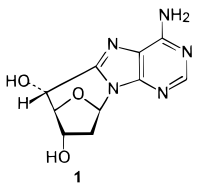
Site-Specific Introduction of (5′*S*)-5′,8-Cyclo-2′-deoxyadenosine into Oligodeoxyribonucleotides

Anthony Romieu, Didier Gasparutto, Didier Molko, and Jean Cadet*

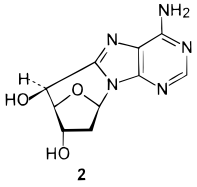
Département de Recherche Fondamentale sur la Matière Condensée, SCIB/Laboratoire des lésions des Acides Nucléiques, CEA Grenoble 17, avenue des Martyrs/F-38054 Grenoble Cedex 9 France

Received January 16, 1998 (Revised Manuscript Received April 24, 1998)

5′,8-Cyclo-2′-deoxyadenosine (CyclodAdo) is one of the main radiation-induced decomposition products of 2′-deoxyadenosine (dAdo).[1] The formation of CyclodAdo is explained in terms of intramolecular attack of the C-8, N-7 double bond of the purine base by the C-5′ carbon radical, produced by the action of •OH radicals formed by the radiolysis of water.[2] The reaction is highly stereoselective since only the (5′*R*)-diastereomer **1** is



1



2

generated, at least, in detectable amount. On the other hand, the radiation-induced decomposition of 5′-AMP led to the (5′*S*)-diastereomer.[2,3] CyclodAdo, which may be considered as a tandem DNA lesion, the 2-deoxyribose and the adenine residues being both altered, is likely to have a significant biological impact. To assess the conformational changes and the biochemical features associated with the formation of CyclodAdo in DNA, it is a requisite to prepare oligonucleotides that contain this double lesion at defined sites.

We report herein the first site-specific incorporation of (5′*S*)-CyclodAdo **2** into oligonucleotides using the phosphoramidite chemistry. Prior to the preparation of the phosphoramidite synthon **12**, the stability of CyclodAdo was checked at room temperature under the three main experimental conditions used during the solid support synthesis. These include treatments with 32% aqueous ammonia, 80% acetic acid, and a commercial oxidizing solution of iodine. Authentic samples of (5′*R*)-CyclodAdo **1** and (5′*S*)-CyclodAdo **2**, obtained by photoreaction of a deaerated aqueous solution of 8-bromo-2′-deoxyadenosine at 254 nm in 12% and 2% yields, respectively, were used for these stability studies.[4] Aliquots of the reaction mixtures were taken up at increasing periods of time and analyzed by reverse phase HPLC (system B). No detectable degradation including epimerization at C-5′ of CyclodAdo was observed after 24 h of incubation in the three reactional solutions.

The preparation of the phosphoramidite synthon **12** has required the development of an efficient method for the synthesis of CyclodAdo. The above-mentioned photochemical method for the synthesis of CyclodAdo is not applicable to preparative purpose. In particular, the yield (roughly 15% for the two diastereomers) was rather unsatisfactory for the purpose of a large scale preparation. Due to the unusual structure of cyclonucleosides with two secondary alcohol functions within the sugar moiety, the selective introduction of the 4,4′-dimethoxytrityl (DMTr) group on the 5′-OH residue represents a difficult challenge. This has to be taken into consideration in the strategy adopted for the synthesis of CyclodAdo. A survey of the literature revealed a variety of chemical and photochemical methods for the preparation of cyclonucleosides, particularly in the RNA series.[5] The methodology developed by Matsuda et al.[6,7] for the synthesis of (5′*S*)-5′,8-cyclopurine nucleosides was chosen. In fact, the radical intramolecular cyclization proceeded in good yields. In addition, the postfunctionalization of C-5′ by the oxidation−reduction procedure worked out the issue of the selective introduction of the DMTr group. The synthesis of the targeted phosphoramidite **12** (Scheme 1) required for the incorporation of compound **2**, started with N^6-benzoyl-2′-deoxyadenosine **3**.[8] The latter nucleoside was converted into the 5′-thiophenyl derivative **5** via the tosyl precursor **4**.[9] This was accomplished by treating **3** with tosyl chloride in pyridine at −20 °C. Compound **4** was then reacted with thiophenol in the presence of sodium methylate under refluxing methanol, giving nucleoside **5** in a moderate yield (42% from compound **3**). The key intermediate, N^6-benzoyl-5′,8-cyclo-2′,5′-dideoxyadenosine **6**, was synthesized by far-UV irradiation of a deaerated acetonitrile solution of **5** in the presence of 10 equiv of triethyl phosphite, a trapping agent for the released phenylthiyl radical.[10] After 10 h of irradiation, which led to the complete degradation of **5**, the anhydronucleoside **6** was obtained by crystallization from hot methanol in 51% yield. To avoid side reactions from occurring during the oxidation step and the reaction with DMTr chloride during the tritylation step, the 3′-OH residue was protected with a *tert*-butyldimethylsilyl (TBDMS) group.[11] Thus, **6** was treated with TBDMS chloride in DMF in the presence of imidazole for 20 h at room temperature. The expected TBDMS ether **7** was isolated in 79% yield by silica gel chroma-

* To whom correspondence should be addressed. Phone: (33) 4-76-88-49-87. Fax: (33) 4-76-88-50-90. E-mail: cadet@drfmc.ceng.cea.fr.

(1) Mariaggi, N.; Cadet, J.; Téoule, R. *Tetrahedron* **1976**, *32*, 2385−2387.

(2) Cadet, J.; Berger, M. *Int. J. Radiat. Biol.* **1985**, *47*, 127−143.

(3) Raleigh, J. A.; Fuciarelli, A. F. *Radiat. Res.* **1985**, *102*, 165−175.

(4) Combat, F.; Berger, M.; Cadet, J. Unpublished results.

