JOC Article

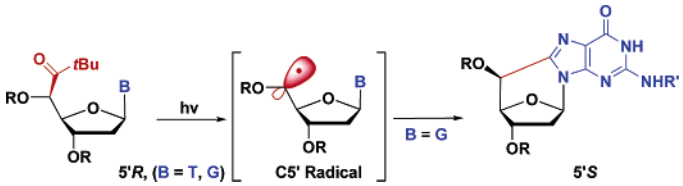
Independent Generation of C5′-Nucleosidyl Radicals in Thymidine and 2′-Deoxyguanosine

Antonio Manetto,[†,‡] Dimitris Georganakis,[§] Leondios Leondiadis,[§,||] Thanasis Gimisis,*[,§] Peter Mayer,[‡] Thomas Carell,[‡] and Chryssostomos Chatgilialoglu*[,†]

ISOF, Consiglio Nazionale delle Ricerche Via P. Gobetti 101, 40129 Bologna, Italy, Department of Chemistry and Biochemistry, Ludwig-Maximilians University Munich, 81377 Munich, Germany, MS and Dioxin Analysis Laboratory, IRRP, National Centre for Scientific Research 'Demokritos', Athens 153 10, Greece, and Department of Chemistry, University of Athens, Panepistimiopolis, 15771 Athens, Greece

gimisis@chem.uoa.gr; chrys@isof.cnr.it

Received December 8, 2006



The synthesis of the C5′ *tert*-butyl ketone of thymidine **1a** and 2′-deoxyguanosine **2** is achieved by reaction of 5′-*C*-cyano derivatives with *tert*-butyl lithium followed by acid hydrolysis. The 5′*R* configuration is assigned by X-ray crystal structure determination of an opportunely protected derivative of **1a**. The (5′*S*)-isomers of both nucleosides are not stable, and a complete decomposition occurs in the reaction medium. The photochemistry of **1a** and **2** effectively produced the thymidin-5′-yl radical and the 2′-deoxyguanosin-5′-yl radical, respectively. In the thymidine system, the C5′ radical is fully quenched in the presence of a physiological concentration of thiols. In the 2′-deoxyguanosine system, the C5′ radical undergoes intramolecular attack onto the C8−N7 double bond of guanine leading ultimately to the 5′,8-cyclo-2′-deoxyguanosine derivative. The cyclization of the 2′-deoxyguanosin-5′-yl radical occurs with a rate constant of ca. 1×10^6 s^{-1} and is highly stereoselective affording only the (5′*S*)-diastereomer.

Introduction

Hydrogen abstraction from the 2-deoxyribose moiety of DNA produces carbon-centered radicals whose fate depends upon the environment.[1] The accessibility of the C−H bond in sugar moieties determines the preferential site of attack even by the highly reactive HO• radicals. In B-DNA, the H5′ and H5″ are the most exposed ones, and therefore, the preference for abstraction of these hydrogens is higher.[2,3] Hydrogen abstraction from the 5′-position of purine nucleosides can lead to 5′,8-cyclonucleosides. They have been observed among the decomposition products of DNA, when it is exposed to ionizing radiations[4−6] or treated chemically by highly oxidizing radical species.[7] They have also been identified in mammalian cellular DNA in vivo, where their level is enhanced by conditions of oxidative stress.[8,9] The difficulty of repair and the amenability to mutation render these lesions biologically significant and the study of their formation necessary.[10−12] Under aerobic conditions, the fate of the C5′ radical is not well understood. All the

[†] Consiglio Nazionale delle Ricerche.
[‡] Ludwig-Maximilians University Munich.
[§] National & Kapodistrian University of Athens.
[||] National Centre for Scientific Research 'Demokritos'.

(1) von Sonntag, C. *Free-Radical-Induced DNA damage and its Repair*; Springer-Verlag: Berlin, 2006; pp 366−371.
(2) Pogozelski, W. K.; Tullius, T. D. *Chem. Rev.* **1998**, *98*, 1089−1107.
(3) Sy, D.; Savoye, C.; Begusova, M.; Michalik, V.; Charlier, M.; Spotheim-Maurizot, M. *Int. J. Radiat. Biol.* **1997**, *72*, 147−155.
(4) Dirksen, M. L.; Blakely, W. F.; Holwitt, E.; Dizdaroglu, M. *Int. J. Radiat. Biol.* **1988**, *54*, 195−204.
(5) Dizdaroglu, M.; Jaruga, P.; Rodriguez, H. *Free Radical Biol. Med.* **2001**, *30*, 774−784.
(6) Jaruga, P.; Birincioglu, M.; Rodriguez, H.; Dizdaroglu, M. *Biochemistry* **2002**, *41*, 3703−3711.
(7) Birincioglu, M.; Jaruga, P.; Chowdhury, G.; Rodriguez, H.; Dizdaroglu, M.; Gates, K. S. *J. Am. Chem. Soc.* **2003**, *125*, 11607−11615.
(8) Randerath, K.; Zhou, G. D.; Somers, R. L.; Robbins, J. H.; Brooks, P. J. *J. Biol. Chem.* **2001**, *276*, 36051−36057.
(9) D'Errico, M.; Parlanti, E.; Teson, M.; de Jesus, B. M. B.; Degan, P.; Calcagnile, A.; Jaruga, P.; Bjoras, M.; Crescenzi, M.; Pedrini, A. M.; Egly, J. M.; Zambruno, G.; Stefanini, M.; Dizdaroglu, M.; Dogliotti, E. *EMBO J.* **2006**, *25*, 4305−4315.
(10) Kuraoka, I.; Bender, C.; Romieu, A.; Cadet, J.; Wood, R. D.; Lindahl, T. *Proc. Natl. Acad. Sci. U.S.A.* **2000**, *97*, 3832−3837.

10.1021/jo062518c CCC: $37.00
Published on Web 04/11/2007

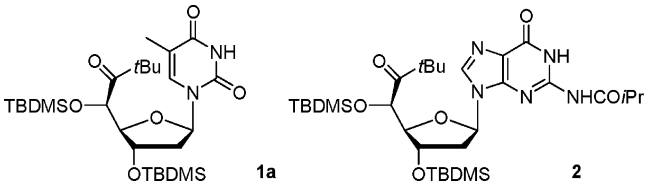


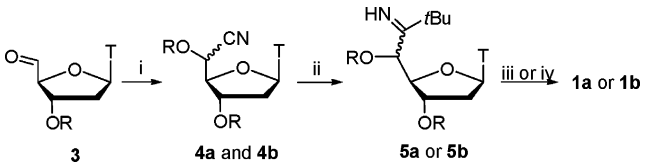
FIGURE 1. Photolabile precursors of C5′ radicals.

proposed intermediates are based on rationalization of the products observed in DNA degradation by the neocarzinostatin chromophore (NCS-chromophore) in the presence of glutathione,[1,13,14] although related studies with metalloporphyrins have also been reported.[2,15] Moreover, strand breakage was observed to be base-selective, preferentially occurring (75%) on the thymidine unit.

The chemistry of carbon-centered radicals resulting from hydrogen atom abstraction from the sugar moieties has been the subject of many recent studies. Selective generation of these species is mainly obtained by photoreactive precursors using nucleosides or oligonucleotides (ODNs). Indeed, generation of a single radical species on duplex ODNs provides a powerful tool for elucidating the role of reactive intermediates in the formation of nucleic acid lesions. For example, C1′ and C4′ positions have been studied in detail by photolysis of the corresponding *tert*-butyl ketones.[16−20] Photolabile precursors of C5′ radicals are missing.

It was recently found that 8-bromo-2′-deoxyadenosine captures electrons and rapidly loses a bromide ion to give the corresponding C8 radical. This intermediate abstracts intramolecularly a hydrogen atom from the C5′ position affording selectively the 2′-deoxyadenosin-5′-yl radical. This allowed us for the first time to verify that the C5′ radical attacks intramolecularly the double bond of the base moiety, and this occurs with a rate constant of $k_c = 1.6 \times 10^5\ s^{-1}$ to form, after oxidation, a 5′,8-cyclo-2′-deoxyadenosine as the final product.[21−23] The analogous sequence of 8-bromo-2′-deoxyguanosine does not operate because the electron adduct undergoes fast protonation at C8 ejecting Br^- and affording the one-electron oxidized 2′-deoxyguanosine transient species.[24,25] Interestingly, the 8-bromo-2′-deoxyadenosine moieties in a series of DNA hairpins containing a light-dependent flavin electron injector in the loop region of the hairpin are found to capture electrons with quantitative formation of the corresponding debrominated oligonucleotides, similar to the analogous 8-bromo-2′-deoxyguanosine derivatives.[26]

SCHEME 1. Synthesis of the Ketones 1a and 1b[a]



[a] Conditions: (i) TBDMS−CN, LiOEt, THF, 0 °C, 30 min, 82%; (ii) *t*BuLi, THF, −78 °C, 2−5 min, 43% (**5a**), 38% (**5b**); (iii) for **1a**, THF/H_2O/2 N HCl (40:20:1), 3 h, 88%; (iv) for **1b**, $CDCl_3$, 30 min, 70%. R = *tert*-butyldimethylsilyl (TBDMS). T = thymine.

In the present paper, we report the synthesis of 5′-keto derivatives as photolabile precursors for selective generation of the 5′-nucleosidyl radical. In particular, compounds **1a** and **2** having thymine or guanine as the base are chosen to evaluate the occurrence of a C5′ radical attack on the pyrimidine or purine moieties (Figure 1).

