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Facile synthesis of a *cis–syn* thymine dimer building block and its incorporation into oligodeoxynucleotides

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Abstract—The synthesis of a building block containing the photobiologically relevant *cis–syn* thymine cyclobutane photoproduct and its incorporation into oligonucleotides by the phosphoramidite-based solid-phase synthesis is reported. Compared to previous syntheses, this route is extremely short and allows such modified oligonucleotides to be easily available for biological studies.

1. Introduction

Cyclobutane pyrimidine dimers (CPD) constitute the most abundant photoproducts generated in DNA upon exposure to UV light.[1] Due to their harmful biological effects, these photolesions have been extensively studied.[2] However, for a long period of time, it has been nearly impossible to unambiguously assess the biological properties of this type of damage since studies were performed on UV-irradiated DNA containing complex mixtures of different types of photodamage.[3a] A considerable knowledge of the molecular and structural biology of CPD repair[4] and translesion synthesis[5] has been gained consecutively to the pioneer work of Taylor who, in 1987, reported the first synthesis of the *cis–syn* thymine dimer-containing phosphoramidite allowing the automated solid-phase synthesis of DNA portions containing a unique CPD at a predefine site.[3a] This work rapidly sparkled several other syntheses that rigorously reproduced Taylor's strategy but differed in the choice of the protecting groups and in the 3′-phosphoramidite nature.[6–9] At present, CPD are still currently the matter of intensive research because of the continuous discovery of biological processes triggered or impaired by this class of damage particularly in the fields of transcription,[7,10] UV-induced signal transduction[11] and immune responses.[12] Nevertheless, all the syntheses of *cis–syn* thymine dimer-containing phosphoramidites developed thus far[3,6–9] require protection/deprotection steps of the precious cyclobutane photoproduct intermediate. An easier access to oligonucleotides harboring, site specifically, the *cis–syn* cyclobutane thymine dimer would dramatically facilitate the investigation of their biological properties.

Herein, we describe the extremely short, efficient and simple synthesis of an alternative phosphoramidite building block containing the *cis–syn* cyclobutane thymine dimer **1** and its successful incorporation into a 10 and a 32mer oligodeoxynucleotide (ODN) by solid-phase synthesis. Our strategy obviates the need to manipulate photoproduct intermediates.

2. Results and discussion

2.1. Synthetic strategy

All the photobiologically relevant *cis–syn* thymine dimer-containing phosphoramidite syntheses hitherto developed only slightly diverge in the choice of the protecting groups and the reaction sequence order (Scheme 1). The strategy makes use of a thymine dinucleotide derivative to be photolyzed (**2a–b**) in which the 5′-hydroxyl function is unsubstituted and the 3′-hydroxyl and internucleoside phosphate groups are protected (Scheme 1). After acetone photosensitization of **2**, the *cis–syn* photoproduct derivative **3** is isolated then 5′-dimethoxytritylated and deprotected at the 3′-end (the order of these two steps can be reversed). The resultant compound is finally converted into its corresponding phosphoramidite **4**. Indeed, a shorter route, consisting to directly photolyze the 5′-dimethoxytritylated derivative **2c** was initially envisioned but the dimethoxytrityl (Dmt) group turned out to be incompatible with the photolysis step.[3a] The necessary protection/deprotection steps of **3a–b**, lead to an overall loss of 40 to 56% of the valuable phosphoramidite **4**.[3a,6] Although the Dmt compatibility was

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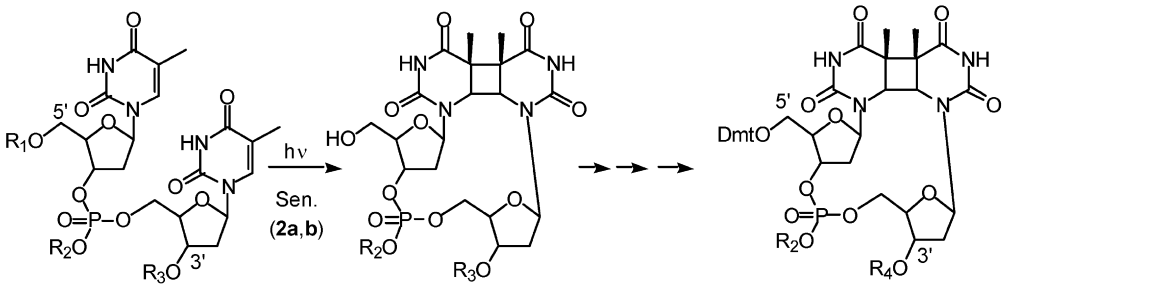