(5) For examples: (a) Harper, P. J.; Hampton, A. *J. Org. Chem.* **1972**, *37*, 795−797. (b) Duong, K. N. V.; Gaudemer, A.; Johnson, M. D.; Quillivic, R.; Zybler. J. *Tetrahedron Lett.* **1975**, *34*, 2997−3000. (c) Matsuda, A.; Tezuka, M.; Ueda, T. *Tetrahedron* **1978**, *34*, 2449−2452. (d) Sugawara, T.; Otter, B. A.; Ueda, T. *Tetrahedron Lett.* **1988**, *29*, 75−78. (e) Haraguchi, K.; Tanaka, H.; Saito, S.; Kinoshima, S.; Hosoe, M.; Kanmuri, K.; Yamaguchi, K.; Miyasaka, T. *Tetrahedron* **1996**, *52*, 9469−9484.

(6) Matsuda, A.; Muneyama, K.; Nishida, T.; Sato, T.; Ueda, T. *Nucleic Acids Res.* **1976**, *3*, 3349−3357.

(7) Matsuda, A.; Tezuka, M.; Niizuma, K. Sugiyama, E.; Ueda, T. *Tetrahedron* **1978**, *34*, 2633−2637.

(8) Ti, G. S.; Gaffney, B. L.; Jones, R. A. *J. Am. Chem. Soc.* **1982**, *104*, 1316−1319.

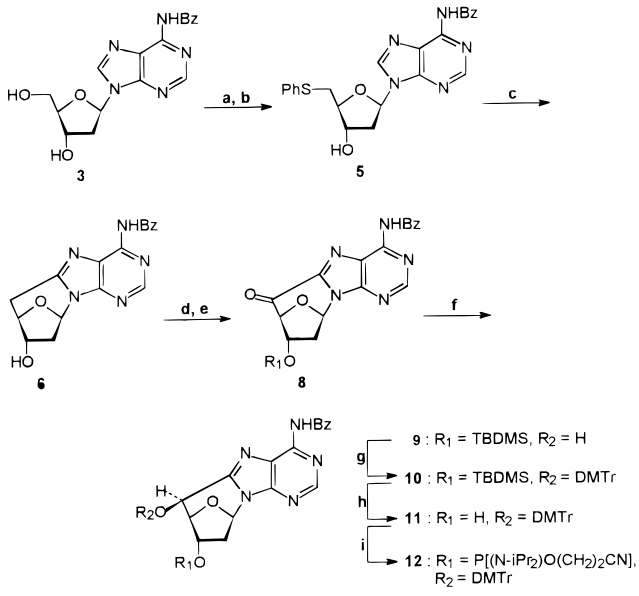
(9) Kuhn, R.; Jahn, W. *Chem. Ber.* **1965**, *98*, 1699−1704.

(10) Walling, C.; Rabinowitz, R. *J. Am. Chem. Soc.* **1959**, *81*, 1243−1249.

(11) Ogilvie, K. K. *Can. J. Chem.* **1973**, *51*, 3799−3807.

S0022-3263(98)00083-8 CCC: $15.00
Published on Web 07/07/1998

Scheme 1. Synthetic Reactions Used for the Preparation of the Phosphoramidite Synthon of (5′*S*)-CyclodAdo[a]



[a] (a) TsCl (1.2 equiv), pyridine, −20 °C, 24 h, 82%; (b) PhSH (2.3 equiv), CH_3ONa (1.5 equiv), CH_3OH, reflux, 2 h, 51%; (c) λ = 254 nm, $(EtO)_3P$ (10 equiv), CH_3CN, argon, 10 h, 51%; (d) TBDMSCl (2 equiv), imidazole (4.4 equiv), DMF, 20 h, 79%; (e) SeO_2 (2 equiv), 1,4-dioxane, reflux, 30 min, 87%; (f) $NaBH_4$ (2 equiv), CH_3OH, 1 h, 90%; (g) DMTrCl (2 equiv), pyridine, 70 °C, 4 h, 60%; (h) TBAF (2 equiv), THF, 4 h, 72%; (i) $P(N\text{-}^iPr_2)_2\text{-}O(CH_2)_2CN$ (1.5 equiv), N,N′-diisopropylammonium tetrazolate (0.5 equiv), CH_2Cl_2, argon, 4 h, 62%.

tography. Sequential treatment of **7** with selenium oxide in refluxing 1,4-dioxane and $NaBH_4$ in methanol gave the (5′*S*)-5′,8-cyclonucleoside **9** stereoselectively and in high yield (78% from compound **7**). Compound **9** was then converted into the DMTr-ether **10** (60% yield) by treatment with an excess of DMTr chloride in pyridine at 70 °C for 4 h.[12] Further treatment of compound **10** with a 1 M solution of tetrabutylammonium fluoride (TBAF) in THF then afforded the free 3′-hydroxyl derivative **11** in 72% yield after silica gel chromatography.[13] Thereafter, the targeted phosphoramidite **12** was obtained from the alcohol **11** in 62% yield using a standard method of phosphitylation.[14]

