Synthesis of Photolabile 5′-*O*-Phosphoramidites for the Photolithographic Production of Microarrays of Inversely Oriented Oligonucleotides

by **Markus Beier**[1]), **Achim Stephan**, and **Jörg D. Hoheisel***

Functional Genome Analysis, Deutsches Krebsforschungszentrum, Im Neuenheimer Feld 506, D-69120 Heidelberg, Germany (fax: +49-6221-424682; e-mail: j.hoheisel@dkfz.de)

The photolabile 3′-*O*-{[2-(2-nitrophenyl)propoxy]carbonyl}-protected 5′-phosphoramidites (**16**–**18**) were synthesized (see *Scheme*) for an alternative mode of light-directed production of oligonucleotide arrays. Because of the characteristics of these monomeric building blocks, photolithographic *in situ* DNA synthesis occurred in 5′ → 3′ direction, in agreement with the orientation of enzymatic synthesis. Synthesis yields were as good as those of conventional reactions. The resulting oligonucleotides are attached to the surface *via* their 5′-termini, while the 3′-hydroxy groups are available as substrates for enzymatic reactions such as primer extension upon hybridization of a DNA template (see *Fig. 2*). The production of such oligonucleotide chips adds new procedural avenues to the growing number of applications of DNA microarrays.

Introduction. – In recent years, DNA microarrays have become a diagnostic assay system of ever increasing importance to a wide range of biotechnical and biomedical applications [1]. Among the various technologies used for their production, the photolithographically controlled *in situ* synthesis of oligonucleotide arrays [2–6] proved to be especially versatile. Based on sequence information, they can readily be made without the cumbersome preparative steps involved in the production of other array formats. However, the photolithographic approach was problematic because of the low degree of flexibility in mask production – with a particular set of masks needed for each individual class of DNA chip – and the relatively low efficiency of the photochemistry. With current developments in flexible mask design [7], the former problem should soon be overcome, however. Also, a new set of photolabile compounds was recently introduced. Nucleoside building blocks bearing 5′-*O*-{[2-(2-nitrophenyl)-propoxy]carbonyl} (5′-*O*-nppoc) groups [8][9] were found to be notably superior under appropriate reaction conditions compared to other photoprotecting groups, resulting in quantitative yields per condensation [10].

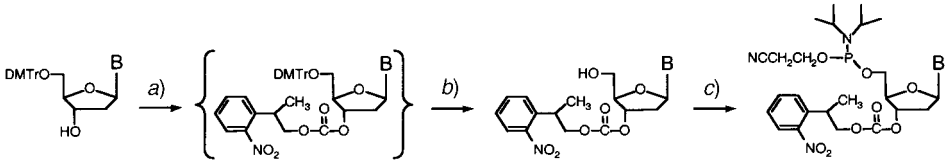
Nevertheless, the methodology permitted oligonucleotide synthesis only in the 3′ → 5′ direction, thus linking the 3′-terminus to the solid support. For several enzymatic reactions and especially polymerase extension, however, the availability of the 3′-hydroxy group is a prerequisite. Thus, reversing the synthesis direction is advantageous or even essential to various chip-based applications, such as highly parallel DNA sequencing [11] or the creation of microarrays containing double-stranded DNA probes [12], for example. Other approaches to produce oligonucleotide arrays with free 3′-ends relied on the hybridization of secondary primers [12] or the spotting of pre-

[1]) Current address: *FeBiT Ferrarius Biotechnology*, Käfertalerstr. 190, D-68167 Mannheim (e-mail: markus.beier@febit.de)

synthesized, mostly 5′-amino-attached molecules [13]. Also, an *in situ* inversion of the orientation of molecules produced initially in the 3′ → 5′ direction was reported [14]. Direct synthesis in the 5′ → 3′ direction is known for conventional synthesis chemistry [15]. Surprisingly, however, there had been no reports of attempted light-directed *in situ* synthesis. Therefore, we set out to synthesize the 3′-photoprotected 5′-phosphoramidites needed for photolithographic *in situ* oligonucleotide synthesis in the 5′ → 3′ direction. As before [10], the nppoc moiety was used as the photoprotecting group of choice.

