interconversion into the corresponding 3′-(2-cyanoethyl *N*,*N*-diisopropylphosphoramidite) **67**.

Structures. – The structures of the newly synthesized compounds were confirmed by elemental analyses as well as UV and NMR spectra reported in the *Exper. Part*.

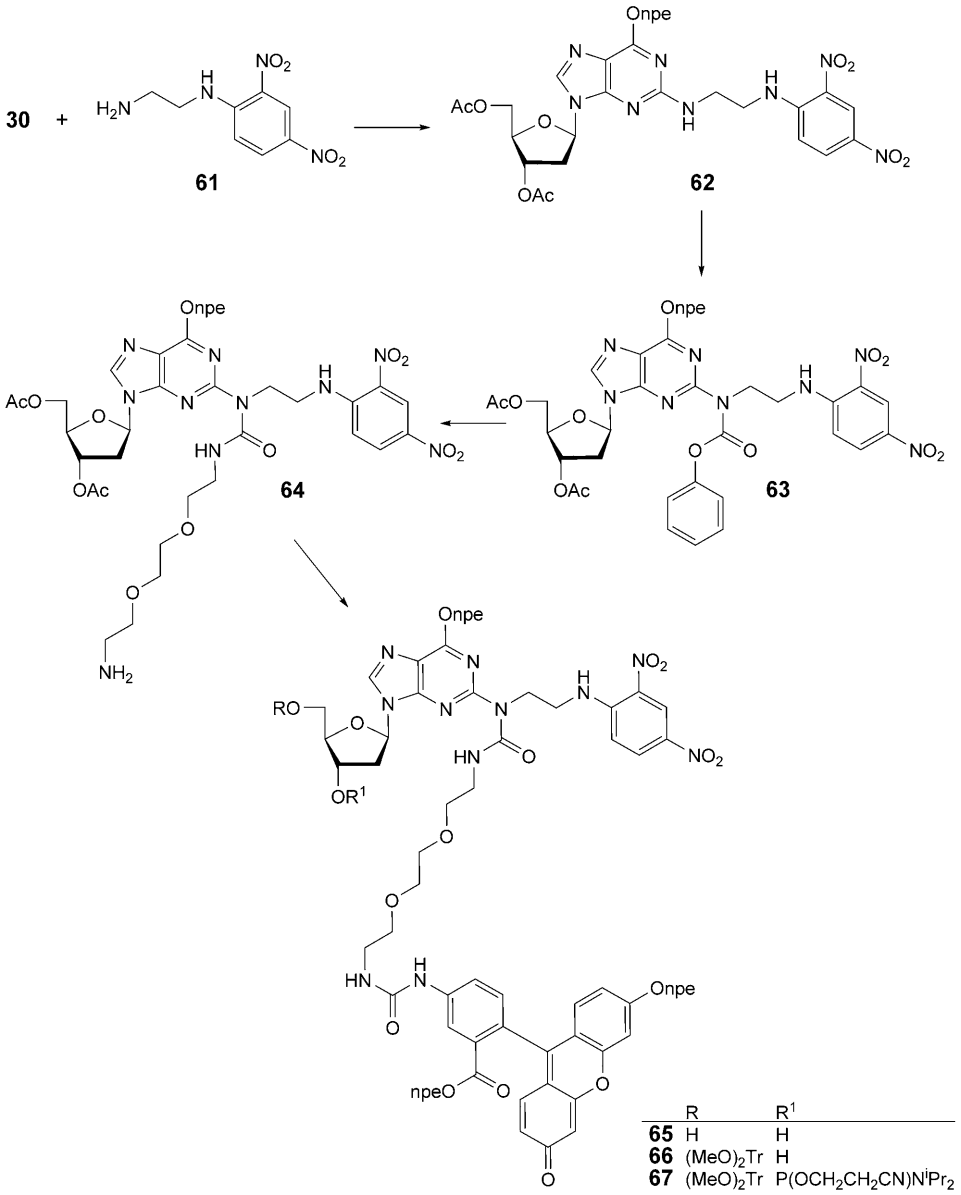
Oligonucleotide Syntheses. – The syntheses of the doubly labeled 2′-deoxyguanosines were based on the idea that in a single-strand oligo-2′-deoxynucleotide a 'random-coil' conformation exists allowing the quencher and fluorophor to adopt a close location to quench the fluorescence. In the corresponding duplex, both functionalities should be so far apart that the quench effect is limited. To corroborate this hypothesis, a series of marked oligo-2′-deoxynucleotides, **68**–**72**, were assembled in a DNA synthesizer (*Table 5*). These oligomers were paired with the unlabeled complementary sequence and their melting points determined and compared with those of the unmodified duplexes which showed as expected, a T_m higher by 8–10° (*Table 5*).

Comparing the relative fluorescence quantum yields of the modified single strands with their duplexes, we see in the case of **68**, **70**, and **72** an increase of the quantum yield by a factor of 1.6–1.8, and in the case of **69** and **71** a remarkably higher increase by a factor of 2.9 and 4.4, respectively (*Table 6*). This effect was temperature-dependent, and close to the T_m, the increased fluorescence in the duplexes was reduced to the level of the single strain (*Fig. 2*).

Experimental Part

General. Products were dried under high vacuum. All solvents used were of anh. grade. DNA Syntheses: *Eppendorf-Biotronik-Ecosyn-D-300* and *Applied-Biosystems-392* synthesizer. TLC: precoated silica gel thin-layer sheets *60 F254* (*Merck*). Flash chromatography (FC): silica gel (30–60 μm; *Baker*); 0.2–0.3 bar. Column chromatography = CC. M.p.: *Büchi-510* melting-point apparatus; no corrections. UV/VIS: *Perkin-Elmer Lambda 15*; λ_{max} in nm (log ε). Temp.-dependent UV/VIS: *Perkin-*

Scheme 7



Elmer Lambda 2 with *PTP-6* (*Peltier* temp. programmer). ^{1}H-NMR: *Bruker AC 250*; δ in ppm rel. to Me_4Si, or $CDCl_3$, or (D_6)DMSO) as internal standard. ^{31}P-NMR: *Joel JNM-GX400.*

1. N-*Propyl-2,4-dinitrobenzenamine* (**2**). A soln. of 1-chloro-2,4-dinitrobenzene (1.01 g, 5 mmol) in dry CH_2Cl_2 (10 ml) was treated with propan-1-amine (0.325 g, 5.5 mmol) at r.t. for 1 h. The mixture was concentrated, then AcOH (1.5 ml) added, and the precipitate collected and recrystallization from EtOH/

Table 5. *Melting Points of Modified and Unmodified Oligo-2′-deoxynucleotide Duplexes.* Dnp = *N*-Substituted 2,4-dinitroaniline; Flu = fluorescein. Phosphate buffer pH 8, $c(Na^+) = 0.12$M.

		T_m [°]	
		modified	unmodified
68	5′-d(AAA GGG AAC AAA AG$^{1\text{-Dnp},N^2\text{-Flu}}$C TGC GTA)-3′ 3′-d(TTT CCC TTG TTT TC G ACC CAT)-5′	52.5	60.5
69	5′-d(AAA GGG AAC AAA AG$^{N^2\text{-Dnp},N^2\text{-Flu}}$C TGG GTA)-3′ 3′-d(TTT CCC TTG TTT TC G ACC CAT)-5′	51.1	60.5
70	5′-d(ACT GCT GTC G$^{1\text{-Dnp},N^2\text{-Flu}}$AT TTC CCA C)-3′ 3′-d(TGA CGA CAG C TA AAG GGT G)-5′	53.2	62.7
71	5′-d(ACT GCT GTC G$^{N^2\text{-Dnp},N^2\text{-Flu}}$AT TTC CCA C)-3′ 3′-d(TGA CGA CAG C TA AAG GGT G)-5′	51.9	62.7
72	5′-d(CGA CCC CCA TG$^{1\text{-Dnp},N^2\text{-Flu}}$G AGC CCC GC)-3′ 3′-d(GCT GGG GGC AC C TCG GGG CG)-5′	62.6	70.0

Table 6. *Relative Fluorescence Quantum Yields of Modified Oligo-2′-deoxynucleotides.* Dnp = *N*-Substituted 2,4-dinitroaniline; Flu = fluorescein. Phosphate buffer pH 8, $c(Na^+) = 0.12$M. Temperature 5°.

		Relative quantum yield	Factor
68	5′-d(AAA GGG AAC AAA AG$^{1\text{-Dnp},N^2\text{-Flu}}$C TGC GTA)-3′	0.30	
68	5′-d(AAA GGG AAC AAA AG$^{1\text{-Dnp},N^2\text{-Flu}}$C TGC GTA)-3′ 3′-d(TTT CCC TTG TTT TC G ACC CAT)-5′	0.48	1.6
69	5′-d(AAA GGG AAC AAA AG$^{N^2\text{-Dnp},N^2\text{-Flu}}$C TGG GTA)-3′	0.14	
69	5′-d(AAA GGG AAC AAA AG$^{N^2\text{-Dnp},N^2\text{-Flu}}$C TGG GTA)-3′ 3′-d(TTT CCC TTG TTT TC G ACC CAT)-5′	0.41	2.9
70	5′-d(ACT GCT GTC G$^{1\text{-Dnp},N^2\text{-Flu}}$AT TTC CCA C)-3′	0.31	
70	5′-d(ACT GCT GTC G$^{1\text{-Dnp},N^2\text{-Flu}}$AT TTC CCA C)-3′ 3′-d(TGA CGA CAG C TA AAG GGT G)-5′	0.42	1.4
71	5′-d(ACT GCT GTC G$^{N^2\text{-Dnp},N^2\text{-Flu}}$AT TTC CCA C)-3′	0.11	
71	5′-d(ACT GCT GTC G$^{N^2\text{-Dnp},N^2\text{-Flu}}$AT TTC CCA C)-3′ 3′-d(TGA CGA CAG C TA AAG GGT G)-5′	0.48	4.4
72	5′-d(CGA CCC CCA TG$^{1\text{-Dnp},N^2\text{-Flu}}$G AGC CCC GC)-3′	0.33	
72	5′-d(CGA CCC CCA TG$^{1\text{-Dnp},N^2\text{-Flu}}$G AGC CCC GC)-3′ 3′-d(GCT GGG GGC AC C TCG GGG CG)-5′	0.52	1.6

H_2O with charcoal: 1.1 g (98%) of **2**. Yellow crystals. M.p. 97–99°. UV (MeOH): 212 (4.08), 259 (3.93), 348 (4.22), 406 (sh, 3.79). ^{1}H-NMR ((D_6)DMSO): 8.81 (*t*, NH); 8.76 (*d*, H–C(3)); 8.16 (*dd*, H–C(5)); 7.15 (*d*, H–C(6)); 3.41 (*q*, $MeCH_2CH_2$); 1.63 (*sext.*, $MeCH_2CH_2$); 0.93 (*t*, $MeCH_2CH_2$). Anal. calc. for $C_9H_{11}N_3O_4$ (225.2): C 48.00, H 4.92, N 18.65; found: C 47.94, H 4.92, N 18.52.

2. *3-[(2,4-Dinitrophenyl)amino]propan-1-ol* (**3**). To a mixture of 3-aminopropan-1-ol (2.25 g, 30 mmol) and Et_3N (13 ml) in dry CH_2Cl_2 (100 ml) was added dropwise a soln. of 1-chloro-2,4-dinitrobenzene (2.02 g, 10 mmol) in CH_2Cl_2 (10 ml) and stirred at r.t. for 2 h. After evaporation, the

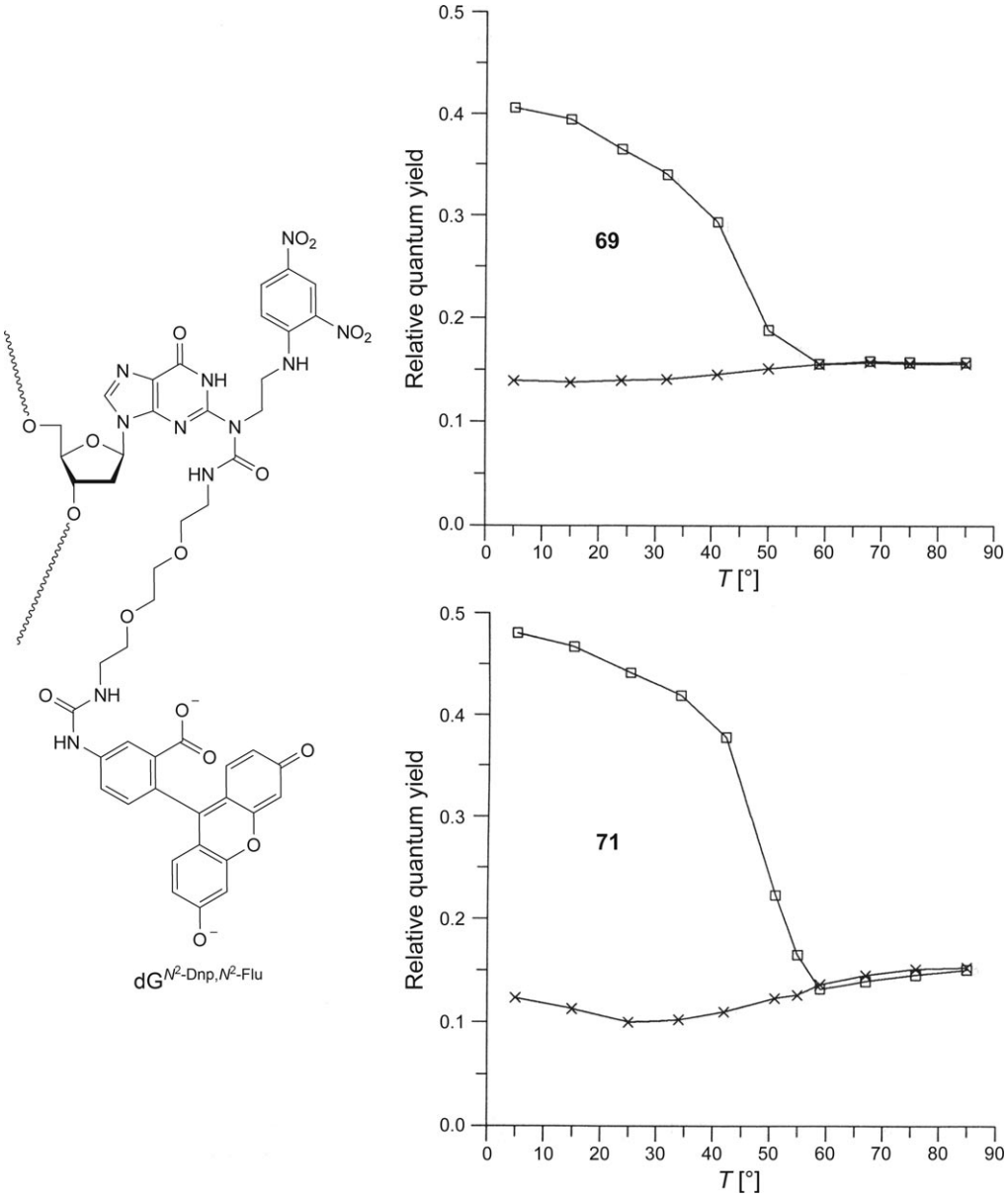


Fig. 2. *Temperature-dependent fluorescence quantum yields of* **69** *and* **71** *in their single strain* (×) *and duplex* (□)

residue was dissolved again in CH_2Cl_2 (50 ml) and the soln. washed with $NaHCO_3$ soln. (3×), dried (Na_2SO_4), and concentrated. The red syrup was dissolved in little EtOH and kept in an icebox to give 2.0 g (83%) of **3**. Orange-red crystals. M.p. 73°. UV (MeOH): 212 (4.11), 260 (3.94), 349 (4.23), 400 (sh, 3.82). ^{1}H-NMR ((D$_6$)DMSO): 9.01 (*t*, NH); 8.80 (*d*, H–C(3)); 8.22 (*dd*, H–C(5)); 7.12 (*d*, H–C(6));

4.80 (br. *t*, OH); 3.55 (*m*, C*H*$_2$CH$_2$C*H*$_2$OH); 1.80 (*m*, CH$_2$C*H*$_2$CH$_2$OH). Anal. calc. for $C_9H_{11}N_3O_5$ (241.2): C 44.82, H 4.60, N 17.41; found: C 44.91, H 4.60, N 17.33.

3. *4-[(2,4-Dinitrophenyl)amino]butan-1-ol* (**4**). As described for **3**, with 4-aminobutan-1-ol (2.7 g, 30 mmol). The oily residue was crystallized from EtOH: 2.0 g (78%) of **4**. Orange crystals. M.p. 94–96°. UV (MeOH): 212 (4.13), 259 (3.94), 348 (4.23), 399 (sh, 3.82). ^{1}H-NMR ((D$_6$)DMSO): 8.88 (*t*, NH); 8.81 (*d*, H–C(3)); 8.21 (*dd*, H–C(5)); 7.21 (*d*, H–C(6)); 4.53 (br. *t*, OH); 3.53–3.42 (*m*, C*H*$_2$CH$_2$CH$_2$C*H*$_2$OH); 1.71–1.42 (*m*, CH$_2$C*H*$_2$C*H*$_2$CH$_2$OH). Anal. calc. for $C_{10}H_{13}N_3O_5$ (255.2): C 47.06, H 5.13, N 16.46; found: C 47.43, H 5.21, N 16.70.