Two oligodeoxynucleotides, namely [5′−d(ATC GTG XCT GAT CT)-3′)] (14-mer, **13**) and [5′-d(CAC TTC GGX TCG TGA CTG ATC T)-3′] (22-mer, **14**), where X = (5′*S*)-CycloA **2**, were then prepared following the usual phosphoramidite protocol (1 μmol scale). However, the duration of the condensation of the modified monomer **12** was increased by a factor of 2 compared with that of standard monomers. Furthermore, the duration of the coupling of the next nucleoside involved in the oligonucleotide sequences (2′-deoxyguanosine, dGuo) was also increased (330 s instead of 30 s for normal nucleoside phosphoramidites). This allowed a coupling efficiency of more than 85%. After standard deprotection with aqueous ammonia at 55 °C for 17 h, the crude 5′-DMTr-oligomers were purified by reverse phase HPLC, detritylated on the HPLC column, and again purified by reverse phase HPLC (system C). The purity and homogeneity of the collected fractions were controlled by HPLC (system D) and gel electrophoresis. Respectively, 16.5 $AU_{260\ nm}$ and 18 $AU_{260\ nm}$ of purified oligomers **13** and **14** were obtained. The molecular weight of the oligonucleotides **13** and **14** was inferred from electrospray mass spectrometry analyses in the negative mode (calcd: 4251.80 Da, found: 4250.50 ± 0.62 Da, **13**; and calcd: 6699.40 Da, found 6697.61 ± 1.53 Da, **14**). These results confirmed the incorporation of (5′*S*)-CyclodAdo **2** and the purity of the oligomers **13**, **14**. A fraction of **13** was enzymatically hydrolyzed with nuclease P_1, followed by bacterial alkaline phosphatase. The resulting mixture of 2′-deoxyribonucleosides was analyzed by reverse phase HPLC (system E) provided dCyd, dGuo, Thd, dAdo, and a trimer [5′-d(GXC)-3′] **15** in a 2:2:5:2:1 ratio, confirming the structure of the modified oligonucleotide (Figure 1a). The lack of the free modified nucleoside **2** received further confirmation by means of coinjection of the enzymatic digestion with an authentic sample of (5′*S*)-CyclodAdo **2** and HPLC analysis (Figure 1b). The structure of **15** was confirmed by electrospray mass measurement of the collected peak (calcd 867.60 Da, found 867.60 ± 0.1 Da). Further treatment of **15** with nuclease P_1 and alkaline phosphatase did not provide the free 2′-deoxyribonucleosides dCyd, dGuo, and (5′*S*)-CyclodAdo **2**, even after 24 h of incubation. Due to the occurrence of significant conformational changes associated with the presence of **2**, phosphodiester linkages with dCyd and dGuo were not hydrolyzed by nuclease P_1; therefore, the modified nucleoside **2** was not released in the enzymatic hydrolysate. The high stability of phosphodiester linkages between **2** and normal 2′-deoxyribonucleosides has found further confirmation with the use of other enzymes. The enzymatic digestions of **13** by snake venom phosphodiesterase (3′-exo) and calf spleen phosphodiesterase (5′-exo) were followed by MALDI-TOF mass spectrometry.[15] To obtain an optimal distribution of cleavage products for the oligonucleotide **13** and a given batch of enzyme, aliquots were removed from the digestion reactions at increasing time intervals and directly analyzed by MALDI-TOF mass spectrometry. The mass spectrum after 2 h of enzymatic digestion with 3′-exo exhibited a single peak at m/z = 2119.50 ± 0.1 Da. This peak corresponds to the 7-mer [5′-d(ATC GTG X)-3′] **16** (calcd 2118.4 Da). Interestingly, the latter modified 7-mer is not affected by prolonged enzymatic treatment. The snake venom phosphodiesterase sequentially degrades the oligonucleotide **13** from the 3′-end until it reaches the (5′*S*)-CyclodAdo **2** that resists further cleavage. On the other hand, the treatment of **13** with 5′-exo provided the 9-mer [5′-(GXC TGA TCT)-3′] **17** (calcd 2711.80 Da). Indeed, the mass spectrum after 2 h of enzymatic digestion with 5′-exo exhibited a single peak at m/z = 2710.86 ± 0.1 Da. The calf spleen phosphodiesterase induced the release of the first five nucleotides at the 5′-end of the oligonucleotide sequence but failed to cleave phosphodiester linkages between **2** and dGuo and dCyd, even after prolonged treatment. With respect to the possible destabilizing structural effect of (5′*S*)-CyclodAdo **2**, the thermal stability of a (5′*S*)-CycloA-T base pair has been studied. The UV melting behavior of oligomer **13** was

(12) Sekine, M.; Hata, T. *J. Org. Chem.* **1983**, *48*, 3011−3014.
(13) Corey, E. J.; Venkateswarlu, A.*J. Am. Chem. Soc.* **1972**, *94*, 6190−6191.
(14) Barone, A. D.; Tang, J.-Y.; Caruthers, M. H. *Nucleic Acids Res.* **1984**, *12*, 4051−4061.
(15) Pieles, U.; Zürcher, W.; Schär, M.; Moser, H. E. *Nucleic Acids Res.* **1993**, *21*, 3191−3196.

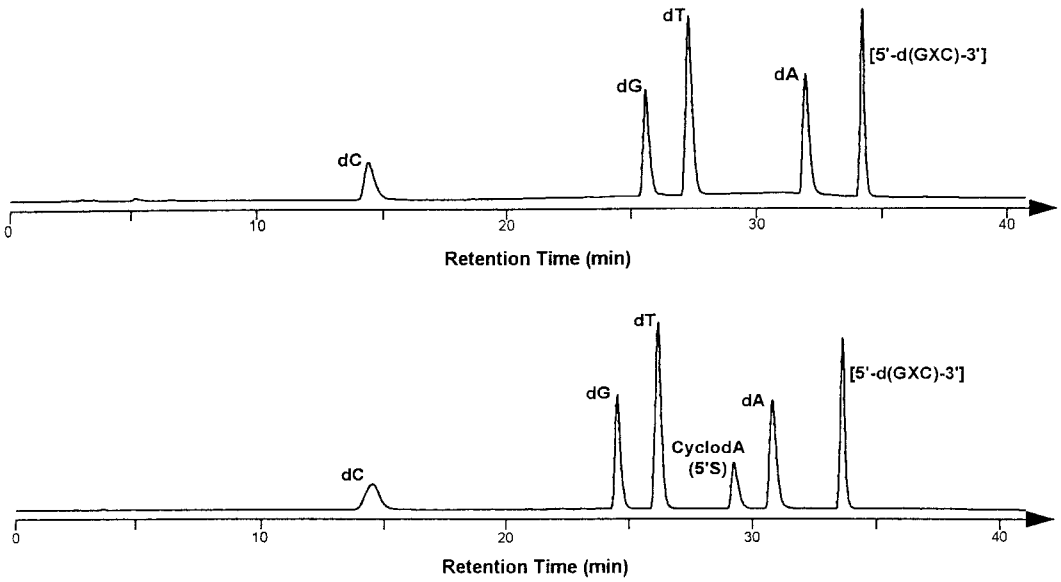


Figure 1. (a) HPLC elution profile of the enzymatic digestion mixture (14-mer, **13**). (b) HPLC elution profile of (5′*S*)-CyclodAdo and the enzymatic digestion mixture (14-mer, **13**).

compared with that of the corresponding parent dAdo containing oligomer. The melting temperature of the duplex that contained (5′*S*)-CyclodAdo **2** (T_m = 48 ± 1 °C) was lower than that of the unmodified duplex (T_m = 54 ± 1 °C). The noticeable decrease in the melting temperature (ΔT_m = 6 °C) suggests that the incorporation of (5′*S*)-CyclodAdo **2** significantly induces a local destabilization of the duplex DNA structure.

