Synthesis of Oligonucleotides Containing 5-Carboxy-2′-deoxyuridine at Defined Sites

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5-Carboxy-2′-deoxyuridine (**2**) is an oxidized nucleoside that may result from 2-methyl-1,4-naphthoquinone-mediated (menadione) photosensitization of thymidine in aerated aqueous solution.[1] Furthermore, CdU **2** has been detected as a decomposition product of thymidine upon exposure to either 254 nm radiation or HO• radicals.[1] Moreover, Thornburg and his co-workers reported that 5-carboxyuracil is generated through the catalyzed oxidation of thymine residues by thymine hydroxylase in DNA.[2] In another respect, it has been shown that CdU **2** is an inhibitor of the *de novo* pyrimidine biosynthetic pathway involving orotate phosphoribosyl transferase and orotidine 5′-phosphate decarboxylase.[3] It should be noted that other methyl oxidation products of thymidine, including 5-(hydroxymethyl)-2′-deoxyuridine[4] and 5-formyl-2′-deoxyuridine,[5−7] have been recently inserted into synthetic oligonucleotides.

Therefore, it would be important to delineate the possible biological role of CdU **2** when present in DNA. This could be achieved, at least partly, by using modified oligonucleotides as substrates for replication and mutagenesis studies. We report herein an efficient method for the solid-phase synthesis of oligonucleotides containing CdU residues at selected sites.

5-Carboxy-2′-deoxyuridine (**2**) was synthesized by photosensitized oxidation of an aqueous solution of thymidine (**1**) at 350 nm in the presence of 0.1 equiv of menadione (Figure 1). After 16 h of irradiation, which led to the complete degradation of thymidine (**1**), the crude mixture was concentrated under reduced pressure. Then, CdU **2** was purified by preparative anion-exchange liquid chromatography (system A).

The acidic function of CdU **2** has to be protected prior to the chemical incorporation of **2** into DNA fragments. In addition, the presence of the carboxylic group requires that the concentrated aqueous solution of ammonia is substituted by a solution of 0.2 N NaOH/MeOH,[8,9] in order to avoid the formation of an amide function during the deprotection step involving alkali treatment.[10] The ethyl group was chosen for the protection of the carboxylic acid function with regard to the work of Bender[11] on the hydrolysis kinetic of benzoic acid esters in NaOH. This author showed that the rate constant of hydrolysis of ethyl benzoate was similar to that of the half-life 2-isobutyryl-2′-deoxyguanosine[8] (271 min) under the latter conditions. The esterification of the carboxyl group of CdU **2** was achieved without protecting the hydroxyl functions of the sugar moiety using dicyclohexylcarbodiimide and 1-hydroxybenzotriazole in anhydrous ethanol.[12] The expected ester **3** was isolated in 77% yield by silica gel chromatography. The FAB-mass spectrometry analysis of **3** in the positive mode showed a protonated molecule at $m/z = 301 \pm 0.1$ Da. This is indicative of a monoethylated product. Furthermore, the structure was confirmed by NMR analyses. In particular, the ^{1}H-NMR spectrum exhibits two multiplets at 1.38 and 4.36 ppm corresponding to the CH_3 and the CH_2 (ABX_3 system) of the protecting group of the carboxylic function, respectively. It should be noted that the integrity of the base moiety was inferred from the presence of a singlet at 8.96 ppm corresponding to the pyrimidine H_6 proton. This received further support from the presence of a signal at 164.27 ppm in the ^{13}C-NMR spectrum that is characteristic of the carboxyl group of an ester function.

Prior to the preparation of the phosphoramidite synthon of CdU **5**, the stability of the compound **3** was checked at room temperature under two conditions. These included incubation of **3** in 80% acetic acid and a commercially available solution of iodine. These two treatments correspond to the detritylation and the oxidation of the internucleotide link steps, respectively. For this purpose, aliquots of the reaction mixtures were taken up at increasing periods of time and analyzed by RPLC (system C), using thymidine as an internal standard. No detectable degradation of **3** was observed after 24 h of incubation. It should be noted that similar conclusions were reached for CdU **2**. Furthermore, **3** was quantitatively converted into the parent compound, CdU **2** (half-life = 200 min) upon treatment with 0.2 N NaOH/MeOH for 16 h at room temperature.

