Fluoride-Labile Protecting Groups for the Synthesis of Base-Sensitive Methyl-SATE Oligonucleotide Prodrugs

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Keywords: Oligonucleotides / Protecting groups / Prodrugs

Base-sensitive methyl-SATE oligonucleotide prodrugs (prooligos) were synthesized using fluoride-labile protection groups on the nucleobases and on certain internucleosidic phosphate linkages. The combination of [(*tert*-butyl)(diphenyl)silyloxymethyl]benzoyl (SiOMB) and (trimethylsilyl)ethyl (TSE) groups is well adapted for synthesis of these compounds using phosphoramidite chemistry on a solid support. The prooligo was anchored on the support via a phosphoramidate linkage. A final treatment with $Et_3N{\cdot}3HF$ in THF efficiently releases the prooligo from the solid support and cleaves the SiOMB and TSE groups.

Introduction

Prodrugs of oligonucleotides (prooligos)[1] have been designed to overcome the instability of oligonucleotides towards phosphodiesterases,[2] reduce nonspecific protein interactions,[3] and enhance their cellular uptake.[4−6] In our prooligonucleotide approach, the majority of the negatively charged internucleosidic linkages of the oligonucleotides were transiently protected with the enzymolabile 2*S*-*A*cyl-*T*hio*E*thyl (SATE) group previously introduced in mononucleotide chemistry.[7,8] In cell extracts, prooligos were fully deprotected through a carboxyesterase-mediated mechanism, to deliver free oligonucleotides.[9,10] This deprotection proceeds by thioester hydrolysis, releasing an unstable 2-mercaptoethyl phosphotriester that rapidly decomposes to ethylene sulfide and the corresponding ionic phosphodiester or phosphorothioate linkages. This hydrolysis also occurs upon base [11] or nucleophile treatment. Prooligos therefore cannot be synthesized under the common conditions of solid-supported DNA synthesis, as they rapidly decompose during the standard ammonia treatment.[2] A new strategy was therefore designed for the solid-phase synthesis of these base-sensitive analogs, with the aid of a photolabile solid support [12] and thymidine SATE phosphoramidites. Prothymidine models were synthesized by mixed phosphoramidite and H-phosphonate chemistries. The synthesis of hetero prooligonucleotides requires protecting groups that could be removed without basic and nucleophilic treatments. Our group has already tried different protecting groups for that purpose.[13−18] Our last attempt was the allylic strategy[19] with a deprotection using Pd^0. In this way we synthesized hetero prooligos but they were contaminated by traces of Pd.[20] To avoid palladium contamination, we developed a new strategy using fluoride-labile protecting groups both on the nucleobase and on the phosphate. Indeed we have shown that prooligos need to have 30 to 50% of phosphodiester linkages to be soluble in aqueous media while keeping their ability to permeate cells.[6] The [(*tert*-butyl)(diphenyl)silyloxymethyl]benzoyl (SiOMB) group [21] (Figure 1) has already been described as a protecting group for nucleobases in the synthesis of RNA [22] and nucleopeptides.[23] It was removed by means of tetrabutylammonium fluoride (TBAF) in solution in THF, which was also used to remove the TBDMS on the 2′-hydroxy groups. Furthermore, diphenylmethylsilylethyl (DPSE) [24] and trimethylsilylethyl (TSE)[25,26] groups have been reported as alternative means of protecting the phosphate. Once again they were removed by means of TBAF. A TBAF treatment is strong enough to also hydrolyze acetyl, so such a treatment must be avoid with prooligos. However, various milder treatments are available to remove silyl groups.[27]

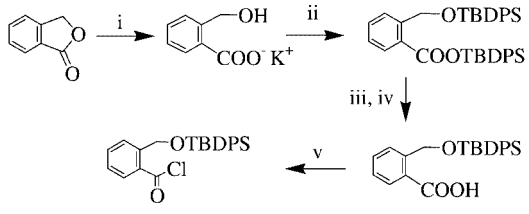

Figure 1. Synthesis of [(*tert*-butyl)(diphenyl)silyloxymethyl]benzoyl chloride i: KOH, aqueous 85% methanol; ii: TBDPSCl dry pyridine; iii: K_2CO_3, H_2O, THF methanol; iv: 1 M $KHSO_4$ to pH 4−5; v: oxalyl chloride, dry toluene

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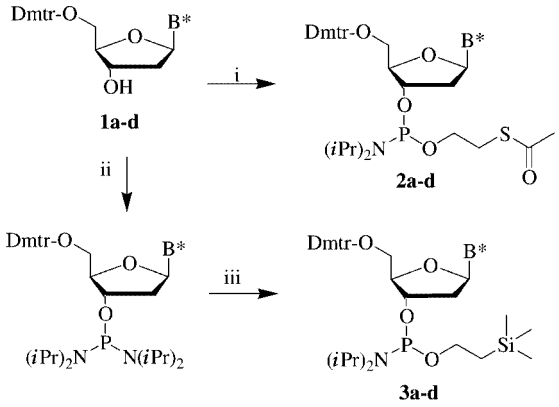
Here we present a strategy using SiOMB on the nucleobases and TSE on certain phosphates for the synthesis of hetero Me-SATE prooligos containing the four bases, and a final treatment with $Et_3N{\cdot}3HF$ to deprotect them without any detrimental effect on Me-SATE groups. Furthermore, a phosphoramidate linker that was cleaved under the same fluoride treatment recently replaced the photolabile linker between the prooligo and the solid support.

Results and Discussion

Synthesis of Phosphoramidite Building Blocks

In the original synthesis,[23] the SiOMB chloride was obtained in four steps starting from 2-bromobenzyl alcohol using a Grignard reaction. We developed an easier and less costly synthesis starting from the cheap phthalide that gave the SiOMB chloride in four steps with an overall yield of 67% (Figure 1). This procedure did not require any chromatography but a simple crystallization to get the carboxylic derivative, which was quantitatively converted into its acid chloride by an oxalyl chloride treatment.[28]

The *N*-SiOMB-5′-*O*-Dmtr-nucleosides of A, C and G (**1a**−**c**) were synthesized according to ref.[23,29] using transient protection[30] of the 3′ and 5′ hydroxy groups with trimethylsilyl chloride and then protection of the exocyclic amino group with SiOMB chloride. However, in our hands, yields were always lower (26 to 61%) than those claimed in the original papers (68 to 82%). Then, they were converted into Me-SATE phosphoramidites (**2a**−**c**, 70%, 82%, and 84% respectively) using Me-SATE bis(diisopropylamino)-phosphane (Figure 2) and diisopropylammonium tetrazolide as activator. The synthesis of the Me-SATE phosphoramidite of thymidine (**2d**) has already been reported elsewhere.[2]



a: B* = N^6-SiOMB-adenine **b**: B* = N^4-SiOMB-cytosine
c: B* = N^2-SiOMB-guanine **d**: B* = thymine

Figure 2. Synthesis of Me-SATE and (trimethylsilyl)ethyl phosphoramidite derivatives i: [2-(acetylthio)ethyloxy]bis(diisopropylamino)phosphane, diisopropylammonium tetrazolide, CH_2Cl_2, ii: bis(diisopropylamino)chlorophosphane, (diisopropyl)ethylamine, CH_2Cl_2, iii: (trimethylsilyl)ethyl alcohol, diisopropylammonium tetrazolide.