Conclusion. The synthesis reported herein provides a facile method for the preparation of oligonucleotides containing (5′*S*)-CyclodAdo **2** at specific positions. We have also demonstrated the high stability of phosphodiester linkages, between **2** and normal 2′-deoxyribonucleosides, toward the enzymatic hydrolysis and that the (5′*S*)-CycloA−T base pair is less stable than the A−T base pair. These modified DNA fragments are suitable for further studies aimed at determining both the biochemical (mutagenesis, repair) and conformational features of (5′*S*)-CyclodAdo **2** into DNA fragments. Furthermore, this versatile synthetic methodology can be used for the incorporation into oligonucleotides of other purine cyclonucleosides.[16]

Experimental Section

General. The photoreactions at 254 nm were performed with a Rayonet photochemical reactor (14x15W). The silica gel (70−200 μm) used for the low-pressure column chromatography was purchased from SDS (Peypin, France). TLC was carried out on Merck DC Kieselgel 60 F-254 plastic sheets. Nuclease P_1 (*Penicilium citrinium*) and 0.3 M sodium acetate/$ZnCl_2$ buffer (pH = 5.3) were purchased from Sigma (St Louis, MO). Alkaline phosphatase, snake venom phosphodiesterase, calf spleen phosphodiesterase and 1 M Tris-HCl/$MgCl_2$/$ZnCl_2$ buffer (10×, pH = 9) were obtained from Boehringer Mannheim (Mannheim, Germany). Anhydrous solvents were purchased from SDS. The HPLC-grade solvents (acetonitrile and methanol) were obtained from Carlo Erba (Milan, Italy). Buffers for high performance liquid chromatography (HPLC) were prepared using water purified with a Milli-Q system (Milford, MA).

High-Performance Liquid Chromatography Separations. Several chromatographic systems were used for the analytical experiments and the purification steps System A semipreparative reverse phase HPLC (Macherey-Nagel Nucleosil C_{18} column, 7 μm, 21 × 250 mm) with acetonitrile and triethylammonium acetate buffer (TEAA, 25 mM, pH = 7) as the eluents [100% TEAA (4 min), linear gradient from 0 to 30% of acetonitrile (50 min)] at a flow rate of 4 mL/min. The detection was achieved at 268 nm System B: reverse phase HPLC (Merck LiChrocart LiChrospher 100RP-18e column, 5 μm, 4 × 125 mm) with acetonitrile and TEAA (25 mM, pH = 7) as the eluents [100% TEAA (10 min), linear gradient from 0 to 30% of acetonitrile (35 min)] at a flow rate of 1 mL/min. The detection was achieved at 268 nm. System C: purification of 5′-DMTr-oligomers by reverse phase HPLC (Hamilton PRP3, polymeric phase, 10 μm, 7 × 305 mm) with acetonitrile and TEAA (25 mM, pH = 7) as the eluents using a nonlinear gradient [100% TEAA (5 min), then isocratic TEAA/acetonitrile [92/8] v/v (10 min)] to remove the DMTr group, the oligomers were treated with an 1% aqueous solution of trifluoroacetic acid [isocratic 100% TFA (1%) (6 min)]. The resulting deprotected oligomers were again purified using the same eluents [gradient from 2 to 10% of acetonitrile (24 min)]. The flow rate was 2.5 mL/min and the detection was achieved at 254 nm. System D: reverse phase HPLC (Hypersil C18, 5 μm, 4.6 × 250 mm) with acetonitrile and (TEAA, 25 mM, pH = 7) as the eluents [100% TEAA (2 min), linear gradient from 0 to 12% of acetonitrile (25 min)] at a flow rate of 1 mL/min. The detection was achieved at 260 nm. System E: system D with a new gradient of acetonitrile and TEAA (25 mM, pH = 7) [100% TEAA (10 min), linear gradient from 0 to 10% of acetonitrile (30 min)].

(5′*R*)-5′,8-Cyclo-2′-deoxyadenosine (1). 8-Bromo-2′-deoxyadenosine[17] (30 mg, 0.09 mmol) was dissolved in 100 mL of water and the resulting solution was deaerated by a stream of argon for 45 min. The solution was irradiated at 254 nm for 12 h, under an argon atmosphere, in a quartz reactor. Then, the reaction mixture was concentrated to dryness. The resulting residue was taken up in 5 mL of water and purified by semipreparative reverse phase HPLC (system A). The appropriate fraction (38 min < t_R < 40 min) was lyophilized, giving 2.7 mg of (5′*R*)-5′,8-cyclo-2′-deoxyadenosine as a white powder (yield 12%). FAB-MS (positive mode): m/z $[M + H]^+$ = 250.2 ± 0.1 Da. ^{1}H NMR (200.13 MHz, D_2O) δ: 8.28 (s, 1H, H-2′); 6.67 (d, $J_{1'2''}$ = 4.9 Hz, H-1′); 4.99 (s, 1H, H-5′); 4.85 (s, 1H, H-4′); 4.56 (q, $J_{3'2'}$ = 7.4 Hz, $J_{3'2''}$ = 3.9 Hz, 1H, H-3′); 2.69 (q, $J_{2'2''}$ = −13.9 Hz, 1H, H-2′); 2.31 (m, 1H, H-2″).

(16) Several oligonucleotides containing (5′*R*)-CyclodAdo **1** and the (5′*R*)- and (5′*S*)-diastereomers of 5′,8-cyclo-2′-deoxyguanosine are in preparation.

(17) Wood, M. L.; Esteve, A.; Morningstar, M. L.; Kuziemko, G. M. Essigmann, J. M. *Nucleic Acids Res.* **1992**, *20*, 6023−6032.