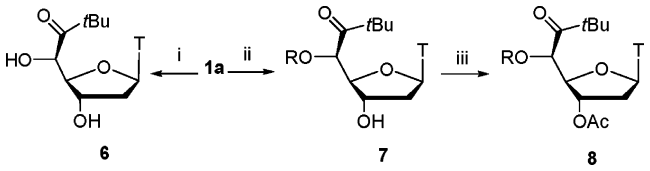
Results and Discussion

Synthesis of Thymidine 5′-*tert*-Butyl Ketone 1a. The synthesis of **1a** is shown in Scheme 1. Specifically, the aldehyde **3**[27−30] was converted in the two 5′-isomers of **4** with a yield of 82%. The crude product consisted of a 3:2 mixture of two isomers, **4a** and **4b**. After separation by silica gel chromatography, the two diastereomers were obtained as pure white foams and were fully characterized, although from the data it was not possible to assign the configuration at the 5′ position, even by 2D NMR experiments. During the α-cyanohydrin formation, under not perfectly anhydrous conditions or in the presence of an excess of LiOEt, the 5′-hydroxyl position resulted unprotected. In this case, an additional step for the in situ 5′-TBDMS protection was required by classical methods (TBDMS−Cl, $AgNO_3$, imidazole).

With a mixture of **4a** and **4b**, the addition of *tert*-butyl lithium (*t*BuLi) was carried out at −78 °C, leading, after quenching the reaction with acidic cold water, to a single product in 55% yield, identified as the *tert*-butyl ketone **1a**. Its configuration (5′*R*) was later assigned by X-ray crystal structure determination of the corresponding 3′-acetate (vide infra). The product yield together with the absence of the (5′*S*)-isomer **1b** indicated that the addition reaction products exhibit different stability when originating from either diastereomer **4a** or **4b**. To further investigate their reactivity, each isomer was used for the addition

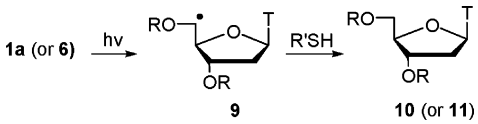
(11) Brooks, P. J.; Wise, D. S.; Berry, D. A.; Kosmoski, J. V.; Smerdon, M. J.; Somers, R. L.; Mackie, H.; Spoonde, A. Y.; Ackerman, E. J.; Coleman, K.; Tarone, R. E.; Robbins, J. H. *J. Biol. Chem.* **2000**, *275*, 22355−22362.
(12) Kuraoka, I.; Robins, P.; Masutani, C.; Hanaoka, F.; Gasparutto, D.; Cadet, J.; Wood, R. D.; Lindahl, T. *J. Biol. Chem.* **2001**, *276*, 49283−49288.
(13) Goldberg, I. H. *Acc. Chem. Res.* **1991**, *24*, 191−198.
(14) Chatgilialoglu, C.; O'Neill, P. *Exp. Gerontol.* **2001**, *36*, 1459−1471.
(15) Pratviel, G.; Pitie, M.; Bernadou, J.; Meunier, B. *Angew. Chem., Int. Ed. Engl.* **1991**, *30*, 702−704.
(16) Chatgilialoglu, C.; Gimisis, T. *Chem. Commun.* **1998**, 1249−1250.
(17) Emanuel, C. J.; Newcomb, M.; Ferreri, C.; Chatgilialoglu, C. *J. Am. Chem. Soc.* **1999**, *121*, 2927−2928.
(18) Chatgilialoglu, C.; Ferreri, C.; Bazzanini, R.; Guerra, M.; Choi, S. Y.; Emanuel, C. J.; Horner, J. H.; Newcomb, M. *J. Am. Chem. Soc.* **2000**, *122*, 9525−9533.
(19) Tronche, C.; Goodman, B. K.; Greenberg, M. M. *Chem. Biol.* **1998**, *5*, 263−271.
(20) Meggers, E.; Michel-Beyerle, M. E.; Giese, B. *J. Am. Chem. Soc.* **1998**, *120*, 12950−12955.
(21) Chatgilialoglu, C.; Guerra, M.; Mulazzani, Q. G. *J. Am. Chem. Soc.* **2003**, *125*, 3839−3848.
(22) Flyunt, R.; Bazzanini, R.; Chatgilialoglu, C.; Mulazzani, Q. G. *J. Am. Chem. Soc.* **2000**, *122*, 4225−4226.
(23) Jimenez, L. B.; Encinas, S.; Miranda, M. A.; Navacchia, M. L.; Chatgilialoglu, C. *Photochem. Photobiol. Sci.* **2004**, *3*, 1042−1046.
(24) Chatgilialoglu, C.; Caminal, C.; Guerra, M.; Mulazzani, Q. G. *Angew. Chem., Int. Ed.* **2005**, *44*, 6030−6032.
(25) Chatgilialoglu, C.; Caminal, C.; Altieri, A.; Vougioukalakis, G. C.; Mulazzani, Q. G.; Gimisis, T.; Guerra, M. *J. Am. Chem. Soc.* **2006**, *128*, 13796−13805.
(26) Manetto, A.; Breeger, S.; Chatgilialoglu, C.; Carell, T. *Angew. Chem., Int. Ed.* **2006**, *45*, 318−321.
(27) Montevecchi, P. C.; Manetto, A.; Navacchia, M. L.; Chatgilialoglu, C. *Tetrahedron* **2004**, *60*, 4303−4308.
(28) Chen, M. Y.; Patkar, L. N.; Lu, K. C.; Lee, A. S. Y.; Lin, C. C. *Tetrahedron* **2004**, *60*, 11465−11475.
(29) Tronchet, J. M. J.; Kovacs, I.; Dilda, P.; Seman, M.; Andrei, G.; Snoeck, R.; De Clereq, E.; Balzarini, J. *Nucleosides Nucleotides Nucleic Acids* **2001**, *20*, 1927−1939.
(30) Angeloff, A.; Dubey, I.; Pratviel, G.; Bernadou, J.; Meunier, B. *Chem. Res. Toxicol.* **2001**, *14*, 1413−1420.

SCHEME 2. Deprotection−Protection Strategy for the Synthesis of 8[a]



[a] Conditions: (i) TBAF, MeOH, reflux, 5 h, 60%; (ii) TBAF, THF, −15 °C, 24 h, 55%; (iii) Ac_2O, Py, rt, 24 h, 82%. R = TBDMS. T = thymine.

SCHEME 3. Generation of the C5′ Radical from Photolysis of 1a and 6[a]



[a] R′SH = BuSH for **1a** or GSH for **6**; R = TBDMS for **10**. R = H for **11**. T = thymine.

under identical conditions and a careful monitoring was carried out by TLC, together with ^{1}H NMR analysis of the final products. This led to the identification of two intermediate imine products **5a** and **5b** (Scheme 1), fully characterizable, using acetone-d_6 as the NMR acid-free solvent, from the crude products, after water quenching and workup of the crude *t*BuLi addition. The imine **5b** readily decomposed under slightly acidic aqueous conditions or in weakly acidic solvents such as $CDCl_3$, when used as the NMR solvent. The corresponding ketone (**1b**) cannot be isolated, as it also slowly decomposes to unidentified fragments. A complete decomposition of the mixture containing **5b** and **1b** also occurred in acidic solution (THF/H_2O/2 N HCl, 40:20:1). On the other hand, acidic hydrolysis conditions applied to the imine **5a** led to its slow but quantitative conversion to the stable ketone **1a**.

The full deprotection of bissilylether **1a** led to the ketone **6** as described in Scheme 2. Under specific conditions, selective deprotection of the ketone **1a** in the 3′ position was also possible, providing the ketone **7**.[31] This compound was then protected to the 3′-acetate **8**, and single crystals, suitable for X-ray crystallography, were obtained from a saturated solution in ethyl acetate. One of the two independent molecules (**8**) present in the crystal asymmetric unit is reported in the Supporting Information. Both of the molecules are in the 5′*R* configuration, and in both, the furanose rings are $C_{2'endo}$ puckered, as evidenced by the Cremer and Pople puckering parameters ($\phi \approx 70°$ for both molecules).[32] The $C_{2'endo}$ puckered form, predominant in solution,[33] is also maintained in the solid state of these molecules. Since the 5′-stereocenter was not involved in the deprotection−protection steps, we could extrapolate the assignment of the same 5′*R* configuration to the ketone **1a** and a 5′*S* configuration to the imine **5a** and cyanohydrin **4a**, due to a

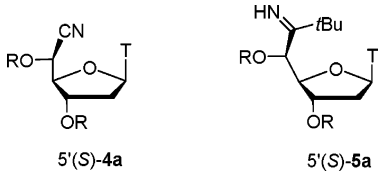
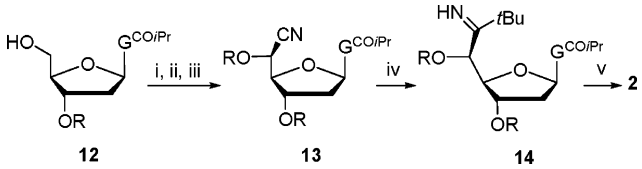


FIGURE 2. Configuration of the major cyanohydrin **4a** and imine **5a**. R = TBDMS. T = thymine.

SCHEME 4. Synthesis of 5′-*tert*-Butyl Ketone 2a[a]



[a] Conditions: (i) EDC, pyridine, CF_3CO_2H, toluene/DMSO (2:1), 95%; (ii) TMSCN, ZnI_2, CH_2Cl_2, 5 h, 88%; (iii) TBDMSCl, imidazole, DMF, overnight, 25%; (iv) *t*BuLi, THF, −78 °C, 5 min, 32%; (v) CH_3CO_2H/THF/H_2O (3:1:1), 1 h, 78%.

priority change of the substituents as a consequence of the Cahn−Ingold−Prelog priority rules (CIP rules) (Figure 2).

