Synthesis of Racemic and Enantiomerically Pure 2'-Thia-2',3'-dideoxycytidine as Potential Anti-Hepatitis B Virus Agents

Bai-Chuan Pan, Yung-Chi Cheng and Shih-Hsi Chu*

Division of Biology and Medicine, Brown University, Providence, RI 02912
School of Medicine, Yale University, New Haven, CT 06510

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A novel class of nucleosides with the $C_{1'}$ atom bonded to three hetero atoms was synthesized. 2'-Thia-2',3'-dideoxycytidine was the pilot compound of this series. (±)-β-2'-Thia-1',3'-dideoxycytidine (**6**) and (±)-α-2'-thia-2',3'-dideoxycytidine (**7**) were synthesized from (±)-3-mercapto-1,2-propanediol. The synthesis of the enantiomerically pure 2'-thia-2',3'-dideoxycytidines (α-D-form, β-D-form, α-L-form and β-L-form) from optically pure (*S*)-(2,2-dimethyl-1,3-dioxalan-yl)methyl *p*-toluenesulfonate (**8**) and its (*R*)-isomer **18** was also described. The preliminary biological results showed that (+)-β-D-2'-thia-2',3'-dideoxycytidine (**26**) was the most active against human hepatitis B virus with an ED_{50} of 3 μ*M*.

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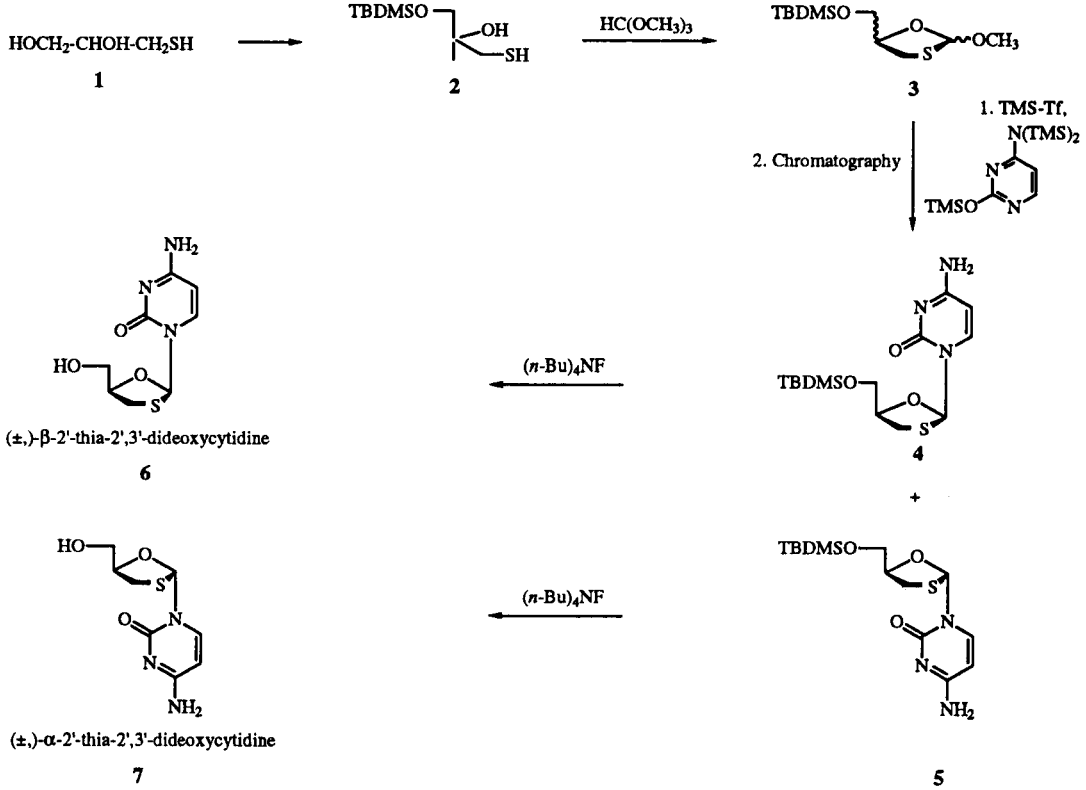
The natural nucleosides such as uridine, thymidine, cytidine *etc.* and nucleoside analogs with antiviral activity such as 3'-deoxy-3'-azidothymidine (AZT), 2',3'-dideoxycytidine, 2',3'-dideoxyinosine, and 2',3'-didehydro-3'-deoxythymidine, contain a "hemi-aminal" carbon atom ($C_{1'}$) with two hetero atoms (N, O) bonded to it. The "hemi-aminal" structures of these nucleosides are making them acid labile and/or substrates for phosphorylytic hydrolysis. The $C_{1'}$ of thianucleosides is also bonded to two hetero atoms (N,S). Theoretically, alternations at $C_{1'}$ hemiaminal structure could lead to increased efficacy and availability of the resulting derivatives, by preventing acid and enzymatic hydrolysis. For example, the isomeric structure replacement of oxygen by a methylene group in either cyclic or acyclic systems has led to remarkably effective and stable antiviral carbocyclic nucleosides. Similarly, formycines, which lack of nitrogen of base bonded to $C_{1'}$, show interesting biological activities and chemical stability. C1' atom of these nucleosides is bonded only to one hetero atom (O or N).

