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Synthesis Of Phosphorodithioate DNA by the H-Phosphonothioate Method[1]

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Abstract: A novel method for the efficient synthesis phosphorodithioate DNA using H-phosphonothioate synthons is described. The 5'-DMT-nucleoside H-phosphonothioate monomers were prepared in two high yielding steps from commercially available phosphoramidites. This strategy for the synthesis of H-phosphonothioate monomers proved versatile and allowed for the synthesis of cholesterol H-phosphonothioate synthons. Synthetic protocols for the assembly of phosphorodithioate DNA using an automated synthesizer were developed. The H-phosphonothioate solid-phase synthesis method facilitates the preparation of oligonucleotide conjugates, as demonstrated by the example of attachment of 5'-cholesterol oligonucleotides. © 1999 Elsevier Science Ltd. All rights reserved.

Keywords: Nucleic acid analogs; phosphorus compounds.

INTRODUCTION

Oligonucleotide analogs bearing modified phosphodiester linkages have been the focus of considerable interest in the antisense field owing to their nuclease resistance and thus prolonged biological half-lives.[2,3] The substitution of both non-bridging oxygen atoms with sulfur gives rise to a phosphorodithioate linkage, which, like natural DNA, is achiral at phosphorus. The dithioate linkage has been found to be highly stable towards enzymatic hydrolysis.[4,5] Recent studies have found that dithioate DNA oligomers have biophysical and biological properties which make them ideally suited for antisense and other biological applications.[6-9]

Since the initial report of a solid-phase synthesis of dithioate DNA,[10] there have been numerous synthetic efforts directed at further refinement of the chemistry.[8,9,11-15] The solid-phase synthesis of phosphorodithioate DNA is compatible with the synthesis of natural oligodeoxynucleotides using the phosphoramidite approach. The major limitation using this strategy is the synthesis of the thiophosphoramidite monomers. Due to the high reactivity of these compounds, purification by silica column chromatography has not been possible and precipitation is the only means of purification. The preparation of pure thioamidites which cannot be precipitated has not been successful to date. Using these phosphoramidites, stepwise coupling yields of 96-98% have been obtained. Still, side products such as phosphorothioates (2-4%) and crosslinked species (<1%) have not been completely eliminated.[16]

As an alternative to the phosphoramidite method for the synthesis of oligonucleotides the H-phosphonate method has gained importance during recent years.[17] Advantages of this approach are the

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stability of the H-phosphonate monomers to hydrolysis and oxidation, short cycle times, and the possibility of oxidizing not after every step, but only at the end of the synthesis as a simple end procedure.[17]

Stawinski and coworkers[18] recently reported extensive studies on the synthesis of deoxynucleoside H-phosphonothioates and their use in the synthesis of dinucleoside H-phosphonothioates.[19] In an extensive study on the synthesis of dinucleotide diesters they reported ^{31}P NMR studies on the condensation reactions using nucleoside H-phosphonothioates as well as nucleoside H-phosphonodithioates. While coupling of the latter compounds with a variety of activators including pivaloyl chloride (Piv-Cl), adamantoyl chloride (Ad-Cl), diisopropylcarbodiimide (DICI), and diphenylchlorophosphate (DPCP) proved unsuccessful, the H-phosphonothioate monomers could be selectively activated at oxygen and coupled, when DPCP or Ad-Cl were used as activators. The formation of desulfurized diesters during couplings employing Piv-Cl and DICI and nucleoside H-phosphonothioates was examined by ^{31}P NMR spectroscopy.[20]

We have studied several approaches for the synthesis of phosphorodithioate nucleic acids as part of an ongoing program concerned with the synthesis and biochemical evaluation of novel nucleic acid analogs. Most recently we reported the first successful synthesis of phosphorodithioate RNA by the H-phosphonothioate method.[21] Here we report our efforts on the synthesis of dithioate DNA using the H-phosphonothioate approach. A novel synthetic scheme for the preparation of deoxynucleoside H-phosphonothioates starting from commercially available deoxynucleoside methoxy-phosphoramidites in two high yielding steps is presented. The synthesis of phosphorodithioate DNA was accomplished on an automated DNA synthesizer by using DPCP as an activator and the incorporation of a novel oxidation step into each coupling cycle. Furthermore, the synthesis of cholesterol H-phosphonothioate synthons which allow the attachment of cholesterol to the 5'-terminus of an oligonucleotide via a phosphorodithioate linkage is described.

RESULTS AND DISCUSSION

The Synthesis of Deoxynucleoside H-Phosphonothioates, -Thiophosphates and -Dithiophosphates via a Common Intermediate. Recently we reported the synthesis of 2'-deoxynucleoside-dithiophosphates via the oxidative coupling of nucleoside-H-phosphonodithioates with 9-fluorenemethanol (Fmol) as an easily removable novel phosphate protecting group.[22] Others have since developed new synthetic routes to these phosphate analogs[23] and the Fmol protecting group has been readily applied in the synthesis of nucleoside phosphates and phosphate analogs.[24]

In an effort to demonstrate the synthetic versatility of the Fmol protecting group, it was discovered that the O-(5'-O-(4,4'-dimethoxytrityl)thymidine-3'-yl) O-(9-fluorenemethyl) H-phosphonothioate **2** could be used as an intermediate for the synthesis of O-(5'-O-(4,4'-dimethoxytrityl)thymidine-3'-yl) dithiophosphate **5**, the O-(5'-O-(4,4'-dimethoxytrityl)thymidine-3'-yl) H-phosphonothioate **3**, and O-(5'-O-(4,4'-dimethoxytrityl)thymidine-3'-yl) thiophosphate **7**.

When O-(5'-O-(4,4'-dimethoxytrityl)thymidine (DMT-T, **1**) was reacted with tris-pyrrolidinylphosphine in the presence of 1-H-tetrazole as a catalyst and 9-fluorenemethanol, the Fmol-pyrrolidino-phosphoramidite intermediate was formed. The addition of hydrogensulfide in the presence of 1-H-tetrazole, yielded the desired product **2**. However, **2** was accompanied by small amounts of hydrolyzed phosphine byproduct as judged by ^{31}P NMR analysis. Oxidation of **2** with elemental sulfur in carbon

disulfide/pyridine resulted in the formation of O-(5'-O-(4,4'-dimethoxytrityl)thymidine-3'-yl) O-(9-fluorenemethyl) phosphoro-dithioate **4** exclusively. Treatment of **4** with a solution of piperidine in dichloromethane yielded **5** as the only product after precipitation from hexane.