2a: R_1=H, R_2=CH_3, R_3=TBDMS
2b: R_1=H, R_2=C_2H_4CN, R_3=Lev
2c: R_1=Dmt, R_2=CH_3, R_3=H

3a: R_2=CH_3, R_3=TBDMS
3b: R_2=C_2H_4CN, R_3=Lev

4a: R_2=CH_3, R_4=$P(OCH_3)(NC_4H_8O)$[3a]
4b: R_2=CH_3, R_4= $P(OC_2H_4CN)N(CH(CH_3)_2)_2$[3b,8]
4c: R_2=C_2H_4CN, R_4= $P(OC_2H_4CN)N(CH(CH_3)_2)_2$[6]
4d: **4c** with monomethoxytrityl instead of Dmt[7]

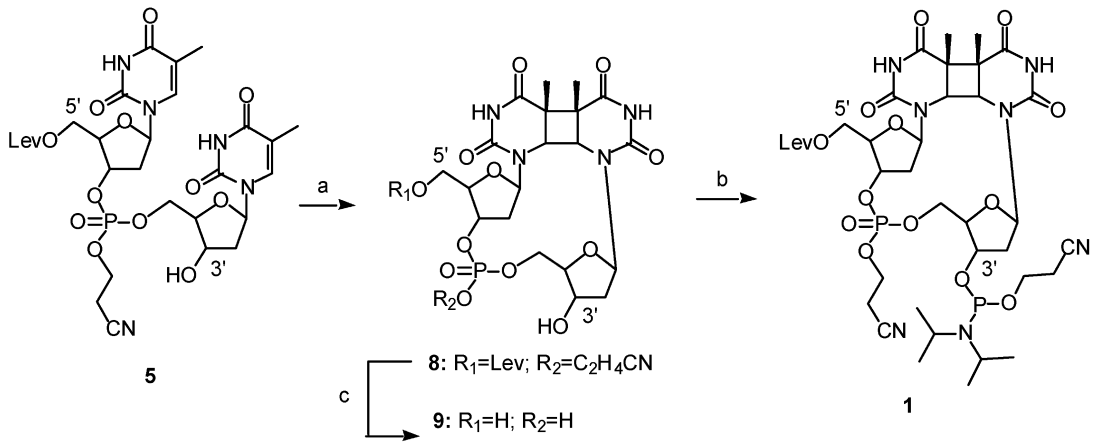
Scheme 1.

later re-evaluated and suggested opposite results,[13,14] all the syntheses of natural CPD building blocks published so far have been achieved by re-introducing the Dmt group after the photolysis step and therefore follow the tedious protection/deprotection procedure of photoproduct intermediates.[3,6–8]

We reasoned that all these difficulties could be avoided by photolyzing, instead of **2**, a thymine dinucleotide derivative (**5**) containing a 5′-hydroxyl protection compatible with the photolysis step and the solid-phase synthesis conditions and a 3′-unprotected alcohol in order to circumvent the protection/deprotection steps before the functionalization of the resulting photoproduct (**8**) into the corresponding phosphoramidite (**1**) (Scheme 2). In addition, for the synthesis of **5**, the 5′-and 3′-protecting groups of each precursor (**6** and **7**) would have to be orthogonal. Finally, for oligonucleotide chain elongation, cleavage of the 5′-protecting group would have to be achieved using conditions respecting the integrity of the cyclobutane photoproduct.