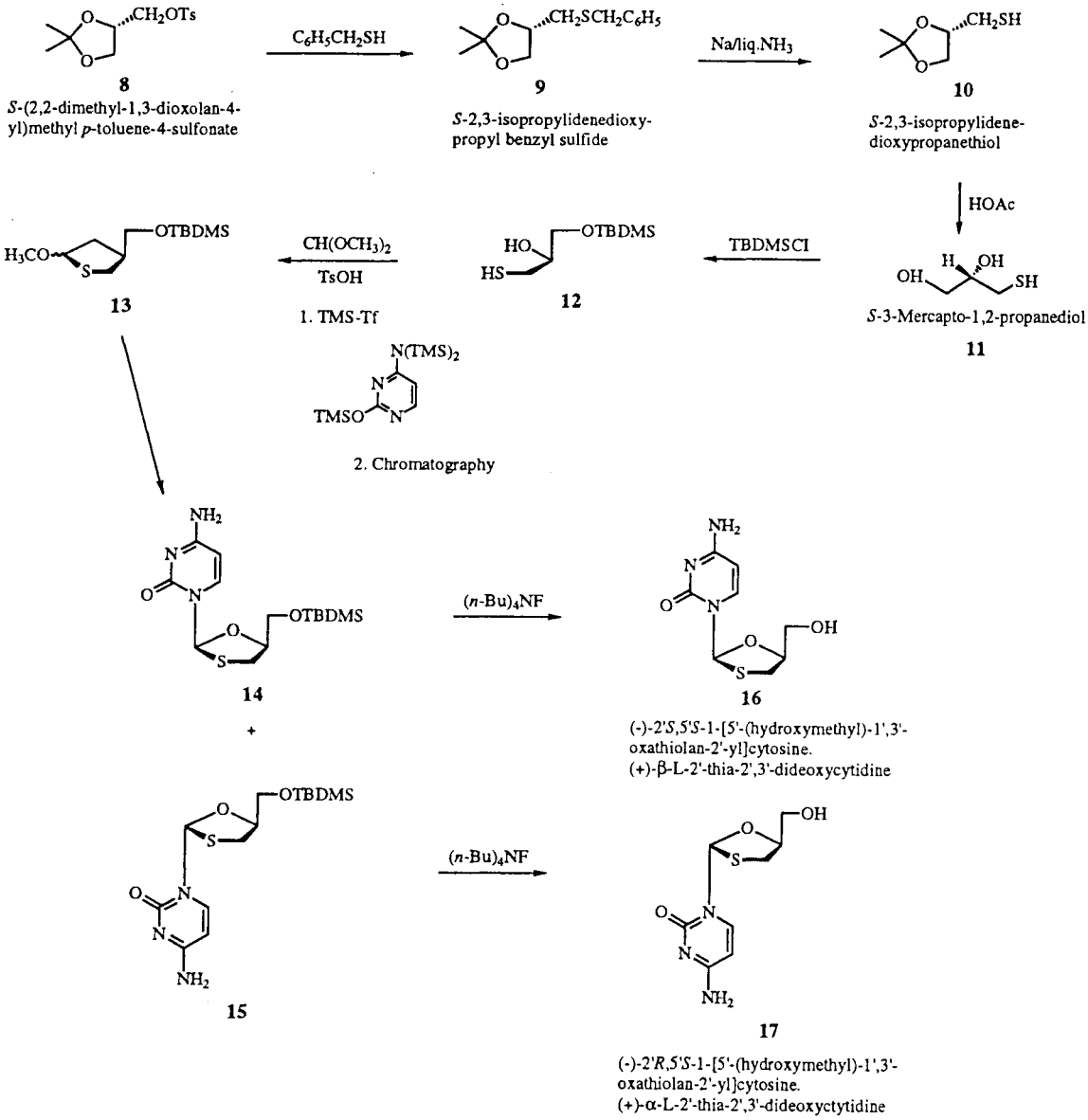
Scheme 1



Recently (+)-(2*R*,4*S*)-1-[4-(hydroxymethyl)-1,3-dioxolan-2yl]-5-fluorouracil was synthesized [1], in which C1' bonded to O, O, N three hetero atoms. We wish to report the synthesis of a novel class of nucleosides with the $C_{1'}$ atom bonded to O, S, N three hetero atoms. 2'-Thia-2',3'-dideoxycytidine (1-(5'-hydroxymethyl-1',3'-dioxathiolan-2'-yl)cytosine) was chosen to be the pilot compound of this series because of the highly potent activity of 3'-thia-2',3'-dideoxycytidine against human immunodeficiency virus (HIV) and hepatitis B virus (HBV) [2-4]. The preliminary biological results indicated that the racemic and enantiomerically pure 2'-thia-2',3'-dideoxycytidine were potent inhibitors of hepatitis B virus.

In this paper we wish to report the synthesis of (±)-α-2'-thia-2',3'-dideoxycytidine, and (±)-β-2'-thia-2',3'-dideoxycytidine from the starting material (±)-3-mercapto-1,2-propanediol (**1**) as shown in Scheme 1. (±)-3-Mercapto-1,2-propanediol (**1**) was reacted with *t*-butyldimethylsilyl chloride and imidazole to give 1-[(*t*-butyldimethylsilyl)oxy]-3-mercapto-2-propanol (**2**). The silylated compound **2** was condensed with trimethyl orthoformate in the presence of *p*-toluenesulfonic acid to form 2-methoxy-5-[(*t*-butyldimethylsilyl)oxy]methyl-1,3-dioxathiolane (**3**). Condensation of **3** with silylated cytosine in methylene chloride in the presence of trimethylsilylmethyl triflate gave a mixture of (±)-β-1-{5'-[[(*t*-butyldimethylsilyl)oxy]methyl]-1',3'-dioxathiolan-2'yl}cytosine (**4**) and (±)-α-enantiomer **5**. The α and β isomers could be carefully separated by silica gel column chromatography. Desilylation of **4** and **5** with tetra-*n*-

