Nucleotides

Part LXVII[1])

The 2-Cyanoethyl and (2-Cyanoethoxy)carbonyl Group for Base Protection in Nucleoside and Nucleotide Chemistry

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The amino functions of the common 2′-deoxyribo- and ribonucleosides were blocked by the (2-cyanoethoxy)carbonyl group on treatment with 2-cyanoethyl carbonochloridate (**5**) or 1-[(2-cyanoethoxy)carbonyl]-3-methyl-1*H*-imidazolium chloride (**6**) leading to **7**, **18**, **8**, **19**, **9**, and **20**. In 2′-deoxyguanosine, the amide group was additionally blocked at the O^6 position by the 2-cyanoethyl (→ **27**) and 2-(4-nitrophenyl)ethyl group (→ **31**, **32**). Comparative kinetic studies regarding the cleavage of the ce/ceoc and npe/npeoc group by β-elimination revealed valuable information about the ease and sequential deprotection of the various blocking groups at different sites of the nucleobases. Besides the 5′-*O*-(dimethoxytrityl)-protected 3′-(2-cyanoethyl diisopropylphosphoramidites) **38** and **39** of N^4-[(2-cyanoethoxy)carbonyl]-2′-deoxycytidine and N^6-[(2-cyanoethoxy)carbonyl]-2′-deoxyadenosine, respectively, the N^2-[(2-cyanoethoxy)carbonyl]-2′-deoxy-O^6-[2-(4-nitrophenyl)ethyl]guanosine analog **40** is recommended as building block for oligo-2′-deoxyribonucleotide synthesis.

Introduction. – The development of the 2-(4-nitrophenyl)ethyl (npe) and [2-(4-nitrophenyl)ethoxy]carbonyl (npeoc) groups as versatile protective groups for nucleobases [2], phosphate [3][4], and phosphite functions [5][6] broadened the strategy of nucleoside and nucleotide protection in oligonucleotide synthesis universally. The great advantange of these groups is their high stability under acidic and mild basic hydrolytic conditions as, for example, in the presence of ammonia and amines in MeOH, dioxane, or H_2O, whereas their cleavage can easily and quantitatively be achieved by 1,8-diazabicyclo[5.4.0]undecene (DBU) in aprotic solvents by a β-elimination process.

The 2-cyanoethyl (ce) group [7][8] is the most common blocking group for the phosphate and phosphite moiety of nucleotides, but strangely enough, the ce and its corresponding (2-cyanoethoxy)carbonyl (ceoc) group have not been applied for base protection in analogy to the npe/npeoc couple. Several years ago, we [9][10] developed the ce/ceoc strategy as an alternative approach for the synthesis of special oligonucleotides. Details about the nucleobase protection by the ce and ceoc group will now be reported since *N*-{[(2-cyanoethoxy)carbonyl]oxy}succinimide has recently been described as a new reagent for protection of amino groups in oligonucleotides [11]. We protected the amino group in cytidine, adenosine, and guanosine as well as in the corresponding 2′-deoxynucleosides with the ceoc group and utilized the ce group

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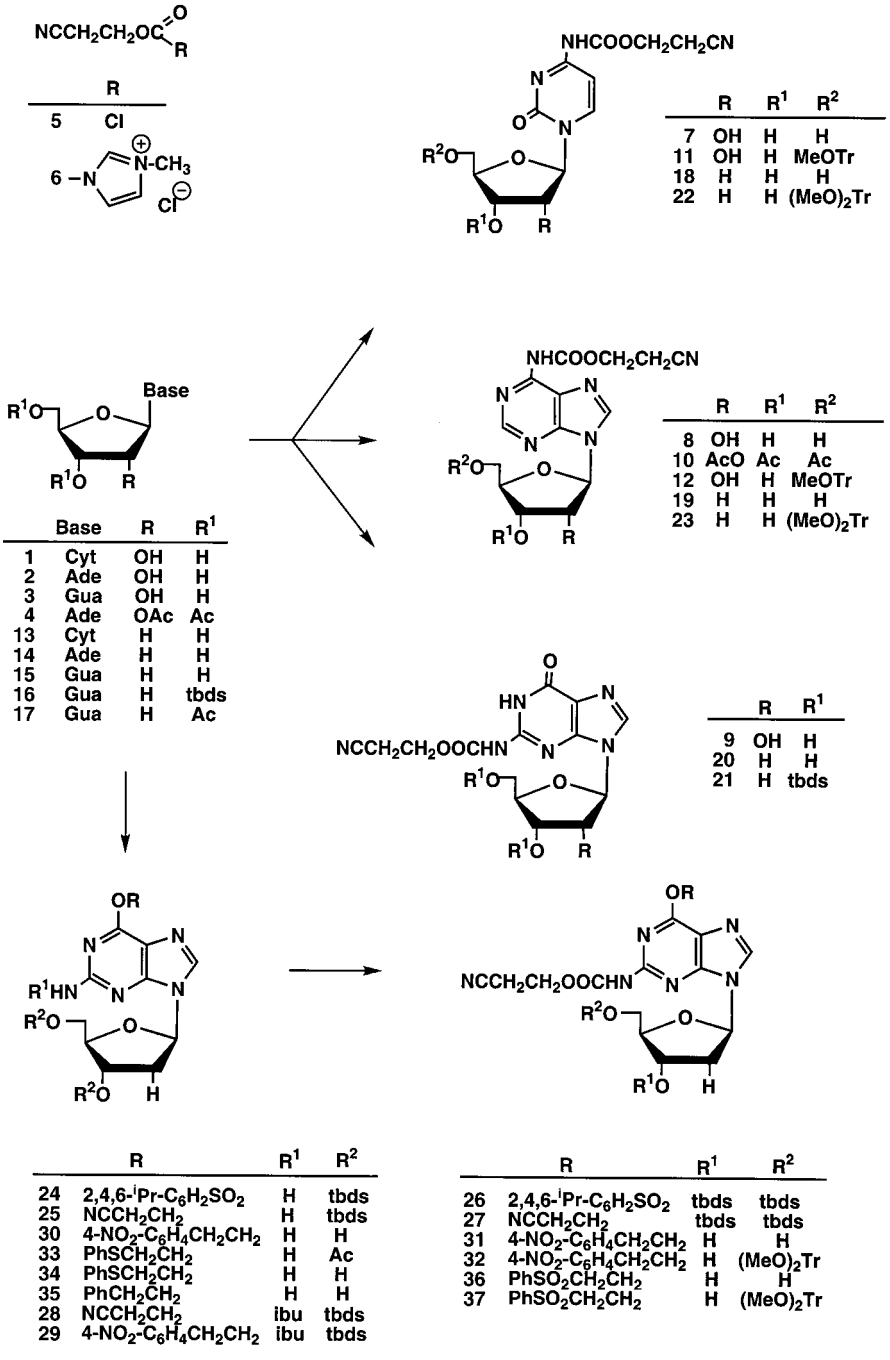
for blocking of the O^6-function in 2′-deoxyguanosine in order to study its stability regarding DBU cleavage in comparison to the npe and 2-(phenylsulfonyl)ethyl (pse) group.

Synthesis. – The (2-cyanoethoxy)carbonylation reactions were achieved either by 2-cyanoethyl carbonochloridate (**5**), which was prepared by an improved synthesis according to *Kondratenko* and *Khaskin* [12] from 3-hydroxypropanenitrile and phosgene, or by 1-[(2-cyanoethoxy)carbonyl]-3-methyl-1*H*-imidazolium chloride (**6**), resulting from **5** and 1-methyl-1*H*-imidazole, in 94% yield. Cytidine (**1**), adenosine (**2**), and guanosine (**3**) were acylated by **5** after transient protection [13] at the sugar moiety by trimethylsilylation to give, after workup, N^4-[2-(cyanoethoxy)carbonyl]cytidine (**7**) in 68%, N^6-[(2-cyanoethoxy)carbonyl]adenosine (**8**) in 80%, and N^2-[(2-cyanoethoxy)-carbonyl]guanosine (**9**) in 54% yield (*Scheme*). Also 2′,3′,5′-tri-*O*-acetyladenosine (**4**) was treated with a mixture of **5** and 1-methyl-1*H*-imidazole in MeCN to give 2′,3′,5′-tri-*O*-acetyl-N^6-[(2-cyanoethoxy)carbonyl]adenosine (**10**) in 90% isolated yield. Monomethoxytritylation of **7** and **8** worked also very well yielding the corresponding 5′-*O*-(monomethoxytrityl) derivatives **11** and **12**.

In the 2′-deoxyribonucleoside series, transient protection of the sugar moiety of 2′-deoxycytidine (**13**) was not necessary due to the high nucleophilicity of its amino group, forming, under otherwise analogous conditions, N^4-[(2-cyanoethoxy)carbonyl]-2′-deoxycytidine (**18**) in a highly selective reaction in 79% yield. The 2′-deoxyadenosine (**14**) reacted, after transient silylation, to N^6-[(2-cyanoethoxy)carbonyl]-2′-deoxyadenosine (**19**), whereas 2′-deoxyguanosine (**15**) expectedly caused problems for solubility reasons, to give only 19% of N^2-[2-(cyanoethoxy)carbonyl]-2′-deoxyguanosine (**20**), and starting from 3′,5′-bis-O-[(*tert*-butyl)dimethylsilyl)]-2′-deoxyguanosine (**16**), to form **21** in 22% yield. Monotritylation of **18** and **19** gave the 5′-protected derivatives **22** and **23**, respectively.

The direct introduction of the 2-cyanoethyl group onto O^6 of **15** by a *Mitsunobu* reaction starting from **16** or from its N^2-isobutyryl- and N^2-(dimethoxytrityl) derivative was unsuccessful because elimination of acrylonitrile from the intermediary (2-cyanoethoxy)triphenylphosphonium ion is much faster than substitution at the O^6-position by alkylation. Also an attempted alkylation with 3-iodopropanenitrile did not proceed in the anticipated manner. Finally, the method of *Jones* and co-workers [14][15] was applied, converting **16** by 2,4,6-triisopropylbenzenesulfonyl chloride in the presence of Et_3N and *N*,*N*-dimethylpyridin-4-amine (DMAP) to 3′,5′-bis-*O*-[(*tert*-butyl)dimethylsilyl]-2′-deoxy-O^6-[(2,4,6-triisopropylphenyl)sulfonyl]guanosine (**24**), which reacted with 3-hydroxypropanenitrile in presence of Et_3N in a DABCO-catalyzed reaction to 3′,5′-bis-*O*-[(*tert*-butyl)dimethylsilyl]-O^6-(2-cyanoethyl)-2′-deoxyguanosine (**25**) in 63% yield (DABCO = 1,4-diazabicyclo[2.2.2]octane). Transformation of **25** into its N^2-[(2-cyanoethoxy)carbonyl] derivative **27** proceeded well in a stepwise reaction first with chlorotrimethylsilane and then with 2-cyanoethyl carbonochloridate (**5**) in pyridine to give **27** in 92% yield. In a second, less effective two-step approach, **27** was prepared from **24** by (2-cyanoethoxy)carbonylation to give 3′,5′-bis-*O*-[(*tert*-butyl)dimethylsilyl]-N^2-[(2-cyanethoxy)carbonyl]-2′-deoxy-O^6-[(2,4,6-triisopropylbenzene)sulfonyl]guanosine (**26**) in 17% yield, which was first treated with Me_3N and then with 2-hydroxypropanenitrile and DBU for 10 min, yielding 53% of **27**.

