Nucleotides

Part LX[1])

Synthesis and Characterization of New 2′-*O*-Methylriboside 3′-*O*-Phosphoramidites Useful for the Solid-Phase Synthesis of 2′-*O*-Methyloligoribonucleotides

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A series of new 2′-*O*-methylribonucleoside 3′-*O*-[2-(4-nitrophenyl)ethyl dialkylphosphoramidites] **27**–**31**, **33**–**38**, **40**–**44**, and **45**–**50** were synthesized and their stability and reactivity compared in automated oligonucleotide synthesis with the standard 2′-*O*-methylribonucleoside 3′-*O*-(β-cyanoethyl diisopropylphosphoramidites) **32**, **39**, **45**, and **51**, respectively. The 2-(4-nitrophenyl)ethyl (npe) and 2-(4-nitrophenyl)ethoxycarbonyl (npeoc) groups were used for the protection of the base moieties.

1. Introduction. – The first nucleoside phosphoramidites as building blocks for oligodeoxyribonucleotide synthesis which were introduced by *Caruthers* and coworkers in the beginning of the 1980's [2–5] were very reactive and thus difficult to work with. The stability of the phosphoramidites were subsequently increased by the replacement of the dimethylamino with the diisopropylamino group in the phosphoramidite moiety. The introduction of 2-cyanoethyl as phosphate-protecting group by *Köster* and coworkers [6][7] led to the 2-cyanoethyl diisopropylphosphoramidites, which are so successful that they are still predominant today.

In oligoribonucleotide synthesis, the additional 2′-protecting group makes the phosphoramidites less reactive. The replacement of the diisopropylamino group by smaller dialkylamino groups, such as the diethylamino group, may make the phosphoramidites more reactive [8][9]; however, this leads to a less stable phosphoramidite as well. Therefore, *Stengele* [10], in a search for other β-eliminating phosphate groups which would stabilize the diethyl phosphoramidites, reported equally reactive but more stable phosphoramidites when using 2-(4-nitrophenyl)ethyl instead of 2-cyanoethyl as phosphate-protecting group. The 2-(4-nitrophenyl)ethyl group was introduced by *Pfleiderer* and coworkers [11–15] as a phosphate-protecting group.

Inspired by the good results of the 2-(4-nitrophenyl)ethyl phosphoramidites in oligoribonucleotide synthesis reported by *Stengele*, we synthesized different 2′-*O*-methylnucleoside 2-(4-nitrophenyl)ethyl phosphoramidites and compared their stability and reactivity with those of the 2-cyanoethyl diisopropyl phosphoramidites in 2′-*O*-methyloligoribonucleotide synthesis. Specifically, we employed for our investigations

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diisopropyl-, diethyl-, isopropyl(methyl)-, and ethyl(isopropyl)phosphoramidites protected as their 2-(4-nitrophenyl)ethyl esters.

The 2-(4-nitrophenyl)ethyl (npe) and 2-(4-nitrophenyl)ethoxycarbonyl (npeoc) groups were also used as base-protecting groups, because they can be removed after oligonucleotide synthesis selectively by 1,8-diazabicyclo[5.4.0]undec-7-ene (DBU) or 1,5-diazabicyclo[4.3.0]non-5-ene (DBN) in aprotic solvents, while the oligonucleotide is still attached to the solid support. This offers the advantage of synthesizing very pure oligonucleotides in a direct manner without further additional purification steps.

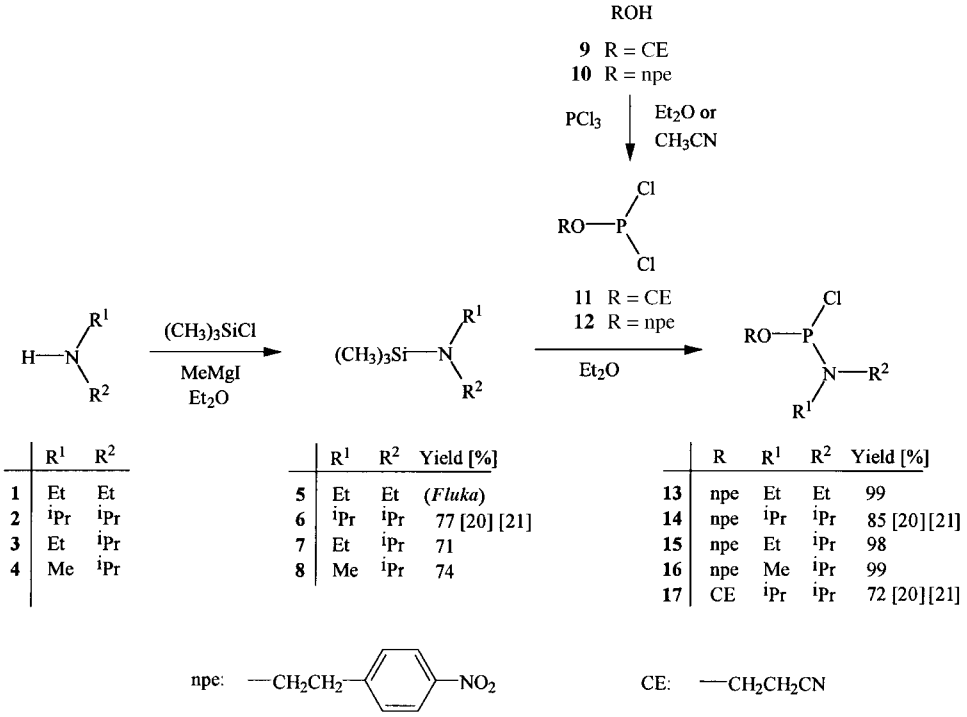
2. Syntheses. – All phosphoramidites were synthesized by phosphitylation using alkyl dialkylphosphoramidochloridites (=alkoxy(chloro)(dialkylamino)phosphanes) which were preferred over alkyl tetraalkylphosphorodiamidites (=alkoxybis(dialkylamino)phosphanes) because of their higher stability when a less bulky *N*,*N*-dialkyl moiety is used [16] and because of the easy removal of excess phosphitylating reagent by reaction with propan-2-ol (^{i}PrOH) [17]. If the reaction is not stopped with ^{i}PrOH, excess phosphitylation reagent hydrolyzes during the workup procedure. Hydrolyzed phosphitylation reagent cannot be removed easily from the product, because it has almost the same chromatographic mobility on silica gel as the phosphoramidite itself. By converting the excess alkyl dialkylphosphoramidochloridite into its alkyl isopropyl diester, separation from the phosphoramidite is achieved easily by silica-gel column chromatography.

The alkyl dialkylphosphoramidochloridites **13**–**17** were prepared by condensing the appropriate *N*,*N*-dialkyl-1,1,1-trimethylsilanamine **5**–**9** with the alkyl phosphorodichloridite **11** or **12** (*Scheme 1*). *N*,*N*-Diethyl-1,1,1-trimethylsilanamine (**5**) is commercially available. The other *N*,*N*-dialkyl-1,1,1-trimethylsilanamines were synthesized by the procedure of *Transjö* [18] in yields ranging from 71 to 77%. The 2-cyanoethyl phosphorodichloridite (**11**) and the 2-(4-nitrophenyl)ethyl phosphorodichloridite (**12**) were prepared by reaction of the appropriate alcohol with excess phosphorus trichloride according to the published procedures [13][19]. Distillation of **12** is not recommended due to possible decomposition. The 2-cyanoethyl diisopropylphosphoramidochloridite (**17**) could be distilled under high vacuum whereas the 2-(4-nitrophenyl)ethyl dialkylphosphoramidochloridites **13**–**16** decomposed during anticipated destillation and were, therefore, used without further purification [20][21].

For the synthesis of the 2′- and 3′-*O*-methylnucleoside phosphoramidites **27**–**51** and **56**–**59**, the appropriate protected nucleosides **18**–**26** and **52**–**55** [22] were treated with the alkyl dialkylphosphoramidochloridites **13**–**17** in the presence of *Hünig*'s base (*Schemes 2* and *3*). Yields after workup and silica-gel flash chromatography were between 70 and 90%. As solvent for the phosphitylation reaction, CH_2Cl_2, which takes up moisture less easily, was usually preferred over tetrahydrofuran (THF) since additional H_2O led to more hydrolyzed phosphitylation reagent.

The phosphitylation reaction of 2′-*O*-methyl-5′-*O*-(monomethoxytrityl)-N^2-[2-(4-nitrophenyl)ethoxycarbonyl]-O^6-[2-(4-nitrophenyl)ethyl]guanosine (**25**) gave rise to a by-product which, according to the ^{1}H- and ^{31}P-NMR spectra, was caused by an additional phosphitylation at the N(2) position of the base in spite of protection with the 2-(4-nitrophenyl)ethoxycarbonyl group. In THF, considerably less by-product was formed than in CH_2Cl_2. For this reason, the guanosine phosphoramidites were synthesized

Scheme 1

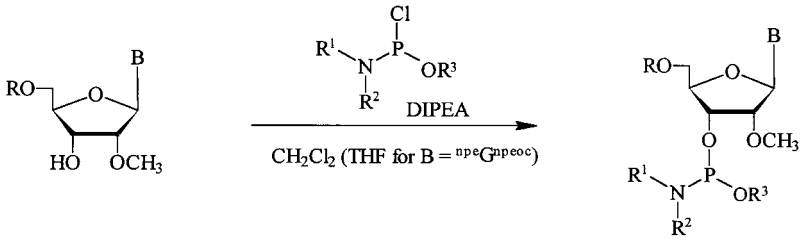


in THF, with the exception of 2′-*O*-methyl-5′-*O*-(monomethoxytrityl)-N^2-[2-(4-nitrophenyl)ethoxycarbonyl]-O^6-[2-(4-nitrophenyl)ethyl]guanosine 3′-[2-(4-nitrophenyl)-ethyl diisopropylphosphoramidite] (**48**) because of the poor solubility of the phosphitylating reagent 2-(4-nitrophenyl)ethyl diisopropylphosphoramidochloridite (**14**) in THF.

The phosphoramidites generally were purified by silica-gel column chromatography and a petroleum ether/AcOEt gradient. With this solvent mixture, the phosphoramidite was eluted prior to the hydrolyzed phosphitylating reagent, except in the case of **28**, **35**, and **41** which were separated from the hydrolyzed phophitylation reagent by a petroleum ether/acetone gradient eluting the H-phosphonate prior to the phosphoramidite. Purification of the npe/npeoc-protected guanosine phosphoramidites was not problematic because of their higher R_f values in comparison to the hydrolyzed phosphitylating reagent.

Solid-phase oligonucleotide synthesis was performed by analogy to published procedures [2–4][23]. Solid-phase synthesis *via* the npe/npeoc approach requires a DBU-stable linkage of the starting nucleoside through a spacer molecule to a glass-bead support [10][24][25]. Therefore, each of the eight succinylated nucleosides **60**–**67** were synthesized by reaction of the appropriate protected nucleoside **18**, **21**, **23**, **25**, and **52**–**55** with succinic anhydride and 4-(dimethylamino)pyridine in CH_2Cl_2 [26] in almost quantitative yields (*Scheme 4*). The succinylated nucleosides **60**–**67** were then reacted with LCMAA-CPG (= long-chain (methylamino)alkyl controlled-pore glass, 500 Å;

Scheme 2

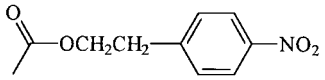


	Base	R
18	Ura	MeOTr
19	Ura	$(MeO)_2Tr$
20	Ura^{An}	MeOTr
21	Cyt^{npeoc}	MeOTr
22	Cyt^{npeoc}	$(MeO)_2Tr$
23	Ade^{npeoc}	MeOTr
24	Ade^{npeoc}	$(MeO)_2Tr$
25	$^{npe}Gua^{npeoc}$	MeOTr
26	$^{npe}Gua^{npeoc}$	$(MeO)_2Tr$

	Base	R	R^1	R^2	R^3	Yield [%]
27	Ura	MeOTr	Et	Et	npe	83
28	Ura	$(MeO)_2Tr$	Et	Et	npe	82
29	Ura	MeOTr	^iPr	^iPr	npe	73
30	Ura	MeOTr	Et	^iPr	npe	81
31	Ura	MeOTr	Me	^iPr	npe	82
32	Ura	MeOTr	^iPr	^iPr	CE	85
33	Ura^{An}	MeOTr	Et	Et	npe	81
34	Cyt^{npeoc}	MeOTr	Et	Et	npe	89
35	Cyt^{npeoc}	$(MeO)_2Tr$	Et	Et	npe	84
36	Cyt^{npeoc}	MeOTr	^iPr	^iPr	npe	72
37	Cyt^{npeoc}	MeOTr	Et	^iPr	npe	90
38	Cyt^{npeoc}	MeOTr	Me	^iPr	npe	83
39	Cyt^{npeoc}	MeOTr	^iPr	^iPr	CE	87
40	Ade^{npeoc}	MeOTr	Et	Et	npe	88
41	Ade^{npeoc}	$(MeO)_2Tr$	Et	Et	npe	80
42	Ade^{npeoc}	MeOTr	^iPr	^iPr	npe	72
43	Ade^{npeoc}	MeOTr	Et	^iPr	npe	84
44	Ade^{npeoc}	MeOTr	Me	^iPr	npe	80
45	Ade^{npeoc}	MeOTr	^iPr	^iPr	CE	81
46	$^{npe}Gua^{npeoc}$	MeOTr	Et	Et	npe	83
47	$^{npe}Gua^{npeoc}$	$(MeO)_2Tr$	Et	Et	npe	80
48	$^{npe}Gua^{npeoc}$	MeOTr	^iPr	^iPr	npe	72
49	$^{npe}Gua^{npeoc}$	MeOTr	Et	^iPr	npe	81
50	$^{npe}Gua^{npeoc}$	MeOTr	Me	^iPr	npe	82
51	$^{npe}Gua^{npeoc}$	MeOTr	^iPr	^iPr	CE	80

CE, npe: see *Scheme 1*

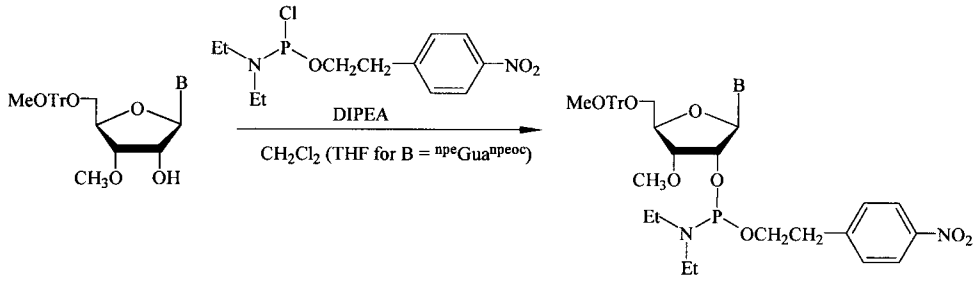
An: 4–$MeOC_6H_4$

npeoc: 

68) with the coupling reagent 2-{[2-(2-cyanoethoxy)-2-oxoethylidene]amino}-1,1,3,3-tetramethyluronium tetrafluoroborate (TOTU) and 4-methylmorpholine in MeCN, followed by a capping process with Ac_2O and 4-(dimethylamino)pyridine in pyridine to give the solid supports **69**–**76** in loadings of 23–38 μmol/g. Loadings were determined according to *Atkinson* and *Smith* [27].