Scheme 2

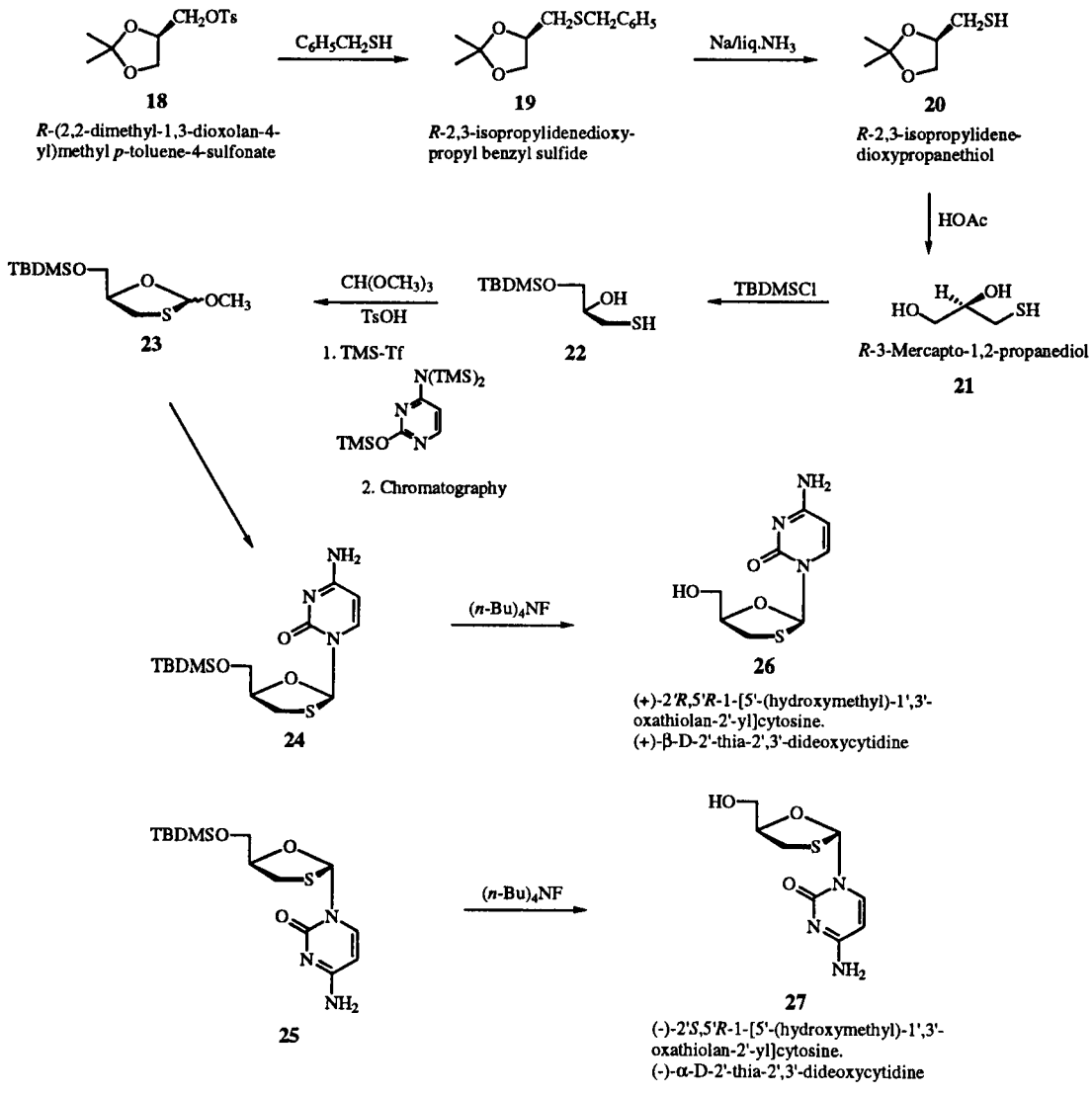


butylammonium fluoride gave (±)-β-2'-thia-1',3'-dideoxycytidine (**6**) and (±)-α-2'-thia-2',3'-dideoxycytidine (**7**).

The assignments of the configurations of (±)-β-2'-thia-2',3'-dideoxycytidine (**6**) and (±)-α-2'-thia-2',3'-dideoxycytidine (**7**) were based on their NOE experiments and characteristic proton nmr spectra. The NOE experiments showed that upon irradiation of 4'-H of **6**, enhancement of 1'-H was observed but no enhancement of 6-H; while upon irradiation of 4'-H of **7**, enhancement of 6-H was observed but no enhancement of 1'-H. 4'-H and 1'-H of **6** and 4'-H and 6-H of **7** were in *cis* orientation, and 4'-H and 1'-H of **7** and 4'H and 6-H of **6** were in *trans* orientation. Therefore, compound **6** was (±)-β-2'-thia-2',3'-dideoxycytidine and compound **7** was (±)-α-2'-thia-2',3'-dideoxycytidine. The proton nmr spectrum supports this assignment. The chemical shift of the 4'-H peak of (±)-β-2'-thia-1',3'-dideoxycytidine (**6**) appears upfield (d = 4.15) from that of (±)-α-2'-thia-2',3'-dideoxycytidine (**7**) (d = 4.55), and the 5'-H peaks of **6** appear at lower field (d = 3.71, 3.65) than those of **7** (d = 3.54, 3.24). [5].

The synthesis of pure enantiomers of 2'-thia-2',3'-dideoxycytidine is shown in Scheme 2 and Scheme 3. The enantiomerically pure 2'-thia-2',3'-dideoxycytidines (α-D- form, β-D-form, α-L-form and β-L-form) were synthesized from optically pure (*S*)-(2,2-dimethyl-1,3-diozolan-yl)methyl *p*-toluenesulfonate (**8**) and its (*R*)-isomer **18**. The sulfonate **8** was converted into (*S*)-2,3-isopropylidenedioxylpropyl benzyl sulfide (**9**) by reacting with benzyl mercaptan and sodium ethoxide. The sulfide **9** was reduced to (*S*)-2,3-isopropylidenedioxypropanethiol (**10**) by sodium in liquid ammonia. Hydrolysis of **10** with 75% acetic acid gave *S*-3-mercapto-1,2-propanediol (**11**). The enantiomeri-

Scheme 3



cally pure **11** was silylated with *t*-butyldimethylsilyl chloride and imidazole to give **12** and then reacted with trimethyl orthoformate to form a mixture of epimers of (5*S*)-2-methoxy-5-[(*t*-butyldimethylsilyl)oxy]methyl-1,3-dioxathiolane (**13**), from which a mixture of β and α-(5'*S*)-1-{5'-[[(*t*-butyldimethylsilyl)oxy]methyl]-1',3'-dioxathiolane-2'-yl}cytosine **14** and **15** was obtained by condensing with silylated cytosine. Separation of the mixture by silica gel column chromatography gave pure **14** and **15**. The enantiomerically pure (-)-β-L-2'-thia-2',3'-dideoxycytidine [(-)-(2'*S*,5'*S*)-1-[5'-(hydroxymethyl)-1',3'-oxathialane-2'yl]cytosine, **16**] and (+)-α-L-2'-thia-2',3'-dideoxycytidine [(+)-(2'*S*,5'*R*)-1-[5'-(hydroxymethyl)-1',3'-oxathialan-2'yl]cytosine, **17**] were obtained by deprotection of **14** and **15** with tetra-*n*-butylammonium fluoride.

The enantiomerically pure (*R*)-3-mercapto-1,2-propanediol (**21**), was synthesized from (*R*)-2,2-dimethyl-1,3-dioxolanylmethyl *p*-toluenesulfonate (**18**) by the similar methods as described above. A mixture of epimers of (5*S*)-2-methoxy-5-[(*t*-butyldimethylsilyl)oxy]methyl-1,3-oxathiolane (**23**) was synthesized by silylation of **21** and followed by condensation with trimethyl orthoformate. (+)-β-D-2'-Thia-2',3'-dideoxycytidine [(+)-2'*R*,5'*R*-1-[5'-(hydroxymethyl)-1',3'-oxathialan-2'yl]cytosine, **26**] and (-)-α-D-2'-thia-2',3'-dideoxycytidine [(-)-2'*S*,5'*R*-1-[5'-(hydroxymethyl)-1',3'-oxathialan-2'yl]cytosine, **27**] were obtained by the same methods for the preparation of **16** and **17**.

The preliminary biological results showed that the racemic 2'-thia-2',3'-dideoxycytidine inhibited human hepatitis B virus DNA replication with an ED_{50} of 7.5 μ*M*. Furthermore, the four pure enantiomers of 2'-thia-2',3'-dideoxycytidine were tested for their anti-hepatitis B virus activity. The results are shown in Table 1. The procedures used were the same as described previously [6]. Among the four enantiomers (+)-β-D-2'-thia-2',3'-dideoxycytidine (**26**) was the most active against hepatitis B virus with an ED_{50} of 3 μ*M*, (-)-β-L-2'-thia-2',3'-dideoxycytidine (**16**) and (+)-α-L-2'-thia-2',3'-dideoxycytidine (**17**) also showed some anti-hepatitis B virus activity.

Table 1

Effect of 2'-Thia-2',3'-dideoxycytidine Enantiomers on Hepatitis B Virus (HBV)

Compound	**26**	**16**	**27**	**17**
	(+)-β-D-form	(-)-β-L-form	(-)-α-D-form	(+)-α-L-form
ED_{50}* (μ*M*)	3	20	>100	100

* Concentration required to inhibit hepatitis B virus DNA (yield in medium by 50% in 2.2.15 cells)

EXPERIMENTAL

Melting points were determined on a Gallenkamp apparatus and were uncorrected. The uv absorption maxima and extinction coefficients were obtained using a Perkin-Elmer Model Lambda 3A recording spectrophotometer. The NMR spectra were run on a Brucker WM-400. Analyses were performed by the Baron Consulting Co., of Orange, CT.