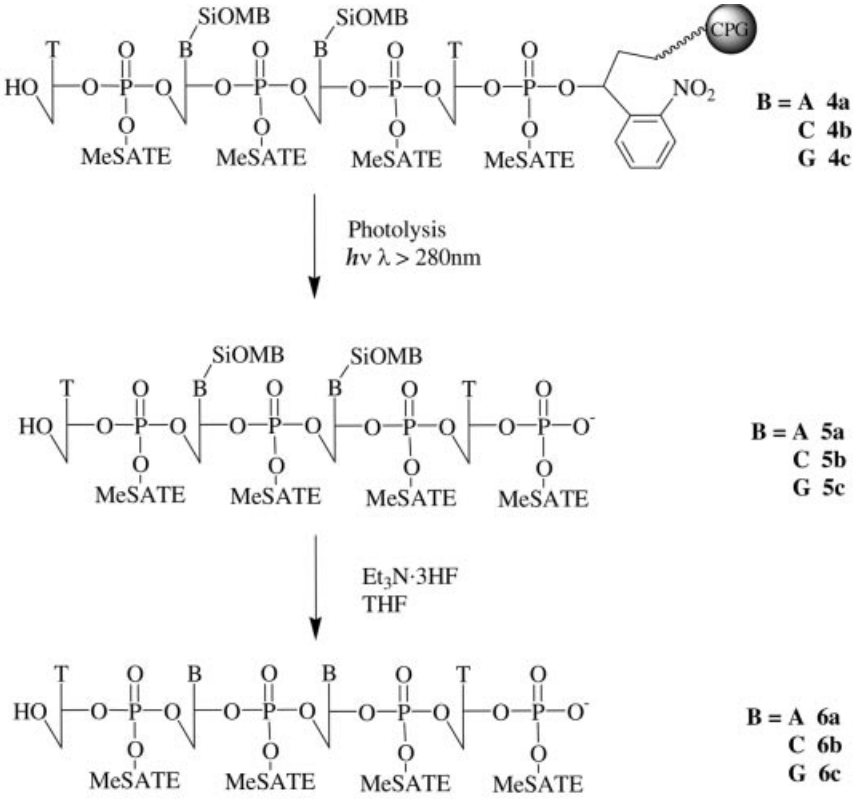


Figure 3. Schematic structure of pro-tetramer models **6a**−**c** and of their derivatives after photolysis and $Et_3N{\cdot}3HF$ treatment

The four TSE phosphoramidites (**3a**−**d**, 59%, 38%, 57%, and 70% yield) were synthesized from compounds **1a**−**d** and bis(diisopropylamino)chlorophosphane in the presence of (diisopropyl)ethylamine (DIEA) to form the corresponding bisamidite nucleosides, which subsequent directly reacted with TSE alcohol in the presence of diisopropylammonium tetrazolide as activator (Figure 2). ^{31}P NMR spectroscopy showed that the desired phosphoramidites were contaminated with the hydrolyzed chlorophosphane (1.3 to 7%) and with a phosphite triester resulting from an over activation of the phosphoramidite formed with TSE alcohol (0 to 18%). As these side compounds will not react during the further internucleosidic linkage formation the mixture was used without purification.

Deprotection of Fluoride-Labile Groups

SiOMB Removal Treatment

To determine a suitable treatment to remove the SiOMB groups on the nucleobases without affecting the Me-SATE groups, we first synthesized three pro-tetramer models constituted by two thymidines and two other nucleotides A, C or G on a photolabile solid support (Figure 3). We found that the *tert*-butyldiphenylsilyl (TBDPS) groups and then the 2-(oxymethyl)benzoyl (OMB) group could be removed on a solid support using trimethylsilyl chloride and water in acetonitrile.[31,32] Such an acidic treatment was efficient with pro-oligo **4b** bearing only C and T[32] but led to some depurination of prooligos bearing A (**4a**) or G (**4c**). Hence this treatment was not used further. A less-acidic treatment with *para*-toluenesulfonic acid in methanol did not lead to depurination but slowly hydrolyzed the TBDPS groups and also led to some hydrolysis of the Me-SATE groups. We abandoned such acidic treatments and looked for fluoride reagents that could remove TBDPS groups under mild conditions. Since the CPG solid support could react with fluoride reagents, the fully protected tetramers were cleaved from the solid support by photolysis. After photolysis, the tetramers in solution were treated with different fluoride reagents. Deprotection of the tetramers was monitored by MALDI-TOF MS allowing a rapid and easy evaluation of the efficacy of each treatment. Treatments with TBAF or HF−pyridine complex were too drastic and led to a total hydrolysis of silyl and Me-SATE groups. In contrast, treatment with $Et_3N{\cdot}3HF$ complex gave a rapid and efficient removal of the TBDPS groups without affecting the Me-SATE ones, as shown by MALDI-TOF MS in negative mode (Figure 4 and 5, Table 1). The treatment of tetramer **5a** (m/z = 2406.9) for 1 h with $Et_3N{\cdot}3HF$ yielded a neat and complete deprotection to give **6a** (m/z= 1660.3) (Figure 4). The HPLC profile of the crude product mainly shows a multiple peak due to the presence of diastereoisomers (2^3). Likewise, tetramer **5c** was treated with $Et_3N{\cdot}3HF$ and the MALDI-TOF spectrum at 15 min showed a major peak at m/z = 1960.5 Da corresponding to **5c** having lost only the two TBDPS groups but not the OMB ones (**5c′**), and a minor peak at m/z = 1826.6 Da corresponding to **6c** still with one OMB group (**5c"**) (Table 1). Longer treatment did not eliminate the OMB groups but this occurred slowly after the reaction was quenched with triethylammonium acetate (TEAAc) buffer for 36 h. The MALDI-TOF spectrum showed a major peak at m/z = 1693.1 Da corresponding to the expected deprotected tetramer **6c** with a small signal at m/z = 1826.6 Da corresponding to **6c** with one OMB group. Owing to the phosphorus chirality of this phosphotriester oligo, the HPLC profile of the crude product exhibited eight major peaks corresponding to the eight diastereoisomers (2^3) (Figure 5).

TSE Removal Treatment

To evaluate the rate of hydrolysis of trimethylsilylethyl (TSE) groups, we synthesized on a photolabile solid sup-