2.2. Synthesis of the new *cis–syn* CPD phosphoramidite 1

The levulinyl (Lev) group was introduced by Iwai and Ohtsuka in 1988 as an alternative 5′-hydroxyl protecting group in the phosphoramidite-based solid-phase synthesis of oligoribonucleotides,[15] and has recently been employed by the group of Cadet to incorporate γ radiation-induced modified nucleosides into ODNs.[16] This protecting group is stable to the photosensitized irradiation conditions and its deprotection under neutral conditions does not interfere with the stability of the cyclobutane photoproduct.[6,7] These characteristics prompted us to use this group at position 5′ of our new phosphoramidite building block **1** assuming that, when at this position, it would not interfere with the photolysis step either through conformational modification or excited state quenching.[3a] In addition, it was also anticipated that the resulting *cis–syn* cyclobutane diastereomers could be easily separated from the *trans–syn* cyclobutane diastereomers. Synthesis of the 5′-levulinylthymidilyl(3′-5′)thymidine 2-cyanoethylphosphotriester **5** was readily achieved using the commercially available phosphoramidite **6** and 3′-*O*-dimethoxytritylthymidine **7**[17] (Scheme 3). Activation of **6** with tetrazole and coupling with **7** afforded the resulting phosphite triester which was oxidized with iodine/THF/lutidine/H_2O to the corresponding phosphate. Then, the 3′-Dmt group was selectively removed by treatment with trifluoroacetic acid in dichloromethane yielding the desired compound **5** in 87% overall yield (three steps). Subsequently, **5** was photolyzed in an aqueous acetonitrile solution using acetone as a photosensitizer.[6,7] Analysis of the ^{1}H NMR (H1′ region) of the crude irradiation mixture showed the total consumption of the starting material together with the formation of several photoproduct derivatives. The two *cis–syn* thymine dimer diastereomers **8**, epimeric at phosphorus, isolated as a 1:1 mixture, were easily separated from the other cyclobutane

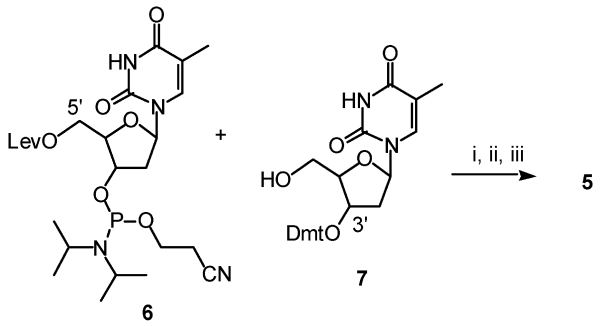


5

8: R_1=Lev; R_2=C_2H_4CN
9: R_1=H; R_2=H

1

Scheme 2. *Reagents*: (a) *hν*, acetone, CH_3CN 30% yield; (b) diisopropylethylamine, chloro-(2-cyanoethyl)-bis-*N*,*N*′-diisopropylphosphoramidite 58% yield; (c) aqueous ammonia.



Scheme 3. *Reagents*: (i) tetrazole; (ii) I_2/THF/lutidine/H_2O; (iii) CF_3COOH/ CH_2Cl_2 87% yield.

diastereomers[18] by preparative reverse-phase HPLC in 30% yield. They were identified by a concentrated-ammonia deprotection of an aliquot which provided a single compound **9** whose ^1H NMR spectrum was identical to that reported for the known *cis–syn* thymine dinucleotide.[19] The mixture of the two *cis–syn* phosphorus diastereomers was then transformed into the corresponding phosphoramidite **1** using Hunig's base and chloro-(2-cyanoethyl)-bis-*N*,*N*′-diisopropylphosphoramidite with 58% yield.

2.3. Synthesis and characterization of oligonucleotides containing T(*cis–syn*)T

The phosphoramidite **1** was used to prepare a 10mer on a 1 μmol scale as a first step using standard phosphoramidites. This decamer sequence was chosen for it and had been previously prepared by UV photosensitization of 5′-d(CGCATTACGC)-3′.[21] We used a modified protocol for the incorporation of **1**. The reaction time for the coupling step was extended to 20 min to ensure chain elongation. A double capping was performed as we previously observed that a single capping following coupling of a bulky building block was not sufficient, affording a significant amount of the 8mer. For chain elongation, we performed an automated levulinyl deprotection on the synthesizer with a solution of hydrazine hydrate for 10 min. Since the efficiency of coupling of the photoproduct could not be measured directly (no Dmt release), we estimated it from subsequent insertion of Dmt-containing phosphoramidite nucleosides as previously reported.[16] The coupling yield of phosphoramidite **1** was estimated to be more than 87% (see Section 3). After chain assembly, the oligonucleotide was simultaneously cleaved from the support and deprotected by treatment with 30% aqueous ammonia at room temperature overnight. The 5′-Dmt-oligomer was purified by reverse-phase HPLC then the Dmt group was removed by treatment with 40% aqueous acetic acid for 2 h. Final purification was performed by reverse-phase HPLC. The quality of the modified oligonucleotide was controlled by HPLC (Fig. 1(A)). Polyacrylamide gel electrophoresis analysis of the 5′-[^{32}P]-labeled fragment indicated a purity of 97% (Fig. 2). To ascertain the identity of the *cis–syn* thymine-containing oligonucleotide, the native 10mer was photolyzed in an aqueous acetone solution.[21] The HPLC chromatogram of the photolysate showed a major peak which was co-eluted with the synthesized ODN (Fig. 1). MALDI MS measurement of the modified synthetic ODN confirmed the incorporation of the *cis–syn* cyclobutane photoproduct (negative mode, *m/z* 2988.7+/−0.5 u). T4 Endonuclease V cleavage of a double-stranded 32mer prepared by ligation of the 10mer containing the *cis–syn* cyclobutane dimer further attested the presence and integrity of the *cis–syn* cyclobutane diastereomer.[22]