(±)-1-[(*t*-Butyldimethylsilyl)oxy]-3-mercapto-2-propanol (**2**).

A solution of 3.24 g (30 mmoles) of (±)-3-mercapto-1,2-propanediolol (**1**) and 2.72 g (40 mmoles) of imidazole in 32 ml of dry DMF was cooled to 0°, to which there was gradually added 4.97 g (33 mmoles) of *t*-butyldimethylsilyl chloride with stirring. The mixture was stirred at room temperature overnight. It was partitioned between water and hexane and then dried over magnesium sulfate. After evaporation of hexane under reduced pressure the residue was purified by column chromatography on silica gel and eluted with 10% ether in hexane to give 6.14 g (92%) of **2** as a clear oil; ^{1}H nmr (deuteriochloroform): δ 0.08 (s, 6H, CH_3Si), 0.90 (s, 9H, *t*-Bu), 1.47 (t, 1H, SH, J = 8.6 Hz), 2.25 (br s, 1H, OH), 2.65 (dd, 2H, CH_2S, J = 5.6, 8.6 Hz), 3.64-3.72 (m, 3H, CH_2O, CHO).

Anal. Calcd. for $C_9H_{22}O_2SSi$: C, 48.60; H, 9.96. Found: C, 48.92; H, 10.12.

(±)-2-Methoxy-5-[(*t*-butyldimethylsilyl)oxy]methyl-1,3-dioxathiolane (**3**).

To a solution of 2.22 g (10 mmoles) of **2** and 2.12 g (20 mmoles) of trimethyl orthoformate in 20 ml of dry ether, 3.8 mg (0.02 mmole) of *p*-toluenesulfonic acid was added. The mixture was stirred at room temperature until tlc in ether:hexane (20:1) showed no starting material remained. To this there was added 100 mg of sodium bicarbonate and the reaction mixture was evaporated to dryness under reduced pressure. The residue was chromatographed on silica gel column and eluted with hexane to give 2.34 g (85%) of the mixture of epimers of (±)-2-Methoxy-5-[(*t*-butyldimethylsilyl)oxy]methyl-1,3-dioxathiolane (**3**) as a colorless oil; ^{1}H nmr (deuteriochloroform): δ 0.08 (s, 6H, CH_3Si), 0.90 (s, 9H, *t*-Bu), 2.65-3.05 (m, 2H, CH_2S), 3.49 (s, ~1.5H, OCH_3, α or β-epimer), 3.78 (s, ~1.5 H, OCH_3, α or β-epimer), 3.57-3.72 (m, 1H, CH_2O), 3.79-3.90 (m, 1H, CH_2O), 4.38-4.55 (m, ~0.5H, CHO, α or β-epimer), 4.96-5.04 (m, ~0.5H, CHO, α or β-epimer), 8.08 (s, ~0.5H, 1'-H, α or β-epimer), 8.11 (s, ~0.5H, 1'-H, α or β-epimer).

Anal. Calcd. for $C_{11}H_{24}O_3SSi$: C, 49.96; H, 9.14. Found: C, 49.67; H, 9.37.

(±)-β-l-{5'-[(*t*-Butyldimethylsilyl)oxy]methyl-1',3'-dioxathiolan-2'-yl}cytosine (**4**) and (±)-α-1-{5'-[(*t*-Butyldimethylsilyl)oxy]methyl-1',3'-dioxathiolan-2'-yl}cytosine (**5**).

A suspension of 445 mg (4 mmoles) of cytosine and one drop of trimethylsilylmethyl triflate in 10 ml of 1,1,1,3,3,3-hexamethyldisilazane was stirred and refluxed under nitrogen until a clear solution was formed. After the excess 1,1,1,3,3,3-hexamethyldisilazane was removed under reduced pressure, the residue was dissolved in 10 ml of dry methylene chloride under nitrogen atmosphere. To this solution, 1.058 g (4 mmoles) of (±)-2-methoxy-5[(*t*-butyldimethylsilyl)oxy]methyl-1,3-dioxathiolane (**3**) was added, cooled to 0°, and then a solution of 945 mg (4 mmoles) of trimethylsilylmethyl triflate in 5 ml of dry methylene chloride

was added which was previously cooled to 0°. The reaction mixture was stirred at 0° for ten minutes and room temperature for one hour. The mixture was then poured into an ice cooled mixture of 10 ml of methylene chloride and 10 ml of saturated sodium bicarbonate solution. The methylene chloride layer was separated and washed with dilute sodium bicarbonate, water and then dried over magnesium sulfate. After removal of solvent under reduced pressure the residue was treated with ether to give 820 mg of a mixture of **4** and **5**. The ethereal mother liquid was evaporated and chromographied on silica gel column using 5% methanol in methylene chloride as an eluent to afford an additional 130 mg of mixture of **4** and **5**, total yield 950 mg (69%) as white crystals. The mixture of **4** and **5** was chromatographied on silica gel column and eluted with methylene chloride: ethanol (100:6) to give (±)-β-1-{5'-[(*t*-butyldimethylsilyl)oxy]methyl-1',3'-dioxathiolan-2'-yl}cytosine (**4**, Rf = 0.192) and (±)-α-1-{5'-[(*t*-butyldimethylsilyl)oxy]methyl-1',3'-dioxathiolan-2'-yl}cytosine (**5**, Rf = 0.185) (α:β = 2:3).

(±)-β-1-{5'-[(*t*-Butyldimethylsilyl)oxy]methyl-1',3'-dioxathiolan-2'yl}cytosine (**4**).

This compound had mp >270° dec; ^{1}H nmr (deuteriochloroform): δ 0.08 (s, 6H, CH_3Si), 0.90 (s, 9H, *t*-Bu), 3.03 (dd, 1H, CH_2S, J = 4.9, 10.7 Hz), 3.21 (t, 1H, CH_2S, J = 3.1, 10.7 Hz), 3.87 (dd, 1H, CH_2O, J = 3.1, 11.3 Hz), 4.04 (dd, 1H, CH_2O, J = 3.6, 11.3 Hz), 4.31 (m, 1H, CHO), 6.33 (d, 1H, 5-H, J = 7.7 Hz), 7.90 (d, 1H, 6-H, J = 7.7 Hz), 7.20 (s, 1H, 1'-H).

Anal. Calcd. for $C_{14}H_{25}N_3O_3SSi$: C, 48.95; H, 7.33; N, 12.23. Found: C, 48.49; H, 7.55; N, 12.19.

(±)-α-1-{5'-[(*t*-Butyldimethylsilyl)oxy]methyl-1',3'-dioxathiolan-2'-yl}cytosine] (**5**).