Results and Discussion. – *Photolabile 3′-*O*-nppoc-Protected Phosphoramidites.* Synthesis of the four monomeric building blocks (**15**–**18**) started from the 5′-*O*-$(MeO)_2$Tr-protected 2′-deoxynucleosides **3**–**6** (*Scheme*). For protection of the exocyclic amino functions, the base-labile [4-(*tert*-butyl)phenoxy]carbonyl group was chosen, which allowed for mild and fast deprotection by a 1 h treatment with ammonia after array synthesis. The nppoc moieties were introduced at the 3′-*O*-position by employing the mild acylating reagent **2** generated from 2-(2-nitrophenyl)propyl carbonochloridate (**1**) and 1-methyl-1*H*-imidazole. When the carbonochloridate **1** was used directly instead of the corresponding 1-methyl-1*H*-imidazolium salt **2**, several by-products were formed and yields dropped significantly. The addition of molecular sieves to the reaction mixture increased the yields further, an effect that has been described before [16]. Because of the ease of chromatographic purification, usually the 5′-*O*-(MeO_2)Tr groups were removed by acid treatment without isolation of the fully protected nucleosides **7**–**10**. The 3′-*O*-nppoc-protected nucleosides **11**–**14** were all obtained in high yields by chromatography. The cytidine derivative **12** was conveniently isolated by precipitation. The final phosphitylation of the 5′-hydroxy group was accomplished in the usual manner by reaction with cyanoethyl tetraisopropylphosphorodiamidite in the presence of pyridine hydrochloride [17] as a mild acidic catalyst instead of the commonly used 1*H*-tetrazole. By this procedure, all four monomeric building blocks **15**–**18** were obtained in high yield. In their NMR spectra, the

Scheme. *Synthesis of Solid Phase 5′-*O*-Phosphoramidites Bearing Photolabile 3′-*O*-nppoc Groups*



	B
3	T
4	^{tac}C
5	^{tac}A
6	^{tac}G

	B	%
7	T	-
8	^{tac}C	74
9	^{tac}A	-
10	^{tac}G	-

	B	%
11	T	93
12	^{tac}C	73
13	^{tac}A	86
14	^{tac}G	81

	B	%
15	T	88
16	^{tac}C	81
17	^{tac}A	65
18	^{tac}G	91

DMTr = $(MeO)_2$Tr = 4,4′-dimethoxytrityl, tac = [4-(*tert*-butyl)phenoxy]acetyl

a) 1.2 Equiv. of 3-{[2-(2-nitrophenyl)propoxy]carbonyl}-1-methyl-1*H*-imidazolium chloride (**2**) (from 1-methyl-1*H*-imidazole and 2-NO_2–C_6H_4–CH(Me)CH_2OCOCl (**1**)). *b*) 10% CCl_3COOH. *c*) 2-Cyanoethyl tetraisopropylphosphorodiamidite, pyridine hydrochloride.

phosphoramidites showed additional peaks due to the additional chiral centre within the nppoc moiety.

Photolithographic Array Synthesis and Hybridization. Light-directed synthesis of oligonucleotide arrays with the new 5′-phosphoramidites was carried out as described earlier [10]. To compensate for the slightly lower reactivity of the secondary 3′-hydroxy group, the highly reactive pyridine hydrochloride was employed as the activator of choice [17]. Furthermore, a condensation protocol was implemented that consisted of two subsequent condensation steps for high coupling yields. All other synthesis steps were performed as described for the corresponding 3′-phosphoramidites [10].

Subsequent to final deprotection, chips were hybridized with complementary target sequences and compared to arrays produced with photolabile 3′-phosphoramidites. Also, oligonucleotides were synthesized in parallel 3′ → 5′ and 5′ → 3′ directed reactions on a single glass chip. On such slides, the first photoactive layer was formed by a photoprotected triethylenglycol-phosphoramidite to provide the same starting conditions for both procedures. Considering the different directions of synthesis, the order of base additions was kept identical, therefore producing oligonucleotides of inversed sequence. Subsequent to deprotection, hybridizations were performed with complementary targets under conditions that reflect the amount of full-length molecules (*Fig. 1*). In all cases, with different sequences, no difference in performance was observed with respect to signal homogeneity, intensity, and signal-to-background ratios.

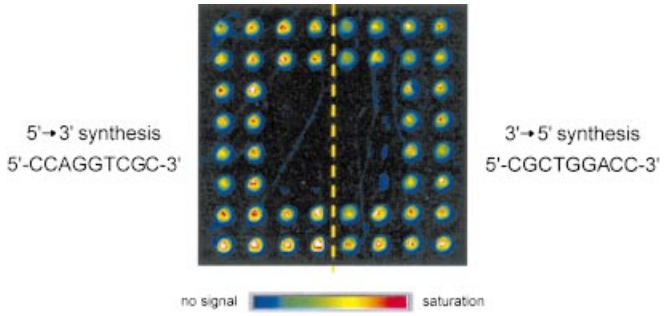


Fig. 1. *Comparison of standard and inverse* in situ *synthesis on a single chip*. On the left: 5′-phosphoramidites were added for the synthesis in 5′ → 3′ direction yielding 5′-CCAGGTCGC-3′; on the right: 3′-phosphoramidites were added for the synthesis in the 3′ → 5′ direction yielding 5′-CGCTGGACC-3′. Signals were produced by a simultaneous hybridization with equimolar amounts of the two complementary oligonucleotides. Hybridizations with one oligonucleotide at a time produced a signal on the respective half of the chip only (not shown). Differences between individual spot signals within either half are due to inhomogeneity of the light source.

