Nucleotides

Part LXI[1])

Phthaloyl Strategy: A New Concept of Oligonucleotide Synthesis

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A new alternative strategy of oligonucleotide synthesis was developed by use of the phthaloyl protecting group for the exocyclic amino functions of the nucleobases (see **9**–**12**). This approach combines the advantages of cheap and easily accessable monomeric building blocks (see **17**–**20**), standard machine-aided oligonucleotide synthesis, and a fast deprotection protocol which is orthogonal to the cleavage procedure from the solid support. The crude oligonucleotides show high purity and require, in general, no further chromatographic purification.

1. Introduction. – In common oligonucleotide synthesis *via* the phosphoramidite approach [2], usually the exocyclic amino functions are protected by a single acyl protecting group. Among the broad variety of blocking groups introduced over the years, only a few such as the benzoyl [3], phenoxyacetyl [4][5], isobutyryl [6], 2-(4-nitrophenyl)ethoxycarbonyl [7], or allyloxycarbonyl [8] groups are applied regularly and are, in part, commercially available. A comparison of the properties of these groups in regard to the machine-aided oligonucleotide synthesis reveals some major differences which are of great importance for the handling of the monomeric building blocks, the assembly of the oligonucleotides, and the isolation and purification procedures. The benzoyl-protected phosphoramidites are currently the reagents of choice due to their low price, but deprotection and the purity of the crude products still present some drawbacks. The slightly more costly oligonucleotide synthesis using the phenoxyacetyl-protected phosphoramidites has the advantage that the deprotection step is faster, but nevertheless, purification by HPLC is necessary to obtain a pure product. On the other hand, working with [2-(4-nitrophenyl)ethoxycarbonyl]- [7][9][10] or (allyloxycarbonyl)-protected phosphoramidites, leads directly to high-purity products after the deprotection step, omitting the time-consuming HPLC-purification procedure.

Since there is a still growing demand for high-purity oligonucleotides in molecular biology-based techniques (*e.g.* PCR [11], expression profiling [12], DNA chips [13] *etc.*), we have been searching for a new protection strategy which would combine some or all the advantages of the protecting groups introduced so far. Especially, we were looking for a protecting group that would feature simple introduction, high-speed synthesis, fast deprotection, and direct generation of pure materials.

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We focussed our attention on bis-acyl-protecting groups to omit any side reaction at the acylamido functions as observed on phosphitylation with highly reactive diethylphosphoramidites, especially at the [2-(4-nitrophenyl)ethoxy-carbonyl]-protected (npeoc) exocyclic amino groups [14]. The side reaction was especially obvious in the case of adenosine- and cytidine-rich sequences when a highly reactive acidic catalyst like pyridine hydrochloride [15] was used. Based on the findings that 2′-deoxy-N^6-phthaloyladenosine derivatives showed an unexpected lability towards non-nucleophilic bases like 1,8-diazabicyclo[5.4.0]undec-7-ene (DBU) [16], we evaluated the possibility to establish a new strategy of oligonucleotide synthesis employing phthaloyl protection for the amino moieties of adenine, cytosine, and guanine (*Fig. 1*) [17].

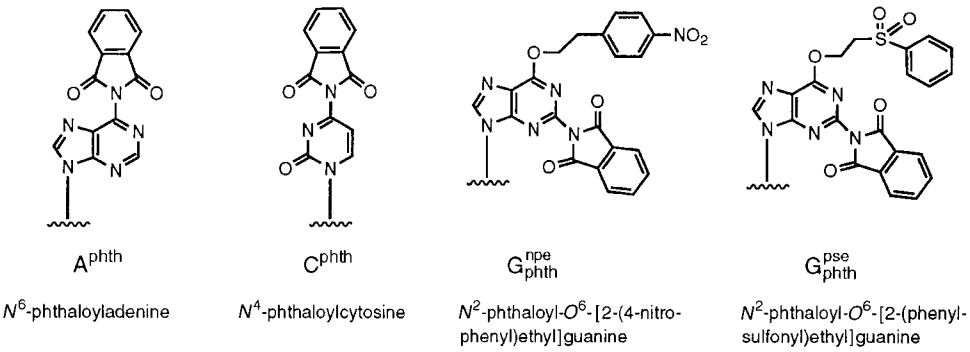


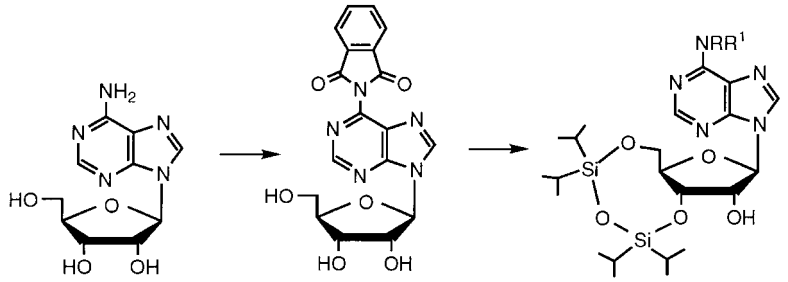
Fig. 1. *Phthaloyl protection for the amino moieties of adenine, cytosine, and guanine*

Phthaloyl-protecting groups were first introduced to nucleic-acid chemistry by *Hata* and coworkers [18][19] to protect deoxyadenosine during the phosphotriester approach, and removal followed the common protocol by the use of nucleophilic bases, *e.g.* hydrazine, conc. ammonia/pyridine, or Et_3N.

2. Synthesis. – In a preliminary study, adenosine (**1**) was converted by *Hata's* procedure [18] *via* transient trimethylsilyl protection [20] into N^6-phthaloyladenosine (**2**) in 59% yield. Further modification with *Markiewicz*'s reagent [21] led in 67% yield to N^6-phthaloyl-3′,5′-*O*-(1,1,3,3-tetraisopropyldisiloxan-1,3-diyl)adenosine (**3**) [22] which could then be compared with the corresponding N^6-[2-(4-nitrophenyl)ethoxycarbonyl]adenosine derivative **4** regarding cleavage with DBU in an aprotic solvent (*Scheme 1*). Surprisingly, the phthaloyl (phth) group was removed much faster than the 2-(4-nitrophenyl)ethoxycarbonyl (npeoc) group, and deprotection was complete within seconds, as shown by TLC monitoring. These findings opened the way for a new oligonucleotide strategy by combining the phthaloyl protection with the acid-labile 5′-*O*-dimethoxytrityl group and a DBU-stable linker system to allow protecting group removal from the oligonucleotide when still attached to the solid support. Final cleavage by ammonia should generate high-quality oligomers without a further purification step.

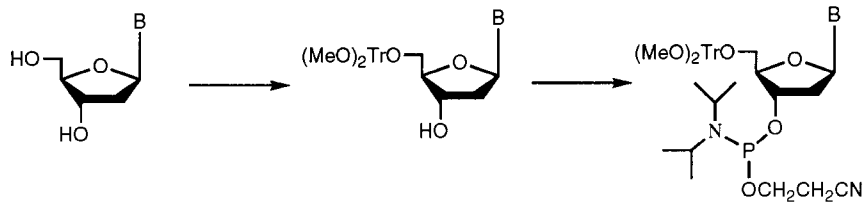
The introduction of the phthaloyl group into 2′-deoxyadenosine (**5**) [18][23] to give **9** started from the fully unprotected nucleoside following the transient protection [20] protocol, by means of persilylation with trimethylsilyl chloride (tms-Cl), acylation with

Scheme 1



phthaloyl chloride, and subsequent removal of the tms groups by hydrolysis (*Scheme 2*). An alternative approach, making use of different sugar-protected deoxyadenosine derivatives and acylation with phthaloyl chloride in pyridine at room temperature or with phthalic anhydride at 100°, did not improve the performance. In a similar manner, 2′-deoxycytidine (**6**) was transformed into 2′-deoxy-N^4-phthaloylcytidine (**10**) in 64% yield. Workup is to some extent more critical since removal of the phthaloyl group can lower the yield.