Scheme



	R	R¹	R²
7	OH	H	H
11	OH	H	MeOTr
18	H	H	H
22	H	H	$(MeO)_2Tr$

	Base	R	R¹
1	Cyt	OH	H
2	Ade	OH	H
3	Gua	OH	H
4	Ade	OAc	Ac
13	Cyt	H	H
14	Ade	H	H
15	Gua	H	H
16	Gua	H	tbds
17	Gua	H	Ac

	R	R¹	R²
8	OH	H	H
10	AcO	Ac	Ac
12	OH	H	MeOTr
19	H	H	H
23	H	H	$(MeO)_2Tr$

	R	R¹
9	OH	H
20	H	H
21	H	tbds

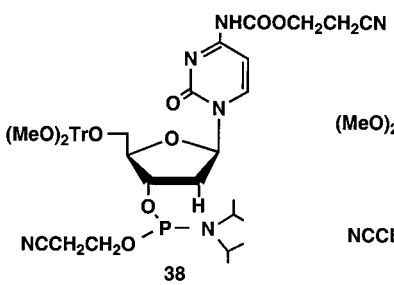
	R	R¹	R²
24	2,4,6-^{i}Pr-$C_6H_2SO_2$	H	tbds
25	$NCCH_2CH_2$	H	tbds
30	4-NO_2-$C_6H_4CH_2CH_2$	H	H
33	$PhSCH_2CH_2$	H	Ac
34	$PhSCH_2CH_2$	H	H
35	$PhCH_2CH_2$	H	H
28	$NCCH_2CH_2$	ibu	tbds
29	4-NO_2-$C_6H_4CH_2CH_2$	ibu	tbds

	R	R¹	R²
26	2,4,6-^{i}Pr-$C_6H_2SO_2$	tbds	tbds
27	$NCCH_2CH_2$	tbds	tbds
31	4-NO_2-$C_6H_4CH_2CH_2$	H	H
32	4-NO_2-$C_6H_4CH_2CH_2$	H	$(MeO)_2Tr$
36	$PhSO_2CH_2CH_2$	H	H
37	$PhSO_2CH_2CH_2$	H	$(MeO)_2Tr$

The known [14] O^6-(2-cyanoethyl)-protected 2′-deoxyguanosine derivative **28** was prepared by a slightly modified procedure in an improved yield of 79% (see *Exper. Part*), whereas the corresponding O^6-[2-(4-nitrophenyl)ethyl] derivative **29** was obtained from the N^2-isobutyryl derivative of **16** with PPh_3 and 2-(4-nitrophenyl)ethanol in the presence of ethyl azodicarboxylate in 51% yield.

The combination of the npe and ceoc group was achieved by transient protection of 2′-deoxy-O^6-[2-(4-nitrophenyl)ethyl]guanosine (**30**) [2] by trimethylsilylation and subsequent reaction with **5** in pyridine/CH_2Cl_2, yielding 75% of N^2-[(2-cyanoethoxy)carbonyl]-2′-deoxy-O^6-[2-(4-nitrophenyl)ethyl]guanosine (**31**). Its 5′-*O*-dimethoxytrityl derivative **32** resulted from treatment with dimethoxytrityl chloride in pyridine/CH_2Cl_2 in 81% yield. A third blocking-group strategy was directed towards the synthesis of N^2-[(2-cyanoethoxy)carbonyl)]-2′-deoxy-O^6-[2-(phenylsulfonyl)ethyl]-guanosine (**36**) starting from 3′,5′-di-*O*-acetyl-2′-deoxy-O^6-[2-(phenylthio)ethyl]guanosine (**33**) [16] prepared in a *Mitsunobu* reaction from **17** with 2-(phenylthio)ethanol. Deacetylation by ammonia converted **33** into 2′-deoxy-O^6-[2-(phenylthio)ethyl]guanosine (**34**), which reacted at the thioether function by 3-chloroperbenzoic acid oxidation to the corresponding sulfone **35**, in 86% yield. Finally, **35** was acylated by 2-cyanoethyl carbonochloridate (**5**) in a similar manner as **30** under transient trimethylsilyl protection to give 85% of **36**. The dimethoxytritylation of the latter worked also well under standard conditions, forming N^2-[(2-cyanoethoxy)carbonyl]-2′-deoxy-5′-*O*-(dimethoxytrityl)-O^6-[2-(phenylsulfonyl)ethyl]guanosine (**37**) in 73% isolated yield.

New monomeric building blocks for oligo-2′-deoxyribonucleotide synthesis *via* the phosphoramidite approach were prepared from the 2′-deoxy-5′-*O*-(dimethoxytrityl)ribonucleosides **22**, **23**, and **32** by 2-cyanoethyl tetraisopropylphosphorodiamidite and 1*H*-tetrazole, leading in very good yields to the corresponding 3′-(2-cyanoethyl diisopropylphosphoramidites) **38**–**40**.



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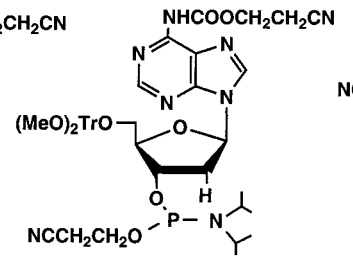

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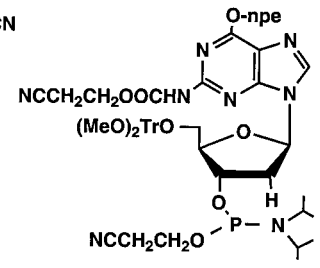

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Cleavage Studies. – In oligonucleotide synthesis, the final cleavage of the various protecting groups is of crucial importance to get clean products in reasonable times. To get a more quantitative understanding of the cleavage rates of the npe/npeoc *vs.* the ce/ceoc blocking groups at the various sites of the common nucleosides, we started some comparative studies, focusing on the β-elimination reactions. The cleavages were done at room temperature with 0.5M DBU in MeCN or for solubility reasons in MeCN/DMF 1:1 and with 15 mol-equiv. of the appropriate nucleoside. Expectedly, there were small differences in the cleavage rates comparing analogous derivatives of the 2′-deoxyribo- and the ribo-series, as *e.g.* **11** *vs.* **22** and **12** *vs.* **23**, respectively, showing a somewhat

higher stability of the latter compounds (see *Table*). Furthermore, small differences were also noticed on changing the solvent from MeCN to MeCN/DMF 1:1 or by various substituents at the sugar moiety, but a drastic increase in the cleavage rates was observed in the presence of 1,5,7-triazabicyclo[4.4.0]dec-5-ene (TBD) as a tetrasubstituted guanidine derivative instead of DBU representing a cyclic amidine. A comparison of the (2-cyanoethoxy)carbonyl (ceoc) *vs.* the [2-(4-nitrophenyl)ethoxy]carbonyl (npeoc) group revealed a substantial acceleration of the β-elimination process with the ceoc-protected compounds.

Table. *Half-lives of Protected Nucleosides*

Unsubstituted nucleoside	Substituents[a]) at						$\tau_{1/2}$ [min]	Solvent	Base
	N^2-	N^4	N^6-	O^6-	3′-*O*	5′-*O*			
dC (see **22**)		ceoc				$(MeO)_2Tr$	5	MeCN	DBU
C (see **11**)		ceoc				MeOTr	7	DMF/MeCN	DBU
							2.5	DMF/MeCN	TBD
dC		npeoc				$(MeO)_2Tr$	42	MeCN	DBU
C		npeoc				MeOTr	50	DMF/MeCN	DBU
							65	MeCN	DBU
							3.5	DMF/MeCN	TBD
dA (see **23**)			ceoc			$(MeO)_2Tr$	13	DMF/MeCN	DBU
A (see **12**)			ceoc			MeOTr	27	DMF/MeCN	DBU
							2	DMF/MeCN	TBD
dA			npeoc			$(MeO)_2Tr$	96	MeCN	DBU
dA			npeoc			MeOTr	97	DMF/MeCN	DBU
							111	MeCN	DBU
							7	DMF/MeCN	TBD
A			npeoc			MeOTr	94	DMF/MeCN	DBU
							126	MeCN	DBU
dG (see **27**)	ceoc			ce	tmbs	tmbs	5700	DMF/MeCN	DBU
G (see **9**)	ceoc						5000	MeCN	DBU
dG (see **31**)	ceoc			npe			27	DMF/MeOH	DBU
dG (see **32**)	ceoc			npe		$(MeO)_2Tr$	32	DMF/MeCN	DBU
dG (see **28**)	ibu			ce	tbds	tbds	0.5	MeCN	DBU
dG	ibu			npe	tbds	tbds	17	DMF/MeCN	DBU
G	ibu			npe		MeOTr	12	DMF/MeCN	DBU
dG	npeoc			npe		$(MeO)_2Tr$	156	DMF/MeCN	DBU
dG (see **37**)	ceoc			pse		$(MeO)_2Tr$	180	DMF/MeCN	DBU

[a]) ce = 2-cyanoethyl, ceoc = 2-(cyanoethoxy)carbonyl, npe = 2-(4-nitrophenyl)ethyl, npeoc = [2-(4-nitrophenyl)-ethoxy]carbonyl, tbds = (*tert*-butyl)dimethylsilyl, MeOTr = monomethoxytrityl, $(MeO)_2Tr$ = dimethoxytrityl, pse = 2-(phenylsulfonyl)ethyl.

The most problematic nucleosides are always seen in the dG and G series, since their specific protection is most difficult and the removal of the protecting groups affords, in general, more severe reaction conditions. Also under our standard ceoc cleavage conditions, the presence of the N^2-ceoc group increased the $\tau_{1/2}$ of the protected dG or G nucleosides to more than 8–9 h (see **9** and **27**), since that anion formation at the nucleobase counteracts the β-elimination process for electronic reasons. On the other hand, it can also be seen from **28** that the O^6-ce group is very labile, it showed the fastest cleavage rate of all protecting groups discussed. This result

limits the combination of the ce and ceoc group in **27**, since the preferential deblocking of the O^6-substituent leads to a stable, partially protected guanosine intermediate. From these findings, it can be concluded that the unified use of β-elimination protecting groups in the guanosine series requires a relatively stable O^6-substituent, such as the npe group combined with the N^2-ceoc function. N^2-[(2-Cyanoethoxy)carbonyl]-2′-deoxy-O^6-2-[(4-nitrophenyl)ethyl]guanosine (**31**) and its 5′-*O*-(dimethoxytrityl)derivative **32** seem to have the anticipated properties, showing a $\tau_{1/2}$ of 27 and 32 min, respectively, which demonstrates the cleavage of the N^2- prior to that of the O^6-substituent, as seen in the HPLC (*Fig.*). The introduction of the O^6-2-(phenylsulfonyl)ethyl (pse) group in **37** is no alternative to npe since its cleavage was also very fast and succeeded the N^2-ceoc group.