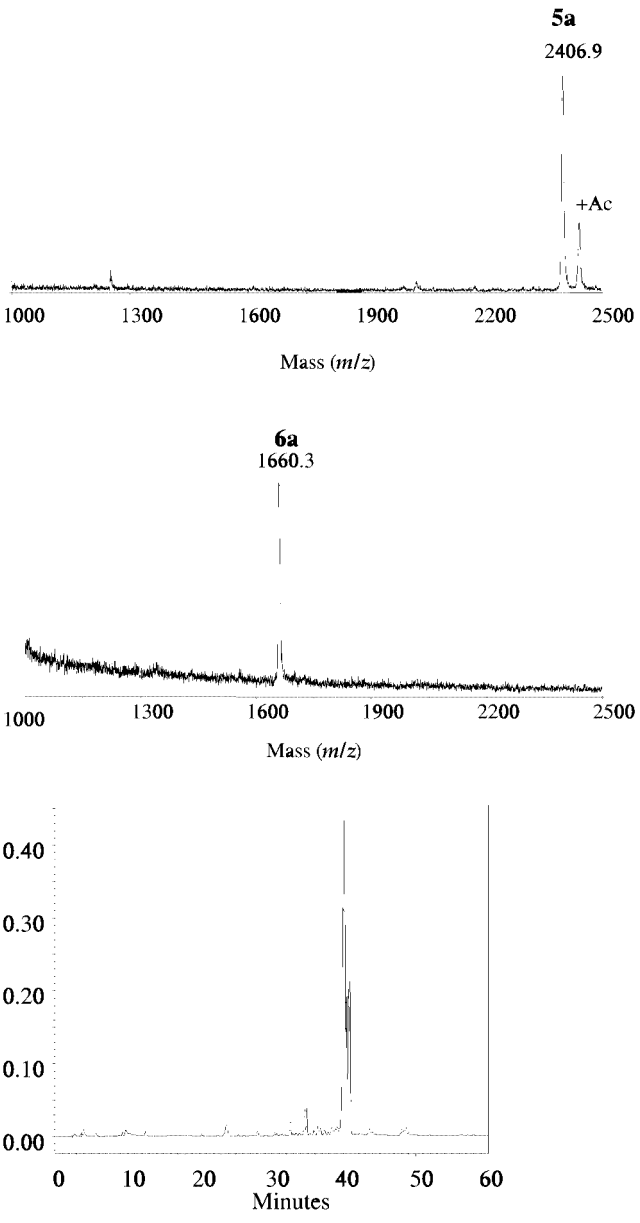


Figure 4. Deprotection of $TA^{SiOMB}A^{SiOMB}T$ Me-SATE **5a** by $Et_3N{\cdot}3HF$ treatment to yield **6a** monitored by MALDI-TOF MS, and (bottom) HPLC profile of the crude **6a**

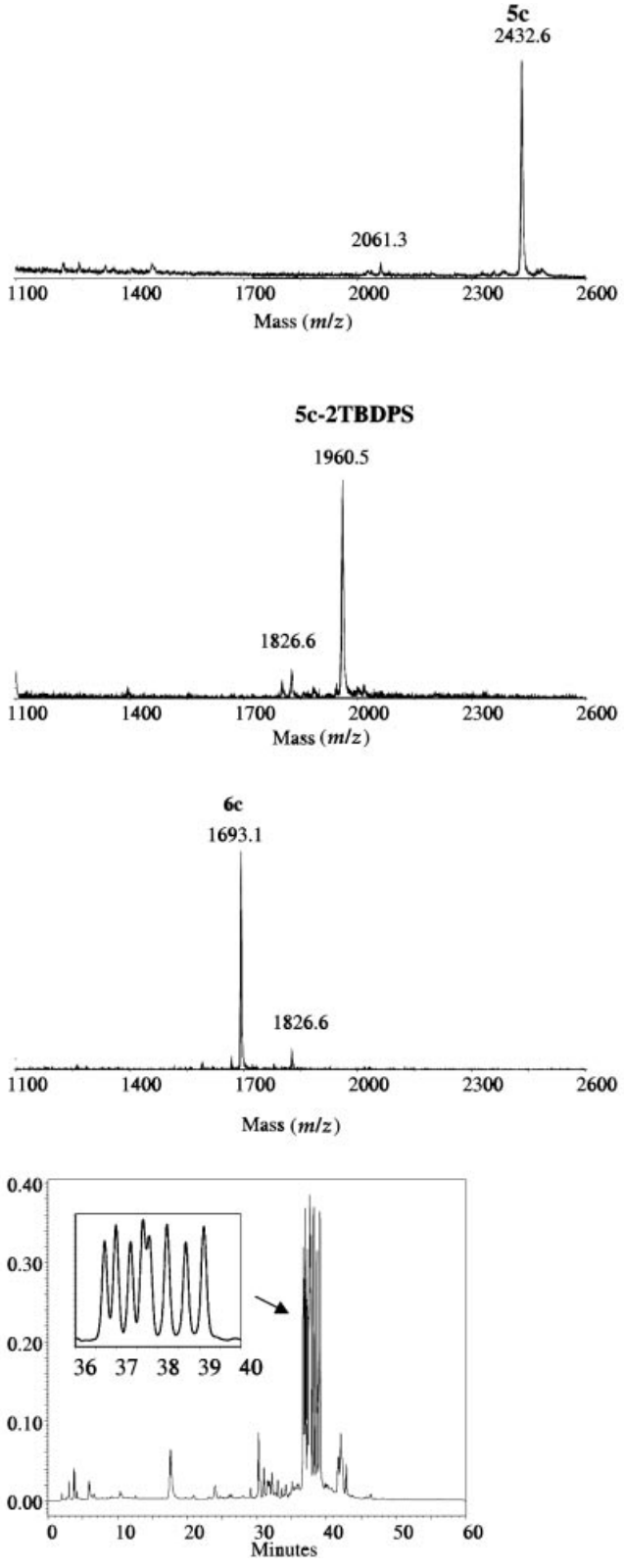


Figure 5. Deprotection of $TG^{SiOMB}G^{SiOMB}T$ Me-SATE **5c** by $Et_3N{\cdot}3HF$ treatment to yield **6c** monitored by MALDI-TOF MS, and (bottom) HPLC profile of the crude **6c**

port a tetramer model **7** (Figure 6, Table 2) bearing two TSE and two Me-SATE groups and exhibiting all the different phosphorus neighboring that we could encounter i.e. TSE and Me-SATE thionotriester linkages and TSE and Me-SATE oxotriester linkages. It was characterized by MALDI-TOF MS directly on the solid support (Figure 7, A) since the UV irradiation of the laser is able to cleave the photolabile linker.[33] We observed two peaks at m/z = 1669.1 and 1568.9 Da, the first corresponding to the expected fully protected tetramer **7** and the second to **7** minus one TSE group. In fact, we had previously observed that the UV irradiation of the laser was able to remove TBDMS groups from alcohols. Furthermore, some other silyl protecting groups are known to be cleaved by photolysis.[34,35] Thus it is likely that some TSE groups could also be cleaved by irradiation. This ability of photolysis to remove TSE groups was confirmed after the photolytic release of the prooligo from the solid support. In this case the MALDI-TOF spectra showed only two peaks, corresponding to the fully TSE deprotected oligo **8** and that bearing only one TSE group (**7**-TSE) (data not shown). This tetramer was then treated with $Et_3N{\cdot}3HF$ for 1 h to give the free prooligo **8** as characterized by MS and HPLC (Figure 7, B and C). It is noteworthy that the same prooligo bearing (diphenyl)-(methyl)silylethyl (DPSE) groups instead of TSE ones was not fully deprotected by the $Et_3N{\cdot}3HF$ treatment within 24 h (data not shown).

Having found a common treatment to remove SiOMB and TSE groups on a Me-SATE prooligo without affecting the Me-SATE groups, we then decided to look for a solid support that could be cleaved by the same treatment. In fact, the photolysis is known to be less efficient as the length of the prooligo increases. Hence, photolysis should be performed at very low concentration and requires long irradiation times that could yield side products.