To verify if this strategy could also be applied to the synthesis of longer oligonucleotides, a 32mer, 5′-d(GATCTCGGCGACATCGGT[*cs*]TCCGTCCTAACTCG)-3′ was prepared on a 1 μmol scale according to the same procedure. HPLC analysis of the modified 30mer (Fig. 3) and polyacrylamide gel electrophoresis of the 5′-[^{32}P]-labeled fragment (Fig. 2) attested its purity (95%). The MALDI MS of the modified ODN confirmed the success of the synthesis (negative mode, *m/z* 9764.3+/−5 u).

In conclusion, our reported strategy for the synthesis of the new *cis–syn* thymine cyclobutane-containing phosphoramidite **1** represents a significant simplification over the previously known syntheses of CPD-containing building blocks. The elimination of the protection/deprotection steps makes our synthetic pathway much shorter (three steps instead of six from the thymidine phosphoramidite) and also much efficient (yield twice higher) and hence very attractive particularly to biochemists. This new phosphoramidite was successfully used to prepare a 10 and a 32mer ODN containing site specifically *cis–syn* thymine cyclobutane diastereomer. These two examples attest the validity of our strategy since the incorporation efficiency of **1** in ODNs was similar to previously reported synthetic approaches[6,7] evidencing that the necessary solid-phase synthesis protocol modification, inherent to the use of the 5′-levulinyl group, did not alter the effectiveness of the ODN syntheses. In addition, the quality of the modified ODNs was found to be very high after reverse-phase HPLC purification which allows their direct use for biochemical applications. We believe that this building block will contribute to render site specifically UV-damaged oligonucleotides more available to biologists.

3. Experimental

3.1. General

(2-Cyanoethyl)-*N*,*N*′-diisopropylchlorophosphoramidite was obtained from Interchim (Montluçon, France). 5′-*O*-Levulinylthymidine-3′-(2-cyanoethyl)-*N*,*N*′-diisopropylphosphoramidite **6** was obtained from ChemGenes Corporation