Scheme 2



	B		B	%		B	%		B	%
5	A	9	A^{phth}	93	13	A^{phth}	46	17	A^{phth}	72
6	C	10	C^{phth}	64	14	C^{phth}	61	18	C^{phth}	72
7	G^{npe}	11	G^{npe}_{phth}	51	15	G^{npe}_{phth}	67	19	G^{npe}_{phth}	87
8	G^{pse}	12	G^{pse}_{phth}	40	16	G^{pse}_{phth}	66	20	G^{pse}_{phth}	73

Low solubility of 2′-deoxyguanosine and reactivity reasons made it necessary to protect the O^6-lactam function before introducing the N^2-phthaloyl moiety. The protection at position O^6 increases both the solubility and the nucleophilic character of the 2-NH_2 group, thus favouring phthaloylation, and additionally reduces side reactions during oligonucleotide synthesis [24][25]. For protection of the lactam function, the 2-(4-nitrophenyl)ethyl (npe) and alternatively the 2-(phenylsulfonyl)ethyl group (pse) were chosen both of which are removable by β-elimination whereby the latter one shows an increased lability towards non-nucleophilic bases, *e.g.*, DBU. Starting from

3′,5′-di-*O*-acetyl-2′-deoxyguanosine [26], the npe group was introduced under *Mitsunobu* conditions [7], and the acetyl groups were removed by transesterification with a catalytic amount of NaCN in MeOH to give 91% of **7**. Similarly, 3′,5′-di-*O*-acetyl-2′-deoxyguanosine was reacted with 2-(phenylthio)ethanol, followed by removal of the acetyl groups and subsequent oxidation, to give 2′-deoxy-O^6-[2-(phenylsulfonyl)-ethyl]guanosine (**8**) [27][28] in 47% yield. Phthaloylation of **7** and **8** proceeded with reasonable yields of 51 (→ **11**) and 40% (→ **12**), respectively, also *via* the transient protection method (*Scheme 2*). The removal of the tms groups could be performed with pyridine/H_2O (15 min), or alternatively with NH_4F in MeOH (3 min) or Bu_4NF in pyridine (5 min), without having a major effect on the overall yields.

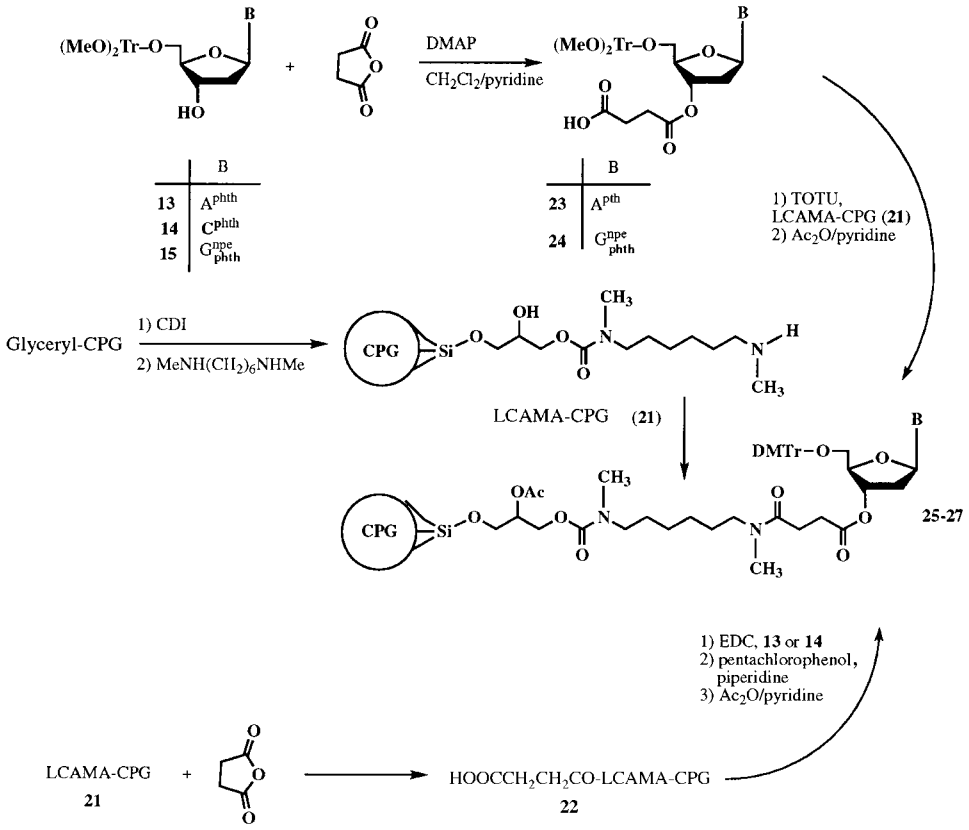
Dimethoxytritylation of compounds **9**–**12** worked well according to the usual procedure. Only the cytidine derivative **14** showed an increased sensitivity towards MeOH during chromatography on silica gel leading to a partial opening of the cyclic phthaloyl protection; therefore, workup was performed in toluene/AcOEt. The final phosphitylation of compounds **13**–**16** also proceeded with 2-cyanoethyl tetraisopropylphosphorodiamidite (= bis(diisopropylamino)(2-cyanoethoxy)phosphane) in the usual manner, except that the yields were slightly improved when pyridine hydrochloride was employed as a mild acidic catalyst instead of the commonly used 1*H*-tetrazole.

For the oligonucleotide synthesis, separating the protecting-group removal from the support-cleavage step, commercially available glyceryl-CPG (CPG = controlled-pore glass) was transformed into the DBU-stable LCAMA-CPG support (**21**) ((long-chain-alkyl)methylamino linker) [9][10] by reaction first with 1,1′-carbonylbis[1*H*-diimidazole] (CDI) and afterwards with *N*,*N*′-dimethylhexane-1,6-diamine (*Scheme 3*).

The 3′-(hydrogen succinates) **23** and **24** of the 2′-deoxyadenosine and 2′-deoxyguanosine derivatives **13** and **15**, respectively, were successfully generated by acylation with succinic anhydride, and subsequent loading of the LCAMA-CPG support (**21**) led to the phth-protected DBU-stable supports **25** and **26**, respectively (*Scheme 3*). The appropiate 3′-(hydrogen succinate) of the 2′-deoxycytidine derivative **14** exhibited a high sensitivity of the dimethoxytrityl residue towards even small amounts of acid and could, therefore, not be isolated by chromatographic means. Therefore, its support had to be built up in the reverse manner, forming first the LCAMA-CPG-derived succinamic acid **22** according to *Pon*'s protocol [29], followed by an EDC-promoted (EDC = 1-[3-(dimethylamino)propyl]-3-ethylcarbodiimide hydrochloride) esterification with 2′-deoxy-5′-*O*-(dimethoxytrityl)-N^4-phthaloylcytidine (**14**) to give the desired loaded solid-support material **27**.

3. Deprotection. – The crucial step of the newly developed phthaloyl strategy was the removal of the phthaloyl groups from the exocyclic amino functions by DBU under aprotic conditions. For solubility reasons, the time course of the deprotection was monitored with the 5′-*O*-(dimethoxytrityl) derivatives **13**–**16** with 1M DBU in MeCN in 200-fold excess as a standard. Aliquots were taken from the reaction solution in distinct time intervals and, after quenching with AcOH, were analyzed by reversed-phase HPLC. The time course of the different deprotection reactions was nicely resolved by HPLC, showing that the phthaloyl groups are removed very fast (5–13 s) under these conditions (*Fig. 2*). Interestingly, the 2′-deoxy-5′-*O*-(dimethoxytrityl)-O^6-[2-(phenyl-

Scheme 3



sulfonyl)ethyl]-N^2-phthaloylguanosine (**16**) was deprotected to 2′-deoxy-5′-*O*-(dimethoxytrityl)guanosine fastest within 5 s, whereas the corresponding O^6-npe derivative **15** showed a comparably slow kinetic. Although the phthaloyl group was cleaved within seconds, removal of the npe group required at least 2 h for completion.