(5′*S*)-5′,8-Cyclo-2′-deoxyadenosine (2). A second fraction (44 min < t_R < 47 min) was collected and then lyophilized, giving 0.5 mg of (5′*S*)-5′,8-cyclo-2′-deoxyadenosine as a white powder (yield 2%). ^{1}H NMR (200.13 MHz, D_2O) δ: 8.26 (s,1H, H-2′); 6.53 (d, $J_{1'2''}$ = 4.8 Hz, H-1′); 5.45 (d, $J_{5'4'}$ = 6.1 Hz, 1H, H-5′); 4.92 (d, 1H, H-4′); 4.84 (q, $J_{3'2'}$ = 7.6 Hz, $J_{3'2''}$ = 4.2 Hz, 1H, H-3′); 2.77 (q, $J_{2'2''}$ = −13.9 Hz, 1H, H-2′); 2.35 (m, 1H, H-2″).

***N*^{6}-Benzoyl-5′-*O*-tosyl-2′-deoxyadenosine (4).** *N*^{6}-Benzoyl-2′-deoxyadenosine[8] **3** (4 g, 11.25 mmol) was dissolved in dry pyridine (10 mL) and evaporated to dryness. The resulting white foam was dissolved in 40 mL of dry pyridine and cooled at −20 °C. Then, tosyl chloride (2.59 g, 13.58 mmol) was added and the mixture was left at −20 °C for 24 h. The reaction was checked for completion by TLC ($CHCl_3$/CH_3OH, 85/15 v/v). Water (20 mL) was added, and after 10 min, the mixture was evaporated to almost dryness. The residue was dissolved in 75 mL of dichloromethane and washed with a saturated solution of NaCl (80 mL). Then, the organic layer was dried over Na_2SO_4 and evaporated to dryness. The resulting residue was purified by chromatography on a silica gel (150 g) column with a step gradient of methanol (0−4%) in chloroform as the mobile phase. The appropriate fractions were pooled and then concentrated to dryness, giving 4.71 g (9.24 mmol) of *N*^{6}-benzoyl-5′-tosyl-2′-deoxyadenosine as a white foam (yield 82%). FAB-MS (positive mode): *m*/*z* M + H]$^+$ = 510.2 ± 0.1 Da; [B + 2H]$^+$ = 240.1 ± 0.1 Da ^{1}H NMR (200.13 MHz, CD_3OD) δ: 8.53 (s, 1H, H-8); 8.35 (s, 1H, H-2); 8.03−7.15 (m, 9H, aromatic-H of Bz and Ts); 6.38 (t, $J_{1'2'}$ = 6.7 Hz, $J_{1'2''}$ = 6.2 Hz, 1H, H-1′); 4.55 (m, 1H, H-3′); 4.23 (m, 2H, H-5′ and H-5″); 4.05 (m, 1H, H-4′); 2.84 and 2.40 (m, 2H, H-2′ and H-2″); 2.27 (s, 3H, CH_3-Ts).

***N*^{6}-Benzoyl-5′-thiophenyl-2′,5′-dideoxyadenosine (5).** A mixture of **4** (3.01 g, 5.91 mmol), thiophenol (1.4 mL, 13.72 mmol), and sodium methylate (0.485 g, 8.97 mmol) in 80 mL of anhydrous methanol was stirred for 2 h under reflux. The reaction was checked for completion by TLC ($CHCl_3$/CH_3OH, 90/10 v/v) and the mixture was evaporated to dryness. The residue was taken up in chloroform (100 mL) and washed with water (100 mL). The organic layer was dried over Na_2SO_4 and evaporated to dryness. The resulting residue was then purified by chromatography on a silica gel (100 g) column with a step gradient of methanol (0−7.5%) in chloroform as the mobile phase. The appropriate fractions were pooled and then concentrated to dryness, giving 1.35 g (3.02 mmol) of *N*^{6}-benzoyl-5′-thiophenyl-2′,5′-dideoxyadenosine as a white foam (yield 51%). FAB-MS (positive mode): *m*/*z* [M + H]$^+$ = 448.2 ± 0.1 Da; [B + 2H]$^+$ = 240.1 ± 0.1 Da. ^{1}H NMR (200.13 MHz, CD_3OD) δ: 8.62 (s, 1H, H-8); 8.42 (s, 1H, H-2); 8.03−7.01 (m, 10H, aromatic-H of Bz and PhS); 6.44 (t, $J_{1'2'}$ = $J_{1'2''}$ = 6.7 Hz, 1H, H-1′); 4.56 (m, 1H, H-3′); 4.09 (m, 1H, H-4′); 3.34 and 3.17 (m, 2H, H-5′ and H-5″); 2.98 and 2.43 (m, 2H, H-2′ and H-2″).

***N*^{6}-Benzoyl-5′,8-cyclo-2′,5′-dideoxyadenosine (6).** Compound **5** (1.35 g, 3.02 mmol) and triethyl phosphite (5 mL, 29.16 mmol) were dissolved in 1 L of anhydrous acetonitrile. Then, argon was bubbled through the solution for 40 min. The oxygen-free solution was irradiated at 254 nm for 10 h, under an argon atmosphere, in a quartz reactor. The reaction was checked for completion by TLC ($CHCl_3$/CH_3OH, 85/15 v/v) and the solution was evaporated to dryness. The resulting residue was crystallized from hot methanol to give 0.52 g (1.54 mmol) of *N*^{6}-benzoyl-5′,8-cyclo-2′,5′-dideoxyadenosine as a yellow powder (yield 51%). FAB-MS (positive mode): *m*/*z* [M + H]$^+$ = 338.1 ± 0.1 Da; ^{1}H NMR (400.13 MHz, DMSO-d_6) δ: 9.09 (bs, 1H, N*H*-Bz); 8.77 (s, 1H, H-2); 8.15−7.64 (m, 5H, aromatic-H of Bz); 6.73 (d, $J_{1'2''}$ = 5.1 Hz, 1H, H-1′); 5.53 (d, $J_{OH-3'}$ = 4.15 Hz, 1H, O*H*-3′); 4.81 (d, $J_{4'5'}$ = 6.3 Hz, 1H, H-4′); 4.48 (m, $J_{3'2'}$ = 7.2 Hz, $J_{3'2''}$ = 3.5 Hz, 1H, H-3′); 3.57 (dd, $J_{5'5''}$ = −18.1 Hz, 1H, H-5′); 3.20 (d, 1H, H-5″); 2.68 (dd, $J_{2'2''}$ = −13.5 Hz, 1H, H-2′); 2.26 (m, 1H, H-2″). ^{13}C NMR (100.61 MHz, DMSO-d_6) δ: 165.6 (1C, C(O) of Bz); 150.9 (1C, C-6); 150.4 (1C, C-4); 148.9 (1C, C-8); 148.2 (1C, C-2); 133.5 (1C, C-1 of Bz); 132.5 (1C, C-4 of Bz); 128.6 (4C, C-2, C-3, C-5, C-6 of Bz); 125.0 (1C, C-5); 84.2 (1C, C-4′); 82.4 (1C, C-1′); 73.2 (1C, C-3′); 46.2 (1C, C-2′); 30.3 (1C, C-5′).

