# 2-(Trimethylsilyl)ethanethiol in Nucleoside Chemistry. A Short Route for Preparing Thionucleosides and Their Methyl Disulfides

Stéphane Chambert,[†] Isabelle Gautier-Luneau,[‡] Marc Fontecave,[§] and Jean-Luc Décout*[,†]

*Laboratoire de Chimie Bio-organique, UMR CNRS 5063, Département de Pharmacochimie Moléculaire, UFR de Pharmacie, Université Joseph Fourier-Grenoble I, Domaine de la Merci, F-38706 La Tronche Cedex, France, LEDSS 2, UMR CNRS 5616, Université Joseph Fourier, BP 53, F-38041 Grenoble Cedex 9, France, and Laboratoire de Chimie et Biochimie des Centres Rédox Biologiques, DBMS-CEA/CNRS/Université Joseph Fourier, Bat. K, 17 avenue des Martyrs, F-38054 Grenoble Cedex 9, France*

*Received May 25, 1999*

## Introduction

Incorporation of a thiol function in nucleosides, nucleotides, or oligonucleotides has led to a number of analogues possessing interesting biological properties.[1] For example, 2′,3′-dideoxy-3′-mercaptonucleoside 5′-triphosphates (T, C, A, G) irreversibly stopped DNA chain elongation by AMV and HIV reverse transcriptase, and the corresponding nucleosides display antiviral activities.[2a,b] Recently, we have demonstrated that another nucleotide possessing a thiol function on the sugar, 2′-deoxy-2′-mercaptouridine 5′-diphosphate strongly inactivates in vitro *E. coli* ribonucleoside diphosphate reductase (RDPR).[3] This enzyme catalyzes the reduction of the four natural ribonucleotides in the corresponding 2′-deoxyribonucleotides and thus is a key enzyme in the synthesis of DNA.[4] The thiol function of the modified nucleotide interacts with a cysteine residue at the active site to lead to a perthiyl radical formed on the enzyme.

Numerous modified oligonucleotides containing a sulfur atom on the base, the sugar, or the phosphoryl group were synthesized and used as tools in studies of nucleic acid structure and function, protein−nucleic acid interactions, and antisense therapy.[5] For examples, 4-thiouridine, a natural constituent of t-RNA used as a photochemical probe in the study of nucleic acids,[6] 2′-deoxy-6-thioguanosine used for the treatment of leukemias,[7] and, more recently, 2′-deoxy-2′-mercaptocytidine[5] were incorporated in oligonucleotides.

The development of new methods for incorporating a thiol function in nucleosides, nucleotides, and oligonucleotides and for preparing their stable useful precursors is thus of interest in the search of bioactive compounds or biological tools. In this regard, mixed disulfides can be interesting precursors that should be reduced efficiently and rapidly under mild conditions for generating the active species. For example, the RDPR inactivator 2′-deoxy-2′-mercaptouridine 5′-diphosphate was obtained in vitro by spontaneous reduction of the corresponding stable mixed propyl disulfide with dithiothreitol (DTT).[3]

We report here a short route for preparing thionucleosides and their corresponding methyl disulfides using 2-(trimethylsilyl)ethanethiol (*β*-ethylsilyl thiol, BEST) as a source of sulfur, a method previously developed by Fuchs and co-workers for synthesizing acyl- and alkyl-substituted thiols.[8] These authors have reported that the 2-(trimethylsilyl)ethyl sulfide intermediate did not afford the corresponding thiol by treatment with fluorides. Reaction with dimethyl(methylthio)sulfonium tetrafluoroborate in the presence of methyl disulfide gave the corresponding acyl- or alkyl-substituted methyl disulfide, which can be reduced with tributylphosphine. Nucleosides in which a 2′-(2-(trimethylsilyl)ethyl)thio group is linked to the sugar were prepared, and their reaction with dimethyl(methylthio)sulfonium tetrafluoroborate led to the corresponding methyl disulfide in high yield. On the contrary, we describe here the first example of direct and quantitative elimination of this group in 2′-deoxy-8-(2-(trimethylsilyl)ethyl)thioadenosine with tetrabutylammonium fluoride at room temperature for obtaining the corresponding thione on the base. Such a deprotection should be of interest in the preparation of modified oligonucleotides.

## Results and Discussion

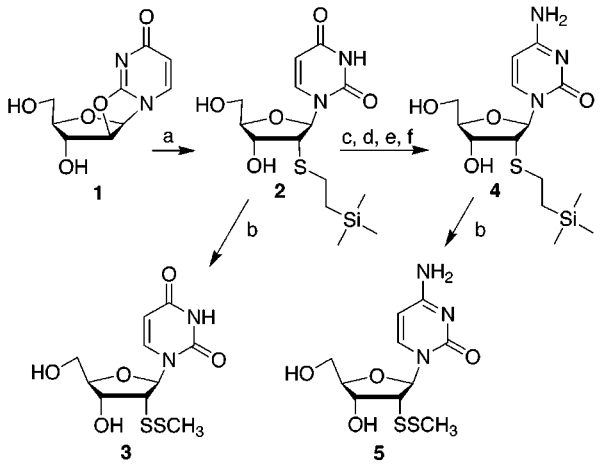
2′-Thioalkyl or -aryl uridine derivatives were prepared by Divakar and Reese by ring-opening of 2,2′-anhydrouridine (**1**) with alkyl or arene thiolates formed with triethylamine or $N^1,N^1,N^3,N^3$-tetramethylguanidine as a base.[9] To prepare 2′-deoxy-2′-(2-(trimethylsilyl)ethyl)-thiouridine (**2**) (Scheme 1), 2,2′-anhydrouridine was heated with 2-(trimethylsilyl)ethanethiol at 120 °C in DMF in the presence of potassium carbonate. After 3 h of heating and purification by chromatography on silica gel, the 2′-sulfide **2** was obtained in 88% yield. In the $^1$H NMR spectrum (DMSO-$d_6$) of compound **2**, the characteristic signals of the (trimethylsilyl)ethyl group were observed at 2.58, 0.80, and −0.01 ppm, respectively. This compound reacted very slowly and incompletely with tetrabutylammonium fluoride in THF at room temperature. Heating at 60 °C, under an argon atmosphere, in the presence of a large excess of tetra-*n*-butylammonium

* To whom correspondence should be addressed. Fax: (33) 04 76 51 86 67. E-mail: jean-luc.decout@ujf-grenoble.fr.
† UMR CNRS 5063, Université Joseph Fourier-Grenoble I.
‡ UMR CNRS 5616, Université Joseph Fourier.
§ DBMS-CEA/CNRS/Université Joseph Fourier.