4. Oligonucleotide Synthesis. – The application of the normal oligonucleotide protocol to the phthaloyl-protected phosphoramidites required the checking of each step of the cycle regarding the stability of the new building blocks under the standard conditions. The acid-catalysed condensation and dimethoxytrityl-cleavage step, as well as the capping procedure, turned out to be compatible with earlier findings, whereas the oxidation procedure had to be changed from the normal I_2 treatment in pyridine/H_2O/THF to a treatment with 1M solution of *tert*-butyl hydroperoxide [30] in MeCN to avoid side reactions (*Table 1*). A series of oligo-2′-deoxyribonucleotides (*Table 2*) were synthesized in a machine-aided manner to give homogeneous products of high purity.

For the phthaloyl deprotection on the solid support, a solution of 1M DBU in MeCN was employed for 12–25 min if the sequence consisted of dT, dA, and dC. The presence of a O^6-npe-protected N^2-phthaloyl-2′-deoxyguanosine moiety in the oligonucleotide

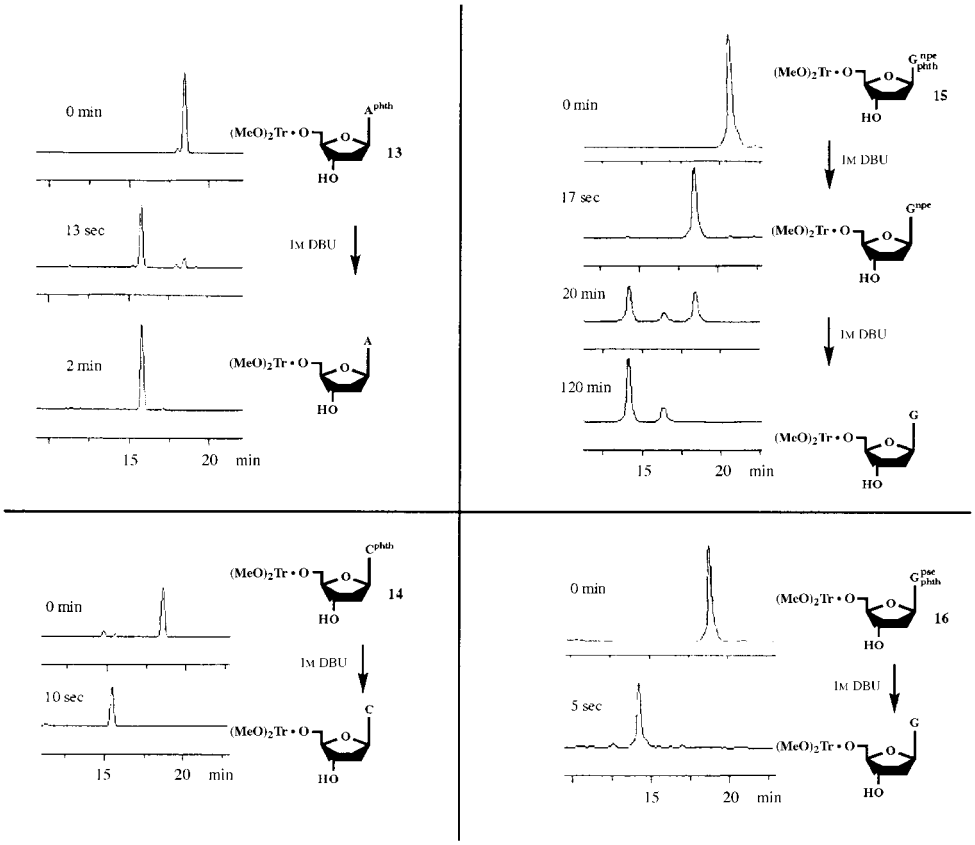


Fig. 2. *Deprotection studies of phth-protected 2′-deoxyribonucleosides monitored by reversed-phase HPLC*

Table 1. *Protocol for Oligonucleotide Synthesis by the Phthaloyl Strategy*

	Reagents	Time
Condensation	0.1M phosphoramidites/0.5M 1*H*-tetrazole 1 : 1	60 s
Oxidation	1M *tert*-butyl hydroperoxide in MeCN	90 s
Capping	A: Ac_2O/2,6-dimethylpyridine/THF 1 : 1 : 8 B: 1-methyl-1*H*-imidazole/THF 16 : 84	15 s
Detritylation	3% CCl_3COOH in CH_2Cl_2	95 s
Deprotection	1M DBU in MeCN	12 min
Cleavage	conc. NH_3 solution	1 h

chain, however, required several hours for complete deprotection. The deprotection was performed either by continuous pumping of 1M DBU over the synthesis column on the synthesizer, or after transfer of the solid support to a 1M DBU solution in a shaker device for prolonged treatment. Afterwards the CPG support with the oligomer still bound was carefully washed with MeCN to remove protecting-group residues, and in the last step, treatment with concentrated aqueous NH_3 solution at room temperature

Table 2. *Oligonucleotide Sequences Synthesized by the Phthaloyl Approach*

5′-Sequence-3′	Deprotection time (1M DBU)
d(AAA AA)	12 h 3 h
d(AAA AAA)	12 h
d(AAA AAA A)	12 h
d(AAA AAA AA)	25 min
d(AAA AAA AAA)	12 h 3 h 15 min
$(dA)_{15}$	12 h 1 h
$(dA)_{20}$	12 h
d(CCC CAT TAT)	25 min
d(AAT CCT A)	12 min
d(ATT CCT A)	12 min
d(ATT TAA TTT AAT TTA A)	12 h 3 h 15 min
d(CCT CCA ATC TAG)	12 h

for 1 h generated the free oligonucleotides as their ammonium salts. The crude products (*Table 2*) were then analyzed by reversed-phase HPLC (*Fig. 3*) and anion-exchange HPLC as well as by capillary electrophoresis, which showed their high purity. Thus, in general, no further purification was required.

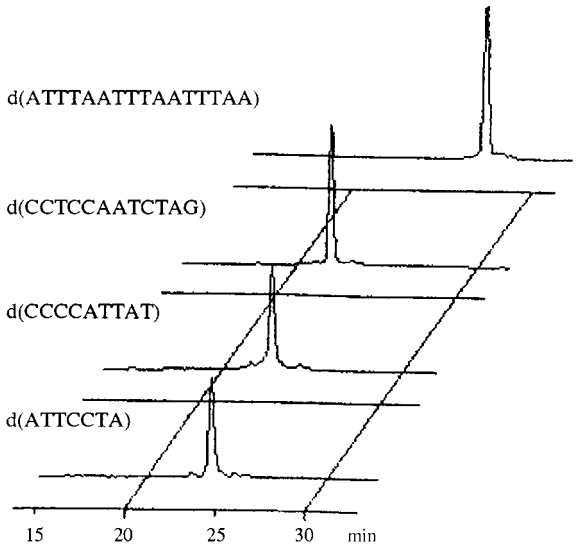


Fig. 3. *Reversed-phase HPLC of DNA sequences synthesized by the phthaloyl strategy*

5. Physical Data. – All newly synthesized compounds were characterized in the usual manner by elemental analysis and UV and ^{1}H-NMR spectra (see *Exper. Part*).

Experimental Part

General. Products were dried under high vacuum. TLC: precoated silica gel thin-layer sheets *F 1500 LS254* from *Schleicher & Schüll*. Flash chromatography (FC): silica gel (*Baker*, 30–60 μm), 0.3–0.4 bar. HPLC: *Merck Hitachi L-6200*, *D-2000* chromatointegrator, detection at 260 nm (*Uvikon 730 SLC*, Fa. *Kontron*);

column *RP-18 LiChrospher* (125 × 4 mm, 5 mm, *Merck*). UV/VIS: *Perkin-Elmer, Lambda 15*; λ_{max} in nm (log ε). ^{1}H-NMR: *Bruker AC 250* and *WM 250*; δ in ppm rel. to DMSO. ^{31}P-NMR: *Jeol-400-MHz* and *Bruker-DRX-600-MHz*; in ppm rel. to H_3PO_4. Gel capillary electrophoresis: *Beckmann Pace System 5000*.

