Photocleavable Protecting Groups as Nucleobase Protections Allowed the Solid-Phase Synthesis of Base-Sensitive SATE-Prooligonucleotides

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The first synthesis of oligodeoxynucleotide heteropolymers carrying base-sensitive *S*-pivaloylthioethyl (*t*-Bu-SATE) phosphotriester linkages has been performed. It is based on the use of 6-nitroveratryloxycarbonyl (NVOC) and 2,2′-bis(2-nitrophenyl)ethoxycarbonyl (diNPEOC) groups as nucleobase protections in combination with photolysis deprotection. The synthesis was realized using the phosphoramidite approach on solid support bearing a 1-(*o*-nitrophenyl)-1,3-propanediol linker. The removal of the protecting groups and the cleavage of the oligonucleotides from the solid support were accomplished in a single photolysis procedure upon UV irradiation at wavelengths >300 nm. Faster deprotection rates were observed for diNPEOC-protected nucleosides and oligomers than with NVOC-protected ones. The synthesis of pentanucleoside *t*-Bu-SATE-phosphotriesters d($^{5'}$T*p*C*p*C*p*C*p*T*p*$^{3'}$), d($^{5'}$T*p*A*p*A*p*A*p*A*p*$^{3'}$), and d($^{5'}$T*p*G*p*G*p*G*p*T*p*$^{3'}$) and of dodecanucleoside *t*-Bu-SATE-phosphotriesters and -phosphorothioate d($^{5'}$A*p*C*p*A*p*C*p*C*p*C*p*A*p*A*p*T*p*T*p*C*p*T*p*$^{3'}$) and d($^{5'}$A*p*G*p*A*p*-A*p*T*p*T*p*G*p*G*p*G*p*T*p*G*p*T*p*$^{3'}$) demonstrated the efficiency of the method.

Introduction

The natural affinity and the specificity of hybridization of antisense oligonucleotides for nucleic acid targets have opened perspectives of new therapies for the treatment of various diseases.[1,2] However, the effectiveness of oligonucleotides is limited because of their instability in serum, their inability to reach their target, their poor cellular uptake, and adverse pharmacokinetics. These limitations are mainly due to the anionic internucleosidic linkages of phosphodiester oligonucleotides and of their phosphorothioate analogues. To circumvent these problems, we have proposed an approach wherein the negative charges of oligonucleotides are temporarily neutralized with enzymolabile protecting groups to form neutral oligonucleotide prodrugs (prooligonucleotide) with phosphotriester linkages.[3] We have shown that thymidylate models with internucleoside phosphodiesters or phosphorothioates protected with *S*-acylthioethyl (SATE) groups are not degraded by nucleases,[4] are stable in human serum, and are selectively hydrolyzed to parent phosphodiester or phosphorothioate oligonucleotides in total cell extract, as a model for intracellular medium.[4−6] The decomposition process is mediated by carboxyesterases (Figure 1).

Prooligonucleotides, being phosphotriester (or thionophosphotriester) derivatives, are sensitive to bases and nucleophiles and decompose during their solid-phase synthesis under the standard basic conditions required for removal of the common heterocyclic *N*-acyl protecting groups[7] and release of the oligonucleotide from the regular succinyl-anchored solid support.[8,9] Therefore, a completely new strategy had to be set for the solid-phase synthesis of these base-sensitive analogues. Requirements for such a synthesis are the achievement of the appropriate *S*-acylthioethyl (SATE) phosphoramidite synthons and the use of a solid support and of nucleobase amino protecting groups which could be cleaved under nonbasic and nonnucleophilic conditions. We recently reported the solid-phase synthesis of dodecathymidine SATE-phosphotriesters[4] using a photolabile linker[10] an-

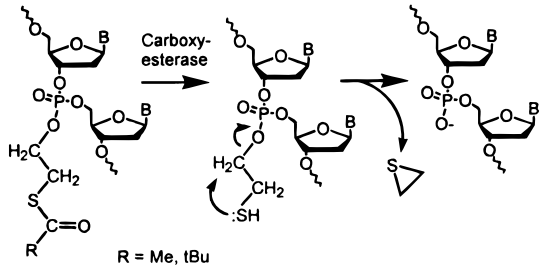

Figure 1. Esterase-dependent decomposition of SATE-prooligonucleotides. Upon incubation in total cell extract, thioester bond breakage induced by carboxyesterases releases an unstable 2-mercaptoethyl phosphotriester which decomposes spontaneously via intramolecular nucleophilic displacement into the corresponding phosphodiester and ethylene sulfide.

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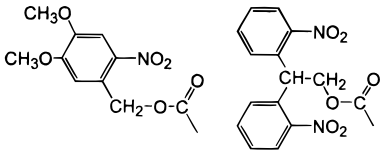
chored to the solid support and thymidine phosphoramidite synthons having a SATE group in place of the regular 2-cyanoethyl group. Our effort is now focused on the solid-supported synthesis by the phosphoramidite approach of SATE-phosphotriester heteropolymers containing the four common nucleobases T, C, A, and G. For this purpose, new *N*-protecting groups of the nucleobase exocyclic amines are required.[11]

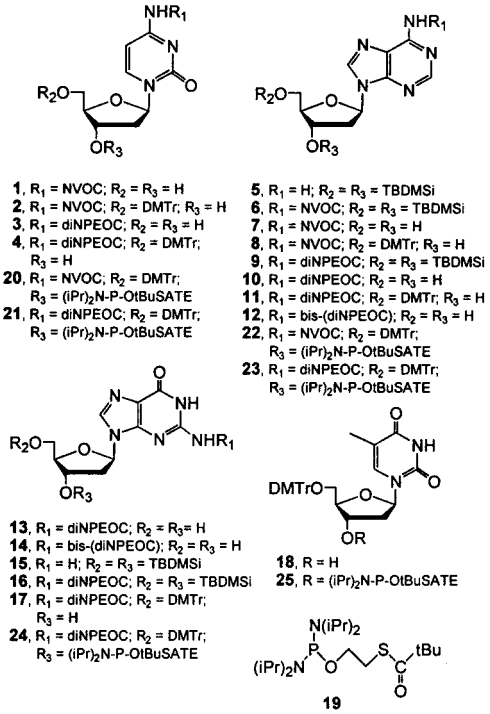
Photolabile protecting groups have been used in nucleic acid chemistry for the protection of primary alcohols of deoxynucleosides[12,13] and of 2′-secondary alcohols of ribonucleosides[14,15] and phosphates.[16,17] The efficiency of photolabile solid-phase supports for the synthesis of oligonucleotides has also been demonstrated.[10,18] Surprisingly, the use of photolabile groups for the protection of exocyclic amino functions of the nucleobases C, G, and A has never been reported.

As part of our oligonucleotide prodrug program, we decided to comparatively evaluate 6-nitroveratryloxycarbonyl (NVOC)[19,20] and 2,2′-bis(2-nitrophenyl)ethoxycarbonyl (diNPEOC)[21] as protecting groups of nucleobases.



We report herein their usefulness in the synthesis of SATE-prooligonucleotides on solid support via the phosphoramidite approach.

Synthesis of the NVOC- and diNPEOC-Protected 2′-Deoxyribonucleoside 3′-*t*-Bu-SATE-phosphoramidites. The NVOC and the diNPEOC groups were introduced on 2′-deoxycytidine through a transient-protection protocol originally developed for the acylation of exocyclic amino functions of deoxynucleosides.[22] The trimethylsilylation of the nucleoside in anhydrous pyridine with trimethylsilyl chloride (5 equiv, with respect to the nucleoside) was followed by the addition of either the *o*-nitroveratryloxycarbonyl chloride (NVOC−Cl) or the 2,2′-bis(2-nitrophenyl)ethyl chloroformate (diNPEOC−Cl)[21] (1.2 equiv, with respect to the nucleoside) and then by the hydrolysis of trimethylsilyl groups with a methanol/water solution. The 4-*N*-NVOC-2′-deoxycytidine (**1**) could be either isolated at this stage with a yield of 45% or dimethoxytritylated by treatment of the crude dried mixture with 4,4′-dimethoxytrityl chloride (1.2 equiv, with respect to the nucleoside) to afford 5′-*O*-DMTr-4-*N*-NVOC-2′-deoxycytidine (**2**) with a yield of 40% from 2′-deoxycytidine. Similarly, 4-*N*-diNPEOC-2′-deoxycytidine (**3**) was isolated with a yield of 78% and then 5′-*O*-tritylated to provide 5′-*O*-DMTr-4-*N*-diNPEOC-2′-deoxycytidine (**4**) with a yield of 83%.



Nitroveratryloxycarbonylation of 5′,3′-di-*O*-TBDMSi-2′-deoxyadenosine (**5**) was performed with in-situ-prepared[23] NVOC-tetrazolide (2 equiv, with respect to the nucleoside) in hot THF, affording 5′,3′-di-*O*-TBDMSi-6-*N*-NVOC-2′-deoxyadenosine (**6**) with a yield of 63%. This compound was quantitatively desilylated with tetrabutylammonium fluoride (TBAF), and the resulting 6-*N*-NVOC nucleoside **7** was dimethoxytritylated to give 5′-*O*-DMTr-6-*N*-NVOC-2′-deoxyadenosine (**8**) with a yield of 53%. Similarly, the reaction of in-situ-prepared[23] diNPEOC-tetrazolide with **5** afforded 5′,3′-di-*O*-TBDMSi-6-*N*-diNPEOC-2′-deoxyadenosine (**9**) in 90% yield. After desilylation, the resulting 5′,3′-diOH nucleoside **10** was dimethoxytritylated to give 5′-*O*-DMTr-6-*N*-diNPEOC-2′-deoxyadenosine (**11**). In comparison with this method, the reaction of free 2′-deoxyadenosine with diNPEOC−Cl following the transient-protection protocol[22] resulted in the concomitant formation of **10** (43% yield) and *N*,*N*-di-(diNPEOC)-2′-deoxyadenosine (**12**, 25% yield).