(1) Wnuk, S. F. *Tetrahedron* **1993**, *49*, 9877−9936.
(2) (a) Yuzhakov, A. A.; Chidgeavadze, Z. G.; Beabealashvilli, R. S. *FEBS* **1992**, *306*, 185−188. (b) Yuzhakov, A. A.; Chidgeavadze, Z. G.; Beabealashvilli, R. Sh.; Kraevskii, A. A.; Galegov, G. A.; Korneeva, M. N.; Nosik, D. N.; Kilesso, T. Y. *Bioorg. Khim.* **1991**, *17*, 504−509; *Chem. Abstr.* **1991**, *115*, 84923g). (c) Dueholm, K. L.; Aly, Y. L.; Joergensen, P. T.; El-Barbary, A. A.; Pedersen, E. B.; Nielsen, C. *Monatsch. Chem.* **1993**, *124*, 37−53.
(3) Le Hir de Fallois, L.; Décout, J.-L.; Fontecave, M. *J. Chem. Soc., Perkin Trans. 1* **1997**, 2587−2595. Covès, J.; Le Hir de Fallois, L.; Lepape, L.; Décout, J.-L.; Fontecave, M. *Biochemistry* **1996**, *35*, 8595−8602.
(4) Fontecave, M. *Cell. Mol. Life Sci. 1* **1998**, 684−695.
(5) Hamm, M. L.; Piccirilli, J. A. *J. Org. Chem.* **1997**, *62*, 3415−3420 and references therein.
(6) Fourrey, J.-L.; Gasche, J.; Fontaine, C.; Guittet, E.; Favre, A. *J. Chem. Soc., Chem. Commun.* **1989**, 1334−1336. Coleman, R. S.; Siedlecki, J. M. *J. Am. Chem. Soc.* **1992**, *114*, 9229−9230.
(7) Christopherson, M. S.; Broom, A. D. *Nucleic Acids Res.* **1991**, *19*, 5719−5724.
(8) Anderson, M. B.; Ranasinghe, M. G.; Fuchs, P. L. *J. Org. Chem.* **1988**, *53*, 3125−3127.
(9) Divakar, K. J.; Reese, C. B. *J. Chem. Soc., Perkin Trans. 1* **1982**, 1625−1628.

10.1021/jo9908492 CCC: $19.00 
Published on Web 12/16/1999

**Scheme 1. Short Preparation of 2′-Deoxyuridin-2′-yl and 2′-Deoxycytidin-2′-yl Methyl Disulfides 3 and 5 Using 2-(Trimethylsilyl)ethanethiol**[a]




[a] Key: (a) 2-(trimethylsilyl)ethanethiol/$K_2CO_3$/DMF, 120 °C, 2 h; (b) dimethyl(methylthio)sulfonium tetrafluoroborate/THF, rt; (c) $Ac_2O$/Py; (d) 1,2,4-triazole/$POCl_3$/$Et_3N$/$CH_3CN$; (e) ammonia/dioxane; (f) concentrated aqueous ammonia/ethanol.

fluoride, converted nearly completely compound **2** in a complex mixture of compounds in which the uracil base was identified as a major component (TLC, HPLC, $^1$H NMR). In this mixture, 2′-deoxy-2′-thiouridine could be identified as a minor component by comparison with a sample synthesized and characterized previously (TLC, Ellman's reagent; HPLC).[3] This result can be explained by the previously observed lability of the glycosidic linkage in 2′-thioribonucleosides.[10] Elimination of the base can result from intramolecular reaction of the thiolate generated on the sugar at the 2′-position.

Finally, the trimethylsilyl sulfide **2** was quantitatively converted to methyl disulfide **3** after reaction with dimethyl(methylthio)sulfonium tetrafluoroborate according to Fuchs and co-workers (Scheme 1).[8] The reaction should be conducted in a large excess of methyl disulfide at room temperature to avoid the formation of the symmetrical disulfide. Compound **3** was obtained in 90% yield after $C_{18}$ reversed-phase chromatography. This method allowed the methyl disulfide **3** to be prepared from 2,2′-anhydrouridine in two steps in 80% yield more efficiently and rapidly than the method previously used for preparing 2′-deoxyuridin-2′-yl propyl disulfide (40% yield).[3] Compound **3** could be crystallized from water to give nice colorless needles.

Compound **2** was converted to its cytosine analogue **4** in four steps using a classical procedure:[11] (i) acetylation of the hydroxyl groups with acetic anhydride in pyridine, (ii) reaction of the crude product with phosphoryl trichloride, triethylamine, and 1,2,4-triazole, (iii) reaction of the triazolo derivative with an excess of ammonia in dioxane, and (iv) deprotection of the hydroxyl groups with ammonia in ethanol. Compound **4** was obtained from compound **2** in 85% yield after chromatography on silica gel. The 2′-(2-(trimethylsilyl)ethyl)thio group is, thus, stable under the conditions of conversion of the uracil base in cytosine. As for compound **2**, deprotection of the thiol group cannot be achieved cleanly and completely by treatment with fluorides. The trimethylsilyl sulfide **4** was quantitatively converted to its methyl disulfide **5** by reaction with dimethyl(methylthio)sulfonium tetrafluoroborate in the presence of a large excess of methyl disulfide at room temperature. It was isolated in 80% yield after $C_{18}$ reversed-phase chromatography. Thus, 2′-deoxycytidin-2′-yl methyl disulfide (**5**) was prepared in 60% yield from 2,2′-anhydrouridine (**1**). The X-ray structure of compound **5** (Figure 1) was determined after crystallization from water (Supporting Information).

