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Universal Solid Supports for the Synthesis of Oligonucleotides via a Transesterification of *H*-phosphonate Diester Linkage

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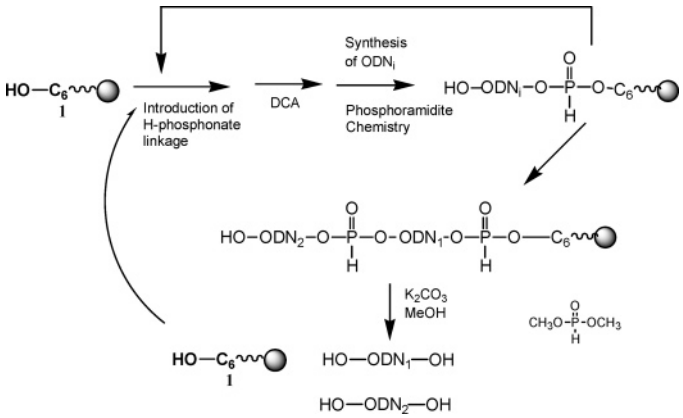
Received June 10, 2005



Three universal solid supports exhibiting an hydroxyl function were prepared. The introduction of a first *H*-phosphonate diester linkage which was kept throughout the elongation allowed the release of 3′-hydroxyl oligonucleotides by a transesterification mechanism. The transesterification was performed in a few minutes with either amino alcohols or K_2CO_3/methanol. Starting from a hydroxyl solid support, tandem oligonucleotides were synthesized and the solid support was easily recyclable. This strategy was extended to the release of an oligonucleotide from the solid support by a nonbasic treatment opening the way to the synthesis of base-sensitive oligonucleotides thanks to the selective deprotection of a hydroxyl in β of the *H*-phosphonate diester linkage.

Introduction

H-Phosphonate diesters are unstable under basic conditions.[1] Furthermore, *H*-phosphonate diesters are subject to intra-transesterification reactions by the vicinal hydroxyl of a ribonucleoside[2] or *cis*-diol[3,4] but also propyl to pentyl diol.[5] The consequence is the removal of the phosphorus group by an intramolecular attack of the free alcohol function on the phosphorus atom with the formation of a cyclic *H*-phosphonate. Moreover, *H*-phosphonate diesters are also subject to transesterification reactions by amino alcohols.[6] In the case of oligonucleotides (oligos), we have employed this behavior to release them from a solid support under different conditions: from basic, mild basic, or neutral according to the design of the solid support.

Herein, we present three universal solid supports, with a hydroxyl function, and among them a reusable one, used in combination with a *H*-phosphonate diester linkage between the oligonucleotide and the solid support. The *H*-phosphonate diester linkage was kept throughout the elongation process. Different conditions were applied to release the oligonucleotide in solution, such as ethanolamine, K_2CO_3/methanol, or $Et_3N{\cdot}3HF$ according to the structure of the solid support.

Two solid supports **1a**,**b** share a structure with a protected diol and triol, and the last (**1c**) is a hexanol

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10.1021/jo051172n CCC: $30.25
Published on Web 10/15/2005

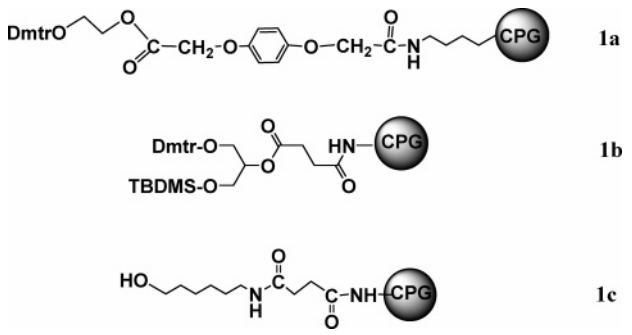
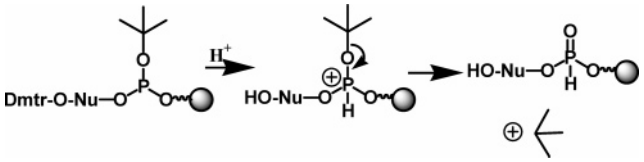


FIGURE 1. Structure of the solid supports, EG-Q **1a**, TBDMS-glycerol **1b**, and hexanol **1c**.

SCHEME 1. Formation of a *H*-Phosphonate Diester Linkage from a *tert*-Butyl Phosphite Triester by an Arbuzov-Type Reaction during the Detritylation Step



solid support (Figure 1). The first solid support **1a** with a Q-linker[7] allows a fast release of oligonucleotides. The second solid support **1b** was designed to release oligonucleotides by a nonbasic $Et_3N{\cdot}3HF$ treatment. The reusable third solid support **1c** was used to synthesize several times the same oligonucleotide or even different oligonucleotides. Furthermore, multiple oligonucleotides were synthesized in a row on the same support.

Results and Discussion

The common principle of the three solid supports is to introduce a *H*-phosphonate diester linkage between the hydroxyl solid support and the oligonucleotide. To do so, two paths are possible. On one hand, classic *H*-phosphonate chemistry with commercial *H*-phosphonate nucleotide and acyl chloride activator could be used. This implies combination of phosphoramidite and H-phosphonate chemistries and specific reagents on the DNA synthesizer (i.e., acyl chloride, pyridine/acetonitrile wash). On the other hand, we applied a "fully phosphoramidite chemistry". A *tert*-butyl phosphoramidite building block was used to generate a *H*-phosphonate diester for the first coupling on the solid suppport. In this case, the coupling step was performed with benzylmercaptotetrazole (BMT) owing to a lower reactivity of *tert*-butyl phosphoramidite building blocks in comparison of the standard cyanoethyl ones. Then the oxidation step was omitted and the *H*-phosphonate diester was formed during the detritylation step by an Arbuzov-type reaction (Scheme 1) as described before with benzyl phosphite triesters.[8] Standard cyanoethyl phosphoramidites were then used for the elongation process of the oligonucleotide.