Similarly, the reaction of 2′-deoxyguanosine with diNPEOC−Cl following the same protocol afforded mono-*N*-2-protected nucleoside **13** (15%) and *N*-2-diprotected nucleoside **14** (20%). Simultaneous mono- and diprotections were reported for dansylethoxycarbonylation of 2′-deoxyguanosine.[24] Better results were obtained when the 3′,5′-di-*O*-silylated nucleoside **15** was reacted with di-

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NPEOC−Cl in the presence of *tert*-butylmagnesium chloride[23] to give **16** with an isolated yield of 50%. Desilylation with TBAF produced **13,** and subsequent dimethoxytritylation afforded 5′-*O*-DMTr-2-*N*-diNPEOC-2′-deoxyguanosine (**17**). The thymidine derivative **18** was easily obtained from thymidine by reaction with dimethoxytrityl chloride.

The 3′-*O*-phosphoramidite monomer units bearing the *S*-pivaloylthioethyl (*t*-Bu-SATE) instead of the standard cyanoethyl were obtained by reaction of the 3′-OH intermediates with the phosphitylating agent (*S*-pivaloylthioethyloxy)bis(diisopropylamino)phosphine[25] (**19**, 1.5 equiv, with respect to the nucleoside), in the presence of diisopropylammonium tetrazolide (0.5 equiv, with respect to the parent nucleoside) in anhydrous dichloromethane. This protocol is more convenient than the one previously described[4] for the synthesis of the 3′-phosphoramidite of thymidine in two steps by reaction with (*N*,*N*-diisopropylamino)chlorophosphine in the presence of *N*,*N*-diisopropylamine, giving rise to an unstable 3′-phosphorodiamidite, which was then reacted in situ with the SATE alcohols in the presence of tetrazole. Utilizing the phosphitylating agent **19**, the isolated yields of **20**, **21**, **22**, **23**, **24**, and **25** after purification were 82, 84, 60, 78, 55, and 80%, respectively. In comparison the thymidine phosphoramidite **25** was obtained with a yield of 55% using the two-step protocol.[4]

Photodeprotection Rates: Nucleoside Level. The removal of photolabile protecting groups such as the NVOC containing the *o*-nitrobenzyl moiety is generally performed by irradiation at wavelengths >300 nm, which do not damage nucleic acids.[26] To avoid eventual side-product formation between the released amino function and the blocking group photoproduct (nitrosoaldehyde), deprotection is carried out under slightly acidic conditions.[26] DiNPEOC deprotection does not require such precautions as the photoproduct is not a nitrosoaldehyde but an *o*-nitrostyrene derivative.[21] However, to compare deprotection rates of NVOC and diNPEOC derivatives, the photolysis experiments were performed in the same medium containing a small amount of acetic acid (1−5%).

These experiments were run in a thermostated cuvette (quartz) at room temperature using a high-pressure Hg lamp. A Pyrex glass filter serves as a 300 nm cutoff filter. Prior to photolysis, argon was bubbled through the solutions (dioxane/water (3/2) + CH_3CO_2H) of the nucleoside samples to remove traces of O_2. The rate of deprotection (Table 1) was determined by HPLC analysis.

Acetic acid percentages varying from 1 to 10% did not affect the rate of NVOC removal; however, experiments with less than 1% acetic acid showed a significant decrease of the photolysis rate (data not shown).

N-4-NVOC-dC (**1**) and *N*-6-NVOC-dA (**7**) were deprotected by photolysis at approximately the same rate. Similarly, no significant difference in the behavior of the diNPEOC derivatives of dC **3** and dA **9** was noticed (Table 1).

The NVOC removal was greatly dependent on the concentration of the starting material. An increase of the initial concentration in starting protected nucleosides **1** and **7** from 0.1 to 1 mM lowered the NVOC removal rate by 6. In comparison, a 4-fold decrease for the removal of diNPEOC protection was observed with the same increase of concentration of the diNPEOC nucleosides **3** and **10**.

Table 1. Photolysis Rates for *N*-Protected Nucleosides 1, 3, 7, 10, 12, 13, and 14 in Dioxane/Water, 3/2, v/v, 5% CH_3CO_2H

no.	nucleoside	*N*-protection	concn (mM)	$t_{completion}$
1	dC	NVOC	1	>4 h ($t_{1/2}$ 28 min)
			0.1	40 min ($t_{1/2}$ 5 min)
3	dC	diNPEOC	1	20 min
			0.1	6 min
7	dA	NVOC	1	>4 h ($t_{1/2}$ 31 min)
			0.1	40 min ($t_{1/2}$ 5 min)
10	dA	diNPEOC	1	20 min
			0.1	6 min
12	dA	bis-(diNPEOC)	1	25 min
13	dG	diNPEOC	1	15 min
			0.1	5 min
14	dG	bis-(diNPEOC)	1	15 min

The diNPEOC derivatives had photolysis rates approximately 5-fold greater than the corresponding NVOC derivatives (Table 1). The effect of the protection on the photolysis rates of *N*-protected nucleosides was consistent with the results described for 5′-*O*-protected deoxythymidines.[21] In this case, it was reported that the rate of photololysis, replacing NVOC by diNPEOC, was enhanced by more than 1 order of magnitude (13×).

Finally, it was more difficult to remove the two diNPEOC protecting groups in *N*,*N*′-6-bis(diNPEOC)-deoxyadenosine (**12**) than in *N*,*N*′-2-bis(diNPEOC)-deoxyguanosine (**14**). One can notice that the deprotection of both diNPEOC groups of the diprotected dG nucleoside **14** was as rapid as the removal of one diNPEOC in the monoprotected dG derivative **13**.

Synthesis of the SATE-Prooligonucleotides on Solid Support. Synthesis of *t*-Bu-SATE-phosphotriester oligonucleotides was performed by use of phosphoramidite methodology on an automated DNA-synthesizer.

To avoid nucleophilic and/or basic conditions used for standard cleavage of oligonucleotides from solid support, the regular succinyl linker was replaced with a photolabile linker bearing a 1-(*o*-nitrophenyl)-1,3-propanediol moiety. This linker was successfully applied to the solid-phase synthesis of oligododecathymidine methylphosphotriesters[10] as well as methyl or *tert*-butyl-SATE-phosphotriesters and -phosphorothiotriesters.[4] Because of their poor solubility in acetonitrile, NVOC- and diNPEOC-protected nucleoside phosphoramidites were solubilized in an acetonitrile/dichloromethane mixture (9/1, v/v).

Despite their high purity, the coupling efficiency with both NVOC- and diNPEOC-protected nucleoside phosphoramidites was low (less than 70% for diNPEOC) compared with >98% for thymidine phosphoramidite **25** even when extended coupling times (one or two times 180 s instead of 30 s coupling for **25**) were applied. These low yields were explained by the presence of residual water and were improved (90−95%) when solutions of the diNPEOC-nucleoside phosphoramidites were dried over 4 Å molecular sieves for 24 h.[27] The same result was

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Table 2. Photolysis Rates for *N*-Protected Oligonucleotides

no.	oligonucleotide	calcd $[M - H]^-$ (*m/z*)	found $[M - H]^-$ (*m/z*)	*N*-protector	concn (mM)	$t_{completion}$
26	d(5′T*p*C*p*T*p*T*p*T*p*3′)	2244.2	2243.6	NVOC	0.05	45 min
					0.1	75 min
				diNPEOC	0.1	10 min[a]
					1	15 min
27	d(5′T*p*A*p*T*p*T*p*T*p*3′)	2268.2	2268.5	NVOC	0.05	45 min
				diNPEOC	0.1	10 min[a]
					1	20 min
28	d(5′T*p*G*p*T*p*T*p*T*p*3′)	2284.2	2283.3	diNPEOC	1	20 min
	d(5′T*p*C*p*C*p*A*p*A*p*T*p*3′)			NVOC	0.05	>5 h
					0.005	2.5 h[b]
29	d(5′T*p*C*p*C*p*C*p*T*p*3′)	2214.2	2214.0			
30	d(5′T*p*A*p*A*p*A*p*T*p*3′)	2286.2	2285.8	diNPEOC	1	20 min
31	d(5′T*p*G*p*G*p*G*p*T*p*3′)	2334.2	2334.3			

[a] Time required for diNPEOC photolysis; a further 5−10 min photolysis time was then applied to totally cleave the oligomer from the solid support. [b] The oligonucleotide was not isolated and not characterized.

observed with extensive drying of the compounds as powders under high vacuum over P_2O_5 and KOH. This problem was not encountered with dT phosphoramidite **25** (coupling yield >98%). Even improved, the coupling yields during solid-phase DNA synthesis were lower than those obtained using standard *N*-acyl nucleoside phosphoramidites.

tert-Butyl hydroperoxide[28,29] in anhydrous conditions was used for the oxidation of phosphite triester intermediates to form *t*-Bu-SATE-phosphotriester linkages. This oxidizer was preferred to the common iodine/water treatment to prevent an eventual loss of the *t*-Bu-SATE protection during aqueous oxidation.[29,30]

First, two pentanucleoside *t*-Bu-SATE-phosphotriesters, d(5′T*p*C*p*T*p*T*p*T*p*3′) **26** and d(5′T*p*A*p*T*p*T*p*T*p*3′) **27**, bearing four *t*-Bu-SATE-phosphotriester internucleoside linkages and one *t*-Bu-SATE-phosphodiester linkage at their 3′-end were synthesized on a 1 μmol scale on a DNA synthesizer using dT phosphoramidite **25** and NVOC-phosphoramidites **20** and **22** as well as diNPEOC-phosphoramidites **21** and **23** to evaluate the conditions of the photolysis on oligonucleotides containing only one adenine or one cytosine in their sequence.

The same protocol as the one described above for the deprotection of the *N*-protected nucleosides was applied to the supported oligonucleotides. Cleavage of these compounds from the solid support was performed upon exposure to the Pyrex-filter output of a high-pressure Hg lamp at 25 °C. Prior to photolysis, argon was bubbled through the suspensions (dioxane/water (3/2) + CH_3-CO_2H) of the samples to remove traces of O_2. At several reaction times, aliquots were withdrawn and analyzed by HPLC/ESI-MS (negative mode) to monitor the course of the deprotection and characterize the compound.