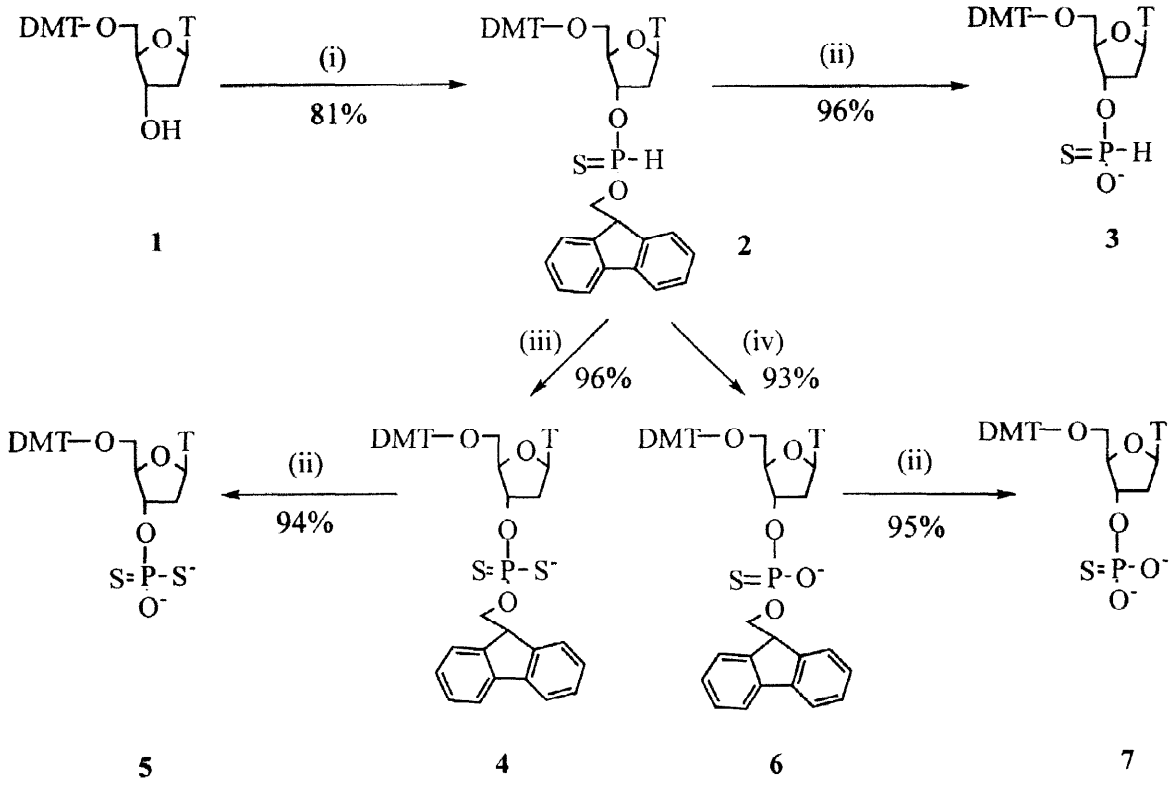
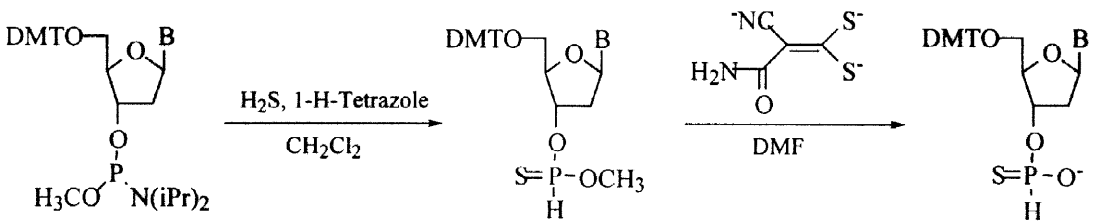


Figure 1. Synthesis of nucleotide analogs via a common intermediate. (i) tris-pyrrolidinylphosphine, 9-fluorenemethanol, H_2S,1-H-tetrazole; (ii) 20% piperidine/CH_2Cl_2; (iii) S_8/CS_2/pyridine; (iv) 0.1 M I_2 in THF/pyridine/H_2O (2/2/1).

Oxidation of intermediate **2** with iodine in the presence of water led to the formation of the O-(5'-O-(4,4'-dimethoxytrityl)thymidine-3'-yl) O-(9-fluorenemethyl) phosphoro-thioate **6** which could be converted to thiophosphate **7** by exposure to piperidine. Treatment of H-phosphonothioate intermediate **2** with a solution of piperidine yielded exclusively the H-phosphonothioate **3**.

Synthesis of 2'-Deoxynucleoside H-Phosphonothioate Monomers. Since the synthesis of nucleoside H-phosphonothioate monomers such as **3** could be accomplished by removal of the oxygen protecting group of a nucleoside H-phosphonothioate diester, the use of other protecting groups in place of Fmol was investigated. The conversion of the β-cyanoethoxy protected nucleoside-bis-diisopropyl phosphoramidite to the corresponding H-phosphonothioate diester proceeded in almost quantitative yields (>95%). Attempts, to remove the β-cyanoethoxy protecting group with a variety of bases (piperidine, tert. butylamine, DBU), resulted not only in formation of the desired H-phosphonothioate but also in a variety of Arbuzov products as well as other side products (as detected by ^{31}P NMR). Alternatively, reaction of the commercially available methoxy protected bisdiisopropyl nucleoside phosphoramidites **8a-d** furnished almost quantitatively the O-(5'-O-(4,4'-dimethoxytrityl)nucleosid-3'-yl) O-(methyl) H-phosphono-thioates **9a-d**.

Removal of the methoxy protecting group was accomplished with 2-carbamoyl-2-cyanoethylene-1,1-dithiolate[23] without formation of any side products. Purification by silica column to remove the dithiolate salt followed by precipitation into hexane yielded the O-(5'-4,4'-dimethoxytrityl-nucleosid-3'-yl) H-phosphonothioates **10a-c, 3** as their sodium salts in yields of greater than 90%.



8 a B: A^{Bz} **9 a** B: A^{Bz} **10 a** B: A^{Bz}
8 b B: C^{Bz} **9 b** B: C^{Bz} **10 b** B: C^{Bz}
8 c B: G^{iBu} **9 c** B: G^{iBu} **10 c** B: G^{iBu}
8 d B: T **9 d** B: T **3** B: T

Figure 2. Synthesis of deoxynucleoside H-phosphonate synthons.

Synthesis of Oligodeoxynucleotide-Phosphorodithioates using Deoxynucleoside H-Phosphonothioates. The central issue to be addressed in order to guarantee a successful solid phase synthesis of phosphorodithioate DNA using H-phosphonothioate synthons is the selective activation of oxygen over sulfur. Exclusive reaction of the activator with oxygen has to be achieved to prevent desulfurization by activation of sulfur.

Two activating agents, Piv-Cl and DPCP were compared for their performance in coupling experiments. Pivaloylchloride activation occurred mainly on sulfur since ^{31}P NMR analysis of a thymidine pentamer revealed the formation of only 5% dithioate linkages, but 70% thioate linkages and 25% phosphate linkages. This result is in agreement with similar activation studies reported by others.[19,20] DPCP proved to be an excellent activator resulting in coupling yields consistently higher than 97.5%. Besides the desired dithiophosphate internucleoside linkage the thioate diester linkage was observed as the main side product by ^{31}P NMR at levels of less than 5%. This contamination could arise due to the reaction of the activator with sulfur of the H-phosphonothioate monomer during coupling or incomplete sulfurization of the thiophosphite diester.[25]

Alternatively, a strategy employing nucleoside H-phosphonodithioates monomers in oligonucleotide synthesis was evaluated.[26] Under this paradigm selectivity during the activation reaction was not an issue as the promoter had to react with a sulfur of the monomer. Activation of sulfur proceeded much slower than with oxygen and the prolonged coupling times resulted in increased side reactions. When using the nucleoside H-phosphonodithioates in coupling experiments on the solid support, with DPCP or Ad-Cl as activators, ^{31}P NMR analysis revealed almost exclusively phosphorothioate diester product in place of the desired dithioate diester linkages.