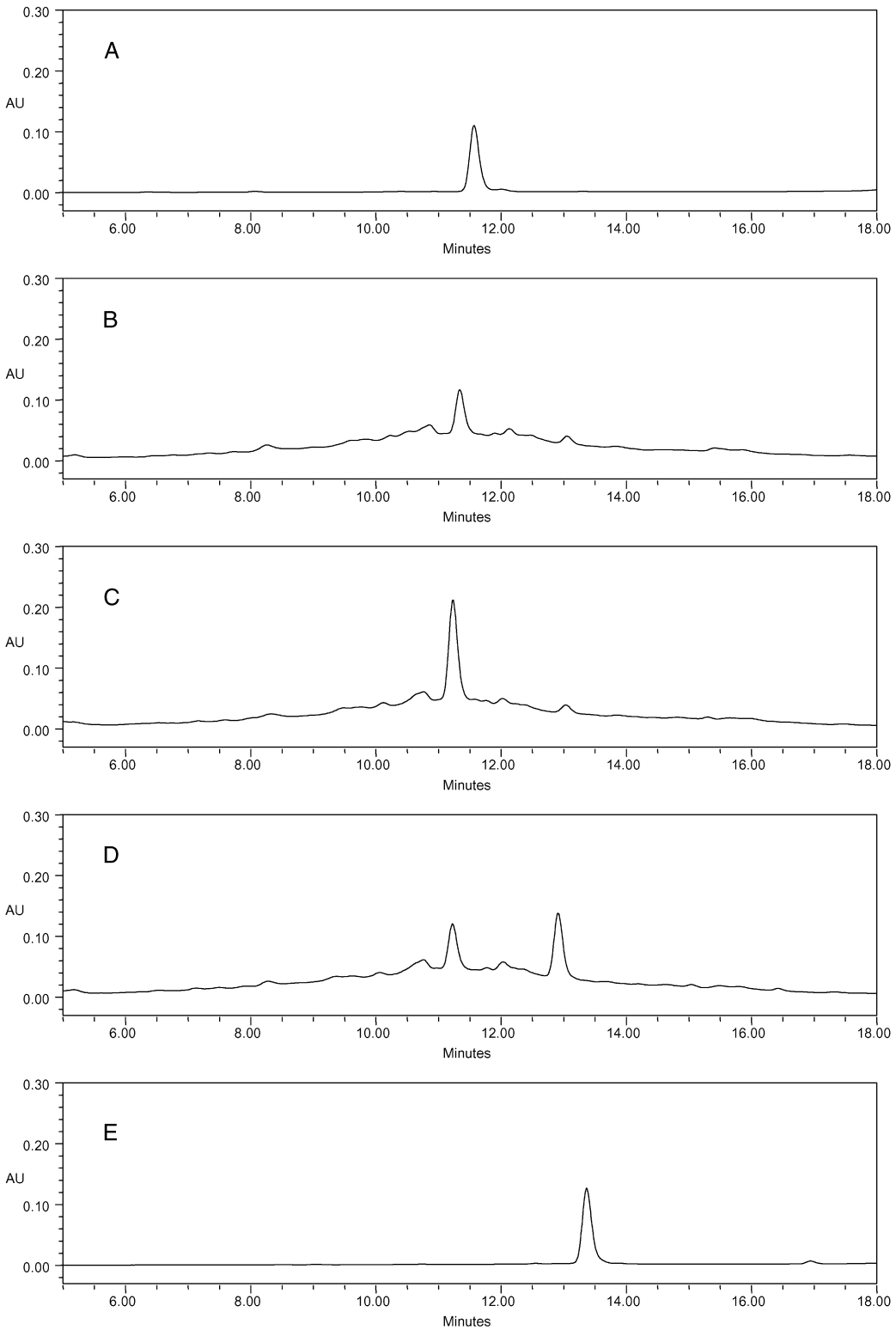


Figure 1. HPLC profile (260 nm) of: (A) d(CGCAT[*cs*]TACGC) obtained by synthesis; (B) photosensitized UV irradiation of d(CGCATTACGC); (C) Co injection of synthetic d(CGCAT[*cs*]TACGC) and photosensitized UV irradiation of d(CGCATTACGC); (D) Co injection of photosensitized UV irradiation of d(CGCATTACGC) and d(CGCATTACGC); (E) d(CGCATTACGC). A Waters Delta-Pak C18 3.9×150 mm (5 μm, 300 Å) column was used with a linear gradient of acetonitrile (5 to 20% during 30 min) in 0.1 M triethylammonium acetate, (pH 7), at a flow rate of 1 mL/min.

(Ashland, MA, USA). 3′-*O*-4,4′-Dimethoxytritylthymidine **7** was prepared as previously described.[17] Compounds **6**, **7** and tetrazole were dried at room temperature in a dessicator over P_2O_5 under vacuum one night prior to use. CH_3CN was dried by heating under reflux with P_2O_5. *N*,*N*′-Diisopropylethylamine and CH_2Cl_2 were dried by distillation from calcium hydride and THF by distillation from sodium/benzophenone. Thin-layer and column chromatography were carried out on silicagel 60 F_{254} 60–15 μm and silicagel 6–35 μm or 35–70 μm respectively, from SDS

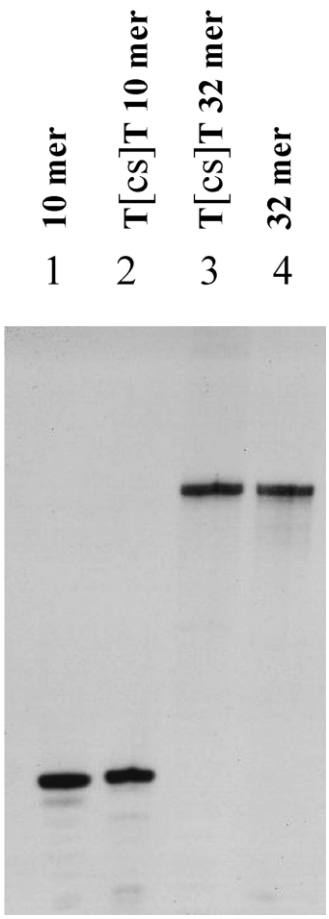


Figure 2. Autoradiogram of a 15% denaturing polyacrylamide electrophoresis gel of the 5′-[^{32}P]-end-labeled 10 and 32mer containing a central *cis–syn* thymine dimer: lane 1, undamaged 10mer; lane 2, 10mer containing the T[*cs*]T photoproduct; lane 3, 32mer containing the T[*cs*]T photoproduct and lane 4, undamaged 32mer.