Phosphoramidate Linkage on Solid Support

Different linkers between the oligos and the solid support could be cleaved by fluoride treatment,[36−38] but it requires the preparation of four solid supports, one for each nucleoside. Hence the use of a universal solid support would be more convenient since only one solid support has to be synthesized. During our researches, we observed that triester phosphoramidate linkages were also cleaved by treatment with $Et_3N{\cdot}3HF$. The literature states that it is the diester phosphoramidate linkage and not the triester phosphoramidate linkage that is cleaved by treatment with acetic acid after standard ammonia treatment.[39] In our case, the cleavage occurred with a triester phosphoramidate linkage and did not need an ammonia treatment. We noticed that when synthesis was done directly on the LCAA−CPG only limited coupling occurred at first. To avoid steric hindrance we introduced an amino linker. The standard 5′-amino-modifier C6 cyanoethyl phosphoramidite was coupled directly on free LCAA−CPG using only six equivalents of phosphoramidite for 180 s, then oxidizing for 70 s with regular iodine water solution, and finally capping the unchanged amino functions using standard acetic anhydride. Loading was 48 μmol/g.

Table 1. MALDI-TOF *m*/*z* (negative mode) of prooligos **5a** and **6a** and their derivatives formed under $Et_3N{\cdot}3HF$ treatment

Prooligos	Elemental composition, (negative mode)	*m*/*z*, calcd.	*m*/*z*, found
$TA^{SiOMB}A^{SiOMB}T$ Me-SATE (**5a**)	$C_{104}H_{124}N_{14}O_{33}P_4S_4Si_2$	2405.6	2406.9
TAAT Me-SATE (**6a**)	$C_{56}H_{76}N_{14}O_{29}P_4S_4$	1660.5	1660.3
$TG^{SiOMB}G^{SiOMB}T$ Me-SATE (**5c**)	$C_{104}H_{124}N_{14}O_{35}P_4S_4Si_2$	2437.5	2432.6
$TG^{OMB}G^{OMB}T$ Me-SATE (**5c′**)	$C_{72}H_{88}N_{14}O_{35}P_4S_4$	1960.7	1960.5
$TG^{OMB}GT$ Me-SATE (**5"**)	$C_{64}H_{81}N_{14}O_{33}P_4S_4$	1826.6	1826.6
TGGT Me-SATE (**6c**)	$C_{56}H_{76}N_{14}O_{31}P_4S_4$	1692.5	1693.1

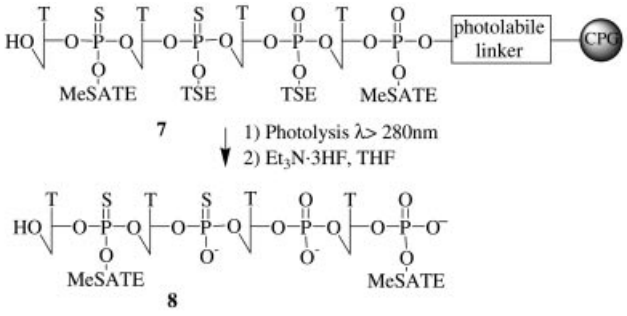


Figure 6. Schematic structure of prooligo **7,** and after photolysis and deprotection by $Et_3N{\cdot}3HF$ to yield **8**

Table 2. MALDI-TOF *m*/*z* (negative mode) of prooligo **7** and its derivatives under $Et_3N{\cdot}3HF$ treatment

Prooligos	Elemental composition (negative mode)	*m*/*z*, calcd.	*m*/*z*, found
7	$C_{58}H_{89}N_8O_{29}P_4S_4Si_2$	1670.7	1669.1
7 − TSE	$C_{53}H_{77}N_8O_{29}P_4S_4Si$	1570.5	1568.9
8	$C_{48}H_{65}N_8O_{29}P_4S_4$	1470.3	1468.9

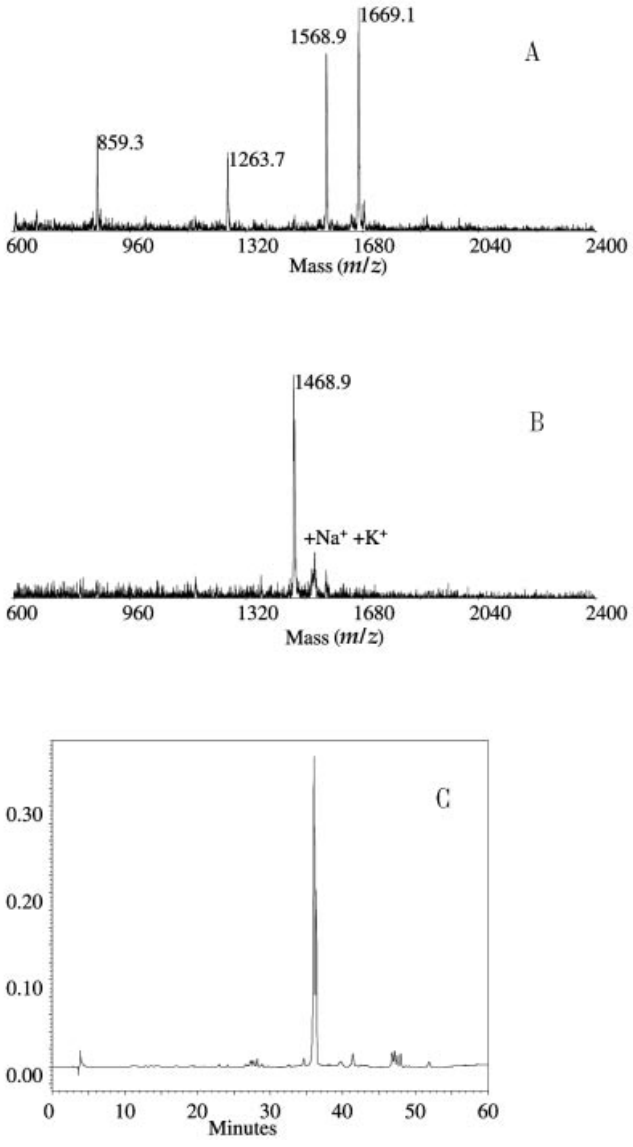


Figure 7. MALDI-TOF analysis **A**) of **7** on solid support, **B**) after photolysis of **7** and its deprotection 1 h with $Et_3N{\cdot}3HF$ and **C**) HPLC profile of the crude **8**

Probably the linker allowed a lower steric hindrance, allowing better coupling for the first nucleosides. Furthermore, using this amino solid support, it was not necessary to synthesize the four solid supports loaded with each *N*-SiOMB nucleoside.