For the assembly of the oligonucleotides, an automated DNA synthesizer was used. A synthesis column filled with the desired starting nucleoside **69**–**76** was attached to the synthesizer. The subsequent oligonucleotide synthesis consisted of a programmed repetitive cycle of chemical reactions such as deprotection of the terminal trityl group with CF_3COOH, coupling with a 0.1M solution of nucleoside phosphoramidite **27**–**51**

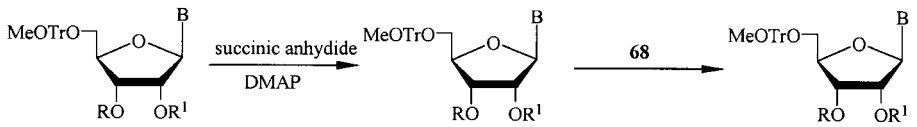
Scheme 3



	Base
52	Ura
53	Cyt^{npeoc}
54	Ade^{npeoc}
55	^{npe}Gua^{npeoc}

	Base	Yield [%]
56	Ura	78
57	Cyt^{npeoc}	79
58	Ade^{npeoc}	77
59	^{npe}Gua^{npeoc}	75

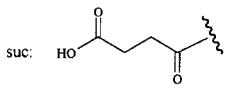
Scheme 4

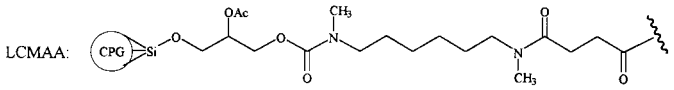


	Base	R	R^1
18	Ura	H	OMe
21	Cyt^{npeoc}	H	OMe
23	Ade^{npeoc}	H	OMe
25	^{npe}Gua^{npeoc}	H	OMe
52	Ura	OMe	H
53	Cyt^{npeoc}	OMe	H
54	Ade^{npeoc}	OMe	H
55	^{npe}Gua^{npeoc}	OMe	H

	Base	R	R^1
60	Ura	suc	OMe
61	Cyt^{npeoc}	suc	OMe
62	Ade^{npeoc}	suc	OMe
63	^{npe}Gua^{npeoc}	suc	OMe
64	Ura	OMe	suc
65	Cyt^{npeoc}	OMe	suc
66	Ade^{npeoc}	OMe	suc
67	^{npe}Gua^{npeoc}	OMe	suc

	Base	R	R^1
69	Ura	LCMAA	OMe
70	Cyt^{npeoc}	LCMAA	OMe
71	Ade^{npeoc}	LCMAA	OMe
72	^{npe}Gua^{npeoc}	LCMAA	OMe
73	Ura	OMe	LCMAA
74	Cyt^{npeoc}	OMe	LCMAA
75	Ade^{npeoc}	OMe	LCMAA
76	^{npe}Gua^{npeoc}	OMe	LCMAA





or **56**–**59**, and 1*H*-tetrazole, capping of any unreacted OH functions by acetylation with Ac_2O, and oxidation of the phosphite triester with I_2 as well as excessive washing steps. The coupling efficiency of each condensation was determined by absorption measurement of the released trityl solutions. The last synthesis cycle was ended after the detritylation step to give a 'trityl-off' product. Because no further purification was anticipated, there is no need for a 'trityl-on' oligonucleotide. To remove all npe and npeoc protecting groups, the support was treated with 1M DBU in dry MeCN for 10 h. Thereafter, the fully deblocked oligonucleotide was cleaved from the support by treatment with concentrated NH_3 solution for 2 h. Finally, the products were

lyophilized in a *Speed-vac* concentrator, and the quality of the crude 2′- and 3′-*O*-methyloligoribonucleotides was analyzed by reversed-phase HPLC.

3. Discussion. – The stability of the phosphoramidites in solution was tested using decoupled ^{31}P-NMR spectroscopy. Thus, 20 mg of the phosphoramidite were dissolved in 0.4 ml of $CDCl_3$ in a NMR tube which was flushed with Ar and stoppered. A first spectrum was measured immediately after dissolution and a second one after 2 weeks at room temperature. Some of the $CDCl_3$ evaporated thereby and was re-added. The stability of phosphoramidite **27** in $CDCl_3$ was compared to its stability in CD_3CN showing that more degradation products were formed in the latter solvent within the same period of time (*Fig. 1*).

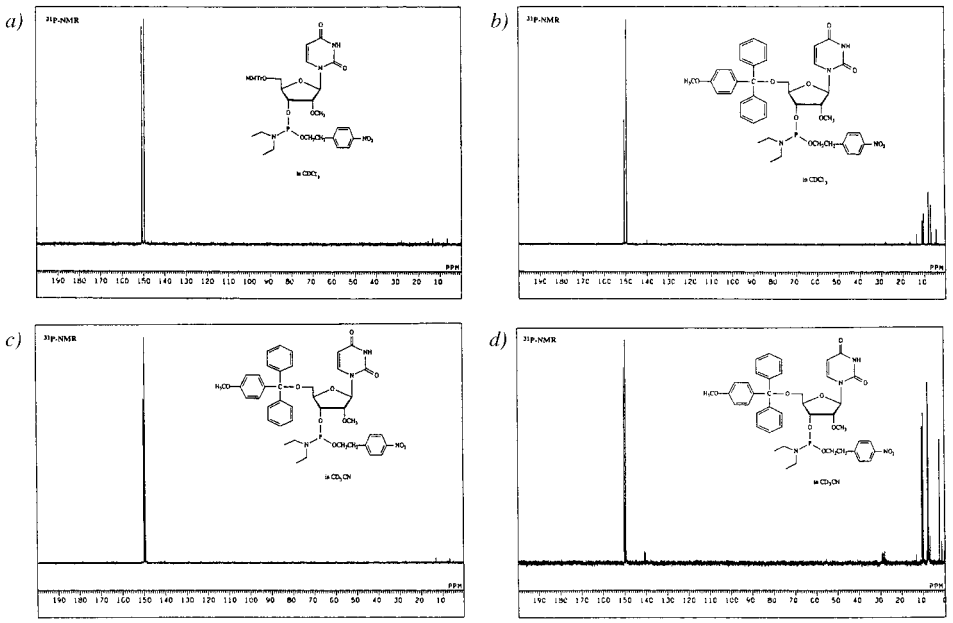


Fig. 1. *Stability of 2′-*O*-methyl-5′-*O*-(monomethoxytrityl)uridine 3′-[2-(4-nitrophenyl)ethyldiethylphosphoramidite]* (**27**) *at room temperature in $CDCl_3$ and in CD_3CN as determined by ^{31}P-NMR spectroscopy:* a) c) *spectra after dissolving in $CDCl_3$ and CDCN, respectively, and* b) d) *spectra after 14 days in $CDCl_3$ and CD_3CN solution, respectively*

The optimal condensation time for each of the phosphoramidites was determined by synthesizing the short homologous oligonucleotides **77**–**90** using a 1-μmol RNA cycle for the synthesis on a 0.6-μmol scale and a 0.2-μmol RNA cycle for the 0.2-μmol scale. Coupling times were altered from 600 to 40 s. The trityl values and the purity of the raw products were taken into consideration for the determination of the average stepwise yield (ASWY) of the different phosphoramidites at different coupling times. When using 5′-(monomethoxytrityl)-2′-*O*-methyluridine 3′-[2-(4-nitrophenyl)ethyl diethylphosphoramidite] (**27**), 5′-(monomethoxytrityl)-2′-*O*-methyluridine 3′-[2-(4-nitrophenyl)ethyl ethyl(isopropyl)phosphoramidite] (**30**), 5′-(monomethoxytrityl)-2′-*O*-methyluridine 3′-[2-(4-nitrophenyl)ethyl isopropyl(methyl)phosphoramidite] (**31**),

or 5′-(monomethoxytrityl)-2′-*O*-methyluridine 3′-(2-cyanoethyl diisopropylphosphoramidite) (**32**), coupling times of 40 s were already sufficient to give ASWYs of >99% for the synthesis of the decakis(2′-*O*-methyluridylate) (**77**), whereas the same synthesis with 5′-(monomethoxytrityl)-2′-*O*-methyluridine 3′-[2-(4-nitrophenyl)ethyl diisopropylphosphoramidite] (**29**) and a coupling time of 120 s gave only a ASWY below 98% (*Table*). Similar results were obtained for the corresponding adenosine phosphoramidites (→ **81**–**83**) whereas the cytidine and guanosine derivatives showed less reactivity (→ **79** and **80**, and **84**–**86**, resp.).

Thus, a coupling time of 300 s was needed for an almost quantitative condensation of cytidine phosphoramidites **36**, **37**, and **38** (→ **80**), whereas a coupling time of 120 s gave only an ASWY of *ca.* 97% (see *Fig. 2*), except in the case of 2′-*O*-methyl-5′-(monomethoxytrityl)-N^4-[2-(4-nitrophenyl)ethoxycarbonyl]cytidine 3′-[2-(4-nitrophenyl)ethyl diisopropylphosphoramidite] (**36**) which showed only an ASWY of 92.1% under the same conditions (*Table*).

The 2′-*O*-methyl-5′-*O*-(monomethoxytrityl)-N^2-[2-(4-nitrophenyl)ethoxycarbonyl]-O^6-[2-(4-nitrophenyl)ethyl]guanosine 3′-[2-(4-nitrophenyl)ethyl diisopropylphosphoramidite] (**48**) was again less reactive than all other tested guanosine phosphoramidites and gave only a ASWY of 97.6% when coupled for 300 s (→ **84**) in comparison to a ≥99% ASWY for phosphoramidites **46** and **51** (→ **84**–**86**).

No differences in the quality of synthesized oligonucleotides were found when changing from the 1.0-μmol RNA cycle for the synthesis on the 0.6-μmol scale to the 0.2-μmol RNA cycle and 0.2-μmol scale, maintaining otherwise the same conditions. As discussed above, 2-(4-nitrophenyl)ethyl phosphoramidites derived from different bases each bearing two corresponding alkyl groups did not show the same reactivity. Therefore, coupling times in correlation to the base (A, U shorter, G and C longer) as described by *Lyttle et al.* for oligoribonucleotide synthesis [9] could reduce the time needed for a 2′-*O*-methyloligoribonucleotide synthesis without loss of product purity.

As expected, the exchange of the 2-cyanoethyl by the 2-(4-nitrophenyl)ethyl group stabilized the phosphoramidite. At the same time, this made the phosphoramidite less reactive in contrast to the reported findings of *Stengele* who claimed equivalent reactivity for 2′-*O*-{[2-(4-nitrophenyl)ethyl]sulfonyl}ribonucleoside 3′-phosphoramidites [10]. The 2′-*O*-methylnucleoside 3′-[2-(4-nitrophenyl)ethyl diisopropylphosphoramidites] are very stable, and they did not show any detectable degradation after 14 days in comparison with a 5 to 10% degradation of the 2-cyanoethyl diisopropylphosphoramidites under the same conditions. But as shown by the synthesis of homo-oligonucleotides, it turned out that the 2-(4-nitrophenyl)ethyl diisopropylphosphoramidites needed longer coupling times for the condensation reaction than the equivalent cyanoethyl diisopropylphosphoramidites.

4. Conclusion. – The exchange of the 2-cyanoethyl by the 2-(4-nitrophenyl)ethyl group in 2-cyanoethyl diisopropylphosphoramidites makes the phosphoramidites more stable towards decomposition but less reactive. To obtain equally reactive phosphoramidites the diisopropylamino moiety can be replaced by a diethylamino, isopropyl(methyl)amino, as well as an ethyl(isopropyl)amino moiety. All those phosphoramidites show about the same reactivity in oligonucleotide synthesis and the same stability in $CHCl_3$ solution. An advantage of the 2-(4-nitrophenyl)ethyl phosphoramidites over

Table. *Synthesized 2′- and 3′-O-Methyloligoribonucleotides*

Sequence	Amidite	Scale [μmol]	Coupling time [s]	ASWY [%][a])	Yield [OD_{260}]
5′-$(U_m)_{10}$-3′ (**77**)	**27**	0.6	600	100	46
	27	0.6	300	100	42
	32	0.6	300	100	46
	27	0.6	120	99.5	43
	29	0.6	120	97.6	40
	30	0.6	120	99.1	45
	31	0.6	120	100	41
	32	0.6	60	100	45
	32	0.2	60	100	20
	27	0.2	40	100	15
	30	0.2	40	100	14
	31	0.2	40	99.1	14
	32	0.2	40	99.2	13
5′-$(U_m)_{18}$-3′ (**78**)	**31**	0.6	120	100	40
	33	0.5	120	98.1	79[b])
5′-$(C_m)_6$-3′ (**79**)	**39**	0.6	300	100	21
5′-$(C_m)_{10}$-3′ (**80**)	**34**	0.6	600	99.9	38
	36	0.6	600	100	53
	37	0.6	600	99.5	45
	38	0.6	600	100	36
	36	0.6	300	100	47
	37	0.6	300	100	42
	38	0.6	300	100	49
	34	0.2	120	97.3	8
	36	0.2	120	92.1	10.6
	37	0.2	120	96.4	8.5
	38	0.2	120	97.3	9
	39	0.2	120	97.0	10
	39	0.6	120	97.1	42
5′-$(A_m)_5$-3′ (**81**)	**44**	0.2	40	100	10
5′-$(A_m)_{10}$-3′ (**82**)	**42**	0.6	120	98.5	57
	44	0.2	120	99.9	18
5′-$(A_m)_{18}$-3′ (**83**)	**43**	0.2	120	99.7	30
5′-$(G_m)_6$-3′ (**84**)	**48**	0.2	300	97.6	17
	51	0.2	300	99.0	17
5′-$(G_m)_8$-3′ (**85**)	**51**	0.2	300	99.1	23
	51	0.2	120	97.8	22
5′-$(G_m)_{10}$-3′ (**86**)	**46**	0.6	600	99.4	66
	46	0.6	300	99.4	52
5′-$(U_m)_{10}$-2′ (**87**)	**56**	0.6	300	99.9	34
5′-$(C_m)_{10}$-2′ (**88**)	**57**	0.6	300	95.6	30
5′-$(A_m)_{10}$-2′ (**89**)	**58**	0.2	120	100	21
5′-$(G_m)_{10}$-2′ (**90**)	**59**	0.2	120	95.5	17

[a]) ASWY: average stepwise yield. [b]) Raised *OD* value due to contamination with anisamide.

the 2-cyanoethyl phosphoramidite is the possible TLC detection by UV light of hydrolyzed phosphitylating agent during their synthesis. This makes the separation of hydrolyzed phosphitylating agent from the target phosphoramidite during the purification by column chromatography (silica gel) easier.