Photochemistry in the Thymidine System. Compounds **1a** and **6** were used as precursors of the C5′ radical. UV irradiation (1000 W Xe-lamp, 320 nm cut-off filter) of **1a** in the presence of a hydrogen donor such as 2.5 mM 1-butanethiol in MeCN and of **6** in the presence of 2.5 mM gluthathione (GSH) in water, under deaerated conditions, led quantitatively to the formation of thymidine **10** or **11**, respectively (Scheme 3). Figure 3 shows the analysis by reversed-phase HPLC of aliquots taken during the photolysis of **1a** (0.12 mM in MeCN) in the presence of 1-butanethiol (2.5 mM) for 30 min. Under these conditions, the half-life of **1a** was calculated to be $t_{1/2}$ = 6.6 min and no cyclization product was observed. The resulting C5′ radical **9** could be obtained either by Norrish type I photocleavage or by initial formation of an acyl radical that decarbonylates with a rate constant in the range of 10^5−10^6 s^{-1}.[34] A physiological concentration of thiol (e.g., 2.5 mM of GSH) is able to trap the C5′ radical preventing subsequent reactions, such as the intramolecular attack onto the C6−C5 double bond of thymine.[35]

Synthesis of 2′-Deoxyguanosine 5′-*tert*-Butyl Ketone 2. The synthesis of **2** is shown in Scheme 4. The intermediate 5′-aldehyde[36,37] was prepared by applying a modification of the Moffatt oxidation procedure[33] on the starting alcohol **12**.[38] It appeared as a complicated mixture of three possible diastereomeric hemiacetals that form by hydration of the 5′-aldehyde, when in contact with an aqueous solution. When the above mixture of diastereomeric hemiacetals was allowed to react, in the presence of TMSCN and ZnI_2 in dry dichloromethane[39,40] containing 4 Å molecular sieves, it was converted into a pair

(31) The availability of **7** offers a pathway for accessing the corresponding phosphoramidites, required for modified oligonucleotide synthesis. Nevertheless, it is apparent that the secondary hydroxyl at the 5′-position will require modified conditions for protection, deprotection, and reactivity. Similar problems have been tackled by other researchers, and alternatives to the DMT group, e.g., levulinic acid, have been reported (see: Romieu, A.; Gasparutto, D.; Cadet, J. *J. Chem. Soc., Perkin Trans. 1* **1999**, 1257−1263).

(32) Cremer, D.; Pople, J. A. *J. Am. Chem. Soc.* **1975**, *97*, 1354−1358.

(33) Jardetzky, C. D. *J. Am. Chem. Soc.* **1962**, *84*, 62−66.

(34) Chatgilialoglu, C.; Crich, D.; Komatsu, M.; Ryu, I. *Chem. Rev.* **1999**, *99*, 1991−2069.

(35) Navacchia, M. L.; Manetto, A.; Montevecchi, P. C.; Chatgilialoglu, C. *Eur. J. Org. Chem.* **2005**, 4640−4648.

(36) Kers, A.; Szabo, T.; Stawinski, J. *J. Chem. Soc., Perkin Trans. 1* **1999**, 2585−2590.

(37) Brown, P.; Richardson, C. M.; Mensah, L. M.; O'Hanlon, P. J.; Osborne, N. F.; Pope, A. J.; Walker, G. *Bioorg. Med. Chem.* **1999**, *7*, 2473−2485.

(38) Flockerzi, D.; Schlosser, W.; Pfleiderer, W. *Helv. Chim. Acta* **1983**, *66*, 2069−2085.

(39) Evans, D. A.; Carroll, G. L.; Truesdale, L. K. *J. Org. Chem.* **1974**, *39*, 914−917.

(40) Hon, Y. S.; Yan, J. L. *Tetrahedron* **1998**, *54*, 8525−8542.

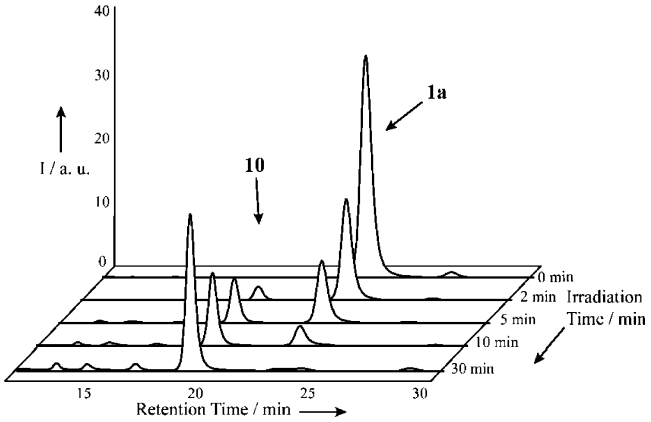


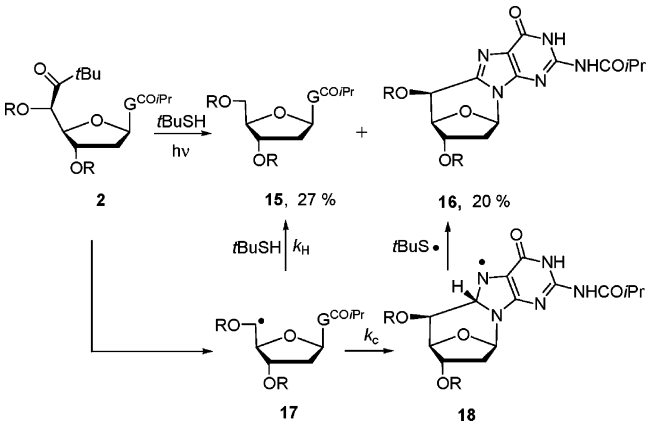
FIGURE 3. Time-dependent HPLC chromatogram of **1a**'s conversion to **10** during photolysis. Normalized peaks.

of diastereomers. No products were observed when KCN and 18-C-6 were used in the presence of TBDMSCl for the trapping of the cyanohydrin produced.[41] The diastereomeric mixture of cyanohydrins proved unstable and reverted back into the aldehyde when column chromatography purification or storage was attempted. For this reason, the crude product was immediately protected with TBDMSCl and was then further purified. A chromatographically inseparable mixture of protected α-cyanohydrins was isolated from the column in 52% yield (Scheme 4). When this mixture was treated with diethyl ether, a single diastereomer **13** precipitated in crystalline form in 25% yield. The filtrate containing the other diastereomer together with other impurities could not be further purified. A third nucleosidic product was present, isolable in yields up to 30%. After considerable experimentation, the product was characterized as a 5′-*O*-*tert*-butyldimethylsilylimidazolyl aminal arising from the reaction of unreacted aldehyde under the TBDMSCl protection conditions. The chemistry and reactivity of this interesting intermediate have been reported elsewhere.[42]

Although NOESY experiments did not provide any useful contacts for assignment purposes also in the case of **13**, as in the thymidine series, the (5′*S*) stereochemistry was assigned to the crystalline protected cyanohydrin isolated. Evidence came from the anisotropy effect observed for the 2′*β*-H in the ^{1}H NMR spectrum, due to the nearby situated multiple bond of the nitrile. In this geometry, this proton experiences the negative side of the nitrile anisotropy cone and, as observed, is shifted by 0.72 ppm to a lower field than the 2′α-H.[43] Further evidence supporting our assignment came from the observed reactivity of the isolated diastereomer (vide infra), which associates it with the corresponding thymidine diastereomer (vide supra).

In the key step of the synthesis, fast *t*BuLi addition to **13**, in THF at low temperature, provided after aqueous workup the imine **14** in 32% yield (Scheme 4). The reaction was never left to completion as prolonged reaction times increased decomposition and base release. After a 5 min reaction, and the above-reported yields of products, there was ca. 40% of unreacted

SCHEME 5. Products and Proposed Mechanism of Formation in the Photolysis of *tert*-Butyl Ketone 2[a]



[a] R = TBDMS.

starting material together with ca. 20% of N^2-isobutyroylguanine present in the product mixture. To optimize the reaction conditions, we attempted several variations but we were never able to obtain better yields. For example, addition of CuI,[44] to mediate the strength of the organometallic reagent, led to prolonged reactions and diminished yields. Once more, the imine **14** exhibited remarkable stability and could be purified through column chromatography without any major loss of material. A characteristic absorption at 186 ppm in the ^{13}C NMR spectrum was assigned to the C═NH carbon. Nevertheless, a slow transformation was observed during ^{13}C NMR in $CDCl_3$, indicating an acid-catalyzed hydrolysis. Mild aqueous acid treatment (CH_3CO_2H/THF/H_2O = 4:1:1) of **14** led to a good yielding (78%) conversion to **2** (Scheme 4). The new product was fully characterized, and HRMS was in agreement with the proposed transformation. Attempts to deprotect both TBDMS groups of **2** were not successful. For example, NH_4F/MeOH or TBAF·SiO_2 led to decomposition with glycosidic bond scission and formation of the aglycon.[45]

Photochemistry in the 2′-Deoxyguanosine System. A variable-intensity 1000 W Xe lamp equipped with a UV-A filter was utilized in the photolysis studies. Photolysis of a THF solution of **2**, in a pyrex vial, in the presence of excess *tert*-butyl thiol, under deaerated conditions, at 110 mW/cm^2 local intensity, led to the complete consumption of the starting material, within 60 min, at analytical conditions (0.5 mL, 13 mmol) and partial conversion at higher concentration (2 mL, 27 mmol). PLC purification of the reaction mixture produced the reduced protected 2′-deoxyguanosine **15** and a new product that was characterized, by comparison with literature data,[46,47] as the protected (5′*S*)-5′,8-cyclo-2′-deoxyguanosine **16**. The stereochemistry of the 5′-carbon was inferred from the $J_{5',4'}$ = 6.2 Hz value, in the ^{1}H NMR spectrum, as has been described.[46] No other diastereomeric cyclic product could be detected in the reaction mixture.