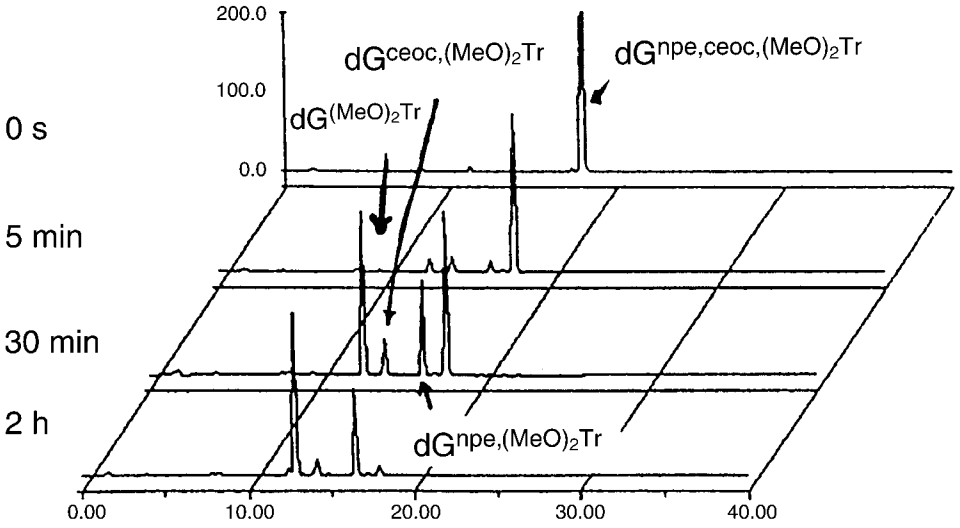

Figure. *Time-dependent cleavage of the* N^2*-ceoc and* O^6*-npe protecting groups of* **32** *by DBU in DMF/MeCN at room temperature*

Experimental Part

General. TLC: precoated silica-gel thin-layer sheets *60 F254* from *Merck*. Prep. column chromatography: silica gel 0.04 mm from *Baker*. HPLC: *Merck-Hitachi L6200* and L6200-A, column *RP-18 LiChrospher* (*Merck*, 125 × 4 mm, 5 μm), flow rate 1 ml/min, mobile phase 0.1M $AcONH_4$/MeCN. FC = flash chromatography. UV/VIS: *Perkin-Elmer Lambda-15*; λ_{max} in nm (lg ε). ^{1}H-NMR: *Bruker AC-250*; δ in ppm rel. to $SiMe_4$. ^{31}P-NMR: *JEOL-400*; δ in ppm rel. to H_3PO_4.

1. *2-Cyanoethyl Carbonochloridate* (ceoc-Cl; **5**) [12]. Phosgene (14.85 g, 150 mmol) was condensed at −50° into a flask and then diluted with THF (34 ml). A soln. of 3-hydroxypropanenitrile (6.82 g, 96 mmol) in THF (140 ml) was added dropwise within 1.5 h under N_2. The soln. was stirred for 1.5 h at −30° and another 3 h at r.t. The excess phosgene together with the THF was condensed off under high vacuum into a cooling trap. The colorless viscous product still contained *ca*. 5% of 3-hydroxypropanenitrile. Because of the similar boiling points of educt and product, a further purification was not possible, and **5** containing 5% of educt was used in the following: 10.61 g (83%) of crude **5**. Colorless viscous liquid. B.p. 80–82°/0.2 Torr ([12]: 110–112°/11 Torr). ^{1}H-NMR ($CDCl_3$): 2.85 (*t*, CH_2CN); 4.52 (*t*, CH_2O).

2. *1-[(2-Cyanoethoxy)carbonyl]-3-methyl-1*H*-imidazolium Chloride* (**6**). To an ice-cold soln. of **5** (4.67 g, 35 mmol) in CH_2Cl_2 (30 ml), 1-methyl-1*H*-imidazole (2.87 g, 35 mmol) in CH_2Cl_2 (2.5 ml) was added within

5 min. The mixture was stirred for 10 min at 0° and 3 h at r.t. The colorless precipitate was filtered off, washed with CH_2Cl_2 (3 ×) and dried *in vacuo:* 7.1 1 g (94%) of colorless powder. M.p. 101°. ^{1}H-NMR ((D$_6$)DMSO): 3.12 (*t*, OCH$_2$C*H*$_2$CN); 3.95 (*s*, MeN); 4.7 (*t*, 2 H, OC*H*$_2$CH$_2$CN); 7.95 (*s*, H–C(5)); 8.15 (*s*, H–C(4)); 10.1 (*s*, H–C(2)). Anal. calc. for $C_8H_{10}ClN_3O_3$ (215.6): C 44.56, H 4.67, N 19.49; found: C 43.99, H 4.75, N 19.05.

3. N^4-*[(2-Cyanoethoxy)carbonyl]cytidine* (**7**). A mixture of cytidine (**1**; 0.487 g, 2 mmol) and a few crystals of ammonium sulfate was heated with hexamethyldisilazane (HMDS; 5 ml) in dry dioxane (5 ml) for 3 h under reflux. After evaporation, the residue was treated with toluene (50 ml), the mixture filtered, and the filtrate evaporated. The solid was dissolved in dry CH_2Cl_2 (40 ml), then **6** (0.56 g, 2.6 mmol) was added and stirred at r.t. for 24 h. After evaporation, MeOH (30 ml) was added, the mixture stirred for 24 h, and then the soln. concentrated to a smaller volume. The product started to crystallize and was collected after standing in the ice-box overnight. Washing with cold MeOH (10 ml) and Et_2O and drying under high vacuum yielded colorless crystals: 0.579 g (85%) of **7**. M.p. 156–157°. UV (MeOH): 211 (4.31), 239 (4.15), 293 (3.87). ^{1}H-NMR ((D$_6$)DMSO): 10.90 (*s*, NH); 8.41 (*d*, H–C(6)); 6.98 (*d*, H–C(5)); 5.75 (*d*, H–C(1′)); 5.40 (*d*, OH–C(2′)); 5.16 (*t*, OH–C(5′)); 5.04 (*d*, OH–C(3′)); 4.28 (*t*, OC*H*$_2$CH$_2$CN); 3.96–3.89 (*m*, H–C(2′), H–C(3′), H–C(4′)); 3.74–3.53 (*m*, 2 H–C(5′)); 2.92 (*t*, OCH$_2$C*H*$_2$CN). Anal. calc. for $C_{13}H_{16}N_4O_7$ (340.3): C 45.88, H 4.74, N 16.46; found: C 45.65, H 4.82, 16.28.

4. N^4-*2-[(Cyanoethoxy)carbonyl]-5′*-O-*(4-methoxytrityl)cytidine* (**11**). Compound **7** (0.5 g, 1.47 mmol) was co-evaporated with dry pyridine (3 × 5 ml), the residue suspended in dry pyridine (7 ml), monomethoxytrityl chloride (0.543 g, 1.76 mmol) added, and the mixture stirred at r.t. for 24 h. After evaporation and co-evaporation with toluene (3 × 12 ml), the residue was dissolved in $CHCl_3$ and then extracted with phosphate buffer pH 7 (2 × 50 ml) and H_2O (50 ml). The org. phase was dried (Na_2SO_4), evaporated, and purified by CC (silica gel (50 g, 3 × 16 cm), 0–5% MeOH/$CHCl_3$). The main fraction yielded, on evaporation and drying under high vacuum, a colorless solid: 0.686 g (76%) of **11**. UV (MeOH): 205 (4.76), 234 (4.39), 294 (3.89). ^{1}H-NMR ((D$_6$)DMSO): 10.91 (*s*, NH); 8.25 (*d*, H–C(6)); 7.40–7.23 (*m*, 12 arom. H); 6.90 (*d*, 2 H *o* to MeO); 6.78 (*d*, H–C(5)); 5.74 (*s*, H–C(1′)); 5.64 (*d*, OH–C(2′)); 5.11 (*d*, OH–C(3′)); 4.28 (*t*, OC*H*$_2$CH$_2$CN); 4.14 (*m*, H–C(2′)); 4.01 (*m*, H–C(3′), H–C(4′)); 3.74 (*s*, MeO); 2.91 (*t*, OCH$_2$C*H*$_2$CN). Anal. calc. for $C_{33}H_{32}N_4O_8 \cdot 0.35\ CHCl_3$ (654.4): C 61.21, H 4.98, N 8.56; found: C 61.24, H 5.09, N 9.14.

5. N^4-*[(2-Cyanoethoxy)carbonyl]-2′-deoxycytidine* (**18**). To a suspension of 2′-deoxycytidine hydrochloride (**13** · HCl; 2.64 g, 10 mmol) and **6** (2.59 g, 12 mmol) in DMF (100 ml), ^{i}Pr$_2$NEt (1.7 ml, 10 mmol) was added and shaken for *ca.* 3 min to give a homogeneous soln. After stirring for 3 h at r.t., the solvent was distilled off under high vacuum at 30° bath temp. The residue was purified by FC (silica gel, 0–20% MeOH/CH_2Cl_2 (2 l)). The main fraction gave a solid, which was recrystallized from ^{i}PrOH: 2.57 g (79%) of **18**. Colorless crystals. TLC ($CHCl_3$/MeOH 4:1): R_f 0.44. M.p. 150–151°. UV (MeOH): 294 (3.89). ^{1}H-NMR ((D$_6$)DMSO): 2.0 (*m*, H–C(2′)); 2.25 (*m*, 1 H–C(2′)); 2.9 (*t*, OCH$_2$C*H*$_2$CN); 3.60 (*m*, 2 H–C(5′)); 3.88 (*m*, H–C(4′)); 4.2 (*m*, H–C(3′)); 4.3 (*t*, OC*H*$_2$CH$_2$CN); 5.11 (*t*, OH–C(5′)); 5.28 (*d*, OH–C(3′)); 6.08 (*t*, H–C(1′)); 7.0 (*d*, H–C(5)); 8.33 (*d*, H–C(6)); 10.9 (*s*, NHCOO). Anal. calc. for $C_{13}H_{16}N_4O_6$ (324.3): C 48.15, H 4.97, N 17.28; found: C 48.18, H 5.03, N 16.79.

6. N^4-*[(2-Cyanoethoxy)carbonyl]-2′-deoxy-5′*-O-*(4,4′-dimethoxytrityl)cytidine* (**22**). A soln. of **18** (2.57 g, 7.93 mmol) in pyridine (10 ml) was co-evaporated twice and the residue then dissolved in pyridine (30 ml). Dimethoxytrityl chloride (2.95 g, 8.7 mmol) was added and stirred for 16 h. The reaction was quenched with MeOH (10 ml), the mixture evaporated, and the residue dissolved in CH_2Cl_2 (200 ml). After washing with sat. $NaHCO_3$ soln. (2 × 200 ml), the org. phase was dried (Na_2SO_4) and evaporated. The residue was recrystallized from AcOEt. From the filtrate, a second crop was obtained by FC (silica-gel (20 g), toluene/AcOEt/MeOH 1:2:0 → 5:5:1): 4.15 g (84%) of **15**. Colorless crystals. TLC ($CHCl_3$/MeOH 9:1): R_f 0.34. UV (MeOH): 283 (3.95), 294 (sh, 3.92). ^{1}H-NMR ((D$_6$)DMSO): 2.15 (*m*, 1 H–C(2′)); 2.35 (*m*, 1 H–C(2′)); 2.95 (*t*, OCH$_2$C*H*$_2$CN); 3.25 (*m*, 2 H–C(5′)); 3.75 (*s*, 2 MeO); 3.95 (*m*, H–C(4′)); 4.21 (*m*, H–C(3′)); 4.3 (*t*, OC*H*$_2$CH$_2$CN); 5.35 (*d*, OH–C(3′)); 6.1 (*t*, H–C(1′)); 6.9 (*d*, H–C(5)); (*d*, 4 H *o* to MeO); 7.2–7.4 (*m*, 9 arom. H); 8.15 (*d*, H–C(6)); 10.9 (*s*, NHCOO). Anal. calc. for $C_{34}H_{34}N_4O_8 \cdot H_2O$ (644.7): C 63.35, H 5.63, N 8.69; found: C 63.81, H 5.58, N 8.52.