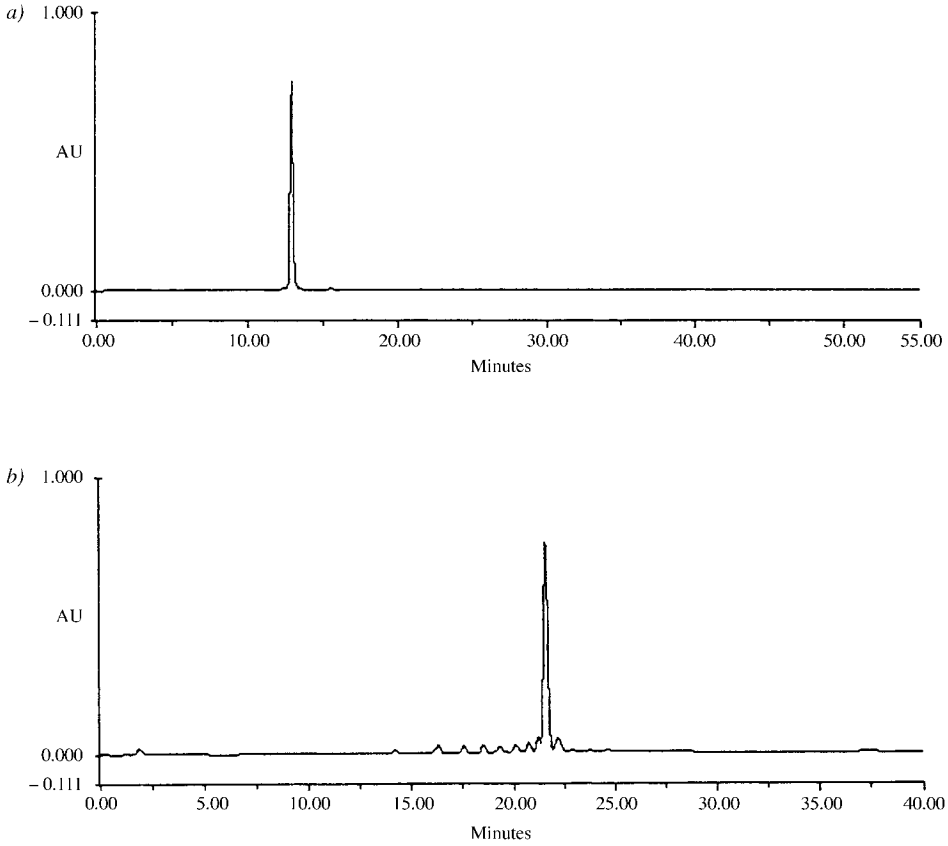


Fig. 2. *Reversed-phase HPLC of 5′-$(C_m)_{10}$-3′* (**80**), *with* **37** *as phosphoramidite:* a) *coupling time 300 s and* b) *coupling time 120 s.* Column: *LiChrospher 100 RP-18,* 5 μm, 4 × 125 mm (*Merck*); gradient: 2.5% MeCN in 0.1M (Et_3NH)Ac (pH 7) for 2 min and then 2.5–20% MeCN in 0.1M (Et_3NH)Ac (pH 7) within 30 min; flow 1 ml/min.

5. Physical Data. – The structural assignments of the newly synthesized compounds are based on elemental analyses, UV, ^{1}H-NMR, and ^{31}P-NMR spectra.

The silylamines **5**–**8** do not have a chromophore and, therefore, do not show a characteristic UV spectrum. Due to their volatility, no elemental analysis data were obtained either. The UV and ^{1}H-NMR spectra of the 2-(4-nitrophenyl)ethyl dialkylphosphoramidochloridites **13**–**16** are dominated by the 2-(4-nitrophenyl)ethyl group. The phosphoramidites **27**–**51** and **56**–**59** show similar UV spectra as their starting nucleosides. The chemical shifts of the ^{1}H-NMR spectra do not show much change in comparison to the starting nucleosides. Additional signals can be seen from the phosphoramidite's alkyl groups. The introduction of the phosphorus(III) center leads to two diastereoisomeric products. This results in a doubling of most of the ^{1}H-NMR signals and makes the interpretation difficult, especially in the range from 2.5 to 5 ppm. The ^{31}P-NMR spectra show with how many P-containing products the phosphoramidite is contaminated. The phosphoramidites exhibit two characteristic

signals (diastereoisomers) with chemical shifts at *ca.* 150 ppm, whereas degradation products from the phosphoramidite as well as the phosphitylating reagent have chemical shifts in the range of 0 to 35 ppm.

Experimental Part

General: Products were dried under high vacuum. TLC: precoated silica-gel thin-layer sheets *F1500 LS 254* from *Schleicher & Schuell* or *60* F_{254} from *Merck*. Flash chromatography (FC): silica gel (*Baker*, 30–60 μm); 0.2–0.3 bar. M.p.: *Gallenkamp* or *Büchi-510* melting-point apparatus; no corrections. UV/VIS: *Perkin-Elmer, Lambda 15*; λ_{max} in nm (log ε). ^{1}H-NMR: *Bruker AC 250*; in ppm rel. to $SiMe_4$ or $CDCl_3$ ((D_6)DMSO, D_2O) as internal standard. ^{31}P-NMR: *Joel 400 MHz*; in ppm rel. to H_3PO_4.

1. N-*Ethyl*-N-*isopropyl-1,1,1-trimethylsilanamine* (**7**). To Mg chips (5.47 g, 0.225 mol) suspended in anh. Et_2O (60 ml), MeI (14.1 ml, 32 g, 0.225 mol) in anh. Et_2O (30 ml) was added dropwise with stirring within *ca.* 40 min so that the soln. was refluxed slightly. The mixture was refluxed for an additional 50 min, and then *N*-ethyl-*N*-isopropylamine (**3**) (25 ml, 18 g, 0.21 mol) was added dropwise. The mixture was refluxed for 1 h, and after cooling to 0°, trimethylsilyl chloride (Me_3SiCl; 28.6 ml, 24.4 g, 0.225 mol) was added within 10 min under vigorous stirring. After refluxing for 20 h, the mixture was allowed to cool. The colorless liquid was decanted from the brownish residue. The residue was washed with anh. Et_2O (3 × 30 ml), and the combined liquids were evaporated. The remaining colorless liquid was fractionally distilled *in vacuo* under ice-cooling of the receiving flasks: 23.4 g (71%) of **7**. Colorless liquid. B.p. 27°/8 Torr. ^{1}H-NMR ($CDCl_3$): 3.20 (*sept.*, Me_2CH); 2.72 (*q*, $MeCH_2$); 1.02 (*d*, Me_2CH); 0.92 (*t*, $MeCH_2$); 0.02 (*s*, Me_3Si).

2. N-*Isopropyl*-N,*1,1,1-tetramethylsilanamine* (**8**). As described for **7**, with Mg chips (7.3 g, 0.3 mol), Et_2O (80 ml), MeI (18.8 ml, 43 g, 0.3 mol), and Et_2O (40 ml; reflux for an additional 60 min), *N*-methylisopropylamine (**4**; 28.7 ml, 20 g, 0.275 mol), and Me_3SiCl (38.1 ml, 32.6 g, 0.3 mol; added within 20 min): 29.3 g (73%) of **8**. Colorless liquid. B.p. 22°/11 Torr or 122°/760 Torr. ^{1}H-NMR ($CDCl_3$): 3.24 (*sept.*, Me_2CH); 2.30 (*s*, Me); 1.00 (*d*, Me_2CH); 0.01 (*s*, Me_3Si).

3. *2-(4-Nitrophenyl)ethyl Diethylphosphoramidochloridite* (**13**) [10]. To a soln. of crude, slightly yellow 2-(4-nitrophenyl)ethyl phosphorodichloridite (**12**) [13][20][21] (synthesized from PCl_3 and 2-(4-nitrophenyl)-ethanol according to [20][21]; 26.7 g, 100 mmol) in anh. Et_2O (150 ml) at 0°, *N,N*-diethyl-1,1,1-trimethylsilanamine (**5**) (20.8 ml, 16.0 g, 110 mmol) in anh. Et_2O (100 ml) were added dropwise within 60 min. After stirring for 2 h at r.t., the Et_2O and excess amine were evaporated. The remaining yellowish oil was dried for 5 h under high vacuum: 30.17 g (99%) of **13**. UV (MeOH): 271 (3.98), 212 (3.86), 202 (4.01). ^{1}H-NMR ($CDCl_3$): 8.17 (*d*, 2 H *o* to NO_2); 7.41 (*d*, 2 H *m* to NO_2); 4.15 (*q*, CH_2CH_2O); 3.19–2.98 (*m*, CH_2CH_2O, 2 $MeCH_2$); 1.10 (*t*, 2 $MeCH_2$). ^{31}P-NMR ($CDCl_3$): 177.55.

4. *2-(4-Nitrophenyl)ethyl Ethyl(isopropyl)phosphoramidochloridite* (**15**). As described for **13**, with **12** (12.26 g, 45 mmol), Et_2O (75 ml), and *N*-ethyl-*N*-isopropyl-1,1,1-trimethylsilanamine (**7**; 7.97 g, 50 mmol) in Et_2O (50 ml; added within 20 min): 14.05 g (98%) of **15**. UV (MeOH): 272 (3.99), 213 (3.87), 203 (4.03). ^{1}H-NMR (CD_3CN): 8.11 (*d*, 2 H *o* to NO_2); 7.45 (*d*, 2 H *m* to NO_2); 4.12 (*q*, CH_2CH_2O); 3.63 (*m*, Me_2CH); 3.11–2.95 (*m*, CH_2CH_2O, $MeCH_2$); 1.12 (*d*, Me_2CH); 1.03 (*t*, $MeCH_2$). ^{31}P-NMR (CD_3CN): 176.80. Anal. calc. for $C_{13}H_{20}ClN_2O_3P$ (318.74): C 48.99, H 6.32, N 8.79; found: C 49.31, H 6.54, N 9.00.

5. *2-(4-Nitrophenyl)ethyl Isopropyl(methyl)phosphoramidochloridite* (**16**). As described for **13**, with **12** (14.10 g, 52 mmol) Et_2O (75 ml), and *N*-isopropyl-*N*-1,1,1-tetramethylsilanamine (**8**; 8.28 g, 57 mmol) in Et_2O (50 ml; added within 20 min). 15.7 g (99%) of **16**. UV (MeOH): 270 (3.98), 212 (3.85), 202 (3.99). ^{1}H-NMR (CD_3CN): 8.11 (*d*, 2 H *o* to NO_2); 7.45 (*d*, 2 H *m* to NO_2); 4.11 (*q*, CH_2CH_2O); 3.70 (*sept.*, Me_2CH); 3.07 (*t*, CH_2CH_2O); 2.48 (*d*, MeN); 1.09 (*d*, Me_2CH). ^{31}P-NMR (CD_3CN): 181.70. Anal. calc. for $C_{12}H_{18}ClN_2O_3P$ (304.71): C 47.30, H 5.95, N 9.19; found: C 47.43, H 6.11, N 9.00.

6. *2′-O-Methyl-5′-O-(monomethoxytrityl)uridine 3′-[2-(4-Nitrophenyl)ethyl Diethylphosphoramidite]* (**27**). To a soln of 2′-*O*-methyl-5′-*O*-(monomethoxytrityl)uridine (**18**) [22] (531 mg, 1.0 mmol) in dry CH_2Cl_2 (4 ml), abs. (^{i}Pr)$_2$EtN (0.6 ml, 3.5 mmol) and **13** (488 mg, 1.6 mmol) were added. The mixture was stirred under Ar for 50 min at r.t. and then quenched with dry ^{i}PrOH (200 μl). After stirring for another 15 min, the mixture was poured on CH_2Cl_2/phosphate buffer (pH 7) 1:1 (40 ml). The aq. layer was extracted twice with CH_2Cl_2 (20 ml), the combined org. phase dried (Na_2SO_4) and evaporated, and the residue taken up in CH_2Cl_2/petroleum ether 1:1 and purified by FC (silica gel (10 g), 11 × 2 cm, petroleum ether/AcOEt 1:1 (30 ml), 1:2 (30 ml), and 1:3 (30 ml), all with 1%) of Et_3N): 667 mg (83%) of **27**. Colorless foam. TLC (SiO_2, petroleum ether/AcOEt/Et_3N 1:9:1): R_f 0.64. UV (MeOH): 265 (4.31), 229 (sh, 4.29), 204 (4.87). ^{1}H-NMR ($CDCl_3$): 8.98 (br., NH); 8.16–

7.97 (*m*, H–C(6), 2 H *o* to NO_2); 7.42–7.21 (*m*, 14 H, MeO*Tr*, 2 H *m* to NO_2); 6.87–6.80 (*m*, 2 H *o* to MeO); 6.02, 5.96 (*2d*, H–C(1′)); 5.20 (*d*, H–C(5)); 4.58, 4.41 (*2m*, H–C(3′)); 4.20 (*m*, H–C(2′)); 4.00–3.67 (*m*, CH_2CH_2O); 3.80, 3.79 (*2s*, *Me*OTr); 3.73 (*m*, H–C(4′)); 3.63 (*dd*, 1 H–C(5′)); 3.55, 3.53 (*2s*, MeO–C(2)); 3.43 (*m*, 1 H–C(5′)); 3.14–2.79 (*m*, CH_2CH_2O, 2 $MeCH_2$); 1.02, 0.92 (*2t*, 2 *Me*CH_2). ^{31}P-NMR ($CDCl_3$): 150.70, 149.52. Anal. calc. for $C_{42}H_{47}N_4O_{10}P \cdot 0.5\ H_2O$ (807.84): C 62.45, H 5.99, N 6.94; found: C 62.25, H 5.98, N 6.86.

7. *5′-*O*-(Dimethoxytrityl)-2′-*O*-methyluridine 3′-[2-(4-Nitrophenyl)ethyl Diethylphosphoramidite]* (**28**). As described in *Exper. 6*, with 5′-*O*-(dimethoxytrityl)-2′-*O*-methyluridine (**19**) [22] (561 mg, 1.0 mmol): 676 mg (82%) of **28**. Colorless foam. TLC (SiO_2, petroleum ether/AcOEt/Et_3N 1:9:1): R_f 0.64. UV (MeOH): 265 (4.30), 234 (4.39), 204 (4.87). ^{1}H-NMR ($CDCl_3$): 9.60 (br., NH); 8.18–8.02 (*m*, H–C(6), 2 H *o* to NO_2); 7.41–7.23 (*m*, 12 H, $(MeO)_2$Tr, 2 H *m* to NO_2); 6.86–6.79 (*m*, 4 H *o* to MeO); 6.04, 5.98 (*2d*, H–C(1′)); 5.24 (*d*, H–C(5)); 4.57, 4.41 (*2m*, H–C(3′)); 4.21 (*m*, H–C(2′)); 4.01–3.70 (*m*, CH_2CH_2O, H–C(4′)); 3.80 (*s*, 3 H, $(MeO)_2$Tr); 3.79, 3.78 (*2s*, 3 H, $(MeO)_2$Tr); 3.64 (*dd*, 1 H–C(5′)); 3.56, 3.54 (*2s*, MeO–C(2′)); 3.42 (*m*, 1 H–C(5′)); 3.15–2.79 (*m*, CH_2CH_2O, 2 $MeCH_2$); 1.02, 0.92 (*2t*, 2 *Me*CH_2). ^{31}P-NMR ($CDCl_3$): 150.75, 149.51. Anal. calc. for $C_{43}H_{49}N_4O_{11}P \cdot 0.5\ H_2O$ (837.86): C 61.64, H 6.02, N 6.69; found: C 61.41, H 6.02, N 6.44.