(Peypin, France). Standard phosphoramidites were purchased from Applied Biosystems (Courtaboeuf, France). ^{1}H, ^{13}C NMR spectra were recorded on Bruker AM300 or AMX400 instruments. ^{1}H Chemical shifts (δ) are reported in ppm relative to residual solvent peak (HOD δ 4.80, MeOD δ 3.30). ^{13}C Chemical shifts are reported in ppm relative to solvent peak (CD_3OD δ 49.0). ^{31}P NMR spectra were recorded on Bruker AM300-P or Avance 600 instruments. Chemical shifts are reported relative to an external capillary standard of 85% phosphoric acid. FABHRMS were carried out using a ZabSpec/T spectrometer (Micromass, Manchester, UK) using a glycerol matrix. Matrix assisted laser desorption ionisation (MALDI) mass spectra were obtained with a Voyager Elite MALDI-TOF mass spectrometer (PerSeptive Biosystems, Framingham, MA, USA) using trihydroxyacetophenone as matrix. Photolysis was conducted in a pyrex immersion well reactor with a Hanau Q81, 80 W high pressure mercury lamp under tap water cooling. Solid-phase DNA synthesis was performed on an Applied Biosystems, 392 DNA/RNA synthesizer. The undamaged 10 and 32mer were synthesized by Eurogentec (Herstal, Belgium).

3.1.1. 5′-*O*-Levulinyl-*P*-(2-cyanoethyl)-thymidilyl(3′-5′)thymidine 5. To a CH_3CN solution (20 mL) of 3′-*O*-(4,4′-dimethoxytrityl)thymidine **7** (168 mg; 0.31 mmol) were added a solution of tetrazole (71 mg; 1.02 mmol) in 10 mL of CH_3CN and 5′-*O*-levulinyl-3′-(2-cyanoethyl)-*N*,*N*′-diisopropylphosphoramidite **6** (250 mg; 0.46 mmol) in 20 mL of CH_3CN under argon. After stirring for 30 min at room temperature, a 0.36 M solution of iodine in tetrahydrofuran-2,6-lutidine-water (2:1:1, v/v/v) (2.5 mL) was added, and the mixture was stirred for 20 min. After evaporation, the residue was diluted with dichloromethane and a saturated sodium thiosulfate solution was added until the solution became colorless, and water was added up to equalization of the two phases. The organic phase was dried with sodium sulfate and concentrated to dryness under reduced pressure. To the residue was added a 3% CF_3CO_2H solution in anhydrous CH_2Cl_2 (2.5 mL). The mixture was stirred for 10 min then coevaporated three times with methanol. The crude product was purified by chromatography using a gradient of methanol in dichloromethane (0–10%) to give compound **5** (87%, 194 mg). ^{1}H NMR (300 MHz, CD_3OD):† δ 7.53 and 7.51 (1H, s, H6 Tp^{a}); 7.47 (1H, s, H6 pT^{a}); 6.26 (1H, t, J=6.8 Hz, H1′ pT); 6.23 (1H, t, J=7.0 Hz, H1′ Tp); 5.12 (1H, m, H3′ Tp); 4.49–4.25 (8H, m, H3′ pT, H4′ Tp^{b}, H5′H5″ pT, H5′H5″ Tp, OCH_2 CH_2CN); 4.08 (1H, s, H4′ pT^{b}); 2.93 (2H, m, OCH_2CH_2CN); 2.82 (2H, m, $OCOCH_2CH_2COMe$); 2.70–2.80 (3H, m, $OCOCH_2CH_2COMe$, H2′ pT^{c}); 2.47 (1H, m, H2″ Tp^{c}); 2.29 (2H, m, H2′H2″ pT); 2.16 (3H, s, $OCOCH_2CH_2COMe$); 1.89 (3H, s, Me pT^{d}); 1.88 (3H, s, Me Tp^{d}). ^{13}C NMR (75 MHz, CD_3OD):† δ 209.6 ($OCOCH_2CH_2COMe$); 174.3 ($OCOCH_2CH_2COMe$); 166.4 (C4 Tp, C4 pT); 152.4 (C2 Tp^{a}); 152.3 (C2 pT^{a}); 138.0 (C6 Tp^{b}); 137.6 (C6 pT^{b}); 118.0 (CN); 112.2 (C5 Tp, C5 pT); 86.7 (C1′ Tp, C1′ pT); 86.1 (C4′ pT^{c}); 84.2 (C4′ Tp^{c}); 80.1 (C3′ Tp); 71.8 (C3′ pT); 69.5 (C5′ pT); 64.7 (C5′ Tp, OCH_2CH_2CN); 40.5 (C2′ pT); 39.1 (C2′ Tp); 38.8 ($COCH_2CH_2COMe$); 29.9