To avoid oxidation of the *H*-phosphonate linkage during the elongation process, the oxidation step of the further cyanoethyl phosphite triesters was performed with peroxides (*tert*-butyl hydroperoxide[9] or butanone peroxide[10]) or with the Beaucage reagent[11] to end up with phosphodiester or phosphorothioate oligonucleotides, respectively. Indeed, since *H*-phosphonate diester linkages do not have an electron pair on the phosphorus atom, they do not react with these reagents.[12−15]

A first universal solid support (**1a**) with a dimethoxytrityl-*O*-ethylene glycol-*O,O′*-hydroquinone diacetic linker (EG-Q-linker) was synthesized (Scheme 2). Ethylene glycol was monotritylated using dimethoxytrityl chloride in anhydrous pyridine and then converted to the dimethoxytrityl-*O*-ethylene glycol-*O,O′*-hydroquinone *O′*-hemiester [7] using hydroquinone-*O,O′*-diacetic acid and DEC/DMAP. This hemiester was loaded on a long-chain alkylamino (LCAA) CPG using DEC/DMAP. After capping with acetic anhydride in the presence of *N*-methyl imidazole, a loading of 49 μmol per gram was determined by trityl assay.

Applying the cycle described in Table 1 (see the Supporting Information), without the capping step, a T_6 exhibiting a 3′-*H*-phosphonate (HP) diester linkage (**A**) was synthesized on this new solid support (Figure 2). For comparison, a T_6 (**B**) using a standard elongation with cyanoethyl phosphoramidite was also performed on this support (Figure 2), and then several basic treatments were performed.

Treatment of supported oligonucleotide **B**, bearing only CNE phosphotriester linkages, with ammonia at room temperature for 2 h or at 55 °C for 2 h yielded a mixture of T_6-OH and $T_6pOCH_2CH_2OH$ in a 4:6 ratio (characterized by MALDI-TOF MS and quantified by HPLC) showing that the elimination of the 3′-phosphorus group by an intramolecular attack of the vicinal hydroxyl function with the formation of a cyclic phosphate was not efficient owing to the flexibility of the ethyl linker.[16] Indeed, it has been shown that the removal of 3′-phosphate is more efficient when the alcohol is constrained.[17,18]

Supported oligonucleotide **A**, bearing a 3′-HP diester linkage, was treated with concentrated ammonia or with an ethanol/ammonia solution (3:1, v/v) for 2 h at room temperature. HPLC profiles showed the formation of T_6 with a 3′-HP monoester (t_R 15.06 min) in 24% and 14% yield, respectively, and the expected T_6 (t_R 15.28 min) being produced in yields of 76% and 86% (Figure 3a,b). Each compound was isolated and characterized by MALDI-TOF MS. This result could be explained by an attack of

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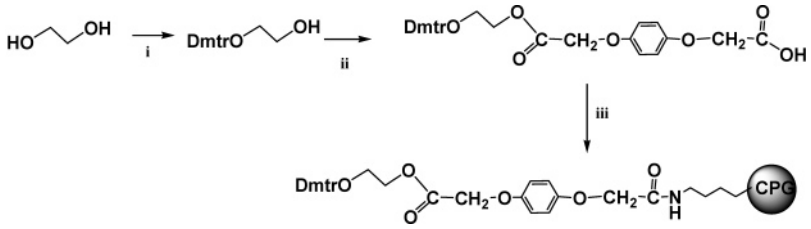
SCHEME 2[a]



[a] Reagents: (i) DMTrCl, anhydrous pyridine; (ii) *O,O′*-hydroquinone diacetic acid, DMAP, DEC, anhydrous pyridine; (iii) activated LCAA-CPG, DMAP, DEC, Et_3N, anhydrous pyridine.

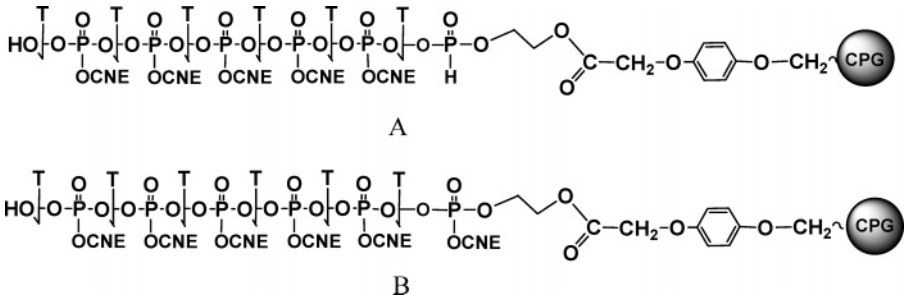


FIGURE 2. Schematic structure of T_6 on the EG-Q-solid support **1a**: (A) with a 3′-*H*-phosphonate diester linkage and (B) with a standard 3′-cyanoethylphosphotriester linkage.

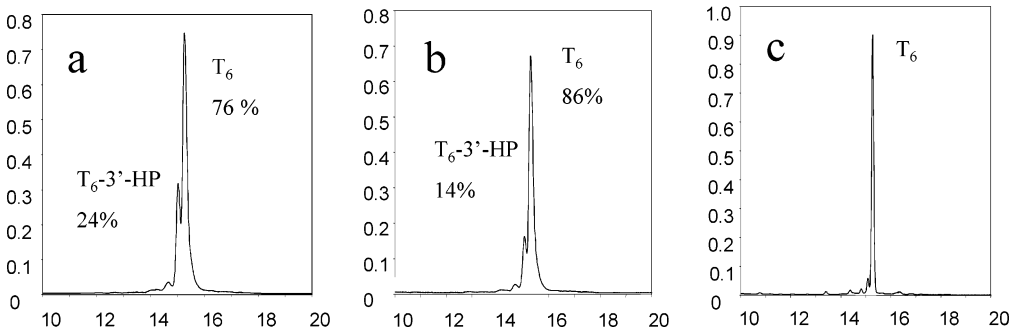
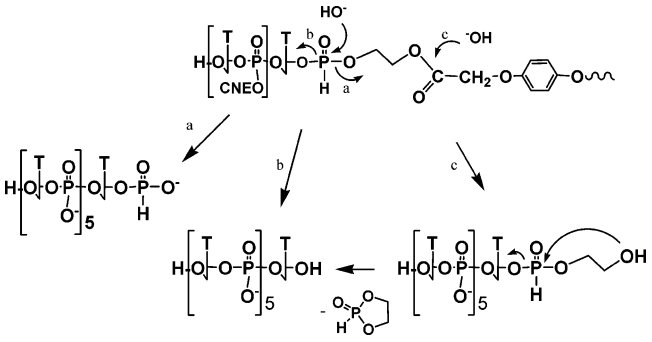


FIGURE 3. HPLC profiles of supported oligonucleotide **A** treated with (a) NH_4OH, (b) NH_4OH/MeOH 1:3, v/v, and (c) K_2CO_3/MeOH.