4. *3-[(2,4-Dinitrophenyl)amino]propyl 2-(4-Nitrophenyl)ethyl* N,N-*Diisopropylphosphoramidite* (**5**). A mixture of **3** (0.12 g, 0.5 mmol) and sublimed 1*H*-tetrazole (17.5 mg, 0.25 mmol) in MeCN/CH$_2$Cl$_2$ 1:1 (4 ml) was treated with N$_2$ for 5 min. Then, 2-(4-nitrophenyl)ethyl *N,N,N′,N′*-tetraisopropylphosphorodiamidite (0.338 g, 0.85 mmol) was added, and after stirring at r.t. for 2 h, the mixture was diluted with CH$_2$Cl$_2$. The soln. was washed with sat. NaHCO$_3$/NaCl soln., dried (Na$_2$SO$_4$), and concentrated. The residue was dissolved in little hexane/acetone and subjected to FC (silica gel (3 × 20 cm), hexane/acetone 4:1 (200 ml) and hexane/acetone 1:1 (400 ml) containing 1.5% Et$_3$N). The residue of the product fractions was co-concentrated with CH$_2$Cl$_2$: 0.18 g (67%) of **5**. Red oil. UV (MeOH): 210 (4.40), 264 (4.26), 348 (4.22), 398 (sh, 3.82). ^{1}H-NMR (CDCl$_3$): 9.12 (*d*, H–C(3)); 8.69 (*t*, NH); 8.70 (*dd*, H–C(5)); 8.12 (*d*, 2 H *o* to NO$_2$); 7.43 (*d*, 2 H *m* to NO$_2$); 6.45 (*d*, H–C(6)); 3.96–3.60 (*m*, 2 Me$_2$C*H*, CH$_2$C*H*$_2$O); 3.59–3.51 (*m*, C*H*$_2$CH$_2$C*H*$_2$O); 3.02 (*t*, C*H*$_2$CH$_2$O); 2.03 (*m*, CH$_2$C*H*$_2$CH$_2$O); 1.11 (*m*, 2 *Me*$_2$CH). ^{31}P-NMR (CDCl$_3$): 147.2.

5. *4-[(2,4-Dinitrophenyl)amino]butyl 2-(4-Nitrophenyl)ethyl* N,N-*Diisopropylphosphoramidite* (**6**). As described for **5**, with **4** (0.5 g, 2 mmol), 1*H*-tetrazole (35 mg, 0.5 mmol), CH$_2$Cl$_2$/MeCN 1:1 (6 ml), and 2-(4-nitrophenyl)ethyl *N,N,N′,N′*-tetraisopropylphosphorodiamidite (1.2 g, 3 mmol) (stirring overnight). FC (hexane/acetone 9:1 (200 ml), hexane/acetone 4:1 (100 ml), and hexane/acetone 7:3 (400 ml) containing 1.5% Et$_3$N), and co-evaporation gave 0.75 g (68%) of **6**. Red oil. UV (MeOH): 210 (4.41), 263 (4.28), 347 (4.23), 399 (sh, 3.83). ^{1}H-NMR (CDCl$_3$): 9.11 (*d*, H–C(3)); 8.71 (*t*, NH); 8.70 (*dd*, H–C(5)); 8.10 (*d*, 2 H *o* to NO$_2$); 7.45 (*d*, 2 H *m* to NO$_2$); 6.47 (*d*, H–C(6)); 3.98–3.60 (*m*, 2 Me$_2$C*H*, CH$_2$C*H*$_2$O); 3.61–3.50 (*m*, C*H*$_2$CH$_2$CH$_2$C*H*$_2$O); 3.00 (*t*, C*H*$_2$CH$_2$O); 2.05 (*m*, CH$_2$C*H*$_2$C*H*$_2$CH$_2$O); 1.09 (*m*, 2 *Me*$_2$CH). ^{31}P-NMR (CDCl$_3$): 148.1. Anal. calc. for $C_{24}H_{34}N_5O_8P$ (551.5): C 52.27, H 6.21, N 12.70; found: C 52.78. H 6.22, N 13.00.

6. *2-Amino*-N-*propylbenzamide* (**8**). To a soln. of 3*H*-1,3-benzoxazine-2,4(1*H*)-dione (**7**; 4.09 g, 25 mmol) in dry DMF (40 ml) was added dropwise propan-1-amine (2.07 g, 25 mmol) in DMF (5 ml) within 20 min. The mixture was stirred for 3 h and then concentrated. The residue was recrystallized from H$_2$O (50 ml): 4.0 g (89%) of **8**. Colorless crystals. M.p. 104°. UV (MeOH): 213 (4.33), 247 (3.92), 322 (3.57). ^{1}H-NMR (CDCl$_3$): 7.33–7.20 (2*d*, H–C(3), H–C(5)); 6.75–6.62 (*m*, H–C(4), H–C(6)); 6.08 (br. *s*, NH); 3.37 (*q*, MeCH$_2$C*H*$_2$); 1.65 (*sext.*, MeC*H*$_2$CH$_2$); 1.00 (*t*, *Me*CH$_2$CH$_2$). Anal. calc. for $C_{10}H_{14}N_2O$ (179.1): C 67.39, H 7.92, N 15.71; found: C 67.02, H 7.81, N 15.60.

7. *4-Oxo-4-{[2-[(propylamino)carbonyl]phenyl}amino}butanoic Acid* (**9**). A suspension of **8** (1.07 g, 6 mmol) in dry toluene (25 ml) was treated with succinic anhydride (0.8 g, 8 mmol) at 40° for 3 h. The precipitate was recrystallized from little H$_2$O: 1.4 g (85%) of **9**. Colorless crystals. M.p. 167°. UV (MeOH): 213 (4.37), 251 (4.16), 296 (3.43). ^{1}H-NMR ((D$_6$)DMSO): 12.19 (br. *s*, COOH); 11.34 (br. *s*, NH); 8.69 (*t*, PrN*H*); 8.36 (*d*, H–C(3)); 7.72 (*dd*, H–C(6)); 7.46 (*dd*, H–C(5)); 7.12 (*t*, H–C(4)); 3.21 (*q*, C(O)C*H*$_2$CH$_2$COOH); 2.55 (*m*, C(O)CH$_2$C*H*$_2$COOH, MeCH$_2$C*H*$_2$NH); 1.58 (*sext.*, MeC*H*$_2$CH$_2$NH); 0.91 (*t*, *Me*CH$_2$CH$_2$NH). Anal. calc. for $C_{14}H_{18}N_2O_4$ (278.3): C 60.42, H 6.52, N 10.06; found: C 60.23, H 6.46, N 10.04.

8. N^1-*[2-(4-Nitrophenyl)ethyl]*-N^4-*{2-[(propylamino)carbonyl]phenyl}butanediamide* (**10**). A mixture of **9** (0.278 g, 1 mmol) and 2-(4-nitrophenyl)ethanamine hydrochloride (0.202 g, 1 mmol) in dry CH$_2$Cl$_2$/DMF 1:1 (10 ml) was treated with TOTU (0.328 g, 1 mmol), followed by *N*-methylmorpholine (0.22 ml, 2 mmol). After stirring at r.t. for 2 h, the mixture was concentrated and the residue dissolved in CH$_2$Cl$_2$ (30 ml). The soln. was washed with sat. NaHCO$_3$ soln. (3 ×), dried (Na$_2$SO$_4$), and concentrated and the residue subjected to FC (silica gel (2.5 × 20 cm), toluene/AcOEt 1:1 (200 ml), toluene/AcOEt/MeOH 49:49:2 (200 ml), toluene/AcOEt/MeOH 48.5:48.5:3 (200 ml), toluene/AcOEt/MeOH 48:48:4 (200 ml), and toluene/AcOEt/MeOH 47.5:47.5:5 (200 ml)). The residue of the product

fractions was recrystallized from toluene (20 ml): 0.388 g (91%) of **10**. Colorless crystals. M.p. 159–160°. UV (MeOH): 213 (4.47), 254 (4.30), 285 (sh, 4.05). ^{1}H-NMR ($CDCl_3$): 11.22 (br. *s*, 2 NH); 8.49 (*d*, H–C(6)); 8.05 (*d*, 2 H *o* to NO_2); 7.45 (*m*, H–C(3), H–C(5)); 7.33 (*d*, 2 H *m* to NO_2); 7.09 (*t*, H–C(4)); 6.44 (br. *t*, NHCO); 6.28 (*s*, ArN*H*CO); 3.56 (*q*, CH_2NH); 3.39 (*q*, CH_2NH); 2.94 (*t*, ArCH_2); 2.74 (*t*, CH_2(Suc)); 2.52 (*t*, CH_2(Suc)); 1.70–1.60 (*sext.*, MeCH_2CH_2); 1.01 (*t*, *Me*CH_2CH_2). Anal. calc. for $C_{22}H_{26}N_4O_5$ (426.5): C 61.96, H 6.14, N 13.14; found: C 61.89, H 6.15, N 13.37.

9. *2-Oxo-2-{[2-[(propylamino)carbonyl]phenyl}amino}acetic Acid* (**11**). A mixture of **8** (1.06 g, 6 mmol) and oxalic acid dihydrate (7.56 g, 60 mmol) in toluene (100 ml) was heated under reflux under a water separator for 3 h. The toluene was evaporated, the residue dissolved in little DMF and then diluted with H_2O (100 ml). The resulting precipitate was recrystallized from EtOH/H_2O 1:1 (100 ml): 1.1 g (73%) of **11**. Colorless crystals. M.p. 186° (dec.). UV (MeOH): 209 (4.16), 225 (4.10), 263 (4.03), 296 (3.82). ^{1}H-NMR ((D_6)DMSO): 14.40 (br. *s*, COOH); 12.67 (br. *s*, ArN*H*CO); 8.83 (br. *s*, PrN*H*CO); 8.51 (*d*, H–C(3)); 7.77 (*d*, H–C(6)); 7.55 (*t*, H–C(5)); 7.23 (*t*, H–C(4)); 3.23 (*q*, MeCH_2CH_2); 1.55 (*sext.*, MeCH_2CH_2); 0.92 (*t*, *Me*CH_2CH_2). Anal. calc. for $C_{12}H_{14}N_2O_4$ (250.2): C 57.60, H 5.64, N 11.19; found: C 57.25, H 5.64, N 11.09.

10. N-*(3-Hydroxypropyl)-2-[(2,2,2-trifluoroacetyl)amino]benzamide* (**14**). To a soln. of **7** (8.18 g, 50 mmol) in dry DMF (30 ml) was added dropwise 3-aminopropan-1-ol (4.5 g, 60 mmol) in DMF (5 ml). After stirring for 3 h, the mixture was concentrated and the residue of **12** dissolved in CH_2Cl_2 (30 ml). The soln. was cooled to 0°, Et_3N (10 ml) and then dropwise 2,2,2-trifluoroacetic anhydride (15 ml, 110 mmol) were added. The soln. was slowly warmed to r.t. and then concentrated to give crude **13**. The solid was dissolved in pyridine/ H_2O 1:1 (100 ml), then Et_3N (10 ml) was added and the mixture kept overnight to cleave the ester bond. The mixture was concentrated and co-concentrated four times with toluene. The residue was dissolved in CH_2Cl_2 (150 ml), the soln. washed with sat. $NaHCO_3$ soln. (3×), dried (Na_2SO_4), and then again concentrated. The solid was dissolved in toluene/CH_2Cl_2 and subjected to FC (silica gel (4 × 40 cm), toluene/AcOEt 1:1 (200 ml), toluene/AcOEt 1:1 + 2% MeOH (200 ml), toluene/AcOEt 1:1 + 4% MeOH (200 ml), toluene/AcOEt 1:1 + 6% MeOH (200 ml), toluene/AcOEt 1:1 + 8% MeOH (400 ml), and toluene/AcOEt 1:1 + 10% MeOH (400 ml)). The residue of the product fractions was recrystallized from MeOH/H_2O: 9.3 g (65%) of **14**. Colorless fine needles. M.p. 88–89°. UV (MeOH): 215 (4.31), 251 (4.19), 293 (3.66). ^{1}H-NMR ((D_6)DMSO): 13.32 (br. *s*, CF_3CON*H*); 8.97 (br. *s*, PrN*H*CO); 8.34 (*d*, H–C(3)); 7.87 (*d*, H–C(6)); 7.60 (*t*, H–C(5)); 7.23 (*t*, H–C(4)); 4.51 (*t*, OH); 3.46 (*t*, $CH_2CH_2CH_2$OH); 3.32 (*t*, C$H_2CH_2CH_2$OH); 1.69 (*sext.*, $CH_2CH_2CH_2$OH). Anal. calc. for $C_{12}H_{13}F_3N_2O_3$ (291.3): C 49.66, H 4.51, N 9.65; found: C 49.77, H 4.56, N 9.66.

11. *2-Amino*-N-*{3-{[dimethyl(1,1,2-trimethylpropyl)silyl]oxy}propyl}benzamide* (**16**). Compound **14** (2.9 g, 10 mmol) was co-concentrated twice with dry pyridine and then dissolved in this solvent (30 ml). Chlorodimethyl(1,1,2-trimethylpropyl)silane (2.55 ml, 13 mmol) was added and the mixture stirred at r.t. for 3 h. After evaporation and co-evaporation with toluene (3×), the residue was dissolved in CH_2Cl_2 (100 ml), the soln. washed with sat. $NaHCO_3$ soln. (2×) and H_2O, dried (Na_2SO_4), and again concentrated. The residue of **15** was dissolved in MeOH/H_2O 9:1 (100 ml), then K_2CO_3 (5.0 g, 36 mmol) was added and the mixture heated under reflux for 3 h. After cooling, the mixture was neutralized with AcOH and concentrated, the residue suspended in CH_2Cl_2 (100 ml), the mixture washed with sat. $NaHCO_3$ soln. (2×), the org. phase dried (Na_2SO_4) and concentrated, and the residue dissolved in little toluene and subjected to FC (silica gel (4 × 30 cm), toluene (200 ml), toluene/AcOEt 95:5 (200 ml), toluene/AcOEt 9:1 (200 ml), toluene/AcOEt 4:1 (400 ml), toluene/AcOEt 3:2 (400 ml), and toluene/AcOEt 5:5 (200 ml)): 3.0 g (89%) of **16**. Colorless oil. UV (MeOH): 212 (4.32), 247 (sh, 3.94), 323 (3.58). ^{1}H-NMR ((D_6)DMSO): 8.12 (br. *t*, N*H*CO); 7.45 (*d*, H–C(6)); 7.08 (*t*, H–C(4)); 6.66 (*d*, H–C(3)); 6.49 (*t*, H–C(5)); 6.34 (br. *s*, NH_2); 3.61 (*t*, $CH_2CH_2CH_2$O); 3.26 (*q*, C$H_2CH_2CH_2$O); 1.71 (*m*, $CH_2CH_2CH_2$O); 1.58 (*sept.*, Me_2CH); 0.86 (*m*, *Me*CHC(*Me*)$_2$Si); 0.06 (2*s*, Me_2Si). Anal. calc. for $C_{18}H_{32}N_2O_2Si$ (336.5): C 64.24, H 9.58, N 8.52; found: C 64.16, H 9.48, N 8.31.

12. *2-[(2-Hydroxyethyl)amino]*-N-*{3-{[dimethyl(1,1,2-trimethylpropyl)silyl]oxy}propyl}benzamide* (**17**). To a cold soln. (0°) of **16** (2.35 g, 7 mmol) in THF/H_2O 2:1 (30 ml) was added AcOH (1.8 g, 30 mmol), then oxirane (3.3 ml, 70 mmol, prepared at −70°) was added at 0°, and the mixture was stirred overnight at r.t. The mixture was concentrated, the residue dissolved in CH_2Cl_2 (100 ml), the soln. washed with sat. $NaHCO_3$ soln. (2×), dried (Na_2SO_4), and again concentrated, and the residue purified

by FC (silica gel (2.5 × 35 cm), toluene/AcOEt 1:1 (150 ml), toluene/AcOEt 1:1 + 2% MeOH (150 ml), toluene/AcOEt 1:1 + 4% MeOH (150 ml), toluene/AcOEt 1:1 + 7% MeOH (300 ml), and toluene/AcOEt 1:1 + 10% MeOH (300 ml): 1.7 g (53) of **17**. Colorless oil. UV (MeOH): 215 (4.37), 256 (4.07), 338 (3.68). ^{1}H-NMR ((D$_6$)DMSO): 8.20 (br. *t*, N*H*CO); 7.81 (*t*, ArN*H*); 7.47 (*d*, H–C(6)); 7.20 (*t*, H–C(4)); 6.63 (*d*, H–C(3)); 6.51 (*t*, H–C(5)); 4.77 (*t*, OH); 3.70–3.60 (*m*, $CH_2CH_2CH_2O$, CH_2CH_2OH); 3.28 (*q*, CH_2NH); 3.12 (*q*, CH_2NH); 1.74 (*m*, $CH_2CH_2CH_2O$); 1.56 (*sept.*, Me_2CH); 0.88 (*m*, $Me_2CHC(Me)_2Si$); 0.10 (2*s*, Me_2Si). Anal. calc. for $C_{20}H_{36}N_2O_3Si$ (380.6): C 63.11, H 9.53, N 7.36; found: C 63.04, H 9.54, N 8.03.