After 5 min, the HPLC analysis (Figure 2) revealed the presence of the undeprotected NVOC-dC oligonucleotide (R_T = 31.2 min, *m/z* = 2484) together with a lower amount of the deprotected oligonucleotide **26** (R_T = 28.6 min, *m/z* = 2243.6) and of a failure sequence, 5′-*O*-acetyl-d(5′T*p*T*p*T*p*3′) (R_T = 25.9 min, *m/z* = 1301.7). This truncated sequence was caused by the low coupling yield of NVOC-dC phosphoramidite **20** (85%) during oligonucleotide elongation. As observed with NVOC-protected nucleosides, the removal of the protecting group was dependent on the concentration of the starting material (Table 2). Completion of the photolysis of the NVOC-protected oligomer was observed after 45 and 75 min with 0.05 and 0.1 mM concentrations of oligonucleotide, respectively. In comparison, the photolysis rate of the NVOC-dC nucleoside was approximately 2-fold greater than that of the NVOC-dC oligonucleotide. The same result was observed with the NVOC-dA incorporated oligonucleotide. A 45 min photolysis reaction time was necessary to ensure complete conversion of the protected NVOC-dA oligonucleotide (R_T = 31.3 min) into the deprotected oligomer **27** (R_T = 29.3 min, *m/z* = 2268.5).

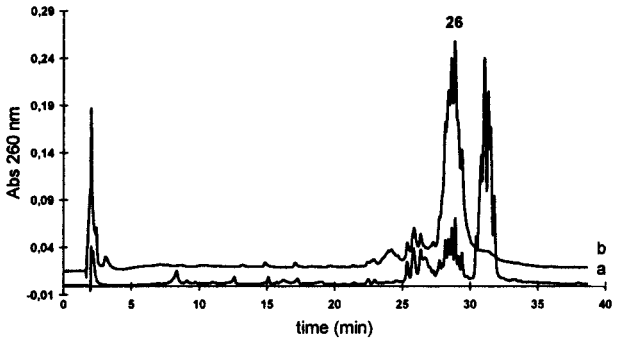

Figure 2. RP-HPLC profiles of the reaction mixture for NVOC deprotection of the oligonucleotide d(T*p*C*p*T*p*T*p*T*p*) **26** (a) after 5 min of photolysis and (b) after 45 min of photolysis.

Using the same experimental conditions as those described for NVOC-oligonucleotides, much faster deprotection rates were observed for diNPEOC oligomers. After 10 min of photolysis of the corresponding solid-supported oligonucleotides, the diNPEOC-protected intermediates were not detected by HPLC/MS of the crude mixtures. However, the photolysis was carried out for a further 5−10 min to ensure complete cleavage of the oligonucleotides **26**, **27**, and **28** from the solid support.[4,10] As observed for the nucleoside derivatives, no significant difference in the behavior toward photolysis of the diNPEOC-oligonucleotides containing dC, dA, or dG was noticed. More interestingly, the time of completion was slightly influenced when the concentration was raised from 0.1 to 1 mM.

Use of NVOC protection was not pursued after an attempt to obtain d(5′T*p*C*p*C*p*A*p*A*p*T*p*3′) from NVOC-units assembly was unsuccessful. The deprotection of the heterocyclic amino functions of this oligomer was not

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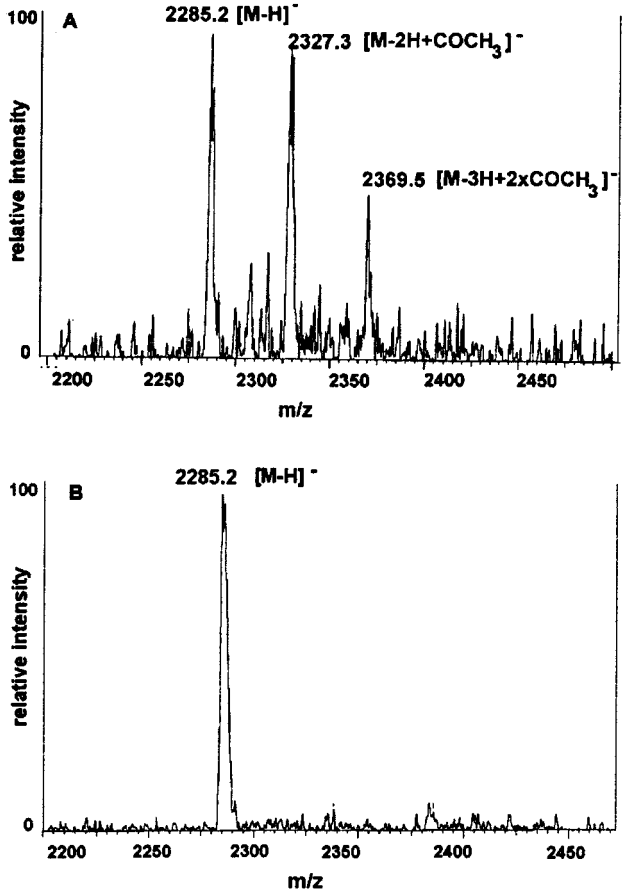

Figure 3. Negative ion ESI mass spectra of d(5′T*p*A*p*A*p*A*p*T*p*3′) **30**, showing deprotonated monocharged ions: (A) use of Ac_2O/THF/2,6-lutidine (1/8/1, v:v:v) and 10% *N*-methylimidazole/THF for the capping step during oligonucleotide elongation; (B) use of diethyl *N*,*N*-diisopropyl phosphoramidite in the presence of tetrazole as capping reagent.

complete even after 5 h of photolysis at a concentration of 0.05 mM. Lowering the concentration from 50 to 5 μM reduced the reaction time to 2.5 h. However, this reaction time was too long, and HPLC analysis of the crude mixture revealed numerous side products. Our attention then focused on diNPEOC protection.

Three other pentanucleoside *t*-Bu-SATE-phosphotriesters, i.e., d(5′T*p*C*p*C*p*C*p*T*p*3′) **29**, d(5′T*p*A*p*A*p*A*p*T*p*3′) **30**, and d(5′T*p*G*p*G*p*G*p*T*p*3′) **31**, were prepared on a 1 μmol scale using diNPEOC-phosphoramidites **21**, **23**, and **24**, respectively, to study the photolysis on oligonucleotides containing three diNPEOC-protected nucleosides in their sequence. At 1 mM concentration in oligonucleotide, photolysis was carried out for 20 min. Longer reaction times did not modify the HPLC profile of the crude mixtures. The oligomers were then purified by reversed-phase HPLC and characterized by ESI-MS analysis (negative mode). Surprisingly, while the ESI-MS analysis mass of the isolated oligomer **29** was in accord with its calculated mass (*m*/*z* calcd 2214.2, found 2214.0), the analysis of the isolated oligomer **30** revealed a mixture of the expected oligomer (*m*/*z* 2285.2) with adducts at *m*/*z* 2327.8 and 2369.5.0 (Figure 3). One possible explanation might be partial acetylation (+42 and +2 × 42) of the oligomer under the capping step conditions used during the oligomer elongation. Standard capping is generally performed with a mixture of Ac_2O/THF/2,6-lutidine (1/8/1, v/v/v) and 10% *N*-methylimidazole/THF for 20 s per cycle incorporation (1 μmol scale). This indicates that Ac_2O capping treatment could not be applied in the synthesis of dA-containing heteropolymers. Therefore, this capping treatment was replaced by the less common one using diethyl *N*,*N*-diisopropyl phosphoramidite in the presence of tetrazole.[31] Under such conditions, the synthesis of the oligonucleotide **30** containing three adjacent deoxyadenosines gave us the expected compound without any detectable adduct as determined by ESI-MS analysis (Figure 3). The synthesis of d(5′T*p*G*p*G*p*G*p*T*p*3′) **31** containing three adjacent deoxyguanosines was carried out using the same capping conditions as for oligomer **30**. Indeed, careful examination of the ESI spectrum of isolated d(5′T*p*G*p*T*p*T*p*T*p*3′) **28** obtained with acetic anhydride as the capping reagent also revealed a small amount of an acetylated oligonucleotide ($[M - 2H + CH_3CO]^-$, *m*/*z* 2326.2; $[M - 3H + CH_3CO]^{2-}$, *m*/*z* 1162.1). In addition, hydrolysis of the *t*-Bu-SATE oligomers **29**, **30**, and **31** with concentrated ammonia yielded the fully unmasked phosphodiester oligonucleotides as the main compounds.

Finally, the *t*-Bu-SATE-phosphotriester and -phosphorothionotriester analogues **32** and **33** of a dodecanucleoside, d(5′ApCpApCpCpCpApApTpTpCpTp3′), complementary to the splice acceptor site of mRNA coding for HIV-1 tat protein were prepared. Their complementary sequence analogue d(5′A*p*G*p*A*p*A*p*T*p*T*p*G*p*G*p*G*p*T*p*G*p*T*p*3′) with phosphotriester linkages **34** was also synthesized. *tert*-Butyl hydroperoxide[28] was used as oxidizer for the formation of phosphotriester linkages in **32** and **34**. Beaucage reagent[32] was used for oxidation of phosphitetriesters into thionophosphotriester internucleoside linkages for oligonucleotide **33**. Diethyl *N*,*N*-diisopropyl phosphoramidite in the presence of tetrazole[31] was used as the capping reagent. After 40 min of photolysis, the oligomers were purified by reversed-phase HPLC. In each case, broad peaks were observed (**32**, R_t = 21.2 min, 9.2 min from start to end of peak (Figure 4); **33**, R_t = 27.4 min over 5.4 min; **34**, R_t = 22.2 min over 8.6 min), reflecting the presence of diastereomeric mixtures due to Rp and Sp isomers at each internucleotide linkage. The integrity of the oligonucleotides **32**, **33**, and **34** was confirmed by ESI-MS analysis performed in positive mode using solutions of the oligomers in CH_3CN/H_2O (7/3, v/v) containing a small amount of formic acid (1%).[33] The mass spectrum (Figure 4) for an aqueous solution of oligomer **32** (1 $OD_{260\ nm}$ in 500 μL of CH_3CN/H_2O (7/3) containing 5 μL of HCO_2H) generated charge states $[M + 4H]^{4+}$ at *m*/*z* 1340.6 (calcd 1341.1) and $[M + 3H]^{3+}$ at *m*/*z* 1787.9 (calcd 1787.7). The measured mass of 5361 was consistent with the expected structure (calculated mass 5360.2). Similarly, the mass spectrum of oligomer **33** showed $[M + 5H]^{5+}$ at *m*/*z* 1111.7 (calcd 1111.6), $[M + 4H]^{4+}$ at *m*/*z* 1389.8 (calcd 1389.3), and $[M + 3H]^{3+}$ at *m*/*z* 1851.9 (calcd 1852.0), and that of oligomer **34** showed $[M + 4H]^{4+}$ at *m*/*z* 1839.2 (calcd 1388.8) and $[M + 3H]^{3+}$ at *m*/*z* 1851.1 (calcd 1851.4). Moreover, hydrolysis of **32**, **33**, and **34** with concentrated aqueous ammonia for 1 h at 55 °C afforded main compounds