SCHEME 3. Competitive Formation of T_6 and T_6-3′-HP



the base on the phosphorus atom and the release of T_6-3′-HP monoester or T_6 (Scheme 3: paths a and b). This does not preclude the nucleophilic attack of the base on the Q linker with the formation of the T_6-3′HP-CH_2CH_2OH followed by an intramolecular attack of the hydroxyl function on the phosphorus atom releasing the cyclic *H*-phosphonate and the expected T_6 (Scheme 3, path c). In such a case, reducing the basic conditions will favor the hydrolysis of the Q-linker and will give more T_6.

It is noteworthy that treatment with 50 mM K_2CO_3 in methanol hydrolyzes the Q linker within 2 min.[7] Here, when the supported oligonucleotide **A** was treated with a solution of K_2CO_3 in methanol (10 mM or 5 mM) for 30 min, the T_6 was obtained (Figure 3c) as characterized by MALDI-TOF MS.

Then a pentamer d(TCAGA), bearing the four nucleobases of DNA, was synthesized on this solid support according to the elongation cycle described in Table 1 without the capping step and using the *tert*-butyl phosphoramidite of deoxyadenosine. A few milligrams of support were treated with a 5 mM K_2CO_3/ methanol solution, and the release of the oligonucleotide from the solid support was monitored by UV at 260 nm. A plateau was reached after 2 min indicating that the release from the solid support is fast even with a low concentration of K_2CO_3. MALDI-TOF MS analysis showed that cyanoethyl groups were removed but the benzoyl and isobutyryl nucleobase protections were partially removed, so an ammonia treatment was applied to complete the deprotection (5 h, 55 °C). For the synthesis of these short oligonucleotides no capping step was applied. Since acetic anhydride could give rise to a side reaction with a *H*-phosphonate diester linkage, we used a phosphor-

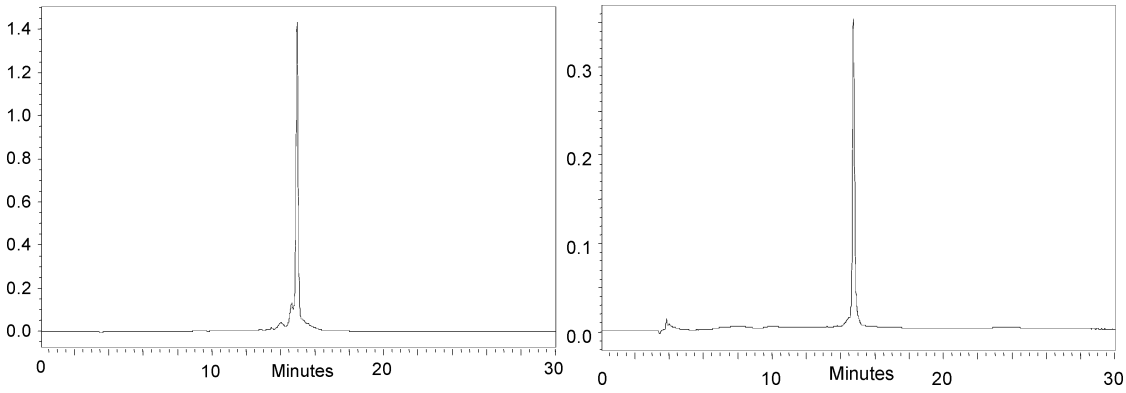
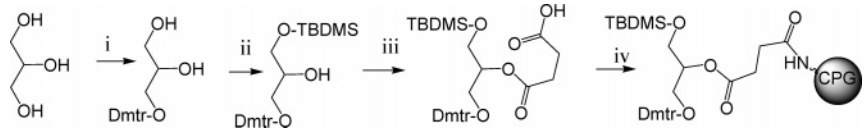


FIGURE 4. HPLC profiles of d(TGACAGTGTCATGTACGA): (left) crude, (right) pure.

SCHEME 4[a]



[a] Reagents: (i) DMTrCl, pyridine; (ii) TBDMSCl, pyridine; (ii) succinic anhydride; (iv) LCAA-CPG, DMAP, EDC, pyridine.

amidite as capping agent in the following syntheses. We chose the dicyanoethyl *N,N*-diisopropyl phosphoramidite since it will yield more hydrophilic shorter mers bearing a 5′-phosphate monoester. Hence, shorter mers will be first eluted on a reversed-phase column and could be eliminated more easily.

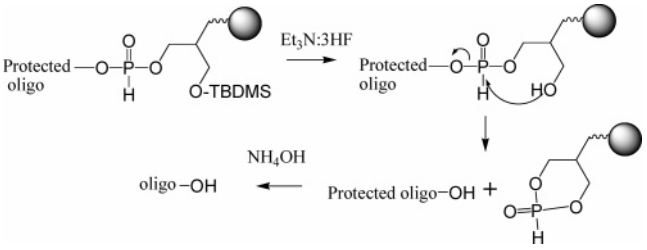
An 18-mer d(TGACAGTGTCATGTACGA) was synthesized by applying the elongation cycle reported in Table 1. After elongation, the solid support was treated with a 5 mM K_2CO_3 methanol solution for 30 min. MS analysis showed the total removal of cyanoethyl groups and partial removal of the benzoyl and isobutyryl groups. Thus, the supernatant was treated with concentrated ammonia for 5 h at 55 °C. After evaporation, the crude (86 OD^{260nm}) was analyzed and purified by HPLC (Figure 4), and characterized by MALDI-TOF MS (*m/z* calcd 5537.70, found 5537.46).

Finally, to obtain easily a fully deprotected oligonucleotide, a decamer d(TCATGTACGT) was synthesized using UltraMild phosphoramidites (i.e., N^4-Ac-dC, N^6-pac-dA, and N^2-isopro-pac-dG).[19,20] After elongation, a single treatment with 50 mM K_2CO_3/methanol for 20 min at 50 °C led to the fully deprotected oligonucleotide (38 OD^{260nm}) as confirmed by MALDI-TOF MS (negative mode *m/z* calcd 3017.04, found 3016.69) with 83% spectroscopic purity at 260 nm (see the Supporting Information).

This universal solid support **1a,** with an EG-Q linker was easily synthesized with a high loading. The 3′-OH oligonucleotides were rapidly released from the solid support by a treatment with 5 mM K_2CO_3 in methanol.

The second solid support **1b** was synthesized from glycerol.[21] The primary alcohols were respectively protected with a dimethoxytrityl and a *tert*-butyldimethylsilyl group, and then the secondary alcohol reacted with succinic anhydride to form the hemiester which was finally loaded on LCAA-CPG using EDC DMAP (loading 59 μmol/g) (Scheme 4).