For the preparation of the phosphoramidite synthon, the ethyl ester of CdU **3** was essentially treated as thymidine. The 5′-*O*-(4,4′-dimethoxytrityl)- **4** and 3′-*O*-(2-cyanoethyl)-*N*,*N*-diisopropylphosphoramidite **5** derivatives were prepared by standard methods[13,14] (Figure 1).

Two oligodeoxynucleotides, namely 5′-d(CCT CTA CXA T) (decamer **6**) and 5′-d(ACC CTG XAT T) (decamer **7**) were then prepared by phosphoramidite solid-phase synthesis on a 1 mmol scale. All coupling yields, including that with the CdU synthon **5**, were higher than 97%. Following the synthesis, the detritylated oligonucleotides were deprotected in a solution of 0.2 N NaOH/MeOH (1:1 v:v) and then purified by RPLC. The separation was achieved by using 25 mM triethylammonium buffer and acetonitrile as the eluent (system D). The homogeneity

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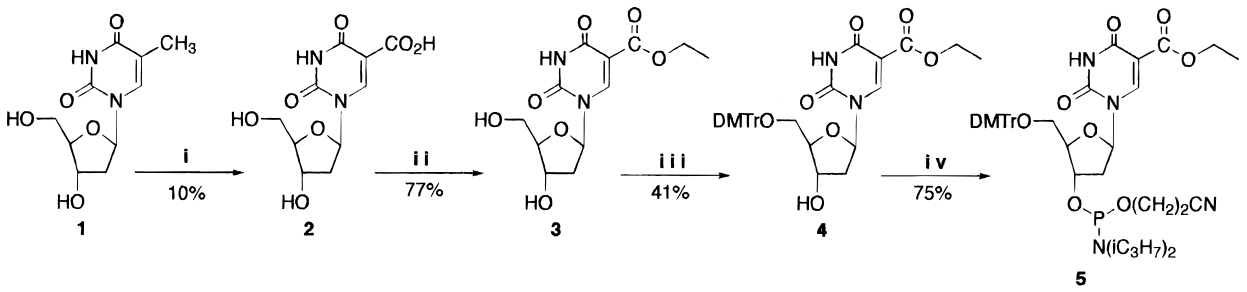

i : Menadione, λ= 365 nm, H_2O, O_2 ; **ii** : DCCI, 1-hydroxybenzotriazole, Bu_3N, EtOH ; **iii** : DMTr-Cl, pyridine ;
iv : N,N'-diisopropylammonium tetrazolate, cyano-2-ethoxy tetra-N,N,N',N'-diisopropyl phosphorodiamidite, CH_2Cl_2, argon.

Figure 1. Synthetic reactions used for the preparation of the phosphoramidite synthon of CdU **2**.

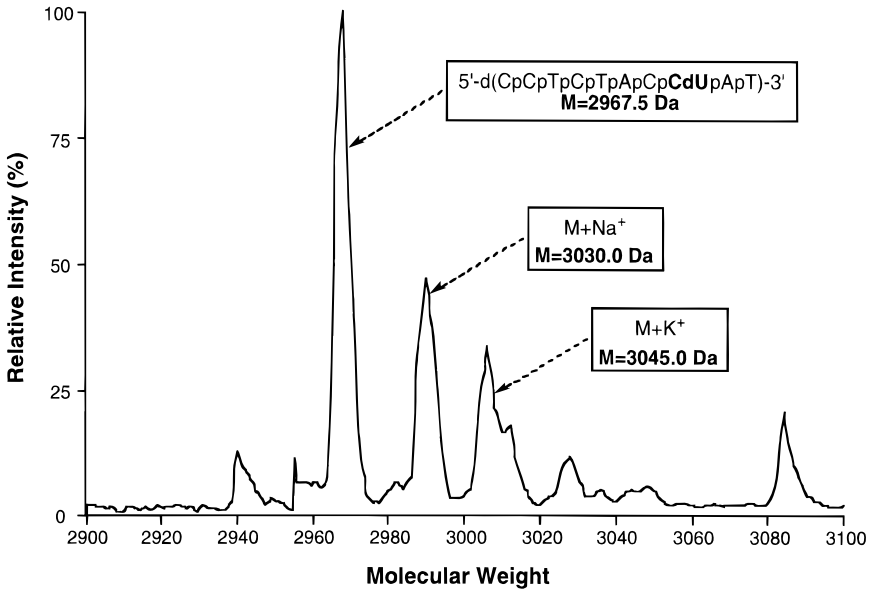

Figure 2. ESI-MS of the decamer **6** deprotected in 0.2 NaOH/MeOH (1:1, v/v) and purified by RPLC (system D).

of the purified oligonucleotides was checked by HPLC on an anion-exchange column (system E).