1. N^6-*Phthaloyladenosine* (**2**) [22]. After codistillation with anh. pyridine, a mixture of adenosine (**1**; 5 g, 18.7 mmol), chlorotrimethylsilane (18 ml, 142 mmol) in anh. pyridine (75 ml) was stirred for 30 min, and then phthaloyl chloride (5.4 ml, 37.4 mmol) was added with stirring. After 1 h the reaction was quenched by addition of ice-water, and stirring was continued for 10 min. Then the mixture was extracted with CH_2Cl_2 (100 ml) and H_2O (100 ml). The org. phase was washed with H_2O (50 ml) and $NaHCO_3$ soln. (100 ml), evaporated, and purified by CC (0–10% MeOH/$CHCl_3$): 4.41 g (59%) of **2**. R_f ($CHCl_3$/MeOH 9:1) 0.22. UV (MeCN): 270 (4.07), 299 (sh, 3.58). ^{1}H-NMR ((D$_6$)DMSO): 9.08 (*s*, H–C(2)); 8.95 (*s*, H–C(8)); 8.00 (*m*, 4 H, phth); 6.11 (*s*, H–C(1′)); 5.64 (*d*, HO–C(2′)); 5.28 (*d*, HO–C(3′)); 5.09 (*t*, HO–C(5′)); 4.70 (*m*, H–C(2′)); 4.23 (*m*, H–C(3′)); 4.01 (m, H–C(4′)); 3.60 (*m*, 2 H–C(5′)). Anal. calc. for $C_{18}H_{15}N_5O_6 \cdot 0.5\ H_2O$ (406.36): C 53.20, H 3.97, N 17.23; found: C 53.78, H 4.18, N 15.09.

2. N^6-*Phthaloyl-3′,5′*-O-*(1,1,3,3-tetraisopropyldisiloxan-1,3-diyl)adenosine* (**3**) [22]. To a soln. of **2** (3.17 g, 8 mmol) in anh. pyridine (50 ml), 1,3-dichloro-1,1,3,3-tetraisopropyldisiloxane (2.75 ml, 8.77 mmol) was added with stirring. After stirring overnight, the mixture was evaporated, the residue dissolved in CH_2Cl_2 (100 ml) and then treated with a sat. $NaHCO_3$ soln. (100 ml). The org. phase was dried ($MgSO_4$), evaporated, and purified by CC (25–33% AcOEt/toluene): 3.43 g (67%) of **3**. R_f (toluene/AcOEt 1:1) 0.39. UV (MeCN): 299 (sh, 3.21), 271 (4.10), 218 (4.57). ^{1}H-NMR ((D$_6$)DMSO): 8.98 (*s*, H–C(2)); 8.73 (*s*, H–C(8)); 8.00 (*m*, 4 H, phth); 6.06 (*s*, H–C(1′)); 5.74 (*d*, HO–C(2′)); 4.75 (*m*, H–C(2′), H–C(3′)); 4.00 (*m*, H–C(4′), 2 H–C(5′)); 1.04 (*m*, Me_2CH). Anal. calc. for $C_{30}H_{41}N_5O_7Si_2 \cdot 0.5\ H_2O$ (648.87): C 55.53, H 6.52, N 10.79; found: C 55.41, H 6.53, N 10.33.

3. N^6-*[2-(4-Nitrophenyl)ethoxycarbonyl]-3′,5′*-O-*(1,1,3,3-tetraisopropyldisiloxan-1,3-diyl)adenosine* (**4**) [31].

4. *2′-Deoxy*-O^6-*[2-(4-nitrophenyl)ethyl]guanosine* (**7**) [7]. From 3′,5′-di-*O*-acetyl-2′-deoxyguanosine as described in 91% yield.

5. *2′-Deoxy*-O^6-*[2-(phenylsulfonyl)ethyl]guanosine* (**8**) [27]. To a suspension of 3′,5′-di-*O*-acetyl-2′-deoxyguanosine [26] (3.0 g, 8.55 mmol) in dioxane (160 ml), PPh_3 (3.36 g, 12.8 mmol) and 2-(phenylthio)-ethanol (2.0 g, 12.9 mmol) were added and then heated to 50°. After stirring for 40 min, ethyl azodicarboxylate (= ethyl diazenedicarboxylate; 2.38 g, 13.7 mmol) was added, and heating at 50° continued for 7 h (TLC monitoring). Then more 2-(phenylthio)ethanol (0.45 g, 3 mmol) and ethyl azodicarboxylate (0.45 g, 2.58 mmol) were added and heating continued for another 60 min. The mixture was evaporated and purified by FC (silica gel (50 g), toluene/AcOEt 5:1 and 3:1, then toluene/AcOEt/MeOH 15:5:1): 3.23 g of 3′,5′-di-*O*-acetyl-2′-deoxy-*O*^{6}-[2-(phenylthio)ethyl]guanosine containing 10% of PPh_3(=O). The crude material (3.23 g) was dissolved in dioxane (100 ml), MeOH (100 ml), and conc. NH_3 soln. (50 ml) and stirred at r.t. for 20 h. The mixture was evaporated and then co-evaporated with toluene (20 ml), MeOH (20 ml), and CH_2Cl_2 (20 ml), and the residue was purified by FC (silica gel, (60 g) 0–5%/MeOH/CH_2Cl_2): 2.03 g (58% over two steps) of 2′-deoxy-*O*^{6}-[2-(phenylthio)ethyl]guanosine. UV (MeOH): 281 (4.08), 250 (4.25), 210 (sh, 4.49). ^{1}H-NMR ((D$_6$)DMSO): 8.1 (*s*, H–C(8)); 7.45 (*m*, 2 H *m* to SCH_2); 7.32 (*m*, 2 H *o* to SCH_2); 7.18 (*m*, H *p* to SCH_2); 6.45 (*s*, NH_2); 6.18 (*t*, H–C(1′)); 5.28 (*d*, HO–C(3′)); 5.0 (*t*, HO–C(5′)); 4.55 (*t*, C*H*$_2$C*H*$_2$O); 4.38 (*m*, H–C(3′)); 3.82 (*m*, H–C(4′)); 3.52 (*m*, 2 H–C(5′)); 3.45 (*t*, C*H*$_2$CH$_2$O); 2.6 (*m*, 1 H–C(2′)); 2.2 (*m*, 1 H–C(2′)). Anal. calc. for $C_{18}H_{21}N_5O_4S \cdot 0.5\ H_2O$ (412.5): C 52.42, H 5.38, N 16.98; found: C 52.62, H 5.30, N 16.71.

A soln. of 2′-deoxy-*O*^{6}-[2-(phenylthio)ethyl]guanosine (0.42 g, 1.04 mmol) in CH_2Cl_2 (50 ml) was cooled to 0° and then 3-chloroperbenzoic acid (0.65 g, 2.08 mmol) was added with stirring. After 1 h, the soln. was concentrated to 10 ml and purified by FC (silica gel (40 g), CH_2Cl_2 and CH_2Cl_2/MeOH 98:2): 0.41 g (91%) of **8**. UV (MeOH): 283 (3.93), 272 (4.40), 247 (3.96), 212 (sh, 4.42). ^{1}H-NMR ((D$_6$)DMSO): 8.05 (*s*, H–C(8)); 7.90 (*m*, 2 H *o* to SO_2); 7.7–7.55 (*m*, 2 H *m* + 1 H *p* to SO_2); 6.43 (*s*, NH_2); 6.18 (*t*, H–C(1′)); 5.25 (*d*, OH–C(3′)); 4.95 (*t*, OH–C(5′)); 4.65 (*t*, CH_2CH_2O); 4.35 (*m*, H–C(3′)); 3.95 (*t*, C*H*$_2$CH$_2$O); 3.80 (*m*, H–C(4′)); 2.55 (*m*, 1 H–C(2′)); 2.2 (*m*, 1 H–C(2′)). Anal. calc. for $C_{18}H_{21}N_5O_6S$ (435.5): C 49.95, H 4.86, N 16.08; found: C 49.76, H 4.78, N 15.91.