SCHEME 5. Release of Protected Oligonucleotide from Solid Support 1b and Deprotection



The 12-mer d(AGATTCCGTCAT) was synthesized from the solid support **1b**, according to the elongation cycle described in Table 1 without capping step. The solid supported 12-mer was then treated with a solution of $Et_3N{\cdot}3HF$ for 2.5 h at room temperature. MALDI-TOF MS analysis showed that the fully protected 3′-OH oligonucleotide was obtained (positive mode *m/z* calcd 4969.99, found 4972.45).

The protecting groups (i.e., cyanoethyl, benzoyl, and isobutyryl) were finally removed by an ammonia treatment at 55 °C for 5 h (Scheme 5). The crude was analyzed by HPLC (see the Supporting Information) and characterized by MS (negative mode *m/z* calcd 3619.44, found 3618.86). MS analysis showed that the 12-mer was slightly contaminated with oligonucleotide N-1 (*m/z* calcd 3307.24, found 3305.87).

When the same supported oligonucleotide was treated with HF/pyridine instead of $Et_3N{\cdot}3HF$, both 3′-OH and 3′-HP 12-mers were obtained in a 7:3 ratio. This solid support with a protected β-hydroxyl in combination with the introduction of a first *H*-phosphonate diester linkage is particularly interesting because it opens the way to the synthesis of base-sensitive oligonucleotides[22,23] This

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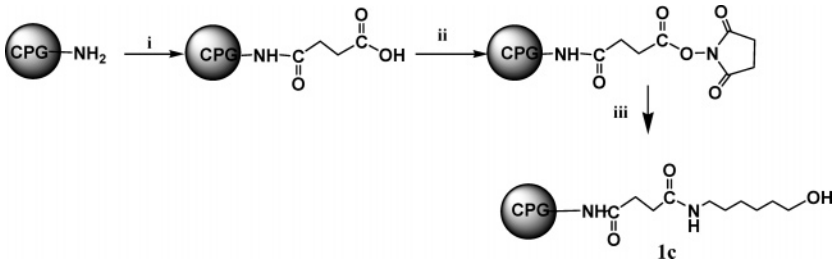
SCHEME 6[a]



[a] Reagents: (i) succinic anhydride, DMAP, pyridine; (ii) EDC, *N*-hydroxysuccinimide, CH_2Cl_2, Et_3N; (iii) 6-amino-1-hexanol, CH_2Cl_2.

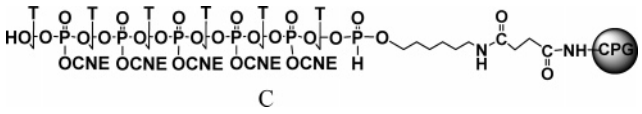


FIGURE 5. Schematic structure of a T_6 on hexanol solid support with a 3′-HP diester linkage.

solid support, using suitable protecting groups on nucleobases,[24,25] was used for the synthesis of prooligonucleotides,[23] and their synthesis will be described elsewhere.

The third solid support **1c** was designed to be recycled by simple washings and dryings before the next synthesis. It consists of an aminohexanol linker anchored on the LCAA-CPG through a succinyl spacer. The resulting amide function is stable under moderate basic conditions. Thus, LCAA-CPG was succinylated, and the hemiester was activated as an *N*-hydoxysuccinimide derivative that finally reacted with 6-amino-1-hexanol to give the desired solid support **1c** (Scheme 6). A loading of 51 μmol/g was determined by trityl assay after a first coupling.

Starting from the solid support **1c**, a T_6 (**C**) was synthesized according to the elongation cycle in Table 1 without the capping step (Figure 5).

Likewise, when oligonucleotide **C** was treated with concentrated ammonia at room temperature or at 55 °C for 2 h, the T_6 and T_6-3′-HP monoester were obtained in 82% and 18% yield, respectively. In contrast, when oligonucleotide **C** was treated for 30 min with a 10% solution of 2-aminoethanol or 3-amino-1-propanol in pyridine the T_6 was quantitatively obtained. The cleavage of the oligonucleotide from the solid support proceeds by a transesterification of the amino alcohols.[6] Likewise, when a treatment with 5 mM K_2CO_3 methanol was applied the T_6 was quantitatively obtained. Since this behavior was not reported in the literature, we further investigated this cleavage with 5 mM K_2CO_3 in methanol and in water. The release of T_6 from the support was monitored by UV at 260 nm. A plateau was reached after 8 and 60 min with the methanol and water solution, respectively. MALDI-TOF MS analysis showed that all cyanoethyl groups were removed in the methanol solution while a few of them remained in the aqueous solution.

A decamer d(TCATGTACGA) phosphodiester (PO) and phosphorothioate (PS) were synthesized on the solid support **1c** starting with the deoxyadenosine *tert*-butyl phosphoramidite according to the elongation cycle with a capping step (Table 1) and using Beaucage's reagent as oxidizer for the latter oligonucleotide. The oligonucleotides were released from the solid support by treatment with 10 mM K_2CO_3 methanol for 30 min, and after evaporation the residues were treated with concentrated ammonia at 55 °C for 5 h to removed the isobutyryl and the remaining benzoyl groups. The oligonucleotides were analyzed by HPLC (Figure 6) and characterized by MALDI-TOF MS (*m/z* for PO oligonucleotide calcd 3026.06, found 3026.91; for PS oligonucleotide calcd 3170.64, found 3170.44).

No difference was found between the phosphotriester and the phosphorothioate oligonucleotide synthesis in term of efficacy to obtain the expected 3′-hydroxyl oligonucleotide.

Recycling. Treatments with an amino alcohol or K_2CO_3/methanol yielded to the transesterification of the *H*-phosphonate diester with the formation of the expected 3′-OH oligonucleotide, the bis alkyl *H*-phosphonate diester and the recovery of the starting hexanol solid support **1c** (Scheme 7). Hence, the solid support could be reused for a new synthesis.

To evaluate the recovery of the solid support, a hexamer d(TGAGAT) was synthesized on the solid support hexanol **1c** according to the elongation cycle reported in Table 1 with a capping step. The supported hexamer was treated with 10% ethanolamine in pyridine for 30 min, and the supernatant after evaporation was treated with ammonia. For recycling, the solid support was washed with pyridine (2 mL), acetonitrile/water (1:1, v/v, 2 mL), and acetonitrile (10 mL) and dried under vacuum. The same hexamer was synthesized on the same solid support three more times (named 1, ×1, ×2, ×3). The amount of each oligonucleotide was 38, 41, 32 and 38 OD^{260nm}, respectively. MALDI-TOF MS visualized only the expected hexamer (*m/z* calcd 1830.28, found 1830.50, 1830.18, 1829.97, 1830.48). HPLC profiles of the crude hexamer were similar for all of them and similar to that of the same oligonucleotide synthesized according to a standard protocol on a commercial succinyl solid support loaded with thymidine (see the Supporting Information).