This compound had mp >250° dec; ^{1}H nmr (deuteriochloroform): δ 0.08 (s, 6H, CH_3Si), 0.90 (s, 9H, *t*-Bu), 3.16 (dd, 1H, CH_2S, J = 6.0, 10.9 Hz), 3.24 (dd, 1H, CH_2S, J = 5.7, 10.9 Hz), 3.77 (m, 2H, CH_2O), 4.62 (m, 1H, CHO), 6.44 (d, 1H, 5-H, J = 7.7 Hz), 7.72 (d, 1H, 6-H, J = 7.7 Hz), 7.12 (s, 1H, 1'-H).

Anal. Calcd. for $C_{14}H_{25}N_3O_3SSi$: C, 48.95; H, 7.33; N, 12.23. Found: C, 48.72; H, 7.51; N, 12.04.

(±)-β-2'-Thia-2',3'-dideoxycytidine [(±)-β-1-(5'-Hydroxymethyl-1',3'-dioxathiolan-2'-yl)cytosine] (**6**).

A solution of 68.7 mg (0.2 mmole) of (±)-β-1-{5'-[(*t*-butyldimethylsilyl)oxy]methyl-1',3'-dioxathiolan-2'-yl]cytosine (**4**) in 0.7 ml of dry THF was treated with 0.2 ml of 1 *M* tetra-*n*-butylammonium fluoride in THF at 0° with stirring. The reaction mixture was allowed to stand in the refrigerator overnight. The separated crystals were filtered and recrystallized from methanol to give 35.7 mg (78%) of **6**, mp >250° dec; ^{1}H nmr (DMSO-d_6): δ 3.01-3.14 (m, 2H, CH_2S), 3.62-3.76 (m, 2H, CH_2O), 4.13-4.20 (m, 1H, CHO), 5.10 (t, 1H, OH, J = 5.8 Hz), 5.81 (d, 1H, 5-H, J = 7.5 Hz), 7.79 (d, 1H, 6-H, J = 7.5 Hz), 7.15 (s, 1H, 1'-H), 7.30 (br s, 1H, NH_2), 7.35 (br s, 1H, NH_2).

Anal. Calcd. for $C_8H_{11}N_3O_3S$: C, 41.91; H, 4.83; N, 18.33. Found: C, 41.78; H, 5.08; N, 18.07.

(±)-α-2'-Thia-2',3'-dideoxycytidine [(±)-α-1-(5'-Hydroxymethyl-1',3'-dioxathiolan-2'-yl)cytosine] (**7**).

This compound was prepared by desilylation of (±)-α-1-{5'-[(*t*-butyldimethylsilyl)oxy]methyl-1',3'-dioxathiolan-2'-yl}cytosine (**5**) with tetra-*n*-butylammonium fluoride as described above, mp >250° dec; ^{1}H nmr (DMSO-d_6): δ 3.01-3.14 (m, 2H, CH_2S), 3.62-3.76 (m, 2H, CH_2O), 4.13-4.20 (m, 1H, CHO), 5.10 (t, 1H, OH, J = 5.8 Hz), 5.81 (d, 1H, 5-H, J = 7.5 Hz), 7.79 (d, 1H, 6-H, J = 7.5 Hz), 7.15 (s, 1H, 1'-H), 7.30 (br s, 1H, NH_2), 7.35 (br s, 1H, NH_2).

Anal. Calcd. for $C_8H_{11}N_3O_3S$: C, 41.91; H, 4.83; N, 18.33. Found: C, 42.01; H, 5.09; N, 18.15.

(-)-(*S*)-(2,3-Isopropylidenedioxy)propyl Benzyl Sulfide (**9**).

Sodium (2.53 g, 110 mmoles) was dissolved in 100 ml of absolute ethanol and cooled to 5°. To the sodium ethoxide solution, there was gradually added 14.9 g (120 mmoles) of benzyl mercaptan with stirring. After the addition was complete the reaction mixture was stirred at 5° for 20 minutes and followed by the addition of 28.63 g (100 mmoles) of (-)-(*S*)-(2,2-dimethyl-1,3-dioxolan-2-yl)methyl *p*-toluenesulfonate (**8**) at 5°. The reaction mixture was gradually heated to reflux until the reaction was complete (by tlc). The solvent was evaporated under reduced pressure and the residue was partitioned between hexane and water. The hexane solution was washed with water, dried over magnesium sulfate and then evaporated under reduced pressure. The oily residue was distilled in vacuum to give 19.6 g (82%) of (-)-(*S*)-(2,3-isopropylidenedioxy)propyl benzyl sulfide (**11**), bp 130-132°/1 mm Hg; $[\alpha]_D^{25}$ = -51.4° (c = 0.2, ethanol), ^{1}H nmr (deuteriochloroform): δ 1.34 (s, 3H, CH_3), 1.41 (s, 3H, CH_3), 2.52 (dd, 1H, CH_2S, J = 7.1, 13.5 Hz), 2.66 (dd, 1H, CH_2S, J = 5.6, 13.5 Hz), 3.66 (dd, 1H, CH_2O, J = 6.4, 8.3 Hz), 3.77 (dd, 2H, CH_2 of Bn, J = 13.5, 16.0 Hz), 4.05 (dd, 1H, CH_2O, J = 6.1, 8.3 Hz), 4.9 (m, 1H, CHO), 7.22-7.32 (m, 5H, ArH).

Anal. Calcd. for $C_{13}H_{18}O_2S$: C, 65.51; H, 7.61. Found: C, 65.71; H, 7.86.

(+)-(*R*)-(2,3-Isopropylidenedioxy)propyl Benzyl Sulfide (**19**).

This compound was synthesized by the condensation of (-)-(*R*)-(2,2-dimethyl-1,3-dioxolan-2-yl)methyl *p*-toluenesulfonate (**18**) with benzyl mercaptan in sodium ethoxide solution as indicated above, bp 130-133°, $[\alpha]_D^{25}$ = +53.5° (c = 0.2, ethanol); ^{1}H nmr (deuteriochloroform): δ 1.34 (s, 3H, CH_3), 1.41 (s, 3H, CH_3), 2.52 (dd, 1H, CH_2S, J = 7.1, 13.5 Hz), 2.66 (dd, 1H, CH_2S, J = 5.6, 13.5 Hz), 3.66 (dd, 1H, CH_2O, J = 6.4, 8.3 Hz), 3.77 (dd, 2H, CH_2 of Bn, J = 13.5, 16.0 Hz), 4.05 (dd, 1H, CH_2O, J = 6.1, 8.3 Hz), 4.9 (m, 1H, CHO), 7.22-7.32 (m, 5H, Ar H).

Anal. Calcd. for $C_{13}H_{18}O_2S$: C, 65.51; H, 7.61. Found: C, 65.12; H, 7.92.

(-)-(*S*)-(2,3-Isopropylidenedioxy)propanethiol (**10**).