6. *2′-Deoxy*-N^6-*phthaloyladenosine* (**9**) [17][19]. After codistillation with anh. pyridine, a soln. of 2′-deoxyadenosine (**5**; 5 g, 20 mmol) and chlorotrimethylsilane (6 ml, 50 mmol) in anh. pyridine (80 ml) was stirred for 30 min. Then phthaloyl chloride (4 ml, 28 mmol) was added, and after 2 h stirring at r.t., the mixture was hydrolyzed with ice-water. After 15 min, the mixture was diluted with AcOEt (150 ml) and extracted with $NaHCO_3$ soln. (4 ×), the aq. phases were washed with AcOEt (4 × 50 ml), the combined org. phase was dried

($MgSO_4$) and evaporated and the residue dissolved in CH_2Cl_2 (50 ml). Precipitation from petroleum ether (1 l) gave 7.37 g (93%) of **9**. R_f (toluene/AcOEt/MeOH 5 : 4 : 1) 0.16.

7. *2′-Deoxy-*N^6*-phthaloylcytidine* (**10**). After codistillation with anh. pyridine, a soln. of 2′-deoxycytidine (**6**) (5.8 g, 22 mmol) and chlorotrimethylsilane (7 ml, 55 mmol) in anh. pyridine (80 ml) was stirred for 60 min. Then 4-(dimethylamino)pyridine (DMAP, 50 mg) was added, followed within 1 h (dropwise) by phthaloyl chloride (4.4 ml, 33 mmol) in anh. dioxane (10 ml). After 2 h, the mixture was hydrolyzed with ice-water, and stirring was continued for 15 min. Then the mixture was partitioned between 10% pyridine in CH_2Cl_2 and H_2O, the org. layer extracted with H_2O (100 ml), the combined aq. phase washed twice with 10% pyridine in CH_2Cl_2 (50 ml), the org. phase dried ($MgSO_4$), evaporated, and codistilled with toluene, and the residue washed with CH_2Cl_2: 5.07 g (64%) of **10**. R_f (toluene/AcOEt/MeOH 5 : 4 : 1) 0.10. UV (MeCN): 331 (sh, 3.68), 316 (3.80), 306 (sh, 3.79), 218 (4.51). ^{1}H-NMR ((D$_6$)DMSO): 8.63 (*d*, H–C(6)); 8.01 (*m*, 4 H, phth); 6.69 (*d*, H–C(5)); 6.10 (*t*, H–C(1′)); 5.30 (*d*, HO–C(3′)); 5.11 (*t*, HO–C(5′)); 4.23 (*m*, H–C(3′)); 3.91 (*m*, H–C(4′)); 3.63 (*m*, 2 H–C(5′)); 2.38 (*m*, 1 H–C(2′)); 2.14 (*m*, 1 H–C(2′)). Anal. calc. for $C_{17}H_{15}N_3O_6$ (357.32): C 57.14, H 4.23, N 11.76; found: C 57.21, H 4.45, N 11.66.

8. *2′-Deoxy-*O^6-[2-(4-nitrophenyl)ethyl]-N^2*-phthaloylguanosine* (**11**). A mixture of **7** (833 mg, 2 mmol) and chlorotrimethylsilane (0.63 ml, 5 mmol) was stirred for 30 min in anh. pyridine (15 ml). Then phthaloyl chloride (0.43 ml, 3 mmol) in anh. dioxane (5 ml) was added dropwise within 15 min. After 2 h, the reaction was quenched with ice-water, and stirring was continued for 15 min. Then extraction with CH_2Cl_2 (50 ml) and $NaHCO_3$ soln. (50 ml) and reextraction of the aq. phase with CH_2Cl_2 (50 ml) was followed by drying ($MgSO_4$) and evaporation of the org. layer. Purification by CC (toluene/AcOEt 5 : 4 containing 0–5% of MeOH): 0.56 g (51%) of **7**. R_f ($CHCl_3$/MeOH 9 : 1) 0.42. UV (MeCN): 262 (4.34), 219 (4.68). ^{1}H-NMR ((D$_6$)DMSO): 8.71 (*s*, H–C(8)); 8.14 (*d*, 2 H *o* to NO_2); 8.00 (m, 4 H, phth); 7.61 (*d*, 2 H *m* to NO_2); 6.39 (*t*, H–C(1′)); 5.32 (*d*, OH–C(3′)); 4.91 (*t*, OH–C(5′)); 4.83 (*t*, CH_2CH_2O); 4.40 (*m*, H–C(3′)); 3.85 (*m*, H–C(4′)); 3.58–3.51 (*m*, 2 H–C(5′)); 3.32 (t, CH_2CH_2O); 2.74 (*m*, 1 H–C(2′)); 2.32 (*m*, 1 H–C(2′)). Anal. calc. for $C_{26}H_{22}N_6O_8$ (546.5): C 57.14, H 4.06, N 15.38; found: C 57.27, H 4.37, N 15.11.

9. *2′-Deoxy-*O^6*-[2-(phenylsulfonyl)ethyl]-*N^2*-phthaloylguanosine* (**12**). After co-evaporation with anh. pyridine, **8** (0.87 g, 2 mmol) was stirred with chlorotrimethylsilane (0.56 ml, 4.4 mmol) in anh. MeCN (15 ml) and anh. pyridine (1 ml). After 30 min, phthaloyl chloride (0.29 ml, 2 mmol) was added, and stirring was continued for 35 min. The mixture was evaporated and codistilled with toluene. The residue was dissolved in MeOH (25 ml) and stirred with NH_4F (160 mg, 4.3 mmol) for 3 min. The mixture was extracted twice with $NaHCO_3$ soln. (100 ml) and CH_2Cl_2 (100 ml), the aq. layer washed with CH_2Cl_2 (50 ml), and the combined org. phases dried ($MgSO_4$) and evaporated. Purification by CC (30–75% acetone/petroleum ether): 0.45 g (40%) of **12**. R_f (petroleum ether/acetone 1 : 2) 0.27. UV (MeCN): 294 (sh, 3.50), 259 (4.26), 219 (4.73). ^{1}H-NMR ((D$_6$)DMSO): 8.65 (*s*, H–C(8)); 8.1–7.96 (*m*, 4 H, phth); 7.82 (*m*, 2 H *o* to SO_2); 7.5–7.36 (*m*, 2 H *m* + 1 H *p* to SO_2); 6.37 (*t*, H–C(1′)); 5.32 (*d*, HO–C(3′)); 4.92 (*t*, HO–C(5′)); 4.80 (*t*, CH_2CH_2O); 4.41 (*m*, H–C(3′)); 4.12 (*t*, CH_2CH_2O); 3.86 (*m*, H–C(4′)); 3.65–3.45 (*m*, 2 H–C(5′)); 2.72 (*m*, 1 H–C(2′)); 2.33 (*m*, 1 H–C(2′)). Anal. calc. for $C_{26}H_{23}N_5O_8S \cdot H_2O$ (583.58): C 53.51, H 4.32, N 12.00; found: C 53.95, H 4.45, N 11.72.

10. *2′-Deoxy-5′-*O*-(4,4′-dimethoxytrityl)-*N^6*-phthaloyladenosine* (**13**) [19]. After codistillation with anh. pyridine, **9** (4 g, 10 mmol) was dissolved in anh. pyridine/CH_2Cl_2 1 : 1 (100 ml), and 4,4′-dimethoxytrityl chloride (2.72 g, 8 mmol), DMAP (20 mg, 0.16 mmol) and molecular sieves 4 Å were added. After stirring for 2.5 h, the mixture was partitioned between CH_2Cl_2 (100 ml) and $NaHCO_3$ soln. (100 ml). The org. layer was dried ($MgSO_4$), evaporated, and purified by CC (40–80% AcOEt/toluene): 3.12 g (46%) of **13**.