13. *4-{2-{{2-{[(3-Hydroxypropyl)amino]carbonyl}phenyl}amino}ethoxy}-4-oxobutanoic Acid* (**18**). A mixture of **17** (1.14 g, 3 mmol) and DMAP (0.55 g, 4.5 mmol) was co-concentrated twice with toluene. Then, the residue was dissolved in CH_2Cl_2 (10 ml), succinic anhydride (0.42 g, 4.2 mmol) added, and the mixture stirred for 24 h at r.t. The soln. was diluted with CH_2Cl_2 (50 ml) and washed with 10% aq. citric acid (2 ×) and H_2O (2 ×). The aq. layers were re-extracted with CH_2Cl_2 (50 ml). Then, the combined org. phase was dried (Na_2SO_4) and concentrated, the residue dissolved in MeOH (15 ml), NH_4F (0.222 g, 6 mmol) added, and the mixture heated under reflux for 5 h. AcOH (1 ml) was added, the mixture concentrated, and the residue subjected to FC (silica gel (2.5 × 30 cm), CH_2Cl_2 + 1% AcOH (100 ml), CH_2Cl_2/MeOH 98:2 + 1% AcOH (100 ml), CH_2Cl_2/MeOH 96:4 + 1% AcOH (200 ml), and CH_2Cl_2/MeOH 94:6 + 1% AcOH (200 ml): 0.75 g (75%) of **18**. Colorless oil. UV (MeOH): 214 (4.35), 256 (4.06), 332 (3.66). ^{1}H-NMR ($CDCl_3$): 7.32 (*m*, H–C(6), H–C(4)); 7.20–6.50 (*m*, 2 OH, 2 NH); 6.63 (*d*, H–C(3)); 6.52 (*t*, H–C(5)); 4.31 (*t*, CH_2CH_2OCO); 3.71 (*t*, $CH_2CH_2CH_2OH$); 3.57 (*q*, CH_2CH_2OCO); 3.43 (*t*, $CH_2CH_2CH_2OH$); 2.61 (*m*, 4 H, Suc); 1.71 (*m*, $CH_2CH_2CH_2OH$). Anal. calc. for $C_{16}H_{22}N_2O_6$ (338.4): C 56.80, H 6.55, N 8.28; found: C 56.76, H 6.54, N 8.30.

14. *Triethylammonium 4-{2-{{2-{{{3-[(4,4′-Dimethoxytrityl)oxy]propyl}amino}carbonyl}phenyl}-amino}ethoxy}-4-oxobutanoate* (**19**). Compound **18** (0.6 g, 1.78 mmol) in dry pyridine was concentrated twice. Then, the residue was dissolved in dry pyridine (50 ml), 4,4′-dimethoxytrityl chloride (0.663 g, 1.96 mmol) added, and the mixture stirred overnight. After evaporation, Et_3N (5 ml) was added and the mixture concentrated twice with toluene. The solid was dissolved in little CH_2Cl_2 and subjected to FC (silica gel (2 × 25 cm), CH_2Cl_2/MeOH 99:1 (100 ml), CH_2Cl_2/MeOH 98:2 (100 ml), CH_2Cl_2/MeOH 96:4 (200 ml), CH_2Cl_2/MeOH 95:5 (100 ml), and CH_2Cl_2/MeOH 93:7 (200 ml)). The residue of the product fractions was dissolved in little CH_2Cl_2 and at 0° added dropwise to Et_2O/Et_3N 39:1. All triethylammonium salts different from **19** precipitated. After filtration, the soln. was evaporared: 1.2 g (91%) of **19**. Colorless solid foam. UV (MeOH): 205 (4.76), 253 (sh, 4.18), 334 (3.68). ^{1}H-NMR ((D$_6$)DMSO): 8.20 (br. *t*, Et_3NH); 7.48 (*m*, H–C(6)); 7.45–7.25 (*m*, Ph, 4 H *m* to MeO, H–C(4)); 6.88 (2*d*, 4 H *o* to MeO); 6.79 (*d*, H–C(3)); 6.58 (*t*, H–C(5)); 4.22 (*t*, CH_2CH_2OCO); 3.66 (2*s*, 2 MeO); 3.41 (*t*, CH_2CH_2OCO); 3.38 (*q*, $CH_2CH_2CH_2OTr(OMe)_2$); 3.05 (*t*, $CH_2CH_2CH_2OTr(MeO)_2$); 2.63 (*q*, $(MeCH_2)_3N$); 2.54–2.42 (*m*, 4 H, Suc); 1.81 (*m*, $CH_2CH_2CH_2OTr(OMe)_2$); 1.01 (*t*, $(MeCH_2)_3N$). Anal. calc. for $C_{43}H_5N_3O_8 \cdot 0.3\ H_2O$ (747.9): C 68.82, H 7.52, N 5.60; found: C 69.05, H 7.50, N 5.61.

15. *N-(2-Chloroethyl)-2,4-dinitrobenzenamine* (**24**). The 2-chloroethanamine hydrochloride (21.0 g, 0.18 mol) was co-concentrated with dry DMF and then dissolved in DMF (300 ml). Then, 1-chloro-2,4 dinitrobenzene (36.5 g, 0.18 mol) was added. Under vigorous stirring, Et_3N (75 ml, 0.54 mol) was added slowly and dropwise within 1 h, and after another 3 h stirring at r.t., the mixture was concentrated. The red oily residue was treated with CH_2Cl_2 (250 ml), the soln. washed with sat. aq. $NaHCO_3$ soln. (2 × 400 ml) and H_2O, dried (Na_2SO_4), and again concentrated. The residue was recrystallized from EtOH/acetone 95:5 (100 ml): 33.1 g (75%) of **24**. Yellow crystalline powder. M.p. 91°. UV (MeOH): 211 (4.11), 259 (3.94), 347 (4.22), 392 (sh, 3.82). ^{1}H-NMR (((D$_6$)DMSO): 8.91 (br. *t*, NH); 8.82 (*d*, H–C(3)); 8.22 (*dd*, H–C(5)); 7.30 (*d*, H–C(6)); 3.86 (*s*, CH_2CH_2). Anal. calc. for $C_8H_8ClN_3O_4$ (245.6): C 39.12, H 3.28, N 17.10; found: C 39.26, H 3.32, N 16.68.

16. *3-{2-[(2,4-Dinitrophenyl)amino]ethyl}thymidine* (**25**). A mixture of thymidine (2.42 g, 10 mmol) and **24** (3.7 g, 15 mmol) was co-concentrated with dry DMF under high vacuum. The residue was dissolved in dry DMF at 100°, dry K_2CO_3 (4.15 g, 30 mmol) added, and the mixture stirred at 100° for 3 h. After cooling, AcOH (3.6 ml, 60 mmol) was added, and the mixture concentrated under high vacuum. The residue was recrystallized from H_2O/EtOH 1:4: 4.1 g (89%) of **25**. Yellow crystals. M.p. 200–201°. UV (MeOH): 210 (4.31), 263 (4.21), 347 (4.23), 390 (sh, 3.84). ^{1}H-NMR ((D$_6$)DMSO): 8.95 (br. *t*, NH);

8.82 (*s*, H–C(3) (Ar)); 8.25 (*d*, H–C(5) (Ar)); 7.78 (*s*, H–C(6)); 7.30 (*d*, H–C(6) (Ar)); 6.15 ('*t*', H–C(1')); 5.23 (*s*, OH–C(3')); 5.04 (*dd*, OH–C(5')); 4.23 (*m*, H–C(3')); 4.09 (*m*, CH_2(5')); 3.77 (*m*, H–C(4')); 3.74 (*q*, CH_2CH_2NH); 3.52 (*m*, CH_2CH_2NH); 2.12–2.01 (*m*, CH_2(2')); 1.80 (*s*, Me–C(5)). Anal. calc. for $C_{18}H_{21}N_5O_9$ (451.4): C 47.90, H 4.68, N 15.15; found: C 47.47, H 4.63, N 15.17.

17. *5'-O-(4,4'-Dimethoxytrityl)-3-[2-[(2,4-dinitrophenyl)amino]ethyl]thymidine* (**26**). Compound **25** (4.0 g, 8.9 mmol) was twice co-concentrated with dry pyridine and then the residue dissolved in dry pyridine (30 ml). Dimethoxytrityl chloride (3.3 g, 9.8 mmol) was added and the mixture stirred at 4° for 3 days. After evaporation, the mixture was twice co-concentrated with toluene, the residue dissolved in CH_2Cl_2 (100 ml) and the soln. washed with sat. aq. $NaHCO_3$ soln. (2 × 300 ml). The aq. layer was reextracted with CH_2Cl_2 (50 ml) and the combined CH_2Cl_2 phase dried (Na_2SO_4) and concentrated. The residue was dissolved in little CH_2Cl_2 and subjected to FC (silica gel (2 × 40 cm), toluene/AcOEt 1:1 (80 ml), toluene/AcOEt 1:1 + 1% MeOH (80 ml), toluene/AcOEt 1:1 + 2% MeOH (80 ml), toluene/AcOEt 1:1 + 3% MeOH (80 ml), and toluene/AcOEt 1:1 + 4% MeOH (80 ml)). The residue of the product fractions was dissolved in CH_2Cl_2 and the soln. filtered through cellulose and again concentrated: 5.9 g (89%) of **26**. Yellow foam. UV (MeOH): 204 (sh, 4.87), 230 (sh, 4.51), 264 (4.25), 347 (4.23), 393 (sh, 3.83). ^{1}H-NMR ($CDCl_3$): 9.11 (*d*, H–C(3) (Ar)); 8.86 (br. *t*, NH); 8.29 (*dd*, H–C(5) (Ar)); 7.65 (*s*, H–C(6)); 7.42–7.18 (*m*, 5 arom. H, 4 H *o* to MeO); 7.12 (*d*, H–C(6) (Ar)); 6.84 (2*d*, 4 H *m* to MeO); 6.20 (*dd*, H–C(1')); 4.61 (*m*, H–C(3')); 4.32 (*m*, CH_2CH_2NH); 4.03 (*m*, H–C(4')); 3.87 (*d*, 1 H–C(5')); 3.80 (*d*, 1 H–C(5')); 3.78 (2*s*, 2 MeO); 3.73 (*q*, CH_2CH_2NH); 2.41–2.35 (*m*, CH_2(2')); 1.53 (*s*, Me–C(5)). Anal. calc. for $C_{39}H_{39}N_5O_{11} \cdot 0.3\ H_2O$ (759.7): C 61.66, H 5.19, N 9.22; found: C 61.42, H 5.19, N 9.14.

18. *5'-O-(4,4'-Dimethoxytrityl)-3-[2-[(2,4-dinitrophenyl)amino]ethyl]thymidine 3'-(2-Cyanoethyl N,N-Diisopropylphosphoramidite)* (**27**). Dry **26** (1.28 g. 1.7 mmol) was dissolved in CH_2Cl_2/MeCN 1:3 (10 ml) and then, under N_2, 2-cyanoethyl *N,N,N',N'*-tetraisopropylphosphorodiamidite (1.0 g, 3.4 mmol) and 1*H*-tetrazole (0.256 g, 0.85 mmol) were added. After stirring for 12 h, the mixture was diluted with CH_2Cl_2 (50 ml) and the soln. washed with sat. aq. $NaHCO_3$ soln. (2 × 150 ml), dried (Na_2SO_4), and concentrated. The residue was dissolved in little toluene and subjected to FC (silica gel (2 × 20 cm), toluene/AcOEt 3:1 (300 ml) and toluene/AcOEt 1:1 (300 ml)): 1.24 g (76%) of **27**. Yellow solid foam. UV (MeOH): 204 (4.88), 231 (sh, 4.50), 265 (4.37), 347 (4.21), 390 (sh, 3.83). ^{1}H-NMR ($CDCl_3$): 9.13 (*d*, H–C(3) (Ar)); 8.82 (br. *t*, NH); 8.31 (*dd*, H–C(5) (Ar)); 7.70 (*s*, H–C(6)); 7.41–7.15 (*m*, 5 arom. H, 4 H *o* to MeO); 7.12 (*d*, H–C(6) (Ar)); 6.84 (2*d*, 4 H *m* to MeO); 6.41 (*dd*, H–C(1')); 4.68 (*m*, H–C(3')); 4.32 (*m*, CH_2CH_2NH); 4.19 (*m*, H–C(4')); 3.73 (2*s*, 2 MeO); 3.88–3.29 (*m*, CH_2CH_2NH, CH_2(5'), OCH_2CH_2CN, 2 Me_2CH); 2.65–2.30 (*m*, CH_2(2'), OCH_2CH_2CN); 1.48 (*s*, Me–C(5)); 1.24–1.00 (*m*, 2 Me_2CH). ^{31}P-NMR ($CDCl_3$): 149.5; 149.26. Anal. calc. for $C_{48}H_{56}N_7O_{12}P$ (953.9): C 60.44, H 5.92, N 10.27; found: C 60.51, H 5.92, N 9.91.

19. *5'-O-(4,4'-Dimethoxytrityl)-3-[2-[(2,4-dinitrophenyl)amino]ethyl]thymidine 3'-(Hydrogen Butanoate)* (**28**). A mixture of dry **26** (0.6 g, 0.8 mmol) and DMAP (0.176 g, 1.44 mmol) in dry CH_2Cl_2 (10 ml) was treated with succinic anhydride (0.136 g, 1.36 mmol) for 3 days at r.t. The mixture was diluted with CH_2Cl_2 (30 ml) and washed with 10% citric acid soln. (3 × 50 ml). The aq. phases were reextracted with CH_2Cl_2 (20 ml) and the combined CH_2Cl_2 phase was washed with H_2O (2×), dried (Na_2SO_4), and concentrated: 0.65 g (95%) of **28**. Yellow foam. UV (MeOH): 205 (sh, 4.82), 231 (sh, 4.42), 263 (4.28), 347 (4.23), 401 (sh, 3.81). ^{1}H-NMR ($CDCl_3$): 9.16 (*d*, H–C(3) (Ar)); 8.88 (br. *t*, NH); 8.29 (*dd*, H–C(5) (Ar)); 7.67 (*s*, H–C(6)); 7.41–7.19 (*m*, 5 arom. H, 4 H *o* to MeO); 7.12 (*d*, H–C(6) (Ar)); 6.83 (2*d*, 4 H *m* to MeO); 6.42 (*dd*, H–C(1')); 5.49 (*m*, H–C(3')); 4.39 (*m*, CH_2CH_2NH); 4.17 (*m*, H–C(4')); 3.78 (2*s*, 2 MeO); 3.70 (*q*, CH_2CH_2NH); 3.49 (*m*, CH_2(5')); 2.73 (*m*, 4 H, Suc); 2.53–2.40 (*m*, CH_2(2')); 1.43 (*s*, Me–C(5)). Anal. calc. for $C_{43}H_{43}N_5O_{14} \cdot 0.5\ H_2O$ (862.8): C 59.85, H 5.06, N 8.11; found: C 59.58, H 5.11, N 8.15.

20. *3',5'-O-Diacetyl-2'-deoxy-2-fluoro-O^6-[2-(4-nitrophenyl)ethyl]inosine* (**30**). In a *Teflon* flask, 70% HF in pyridine (10 ml) was cooled to −50°. Then, dry pyridine (1.9 ml) was dropwise added under stirring for 30 min, followed by addition of 3',5'-di-*O*-acetyl-2'-deoxy-*O*^{6}-[2-(4-nitrophenyl)ethyl]guanosine [7] (**29**; 1.5 g, 3 mmol). After 3 min, 95% *tert*-butyl nitrite in ^{t}BuOH (0.53 ml, 0.433 g, 4.2 mmol) was added and stirred for 30 min at −50°. The reaction was stopped by ice/H_2O 1:1 (200 ml). Then, the mixture was extracted with CH_2Cl_2 (2 × 80 ml) and the org. phase again extracted with ice/phosphate

buffer (pH 7) 1:10 (3 × 200 ml), dried (Na_2SO_4), and concentrated. The residue was dissolved in ^{i}PrOH and the soln. concentrated and kept at −10° for crystallization. The precipitate was washed with cold EtOH and Et_2O: 1.4 g (93%) of **30**. Colorless crystals from EtOH. M.p. 131°. UV (MeOH): 203 (4.46), 257 (4.29), 276 (sh, 4.05). ^{1}H-NMR ((D$_6$)DMSO): 8.58 (*s*, H−C(8)); 8.16 (*d*, 2 H *o* to NO_2); 7.61 (*d*, 2 H *m* to NO_2); 6.35 (*dd*, H−C(1′)); 5.39 (*m*, H−C(3′)); 4.82 (*t*, CH_2CH_2O); 4.30–4.15 (*m*, H−C(4′), CH_2(5′)); 3.32 (*t*, CH_2CH_2O); 3.12–3.00 (*m*, H_a−C(2′)); 2.60–2.50 (*m*, H_b−C(2′)); 2.10 (*s*, Ac); 2.00 (*s*, Ac). Anal. calc. for $C_{22}H_{22}FN_5O_8$ (503.4): C 52.49, H 4.41, N 13.91; found: C 52.52, H 4.44, N 13.62.