Synthesis of Heteropolymer SATE Prooligo

A SATE prooligo **9** bearing the four usual nucleobases was synthesized as a model on a 1-μmol scale (Figure 8) on the previously prepared Mmtr-amino-C6 solid support. An extended trichloroacetic acid treatment of 8 min was required to remove the monomethoxytrityl group on the amino function. The *N*-SiOMB nucleoside SATE or TSE phosphoramidite solutions were used at 0.09 M concentration in anhydrous acetonitrile. The synthesis was performed using regular tetrazole as activator and *tert*-butyl hydroperoxide[40] as oxidizer[2] instead of standard iodine water. The first coupling was extended to 360 s and then reduced to 180 s for the next couplings. The first capping step was extended to 300 s to cap the unchanged amino functions borne by the solid support and then reduced to 15 s. The average yield per cycle calculated from the trityl assay was 95%. After the synthesis the resin was treated with $Et_3N{\cdot}3HF$ (300 equivalents in THF) for 4 h then

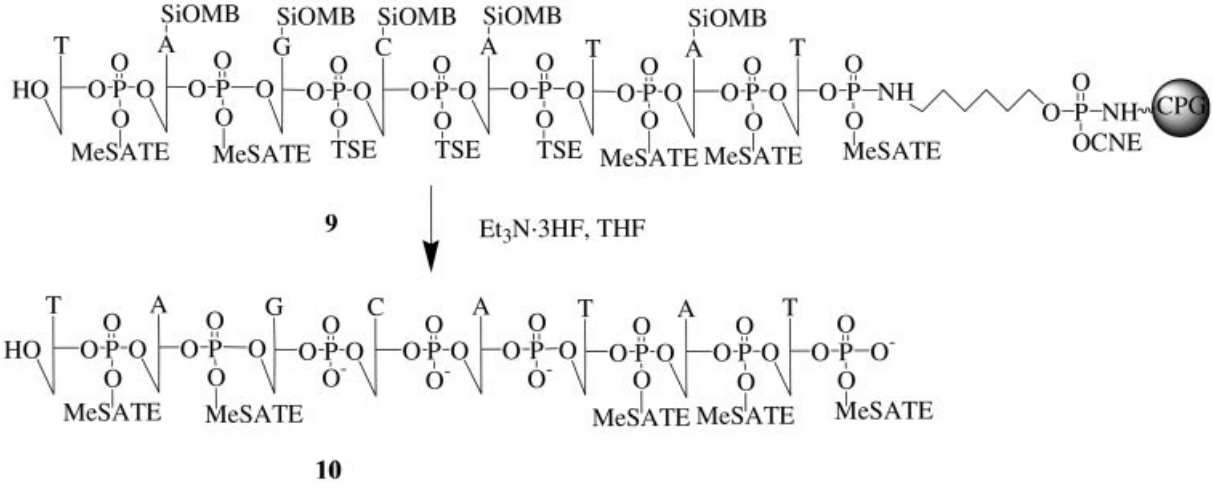


Figure 8. Schematic structure of the hetero prooligo **9** on aminolinker solid support and its deprotection by a single treatment with 3HF·Et_3N 4 h, then 50 mM TEAAc 36 h to yield the fully deprotected Me-SATE prooligo **10**

50 mM TEAAc buffer was added for 36 h to release the OMB group on G. After evaporation the resulting fully deprotected SATE prooligo was purified by HPLC (16 OD^{260nm} of pure material) and characterized by MALDI-TOF MS (*m*/*z* calcd. 2998.4; found 2998.4) (Figure 9). The HPLC profile exhibited a broad peak due to the presence of diastereoisomers (2^4). In the mass spectrometry we also observed a peak corresponding to the prooligo having lost the 3′- SATE phosphate (*m*/*z* calcd. 2816.3; found 2816.5).

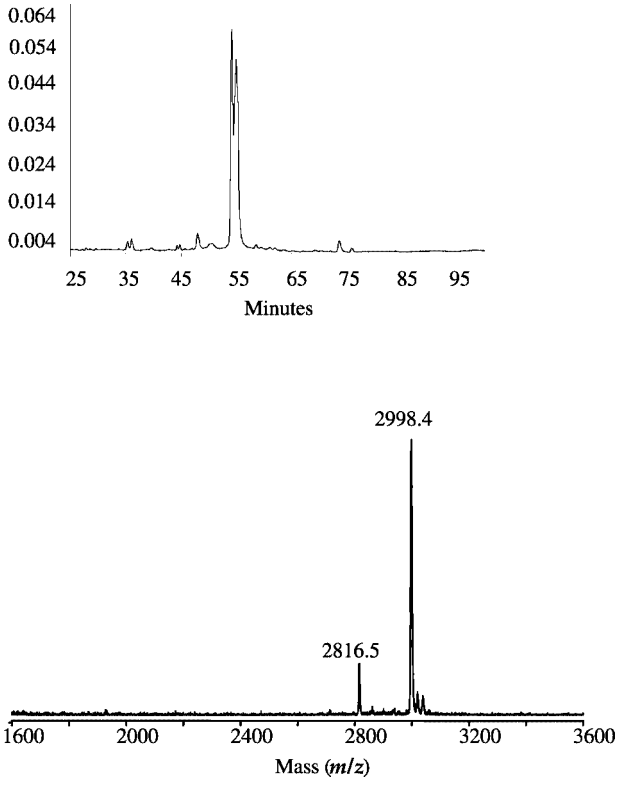


Figure 9. HPLC profile of the purified prooligo **10** (top) and its MALDI-TOF analysis (bottom)

Conclusion

A prooligo exhibiting the four usual nucleobases, Me-SATE phosphotriester and phosphodiester linkages was synthesized thanks to the use of SiOMB protecting groups on the nucleobases and TSE protecting groups on certain phosphates. Both these protecting groups were cleaved by means of a Et_3N·3HF solution in THF. Hence using Et_3N·3HF as a single treatment we were able to cleave the prooligo from the solid support and remove the SiOMB and the TSE groups without any hydrolysis of Me-SATE groups. This combination of fluoride-labile protecting groups is well adapted for the synthesis of Me-SATE prooligos but could also be applied to any other base-sensitive oligonucleotides such as nucleopeptides, RNAs or oligos containing special base-sensitive nucleobases.

Experimental Section

General Remarks: All commercial chemicals were reagent grade and were used without further purification. DNA synthesis reagents, except for the oxidizer, were from Applied Biosystems Inc. (Voisins le Bretonneux, France). Anhydrous *tert*-butyl hydroperoxide (5.5 M in decane) was obtained from Fluka and was diluted with anhydrous dichloromethane (Aldrich). 5′-*O*-(4,4′-Dimethoxytrityl)thymidine 3′-*O*-[2-(acetylthio)ethyl] diisopropylphosphoramidite was prepared as described previously.[2]

MALDI-TOF MS Analysis: MALDI-TOF mass spectra were recorded with a Voyager DE mass spectrometer (PerSeptive Biosystems) equipped with an N_2 laser (337 nm). MALDI conditions were: accelerating voltage 24000 V; guide wire, 0.05% of accelerating voltage; grid voltage, 94% of the accelerating voltage; delay extraction time, 550 ns. For direct analysis of the CPG-supported

prooligonucleotides, the solid materials (0.2 mg) were suspended in 5−10 μL of a saturated solution of the matrix 2,4,6-(trihydroxy)-acetophenone (THAP, 45 mg, ammonium citrate, 4 mg) in 500 μL of acetonitrile/water (1:1, v/v). The mixture (1 μL) was spotted onto the stainless steel probe plate and allowed to air-dry before analysis. For analysis of prooligonucleotides released from the solid support, each of the samples [1 μL, 0.1 OD^{260nm} in 100 μL water/acetonitrile (1:1, v/v)] was exchanged on DOWEX 50 W X8 resin (ammonium form) prior to the addition of the THAP matrix solution (5−10 μL). The resulting solution (1 μL) was then spotted onto the stainless steel probe plate and allowed to air-dry before analysis.