In standard H-phosphonate synthesis protocols, oxidation is performed after completion of oligonucleotide assembly, since the H-phosphonate diesters are not labile to either coupling or deprotection

conditions. When the oxidation with elemental sulfur was performed at the end of the synthesis, significant amounts of desulfurized material were detected in the final product by ^{31}P NMR. Inclusion of an oxidation step with sulfur in every cycle did not prevent the formation of desulfurized products as detected by ^{31}P NMR. From our previous studies on the synthesis of dithioate DNA it was known that the phosphorodithioate triester was stable to all steps during synthesis.[16] Use of the previously described[27] 2,4-dichlorobenzyl-thiosuccinimide in the oxidation step, furnished in each cycle a phosphorodithioate triester which is stable during the course of the synthesis (Fig. 3).

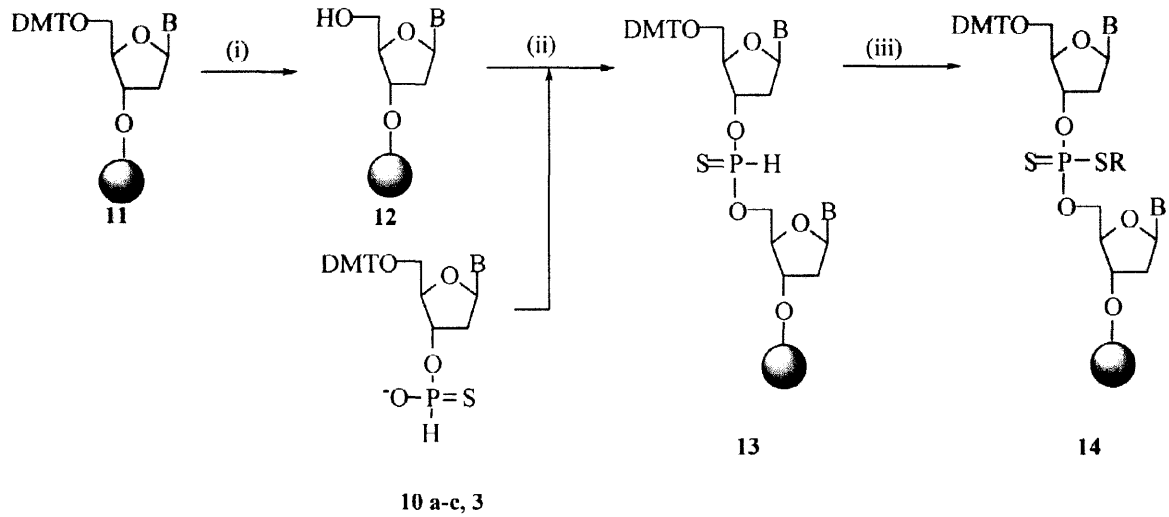


Figure 3. Synthesis of deoxynucleotide dithioates by the H-phosphonate approach. (i) 3% trichloroacetic acid; (ii) DPCP; (iii) 2,4-dichlorobenzyl thiosuccinimide. R: 2,4-dichlorobenzyl.

Based on these studies of the coupling and oxidation steps, a coupling cycle was developed (Table 1). Optimization of activator and monomer concentrations and the number of deliveries, as well as the wait time after delivery, resulted in average coupling yields of greater than 97.5% (as assessed by UV assay of the released DMT). Detritylation with 3% trichloroacetic acid was followed by washing of the synthesis column with CH_2Cl_2 and CH_3CN/pyridine to remove all acid from the solid support. A 0.1 M solution of nucleoside H-phosphonothioate monomer in CH_3CN/pyridine (50/50) was delivered simultaneously with a 0.1 M solution of DPCP in CH_3CN/pyridine (95/5) in two steps of 5 seconds each onto the synthesis column. A reaction time of 40 seconds resulted in virtually complete coupling. After washing the synthesis column with CH_3CN/pyridine and CH_2Cl_2, sulfurization with a 0.1 M solution of 2,4-dichlorobenzyl thiosuccinimide, delivered simultaneously with 5% triethylamine followed. Another washing step was followed by entry into the next coupling cycle and detritylation. After the entire oligodeoxynucleotide was assembled, the 2,4-dichlorobenzyl protecting groups were removed by treatment with a 0.1 M solution of 2-carbamoyl-2-cyanoethylene-1,1-dithiolate in DMF for 16 h, followed by cleavage from the solid support by treatment with concentrated ammonium hydroxide for 45 minutes.

Table 1. Chemical Steps for the Synthesis of Dithioate DNA by the H-Phosphonothioate Approach

Step	Reagent or Solvent	Purpose	Time (s)
(i)	a. Trichloroacetic acid in CH_2Cl_2 (3% w/v)	Detritylation	50
	b. CH_2Cl_2	Wash	100
	c. CH_3CN/pyridine (1/1, v/v)	Wash	60
(ii)	a. Activated nucleotide in CH_3CN/pyridine	Add nucleotide	5
	b. Repeat step a.	Complete nucleotide addition	5
	c. Wait	Coupling	40
	d. CH_3CN/pyridine (50/50)	Wash	20
	e. CH_2Cl_2	Wash	40
(iii)	a. 0.1 M 2,4-dichlorobenzyl thiosuccinimide and 1% TEA in CH_2Cl_2	Sulfurization/protection	300
	b. CH_2Cl_2	Wash	30

Using this protocol, a phosphorodithioate thymidine hexamer (S_2T_6) was assembled. The crude product was analyzed by ^{31}P NMR (Figure 4) and showed 6% phosphorothioate contamination (56 ppm) as well as 1% phosphodiester impurities (-10 ppm). These results suggest that the H-phosphonate approach allows for the synthesis of phosphorodithioate DNA of a quality comparable to that obtained by the phosphoramidite method. In order to further assess the full potential of this synthetic approach, studies involving the synthesis of longer heterosequences containing all four nucleosides will be necessary.

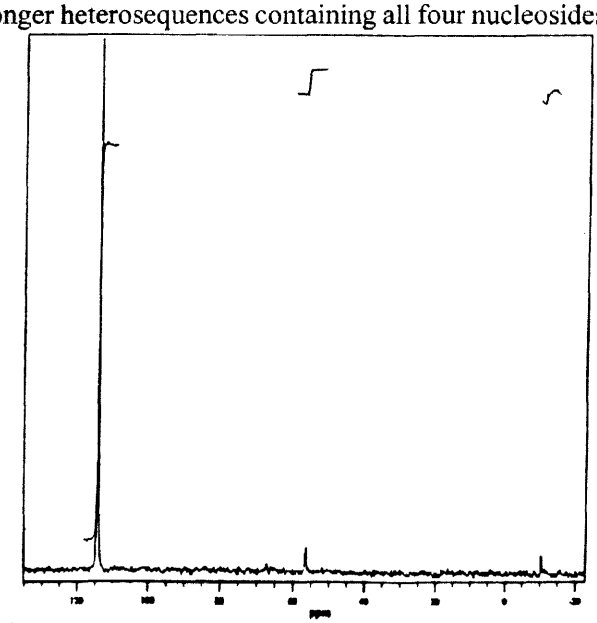


Figure 4. ^{31}P NMR of crude phosphorodithioate thymidine hexamer (S_2T_6).