During the release, the ethanolamine could give some transamination reaction with N^4-benzoyl dC to yield the N^4-hydroxyethyl dC.[26] To avoid this side reaction, the

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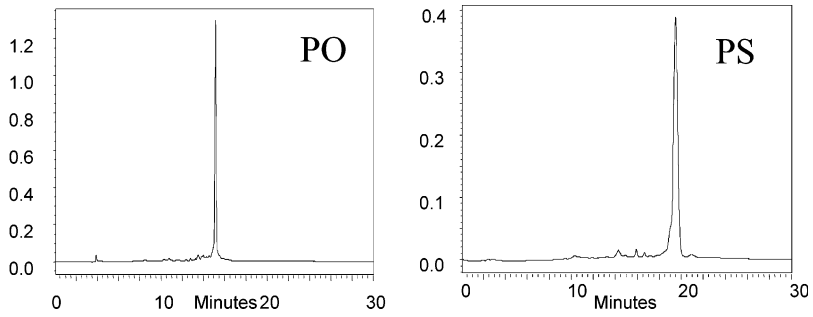
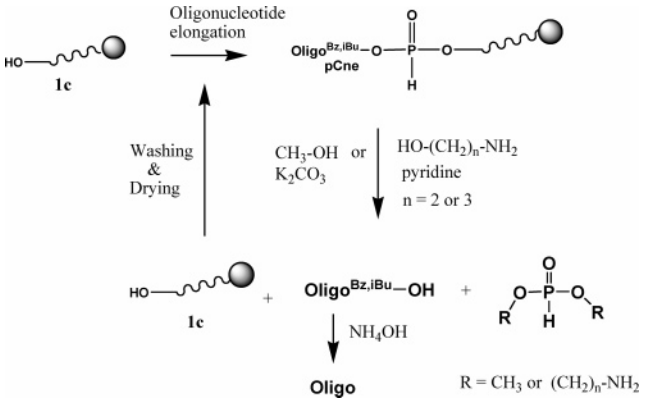


FIGURE 6. HPLC profiles of crude d(TCATGTACGA) phosphodiester (left) and phosphorothioate (right) released from solid support with 10 mM K_2CO_3 methanol for 30 min and then deprotected with NH_4OH, 5 h, 55 °C.

SCHEME 7. Synthesis of Oligonucleotides on a Universal and Reusable Solid Support 1c Released by Transesterification by Amino Alcohol or K_2CO_3/Methanol



standard benzoyl protecting group should by replaced by an acetyl, isobutyryl, or *tert*-butylphenoxyacetyl group. (For all of them, the corresponding phosphoramidites are now commercially available.). Furthermore, removal of ethanolamine (bp 170 °C) and pyridine (bp 113 °C) is not convenient owing to their high boiling points. An alternative was to use the treatment with K_2CO_3/methanol to do the transesterification of the *H*-phosphonate diester linkage. The synthesis of a heptamer d(TGACGAT) was performed on a solid support **1c** according to the elongation cycle reported in Table 1 with a capping step. The supported heptamer was treated with 10 mM K_2CO_3 methanol for 30 min, and the supernatant after evaporation was treated with ammonia since MS analysis showed that isobutyryl groups were only partially removed by K_2CO_3 treatment. For recycling, the solid support was washed with methanol/water (1:1, v/v, 2 mL), acetonitrile/water (1:1, v/v, 2 mL), and acetonitrile (10 mL) and dried under vacuum. The same oligonucleotide was synthesized on the same solid support three more times (named 1′, ×1′, ×2′, ×3′). The amount of each oligonucleotide was 37, 38, 36, and 36 OD^{260nm}, respectively. MS analysis visualized only the expected heptamer (calcd 2119.46, found 2120.00, 2120.07, 2118.16, 2121.10). HPLC profiles were similar for all of them and similar to that of the same heptamer synthesized according to a standard protocol (Figure 7).

For comparison, the heptamers d(TGACGAX) with X = C, G, or A were synthesized starting from the corresponding *tert*-butyl phosphoramidites. After release by treatment with 10 mM K_2CO_3 MeOH and then ammonia, HPLC and MS analyses showed profiles similar to those obtained for d(TGACGAT). For X = C: 42 $OD^{260\ m}$ 93% purity, for X = G: 30 OD^{260nm} 87% purity and for X = A: 37 OD^{260nm} 90% purity (see the Supporting Information). All *tert*-butyl phosphoramidites showed good coupling and no difference of transesterification of the corresponding *H*-phosphonate diester linkage.

To avoid two treatments for the release and the deprotection of the oligonucleotide, we performed the synthesis of d(TCATGTACGT) using UltraMild phosphoramidites. After elongation a single treatment with 50 mM K_2CO_3 methanol for 20 min at 50 °C led to the fully deprotected oligonucleotide (40 OD^{260nm}) as confirmed by MALDI-TOF MS (*m/z* calcd 3017.04, found 3017.35) with a 82% spectroscopic purity at 260 nm (see the Supporting Information).

With the both ethanolamine or K_2CO_3 methanol treatments the amount of oligonucleotide produced was stable from one synthesis to another, showing no decrease of available hydroxyl functions on the solid support. Among the both treatments, the latter was more convenient since standard phosphoramidite with a benzoyl group on deoxycytidine could be used and it avoids high-boiling solvents.

Note that the recycling of the support was rapid and straight since only washings and a drying of the solid support were required. Two articles report universal and reusable solid supports, but unfortunately, in one case the coupling of the first nucleoside derivative required, besides its preparation, the use of specific reagents (i.e., HBTU) to form an ester linkage with the hydroxyl solid support,[27] and in the second case, recycling proceeded with a tedious protocol in four steps with some noncommercial reagents.[28]

Multiple Synthesis. Since many applications use more than one oligonucleotides at a time, synthesis of defined mixtures of oligonucleotides is a useful way to improve synthesis productivity. Synthesis of two oligonucleotides in a row is particularly interesting in the case of primers for PCR since it will reduce the manipulations. Two versatile methods were reported to produce several oligonucleotides in a row. The first method uses a phosphoramidite derivative comprising two 1,4-anhydroerythritols linked by a succinyl arm (Figure 8, left, TOPS). However a dephosphorylation step under harsh

(27) Pon, R. T.; Yu, S. Y.; Guo, Z. Q.; Sanghvi, Y. S. *Nucleic Acids Res.* **1999**, *27*, 1531−1538.
(28) Kumar, P.; Mahajan, S.; Gupta, K. C. *J. Org. Chem.* **2004**, *69*, 6482−6485.