11. *2′-Deoxy-5′-*O*-(4,4′-dimethoxytrityl)-*N^6*-phthaloylcytidine* (**14**). After codistillation with anh. pyridine, **10** (3 g, 8.4 mmol) was dissolved in anh. pyridine (60 ml), and 4,4′-dimethoxytrityl chloride (3.13 g, 9.23 mmol), DMAP (50 mg, 0.41 mmol) and molecular sieves 4 Å were added. After stirring for 2 h, workup as described for **13**, and CC (33–75% AcOEt/toluene) gave 3.35 g (61%) of **10**. R_f 0.39 (toluene/AcOEt/MeOH 5 : 4 : 1) 0.39. UV (MeCN): 331 (sh, 3.68), 317 (3.84), 306 (sh, 3.83), 283 (3.72), 275 (sh, 3.69), 232 (4.65). ^{1}H-NMR ((D$_6$)DMSO): 8.42 (*d*, H–C(6)); 7.99 (*m*, 4 H, phth); 7.30 (*m*, 9 H, $(MeO)_2Tr$); 6.92 (*m*, 2 H *o* to MeO); 6.53 (*d*, H–C(5)); 6.11 (*t*, H–C(1′)); 5.40 (*d*, HO–C(3′)); 4.31 (*m*, H–C(3′)); 4.01 (*m*, H–C(4′)); 3.73 (*s*, MeO); 3.33 (*m*, 2 H–C(5′)); 2.40 (*m*, 1 H–C(2′)); 2.20 (*m*, 1 H–C(2′)). Anal. calc. for $C_{38}H_{33}N_3O_8$ (659.7): C 69.19, H 5.04, N 6.37; found: C 69.29, H 5.35, N 6.17.

12. *2′-Deoxy-5′-*O*-(4,4′-dimethoxytrityl)-*O^6*-[2-(4-nitrophenyl)ethyl]-*N^2*-phthaloylguanosine* (**15**). After codistillation with anh. pyridine, **11** (0.99 g, 1.81 mmol) was dissolved in anh. pyridine (25 ml), and then 4,4′-dimethoxytrityl chloride (675 mg, 2 mmol) was added. After stirring for 3 h, workup as described for **13** and CC (20–66% AcOEt/toluene) gave 1.0 g (67%) of **15**. R_f (toluene/AcOEt 1 : 6) 0.57. UV (MeCN): 262 (4.38), 218 (4.78). ^{1}H-NMR ((D$_6$)DMSO): 8.59 (*s*, H–C(8)); 8.13 (*d*, 2 H *o* to NO_2); 8.00 (*m*, 4 H, phth); 7.60 (*d*, 2 H *m*

to NO_2); 7.26–7.10 (*m*, 9 H, $(MeO)_2$*Tr*); 6.68 (*m*, 2 H *o* to MeO); 6.43 (*m*, H–C(1′)); 5.36 (*d*, HO–C(3′)); 4.81 (*t*, CH_2CH_2O); 4.46 (*m*, H–C(3′)); 3.95 (*m*, H–C(4′)); 3.67 (*s*, 2 MeO); 3.32 (*t*, CH_2CH_2O); 3.25–3.05 (*m*, 2 H–C(5′)); 2.85 (*m*, 1 H–C(2′)); 2.38 (*m*, 1 H–C(2′)). Anal. calc. for $C_{47}H_{40}N_6O_{10}$ (848.87): C 66.50, H 4.75, N 9.90; found: C 66.65, H 4.90, N 9.52.

13. *2′-Deoxy-5′*-O-*(4,4′-dimethoxytrityl)*-O^6-*[2-(phenylsulfonyl)ethyl]*-N^2-*phthaloylguanosine* (**16**). After codistillation with anh. pyridine **12** (0.56 g, 1 mmol) was dissolved in anh. pyridine (20 ml) and then 4,4′-dimethoxytrityl chloride (373 mg, 1.1 mmol) added. After stirring for 16 h, workup as described for **13** and CC (30–80% AcOEt/toluene) gave 0.57 g (66%) of **16**. R_f (toluene/AcOEt/MeOH 5:4:1) 0.39. UV (MeCN): 261 (4.26), 217 (4.80). ^{1}H-NMR ((D$_6$)DMSO): 8.55 (*s*, H–C(8)); 8.07–8.01 (*m*, 4 H, phth); 7.82 (*m*, 2 H *o* to SO_2); 7.35 (*m*, 2 H *m* + 1 H *p* to SO_2); 7.27–7.05 (*m*, 9 H, $(MeO)_2$*Tr*); 6.70 (*m*, 4 H *o* to MeO); 6.41 (*m*, H–C(1′)); 5.37 (*d*, HO–C(3′)); 4.79 (*t*, CH_2CH_2O); 4.46 (*m*, H–C(3′)); 4.10 (*t*, CH_2CH_2O); 3.96 (*m*, H–C(4′)); 3.68 (*s*, 2 MeO); 3.25–3.09 (*m*, 2 H–C(5′)); 2.81 (*m*, 1 H–C(2′)); 2.35 (*m*, 1 H–C(2′)). Anal. calc. for $C_{47}H_{41}N_5O_{10}S$ (867.93): C 65.04, H 4.76, N 8.07; found: C 64.97, H 4.82, N 7.88.

14. *2′-Deoxy-5′*-O-*(4,4′-dimethoxytrityl)*-N^6-*phthaloyladenosine 3′-(2-Cyanoethyl Diisopropylphosphoramidite)* (**17**). To a soln. of **13** (684 mg, 1 mmol) in anh. MeCN (5 ml), 2-cyanoethyl tetraisopropylphosphorodiamidite (331 mg, 1.1 mmol) and anh. 0.5M pyridine hydrochloride (1 ml, 0.5 mmol) in MeCN were added. After strirring for 1.5 h, the mixture was partitioned between CH_2Cl_2 (100 ml) and $NaHCO_3$ soln. (100 ml) and the org. layer dried ($MgSO_4$) and evaporated. Purification by CC (30–50% AcOEt/toluene): 0.64 g (72%) of **17**. R_f (toluene/AcOEt 1:1) 0.33, 0.42. ^{1}H-NMR ((D$_6$)DMSO): 8.92 (*s*, H–C(2)); 8.82 (*s*, H–C(8)); 8.09 (*m*, 4 H, phth); 7.33 (*m*, 2 H, $(MeO)_2$*Tr*); 7.21 (*m*, 7 H, $(MeO)_2$*Tr*), 6.82 (*m*, 4 H *o* to MeO); 6.55 (*m*, H–C(1′)); 4.82 (*m*, H–C(3′)); 4.27 (*m*, H–C(4′)); 3.65 (*m*, CH_2CH_2O, MeO, 2 H–C(5′)); 3.20 (*m*, Me_2CH, 2 H–C(2′)); 1.10 (*m*, 2 Me_2CH). ^{31}P-NMR ((D$_6$)DMSO): 148.51, 147.99 (2 *s*). Anal. calc. for $C_{48}H_{50}N_7O_8P$ (883.95): C 65.22, H 5.70, N 11.09; found: C 64.94, H 5.78, N 10.87.