Synthesis of 5'-Cholesterol-Phosphorodithioate Oligonucleotides. The transport of oligodeoxynucleotides into cells has been facilitated by linking these oligonucleotides to lipophilic carriers. Due to its highly hydrophobic character cholesterol has been incorporated to anchor the oligomer, at least transiently, in the cell membrane.[28] The synthesis of pure cholesterol-thiophosphoramidites had previously not been achieved since precipitation was not feasible.[29] The synthesis of a cholesterol H-phosphonothioate monomer serves as an example for the versatility of the synthesis approach outlined thus far. During the initial synthetic studies O-(cholesteryl) O-(9-fluorenemethyl) H-phosphonothioate **16a** was prepared. The yields for the synthesis of **16a** and deprotection with 20% piperidine to form **17** were low. In contrast, the synthesis of the O-(cholesteryl) O-(methyl) H-phosphonothioate **16b** was straightforward, as was the removal of the methoxy group with 2-carbamoyl-2-cyanoethylene-1,1-dithiolate yielding **17**. The final product **17** was purified by silica column chromatography.

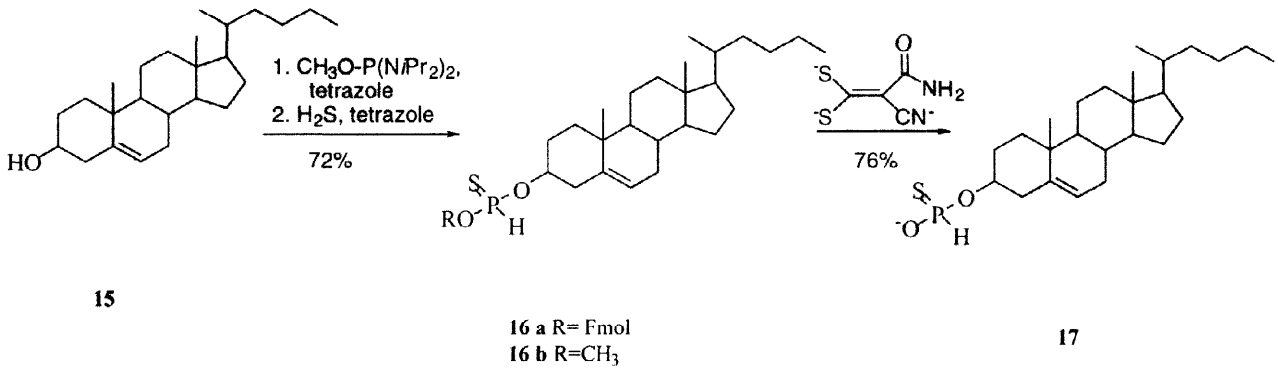


Figure 5. Synthesis of cholesterol H-phosphonothioate.

To study the performance of cholesterol H-phosphonothioate **17** in solid support synthesis, 5'-cholesterol oligonucleotide conjugates were prepared. A thymidine decamer (dT_{10}) mer and a dithioate cytidine pentadecamer (S_2dC_{15}) mer were synthesized by standard phosphoramidite chemistry on the solid support and **17** was coupled to the 5'-hydroxyl of the oligonucleotide by activation with DPCP in the synthesis cycle outlined in Table 3. Sulfurization with 1,4-dichlorobenzyl thiosuccinimide was followed by removal of the protecting groups in 1.0 M dithiolate solution (16 h) and subsequent cleavage of the cholesterol-oligonucleotide conjugate from the solid support by treatment with concentrated ammonium hydroxide (16 h). The crude product was characterized by ^{31}P-NMR; a peak at 117 ppm corresponds to the phosphorodithioate internucleoside bonds, while the nucleoside-cholesterol phosphorodithioate linkages show a distinct peak at 112 ppm. Phosphorothioate internucleoside bonds were the only impurity identified by ^{31}P NMR at 54 ppm (2.5%). The crude cholesterol conjugate was purified by reverse-phase HPLC. The conjugate was distinctly separated from a series of side-products, which constitute failure sequences of the oligonucleotide synthesis.

Conclusion. This study describes a new and convenient method for the synthesis of H-phosphonothioate synthons. These compounds were used in the solid support synthesis of a thymidine phosphorodithioate hexamer by the H-phosphonate approach. Different aspects of the synthesis protocol

were extensively studied and a novel oxidation/protection step was incorporated into every synthesis cycle resulting in oligonucleotide products containing less side products than oligomers synthesized using the phosphoramidite approach. Further utility of this approach was demonstrated with the synthesis of a 5'-cholesterololigonucleotideconjugate.

The H-phosphonothioate approach has proven very useful for the solid support synthesis of phosphorodithioate DNA, RNA[21] and oligonucleotide conjugates. Versatility, high coupling yields and products of high purity are the hallmarks of this approach which uses monomers which are easy to handle and can be purified by silica column. The H-phosphonothioate chemistry described herein complements the phosphoramidite method as it allows for access to conjugates which could otherwise not be prepared but may be biologically quite interesting.

EXPERIMENTAL

Materials and Methods

All chemicals used were reagent grade and used as supplied. 9-Fluorenemethanol was recrystallized from anh. dichloromethane before use. Tris-pyrrolidinylphosphine was synthesized as described previously[7]. Pyridine and dichloromethane were refluxed over CaH_2 and distilled prior to use.

Solid support syntheses were carried out on a Applied Biosystems Inc. Model 380A or 394 automated DNA synthesizer.

^1H NMR spectra were recorded on a Varian VXR-300S and a General Electrics GE 300 in the solvent indicated. ^{31}P NMR spectra were recorded on a Varian VXR-300S spectrometer operating at 121.4 Hz, a Bruker AM-400 spectrometer operating at 162.0 Hz and a General Electrics GE 300 spectrometer with broad band decoupling referenced to 85% H_3PO_4 as an external standard. Reverse-phase HPLC was performed with a Waters dual-pump 6000A system in combination with a Maxima 820 gradient control system and a Model 440 UV detector operating at 254 nm. Columns were packed with PRP-1 (5μm, from Hamilton) and eluted with 50 mM triethylammonium bicarbonate (TEAB), pH 7.5 containing a linear gradient of acetonitrile.

TLC was performed on Kieselgel 60 Platten (Merck, Darmstadt) and eluted with CH_2Cl_2/MeOH (98/2 (v/v)) (system I),CH_2Cl_2/MeOH (95/5 (v/v)) (system II), CH_2Cl_2/MeOH (90/10 (v/v)) (system III), CH_2Cl_2/MeOH (80/20 (v/v)) (system IV).

Synthesis of *O*-(5'-*O*-(4,4'-Dimethoxytrityl)thymidine-3'-yl) *O*-(9-Fluorenemethyl) H-phosphonothioate (2). To a solution of 544.6 mg (1.0 mmol) 5'-DMT-thymidine in 100 mL anh. CH_2Cl_2 under argon (Ar) atmosphere, 230 μL (1.0 mmol (1 eq.)) tris-pyrrolidinylphosphine were added. In seven portions over seven minutes, 0.7 mL (0.35 mmol (0.35 eq.)) of a 0.5 M solution of 1-H-tetrazole in anh. acetonitrile were added. The solution was stirred for ten minutes, and 196 mg (1 mmol) 9-fluorenemethanol in 10 mL anh. CH_2Cl_2 and 5.4 ml (2.7 mmol (2.7 eq.) 0.5 M 1-H-tetrazole solution were added simultaneously and stirred for 10 min. Hydrogensulfide was bubbled through the solution for 5 min and was stirred under Ar for an additional 30 min. The solution was purged with Ar and extracted with 5 % $NaHCO_3$ solution (2 x 30 mL), followed by sat. NaCl (1 x 30 mL). The aqueous phases were combined and reextracted with 15 mL