A fraction of the latter modified oligonucleotides was analyzed by electrospray ionization mass spectrometry. As shown in Figure 2, the mass spectra of the decamers **6** and **7** exhibited the expected molecular peaks at 2967.5 ± 0.5 Da and 3008.0 ± 0.5 Da, respectively. Furthermore, the presence of intact CdU **2** in the synthesized oligonucleotides was confirmed by the analysis of their enzymatic digestion mixtures by Micellar Electrokinetic Chromatography (system F). This was achieved by comparison of the retention time (t_R = 5.43 min) and UV spectroscopic features with those of the authentic CdU **2**.

Conclusion. The synthesis reported herein provides a facile method for the preparation of oligonucleotides containing 5-carboxy-2′-deoxyuridine (**2**) at specific positions. These modified DNA fragments are suitable to study both the biochemical and conformational features of CdU **2** when inserted into DNA fragments.

Materials and Methods

Chemicals. Thymidine (**1**) was purchased from Pharma-Waldhof (Düsseldorf, Germany). 2-Methyl-1,4-naphthoquinone (menadione) and boric acid were from Merck (Darmstadt, FR, Germany). Triethylamine and sodium were obtained from Prolabo (Paris, France). Dicyclohexylcarbodiimide and 1-hydroxybenzotriazole were from Fluka (Buchs, Switzerland). Acetic acid, ethanol, and HPLC-grade methanol were obtained from Carlo Erba (Milan, Italy). Dichloromethane, hexane, chloroform, toluene, and NaOH were from SDS (France). Ammonium formate was from BDM Laboratory Supplies, Poole (U.K.). Buffers for high performance liquid chromatography (HPLC) and capillary electrophoresis (CE) were prepared using water purified with a Milli-Q system (Milford, MA).

High-Performance Liquid Chromatography Separations. CdU **2** was purified by preparative HPLC. Samples were injected onto a preparative column filled with 70 g of Amicon Matrex Silica PAE 300 (40 mm, Pore diameter 300 Å, particle size 50 μm). The eluent was a step gradient of 25 mM triethylammonium acetate buffer at pH = 7 (TEAA) in water, at an elution pressure of 4 bars. Compounds were detected at 260 or 300 nm (System A).

HPLC was performed using an injector loop of 20 μL for the analytical experiments and 500 μL for the purification steps. Several chromatographic systems were used. System B: reversed-phase high performance liquid chromatography in the ion suppression mode (RPLC); column, Merck LiChrocart LiChrospher 100RP-18e (5 μm, 125 × 4 mm); eluent, 25 mM TEAA/acetonitrile (96:4 v:v) at a flow rate of 1 mL/min. System C: RPLC; column, Merck LiChrocart LiChrospher 100RP-18e (5 μm, 125 × 4 mm); eluent, 25 mM TEAA/methanol (95:5, v:v) at a flow rate of 1 mL/min. System D: RPLC in the ion suppression mode; column, Merck LiChrocart LiChrospher 100RP-18e (5 μm, 125 × 4 mm); eluent, a gradient of 0−12.5% of acetonitrile in 25 mM TEAA at a flow rate of 1 mL/min. System E: column, anion-exchange Partisil P10 SAX 25F (5 μm, 250 × 4 mm); eluent, a gradient of 20−70% of 0.2 M $NaHPO_4$ buffer (pH 6.7) containing 30% of acetonitrile in a mixture of H_2O/acetonitrile (70:30, v:v) at a flow rate of 1 mL/min.

k' represented the capacity factor of the different products in the conditions described previously, and t_R is the abbreviation for the retention time.