A solution of 11.9 g (50 mmoles) of (-)-(*S*)-(2,3-isopropylidenedioxy)propyl benzyl sulfide (**9**) in 80 ml of dry ether was added to 160 ml of dry liquid ammonia at -78° under nitrogen. To the mixture there was gradually added sodium with stirring at -78° until the color of the solution became blue and the stirring was continued for 20 minutes more at -78°. It was quenched by adding a small amount of water. Ammonia was evaporated, and the residue was diluted with 50 ml of water. Carbon dioxide was bubbled through the mixture at 0° until it was saturated. The ethereal solution was separated and the aqueous solution was extracted with ether. The combined ethereal solution was washed with a small amount of water and then dried over magnesium sulfate. After removal of ether the residue was distilled under reduced pressure to give 5.6 g (75%) of (+)-(*R*)-(2,3-isopropylidenedioxy)propanethiol (**10**), bp 65°/15 mm Hg; $[\alpha]_D^{25}$ = -29.3° (c = 0.2, ethanol); ^{1}H nmr (deuteriochloroform): δ 1.36 (s, 3H, CH_3), 1.44 (s, 3H, CH_3), 2.57-2.66 (m, 1H, CH_2S), 2.70-2.79 (m,

1H, CH_2S), 3.77 (dd, 1H, CH_2O, J = 6.0, 8.4 Hz), 4.11 (dd, 1H, CH_2O, J = 6.0, 9.3 Hz), 4.21 (m, 1H, CHO).

Anal. Calcd. for $C_6H_{12}O_2S \cdot 1/3\ H_2O$: C, 46.72; H, 8.27. Found: C, 46.92; H, 8.46.

(+)-(*R*)-(2,3-Isopropylidenedioxy)propanethiol (**20**).

This compound was prepared from (+)-(*R*)-(2,3-isopropylidenedioxy)propyl benzyl sulfide (**19**) by the method described above, bp 68°/15 mm Hg; $[\alpha]_D^{25}$ = +30.2°(c = 0.2, ethanol) [7]; ^1H nmr (deuteriochloroform): δ 1.36 (s, 3H, CH_3), 1.44 (s, 3H, CH_3), 2.57-2.66 (m, 1H, CH_2S), 2.70-2.79 (m, 1H, CH_2S), 3.77 (dd, 1H, CH_2O, J = 6.0, 8.4 Hz), 4.11 (dd, 1H, CH_2O, J = 6.0, 9.3 Hz), 4.21 (m, 1H, CHO).

Anal. Calcd. for $C_6H_{12}O_2S \cdot 1/3\ H_2O$: C, 46.72; H, 8.27. Found: C, 47.17; H, 8.77.

(+)-(*S*)-3-Mercapto-1,2-propanediol (**11**).

A solution of 4.63 g (30 mmoles) of (-)-(*S*)-(2,3-isopropylidenedioxy)propanethiol (**10**) in 50 ml of 75% acetic acid was stirred at 70° for about 4 hours until the reaction was complete (by tlc). The solvent was then evaporated under reduced pressure and the residue was chromatographied on silica gel eluted with 5% methanol in methylene chloride to form 3.02 g (93%) of (-)-(*S*)-3-mercapto-1,2-propanediol (**11**) as a colorless oil; $[\alpha]_D^{25}$ = +8.4° (c = 0.1, ethanol), ^1H nmr (deuteriochloroform): δ 1.47 (t, 1H, SH, J = 8.6 Hz), 2.25 (br s, 1H, OH), 2.58-2.67 (m, 1H, CH_2S), 2.69-2.76 (m, 1H, CH_2S), 2.85 (br s, 1H, OH), 3.61 (dd, 1H, CH_2O, J = 7.0, 12.1 Hz), 3.72-3.79 (m, 3H, CH_2O, CHO).

Anal. Calcd. for $C_3H_8O_2S$: C, 33.32; H, 7.45. Found: C, 33.28; H, 7.81.

(-)-(*R*)-3-Mercapto-1,2-propanediol (**21**).

This compound was prepared from (+)-(*R*)-(2,3-isopropylidenedioxy)propanethiol (**20**) by the method described above, $[\alpha]_D^{25}$ = -9.3° (c = 0.1, ethanol) [7]; ^1H nmr (deuteriochloroform): δ 1.47 (t, 1H, SH, J = 8.6 Hz), 2.58-2.67 (m, 1H, CH_2S), 2.69-2.76 (m, 1H, CH_2S), 2.95 (br s, 1H, OH), 3.45 (br s, 1H, OH), 3.61 (dd, 1H, CH_2O, J = 7.0, 12.1 Hz), 3.72-3.79 (m, 3H, CH_2O, CHO).

Anal. Calcd. for $C_3H_8O_2S$: C, 33.32; H, 7.45. Found: C, 33.06; H, 7.70.

(+)-(*S*)-1-[(*t*-Butyldimethylsilyl)oxy]-3-mercapto-2-propanol (**12**).

This compound was prepared from (+)-(*S*)-3-mercapto-1,2-propanediol (**11**) in 91% yield by the method used for the synthesis of **2**; $[\alpha]_D^{25}$ = +15.3° (c = 0.5, ethanol); ^1H nmr (deuteriochloroform): δ 0.08 (s, 6H, CH_3Si), 0.90 (s, 9H, *t*-Bu), 1.47 (t, 1H, SH, J = 8.6 Hz), 2.25 (br s, 1H, OH), 2.65 (dd, 2H, CH_2S, J = 5.6, 8.6 Hz), 3.64-3.72 (m, 3H, CH_2O, CHO).

Anal. Calcd. for $C_9H_{22}O_2SSi$: C, 48.60; H, 9.96. Found: C, 48.84; H, 10.18.

(-)-(*R*)-1-[(*t*-Butyldimethylsilyl)oxy]-3-mercapto-2-propanol (**22**).

This compound was prepared from (-)-(*R*)-3-mercapto-1,2-propanediol (**21**) in 90% yield by the method used for the synthesis of **2**; $[\alpha]_D^{25}$ = -16.6° (c = 0.5, ethanol); ^1H nmr (deuteriochloroform): δ 0.08 (s, 6H, CH_3Si), 0.90 (s, 9H, *t*-Bu), 1.47 (t, 1H, SH, J = 8.6 Hz), 2.25 (br s, 1H, OH), 2.65 (dd, 2H, CH_2S, J = 5.6, 8.6 Hz), 3.64-3.72 (m, 3H, CH_2O, CHO).

Anal. Calcd. for $C_9H_{22}O_2SSi$: C, 48.60; H, 9.96. Found: C, 48.29; H, 10.06.

(5*S*)-2-Methoxy-5-[(*t*-butyldimethylsilyl)oxy]methyl-1,3-dioxathiolane (**13**).

This compound was prepared from (-)-(*S*)-1-[(*t*-butyldimethylsilyl)oxy]-3-mercapto-2-propanol (**12**) in 81% yield by the method used for the synthesis of **3**, ^1H nmr (deuteriochloroform): δ 0.08 (s, 6H, CH_3Si), 0.90 (s, 9H, *t*-Bu), 2.65-3.05 (m, 2H, CH_2S), 3.49 (s, 1.5H, OCH_3, α or β-epimer), 3.78 (s, 1.5H, OCH_3, α or β-epimer), 3.57-3.72 (m, 1H, CH_2O), 3.79-3.90 (m, 1H, CH_2O), 4.38-4.55 (m, 0.5H, CHO, α or β-epimer), 4.96-5.04 (m, 0.5H, CHO, α or β-epimer), 8.08 (s, 0.5H, 1'-H, α or β-epimer), 8.11 (s, ~0.5H, 1'-H, α or β-epimer).