On-Chip Enzymatic Reactions. To establish the availability of the oligonucleotides 3′-ends, on-chip enzymatic reactions were performed, by means of a re-designed PCR machine for temperature control. Template DNA was added to the oligonucleotide slide and heat-denatured, followed by 15 min of annealing at 15°. Primer extension was carried out with either *Klenow* fragment of DNA polymerase I at 37° or *Taq* polymerase at 55°. Subsequently, the template DNA was completely removed by washing at 95°. Successful extension of the surface-bound primer was detected by a simultaneous hybridization of two probe molecules, labelled with either Cy3 or Cy5, that were specific to the primer sequence or the newly synthesized DNA (*Fig. 2*). In the

absence of any DNA template during the polymerase reaction or upon addition of a fragment of no complementarity to the oligonucleotide primers, no signal could be observed with the latter probe.

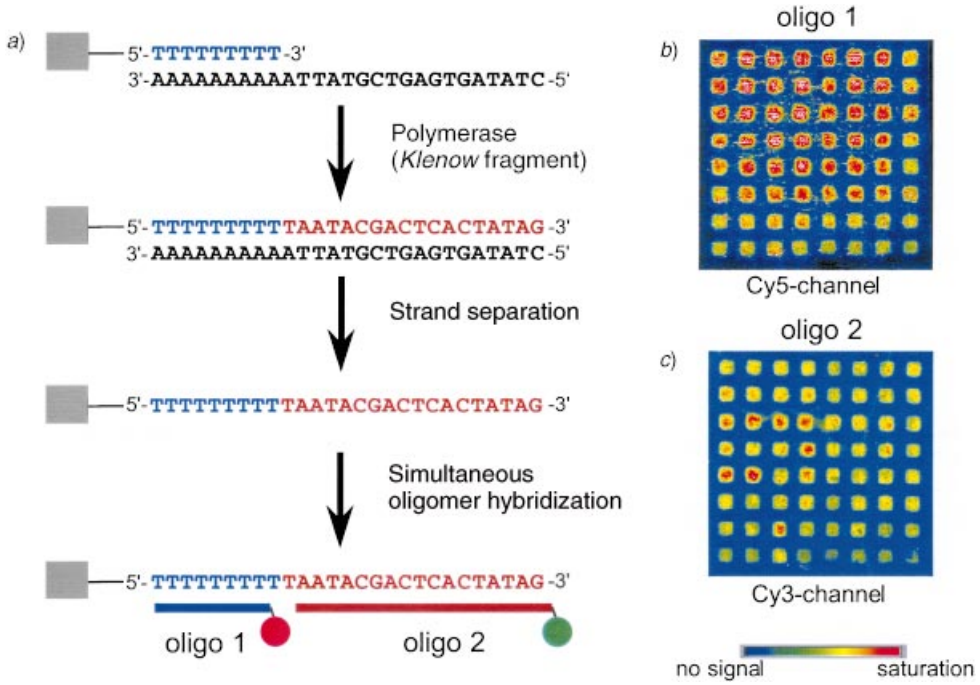


Fig. 2. *Generation of an array of d(T_9) sequences by light-directed* in situ *synthesis in the 5′ → 3′ direction on a microscope glass slide. a*) Subsequent to the annealing of template d(CTATAGTGAGTCGTATTAAAAAAAAAA), a primer extension reaction was carried out with the *Klenow* fragment, followed by the removal of the template. *b*) *c*) The surface-bound oligomer sequences were made visible by a simultaneous hybridization with d(A_{16}), labelled with dye Cy5 (*b*), and Cy3-labelled d(TTATGCTGAGTGATATC) (*c*).

Conclusion. – An efficient route for the synthesis of 3′-*O*-photoprotected 5′-*O*-phosphoramidites was established starting from commercially available precursors. These monomers were utilized for light-directed *in situ* synthesis of oligonucleotides on microarrays. Because of the inverse synthesis direction, the oligomers' 3′-hydroxy groups are exposed and can act as substrates for on-chip enzymatic reactions. This feature opens new avenues in the ever growing field of applications of *in situ* synthesized oligonucleotide arrays.