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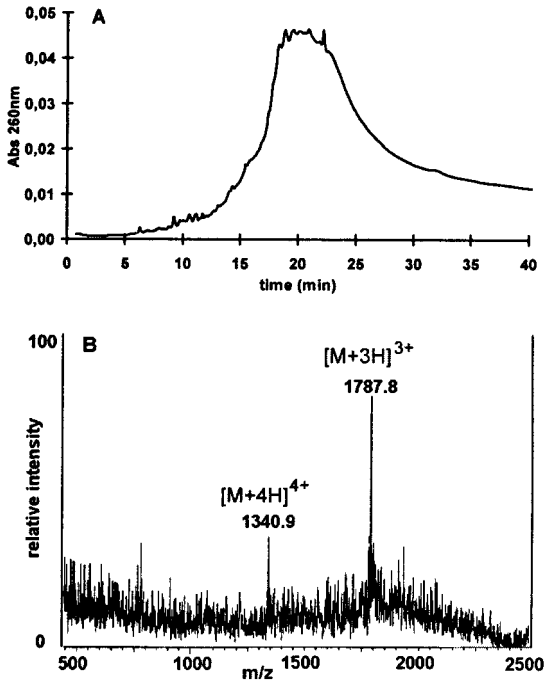

Figure 4. (A) RP-HPLC profile of the purified oligonucleotide **32** d(A*p*C*p*A*p*C*p*C*p*C*p*A*p*A*p*T*p*T*p*C*p*T*p*) obtained after 40 min of photolysis. (B) Positive ion ESI mass spectrum of d(A*p*C*p*-A*p*C*p*C*p*C*p*A*p*A*p*T*p*T*p*C*p*C*p*T*p*) **32** showing protonated ions with charges of 3 and 4. The measured mass was 5361, and the averaged calculated mass is 5360.2.

which comigrated on reversed-phase HPLC with authentic samples of dodecanucleoside phosphodiesters d(ApCpApCpCpCpApApTpTpCpTp), its phosphorothioate analogue, and d(ApGpApApTpTpGpGpGpTpGpTp), respectively.

Conclusion

For the first time, photocleavable protecting groups for nucleobases combined with an anchored photolabile solid support allowed the synthesis of base-sensitive SATE-phosphotriester oligonucleotides of various sequences. The diNPEOC protecting groups are removed, and the oligonucleotides are cleaved from the solid support in a single deprotection step by UV irradiation at wavelengths >300 nm without affecting the phosphotriester internucleoside linkages.

Experimental Section

General Methods. Commercial chemical reagents were reagent grade and were used without purification except where otherwise stated. *o*-Nitroveratryloxycarbonyl chloride (NVOC−Cl), *N*,*N*-bis(diisopropylamino)chlorophosphine, tetrazole (>99%), and redistilled *N*,*N*-diisopropylethylamine were obtained from Aldrich (St. Quentin Fallavier, France). Methylene chloride (Aldrich) was dried over P_2O_5 followed by distillation. DNA synthesis reagents, except oxidizers, were from Perseptive Biosystems Ltd. (Voisins le Bretonneux, France). Anhydrous *tert*-butyl hydroperoxide (3 M in toluene) was from Fluka and was diluted with anhydrous methylene chloride. 3*H*-1,2-Benzodithiol-3-one-1,1-dioxide (Beaucage reagent) was a gift from Isis Pharmaceuticals (Carlsbad, CA). Diethyl *N*,*N*-diisopropyl phosphoramidite was from ChemGenes (Waltham, MA). 2,2′-Bis(2-nitrophenyl)ethyl chloroformate (diNPEOC−Cl) was prepared according to a published procedure.[31] Analytical TLC was performed on precoated silica gel plates (Kieselgel 60 F_{254}, Merck), and column flash chromatography was run on silica gel Kieselgel Merck 60, 40−63 μm. Elution for TLC was performed with methanol/dichloromethane mixtures (2/98 to 20/80, v/v) except where otherwise stated. ^{1}H NMR spectra were recorded at 200, 250, or 400 MHz. Tetramethylsilane, CHD_2CN, $CHCl_3$, and DMSO-d_5 were used as internal standards. ^{31}P NMR spectra were recorded at room temperature at 80 or 100 MHz relative to phosphoric acid as an external standard. Glycerol, glycerol/thioglycerol (1/1, v/v, GT), 3-nitrobenzylic alcohol (NOBA),and poly(ethylene glycol) (PEG) were used as the sample matrix for fast-atom-bombardment mass spectra (FAB-MS). Electrospray mass spectra (ESI-MS) were recorded on a quadrupole mass spectrometer. Oligonucleotides with molecular weight below 2500 were analyzed in negative mode. Crude materials were analyzed by HPLC/ESI-MS. Purified samples (1 $OD_{260\ nm}$) were dissolved in acetonitrile/water (500 μL, v/v, 1/1). Longer oligonucleotides were analyzed by ESI-MS in positive mode, after HPLC purification. Samples (1 $OD_{260\ nm}$) were dissolved in acetonitrile/water (500 μL, v/v, 7/3) containing formic acid (5 μL, 1%) and introduced into the mass spectrometer at a 10 μL/min flow rate with a Harvard Apparatus 22 pump model. HPLC analyses were performed on an instrument equipped with a photodiode array UV detector. Reversed-phase HPLC analysis of reaction mixtures and purified compounds as well as HPLC purification of the oligonucleotides were performed on a C_{18} (5 μm) Nucleosil column (150 × 4.6 mm, Macherey-Nagel, Germany) at a flow rate of 1 mL/min. Solid-phase syntheses of the prooligonucleotides were performed using a photolabile CPG solid support[13,14] (63.6 μmol/g) on a 1 μmol scale (15−16 mg of solid support) using a DNA synthesizer. Photolyses were conducted upon exposition of the samples in dioxane/water in a 1 cm path length quartz cell to the Pyrex-filtered output of a high-pressure Hg lamp (HPK 125, Philips, Nederlands).

4-*N*-(6-Nitroveratryloxycarbonyl)-2′-deoxycytidine (1). Prior to use, the 2′-deoxycytidine hydrochloride (300 mg, 1.13 mmol) was dried three times by coevaporation of anhydrous pyridine and then suspended in anhydrous pyridine (10 mL). To that suspension was added under argon trimethylchlorosilane (0.72 mL, 5.67 mmol). After the mixture was stirred for 30 min to 1 h, NVOC−Cl (376 mg, 1.36 mmol) was added, and the reaction mixture was stirred at room temperature overnight. Water/methanol (1/1, v/v, 5 mL) was added and the reaction mixture was stirred for 30 min and then concentrated under reduced pressure. Dichloromethane (100 mL) was added, and the resulting mixture was washed with saturated aqueous $NaHCO_3$ (80 mL) and then with brine (80 mL). The organic layer was dried (Na_2SO_4), filtered, and evaporated to dryness. Residual pyridine was removed by coevaporations with toluene (3×). The residue was purified by flash column chromatography (silica gel, gradient 0−10% MeOH/CH_2Cl_2). The appropriate fractions were combined and evaporated to dryness. The resulting foam was dissolved in dioxane and lyophilized to give **1** (237 mg, 45%) as a colorless powder: ^{1}H NMR (pyridine-d_5) δ 8.86 (d, J = 7.5 Hz, 1 H, H-6), 7.77 (s, 1 H, H ortho to NO_2 of NVOC), 7.54 (s, 1 H, H meta to NO_2 of NVOC), 7.45 (d, J = 7.5 Hz, 1 H, H-5), 7.26 (d, 1 H, OH-3′), 7.01 (t, 1 H, OH-5′), 6.93 (m, 1 H, H-1′), 5.86 (s, 2 H, CO_2CH_2), 5.00 (m, 1 H, H-3′), 4.52 (m, 1 H, H-4′), 4.22−4.18 (m, 2 H, H-5′, H-5″), 3.84 and 3.79 (2 s, 6 H, 2 OCH_3), 2.95 and 2.62 (2 m, 2 H, H-2′, H-2″); FABMS (positive mode) *m*/*z* 467 $(MH)^+$, 351 $(4\text{-}N\text{-NVOC-Cyt} + H)^+$, 228 $(M - NVOC + 2H)^+ = (dC + H)^+$; HRMS calcd for $C_{19}H_{23}N_4O_{10}$ 467.1414, found 467.1489; FABMS (negative mode) *m*/*z* 465 $(M - H)^-$, 226 $(M - NVOC)^- = (dC - H)^-$.

4-*N*-(2,2′-Bis(2-nitrophenyl)ethyloxycarbonyl)-2′-deoxycytidine (3). 2′-Deoxycytidine hydrochloride (204 mg, 0.77 mmol) was treated with the same protocol as for preparation of **1**, except the NVOC−Cl was replaced with diNPEOC−Cl (330.5 mg, 0.94 mmol). After purification by flash column chromatography (silica gel, gradient 2−6% MeOH/CH_2Cl_2), the appropriate fractions were combined and evaporated to dryness. The resulting foam was dissolved in dioxane and lyophilized to give **3** (325 mg, 78%) as a colorless powder: ^{1}H NMR

(CD_3CN) δ 8.44 (s, 1 H, NH), 8.27 (d, J = 7.52 Hz, 1 H, H-6), 7.97 (dd, J_{ortho} = 8.04 Hz, J_{meta} = 1.06 Hz, 2 H, H ortho to NO_2 of diNPEOC), 7.69−7.51 (m, 6 H, H meta and para to NO_2 of diNPEOC), 7.08 (d, J = 7.50 Hz, 1 H, H-5), 6.10 (m, 1 H, H-1′), 5.55 (t, J = 6.17 Hz, 1 H, CO_2CH_2CH), 4.90 (d, J = 6.14 Hz, 2 H, CO_2CH_2CH), 4.30 (m, 1 H, H-3′), 3.96 (m, 1 H, H-4′), 3.70 (m, 2 H, H-5′, H-5″), 3.40 (d, 1 H, OH-3′), 3.26 (t, 1 H, OH-5′), 2.39 and 2.14 (2 m, 2 H, H-2′, H-2″); FABMS (positive mode) m/z 542 $(MH)^+$, 426 (4-*N*-diNPEOC−Cyt + H)$^+$; HRMS calcd for $C_{24}H_{24}N_5O_{10}$ 542.1523, found 542.1520; FABMS (negative mode) m/z 540 $(M - H)^-$, 226 $(M - diNPEOC)^- = (dC - H)^-$.