***N*^{6}-Benzoyl-3′-*O*-(*tert*-butyldimethylsilyl)-5′,8-cyclo-2′,5′-dideoxyadenosine (7).** Compound **6** (0.452 g, 1.34 mmol) and imidazole (0.388 g, 5.70 mmol) were dissolved in dry pyridine (5 mL) and evaporated to dryness. The resulting residue was dissolved in 10 mL of anhydrous DMF, and *tert*-butyldimethylsilyl chloride (0.481 g, 3.19 mmol) was added. The mixture was left at room temperature for 20 h. The reaction was checked for completion by TLC ($CHCl_3$/CH_3OH, 90/10 v/v) and the mixture was cooled at 5 °C. Then, water (5 mL) was added and after 10 min, the mixture was evaporated to almost dryness. The residue was taken up in chloroform (40 mL) and washed with 5% $NaHCO_3$ (30 mL). The organic layer was dried over Na_2SO_4 and evaporated to dryness. The resulting residue was then purified by chromatography on a silica gel (50 g) column with a step gradient of methanol (0−4%) in chloroform as the mobile phase. The appropriate fractions were pooled and then concentrated to dryness, giving 0.477 g (1.06 mmol) of *N*^{6}-benzoyl-3′-*O*-(*tert*-butyldimethylsilyl)-5′,8-cyclo-2′,5′-dideoxyadenosine as a yellow foam (yield 79%). EI-MS (positive mode): *m*/*z* [M +•] = 451.0 ± 0.1 Da. ^{1}H NMR (200.13 MHz, DMSO-d_6) δ: 11.22 (bs, 1H, N*H*-Bz); 8.77 (s, 1H, H-2); 8.16−7.62 (m, 5H, aromatic-H of Bz); 6.77 (d, $J_{1'2''}$ = 4.8 Hz, 1H, H-1′); 4.82 (d, $J_{4'5'}$ = 6.0 Hz, 1H, H-4′); 4.68 (q, $J_{3'2'}$ = 7.0 Hz, $J_{3'2''}$ = 3.2 Hz, 1H, H-3′); 3.60 (dd, $J_{5'5''}$ = −18.4 Hz, 1H, H-5′); 3.25 (d, 1H, H-5″); 2.78 (dd, $J_{2'2''}$ = −13.6 Hz, 1H, H-2′); 2.27 (m, 1H, H2″); 0.99 (s, 9H, tBu-TBDMS); 0.21 and 0.18 (s, 6H, CH_3-TBDMS).

***N*^{6}-Benzoyl-3′-*O*-(*tert*-butyldimethylsilyl)-5′,8-cyclo-5′-oxo-2′-deoxyadenosine (8).** A mixture of **7** (0.477 g, 1.06 mmol) and selenium oxide (0.254 g, 2.29 mmol) in 140 mL of anhydrous 1,4-dioxane was stirred for 1 h under reflux. The reaction was checked for completion by TLC ($CHCl_3$/CH_3OH, 95/5 v/v). Then, the mixture was cooled at room temperature and passed through a Celite column. Eluates were evaporated to dryness. The resulting residue was purified by chromatography on a silica gel (50 g) column with a step gradient of methanol (0−2%) in chloroform as the mobile phase. The appropriate fractions were pooled and then concentrated to dryness, giving 0.430 g (0.92 mmol) of *N*^{6}-benzoyl-3′-*O*-(*tert*-butyldimethylsilyl)-5′,8-cyclo-5′-oxo-2′-deoxyadenosine as a pink powder (yield 87%). EI-MS (positive mode): *m*/*z* [M +•] = 465.0 ± 0.1 Da. ^{1}H NMR (200.13 MHz, $CDCl_3$) δ: 8.93 (s, 1H, H-2); 8.02−7.48 (m, 5H, aromatic-H of Bz); 6.84 (d, $J_{1'2''}$ = 5.0 Hz, 1H, H-1′); 4.92 (s, 1H, H-4′); 4.66 (q, $J_{3'2'}$ = 6.0 Hz, $J_{3'2''}$ = 2.4 Hz, 1H, H-3′); 2.70 (dd, $J_{2'2''}$ = −14.1 Hz, 1H, H-2′); 2.52 (m, 1H, H2″); 0.91 (s, 9H, tBu-TBDMS); 0.14 and 0.11 (s, 6H, CH_3-TBDMS).

(5′*S*)-*N*^{6}-Benzoyl-3′-*O*-(*tert*-butyldimethylsilyl)-5′,8-cyclo-2′-deoxyadenosine (9). Compound **8** (0.430 g, 0.92 mmol) was dissolved in 40 mL of methanol. $NaBH_4$ (67 mg, 1.77 mmol) was added and the mixture was stirred for 1 h at room temperature. The reaction was checked for completion by TLC ($CHCl_3$/CH_3OH, 95/5 v/v) and then neutralized with 1 N HCl (3 mL). After 5 min, the mixture was evaporated to dryness. The resulting residue was purified by chromatography on a silica gel (50 g) column with a step gradient of methanol (0−4%) in chloroform as the mobile phase. The appropriate fractions were pooled and then concentrated to dryness, giving 0.387 g (0.83 mmol) of (5′*S*)-*N*^{6}-benzoyl-3′-*O*-(*tert*-butyldimethylsilyl)-5′,8-cyclo-2′-deoxyadenosine as a white foam (yield 90%). FAB-MS (positive mode): *m*/*z* [M + Na]$^+$ = 490.4 ± 0.1 Da; [M + H]$^+$ = 468.2 ± 0.1 Da; ^{1}H NMR (200.13 MHz, $CDCl_3$) δ: 8.71 (s, 1H, H-2); 8.03−7.48 (m, 5H, aromatic-H of Bz); 6.51 (d, $J_{1'2''}$ = 4.4 Hz, 1H, H-1′); 5.47 (d, $J_{5'4'}$ = 5.8 Hz, 1H, H-5′); 5.29 (s, 1H, O*H*-5′); 4.82 (q, $J_{3'2'}$ = 7.2 Hz, $J_{3'2''}$ = 3.8 Hz, 1H, H-3′); 4.51 (d, 1H, H-4′); 2.54 (dd, $J_{2'2''}$ = −13.1 Hz, 1H, H-2′); 2.26 (m, 1H, H-2″); 0.85 (s, 9H, tBu-TBDMS); 0.04 and 0.02 (s, 6H, CH_3-TBDMS).