CH_2Cl_2. The combined organic phases were dried over Na_2SO_4 and the solvent was removed. Purification by silica column flash chromatography followed by precipitation into hexane yielded the pure product **2** as a white powder. Yield: 671 mg (81%); TLC (system I): R_f= 0.15; ^{31}P NMR ($CDCl_3$): δ 71.0, 70.6; ^1H NMR ($CDCl_3$): δ 1.51 (s, 3H, (CH_3)); 2.03-2.58 (m, 2H, H2'); 3.40 (m, 2H, H5'); 3.79 (s, 6H, OCH_3); 3.97-4.59 (m, 4H, H4'); 5.34 (m, 1H, H3'); 6.36-6.40 (dd, J = 6.6 6.5 Hz, 1H, H1'); 6.78-6.95 (m, 4H); 7.20-7.81 (m, 17H); 8.84 (s, 1H, NH); 9.13 (d, J = 542 Hz, 1H, P-H); ^{13}C NMR ($CDCl_3$): δ 12.0 (CH_3); 39.4 ($C_{2'}$); 48.2; 55.5 (OCH_3); 63.3 ($C_{5'}$); 68,4; 84.7 ($C_{1'}$); 85.1 ($C_{4'}$); 87.6; 111.9 (C(4)); 113.6; 120.4; 125.3; 127.5; 128.4; 130.4; 135.6 (C(5)); 141.8; 143.3; 144.6; 150.8 (C(2)); 159.2; 164.2 (C(6)); MS (FAB+; NOBA): 803 (M^+); MS (FAB-; NOBA): 801 (M^-).

Synthesis of *O*-(5'-*O*-(4,4'-Dimethoxytrityl)thymidine-3'-yl) H-phosphonothioate (3). 207 mg (0.25 mmol) of **2** were dissolved in 5 mL of a 20 % solution of piperidine in CH_2Cl_2 and stirred for 20 min. The solvents were removed *in vacuo* and the crude product was purified by silica column chromatography followed by precipitation into hexane to yield the pure product **3** as a white powder. Yield: 171 mg (97%); TLC (system II):R_f= 0.19; ^{31}P NMR ($CDCl_3$): δ 55.8, 55.2; ^{31}P NMR (pyridine-d5): δ 53.00, 52.04; ^1H NMR (pyridine-d5): δ 1.68 (d, J = 8.06 Hz, 3H, (CH_3)); 2.78-2.82 (m, 2H, H2'); 3.68 (d, J = 1.2 Hz, 6H, OCH_3); 3.71-3.74 (m, 3H, H4'/H5'); 4.82 (m, 1H, H3'); 6.10-6.20 (m, 1H, H1'); 6.96-7.86 (m, 14H, H(6)); 9.23 (d, J = 581 Hz, 1H, P-H); ^{13}C NMR (pyridine-d5): δ 12.3 (CH_3); 40.2 ($C_{2'}$); 55.2 (OCH_3); 64.8 ($C_{5'}$); 76.2 ($C_{3'}$); 85.1 ($C_{1'}$); 85.5 ($C_{4'}$); 87.5 (DMT); 111.4 (C(4)); 114.1; 127.5; 128.7; 128.8; 130.9; 135.7 (C(5)); 151.9 (C(2)); 159.4; 165.0 (C(6)); MS (FAB+; Glycerol): 625 (M^+); MS (FAB-; Glycerol): 623 (M^-).

Synthesis of *O*-(5'-*O*-(4,4'-Dimethoxytrityl)thymidine-3'-yl) *O*-(9-Fluorenemethyl) Phosphorodithioate (4). 207 mg (0.25 mmol) **2** were dissolved in 2.5 ml S_8/CS_2/pyridine solution and stirred for 10 min under Ar. The solvents were removed *in vacuo* and purification by silica column flash chromatography followed by precipitation into hexane yielded the pure product **4** as a white powder. Yield: 206 mg (97%); TLC (system III): R_f= 0.18; ^{31}P NMR ($CDCl_3$): δ 112.5; ^1H NMR (CD_3OD): δ 1.75 (s, (CH_3)); 2.74-2.52 (m, 2H, H2'); 3.62-3.94 (m, 2H, H5'); 4.10 (s, 6H, OCH_3); 4.59-4.78 (m, 4H, H4'); 5.97-6.03 (m, 1H, H3'); 6.77-6.81 (dd, J = 6.6 6.5 Hz, 1H, H1'); 7.20 (m, 4H); 7.58-8.19 (m, 18H, H(6)); ^{13}C NMR (CD_3OD): δ 12.2 (CH_3); 40.6 ($C_{2'}$); 56.0 (OCH_3); 65.2 ($C_{5'}$); 69.2 (Fmol); 78.0 ($C_{3'}$); 86.3 ($C_{1'}$); 87.1 ($C_{4'}$); 88.5; 112.1 (C(4)); 114.6; 121.0; 126.8; 128.2; 128.7; 131.7; 137.1 (C(5)); 142.7; 146.3; 152.6 (C(2)); 160.4; 166.6 (C(6)); MS (FAB+; NOBA): 876 (M^++K^+); MS (FAB-; NOBA): 833 (M^-).

Synthesis of *O*-(5'-*O*-(4,4'-Dimethoxytrityl)thymidine-3'-yl) Dithiophosphate Piperidinium Salt (5). 86 mg (0.1mmol) of **4** were dissolved in 5 mL of a 20 % solution of piperidine in CH_2Cl_2 and stirred for 20 min. The solvents were removed *in vacuo,* and the crude product was purified by precipitation into hexane to yield the pure product **5** as a white powder in form of its piperidinium salt. Yield: 70 mg (94%); ^{31}P NMR (pyridine-d5): δ 94.7; ^1H NMR (pyridine-d5): δ 1.09 (br, 4H, H4-piperidine); 1.25 (br, 4H, H3-piperidine); 1.48 (s, 3H, (CH_3)); 2.41-2.44 (m, 1H, H2'); 3.23-3.29 (br, 4H, H2-piperidine); 3.31 (s, 6H, OCH_3); 3.57-3.60 (m, 1H, H2'); 4.76 (m, 1H, H3'); 5.85-5.89 (m, 1H, H5'); 6.64-7.50 (m, 14 H); 8.35 (br, 1H, N-H); ^{13}C NMR (pyridine-d5): δ 12.5 (CH_3); 22.8 (piperidine); 23.1 (piperidine); 41.2 ($C_{2'}$); 44.7 (piperidine); 55.2 (OCH_3); 64.7 ($C_{5'}$); 71.7 ($C_{3'}$); 85.1 ($C_{1'}$); 87.0 ($C_{4'}$); 87.1; 110.9 (C(4)); 113.8; 127.4; 128.4; 128.7; 130.7; 145.7; 151.8 (C(2)); 159.2; 165.0 (C(6)); MS (FAB-; NOBA): 655 (M^-).