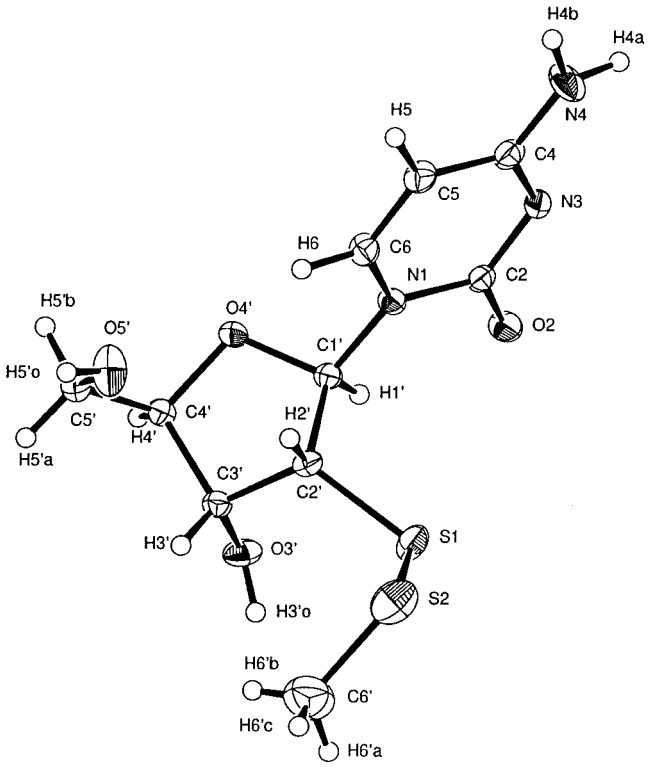



**Figure 1.** X-ray structure of 2′-deoxycytidin-2′-yl methyl disulfide (**5**).

3′-Thiothymidine 5′-triphosphate was reported as a highly effective terminator of DNA synthesis that is catalyzed by HIV reversed transcriptase.[2b,c] However, the antiviral activity of the corresponding nucleoside was controverted.[2c] To prepare 3′-deoxythymidin-3′-yl methyl disulfide (**9**), which is a potent antiviral agent and a precursor of 3′-thiothymidine,[2b,c] we used the approach developed for the synthesis of methyl disulfides **3** and **5**. 5′-Protected derivatives of 3′-thiothymidine were prepared previously by reaction of 2,3′-anhydro-5′-tritylthymidine or 1-(2-deoxy-3-mesyl-5-(4-monomethoxytrityl)-$\beta$-D-lyxosyl)thymine with potassium or sodium thiobenzoate,[12] and a very fast oxidation of 3′-thiothymidine in the corresponding symmetrical disulfide was observed.[12a]

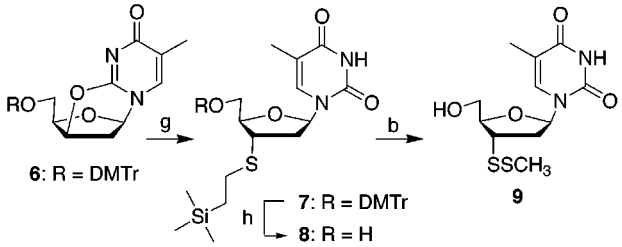
To prepare the methyl disulfide of this nucleoside, 2,3′-anhydro-5′-(4,4′-dimethoxytrityl)thymidine (**6**) was prepared from thymidine. Reaction of this anhydro derivative with 2-(trimethylsilyl)ethanethiol at 140 °C in DMF in the presence of potassium carbonate resulted in the formation of the trimethylsilyl derivative **7** in a low 42%

(10) Johnson, R.; Reese, C. B.; Pei-Zhuo, Z. *Tetrahedron* **1995**, *51*, 5093−5098.

(11) Divakar, K. J.; Mottoh, A.; Reese, C. B. *J. Chem. Soc., Perkin Trans. 1* **1990**, 969−974.

(12) (a) Miller, N.; Fox, J. J. *J. Org. Chem.* **1964**, *29*, 1772−1776. (b) Cosstick, R.; Vyle, J. S. *J. Chem. Soc., Chem. Commun.* **1988**, 992−993.

**Scheme 2. Synthesis of 3′-Deoxythymidin-3′-yl Methyl Disulfide Using 2-(Trimethylsilyl)ethanethiol**[a]




[a] Key: (g) 2-(trimethylsilyl)ethanethiol/NaH/DMF, 90 °C, 1 h; (h) 2% DCA/$CH_2Cl_2$, aqueous $NaHCO_3$; (b) dimethyl(methylthio)-sulfonium tetrafluoroborate/THF, rt.

yield. Surprisingly, the detritylated analogue of **6**, 2,3′-anhydrothymidine, did not react with the thiol under these conditions. To increase the yield of compound **7**, sodium 2-(trimethylsilyl)ethanethiolate was prepared in DMF by treatment of the corresponding thiol with 1.05 equiv of sodium hydride, and then the mixture was heated with the anhydro derivative **6** at 90 °C for 1 h. Under these conditions, the (2-(trimethylsilyl)ethyl)thio derivative **7** was formed as the major product, whereas a minor compound formed was identified as 3′-deoxy-2′,3′-didehydro-5′-(4,4′-dimethoxytrityl)thymidine (5′-tritylated D4T; $R_f^D$ 0.53) by detritylation ($^1$H NMR, mass spectrometry; $R_f^B$ 0.62). This elimination with synchronous decyclization has been previously observed in high yield at room temperature with 2,3′-anhydro-5′-tritylthymidine in the presence of potassium *tert*-butoxide in dimethyl sulfoxide.[13]

The crude mixture isolated after ring-opening of the anhydro derivative **6** with sodium 2-(trimethylsilyl)-ethanethiolate was detritylated with 2% dichloroacetic acid in dichloromethane for obtaining the (2-(trimethylsilyl)ethyl)thio derivative **8** in 62% yield for the two steps. 3′-Deoxythymidin-3′-yl methyl disulfide (**9**) was finally obtained from compound **8** in 90% yield after reaction at room temperature with dimethyl(methylthio)sulfonium tetrafluoroborate in the presence of a large excess of methyl disulfide (Scheme 2). After crystallization of this compound from water, the X-ray structure (Figure 2) of its crystalline monohydrate was obtained (Supporting Information).