Anal. Calcd. for $C_{11}H_{24}O_3SSi$: C, 49.96; H, 9.14. Found: C, 50.04; H, 9.14.

(5*R*)-2-Methoxy-5-[(*t*-butyldimethylsilyl)oxy]methyl-1,3-dioxathiolane (**23**).

This compound was prepared from (-)-(*R*)-1-[(*t*-butyldimethylsilyl)oxy]-3-mercapto-2-propanol (**22**) in 78% yield by the method used for the synthesis of **3**; ^1H nmr (deuteriochloroform): δ 0.08 (s, 6H, CH_3Si), 0.90 (s, 9H, *t*-Bu), 2.65-3.05 (m, 2H, CH_2S), 3.49 (s, 1.5H, OCH_3, α or β-epimer), 3.78 (s, 1.5H, OCH_3, α or β-epimer), 3.57-3.72 (m, 1H, CH_2O), 3.79-3.90 (m, 1H, CH_2O), 4.38-4.55 (m, 0.5H, CHO, α or β-epimer), 4.96-5.04 (m, 0.5H, CHO, α or β-epimer), 8.08 (s, 0.5H, 1'-H, α or β-epimer), 8.11 (s, 0.5H, 1'-H, α or β-epimer).

Anal. Calcd. for $C_{11}H_{24}O_3SSi$: C, 49.96; H, 9.14. Found: C, 49.59; H, 9.52.

(-)-(2'*S*,5'*S*)-1-{5'-[(*t*-Butyldimethylsilyl)oxy]methyl-1',3'-dioxathiolan-2'-yl}cytosine (**14**).

This compound was prepared from (5*S*)-2-methoxy-5-[(*t*-butyldimethylsilyl)oxy]methyl-1,3-dioxathiolane (**13**) and silylated cytosine by the method used for the synthesis of **4**, mp >250° dec; $[\alpha]_D^{25}$ = -184.2° (c = 0.02, ethanol); ^1H nmr (deuteriochloroform): δ 0.08 (s, 6H, CH_3Si), 0.90 (s, 9H, *t*-Bu), 3.03 (dd, 1H, CH_2S, J = 4.9, 10.7 Hz), 3.21 (t, 1H, CH_2S, J = 3.1, 10.7 Hz), 3.87 (dd, 1H, CH_2O, J = 3.1, 11.3 Hz), 4.04 (dd, 1H, CH_2O, J = 3.6, 11.3 Hz), 4.31 (m, 1H, CHO), 6.33 (d, 1H, 5-H, J = 7.7 Hz), 7.90 (d, 1H, 6-H, J = 7.7 Hz), 7.20 (s, 1H, 1'-H).

Anal. Calcd. for $C_{14}H_{25}N_3O_3SSi$: C, 48.95; H, 7.33; N, 12.23. Found: C, 49.07; H, 7.06; N, 12.10.

(+)-(2'*R*,5'*R*)-1-{5'-[(*t*-Butyldimethylsilyl)oxy]methyl-1',3'-dioxathiolan-2'-yl}cytosine (**24**).

This compound was prepared from (5*R*)-2-methoxy-5-[(*t*-butyldimethylsilyl)oxy]methyl-1,3-dioxathiolane (**23**) and silylated cytosine by the method used for the synthesis of **4**, mp >250° dec; $[\alpha]_D^{25}$ = +189.9° (c = 0.02, ethanol); ^1H nmr (deuteriochloroform): δ 0.08 (s, 6H, CH_3Si), 0.90 (s, 9H, *t*-Bu), 3.03 (dd, 1H, CH_2S, J = 4.9, 10.7 Hz), 3.21 (t, 1H, CH_2S, J = 3.1, 10.7 Hz), 3.87 (dd, 1H, CH_2O, J = 3.1, 11.3 Hz), 4.04 (dd, 1H, CH_2O, J = 3.6, 11.3 Hz), 4.31 (m, 1H, CHO), 6.33 (d, 1H, 5-H, J = 7.7 Hz), 7.90 (d, 1H, 6-H, J = 7.7 Hz), 7.20 (s, 1H, 1'-H).

Anal. Calcd. for $C_{14}H_{25}N_3O_3SSi$: C, 48.95; H, 7.33; N, 12.23. Found: C, 48.67; H, 7.45; N, 12.28.

(+)-(2'*R*,5'*S*)-1-{5'-[(*t*-Butyldimethylsilyl)oxy]methyl-1',3'-dioxathiolan-2'-yl}cytosine (**15**).

This compound was prepared from (5*S*)-2-methoxy-5-[(*t*-butyldimethylsilyl)oxy]methyl-1,3-dioxathiolane (**13**) and silylated cytosine by the method used for the synthesis of **5**, mp >250° dec; $[\alpha]_D^{25}$ = +213.9° (c = 0.02, ethanol); ^1H nmr

(deuteriochloroform): δ 0.08 (s, 6H, CH_3Si), 0.90 (s, 9H, *t*-Bu), 3.16 (dd, 1H, CH_2S, J = 6.0, 10.9 Hz), 3.24 (dd, 1H, CH_2S, J = 5.7, 10.9 Hz), 3.77 (m, 2H, CH_2O), 4.62 (m, 1H, CHO), 6.44 (d, 1H, 5-H, J = 7.7 Hz), 7.72 (d, 1H, 6-H, J = 7.7 Hz), 7.12 (s, 1H, 1'-H).

Anal. Calcd. for $C_{14}H_{25}N_3O_3SSi$: C, 48.95; H, 7.33; N, 12.23. Found: C, 48.73; H, 7.34; N, 12.08.

(-)-(2'*S*,5'*R*)-1-{5'-[(*t*-Butyldimethylsilyl)oxy]methyl-1',3'-dioxathiolan-2'-yl}cytosine (**25**).

This compound was prepared from (5*R*)-2-methoxy-5-[(*t*-butyldimethylsilyl)oxy]methyl-1,3-dioxathiolane (**23**) and silylated cytosine by the method used for the synthesis of **5**, mp >250° dec; $[\alpha]_D^{25}$ = -217.2° (c = 0.02, ethanol); ^1H nmr (deuteriochloroform): δ 0.08 (s, 6H, CH_3Si), 0.90 (s, 9H, *t*-Bu), 3.16 (dd, 1H, CH_2S, J = 6.0, 10.9 Hz), 3.24 (dd, 1H, CH_2S, J = 5.7, 10.9 Hz), 3.77 (m, 2H, CH_2O), 4.62 (m, 1H, CHO), 6.44 (d, 1H, 5-H, J = 7.7 Hz), 7.72 (d, 1H, 6-H, J = 7.7 Hz), 7.12 (s, 1H, 1'-H).