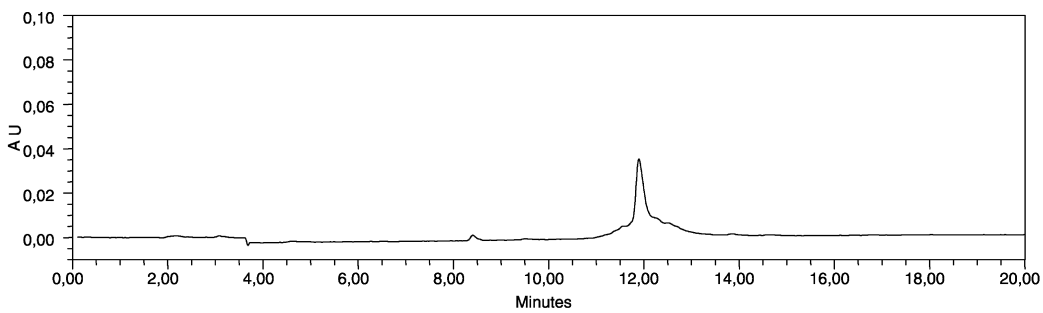


Figure 3. HPLC profile (260 nm) of 5′-d(GATCTCGGCGACATCGGT[*cs*]TCCGTCCTAACTCG)-3′. HPLC conditions are those of Figure 1 except for the linear gradient of acetonitrile (5 to 40% during 30 min).

($OCOCH_2CH_2CO$*Me*) 28.9 ($OCOCH_2$*C*H_2COMe); 20.4 (OCH_2*C*H_2CN); 12.7 (CH_3 pT, CH_3 Tp). ^{31}P NMR (121 MHz, CD_3OD): δ −1.65. HRMS (FAB): $(M+H)^+$ calcd for $C_{28}H_{37}O_{14}N_5P$ 698.2074, found 698.2092.

3.1.2. 5′-*O*-Levulinyl-*P*-(2-cyanoethyl)-T(*cis*–*syn*)T 8. An argon purged 30% aqueous acetonitrile solution (400 mL) of **5** (200 mg, 0.28 mmol) containing 5% of acetone was photolyzed for 4 h. After evaporation, the photoproducts were isolated by preparative reverse phase HPLC, monitored at 230 nm, on a Waters Preppak Cartrige, bondapak C18, 125 Å (15–20 μm), 47×300 mm column with a linear gradient of acetonitrile in water (10 to 50% during 65 min) at a flow rate of 50 mL/min.

Fraction of retention time 54 min was concentrated to dryness affording 60 mg of a colorless product **8** (30% yield). ^1H NMR (400 MHz, D_2O) (H1′ and methyl region):† δ 6.25 (0.5H, t, *J*=6.5 Hz, H1′ pT); 6.06 (0.5H, m, H1′ pT); 5.74 (0.5H, dd, *J*=10.4, 4.2 Hz, H1′ Tp); 5.38 (0.5H, dd, *J*=10.2, 4.2 Hz, H1′ Tp); 5.11 and 5.07 (1H, brs, H3′ Tp); 1.61 (3H, s, CH_3 Tp[a]); 1.54 (3H, s, CH_3 pT[a]). ^{31}P NMR (243 MHz, D_2O): δ −4.02; −4.52. HRMS (FAB): $(M+H)^+$ calcd $C_{28}H_{37}O_{14}N_5P$ 698.2074, found 698.2076.

Structure assignment of **8**. Treatment of the fraction of retention time 54 min with a 30% aqueous ammonia solution at room temperature overnight afforded the completely deprotected photoproduct **9**. ^1H NMR (300 MHz, D_2O):† δ 5.98 (1H, dd, *J*=5.7, 8.8 Hz, H1′ pT); 5.66 (1H, dd, *J*=5.3, 8.6 Hz, H1′ Tp); 4.65 (1H, m, H3′ Tp); 4.33 (2H, m, H6 pT, H3′ pT); 4.25 (1H, d, *J*=5.9 Hz, H6 Tp); 4.19–3.89 (4H, m, H4′ Tp, H4′ pT, H5′ H5″ pT); 3.70 (2H, m, H5′ H5″ Tp); 2.61 (1H, ddd, *J*=13.8, 8.6, 5.3 Hz, H2′ Tp); 2.43–2.25 (2H, m, H2″ Tp, H2′ pT); 2.09 (1H, ddd, *J*=13.6, 5.7, 3.5 Hz, H2″ pT); 1.52 (3H, s, Me Tp); 1.48 (3H, s, Me pT). Data in accordance with Ref. 19.