6-*N*-(2,2′-Bis(2-nitrophenyl)ethyloxycarbonyl)-2′-deoxyadenosine (10) and 6-*N*-Bis(2,2′-bis(2-nitrophenyl)ethyloxycarbonyl)-2′-deoxyadenosine (12). 2′-Deoxyadenosine (200 mg, 0.79 mmol) was treated as 2′-deoxycytidine for the preparation of **3**. After purification by flash column chromatography (silica gel, gradient 0−5% MeOH/CH_2Cl_2), the appropriate fractions were combined and evaporated to dryness. The resulting foams were dissolved in dioxane and lyophilized to afford **10** (192 mg, 43%) and **12** (173 mg, 25%) as colorless powders. Compound **10**: ^{1}H NMR (CD_3CN) δ 8.90 (s, 1 H, NH), 8.57 (s, 1 H, H-8), 8.25 (s, 1 H, H-2), 7.93 (dd, J_{ortho} = 8.11 Hz, J_{meta} = 1.07 Hz, 2 H, H ortho to NO_2 of diNPEOC), 7.66−7.57 (m, 6 H, H meta and para to NO_2 of diNPEOC), 6.40 (m, 1 H, H-1′), 5.60 (t, J = 6.32 Hz, 1 H, CO_2CH_2CH), 4.95 (d, J = 6.25 Hz, 2 H, CO_2CH_2CH), 4.56 (m, 2 H, H-3′, OH-3′), 4.04 (m, 1 H, H-4′), 3.74 (m, 2 H, H-5′, H-5″), 3.47 (m, 1 H, OH-5′), 2.79 and 2.39 (2 m, 2 H, H-2′, H-2″); FABMS (positive mode) m/z 566 $(MH)^+$, 450 (6-*N*-diNPEOC-Ade + H)$^+$; HRMS calcd for $C_{25}H_{24}N_7O_9$ 566.1636, found 566.1611; FABMS (negative mode) m/z 564 $(M - H)^-$, 448 (6-*N*-diNPEOC-Ade − H)$^-$. Compound **12**: ^{1}H NMR (CD_3CN) δ, 8.52 (s, 1 H, H-8), 8.13 (s, 1 H, H-2), 7.88 (m, 4 H, H ortho to NO_2 of diNPEOC), 7.49−7.22 (m, 12 H, H meta and para to NO_2 of diNPEOC), 6.42 (m, 1 H, H-1′), 5.47 (t, 2 H, 2 CO_2CH_2CH), 5.05−4.85 (m, 6 H, 2 CO_2CH_2CH, H-3′, OH-5′), 4.29 (m, 1 H, H-4′), 3.96 (m, 2 H, H-5′, H-5″), 2.99 and 2.37 (2 m, 2 H, H-2′, H-2″); FABMS (positive mode) m/z 880 $(MH)^+$, 764 (6-*N*-bis-diNPEOC-Ade + H)$^+$, 566 (6-*N*-diNPEOC-dA + H)$^+$, 450 (6-*N*-diNPEOC-Ade + H)$^+$; HRMS calcd for $C_{40}H_{34}N_9O_{15}$ 880.2174, found 880.2140; FABMS (negative mode) m/z 878 $(M - H)^-$, 762 (6-*N*-bis-diNPEOC-Ade − H)$^-$, 564 (6-*N*-diNPEOC-dA − H)$^-$, 448 (6-*N*-diNPEOC-Ade − H)$^-$.

2-*N*-(2,2′-Bis(2-nitrophenyl)ethyloxycarbonyl)-2′-deoxyguanosine (13) and 2-*N*-Bis(2,2′-bis(2-nitrophenyl)-ethyloxycarbonyl)-2′-deoxyguanosine (14). The procedure applied to 2′-deoxyadenosine in the preparation of **10** and **12** was modified for 2′-deoxyguanosine (342 mg, 1.28 mmol). After standing overnight with diNPEOC−Cl (583 mg, 1.66 mmol), the mixture was treated for 15 min with 10% aqueous ammonia (1.5 mL) at room temperature and then filtered. The filtrate was evaporated, redissolved in CH_2Cl_2/MeOH (95/5, v/v), and purified by flash column chromatography (silica gel, gradient 5−10% MeOH/1% Et_3N/CH_2Cl_2), and the appropriate fractions were combined and evaporated to dryness. The resulting foams were dissolved in dioxane and lyophilized to afford **13** (103 mg, 14%) and **14** (209 mg, 18%) as colorless powders. Compound **13**: ^{1}H NMR (DMSO-d_6) δ 8.12 (s, 1 H, H-8), 7.99 (dd, J_{ortho} = 7.71 Hz, J_{meta} = 0.97 Hz, 2 H, H ortho to NO_2 of diNPEOC), 7.76−7.53 (m, 6 H, H meta and para to NO_2 of diNPEOC), 6.15 (m, 1 H, H-1′), 5.40 (t, J = 6.69 Hz, 1 H, CO_2CH_2CH), 5.31 (m, 1 H, OH-3′), 5.04 (m, 1 H, OH-5′), 4.86 (d, J = 6.50 Hz, 2 H, CO_2CH_2CH), 4.35 (m, 1 H, H-3′), 3.82 (m, 1 H, H-4′), 3.57−3.47 (m, 2 H, H-5′, H-5″), 2.21 (m, 2 H, H-2′, H-2″); FABMS (positive mode) m/z 582 $(MH)^+$, 466 (2-*N*-diNPEOC-Gua + H)$^+$; HRMS calcd for $C_{25}H_{24}N_7O_{10}$ 582.1585, found 582.1583; FABMS (negative mode) m/z 580 $(M - H)^-$, 464 (2-*N*-diNPEOC-Gua − H)$^-$. Compound **14**: ^{1}H NMR (DMSO-d_6) δ 8.21 (s, 1 H, H-8), 7.90 (m, 4 H, H ortho to NO_2 of diNPEOC), 7.46−7.18 (m, 12 H, H meta and para to NO_2 of diNPEOC), 6.08 (m, 1 H, H-1′), 5.33 (m, 3 H, 2 CO_2CH_2CH, OH-5′), 4.86 (m, 5H, 2 CO_2CH_2CH, OH-3′), 4.23 (m, 1 H, H-3′), 3.86 (m, 1 H, H-4′), 3.55−3.42 (m, 2 H, H-5′, H-5″), 2.09 (m, 2 H, H-2′, H-2″); FABMS (positive mode) m/z 896 $(MH)^+$, 780 (2-*N*-bis-diNPEOC-Gua + H)$^+$; 582 (2-*N*-diNPEOC-dG + H)$^+$, 466 (2-*N*-diNPEOC-Gua + H)$^+$; HRMS calcd for $C_{40}H_{34}N_9O_{16}$ 896.2124, found 896.2155; FABMS (negative mode) m/z 894 $(M - H)^-$, 778 (2-*N*-bis-diNPEOC-Gua − H)$^-$, 580 (2-*N*-diNPEOC-dG − H)$^-$, 464 (2-*N*-diNPEOC-Gua − H)$^-$.

5′,3′-Bis(*O*-*tert*-butyldimethylsilyl)-6-*N*-(6-nitroveratryloxycarbonyl)-2′-deoxyadenosine (6). A THF solution of 6-nitroveratryloxycarbonyltetrazolide was prepared by adding NVOC−Cl (500 mg, 1.81 mmol) to a mixture of 1*H*-tetrazole (113, 1.6 mmol) and Et_3N (242 μL, 1.73 mmol) in anhydrous THF (20 mL) at 0 °C. The resulting mixture was stirred at 0 °C for 10 min and then at room temperature for 30 min. The precipitate was filtered off through a pad of Celite and washed with THF. The THF solution of NVOC-tetrazole was concentrated and added to 5′,3′-bis(*O*-*tert*-butyldimethylsilyl)-2′-deoxyadenosine (**5**) (385 mg, 0.8 mmol) in anhydrous THF (15 mL). The resulting mixture was heated to 70 °C for 3 h and then kept at room temperature overnight. After concentration under reduced pressure, the oil residue was diluted with ethyl acetate (100 mL) and washed with saturated aqueous $NaHCO_3$ (80 mL) and then with brine (80 mL). The organic layer was dried (Na_2SO_4) and then evaporated to dryness. The residue was purified by flash column chromatography (silica gel, gradient 70/30 to 100/0, v/v, of CH_2Cl_2/cyclohexane), and the appropriate fractions were combined and evaporated to dryness. The resulting foam was dissolved in dioxane and lyophilized to afford **6** (362 mg, 63%): ^{1}H NMR ($CDCl_3$) δ 8.77 (s, 1 H, H-8), 8.36 (s, 1 H, H-2), 7.76 (s, 1 H, H ortho to NO_2 of NVOC), 7.27 (s, 1 H, H meta to NO_2 of NVOC), 6.52 (m, 1 H, H-1′), 5.75 (s, 2 H, CO_2CH_2), 4.63 (m, 1 H, H-3′), 4.06 (m, 1 H, H-4′), 4.02 and 3.99 (2 s, 6H, 2 OCH_3), 3.99−3.76 (m, 2 H, H-5′, H-5″), 2.66 and 2.50 (2 m, 2 H, H-2′, H-2″), 0.93 (m, 18 H, 2 $C(CH_3)_3$), 0.12 (m, 12H, 2 $Si(CH_3)_2$); FABMS (positive mode) m/z 719 $(MH)^+$; HRMS calcd for $C_{32}H_{51}N_6O_9Si_2$ 719.3252, found 719.3346; FABMS (negative mode) m/z 717 $(M - H)^-$.