(41) Rawal, V. H.; Rao, J. A.; Cava, M. P. *Tetrahedron Lett.* **1985**, *26*, 4275−4278.

(42) Gimisis, T.; Arsenyan, P.; Georganakis, D.; Leondiadis, L. *Synlett* **2003**, 1451−1454.

(43) Although the diastereomer of **13** could not be isolated in the deoxy series, we were able to isolate both diastereomers in the ribo series (results not presented), whereas an anisotropy effect was observed in only one of the two diastereomers.

(44) Hwang, J. T.; Greenberg, M. M. *J. Am. Chem. Soc.* **1999**, *121*, 4311−4315.

(45) In the presence of BF_3.Et_2O, only the 3′-TBDMS group was chemoselectively removed, thus offering a means of accessing the corresponding phosphoramidite.[31]

(46) Romieu, A.; Gasparutto, D.; Cadet, J. *Chem. Res. Toxicol.* **1999**, *12*, 412−421.

(47) Navacchia, M. L.; Chatgilialoglu, C.; Montevecchi, P. C. *J. Org. Chem.* **2006**, *71*, 4445−4452.

In the proposed mechanism for the above observed reaction, photolysis of the *tert*-butyl ketone **2** generates the 2′-deoxyguanosin-5′-yl radical **17**, which is partitioned between two competitive reaction channels, i.e., a bimolecular reduction leading to 2′-deoxyguanosine **15** and an intramolecular cyclization onto the 8-position of the purine base leading to radical **18** (Scheme 5). The high stereoselectivity of the cyclization reaction can be explained by the steric hindrance between the 5′-*O*-TBDMS substituent and the guanine moiety in analogy with the adenine system.[21,35,47] The cyclization occurs via a chair transition state leading to (5′*S*,8*R*)-**18** followed by rearomatization of the guanine moiety by reaction with the thiyl radical. Under the conditions in which the photolysis was performed (i.e., in the presence of an excess of *t*BuSH), the rate constant of the unimolecular path can be measured by applying free-radical clock methodology.[48,49] Using 0.27 M *t*BuSH, the concentration of thiol during the reaction remained essentially constant (pseudo-first-order conditions) and the following relation (eq 1) is obeyed. A $k_H/k_c = 5.0$ was obtained as an average of two independent experiments at ca. 30 °C. Assuming a $k_H = 5 \times 10^6\ M^{-1}\ s^{-1}$ for the reaction of secondary α-alkoxy carbon radical **17** with *t*BuSH,[50] the cyclization rate constant of $k_c = 1 \times 10^6\ s^{-1}$ can be estimated.[51]

$$\mathbf{[15]/[16]} = (k_H/k_c)[t\mathrm{BuSH}] \qquad (1)$$

Conclusion

We have disclosed a synthetic sequence for the preparation of (5′*R*)-*tert*-butyl ketones **1** and **2**. Our results demonstrate that their photolysis affords selectively the corresponding C5′ radical. The nature of the base plays an important role in the fate of the C5′ radical. In the presence of a physiological concentration of alkanethiol, the thymidin-5′-yl radical is efficiently reduced, whereas the 2′-deoxyguanosin-5′-yl radical adds intramolecularly to the C8−N7 double bond of the guanine moiety with a rate constant of ca. $1 \times 10^6\ s^{-1}$, nearly an order of magnitude faster than the analogous 2′-deoxyadenosin-5′-yl radical.[21]

Our findings furnish a molecular basis for forthcoming experiments involving the incorporation of these photolabile precursors in oligonucleotides and their application in DNA damage mechanistic studies.[52−54] In the absence of O_2, the C5′ radical is partitioned between cyclization and the repair reaction by hydrogen abstraction from glutathione. In the presence of molecular oxygen, O_2 trapping leads to the corresponding peroxyl radical and eventually to a strand break with formation of a 5′-aldehyde, 3′-formyl phosphate, and *trans*-1,4-dioxo-2-butene.[55,56] By taking into account the thiol and molecular oxygen concentrations, the above partitions will be evaluated in relation to restricted conformations of duplex DNA. The photostability of the imines (**5a** or **14**) and their ability to hydrolyze in acidic media to photolabile ketones also make these transformations suitable for biotechnological applications.[57−60] Toward this goal, these systems are currently under investigation.

Experimental Section

(5′*R*)- and (5′*S*)-5′-Cyano-3′,5′-di-*O*-(*tert*-butyldimethylsilyl)-thymidine (4a,b). A solution of aldehyde **3**[27−30] (600 mg, 1.70 mmol) in dry THF (6.0 mL) was added slowly to a mixture of LiOEt (85 μL of 1 M THF, 0.085 mmol) and TBDMS−CN (311 mg, 2.20 mmol) in dry THF (4.0 mL) at 0 °C (ice-bath) under an N_2 atmosphere. The reaction mixture was stirred at room temperature until all starting material was consumed (30−50 min). After workup (EtOAc/water/brine), the organic layer was dried over Na_2SO_4 and concentrated. The resulting yellow oil was purified on silica (from 20% to 30% of EtOAc in *i*-hexane) leading to two main fractions: a major isomer, 5′-cyano-thymidine **4a** (376 mg, 0.76 mmol, 45%), and a minor isomer, 5′-cyano-thymidine **4b** (308 mg, 0.62 mmol, 37%), both as white foams for a total yield of 82% for the pure compounds. ^{1}H NMR (400 MHz, $CDCl_3$), isomer **4a**: $\delta = 8.30$ (s, 1H, NH), 7.46 (d, 1H, H6, $J_{6-Me} = 1.3$ Hz), 6.45 (dd, 1H, H1′, $J_{1'-2'a} = 9.3$ Hz, $J_{1'-2'b} = 5.6$ Hz), 4.64 (d, 1H, H5′, $J_{5'-4'} = 4.0$ Hz), 4.54 (dt, 1H, H3′, $J_{3'-2'b} = 5.2$ Hz, $J_{3'-4'} = 1.4$ Hz), 4.02 (dd, 1H, H4′, $J_{4'-5'} = 4.0$ Hz, $J_{4'-3'} = 1.4$ Hz), 2.25−2.13 (m, 2H, H2′), 1.93 (d, 3H, CH_3-5, $J_{Me-6} = 1.2$ Hz), 0.94 (s, 9H, ^{t}BuSi), 0.90 (s, 9H, ^{t}BuSi), 0.24 (s, 3H, CH_3Si), 0.20 (s, 3H, CH_3Si), 0.11 (s, 3H, CH_3Si), 0.10 (s, 3H, CH_3Si). ^{1}H NMR (400 MHz, $CDCl_3$), isomer **4b**: $\delta = 8.13$ (s, 1H, H3 (NH)), 7.25 (d, 1H, H6, $J_{6-Me} = 1.3$ Hz), 6.27 (dd, 1H, H1′, $J_{1'-2'a} = 8.2$ Hz, $J_{1'-2'b} = 6.0$ Hz), 4.65 (d, 1H, H5′, $J_{5'-4'} = 3.4$ Hz), 4.48 (dt, 1H, H3′, $J_{3'-2'b} = 6.1$ Hz, $J_{3'-4'} = 3.1$ Hz), 4.02 (dd, 1H, H4′, $J_{4'-5'} = 3.3$ Hz, $J_{4'-3'} = 3.3$ Hz), 2.29−2.23 (ddd, 1H, H2$_b$′, $J_{2'b-2'a} = 13.4$ Hz, $J_{2'b-1'} = 6.0$ Hz, $J_{2'b-3'} = 2.9$ Hz), 2.19−2.12 (m, 1H, H2$_b$′, $J_{2'a-2'b} = 13.4$ Hz, $J_{2'a-1'} = 8.1$ Hz), 1.93 (d, 3H, CH_3-5, $J_{Me-6} = 1.2$ Hz), 0.96 (s, 9H, ^{t}Bu−Si), 0.91 (s, 9H, ^{t}Bu−Si), 0.26 (s, 3H, CH_3−Si), 0.20 (s, 3H, CH_3−Si), 0.14 (s, 3H, CH_3−Si), 0.13 (s, 3H, CH_3−Si). ^{13}C NMR (100.6 MHz, $CDCl_3$), isomer **4a**: $\delta = 163.2$ (C), 150.2 (C), 135.0 (CH), 118.7 (C), 111.7 (C), 87.3 (CH), 84.6 (CH), 72.3 (CH), 62.2 (CH), 40.2 (CH_2), 25.7 (CH_3), 25.6 (CH_3), 18.2 (C), 17.9 (C), 12.5 (CH_3), −4.6 (CH_3), −4.8 (CH_3), −5.2 (CH_3), −5.3 ppm (CH_3). ^{13}C NMR (100.6 MHz, $CDCl_3$), isomer **4b**: $\delta = 163.0$ (C), 149.9 (C), 135.4 (CH), 117.7 (C), 111.4 (C), 87.5 (CH), 85.5 (CH), 71.2 (CH), 62.8 (CH), 39.9 (CH_2), 25.6 (CH_3), 25.6 (CH_3), 18.2 (C), 17.8 (C), 12.4 (CH_3), −4.6 (CH_3), −4.8 (CH_3), −5.0 (CH_3), −5.1 (CH_3). HR-ESI-MS isomer **4a**: for $C_{23}H_{42}N_3O_5Si_2^+$ $[M + H]^+$, calcd 496.2658; found 496.2630. HR-ESI-MS isomer **4b**: for $C_{23}H_{42}N_3O_5Si_2^+$ $[M + H]^+$, calcd 496.2658; found 496.2661. R_f **4a** (EtOAc/*i*-Hex 2:3) = 0.58. R_f **4b** (EtOAc/*i*-Hex 2:3) = 0.68.