Anal. Calcd. for $C_{14}H_{25}N_3O_3SSi$: C, 48.95; H, 7.33; N, 12.23. Found: C, 49.14; H, 7.09; N, 11.98.

(-)-β-L-2'-Thia-2',3'-dideoxycytidine [(-)-(2'*S*,5'*S*)-1-(5'-Hydroxymethyl-1',3'-dioxathiolan-2'-yl)cytosine] (**16**).

Desilylation of (-)-(2'*S*,5'*S*)-1-{5'-[(*t*-butyldimethylsilyl)oxy]methyl-1',3'-dioxathiolan-2'-yl}cytosine (**14**) with tetra-*n*-butylammonium fluoride was performed by the method used for preparation of **6**, mp >250° dec; $[\alpha]_D^{25}$ = -257.4° (c = 0.02, ethanol); ^1H nmr (DMSO-d_6): δ 3.01-3.14 (m, 2H, CH_2S), 3.62-3.76 (m, 2H, CH_2O), 4.13-4.20 (m, 1H, CHO), 5.10 (t, 1H, OH, J = 5.8 Hz), 5.81 (d, 1H, 5-H, J = 7.5 Hz), 7.79 (d, 1H, 6-H, J = 7.5 Hz), 7.15 (s, 1H, 1'-H), 7.30 (br s, 1H, NH_2), 7.35 (br s, 1H, NH_2).

Anal. Calcd. for $C_8H_{11}N_3O_3S$: C, 41.91; H, 4.83; N, 18.33. Found: C, 42.23; H, 4.60; N, 18.14.

(+)-β-D-2'-Thia-2',3'-dideoxycytidine [(+)-(2'*R*,5'*R*)-1-(5'-Hydroxymethyl-1',3'-dioxathiolan-2'-yl)cytosine] (**26**).

Desilylation of (+)-(2'*R*,5'*R*)-1-{5'-[(*t*-butyldimethylsilyl)oxy]methyl-1',3'-dioxathiolan-2'-yl}cytosine (**24**) with tetra-*n*-butylammonium fluoride was performed by the method used for preparation of **6**, mp >250° dec; $[\alpha]_D^{25}$ = +260.1° (c = 0.02, ethanol); ^1H nmr (DMSO-d_6): δ 3.01-3.14 (m, 2H, CH_2S), 3.62-3.76 (m, 2H, CH_2O), 4.13-4.20 (m, 1H, CHO), 5.10 (t, 1H, OH, J = 5.8 Hz), 5.81 (d, 1H, 5-H, J = 7.5 Hz), 7.79 (d, 1H, 6-H, J = 7.5 Hz), 7.15 (s, 1H, 1'-H), 7.30 (br s, 1H, NH_2), 7.35 (br s, 1H, NH_2).

Anal. Calcd. for $C_8H_{11}N_3O_3S$: C, 41.91; H, 4.83; N, 18.33. Found: C, 42.24; H, 4.65; N, 18.06.

(+)-α-L-2'-thia-2',3'-dideoxycytidine [(+)-(2'*R*,5'*S*)-1-(5'-Hydroxymethyl-1',3'-dioxathiolan-2'-yl)cytosine] (**17**).

Desilylation of (+)-(2'*R*,5'*S*)-1-{5'-[(*t*-butyldimethylsilyl)oxy]methyl-1',3'-dioxathiolan-2'-yl}cytosine (**15**) with tetra-*n*-butylammonium fluoride was carried out by the method used for preparation of **7**, mp >250° dec; $[\alpha]_D^{25}$ = +224.0° (c = 0.02, ethanol); ^1H nmr (DMSO-d_6): δ 3.01-3.14 (m, 2H, CH_2S), 3.62-3.76 (m, 2H, CH_2O), 4.13-4.20 (m, 1H, CHO), 5.10 (t, 1H, OH, J = 5.8 Hz), 5.81 (d, 1H, 5-H, J = 7.5 Hz), 7.79 (d, 1H, 6-H, J = 7.5 Hz), 7.15 (s, 1H, 1'-H), 7.30 (br s, 1H, NH_2), 7.35 (br s, 1H, NH_2).

Anal. Calcd. for $C_8H_{11}N_3O_3S$: C, 41.91; H, 4.83; N, 18.33. Found: C, 42.20; H, 4.83; N, 18.16.

(-)-α-D-2'-Thia-2',3'-dideoxycytidine ([(-)-(2'*S*,5'*R*)-1-(5'-Hydroxymethyl-1',3'-dioxathiolan-2'-yl)cytosine] (**27**).

Desilylation of (+)-(2'*S*,5'*R*)-1-{5'-[(*t*-butyldimethylsilyl)oxy]methy-1',3'-dioxathiolan-2'-yl}cytosine (**25**) with tetra-*n*-butylammonium fluoride was performed by the method used for preparation of **7**, mp >250° dec; $[\alpha]_D^{25}$ = -226.5° (c = 0.02, ethanol); ^1H nmr (DMSO-d_6): δ 3.01-3.14 (m, 2H, CH_2S), 3.62-3.76 (m, 2H, CH_2O), 4.13-4.20 (m, 1H, CHO), 5.10 (t, 1H, OH, J = 5.8 Hz), 5.81 (d, 1H, 5-H, J = 7.5 Hz), 7.79 (d, 1H, 6-H, J = 7.5 Hz), 7.15 (s, 1H, 1'-H), 7.30 (br s, 1H, NH_2), 7.35 (br s, 1H, NH_2).

Anal. Calcd. for $C_8H_{11}N_3O_3S$: C, 41.91; H, 4.83; N, 18.33. Found: C, 42.11; H, 4.55; N, 18.11.

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REFERENCES AND NOTES

* To whom coresspondence should be addressed.

[1] C. Liang, D. W. Lee, M. G. Newton and C. K. Chu, *J. Org. Chem.*, **60**, 1546 (1995).

[2] S. Belleau, D. Dixit, N. Nguyen-Ba and L. Kraus, Abstr. 5th Int. Conf. AIDS, Montreal, Canada, 1989.

[3] H. Soudeyns, Q. Yao, B. Belleau, J. -L. Kraus, N. Nguyen-Ba, B. Spira, M. A. Wainberg, *Antimicrob. Agents Chemother.*, **35**, 1386 (1991).

[4] C. -N. Chang, V. Skalski, J. H. Zhou and Y. -C. Cheng, *J. Biol. Chem.*, **267**, 22414 (1992).

[5] M. Okabe, R. C. Sun, S. Y. K. Tam, L. J. Todaro, and D. L. Coffen, *J. Org. Chem.*, **53**, 4780 (1988).

[6] S. -L. Doong, C. H. Tsai, R. F. Schinazi, D. C. Liotta and Y. C. Cheng, *Proc. Natl. Acad. Sci.*, USA, **88**, 8495 (1991).

[7] A. K. M. Anisuzaman and L. N. Owen, *J. Chem. Soc. (C)*, 1021 (1967).