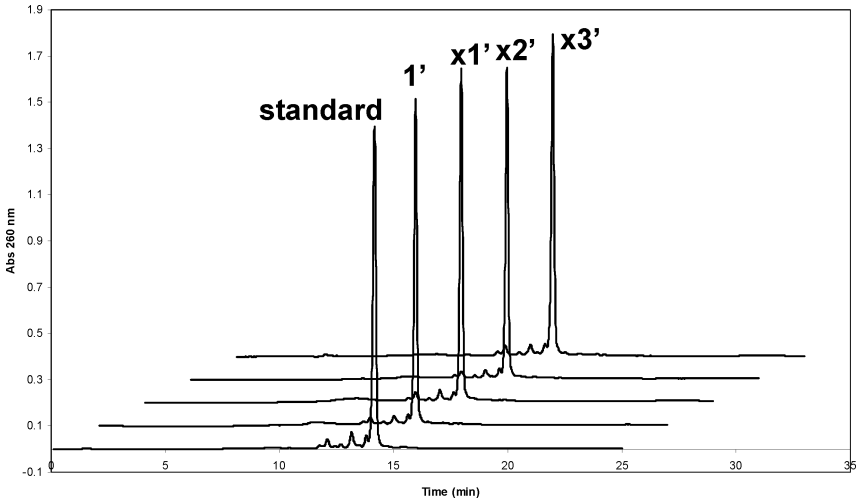
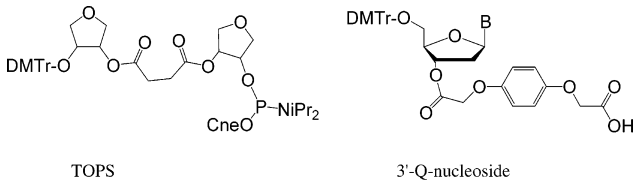


FIGURE 7. HPLC profiles of d(TGACGAT) synthesized four times on the same solid support and released by 10 mM K_2CO_3 MeOH and a standard synthesis: standard (offset: 2 min and 0.1 Abs for each) 1′, ×1′, ×2′, ×3′.



TOPS 3'-Q-nucleoside

FIGURE 8. Reagents for tandem oligonucleotide synthesis.

or prolonged deprotection conditions is required, and nonquantitative dephosphorylation leaves unwanted impurities in the mixture.[29] The second method uses a 3′-*O*-hydroquinone-*O,O′*-diacetic acid derivative of each nucleoside (Figure 8, right, 3′-Q-nucleoside) and requires its coupling with specific reagents (i.e., HBTU/DMAP) usually not used on a DNA synthesizer to form the ester function with the former oligonucleotide on the solid support.[30]

Since a *H*-phosphonate diester linkage could be easily cleaved by transesterification using either ethanolamine or K_2CO_3 methanol, the synthesis of multiple oligonucleotides could be easily performed on the solid support **1c** by starting each synthesis of the oligonucleotide with a *tert*-butyl phosphoramidite without oxidation or with a commercial *H*-phosphonate monoester nucleoside using pivaloyl chloride both will yield a *H*-phosphonate diester linkage (Scheme 8). The limitation will be the size of the pores of the solid support (500 Å in our case) but for multiple and long oligonucleotides a solid support with 2000 Å pore could be easily prepared. Moreover, the solid support is recyclable.

A 18-mer d(TGA CAG TGT CAT GTA CGA) and then a 15-mer d(TAT TCC GTC ATC GCA) were synthesized in a row on the solid support **1c** each of them starting with the coupling of a *tert*-butyl phosphoramidite of deoxyadenosine according to the cycle in Table 1. For comparison, the same tandem was performed on a solid support **1c** using commercial *H*-phosphonate monoester of deoxyadenosine to start each oligonucleotide and using pivaloyl chloride as activator. After elongation, the supported tandem oligonucleotides were treated with a solution of 10 mM K_2CO_3/methanol for 30 min and with concentrated ammonia for 5 h at 55 °C. HPLC profiles showed two main peaks corresponding to the 18-mer and 15-mer (Figure 9). Each peak was collected and characterized by MALDI-TOF MS, and surprisingly, the longer oligonucleotide was first eluted (18-mer t_R 15.0 min; *m/z* calcd 5537.70, found 5537.09; 15-mer t_R 16.7 min *m/z* calcd 4502.01, found 4503.08). However, for the synthesis using *tert*-butyl phosphoramidite, we also observed by HPLC a small peak at 17.6 min which was isolated and characterized as a 32-mer d(TAT TCC GTC ATC GC-TGA CAG TGT CAT GTA CGA) corresponding to an incomplete coupling of the second *tert*-butyl phosphoramidite. Hence, the elongation of the 14 next cyanoethyl phosphoramidite derivatives occurred on the 18-mer. This small peak was not visualized for the second synthesis using *H*-phosphonate monoester since pivaloyl chloride acts also as a capping reagent. Note that since the extinction coefficient (epsilon) of the 32-mer is almost twice as high as the two other oligonucleotides it was detected by HPLC although its low amount. In a PCR, this impurity will lead to some arrest of the polymerization of the strand complementary to the 15-mer; however, since primer are used in large excess this should not affect the final PCR products.

This solid support **1c** in combination with a *H*-phosphonate diester linkage displays is (1) easy to prepare, (2) universal, and (3) reusable and (4) allows multiple synthesis.

Finally, starting from a commercial solid support, the introduction of a H-phosphonate linkage could be used to release tandem synthesized oligonucleotides one after the other. Thus a first oligonucleotide d(TCATGTACGA) was synthesized with a standard protocol on a commercial succinyl solid support then a H-phosphonate linkage was introduced according to our protocol and a second oligonucleotide T_8 was synthesized. A first treatment with K_2CO_3/methanol at 0 °C for 20 min cleaved the H-phosphonate linkage, the T_8 was isolated from the supernatant, and then a standard ammonia treatment

(29) Hardy, P. M.; Holland, D.; Scott, S.; Garman, A. J.; Newton, C. R.; McLean, M. J. *Nucleic Acids Res.* **1994**, *22*, 2998−3004.
(30) Pon, R. T.; Yu, S.; Sanghvi, Y. S. *J Org Chem* **2002**, *67*, 856−64.