Synthesis of *O*-(5'-*O*-(4,4'-Dimethoxytrityl)thymidine-3'-yl) *O*-(9-Fluorenemethyl) Phosphorothioate (6). 200 mg (0.25 mmol) **2** were dissolved in 5 mL of a 0.1 M solution of iodine in THF/pyridine/water (2/2/1) and stirred for 20 min. $Na_2S_2O_3$ solution was added until the iodine color disappeared and stirred for 10 min. After extraction with CH_2Cl_2 (4 x 15 mL) the combined organic phase were washed with 12 mL 5% $NaHCO_3$ and dried over Na_2SO_4. Purification by silica column flash chromatography followed by precipitation into hexane yielded the pure product **6** as a white powder. Yield: 181 mg (93%); TLC (system IV)): R_f= 0.77; ^{31}P NMR ($CDCl_3$): δ 55.65; ^1H NMR (pyridine-d5): δ 1.65 (d, 3H, (CH_3)); 2.70-2.89 (m, 2H, H2'); 3.62 (d, J = 1.0 Hz, 6H, OCH3); 3.65-3.98 (m, 2H, H5'); 4.39-4.84 (m, 4H, Fmol/H4'); 5.47 (m, 1H, H3'); 6.09-6.12 (m, 1H, H1'); 6.92-7.92 (m, 22H); 8.72 (br, 1H, NH); ^{13}C NMR (pyridine-d5): δ 12.3 (CH_3); 39.4 ($C_{2'}$); 49.0; 55.2 (OCH_3); 65.1; 85.2 ($C_{1'}$); 85.8 ($C_{4'}$); 87.5; 111.3 (C(4)); 114.0; 120.5; 125.9; 127.5; 128.1; 128.5; 128.9; 130.8; 137.2 (C(5)); 141.8; 145.1; 145.6; 151.8 (C(2)); 159.4; 164.9 (C(6)); MS (FAB+; Glycerol): 819 (M^+); MS (FAB-; Glycerol): 909 (M^-+Gly); 817 (M^-).

Synthesis of *O*-(5'-*O*-(4,4'-Dimethoxytrityl)thymidine-3'-yl) Thiophosphate Piperidinium Salt (7). **6** (84 mg (0.1mmol)) was dissolved in 5 mL of a 20 % solution of piperidine in CH_2Cl_2 and stirred for 20 min. The solvents were removed *in vacuo* and the crude product was purified by precipitation into hexane to yield the pure product **7** as a white powder in the form of its piperidinium salt. Yield: 69 mg (95%); ^{31}P NMR (CD_3OD): δ 43.84; ^1H NMR (pyridine-d5): δ 1.43 (br, 4H, H4-piperidine); 1.62 (br, 4H, H3-piperidine); 1.78 (s, 3H, (CH_3)); 2.78-2.81 (m, 2H, H2'); 3.32 (m, 4H, H2-piperidine); 3.69 (s, 6H, OCH_3); 3.89-3.93 (m, 1H, H4'); 4.90-5.01 (m, 1H, H3'); 6.02-6.08 (m, 1H, H1'); 6.97-7.92 (m, 14H, DMT/H(6)); 8.72 (br, 1H, NH); ^{13}C NMR (pyridine-d5): δ 12.5 (CH_3); 22.9 (piperidine); 23.2 (piperidine); 39.4 ($C_{2'}$); 44.5 (piperidine); 55.2 (OCH_3); 65.3 ($C_{5'}$); 84.3 ($C_{1'}$); 84.8 ($C_{4'}$); 88.9; 111.7 (C(4)); 113.8; 127.2; 128.7; 131.2; 150.8 (C(2)); 159.5; 165.9 (C(6)); MS (FAB-; NOBA): 639 (M^-).

General Procedure for the Synthesis of *O*-(5'-*O*-(4,4'-Dimethoxytrityl)nucleosid-3'-yl) *O*-(Methyl) H-Phosphonothioates (9a-d). **8a-d** (1 mmol) was dissolved in 100 mL anh. CH_2Cl_2. 10 mL (5 mmol (5 eq.)) of 0.5 M 1-H-tetrazole solution were added and H_2S was bubbled through for 10 min. The mixture was stirred 1 h under Ar. After extraction with 5% $NaHCO_3$ (2 x 15 mL) and drying over Na_2SO_4 the solvent was removed and the crude product was purified by silica column flash-chromatography followed by precipitation from hexane.

O-[(5'-O-(4,4'-Dimethoxytrityl)-6-N-benzoyl-2'-deoxyadenosin-3'-yl] O-(Methyl) H-Phosphonothioate (9a). Yield: 700 mg (90%); TLC (system I): R_f= 0.27; ^{31}P NMR ($CDCl_3$): δ 72.29, 72.24; ^1H NMR ($CDCl_3$): δ 2.64 (dd, J = 8.5 4.4 Hz, 1H, H2'); 3.04 (dd, J =3.6 3.4 Hz, 1H, H2'); 3.43 (s, 3H, P-OCH_3); 3.56-3.66 (m, 2H, H5'); 3.69 (s, 6H, OCH_3); 4.35 (br, 1H, H4'); 6.46 (d, J = 1.4 Hz, H1'); 6.77 (d, J = 8.3 Hz, 4H); 7.12-7.46 (m, 12H, Bz); 7.75 (d, J = 664 Hz, 1H, P-H); 7.95 (d, J = 7.1 Hz, 2H, Bz); 8.15 (d, J = 5.6 Hz, 1H, CH(8)); 9.67 (br, 1H, NH); ^{13}C NMR ($CDCl_3$): δ 40.7 ($C_{2'}$); 52.8 (P-OCH_3); 55.4 (OCH_3); 62.6 ($C_{5'}$); 85.7 ($C_{1'}$); 85.8 ($C_{4'}$); 87.9; 113.7; 127.5; 128.0; 128.4; 129.3; 130.4; 133.0; 133.3; 135.5; 142.0 (C(8)); 144.7; 150.1 (C(4)); 152.0 (C(6)); 152.7 (C(2)); 159.0; 165.5 (CO); MS (FAB+; NOBA): 752 (M^+); MS (FAB-; NOBA): 750 (M^-).

***O*-[(5'-*O*-(4,4'-Dimethoxytrityl)-4-N-benzoyl-2'-deoxycytidin-3'-yl] *O*-(Methyl) H-Phosphonothioate (9b).** Yield: 671mg (92%); TLC (CH_2Cl_2/MeOH=97/3): R_f= 0.32; ^{31}P NMR ($CDCl_3$): δ 72.72, 72.29; ^1H NMR ($CDCl_3$): δ 2.36 (dd, J = 5.4 3.7 Hz, 1H, H2'); 2.85 (dd, J = 3.4 2.9 Hz, 1H, H2'); 3.50

(s, 1H, P-OCH3); 3.66 (m, 2H, H5'); 3.77 (s, 6II, OCH3); 4.35 (d, J = 2.5 Hz, 1H, H4'); 5.40 (m, 1H, H3'); 6.31 (d, J = 4.9 Hz, 1H, H1'); 6.88 (d, J = 7.0 Hz, 4H); 7.23-7.58 (m, 13H, CH(5)); 7.72 (d, J = 674 Hz, 1H, P-H); 7.91 (d, J = 6.6 Hz, 2H, Bz); 8.21 (d, J = 7.6 Hz, 1H, CH(6)); 9.18 (br, 1H, N-H). ^{13}C NMR ($CDCl_3$): δ 40.6 ($C_{2'}$); 52.7 (P-OCH3); 55.5 (OCH3); 62.5 ($C_{5'}$); 76.1 ($C_{3'}$); 85.6 ($C_{1'}$); 85.8 ($C_{4'}$); 87.5; 97.0 (C(5)); 113.7; 127.5; 128.0; 128.2; 129.2; 130.4; 133.4; 135.4; 144.3 (C(6)); 144.5; 155.0 (C(2)); 159.1; 162.8 (CO); 167.2 (C(4)); MS (FAB+; NOBA): 728 (M^+); MS (FAB-; NOBA): 726 (M^-).