General Procedure for the Me-SATE Phosphitylation of *N*-SiOMB-5′-*O*-(4,4′-dimethoxytrityl)-2′-deoxynucleosides (2a−c): Prior to use, the *N*-SiOMB-5′-*O*-Dmtr-2′-deoxynucleosides **1a−c** (1 mmol) and diisopropylammonium tetrazolide (85.5 mg, 0.5 mmol) were separately dried three times by coevaporation with anhydrous acetonitrile, and then mixed and dissolved in anhydrous dichloromethane (7 mL). A solution of [2-(acetylthio)ethyloxy]bis(diisopropylamino)phosphane (421 mg, 1.2 mmol) in dichloromethane (3 mL) was added under argon. The resulting mixture was stirred at room temperature overnight, then diluted with ethyl acetate (50 mL) and washed with saturated aqueous $NaHCO_3$ (25 mL) and then with brine (50 mL). The organic layer was dried (Na_2SO_4), filtered, and the solvents were 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, concentrated to dryness, dissolved in a few mL of toluene, and then precipitated in cold hexane (−78 °C). The resulting precipitate was dried under vacuum to afford the resulting phosphoramidites **2a** (70%), **2b** (82%), or **2c** (84%), as colorless powders.

***N*⁶-{2-[(*tert*-Butyl)(diphenyl)silyloxymethyl]benzoyl}-5′-*O*-(4,4′-dimethoxytrityl)-2′-deoxyadenosine 3′-*O*-[2-(acetylthio)ethyl] Diisopropylphosphoramidite (2a):** 0.77 mmol, 710 mg of **1a** gave 631 mg, 70% yield, TLC (Cyclohexane/EtOAc/Et_3N, 20:70:10, v/v/v), R_f = 0.55. ^1H NMR ($CDCl_3$): δ = 8.37 (s, 1 H, 8-H), 8.09 (s, 1 H, 2-H), 7.63−7.01 (m, 23 H, H aromatic), 6.68 (d, 4 H, H_α of OCH_3), 6.32 (q, 1 H, 1′-H), 4.92 (s, 2 H, CH_2OSi), 4.81−4.68 (m, 1 H, 3′-H), 4.17−4.09 (m, 1 H, 4′-H), 3.62 (s, 6 H, 2 × OCH_3), 3.30−3.15 (m, 4 H, 2 × CH_2), 3.01−2.86 (m, 4 H, 5′-H 5′′-H 2 × CH), 2.58−2.41 (m, 1 H, 2′-H), 2.17 (s, 3 H, $SCOCH_3$), 2.13−2.01 (m, 1 H, 2′′-H), 1.04 [s, 12 H, $N[CH(CH_3)_2]_2$), 0.92 [s, 9 H, $C(CH_3)_3$] ppm. ^{31}P NMR (CD_3CN): δ = 148.9 and 148.7 ppm.

***N*⁴-{2-[(*tert*-Butyl)(diphenyl)silyloxymethyl]benzoyl}-5′-*O*-(4,4′-dimethoxytrityl)-2′-deoxycytidine 3′-*O*-[2-(acetylthio)ethyl] Diisopropylphosphoramidite (2b):** 3.0 mmol, 2.8 g of **1b** gave 2.8 g, 82% yield, TLC (Cyclohexane/EtOAc/Et_3N, 40:60:10, v/v/v): R_f = 0.41 and 0.45. ^1H NMR (CD_3CN): δ = 9.15 (br. s, 1 H, NH), 8.16 (dd, 1 H, 6-H), 6.99−7.61 (m, 24 H, H aromatic), 6.78 (dd, 4 H, H aromatic *meta* of OCH_3), 6.04 (q, 1 H, 1′-H), 4.89 (s, 2 H, CH_2Si), 4.51 (d, 1 H, 3′-H), 4.02 (q, 1 H, 4′-H), 3.65 (s, 6 H, 2 × OCH_3), 3.51 (m, 3 H, CH_2 +2 × CH), 3.32 (m, 2 H, CH_2), 3.01 (m, 1 H, 5′′-H), 2.89 (m, 1 H, 5′-H), 2.50 (m, 1 H, 2′-H), 2.22 (m, 1 H, 2′′-H), 2.13 (s, 3 H, $SCOCH_3$), 1.09 [d, 12 H, $N\{CH(CH_3)_2\}_2$), 0.90 [s, 9 H, $C(CH_3)_3$] ppm. ^{31}P NMR (CD_3CN): δ = 149.16 and 148.90 ppm. +FAB (thioglycerol): *m*/*z* = 1151 $[M + H]^+$.

***N*²-{2-[(*tert*-Butyl)(diphenyl)silyloxymethyl]benzoyl}-5′-*O*-(4,4′-dimethoxytrityl)-2′-deoxyguanosine 3′-*O*-[2-(acetylthio)ethyl] Diisopropylphosphoramidite (2c): 1c** (365.mg, 0.39 mmol) gave 389 mg, 84%, TLC (EtOAc/CH_3CN/Et_3N, 60:30:10, v/v/v). R_f = 0.46. ^1H NMR ($CDCl_3$): δ = 7.89 (s, 1 H, 8-H), 7.75−7.12 (m, 23 H, H aromatic), 6.76 (d, 4 H, H_α of OCH_3), 6.17 (t, 1 H, 1′-H), 4.99 (s, 2 H, CH_2OSi), 4.59 (m, 1 H, 3′-H), 4.11 (d, 1 H, 4′-H), 3.70 (s, 6 H, 2 × OCH_3), 3.55 (m, 4 H, 2 × CH_2), 3.34 (m, 1 H, CH), 3.23 (m, 1 H, CH), 3.02 (m, 1 H, 5-H′), 2.92 (m, 1 H, 5′′-H), 2.81 (m, 1 H, 2′-H), 2.41 (m, 1 H, 2′′-H), 2.19 (s, 3 H, $SCOCH_3$), 1.12 [s, 12 H, $N\{CH(CH_3)_2\}_2$), 0.92 [s, 9 H, $C(CH_3)_3$] ppm. ^{31}P NMR (CD_3CN): δ = 148.91 and 148.86 ppm.

General Procedure for the Trimethylsilylethyl Phosphitylation of *N*-SiOMB-5′-*O* (4,4′-Dimethoxytrityl)-2′-deoxynucleosides (3a−d): A solution of bis(*N*,*N*-diisopropylamino)chlorophosphane (320 mg, 1.2 mmol) in dry CH_2Cl_2 (1.2 mL) was added dropwise over 3 min to a cooled (−20 °C) and magnetically stirred solution of *N*-SiOMB-5′-*O*-Dmtr-2′-deoxynucleoside **1a−d** (1.0 mmol) and (diisopropyl)ethylamine (0.28 mL, 1.3 mmol) in dry CH_2Cl_2 (6.5 mL). The reaction mixture was warmed to room temperature while stirring was maintained (2 h). The 2-trimethylsilyl alcohol (0.17 mL, 1.16 mmol) was added followed by diisopropylammonium tetrazolide (86 mg, 0.5 mmol), and the reaction mixture was stirred overnight. Then CH_2Cl_2 (15 mL) was added and the reaction mixture was washed with saturated aqueous sodium hydrogen carbonate (20 mL) and brine (2 × 20 mL) and dried with anhydrous sodium sulfate. The residue obtained after evaporation of the organic layer under reduced pressure was purified on a silica-gel column prepared with 1% triethylamine in cyclohexane and washed with cyclohexane (1-bed volume) before the products were deposited. Elution was performed with a stepwise gradient of ethyl acetate (0−50%) in cyclohexane, fractions containing the desired product were combined and the solvents evaporated to dryness. The residue was lyophilized from dioxane affording a colorless powder: **3a** (59%), **3b** (38%), **3c** (57%) and **3d** (70%). ^{31}P NMR showed also the presence of few percent of hydrolyzed chlorophosphane (≈ 7 ppm) and phosphite triester (≈140 ppm). As these side compounds will not react, the mixture was used without a second purification. Owing to their high molecular weight FAB MS showed only characteristic fragments of the molecules.