21. *3′,5′-Di*-O-*acetyl-2′-deoxy-2-fluoroinosine* (**31**). Compound **30** (1.0 g, 2 mmol) was dried by co-concentration with dry pyridine (3 × 4 ml) and then dissolved in dry pyridine (4 ml), DBU (0.6 ml, 4 mmol) was added and stirred at r.t. for 4 h. After neutralization with AcOH, the mixture was concentrated and twice co-concentrated with toluene and the residue dissolved in little CH_2Cl_2 and subjected to FC (silica gel (1 × 10 cm) with toluene/AcOEt 1:1 + 10% MeOH (100 ml), toluene/AcOEt 1:1 + 15% MeOH (160 ml), toluene/AcOEt 1:1 + 20% MeOH (80 ml), and toluene/AcOEt 1:1 + 25% MeOH (80 ml)). The oily residue of the product fractions was dissolved in CH_2Cl_2 and the soln. concentrated: 0.39 g (55%) of **31**. Colorless foam. UV (MeOH): 204 (4.15), 262 (3.87), 304 (sh, 4.15). ^{1}H-NMR ((D$_6$)DMSO): 8.31 (*s*, H−C(8)); 6.21 (*dd*, H−C(1′)); 5.32 (*m*, H−C(3′)); 4.30–4.12 (*m*, H−C(4′), CH_2(5′)); 3.05–2.92 (*m*, H_a−C(2′)); 2.60–2.50 (*m*, H_b−C(2′)); 2.08 (*s*, Ac); 2.02 (*s*, Ac). Anal. calc. for $C_{14}H_{15}FN_4O_6 \cdot 0.5\ H_2O$ (363.3): C 46.29, H 4.44, N 15.41; found: C 46.36, H 4.45, N 15.73.

22. *2-[(2,4-Dinitrophenyl)amino]ethanol* (**32**) [8]. A soln. of 1-chloro-2,4-dinitrobenzene (36.5 g, 0.18 mol) in DMF (250 ml) was treated with 2-aminoethanol (14 ml, 0.23 mol), followed by Et_3N (50 nl, 0.36 mol). Reaction took place under warming to 50°, and after stirring for 2 h at r.t., the mixture was evaporated under high vacuum. The oily residue was dissolved in CH_2Cl_2 (200 ml), the soln. washed with H_2O (4 × 150 ml), dried (Na_2SO_4), and concentrated, and the residue recrystallized from EtOH (80 ml): 29 g (72%) of **32**. Yellow crystals. M.p. 89–90°. UV (MeOH): 212 (4.11), 259 (3.93), 347 (4.21), 399 (sh, 3.78). ^{1}H-NMR ((D$_6$)DMSO): 8.88 (br. *t*, NH); 8.79 (*d*, H−C(3)); 8.22 (*dd*, H−C(5)); 7.22 (*d*, H−C(6)); 5.08 (*t*, OH); 3.69 (*d*, CH_2); 3.56 (*q*, CH_2). Anal. calc. for $C_8H_9N_3O_5$ (227.2): C 42.30, H 4.00, N 18.49; found: C 42.28, H 4.02, N 18.39.

23. *3′,5′-Di*-O-*acetyl-2′-deoxy-2-fluoro*-O^6-*{2-[(2,4-dinitrophenyl)amino]ethyl}inosine* (**33**) *and 3-(3′,5′-Di*-O-*acetyl-2′-deoxy-β*-D-*ribofuranosyl)-5-(2,4-dinitrophenyl)-3,5,6,7-tetrahydro-9*H-*imidazo[1,2-a]purin-9-one* (**34**). A mixture of **31** (0.85 g, 2.4 mmol), *N*-(2-iodoethyl)-2,4-dinitroaniline (0.97 mg, 2.9 mmol) and K_2CO_3 (0.8 g, 5.8 mmol) in dry DMF (10 ml) was heated under stirring to 100° for 1 h. After cooling, the solid was filtered off, the filtrate mixed with little flash silica gel and then concentrated under high vacuum, and the residue put onto a FC column (SiO_2 (3 × 25 cm), toluene/AcOEt 1:1 (100 ml), toluene/AcOEt 1:1 + 2% MeOH (100 ml), toluene/AcOEt 1:1 + 5% MeOH (100 ml), toluene/AcOEt 1:1 + 8% MeOH (100 ml), toluene/AcOEt 1:1 + 12% MeOH (100 ml), toluene/AcOEt 1:1 + 15% MeOH (100 ml), toluene/AcOEt 1:1 + 20% MeOH (100 ml), and toluene/AcOEt 1:1 + 30% MeOH (100 ml)). The first product fractions (R_f 0.52; toluene/AcOEt/MeOH 4.5:4.5:1) were concentrated, and the residue was treated with little MeOH: 0.85 g (61%) of **33**. The second product fractions (R_f 0.13) were also concentrated, and the residue was recrystallized from MeOH (20 ml) + acetone (2 ml): 0.19 g (15%) of **34**.

Data of **33**: Amorphous yellow solid. M.p 162°. UV (MeOH): 203 (4.38), 254 (4.28), 346 (4.22), 402 (sh, 3.76). ^{1}H-NMR ((D$_6$)DMSO): 9.00 (br. *t*, NH); 8.82 (*d*, H−C(3) (Ar)); 8.22 (*dd*, H−C(5) (Ar)); 8.08 (*s*, H−C(8)); 7.43 (*d*, H−C(6) (Ar)); 6.32 (*dd*, H−C(1′)); 5.40 (*m*, H−C(3′)); 4.82 (*t*, CH_2CH_2O); 4.30–4.12 (*m*, H−C(4′), CH_2(5′)); 4.02 (*q*, CH_2CH_2O); 3.12–3.00 (*m*, H_a−C(2′)); 2.61–2.50 (*m*, H_b−C(2′)); 2.09 (*s*, Ac); 2.00 (*s*, Ac). Anal. calc. for $C_{22}H_{22}FN_7O_{10}$ (563.4): C 46.90, H 3.94, N 17.39; found: C 47.09, H 4.01, N 16.92.

Data of **34**: Yellow crystals. M.p. >111° (dec.). UV (MeOH): 204 (4.42), 253 (4.37), 336 (3.76), 367 (sh, 3.71). ^{1}H-NMR ((D$_6$)DMSO): 8.80 (*d*, H−C(3) (Ar)); 8.61 (*dd*, H−C(5) (Ar)); 8.06 (*s*, H−C(2)); 7.92 (*d*, H−C(6) (Ar)); 6.01 (*dd*, H−C(1′)); 5.21 (*m*, H−C(3′)); 4.45 (*t*, NCH_2CH_2N); 4.29 (*t*, NCH_2CH_2N); 4.12 (*m*, H−C(4′)); 3.96 (*m*, CH_2(5′)); 2.85–2.72 (*m*, H_a−C(2′)); 2.50–2.41 (*m*, H_b−C(2′)); 2.10 (*s*, Ac); 1.92 (*s*, Ac). Anal. calc. for $C_{22}H_{21}N_7O_{10} \cdot 0.5\ H_2O$ (552.4): C 47.83, H 4.01, N 17.74; found: C 47.93, H 3.94, N 17.66.

24. *2′-Deoxy-*O^6*-{2-[(2,4-dinitrophenyl)amino]ethyl}guanosine* (**35**). A soln. of **36** (0.2 g, 0.36 mmol) in dioxane/MeOH/conc. NH_3 soln. 1:1:1 (50 ml) was stirred at r.t. for 3 h and then concentrated. The residue was dissolved in dioxane/MeOH 1:1 and subjected to FC (silica gel (1 × 10 cm), CH_2Cl_2/MeOH 85:15 (100 ml), CH_2Cl_2/MeOH 8:2 (100 ml), CH_2Cl_2/MeOH 75:25 (50 ml), and CH_2Cl_2/MeOH 7:3 (50 ml)). The product fractions were concentrated till a precipitate separated. After cooling in the icebox overnight, the solid was collected and recrystallized from a mixture of acetone (30 ml), $CHCl_3$ (20 ml), and little MeOH: 0.13 g (76%) of **35**. Yellow crystals. M.p. 171–173° (dec.). UV (MeOH): 211 (4.54), 248 (4.24), 264 (sh, 4.10), 346 (4.21), 397 (sh, 3.79). ^{1}H-NMR ((D$_6$)DMSO): 9.00 (br. *t*, NH); 8.82 (*d*, H–C(3) (Ar)); 8.31 (*dd*, H–C(5) (Ar)); 8.09 (*s*, H–C(8)); 7.41 (*d*, H–C(6) (Ar)); 6.48 (br. *s*, NH_2); 6.21 (*dd*, H–C(1′)); 5.27 (*d*, OH–C(3′)); 4.99 (*dd*, OH–C(5′)); 4.65 (*t*, OCH_2CH$_2$NH); 4.31 (*m*, H–C(3′)); 3.96 (*q*, OCH$_2$CH_2NH); 3.81 (*m*, H–C(4′)); 3.61–3.48 (*m*, CH_2(5′)); 2.62–2.50 (*m*, H$_a$–C(2′)); 2.28–2.18 (*m*, H$_b$–C(2′)). Anal. calc. for $C_{18}H_{20}N_8O_8 \cdot 0.5\ H_2O$ (485.4): C 44.54, H 4.36, N 23.07; found: C 44.40, H 4.41, N 22.85.

25. *3′,5′-Di-*O*-acetyl-2′-deoxy-*O^6*-{2-[(2,4-dinitrophenyl)amino]ethyl}guanosine* (**36**). A mixture of 3′,5′-di-*O*-acetyl-2′-deoxyguanosine (0.705 g, 2 mmol) and **32** (0.909 g, 4 mmol) was treated in dry dioxane (10 ml) by ultrasound. The suspension was concentrated, co-concentrated twice with dry dioxane, and then suspended in dry dioxane (10 ml) by heating to 60°. Triphenylphosphine (0.813 g, 3 mmol) was added, followed by dropwise addition of diisopropyl azodicarboxylate (0.6 ml, 3 mmol). After stirring at 60° for 1 h, the mixture was concentrated and the residue washed with CH_2Cl_2 (10 ml) and Et_2O: 0.98 g (88%) of crude **36**. Recrystallization from acetone/EtOH 1:4 gave 0.78 g (70%) of **36**. Yellow crystals. M.p. 215°. UV (MeOH): 210 (4.53), 248 (4.26), 266 (sh, 4.12), 346 (4.23), 400 (sh, 3.79). ^{1}H-NMR ((D$_6$)DMSO): 9.02 (br. *t*, NH); 8.84 (*d*, H–C(3) (Ar)); 8.31 (*dd*, H–C(5) (Ar)); 8.11 (*s*, H–C(8)); 7.41 (*d*, H–C(6) (Ar)); 6.55 (br. *s*, NH_2); 6.21 (*dd*, H–C(1′)); 5.32 (*m*, H–C(3′)); 4.64 (*t*, OCH_2CH$_2$NH); 4.28–4.10 (*m*, H–C(4′), CH_2(5′)); 3.92 (*q*, OCH$_2$CH_2NH); 3.10–2.90 (*m*, H$_a$–C(2′)); 2.51–2.40 (*m*, H$_b$–C(2′)); 2.08 (*s*, Ac); 2.01 (*s*, Ac). Anal. calc. for $C_{22}H_{24}N_8O_{10}$ (560.4): C 47.15, H 4.32, N 19.98; found: C 46.79, H 4.31, N 19.49.

26. *3′,5′-Di-*O*-acetyl-2′-deoxy-*N^2*-[(dimethylamino)methylene]guanosine* (**37**) *and 2′-Deoxy-*N^2*-[(dimethylamino)methylene]guanosine* (**40**). The 2′-deoxyguanosine dihydrate (0.2 g, 0.36 mmol) was co-concentrated with dry pyridine, the residue suspended in MeOH (8.5 ml), *N,N*-dimethylformamide dimethyl acetal (1.67 g, 14 mmol) added, and the mixture treated by ultrasound for 30 min. After stirring for 30 h, the precipitate of **40** was washed with little MeOH and Et_2O. The solid was co-concentrated with dry pyridine again and then suspended in DMF/pyridine 1:1 (10 ml). Ac_2O (2 ml) was added and the mixture stirred at r.t. for 5 h. After evaporation, the residue was dissolved in CH_2Cl_2 (30 ml), the soln. washed with H_2O, dried (Na_2SO_4), and concentrated, and the residue dissolved in MeOH (5 ml). Et_2O (30 ml) was added and the mixture cooled in an icebox overnight. The crystals were collected: 1.1 g (78%) of **37**. Colorless needles. M.p. 113°. pK_a 11.0. UV (pH 8): 206 (4.15), 232 (4.11), 276 (4.18), 298 (4.31). ^{1}H-NMR ($CDCl_3$): 9.40 (br. *s*, NH); 8.68 (*s*, Me$_2$NC*H*=N)); 7.72 (*s*, H–C(8)); 6.24 (*dd*, H–C(1′)); 5.59 (*m*, H–C(3′)); 4.46–4.20 (*m*, H–C(4′), CH_2(5′)); 3.21 (*s*, MeN); 3.11 (*s*, MeN); 3.11–3.00 (*m*, H$_a$–C(2′)); 2.60–2.50 (*m*, H$_b$–C(2′)); 2.16 (*s*, Ac); 2.11 (*s*, Ac). Anal. calc. for $C_{17}H_{22}N_6O_6$ (406.4): C 50.24, H 5.46, N 20.68; found: C 49.80, H 5.55, N 20.64.

27. *3′,5′-Di-*O*-acetyl-2′-deoxy-*N^2*-[(dimethylamino)methylene]-1-{2-[(2,4-dinitrophenyl)amino]-ethyl}guanosine* (**38**) and *3′,5′-Di-*O*-acetyl-2′-deoxy-*N^2*-[(dimethylamino)methylene]-*O^6*-{2-[(2,4-dinitrophenyl)amino]ethyl}guanosine* (**39**). A mixture of **37** (0.16 g, 0.34 mmol), **32** (0.136 g, 0.6 mmol), and triphenylphosphine (0.157 g, 0.6 mmol) was co-concentrated with dry dioxane (3 × 10 ml) and then the residue dissolved in dry dioxane (10 ml). To the soln., diisopropyl azodicarboxylate (0.12 g, 0.6 mmol) was added and stirred at 50° for 4 h. The suspension was concentrated and the residue dissolved in DMF (20 ml). Flash silica gel was added, the mixture concentrated under high vacuum, and the solid put onto a FC column (SiO_2 (3 × 12 cm), toluene/AcOEt 1:1 + 5% MeOH (200 ml), toluene/AcOEt 1:1 + 7% MeOH (200 ml), toluene/AcOEt 1:1 + 9% MeOH (200 ml), toluene/AcOEt 1:1 + 11% MeOH (200 ml), toluene/AcOEt 1:1 + 15% MeOH (200 ml), and toluene/AcOEt 1:1 + 18% MeOH (200 ml)). The residue of the fractions of the main product (R_f 0.37) was recrystallized from MeOH (10 ml): 0.125 g (60%) of **38**. The product fractions with R_f 0.26 gave, after evaporation and recrystallization from MeOH (30 ml) + H_2O (1 ml), 36 mg (17%) of **39**.

Data of **38**: Yellow crystals. M.p. 118–121°. UV (MeOH): 237 (4.35), 267 (4.26), 315 (4.38), 342 (sh, 4.28), 394 (sh, 3.77). ^{1}H-NMR ((D$_6$)DMSO): 9.02 (br. *t*, NH); 8.82 (*d*, H–C(3) (Ar)); 8.49 (*s*, Me_2NCH=N)); 8.17 (*dd*, H–C(5) (Ar)); 8.05 (*s*, H–C(8)); 7.30 (*d*, H–C(6) (Ar)); 6.26 (*dd*, H–C(1′)); 5.49 (*m*, H–C(3′)); 4.55 (*t*, NCH_2CH_2NH); 4.30–4.10 (*m*, H–C(4′), CH_2(5′)); 3.81 (*m*, NCH_2CH_2NH); 3.18 (*s*, MeN); 2.94 (*s*, MeN); 3.12–3.02 (*m*, H_a–C(2′)); 2.50–2.42 (*m*, H_b–C(2′)); 2.10 (*s*, Ac); 1.98 (*s*, Ac). Anal. calc. for $C_{25}H_{29}N_9O_{10}$ (615.6): C 48.78, H 4.75, N 20.47; found: C 48.65, H 4.76, N 20.19.