8. *2′-*O*-Methyl-5′-*O*-(monomethoxytrityl)uridine 3′-[2-(4-Nitrophenyl)ethyl Diisopropylphosphoramidite]* (**29**). As described in *Exper. 6*, with 2-(4-nitrophenyl)ethyl diisopropylphosphoramidochloridite (**14**; 765 mg, 2.3 mmol). Purification by FC (silica gel (10 g), 11 × 2 cm, soln. in CH_2Cl_2/petroleum ether 1:1, then petroleum ether/acetone 7:2 (100 ml), 3:1 (50 ml), 2:1 (50 ml), and 1:1 (50 ml), all with 1% of Et_3N): 604 mg (73%) of **29**. Colorless foam. TLC (SiO_2, petroleum ether/AcOEt/Et_3N 1:9:1): R_f 0.68. UV (MeOH): 265 (4.29), 230 (sh, 4.27), 205 (4.82). ^{1}H-NMR ($CDCl_3$): 8.11, 7.99 (*2d*, 2 H *o* to NO_2); 7.76, 7.64 (*2d*, H–C(6)); 7.47–7.25 (*m*, 14 H, MeO*Tr*, 2 H *m* to NO_2); 6.86 (*dd*, 2 H *o* to MeO); 5.85 (*2d*, H–C(1′)); 5.25 (*t*, H–C(5)); 4.42, 4.32 (*2m*, H–C(3′)); 4.06 (*m*, H–C(2′)); 3.85 (*m*, CH_2CH_2O); 3.79 (*m*, H–C(4′)); 3.75 (*2s*, *Me*OTr); 3.58–3.28 (*m*, 2 H–C(5′), 2 Me_2CH); 3.42 (*2s*, MeO–C(2′)); 3.00, 2.86 (*2t*, 2 H, CH_2CH_2O); 1.14–0.97 (*m*, 2 Me_2CH). ^{31}P-NMR ($CDCl_3$): 150.53, 149.66. ^{31}P-NMR (CD_3CN): 150.02, 149.62. Anal. calc. for $C_{44}H_{51}N_4O_{10}P$ (826.88): C 63.91, H 6.22, N 6.78; found: C 63.58, H 6.52, N 6.64.

9. *2′-*O*-Methyl-5′-*O*-(monomethoxytrityl)uridine 3′-[2-(4-Nitrophenyl)ethyl Ethyl(isopropyl)phosphoramidite]* (**30**). As described in *Exper. 6*, with **15** (510 mg, 1.6 mmol): 667 mg (81%) of **30**. Colorless foam. TLC (SiO_2, petroleum ether/AcOEt/Et_3N 1:9:1): R_f 0.66. UV (MeOH): 265 (4.31), 230 (4.34), 206 (4.82). ^{1}H-NMR ($CDCl_3$): 8.83 (br., NH); 8.16–7.98 (*m*, H–C(6), 2 H *o* to NO_2); 7.42–7.22 (*m*, 14 H, MeO*Tr*, 2 H *m* to NO_2); 6.84, 6.83 (*2d*, 2 H *o* to MeO); 6.02, 5.97 (*2d*, H–C(1′)); 5.21, 5.18 (*2d*, H–C(5)); 4.56, 4.42 (*2m*, H–C(3′)); 4.20 (*m*, H–C(2′)); 4.02–3.40 (*m*, H–C(4′), CH_2CH_2O, Me_2CH, 2 H–C(5′)); 3.80, 3.78 (*2s*, *Me*OTr); 3.55, 3.53 (*2s*, MeO–C(2′)); 3.11–2.70 (*m*, CH_2CH_2O, $MeCH_2$); 1.17–0.91 (*m*, *Me*CH_2, Me_2CH). ^{31}P-NMR ($CDCl_3$): 151.86, 150.90. Anal. calc. for $C_{43}H_{49}N_4O_{10}P \cdot 0.5\ H_2O$ (821.86): C 62.84, H 6.13, N 6.82; found: C 62.56, H 6.11, N 6.87.

10. *2′-*O*-Methyl-5′-*O*-(monomethoxytrityl)uridine 3′-[2-(4-Nitrophenyl)ethyl Isopropyl(methyl)phosphoramidite]* (**31**). As described in *Exper. 6*, with 2-(4-nitrophenyl)ethyl isopropyl(methyl)phosphoramidochloridite (**16**; 488 mg, 1.6 mmol): 657 mg (82%) of **31**. Colorless foam. TLC (SiO_2, petroleum ether/AcOEt/Et_3N 1:9:1): R_f 0.64. UV (MeOH): 265 (4.29), 230 (sh, 4.29), 206 (4.83). ^{1}H-NMR ($CDCl_3$): 8.92 (br., NH); 8.15–8.00 (*m*, H–C(6), 2 H *o* to NO_2); 7.41–7.21 (*m*, 14 H, MeO*Tr*, 2 H *m* to NO_2); 6.83 (*2d*, 2 H *o* to MeO); 6.01, 5.95 (*2d*, H–C(1′)); 5.21, 5.19 (*2d*, H–C(5)); 4.56, 4.41 (*2m*, H–C(3′)); 4.19 (*m*, H–C(2′)); 4.00–3.38 (*m*, H–C(4′), CH_2CH_2O, Me_2CH, 2 H–C(5′)); 3.80, 3.79 (*2s*, *Me*OTr); 3.55, 3.54 (*2s*, MeO–C(2′)); 3.01–2.89 (*2t*, CH_2CH_2O); 2.42–2.24 (*2t*, MeN); 1.09–1.02 (*2d*, Me_2CH). ^{31}P-NMR ($CDCl_3$): 149.63, 148.23. Anal. calc. for $C_{42}H_{47}N_4O_{10}P \cdot 0.5\ H_2O$ (807.84): C 62.45, H 5.99, N 6.94; found: C 62.07, H 6.13, N 6.77.

11. *2′-*O*-Methyl-5′-*O*-(monomethoxytrityl)uridine 3′-(2-Cyanoethyl Diisopropylphosphoramidite)* (**32**) [17]. As described in *Exper. 6*, with 2-cyanoethyl diisopropyl phosphoramidochloridite (**17**) (379 mg, 1.6 mmol): 621 mg (85%) of **32**. Colorless foam. TLC (SiO_2, petroleum ether/AcOEt/Et_3N 1:9:1): R_f 0.63. UV (MeOH): 262 (4.03), 230 (sh, 4.24), 206 (4.74). ^{1}H-NMR ($CDCl_3$): 8.65 (br., 1 NH); 8.08, 7.96 (*2d*, H–C(6)); 7.44–7.23 (*m*, 12 H, MeOTr); 6.89–6.82 (*m*, 2 H *o* to MeO); 6.02, 5.97 (*2d*, H–C(1′)); 5.20 (*t*, H–C(5)); 4.63, 4.50 (*2m*, H–C(3′)); 4.22 (*m*, H–C(2′)); 3.95–3.78 (*m*, H–C(4′), CH_2CH_2O); 3.81, 3.80 (*2s*, *Me*OTr); 3.70–3.43 (*m*, 2 Me_2CH, 2, H–C(5′)); 3.58 (*2s*, MeO–C(2′)); 2.65, 2.42 (*2t*, CH_2CH_2O); 1.21–1.02 (*m*, 2 Me_2CH). ^{31}P-NMR ($CDCl_3$): 151.30, 150.87. Anal. calc. for $C_{39}H_{47}N_4O_8P \cdot 0.5\ H_2O$ (739.81): C 63.32, H 6.54, N 7.57; found: C 63.44, H 6.59, N 7.63.

12. *N^3-(4-Methoxybenzoyl)-2′-*O*-methyl-5′-*O*-(monomethoxytrityl)uridine 3′-[2-(4-Nitrophenyl)ethyl Diethylphosphoramidite]* (**33**). As described in *Exper. 6*, with *N^3*-(4-methoxybenzoyl)-2′-*O*-methyl-5′-*O*-(mono-

methoxytrityl)uridine (**20**) [22] (665 mg, 1.0 mmol). Purification by FC (silica gel (10 g), 11 × 2 cm, soln. in CH_2Cl_2/petroleum ether 1:1, then petroleum ether/AcOEt 2:1 (30 ml), 3:2 (50 ml), and 1:1 (50 ml), all with 1% of Et_3N): 755 mg (81%) of **33**. Colorless foam. TLC (SiO_2 petroleum ether/AcOEt/Et_3N 3:7:1): R_f 0.74. UV (MeOH): 276 (4.51), 224 (4.50), 204 (4.90). ^{1}H-NMR ($CDCl_3$): 8.23–8.04 (*m*, H–C(6), 2 H *o* to NO_2); 7.90 (*m*, 2 H *o* to CO); 7.43–7.24 (*m*, 14 H, MeO*Tr*, 2 H *m* to NO_2); 6.94 (*d*, 2 H *o* to MeO of An); 6.87–6.83 (*m*, 2 H *o* to MeO of MeOTr); 5.96, 5.91 (*2s*, H–C(1′)); 5.27 (*d*, H–C(5)); 4.62, 4.45 (*2m*, H–C(3′)); 4.23 (*m*, H–C(2′)); 3.99–3.44 (*m*, H–C(4′), CH_2CH_2O, 2 H–C(5′)); 3.86 (*s*, MeO of An); 3.81, 3.79 (*2s*, *Me*OTr); 3.53, 3.51 (*2s*, Me–C(2′)); 3.15–2.80 (*m*, CH_2CH_2O, 2 $MeCH_2$); 1.03, 0.93 (*2t*, 2 *Me*CH_2). ^{31}P-NMR ($CDCl_3$): 150.84, 149.59. Anal. calc. for $C_{50}H_{53}N_4O_{12}P$ (932.96): C 64.37, H 5.73, N 6.00; found: C 63.83, H 5.85, N 5.62.

13. *2′*-O-*Methyl-5′*-O-*(monomethoxytrityl)*-N^4-*[2-(4-nitrophenyl)ethoxycarbonyl]cytidine 3′-[2-(4-Nitrophenyl)ethyl Diethylphosphoramidite]* (**34**). As described in *Exper. 6*, with 2′-*O*-methyl-5′-*O*-(monomethoxytrityl)-*N*^{4}-[2-(4-nitrophenyl)ethoxycarbonyl]cytidine (**21**) [22] (723 mg, 1.0 mmol): 882 mg (89%) of **34**. Colorless foam. TLC (SiO_2, petroleum ether/AcOEt/Et_3N 1:9:1): R_f 0.60. UV (MeOH): 274 (4.39), 235 (4.46), 205 (4.94). ^{1}H-NMR ($CDCl_3$): 8.57 (*m*, H–C(6)); 8.17 (*d*, 2 H *o* to NO_2); 8.11, 8.05 (*2d*, 2 H *o* to NO_2); 7.44–7.25 (*m*, 16 H, MeO*Tr*, 2 × 2 H *m* to NO_2); 6.84 (*m*, 2 H *o* to MeO); 6.73 (*m*, H–C(5)); 5.98 (*d*, H–C(1′)); 4.51–4.25 (*m*, H–C(3′), H–C(2′)); 4.43 (*t*, CH_2CH_2OCO); 3.96–3.64 (*m*, CH_2CH_2OP, H–C(4′)); 3.81, 3.79 (*2s*, *Me*OTr); 3.67, 3.65 (*2s*, MeO–C(2′)); 3.44 (*m*, 2 H–C(5′)); 3.11 (*t*, CH_2CH_2O), 3.04–2.80 (*m*, CH_2CH_2O, 2 $MeCH_2$); 0.98, 0.89 (*2t*, 2 *Me*CH_2). ^{31}P-NMR ($CDCl_3$): 150.55, 149.74. Anal. calc. for $C_{51}H_{55}N_6O_{13}P$ (991.01): C 61.81, H 5.59, N 8.48; found: C 61.21, H 5.54, N 8.24.

14. *5′*-O-*(Dimethoxytrityl)-2′*-O-*methyl*-N^4-*[2-(4-nitrophenyl)ethoxycarbonyl]cytidine 3′-[2-(4-Nitrophenyl)-ethyl Diethylphosphoramidite]* (**35**). As described in *Exper. 6*, with 5′-*O*-(dimethoxytrityl)-2′-*O*-methyl-*N*^{4}-[2-(4-nitrophenyl)ethoxycarbonyl]cytidine (**22**) [22] (753 mg, 1.0 mmol): 858 mg (84%) of **35**. Colorless foam. TLC (SiO_2, petroleum ether/AcOEt/Et_3N 1:9:1): R_f 0.60. UV (MeOH): 274 (4.42), 236 (4.56), 204 (4.96). ^{1}H-NMR ($CDCl_3$): 8.60 (*m*, H–C(6)); 8.17 (*d*, 2 H *o* to NO_2); 8.11, 8.05 (*2d*, 2 H *o* to NO_2); 7.41–7.25 (*m*, 14 H, $(MeO)_2$*Tr*, 2 × 2 H *m* to NO_2); 6.84 (*m*, 4 H *o* to MeO); 6.74 (*m*, H–C(5)); 5.98 (*d*, H–C(1′)); 4.56–4.25 (*m*, H–C(3′), H–C(2′)); 4.43 (*t*, CH_2CH_2OCO); 3.96–3.64 (*m*, CH_2CH_2OP, H–C(4′)); 3.81, 3.79 (*2s*, $(MeO)_2$Tr); 3.66, 3.64 (*2s*, MeO–C(2′)); 3.44 (*m*, 2 H–C(5′)); 3.11 (*t*, CH_2CH_2O); 3.04–2.76 (*m*, CH_2CH_2O, 2 $MeCH_2$); 0.99, 0.90 (*2t*, 2 *Me*CH_2). ^{31}P-NMR ($CDCl_3$): 150.47, 149.31. Anal. calc. for $C_{52}H_{57}N_6O_{14}P \cdot 0.5\ H_2O$ (1030.04): C 60.64, H 5.68, N 8.16; found: C 60.22, H 5.84, N 7.87.

15. *2′*-O-*Methyl-5′*-O-*(monomethoxytrityl)*-N^4-*[2-(4-nitrophenyl)ethoxycarbonyl]cytidine 3′-[2-(4-Nitrophenyl)ethyl Diisopropylphosphoramidite]* (**36**). As described in *Exper. 6*, with **21** (723 mg, 1.0 mmol) and **14** (765 mg, 2.3 mmol). Purification by FC (silica gel (10 g), 11 × 2 cm, soln. in CH_2Cl_2/petroleum ether 1:1, then petroleum ether/acetone 7:2 (100 ml), 3:1 (50 ml), 2:1 (50 ml), and 1:1 (50 ml), all with 1% of Et_3N): 732 mg (72%) of **36**. TLC (SiO_2, petroleum ether/AcOEt/Et_3N 1:9:1): 0.64. Colorless foam. UV (MeOH): 274 (4.42), 235 (4.48), 205 (4.93). ^{1}H-NMR ($CDCl_3$): 8.57 (*m*, 1 H, H–C(6)); 8.18 (*d*, 2 H *o* to NO_2); 8.11, 8.01 (*2d*, 2 H *o* to NO_2); 7.45–7.24 (*m*, 16 H, MeO*Tr*, 2 × 2 H *m* to NO_2); 6.83 (*m*, 2 H *o* to MeO); 6.69 (*m*, H–C(5)); 6.00 (*d*, H–C(1′)); 4.51–4.22 (*m*, H–C(3′), H–C(2′)); 4.43 (*t*, CH_2CH_2OCO); 3.96–3.41 (*m*, CH_2CH_2OP, H–C(4′), 2 Me_2CH, 2 H–C(5′)); 3.81–3.79 (*2s*, *Me*OTr); 3.66, 3.63 (*2s*, MeO–C(2′)); 3.11 (*t*, CH_2CH_2O); 2.98, 2.83 (*2t*, CH_2CH_2O); 1.21–0.95 (*m*, 2 Me_2CH). ^{31}P-NMR ($CDCl_3$): 149.98, 149.57. Anal. calc. for $C_{53}H_{59}N_6O_{13}P \cdot 0.5\ H_2O$ (1026.06): C 62.04, H 5.89, N 8.19; found: C 61.79, H 5.90, N 8.36.