Micellar Electrokinetic Chromatography Analysis. For micellar electrokinetic chromatography (MEKC) analyses, an untreated fused-silica capillary of 57 cm total length × 75 μm i.d. (375 μm o.d.) was used with the detection window located at a distance of 50 cm. The column temperature was maintained at 25 °C during the analysis. The samples were hydrodynamically injected during 1 s (~5 nL). The separation was achieved using a 50 mM sodium borate/25mM SDS buffer adjusted at pH 10.0 ± 0.1 (system F) with a 1 N NaOH solution. The instrument was set at a fixed voltage of 25 kV leading to a constant current of 78 μA. UV data (214 nm or diode array detector) were collected at a rate of 10 points per s.

5-Carboxy-2′-deoxyuridine (2). To 1 L of a 5 mM aqueous solution of thymidine (**1**; 1.21 g) was added 13 mg of menadione (0.5 mM, 0.1 equiv). The resulting solution was exposed to 16 350 nm black lamps (approximatively 4.5 W each with 90% of the emitted light in the 350 nm range) in a Rayonet photochemical reactor at room temperature. A continuous flow of air maintained the solution saturated with oxygen during the irradiation. After the degradation of thymidine (**1**) was completely achieved as controlled by RPLC (system B: k'(dT) = 10.52; k'(CdU) = 2.10), the reaction mixture was concentrated under reduced pressure. The overall process was resumed 10 times in order to photooxidize a total of 12.1 g of thymidine (**1**). CdU **2** was purified by preparative liquid chromatography (system A), and the collected fractions were controlled by analytical RPLC (system A). The appropriate fractions ($20 < k' < 33$) were pooled and then lyophilized. The resulting residue was dissolved in water (100 mL), and the solution was desalted through a column of Dowex 50 (H^+) resin. The acidic fractions that showed a positive 2′-deoxyribonucleoside test were pooled and concentrated under reduced pressure and then lyophilized. 5-Carboxy-2′-deoxyuridine (**2**) (2.04 g, 7.5 mmol) was obtained (yield 15%). UV (λ_{max}; H_2O) 272 nm (e = 9714 $M^{-1}\cdot cm^{-1}$). FAB-MS positive mode: $[M + H]^+ = 273 \pm 0.1$ Da; $[B + 2H]^+ = 157 \pm 0.1$ Da; $[dR]^+ = 117 \pm 0.1$ Da. ESI-MS negative mode: $[M - H]^- = 271 \pm 0.1$ Da. ^{1}H-NMR (499.838 MHz; D_2O) δ: 8.78 (s, 1H, H-6); 6.41 (t, $J_{1',2'}$ = 6.7 Hz, $J_{1',2''}$ = 6.5 Hz, 1H, H-1′); 4.60 (m, $J_{2',3'}$ = 6.7 Hz, $J_{2'',3'}$ = 4.1 Hz, $J_{3',4'}$ = 3.8 Hz, 1H, H-3′); 4.17 (m, $J_{4',5'}$ = 3.5 Hz, $J_{4',5''}$ = 5.1 Hz, 1H, H-4′); 3.96 and 3.89 (m, $J_{5',5''}$ = −12.5 Hz, 2H; H-5′ and H-5″); 2.55 and 2.51 (m, $J_{2',2''}$ = −14.2 Hz, 2H, H-2′ and H-2″). ^{13}C-NMR (100.62 MHz; D_2O) δ: 184.3 (*C*OOH); 163.8 (C-4); 151.1 (C-2); 146.6 (C-6); 108.3 (C-5); 87.1 (C-4′); 86.2 (C-1′); 70.3 (C-3′); 61.1 (C-5′); 39.3 (C-2′).