5′,3′-Bis(*O*-*tert*-butyldimethylsilyl)-6-*N*-(2,2′-bis(2-nitrophenyl)ethyloxycarbonyl)-2′-deoxyadenosine (9). 5′,3′-Bis(*O*-*tert*-butyldimethylsilyl)-2′-deoxyadenosine (**5**) (1.7 g, 3.5 mmol) was treated with the same protocol as for the preparation of **6**, except 2,2′-bis(2-nitrophenyl)ethyloxycarbonyltetrazolide prepared as above from diNPEOC−chloride (1.6 g, 4.56 mmol) replaced 6-nitroveratryloxycarbonyltetrazolide. Compound **9** was in with 90% yield (2.51 g): ^{1}H NMR ($CDCl_3$) δ 8.75 (s, 1 H, H-8), 8.40 (sl, 1 H, NH), 8.30 (s, 1 H, H-2), 8.00 (dd, J_{ortho} = 8.06 Hz, J_{meta} = 1.22 Hz, 2 H, H ortho to NO_2 of diNPEOC), 7.60−7.42 (m, 6 H, H meta and para to NO_2 of diNPEOC), 6.50 (m, 1 H, H-1′), 5.81 (t, J = 6.81 Hz, 1 H, CO_2CH_2CH), 4.99 (d, J = 6.84 Hz, 2 H, CO_2CH_2CH), 4.62 (m, 1 H, H-3′), 4.04 (m, 1 H, H-4′), 3.94−3.75 (m, 2 H, H-5′, H-5″), 2.63 and 2.51 (2 m, 2 H, H-2′, H-2″), 0.93 (m, 18 H, 2 $C(CH_3)_3$), 0.12 (m, 12H, 2 $Si(CH_3)_2$); FABMS (positive mode) m/z 794 $(MH)^+$; HRMS calcd for $C_{37}H_{52}N_7O_9Si_2$ 794.3365, found 794.3455; FABMS (negative mode) m/z 792 $(M - H)^-$, 478 $(M - diNPEOC - H)^-$.

6-*N*-(6-Nitroveratryloxycarbonyl)-2′-deoxyadenosine (7). To a solution of **6** (362 mg, 0.5 mmol) in anhydrous THF (15 mL) was added a 1 M solution of TBAF in THF (1.51 mL, 1.51 mmol), and the resulting mixture was stirred at room temperature for 1.5 h. After evaporation, the residue was purified by flash column chromatography (silica gel, gradient 0−10% MeOH/CH_2Cl_2), and the appropriate fractions were combined and evaporated to dryness. The resulting foam was dissolved in dioxane and lyophilized to afford **7** in quantitative yield. ^{1}H NMR (pyridine-d_5) δ 9.15 (s, 1 H, H-8), 9.02 (s, 1 H, H-2), 7.77 (s, 1 H, H ortho to NO_2 of NVOC), 7.54 (s, 1 H, H meta to NO2 of NVOC), 7.32 (d, 1 H, OH-3′), 6.97 (t, 1 H, OH-5′), 6.77 (m, 1 H, H-1′), 6.01 (s, 2 H, CO_2CH_2), 5.19 (m, 1 H, H-3′), 4.59 (m, 1 H, H-4′), 4.23−4.14 (m, 2 H, H-5′, H-5″), 3.76 and 3.74 (2 s, 6H, 2 OCH_3), 3.15 and 2.77 (2 m, 2 H, H-2′, H-2″); FABMS (positive mode) m/z 491 $(MH)^+$; 252 (M − NVOC + 2H)$^+$ = (dA + H)$^+$; HRMS calcd for $C_{20}H_{23}N_6O_9$ 491.1527, found 491.1572; FABMS (negative mode) m/z 489 $(M - H)^-$, 250 $(M - NVOC)^- = (dC - H)^-$.

6-*N*-(2,2′-Bis(2-nitrophenyl)ethyloxycarbonyl)-2′-deoxyadenosine (10). The TBAF treatment described above for the synthesis of **7** was performed with **9** (2.5 g, 3.16 mmol) and afforded **10** in quantitative yield.

5′,3′-Bis(*O*-*tert*-butyldimethylsilyl)-6-*N*-(2,2′-bis(2-nitrophenyl)ethyloxycarbonyl)-2′-deoxyguanosine (16). To a solution of 5′,3′-bis(*O*-*tert*-butyldimethylsilyl)-2′-deoxyguanosine (**15**) (1 g, 4 mmol) in THF (20 mL) and HMPA (3 mL) was added dropwise a 1 M solution of *tert*-butylmagnesium chloride in THF (5 mL), and the mixture was stirred at 25 °C for 1 h. To this solution was added diNPEOC−Cl (1.5 g, 8 mmol), and the resulting mixture was stirred for 4 h. Methanol (5 mL) was added and the resulting mixture evaporated to an oil, which was dissolved in ether (100 mL). This solution was washed with an aqueous solution of 0.15 M EDTA (80 mL) and then with saturated aqueous $NaHCO_3$ (50 mL) and brine (50 mL). The crude residue was subjected to silica gel column chromatography (silica gel, gradient 0−2% MeOH/CH_2Cl_2), and the appropriate fractions were combined and evaporated to dryness. The resulting foam was dissolved in dioxane and lyophilized to afford **16** in 50% yield: ^{1}H NMR ($CDCl_3$) δ 11.14 (sl, 1 H, NH), 8.02 (dd, J_{ortho} = 8.00 Hz, J_{meta} = 1.41 Hz, 2 H, H ortho to NO_2 of diNPEOC), 7.97 (s, 1 H, H-8), 7.91 (sl, 1 H, NH), 7.66−7.39 (m, 6 H, H meta and para to NO_2 of diNPEOC), 6.20 (m, 1 H, H-1′), 5.81 (t, J = 7.17 Hz, 1 H, CO_2CH_2CH), 4.96 (d, J = 7.16 Hz, 2 H, CO_2CH_2CH), 4.57 (m, 1 H, H-3′), 3.99 (m, 1 H, H-4′), 3.78 (m, 2 H, H-5′, H-5″), 2.39 (2 m, 2 H, H-2′, H-2″), 0.92 (m, 18 H, 2 $C(CH_3)_3$), 0.11 (m, 12H, 2 $Si(CH_3)_2$); FABMS (positive mode) m/z 810 $(MH)^+$, 496 (M − diNPEOC + 2H)$^+$; HRMS calcd for $C_{37}H_{52}N_7O_{10}Si_2$ 810.3314, found 810.3352; FABMS (negative mode) m/z 808 $(M - H)^-$, 494 (M − diNPEOC − H)$^-$.

6-*N*-(2,2′-Bis(2-nitrophenyl)ethyloxycarbonyl)-2′-deoxyguanosine (13). To a solution of **16** (1.5 g, 1.8 mmol) in anhydrous THF (15 mL) was added a 1 M solution of TBAF in THF (5.66 mL, 5.66 mmol), and the resulting mixture was stirred at room temperature for 1.5 h. After concentration, the mixture was extracted with an 8:2 mixture of dichloromethane and pyridine, washed with saturated aqueous $NaHCO_3$ (50 mL) and brine (50 mL), and then dried and concentrated. The crude product was subjected to silica gel column chromatography (silica gel, gradient 0−10% MeOH/CH_2Cl_2), and the appropriate fractions were combined and evaporated to dryness. The resulting foam was dissolved in dioxane and lyophilized to afford **13** in quantitative yield.

General Procedure for the 5′-*O*-(4,4′-Dimethoxy)tritylation of *N*-Protected 2′-Deoxynucleosides. Prior to use, the *N*-protected 2′-deoxynucleosides **1**, **3**, **7**, **10**, and **13** (0.5 mmol) were dried three times by coevaporation of anhydrous pyridine and then dissolved in anhydrous pyridine (10 mL). 4,4′-Dimethoxytrityl chloride (200 mg, 0.6 mmol) and the resulting mixture were stirred at room temperature overnight. Methanol (10 mL) was added, and the mixture was stirred for a further 10 min, concentrated under reduced pressure, then diluted with dichloromethane (100 mL), and washed with saturated aqueous $NaHCO_3$ (80 mL) and then with brine (80 mL). The organic layer was dried (Na_2SO_4), filtered, and evaporated to dryness. Residual pyridine was removed by coevaporations with toluene (3×). The residues were purified by flash column chromatography (silica gel, gradient 0−2% MeOH/CH_2Cl_2 containing 1% Et_3N). The appropriate fractions were combined and evaporated to dryness. The resulting foams were dissolved in dioxane and lyophilized to give **2** (40% yield), **4** (83% yield), **8** (53% yield), **11** (60% yield), and **17** (60% yield), respectively.

5′-*O*-(4,4′-Dimethoxytrityl)-4-*N*-(6-nitroveratryloxycarbonyl)-2′-deoxycytidine (2): ^{1}H NMR ($CDCl_3$) δ 8.24 (d, J = 7.35 Hz, 1 H, H-6), 7.72 (s, 1 H, H ortho to NO_2 of NVOC), 7.28 (m, 9H, aromatic H of DMTr), 7.08 (s, 1 H, H meta to NO_2 of NVOC),6.93−6.69 (m, 5 H, 4 aromatic H, H-5), 6.26 (m, 1 H, H-1′), 5.60 (s, 2 H, CO_2CH_2), 4.49 (m, 1 H, H-3′), 4.12 (m, 1 H, H-4′), 3.96 and 3.94 (2 s, 6 H, 2 OCH_3), 3.77 and 3.68 (2 s, 6 H, 2 OCH_3), 3.44 (m, 2 H, H-5′, H-5″), 2.24 and 1.44 (2 m, 2 H, H-2′, H-2″).

5′-*O*-(4,4′-Dimethoxytrityl)-4-*N*-(2,2′-bis(2-nitrophenyl)ethyloxycarbonyl)-2′-deoxycytidine (4): ^{1}H NMR ($CDCl_3$) δ 8.23 (d, J = 7.55 Hz, 1 H, H-6), 7.99 (dd, J_{ortho} = 8.00 Hz, J_{meta} = 1.34 Hz, 2 H, H ortho to NO_2 of diNPEOC), 7.60−7.26 (m, 15 H, 6 H meta and para to NO_2 of diNPEOC, 9 H DMTr), 6.87 (m, 5 H, 4 H DMTr, H-5), 6.25 (m, 1 H, H-1′), 5.81 (t, J = 6.32 Hz, 1 H, CO_2CH_2CH), 4.93 (d, J = 6.33 Hz, 2 H, CO_2CH_2-CH), 4.50 (m, 1 H, H-3′), 4.12 (m, 1 H, H-4′), 3.82 (s, 6 H, 2 OCH_3), 3.48 (m, 2 H, H-5′, H-5″), 2.70 and 2.25 (2 m, 2 H, H-2′, H-2″); FABMS (positive mode) m/z 844 $(MH)^+$, 426 (4-*N*-diNPEOC-Cyt + H)$^+$, 303 $(DMTr)^+$; HRMS calcd for $C_{45}H_{42}N_5O_{12}$ 844.2830, found 844.2729; FABMS (negative mode) m/z 842 $(M - H)^-$, 528 $(M - DMTr)^-$.