16. *2′*-O-*Methyl-5′*-O-*(monomethoxytrityl)*-N^4-*[2-(4-nitrophenyl)ethoxycarbonyl]cytidine 3′-[2-(4-Nitrophenyl)ethyl Ethyl(isopropyl)phosphoramidite]* (**37**). As described in *Exper. 6*, with **21** [22] (723 mg, 1.0 mmol) and **15** (510 mg, 1.6 mmol): 905 mg (90%) of **37**. Colorless foam. TLC (SiO_2, petroleum ether/AcOEt/Et_3N 1:9:1): R_f 0.62. UV (MeOH): 274 (4.42), 233 (4.50), 205 (4.96). ^{1}H-NMR ($CDCl_3$): 8.57 (*m*, H–C(6)); 8.18 (*d*, 2 H *o* to NO_2); 8.11, 8.03 (*2d*, 2 H *o* to NO_2); 7.44–7.25 (*m*, 16 H, MeO*Tr*, 2 × 2 H *m* to NO_2); 6.84 (*m*, 2 H *o* to MeO); 6.71 (*m*, H–C(5)); 5.98 (*d*, H–C(1′)); 4.51–4.24 (*m*, H–C(3′), H–C(2′)); 4.43 (*t*, CH_2CH_2O-CO); 3.96–3.40 (*m*, CH_2CH_2OP, H–C(4′), Me_2CH, 2 H–C(5′)); 3.81, 3.79 (*2s*, MeO*Tr*); 3.66, 3.64 (*2s*, MeO–C(2′)); 3.11 (*t*, CH_2CH_2O); 3.04–2.83 (*m*, CH_2CH_2O, $MeCH_2$); 1.17–0.89 (*m*, Me_2CH, *Me*CH_2). ^{31}P-NMR ($CDCl_3$): 151.44, 150.70. Anal. calc. for $C_{52}H_{57}N_6O_{13}P \cdot 0.5\ H_2O$ (1014.04): C 61.59, H 5.77, N 8.29; found: C 61.21, H 5.79, N 8.27.

17. *2′*-O-*Methyl-5′*-O-*(monomethoxytrityl)*-N^4-*[2-(4-nitrophenyl)ethoxycarbonyl]cytidine 3′-[2-(4-Nitrophenyl)ethyl Isopropyl(methyl)phosphoramidite]* (**38**). As described in *Exper. 6*, with **21** [22] (723 mg, 1.0 mmol) and **16** (488 mg, 1.6 mmol): 823 mg (83%) of **38**. Colorless foam. TLC (SiO_2, petroleum ether/AcOEt/Et_3N 1:9:1): R_f 0.60. UV (MeOH): 274 (4.42), 234 (4.52), 205 (4.93). ^{1}H-NMR ($CDCl_3$): 8.57 (*m*, H–C(6)); 8.18 (*d*, 2 H *o* to NO_2); 8.11, 8.05 (*2d*, 2 H *o* to NO_2); 7.44–7.25 (*m*, 16 H, MeO*Tr*, 2 × 2 H *m* to NO_2);

6.84 (*m*, 2 H *o* to MeO); 6.74 (*m*, H–C(5)); 5.97 (*d*, H–C(1′)); 4.51–4.25 (*m*, H–C(3′), H–C(2′)); 4.43 (*t*, CH_2CH_2OCO); 3.96–3.58 (*m*, CH_2CH_2OP, H–C(4′), Me_2CH); 3.81–3.79 (*2s*, *Me*OTr); 3.66, 3.64 (*2s*, MeO–C(2′)); 3.44 (*m*, 2 H–C(5′)); 3.10 (*t*, CH_2CH_2O); 2.98, 2.88 (*2t*, CH_2CH_2O); 2.40, 2.22 (*2d*, MeN); 1.09–0.98 (*m*, Me_2CH). ^{31}P-NMR ($CDCl_3$): 149.30, 147.94. Anal. calc. for $C_{51}H_{55}N_6O_{13}P \cdot 0.5\ H_2O$ (1000.02): C 61.25, H 5.64, N 8.40; found: C 60.84, H 5.66, N 8.52.

18. *2′-O-Methyl-5′-O-(monomethoxytrityl)-N⁴-[2-(4-nitrophenyl)ethoxycarbonyl]cytidine 3′-(2-Cyanoethyl Diisopropylphosphoramidite)* (**39**). As described in *Exper. 6*, with **21** [22] (723 mg, 1.0 mmol) and **17** (379 mg, 1.6 mmol): 803 mg (87%) of **39**. Colorless foam. TLC (SiO_2, petroleum ether/AcOEt/Et_3N 1:9:1): R_f 0.59 (0.56, 0.62). UV (MeOH): 281 (4.18), 234 (4.45), 206 (4.84). ^{1}H-NMR ($CDCl_3$): 8.59, 8.49 (*2d*, H–C(6)); 8.18 (*d*, 2 H *o* to NO_2); 7.68 (br., NH); 7.45–7.25 (*m*, 14 H, MeO-*Tr*, 2 H *m* to NO_2); 6.87, 6.84 (*2d*, 2 H *o* to MeO); 6.72, 6.65 (*2d*, H–C(5)); 6.01 (*d*, H–C(1′)); 4.58–4.38 (*m*, H–C(3′), CH_2CH_2OCO); 4.27 (*m*, H–C(2′)); 3.90 (*m*, H–C(4′)); 3.86–3.38 (*m*, CH_2CH_2OP, 2 Me_2CH, 2 H–C(5′)); 3.81 (*s*, *Me*OTr); 3.67 (*s*, Me–C(2′)); 3.09 (*t*, CH_2CH_2O); 2.61, 2.39 (*2t*, CH_2CH_2O); 1.26–0.98 (*m*, 2 Me_2CH). ^{31}P-NMR ($CDCl_3$): 151.10, 150.78. Anal. calc. for $C_{48}H_{55}N_6O_{11}P \cdot 0.5\ H_2O$ (931.98): C 61.86, H 5.95, N 9.02; found: C 61.56, H 6.08, N 8.91.

19. *2′-O-Methyl-5′-O-(monomethoxytrityl)-N⁶-[2-(4-nitrophenyl)ethoxycarbonyl]adenosine 3′-[2-(4-Nitrophenyl)ethyl Diethylphosphoramidite]* (**40**). As described in *Exper. 6*, with 2′-*O*-methyl-5′-*O*-(monomethoxytrityl)-*N*⁶-[2-(4-nitrophenyl)ethoxycarbonyl]adenosine (**23**) [22] (747 mg, 1.0 mmol): 893 mg (88%) of **40**. Colorless foam. TLC (SiO_2, petroleum ether/AcOEt/Et_3N 1:9:1): R_f 0.68. UV (MeOH): 267 (4.60), 232 (sh, 4.40), 204 (4.96). ^{1}H-NMR ($CDCl_3$): 8.66 (*s*, H–C(8)); 8.31 (br., NH); 8.20–8.05 (*m*, H–C(2), 2 × 2 H *o* to NO_2); 7.44–7.18 (*m*, 16 H, MeO*Tr*, 2 × 2 H *m* to NO_2); 6.80 (*m*, 2 H *o* to MeO); 6.14 (*t*, H–C(1′)); 4.65–4.50 (*m*, H–C(3′), H–C(2′)); 4.53 (*t*, CH_2CH_2OCO); 4.37 (*m*, H–C(4′)); 4.02–3.68 (*m*, CH_2CH_2OP); 3.77, 3.76 (*2s*, *Me*OTr); 3.50 (*dd*, 1 H–C(5′)); 3.44, 3.43 (*2s*, MeO–C(2′)); 3.33 (*m*, 1 H–C(5′)); 3.17–2.84 (*m*, 2 CH_2CH_2O, 2 $MeCH_2$; 1.01, 0.94 (*2t*, 2 $MeCH_2$). ^{31}P-NMR ($CDCl_3$): 150.63, 149.85. Anal. calc. for $C_{52}H_{55}N_8O_{12}P$ (1015.03): C 61.53, H 5.46, N 11.04; found: C 60.93, H 5.40, N 10.69.

20. *5′-O-(Dimethoxytrityl)-2′-O-methyl-N⁶-[2-(4-nitrophenyl)ethoxycarbonyl]adenosine 3′-[2-(4-Nitrophenyl)ethyl Diethylphosphoramidite]* (**41**). As described in *Exper. 6*, with 5′-*O*-(dimethoxytrityl)-2′-*O*-methyl-*N*⁶-[2-(4-nitrophenyl)ethoxycarbonyl]adenosine (**24**) [22] (777 mg, 1.0 mmol): 836 mg (80%) of **41**. Colorless foam. TLC (SiO_2, petroleum ether/AcOEt/Et_3N 1:9:1): R_f 0.68. UV (MeOH): 267 (4.59), 236 (4.47), 204 (4.98). ^{1}H-NMR ($CDCl_3$): 8.68 (*s*, H–C(8)); 8.47 (br., NH); 8.18–8.05 (*m*, H–C(2), 2 × 2 H *o* to NO_2); 7.43–7.21 (*m*, 14 H, $(MeO)_2Tr$, 2 × 2 H *m* to NO_2); 6.80 (*d*, 4 H *o* to MeO); 6.15 (*t*, H–C(1′)); 4.70–4.50 (*m*, H–C(3′), H–C(2′)); 4.53 (*t*, CH_2OCO); 4.37, 4.32 (*2m*, H–C(4′)); 4.06–3.80 (*m*, CH_2CH_2OP); 3.77, 3.76 (*2s*, $(MeO)_2Tr$); 3.50 (*dd*, 1 H–C(5′)); 3.45, 3.44 (*2s*, MeO–C(2′)); 3.33 (*m*, 1 H–C(5′)); 3.14 (*t*, CH_2CH_2O); 3.07–2.85 (*m*, CH_2CH_2O, 2 $MeCH_2$); 1.02, 0.95 (*2t*, 2 $MeCH_2$). ^{31}P-NMR ($CDCl_3$): 150.66, 149.80. Anal. calc. for $C_{53}H_{57}N_8O_{13}P \cdot 0.5\ H_2O$ (1054.07): C 60.39, H 5.55, N 10.63; found: C 60.07, H 5.67, N 10.30.

21. *2′-O-Methyl-5′-O-(monomethoxytrityl)-N⁶-[2-(4-nitrophenyl)ethoxycarbonyl]adenosine 3′-[2-(4-Nitrophenyl)ethyl Diisopropylphosphoramidite]* (**42**). As described in *Exper. 6*, with **23** [22] (747 mg, 1.0 mmol) and **14** (765 mg, 2.3 mmol). Purification by FC (silica gel, 10 g, 11 × 2 cm, soln. in CH_2Cl_2/petroleum ether 1:1, then petroleum ether/acetone 7:2 (100 ml), 3:1 (50 ml), 2:1 (50 ml), and 1:1 (50 ml), all with 1% of Et_3N): 751 mg (72%) of **42**. Colorless foam. TLC (SiO_2, petroleum ether/AcOEt/Et_3N 1:9:1): R_f 0.72. UV (MeOH): 267 (4.62), 233 (4.40), 206 (4.95). ^{1}H-NMR ($CDCl_3$): 8.67 (*s*, H–C(8)); 8.33 (br., NH); 8.19–8.01 (*m*, H–C(2), 2 × 2 H *o* to NO_2); 7.44–7.18 (*m*, 16 H, MeO*Tr*, 2 × 2 H *m* to NO_2); 6.79 (*d*, 2 H *o* to MeO); 6.16, 6.12 (*2d*, H–C(1′)); 4.64–4.50 (*m*, H–C(3′), H–C(2′)); 4.53 (*t*, CH_2CH_2OCO); 4.39–4.34 (*m*, H–C(4′)); 3.77, 3.76 (*2s*, *Me*OTr); 4.05–3.28 (*m*, CH_2 CH_2OP, 2 Me_2CH, 2 H–C(5′)); 3.45, 3.43 (*2s*, MeO–C(2′)); 3.14 (*t*, CH_2CH_2O); 3.02, 2.83 (*2t*, CH_2CH_2O); 1.19–1.03 (*m*, 2 Me_2CH). ^{31}P-NMR ($CDCl_3$): 150.64, 149.76. Anal. calc. for $C_{54}H_{59}N_8O_{12}P \cdot 0.5\ H_2O$ (1052.09): C 61.65, H 5.70, N 10.74; found: C 61.40, H 5.74, N 10.58.

22. *2′-O-Methyl-5′-O-(monomethoxytrityl)-N⁶-[2-(4-nitrophenyl)ethoxycarbonyl]adenosine 3′-[2-(4-Nitrophenyl)ethylphosphoramidite]* (**43**). As described in *Exper. 6*, with **23** [22] (747 mg, 1.0 mmol) and **15** (510 mg, 1.6 mmol): 864 mg (84%) of **43**. Colorless foam. TLC (SiO_2, petroleum ether/AcOEt/Et_3N 1:9:1): R_f 0.70. UV (MeOH): 267 (4.57), 235 (4.36), 204 (4.94). ^{1}H-NMR ($CDCl_3$): 8.66 (*s* H–C(8)); 8.34 (*s*, NH); 8.19–8.04 (*m*, H–C(2), 2 × 2 H *o* to NO_2); 7.44–7.21 (*m*, 16 H, MeO*Tr*, 2 × 2 H *m* to NO_2); 6.80 (*m*, 2 H *o* to MeO); 6.15, 6.13 (*2d*, H–C(1′)); 4.66–4.50 (*m*, H–C(3′), H–C(2′)); 4.53 (*t*, CH_2CH_2OCO); 4.35 (*m*, H–C(4′)); 4.04–3.68 (*m*, CH_2CH_2OP); 3.77, 3.76 (*2s*, *Me*OTr); 3.52 (*dd*, 1 H–C(5′), Me_2CH); 3.44, 3.43 (*2s*, MeO–C(2′)); 3.33 (*dd*, 1 H–C(5′)); 3.14 (*t*, CH_2CH_2O); 3.04–2.83 (*m*, CH_2CH_2O, $MeCH_2$); 1.16–0.96 (*m*, Me_2CH, $MeCH_2$).

^{31}P-NMR ($CDCl_3$): 151.94, 151.14. Anal. calc. for $C_{53}H_{57}N_8O_{12}P$ (1029.06): C 61.86, H 5.58, N 10.89; found: C 61.39, H 5.73, N 10.76.