15. *2′-Deoxy-5′*-O-*(4,4′dimethoxytrityl)*-N^6-*phthaloylcytidine 3′-(2-Cyanoethyl Diisopropylphosphoramidite)* (**18**). To a soln. of **14** (660 mg, 1 mmol) in anh. MeCN (7 ml), 2-cyanoethyl tetraisopropylphosphorodiamidite (362 mg, 1.2 mmol) and 1*H*-tetrazole (35 mg, 0.5 mmol) were added. After stirring for 1.5 h, the mixture was partitioned between CH_2Cl_2 (100 ml) and $NaHCO_3$ soln. (60 ml). The org. layer was dried ($MgSO_4$) and evaporated. Purification by CC (40–50% AcOEt/toluene) gave 0.62 g (72%) of **18**. R_f (toluene/AcOEt 1:6) 0.47, 0.51. ^{1}H-NMR ((D$_6$)DMSO): 8.45 (*m*, H–C(6)); 7.99 (*m*, 4 H, phth); 7.30 (*m*, 9 H, $(MeO)_2$*Tr*); 6.91 (*m*, 4 H *o* to MeO); 6.55 (*d*, H–C(5)); 6.15 (*m*, H–C(1′)); 4.52 (*m*, H–C(3′)); 4.17 (*m*, H–C(4′)); 3.72 (*s*, 2 MeO); 3.60 (*m*, 2, H–C(5′)), CH_2CH_2O, Me_2CH)); 2.70 (2*t*, CH_2CH_2O); 2.60 (*m*, 1 H–C(2′)); 2.40 (*m*, 1 H–C(2′)); 1.19–0.97 (*m*, 2 Me_2CH). ^{31}P-NMR ((D$_6$)DMSO): 148.63, 148.38 (2 *s*). Anal. calc. for $C_{47}H_{50}N_5O_9P$ (859.91): C 65.65, H 5.86, N 8.14; found: C 64.16, H 6.04, N 8.23.

16. *2′-Deoxy-5′*-O-*(4,4′-dimethoxytrityl)*-O^6-*[2-(4-nitrophenyl)ethyl]*-N^2-*phthaloylguanosine 3′-(2-Cyanoethyl Diisopropylphosphoramidite)* (**19**). To a soln. of **15** (0.68 g, 0.8 mmol) in anh. MeCN (8 ml), 2-cyanoethyl tetraisopropylphosphorodiamidite (0.29 g, 1 mmol) and anh. 0.5M pyridine hydrochloride (0.8 ml, 0.4 mmol) in MeCN were added. After 3 h, additional 2-cyanoethyl tetraisopropylphosphorodiamidite (0.2 g, 0.66 mmol) and anh. 0.5M pyridine hydrochloride (0.4 ml, 0.2 mmol) in MeCN were added, and stirring was continued for 3.5 h. Then the mixture was extracted with CH_2Cl_2 (50 ml) and $NaHCO_3$ soln. (50 ml) and the org. layer dried ($MgSO_4$) and evaporated. Purification by CC (33–50% AcOEt/toluene): 0.73 g (87%) of **19**. R_f (toluene/AcOEt 1:6) 0.74, 0.82. ^{1}H-NMR ((D$_6$)DMSO): 8.63 (*s*, H–C(8)); 8.13 (*s*, 2 H *o* to NO_2); 8.00 (*m*, 4 H, phth); 7.61 (*s*, 2 H *m* to NO_2); 7.24–7.08 (*m*, 9 H, $(MeO)_2$*Tr*); 6.71–6.63 (*m*, 4 H, $(MeO)_2$*Tr*); 6.45 (*m*, H–C(1′)); 4.80 (*m*, H–C(3′); CH_2CH_2O); 4.10 (*m*, H–C(4′)); 3.66, 3.67 (2*s*, 2 MeO); 3.59–2.95 (*m*, CH_2CH_2O, 2 H–C(5′), 2 Me_2CH, 2 CH_2CH_2O); 2.72 (*m*, 1 H–C(2′)); 2.61 (*m*, 1 H–C(2′)); 1.20–0.90 (*m*, 2 Me_2CH). ^{31}P-NMR ((D$_6$)DMSO): 148.55, 148.05 (2*s*). Anal. calc. for $C_{56}H_{57}N_8O_{11}P$ (1049.09): C 64.11, H 5.48, N 10.68; found: C 63.92, H 5.47, N 10.12.

17. *2′-Deoxy-5′*-O-*(4,4′-dimethoxytrityl)*-O^6-*[2-(phenylsulfonyl)ethyl]*-N^2-*phthaloylguanosine 3′-(2-Cyanoethyl Diisopropylphosphoramidite)* (**20**). As described for **19**, with **16** (0.47 g, 0.54 mmol), MeCN (8 ml), 2-cyanoethyl tetraisopropylphosphorodiamidite (0.2 g, 0.65 mmol), 0.5M pyridine hydrochloride (0.54 ml, 0.27 mmol) in MeCN, then 2-cyanoethyl tetraisopropylphosphoradiamidite (0.15 g, 0.5 mmol), and 0.5M pyridine hydrochloride (0.27 ml, 0.13 mmol) in MeCN. CC (33–50% AcOEt/toluene) gave 0.42 g (73%) of **20**. R_f (petroleum ether/AcOEt/Et_3N 1:9:1) 0.49, 0.60. ^{1}H-NMR ((D$_6$)DMSO): 8.57 (2 *s*, H–C(8)); 8.00 (m, 4 H, phth); 7.81 (*m*, 2 H *o* to SO_2); 7.40–7.31 (*m*, 2 H *m* + 1 H *p* to SO_2); 7.25–7.08 (*m*, 9 H, $(MeO)_2$*Tr*); 6.73–6.39 (*m*, 4 H, $(MeO)_2$*Tr*); 6.43 (*m*, H–C(1′)); 4.78 (*m*, H–C(3′), CH_2CH_2O); 4.10 (*m*, SCH_2CH_2O, H–C(4′)); 3.66, 3.67 (2*s*, 2 MeO); 3.60–3.30 (*m*, CH_2CH_2O, 2 Me_2CH); 3.23 (*m*, 2 H–C(5′)); 3.00 (*m*, 1 H–C(2′)); 2.62,

2.73 (2 *t*, NCCH_2CH_2O); 2.50 (*m*, 1 H–C(2′)); 1.19–0.86 (*m*, 2 *Me*$_2$CH). ^{31}P-NMR ((D$_6$)DMSO): 148.54, 148.06 (2*s*). Anal. calc. for $C_{56}H_{58}N_7O_{11}PS$ (1068.15): C 62.97, H 5.47, N 9.18; found: C 62.25, H 5.65, N 8.82.

18. *2′-Deoxy-5′-*O*-(4,4′-dimethoxytrityl)-*N^6*-phthaloyladenosine 3′-(Hydrogen Butanedioate)* (**23**). To a soln. of **13** (342 mg, 0.5 mmol) in anh. CH_2Cl_2 (10 ml), DMAP (79 mg, 0.65 mmol) and succinic anhydride (= 2,5-dihydrofuran-2,5-dione; 0.1 g, 1 mmol) were added and stirred for 17 h. The mixture was extracted with CH_2Cl_2 (50 ml) and $NaHCO_3$ soln. (30 ml) and the org. layer washed with 10% aq. citric acid, dried ($MgSO_4$), and evaporated: 0.39 g (98%) of **21**. R_f (toluene/AcOEt/MeOH 5:4:1) 0.17. UV (MeCN): 300 (sh, 3.60), 271 (4.17), 220 (sh, 4.72). ^{1}H-NMR ((D$_6$)DMSO): 12.30 (*s*, COOH); 8.91 (*s*, H–C(2)); 8.82 (*s*, H–C(8)); 8.06 (*m*, 4 H, phth); 7.17 (*m*, (MeO)$_2$*Tr*); 6.83 (*m*, 4 H *o* to MeO); 6.56 (*t*, H–C(1′)); 5.45 (*m*, H–C(3′)); 4.25 (*m*, H–C(4′)); 3.70 (*s*, 2 MeO); 3.33 (*m*, 2 H–C(5′)); 2.58 (*m*, 2 H–C(2′), CH_2CH_2). Anal. calc. for $C_{43}H_{37}N_5O_{10}\cdot H_2O$ (801.82): C 64.41, H 4.90, N 8.73; found: C 64.47, H 4.98, N 8.74.