***N*⁶-{2-[(*tert*-Butyl)(diphenyl)silyloxymethyl]benzoyl}-5′-*O*-(4,4′-dimethoxytrityl)-2′-deoxyadenosine 3′-*O*-[(2-trimethylsilyl) ethyl] Diisopropylphosphoramidite (3a): 1a** (552 mg, 0.60 mmol) gave **3a** in 59% yield (413 mg). TLC (EtOAc/cyclohexane/Et_3N, 60:30:10, v/v/v). R_f = 0.57. ^{31}P NMR (CD_3CN): δ = 147.13 and 146.89 ppm, plus 1.3% of hydrolyzed chlorophosphane (δ = 7.1 ppm).

***N*⁴-{2-[(*tert*-Butyl)(diphenyl)silyloxymethyl]benzoyl}-5′-*O*-(4,4′-dimethoxytrityl)-2′-deoxycytidine 3′-*O*-[(2-trimethylsilyl) ethyl] Diisopropylphosphoramidite (3b): 1b** (724 mg, 0.80 mmol) gave **3b** in 38% yield (351 mg). TLC (EtOAc/cyclohexane/Et_3N, 50:40:10, v/v/v). R_f = 0.62. ^{31}P NMR (CD_3CN): δ = 147.31 and 147.14 ppm plus 7% of hydrolyzed chlorophosphane (δ = 7.2 ppm) and 10% phosphite triester (δ = 140.5 ppm).

***N*²-{2-[(*tert*-Butyl)(diphenyl)silyloxymethyl]benzoyl}-5′-*O*-(4,4′-dimethoxytrityl)-2′-deoxyguanosine 3′-*O*-[(2-trimethylsilyl) ethyl] Diisopropylphosphoramidite (3c): 1c** (0.94 mmol, 889 mg) gave **3c** in 57% yield (678 mg). TLC (CH_2Cl_2/EtOAc/Et_3N, 50:40:10, v/v/v). R_f = 0.41. ^{31}P NMR (CD_3CN): δ = 147.17 and 147.09 ppm, plus 5% of hydrolyzed chlorophosphane (δ = 7.0 ppm) and 18% of phosphite triester (δ = 140.0 ppm).

5′-*O*-(4,4′-Dimethoxytrityl)thymidine 3′-*O*-[2-(Trimethylsilyl)ethyl] Diisopropylphosphoramidite (3d): 1d (3.3 mmol, 1.14 g) gave **3d** in 70% yield (1.81 g). TLC (EtOAc/cyclohexane/Et_3N, 50:40:10, v/v/v). R_f = 0.48. ^{31}P NMR (CD_3CN): δ = 147.0 and 146.7 ppm, plus 7% of hydrolyzed chlorophosphane (δ = 7.7 ppm) and 9% of phosphite triester (δ = 140.1 ppm).

Solid Phase Elongation of Prooligos on a Photolabile Solid Support: The prooligonucleotides were synthesized on an ABI 381A DNA synthesizer on a 1-μmol scale, by a cycle involving phosphoramidite chemistry. An 18-fold molar excess of the nucleoside phosphoramidites **2a**−**d** and **3a**−**d** (0.09 M in CH_3CN) in the presence of tetrazole (0.45 M in CH_3CN) was introduced onto the column containing the photolabile CPG solid support (coupling time of 60 s). The intermediate phosphite triesters were oxidized by treatment (60 s) with a 1.1 M solution of *tert*-butyl hydroperoxide [40] in toluene/dichloromethane to afford phosphotriester internucleosidic linkages or with a 0.05 M solution of 3*H* −1,2-benzodithiole-3-one 1,1-dioxide [41] in dry acetonitrile to afford thionophosphorotriester linkages. The coupling efficiency was evaluated by spectrophotometric measurement (λ = 498 nm) of the release of the 4,4′-dimethoxytrityl carbocation at the end of each incorporation cycle. Yields for couplings were between 95% and 98%.

Deprotection and Release of the Prooligonucleotides from the Photolabile Solid Support: At the end of the elongation process, the columns were disassembled. The CPG-supported prooligonucleotides were suspended in a 1-cm path length quartz cell in acetonitrile/water (1:1, v/v, 1 mL) and the magnetically stirred suspensions were exposed to the light of a high-pressure Hg lamp (HPK, 125, Philips), filtered with a Pyrex glass (thickness 2 mm), for 25 min at 20 °C. The glass beads were filtered off with a 0.45-μm Millex filter and washed with the same solvent mixture (2 × 0.5 mL). The prooligonucleotides were purified by reverse-phase HPLC on a Waters-Millipore instrument equipped with a Model 600 E solvent delivery system, a Model U6 K injector, and an ND Model 486 absorbance detector. A reverse-phase C18 (5 μm) Nucleosil column (150 × 4.6 mm, Macherey−Nagel) was used with linear gradients of acetonitrile (0−90%) in 0.05 M aqueous triethylammonium acetate (pH = 7). The appropriate fractions were concentrated and redissolved in water/dioxane (1:1, v/v), and the solvents were removed. This last operation was repeated twice. The residues were then redissolved in water/dioxane and lyophilized to afford colorless powders.

Solid Phase Elongation of Prooligos on C6-Aminolink-LCAA CPG: Commercial LCAA-CPG 500 Å (300 mg, ≈ 27 μmol) was poured into a 10-μmol column and coupled on a 381A DNA synthesizer with a 0.17 M solution of C6 Mmtr-amino link phosphoramidite (6 equiv.) in dry acetonitrile using tetrazole as activator for 180 s, then oxidation was performed for 70 s and finally unchanged amino functions were capped for 180 s with acetic anhydride. Loading 48 μmol/g, was calculated from the release of monomethoxytrityl cation after treatment with a 3% TCA solution in CH_2Cl_2.

First detritylation of Mmtr was extended to 8 min, then first coupling was extended to 360 s then 180 s and first capping was extended to 300 s then 15 s. The prooligo was synthesized as described above.

Deprotection and Release of the Prooligos from the Phosphoramidate Solid Support: The supported fully protected prooligo was treated with commercial $Et_3N{\cdot}3HF$ (200 μL) and dry THF (200 μL) for 4 h. The solution was quenched by addition of TEAAc (500 μL of 50 mM solution) pH 7 for 36 h. The solution was evaporated and the prooligo was purified by HPLC on a C_{18} reverse-phase column.

Acknowledgments

The authors thank Dr J.-J. Vasseur for helpful discussions. This work was supported by grants from the Association pour la Recherche sur le Cancer (ARC). TG thanks the "Société de Secours des Amis des Sciences" for the award of a research studentship.