(5′*S*)-*N*^{6}-Benzoyl-3′-*O*-(*tert*-butyldimethylsilyl)-5′-*O*-(4,4′-dimethoxytrityl)-5′,8-cyclo-2′-deoxyadenosine (10). Compound **9** (0.193 g, 0.41 mmol) was dissolved in dry pyridine (2 mL) and the resulting solution was evaporated to dryness. The operation was repeated twice. The resulting residue was dissolved in 7 mL of dry pyridine, and 4,4′-dimethoxytrityl chloride (0.280 g, 0.82 mmol) was added. The mixture was heated at 70 °C and left at this temperature for 4 h. The reaction was checked for completion by TLC ($CHCl_3$/CH_3OH, 97/3 v/v). The solution was cooled at 5 °C and methanol (1 mL) was added. After 10 min, the mixture was evaporated to dryness. The resulting residue was purified by chromatography on a silica gel (30 g) column with a step gradient of methanol (0−1%) in chloroform/triethylamine (99/1) as the mobile phase. The appropriate fractions were pooled and then concentrated to dryness, giving

0.189 g (0.24 mmol) of (5′*S*)-N^6-benzoyl-3′-*O*-(*tert*-butyldimethylsilyl)-5′-*O*-(4,4′-dimethoxytrityl)-5′,8-cyclo-2′-deoxyadenosine as a yellow foam (yield 60%). FAB-MS (positive mode): *m*/*z* $[M + H]^+ = 770.3 \pm 0.1$ Da; $[DMTr]^+ = 303.1 \pm 0.1$ Da. ^{1}H NMR (200.13 MHz, $CDCl_3$) δ: 8.69 (s, 1H, H-2); 8.14−6.78 (m, 18H, aromatic-H of Bz and DMTr); 6.34 (d, $J_{1'2'} = 4.4$ Hz, 1H, H-1′); 5.26 (d, $J_{4'5'} = 5.2$ Hz, 1H, H-4′); 4.87 (m, 1H, H-3′); 3.76 and 3.74 (s, 6H, CH_3O-DMTr); 3.24 (d, 1H, H-5′); 2.54 and 2.10 (m, 2H, H-2′ and H-2″); 0.82 (s, 9H, tBu-TBDMS); −0.03 (s, 6H, CH_3-TBDMS).

(5′*S*)-N^6-Benzoyl-5′-O-(4,4′-dimethoxytrityl)-5′,8-cyclo-2′-deoxyadenosine (11). Compound **10** (98 mg, 0.12 mmol) was dissolved in 4 mL of anhydrous THF. A solution of TBAF (0.25 mL, 0.25 mmol) in THF (1 M) was added and the mixture was stirred for 4 h at room temperature. The reaction was checked for completion by TLC ($CHCl_3$/CH_3OH, 95/5 v/v) and the mixture was evaporated to dryness. The resulting residue was purified by chromatography on a silica gel (25 g) column with a step gradient of methanol (0−2%) in chloroform/triethylamine (99/1) as the mobile phase. The appropriate fractions were pooled and then concentrated to dryness giving 57 mg (0.08 mmol) of (5′*S*)-N^6-benzoyl-5′-O-(4,4′-dimethoxytrityl)-5′,8-cyclo-2′-deoxyadenosine as a white foam (yield 72%). FAB-MS (positive mode): *m*/*z* $[M + H]^+ = 656.0 \pm 0.1$ Da; $[DMTr]^+ = 303.0 \pm 0.1$. ^{1}H NMR (200.13 MHz, $CDCl_3$) δ: 8.75 (s, 1H, H-2); 8.08−6.88 (m, 18H, aromatic-H of Bz and DMTr); 6.36 (d, $J_{1'2''} = 4.7$ Hz, 1H, H-1′); 5.30 (d, $J_{4'5'} = 5.9$ Hz, 1H, H-4′); 4.82 (m, 1H, H-3′); 3.79 and 3.78 (s, 6H, CH_3O-DMTr); 2.87 (d, 1H, H-5′); 2.60 (dd, $J_{2'2''} = -13.8$ Hz); 2.13 (m, 1H, H-2″).