7. N^6-*[(2-Cyanoethoxy)carbonyl]adenosine* (**8**). Adenosine (**2**; 1.106 g, 4 mmol) and a few crystals of ammonium sulfate were heated with HMDS (16 ml) in dry dioxane (15 ml) for 6 h under reflux. The mixture was evaporated, the resulting syrup treated with dry toluene, the undissolved material filtered off, and then the filtrate evaporated. The residue was dissolved in dry CH_2Cl_2 (80 ml), **6** (1.08 g, 5 mmol) was added and stirred at r.t. for 20 h. After evaporation, the residue was treated with EtOH (60 ml) and MeOH (10 ml) by stirring to give a colorless precipitate. The solid was washed with EtOH and Et_2O and dried under vacuum: 1.165 g (80%) of **8**. M.p. 158–161°. UV (MeOH): 209 (4.45), 266 (4.26), 273 (sh, 4.15). ^{1}H-NMR ((D$_6$)DMSO): 10.82 (*s*, NH); 8.70

(*s*, H–C(2)); 8.65 (*s*, H–C(8)); 5.99 (*d*, H–C(1′)); 5.54 (br. *s*, OH–C(2′)); 5.21 (br. *s*, OH–C(3′), OH–C(5′)); 4.60 (*t*, H–C(2′)); 4.31 (*t*, OCH_2CH$_2$CN); 4.16 (*m*, H–C(3′)); 3.95 (*m*, H–C(4′)); 3.85–3.52 (*m*, 2 H–C(5′)); 2.94 (*t*, OCH$_2$CH_2CN). Anal. calc. for $C_{14}H_{16}N_6O_6$ (364.3): C 46.15, H 4.42, N 23.06; found: C 46.05, H 4.57, N 22.78.

8. *2′,3′,5′-Tri*-O-*acetyl*-N^6-*[(2-cyanoethoxy)carbonyl]adenosine* (**10**). To a cold suspension of 2′,3′,5′-tri-*O*-acetyladenosine (**4**; 0.197 g, 0.5 mmol) in 1M 1-methyl-1*H*-imidazole in MeCN (2 ml), **5** (0.35 g, 2.3 mmol) was added under stirring and ice-cooling. After 30 min, the mixture was warmed to r.t., and stirring continued until the starting material had disappeared. The mixture was diluted with $CHCl_3$ and extracted twice with H_2O (25 ml). The org. phase was dried (Na_2SO_4) and evaporated, and the residue purified by CC (silica gel (20 g, 40 × 2 cm), $CHCl_3$/MeOH 95:5, then 4:1): 0.225 g (90%) of **10**. Colorless solid foam. UV (MeOH): 209 (4.39), 254 (sh, 4.13), 265 (4.22), 272 (sh, 4.12). ^{1}H-NMR ($CDCl_3$): 9.30 (*s*, NHCOO); 8.81 (*s*, H–C(2)); 8.25 (*s*, H–C(8)); 6.25 (*d*, H–C(1′)); 5.97 (*m*, H–C(2′)); 5.7 (*m*, H–C(3′)); 4.55–4.335 (*m*, 2 H–C(5′), H–C(4′), OCH_2CH$_2$CN); 2.85 (*t*, OCH$_2$CH_2CN); 2.15 (3*s*, Ac). Anal. calc. for $C_{20}H_{22}N_6O_9 \cdot 0.5\ H_2O$ (499.4): C 48.10, H 4.64, N 16.83; found: C 48.02, H 4.62, N 16.64.

9. N^6-*[(2-Cyanoethoxy)carbonyl]-5′*-O-*(4-methoxytrityl)adenosine* (**12**). *N*^{6}-[(2-Cyanoethoxy)carbonyl]-adenosine (**8**; 0.5 g, 1.37 mmol) was co-evaporated with dry pyridine (3 × 10 ml), the residue dissolved in the same solvent (5 ml), and then monomethoxytrityl chloride (0.5 g, 1.64 mmol) added. After stirring at r.t. for 24 h, the reaction was quenched by MeOH (1 ml) and stirring for 25 min. The soln. was evaporated and co-evaporated with toluene (2 × 10 ml) and $CHCl_3$ (3 × 10 ml). The residue was dissolved in $CHCl_3$ and extracted with phosphate buffer pH 7 (2 × 50 ml) and H_2O (50 ml). The org. phase was dried (Na_2SO_4) and evaporated and the residue purified by CC (silica gel (50 g, 16 × 3 cm), 0–3% MeOH/$CHCl_3$: 0.751 g (86%) of **12**. Colorless foam: UV (MeOH): 206 (4.80), 232 (sh, 4.25), 266 (4.26), 274 (sh, 4.14). ^{1}H-NMR ((D$_6$)DMSO): 10.83 (*s*, NH); 8.58 (*s*, H–C(2), H–C(8)); 7.37–7.19 (*m*, 12 arom. H); 6.84 (*d*, 2 H *o* to MeO); 6.02 (*d*, H–C(1′)); 5.61 (*d*, OH–C(2′)); 5.27 (*d*, OH–C(3′)); 4.74 (*m*, H–C(2′)); 4.31 (*m*, H–C(3′), OCH_2CH$_2$CN); 4.10 (*m*, H–C(4′)); 3.72 (*s*, MeO); 3.23 (br. *s*, 2 H–C(5′)); 2.92 (*t*, OCH$_2$CH_2CN). Anal. calc. for $C_{34}H_{32}N_6O_7 \cdot 0.4\ CHCl_3$ (684.4): C 60.36, H 4.77, N 12.27; found: C 60.25, H 4.89, N 12.12.

10. N^6-*[(2-Cyanoethoxy)carbonyl]-2′-deoxyadenosine* (**19**). A soln. of 2′-deoxyadenosine (**14**; 0.50 g, 2 mmol) in dioxane (8 ml) was treated with HMDS (8 ml) and some crystals of $(NH_4)_2SO_4$ by heating under reflux for 5 h. The mixture was evaporated and the residue co-evaporated with toluene (5 ml) and then redissolved in CH_2Cl_2 (40 ml). Then **6** (0.538 g, 2.5 mmol) was added and the resulting suspension stirred for 20 h at r.t. The precipitate was filtered off and washed with CH_2Cl_2 (3 × 5 ml), the combined org. soln. evaporated, and the residue redissolved in H_2O/MeOH 1:1 (20 ml). After stirring for 6 h, the solvent was distilled off and the residue co-evaporated with toluene (2 × 5 ml), MeOH (2 × 5 ml), and CH_2Cl_2 (2 × 5 ml). The resulting solid was purified by FC (silica gel (15 g), 0–10% MeOH/CH_2Cl_2 (800 ml)): 0.57 g (82%) of **19**. Colorless foam. TLC ($CHCl_3$/MeOH 4:1): R_f 0.57. UV (MeOH): 266 (4.20), 273 (sh, 4.11). ^{1}H-NMR ((D$_6$)DMSO): 2.30 (*m*, 1 H–C(2′)); 2.75 (*m*, 1 H–C(2′)); 2.95 (*t*, OCH$_2$CH_2CN); 3.55 (*m*, 2 H–C(5′)); 3.88 (*m*, H–C(4′)); 4.30 (*t*, OCH_2CH$_2$CN); 4.42 (*m*, H–C(3′)); 5.01 (*t*, OH–C(5′)); 5.35 (*d*, OH–C(3′)); 6.45 (*t*, H–C(1′)); 8.65 (*s*, H–C(2)); 8.67 (*s*, H–C(8)); 10.8 (*s*, NHCOO). Anal. calc. for $C_{14}H_{16}N_6O_5 \cdot H_2O$ (366.3): C 45.90, H 4.95, N 22.94; found: C 45.63, H 4.62, N 22.51.

11. N^6-*[(2-Cyanoethoxy)carbonyl]-2′-deoxy-5′*-O-*(4,4′-dimethoxytrityl)adenosine* (**23**). A soln. of **19** (3.8 g, 10.9 mmol) in dry pyridine (15 ml) was evaporated, the residue dissolved in pyridine (40 ml), 4,4′-dimethoxytrityl chloride (4 g, 12 mmol) added, and the mixture stirred for 16 h. The soln. was reduced to 1/3 of its volume, diluted with CH_2Cl_2 (300 ml), and washed with sat. $NaHCO_3$ soln. (2 × 300 ml). The org. phase was dried (Na_2SO_4), evaporated, and co-evaporated with toluene (2 × 15 ml), MeOH (2 × 15 ml), and CH_2Cl_2 (2 × 15 ml). The residue was purified by FC (silica-gel (70 g), AcOEt/toluene 3:1 → AcOEt/toluene/MeOH 40:2:1 (1800 ml)): 6.5 g (92%) of **23**. Colorless solid foam. TLC ($CHCl_3$/MeOH 9:1): R_f 0.41. UV (MeOH): 267 (4.23), 274 (sh, 4.14). ^{1}H-NMR ((D$_6$)DMSO): 2.37 (*m*, 1 H–C(2′)); 2.90 (*m*, 1 H–C(2′)); 2.93 (*t*, OCH$_2$CH_2CN); 3.17 (*m*, 2 H–C(5′)); 3.69 (*s*, 2 MeO); 4.01 (*m*, H–C(4′)); 4.31 (*t*, OCH_2CH$_2$CN); 4.50 (*m*, H–C(3′)); 5.42 (*d*, OH–C(3′)); 6.46 (*t*, H–C(1′)); 6.77 (*m*, 4 H *o* to MeO); 7.1–7.35 (*m*, 9 arom. H); 8.56 (*s*, H–C(2), H–C(8)); 10.81 (*s*, NHCOO). Anal. calc. for $C_{34}H_{34}N_6O_7$ (650.7): C 64.61, H 5.27, N 12.92; found: C 64.82, H 5.48, N 12.14.

12. N^2-*[(2-Cyanoethoxy)carbonyl]guanosine* (**9**). A mixture of dry guanosine (**3**) (1.4 g, 5 mmol) and Me_3SiCl (5 ml) in pyridine (40 ml) was stirred at r.t. for 4 h. Then, a soln. of **5** (1.33 g, 10 mmol) in CH_2Cl_2 (10 ml) was added. The resulting mixture was stirred at r.t. for 20 h and the precipitate filtered off. The soln. was evaporated and co-evaporated with toluene (30 ml). The residue was dissolved in MeOH (70 ml), the soln. stirred at r.t. for 18 h and evaporated, and the residue purified by CC (silica gel (3.5 × 5 cm), CH_2Cl_2/MeOH

50:1 → 9:1) and finally crystallized from 90% EtOH: 1.02 g (54%) of **9**. M.p. 190°. UV (MeOH): 256 (4.23), 277 (sh, 4.04). ^{1}H-NMR ((D$_6$)DMSO): 11.62, 11.31 (2*s*, NH); 8.23 (*s*, H–C(8)); 5.77 (*d*, H–C(1′)); 5.46 (*d*, OH–C(2′)); 5.16 (*d*, OH–C(3′)); 4.97 (br. *s*, OH–C(5′)); 4.47 (*t*, OC*H*$_2$CH$_2$ON); 4.12 (*m*, H–C(3′)); 3.87 (*m*, H–C(4′)); 3.57 (*m*, 2 H–C(5′)); 2.96 (*t*, OCH$_2$C*H*$_2$CN). Anal. calc. for C$_{14}$H$_{16}$N$_6$O$_7$ (380.3): C 44.21, H 4.24, N 22.09; found: C 44.01, H 4.34, N 21.67.