***O*-[(5'-*O*-(4,4'-Dimethoxytrityl)-2-N-isobutyryl-2'-deoxyguanosin-3'-yl] *O*-(Methyl) H-Phosphonothioate (9c)**. Yield: 666 mg (91%); TLC (system II): R_f= 0.63; ^{31}P NMR ($CDCl_3$): δ 72.76, 72.60; ^{1}H NMR ($CDCl_3$): δ 1.05 (q, J = 6.8, 6.8, 4.6 Hz, 6II, iBu-CH3); 2.45-2.59 (m, 2H, H2'/CH-iBu); 2.94-2.99 (m, Br, 1H, H2'); 3.32-3.39 (m, br, 1H, H5'); 3.54-3.67 (m, 3H, P-OCH3); 3.71 (s, 6H, OCH3); 4.22 (s, br, 1H, H4'); 5.55 (m, br, 1H, H3'); 6.75 (q, J = 6.8, 2.2, 2.0 Hz, 4H); 7.15-7.39 (m, 9H); 7.65 (d, J = 665 Hz, 1H, P-H); 7.84 (s, 1H, H(8)); 9.67 (d, J = 17 Hz, 1H, N(2)-H); 12.19 (s, br, 1H, N(1)-H); ^{13}C NMR ($CDCl_3$): δ 19.1 (CH3-iBu); 36.3 (iBu); 38.9 ($C_{2'}$); 52.9 (P-OCH3); 55.5 (OCH3); 63.0 ($C_{5'}$); 76.4 ($C_{3'}$); 84.4 ($C_{1'}$); 85.0 ($C_{4'}$); 86.9; 113.5; 121.9 (C(8)); 127.4; 128.3; 130.3; 135.8; 138.1 (C(5)); 144.8; 147.9 (C(2)); 148.1 (C(4)); 156.1 (C(6)); 159.0; 179.8 (CO-iBu); MS (FAB+; NOBA): 734 (M^+); MS (FAB-; NOBA): 732 (M^-).

***O*-(5'-*O*-(4,4'-Dimethoxytrityl)thymidine-3'-yl) *O*-(Methyl) H-Phosphonothioate (9d)**. Yield: 615 mg (96%); TLC (system II): R_f= 0.84; ^{31}P NMR ($CDCl_3$): δ 72.62, 72.22; ^{1}H NMR ($CDCl_3$): δ 1.46 (t, J = 1.9, 0.8 Hz, 3H, CH3); 2.39-2.55 (m, 2H, H2'); 3.45 (m, br, 2H, H5'); 3.62-3.72 (m, 1H, P-OCH3); 3.79 (s, 6H, OCH3); 4.18-4.28 (m, br, 1H, H4'); 5.44-5.50 (m, br, 1H, H3'); 6.41-6.48 (m, br, 1H, H1'); 6.84 (d, J = 7.6 Hz, 4H); 7.24-7.40 (m, 9H); 7.45-7.60 (m, br, 1H, H(6)); 7.74 (d, J = 663 Hz, 1H, P-H); 9.52 (m, br, 1H, NH); ^{13}C NMR ($CDCl_3$): δ 12.9 (CH3); 39.6 ($C_{2'}$); 52.8 (P-OCH3); 55.5 (OCH3); 63.2 ($C_{5'}$); 84.7 ($C_{1'}$); 85.2 ($C_{4'}$); 87.5; 111.9 (C(4)); 113.7; 127.5; 128.4; 130.4; 135.4 ; 144.5; 150.9 (C(2)); 159.1; 164.2 (C(6)); MS (FAB+; NOBA): 639 (M^+); MS (FAB-; NOBA): 637 (M^-).

General Procedure for the Synthesis of O-(5'-O-(4,4'-dimethoxytrityl-nucleosid-3'-yl) H-phosphonothioate Sodium Salts (10a-c, 3). **9a-d** (0.25 mmol) were dissolved in 0.5 mL (2 eq.) of a 1 M solution of 2-carbamoyl-2-cyanoethylene-1,1-dithiolate in DMF. The mixture was stirred for 20 min and the salts were removed by silica column flash chromatography (CH_2Cl_2/MeOH=90/10). The combined product containing fractions were extracted with 5% $NaHCO_3$, dried over Na_2SO_4 and the solvent was removed. The pure product was obtained after precipitation from hexane.

***O*-[(5'-*O*-(4,4'-Dimethoxytrityl)-6-N-benzoyl-2'-deoxyadenosin-3'-yl] H-Phosphonothioate (10a)**. Yield: 185 mg (97%); TLC (system III): R_f= 0.30; ^{31}P NMR (pyridine-d5): δ 52.37, 51.93; ^{1}H NMR (pyridine-d5): δ 2.96-3.15 (m, 1H, H2'); 3.36-3.43 (m, 1H, H2'); 3.74 (s, 6H, OCH3); 3.79-3.95 (m, 1H, H5'); 4.94 (d, J = 58 Hz, 1H, H4'); 5.98-6.17 (m, 1H, H3'); 6.93 (m, 1H, H1'); 6.97-7.05 (m, 4H); 7.31-7.89 (m, 14H); 8.14 (d, J = 0.8 Hz, 1H, CH(8)); 8.41 (d, J = 0.8 Hz); 9.76 (d, J = 9.5 Hz, P-H); 11.72 (s, br, 1H, N-H); ^{13}C NMR (pyridine-d5): δ 37.9 ($C_{2'}$); 53.7 (OCH3); 63.1 ($C_{5'}$); 74.8 ($C_{3'}$); 83.8 ($C_{1'}$); 84.6 ($C_{4'}$); 85.5; 112.3; 125.8; 126.9; 127.3; 127.8; 129.3; 131.2; 141.2 (C(8)); 144.3; 149.2 (C(6)); 151.0 (C(2)); 157.6; 166.3 (CO); MS (FAB+; NOBA): 738 (M^+); MS (FAB-; NOBA): 736 (M^-).

***O*-[(5'-*O*-(4,4'-Dimethoxytrityl)-4-N-benzoyl-2'-deoxycytidin-3'-yl] H-Phosphonothioate (10b).** Yield: 169 mg (95%); TLC (system III): R_f= 0.17; ^{31}P NMR (pyridine-d5): δ 52.60, 51.63 ppm; ^{1}H NMR (pyridine-d5): δ 2.71-2.73 (m, br, 1H, H2'); 3.11-3.28 (m, br, 1H, H2'); 3.57-3.67 (m, br, 1H, H5'); 3.73 (s, 1H, OCH_3); 4.82 (d, J = 65 Hz, 1H, H4'); 6.80 (m, 1H, H1'); 7.05-7.07 (m, 4H); 7.20-7.79 (m, 14H); 8.24 (d, J = 7.4 Hz); 8.50 (t, J = 7.6, 7.3 Hz, 1H, CH(6)); 9.65 (d, J = 4.6 Hz, P-H); ^{13}C NMR (pyridine-d5): δ 39.6 ($C_{2'}$); 53.8 (OCH_3); 62.2 ($C_{5'}$); 73.0 ($C_{3'}$); 84.6 ($C_{1'}$); 85.9; 86.2 ($C_{4'}$); 96.2; 112.5; 127.1; 127.6; 129.1; 131.5; 133.5; 143.1 (C(6)); 143.9; 154.5 (C(2)); 157.8; 162.4 (CO); 167.6 (C(4)); MS (FAB+; NOBA): 714 (M^+); MS (FAB-; NOBA): 865 (M^-+NOBA).