[1] F. Morvan, J.-J. Vasseur, E. Vivès, B. Rayner, J.-L. Imbach, in *Pharmaceutical aspects of oligonucleotides*, vol. 3 (Eds.: P. Couvreur, C. Malvy), Taylor & Francis, London, **2000**, pp. 79−97.
[2] G. Tosquellas, K. Alvarez, C. Dell′Aquila, F. Morvan, J. J. Vasseur, J. L. Imbach, B. Rayner, *Nucleic Acids Res.* **1998**, *26*, 2069−2074.
[3] G. Tosquellas, A. Bryksin, K. Alvarez, F. Morvan, J. J. Vasseur, B. Rayner, E. Rykova, P. Laktionov, V. Vlassov, J. L. Imbach, *Nucleosides Nucleotides Nucleic Acids* **2000**, *19*, 995−1003.
[4] F. Morvan, E. Vivès, J. C. Bologna, C. Dell′Aquila, T. Lioux, J. J. Vasseur, I. Lefebvre, B. Rayner, J. L. Imbach, *Collect. Symp. Ser.* **1999**, *2*, 135−140.
[5] E. Vivès, C. Dell′Aquila, J. C. Bologna, F. Morvan, B. Rayner, J. L. Imbach, *Nucleic Acids Res.* **1999**, *27*, 4071−4076.
[6] J. C. Bologna, E. Vives, J. L. Imbach, F. Morvan, *Antisense Nucleic Acid Drug Dev* **2002**, *12*, 33−41.
[7] C. Perigaud, G. Gosselin, I. Lefebvre, J. L. Girardet, S. Benzaria, I. Barber, J. L. Imbach, *Bioorg. Med. Chem. Lett.* **1993**, *3*, 2521−2526.
[8] C. Périgaud, G. Gosselin, J.-L. Imbach, in *Current Topics in Medicinal Chemistry: Research Trends*, vol. 2, **1997**, pp. 15−29.
[9] T. Lioux, I. Lefebvre, J. J. Vasseur, J. L. Imbach, *Rapid Commun. Mass Spectrom.* **1999**, *13*, 1645−1649.
[10] J. C. Brès, F. Morvan, I. Lefebvre, J. J. Vasseur, A. Pompon, J. L. Imbach, *J. Chromatogr. B* **2001**, *753*, 123−130.
[11] W. T. Wiesler, W. S. Marshall, M. H. Caruthers, in *Protocols for Oligonucleotides and Analogs*, (Ed.: S. Agrawal), Humana Press Inc, Totowa, NJ, **1993**, pp. 191−206.
[12] C. Dell′Aquila, J. L. Imbach, B. Rayner, *Tetrahedron Lett.* **1997**, *38*, 5289−5292.
[13] M. Sekine, K. Seio, *J. Chem. Soc., Perkin Trans. 1* **1993**, 3087−3093.
[14] J. Heikkilä, N. Balgobin, J. Chattopadhyaya, *Acta Chem. Scand., Ser. B* **1983**, *37*, 857−864.
[15] K. Alvarez, I. Tworkowski, J. J. Vasseur, J. L. Imbach, B. Rayner, *Nucleosides, Nucleotides* **1998**, *17*, 365−378.
[16] V. N. R. Pillai, *Synthesis* **1980**, 1−26.
[17] A. Hasan, K.-P. Stengele, H. Giegrich, P. Cornwell, K. R. Isham, R. A. Sachleben, W. Pfleiderer, R. S. Foote, *Tetrahedron* **1997**, *53*, 4247−4264.
[18] K. Alvarez, J. J. Vasseur, T. Beltran, J. L. Imbach, *J. Org. Chem.* **1999**, *64*, 6319−6328.
[19] N. Spinelli, A. Meyer, Y. Hayakawa, J.-L. Imbach, J.-J. Vasseur, *Eur. J. Org. Chem.* **2002**, 49−56.
[20] Y. Hayakawa, S. Wakabayashi, H. Kato, R. Noyori, *J. Am. Chem. Soc.* **1990**, *112*, 1691−1696.
[21] C. M. Dreef-Tromp, P. Hoogerhout, G. A. van der Marel, J. H. van Boom, *Tetrahedron Lett.* **1990**, *31*, 427−430.
[22] C. M. Dreef-Tromp, E. M. A. van Dam, H. van den Elst, J. E. van den Boogaart, G. A. van der Marel, J. H. van Boom, *Recl. Trav. Chim. Pays-Bas* **1991**, *110*, 378−383.
[23] C. M. Dreef-Tromp, E. M. A. Van Dam, H. Van den Elst, G. A. Van der Marel, J. H. Van Boom, *Nucleic Acids Res.* **1990**, *18*, 6491−6495.
[24] V. T. Ravikumar, H. Sasmor, D. L. Cole, *Bioorg. Med. Chem. Lett.* **1993**, *3*, 2637−2640.
[25] T. Wada, M. Sekine, *Tetrahedron Lett.* **1994**, *35*, 757−760.
[26] A. Sawabe, S. A. Filla, S. Masamune, *Tetrahedron Lett.* **1992**, *33*, 7685−7686.
[27] T. D. Nelson, R. D. Crouch, *Synthesis* **1996**, 1031−1069.
[28] T. Guerlavais-Dagland, A. Meyer, F. Morvan, *J. Chem. Res.* **2002**, 606−607.
[29] C. M. Dreef-Tromp, J. C. M. Van der Maarel, H. Van de Nelst, G. A. Van der Marel, J. H. Van Boom, *Nucleic Acids Res.* **1992**, *20*, 4015−4020.

[30] G. S. Ti, B. L. Gaffney, R. A. Jones, *J. Am. Chem. Soc.* **1982**, *104*, 1316−1319.
[31] P. A. Grieco, C. J. Markworth, *Tetrahedron Lett.* **1999**, *40*, 665−666.
[32] T. Guerlavais, A. Meyer, J.-L. Imbach, F. Morvan, *Bioorg. Med. Chem. Lett.* **2001**, *11*, 2813−2816.
[33] A. Meyer, N. Spinelli, J.-L. Imbach, J.-J. Vasseur, *Rapid Commun. Mass Spectrom.* **2000**, *14*, 234−232.
[34] M. A. Brook, C. Gottardo, S. Balduzzi, M. Mohamed, *Tetrahedron Lett.* **1997**, *38*, 6997−7000.
[35] M. C. Pirrung, Y. R. Lee, *J. Org. Chem.* **1993**, *58*, 6961−6963.
[36] A. Routledge, M. P. Wallis, K. C. Ross, W. Fraser, *Bioorg. Med. Chem. Lett.* **1995**, *5*, 2059−2064.
[37] R. T. Pon, S. Y. Yu, *Nucleic Acids Res.* **1997**, *25*, 3629−3635.
[38] A. Kobori, K. Miyata, M. Ushioda, K. Seio, M. Sekine, *Chem. Lett.* **2002**, 16−17.
[39] S. M. Gryaznov, R. L. Letsinger, *Tetrahedron Lett.* **1992**, *33*, 4127−4128.
[40] Y. Hayakawa, M. Uchiyama, R. Noyori, *Tetrahedron Lett.* **1986**, *27*, 4191−4194.
[41] R. P. Iyer, W. Egan, J. B. Regan, S. L. Beaucage, *J. Am. Chem. Soc.* **1990**, *112*, 1253−1254.

Received February 5, 2003