5-Carboxyethyl-2′-deoxyuridine (3). CdU **2** (1.983 g, 7.28 mmol), 0.983 mg of 1-hydroxybenzotriazole (7.28 mmol), and 1.73 mL of tributylamine (7.28 mmol) were dissolved in 200 mL of anhydrous ethanol that was previously desiccated with sodium and then distilled. To the resulting solution was added 1.802 g of dicyclohexylcarbodiimide (8.736 mmol), and the reaction was kept overnight at room temperature. After a TLC control ($CHCl_3/CH_3OH$; 80:20) of the reaction mixture, the solution was evaporated under reduced pressure and then coevaporated twice with chloroform. The resulting residue was then purified by flash-chromatography on a Merck Kieselgel silica gel column PF_{254} (200 g). The mobile phase was a step gradient of 0−5% methanol in chloroform. The appropriate fractions were concentrated to dryness. 5-(Carboxyethyl)-2′-deoxyuridine (**3**) 1.685 g, 5.61 mmol) was obtained (yield 77%). FAB-MS positive mode: $[M + Na]^+ = 323.2 \pm 0.1$ Da; $[M + H]^+ = 301.2 \pm 0.1$ Da; $[BH + Na]^+ = 207.2 \pm 0.1$ Da; $[B + 2H]^+ = 185.2 \pm 0.1$ Da. ^{1}H-NMR (400.135 MHz; D_2O) δ: 8.97 (s, 1H, H-6); 6.32 (t, $J_{1',2'}$ = 5.4 Hz, $J_{1',2''}$ = 6.7 Hz, 1H, H-1′); 4.55 (m, $J_{2',3'}$ = 6.6 Hz, $J_{2'',3'}$ = 5.1 Hz and $J_{3',4'}$ = 4.4 Hz, 1H, H-3′); 4.38 and 4.35 (m, J_{a-CH_3} = 7.2 Hz, J_{b-CH_3} = 7.1 Hz, $J_{a,b}$ = −10.7 Hz, 2H, CH_2CH$_3$); 4.15 (m, $J_{4',5'}$ = 3.2 Hz, $J_{4',5''}$ = 4.4 Hz, 1H, H-4′); 3.97 and 3.86 (m, $J_{5',5''}$ = −12.6 Hz, 2H; H-5′ and H-5″); 2.58 and 2.49 (m, $J_{2',2''}$ = −14.3 Hz, 2H, H-2′ and H-2″); 1.39 (m, 3H, CH_3CH$_2$). ^{13}C-NMR (100.614 MHz; D_2O) δ: 164.3 (*C*OOEt); 162.7 (C-4); 149.0 (C-6); 151.0 (C-2); 104.2 (C-5); 87.0 (C-4′); 86.5 (C-1′); 69.7 (C-3′); 62.0 (*C*H$_2$-CH$_3$); 60.4 (C-5′); 39.6 (C-2′); 13.4 (*C*H$_3$CH$_2$).