Experimental Part

General. $(MeO)_2$)Tr-Protected nucleosides and 2-cyanoethyl tetraisopropylphosphorodiamidite were obtained from *Proligo* (Germany). Other reagents were from *Fluka* (Germany) (FC = flash chromatography). All newly synthesized compounds were characterized in the usual manner by ^{1}H-, ^{31}P-, and ^{13}C-NMR, electrospray (ESI) and high-resolution fast-atom-bombardment (HR-FAB) mass spectroscopy. Only the relevant part of these analyses is presented here, but the remainder can be provided upon request.

*3-{[2-(2-Nitrophenyl)propoxy]carbonyl}-1-methyl-1*H*-imidazolium Chloride* (**2**). At 0°, 2-(2-nitrophenyl)-propyl carbonochloridate (**1**) (1.07 ml, 4.4 mmol) was added dropwise to a soln. of 1-methyl-1*H*-imidazole 1.24 ml (14.7 mmol) in CH_2Cl_2 (40 ml) over molecular sieves of 4 Å. After 30 min stirring, this soln. was used directly in the subsequent reaction.

*N⁴-{[4-(*tert*-Butyl)phenoxy]acetyl}-2′-deoxy-5′-*O*-(4,4′-dimethoxytrityl)-3′-*O*-{[2-(2-nitrophenyl)propoxy]carbonyl}cytidine* (**8**). A soln. of 2.27 g N^4-[[4-tert-butyl)phenoxy]acetyl}-2′-deoxy-5′-*O*-(4,4’-dimethoxytrityl)cytidine (**4**; 3.1 mmol) in CH_2Cl_2 (20 ml) was added dropwise within 10 min to **2** (3.7 mmol, 1.2 equiv.) in CH_2Cl_2 (50 ml), kept over molecular sieves at 0°. The resulting soln. was stirred overnight at 0°, extracted with sat. $NaHCO_3$ soln (100 ml), dried (Na_2SO_4), and evaporated. Purification by FC (0–66% AcOEt/toluene) gave 2.12 g (74%) of **8**. R_f (toluene/AcOEt 2:1) 0.34. ^{1}H-NMR ((D$_6$)DMSO): 10.90 (br., NH); 8.04 (2*d*, H–C(6)); 7.80 (*m*, 1 H *o* to NO_2); 7.67 (*m*, 1 H *m* to NO_2, 1 H *p* to NO_2); 7.47 (*m*, 1 H *m* to NO_2); 7.19–7.34 (*m*, 9 H of $(MeO)_2$*Tr*, 2 H *m* to ^{t}Butyl); 6.99 (2*d*, H–C(5)); 6.84 (*m*, 4 H of $(MeO)_2$*Tr*, 2 H *o* to ^{t}Bu); 6.07 (*m*, H–C(1′)); 5.10 (*m*, H–C(3′)); 4.77 (*s*, $COCH_2O$); 4.14–4.35 (*m*, MeCHCH_2O, H–C(4′)); 3.70 (*m*, 2 MeO); 3.51 (*m*, MeC*H*CH_2O); 3.20–3.30 (*m*, 2 H–C(5′)); 2.51 (*m*, 1 H–C(2′)); 2.31 (*m*, 1 H–C(2′)); 1.28 (*d*, *Me*$CHCH_2O$); 1.24 (*s*, ^{t}Bu). HR-FAB-MS: 927.3826 ($[M+H]^+$, $C_{52}H_{54}N_4O_{12}^+$; calc. 927.3816). ESI-MS: 927 ($[M+H]^+$), 950 ($[M+Na]^+$).