Addition of a nearly stoichiometric amount of DTT (1.1 equiv) to a methanolic solution of one of the three methyl disulfides **3**, **5**, and **9** led immediately and quantitatively to the corresponding thiol evidenced by TLC ($R_f^B$ 0.31, $R_f^D$ 0.33, $R_f^A$ 0.48, respectively; Ellman's reagent) and $^1$H NMR spectrometry after removal of methanethiol formed by argon bubbling.

To demonstrate the potential of 2-(trimethylsilyl)-ethanethiol for incorporating a thione function on the base of nucleosides, 2′-deoxy-8-thioadenosine (**12**) was synthesized using this reagent. This nucleoside was previously used as an intermediate in the synthesis of modified oligonucleotides carrying an 8-thio-substituted adenine base.[14]

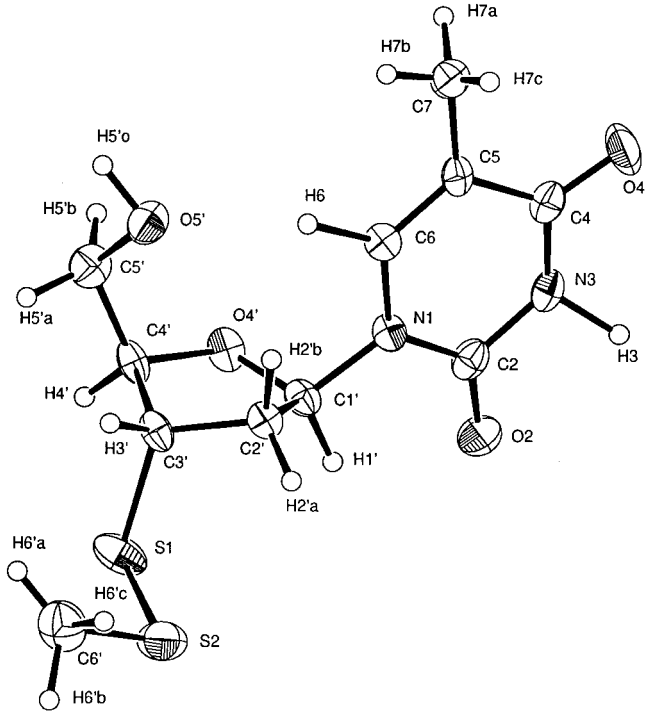
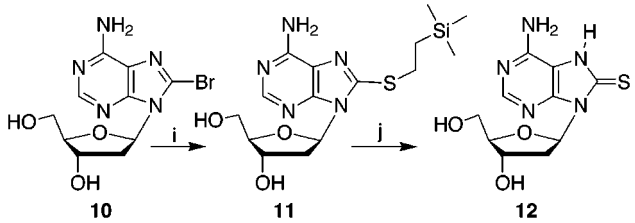



**Figure 2.** X-ray structure of 3′-deoxythymidin-3′-yl methyl disulfide (**9**).

**Scheme 3. Synthesis of 2′-Deoxy-8-thioadenosine Using 2-(Trimethylsilyl)ethanethiol**[a]




[a] Key: (i) 2-(trimethylsilyl)ethanethiol/$K_2CO_3$/DMF, 60 °C, 8 h; (j) 1 M tetrabutylammonium fluoride/THF, rt, 3 h.

8-Bromo-2′-deoxyadenosine (**10**) was prepared by bromination of 2′-deoxyadenosine at the 8-position.[15] Reaction of this nucleoside with 2-(trimethylsilyl)ethanethiol at 60 °C in the presence of potassium carbonate led to the corresponding sulfide **11** in 95% yield (Scheme 3). Deprotection of the thione function was achieved quantitatively by treatment with 1 M tetrabutylammonium fluoride in THF at room temperature for 3 h. After precipitation in water, a mixture containing only the thione and its tetrabutylammonium salt was obtained (1:1; $^1$H NMR and mass spectrometry; 92% yield). It could be transformed to the pure thione form by chromatography on a $C_{18}$ reversed-phase column eluting with aqueous ammonium carbonate, acidification of the resulting solution, and desalting. All the characteristics of the isolated compound appeared identical to those of an authentic sample of 2′-deoxy-8-thioadenosine (**12**) prepared previously.[16]

This result constitutes the first example of mild deprotection with tetrabutylammonium fluoride of a thione

(13) Horwitz, J. P.; Chua, J.; Da Rooge, M. A.; Noel, M.; Klundt, I. L. *J. Org. Chem.* **1966**, *31*, 205−211.

(14) Pieles, U.; Sproat, B. S.; Neuner, P.; Cramer, F. *Nucleic Acids Res.* **1989**, *17*, 8967−8978. Laayoun, A.; Décout, J.-L.; Defrancq, E.; Lhomme, J. *Tetrahedron Lett.* **1994**, *35*, 4991−4994.

(15) Ikehara, M.; Kaneko, M. *Tetrahedron* **1970**, *26*, 4251−4259. Guy, A.; Duplaa, A. M.; Harel, P.; Teoule, R. *Helv. Chim. Acta* **1988**, *71*, 1566−1572.

(16) Laayoun, A.; Décout, J.-L.; Lhomme, J. *Tetrahedron Lett.* **1994**, *35*, 4989−4990.

function protected in the form of 2-(trimethylsilyl)ethyl sulfide. The easy $\beta$-elimination of the trimethylsilylethyl group can be explained by resonance interaction of the unshared electrons of the sulfur atom with the p-electron system of the aromatic adenine ring. Thus, the (trimethylsilyl)ethyl group appears very attractive for protecting an aromatic thione function. 2-(Trimethylsilyl)ethyl sulfide **11** was found to be stable under strong basic conditions in a 50:50 mixture of concentrated ammonia−ethanol for 24 h at 50 °C. Synthesis of oligonucleotides containing such a thione function on the base should be possible with this protecting group.