5′-*O*-(4,4′-Dimethoxytrityl)-6-*N*-(6-nitroveratryloxycarbonyl)-2′-deoxyadenosine (8): ^{1}H NMR ($CDCl_3$) δ 8.70 (s, 1 H, H-8), 8.15 (s, 1 H, H-2), 7.79 (s, 1 H, H ortho to NO_2 of NVOC), 7.43−7.24 (m, 10 H, 9 aromatic H of DMTr, H meta to NO_2 of NVOC), 6.81 (m, 4 H, 4 aromatic H of DMTr), 6.49 (m, 1 H, H-1′), 5.75 (s, 2 H, CO_2CH_2), 4.74 (m, 1 H, H-3′), 4.18 (m, 1 H, H-4′), 4.01 and 3.99 (2 s, 6 H, 2 OCH_3 of NVOC), 3.80 (s, 6 H, 2 OCH_3 of DMTr), 3.44 (m, 2 H, H-5′, H-5″), 2.89 and 2.61 (2 m, 2 H, H-2′, H-2″).

5′-*O*-(4,4′-Dimethoxytrityl)-6-*N*-(2,2′-bis(2-nitrophenyl)ethyloxycarbonyl)-2′-deoxyadenosine (11): ^{1}H NMR ($CDCl_3$) δ 8.68 (s, 1 H, H-8), 8.10 (s, 1 H, H-2), 8.01 (dd, J_{ortho} = 7.98 Hz, J_{meta} = 1.36 Hz, 2 H, H ortho to NO_2 of diNPEOC), 7.60−7.24 (m, 15 H, 6 H meta and para to NO_2 of diNPEOC, 9 H of DMTr), 6.81 (m, 4 H, 4 H of DMTr), 6.48 (m, 1 H, H-1′), 5.82 (t, J = 6.74 Hz, 1 H, CO_2CH_2CH), 4.99 (d, J = 6.79 Hz, 2 H, CO_2CH_2CH), 4.72 (m, 1 H, H-3′), 4.16 (m, 1 H, H-4′), 3.80 (s, 6 H, 2 OCH_3), 3.43 (m, 2 H, H-5′, H-5″), 2.87 and 2.60 (2 m, 2 H, H-2′, H-2″); FABMS (positive mode) m/z 868 $(MH)^+$, 450 (4-*N*-diNPEOC-Ade + H)$^+$, 303 $(DMTr)^+$; HRMS calcd for $C_{46}H_{42}N_7O_{11}$ 868.2942, found 868.2943; FABMS (negative mode) m/z 866 $(M - H)^-$, 552 $(M - DMTr)^-$.

5′-*O*-(4,4′-Dimethoxytrityl)-2-*N*-(2,2′-bis(2-nitrophenyl)ethyloxycarbonyl)-2′-deoxyguanosine (17): ^{1}H NMR ($CDCl_3$) δ 8.39 (s, 1 H, NH), 7.92 (dd, J_{ortho} = 8.08 Hz, J_{meta} = 1.39 Hz, 2 H, H ortho to NO_2 of diNPEOC), 7.76 (s, 1 H, H-8), 7.54−7.18 (m, 15 H, 6 H meta and para to NO_2 of diNPEOC, 9 H DMTr), 6.74 (m, 4 H, DMTr), 6.25 (m, 1 H, H-1′), 5.74 (t, J = 6.78 Hz, 1 H, CO_2CH_2CH), 4.90 (d, J = 6.76 Hz, 2 H, CO_2CH_2-CH), 4.76 (m, 1 H, H-3′), 4.16 (m, 1 H, H-4′), 3.76 (s, 6 H, 2 OCH_3), 3.27 (m, 2 H, H-5′, H-5″), 2.59 (m, 2 H, H-2′, H-2″); FABMS (positive mode) m/z 884 $(MH)^+$, 303 $(DMTr)^+$; HRMS calcd for $C_{46}H_{42}N_7O_{12}$ 884.2891, found 884.2979; FABMS (negative mode) m/z 882 $(M - H)^-$, 568 $(M - DMTr)^-$.

(*S*-Pivaloyl-2-thioethyl)-*N*,*N*-bis(diisopropylamino)-phosphine (19). To a solution at 0 °C of *N*,*N*-bis(diisopropylamino)chlorophosphine (5 g, 18.7 mmol) in diethyl ether (40 mL) was added dropwise a mixture of *S*-pivaloyl-2-thioethanol (3 g, 18.7 mmol) and Et_3N (2.9 mL, 20.6 mmol). The resulting mixture was stirred at room temperature for 3 h and then filtered; the glassware was washed with cyclohexane, and the combined filtrates were concentrated until a viscous oil was obtained. The oil was purified by flash column chromatography (silica gel, elution with cyclohexanes/ethyl acetate/triethylamine, 89/10/1, v/v/v). The appropriate fractions were combined and evaporated to dryness to give **17** as an oil (6.3 g, 86%): ^{31}P NMR ($CDCl_3$) δ 126.01; ^{1}H NMR ($CDCl_3$) δ 3.66 (m, 2 H, OCH_2CH_2S), 3.54 (m, 4 H, $CH(CH_3)_2$), 3.10 (t, J = 6.4 Hz, 2 H, OCH_2CH_2S), 1.25 (s, 9 H, $COC(CH_3)_3$), 1.19 (m, 24 H, $CH(CH_3)_2$); FABMS (positive mode) m/z 393 $(MH)^+$.

General Procedure for the Phosphitylation of 5′-*O*-(4,4′-Dimethoxytrityl) *N*-Protected 2′-Deoxynucleosides. Prior to use, the 5′-*O*-DMTr *N*-protected 2′-deoxynucleosides **2**, **4**, **8**, **11**, **17**, and **18** (0.5 mmol) and diisopropylammonium tetrazolide (43 mg, 0.25 mmol) were separately dried three times by coevaporation of anhydrous acetonitrile and then mixed and dissolved in anhydrous dichloromethane (14 mL). A solution of (*S*-pivaloyl-2-thioethyl)-*N*,*N*-bis(diisopropylamino)phosphine (**19**; 235 mg, 0.6 mmol) in dichloromethane (3 mL) was added under argon. The resulting mixture was stirred overnight at room temperature and then diluted with ethyl

acetate (60 mL) and washed with saturated aqueous $NaHCO_3$ (50 mL) and then with brine (50 mL). The organic layer was dried (Na_2SO_4), filtered, and evaporated to dryness. The residues were purified by flash column chromatography (silica gel, gradient 30−100% ethyl acetate/1% Et_3N/cyclohexane). The appropriate fractions were combined, evaporated to dryness, and dissolved in acetonitrile (20 mL). The resulting solution were filtered on a Millex filter, evaporated to dryness, redissolved in benzene, and lyophilized to, respectively, afford the resulting phosphororamidites **20** (82%), **21** (84%), **22** (60%), **23** (78%), **24** (55%), and **25** (80%) as colorless powders.

5′-*O*-(4,4′-Dimethoxytrityl)-4-*N*-(6-nitroveratryloxycarbonyl)-2′-deoxycytidine 3′-*O*-(*S*-Pivaloyl-2-thioethyl) *N*,*N*-Diisopropylphosphoramidite (20): ^{31}P NMR ($CDCl_3$) δ 148.95, 148.35; ^{1}H NMR ($CDCl_3$) δ 8.35 (m, 1 H, H-6), 7.76 (s, 1 H, H ortho to NO_2 of NVOC), 7.43−7.25 (m, 9H, H of DMTr), 7.04 (s, 1 H, H meta to NO_2 of NVOC), 6.85 (m, 5 H, 4 H of DMTr, H-5), 6.28 (m, 1 H, H-1′), 5.64 (s, 2 H, CO_2CH_2), 4.66 (m, 1 H, H-3′), 4.23 (m, 1 H, H-4′), 4.01 and 3.99 (2 s, 6 H, 2 OCH_3 of NVOC), 3.81 (s, 6 H, 2 OCH_3 of DMTr), 3.75−3.43 (m, 6 H, C*H*$(CH_3)_2$, O*CH*$_2$CH$_2$S, H-5′, H-5″), 3.10−2.94 (m, 2 H, OCH$_2$C*H*$_2$S), 2.76 and 2.32 (2 m, 2 H, H-2′, H-2″), 1.27−1.06 (m, 21 H, CH(C*H*$_3$)$_2$, CH(C*H*$_3$)$_3$); FABMS (positive mode, PEG) *m*/*z* 1060 $(MH)^+$, 303 $(DMTr)^+$.

5′-*O*-(4,4′-Dimethoxytrityl)-4-*N*-(2,2′-bis(2-nitrophenyl)-ethyloxycarbonyl)-2′-deoxycytidine 3′-*O*-(*S*-Pivaloyl-2-thioethyl) *N*,*N*-Diisopropylphosphoramidite (21): ^{31}P NMR (CD_3CN) δ 148.96, 148.65; ^{1}H NMR (CD_3CN) δ 8.42 (s, 1 H, NH), 8.21 and 8.15 (2 d, 1 H, H-6), 7.95 (dd, 2 H, H ortho to NO_2 of diNPEOC), 7.69−7.30 (m, 15 H, 6 H meta and para to NO_2 of diNPEOC, 9 H DMTr), 6.89 (m, 4 H, H DMTr), 6.81 (d, 1 H, H-5), 6.09 (m, 1 H, H-1′), 5.57 (t, 1 H, CO_2CH_2CH), 4.90 (d, J = 6.27 Hz, 2 H, CO_2CH_2CH), 4.60 (m, 1 H, H-3′), 4.11 (m, 1 H, H-4′), 3.79 (s, 6 H, 2 OCH_3), 3.67−3.54 (m, 4 H, 2 C*H*$(CH_3)_2$, O*CH*$_2$CH$_2$S), 3.41 (m, 2 H, H-5′, H-5″), 3.02 (m, 2 H, OCH$_2$C*H*$_2$S), 2.53 and 2.32 (2 m, 2 H, H-2′, H-2″), 1.29−1.05 (m, 21 H, CH(C*H*$_3$)$_2$, CH(C*H*$_3$)$_3$); FABMS (positive mode, PEG) *m*/*z* 1135 $(MH)^+$, 826 $(M - DMTr + 2H)^+$, 303 $(DMTr)^+$; HRMS (NOBA) calcd for $C_{58}H_{67}N_6O_{14}PSNa$ 1157.4071, found 1157.4034; FABMS (negative mode, PEG) *m*/*z* 1133 $(M - H)^-$, 1149, $(M + O - H)^-$.