Data of **39**: Yellow crystals. M.p. >215° (dec.). UV (MeOH): 211 (4.43), 273 (4.53), 290 (4.53), 345 (sh, 4.22), 396 (sh, 3.79). ^{1}H-NMR ((D$_6$)DMSO): 8.97 (br. *t*, NH); 8.81 (*d*, H–C(3) (Ar)); 8.55 (*s*, Me_2NCH=N)); 8.28 (*s*, H–C(8)); 8.21 (*dd*, H–C(5) (Ar)); 7.57 (*d*, H–C(6) (Ar)); 6.32 (*dd*, H–C(1′)); 5.43 (*m*, H–C(3′)); 4.78 (*t*, OCH_2CH_2N); 4.32–4.12 (*m*, H–C(4′), CH_2(5′)); 3.92 (*m*, OCH_2CH_2N); 3.12 (*s*, MeN); 3.14–3.05 (*m*, H_a–C(2′)); 3.03 (*s*, MeN); 2.58–2.48 (*m*, H_b–C(2′)); 2.10 (*s*, Ac); 2.01 (*s*, Ac). Anal. calc. for $C_{25}H_{29}N_9O_{10}$ (615.7): C 48.78, H 4.75, N 20.47; found: C 49.13, H 4.79, N 20.19.

28. *2′-Deoxy-1-{2-[(2,4-dinitrophenyl)amino]ethyl}guanosine* (**41**). To a mixture of **40** (6.65 g, 21.7 mmol) and **24** (16.0 g, 65.1 mmol) in dry DMF (100 ml), K_2CO_3 (18.0 g) was added and the suspension treated with ultrasound and then heated under stirring to 120° for 2 h. After cooling, aq. conc. ammonia (100 ml) was added and the mixture stirred at 55° overnight. After concentration to half of the volume, the mixture was neutralized with AcOH and then concentrated. The residue was suspended in little DMF, flash silica gel added, and the mixture concentrated and put on a FC column (SiO_2 (3 × 20 cm), CH_2Cl_2/MeOH 85:15 (200 ml), CH_2Cl_2/MeOH 82:18 (200 ml), CH_2Cl_2/MeOH 8:2 (200 ml), CH_2Cl_2/MeOH 75:25 (200 ml), and CH_2Cl_2/MeOH 7:3 (200 ml)). The product fractions were concentrated to 1/5 and then kept in an icebox overnight: 7.0 g (75%) of **41**. Yellow crystals. M.p. 174–175°. UV (MeOH): 205 (4.46), 257 (4.34), 346 (4.23), 401 (sh, 3.79). ^{1}H-NMR ((D$_6$)DMSO): 8.98 (br. *t*, NH); 8.83 (*d*, H–C(3) (Ar)); 8.26 (*dd*, H–C(5) (Ar)); 7.93 (*s*, H–C(8)); 7.28 (*d*, H–C(6) (Ar)); 7.23 (br. *s*, NH_2); 6.10 (*dd*, H–C(1′)); 5.29 (*d*, OH–C(3′)); 4.91 (*dd*, OH–C(5′)); 4.33 (*m*, H–C(3′)); 4.21 (*t*, NCH_2CH_2NH); 3.81 (*m*, H–C(4′)); 3.71 (*m*, NCH_2CH_2NH); 3.58–3.49 (*m*, CH_2(5′)); 2.60–2.48 (*m*, H_a–C(2′)); 2.20–2.10 (*m*, H_b–C(2′)). Anal. calc. for $C_{18}H_{20}N_8O_8 \cdot H_2O$ (485.4): C 43.73, H 4.49, N 22.66; found: C 43.68, H 4.27, N 22.38.

29. *3′,5′-Di*-O-*acetyl-2′-deoxy-1-{2-[(2,4-dinitrophenyl)amino]ethyl}guanosine* (**42**). A soln. of **41** (6.5 g, 13.6 mmol) was co-concentrated with dry pyridine (2 × 100 ml) and then dissolved in dry pyridine (200 ml). Under stirring at r.t., Ac_2O (45 ml) was dropwise added, and after 3 h, the mixture was concentrated. The red residue was dissolved in CH_2Cl_2/acetone 95:5 (400 ml), the soln. washed with sat. aq. $NaHCO_3$ soln. (3 × 200 ml) and H_2O (200 ml), the aq. phase reextracted with CH_2Cl_2/acetone 9:1 (100 ml), and the combined org. phase dried (Na_2SO_4) and concentrated. The residue was treated with hot EtOH/H_2O 3:1 (400 ml) and then the mixture kept at −10° for two days: 6.39 g (84%) of **42**. Yellow solid from EtOH. M.p. 124–126° (dec.). UV (MeOH): 205 (4.46), 257 (4.36), 346 (4.23), 399 (sh, 3.81). ^{1}H-NMR ((D$_6$)DMSO): 8.98 (br. *t*, NH); 8.82 (*d*, H–C(3) (Ar)); 8.28 (*dd*, H–C(5) (Ar)); 7.90 (*s*, H–C(8)); 7.31 (*d*, H–C(6) (Ar)); 7.28 (br. *s*, NH_2); 6.10 (*dd*, H–C(1′)); 5.30 (*m*, H–C(3′)); 4.28–4.11 (*m*, H–C(4′), CH_2 (5′)); 4.18 (*t*, NCH_2CH_2NH); 3.71 (*m*, NCH_2CH_2NH); 3.00–2.89 (*m*, H_a–C(2′)); 2.50–2.21 (*m*, H_b–C(2′)); 2.07 (*s*, Ac); 2.01 (*s*, Ac). Anal. calc. for $C_{22}H_{24}N_8O_{10} \cdot 2\,H_2O$ (596.4): C 44.30, H 4.73, N 18.78; found: C 44.68, H 4.66, N 18.17.

30. *3′,5′-Di*-O-*acetyl-2′-deoxy*-N^2-*[(dimethylamino)methylene]-1-methylguanosine* (**43**). Compound **37** (1.5 g, 4 mmol) was co-concentrated with dry DMF and then dissolved in dry DMF (20 ml). K_2CO_3 (1.2 g, 8.8 mmol) was added under stirring, followed by dropwise addition of MeI (0.28 ml, 4.4 mmol). Stirring was continued for 20 h at 35°. The solid was filtered off and washed with DMF, and the filtrates were concentrated under high vacuum. The residue was dissolved in CH_2Cl_2 (50 ml) and the soln. washed with sat. aq. $NaHCO_3$ soln. (2 × 50 ml), dried (Na_2SO_4), and concentrated to a small volume which was put on a FC column (silica gel (3 × 20 cm), toluene/AcOEt 1:1 + 12% MeOH (200 ml), toluene/AcOEt 1:1 + 14% MeOH (200 ml), toluene/AcOEt 1:1 + 16% MeOH (200 ml), and toluene/AcOEt 1:1 + 18% MeOH (200 ml)). The residue of the product fractions was recrystallized from EtOH (30 ml)/Et_2O (70 ml): 1.4 g (82%) of **43**. Colorless crystals. M.p. 171°. UV (MeOH): 202 (4.32), 237 (4.24), 281 (sh, 4.08), 306 (4.30). ^{1}H-NMR ((D$_6$)DMSO): 8.58 (*s*, Me_2NCH=N)); 7.98 (*s*, H–C(8)); 6.25 (*dd*, H–C(1′)); 5.47 (*m*, H–C(3′)); 4.38–4.10 (*m*, H–C(4′), CH_2(5′)); 3.51 (*s*, Me–N(1)); 3.20 (*s*, MeN); 3.09

(*s*, MeN); 3.10–3.00 (*m*, H_a–C(2′)); 2.59–2.40 (*m*, H_b–C(2′)); 2.13 (*s*, Ac); 1.98 (*s*, Ac). Anal. calc. for $C_{18}H_{24}N_6O_6$ (420.4): C 51.43, H 5.75, N 19.98; found: C 51.29, H 5.75, N 20.01.

31. *3′,5′-Di*-O-*acetyl-2′-deoxy*-N^2-*[(dimethylamino)methylene]-1-ethylguanosine* (**44**). As described for **43**, with **37** (1.5 g, 4 mmol) in dry DMF (20 ml), K_2CO_3 (1.2 g, 8.8 mmol), and iodoethane (0.36 ml, 4.4 mmol): 1.5 g (85%) of **44**. Colorless crystals. M.p. 162°. UV (MeOH): 203 (4.30), 238 (4.24), 280 (sh, 4.08), 307 (4.31). ^{1}H-NMR ((D$_6$)DMSO): 8.62 (*s*, Me_2NCH=N)); 8.00 (*s*, H–C(8)); 6.23 (*dd*, H–C(1′)); 5.50 (*m*, H–C(3′)); 4.35–4.10 (*m*, H–C(4′), CH_2(5′), MeCH_2N); 3.21 (*s*, MeN); 3.08 (*s*, MeN); 3.10–3.00 (*m*, H_a–C(2′)); 2.55–2.43 (*m*, H_b–C(2′)); 2.11 (*s*, Ac); 1.96 (*s*, Ac); 1.13 (*t*, *Me*CH_2N). Anal. calc. for $C_{19}H_{26}N_6O_6$ (434.4): C 52.53, H 6.03, N 19.34; found: C 52.53, H 6.05, N 19.45.

32. *2′-Deoxy-1-methylguanosine* (**45**) [12]. Compound **43** (0.84 g, 2 mmol) was stirred in a closed round-bottom flask with a mixture of sat. methanolic ammonia (40 ml) and conc. aq. ammonia (80 ml) at r.t. for 4 days. After evaporation, the residue was dissolved in MeOH (50 ml), and then Et_2O was added until a precipitate started to separate. The mixture was kept in an icebox for 2 days: 0.45 g (80%) of **45**. Colorless crystals. M.p. >230° (dec.). UV (MeOH): 204 (4.31), 256 (4.14), 267 (sh, 4.06). ^{1}H-NMR ((D$_6$)DMSO): 7.92 (*s*, H–C(8)); 7.00 (*s*, NH_2); 6.11 (‘*t*’, H–C(1′)); 5.21 (*d*, OH–C(3′)); 4.93 (*t*, OH–C(5′)); 4.37 (*m*, H–C(3′)); 3.77 (*m*, H–C(4′)); 3.60–3.45 (*m*, CH_2(5′)); 3.28 (*s*, Me–N(1)); 2.60–2.45 (*m*, H_a–C(2′)); 2.22–2.10 (*m*, H_b–C(2′)). Anal. calc. for $C_{11}H_{15}N_5O_4$ (281.2): C 46.98, H 5.38, N 24.89; found: C 46.81, H 5.41, N 25.26.

33. *2′-Deoxy-1-ethylguanosine* (**46**) [12]. As described for **45**, with **44** (0.59 g, 2 mmol): 0.44 g (75%) of **46**. Colorless crystals. M.p. 153–154°. UV (MeOH): 204 (4.36), 257 (4.10), 268 (sh, 4.00). ^{1}H-NMR ((D$_6$)DMSO): 7.88 (*s*, H–C(8)); 7.06 (*s*, NH_2); 6.12 (‘*t*’, H–C(1′)); 5.20 (*d*, OH–C(3′)); 4.93 (*t*, OH–C(5′)); 4.32 (*m*, H–C(3′)); 3.96 (*q*, MeCH_2); 3.79 (*m*, H–C(4′)); 3.56–3.41 (*m*, CH_2(5′)); 2.45 (*m*, H_a–C(2′)); 2.25–2.13 (*m*, H_b–C(2′)); 1.12 (*t*, *Me*CH_2). Anal. calc. for $C_{12}H_{17}N_5O_4 \cdot 0.3\ H_2O$ (301.3): C 47.84, H 5.91, N 23.23; found: C 47.84, H 6.11, N 23.01.

34. N-*(2-Chloroethyl)*-N-*methyl-2,4-dinitrobenzenamine* (**49**). A mixture of 2-(methylamino)ethanol (15.3 g, 0.2 mol) and 1-chloro-2,4-dinitrobenzene (20.2 g, 0.1 mol) was co-concentrated with dry pyridine (2 × 80 ml) and the residue dissolved in dry pyridine (80 ml) and stirred at 70° for 3 h. After evaporation and co-evaporation with toluene (3 × 100 ml), the residue was taken up in CH_2Cl_2 (80 ml) and extracted with sat. aq. $NaHCO_3$ soln. (2 × 100 ml). The org. phase was dried (Na_2SO_4) and concentrated and the red sirup treated with thionyl chloride (20 ml) under reflux for 2 h. The reaction soln. was added dropwise unter vigorous stirring into H_2O (300 ml). The resulting suspension was extracted with CH_2Cl_2 (2 × 100 ml), the extract washed with sat. $NaHCO_3$ soln. (2 × 100 ml), dried (Na_2SO_4), and again concentrated: 18.0 g (70%) of crude **49** which was pure enough for the next reaction to **50**. A sample of crude **49** (1.0 g) in little CH_2Cl_2 was subjected to FC (silica gel (25 g), toluene (400 ml)). The residue of the product fraction gave, on drying under high vacuum, a yellow oil which solidified: 0.85 g of a yellow solid. M.p. 61–63°. UV (MeOH): 206 (4.11), 362 (4.20). ^{1}H-NMR ((D$_6$)DMSO): 8.57 (*d*, H–C(2)); 8.19 (*dd*, H–C(5)); 7.39 (*d*, H–C(6)); 3.91 (*t*, CH_2CH_2Cl); 3.72 (*t*, CH_2CH_2Cl); 2.94 (*s*, MeN). Anal. calc. for $C_9H_{10}ClN_3O_4$ (259.6): C 41.64, H 3.88, N 16.18; found: C 42.01, H 3.87, N 16.07.

35. N-*(2-Iodoethyl)*-N-*methyl-2,4-dinitrobenzenamine* (**50**). A mixture of crude **49** (13.0 g, 0.05 mol), NaI (37.0 g, 0.25 mol), and $NaHCO_3$ (21.0 g, 0.25 mol) was refluxed in dry butan-2-one (350 ml) for 10 h. After cooling, the mixture was filtered and the residue washed with acetone till the solvent was colorless. The filtrate and extract were concentrated, and the residue was suspended in CH_2Cl_2 (80 ml), the suspension washed with H_2O (3 × 200), the org. phase dried (Na_2SO_4) and concentrated, and the resulting solid recrystallized from EtOH (200 ml) with acetone (30 ml): 16.5 g (94%) of **50**. Yellow crystals. M.p. 97°. UV (MeOH): 204 (4.07), 364 (4.18). ^{1}H-NMR ((D$_6$)DMSO): 8.56 (*d*, H–C(2)); 8.21 (*dd*, H–C(5)); 7.36 (*d*, H–C(6)); 3.76 (*t*, CH_2CH_2I); 3.43 (*t*, CH_2CH_2I); 2.92 (*s*, MeN). Anal. calc. for $C_9H_{10}IN_3O_4$ (351.1): C 30.79, H 3.10, N 11.94; found: C 30.81, H 2.85, N 11.98.

36. *3′,5′-Di*-O-*acetyl-2′-deoxy*-N^2-*[(dimethylamino)methylene]-1-{2-[(2,4-dinitrophenyl)methylamino]ethyl}guanosine* (**51**). A mixture of **37** (0.406 g, 1 mmol), **50** (0.421 g, 1.2 mol), and K_2CO_3 (0.331 g, 2.4 mol) was co-concentrated with dry DMF (3 × 20 ml) and then the residue dissolved in dry DMF (20 ml) and heated to 100° for 6 h. After cooling to r.t., the solid was filtered off and washed with DMF, and the filtrates were evaporated. The residue was dissolved in CH_2Cl_2 (30 ml), the soln.

washed with sat. aq. $NaHCO_3$ soln. (3×60 ml), dried (Na_2SO_4), and then concentrated again. The residue, dissolved in little CH_2Cl_2, was subjected to FC (silica gel (2×12 cm), toluene/AcOEt/MeOH 47:47:6 (200 ml), toluene/AcOEt/MeOH 46:46:8 (200 ml), and toluene/AcOEt/MeOH 45:45:10 (200 ml)). The residue of the product fractions was co-concentrated several times with CH_2Cl_2: 0.47 g (76%) of **51**. Yellow solid foam. UV (MeOH): 203 (4.42), 234 (4.33), 311 (4.28), 371 (4.13). ^{1}H-NMR ((D$_6$)DMSO): 8.58 (*s*, $Me_2NCH{=}N$)); 8.49 (*d*, H–C(3) (Ar)); 8.12 (*dd*, H–C(5) (Ar)); 7.98 (*s*, H–C(8)); 7.37 (*d*, H–C(6) (Ar)); 6.22 (*dd*, H–C(1′)); 5.47 (*m*, H–C(3′)); 4.52 (*t*, NCH_2CH_2NMe); 4.37–4.12 (*m*, H–C(4′), CH_2(5′)); 3.73 (*m*, NCH_2CH_2NMe); 3.19 (*s*, MeN); 3.09–2.90 (*m*, H_a–C(2′)); 2.99–2.95 (2*s*, Me_2N); 2.50–2.36 (*m*, H_b–C(2′)); 2.09 (*s*, Ac); 1.98 (*s*, Ac). Anal. calc. for $C_{26}H_{31}N_9O_{10}$ (629.6): C 49.60, H 4.96, N 20.01; found: C 49.70, H 4.96, N 19.75.