5′-*O*-(4,4′-Dimethoxytrityl)-5-(carboxyethyl)-2′-deoxyuridine (4). 5-(Carboxyethyl)-2′-deoxyuridine (**3**) (496 mg, 1.65 mmol) was dissolved in dry pyridine (10mL) and evaporated to dryness. The operation was repeated twice. The resulting oily residue was dissolved in 14 mL of dry pyridine, and 4,4′-dimethoxytrityl chloride (671 mg, 1.98 mmol) was added. The mixture was left at room temperature for 2 h. The reaction was checked for completion by TLC ($CHCl_3/CH_3OH$, 95:5). The solution was cooled in an ice bath, and then ethanol (1 mL) was added. After 10 min, the mixture was dissolved in 50 mL solution of ethanol/toluene (3:1 v/v) and evaporated to almost dryness. Two other coevaporations with the latter solution were performed, and finally the residue was again coevaporated with dichloromethane. The residue was dissolved in 40 mL of dichloromethane and washed with 5% $NaHCO_3$ (2 × 50 mL) and water (2 × 50 mL). The organic layer was dried over Na_2SO_4 and evaporated to dryness. Then, the residue was coevaporated twice with ethanol and finally with chloroform. The resulting residue was then purified by flash chromatography on a Merck Kieselgel silica gel column PF_{254} (60 g) with a step gradient of 0−5% ethanol in chloroform as the mobile phase. The appropriate fractions were pooled and then concentrated to dryness giving 408 mg (0.67 mmol) of 5′-*O*-(4,4′-dimethoxytrityl)-5-(carboxyethyl)-2′-deoxyuridine (**4**, yield 41%). FAB-MS positive mode: $[MNa_2]^+ = 647.7 \pm 0.1$ Da; $[M + Na]^+ = 625.7 \pm 0.1$; $[M + H]^+ = 602.7 \pm 0.1$ Da; $[DMT]^+ = 303.4 \pm 0.1$ Da; $[BH + Na]^+ = 207.3 \pm 0.1$ Da; $[BH + H]^+ = 185.3 \pm 0.1$ Da. ^{1}H-NMR (400.135 MHz; CD_2Cl_2) δ: 8.50 (s, 1H, H-6); 7.44−6.82 (m, 13 H, aromatic-H); 6.20 (t, $J_{1',2'}$ = 7.0 Hz, $J_{1',2''}$ = 5.9 Hz, 1H, H-1′); 4.39 (m, $J_{2',3'}$ = 6.4 Hz, $J_{2'',3'}$ = 3.5 Hz, $J_{3',4'}$ = 3.5 Hz, 1H, H-3′); 4.06 (m, $J_{4',5'}$ = 4.2 Hz, $J_{4',5''}$ = 4.2 Hz, 1H, H-4′); 3.99 and 3.86 (m, J_{a-CH_3} = 7.2 Hz, J_{b-CH_3} = 7.2 Hz, $J_{a,b}$ = −10.81 Hz, 2H, CH_2-CH$_3$); 3.77 (s, 6H, CH_3O); 3.41 and 3.35 (m, $J_{5',5''}$ = −10.5 Hz, 2H; H-5′ and H-5″); 2.48 and 2.23 (m, $J_{2',2''}$ = −13.8 Hz, 2H, H-2′ and H-2″); 1.06 (m, 3H, CH_3CH$_2$). ^{13}C-NMR (100.62 MHz; CD_2Cl_2) δ: 162.1 (*C*OOEt); 158.92 (C-4); 158.9 (C-4, -4′ of DMT); 149.4 (C-2); 146.7 (C-6); 144.8 (C-1″ of DMT); 135.8 and 135.7 (C-1, C-1′ of DMT); 130.4 and 130.2 (C-2, C-2′, C-6, C-6′ of DMT); 128.3−128.1 (C-2″, C-3, C-5″, C-6″ of DMT); 127.1 (C-4″ of DMT); 113.4−113.2 (C-3, C-3′, C-5, C-5′ of DMT); 105.7 (C-5); 87.5 ((MeOPh)$_2$Ph*C*O); 86.8 (C-4′); 86.27 (C-1′); 72.3 (C-3′); 63.5 (C-5′); 61.1 (*C*H$_2$-CH$_3$); 55.4 (*C*H$_3$O); 41.2 (C-2′); 14.0 (*C*H$_3$CH$_2$).

5-Carboxy-2′-deoxyuridine Phosphoramidite Derivative 5. 5′-*O*-(4,4′-Dimethoxytrityl)-5-(carboxyethyl)-2′-deoxyuridine (compound **4**; 0.57 mmole) and diisopropylammonium tetrazolate (0.28 mmol) were dissolved in dry dichloromethane (5 mL) and evaporated to dryness. The operation was repeated twice. The solid residue was then dissolved in dry dichloromethane (2.3 mL) and kept under an argon atmosphere. Subsequently, (cyanoethyl)bis(diisopropylamino)phosphine (200 μL, 0.628 mmol) was added with a syringe through a rubber septum. The reaction mixture was stirred during 35 min. The formation of the desired product was checked by TLC. After dilution in ethyl acetate (20 mL), the mixture was concentrated to dryness. The resulting residue was taken up in ethyl acetate (10 mL) and washed successively with 5% $NaHCO_3$ (2 × 50 mL) and a saturated solution of NaCl (2 × 50 mL). Then, the organic layer was evaporated to dryness. The dry residue was purified by flash-chromatography on a Merck Kieselgel silica gel column PF254 (30 g) with a step gradient of 0−5% AcOEt in dichloromethane/triethylamine (99:1) as the mobile phase. The appropriate fractions were pooled and then concentrated to dryness. The product was dissolved in dry dichloromethane (1 mL) and subsequently precipitated at −78 °C in hexane (40 mL). Filtration gave a white powder corresponding to the phosphoramidite synthon of CdU **5**. The building block was dried under reduced pressure in a desiccator, and then stored under dry argon. Under these conditions, 362 mg (0.45 mmole) of **5** were obtained (yield 79%). FAB-MS positive mode: $[M + K]^+ = 841.0 \pm 0.1$ Da; $[M + Na]^+ = 825.8 \pm 0.1$ Da; $[M + H]^+ = 803.8 \pm 0.1$ Da; $[DMT]^+ = 303.2 \pm 0.1$ Da. In addition, the exact mass measurement of the pseudomolecular ion $[M + Na]^+$ (m/z = 825.3272; D = 3.7 ppm) was inferred from a high-resolution FAB-MS analysis. This is indicative of an empirical formula of $C_{42}H_{51}N_4O_{10}PNa$. ^{1}H-NMR (200.15 MHz; CD_2Cl_2) two diastereomers δ: 8.52 and 8.47 (s, 1H, H-6); 7.43−6.79 (m, 13 H, aromatic-H); 6.18 (t, 1H, H-1′); 4.45 (m, 1H, H-3′); 4.16 (m, 1H, H-4′); 3.9 (m, 2H, CH_2CH$_3$); 3.64 (s, 6H, CH_3O); 3.39 (m, 3H, C*H*iPr and CH_2OP); 3.28−3.23 (m, 2H; H-5′ and H-5″); 2.55 (m, 1H, H-2″); 2.41 (t, 2H, −CH_2CN); 2.21 (m, 1H, H-2′); 1.41−1.03 (m, 15H, CH_3iPr and CH_3CH$_2$). ^{31}P-NMR (101.21 MHz; CD_2Cl_2) δ: 149.63 and 149.37 (two diastereomers).