5′-*O*-(4,4′-Dimethoxytrityl)-6-*N*-(6-nitroveratryloxycarbonyl)-2′-deoxyadenosine 3′-*O*-(*S*-Pivaloyl-2-thioethyl) *N*,*N*-Diisopropylphosphoramidite (22): ^{31}P NMR ($CDCl_3$) δ 149.07, 149.04; ^{1}H NMR ($CDCl_3$) δ 8.70 (s, 1 H, H-8), 8.22 and 8.20 (2 s, 1 H, H-2), 7.76 (s, 1 H, H ortho to NO_2 of NVOC), 7.43−7.20 (m, 10 H, 9 H of DMTr, H meta to NO_2 of NVOC), 6.79 (m, 4 H, H of DMTr), 6.52 (m, 1 H, H-1′), 5.75 (s, 2 H, CO_2CH_2), 4.79 (m, 1 H, H-3′), 4.35 (m, 1 H, H-4′), 4.01 and 3.99 (2 s, 6 H, 2 OCH_3 of NVOC), 3.79 and 3.76 (2 s, 6 H, 2 OCH_3 of DMTr), 3.72−3.44 (m, 6 H, C*H*$(CH_3)_2$, O*CH*$_2$CH$_2$S, H-5′, H-5″), 3.20−2.87 (m, 4 H, OCH$_2$C*H*$_2$S, H-2′, H-2″), 1.29−1.11 (m, 21 H, CH(C*H*$_3$)$_2$, CH(C*H*$_3$)$_3$); FABMS (positive mode, PEG) *m*/*z* 1084 $(MH)^+$, 303 $(DMTr)^+$.

5′-*O*-(4,4′-Dimethoxytrityl)-6-*N*-(2,2′-bis(2-nitrophenyl)-ethyloxycarbonyl)-2′-deoxyadenosine 3′-*O*-(*S*-Pivaloyl-2-thioethyl) *N*,*N*-Diisopropylphosphoramidite (23): ^{31}P NMR (CD_3CN) δ 148.79, 148.64; ^{1}H NMR (CD_3CN) δ 8.73 (s, 1 H, NH), 8.51 (s,1 H, H-8), 8.20(s, 1 H, H-2), 7.93 (dd, 2 H, H ortho to NO_2 of diNPEOC), 7.66−7.42 (m, 6 H, 6 H meta and para to NO_2 of diNPEOC), 7.41−7.18 (m, 9 H, H DMTr), 6.77 (m, 4 H, H DMTr), 6.41 (m, 1 H, H-1′), 5.58 (t, J = 6.21 Hz, 1 H, CO_2CH_2CH), 4.94 (d, J = 6.16 Hz, 2 H, CO_2CH_2CH), 4.88 (m, 1 H, H-3′), 4.22 (m, 1 H, H-4′), 3.74 (s, 6 H, 2 OCH_3), 3.61 (m, 4 H, 2 C*H*$(CH_3)_2$, O*CH*$_2$CH$_2$S), 3.29 (m, 2 H, H-5′, H-5″), 3.03 (m, 3 H, OCH$_2$C*H*$_2$S, H-2′), 2.59 (2 m, 1 H, H-2″), 1.20−1.09 (m, 21 H, CH(C*H*$_3$)$_2$, CH(C*H*$_3$)$_3$); FABMS (positive mode, PEG) *m*/*z* 1159 $(MH)^+$, 1175 $(M + O + H)^+$, 850 $(M - DMTr + 2H)^+$, 303 $(DMTr)^+$; HRMS (NOBA) calcd for $C_{59}H_{68}N_8O_{13}PS$ 1159.4364, found 1159.4333.

5′-*O*-(4,4′-Dimethoxytrityl)-2-*N*-(2,2′-bis(2-nitrophenyl)-ethyloxycarbonyl)-2′-deoxyguanosine 3′-*O*-(*S*-Pivaloyl-2-thioethyl) *N*,*N*-Diisopropylphosphoramidite (24): ^{31}P NMR ($CDCl_3$) δ 148.94, 148.65; ^{1}H NMR ($CDCl_3$) δ 8.03 (dd, 2 H, H ortho to NO_2 of diNPEOC), 7.82 (s,1 H, H-8), 7.68−7.21 (m, 15 H, 6 H meta and para to NO_2 of diNPEOC, 9 H DMTr), 6.78 (m, 4 H, H DMTr), 6.23 (m, 1 H, H-1′), 5.78 (m, 1 H, CO_2CH_2CH), 4.93 (m, 2 H, CO_2CH_2CH), 4.67 (m, 1 H, H-3′), 4.24 (m, 1 H, H-4′), 3.79 (s, 6 H, 2 OCH_3), 3.75−3.51 (m, 4 H, 2 C*H*$(CH_3)_2$, O*CH*$_2$CH$_2$S), 3.30 (m, 2 H, H-5′, H-5″), 3.03 (m, 2 H, OCH$_2$C*H*$_2$S), 2.58 (2 m, 1 H, H-2′, H-2″), 1.28−1.09 (m, 21 H, CH(C*H*$_3$)$_2$, CH(C*H*$_3$)$_3$); FABMS (positive mode, PEG) *m*/*z* 1175 $(MH)^+$, 303 $(DMTr)^+$; HRMS (NOBA) calcd for $C_{59}H_{68}N_8O_{14}PS$ 1175.4313, found 1175.4338; FABMS (negative mode, PEG) *m*/*z* 1173 $(M - H)^-$, 1189, $(M + O - H)^-$.

Solid-Phase Elongation of Prooligonucleotides Using NVOC-Protected Nucleoside Phosphoramidites 20 and 22. Nucleoside phosphoramidites **20** and **22** solutions (0.1 M) in anhydrous acetonitrile/dichloromethane (9/1, v/v) were used. dT phosphoramidite **25** was dissolved in anhydrous acetonitrile. During each coupling step, a 20-fold molar excess of **20**, **22**, and **25** was introduced into the column bearing the photolabile CPG solid support.[13,14] A modified "1 μmol" cycle was applied. Modifications included extended coupling times (180 s "wait") and an extended capping time (300 s "wait") for the first capping step (8 s "wait" for the next capping steps). A solution of *tert*-butyl hydroperoxide (1.1 M) in toluene/dichloromethane (obtained from commercial 3 M solution in toluene) was then used as the oxidizer for the formation of phosphotriester internucleoside linkages (60 s "wait"). The coupling efficiency was evaluated by measuring the release of the 4,4′-dimethoxytrityl carbocation by spectrophotometry (λ 498 nm) at the end of each incorporation cycle. Yields for **20** and **22** couplings were between 87 and 93% whereas the coupling efficiency of **25** was >98%.

Solid-Phase Elongation of Prooligonucleotides Using diNPEOC-Protected Nucleoside Phosphoramidites 21, 23, and 24. Nucleoside amidite **21**, **23**, and **24** solutions (0.06 M) in acetonitrile/dichloromethane (9/1, v/v) were dried over 4 Å molecular sieves for 24 h and then filtered off on a 0.45 μm Millex filter. A 15-fold molar excess of the amidites was introduced into the column bearing the photolabile CPG solid support.[13,14] Modifications of the standard "1 μmol" cycle were extended coupling times (360 s "wait"), replacement of the common capping step by a treatment with diethyl *N*,*N*-diisopropyl phosphoramidite (0.1 M in acetonitrile) in the presence of 0.5 M tetrazole solution in acetonitrile[31] (capping time of 3 × 120 s "wait"). The intermediate phosphite triesters were oxidized by a 60 s treatment with a 1.1 M solution of *tert*-butyl hydroperoxide in toluene/dichloromethane or by a 30 s treatment with a solution of 3-*H*-1,2-benzodithiol-3-one 1,1-dioxide (0.05 M) to afford phosphate triester and thionophosphate triester internucleoside linkages, respectively. The coupling yield of nucleoside amidites **21**, **23**, and **24** was between 90 and 96%.

Deprotection and Release of the Prooligonucleotides from the Solid Support. When the required number of cycles were completed, the columns were flushed with argon and then disassembled and the supported 5′-detritylated prooligonucleotides (~15 mg) were divided into portions (4 mg, ~0.25 μmol). Each portion was suspended within a 1 cm path length quartz cell in the appropriate volume of dioxane/water (3/2) containing acetic acid (5%). To obtain a desired ~0.1 mM theoretical prooligo concentration (C_{theor}), 2.5 mL of solution was used, and 250 μL of solution was used for $C_{theor} \approx 1$ mM. Argon was bubbled through the suspensions, and then the magnetically stirred suspensions were exposed at 20 °C to the light of a high-pressure Hg lamp filtered with a Pyrex glass (2 mm thick). Aliquots of the solutions were withdrawn and analyzed by reversed-phase HPLC. After completion of the photolyses (table), the glass beads were filtered off on a 0.45 μm Millex filter and washed with the same solvent mixture (2 × 0.5 mL). The prooligonucleotides were purified by reversed-phase HPLC using linear gradients of acetonitrile (0−90%) in 0.05 M aqueous triethylammonium acetate (pH 7). The appropriate fractions were concentrated under reduced pressure. Residues were dissolved in dioxane/water (1/1 to 3/2, v/v) and lyophilized to afford colorless powders.

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Supporting Information Available: Copies of the ^{1}H and ^{31}P NMR spectra of all new compounds. This material is available free of charge via the Internet at http://pubs.acs.org.

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