3.1.3. 5′-*O*-Levulinyl-*P*-(2-cyanoethyl)-T(*cis*–*syn*)T-3′-(2-cyanoethyl)-*N*,*N*′-diisopropylphosphoramidite 1. Photoproduct **8** (100 mg, 0.14 mmol), dried overnight under vacuum over P_2O_5 was dissolved in an anhydrous solution of THF/CH_3CN (1:1) (2 mL) under argon. *N*,*N*′-Diisopropylethylamine (100 μL, 0.57 mmol) was added and the reaction stirred for 30 min at room temperature. Then chloro-(2-cyanoethyl)-bis-*N*,*N*′-diisopropylphosphoramidite (64 μL, 0.28 mmol) was added and the reaction stirred for 10 min. The mixture was then concentrated, diluted with CH_2Cl_2, washed with saturated aqueous $NaHCO_3$, and dried over Na_2SO_4. After evaporation of the solvent, phosphoramidite **1** was purified by chromatography on silica gel eluted with a gradient of acetone in ethyl acetate (0–40%). Compound **1** was obtained in 58% yield (75 mg) as a colorless amorphous solid. ^{31}P NMR (243 MHz, CD_3CN): δ 149.78; 149.63; −4.06.

3.2. Oligonucleotide synthesis

The building block **1** was dissolved in a solution of THF/CH_3CN (1:3) at a concentration of 0.1 M. A modified protocol was used for incorporation of **1**. The coupling time was extended to 20 min and was followed by a double capping. Deprotection of the 5′ levulinyl group was achieved using a 0.5 M solution of hydrazine monohydrate in pyridine/acetic acid (3:2, v/v) for 5 min two times followed by five 30 s acetonitrile washes. Coupling yields were evaluated by the conductimetric measure of the Dmt cation released upon the coupling of the phosphoramidites following incorporation of **1** with respect to the phosphoramidites preceding **1**. In the case of the 10mer, the coupling yield for **1** (position 5) and the following phosphoramidite (A6) with respect to the one preceding **1** (A4) is 87% (two couplings). In the case of the 32mer, the coupling yield for **1** (position 14), the preceding (C13) and the two followings (G15 et G16) with respect to C12 was 91% (four couplings). After chain elongation (DMT ON), the oligonucleotides were cleaved from the CPG support and were deprotected simultaneously by treatment with a 30% aqueous ammonia solution at room temperature for 24 h in the dark. The ammonia was removed by evaporation and tritylated oligonucleotides were purified by reverse phase C-18 chromatography on Waters Prepak Delta-Pak C18 25×100 mm (15 μm, 300 Å) column using a linear gradient of acetonitrile (5 to 40% during 55 min) in 0.1 M triethylammonium acetate, (pH 7), at a flow rate of 3 mL/min and detection at 260 nm. After evaporation, the 5′-Dmt oligonucleotides were treated with 40% aqueous acetic acid for 2 h. After concentration to dryness, deprotected oligonucleotides were purified by reverse phase HPLC on a Waters Delta-Pak C18 3.9×150 mm (5 μm, 300 Å) column using a linear gradient of acetonitrile (5 to 40% during 30 min) in 0.1 M triethylammonium acetate, (pH 7), at a flow rate of 1 mL/min and the effluent was monitored at 260 nm.

3.3. Polyacrylamide gel electrophoresis of the modified ODNs

Oligodeoxynucleotides (10 pmol) were 5′-end-labeled with [γ-^{32}P]-ATP (7 μCi/pmol, 3.57 pmol, ICN) using T4 polynucleotide kinase (10 units, 20 min at 37°C, New England Biolabs) and analyzed by PAGE on a 15% denaturing (8 M urea) polyacrylamide gel (45 V/cm). Oligonucleotides were visualized by autoradiography and quantified by Phosphorimager analysis (Molecular Dynamics).

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† Tp refers to the 5′ terminal residue and pT to the 3′ terminal residue; [a–d]interchangeable attributions within a compound.

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