These results point to 2-(trimethylsilyl)ethanethiol as a useful tool in the synthesis of thionucleosides and potentially of thio-nucleotides and -oligonucleotides.

## Experimental Section

**General Procedures.** Melting points are uncorrected. Chemical shifts are reported in parts per million relative to the residual signal of the solvent. Thin-layer chromatographic data ($R_f$ values) were obtained with Macherey Nagel Alugram SIL G/UV$_{254}$ analytical sheets (layer, 0.25 mm) developed with dichloromethane−methanol (95:5 ($R_f^A$), 90:10 ($R_f^B$), 85:15 ($R_f^C$), 80:20 ($R_f^D$)) or dichloromethane−ethyl acetate (75:25 ($R_f^E$)).

**Synthesis.** 2-(Trimethylsilyl)ethanethiol (commercially available) was synthesized from vinyltrimethylsilane (Aldrich) and thiolacetic acid according to procedures described previously.[8,17] Dimethyl(methylthio)sulfonium tetrafluoroborate (commercially available) was prepared from trimethyloxonium tetrafluoroborate in acetonitrile.[18] 2,2′-Anhydrouridine (**1**) was obtained using the method reported by Hampton and Nichol and improved by Mofatt and co-workers.[19] Bromination of 2′-deoxyadenosine leading to 8-bromo-2′-deoxyadenosine (**10**) was performed according to the procedure of Ikehara and Kaneko described for bromination of adenosine.[15]

**2′-Deoxy-2′-(2-(trimethylsilyl)ethyl)thiouridine (2).** To a suspension of 2,2′-anhydrouridine (**1**) (5 g, 22 mmol; $R_f^D$ 0.30) and anhydrous $K_2CO_3$ (11 g, 79 mmol) in DMF (110 mL) was added 2-(trimethylsilyl)ethanethiol (3.5 g, 26 mmol). The mixture was stirred at 120 °C for 3 h under argon. After cooling and filtration to remove the mineral salts, DMF was evaporated off. The residue was washed with hexane and then chromatographed on silica gel in dichloromethane−methanol (95:5) to yield compound **2** (6.9 g, 19 mmol, 88%; $R_f^B$ 0.53): mp 59 °C; $^1$H NMR (300 MHz, $CD_3OD$) $\delta$ 8.00 (1 H, d, $J$ = 8.0 Hz), 6.14 (1 H, d, $J$ = 8.5 Hz), 5.75 (1 H, d, $J$ = 8.0 Hz), 4.32 (1 H, dd, $J$ = 5.4, 2.1 Hz), 4.03 (1 H, m), 3.77 (2 H, m), 3.51 (1 H, dd, $J$ = 8.4, 5.3 Hz), 2.58 (2 H, m), 0.80 (2 H, m), −0.04 (9 H, s); $^{13}$C NMR (75 MHz, $CD_3OD$) $\delta$ 165.8, 152.4, 142.5, 103.3, 90.3, 88.1, 73.8, 63.1, 54.2, 28.1, 18.7, −1.8; LRMS [FAB$^+$, glycerol] $m/z$ = 361 [M + H]$^+$, 249 [M − Uracil + H]$^+$; [FAB$^-$, glycerol] $m/z$ = 359 [M − H]$^-$, 111 [uracil − H]$^-$. Anal. Calcd for $C_{14}H_{24}N_2O_5SSi$: C, 46.64; H, 6.71; N, 7.77; S. 8.89. Found: C, 46.47; H, 6.66; N, 7.58; S, 8.62.

**2′-Deoxy-2′-(2-(trimethylsilyl)ethyl)thiocytidine (4).** To an ice-cold solution of compound **2** (1.92 g, 5.32 mmol) in dry pyridine (25 mL) was added acetic anhydride (10 mL). The resultant solution was stirred at room temperature for 24 h, and then ethanol (20 mL) was added at 0 °C. The solvents were evaporated, and the residue was dissolved in dichloromethane (150 mL). The resultant solution was washed with water (100 mL), and the aqueous layer was extracted with dichloromethane. The combined extracts were dried over $Na_2SO_4$, evaporated, and coevaporated with toluene.

1,2,4-Triazole (5 g, 72 mmol) was added to a $POCl_3$ solution (1.35 mL, 17.7 mmol) in $CH_3CN$ (20 mL). The mixture was stirred for 15 min in an ice bath, and then triethylamine (20 mL, 177 mmol) was added dropwise under argon. After 5 min, a solution of the crude acetylated nucleoside **2** previously prepared in $CH_3CN$ (40 mL) was added. The mixture was stirred for 24 h under argon at room temperature. Water (3 mL) was added, and the solvents were evaporated. The residue was dissolved in $CH_2Cl_2$ (100 mL), and the solution was washed with aqueous $NaHCO_3$ (5%, 5 mL) and then with water. It was dried over $Na_2SO_4$, evaporated to dryness, and then coevaporated with toluene. The residue was dissolved in an ammonia solution in dioxane (40 mL, 0.5 M). The solution was stirred for 24 h under argon and then evaporated to dryness. To a solution of the residue in ethanol (20 mL) was added concentrated aqueous ammonia (30%, 30 mL). The resultant solution was stirred for 12 h and then evaporated and coevaporated with ethanol. The residue was chromatographed on silica gel in $CH_2Cl_2$−MeOH (95:5) and then $CH_2Cl_2$−MeOH (9:1) to yield compound **4** (1.62 g, 4.51 mmol, 85%; $R_f^B$ 0.37): mp 112 °C; $^1$H NMR (300 MHz, DMSO-$d_6$) $\delta$ 7.86 (1 H, d, $J$ = 7.5 Hz), 7.20 (2H, d, $J$ = 6.4 Hz), 6.19 (1 H, d, $J$ = 8.6 Hz), 5.83 (1 H, d, $J$ = 7.4 Hz), 5.60 (1H, d, $J$ = 5.3 Hz), 5.14 (1H, t, $J$ = 5.3), 4.23 (1 H, m), 3.91 (1 H, m), 3.63 (2H, m), 3.38 (1 H, dd, $J$ = 8.7 Hz, $J$ = 5.5 Hz), 2.39 (2 H, m), 0.77 (2 H, m), −0.03 (9 H, s); $^{13}$C NMR (75 MHz, DMSO-$d_6$) $\delta$ 165.4, 155.3, 141.5, 94.6, 89.0, 86.2, 72.3, 61.6, 51.4, 25.9, 17.2, −1.8; LRMS [FAB$^+$, glycerol] $m/z$ = 360 [M + H]$^+$, 248 [M − cytosine]$^+$, 112 [cytosine + H]$^+$. Anal. Calcd for $C_{14}H_{25}N_3O_4SSi\cdot 1.5H_2O$: C, 43.50; H, 7.30; N, 10.87; S, 8.29. Found: C, 43.49; H, 6.99; N, 10.95; S, 8.32.