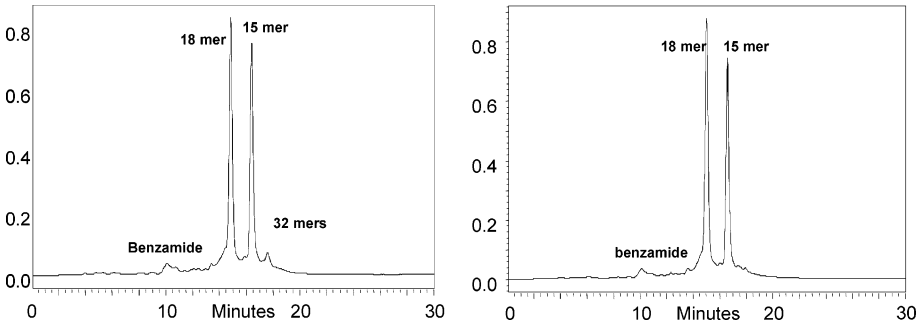
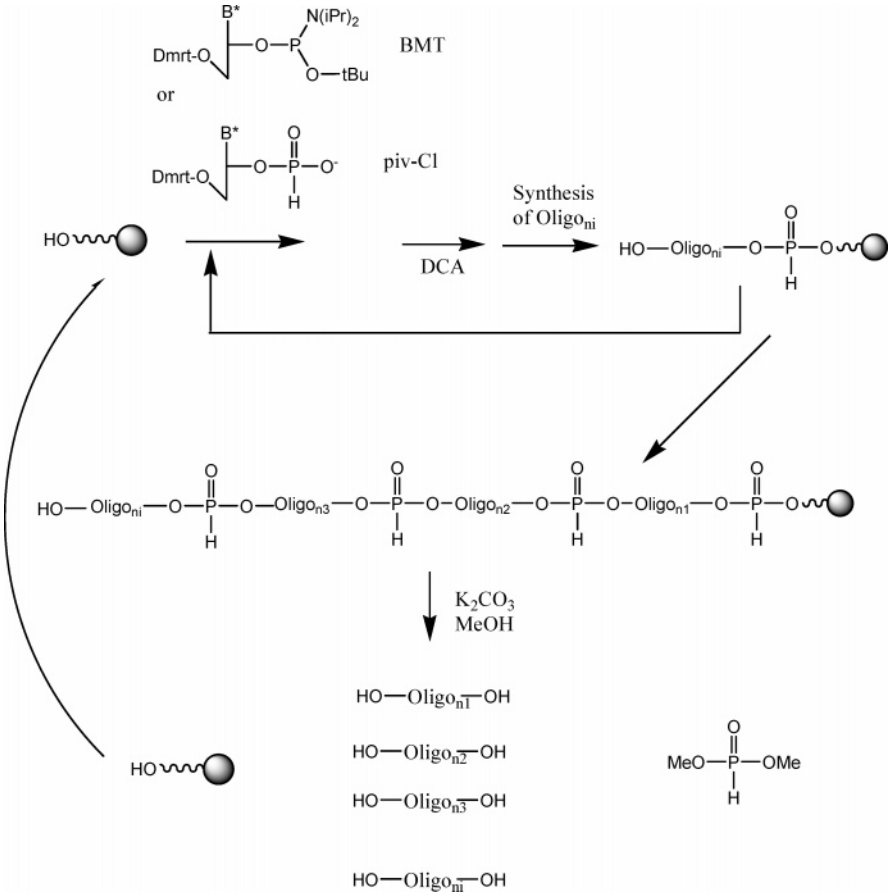


FIGURE 9. HPLC profiles of crude tandem synthesis of 18- and 15-mers using (left) *tert*-butyl phosphoramidite or (right) *H*-phosphonate monoester derivatives. Gradient: 8% to 24% CH_3CN in 50 mM TEAAc over 30 min.

SCHEME 8. Principle of Multiple Synthesis of Oligonucleotide on Universal and Reusable Solid Support 1c



of the CPG beads released the d(TCATGTACGA) (Supporting Information).

Conclusion

All of the solid supports presented herein in combination with a first incorporation of a *H*-phosphonate diester linkage are universal. Among them, the second one **1b** opens the way for the synthesis of base-sensitive oligonucleotides and the third one **1c** exhibits the advantage to be recyclable by a straight and easy protocol.

For all of them, the major point to highlight is that a *H*-phosphonate diester linkage allows a clean and rapid transesterification yielding 3′ and 5′ hydroxyl oligonucleotides. This transesterification of the H-phosphonate diester linkage allowed the synthesis of multiple oligonucleotides per synthesis. The introduction of the *H*-phosphonate could either be done by a "fully phosphoramidite chemistry"[8] using *tert*-butyl phosphoramidite derivatives or with commercial *H*-phosphonate monoester nucleosides using *H*-phosphonate chemistry.

This approach could also be applied to the RNA synthesis since the transesterification proceeded under mild basic conditions unlike the standard universal solid supports that required harsh basic treatments. Thus, the tandem synthesis of RNA for a RNAi purpose is possible and works along this line is in progress.

Experimental Section

Solid-Support EG-Q-CPG. 2-*O*-(4,4′-Dimethoxytrityl)-1-hydroxyethane. To dry ethylene glycol (1.90 g; 30.6 mmol)

dissolved in 20 mL of dry pyridine was added 4,4′-dimethoxytrityl chloride (2.10 g; 6.2 mmol) at room temperature. The mixture was stirred for 0.5 h, diluted with CH_2Cl_2 (50 mL), and washed with $NaHCO_3$ (2 × 150 mL). The organic layers was dried over Na_2SO_4 and evaporated to dryness. The residue was purified by flash column chromatography (silica gel; gradient 20−100% dichloromethane/2% Et_3N/in cyclohexane). The appropriate fractions were combined and concentrated to dryness to afford 1.6 g (70%) of the product as a yellow oil. TLC (cyclohexane/ether/Et_3N 1:8:1, v/v/v) R_f: 0.4. ^1H NMR ($CDCl_3$) δ ppm: 2.1 (bs, 1 H), 3.25 (m, 2 H), 3.7 (m, 2 H), 3.8 (s, 6 H), 6.8−6.9 (m, 4 H), 7.2−7.6 (m, 9 H).