*3′-*O*-{[2-(2-Nitrophenyl)propoxy]carbonyl}thymidine* (**11**). A soln. of 5′-*O*-(dimethoxytrityl)thymidine (**3**; 2 g, 3.67 mmol) in CH_2Cl_2 (20 ml) was added dropwise within 10 min to **2** (4.4 mmol, 1.2 equiv.) in CH_2Cl_2 (30 ml) over molecular sieves at 0′. The resulting soln. was stirred overnight at 0°, extracted with 100 ml 0.5% HCl soln., and dried (Na_2SO_4). Then 10% CCl_3COOH soln. (70 ml) in CH_2Cl_2 was added to the org. phase and stirred for 2 min. The red soln. was washed with sat. $NaHCO_3$ soln. (2 × 100 ml), dried (Na_2SO_4), and evaporated. Purification by FC (0–10% MeOH in toluene/AcOEt 5:4) gave 1.53 g (93%) of **11**. R_f (toluene/AcOEt 1:2) 0.21. ^{1}H-NMR ((D$_6$)DMSO): 11.26 (br., NH); 7.82 (*m*, 1 H *o* to NO_2); 7.69 (*m*, H–C(6), 1 H *m* to NO_2, 1 H *p* to NO_2); 7.49 (*m*, 1 H *m* to NO_2); 6.11 (*m*, H–C(1′)); 5.09 (*m*, H–C(3′), OH–C(5′)); 4.33 (*m*, MeCHCH_2O); 3.96 (*m*, H–C(4′)); 3.59 (2 *m*, 2 H–C(5′)); 3.52 (*m*, MeC*H*CH_2O); 2.24 (*m*, 2 H–C(2′)); 1.77 (2 *s*, Me–C(5)); 1.29 (*d*, *Me*$CHCH_2O$). HR-FAB-MS: 450.1524; ($[M+H]^+$, $C_{20}H_{23}N_3O_9^+$; calc.: 450.1512). ESI-MS: 450 ($[M+H]^+$), 472 ($[M+Na]^+$), 899 ($[2M+H]^+$), 921 ($[2M+Na]^+$).

N^4*-{[4-(*tert*-Butyl)phenoxy]acetyl}-2′-deoxy-3′-*O*-{[2-(2-nitrophenyl)propoxy]carbonyl}cytidine* (**12**). As described for **11**, with **4** (5 g, 6.93 mmol), CH_2Cl_2 (50 ml), **2** (2.71 g, 8.32 mmol), and CH_2Cl_2 (50 ml). Purification by precipitation with toluene gave 3.16 g (73%) of **12**. R_f (toluene/AcOEt 1:4) 0.50. ^{1}H-NMR ((D$_6$)DMSO): 10.87 (br., NH); 8.29 (*d*, H–C(6)); 7.82 (*m*, 1 H *o* NO_2); 7.69 (*m*, 1 H *m* to NO_2, 1 H *p* to NO_2); 7.48 (*m*, 1 H *m* to NO_2); 7.29 (*m*, 2 H *m* to ^{t}Bu); 7.13 (*d*, H–C(5)); 6.84 (*m*, 2 H *o* to ^{t}Bu); 6.08 (*m*, H–C(1′)); 5.10 (*m*, H–C(3′), OH–C(5′); 4.77 (*s*, $COCH_2O$); 4.33 (*m*, MeCH_2O); 4.10 (*m*, H–C(4′)); 3.62 (*m*, 2 H–C(5′)); 3.53 (*m*, MeC*H*CH_2O); 2.48 (*m*, 1 H–C(2′)); 2.21 (*m*, 1 H–C(2′)); 1.29 (*d*, *Me*$CHCH_2O$); 1.24 (*s*, ^{t}Bu). HR-FAB-MS: 625.2495 ($[M+H]^+$, $C_{31}H_{36}N_4O_{10}^+$: calc. 625.2509). ESI-MS: 625 ($[M+H]^+$), 647 ($[M+Na]^+$), 1249 ($[2M+H]^+$), 1271 ($[2M+Na]^+$).

N^6*-{[4-(*tert*-Butyl)phenoxy]acetyl}-2′-deoxy-3′-*O*-{[2-(2-nitrophenyl)propoxy]carbonyl}adenosine* (**13**). As described for **11**, with N^6-{[(*tert*-butyl)phenoxy]acetyl}-2′-deoxyadenosine (**5**; 2.73 g, 3.67 mmol), CH_2Cl_2 (50 ml), **2** (1.31 g, 4.40 mmol), and CH_2Cl_2 (50 ml). Purification by FC (0–4% MeOH toluene/AcOEt 1:1) gave 2.05 g (86%) of **13**. R_f (toluene/AcOEt 1:1) 0.17. ^{1}H-NMR ((D$_6$)DMSO): 10.78 (br., NH); 8.66 (*m*, H–C(2), H–C(8)); 7.83 (*m*, 1 H *o* to NO_2); 7.70 (*m*, 1 H *m* to NO_2, 1 H *p* to NO_2); 7.49 (*m*, 1 H *m* to NO_2); 7.30 (*m*, 2 H *o* to ^{t}Bu); 6.89 (*m*, 2 H *m* to ^{t}Bu); 6.43 (*m*, H–C(1′)); 5.28 (*m*, H–C(3′)); 5.14 (*m*, OH–C(5′)); 4.98 (*s*, $COCH_2O$); 4.34 (*m*, MeCHCH_2O); 4.12 (*m*, H–C(4′)); 3.60 (*m*, 2 H–C(5′), MeC*H*CH_2O); 3.02 (*m*, 1 H–C(2′)); 2.57 (*m*, 1 H–C(2′)); 1.31 (*d*, MeCHCH_2O); 1.24 (*s*, ^{t}Bu). HR-FAB-MS: 649.2644 ($[M+H]^+$, $C_{32}H_{36}N_6O_9^+$; calc. 649.2621). ESI-MS: 649 ($[M+H]^+$), 671 ($[M+Na]^+$), 1297 ($[2M+H]^+$), 1319 ($[2M+Na]^+$).