***O*-[(5'-*O*-(4,4'-Dimethoxytrityl)-2-N-isobutyryl-2'-deoxyguanosin-3'-yl] H-Phosphonothioate (10c).** Yield: 169 mg (92%); TLC (system III): R_f= 0.21; ^{31}P NMR (pyridine-d5): δ 52.81, 52.51; ^{1}H NMR (DMSO-d6): δ 1.21 (d, J = 6.6 Hz, iBu-CH_3); 2.49-2.61(m, 1H, H2'); 2.82-2.98 (m, 1H, H2'); 3.25-3.51 (m, 2H, H5'); 3.83 (s, 6H, OCH_3); 4.30 (m, br, 1H, H4'); 5.13 (m, br, 1H, H3'); 6.39 (m, br, 1H, H1'); 6.94 (d, J = 7.6 Hz, 4H, DMT); 7.28-7.48 (m, 9H, DMT); 7.28-7.48 (m, 9H, DMT); 7.96 (d, J = 656 Hz, 1H, P-H); 8.06 (br, 1H, N-H); ^{13}C NMR (DMSO-d6): δ 19.3 (CH_3-iBu); 35.0 (iBu); 38.8 (C2'); 55.2 (OCH_3); 64.1 (C5'); 74.8 (C3'); 82.1 (C1'); 84.5 (C4'); 85.8 (DMT); 113.4 (DMT); 120.0 (C(8)); 125.5 (DMT); 126.9 (DMT); 127.8 (DMT); 128.4 (DMT); 129.9 (DMT); 135.7 (DMT); 137.6 (C(5)); 145.0 (DMT); 149.4 (C(2)); 150.1 (C(4)); 158.3 (DMT); MS (FAB-; NOBA): 718 (M^-).

***O*-(5'-*O*-(4,4'-Dimethoxytrityl)thymidine-3'-yl) H-Phosphonothioate (3).** Yield: 156 mg (97%).

Synthesis of Oligodeoxynucleotide Phosphorodithioates. Oligonucleotide synthesis was performed on controlled pore glass support, with the first nucleotide attached. All syntheses were performed on a 1 μM scale. The coupling cycle outlined in Table 3 was used, after removal of the 5' protecting group with 3% trichloroacetic acid in CH_2CL_2, 5'-DMT nucleoside H-phosphonothioates as a 0.1 M solution in CH_2Cl_2/pyridine (1/1 v/v) and activator, as a 0.1 M solution in CH_2Cl_2/pyridine (95/5 v/v), were delivered simultaneously to the synthesis column for 5 seconds, followed by a 40 s wait. After several washing steps with CH_2Cl_2/pyridine (1/1 v/v), oxidation/protection with a 0.1 M solution of 2,4-dichlorobenzyl thiosuccinimide in CH_2Cl_2 followed. After several washing steps the next cycle began by detritylation. After the complete oligonucleotide sequence was assembled, the 2,4-dichlorobenzyl protecting group was removed by treatment with a 1 M solution of 2-carbamoyl-2-cyanoethylene-1,1-dithiolate in DMF for 16 h, followed by cleavage from the solid support, using conc. ammonium hydroxide at 55°C for 16 h. Purity of the oligomer was assessed by ^{31}P NMR and reverse phase HPLC.

Synthesis of *O*-(Cholesteryl) *O*-(Methyl) H-Phosphonothioate 16b. 336 mg (1 mmol) cholesterol **15** were dissolved in 100 mL anh. CH_2Cl_2 and 193 μL (1 mmol (1 eq.)) methoxy-bisdiisopropylphosphine[30] and 2.7 mL (1.35 mmol (1.35 eq.)) of 0.5 M 1-H-tetrazole solution were added and stirred for 1 h. 10 mL (5 mmol (5 eq.)) of 0.5 M 1-H-tetrazole solution was added and H_2S was bubbled through for 10 min. The mixture was stirred 1 h under Ar. After extraction with 5% $NaHCO_3$ (2 x 15 mL) and drying over Na_2SO_4 the solvent was removed and the crude product was purified by silica column flash-chromatography. Yield: 310 mg (72%); TLC (CH_2Cl_2): R_f= 0.92; ^{31}P NMR ($CDCl_3$): δ 70.25, 70.22; ^{1}H NMR ($CDCl_3$): δ 0.86-2.06 (m, 41H), 2.42-2.50 (m, 2H); 3.78 (d, J = 14.4 Hz, 6H, P-OCH_3); 4.44 (m, 1H); 5.43 (s, 1H); 7.79 (d, J = 649.5 Hz, 1H, P-H); ^{13}C NMR ($CDCl_3$): δ 12.1; 19.3; 21.2; 22.9; 24.3; 28.3; 29.9; 32.1; 36.7; 39.7; 42.5; 50.2; 52.2

(P-OCH_3); 56.6; 123.5; 139.6; MS (FAB+; NOBA): 469 (M^++K^+); MS (FAB-; NOBA): 862 (M^-+M^-); 479 (M^-+K^+).

Synthesis of O-(Cholesteryl) H-Phosphonothioate Sodium Salt 17. **16b** (0.25 mmol) was dissolved in 0.5 mL of a 1 M solution (2 eq.) of 2-carbamoyl-2-cyanoethylene-1,1-dithiolate in DMF. The mixture was stirred for 20 min and the salts were removed by silica column flash chromatography (CH_2Cl_2/MeOH=90/10). The product containing fractions were combined and the solvent was removed. Yield: 79.4 mg (76%); TLC (system III): R_f= 0.87; ^{31}P NMR (pyridine-d5): δ 49.78; ^1H NMR (pyridine-d5): δ 0.84-2.08 (m, 41H); 2.41-2.49 (m, 2H); 4.78 (m, br, 1H); 5.38 (s, br, 1H); 8.76 (d, J = 568.4 Hz, 1H, P-H); ^{13}C NMR (pyridine-d5): δ 11.9; 19.1; 21.2; 22.7; 24.2; 28.3; 32.0; 36.6; 39.7; 42.5; 50.2; 56.5; 75.3; 122.2; 140.7; MS (FAB+; NOBA): 467 (M^+); MS (FAB-; NOBA): 465 (M^-).

Synthesis of 5'-Cholesterol Oligonucleotide Conjugates. General Procedure. Oligonucleotides were synthesized on a 1 μM scale on CPG support using standard protocols. A 0.1 M solution of **17** in CH_2Cl_2/pyridine (1/1 v/v) and a solution of the activator, 0.1 M in CH_2Cl_2/pyridine (95/5 v/v), were delivered simultaneously to the synthesis column, followed by a 40 s wait. Sulfurization with 1.56 M sulfur in CS_2/pyridine/TEA (95/95/10 v/v/v) for 1 h, followed by extensive washes with CS_2 and CH_2Cl_2. Depending on the analog, deprotection and cleavage off the solid support with conc. ammonium hydroxide took 16 h. Purity of the conjugate was assessed by ^{31}P NMR and reverse phase HPLC.

5'-Cholesteryl-T_{10}. ^{31}P NMR (D_2O): δ 112.2 (1 P, cholesterol-O-PS_2-OR), 0.2 (9 P, phosphodiester linkages) ppm.

5'-Cholesteryl-S_2dC_{15}. ^{31}P NMR (D_2O): δ 114.5 (14 P, RO-PO_2-OR), 112.2 (1 P, cholesterol-O-PS_2-OR), 54.5 (2.5 % thioate (contamination).

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REFERENCES AND NOTES

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