DMTr-EG-HQDA. 2-*O*-(4,4′-Dimethoxytrityl)-1-hydroxyethane (0.71 g; 2 mmol), hydroquinone-*O,O′*-diacetic acid (HQDA) (0.529 g; 2.4 mmol), 1-(3-dimethylaminopropyl)-3-ethylcarbodiimide (DEC) (0.373 g, 2 mmol), and 4-(dimethylamino)pyridine (DMAP) (0.024 g, 0.1 mmol) were stirred in anhydrous pyridine at room temperature for 5 h. Then, the solvent was distilled off and the residue diluted with 100 mL of CH_2Cl_2. The organic layer was washed with water (2 × 100 mL), and the solvent was evaporated to dryness. The residue was purified by flash column chromatography (silica gel; gradient 1−15% MeOH/1% Et_3N/in dichloromethane). The appropriate fractions were combined and concentrated to dryness to afford 0.48 g (42%) of the product as an oil. TLC (5% CH_3OH/CH_2Cl_2) R_f: 0.3. ^1H NMR ($CDCl_3$) δ ppm: 3.23−3.27 (m, 2 H), 3.75 (s, 6 H), 4.29−4.32 (m, 2 H), 4.42 (s, 2 H), 4.55 (s, 2 H), 6.74−6.79 (m, 8 H), 7.15−7.4 (m, 9 H).

Preparation of DMTr-EG-Q-CPG (LCAA-CPG 500 A, 80−120 mesh, 80−90 μmol/g). LCAA-CPG (1.00 g), EG-HQDA (0.114 g, 0.2 mmol), EDC (0.191 g, 1 mmol), DMAP (0.012 g, 0.1 mmol), and Et_3N (0.1 mL) were shaken in anhydrous pyridine (5 mL) at room temperature for 5 h. The solid support was filtered off, washed with MeOH and CH_2Cl_2, and dried. A capping step with standard Cap A and Cap B solutions was applied for 1 h, and the solid support was filtered off, washed with MeOH and CH_2Cl_2, and dried. The trityl assay indicated a loading of 48 μmol/g.

Solid Support from Glycerol. Synthesized according De Napoli et al.[21] Loading 59 μmol/g.

Hexanol Solid Support. LCAA-CPG (1.00 g), succinic anhydride (0.2 g, 2 mmol), and *N,N*-(dimethylamino)pyridine (DMAP) (0.04 g, 0.33 mmol) were added in a flask with anhydrous pyridine (6 mL) and shaken overnight at room temperature. The succinylated support was filtered off, washed with pyridine and CH_2Cl_2, and dried under vacuum. The succinylated CPG, 3-ethyl 1-[3-(dimethylamino)propyl]carbodiimine (EDC) (0.191 g, 1 mmol), *N*-hydroxysuccinimide (0.06 g, 0.5 mmol), and Et_3N (40 μL) were added in CH_2Cl_2 (5 mL), and the flask was shaken at room temperature for 4 h. Then, 6-aminohexan-1-ol (6 mg, 0.05 mmol) was added, and shaking was continued overnight. Finally, piperidine (5 mL) was added, and after 15 min, hexanol-CPG was filtered, washed with MeOH and CH_2Cl_2, and dried under vacuum. The trityl assay determined after the coupling of one standard phosphoramidite indicated a loading of 51 μmol/g.

Oligonucleotide Synthesis. Oligonucleotides (1 μmol scale) were synthesized on an ABI 381A or 394 DNA synthesizer using a cycle involving phosphoramidite chemistry. Detritylation was performed with 3% DCA in CH_2Cl_2 for 60 s. Coupling step: BMT (0.3 M in dry acetonitrile) was used as activator; *tert*-butyl phosphoramidites (0.09M in CH_3CN) were introduced with a 45 s coupling time and the oxidation step was omitted; commercially available phosphoramidites (0.09 M in CH_3CN) were introduced with a 15 s coupling time. The capping step was performed with biscyanoethyl diisopropyl phosphoramidite (0.03 M in dry acetonitrile) + BMT for 15 s. Oxidation were performed with 1.1 M *tert*-butyl hydroperoxide in CH_2Cl_2 or 0.05 M 3*H*-1,2-benzodithiol-3-one 1,1-dioxide in dry acetonitrile for 60 s.

Release from solid supports and deprotection:

With EG-Q-Solid Support 1a. The column containing the supported oligonucleotide was treated back and forth with 2 mL of 5 mM K_2CO_3 methanol for 30 min using two syringes. Then beads were washed with methanol (2 mL) and water (2 mL). After evaporation, the residue was treated with ammonia for 5 h at 55 °C.

With TBDMS-Glycerol Solid Support 1b. The beads were transferred to an Eppendorf tube and treated with 200 μL of commercial $Et_3N{\cdot}3HF$ in 200 μL of THF solution for 2 h 30 at room temperature, and then 50 mM TEAAc pH 7 buffer was added (1 mL). After evaporation, the residue was treated with concentrated ammonia for 5 h at 55 °C.

With Hexanol Solid Support 1c. Protocol A. The column containing the supported oligonucleotide was treated back and forth with 2 mL of 10% ethanolamine in pyridine for 30 min using two syringes. Then beads were washed with pyridine (2 mL), pyridine/water (2 mL, 1:1, v/v), water (2 mL). After evaporation, the residue was treated with ammonia for 5 h at 55 °C.

Protocol B. The same treatment used for support **1a** using K_2CO_3 methanol was applied.

For Ultramild Protecting Groups. The supported oligonucleotide was treated with 50 mM K_2CO_3/MeOH for 20 min at 50 °C.

Recycling of Solid Support 1c. After Treatment According to Protocol A. The solid support was washed with pyridine (2 mL), acetonitrile/water (1:1, v/v, 2 mL), and dry acetonitrile (10 mL) and dried under vacuum for 5 h under P_2O_5.

After Treatment According to Protocol B. The solid support was washed with methanol/water (1:1, v/v, 2 mL), acetonitrile/water (1:1, v/v, 2 mL), and dry acetonitrile (10 mL) and dried under vacuum for 5 h under P_2O_5.

Acknowledgment. F.F. thanks the Ministère de la Recherche et de la Technologie for the award of a research studentship.

Supporting Information Available: Preparation of *tert*-butyl phosphoramidites. Elongation cycle. HPLC profiles of oligonucleotides synthesized on solid support **1a**−**c**. This material is available free of charge via the Internet at http://pubs.acs.org.

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