13. N^2-*[(2-Cyanoethoxy)carbonyl]-2′-deoxyguanosine* (**20**). Dry 2′-deoxyguanosine (**15**; 0.2 g, 0.75 mmol) was twice co-evaporated with dry pyridine (5 ml). The residue was suspended in dry pyridine (1.5 ml), Me$_3$SiCl (0.5 ml, 4 mmol) added, the mixture stirred for 90 min, and then a soln. of **5** (0.14 g, 1.05 mmol) in CH$_2$Cl$_2$ (1.5 ml) dropwise added. Stirring was continued at r.t. for 7 h, the solid pyridinium chloride filtered off and washed twice with CH$_2$Cl$_2$ (2.5 ml), and then the filtrate evaporated. The residue was dissolved in MeOH (5 ml), the soln. stirred for 20 min and evaporated, the solid dissolved in AcOEt (15 ml), the soln. washed with H$_2$O (15 ml), the aq. phase re-extracted with AcOEt (3 × 15 ml), the combined org. phase dried (Na$_2$SO$_4$) and evaporated, and the residue purified by CC (silica gel (8 g; 2.5 cm), CH$_2$Cl$_2$/MeOH 9:1): 51 mg (19%). TLC (CHCl$_3$/MeOH 6:1): R_f 0.21. ^{1}H-NMR ((D$_6$)DMSO): 11.58 (*s*, NH); 11.30 (*s*, NH); 8.21 (*s*, H–C(8)); 6.17 (‘*t*’, H–C(1′)); 5.25 (*d*, OH–C(3′)); 4.92 (*t*, OH–C(5′)); 4.38 (*m*, H–C(3′), OC*H*$_2$CH$_2$CN); 3.80 (*m*, H–C(4′)); 3.55 (*m*, 2 H–C(5′)); 2.95 (*t*, OCH$_2$C*H*$_2$CN); 2.65 (*m*, 1 H–C(2′)); 2.25 (*m*, 1 H–C(2′)).

14. *3′,5′-Bis*-O-*[(*tert*-butyl)dimethylsilyl]*-N^2-*[(2-cyanoethoxy)carbonyl]-2′-deoxyguanosine* (**21**). A mixture of 3′,5′-bis-O-[(*tert*-butyl)dimethylsilyl]-2′-deoxyguanosine (**16**) [17] (0.14 g, 0.28 mmol) in dry pyridine (2 ml) was evaporated twice. The residue was dissolved in CH$_2$Cl$_2$ (3 ml), and then Et$_3$N (0.16 ml, 1.15 mmol) and Me$_3$SiCl (0.1 ml, 0.8 mmol) were added. After stirring for 15 min, the mixture was evaporated and co-evaporated twice with dry pyridine (2 ml), the residue dissolved in CH$_2$Cl$_2$ (2 ml) and pyridine (2 ml), and **5** (38 mg, 0.29 mmol) added. The mixture was stirred at r.t. for 15 h and then evaporated, the residue dissolved in CH$_2$Cl$_2$ (10 ml), the soln. extracted with H$_2$O (5 ml), the aq. phase re-extracted with CH$_2$Cl$_2$ (3 ×), the combined org. phase dried (Na$_2$SO$_4$) and evaporated, and the residue purified by CC (silica gel (6 g), 0.1–6% MeOH/CH$_2$Cl$_2$): 29 mg (22%) of **21**. Colorless solid foam. R_f (CHCl$_3$/MeOH 9:1) 0.22. ^{1}H-NMR ((D$_6$)DMSO): 11.55 (*s*, NH); 11.40 (*s*, NH); 8.20 (*s*, H–C(8)); 6.21 (‘*t*’, H–C(1′)); 4.50 (*m*, H–C(3′)); 4.43 (*t*, OC*H*$_2$CH$_2$CN); 3.81 (*m*, H–C(4′)); 3.65 (*m*, 2 H–C(5′)); 2.95 (*t*, OCH$_2$C*H*$_2$CN); 2.75 (*m*, 1 H–C(2′)); 2.40 (*m*, 1 H–C(2′)); 0.88 (*s*, 1 ^{t}Bu); 0.85 (*s*, ^{t}Bu); 0.10 (*s*, Me$_2$Si); 0.00 (*s*, Me$_2$Si).

15. *3′,5′-Bis*-O-*[(*tert*-butyl)dimethylsilyl]*-O^6-*(2-cyanoethyl)-2′-deoxy*-N^2-*isobutyrylguanosine* (**28**) [14]. 15.1. To a soln. of 3′,5′-bis-*O*-[(*tert*-butyl)dimethylsilyl]-2′-deoxy-*N*^{2}-isobutyryl-*O*^{6}-[(2,4,6-triisopropylphenyl)-sulfonyl]guanosine [14] (1.27 g, 1.46 mmol) and 3-hydroxypropanenitrile (1.35 ml, 19.8 mmol) in dry CH$_2$Cl$_2$ (25 ml) under N$_2$, Me$_3$N (1.27 g, 21.5 mmol) was added under cooling. After stirring for 10 min, DBU (0.45 g, 0.445 mmol) was added, and, after another 10 min, the reaction was quenched by diluting with CH$_2$Cl$_2$ (25 ml) and extraction with sat. NH$_4$Cl soln. (2 × 25 ml) and H$_2$O (25 ml). The org. phase was dried (Na$_2$SO$_4$) and evaporated, and the residue purified by CC (silica gel (20 g; 5 × 2.5 cm), CH$_2$Cl$_2$): 0.71 g (79%) of **28**. Yellowish solid foam.

15.2. As described in *15.1*, with small amounts of DABCO (3 mg) instead of DBU, and with Et$_3$N instead of Me$_3$N, and with stirring at r.t. for 28 h. Workup yielded 64% of **28**. UV (MeOH): 219 (sh, 4.32), 268 (4.22), 279 (4.06). ^{1}H-NMR ((D$_6$)DMSO): 10.45 (*s*, NH); 8.41 (*s*, H–C(8)); 6.30 (‘*t*’, H–C(1′)); 4.70 (*m*, H–C(3′), OC*H*$_2$CN$_2$CN); 3.81 (*m*, 2 H–C(5′)); 3.75 (*m*, H–C(4′)); 3.20 (*t*, OCH$_2$C*H*$_2$CN); 2.95 (*sept.*, Me$_2$C*H*); 2.80 (*m*, 1 H–C(2′)); 2.21 (*m*, 1 H–C(2′)); 1.08 (*d*, *Me*$_2$CH); 0.90 (*s*, 1 ^{t}Bu); 0.82 (*s*, 1 ^{t}Bu); 0.12 (*s*, Me$_2$Si); –0.02 (*s*, 1 Me$_2$Si). Anal. calc. for C$_{29}$H$_{50}$N$_6$O$_5$Si$_2$ (618.9): C 56.27, H 8.14, N 13.58; found: C 56.32, H 8.21, N 13.42.

16. *3′,5′-Bis*-O-*[(*tert*-butyl)dimethylsilyl]-2′-deoxy*-N^2-*isobutyryl*-O^6-*[2-(4-nitrophenyl)ethyl]guanosine* (**29**). After co-evaporation of 3′,5′-bis-*O*-[(*tert*-butyl)dimethylsilyl]-2′-deoxy-*N*^{2}-isobutyrylguanosine (0.5 g, 0.88 mmol) with dry dioxane (2 × 8 ml), the residue was dissolved in the same solvent (18 ml). Triphenylphosphine (0.37 g, 1.41 mmol) and 2-(4-nitrophenyl)ethanol (0.222 g, 1.32 mmol) were added. After stirring for 15 min at r.t., ethyl azodicarboxylate (= diethyl diazenedicarboxylate; 0.25 g, 1.42 mmol) was added, the mixture stirred for 3 h, and again some ethyl azodicarboxylate (30 µl) added to complete the reaction within 1 h. The soln. was evaporated, the residue dissolved in CH$_2$Cl$_2$ (3.5 ml) and the soln. cooled overnight. The precipitate of diethyl hydrazine-1,2-dicarboxylate was washed with CH$_2$Cl$_2$, the combined filtrate evaporated and the residue again treated with CH$_2$Cl$_2$ to give a second crop of diethyl hydrazine-1,2-dicarboxylate. Purification by CC (silica gel (18 g; 6 × 2.5 cm), 0–12% AcOEt/toluene) yielded a crude product, which still contained some diethyl hydrazine-1,2-dicarboxylate: 0.322 g (51%). A pure anal. sample of **29** was obtained by prep. TLC (silica gel, toluene/AcOEt 3:1). UV (MeOH): 206 (4.38), 217 (sh, 4.46), 269 (4.42), 281 (4.29). ^{1}H-NMR ((D$_6$)DMSO): 10.38 (*s*, NH); 8.35 (*s*, H–C(8)); 8.15 (*d*, 2 H *o* to NO$_2$); 7.65 (*d*, 2 H *m* to NO$_2$); 6.30 (‘*t*’, H–C(1′)); 4.81 (*t*, ArCH$_2$C*H*$_2$O); 4.7 (*m*, H–C(3′)); 3.79 (*m*, 2 H–C(5′)); 3.68 (*m*, H–C(4′)); 3.32

(*t*, ArCH_2CH$_2$O); 3.00 (*sept.*, Me$_2$C*H*); 2.79 (*m*, 1 H–C(2′)); 2.25 (*m*, 1 H–C(2′)); 1.10 (*d*, *Me*$_2$CH); 0.91 (*s*, 1 *t*Bu); 0.82 (*s*, 1 *t*Bu); 0.12 (*s*, Me$_2$Si); –0.05 (*s*, 1 Me$_2$Si). Anal. calc. for $C_{34}H_{54}N_6O_7Si_2$ (715.0): C 57.11, H 7.61, N 11.75; found: C 56.54, H 7.51, N 11.54.