N^2*-{[4-(*tert*-Butyl)phenoxy]acetyl}-2′-deoxy-3′-*O*-{[2-(2-nitrophenyl)propoxy]carbonyl}guanosine* (**14**). As described for **11**, with N^2-{[4-(*tert*-butyl)phenoxy]acetyl}-2′-deoxyguanosine (**6**; 5 g, 6.58 mmol), CH_2Cl_2 (50 ml), **2** (2.57 g, 7.9 mmol), and CH_2Cl_2 (50 ml). Purification by FC (0–10% MeOH in toluene/AcOEt 1:1) gave 3.53 g (81%) of **14**. R_f (AcOEt/MeOH 3:1) 0.18. ^{1}H-NMR ((D$_6$)DMSO): 11.74 (br., 2 NH); 8.23 (2 *s*, H–C(8)); 7.83 (*m*, 1 H *o* to NO_2); 7.70 (*m*, 1 H *m* to NO_2, 1 H *p* to NO_2); 7.49 (*m*, 1 H *m* to NO_2); 7.30 (*m*, 2 H *o* to ^{t}Bu); 6.89 (*m*, 2 H *m* to ^{t}Bu); 6.18 (*m*, H–C(1′)); 5.13 (*m*, H–C(3′)); 5.06 (*m*, OH–C(5′)); 4.81 (2 *s*, MeCHCH_2O); 4.34 (*m*, MeCHH_2O); 4.04 (*m*, H–C(4′)); 3.55 (*m*, 2 H–C(5′), MeC*H*CH_2O); 2.83 (*m*, 1 H–C(2′)); 2.49 (*m*, 1 H–C(2′)); 1.29 (*d*, *Me*CHCH_2O); 1.25 (*s*, ^{t}Bu). HR-FAB-MS: 665.2582 ($[M+H]^+$, $C_{32}H_{36}N_6O_{10}^{2+}$; calc. 665.2570). ESI-MS: 665 ($[M+H]^+$), 687 ($[M+Na]^+$), 1329 ($[2M+H]^+$), 1351 ($[2M+Na]^+$).

3′-O-{[2-(2-Nitrophenyl)propoxy]carbonyl}thymidine 5′-(2-Cyanoethyl Diisopropylphosphoramidite) (**15**). A soln. of 2-cyanoethyl tetraisopropylphosphorodiamidite (1.2 ml, 3.98 mmol) and 0.5M pyridine hydrochloride (3.4 ml, 1.7 mmol) in MeCN was added to a soln. of **11** (1.53 g, 3.4 mmol) in MeCN (15 ml). After stirring for 1 h, the mixture was partitioned between CH_2Cl_2 (100 ml) and sat. $NaHCO_3$ soln. (100 ml). The org. layer was washed with sat. NaCl soln. (100 ml), dried (Na_2SO_4) and evaporated. Purification by FC (0–30% AcOEt// toluene) gave 1.94 g (88%) of **15**. R_f (toluene/AcOEt 1:1) 0.37. ^{1}H-NMR ((D$_6$)DMSO): 11.26 (br., NH); 7.82 (*m*, 1 H *o* to NO_2); 7.69 (*m*, 1 H *m* to NO_2, 1 H *p* to NO_2); 7.47–7.55 (*m*, H–C(5), 1 H *m* to NO_2); 6.08 (*m*, H–C(1′)); 5.09 (*m*, H–C(3′)); 4.27–4.35 (*m*, MeCHCH_2O); 4.12 (*m*, H–C(4′)); 3.70–3.83 (*m*, 2 H–C(5′), OCH_2CH$_2$CN); 3.49–3.59 (*m*, 2 Me$_2$C*H*, MeC*H*CH$_2$O); 2.75 (*m*, OCH$_2$CH_2CN); 2.29 (*m*, 2 H–C(2′)); 1.78 (*m*, Me); 1.28 (*d*, Me); 1.09–1.24 (*m*, 7 Me). ^{31}P-NMR ((D$_6$)DMSO): 149.36; 149.33; 149.29. ESI-MS: 649 ([*M* + H]$^+$, 672 ([*M* + Na]$^+$), 1321 ([2*M* + Na]$^+$). HR-FAB-MS: 650.2576 ([*M* + H]$^+$, $C_{29}H_{40}N_5O_{10}P^+$; calc. 650.2590).