(5′*S*)-3′,5′-Di-*O*-(*tert*-butyldimethylsilyl)-5′-(2,2-dimethylpropanimidoyl)thymidine 5a. To a solution of **4a** (300 mg, 0.60 mmol) in THF (12 mL) at −78 °C was slowly added *tert*-BuLi (1.5 M, 1.5 mL, 2.4 mmol). After 2 min of vigorous stirring at −78 °C, the reaction was quenched by water addition and allowed to warm to room temperature. The mixture was diluted with EtOAc, and the organic phases were then washed with water until neutrality and finally with brine. Anhydrification on $MgCl_2$ and subsequent

(48) Griller, D.; Ingold, K. U. *Acc. Chem. Res.* **1980**, *13*, 317−323.
(49) Newcomb, M. *Tetrahedron* **1993**, *49*, 1151−1176.
(50) Tronche, C.; Martinez, F. N.; Horner, J. H.; Newcomb, M.; Senn, M.; Giese, B. *Tetrahedron Lett.* **1996**, *37*, 5845−5848.
(51) In recent studies of 8-bromo-2′-deoxyguanosine photolysis or 2′-deoxyguanosine radiolysis, the cyclization of the fully deprotected 2′-deoxyguanosin-5′-yl radical in aqueous media has been observed (Chatgilialoglu, C.; Bazzanini, R.; Jimenez, L. B.; Miranda, M. A., unpublished results).
(52) Gimisis, T.; Cismas, C. *Eur. J. Org. Chem.* **2006**, 1351−1378.
(53) Neeley, W. L.; Essigmann, J. M. *Chem. Res. Toxicol.* **2006**, *19*, 491−505.
(54) Pratviel, G.; Meunier, B. *Chem.−Eur. J.* **2006**, *12*, 6018−6030.
(55) Chen, B. Z.; Bohnert, T.; Zhou, X. F.; Dedon, P. C. *Chem. Res. Toxicol.* **2004**, *17*, 1406−1413.
(56) Chen, B. Z.; Vu, C. C.; Byrns, M. C.; Dedon, P. C.; Peterson, L. A. *Chem. Res. Toxicol.* **2006**, *19*, 982−985.
(57) Cecchini, S.; Masson, C.; La Madeleine, C.; Huels, M. A.; Sanche, L.; Wagner, J. R.; Hunting, D. J. *Biochemistry* **2005**, *44*, 16957−16966.
(58) Greenberg, D. A.; Jin, K. L. *Nature* **2005**, *438*, 954−959.
(59) Galanski, M.; Baumgartner, C.; Meelich, K.; Arion, V. B.; Fremuth, M.; Jakupec, M. A.; Schluga, P.; Hartinger, C. G.; Von Keyserlingk, N. G.; Keppler, B. K. *Inorg. Chim. Acta* **2004**, *357*, 3237−3244.
(60) Ulbrich, K.; Etrych, T.; Chytil, P.; Pechar, M.; Jelinkova, M.; Rihova, B. *Int. J. Pharm.* **2004**, *277*, 63−72.

rotary evaporation of the solvent lead to 144 mg (0.27 mmol, 43%) of yellow oil, analyzed as crude product. ^{1}H NMR (400 MHz, acetone-d_6): δ = 9.99 (s, 1H, NH), 7.51 (bs, 1H, H6), 6.28 (dd, 1H, H1′, $J_{1'-2'a}$ = 8.9 Hz, $J_{1'-2'b}$ = 5.5 Hz), 4.78 (d, 1H, H5′, J = 1.5 Hz), 4.53 (d, 1H, H3′, J = 5.5 Hz), 4.21 (bs, 1H, H4′), 2.25−2.11 (m, 2H, H2′), 1.90 (d, 3H, CH_3-5, J = 1.1 Hz), 1.25 (s, 9H, ^{t}Bu−CO), 1.02 (s, 9H, ^{t}Bu−Si), 0.94 (s, 9H, ^{t}Bu−Si), 0.24 (s, 3H, CH_3−Si), 0.18 (s, 3H, CH_3−Si), 0.17 (s, 3H, CH_3−Si), 0.10 (s, 3H, CH_3−Si). ^{13}C NMR (100.5 MHz, acetone-d_6): δ = 185.8 (q, C=NH), 165.5 (C), 152.0 (C), 136.5 (CH), 112.0 (C), 89.3 (CH), 86.0 (CH), 73.1 (CH), 70.2 (CH), 42.5 (CH_2), 39.8 (C), 30.2 (CH_3), 27.4 (CH_3), 27.1 (CH_3), 19.9 (C), 19.6 (C), 13.7 (CH_3), −3.3 (CH_3), −3.5 (CH_3), −3.6 (CH_3), −3.6 (CH_3). HR-ESI-MS for $C_{27}H_{52}N_3O_5Si_2^+$ [M + H]$^+$, calcd 554.3440; found 554.3420. R_f (EtOAc/*i*-Hex 2:3) = 0.36.

(5′*R*)-3′,5′-Di-*O*-(*tert*-butyldimethylsilyl)-5′-(*tert*-butylcarbonyl)thymidine 1a. A portion of 10 mL of an acidic solution (THF/H_2O/2 N HCl, 40:20:1) was added to the imine **5a** (100 mg, 0.20 mmol), and the mixture was stirred for 3 h at room temperature. The phases were then separated after dilution with 10 mL of EtOAc, and the organic layer was washed with water until neutrality and finally with brine. Anhydrification on $MgCl_2$ and subsequent rotary evaporation of the solvent yielded an orange oil. Purification on silica gel (from 1% to 3% of MeOH in $CHCl_3$) provided the *tert*-butyl ketone **1a** as a yellow oil (98 mg, 0.18 mmol, 88%). ^{1}H NMR (400 MHz, acetone-d_6): δ = 9.89 (s, 1H, NH), 7.85 (d, 1H, H6, J = 1.2 Hz), 6.30 (dd, 1H, H1′, $J_{1'-2'a}$ = 9.1 Hz, $J_{1'-2'b}$ = 5.4 Hz), 5.17 (d, 1H, H5′, J = 2.4 Hz), 4.61 (d, 1H, H3′, J = 4.8 Hz), 4.57 (d, 1H, H4′, J = 2.2 Hz), 2.29−2.14 (m, 2H, H2′), 1.93 (d, 3H, CH_3-5, J = 1.1 Hz), 1.24 (s, 9H, ^{t}Bu−CO), 0.96 (s, 9H, ^{t}Bu−Si), 0.95 (s, 9H, ^{t}Bu−Si), 0.18 (s, 3H, CH_3−Si), 0.17 (s, 3H, CH_3−Si), 0.15 (s, 3H, CH_3−Si), 0.11 (s, 3H, CH_3−Si). ^{1}H NMR (600 MHz, $CDCl_3$): δ = 8.12 (s, 1H, H3 (NH)), 7.85 (bs, 1H, H6), 6.32 (dd, 1H, H1′, $J_{1'-2'a}$ = 9.0 Hz, $J_{1'-2'b}$ = 5.4 Hz), 4.86 (d, 1H, H5′, $J_{5'-4'}$ = 2.0 Hz), 4.47 (d, 1H, H3′, $J_{3'-2'b}$ = 5.0 Hz), 4.44 (d, 1H, H4′, J = 1.5 Hz), 2.25 (dd, 1H, H2$_b$′, $J_{2b'-2'a}$ = 12.8 Hz, $J_{2'b-1'}$ = 5.4 Hz), 2.02 (bs, 3H, CH_3-5), 2.00−1.98 (m, 1H, H2a′, $J_{2'a-2'b}$ = 12.9 Hz, $J_{2'a-1'}$ = 9.0 Hz, $J_{2'a-3'}$ = 4.9 Hz), 1.23 (s, 9H, ^{t}Bu−CO), 0.92 (s, 9H, ^{t}Bu−Si), 0.91 (s, 9H, ^{t}Bu−Si), 0.11 (s, 6H, $(CH_3)_2$−Si), 0.10 (s, 3H, CH_3−Si), 0.02 (s, 3H, CH_3−Si). ^{13}C NMR (150.8 MHz, $CDCl_3$): δ = 210.6 (C), 163.7 (C), 150.1 (C), 136.5 (CH), 110.7 (C), 88.1 (CH), 86.1 (CH), 75.2 (CH), 74.8 (CH), 43.1 (C), 41.5 (CH_2), 27.5 (CH_3), 25.8 (CH_3), 25.8 (CH_3), 18.4 (C), 18.1 (C), 12.4 (CH_3), −4.5 (CH_3), −4.6 (CH_3), −4.8 (CH_3), −5.5 (CH_3). HR-ESI-MS for $C_{27}H_{51}N_2O_6Si_2^+$ [M + H]$^+$, calcd 555.3280; found 555.3277. UV λ_{max} = 264 nm; ϵ_{260} = 1049 ± 64 M^{-1} cm^{-1}. R_f (EtOAc/*i*-Hex 2:3) = 0.78.