**2,3′-Anhydro-5′-(4,4′-dimethoxytrityl)thymidine (6).** To a solution of thymidine (5.01 g, 20.7 mmol) in dry pyridine (50 mL) was added 4,4′-dimethoxytrityl chloride (7.72 g, 22.8 mmol) at 0 °C under argon. The resultant solution was stirred at room temperature for 20 h. Methanesulfonyl chloride (1.91 mL, 24.7 mmol) was added, and the mixture was stirred for 4 h under argon at room temperature. Water (5 mL) was added, and the solvents were evaporated. The residue was dissolved in $CH_2Cl_2$, and the solution was washed with water, dried over $Na_2SO_4$, and then evaporated. The colorless foam obtained was dissolved in dry $CH_3CN$ (100 mL), and potassium carbonate (10 g) was added. The mixture was stirred at room temperature for 24 h under argon, and then the mineral salts were removed by filtration. The filtrate was evaporated, and the residue was dissolved in dichloromethane (150 mL). The resultant solution was washed with water, dried over $Na_2SO_4$, and filtered, and the solvent was evaporated. The residue was chromatographed on alumina gel in $CH_2Cl_2$−MeOH (95:5) to lead to compound **6** (8.34 g, 15.8 mmol, 75%; $R_f^A$ 0.42): mp 134 °C; $^1$H NMR (300 MHz, DMSO-$d_6$) $\delta$ 7.64−7.20 (9 H, m), 6.85 (4 H, m), 5.90 (1 H, d, $J$ = 3.5 Hz), 5.30 (1 H, m), 4.41 (1 H, m), 3.30 (6 H, s), 3.17−3.05 (3 H, m), 2.60−2.40 (2 H, m), 1.78 (3 H, s); $^{13}$C NMR (75 MHz, DMSO-$d_6$) $\delta$ 170.8, 158.1, 153.3, 144.6, 135.3, 135.1, 129.7, 127.8, 127.7, 126.7, 116.2, 113.2, 86.8, 85.8, 83.5, 77.1, 62.3, 55.0, 32.7, 13.0; LRMS [DCI, $NH_3$/isobutane] $m/z$ = 527 [M + H]$^+$, 303 [DMTr]$^+$, 225 [M − DMTr + H]$^+$, 127 [thymine + H]$^+$.

**3′-Deoxy-3′-(2-(trimethylsilyl)ethyl)thiothymidine (8).** To a stirred suspension of sodium hydride (60%; 215 mg, 5.38 mmol) in dry DMF (10 mL) was added a solution of 2-(trimethylsilyl)-ethanethiol (0.69 g, 5.12 mmol) in dry DMF (10 mL). The solution was stirred for 15 min under argon, and then 2,3′-anhydro-5′-(4,4′-dimethoxytrityl)thymidine (**6**) (2.57 g, 4.88 mmol; $R_f^E$ 0.54) was added. After being heated at 90 °C for 1 h under argon, the mixture was filtered and DMF was evaporated off. The residue was washed with pentane and then dissolved in dichloromethane (150 mL). The solution was washed with aqueous $NaH_2PO_4$ (10%, 10 mL) and then with water, dried over $Na_2SO_4$, and then evaporated.

The residue was dissolved in 2% dichloroacetic acid solution in dichloromethane (100 mL). The resultant orange solution was stirred for 30 min under nitrogen at room temperature, and aqueous $NaHCO_3$ (5%, 80 mL) was added. The aqueous solution was extracted with dichloromethane (2 × 50 mL), and then the combined organic extracts were dried over $Na_2SO_4$ and evaporated. Compound **8** was purified by chromatography on silica gel in $CH_2Cl_2$−AcOEt (6:4) (1.08 g, 3.02 mmol; 62%; $R_f^A$ 0.48): mp 49 °C; $^1$H NMR (300 MHz, $CDCl_3$) $\delta$ 8.48 (1 H, s), 7.52 (1H, s), 6.07 (1 H, dd, $J$ = 7.0, $J$ = 4.1 Hz), 4.05 (1 H, m), 3.85 (2 H, m), 3.47 (1 H, m), 2.64−2.50 (3 H, m), 2.41 (1H, m), 2.07 (1H, s),

(17) Gornowicz, G. A.; Ryan, J. W.; Speier, J. L. *J. Org. Chem.* **1968**, *33*, 2918−2924.

(18) Helmkamp, G. K.; Cassey, H. N.; Olsen, B. A.; Pettitt, D. J. *J. Org. Chem.* **1965**, *30*, 933−935. Smallcombe, S. H.; Caserio, M. C. *J. Am. Chem. Soc.* **1971**, *93*, 5826−5832.

(19) Hampton, A.; Nichol, A. W. *Biochemistry* **1966**, *5*, 2076−2082. Verheyden, J. P. H.; Wagner, D.; Moffatt, J. G. *J. Org. Chem.* **1971**, *36*, 250−254.