17. *3′,5′-Bis*-O-*[(*tert-*butyl)dimethylsilyl]-2′-deoxy*-O^6-*[(2,4,6-triisopropylphenyl)sulfonyl]guanosine* (**24**). A soln. of 3′,5′-bis-*O*-[(*tert*-butyl)dimethylsilyl]-2′-deoxyguanosine (**16**; 1 g, 2.02 mmol) in dry pyridine (8 ml) was twice co-evaporated and then dissolved in CH_2Cl_2 (15 ml) and pyridine (15 ml). After addition of 2,4,6-triisopropylbenzenesulfonyl chloride (1.27 g, 4.2 mmol), DMAP (25 mg, 0.2 mmol), and Et_3N (1.5 ml, 10.6 mmol), the soln. was stirred for 14 h at 50° to give a colorless precipitate of (Et_3NH)Cl. The soln. was diluted with CH_2Cl_2 (30 ml) and washed with H_2O (3 × 20 ml), the aq. phase extracted with CH_2Cl_2 (20 ml), and then the combined org. phases dried (Na_2SO_4) and evaporated. Purification by FC (silica-gel (30 g), toluene/AcOEt 10:1) gave 0.907 g (59%) of **24**. Lightly orange foam. TLC ($CHCl_3$/MeOH 49:1): R_f 0.41. UV (MeOH): 291 (3.98), 252 (sh, 3.93), 207 (4.72). ^{1}H-NMR ((D$_6$)DMSO): –0.05 (*s*, 1 Me$_2$Si); 0.00 (*s*, 1 Me$_2$Si); 0.80 (*s*, 1 *t*Bu); 0.84 (*s*, 1 *t*Bu); 1.20 (*d*, 3 *Me*$_2$CH); 2.25 (*m*, 1 H–C(2′)); 2.75 (*m*, 1 H–C(2′)); 2.95 (*sept.*, 1 Me$_2$C*H*); 3.65 (*m*, 2 H–C(5′)); 3.81 (*m*, H–C(4′)); 4.1 (*sept.*, 2 Me$_2$C*H o* to SO_2); 4.5 (*m*, H–C(3′)); 6.20 (*t*, H–C(1′)); 6.65 (*s*, NH_2); 7.33 (*s*, 2 arom. H); 8.20 (*s*, H–C(8)). Anal. calc. for $C_{37}H_{63}N_5O_6SSi_2$ (762.2): C 58.31, H 8.33, N 9.19; found: C 58.22, H 8.44, N 9.17.

18. *3′,5′-Bis*-O-*[(*tert-*butyl)dimethylsilyl]*-O^6-*(2-cyanoethyl)-2′-deoxyguanosine* (**25**). After co-evaporation of **24** (0.2 g, 0.26 mmol) with CH_2Cl_2 (2 × 5 ml), the residue was dissolved in CH_2Cl_2 (8 ml), and after addition of Et_3N (0.3 ml, 2.11 mmol), 3-hydroxypropanenitrile (0.1 ml, 1.42 mmol), and a cat. amount of DABCO, the mixture was stirred for 48 h at r.t. The soln. was diluted with CH_2Cl_2 (10 ml), washed twice with sat. NH_4Cl soln., the org. phase dried (Na_2SO_4) and evaporated, and the resulting solid purified by FC (silica gel (8 g), CH_2Cl_2 (300 ml), CH_2Cl_2/MeOH 49:1 (200 ml)): 90 mg (63%) of **29**. Yellowish foam. TLC ($CHCl_3$/MeOH 25:1): R_f 0.34. UV (MeOH): 283 (3.98), 247 (4.00). ^{1}H-NMR ((D$_6$)DMSO): 0.05 (*s*, 1 Me$_2$Si); 0.14 (*s*, 1 Me$_2$Si); 0.86 (*s*, *t*Bu); 0.88 (*s*, *t*Bu); 2.25 (*m*, 1 H–C(2′)); 2.75 (*m*, 1 H–C(2′)); 3.1 (*t*, OCH$_2$C*H*$_2$CN); 3.7 (*m*, 2 H–C(5′)); 3.85 (*m*, H–C(4′)); 4.52 (*m*, H–C(3′)); 4.58 (*t*, OC*H*$_2$CH$_2$CN); 6.20 (*t*, H–C(1′)); 6.55 (*s*, NH_2); 8.12 (*s*, H–C(8)). Anal. calc. for $C_{25}H_{44}N_6O_4Si_2$ (548.8): C 54.72, H 8.08, N 15.31; found: C 54.20, H 8.08, N 14.76.

19. *2′-Deoxy*-O^6-*[2-(phenylthio)ethyl]guanosine* (**34**). A soln. of 3′,5′-bis-*O*-acetyl-2′-deoxy-*O*^{6}-[2-(phenylthio)ethyl]guanosine (**33**) [16] (3.23 g, 5.66 mmol; contaminated with 16% of triphenyl phosphine) in dioxane (100 ml), MeOH (100 ml), and conc. ammonia (50 ml) was stirred for 20 h at r.t. The solvents were distilled off, and the residue was co-evaporated with toluene (2 × 20 ml), MeOH (2 × 20 ml), and CH_2Cl_2 (20 ml). The solid was purified by FC (silica gel (60 g), 0–5% MeOH/CH_2Cl_2 (1500 ml)): 2.03 g (89%) of **34**. Yellowish foam. TLC ($CHCl_3$/MeOH 9:1): R_f 0.42. UV (MeOH): 281 (4.08), 250 (4.26). ^{1}H-NMR ((D$_6$)DMSO): 2.20 (*m*, 1 H–C(2′)); 2.61 (*m*, 1 H–C(2′)); 3.45 (*t*, OCH$_2$C*H*$_2$S); 3.52 (*m*, 2 H–C(5′)); 3.82 (*m*, H–C(4′)); 4.38 (*m*, H–C(3′)); 4.55 (*t*, OC*H*$_2$CH$_2$S); 5.00 (*t*, OH–C(5′)); 5.28 (*d*, OH–C(3′)); 6.18 (*t*, H–C(1′)); 6.45 (*s*, NH_2); 7.18 (*m*, H *p* to SCH_2); 7.32 (*m*, 2 H *o* to SCH_2); 7.45 (*m*, 2 H *m* to SCH_2); 8.10 (*s*, H–C(8)). Anal. calc. for $C_{18}H_{21}N_5O_4S \cdot H_2O$ (421.5): C 51.30, H 5.5, N 16.62; found: C 51.88, H 5.55, N 17.10.

20. *2′-Deoxy*-O^6-*[2-(phenylsulfonyl)ethyl]guanosine* (**35**). A soln. of **34** (42 mg, 0.104 mmol) in CH_2Cl_2 (5 ml) was treated with 55% 3-chloroperbenzoic acid (65 mg, 0.208 mmol) at 0° for 1 h. The soln. was concentrated *in vacuo* to 1/5 of its volume and then the product isolated by FC (silica gel (4 g), CH_2Cl_2 (200 ml), CH_2Cl_2/MeOH 98:2 (100 ml)): 41 mg (91%) of **35**. Colorless foam. TLC ($CHCl_3$/MeOH 9:1): R_f 0.38. UV (MeOH): 283 (3.93), 272 (4.40). ^{1}H-NMR ((D$_6$)DMSO): 2.2 (*m*, 1 H–C(2′)); 2.55 (*m*, 1 H–C(2′)); 3.55 (*m*, 2 H–C(5′)); 3.80 (*m*, H–C(4′)); 3.95 (*t*, OCH$_2$CH$_2$SO$_2$); 4.35 (*m*, H–C(3′)); 4.65 (*t*, OCH$_2$CH$_2$SO$_2$); 4.95 (*t*, OH–C(5′)); 5.25 (*d*, OH–C(3′)); 6.18 (*t*, H–C(1′)); 6.43 (*s*, NH_2); 7.55–7.70 (*m*, 3 arom. H); 7.90 (*m*, 2 arom. H); 8.05 (*s*, H–C(8)). Anal. calc. for $C_{18}H_{21}N_5O_6S \cdot 0.75\ H_2O$ (448.9): C 48.16, H 5.05, N 15.59; found: C 48.26, H 4.92, N 15.01.

21. *3′,5′-Bis*-O-*[(*tert-*butyl)dimethylsilyl]*-N^2-*2-[(cyanoethoxy)carbonyl]-2′-deoxy*-O^6-*[(2,4,6-triisopropylphenyl)sulfonyl]guanosine* (**26**). A soln. of 3′,5′-bis-*O*-[(*tert*-butyl)dimethylsilyl]-2′-deoxy-*O*^{6}-[(2,4,6-triisopropylphenyl)sulfonyl]guanosine (**24**; 0.3 g, 0.394 mmol) in dry pyridine (2 × 3 ml) was co-evaporated and then dissolved in a mixture of CH_2Cl_2 (3 ml) and pyridine (3 ml). Me_3SiCl (0.27 ml, 2.16 mmol) was added, the soln. stirred for 30 min, then **5** (158 mg, 1.18 mmol) added, and the resulting suspension stirred for 4 h at 40°. The reaction was quenched by the addition of H_2O (4 ml) followed by CH_2Cl_2 (10 ml), the mixture stirred for 5 min and then extracted twice with H_2O, the org. phase dried (Na_2SO_4) and evaporated, and the residue co-evaporated with toluene (2 × 5 ml), MeOH (2 × 5 ml), and CH_2Cl_2 (2 × 5 ml). The resulting solid was purified by FC (silica-gel (8 g), 10–30% AcOEt/toluene (400 ml)): 50 mg (17%) of **26**. TLC ($CHCl_3$/MeOH 49:1): R_f 0.32. UV (MeOH): 278 (4.10), 238 (sh, 4.25). ^{1}H-NMR ((D$_6$)DMSO): –0.05 (*s*, 1 Me$_2$Si); 0.10 (*s*, 1 Me$_2$Si); 0.80 (*s*, 1 *t*Bu); 0.9 (*s*, *t*Bu); 1.2 (*d*, 3 *Me*$_2$CH); 2.35 (*m*, 1 H–C(2′)); 2.95–3.05 (*m*, 1 H–C(2′), OCH$_2$C*H*$_2$CN,

1 Me_2CH); 3.65–3.90 (*m*, 2 H–C(5′), H–C(4′)); 4.15 (*sept.*, 2 Me_2CH); 4.25 (*t*, OCH_2CH_2CN); 4.65 (*m*, H–C(3′)); 6.35 (*t*, H–C(1′)); 7.35 (*s*, 2 arom. H); 8.55 (*s*, H–C(8)); 10.68 (*s*, NH–C(2)). Anal. calc. for $C_{41}H_{66}N_6O_8SSi_2 \cdot H_2O$ (877.3): C 56.14, H 7.81, N 9.57; found: C 56.40, H 7.83, N 8.84.

22. *3′,5′-Bis*-O-*[(*tert*-butyl)dimethylsilyl]*-N^2-*2-[(cyanoethoxy)carbonyl]*-O^6-*(2-cyanoethyl)-2′deoxyguanosine* (**27**). 22.1. Into a soln. of **26** (80 mg, 0.09 mmol) in CH_2Cl_2 (3 ml), Me_3N (130 mg, 0.106 mmol) was condensed at 0°. After stirring for 10 min at 0°, 3-hydroxypropanenitrile (0.166 g, 2.34 mmol) and DBU (44 mg, 0.29 mmol) were added, and the mixture was stirred for max. 10 min at 0° and then diluted with CH_2Cl_2 (4 ml). The soln. was washed with NH_4Cl soln. (3×), the org. phase dried (Na_2SO_4) and evaporated, and the residue purified by FC (silica gel (4 g), CH_2Cl_2 (200 ml), CH_2Cl_2/MeOH 49:1 (200 ml)): 31 mg (53%) of **27**. Yellowish foam.