19. *2′-Deoxy-5′-*O*-(4,4′-dimethoxytrityl)-*O^6*-[2-(4-nitrophenyl)ethyl]-*N^2*-phthaloylguanosine 3′-(Hydrogen Butanedioate)* (**24**). To a soln. of **15** (212 mg, 0.25 mmol) in anh. CH_2Cl_2 (5 ml), DMAP (40 mg, 0.32 mmol) and succinic anhydride (50 mg, 0.5 mmol) were added. After stirring for 24 h, the mixture was partitioned between CH_2Cl_2 (60 ml) and $NaHCO_3$ soln. (60 ml). The org. layer was washed with 10% aq. citric acid (40 ml), dried ($MgSO_4$), and evaporated: 0.21 g (88%) of **24**. UV (MeCN): 262 (4.37), 216 (4.78). ^{1}H-NMR ((D$_6$)DMSO): 12.27 (*s*, COOH); 8.61 (*s*, H–C(8)); 8.14 (*d*, 2 H *o* to NO_2); 7.80–7.96 (*m*, 4 H, phth); 7.61 (*d*, 2 H *m* to NO_2); 7.27–7.05 (*m*, 9 H, (MeO)$_2$*Tr*); 6.71–6.65 (*m*, *o* to MeO); 6.44 (*t*, H–C(1′)); 5.36 (*m*, H–C(3′)); 4.82 (*t*, CH_2CH_2O); 4.14 (*m*, H–C(4′)); 3.67 (*s*, 2 MeO); 3.42–3.16 (*m*, 2 H–C(5′)), CH_2CH_2O); 2.60–2.45 (*m*, 2 H–C(2′), CH_2CH_2).

20. *LCAMA-CPG Support* (**21**). Dry glyceryl-CPG material (1 g, 1000 Å or 1400 Å) was shaken for 6 h with 1,1′-carbonylbis[1*H*-diimidazole] (1 g, 12.4 mmol) in anh. CH_2Cl_2 (20 ml). The supernatant was decanted, and the activated support was washed 3 times with anh. CH_2Cl_2 (15 ml). Then the support was suspended in anh. CH_2Cl_2 (15 ml), *N,N′*-dimethylhexane-1,6-diamine (1 ml, 5.8 mmol) added, and the mixture shaken for 3 h. The supernatant was decanted and the LCAMA-CPG washed subsequently with pyridine, DMF, MeOH, acetone, and Et_2O.

21. *LCAMA-CPG-Derived Succinamic Acid* **22**. LCAMA-CPG (0.5 g, 1400 Å) in pyridine (4 ml)was shaken for 24 h with DMAP (12 mg, 0.1 mmol) and succinic anhydride (400 mg). The support was filtered off and washed with pyridine and CH_2Cl_2.

22. *Loading of LCAMA-CPG: Supports* **25** *and* **26**. A mixture of LCAMA-CPG (400 mg), TOTU (7 mg, 21 μmol), 4-methylmorpholine (3 μl, 27 μmol), und succinate **23** or **24** (23 μmol), in anh. MeCN (5 ml) was shaken for 2 h. Then the support was filtered and subsequently washed with DMF, MeOH, acetone and Et_2O. Afterwards the support was capped by shaking in Ac_2O/pyridine/1-methyl-1*H*-imidazole 1:5:1 (3.5 ml) for 30 min. The support was filtered and subsequently washed with DMF, MeOH, acetone, and Et_2O. Loadings obtained: **25** (13 μmol/g) and **26** (11 μmol/g).

23. *Loading of LCAMA-CPG-Derived Succinamic Acid: Supports* **25** and **27**. A suspension of **22** (150 mg, 1400 Å), DMAP (1.8 mg, 0.015 mmol), 1-[3-(dimethylamino)propyl]-3-ethylcarbodiimide hydrochloride (29 mg, 0.15 mmol), and 5′-*O*-(MeO)$_2$Tr-protected nucleoside **13** or **14** (0.015 mmol) in anh. pyridine (3 ml) and Et_3N (12 μl) was shaken for 24 h. Then pentachlorophenol (20 mg, 0.07 mmol) was added, and shaking was continued for 23 h. Afterwards, piperidine (0.75 ml) was added and after 5 min, the support was filtered immediately and washed with CH_2Cl_2 and Et_2O. The support was then capped by shaking in Ac_2O/pyridine/1-methyl-1*H*-imidazole 1:5:1 (3.5 ml) for 1.5 h, filtered, and subsequently washed with DMF, MeOH, acetone, and Et_2O. Loadings obtained: **25** (2 μmol/g) and **27** (3 μmol/g).

REFERENCES

[1] H. Cramer, W. Pfleiderer, *Helv. Chim. Acta* **1999**, *82*, in press.
[2] L. J. McBride, M. H. Caruthers, *Tetrahedron Lett.* **1983**, *24*, 245.
[3] H. G. Khorana, *Bioorg. Chem.* **1978**, *1*, 351.
[4] F. Benseler, L. W. McLaughlin, *Synth. Commun.* **1986**, 45.
[5] J. C. Schulhof, D. Molko, R. Teoule, *Nucleic Acids Res.* **1987**, *15*, 3.
[6] H. Büchi, H. G. Khorana, *J. Mol. Biol.* **1972**, *72*, 251.
[7] F. Himmelsbach, B. Schulz, T. Trichtinger, R. Charubala, W. Pfleiderer, *Tetrahedron* **1984**, *40,* 59.
[8] K. P. Stengele, W. Pfleiderer, *Nucleic Acids Symp. Ser.* **1989**, *21*, 101.
[9] K. P. Stengele, W. Pfleiderer, *Tetrahedron Lett.* **1990,** *31*, 2549.

[10] Y. Hayakawa, S. Wakabayashi, H. Kato, R. Noyori, *J. Am. Chem. Soc.* **1990**, *112*, 1691.
[11] K. B. Mullis, F. A. Fallona, *Methods Enzymol*. **1987**, *155*, 335.
[12] N. Hauser, J. D. Hoheisel, *Yeast* **1999**, submitted.
[13] G. H. McGall, A. D. Barone, M. Diggelmann, S. P. A. Fodor, E. Gentalen, N. Ngo, *J. Org. Chem.* **1997**, *119*, 5081.
[14] M. Beier, Ph.D. Thesis, Konstanz University, 1996.
[15] M. Beier, W. Pfleiderer, in preparation.
[16] S. Waldvogel, W. Pfleiderer, *Helv. Chim. Acta* **1998**, *81*, 46.
[17] M. Beier, W. Pfleiderer, *Nucleosides Nucleotides*, **1997**, *16*, 1621.
[18] A. Kume, M. Sekine, T. Hata, *Tetrahedron Lett*. **1982**, *23*, 4365.
[19] A. Kume, K. Koura, H. Tanimura, T. Kamimura, J. Matsuzaki, M. Sekine, T. Hata, *Nucleic Acids Symp. Ser*. **1982**, *11*, 25.
[20] G. S. Ti, B. L. Gaffney, R. Jones, *J. Am. Chem. Soc*. **1982**, *104*, 1316.
[21] W. T. Markiewicz, *J. Chem. Res.* **1979**, 24.
[22] H. Bazin, J. Heikkilä, J. Chattopadhyaya, *Acta Chem. Scand., Ser. B* **1985**, *39*, 391.
[23] J. Matsuzaki, K. Khono, S. Tahara, M. Sekine, T. Hata, *Bull. Chem. Soc. Jpn.* **1987**, *60*, 1407.
[24] R. T. Pon, N. Usman, M. J. Damha, K. K. Ogilvie, *Nucleic Acids Res.* **1986**, *14*, 6453.
[25] A. P. Guzaev, *Nucleosides Nucleotides* **1992**, *11*, 1463.
[26] H. Schaller, G. Weimann, B. Lerch, H. G. Khorana, *J. Am. Chem. Soc.* **1963**, *85*, 3821.
[27] I. Kieper, T. Schmidt, B. Fera, H. Rüterjans, *Nucleosides Nucleotides* **1988**, *7*, 821.
[28] B. L. Gaffney, R. A. Jones, *Tetrahedron Lett*. **1982**, *23*, 2257.
[29] R. T. Pon, in 'Protocols for Oligonucleotides and Analogs', Humana Press, 1993, 465.
[30] J. Weiler, W. Pfleiderer, *Nucleosides Nucleotides* **1995**, *14*, 917.
[31] F. Bergmann, W. Pfleiderer, *Helv. Chim. Acta* **1994**, *77*, 203.

Received February 3, 1999