1.90 (3 H, s), 0.84 (2 H, m), −0.02 (9 H, s); $^{13}$C NMR (75 MHz, $CDCl_3$) $\delta$ 164.3, 150.4, 136.8, 110.6, 85.9, 85.6, 61.2, 40.4, 40.2, 27, 17.3, 12.4, −1.8; LRMS [FAB$^+$, glycerol] $m/z$ = 359 [M + H]$^+$, 233 [M − thymine]$^+$, 127 [thymine + H]$^+$, [FAB$^-$, glycerol] $m/z$ = 357 [M − H]$^-$. Anal. Calcd for $C_{15}H_{26}N_2O_4SSi\cdot0.25H_2O$: C, 49.63; H, 7.36; N, 7.72; S, 8.83. Found: C, 49.47; H, 7.15; N, 7.48; S, 8.91.

**General Procedure for Preparing the Methyl Disulfides 3, 5, and 9.** To a solution of nucleoside **2**, **4**, or **8** (200 mg; **2**, 0.55 mmol; **4**, 0.55 mmol; **8**, 0.56 mmol) and methyl disulfide (**2**, 1.4 mL, 16 mmol; **4**, 2.5 mL, 28 mmol; **8**, 1.4 mL, 16 mmol) in dry THF (10 mL) was added dropwise a solution of dimethyl(methylthio)sulfonium tetrafluoroborate (**2**, 271 mg, 1.4 mmol; **4**, 368 mg, 1.9 mmol; **8**, 223 mg, 1.2 mmol) in THF (30 mL). The mixture was stirred for 15 h under argon, and then it was neutralized by addition of aqueous $NaHCO_3$ (10%, 1 mL). After evaporation, the residue dissolved in a minimal volume of water was chromatographed on a Sep-Pak C18 cartridge (1 g) eluting with $H_2O$ and then $H_2O$−MeOH (95:5−90:10) to afford the corresponding methyl disulfide **3** (154 mg, 0.50 mmol, 90%; $R_f^B$ 0.43), **5** (134 mg, 0.44 mmol, 80%; $R_f^C$ 0.53), or **9** (152 mg, 0.50 mmol, 90%; $R_f^A$ 0.43). These compounds were crystallized from water.

**Data for 2′-Deoxyuridin-2′-yl Methyl Disulfide (3):** mp 175 °C dec; $^1$H NMR (300 MHz, $CD_3OD$) $\delta$ 8.01 (1 H, d, $J$ = 8.1 Hz), 6.28 (1 H, d, $J$ = 8.7 Hz), 5.75 (1 H, d, $J$ = 8.1 Hz), 4.39 (1 H, m), 4.00 (1 H, m), 3.74 (2 H, m), 3.69 (1 H, dd, $J$ = 8.7, $J$ = 5.5 Hz), 2.35 (3 H, s); $^{13}$C NMR (75 MHz, $CD_3OD$) $\delta$ 165.9, 152.6, 142.7, 103.4, 90.2, 88.3, 74.1, 63.1, 60.0, 23.6; LRMS [FAB$^+$, glycerol] $m/z$ = 307 [M + H]$^+$, [FAB$^-$, glycerol] $m/z$ = 305 [M − H]$^-$. Anal. Calcd for $C_{10}H_{14}N_2O_5S_2$: C, 39.21; H, 4.61; N, 9.14; S, 20.93. Found: C, 39.30; H, 4.74; N, 9.01; S, 21.14.

**Data for 2′-Deoxycytidin-2′-yl Methyl Disulfide (5):** mp 145 °C dec; $^1$H NMR (300 MHz, DMSO-$d_6$) $\delta$ 7.79 (1 H, d, $J$ = 7.5 Hz), 7.26 (2 H, d, $J$ = 9.6 Hz), 6.21 (1 H, d, $J$ = 8.9 Hz), 5.82 (1 H, d, $J$ = 5.3), 5.77 (1 H, d, $J$ = 7.5 Hz), 5.07 (1 H, t, $J$ = 5.2 Hz), 4.24 (1 H, m), 3.83 (1 H, m), 3.63 (1 H, dd, $J$ = 8.9, 5.4 Hz), 3.52 (2H, m), 2.24 (3H, s); $^{13}$C NMR (75 MHz, DMSO-$d_6$) $\delta$ 165.4, 155.4, 141.7, 94.8, 88.3, 86.2, 72.3, 61.5, 58.8, 23.1; LRMS [FAB$^+$, glycerol] $m/z$ = 306 [M + H]$^+$. Anal. Calcd for $C_{10}H_{15}N_3O_4S_2$: C, 39.33; H, 4.95; N, 13.76; S, 21.00. Found: C, 39.23; H, 4.96; N, 13.65; S, 21.19.

**Data for 3′-Deoxythymidin-3′-yl Methyl Disulfide (9):** mp 83 °C; $^1$H NMR (300 MHz, $CDCl_3$) $\delta$ 8.46 (1 H, s), 7.51 (1 H, d, $J$ = 1.0 Hz), 6.09 (1 H, dd, $J$ = 7.0, 4.5 Hz), 4.10−3.84 (3 H, m), 3.61 (1 H, m), 2.54 (2 H, m), 2.43 (3 H, s), 2.35 (1 H, t, $J$ = 6.0 Hz), 1.89 (3H, d, $J$ = 1.0 Hz); $^{13}$C NMR (75 MHz, $CDCl_3$) $\delta$ 163.4, 150.1, 136.5, 110.9, 85.8, 85.3, 61.3, 44.8, 38.1, 24.4, 12.6; LRMS [FAB$^+$, glycerol] $m/z$ = 305 [M + H]$^+$, 179 [M − thymine + H]$^+$, 127 [thymine + H]$^+$, [FAB$^-$, glycerol] $m/z$ = 303 [M − H]$^-$, 125 [thymine − H]$^-$. Anal. Calcd for $C_{11}H_{16}N_2O_4S_2\cdot H_2O$: C, 40.98; H, 5.63; N, 8.69; S, 19.89. Found: C, 41.15; H, 5.62; N, 8.51; S, 20.08.