(5′*R*)-3′,5′-Di-*O*-(*tert*-butyldimethylsilyl)-5′-(2,2-dimethylpropanimidoyl)thymidine 5b. To a solution of **4b** (300 mg, 0.60 mmol) in THF (12 mL) at −78 °C was slowly added *tert*-BuLi (1.5 M, 1.5 mL, 2.4 mmol). After 2 min of vigorous stirring at −78 °C, the reaction was quenched by water addition and left to warm to room temperature. The mixture was diluted with EtOAc, and the organic phases were then washed with water until neutrality and finally with brine. Anhydrification on $MgCl_2$ and subsequent rotary evaporation of the solvent led to 125 mg (0.22 mmol, 38%) of yellow oil, analyzed as crude product. ^{1}H NMR (400 MHz, acetone-d_6): δ = 10.06 (s, 1H, NH), 7.34 (bs, 1H, H6), 6.21 (bdd, 1H, H1′, J = 7.2 Hz), 4.73 (d, 1H, H5′, J = 1.4 Hz), 4.61 (m, 1H, H3′), 4.04 (dd, 1H, H4′, $J_{4'-3'}$ = 3.4 Hz, $J_{4'-5'}$ = 1.3 Hz), 2.21−2.16 (m, 2H, H2′), 1.86 (bs, 3H, CH_3-5), 1.27 (s, 9H, ^{t}Bu−CO), 1.00 (s, 9H, ^{t}Bu−Si), 0.88 (s, 9H, ^{t}Bu−Si), 0.14 (s, 3H, CH_3−Si), 0.10 (s, 3H, CH_3−Si), 0.07 (s, 3H, CH_3−Si), 0.03 (s, 3H, CH_3−Si). ^{13}C NMR (100.5 MHz, acetone-d_6): δ = 187.2 (q, C=NH), 165.0 (C), 156.9 (C), 137.0 (CH), 111.9 (C), 89.3 (CH), 88.2 (CH), 75.9 (CH), 70.5 (CH), 49.1 (CH_2), 40.1 (C), 30.6 (CH_3), 27.4 (CH_3), 27.2 (CH_3), 19.8 (C), 19.3 (C), 13.5 (CH_3), −2.2 (CH_3), −3.2 (CH_3), −3.4 (CH_3), −3.6 (CH_3). HR-ESI-MS for $C_{27}H_{52}N_3O_5Si_2^+$ [M + H]$^+$, calcd 554.3440; found 554.3431. R_f (EtOAc/*i*-Hex 2:3) = 0.34.

(5′*S*)-3′,5′-Di-*O*-(*tert*-butyldimethylsilyl)-5′-(*tert*-butylcarbonyl)thymidine 1b. A portion of 10 mL of $CDCl_3$ was added to the oil of the crude product containing **5b** (100 mg, 0.20 mmol), and the solution was stirred for 30 min at room temperature. The reaction was followed by ^{1}H NMR. The hydrolysis of **5b** to **1b** was complete in 30 min. Stirring of this solution for a longer time led to the degradation of the product of reaction with the replacement of the NMR peaks of **1b** by many unknown peaks. $CDCl_3$ was removed by rotary evaporation followed by high vacuum, and the obtained yellow oil (78 mg, 70%) was dissolved in acetone-d_6 for NMR characterization. ^{1}H NMR (400 MHz, acetone-d_6): δ = 10.03 (s, 1H, NH), 7.32 (s, 1H, H6), 6.27 (bdd, 1H, H1′, J = 7.3 Hz), 5.00 (d, 1H, H5′, $J_{5'-4'}$ = 2.5 Hz), 4.72 (m, 1H, H3′), 4.13 (bt, 1H, H4′, J = 2.5 Hz), 2.15 (m, 2H, H2′), 1.85 (bs, 3H, CH_3-5), 1.25 (s, 9H, ^{t}Bu−CO), 0.97 (s, 9H, ^{t}Bu−Si), 0.89 (s, 9H, ^{t}Bu−Si), 0.13 (s, 3H, CH_3−Si), 0.12 (s, 3H, CH_3−Si), 0.09 (s, 3H, CH_3−Si), 0.08 (s, 3H, CH_3−Si). ^{13}C NMR (150.8 MHz, acetone-d_6): δ = 214.4 (C), 152.4 (C), 147.2 (C), 136.9 (CH), 111.9 (C), 88.7 (CH), 85.2 (CH), 76.7 (CH), 72.2 (CH), 45.3 (C), 41.8 (CH_2), 28.6 (CH_3), 27.4 (CH_3), 27.2 (CH_3), 19.9 (C), 19.4 (C), 13.5 (CH_3), −3.1 (CH_3), −3.2 (CH_3), −3.3 (CH_3), −3.5 (CH_3). HR-ESI-MS for $C_{27}H_{50}N_2NaO_6Si_2^+$ [M + Na]$^+$, calcd 577.3100; found 577.3068. HR-ESI-MS for $C_{27}H_{50}ClN_2O_6Si_2^-$ [M + Cl]$^-$, calcd 589.2901; found 589.2945. R_f (EtOAc/*i*-Hex 2:3) = 0.76.

(5′*R*)-5′-(*tert*-Butylcarbonyl)thymidine 6. To a solution of 3′,5′-(*R*)-TBDMS-ketone **1a** (170 mg, 0.3 mmol) in MeOH (10 mL) was added a solution of TBAF (1 M) in THF (1 mL, 1.0 mmol). The mixture was refluxed for 5 h and then stirred at room temperature for 24 h. MeOH was then removed by reduced pressure, and the crude product was purified on silica gel (EtOAc in *i*-hexane from 30% to 90%). A white foam (61 mg, 0.19 mmol, 60%) was isolated from the fractions and identified as 3′,5′-OH ketone **6** by NMR and mass analysis. A less polar fraction was collected leading to 10 mg (7.3% from the bis-protected ketone) of white powder analyzed by NMR and mass spectral analyses and identified as 3′-OH, 5′-TBDMS ketone **7**. ^{1}H NMR (600 MHz, $CDCl_3$): δ = 8.93 (bs, 1H, H3 (NH)), 7.36 (bs, 1H, H6), 6.26 (dd, 1H, H1′, $J_{1'-2'a}$ = 7.5 Hz, $J_{1'-2'b}$ = 6.2 Hz), 4.77 (d, 1H, H5′, J = 1.6 Hz), 4.72 (dt, 1H, H3′, J = 5.7 Hz, J = 2.7 Hz), 4.46 (m, 1H, H4′), 2.37−2.34 (ddd, 1H, H2$_b$′, $J_{2b'-2'a}$ = 13.5 Hz, $J_{2'b-1'}$ = 5.9 Hz, J = 2.9 Hz), 2.23−2.18 (m, 1H, H2a′, $J_{2'a-2'b}$ = 13.7 Hz, $J_{2'a-1'}$ = 7.2 Hz), 1.96 (bs, 3H, CH_3-5), 1.27 (s, 9H, ^{t}Bu−CO). ^{13}C NMR (150.8 MHz, $CDCl_3$): δ = 214.1 (q, C5″), 163.7 (C), 150.4 (C), 135.5 (CH, C6), 111.2 (q, C5), 86.3 (CH, C4′), 85.4 (CH, C1′), 73.3 (CH, C5′), 72.5 (CH, C3′), 42.9 (q, C6″), 40.6 (CH_2, C2′), 27.1 (CH_3, C6″-^{t}Bu), 12.6 (CH_3, C5-Me). HR-ESI-MS for $C_{15}H_{21}N_2O_6^-$ [M − H]$^-$, calcd 325.1405; found 325.1409. R_f (EtOAc/*i*-Hex 1:1) = 0.11.

(5′*R*)-5′-*O*-(*tert*-Butyldimethylsilyl)-5′-(*tert*-butylcarbonyl)thymidine 7. An independent synthesis of **7** was achieved as follows: 170 mg (0.3 mmol) of ketone **1a** was dissolved in THF (10 mL), and TBAF (1 M in THF, 1 mL, 1.0 mmol) was added to this mixture at low temperature (−15 °C) and stirred for 24 h at −15 °C. Under these conditions, the relative yields after purification on silica gel (EtOAc in *i*-hexane from 30% to 90%) of **6** and **7** were 10% and 55%, respectively. ^{1}H NMR (600 MHz, DMSO-d_6): δ = 11.27 (s, 1H, H3 (NH)), 7.80 (bs, 1H, H6), 6.12 (dd, 1H, H1′, $J_{1'-2'a}$ = 8.5 Hz, $J_{1'-2'b}$ = 5.4 Hz), 5.07 (d, 1H, H5′, J = 2.2 Hz), 4.41 (bs, 1H, H3′), 4.30 (d, 1H, H4′, J = 4.8 Hz), 2.15 (dd, 1H, H2′, $J_{2'b-2'a}$= −13.2 Hz, $J_{2'b-1'}$ = 5.7 Hz), 1.93 (ddd, 1H, H2′, $J_{2'a-2'b}$ = −13.8 Hz, $J_{2'a-1'}$ = 8.9 Hz, $J_{2'a-3'}$ = 5.4 Hz), 1.83 (bs, 3H, CH_3-5), 1.16 (s, 9H, ^{t}Bu−CO), 0.87 (s, 9H, ^{t}Bu−Si), 0.04 (s, 3H, CH_3−Si), 0.03 (s, 3H, CH_3−Si). ^{13}C NMR (150.8 MHz, DMSO-d_6): δ = 210.8 (C), 163.2 (C), 149.7 (C), 135.4 (CH), 108.5 (C), 86.6 (CH, C4′), 84.7 (CH, C1′), 74.3 (CH, C5′), 71.9 (CH, C3′), 42.2 (CH_2, C2′), 26.6 (CH_3), 25.1 (CH_3), 17.5 (C), 11.8 (CH_3), −5.3 (CH_3), −5.9 (CH_3). HR-ESI-MS for $C_{21}H_{37}N_2O_6Si^+$ [M + H]$^+$, calcd 441.2415; found 441.2396. HR-ESI-MS for $C_{21}H_{36}ClN_2O_6Si^-$ [M + Cl]$^-$, calcd 475.2037; found 475.2078. R_f = (EtOAc/*i*-Hex 1:1) = 0.50.