22.2. After co-evaporation of **25** (0.15 g, 0.27 mmol) with dry pyridine (2 × 2 ml), the residue was dissolved in pyridine (1.5 ml) and CH_2Cl_2 (3 ml). Me_3SiCl (88 mg, 0.9 mmol) in CH_2Cl_2 (0.5 ml) was added, and the soln. was stirred for 30 min at r.t. Then **5** (57 m, 0.425 mmol) was added and stirring continued for another 4.5 h. The reaction was quenched by the addition of MeOH (8 ml), the mixture stirred for 15 min and then evaporated, and the residue treated with H_2O (10 ml) and AcOEt (10 ml). The aq. phase was extracted with AcOEt (3 × 10 ml), the combined org. phase dried (Na_2SO_4) and evaporated, and the resulting solid purified by FC (silica-gel (6 g), CH_2Cl_2 (400 ml), then CH_2Cl_2/MeOH 400:1 (200 ml)): 0.161 g (92%) of **27**, identical with the product from *22.1*. Yellowish foam. TLC ($CHCl_3$/MeOH 9:1): R_f 0.68. UV (MeOH): 268 (4.13), 277 (sh, 3.96). ^{1}H-NMR ((D_6)DMSO): 0.05 (*s*, 1 Me_2Si); 0.1 (*s*, 1 Me_2Si); 0.85 (*s*, 1 ^{t}Bu); 0.9 (*s*, 1 ^{t}Bu); 2.30 (*m*, 1 H–C(2′)); 3.0 (*m*, 1 H–C(2′)); 2.95 (*t*, OCH_2CH_2CN); 3.15 (*t*, OCH_2CH_2CN); 3.60 (*m*, H–C(4′)); 3.81 (*m*, 2 H–C(5′)); 4.28 (*t*, OCH_2CH_2CN); 4.70 (*m*, H–C(3′)), OCH_2CH_2CN); 6.35 (*t*, H–C(1′)); 8.45 (*s*, H–C(8)); 10.62 (*s*, NH–C(2)). Anal. calc. for $C_{29}H_{47}N_7O_6Si_2$ (645.9): C 53.93, H 7.33, N 15.18; found: C 53.82, H 7.15, N 14.83.

23. N^2-*[(2-Cyanoethoxy)carbonyl]-2′-deoxy*-O^6-*[2-(4-nitrophenyl)ethyl]guanosine* (**31**). A soln. of 2′-deoxy-O^6-[2-(4-nitrophenyl)ethyl]guanosine (**30**) [2] (0.90 g, 2.16 mmol) in dry pyridine (2 × 5 ml) was co-evaporated and then the residue dissolved in a mixture of dry pyridine (8.5 ml) and CH_2Cl_2 (11 ml). After addition of Me_3SiCl (1.6 ml, 12.8 mmol) and stirring for 20 min, a precipitate of pyridinium chloride was formed. To this mixture, **5** (0.414 g, 3.1 mmol) in CH_2Cl_2 (2 ml) was added, and stirring was continued at r.t. for 4 h. The reaction was quenched by addition of MeOH (20 ml) to hydrolyze the Me_3Si groups. After stirring for 20 min and evaporation, the residue was treated with H_2O (40 ml) and AcOEt (40 ml). The aq. phase was extracted with AcOEt (3×) and the combined org. phase evaporated and co-evaporated with toluene (2 × 15 ml), MeOH (2 × 15 ml), and CH_2Cl_2 (3 × 15 ml). The resulting residue was suspended in CH_2Cl_2, then the insoluble material filtered off and dried: 0.836 g (75%) of **31**. Yellowish powder. TLC ($CHCl_3$/MeOH 9:1): R_f 0.37. UV (MeOH): 267 (4.38), 275 (sh, 4.32). ^{1}H-NMR ((D_6)DMSO): 2.25 (*m*, 1 H–C(2′)); 2.75 (*m*, 1 H–C(2′)); 2.95 (*t*, OCH_2CH_2CN); 3.32 (*t*, $ArCH_2CH_2O$); 3.65 (*m*, 2 H–C(5′)); 3.85 (*m*, H–C(4′)); 4.30 (*t*, CH_2O)); 4.4 (*m*, H–C(3′)); 4.75 (*t*, CH_2O); 4.88 (*t*, OH–C(5′)); 5.32 (*d*, OH–C(3′)); 6.32 (*t*, H–C(1′)); 7.65 (*d*, 2 H *m* to NO_2); 8.20 (*d*, 2 H *o* to NO_2); 8.42 (*s*, H–C(8)); 10.65 (*s*, NH–C(2)). Anal. calc. for $C_{22}H_{23}N_7O_8$ (513.5): C 51.46, H 4.51, N 19.10; found: C 51.52, H 4.66, N 18.66.

24. N^2-*[(2-Cyanoethoxy)carbonyl]-2′-deoxy-5′*-O-*(4,4′-dimethoxytrityl)*-O^6-*[2-(4-nitrophenyl)ethyl]guanosine* (**32**). Compound **31** (217 mg, 0.41 mmol) was co-evaporated twice with dry pyridine (2 × 2.5 ml) and then dissolved in a mixture of pyridine (0.5 ml) and CH_2Cl_2 (1.5 ml). Dimethoxytrityl chloride (172 mg, 0.51 mmol) was added and the soln. stirred for 2 h at r.t. After addition of MeOH (0.5 ml), the mixture was evaporated and the residue redissolved in CH_2Cl_2 (10 ml). The soln. was extracted with sat. $NaHCO_3$ soln. (2 × 5 ml), the org. phase evaporated and co-evaporated with toluene (2 × 5 ml), MeOH (2 × 5 ml), and CH_2Cl_2 (2 × 5 ml), and the residue purified by FC (silica gel (8 g), CH_2Cl_2 (600 ml)): 278 mg (81%) of **32**. Yellowish solid foam. TLC ($CHCl_3$/MeOH 9:1): R_f 0.55. UV (MeOH): 269 (4.41), 277 (sh, 4.34). ^{1}H-NMR ((D_6)DMSO): 2.35 (*m*, 1 H–C(2′)); 2.90 (*m*, 1 H–C(2′)); 2.95 (*t*, OCH_2CH_2CN); 3.15 (*m*, 2 H–C(5′)); 3.30 (*t*, $ArCH_2CH_2O$); 3.70 (*s*, 2 MeO); 3.95 (*m*, H–C(4′)); 4.31 (*t*, OCH_2CH_2CN); 4.55 (*m*, H–C(3′)); 4.75 (*t*, $ArCH_2CH_2O$); 5.3 (*d*, OH–C(3′)); 6.35 (*t*, H–C(1′)); 6.75 (*m*, 4 H *o* to MeO); 7.15–7.3 (*m*, 9 arom. H); 7.65 (*d*, 2 H *m* to NO_2); 8.18 (*d*, 2 H *o* to NO_2); 8.3 (*s*, H–C(8)); 10.48 (*s*, NH–C(2)). Anal. calc. for $C_{43}H_{41}N_7O_{10}$ (815.8): C 63.31, H 5.07, N 12.02; found: C 63.18, H 5.09, N 11.99.

25. N^2-*[(2-Cyanoethoxy)carbonyl]-2′-deoxy*-O^6-*[2-(phenylsulfonyl)ethyl]guanosine* (**36**). After co-evaporation of **35** (0.25 g, 0.57 mmol) in dry pyridine (3 × 2 ml), the residue was dissolved in a mixture of dry pyridine (2 ml) and CH_2Cl_2 (2 ml). Then Me_3SiCl (0.385 ml, 3.08 mmol) was added and stirred for 20 min to give a colorless precipitate of pyridinium chloride. To this suspension, **5** (0.115 g, 0.86 mmol) in CH_2Cl_2 (1 ml) was added, then the mixture stirred for 6 h, the reaction quenched by the addition of MeOH (15 ml), and the mixture evaporated after stirring for 20 min. The residue was treated with H_2O (20 ml) and AcOEt (20 ml), the

aq. phase extracted with AcOEt (3×20 ml), the combined org. phase evaporated and co-evaporated with toluene (2×10 ml), MeOH (2×20 ml), and CH_2Cl_2 (2×20 ml), and the residue purified by FC (silica gel (5 g) 0–5% MeOH in CH_2Cl_2 (700 ml)): 0.26 g (85%) of **36**. Colorless foam. TLC ($CHCl_3$/MeOH 9:1): R_f 0.32. UV (MeOH): 265 (4.15), 277 (sh, 4.0). ^{1}H-NMR ((D$_6$)DMSO): 2.30 (*m*, 1 H–C(2′)); 2.70 (*m*, 1 H–C(2′)); 2.95 (*t*, OCH_2CH_2CN); 3.55 (*m*, 2 H–C(5′)); 3.85 (*m*, H–C(4′)); 4.15 (*t*, $SO_2CH_2CH_2O$); 4.30 (*t*, OCH_2CH_2CN); 4.40 (*m*, H–C(3′)); 4.72 (*t*, $SO_2CH_2CH_2O$); 4.90 (*t*, OH–C(5′)); 5.31 (*d*, OH–C(3′)); 6.28 (*t*, H–C(1′)); 7.45–7.60 (*m*, 2 H *m* and 1 H *p* to SO_2); 7.85 (*m*, 2 H *o* to SO_2); 8.45 (*s*, H–C(8)); 10.55 (*s*, NH–C(2)). Anal. calc. for $C_{22}H_{24}N_6O_8S \cdot 0.5\ H_2O$ (541.5): C 48.80, H 4.28, N 15.52; found: C 48.99, H 4.52, N 15.35.

26. N^2-*[(2-Cyanoethoxy)carbonyl]-2′-deoxy-5′*-O-*(4,4′-dimethoxytrityl)*-O^6-*[2-(phenylsulfonyl)ethyl]guanosine* (**37**). After co-evaporation of **36** (0.25 g, 0.47 mmol) in dry pyridine (2×2 ml), the residue was dissolved in pyridine (2 ml) and CH_2Cl_2 (1.7 ml). Then dimethoxytrityl chloride (0.192 g, 0.57 mmol) was added and stirred for 50 min at r.t. The reaction was quenched with MeOH (0.5 ml), the mixture evaporated, and the residue redissolved in AcOEt (7.5 ml). The soln. was washed with sat. $NaHCO_3$ soln. (2×5 ml), dried (Na_2SO_4), evaporated, and co-evaporated with toluene (2×5 ml), MeOH (2×5 ml), and CH_2Cl_2 (2×5 ml), and the residue purified by FC (silica-gel (8 g), CH_2Cl_2 (250 ml), then CH_2Cl_2/MeOH 49:1 (200 ml)): 0.29 g (73%) of **37**. Yellowish foam. TLC ($CHCl_3$/MeOH 9:1): R_f 0.5. UV (MeOH): 267 (4.20), 281 (sh, 3.95). ^{1}H-NMR ((D$_6$)DMSO): 2.32 (*m*, 1 H–C(2′)); 2.83 (*m*, 1 H–C(2′)); 2.97 (*t*, OCH_2CH_2CN); 3.12 (*m*, 1 H–C(5′)); 3.29 (*m*, 1 H–C(5′)); 3.69 (*s*, 2 MeO); 3.98 (*m*, H–C(4′)); 4.12 (*t*, $SO_2CH_2CH_2O$); 4.28 (*t*, OCH_2CH_2CN); 4.53 (*m*, H–C(3′)); 4.73 (*t*, $SO_2CH_2CH_2O$); 5.30 (*d*, OH–C(3′)); 6.30 (*t*, H–C(1′)); 6.75 (*m*, 4 H *o* to MeO); 7.10–7.33 (*m*, 9 arom. H); 7.42–7.60 (*m*, 2 H *m* and 1 H *p* to SO_2); 7.88 (*m*, 2 H *o* to SO_2); 8.27 (*s*, H–C(8)); 10.47 (*s*, NH–C(2)). Anal. calc. for $C_{43}H_{42}N_6O_{10}S \cdot 0.5\ H_2O$ (843.8): C 61.20, H 5.02, N 9.96; found: C 61.17, H 5.05, N 9.85.