23. *2′-*O*-Methyl-5′-*O*-(monomethoxytrityl)-*N*^{6}-[2-(4-nitrophenyl)ethoxycarbonyl]adenosine 3′-[2-(4-Nitrophenyl)ethyl Isopropyl(methyl)phosphoramidite]* (**44**). As described in *Exper. 6*, with **23** [22] (747 mg, 1.0 mmol) and **16** (488 mg, 1.6 mmol): 812 mg (80%) of **44**. Colorless foam. TLC (SiO_2, petroleum ether/AcOEt/Et_3N 1:9:1): R_f 0.68. UV (MeOH): 267 (4.58), 234 (sh, 4.38), 206 (4.93). ^{1}H-NMR ($CDCl_3$): 8.67 (*s*, H–C(8)); 8.41 (br., NH); 8.18–8.05 (*m*, H–C(2), 2 × 2 H *o* to NO_2); 7.44–7.18 (*m*, 16 H, MeO*Tr*, 2 × 2 H *m* to NO_2); 6.80, 6.79 (*2d*, 2 H *o* to MeO); 6.16, 6.14 (*2d*, H–C(1′)); 4.68–4.50 (*m*, H–C(3′), H–C(2′)); 4.53 (*t*, CH_2, CH_2OCO); 4.38, 4.32 (*2m*, H–C(4′)); 3.99–3.48 (*m*, CH_2CH_2OP, Me_2CH); 3.77, 3.76 (*2s*, *Me*OTr); 3.50 (*dd*, 1 H–C(5′)); 3.45, 3.44 (*2s*, Me–C(2′)); 3.33 (*m*, 1 H–C(5′)); 3.14 (*t*, CH_2CH_2O); 3.02, 2.88 (*2t*, CH_2CH_2O); 2.41, 2.32 (*2d*, MeN); 1.10–0.98 (*m*, Me_2CH). ^{31}P-NMR ($CDCl_3$): 149.48, 148.75. Anal. calc. for $C_{52}H_{55}N_8O_{12}P \cdot 0.5\ H_2O$ (1024.04): C 61.65, H 5.70, N 10.74; found: C 61.40, H 5.74, N 10.58.

24. *2′-*O*-Methyl-5′-*O*-(monomethoxytrityl)-*N*^{6}-[2-(4-nitrophenyl)ethoxycarbonyl]adenosine 3′-(2-Cyanoethyl Diisopropylphosphoramidite)* (**45**). As described in *Exper. 6*, with **23** [22] (747 mg, 1.0 mmol) **17** (379 mg, 1.6 mmol): 768 mg (81%) of **45**. Colorless foam. TLC (SiO_2, petroleum ether/AcOEt/Et_3N 1:9:1): R_f 0.66 (0.63, 0.69). UV (MeOH): 267 (4.44), 234 (4.29), 204 (4.90). ^{1}H-NMR ($CDCl_3$): 8.68, 8.65 (*2s*, H–C(8)); 8.49 (br, NH); 8.21–8.13 (*m*, H–C(2), 2 H *o* to NO_2); 7.47–7.19 (*m*, 14 H, MeO*Tr*, 2 H *m* to NO_2); 6.80 (*m*, 2 H *o* to MeO); 6.15, 6.14 (*2d*, H–C(1′)); 4.70 (*m*, H–C(3′)); 4.60 (*m*, H–C(2′)); 4.52 (*t*, CH_2CH_2O-CO); 4.42, 4.36 (*2m*, H–C(4′)); 3.96–3.82 (*m*, CH_2CH_2OP); 3.78, 3.77 (*2s*, *MeO*Tr); 3.71–3.51 (*m*, 2 Me_2CH, 1 H–C(5′)); 3.48 (*s*, MeO–C(2′); 3.36 (*m*, 1 H–C(5′)); 3.13 (*t*, $ArCH_2CH_2O$); 2.65, 2.37 (*2t*, $NCCH_2CH_2O$); 1.22–1.06 (*m*, Me_2CH). ^{31}P-NMR ($CDCl_3$): 151.65, 150.93. Anal. calc. for $C_{49}H_{55}N_8O_{10}P \cdot H_2O$ (965.02): C 60.99, H 5.95, N 11.61; found: C 61.04, H 5.93, N 11.61.

25. *2′-*O*-Methyl-5′-*O*-(monomethoxytrityl)-*N*^{2}-[2-(4-nitrophenyl)ethoxycarbonyl]-*O*^{6}-[2-(4-nitrophenyl)ethyl]guanosine 3′-[2-(4-Nitrophenyl)ethyl Diethylphosphoramidite]* (**46**). As described in *Exper. 6*, with 2′-*O*-methyl-5′-*O*-(monomethoxytrityl)-*N*^{2}-[2-(4-nitrophenyl)ethoxycarbonyl]-*O*^{6}-[2-(4-nitrophenyl)ethyl]guanosine (**25**) [22] (912 mg, 1.0 mmol) and dry THF as solvent. Purification by FC (silica gel (10 g), 11 × 2 cm, soln. in CH_2Cl_2/petroleum ether 1:1, then petroleum ether/AcOEt 2:1 (30 ml), 1:1 (30 ml), and 1:2 (70 ml), all with 1% of Et_3N): 980 mg (83%) of **46**. Colorless foam. TLC (SiO_2, petroleum ether/AcOEt/Et_3N 3:7:1): R_f 0.70. UV (MeOH): 269 (4.64), 236 (4.41), 205 (4.95). ^{1}H-NMR ($CDCl_3$): 8.17–7.97 (*m*, 3 × 2 H *o* to NO_2, H–C(8), NH); 7.54–7.22 (*m*, 18 H, MeO*Tr*, 3 × 2 H *m* to NO_2); 6.78 (*d*, 2 H *o* to MeO); 6.07 (*2d*, H–C(1′)); 4.82 (*t*, CH_2CH_2O); 4.61–4.47 (*m*, H–C(3′), H–C(2′)); 4.41 (*t*, CH_2CH_2OCO); 4.30, 4.26 (*2m*, H–C(4′)); 4.06–3.70 (*m*, CH_2CH_2OP); 3.70 (*s*, MeO*Tr*); 3.44 (*s*, MeO–C(2′), 1 H–C(5′)); 3.32 (*t*, CH_2CH_2O, 1 H–C(5′)); 3.10–2.82 (*m*, 2 CH_2CH_2O, 2 $MeCH_2$); 1.14, 1.00 (*2t*, 2 *Me*CH_2). ^{31}P-NMR ($CDCl_3$): 150.48, 149.94. Anal. calc. for $C_{60}H_{62}N_9O_{15}P \cdot H_2O$ (1198.20): C 60.15, H 5.38, N 10.52; found: C 59.61, H 5.37, N 10.17.

26. *5′-*O*-(Dimethoxytrityl)-2′-*O*-methyl-*N*^{2}-[2-(4-nitrophenyl)ethoxycarbonyl]-*O*^{6}-[2-(4-nitrophenyl)ethyl]-guanosine 3′-[2-(4-Nitrophenyl)ethyl Diethylphosphoramidite]* (**47**). As described in *Exper. 6*, with 5′-*O*-(dimethoxytrityl)-2′-*O*-methyl-*N*^{2}-[2-(4-nitrophenyl)ethoxycarbonyl]-*O*^{6}-[2-(4-nitrophenyl)ethyl]guanosine (**26**) [22] (942 mg, 1.0 mmol) and dry THF as solvent. Purification by FC (silica gel (10 g), 11 × 2 cm, soln. in CH_2Cl_2/petroleum ether 1:1, then petroleum ether/AcOEt 2:1 (30 ml), 1:1 (30 ml), and 1:2 (70 ml), all with 1% of Et_3N): 968 mg (80%) of **47**. Colorless foam. TLC (SiO_2, petroleum ether/AcOEt/Et_3N 3:7:1): R_f 0.70. UV (MeOH): 269 (4.66), 236 (4.52), 204 (5.01). ^{1}H-NMR ($CDCl_3$): 8.17–7.97 (*m*, 3 × 2 H *o* to NO_2, H–C(8)); 7.54–7.22 (*m*, 16 H, $(MeO)_2Tr$, 3 × 2 H *m* to NO_2); 6.78 (*d*, 4 H *o* to MeO); 6.07 (*m*, H–C(1′)); 4.82 (*t*, CH_2CH_2O); 4.61–4.47 (*m*, H–C(3′), H–C(2′)); 4.41 (*t*, CH_2CH_2OCO); 4.30, 4.26 (*2m*, H–C(4′)); 4.06–3.70 (*m*, CH_2CH_2OP); 3.77, 3.75 (*2s*, $(MeO)_2Tr$); 3.44 (*s*, 2′ MeO–C(2′), 1 H–C(5′)); 3.32 (*t*, CH_2CH_2O, 1 H–C(5′)); 3.10–2.82 (*m*, 2 CH_2CH_2O, 2 $MeCH_2$); 1.14, 1.00 (*2t*, 2 *Me*CH_2). ^{31}P-NMR ($CDCl_3$): 150.50, 149.90. Anal. calc. for $C_{61}H_{64}N_9O_{16}P \cdot 0.5\ H_2O$ (1219.21): C 60.09, H 5.29, N 10.34; found: C 59.70, H 5.40, N 9.81.

27. *2′-*O*-Methyl-5′-*O*-(monomethoxytrityl)-*N*^{2}-[2-(4-nitrophenyl)ethoxycarbonyl]-*O*^{6}-[2-(4-nitrophenyl)-ethyl]guanosine 3′-[2-(4-Nitrophenyl)ethyl Diisopropylphosphoramidite]* (**48**). As described in *Exper. 6*, with **25** [22] (912 mg, 1.0 mmol) and **14** (765 mg, 2.3 mmol). Purification by FC (silica gel (10 g), 11 × 2 cm, soln. in CH_2Cl_2/petroleum ether 1:1, then petroleum ether/AcOEt 2:1 (30 ml), 3:2 (30 ml), 1:1 (50 ml), and 1:2 (50 ml), all with 1% of Et_3N): 870 mg (72%) of **48**. Colorless foam. TLC (SiO_2, petroleum ether/AcOEt/Et_3N 3:7:1): R_f 0.74. UV (MeOH): 269 (4.65), 236 (4.43), 206 (4.92). ^{1}H-NMR ($CDCl_3$): 8.19–7.97 (*m*, 3 × 2 H *o* to NO_2, H–C(8)); 7.55–7.17 (*m*, 18 H, MeO*Tr*, 3 × 2 H *m* to NO_2); 6.82–6.76 (*m*, 2 H *o* to MeO); 6.09, 6.03 (*2d*, H–C(1′)); 4.83 (*t*, CH_2CH_2O); 4.59–4.48 (*m*, H–C(3′), H–C(2′)); 4.40 (*t*, CH_2CH_2OCO); 4.33, 4.29 (*2m*, H–C(4′)); 4.04–3.69 (*m*, CH_2CH_2OP); 3.77, 3.75 (*2s*, *Me*OTr); 3.65–3.27 (*m*, 2Me_2CH, 2 H–C(5′));

3.44 (*s*, MeO–C(2′)); 3.33 (*t*, CH_2CH$_2$O); 3.04–2.79 (*m*, CH_2CH$_2$O); 1.17–0.98 (*m*, 2 *Me*$_2$CH). ^{31}P-NMR ($CDCl_3$): 150.49, 149.91. Anal. calc. for $C_{62}H_{66}N_9O_{15}P \cdot H_2O$ (1226.25): C 60.72, H 5.59, N 10.28; found: C 60.65, H 5.59, N 10.06.

28. *2′-*O*-Methyl-5′-*O*-(monomethoxytrityl)-*N^2*-[2-(4-nitrophenyl)ethoxycarbonyl]-*O^6*-[2-(4-nitrophenyl)-ethyl]guanosine 3′-[2-(4-Nitrophenyl)ethyl Ethyl(isopropyl)phosphoramidite]* (**49**). As described in *Exper. 6*, with **25** [22] (912 mg, 1.0 mmol), **15** (510 mg, 1.6 mmol), and dry THF as solvent. Purification by FC (silica gel (10 g), 11 × 2 cm, soln. in CH_2Cl_2/petroleum ether 1:1, then petroleum ether/AcOEt 2:1 (30 ml), 1:1 (30 ml), and 1:2 (70 ml), all with 1% of Et_3N): 967 mg (81%) of **49**. Colorless foam. TLC (SiO_2, petroleum ether/AcOEt/Et_3N 3:7:1): R_f 0.74. UV (MeOH): 269 (4.64), 236 (4.42), 204 (4.97). ^{1}H-NMR ($CDCl_3$): 8.18–7.99 (*m*, 3 × 2 H *o* to NO$_2$, H–C(8)); 7.54–7.18 (*m*, 18 H, MeO*Tr*, 3 × 2 H *m* to NO$_2$); 6.79 (*m*, 2 H *o* to MeO); 6.09, 6.04 (*2d*, H–C(1′)); 4.83 (*t*, CH$_2$CH_2O); 4.60–4.47 (*m*, H–C(3′), H–C(2′)); 4.40 (*t*, CH$_2$CH_2O-CO); 4.32, 4.28 (*2m*, H–C(4′)); 4.04–3.65 (*m*, CH$_2$CH_2OP); 3.77, 3.75 (*2s*, *Me*OTr); 3.63–3.46 (*m*, Me$_2$C*H*, 1 H–C(5′)); 3.44 (*s*, MeO–C(2′)); 3.32 (*t*, CH_2CH$_2$O, 1 H–C(5′)); 3.10–2.80 (*m*, 2 CH_2CH$_2$O, MeCH$_2$); 1.15–0.93 (*m*, *Me*$_2$CH, *Me*CH$_2$). ^{31}P-NMR ($CDCl_3$): 151.80, 151.27. Anal. calc. for $C_{61}H_{64}N_9O_{15}P$ (1194.21): C 61.35, H 5.40, N 10.56; found: C 60.81, H 5.41, N 10.25.

29. *2′-*O*-Methyl-5′-*O*-(monomethoxytrityl)-*N^2*-[2-(4-nitrophenyl)ethoxycarbonyl]-*O^6*-[2-(4-nitrophenyl)-ethyl]guanosine 3′-[2-(4-Nitrophenyl)ethyl Isopropyl(methyl)phosphoramidite]* (**50**). As described in *Exper. 6*, with **25** [22] (912 mg, 1.0 mmol), **16** (488 mg, 1.6 mmol), and dry THF as solvent. Purification by FC (silica gel (10 g), 11 × 2 cm, soln. in CH_2Cl_2/petroleum ether 1:1, then petroleum ether/AcOEt 2:1 (30 ml), 1:1 (30 ml), and 1:2 (70 ml), all with 1% of Et_3N): 968 mg (82%) of **50**. Colorless foam. TLC (SiO_2, petroleum ether/AcOEt/Et_3N 3:7:1): R_f 0.70. UV (MeOH): 269 (4.62), 236 (4.38), 204 (4.94). ^{1}H-NMR ($CDCl_3$): 8.18–7.99 (*m*, 3 × 2 H *o* to NO$_2$, H–C(8)); 7.58–7.20 (*m*, 18 H, MeO*Tr*, 3 × 2 H *m* to NO$_2$); 6.79 (*m*, 2 H *o* to MeO); 6.09, 6.05 (*2d*, H–C(1′)); 4.83 (*t*, CH$_2$CH_2O); 4.60 (*m*, H–C(3′)); 4.52–4.38 (*m*, H–C(2′), CH$_2$CH_2O-CO); 4.32, 4.26 (*2m*, H–C(4′)); 4.01–3.42 (*m*, CH$_2$CH_2OP, Me$_2$C*H*, 1 H–C(5′)); 3.77, 3.76 (*2s*, *Me*OTr); 3.44 (*s*, MeO–C(2′)); 3.32 (*t*, CH$_2$OCH_2, 1 H–C(5′)); 3.10–2.85 (*m*, 2CH_2CH$_2$O); 2.39, 2.29 (*2d*, MeN); 1.09–0.94 (*m*, *Me*$_2$CH). ^{31}P-NMR ($CDCl_3$): 149.33, 148.88. Anal. calc. for $C_{60}H_{62}N_9O_{15}P$ (1180.18): C 61.06, H 5.30, N 10.68; found: C 60.95, H 5.34, N 10.37.