General Coupling Procedures for the Assembly of Oligonucleotides. The preparation of oligonucleotides containing-CdU was performed on a DNA synthesizer using a 1 μmol protected nucleoside functionnalized on a chemical modified CPG polymer support. For optimal coupling efficiency, the flow rate delivery of reagents was fixed at 2.3 mL/min. Synthesized amidite **5** and commercially available amidites were used in 0.08 M dry acetonitrile solution. Standard 0.2 μmol scale synthesis cycles were used. The coupling yield of the CdU unit **5** was 97% estimated by quantitation of the dimethoxytrityl cation before and after the CdU cycle.

Removal of the Oligomer from the CPG Beads, Deprotection, and Purification. The oligonucleotides bound to the support were treated with 0.2 N NaOH/MeOH (1:1 v/v) solution. Then, the collected fractions were neutralized with 1 M aqueous acetic acid, and the resulting solutions were evaporated to dryness. The decamers **6** and **7** were further purified by RPLC/system D (t_R(decamer **6**) = 40.2 min; t_R(decamer **7**) = 41.51 min) and the homogeneity of the purified decamers was checked by HPLC/system E (t_R(decamer **6**) = 51.4 min; t_R(decamer **7**) = 55.05 min).

Decamer **6** [5′-d(CCT CTA CXA T)-3′]: MW = 2967.5 ± 0.5 Da. Decamer **7** [5′-d(ACC CTG XAT T)-3′]: MW = 3008.0 ± 0.5 Da.

Enzymatic Digestion of Decamers 6 and 7. 0.1 $AUFS^{260nm}$ of purified decamer **6** and decamer **7** were taken up in 30 μL of 0.3 M sodium acetate buffer. Nuclease P_1 (10 μL, 1 mg) was added, and the resulting mixture was incubated at 37 °C for 1 h. Subsequently, 33 μL of Tris−HCl/$MgCl_2$ buffer (pH = 9) and 20 U of alkaline phosphatase were added, and the resulting mixture was incubated for 2 h at 37 °C. After lyophilization, the residues obtained were taken up in 1 mL of running buffer and analyzed by MEKC/system F. The UV detector was set at 214 nm (t_R(dC) = 3.15 min; t_R(dA) = 3.29 min; t_R(dT-**1**) = 3.73 min; t_R (Nuclease P1) = 3.87 min; t_R(dG) = 4.06 min; t_R(CdU-**2**) = 5.43 min).

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Supporting Information Available: Copies of ^{1}H-,^{13}C-NMR and FAB mass spectra of **2**−**4**, UV spectrum of **2**, ^{31}P-NMR and FAB mass spectra of **5**, and electrospray ionization mass spectrum of decamer **7** (15 pages). This material is contained in libraries on microfiche, immediately follows this article in microfilm version of the journal, and can be ordered from the ACS; see any current masthead page for ordering information.

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