37. *2′-Deoxy-*N^2*-[(dimethylamino)methylene]-1-{2-[(2,4-dinitrophenyl)methylamino]ethyl}guanosine* (**52**). A soln. of **51** (0.651 g, 1 mmol) in dioxane (10 ml), MeOH (10 ml), and conc. aq. NH_3 soln. was kept in an icebox overnight and then concentrated. The residue was treated with MeOH (10 ml): 0.53 g (95%) of **52**. Yellow fine crystals. M.p. 219°. UV (MeOH): 205 (4.43), 233 (4.35), 311 (4.30), 370 (4.14). ^{1}H-NMR ((D$_6$)DMSO): 8.53 (*s*, $Me_2NCH{=}N$)); 8.51 (*d*, H–C(3) (Ar)); 8.09 (*dd*, H–C(5) (Ar)); 8.00 (*s*, H–C(8)); 7.38 (*d*, H–C(6) (Ar)); 6.21 (*dd*, H–C(1′)); 5.33 (*d*, OH–C(3′)): 4.92 (*t*, OH–C(5′)); 4.53 (*t*, NCH_2CH_2NMe); 4.41 (*m*, H–C(3′)); 3.82 (*m*, H–C(4′)); 3.77 (*m*, NCH_2CH_2NMe); 3.54 (*m*, CH_2(5′)); 3.21 (*s*, MeN); 3.03–3.00 (2*s*, Me_2N); 2.62–2.50 (*m*, H_a–C(2′)); 2.29–2.18 (*m*, H_b–C(2′)). Anal. calc. for $C_{22}H_{27}N_9O_8$ (545.5): C 48.44, H 4.99, N 23.10; found: C 48.56, H 4.96, N 22.86.

38. *3′,5′-Di-*O*-acetyl-2′-deoxy-1-{2-[(2,4-dinitrophenyl)methylamino]ethyl}guanosine* (**53**). To a soln. of **52** (2.65 g, 5 mmol) in DMF (300 ml), conc. aq. NH_3 soln. (300 ml) was added and heated to 55° for 6 h. After concentration to half of the volume, more conc. aq. NH_3 soln. (300 ml) was added and heating to 55° continued for 12 h. The mixture was concentrated, the residue co-concentrated with dry pyridine (3×150 ml) and then dissolved in dry pyridine (150 ml), and Ac_2O (5.1 g, 50 mmol) added. After stirring at r.t. for 3 h, the mixture was concentrated, the residue dissolved in CH_2Cl_2 (100 ml), the soln. washed with sat. aq. $NaHCO_3$ soln. (2×150 ml), dried, (Na_2SO_4), and concentrated, and the residue purified by FC (silica gel (2.5×30 cm), CH_2Cl_2 (150 ml), CH_2Cl_2/MeOH 97:3 (150 ml), CH_2Cl_2/MeOH 95:5 (150 ml), CH_2Cl_2/MeOH 93:7 (150 ml), and CH_2Cl_2/MeOH 9:1 (150 ml)). The residue of the product fractions was treated with little MeOH to give 1.8 g (63%) of **53**. Fine yellow crystals. M.p. 115° (dec.). UV (MeOH): 204 (4.40), 251 (4.24), 369 (4.13). ^{1}H-NMR ((D$_6$)DMSO): 8.48 (*d*, H–C(3) (Ar)); 8.04 (*dd*, H–C(5) (Ar)); 7.83 (*s*, H–C(8)); 7.41 (*d*, H–C(6) (Ar)); 7.20 (br. *s*, NH_2); 6.04 (*dd*, H–C(1′)); 5.29 (*d*, H–C(3′)); 4.34–4.13 (*m*, H–C(4′), CH_2(5′), NCH_2CH_2NMe); 3.67 (*m*, NCH_2CH_2NMe); 3.09 (*s*, MeN); 3.00–2.86 (*m*, H_a–C(2′)); 2.46–2.32 (*m*, H_b–C(2′)); 2.08 (*s*, Ac); 2.03 (*s*, Ac). Anal. calc. for $C_{23}H_{26}N_8O_{10} \cdot 0.5\ H_2O$ (583.5): C 47.35, H 4.66, N 19.19; found: C 47.42, H 4.72, N 18.74.

39. *3′,5′-Di-*O*-acetyl-2′-deoxy-2-fluoro-1-{2-[(2,4-dinitrophenyl)methylamino]ethyl}inosine* (**54**). In a *Teflon* flask, 70% HF in pyridine (8 ml) was cooled to −50°. Then, dry pyridine (1.5 ml) was added dropwise under stirring within 30 min, followed by the addition of **53** (0.862 g, 1.5 mmol). After 3 min, 95% *tert*-butyl nitrite in ^{t}BuOH (1.87 ml, 15 mmol) was added and stirred for 30 min at −50° with occasional shaking. Then, cold dry Et_3N (17 ml) was added dropwise within 15 min. The reaction was stopped by ice (50 g). Then, the mixture was extracted twice with CH_2Cl_2 (2×50 ml), the org. phase washed with cold phosphate buffer (pH 7) (250 ml) and sat. aq. NaCl soln. followed by reextraction of the aq. phases with CH_2Cl_2. The combined org. phase was dried (Na_2SO_4), and concentrated and the residue dissolved in ^{i}PrOH (50 ml). The soln. was concentrated until the beginning of crystallization and then kept in an icebox overnight. The yellow crystals were collected and recrystallized from EtOH (100 ml) by addition of acetone (40 ml): 0.675 g (78%) of **53** which was pure enough for the subsequent reactions. A sample of **53** (0.3 g) was purified by FC (silica gel (1×10 cm), toluene/AcOEt 1:1 (50 ml), then toluene/AcOEt/MeOH 49:49:2 → 47:47:6). The residue of the product fractions was recrystallized from EtOH/acetone: 0.25 g of **54**. Fine yellow crystals. M.p. 174–175° (dec.). UV (MeOH): 203 (4.40), 238 (4.22), 364 (4.11). ^{1}H-NMR ((D$_6$)DMSO): 8.52 (*d*, H–C(3) (Ar)); 8.31 (*s*, H–C(8)); 8.20 (*dd*, H–C(5) (Ar)); 7.43 (*d*, H–C(6) (Ar)); 6.19 (*dd*, H–C(1′)); 5.32 (*d*, H–C(3′)): 4.31–4.14 (*m*, H–C(4′), CH_2(5′), NCH_2CH_2NMe); 3.67 (*m*, NCH_2CH_2NMe); 2.95 (*s*, MeN); 3.00–2.88 (*m*, H_a–C(2′)); 2.61–2.50 (*m*, H_b–C(2′)); 2.08 (*s*, Ac); 2.01 (*s*, Ac). Anal. calc. for $C_{23}H_{24}FN_7O_{10}$ (577.4): C 47.48, H 4.19, N 16.96; found: C 47.85, H 4.19, N 16.81.

40. *2-(4-Nitrophenyl)ethyl 2-{6-[2-(4-Nitrophenyl)ethoxy]-3-oxo-3*H*-xanthen-9-yl}-5-[(phenoxycarbonyl)amino]benzoate* (**55**). To a mixture of 2-(4-nitrophenyl)ethyl 5-amino-2-{6-[2-(4-nitrophenyl)-ethoxy]-3-oxo-3*H*-xanthen-9-yl}benzoate [1] (1.0 g, 1.55 mmol) and DMAP (0.244 g, 2 mmol) in dry pyridine (50 ml) at 0°, phenyl carbonochloridate (0.19 ml, 1.55 mmol) was added dropwise and the mixture and stirred at r.t. for 3 h. After evaporation and co-evaporation with toluene (3 × 20 ml), the residue was suspended in CH_2Cl_2/MeOH 1:1 (40 ml) by ultrasound. The suspension was cooled to 0° and the red solid collected and washed with EtOH: 1.1 g (93%) of **55**. Red powder. UV (CH_2Cl_2/MeOH 1:1): 227 (4.80), 250 (4.73), 299 (sh, 4.31), 357 (3.96), 438 (sh, 4.26), 460 (4.42), 487 (sh, 4.31). ^{1}H-NMR ((D$_6$)DMSO): 10.80 (br. *s*, NH); 8.43 (*d*, H–C(6)); 8.18 (*d*, 2 H *o* to NO_2); 8.00 (*d*, 2 H *o* to NO_2); 7.87 (*dd*, H–C(4)); 7.63 (*d*, 2 H *m* to NO_2); 7.46 (*d*, 2 H *m* to NO_2); 7.38 (*d*, H–C(3)); 7.37–7.27 (*m*, Ph); 7.18 (*d*, H–C(5) (Xan)); 6.85–6.80 (*m*, H–C(8), H–C(7), H–C(1) (all Xan)); 6.36 (*dd*, H–C(2) (Xan)); 6.18 (*d*, H–C(4) (Xan)); 4.46 (*t*, CH_2CH_2O); 4.25 (*t*, CH_2CH_2O); 3.27 (*t*, CH_2CH_2O); 2.81 (*t*, CH_2CH_2O). Anal. calc. for $C_{43}H_{31}N_3O_{11}$ (765.7): C 67.48, H 4.08, N 5.48; found: C 67.01, H 4.18, N 5.48.

41. *N^2-{[2-[2-(2-Aminoethoxy)ethoxy]ethyl}amino}-2′-deoxy-1-{2-[(2,4-dinitrophenyl)methylamino]ethyl}guanosine* (**56**). To a soln. of **54** (2.31 g, 4 mmol) in dry CH_2Cl_2 (20 ml) at 0°, 2,2′-[ethane-1,2-diylbis(oxy)]bis[ethanamine] (2.37 g, 16 mmol) was added and stirred vigorously for 10 min. The yellow soln. was then treated with sat. NH_3/MeOH (80 ml) and, in a closed system, stirred at 40° for 24 h. The mixture was concentrated and the residue, dissolved in little CH_2Cl_2, subjected to FC (silica gel (2.5 × 20 cm), CH_2Cl_2/MeOH 85:15 + 2% Et_3N (200 ml), CH_2Cl_2/MeOH 8:2 + 2% Et_3N (200 ml), and CH_2Cl_2/MeOH 75:25 + 2% Et_3N (400 ml)). The residue of the product fractions was dissolved in CH_2Cl_2 and the soln. filtered through cellulose powder and concentrated: 1.9 g (77%) of **56**. Yellowish solid foam. UV (MeOH): 204 (4.43), 251 (4.23), 284 (sh., 4.00), 3.67 (4.14). ^{1}H-NMR ((D$_6$)DMSO): 8.41 (*d*, H–C(3) (Ar)); 7.96 (*dd*, H–C(5) (Ar)); 7.72 (*s*, H–C(8)); 7.31 (*d*, H–C(6) (Ar)); 7.25 (*t*, NH–C(2) (Pur)); 6.02 (*dd*, H–C(1′)); 4.30 (*d*, H–C(3′), NCH_2CH_2NMe); 3.71 (*m*, H–C(4′)); 3.70–3.20 (*m*, CH_2(5′), NCH_2CH_2NMe, CH_2CH_2O, OCH_2CH_2O); 3.32 (*t*, $OCH_2CH_2NH_2$); 3.01 (*s*, MeN); 2.61 (*t*, $OCH_2CH_2NH_2$); 2.60–2.50 (*m*, H_a–C(2′)); 2.18–2.11 (*m*, H_b–C(2′)).

42. *2-(4-Nitrophenyl)ethyl 5-{{{{2-[2-[2-{[9-(2′-Deoxy-β-D-ribofuranosyl)-1-{2-[(2,4-dinitrophenyl)-methylamino]ethyl}-6,9-dihydro-6-oxo-1*H*-purin-2-yl}amino}ethoxy}ethoxy]ethyl}amino}carbonyl}amino}-2-{6-[2-(4-nitrophenyl)ethoxy]-3-oxo-3*H*-xanthen-9-yl}benzoate* (**57**). A mixture of **55** (1.53 g, 2 mmol) and DMAP (0.488 g, 4 mmol) in dry DMF (50 ml) was concentrated and then the residue dissolved in dry DMF (50 ml) and dry pyridine (20 ml). After addition of **56** (1.0 g, 1.6 mmol), the mixture was heated to 70° for 45 min. After evaporation under high vacuum to remove DMF completely, the residue was dissolved in CH_2Cl_2 (8 ml) and the soln. subjected to FC (silica gel (3 × 30 cm), CH_2Cl_2/MeOH 95:5 (100 ml), CH_2Cl_2/MeOH 93:7 (100 ml), CH_2Cl_2/MeOH 9:1 (100 ml), CH_2Cl_2/MeOH 87:13 (200 ml), and CH_2Cl_2/MeOH 85:15 (200 ml)). The product fractions which contained also some DMAP, were concentrated, and the red sirup was dissolved in little CH_2Cl_2/MeOH 95:5, the soln. filtered through cellulose powder, and the filtrate added dropwise under stirring to cold MeOH (0°). The resulting suspension was kept in an icebox overnight and the precipitate collected and dried in a vacuum desiccator: 1.7 g (84%) of **57**. Orange-red powder. UV (CH_2Cl_2/MeOH 1:1): 230 (4.88), 260 (4.81), 304 (sh, 4.23), 364 (4.37), 437 (sh, 4.33), 459 (4.45), 487 (sh, 4.34). ^{1}H-NMR ((D$_6$)DMSO): 9.08 (br. *s*, NHCONH); 8.38 (*d*, H–C(3) (Ar)); 8.31 (*s*, H–C(2) (Ar)); 8.18 (*d*, 2 H *o* to NO_2); 8.03 (*d*, 2 H *o* to NO_2); 7.96 (*d*, H–C(5) (Ar)); 7.82 (*s*, H–C(8) (Pur)); 7.68 (*d*, H–C(6) (Ar)); 7.63 (*d*, 2 H *m* to NO_2); 7.35–7.21 (*m*, H–C(5) (Ar), H–C(6) (Ar), 2 H *m* to NO_2, NH–C(2) (Pur)); 7.38 (*d*, H–C(3)); 7.16 (*d*, H–C(5) (Xan)); 6.90–6.77 (*m*, H–C(8), H–C(7), H–C(1) (all Xan)); 6.36 (*dd*, H–C(2) (Xan); *t*, NHCONH); 6.13 (*d*, H–C(4)); 6.06 (‘*t*’, H–C(1′)); 5.31 (br. *d*, OH–C(3′)); 4.82 (br. *t*, OH–C(5′)); 4.50–4.22 (*m*, OCH_2CH_2, NCH_2CH_2, H–C(3′)); 4.18 (*t*, OCH_2CH_2); 3.81 (*m*, H–C(4′)); 3.70–3.45 (*m*, NCH_2CH_2NMe, CH_2(5′), $NHCH_2CH_2OCH_2CH_2OCH_2CH_2NH$); 3.25 (*m*, CH_2CH_2O, OCH_2CH_2NH); 3.04 (*s*, MeN); 2.82 (*t*, CH_2CH_2O); 2.20–2.10 (*m*, CH_2(2′)). Anal. calc. for $C_{62}H_{60}N_{12}O_{20}$ (1293.2): C 57.59, H 4.68, N 12.99; found: C 57.50, H 4.62, N 12.75.