**2′-Deoxy-8-(2-(trimethylsilyl)ethyl)thioadenosine (11).** To a suspension of 8-bromo-2′-deoxyadenosine (**10**) (2.5 g, 7.6 mmol; $R_f^B$ 0.43) and anhydrous $K_2CO_3$ (3 g, 21.7 mmol) in DMF (25 mL) was added 2-(trimethylsilyl)ethanethiol (1.2 g, 8.9 mmol). The mixture was stirred at 60 °C for 8 h under argon. After cooling and filtration, DMF was evaporated off. The residue was washed with hexane and dried. After addition of water and filtration, the solid was washed with water to obtain compound **11** (2.8 g, 7.3 mmol, 95%; $R_f^B$ 0.59): mp 170 °C; $^1$H NMR (300 MHz, DMSO-$d_6$) $\delta$ 8.04 (1 H, s), 7.22 (2 H, s), 6.23 (1 H, dd, $J$ = 8.3, 6.3 Hz), 5.47 (1 H, dd, $J$ = 8.1, 4.0 Hz), 5.33 (1 H, d, $J$ = 4.0 Hz), 4.43 (1 H, m), 3.87 (1 H, m), 3.67 (1 H, m), 3.49 (1 H, m), 3.30 (2 H, m), 3.10 (1 H, m), 2.09 (1 H, m), 1.00 (2 H, m), 0.04 (9 H, s); $^{13}$C NMR (75 MHz, DMSO-$d_6$) $\delta$ 154.5, 151.3, 150.4, 148.2, 119.7, 88.3, 84.9, 71.4, 62.3, 37.4, 29.1, 16.74, −1.7; LRMS [FAB$^+$, glycerol] $m/z$ = 384 [M + H]$^+$, [FAB$^-$, glycerol] $m/z$ = 382 [M − H]$^-$, 282 [M − 2H − $C_2H_4SiMe_3$]$^-$. Anal. Calcd for $C_{15}H_{25}N_5O_3SSi$: C, 46.97; H, 6.57; N, 18.26; S, 8.36. Found: C, 46.57; H, 6.68; N, 18.18; S, 8.27.

**2′-Deoxy-8-thioadenosine (12).** To a solution of compound **11** (200 mg, 0.52 mmol) in anhydrous THF (2 mL) was added a solution of tetrabutylammonium fluoride in THF (1 M, 2 mL). The resultant solution was stirred for 3 h at room temperature and then was evaporated. To the oily residue was added ether, and the white solid obtained was filtered off and washed with ether and then with water (5 mL at 0 °C). NMR analysis of this dried solid revealed the presence of a 1:1 mixture of thione **12** and its tetrabutylammonium salt (194 mg, 92%; $R_f^B$ 0.24): .$^1$H NMR (300 MHz, DMSO-$d_6$) $\delta$ 7.90 (1 H, s), 6.84 (1 H, dd, $J$ = 8.9, 6.0 Hz), 6.66 (2 H, s), 5.98 (1 H, s), 5.18 (1 H, d), 4.40 (1 H, m), 3.85 (1 H, m), 3.65 (1 H, m), 3.52 (1 H, m), 3.15 (4 H, m), 2.84 (1 H, m), 1.95 (1 H, m), 1.55 (4 H, m), 1.32 (4 H, m), 0.92 (6 H, t, $J$ = 7.3 Hz); LRMS [FAB$^+$, glycerol] $m/z$ = 284 [M + H]$^+$, [FAB$^-$, glycerol] $m/z$ = 282 [M − H]$^-$.

**Isolation of the Pure Thione (12).** The obtained mixture of thione **12** and the corresponding tetrabutylammonium salt dissolved in aqueous $NH_4HCO_3$ (5%, 25 mL) was chromatographed on a Sep-Pak C18 cartridge (1 g) with a mixture of 2% aqueous $NH_4HCO_3$−MeOH (9:1). Fractions containing compound **12** were evaporated, giving a white residue which was dissolved in aqueous HCl (0.2 M, 5 mL). The solution was chromatographed on a Sep-Pak C18 cartridge (1 g) with $H_2O$ and then $H_2O$−MeOH (8:2) to lead to the thione **12**: mp 141 °C; $^1$H NMR (400 MHz, DMSO-$d_6$) $\delta$ 12.55 (1 H, s), 8.18 (1 H, s), 6.95 (2 H, s), 6.28 (1 H, t, $J$ = 6.4 Hz), 5.35 (1 H, s), 5.23 (1 H, s), 4.46 (1 H, m), 3.68 (1H, m), 3.53 (1H, m), 2.95 (1H, m), 2.07 (1 H, m); $^{13}$C NMR (75 MHz, DMSO-$d_6$) $\delta$ 167.1, 151.9, 148.1, 107.5, 88.1, 85.1, 71.5, 62.4, 39.9, 36.8; IR (KBr) 3200, 2920, 1650, 1580, 1450, 1100 (C═S) cm$^{-1}$; LRMS [FAB$^+$, glycerol] $m/z$ = 284 [M + H]$^+$, [FAB$^-$, glycerol] $m/z$ = 282 [M − H]$^-$. Anal. Calcd for $C_{10}H_{13}N_5O_3S$: C, 39.86; H, 5.02; N, 23.24; S, 10.64. Found: C, 40.09; H, 5.05; N, 22.90; S, 10.58.

**Acknowledgment.** This work was supported by a grant from the Ligue Nationale contre le Cancer, Comité de la Drôme.

**Supporting Information Available:** Crystallographic data for 2′-deoxycytidin-2′-yl methyl disulfide (**5**) and 3′-deoxythymidin-3′-yl methyl disulfide (**9**) (tables of crystallographics details, positional parameters, *B*(eq), intramolecular distances, intramolecular bond angles, anisotropic displacement parameters, and least-squares planes). This material is available free of charge via the Internet at http://pubs.acs.org.

JO9908492