N^4*-{[4-(*tert*-Butyl)phenoxy]acetyl}-2′-deoxy-3′-O-{[2-(2-nitrophenyl)propoxy]carbonyl}cytidine 5′-(2-Cyanoethyl Diisopropylphosphoramidite)* (**16**). As described for **15**, with **12** (1.51 g, 2.41 mmol), 2-cyanoethyl tetraisopropylphosphorodiamidite (0.9 ml, 2.84 mmol) and 0.5M pyridine hydrochloride (2.6 ml, 1.3 mmol). Purification by FC (0–30% AcOEt/toluene) gave 1.61 g (81%) of **16**. R_f (toluene/AcOEt 1:1) 0.43. ^{1}H-NMR ((D$_6$)DMSO): 10.90 (br., NH); 8.14 (*m*, H–C(6)); 7.82 (*m*, 1 H *o* to NO_2); 7.69 (*m*, 1 H *m* to NO_2, 1 H *p* to NO_2); 7.48 (*m*, 1 H *m* to NO_2); 7.29 (*m*, 2 H *o* to ^{t}Bu); 7.14 (*m*, H–C(5)); 6.83 (*m*, 2 H *m* to ^{t}Bu); 6.07 (*m*, H–C(1′)); 5.10 (*m*, H–C(3′)); 4.77 (*s*, COCH_2O); 4.30 (*m*, MeCHCH_2O, H–C(4′)); 3.75 (*m*, 2 H–C(5′), COCH_2CH$_2$CN); 3.55 (*m*, 2 MeC*H*, MeCH_2O); 2.73 (*m*, OCH$_2$CH_2CN); 2.55 (*m*, 1 H–C(2′)); 2.25 (*m*, 1 H–C(2′)); 1.29 (*m*, Me); 1.15 (*m*, Me). ^{31}P-NMR ((D$_6$)DMSO): 149.35. HR-FAB-MS: 825.3568 ([*M* + H]$^+$, $C_{40}H_{53}N_6O_{11}P^+$; calc. 825.3587). ESI-MS: 825 ([*M* + H]$^+$), 847 ([*M* + Na]$^+$), 1649 ([2*M* + H]$^+$), 1671 ([2*M* + Na]$^+$).

N^6*-{[4-(*tert*-Butyl)phenoxy]acetyl}-2′-deoxy-3′-O-{[2-(2-nitrophenyl)propoxy]carbonyl}adenosine 5′-(2-Cyanoethyl Diisopropylphosphoramidite)* (**17**). As described for **15**, with **13** (1.90 g, 2.93 mmol), 2-cyanoethyl tetraisopropylphosphorodiamidite (1.2 ml, 3.78 mmol), and 0.5M pyridine hydrochloride (2.9 ml, 1.45 mmol). Purification by FC (0–30% AcOEt/toluene) gave 1.9 g (65%) of **17**. R_f (toluene/AcOEt 1:1) 0.53. ^{1}H-NMR ((D$_6$)DMSO): 11.70 (br., NH); 8.64 (*m*, H–C(2), H–C(8)); 7.83 (*m*, 1 H *o* to NO_2); 7.71 (*m*, 1 H *m* to NO_2, 1 H *p* to NO_2); 7.48 (*m*, 1 H *m* to NO_2); 7.29 (*m*, 2 H *o* to ^{t}Bu); 6.88 (*m*, 2 H *m* to ^{t}Bu); 6.45 (*m*, H–C(1′)); 5.33 (*m*, H–C(3′)); 4.98 (*s*, COCH_2O); 4.36 (*m*, MeCHCH_2O); 4.24 (*m*, H–C(4′)); 4.78 (*m*, 2 H–C(5′), OCH_2CH$_2$CN); 3.53 (*m*, 2 Me$_2$C*H*, MeC*H*CH$_2$O); 3.12 (*m*, 1 H–C(2′)); 2.74 (*m*, OCH$_2$CH_2CN); 2.60 (*m*, 1 H–C(2′)); 1.30 (*d*, Me); 1.11 (*m*, 7 Me). ^{31}P-NMR ((D$_6$)DMSO): 149.24; 149.20; 149.16. HR-FAB-MS: 849.3723 ([*M* + H]$^+$; $C_{41}H_{53}N_8O_{10}P^+$; calc. 849.3700). ESI-MS: 849 ([*M* + H]$^+$), 871 ([*M* + Na]$^+$), 1697 ([2*M* + H]$^+$), 1719 ([2*M* + Na]$^+$).