43. *2-(4-Nitrophenyl)ethyl 5-{{{{2-[2-[2-{[9-[2′-Deoxy-5′-*O*-(4,4′-dimethoxytrityl)-β-D-ribofuranosyl]-1-{2-[(2,4-dinitrophenyl)methylamino]ethyl}-6,9-dihydro-6-oxo-1*H*-purin-2-yl}amino}ethoxy}ethoxy}ethyl}amino}carbonyl}amino}-2-{6-[2-(4-nitrophenyl)ethoxy]-3-oxo-3*H*-xanthen-9-yl}benzoate* (**58**). Compound **57** (1.5 g, 1.2 mmol) was co-concentrated with dry pyridine (3 × 30 ml) and then dissolved in

the same solvent (30 ml). After addition of 4,4′-dimethoxytrityl chloride (0.474 g, 1.4 mmol), the mixture was stirred overnight, then concentrated, and co-concentrated with toluene (50 ml). The residue was dissolved in CH_2Cl_2 (100 ml), the soln. washed with sat. aq. $NaHCO_3$ soln. (3 × 200 ml), dried (Na_2SO_4), and concentrated, and the residue dissolved in little CH_2Cl_2/MeOH and subjected to FC (silica gel (2.5 × 30 cm), CH_2Cl_2 (100 ml), CH_2Cl_2/MeOH 98:2 (100 ml), CH_2Cl_2/MeOH 96:4 (100 ml), CH_2Cl_2/MeOH 94:6 (100 ml), CH_2Cl_2/MeOH 92:8 (100 ml), and CH_2Cl_2/MeOH 99:1 (100 ml)). The red residue of the product fractions was dissolved in CH_2Cl_2/MeOH 95:5, the soln. filtered through cellulose powder, and the filtrate dropwise added to cold MeOH (50 ml) at 0°. The resulting suspension was kept in an icebox overnight, the solid collected, washed with Et_2O, and dried: 1.64 g (88%) of **58**. Orange-red powder. UV (CH_2Cl_2/MeOH 1:1): 231 (4.96), 260 (4.83), 304 (sh, 4.22), 367 (4.38), 436 (sh, 4.33), 458 (4.46), 488 (sh, 4.34). ^{1}H-NMR ((D_6)DMSO): 9.08 (br. *s*, NHCONH); 8.39 (*d*, H–C(3) (Ar)); 8.33 (*s*, H–C(2) (Ar)); 8.19 (*d*, 2 H *o* to NO_2); 8.06 (*d*, 2 H *o* to NO_2); 7.96 (*d*, H–C(5) (Ar)); 7.80 (*s*, H–C(8) (Pur)); 7.68 (*d*, H–C(6)); 7.65 (*d*, 2 H *m* to NO_2); 7.41–7.14 (*m*, H–C(5) (Ar), H–C(6) (Ar), H–C(5) (Xan), H–C(3) (Ar), 2 H *m* to NO_2, 4 H *o* to MeO, C_6H_5 ($(MeO)_2$Tr), NH–C(2) (Pur)); 6.90–6.72 (*m*, H–C(8), H–C(7), H–C(1) (all Xan), 4 H *m* to MeO); 6.35 (*dd*, H–C(2) (Xan); *t*, NHCONH); 6.15 (*d*, H–C(4) (Xan)); 6.12 ('*t*', H–C(1′)); 5.35 (br. *d*, OH–C(3′)); 4.48–4.25 (*m*, CH_2CH_2O, NCH_2CH_2NMe, H–C(3′)); 4.21 (*t*, CH_2CH_2O); 3.92 (*m*, H–C(4′)); 3.70–3.00 (*m*, NCH_2CH_2NMe, CH_2(5′), $NHCH_2CH_2OCH_2CH_2OCH_2CH_2NH$, 2 MeO, OCH_2CH_2NH, CH_2CH_2O); 3.03 (*s*, MeN); 2.81 (*t*, CH_2CH_2O); 2.75–2.65 (*m*, H_a–C(2′)); 2.30–2.15 (*m*, H_b–C(2′)). Anal. calc. for $C_{83}H_{78}N_{12}O_{22}$ (1595.5): C 62.48, H 4.93, N 10.52; found: C 62.43, H 4.93, N 10.34.

44. *2-(4-Nitrophenyl)ethyl 5-{{{{2-{2-{2-{{9-[3′-*O*-(3-Carboxy-1-oxopropyl)-2′-deoxy-5′-*O*-(4,4′-dimethoxytrityl)-β-*D*-ribofuranosyl]-1-{2-[(2,4-dinitrophenyl)methylamino]ethyl}-6,9-dihydro-6-oxo-1*H*-purin-2-yl}amino}ethoxy}ethoxy}ethyl}amino}carbonyl}amino}-2-{6-[2-(4-nitrophenyl)ethoxy]-3-oxo-3*H*-xanthen-9-yl}benzoate* (**59**). A mixture of **58** (0.109 g, 0.07 mmol) and DMAP (34 mg, 0.28 mmol) was dissolved in dry CH_2Cl_2 (5 ml), succinic anhydride (20 mg, 0.2 mmol) added, and the mixture stirred at r.t. for 24 h. The mixture was diluted with CH_2Cl_2 (10 ml), the soln. washed with 10% citric acid soln. (3 × 30 ml) and H_2O (2 × 20 ml), dried (Na_2SO_4), and concentrated. The red residue was dissolved in little CH_2Cl_2/MeOH 95:5 and dropwise added to MeOH (10 ml) at 0°. The precipitate was collected, washed with Et_2O, and dried under vacuum: 0.114 g (98%) of **59**. Orange-red powder. UV (CH_2Cl_2/MeOH 1:1): 230 (4.93), 260 (5.23), 304 (sh, 4.25), 365 (4.34), 433 (sh, 4.32), 458 (4.44), 486 (sh, 4.32). ^{1}H-NMR ((D_6)DMSO): 12.21 (*s*, COOH); 9.09 (br. *s*, NHCONH); 8.37 (*d*, H–C(3) (Ar)); 8.31 (*s*, H–C(2) (Ar)); 8.15 (*d*, 2 H *o* to NO_2); 8.03 (*d*, 2 H *o* to NO_2); 7.98 (*d*, H–C(5) (Ar)); 7.78 (*s*, H–C(8) (Pur)); 7.68 (*d*, H–C(6)); 7.62 (*d*, 2 H *m* to NO_2); 7.37–7.12 (*m*, H–C(5), H–C(6) (Ar), H–C(5) (Xan), 2 H *m* to NO_2, 4 H *o* to MeO, C_6H_5 ($(MeO)_2$Tr), NH–C(2) (Pur)); 6.88–6.77 (*m*, H–C(8), H–C(7), H–C(1) (all Xan), 4 H *m* to MeO); 6.38 (*dd*, H–C(2) (Xan); *t*, NHCONH); 6.18–6.10 (*m*, H–C(1′), H–C(4) (Xan)); 5.31 (*m*, H–C(3′)); 4.50–4.21 (*t*, CH_2CH_2O, NCH_2CH_2NMe); 4.20 (*t*, CH_2CH_2O); 4.08 (*m*, H–C(4′)); 3.70–3.00 (*m*, NCH_2CH_2NMe, CH_2(5′), $NHCH_2CH_2OCH_2$-$CH_2OCH_2CH_2NH$, 2 *Me*O, OCH_2CH_2NH, CH_2CH_2O); 3.02 (*s*, MeN); 2.82 (*t*, CH_2CH_2O); 2.60–2.50 (*m*, H_a–C(2′), C(O)CH_2CH_2COOH); 2.45–2.35 (*m*, H_b–C(2′)). Anal. calc. for $C_{87}H_{82}N_{12}O_{25}$ (1695.6): C 61.63, H 4.87, N 9.90; found: C 61.79, H 4.86, N 9.84.

45. *2-(4-Nitrophenyl)ethyl 5-{{{{2-{2-{2-{{9-[3′-*O*-[(2-Cyanoethoxy)(diisopropylamino)phosphino]-2′-deoxy-5′-*O*-(4,4′-dimethoxytrityl)-β-*D*-ribofuranosyl}-1-{2-[(2,4-dinitrophenyl)methylamino]ethyl}-6,9-dihydro-6-oxo-1*H*-purin-2-yl}amino}ethoxy}ethoxy}ethyl}amino}carbonyl}amino}-2-{6-[2-(4-nitrophenyl)ethoxy]-3-oxo-3*H*-xanthen-9-yl}benzoate* (**60**). A mixture of **59** (0.45 g, 0.3 mmol), sublimed 1*H*-tetrazole (10.5 mg, 0.15 mmol), and 2-cyanoethyl *N,N,N′,N′*-tetraisopropylphosphorodiamidite (0.165 g, 0.55 mmol) in dry MeCN (5 ml) was stirred under N_2 at r.t. for 3 h. After dilution with CH_2Cl_2 (50 ml), the soln. was washed with NaCl-sat. $NaHCO_3$ soln. (2 × 50 ml), dried (Na_2SO_4), and concentrated and the residue dissolved in little CH_2Cl_2 and subjected to FC (flash silica gel (3 × 8 cm), hexane/acetone 1:1 (200 ml), hexane/acetone 9:1 (200 ml), and acetone (100 ml)). The oily sirup of the product fractions was dissolved again in little CH_2Cl_2 and dropwise added to dry Et_2O at 0°. The precipitate was collected under N_2 and dried under vacuum: 0.454 g (77%) of **60**. UV (CH_2Cl_2/MeOH 1:1): 231 (4.97), 261 (4.85), 302 (sh, 4.30), 367 (4.40), 435 (sh, 4.36), 458 (4.46), 486 (sh, 4.35). ^{1}H-NMR ((D_6)DMSO): 9.09 (br. *s*, NHCONH); 8.36 (*d*, H–C(3) (Ar)); 8.31 (*s*, H–C(2) (Ar)); 8.17 (*d*, 2 H *o* to NO_2); 8.02 (*d*, 2 H *o* to

NO_2); 8.00 (*s*, H–C(8) (Pur)); 7.96 (*d*, H–C(5) (Ar)); 7.69 (*d*, 2 H *m* to NO_2); 7.65 (*d*, H–C(6)); 7.37–7.17 (*m*, H–C(5), H–C(6) (Ar), H–C(5) (Xan), 2 H *m* to NO_2, 4 H *o* to MeO, C_6H_5 ($(MeO)_2Tr$), NH–C(2) (Pur)); 6.86–6.75 (*m*, H–C(8), H–C(7), H–C(1) (all Xan), 4 H *m* to MeO); 6.31 (*dd*, H–C(2) (Xan); *t*, NHCONH); 6.15 (*m*, H–C(1′), H–C(4) (Xan)); 4.65 (*m*, H–C(3′)); 4.48–4.25 (*t*, CH_2CH_2O, NCH_2CH_2NMe); 4.33 (*m*, OCH_2CH_2CN); 4.21 (*t*, CH_2CH_2O); 4.08 (*m*, H–C(4′)); 3.70–3.00 (*m*, NCH_2CH_2NMe, $CH_2(5')$, $NHCH_2CH_2OCH_2CH_2OCH_2CH_2NH$, 2 MeO, Me_2CH, OCH_2CH_2NH, 2 CH_2CH_2O); 3.03 (*s*, MeN); 2.81 (*t*, CH_2CH_2O); 2.82 (*m*, H_a–C(2′)); 2.50 (*m*, H_b–C(2′)); 1.26–1.00 (*m*, 2 Me_2CH). Anal. calc. for $C_{92}H_{95}N_{14}O_{23}P$ (1795.7): C 61.54, H 5.33, N 10.91; found: C 60.55, H 5.37, N 10.66.

46. *N¹-(2,4-Dinitrophenyl)ethane-1,2-diamine* (**61**). To a mixture of ethane-1,2-diamine dihydrochloride (33.3 g, 0.25 mol) and Et_3N (124.5 ml, 0.9 mol) in DMF/H_2O 1:1 (400 ml) was added dropwise at 0° under vigorous stirring a soln. of 1-chloro-2,4-dinitrobenzene (10.1 g, 0.05 mol) in DMF (50 ml). After stirring at r.t. for 2 h, the mixture was concentrated under high vacuum. The residue was heated with H_2O (500 ml) and conc. HCl soln. (50 ml) to boiling, and then the hot soln. was filtered. The precipitate contained the bis-substituted less soluble by-product and was, therefore, treated with hot MeOH/acetone, the mixture filtered, and the filtrate co-concentrated with the firstly obtained filtrate. After concentration to 80 ml, the soln. was cooled, the resulting solid (HCl salt) collected and treated with H_2O (90 ml) and conc. NH_3 soln. (10 ml). The soln. was kept overnight in an icebox, the precipitate filtered off by suction, washed with little H_2O, and dried in a vacuum desiccator: 7.8 g (69%) of **61**. Yellow powder. Mp. 92°. ^{1}H-NMR ((D_6)DMSO): 8.80 (*d*, H–C(3)); 8.19 (*d*, H–C(5)); 7.20 (*d*, H–C(6)); 3.49 (*t*, CH_2); 2.82 (*t*, CH_2).

Data of **61**·HCl: UV (MeOH): 209 (4.07), 259 (3.93), 349 (4.20), 393 (sh, 3.77). ^{1}H-NMR ((D_6)DMSO): 8.87 (br. *t*, NH); 8.83 (*d*, H–C(3)); 8.22 (br. *s*, $CH_2NH_3^+$); 8.20 (*d*, H–C(5)); 7.41 (*d*, H–C(6)); 3.84 (*q*, $NHCH_2CH_2NH_3^+$); 2.93 (*t*, $NHCH_2CH_2NH_3^+$). Anal. calc. for $C_8H_{10}N_4O_4 \cdot HCl$ (262.6): C 36.59, H 4.22, N 21.37; found: C 36.67, H 4.33, N 21.34.

47. *3′,5′-Di-*O*-acetyl-2′-deoxy-*N²*-{2-[(2,4-dinitrophenyl)amino]ethyl}-*O⁶*-[2-(4-nitrophenyl)ethyl]-guanosine* (**62**). A mixture of **61** (1.1 g, 4.8 mmol) and **30** (1.0 g, 2 mmol) was co-concentrated with dry pyridine (30 ml). The residue was dissolved in dry pyridine (10 ml) and then heated to 80° for 8 h. After cooling to r.t., the mixture was concentrated and co-concentrated with toluene (3 × 20 ml), the residue dissolved in CH_2Cl_2 (50 ml), and the soln. washed with sat. aq. $NaHCO_3$ soln. (3 × 80 ml). The aq. phase was re-extracted with CH_2Cl_2 (2 × 20 ml), the combined org. phase dried (Na_2SO_4) and concentrated, and the residue purified by FC (silica gel (2 × 25 cm), CH_2Cl_2/MeOH 99:1). The residue of the product fractions was recrystallized from MeOH (80 ml)/acetone (2 ml): 1.05 g (74%) of **62**. Red powder. UV (MeOH): 211 (4.63), 254 (4.48), 350 (4.23), 407 (sh, 3.82). ^{1}H-NMR ((D_6)DMSO; measured at 60°): 8.89 (br. *t*, NH); 8.82 (*d*, H–C(3) (Ar)); 8.20 (*d*, H–C(5) (Ar)); 8.13 (*d*, 2 H *o* to NO_2); 8.04 (*s*, H–C(8)); 7.60 (*d*, 2 H *m* to NO_2); 7.31 (*d*, H–C(6) (Ar)); 7.17 (br. *t*, NH); 6.26 (*dd*, H–C(1′)); 5.43 (*m*, H–C(3′)); 4.70 (*t*, CH_2CH_2O); 4.30–4.13 (*m*, H–C(4′), $CH_2(5')$); 3.71 (*t*, CH_2); 3.65 (*t*, CH_2); 3.22 (*t*, CH_2CH_2O); 3.11–3.02 (*m*, H_a–C(2′)); 2.55–2.45 (*m*, H_b–C(2′)); 2.08 (*s*, Ac); 2.00 (*s*, Ac). Anal. calc. for $C_{30}H_{31}N_9O_{12}$ (709.6): C 50.78, H 4.40, N 17.76; found: C 50.67, H 4.48, N 17.37.

48. *Phenyl* N-*{9-(3′,5′-Di-*O*-acetyl-2′-deoxy-β-*D*-ribofuranosyl)-6-[2-(4-Nitrophenyl)ethoxy]-9*H*-purin-2-yl}-*N*-{2-[(2,4-dinitrophenyl)amino]ethyl}carbamate* (**63**). Compound **62** (0.4 g, 0.56 mmol) was co-concentrated with dry pyridine (3 × 10 ml) and the residue dissolved in dry pyridine (2 ml) and CH_2Cl_2 (10 ml). A small amount of molecular sieve (4Å) and phenyl carbonochloridate (0.22 ml, 1.68 mmol) was added at 0° and the mixture stored for 5 days in an icebox (0°). After evaporation and co-evaporation with toluene (3 × 10 ml), the residue was purified by FC (silica gel (1.5 × 20 cm), CH_2Cl_2/MeOH 97:3): 0.17 g (37%) of **63**. Yellow foam. UV (MeOH): 202 (4.68), 218 (sh, 4.58), 262 (4.50), 348 (4.13), 403 (sh, 3.65). ^{1}H-NMR ((D_6)DMSO): 8.92 (br. *t*, NH); 8.67 (*d*, H–C(3) (Ar)); 8.50 (*s*, H–C(8)); 8.20 (*d*, H–C(5) (Ar)); 8.12 (*d*, 2 H *o* to NO_2); 7.50 (*d*, 2 H *m* to NO_2); 7.57–7.10 (*m*, C_6H_5, H–C(6) (Ar)); 6.28 (*dd*, H–C(1′)); 5.32 (*m*, H–C(3′)); 4.68 (*t*, CH_2CH_2O); 4.35 (*m*, $NHCH_2CH_2N$–C(2)); 4.30–4.18 (*m*, H–C(4′), $CH_2(5')$); 3.91 (*q*, $NHCH_2CH_2N$–C(2)); 3.20 (*t*, CH_2CH_2O); 3.14–3.02 (*m*, H_a–C(2′)); 2.55–2.49 (*m*, H_b–C(2′)); 2.08 (*s*, Ac); 1.99 (*s*, Ac). Anal. calc. for $C_{37}H_{35}N_9O_{14}$ (829.7): C 53.56, H 4.25, N 15.19; found: C 53.61, H 4.25, N 14.97.