Synthesis of (5′*R*)-3′-*O*-Acetyl-5′-*O*-(*tert*-butyldimethylsilyl)-5′-(*tert*-butylcarbonyl)thymidine 8. To a solution of 3′-OH, 5′-TBDMS-ketone **7** (73 mg, 0.2 mmol) in dry pyridine (2 mL) was added 35 μL (0.38 mmol) of acetic anhydride. The mixture was stirred at room temperature for 24 h. The solvent was removed by rotary evaporation, and the residue was coevaporated three times with 1 mL of toluene. The ketone **8** was obtained in 82% yield as a white powder and was characterized. The 5′(*R*) configuration was assigned by resolution of the crystal obtained from a saturated EtOAc solution of **8**. ^{1}H NMR (600 MHz, $CDCl_3$): δ = 8.06 (s, 1H, H3 (NH)), 7.87 (d, 1H, H6, J = 1.1 Hz), 6.30 (dd, 1H, H1′, $J_{1'-2'a}$ = 9.2 Hz, $J_{1'-2'b}$ = 5.4 Hz), 5.22 (d, 1H, H3′, J = 5.9 Hz), 5.08 (d, 1H, H5′, J = 2.1 Hz), 4.51 (d, 1H, H4′, J = 1.8 Hz), 2.48 (dd, 1H, H2′, $J_{2'b-2'a}$ = −13.8 Hz, $J_{2'b-1'}$ = 5.4 Hz), 2.13 (m, 1H, H2′), 2.14 (s, 3H, Ac), 2.03 (d, 3H, CH_3-5, J = 1.0 Hz), 1.21 (s, 9H, ^{t}Bu−CO), 0.92 (s, 9H, ^{t}Bu−Si), 0.11 (s, 3H, CH_3−Si), 0.06 (s, 3H, CH_3−Si). ^{13}C NMR (150.8 MHz, $CDCl_3$): δ = 210.6 (C), 171.0 (C), 163.5 (C), 150.1 (C), 136.0 (CH), 111.1 (C), 85.5 (CH), 85.5 (CH), 74.8 (CH), 43.1 (C), 37.6 (CH_2), 27.4 (CH_3), 25.8 (CH_3), 21.0 (CH_3), 18.4 (C), 12.5 (CH_3), −4.5 (CH_3), −5.4 (CH_3). HR-ESI-MS for $C_{23}H_{39}N_2O_7Si^+$ [M + H]$^+$, calcd 483.2521; found 483.2502. HR-ESI-MS for $C_{23}H_{37}N_2O_7Si^-$ [M − H]$^-$, calcd 481.2376; found 481.2425. R_f (EtOAc/*i*-Hex, 2:3) = 0.42.

X-Ray Crystal Structure Determination. X-ray data were collected at 200 K with a diffractometer equipped with a rotating anode and graded multilayer X-ray optics to get monochrome Mo Kα radiation (λ = 0.71073 Å). The structure was solved with direct methods with SIR97[61] and refined with SHELXL-97.[62] Hydrogen atoms were calculated in idealized positions riding on their parent atoms. One of the *tert*-butyl groups is disordered; a split model was applied with isotropic refinement of the split atoms. Data for compound **8**: $C_{23}H_{38}N_2O_7Si$, fw = 482.643, colorless block, 0.18 × 0.15 × 0.11 mm^3, monoclinic, *P*21 (No. 4), a = 14.1327(4) Å, b = 10.9042(3) Å, c = 17.7449(6) Å, β = 93.3188(11)°, V = 2730.01(14) Å,^{3} Z = 47 119 unique reflections, 593 parameters were refined, R1 ($I > 2\sigma(I)$) = 0.0621, wR2 (all reflections) = 0.1774, S = 1.062. Residual electron density between 0.763 and −0.316 e Å^{-3}. Further details are available under the depository number CCDC 618360 from the Cambridge Crystallographic Data Centre.

(5′*S*)-2-*N*-Isobutyryl-3′,5′-di-*O*-(*tert*-butyldimethylsilyl)-5′-cyano-2′-deoxyguanosine (13). To a stirred solution of compound **12**[38] (2.40 g, 5.31 mmol), under an inert atmosphere, in dry toluene/DMSO (2:1, 48 mL), at room temperature, were added solid EDC (3.03 g, 15.9 mmol), pyridine (1.48 mL, 18.3 mmol), and CF_3-COOH (0.20 mL, 2.65 mmol). After the last addition, the cleared solution was stirred for 15 min, then diluted with CH_3Cl and washed with water and brine (each 50 mL). After drying over Na_2SO_4, the organic phase was condensed under a vacuum and the well-dried foamy residue (2.26 g, 95%) was applied directly to the next step. ^{1}H NMR (200 MHz, $CDCl_3$): characteristic peaks at δ = 9.76 (s, H, C*H*O), 7.79 (s, H, H8), 6.33 (m, H, H1′). R_f (CH_2Cl_2/MeOH, 9:1) = 0.65. To a stirred solution containing the above foam (1.87 g, 4.16 mmol) and ZnI_2 (70 mg) in dry CH_2Cl_2 (14 mL), under an inert atmosphere, was added dropwise TMSCN (1.35 mL, 10.12 mmol), at room temperature. After 5 h or upon disappearance of starting material on TLC, the mixture was diluted with EtOAc and washed with sat. aq $NaHCO_3$ and brine (each 50 mL). After drying over Na_2SO_4, the organic phase was condensed under a vacuum and the well-dried product foam (1.74 g, 88%) was applied directly to the next step. ^{1}H NMR (200 MHz, $CDCl_3$): characteristic peaks at δ = 7.86 (s, H, H8β), 7.78 (s, H, H8α), 6.19 (m, 2H, H1′). R_f (CH_3Cl/MeOH, 9:1) = 0.44. To a stirred solution containing the above cyanohydrin (1.50 g, 2.71 mmol) and imidazole (680 mg, 9.50 mmol) in dry DMF (15 mL), under an inert atmosphere, was added TBDMSCl (1.22 g, 8.14 mmol), at room temperature. After the initial gas evolution had subsided, the reaction was stirred overnight and then diluted with EtOAc and washed with sat. aq $NaHCO_3$ and brine (each 50 mL). After drying over Na_2SO_4, the organic phase was filtered, condensed under a vacuum, purified by column chromatography ($CHCl_3$/MeOH, 95:5), and precipitated from an ether/hexane mixture to yield **13** (400 mg, 25%) as a white solid. mp: 134−136 °C. ^{1}H NMR (200 MHz, $CDCl_3$): δ = 12.05 (bs, 1H, N*H*), 9.41 (bs, 1H, N*H*), 7.92 (s, 1H, H8), 6.15 (dd, 1H, H1′, $J_{1'-2'a}$ = 9.6 Hz, $J_{1'-2'b}$ = 5.0 Hz), 4.69 (d, 1H, H3′, J = 5.2 Hz), 4.63 (d, 1H, H5′, J = 4.2 Hz), 4.05 (dd, 1H, H4′, $J_{4'-5'}$ = 4.2 Hz, $J_{4'-3'}$ = 1.4 Hz), 2.61 (hpt, 1H, ^{i}Pr), 3.01 (ddd, 1H, H2′α), 2.29 (dd, 1H, H2′β, $J_{2'b-2'a}$ = −13.2 Hz, $J_{2'b-1'}$ = 5.2 Hz), 1.26, 1.23 (each s, 3H, ^{i}Pr), 0.92 (d, 18H, SitBu), 0.21, 0.18 (each s, 3H, SiMe), 0.14 (s, 6H, SiMe). ^{13}C NMR (50 MHz, $CDCl_3$): δ = 178.7, 155.8, 148.4, 147.6 (each C), 138.2 (CH), 122.1, 119.0 (each C), 88.5, 85.0, 82.8, 72.4 (each CH), 43.4 (CH_2), 36.6 (CH), 25.8 (6 × CH_3), 19.0 (2 × CH_3), 18.4, 18.0 (each C), −4.3, −4.9 (each CH_3). IR (NaCl) 2332 (C≡N), 1713 (C═O), 1688 cm^{-1} (C═O). HR-ESI-MS for $C_{27}H_{45}N_6O_5Si_2^-$ [M − H]$^-$, calcd 589.2995; found 589.2997. R_f (CH_2Cl_2/MeOH, 9:1) = 0.49.