(5′*S*)-5′,8-Cyclo-2′-deoxyadenosine Phosphoramidite Derivative (12). Compound **11** (57 mg, 0.08 mmol) and diisopropylammonium tetrazolate (7 mg, 0.04 mmol) were dissolved in dry dichloromethane (3 mL), and the resulting solution was evaporated to dryness. The operation was repeated twice. The resulting residue was then dissolved in dry dichloromethane (3 mL) and kept under an argon atmosphere. Subsequently, (cyanoethyl)bis(diisopropylamino)phosphine (41 μL, 0.13 mmol) was added with a syringe through a rubber septum. The reaction mixture was stirred during 4 h. The formation of the desired product was checked by TLC (AcOEt/Hexane/TEA, 70/30/1 v/v/v). The mixture was evaporated to dryness. The resulting residue was taken up in ethyl acetate (20 mL) and washed successively with 5% $NaHCO_3$ (30 mL) and a saturated solution of NaCl (25 mL). The organic layer was dried over Na_2SO_4 and evaporated to dryness. The resulting residue was purified by chromatography on a silica gel (25 g) column with a step gradient of AcOEt (15−60%) in hexane/triethylamine (98/2) as the mobile phase. The appropriate fractions were pooled and then concentrated to dryness, giving 46 mg (0.05 mmol) of the phosphoramidite synthon **12** as a white foam (yield 62%). FAB-MS (positive mode): *m*/*z* $[M + H]^+ = 856.0 \pm 0.1$ Da; $[DMTr]^+ = 303.0 \pm 0.1$ Da. ^{1}H NMR (200.13 MHz, acetone-d_6) two diastereomers δ: 8.71 and 8.59 (s, 1H, H-2); 8.38−7.00 (m, 18H, aromatic-H of Bz and DMTr); 6.58 and 6.53 (d, $J_{1'2''} = 4.7$ Hz and $J_{1'2''} = 4.4$ Hz, 1H, H-1′); 5.41 and 5.35 (d, $J_{4'5''} = 5.6$ Hz, 1H, H-4′); 5.17 (m, 1H, H-3′); 3.90 and 3.89 (s, 6H, CH_3O-DMTr); 3.83 (s, 6H, CH_3O-DMTr); 4.38−3.49 (m, 4H, CH-^{i}Pr and CH_2-OP); 3.33 and 3.21 (d, 1H, H-5′); 2.87−2.61 (m, 4H, H-2′, H-2″ and $-CH_2CN$); 1.40−1.23 (m, 12H, CH_3-^{i}Pr). ^{31}P NMR (100.21, acetone-d_6) δ: 150.8 and 148.7 (two diastereomers).

Solid-Phase Synthesis of Oligonucleotides. Oligonucleotides containing (5′*S*)-CyclodAdo were prepared by phosphoramidite solid-phase synthesis, using an acetyl protective group for dCyd, a benzoyl protective group for dAdo, and an isobutyryl protective group for dGuo. The synthesized amidite **12** (46 mg, 0.05 mmol) was dissolved in 400 μL of dry dichloromethane and placed in the additional ports of a model 392 DNA synthesizer (ABI). The standard 1 μmol synthesis scale with retention of the 5′ terminal DMTr group (trityl-on mode) was used with the modifications previously mentioned.

Deprotection and Purification of Oligonucleotides. Following synthesis, the solid support was placed in concentrated aqueous ammonia (32%) in a sealed vial and heated at 55 °C for 17 h. The crude 5′-DMTr oligomers were purified and deprotected online by reverse phase HPLC (system C).

Enzymatic Digestion of Oligomers 13 and 14. A 0.5 $AU_{260\ nm}$ portion of purified oligomers **13** and **14** was taken up in 45 μL of water. A solution (5 μL) of nuclease P_1 in acetate buffer (1unit/1μL) was added. The resulting mixture was incubated at 37 °C for 2 h. Subsequently, 5 μL of Tris-HCl buffer (10×) and 2 units of alkaline phosphatase were added, and the resulting mixture was incubated for additional 1 h at 37 °C. Subsequently, the mixture was taken up in 40 μL of TEAA (25 mM, pH = 7) and the content was analyzed by reverse phase HPLC (system E).

Snake Venom Phosphodiesterase (3′-Exo) Digestion. A 0.2 $AU_{260\ nm}$ portion of purified oligomer **13** were taken up in 18 μL of water. Then, 2 μL of ammonium citrate buffer (0.2M, pH = 9) and 3×10^{-4} unit of 3′-exo were added. The resulting mixture was incubated at 37 °C and aliquots of 2 μL were taken up at increasing periods of time. To stop the enzymatic reaction, each aliquot was diluted with 50 μL of water and frozen in liquid nitrogen. After lyophilization, the residues obtained were taken up in 20 μL of 0.1% aqueous solution of trifluoroacetic acid and analyzed by MALDI-TOF mass spectrometry (matrix: 3-hydroxypicolinic acid).

Calf Spleen Phosphodiesterase (5′-exo) Digestion. A similar procedure as described (*vide supra*) was used with the following modifications: 1×10^{-3} unit of 5′-exo and ammonium citrate buffer (0.2M, pH = 5) were used.

Thermal Denaturation Studies. The oligodeoxynucleotides [5′-d(ATC GTG XCT GAT CT)-3′], where X = A or (5′*S*)-CycloA (0.25 $AU_{260\ nm}$), and its complement [3′-d(TAG CAC TGA CTA GA)-5′] (0.27 $AU_{260\ nm}$) were mixed in 200 μL of a buffer containing 0.01 M sodium phosphate, 0.1 M NaCl, 0.001 M EDTA, pH = 7. DNA was annealed by heating to 85 °C for 5 min followed by slow cooling to 4 °C (3 h). The hybridization solutions were diluted with 400 μL of buffer and degassed prior to each melting experiment. Measurements of UV absorbance were made in a 0.8 mL quartz cell (0.2 cm path length) with a UV/vis spectrometer equipped with a Peltier temperature controller. The absorbance of the sample was monitored at 260 nm from 15 to 85 °C at a heating rate of 1 °C/min. Data reflect the average of three melting curves per oligodeoxynucleotide duplex.

Acknowledgment. The contributions of Colette Lebrun (SCIB/LRI/CEA-Grenoble), Daniel Ruffieux (Biochimie C/CHRU Grenoble) and Michel Jaquinod (LSMP/IBS/CEA/CNRS-Grenoble) to the mass spectrometry measurements are greatly acknowledged. We thank Dr. Thierry Douki and Thierry Delatour for helpful discussions concerning the photoreactions of 8-bromo-2′-deoxyadenosine and the thiophenyl derivative **5**. This work was supported, in part, by the French Ministry of Science and Research (ACC-SV no. 8-MESR 1995) and Electricité de France (Comité de Radioprotection).

Supporting Information Available: Copies of ^{1}H-, ^{13}C NMR, and FAB mass spectra of **6**, ^{1}H NMR spectrum of **9**, FAB mass spectrum of **12**, electrospray ionization mass spectra of oligomers **13**−**15**, MALDI-TOF mass spectra of the 3′-exo and 5′-exo enzymatic digestions of **13**, and melting curves of the normal and modified duplexes (11 pages). This material is contained in libraries on microfiche, immediately follows this article in the microfilm version of the journal, and can be ordered from the ACS; see any current masthead page for ordering information.

JO980083Q