30. *2′-*O*-Methyl-5′-*O*-(monomethoxytrityl)-*N^2*-[2-(4-nitrophenyl)ethoxycarbonyl]-*O^6*-[2-(4-nitrophenyl)-ethyl]guanosine 3′-(2-Cyanoethyl Diisopropylphosphoramidite)* (**51**). As described in *Exper. 6*, with **25** [22] (912 mg, 1.0 mmol), **17** (379 mg, 1.6 mmol), and dry THF as solvent. Purification by FC (silica gel (10 g), 11 × 2 cm, soln. in CH_2Cl_2/petroleum ether 1:1, then petroleum ether/AcOEt 2:1 (30 ml), petroleum ether/AcOEt 1:1 (30 ml), and petroleum ether/AcOEt 1:2 (70 ml), all with 1% of Et_3N): 890 mg (80%) of **51**. Colorless foam. TLC (SiO_2, petroleum ether/AcOEt/Et_3N 3:7:1): R_f 0.68 (0.72, 0.65). UV (MeOH): 269 (4.62), 235 (4.40), 206 (4.92). ^{1}H-NMR ($CDCl_3$): 8.16 (*d*, 2 × 2 H *o* to NO$_2$); 8.02, 7.98 (*2s*, H–C(8)); 7.55–7.18 (*m*, 16 H, MeO*Tr*, 2 × 2 H *m* to NO$_2$); 6.82–6.78 (*m*, 2 H *o* to MeO); 6.08, 6.03 (*2d*, H–C(1′)); 4.82 (*t*, CH$_2$CH_2O); 4.62–4.53 (*m*, H–C(3′), H–C(2′)); 4.41 (*t*, CH$_2$CH_2OCO); 4.29 (*m*, H–C(4′)); 3.96–3.84 (*m*, CH$_2$CH_2OP); 3.77 (*2s*, MeOTr); 3.66–3.36 (*m*, 2 Me$_2$C*H*, 2 H–C(5′)); 3.47 (*s*, MeO–C(2′)); 3.32 (*t*, ArCH_2CH$_2$O); 3.07 (*t*, ArCH_2CH$_2$O); 2.68, 2.34 (*2t*, NCCH$_2$CH$_2$O); 1.20–1.02 (*m*, 2*Me*$_2$CH). ^{31}P-NMR ($CDCl_3$): 151.38, 151.04. Anal. calc. for $C_{57}H_{62}N_9O_{13}P \cdot H_2O$ (1130.16): C 60.57, H 5.71, N 11.15; found: C 60.64, H 5.75, N 11.16.

31. *3′-*O*-Methyl-5′-*O*-(monomethoxytrityl)uridine 2′-[2-(4-Nitrophenyl)ethyl Diethylphosphoramidite]* (**56**). As described in *Exper. 6*, with 3′-*O*-Methyl-5′-*O*-(monomethoxy)trityluridine (**52**) [22] (531 mg, 1.0 mmol): 623 mg (78%) of **56**. Colorless foam. TLC (SiO_2, petroleum ether/AcOEt/Et_3N 1:9:1): R_f 0.64. UV (MeOH): 265 (4.33), 229 (sh, 4.33), 206 (4.85). ^{1}H-NMR ($CDCl_3$): 8.12 (*d*, 2 H *o* to NO$_2$); 8.02 (*t*, H–C(6)); 7.41–7.23 (*m*, 14 H, MeO*Tr*, 2 H *m* to NO$_2$); 6.86 (*dd*, 2 H *o* to MeO); 5.98, 5.92 (*2d*, H–C(1′)); 5.31 (*2d*, H–C(5)); 4.35–4.42 (*m*, H–C(2′)); 4.18 (*m*, H–C(4′)); 4.01–3.86 (*m*, H–C(3′), CH$_2$CH_2OP); 3.81 (*2s*, *Me*OTr); 3.56 (*m*, 1 H–C(5′)); 3.46 (*m*, 1 H–C(5′)); 3.41 (*2s*, MeO–C(3′)); 3.12–2.93 (*m*, CH_2CH$_2$O, 2 MeCH_2); 1.02 (*m*, 2 *Me*CH$_2$). ^{31}P-NMR ($CDCl_3$): 152.01, 151.12. Anal. calc. for $C_{42}H_{47}N_4O_{10}P \cdot 0.5\ H_2O$ (807.84): C 62.45, H 5.99, N 6.94; found: C 61.94, H 6.02, N 6.33.

32. *3′-*O*-Methyl-5′-*O*-(monomethoxytrityl)-*N^4*-[2-(4-nitrophenyl)ethoxycarbonyl]cytidine 2′-[2-(4-Nitrophenyl)ethyl Diethylphosphoramidite]* (**57**). As described in 3′-*O*-methyl-5′-*O*-(monomethoxytrityl)-*N*^{4}-[2-(4-nitrophenyl)ethoxycarbonyl]cytidine (**53**) [22] (723 mg, 1.0 mmol): 783 mg (79%) of **57**. Colorless foam. TLC (SiO_2, petroleum ether/AcOEt/Et_3N 1:9:1): R_f 0.60. UV (MeOH): 274 (4.39), 236 (4.46), 205 (4.90). ^{1}H-NMR (CD_3CN): 8.30 (*m*, H–C(6)); 8.13 (*m*, 2 × 2 H *o* to NO$_2$); 7.53–7.28 (*m*, 16 H, MeO*Tr*, 2 × 2 H *m* to NO$_2$); 6.88 (*dd*, 2 H *o* to MeO); 6.78 (*m*, H–C(5)); 5.78 (*d*, H–C(1′)); 4.57, 4.47 (*2m*, H–C(2′)); 4.40 (*t*, CH$_2$CH_2OCO); 4.11–3.86 (*m*, CH$_2$CH_2OP, H–C(3′), H–C(4′)); 3.76 (*2s*, *Me*OTr);

3.37 (*s*, 2 H–C(5′)); 3.32 (*2s*, MeO–C(3′)); 3.09–2.97 (*m*, 2CH_2CH$_2$O, 2 MeCH_2); 0.98 (*m*, 2 *Me*CH$_2$). ^{31}P-NMR (CD_3CN): 152.40, 151.32. Anal. calc. for $C_{51}H_{55}N_6O_{13}P \cdot 0.5\ H_2O$ (1000.02): C 61.25, H 5.64, N 8.40; found: C 61.08, H 5.63, N 8.11.

33. *3′-O-Methyl-5′-O-(monomethoxytrityl)-*N^6*-[2-(4-nitrophenyl)ethoxycarbonyl]adenosine 2′-[2-(4-Nitrophenyl)ethyl Diethylphosphoramidite]* (**58**). As described in *Exper. 6*, with 3′-*O*-methyl-5′-*O*-(monomethoxytrityl)-*N*^{6}-[2-(4-nitrophenyl)ethoxycarbonyl]adenosine (**54**) [22] (747 mg, 1.0 mmol): 782 mg (77%) of **58.** Colorless foam. TLC (SiO_2, petroleum ether/AcOEt/Et_3N 1:9:1): R_f 0.68. UV (MeOH): 267 (4.60), 232 (sh, 4.40), 206 (4.93). ^{1}H-NMR ($CDCl_3$): 8.65 (*s*, H–C(8)); 8.23–8.01 (*m*, NH, H–C(2), 2 × 2 H *o* to NO$_2$); 7.46–7.19 (*m*, 16 H, MeO*Tr*, 2 × 2 H *m* to NO$_2$); 6.81 (*d*, 2 H *o* to MeO); 6.14, 6.11 (*2d*, H–C(1′)); 5.30–5.07 (*m*, H–C(2′)); 4.53 (*t*, CH$_2$CH_2OCO); 4.32 (*m*, H–C(4′)); 4.11, 4.04 (*2t*, H–C(3′)); 3.96–3.32 (*m*, CH$_2$CH_2OP, 2 H–C(5′)); 3.78 (*s*, MeOTr); 3.42 (*s*, MeO–C(3′)); 3.15 (*t*, CH_2CH$_2$O); 3.03–2.75 (*m*, 6 H, CH_2CH$_2$O, 2 MeCH_2); 0.97 (*t*, *Me*CH$_2$); 0.86 (*t*, *Me*CH$_2$). ^{31}P-NMR ($CDCl_3$): 150.78, 150.04. Anal. calc. for $C_{52}H_{55}N_8O_{12}P \cdot 0.5\ H_2O$ (1024.04): C 60.99, H 5.51, N 10.94; found: C 60.94, H 5.58, N 10.86.

34. *3′-O-Methyl-5′-O-(monomethoxytrityl)-*N^2*-[2-(4-nitrophenyl)ethoxycarbonyl]-*O^6*-[2-(4-nitrophenyl)-ethyl]guanosine 2′-[2-(4-Nitrophenyl)ethyl Diethylphosphoramidite]* (**59**). As described in *Exper. 6*, with 3′-*O*-methyl-5′-*O*-(monomethoxytrityl)-*N*^{2}-[2-(4-nitrophenyl)ethoxycarbonyl]-*O*^{6}-[2-(4-nitrophenyl)ethyl]guanosine (**55**) [22] (912 mg, 1.0 mmol) and dry THF as solvent. Purification by FC (silica gel (10 g), 11 × 2 cm, soln. in CH_2Cl_2/petroleum ether 1:1, then 2:1 (30 ml), 1:1 (30 ml), and 1:2 (70 ml), all with 1% of Et_3N): 885 mg (75%) of **59**. Colorless foam. TLC (SiO_2, petroleum ether/AcOEt/Et_3N 3:7:1): R_f 0.70. UV (MeOH): 269 (4.62), 235 (4.40), 204 (4.95). ^{1}H-NMR (CD_3CN): 8.25 (br., NH); 8.10–7.86 (*m*, 3 × 2 H *o* to NO$_2$, H–C(8)); 7.53–7.15 (*m*, 18 H, MeOTr, 3 × 2 H *m* to NO$_2$); 6.71 (*d*, 2 H *o* to MeO); 5.93, 5.88 (*2d*, H–C(1′)); 5.13 (*m*, H–C(2′)); 4.76 (*m*, CH$_2$CH_2O); 4.42 (*m*, H–C(4′)); 4.34 (*m*, CH$_2$CH_2OCO); 4.07 (*d*, H–C(3′)); 3.83, 3.63 (*2t*, CH$_2$CH_2OP); 3.69 (*s*, *Me*OTr); 3.37, 3.36 (*2s*, MeO–C(3′)); 3.39–3.20 (*m*, 2 H–C(5′)); 3.27 (*t*, CH_2CH$_2$O); 3.04–2.69 (*m*, 2CH_2CH$_2$O, 2 MeCH_2); 0.94 (*t*, *Me*CH$_2$); 0.75 (*t*, *Me*CH$_2$). ^{31}P-NMR (CD_3CN): 150.70, 149.62. Anal. calc. for: $C_{60}H_{62}N_9O_{15}P \cdot H_2O$ (1198.20): C 60.15, H 5.38, N 10.52; found: C 59.92, H 5.39, N 10.30.

35. *2′-O-Methyl-5′-O-(monomethoxytrityl)uridine 3′-(Hydrogen Butanedioate)* (**60**). To a soln. of 2′-*O*-methyl-5′-*O*-(monomethoxytrityl)uridine (**18**) (265 mg, 0.50 mmol) in CH_2Cl_2 (1 ml), succinic anhydride (= dihydrofluran-2,5-dione; 100 mg, 1.0 mmol) and DMAP (= 4-(dimethylamino)pyridine; 80 mg, 0.65 mmol) were added, and the soln. was stirred for 1 h at r.t. The mixture was diluted with CH_2Cl_2 (20 ml) and washed with sat. $NaHCO_3$ soln. (20 ml). The aq. phase was extracted twice with CH_2Cl_2 (10 ml), the combined org. phase washed with sat. $NaHCO_3$ soln. (20 ml), and the aq. phase extracted back with CH_2Cl_2 (10 ml). After a final wash with 10% citric acid (25 ml), the combined org. phase was dried (Na_2SO_4) and evaporated and the residue dried for 24 h at 40°: 312 mg (99%) of **60**. Colorless foam. TLC (SiO_2, CH_2Cl_2/MeOH 20:1): R_f 0.47. UV (MeOH): 261 (4.03), 231 (4.22), 205 (4.78). ^{1}H-NMR ($CDCl_3$): 10.51 (*s*, NH); 8.08 (*d*, H–C(6)); 7.39–7.25 (*m*, 12 H, MeO*Tr*); 6.84 (*d*, 2 H *o* to MeO); 5.91 (*s*, H–C(1′)); 5.30 (*m*, H–C(3′), H–C(5)); 4.30 (*d*, H–C(2′)); 3.97 (*m*, H–C(4′)); 3.80 (*s*, *Me*OTr); 3.66 (*dd*, 1 H–C(5′)); 3.56 (*s*, MeO–C(2′)); 3.41 (*dd*, 1 H–C(5′)); 2.82–2.52 (*m*, CH$_2$CH$_2$). Anal. calc. for: $C_{34}H_{34}N_2O_{10}$ (630.65): C 64.75, H 5.43, N 4.44; found: C 64.32, H 5.59, N 4.21.

36. *2′-O-Methyl-5′-O-(monomethoxytrityl)-*N^4*-[2-(4-nitrophenyl)ethoxycarbonyl]cytidine 3′-(Hydrogen Butanedioate)* (**61**). As described in *Exper. 35,* with **21** (361 mg, 0.50 mmol): 406 mg (99%) of **61**. Colorless foam. TLC (SiO_2, CH_2Cl_2/MeOH 20:1): R_f 0.43. UV (MeOH): 280 (4.21), 235 (4.44), 205 (4.84). ^{1}H-NMR ($CDCl_3$): 8.57 (*d*, H–C(6)); 8.16 (*d*, 2 H *o* to NO$_2$); 7.10–7.26 (*m*, 14 H, MeO*Tr*, 2 H *m* to NO$_2$); 6.95 (*d*, 1 H–C(5)); 6.85 (*d*, 2 H *o* to MeO); 5.98 (*s*, H–C(1′)); 5.35 (*m*, H–C(3′)); 4.35 (*m*, H–C(4′), CH$_2$CH_2O-CO); 3.97 (*d*, H–C(2′)); 3.80 (*s*, MeOTr); 3.66 (*s*, MeO–C(2′), 1 H–C(5′)); 3.38 (*dd*, 1 H–C(5′)); 3.04 (*t*, CH_2CH$_2$O); 2.91–2.49 (*m*, CH$_2$CH$_2$). Anal. calc. for $C_{43}H_{42}N_4O_{13}$ (822.82): C 62.77, H 5.15, N 6.64; found: C 62.62, H 5.29, N 6.64.