(5′*S*)-2-*N*-Isobutyryl-3′,5′-di-*O*-(*tert*-butyldimethylsilyl)-5′-(2,2-dimethylpropanimidoyl)-2′-deoxyguanosine (14). To a solution of the nitrile **13** (450 mg, 0.76 mmol) in dry THF (4 mL), at −78^{o}C, under an argon atmosphere, was added dropwise *t*-BuLi (1.34 M in pentane, 2.84 mL, 3.8 mmol). After 5 min of vigorous stirring, the bright red reaction mixture was quenched by addition of methanol (1 mL) followed by CH_3COOH (0.5 mL) and H_2O (1 mL) and left to slowly warm to room temperature. Dilution with EtOAc (30 mL), washing with H_2O, sat. aq $NaHCO_3$, and brine (each 30 mL), drying over Na_2SO_4, filtering, and evaporating under reduced pressure furnished the crude product that was further purified by silica gel flash column chromatography ($CHCl_3$/MeOH, 97:3) to yield the imine **14** (160 mg, 32.4%) as an off-white foam, together with unreacted starting material **13** (180 mg, 40%) and 2-*N*-isobutyroylguanine (38 mg, 20%). ^{1}H NMR (200 MHz, CD_2Cl_2): δ = 11.95 (bs, 1H, N*H*), 10.20 (bs, 1H, N*H*), 8.00 (s, 1H, H8), 6.20 (dd, 1H, H1′, $J_{1'-2'a}$ = 6.3 Hz, $J_{1'-2'b}$ = 6.1 Hz), 4.72 (d, 1H, H5′, J = 1.8 Hz), 4.49 (m, 1H, H3′), 4.03 (dd, 1H, H4′, $J_{4'-3'}$ = 3.5 Hz, $J_{4'-5'}$ = 1.7 Hz), 2.72 (hpt, 1H, ^{i}Pr), 2.29−2.35 (m, 2H, H2′), 1.26 (s, 9H, ^{t}Bu), 1.16 (s, 3H, ^{i}Pr), 1.15 (s, 3H, ^{i}Pr), 0.92, 0.88 (each s, 9H, Si^{t}Bu), 0.11, 0.09, 0.07, 0.04 (each s, 3H, SiMe). ^{13}C NMR (125 MHz, $CDCl_3$): δ = 186.4, 179.7, 156.2, 148.4, 148.2 (each C), 136.6 (CH), 121.2 (C), 87.8, 83.7, 72.5, 70.5 (each CH), 43.2 (CH_2), 38.9 (C), 36.4 (CH), 29.4 (CH_3), 26.2 (6 × CH_3), 19.3 (2 × CH_3), 18.6, 18.3 (each C), −3.7, −4.3 (each 2 × CH_3). LR-ESI-MS: 649 [M + H]$^+$, 1297 [2M + H]$^+$, 209 [B + H]$^+$. HR-ESI-MS: for $C_{31}H_{55}N_6O_5Si_2^-$ [M − H]$^-$, calcd 647.3778; found 647.3757. R_f (CH_2Cl_2/MeOH, 9:1) = 0.43.

(5′*R*)-2-*N*-Isobutyryl-3′,5′-di-*O*-(*tert*-butyldimethylsilyl)-5′-(*tert*-butylcarbonyl)-2′-deoxyguanosine (2). A solution of the *tert*-butyl imine **14** (50 mg, 0.077 mmol) in THF/CH_3CO_2H/H_2O (1:4:1, 3 mL) was stirred for 1 h at room temperature. Dilution with EtOAc (10 mL), washing with H_2O, sat. aq $NaHCO_3$, and brine (each 10 mL), drying over Na_2SO_4, filtering, and evaporating under reduced pressure furnished the crude product that was further purified by silica gel flash column chromatography (CHCl3/MeOH, 97:3) to yield the product **2** (39 mg, 78.1%) as a white foam. ^{1}H NMR (200 MHz, $CDCl_3$): δ = 11.85 (bs, 1H, N*H*), 8.65 (bs, 1H, N*H*), 8.25 (s, 1H, H8), 6.21 (dd, 1H, H1′, $J_{1'-2'a}$ = $J_{1'-2'b}$ = 6.0 Hz), 4.89 (d, 1H, H5′, J = 2.6 Hz), 4.63 (m, 1H, H3′), 4.45 (dd, 1H, H4′, $J_{4'-3'}$ = 3.3 Hz, $J_{4'-5'}$ = 2.6 Hz), 2.62 (hpt, 1H, ^{i}Pr), 2.30−2.37 (m, 2H, H2′), 1.28 (s, 3H, ^{i}Pr), 1.24 (s, 3H, ^{i}Pr), 1.20 (s, 9H, CO^{t}Bu), 0.93, 0.88 (each s, 9H, Si^{t}Bu), 0.13 (s, 6H, SiMe), 0.12, 0.03 (each s, 3H, SiMe). ^{13}C NMR (50 MHz, $CDCl_3$): δ = 210.3, 185.9, 156.4, 148.5, 143.7 (each C), 136.8 (CH), 121.1 (C), 90.6,

(61) Altomare, A.; Burla, M. C.; Camalli, M.; Cascarano, G. L.; Giacovazzo, C.; Guagliardi, A.; Moliterni, A. G. G.; Polidori, G.; Spagna, R. *J. Appl. Crystallogr.* **1999**, *32*, 115−119.

(62) Sheldrick, G. M. *SHELXL-97*, 97-2 version; University of Göttingen: Germany.

(63) Nagatsugi, F.; Uemura, K.; Nakashima, S.; Maeda, M.; Sasaki, S. *Tetrahedron* **1997**, *53*, 3035−3044.

83.6, 80.9, 74.7 (each CH), 46.0 (C), 38.9 (CH_2), 36.4 (CH), 29.6, 26.3, 26.1 (each 3 × CH_3), 19.4 (2 × CH_3), 18.5, 18.3 (each C), −3.9, −4.2 (each 2 × CH_3). UV $\lambda = 253$ ($\epsilon = 9973$), 281 nm ($\epsilon = 7748$ M^{-1} cm^{-1}). HR-ESI-MS: for $C_{31}H_{54}N_5O_6Si_2^-$ [M − H]$^-$, calcd 648.3618; found 648.3603. R_f (CH_2Cl_2/MeOH, 9:1) = 0.34.

(5′*S*)-2-*N*-Isobutyryl-3′,5′-di-*O*-(*tert*-butyldimethylsilyl)-5′,8-cyclo-2′-deoxyguanosine (16). To a solution of **2** (35 mg, 0.054 mmol) in THF (2 mL) in a 5-mL pyrex vial was added *t*-BuSH (30 μL, 0.54 mmol). After 15 min of argon bubbling, the solution was irradiated at 5 °C with an Xe lamp at 1000 W, delivering 110 mW/cm^2 local intensity of UV-A radiation on the sample. After 60 min of irradiation, the solvent was evaporated under reduced pressure and the residue was purified through a short path column chromatography ($CHCl_3$) to yield starting material **2** (15 mg, 43%), the reduced 2′-deoxyguanosine **15** (8 mg, 27%), and the cyclized title compound **16** (6 mg, 20%). **15.**[63] ^{1}H NMR (200 MHz, $CDCl_3$): $\delta = 11.50$ (bs, 1H, N*H*), 8.20 (bs, 1H, N*H*), 7.95 (s, H, H8), 6.21 (dd, 1H, H1′, $J_{1'-2'a} = J_{1'-2'b} = 6.6$ Hz), 4.56 (m, 1H, H3′), 3.96 (dd, 1H, H4′, $J_{4'-3'} = 7.2$, $J_{4'-5'} = 3.6$ Hz), 3.75 (d, 2H, H5′, $J = 3.6$ Hz), 2.59 (hpt, 1H, C*H*Me_2, $J = 7.2$ Hz), 2.35−2.45 (m, 2H, H2′), 1.28 & 1.25 (each d, 3H, ^{i}Pr, $J = 7.2$ Hz), 0.90 (s, 18H, 2 × ^{t}Bu), 0.09, 0.07 (each s, 6H, $SiMe_2$). **16.** ^{1}H NMR (200 MHz, $CDCl_3$): $\delta = 11.85$ (bs, 1H, N*H*), 8.60 (bs, 1H, N*H*), 6.17 (d, 1H, H1′, $J = 4.8$ Hz), 5.16 (d, 1H, H5′, $J = 6.2$ Hz), 4.85 (m, 1H, H3′), 4.56 (d, 1H, H4′, $J = 5.8$ Hz), 2.60−2.85 (hpt, 1H, ^{i}Pr), 2.45−2.65 (m, 1H, H2′α), 2.20−2.35 (m, 1H, H2′*β*), 1.27, 1.25 (each s, 3H, ^{i}Pr), 0.96 (s, 9H, Si^{t}Bu), 0.88 (s, 9H, Si^{t}Bu), 0.28, 0.27, 0.05, 0.04 (each s, 3H, SiMe). **16.** ^{13}C NMR (50 MHz, $CDCl_3$): $\delta = 186.1$, 158.4, 154.7, 150.3, 147.5, 111.8 (each C), 87.3, 85.2, 71.0, 63.4 (each CH), 41.0 (CH_2), 37.0 (CH), 26.1, 25.9 (each 3 × CH_3), 19.1 (2 × CH_3), 18.5, 18.4 (each C), −3.9, −4.2 (each CH_3), −4.7 (2 × CH_3). HR-ESI-MS: for $C_{26}H_{44}N_5O_5Si_2^-$ [M − H]$^-$, calcd 562.2886; found 562.2875. R_f (CH_3Cl:MeOH, 9:1) = 0.53.

Acknowledgment. This work was supported in part by the European Community's Marie Curie Research Training Network under contract MRTN-CT-2003-505086 [CLUSTOXDNA]. A.M. thanks also the "Marco Polo" program (Alma Mater Studiorum, University of Bologna).

Supporting Information Available: ^{1}H NMR spectra of **1a**/**b**, **2**, **4a**/**b**, **5a**/**b**, **7**, **8**, **13**, **14**, and **16**; COSY and HMQC spectra of **14**; and X-ray crystallographic structure and data of **8**. This material is available free of charge via the Internet at http://pubs.acs.org.

JO062518C