27. N^4-*2-[(Cyanoethoxy)carbonyl]-2′-deoxy-5′*-O-*(4,4′-dimethoxytrityl)cytidine 3′-(2-Cyanoethyl Diisopropylphosphoramidite)* (**38**). A soln. of **22** (1 g, 1.59 mmol) in dry CH_2Cl_2 (10 ml) was evaporated and dissolved in CH_2Cl_2 (10 ml). Then 1*H*-tetrazole (54 mg, 0.775 mmol) and 2-cyanoethyl tetraisopropylphosphorodiamidite (980 mg, 3.25 mmol) were added and stirred for 1 h under N_2. After dilution with CH_2Cl_2 (40 ml) and washing with 5% $NaHCO_3$ soln. (40 ml), the aq. phase was extracted with CH_2Cl_2 (2×40 ml), the combined org. phase dried (Na_2SO_4) and evaporated, and the resulting solid purified by FC (silica gel (17 g), toluene/AcOEt 1:1). The product fractions (>150 ml) containing both diastereoisomers were evaporated and co-evaporated with CH_2Cl_2 ($3\times$): 1.06 g (83%) of **38**. Solid foam. TLC ($CHCl_3$/MeOH 9:1): R_f 0.64, 0.69 (2 diastereoisomers). UV (MeOH): 282 (4.09), 303 (sh, 3.93), 235 (sh, 4.60), 206 (sh, 4.89). ^{1}H-NMR ($CDCl_3$): 1.29 (*d*, 2 Me_2CH); 2.34 (*m*, 1 H–C(2′)); 2.48 (*t*, CH_2CN); 2.67 (*m*, 1 H–C(2′)); 2.80 (*m*, 4 H, CH_2O, CH_2CN); 3.39–3.71 (*m*, 2 Me_2CH, 2 H–C(5′)); 3.80 (*s*, 2 MeO); 4.21 (*m*, H–C(4′)); 4.41 (*t*, CH_2O); 4.68 (*m*, H–C(3′)); 6.27 (*t*, H–C(1′)); 6.85 (*m*, 4 H *o* to MeO); 7.23–7.45 (*m*, 7 arom. H, H–C(5), H–C(6)); 8.35 (*s*, NH–C(4)). ^{31}P-NMR ($CDCl_3$): 149.96; 149.45. Anal. calc. for $C_{43}H_{51}N_6O_9P$ (826.9): C 62.46, H 6.22, N 10.16; found: C 61.86, H 6.79, N 10.20.

28. N^6-*[(2-Cyanoethoxy)carbonyl]-2′-deoxy-5′*-O-*(4,4′-dimethoxytrityl)adenosine 3′-(2-Cyanoethyl Diisopropylphosphoramidite)* (**39**). A soln. of **23** (1.5 g, 2.3 mmol) in CH_2Cl_2 (10 ml) was evaporated and the residue dissolved in CH_2Cl_2 (12 ml). Then 1*H*-tetrazole (80.7 mg, 1.15 mmol) and 2-cyanoethyl tetraisopropylphosphorodiamidite (1.38 g, 4.58 mmol) were added and stirred under N_2 at r.t. for 1 h. The mixture was diluted with CH_2Cl_2 (50 ml) and washed with 5% $NaHCO_3$ soln. (50 ml), the aq. phase extracted with CH_2Cl_2 (2×50 ml), the combined org. phase dried (Na_2SO_4) and evaporated, and the residue purified by FC (silica gel (30 g), toluene/AcOEt 2:3 (600 ml)). The combined fractions of both diastereoisomers were evaporated and co-evaporated with CH_2Cl_2: 1.70 g (87%) of **39**. Colorless foam. TLC ($CHCl_3$/MeOH 25:1): R_f 0.16, 0.19. UV (MeOH): 267 (4.35), 276 (4.22). ^{1}H-NMR ($CDCl_3$): 1.20 (*d*, 2 Me_2CH); 2.47 (*t*, CH_2CN); 2.62 (*t*, CH_2CN); 2.81 (*t*, CH_2O); 2.60–2.95 (*m*, 2 H–C(2′)); 3.30–3.80 (*m*, 2 Me_2CH, 2 H–C(5′)); 3.78 (*s*, 2 MeO); 4.32 (*m*, H–C(4′)); 4.47 (*t*, CH_2O); 4.80 (*m*, H–C(3′)); 6.48 (*t*, H–C(1′)); 6.78 (*d*, 4 H *o* to MeO); 7.15–7.45 (*m*, 9 arom. H); 8.19 (*s*, H–C(8)); 8.38 (*s*, H–C(2)); 8.71 (*s*, NH–C(6)). ^{31}P-NMR ($CDCl_3$): 149.544; 149.420. Anal. calc. for $C_{44}H_{51}N_8O_8P$ (850.9): C 62.11, H 6.04, N 13.17; found: C 62.05, H 6.28, N 12.44.

29. N^2-*[(2-Cyanoethoxy)carbonyl]-2′-deoxy-5′*-O-*(4,4′-dimethoxytrityl)*-O^6-*[2-(4-nitrophenyl)ethyl]guanosine 3′-(2-Cyanoethyl Diisopropylphosphoramidite)* (**40**). A soln. of **32** (0.80 g, 0.98 mmol) in CH_2Cl_2 (3 ml) was evaporated and the residue dissolved in CH_2Cl_2 (5 ml). Then, 1*H*-tetrazole (34.5 mg, 0.49 mmol) and 2-cyanoethyl tetraisopropylphosphorodiamidite (591 mg, 1.95 mmol) were added. After stirring at r.t. for 1 h, the soln. was diluted with CH_2Cl_2 (20 ml) and washed with 5% $NaHCO_3$ soln. (20 ml). The aq. phase was extracted with CH_2Cl_2 (2×20 ml), the combined org. phase dried (Na_2SO_4) and evaporated, and the resulting residue purified by FC (silica gel (18 g), toluene/AcOEt 2:3 (400 ml)): 0.836 g (84%) of **40**. Yellowish solid foam. TLC (toluene/AcOEt 1:1): R_f 0.28, 0.40. UV (MeOH): 269 (4.38), 236 (sh, 4.42, 206 (sh, 4.83). ^{1}H-NMR ($CDCl_3$):

1.17 (*d*, 2 *Me*$_2$CH); 2.60–2.95 (*m*, 2 H–C(2′)); 2.64 (*t*, CH_2CN); 2.76 (*t*, CH_2CN); 3.33 (*t*, ArCH_2, CH_2O); 3.50–3.90 (*m*, 2 Me_2CH, 2 H–C(5′)); 3.79 (*s*, 2 MeO); 4.38 (*m*, H–C(4′)); 4.39 (*t*, CH_2O); 4.84 (*t*, CH_2O–C(6), H–C(3′)); 6.38 (*t*, H–C(1′)); 6.77 (*d*, 4 H *o* to MeO); 7.15–7.40 (*m*, 9 arom. H); 7.55 (*d*, 2 H *m* to NO_2); 8.18 (*d*, 2 H *o* to NO_2); 7.99 (*s*, H–C(8)). ^{31}P-NMR ($CDCl_3$): 149.45; 149.31. Anal. calc. for $C_{52}H_{58}N_9O_{11}P$ (1016.1): C 61.47, H 5.75, N 12.41; found: C 61.05, H 6.44, N 11.92.

30. *Kinetics.* An exact amount of nucleoside was weighed into a screw-cap glass vial (5 ml) and mixed with a 15-fold molar excess of 0.5M DBU/MeCN. This soln. was stirred at 22–23°. Samples were taken with a 25-µl *Eppendorf* pipette and quenched by the addition to 0.25M AcOH (0.5 ml). The samples were stored in a refrigerator until the measurement was performed. The nucleosides 2′-deoxy-5′-*O*-(4,4′-dimethoxytrityl)-N^4-{[2-(4-nitrophenyl)ethoxy]carbonyl}cytidine (C^{npeoc}), 2′-deoxy-5′-*O*-(4,4′-dimethoxytrityl)-N^6-{[2-(4-nitrophenylethyl)-ethoxy]carbonyl}adenosine (A^{npeoc}) and 2′-deoxy-5′-*O*-(4,4′-dimethoxytrityl) -O^6-[2-(4-nitrophenyl)ethyl]-N^2-{[2-(4-nitrophenyl)ethoxy]carbonyl}guanosine ($G^{npe,npeoc}$) used for comparison with the ce/ceoc-substituted nucleosides were prepared as described in [2].

REFERENCES

[1] T. Reiner, E. Kvasyuk, W. Pfleiderer, *Helv. Chim. Acta* **2000**, *83*, 3053.
[2] F. Himmelsbach, B. S. Schulz, T. Trichtinger, R. Charubala, W. Pfleiderer, *Tetrahedron* **1984**, *40*, 59.
[3] E. Uhlmann, W. Pfleiderer, *Helv. Chim. Acta* **1981**, *64*, 1688.
[4] M. Ichiba, R. Charubala, R. S. Varma, W. Pfleiderer, *Helv. Chim. Acta* **1986**, *69*, 1768.
[5] L. J. McBride, M. M. Caruthers, *Tetrahedron Lett*. **1983**, *24*, 245.
[6] A. H. Beiter, W. Pfleiderer, *Tetrahedron Lett*. **1984**, *25*, 1975.
[7] G. M. Tener, *J. Am. Chem. Soc*. **1961**, *83*, 159.
[8] N. D. Sinha, J. Bierat, H. Köster, *Tetrahedron Lett*. **1983**, *24*, 5843.
[9] C. Merk, Diploma Thesis, University of Konstanz, 1994.
[10] W. Pfleiderer, C. Merk, German Pat. DE 97-19745708 A 1, 30.4.1998.
[11] M. Manoharan, T. P. Prakash, I. Barber-Peocv'h, B. Bhat, G. Vasquez, B. S. Ross, P. D. Cook, *J. Org. Chem*. **1999**, *64*, 6468.
[12] V. I. Kondratenko, I. G. Khaskin, *J. Org. Chem. U.S.S.R.* **1970**, *6*, 2231.
[13] G. S. Ti, B. L. Gaffney, R. A. Jones, *J. Am. Chem. Soc*. **1982**, *104*, 1316.
[14] B. L. Gaffney, L. A. Marky, R. A. Jones, *Tetrahedron* **1984**, *40*, 3.
[15] X. Gao, B. L. Gaffney, S. Hadden, R. A. Jones, *J. Org. Chem*. **1986**, *51*, 755.
[16] I. Kieper, T. Schmidt, B. Fera, H. Rüterjans, *Nucleosides Nucleotides* **1988**, *7*, 821.
[17] R. T. Pon, N. Usman, M. H. Damha, K. K. Ogilvie, *Nucleic Acids Res.* **1986**, *14*, 6453.

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