37. *2′-O-Methyl-5′-O-(monomethoxytrityl)-*N^6*-[2-(4-nitrophenyl)ethoxycarbonyl]adenosine 3′-(Hydrogen Butanedioate)* (**62**). As described in *Exper. 35,* with **23** (373 mg, 0.50 mmol): 408 mg (96%) of **62**. Colorless foam. TLC (SiO_2, CH_2CO_2/MeOH 20:1): R_f 0.48. UV (MeOH): 266 (4.46), 234 (4.31), 205 (4.87). ^{1}H-NMR ($CDCl_3$): 8.64 (*s*, H–C(8)); 8.16 (*d*, H–C(2), 2 H *o* to NO$_2$); 7.45–7.19 (*m*, 14 H, MeO*Tr*, 2 H *m* to NO$_2$); 6.81 (*d*, 2 H *o* to MeO); 6.12 (*d*, H–C(1′)); 5.54 (*t*, H–C(3′)); 4.77 (*m*, H–C(2′)); 4.51 (*t*, CH$_2$CH_2OCO); 4.36 (*m*, H–C(4′)); 3.77 (*s*, *Me*OTr); 3.54 (*dd*, 1 H–C(5′)); 3.43 (*dd*, 1 H–C(5′)); 3.36 (*s*, MeO–C(2′)); 3.14 (*t*, CH_2CH$_2$O); 2.73 (*s*, CH$_2$CH$_2$). Anal. calc. for $C_{44}H_{42}N_6O_{12}$ (846.85): C 62.41, H 5.00, N 9.92; found: C 61.92, H 5.11, N 9.68.

38. *2′-O-Methyl-5′-O-(monomethoxytrityl)-N²-[2-(4-nitrophenyl)ethoxycarbonyl]-O⁶-[2-(4-nitrophenyl)-ethyl]guanosine 3′-(Hydrogen Butanedioate)* (**63**). As described in *Exper. 35* with **25** (456 mg, 0.5 mmol): 491 mg (97%) of **63**. Colorless foam. TLC (SiO_2, CH_2Cl_2/MeOH 20:1): R_f 0.51. UV (MeOH): 269 (4.56), 234 (4.38), 205 (4.85). ^{1}H-NMR ($CDCl_3$): 8.15 (*dd*, 2 × 2 H *o* to NO_2); 7.97 (*s*, H−C(8)); 7.51–7.21 (*m*, 16 H, MeO*Tr*, 2 × 2 H *m* to NO_2); 6.80 (*d*, 2 H *o* to MeO); 6.10 (*d*, H−C(1′)); 5.54 (*m*, H−C(3′)); 4.80 (*t*, CH_2CH_2O); 4.65 (*2d*, H−C(2′)); 4.42 (*t*, CH_2CH_2OCO); 4.30 (*m*, H−C(4′)); 3.77 (*s*, MeO*Tr*); 3.48 (*dd*, 1 H−C(5′)); 3.41 (*dd*, 1 H−C(5′)); 3.34 (*s*, MeO−C(2′)); 3.30 (*t*, CH_2CH_2O); 3.07 (*t*, CH_2CH_2O); 2.72 (*m*, CH_2CH_2). Anal. calc. for $C_{52}H_{49}N_7O_{15}\cdot H_2O$ (1030.02): C 60.64, H 4.99, N 9.52; found: C 60.35, H 4.91, N 9.39.

39. *3′-O-Methyl-5′-O-(monomethoxytrityl)uridine 2′-(Hydrogen Butanedioate)* (**64**). As described in *Exper. 35* with **52** (265 mg, 0.50 mmol): 493 mg (99%) of **64**. Colorless foam. TLC (SiO_2, CH_2Cl_2/MeOH 20:1): R_f 0.43. UV (MeOH): 260 (4.00), 230 (4.21), 204 (4.73). ^{1}H-NMR ($CDCl_3$): 10.26 (*s*, NH); 7.86 (*d*, H−C(6)); 7.41–7.18 (*m*, 12 H, MeO*Tr*); 6.86 (*d*, 2 H *o* to MeO); 6.10 (*d*, H−C(1′)); 5.47 (*m*, H−C(2′)); 5.40 (*d*, H−C(5)); 4.12 (*m*, H−C(3′), H−C(4′)); 3.80 (*s*, *Me*OTr); 3.55 (*d*, 1 H−C(5′)); 3.42 (*dd*, 1 H−C(5′)); 3.39 (*s*, MeO−C(3′)); 2.71 (*m*, CH_2CH_2). Anal. calc. for: $C_{34}H_{34}N_2O_{10}$ (630.65); C 64.75, H 5.43, N 4.44; found: C 64.29, H 5.52, N 4.29.

40. *3′-O-Methyl-5′-O-(monomethoxytrityl)-N⁴-[2-(4-nitrophenyl)ethoxycarbonyl]cytidine 2′-(Hydrogen Butanedioate)* (**65**). As described in *Exper. 35,* with **53** (361 mg, 0.50 mmol): 393 mg (96%) of **65**. Colorless foam. TLC (SiO_2, CH_2Cl_2/MeOH 20:1): 0.40. UV (MeOH): 280 (4.19), 235 (4.43), 204 (4.84). ^{1}H-NMR ($CDCl_3$): 8.34 (*d*, H−C(6)); 8.16 (*d*, 2 H *o* to NO_2); 7.43–7.21 (*m*, 14 H, MeO*Tr*, 2 H *m* to NO_2); 6.98 (*d*, H−C(5)); 6.85 (*d*, 2 H *o* to MeO); 6.02 (*s*, H−C(1′)); 5.52 (*m*, H−C(2′)); 4.38 (*t*, CH_2CH_2OCO); 4.12 (*s*, H−C(3′), H−C(4′)); 3.80 (*s*, *Me*OTr); 3.57 (*d*, 1 H−C(5′)); 3.40 (*d*, 1 H−C(5′)); 3.35 (*s*, MeO−C(3′)); 3.07 (*t*, CH_2CH_2O); 2.72 (*m*, CH_2CH_2). Anal. calc. for $C_{43}H_{42}N_4O_{13}\cdot H_2O$ (840.84): C 61.42, H 5.27, N 6.66; found: C 61.58, H 5.23, N 6.56.

41. *3′-O-Methyl-5′-O-(monomethoxytrityl)-N⁶-[2-(4-nitrophenyl)ethoxycarbonyl]adenosine 2′-(Hydrogen Butanedioate)* (**66**). As described in *Exper. 35,* with **54** (373 mg, 0.50 mmol): 412 mg (97%) of **66**. Colorless foam. TLC (SiO_2, CH_2Cl_2/MeOH 20:1): R_f 0.45. UV (MeOH): 266 (4.46), 233 (4.30), 206 (4.85). ^{1}H-NMR ($CDCl_3$): 9.32 (br., NH); 8.65 (*s*, H−C(8)); 8.18 (*s*, H−C(2)); 8.12 (*d*, 2 H *o* to NO_2); 7.41–7.16 (*m*, 14 H, MeO*Tr*, 2 H *m* to NO_2); 6.79 (*d*, 2 H *o* to MeO); 6.23 (*d*, H−C(1′)); 5.98 (*m*, H−C(2′)); 4.48–4.38 (*m*, CH_2CH_2OCO, H−C(3′)); 4.29 (*m*, H−C(4′)); 3.75 (*s*, *Me*OTr); 3.50 (*m*, H−C(5′)); 3.39 (*s*, MeO−C(3′)); 3.36 (*m*, 1 H−C(5′)); 3.09 (*t*, CH_2CH_2O); 2.67 (*s*, CH_2CH_2). Anal. calc. for $C_{44}H_{42}N_6O_{12}\cdot H_2O$ (864.87): C 61.10, H 5.13, N 9.72; found: C 60.78, H 5.10, N 9.85.

42. *3′-O-Methyl-5′-O-(monomethoxytrityl)-N²-[2-(4-nitrophenyl)ethoxycarbonyl]-O⁶-[2-(4-nitrophenyl)-ethyl]guanosine 2′-(Hydrogen Butanedioate)* (**67**). As described in *Exper. 35,* with **55** (228 mg, 0.25 mmol), succinic anhydride (50 mg, 0.5 mmol), and DMAP (40 mg, 0.33 mmol): 244 mg (96%) of **67**. Colorless foam. TLC (SiO_2, CH_2Cl_2/MeOH 20:1): R_f 0.43. UV (MeOH): 269 (4.54), 235 (4.36), 206 (4.86). ^{1}H-NMR ($CDCl_3$): 8.17–8.12 (*m*, 2 × 2 H *o* to NO_2); 8.04 (*s*, H−C(8)); 7.50–7.15 (*m*, 16 H, MeO*Tr*, 2 × 2 H *m* to NO_2); 6.77 (*d*, 2 H *o* to MeO); 6.14 (*d*, H−C(1′)); 5.85 (*m*, H−C(2′)); 4.76 (*t*, CH_2CH_2O); 4.59 (*t*, H−C(3′)); 4.41 (*t*, CH_2CH_2OCO); 4.18 (*m*, H−C(4′)); 3.76 (*s*, *Me*OTr); 3.49 (*dd*, 1 H−C(5′)); 3.40 (*s*, 3 MeO−C(3′)); 3.39 (*dd*, 1 H−C(5′)); 3.27 (*t*, CH_2CH_2O); 3.05 (*t*, CH_2CH_2O); 2.69 (*m*, CH_2CH_2). Anal. calc. for $C_{52}H_{49}N_7O_{15}$ (1012.00): C 61.17, H 4.88, N 9.69; found: C 61.43, H 5.11, N 9.39.

43. *Long-Chain (Methylamino)alkyl Controlled-Pore Glass* (LCMAA-CPG; **68**). To a soln. of 1.1′-Carbonylbis[1*H*-diimidazole] (5 g, 62 mmol) in abs. CH_2Cl_2 (150 ml) under Ar, glyceryl-CPG 500 Å (*Bioran*, *Schott*; 5 g, dried for several hours under high vacuum) was added and shaken at r.t. for 6 h. Then, the material was collected in a glass suction filter and washed with CH_2Cl_2 (4 ×) by taking up the CPG in CH_2Cl_2 (50 ml) and suction-filtering. Thereafter, the CPG material was taken up in CH_2Cl_2 (50 ml), *N*,*N*′-dimethylhexane-1,6-diamine (5 ml, 57 mmol) was added, and the mixture was shaken for 18 h at r.t. The LCMAA-CPG (**68**) was isolated in a glass-frit suction funnel, washed with MeOH, DMF, pyridine, H_2O, MeOH, acetone, and Et_2O, and dried at 40° under high vacuum.

44. *Derivatives* **69**–**76** *of LCMAA-CPG 500 Å* (**68**) *and Nucleoside 3′-or 2′-(Hydrogen Butanedioate)* **60**–**67**. To a soln. of 18 μmol of the nucleoside 3′- or 2′-hydrogen butanedioate (11.3 mg of **60** or **64**; 14.8 mg of **61** or **65**; 15.2 mg of **62** or **66**; 18.5 mg of **63** or **67**) in abs. MeCN (2 ml), LCMAA-CPG (**68**; 250 mg), 2-{[2-(2-(cyanoethoxy)-2-oxoethylidene]amino}-1,1,3,3-tetramethyl]uronium tetrafluoroborate (TOTU; 8 mg, 24 mmol), and methylmorpholine (5 mg, 45 mmol) were added. After shaking for 4 h, the CPG material was collected in a glass-frit suction funnel and washed with MeOH, DMF, MeOH, acetone and Et_2O. For the capping procedure, the nucleoside-functionalized CPG, abs. pyridine (5 ml), Ac_2O (100 ml, 1.06 mmol) and DMAP (5 mg, 0.04 mmol) were kept at r.t. for 30 min. The nucleoside-functionalized CPG (**69**–**76**) was isolated in a

glass suction filter, washed with MeOH, DMF, H_2O, MeOH, acetone, and Et_2O, and dried at 40° under high vacuum. Determination of loading: A defined amount of **69**–**76** (3–7 mg) was weighed into a 10-ml measuring flask. After addition of 10 ml of 0.2M TsOH in MeCN, the absorption of the trityl cation was measured at 478 nm against 0.2M TsOH in MeCN. The loading was calculated by the formula L [μmol/g] = 14.4 · A/m (L = loading, A = absorbance at 478 nm, m = weighed CPG **69**–**76** in mg): **69**, L = 26.7 μmol/g; **70**, L = 38.0 μmol/g; **71**, L = 28.7 μmol/g; **72**, L = 23.3 μmol/g; **73**, L = 30.9 μmol/g; **74**, L = 28.4 μmol/g; **75**, L = 28.4 μmol/g; **76**, L = 24.9 μmol/g.

45. *Assembly of Oligonucleotides* **77**–**90**. The syntheses were carried out with an *Applied Biosystems 380B* or *392* DNA synthesizer. Nucleoside-functionalized CPG material **69**–**76** (0.6 or 0.2 μmol) was packed into a 1-mmol *ABI* crimp column. Cycles of nucleotide addition were carried out by a programmed series of reagent and solvent washes based on recommended procedures with the following main steps: 1) 5′-*O*-MeOTr Deprotection: 5% CCl_3COOH (for $(MeO)_2Tr$ deprotection, 3% CCl_3COOH) in CH_2Cl_2, delivered in 3 15-s and 2 10-s bursts with intermediate 4-s block flushes (*ABI 380*), 4 6-s bursts with intermediate 5-s trityl flushes (*ABI 392*); the eluate from this step was collected and the absorbance at 478 nm (498 nm for $(MeO)_2Tr$) measured to determine the condensation yields. 2) Coupling: 0.1M phosphoramidite and 0.5M 1*H*-tetrazole in dry MeCN, delivered in alternating reagent pulses with a subsequent waiting time of 40 to 600 s. 3) Capping: Ac_2O/2,6-dimethylpyridine/THF 1:1:8 and 1-methyl-1*H*-imidazole/THF 16:84, delivered in one 15-s burst with a subsequent wait time of 10 s (*ABI 380*), 10-s burst and wait time of 5 s (*ABI 392*). 4) Oxidation: 0.05M I_2 in THF/H_2O/pyridine 7:2:1, delivered in one 15-s burst with a subsequent wait time of 15 s (*ABI 380*), 8-s burst and wait time of 15 s (*ABI 392*).

Deprotection and cleavage program: 1) npe/npeoc Deprotection for the *ABI 380B:* 1M DBU in MeCN delivered in a 100-s burst with a consecutive waiting time of 900 s and then 9 more 60-s bursts followed by waiting times of 900 s, 1800 s, 2 × 3600 s, and 5 × 5400 s (total waiting time 10.5 h). *ABI 392:* burst times reduced to 30 s for the first and to 25 s for the consecutive ones. To assure complete removal of all DBU, the column was additionally washed with CF_3COOH for trityl-off products in a detritylation step before final cleavage. 2) Cleavage from the support for the *ABI 380B:* conc. NH_3 delivered in a 22-s burst with a consecutive waiting time of 1800 s repeated 3 times (total waiting time 2 h). *ABI 392:* burst times reduced to 11 s.

The NH_3 soln. was collected and lypholized in a *Speed-vac* concentrator under high vacuum. The isolated amount of oligonucleotide was determined by measuring the absorbance at 260 nm.

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