49. N′-*{2-[2-(2-Aminoethoxy)ethoxy]ethyl}*-N-*{9-(3′,5′-di*-O-*acetyl-2′-deoxy-β*-D-*ribofuranosyl}-6-[2-(4-nitrophenyl)ethoxy]-9*H-*purin-2-yl}*-N-*{2-[(2,4-dinitrophenyl)amino]ethyl}urea* (**64**). Compound **63** (0.25 g, 0.3 mmol) was co-concentrated with dry pyridine (3 × 10 ml) and then dissolved in dry pyridine (10 ml). Then, 2,2′-[ethane-1,2-diylbis(oxy)]bis[ethanamine] (0.18 ml, 1.2 mmol) was added and stirred at 50° for 30 min. After evaporation and co-evaporation with toluene (2 × 10 ml), the residue was purified by FC (SiO_2 (1.5 × 30 cm), CH_2Cl_2/MeOH 97:3 + 1% Et_3N (100 ml), CH_2Cl_2/ MeOH 92:8 + 1% Et_3N (100 ml), CH_2Cl_2/MeOH 85:15 + 1% Et_3N (200 ml), and CH_2Cl_2/MeOH 8:2 + 1% Et_3N (200 ml)). The residue of the product fractions was treated with little MeOH and the solid collected and dried to give 0.145 g (56%) of **64**. UV (MeOH): 205 (4.63), 254 (4.51), 349 (4.23), 402 (sh, 3.84). ^{1}H-NMR ((D_6)DMSO): 9.62 (br. *t*, NH); 9.00 (br. *t*, NH); 8.78 (*d*, H–C(3) (Ar)); 8.41 (*s*, H–C(8)); 8.20 (*d*, H–C(5) (Ar)); 8.13 (*d*, 2 H *o* to NO_2); 7.61 (*d*, 2 H *m* to NO_2); 7.29 (*m*, H–C(6) (Ar)); 6.36 (*dd*, H–C(1′)); 5.36 (*m*, H–C(3′)); 4.80 (*t*, CH_2CH_2O); 4.51 (*m*, $NHCH_2CH_2N$–C(2)); 4.40–4.10 (*m*, H–C(4′), CH_2(5′)); 3.83 (*q*, $NHCH_2CH_2N$–C(2)); 3.60–3.20 (*m*, CH_2CH_2O, $H_2NCH_2CH_2OCH_2$-$CH_2OCH_2CH_2$); 3.09–2.95 (*m*, H_a–C(2′)); 2.60–2.49 (*m*, H_b–C(2′)); 2.08 (*s*, Ac); 1.98 (*s*, Ac). Anal. calc. for $C_{37}H_{45}N_{11}O_{15}$ (883.8): C 50.28, H 5.13, N 17.43; found: C 50.23, H 5.14, N 17.08.

50. *2-(4-Nitrophenyl)ethyl 5-{{{{2-{2-{2-{{{{9-(2′-Deoxy-β*-D-*ribofuranosyl)-6-[2-(4-nitrophenyl)-ethoxy]-9*H-*purin-2-yl}{2-(2,4-dinitrophenyl)amino]ethyl}amino}carbonyl}amino}ethoxy}ethoxy}ethyl}-amino}carbonyl}amino}-2-{6-[2-(4-nitrophenyl)ethoxy]-3-oxo-3*H-*xanthen-9-yl}benzoate* (**65**). A soln. of **64** (0.15 g, 0.177 mmol) in conc. NH_3 soln./H_2O/dioxane 1:1:1 (20 ml) was kept overnight in an icebox. After evaporation, the residue and DMAP (65 mg, 0.27 mmol) was co-concentrated with dry DMF and dissolved in dry DMF (4 ml) and dry pyridine (1 ml). Then, **55** (0.203 g, 0.27 mmol) was added and heated to 60° for 4 h. The mixture was concentrated, and the oily sirup dissolved in little CH_2Cl_2/MeOH 95:5 and subjected to FC (silica gel (1 × 10 cm), CH_2Cl_2/MeOH 95:5 (80 ml), CH_2Cl_2/MeOH 93:7 (50 ml), CH_2Cl_2/MeOH 9:1 (100 ml), and CH_2Cl_2/MeOH 85:15 (100 ml)). The residue of the product fractions was treated with cold MeOH and kept in an icebox to give 0.2 g (78%) of **65**. Orange-red solid. UV (CH_2Cl_2/MeOH 1:1): 205 (4.60), 211 (4.65), 217 (4.68), 225 (4.93), 262 (4.90), 302 (sh, 4.36), 354 (4.45), 436 (sh, 4.38), 459 (4.46), 487 (sh, 4.37). ^{1}H-NMR ((D_6)DMSO): 9.69 (br. *t*, NH); 9.07 (br. *t*, NH); 9.02 (br. *t*, NH); 8.75 (*d*, H–C(3) (Ar)); 8.44 (*s*, H–C(8) (Pur)); 8.28 (*d*, H–C(2)); 8.21 (*d*, H–C(5) (Ar)); 8.17 (*d*, 6 H *o* to NO_2); 7.69 (*d*, 2 H *m* to NO_2); 7.66–7.58 (2*d*, 2 H *m* to NO_2); 7.31 (*m*, 2 H *m* to NO_2, H–C(5) (Ar)); 7.30 (*m*, H–C(6) (Ar)); 7.12 (*d*, H–C(5) (Xan)); 6.88–6.78 (*m*, H–C(8), H–C(7), H–C(1) (all Xan)); 6.36–6.25 (*m*, H–C(1′), H–C(2) (Xan), NH); 6.13 (*d*, H–C(4) (Xan)); 5.32 (*d*, OH–C(3′)); 4.94 (br. *t*, OH–C(5′)); 4.79 (*t*, CH_2CH_2O); 4.48 (*m*, $NHCH_2CH_2N$–C(2) (Pur)); 4.45 (*m*, CH_2CH_2O, H–C(3′)); 4.21 (*t*, CH_2CH_2O); 3.89 (*m*, H–C(4′), $NHCH_2CH_2N$–C(2) (Pur)); 3.60–3.20 (*m*, 2 CH_2CH_2O, CH_2(5′), $H_2NCH_2CH_2OCH_2CH_2OCH_2CH_2$); 2.82 (*t*, CH_2CH_2O); 2.70–2.59 (*m*, H_a–C(2′)); 2.39–2.28 (*m*, H_b–C(2′)). Anal. calc. for $C_{70}H_{66}N_{14}O_{23}$ (1471.3): C 57.14, H 4.52, N 13.32; found: C 56.77, H 4.63, N 13.20.

51. *2-(4-Nitrophenyl)ethyl 5-{{{{2-{2-{2-{{{{9-[2′-Deoxy-5′*-O-*(4,4′-dimethoxytrityl)-β*-D-*ribofurano-syl]-6-[2-(4-nitrophenyl)ethoxy]-9*H-*purin-2-yl}{2-[(2,4-dinitrophenyl)amino]ethyl}amino}carbonyl}-amino}ethoxy}ethoxy}ethyl}amino}carbonyl}amino}-2-{6-[2-(4-nitrophenyl)ethoxy]-3-oxo-3*H-*xanthen-9-yl}benzoate* (**66**). As described for **58**, with **65** (1.5 g, 1 mmol) in dry pyridine (30 ml) and 4,4′-dimethoxytrityl chloride 0.45 g, 1,35 mmol). Purification by FC (silica gel (2.5 × 30 cm), CH_2Cl_2/MeOH 97:3 (300 ml) and CH_2Cl_2/MeOH 96:4 (400 ml)) gave 1.45 g (80%) of **66**. Orange red crystals. UV (CH_2Cl_2/MeOH 1:1): 207 (4.64), 226 (5.03), 262 (4.90), 306 (sh, 4.30), 353 (4.43), 436 (sh, 4.36), 459 (4.45), 487 (sh, 4.34). ^{1}H-NMR ((D_6)DMSO): 9.71 (br. *s*, NHCONH); 8.96 (br. *t*, NH); 8.77 (*d*, H–C(3) (Ar)); 8.34 (*s*, H–C(8) (Pur)); 8.29 (*s*, H–C(2) (Ar)); 8.18 (*dd*, H–C(5) (Ar)); 8.15 (*d*, 4 H *o* to NO_2); 8.02 (*d*, 2 H *o* to NO_2); 7.69 (*d*, H–C(6)); 7.66–7.58 (2*d*, 4 H *m* to NO_2); 7.30–7.08 (*m*, H–C(5) (Ar), H–C(6) (Ar), H–C(5) (Xan), H–C(3) (Ar), 2 H *m* to NO_2, 4 H *o* to MeO, C_6H_5 ($(MeO)_2$Tr)); 6.8–6.67 (*m*, H–C(8) (Xan), H–C(7), H–C(1) (Xan), 4 H *m* to MeO); 6.38 (*dd*, H–C(1′)); 6.33 (*dd*, H–C(2) (Xan)); 6.21 (*t*, NHCONH); 6.13 (*d*, H–C(4) (Xan)); 5.32 (br. *d*, OH–C(3′)); 4.79 (*m*, CH_2CH_2O); 4.42 (*m*, $NHCH_2CH_2N$–C(2) (Pur), CH_2CH_2O, H–C(3′)); 4.21 (*t*, CH_2CH_2O); 3.99 (*m*, H–C(4′)); 3.78 (*m*, $NHCH_2CH_2N$–C(2) (Pur)); 3.68 (2*s*, 2 MeO); 3.60–3.18 (*m*, $NHCH_2CH_2OCH_2$-$CH_2OCH_2CH_2NH$, OCH_2CH_2NH, CH_2CH_2O); 3.16 (*m*, CH_2(5′)); 2.81 (*t*, CH_2CH_2O); 2.85–2.73 (*m*,

H_a–C(2′)); 2.50–2.38 (*m*, H_b–C(2′)). Anal. calc. for $C_{91}H_{84}N_{14}O_{25}\cdot H_2O$ (1782.7): C 61.31, H 4.86, N 10.99; found: C 61.02, H 4.85, N 10.84.

52. *2-(4-Nitrophenyl)ethyl 5-{{{{2-{2-{2-{{{{9-{3′-*O*-[(2-Cyanoethoxy)(diisopropylamino)phosphino]-2′-deoxy-5′-*O*-(4,4′-dimethoxytrityl)-β-*D*-ribofuranosyl}-6-[2-(4-nitrophenyl)ethoxy]-9*H*-purin-2-yl}{2-[(2,4-dinitrophenyl)amino]ethyl}amino}carbonyl}amino}ethoxy}ethoxy}ethyl}amino}carbonyl}-amino}-2-{6-[2-(4-nitrophenyl)ethoxy]-3-oxo-3*H*-xanthen-9-yl}benzoate* (**67**). As described for **60**, with **66** (0.25 g, 0.14 mmol), 1*H*-tetrazole (2.4 mg, 0.035 mmol), and 2-cyanoethyl *N*,*N*,*N*′,*N*′-tetraisopropylphosphorodiamidite (77 mg, 0.25 mmol): 0.2 g (72% of **67**. Red solid foam. UV (CH_2Cl_2/MeOH 1:1): 228 (4.99), 263 (4.91), 299 (sh, 4.40), 354 (4.45), 435 (sh, 4.37), 458 (4.46), 486 (sh, 4.35). ^{1}H-NMR ((D$_6$)DMSO): 9.80 (br. *s*, NHCONH); 9.08 (br. *s*, NH); 9.00 (br. *t*, NH); 8.72 (*d*, H–C(3) (Ar)); 8.32 (*s*, H–C(8) (Pur)); 8.28 (*s*, H–C(2) (Ar)); 8.18 (*m*, H–C(5) (Ar), 4 H *o* to NO_2); 8.01 (*d*, 2 H *o* to NO_2); 7.96 (*d*, H–C(5) (Ar)); 7.64 (*d*, H–C(6)); 7.62–7.54 (2*d*, 4 H *m* to NO_2); 7.30–7.08 (*m*, H–C(5) (Ar), H–C(6) (Ar), H–C(5) (Xan), 2 H *m* to NO_2, 4 H *o* to MeO, C_6H_5 ((MeO)$_2$Tr)); 6.85–6.70 (*m*, H–C(8), H–C(7), H–C(1) (all Xan), 4 H *m* to MeO); 6.41 (*m*, H–C(1′)); 6.32 (*dd*, H–C(2) (Xan)); 6.30 (br. *t*, NHCONH); 6.12 (*d*, H–C(4) (Xan)); 4.81 (*t*, CH_2CH_2O); 4.70–4.60 (*m*, H–C(3′)); 4.42 (*m*, CH_2CH_2O, $NHCH_2CH_2N$–C(2) (Pur)); 4.25 (*t*, OCH_2CH_2CN); 4.20 (*t*, CH_2CH_2O); 4.11 (*m*, H–C(4′)); 3.79 (*m*, $NHCH_2CH_2N$–C(2) (Pur)). 3.66 (2*s*, MeO); 3.69–3.15 (*m*, CH_2(5′), $NHCH_2CH_2OCH_2$-$CH_2OCH_2CH_2NH$, OCH_2CH_2CN, 2 CH_2CH_2O); 3.00–2.90 (*m*, H_a–C(2′)); 2.89–2.50 (*m*, H_b–C(2′), CH_2CH_2O, OCH_2CH_2CN, 2 Me_2CH); 1.18–1.00 (*m*, 2 Me_2CH). ^{31}P-NMR ((D$_6$)DMSO): 148.4; 149.0. Anal. calc. for $C_{100}H_{101}N_{16}O_{26}P$ (1973.9): C 60.85, H 5.16, N 11.35; found: C 60.15, H 5.26, N 11.04.

53. *Oligonucleotide Synthesis.* Solid-support material 500 Å LCAMA-CPG [4] (200 mg) was loaded with the appropriate nucleoside 3′-succinate (30 mmol) by TOTU (10 mg, 30 mmol) in dry MeCN (3 ml) and *N*-methylmorpholine (8 μl, 80 μml) 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 Et_2O. Capping procedure: The nucleoside-functionalized CPG was treated with a mixture of DMAP (50 mg, 0.41 mmol), abs. pyridine (10 ml), and Ac_2O (1 ml, 10.6 mmol) for 1 h at r.t. by gently shaking. The material was collected, washed with MeOH, DMF, MeOH, acetone, and Et_2O and dried in a vacuum desiccator. Assembly of oligodeoxynucleotides: The syntheses were carried out in an *Eppendorf-Biotronik-Ecosyn-D-300* or *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 a programmed series of reagents and solvent washes based on recommended procedures with the following main steps: 1) 5′-*O*-(MeO)$_2$Tr deprotection in 135 s. 2) Coupling: 0.1M phosphoramidite and 0.5M 1*H*-tetrazole in dry MeCN, delivered in alternating reagent pushes with a subsequent wait time of 60 s. 3) Capping: Ac_2O/2,6-dimethylpyridine/THF 1:1:8 and 1-methyl-1*H*-imidazole/THF 16:84, delivered in one 10 s push with a subsequent wait time of 5 s. 4) Oxidation: 0.05M I_2 in THF/H_2O/pyridine 7:2:1, delivered in one 10 s push with a subsequent wait time of 15 s. Then, a cleavage program was carried out: 1) Cleavage of npe groups by 1M DBU in MeCN delivered in several pushes and following wait steps (total wait time 20 min). 2) Cleavage of the base-labile groups and cleavage from the support: conc. NH_3 soln. delivered in one push with a consecutive wait time of 1 h. The reaction soln. was collected and lyophilized in a *Speed-vac* concentrator under high vacuum.

REFERENCES

[1] T. Maier, W. Pfleiderer, *Heterocycles*, in press.

[2] M. C. F. Oliveira, Y. I. Hirata, J. R. Chagas, P. Boschcov, R. A. Gomes, A. F. S. Figueiredo, L. Juliano, *Anal. Biochem.* **1992**, *203*, 34.

[3] O. Zelenko, U. Neumann, W. Brill, U. Pieles, H. Moser, J. Hofsteenge, *Nucleic Acids Res.* **1994**, *22*, 2731.

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

[5] T. Förster, *Ann. Phys.* **1948**, *437*, 55.

[6] T. G. Durey, 'Biophysical and Biochemical Aspects of Fluorescence Spectroscopy', Plenum Press, 1991.

[7] H. Sigmund, T. Maier, W. Pfleiderer, *Nucleosides, Nucleotides Nucleic Acids* **1997**, *16*, 685.
[8] M. J. Robins, B. Uznański, *Can. J. Chem.* **1981**, *59*, 2608.
[9] H. Hippchen, *Chem. Ber.* **1947**, *80*, 263.
[10] Q. Yao, W. Pfleiderer, *Helv. Chim. Acta* **2003**, *86*, 1.
[11] L. J. McBride, R. Kierzek, S. L. Beaucage, M. H. Caruthers, *J. Am. Chem. Soc.* **1986**, *108*, 2040.
[12] B. Singer, *Biochemistry* **1972**, *11*, 3939.

Received July 5, 2010