N^2*-{[4-(*tert*-Butyl)phenoxy]acetyl}-2′-deoxy-3′-O-{[2-(2-nitrophenyl)propoxy]carbonyl}guanosine 5′-(2-Cyanoethyl Diisopropylphosphoramidite)* (**18**). As described for **15**, with **14** (1.0 g, 1.50 mmol), 2-cyanoethyl tetraisopropylphosphorodiamidite (0.9 ml, 2.84 mmol) and 0.5M pyridine hydrochloride (1.75 ml, 0.87 mmol). Purification by FC (0–30% AcOEt/toluene) gave 1.19 g (91%) of **18**. R_f (toluene/AcOEt/MeOH 5:4) 0.39. ^{1}H-NMR ((D$_6$)DMSO): 11.45 (br., 2 NH); 8.15 (*m*, H–C(8)); 7.83 (*m*, 1 H *o* to NO_2); 7.70 (*m*, 1 H *m* to NO_2, 1 H *p* to NO_2); 7.49 (*m*, 1 H *m* to NO_2); 7.29 (*m*, 2 H *o* to ^{t}Bu); 6.88 (*m*, 2 H *m* to ^{t}Bu); 6.19 (*m*, H–C(1′)); 5.23 (*m*, H–C(3′)); 4.79 (*s*, COCH_2O); 4.36 (*m*, MeCHCH_2O); 4.20 (*m*, H–C(4′)); 3.73 (*m*, 2 H–C(5′), OCH_2CH$_2$CN); 3.55 (*m*, 2 Me$_2$C*H*, *Me*CHCH$_2$O); 2.87 (*m*, OCH$_2$CH_2CN); 2.76 (*m*, 1 H–C(2′)); 2.57 (*m*, 1 H–C(2′)); 1.30 (*d*, Me); 1.15 (*m*, 7 Me). ^{31}P-NMR ((D$_6$)DMSO): 149.46; 149.41. HR-FAB-MS: 865.3660 ([*M* + H]$^+$, $C_{41}H_{53}N_8O_{11}P^+$; calc. 865.3649). ESI-MS: 865 ([*M* + H]$^+$), 887 ([*M* + Na]$^+$), 1751 ([2*M* + Na]$^+$).

Array Synthesis and Hybridization. Photolithographic synthesis of oligonucleotide arrays was carried out as described earlier [10]. Coupling was done twice for 120 s with 50 mM phosphoramidite soln. in MeCN and 0.5M pyridine hydrochloride as the activator of choice [17]. The temporary 3′-nppoc groups were removed by light exposure (100-W Hg lamp; 5 min at 365 nm) in presence of 50 mM ^{i}Pr$_2$NEt. Final deprotections were with conc. ammonia for 1 h at r.t. Hybridizations were carried out with 200 nM of fluorescently labelled oligomer probe (*Ark Scientific*, Germany) in 600 mM NaCl, 60 mM sodium citrate, 7.2% (*v*/*v*) sodium sarcosyl. Typically, 50 µl were applied to a microscope slide and spread evenly by a coverslip; hybridization temp. was 15°. After washing, the arrays were scanned on a *ScanArray-3000* system (*GSI Lumonics*, USA).

On-Chip Enzymatic Reactions. Reactions were performed in 25-µl *EasiSeal* chambers (*Hybaid*, Germany) mounted directly on the glass slide. Template DNA was denatured at 95° for 5 min and chilled on ice prior to being filled into a chamber. Hybridization took place at 15° in 16.5 mM *Tris*·HCl (=2-amino-2-(hydroxymethyl)propane-1,3-diol hydrochloride), pH 7.5, 12.5 mM $MgCl_2$, 12.5 mM DTT (=DL-dithiothreitol), 0.4 mM of each nucleotide triphosphate. Polymerase elongation was performed by adding 1 U/µl *Klenow* fragment of

DNA polymerase I (*New England Biolabs*, Germany) or 0.4 U/µl *Taq* polymerase. Incubation was at 37° or at 55°, resp., placing the slide in a custom-built PCR machine for temp. control. Subsequently, the arrays were washed with H_2O and remaining template was removed by a 30-s treatment with 2.5 mM Na_2HPO_4 and 0.1% (*v*/*v*) SDS (=sodium dodecyl sulfate) at 95°. Successful extension of the chip-bound oligomers was detected by hybridizing oligonucleotides known to be complementary to the newly synthesized 3′-end sequences and thus specifically binding to the elongated molecules only.

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