(br s, 1 H), 5.52 (br s, 1 H); high-resolution mass spectrum calcd for $C_{12}H_{18}O_3$ m/e 210.125 60, found m/e 210.126 05.

(±)-(4aα,7α,8aβ)-4,7-Dimethyl-4a,7-ethano-4a,7,8,8a-tetrahydro-2*H*,5*H*-pyrano[4,3-*b*]pyran-3(4*H*)-one (20). Phenylselenyl chloride (0.086 g, 0.56 mmol) in dichloromethane (1.0 mL) was added dropwise to a solution of keto alcohol **3b** in dichloromethane (2.0 mL) cooled to −78 °C under nitrogen. At the completion of addition, the cooling bath was removed and the light orange solution allowed to warm to room temperature. Removal of solvents and chromatography (silica gel, 10:1 hexanes–EtOAc) afforded the crude selenide which was dissolved in toluene (5 mL) and heated to reflux with tri-*n*-butyltin hydride (0.22 mL, 0.82 mmol) and a catalytic amount of azobis(isobutyrylnitrile). After 60 min, the solution was cooled to room temperature. Concentration and chromatography (silica gel, 10:1 hexanes–EtOAc) provided ether **20** as a colorless oil: R_t 9.38 min (175 °C, isothermal, 6 ft × $^1/_4$ in., 5% SE-30 capillary column gas chromatograph); IR (film 2960, 1725, 1150 cm^{-1}; 100-MHz NMR ($CDCl_3$) δ 0.98 (d, J = 7 Hz, 3 H), 1.14 (s, 3 HO), 1.3–1.8 (m, 6 H), 2.30 (q, J = 7 Hz, 1 H), 3.60 (m, 1 H), 4.1 (m, 4 H); high-resolution mass spectrum calcd for $C_{12}H_{18}O_3$ m/e 210.125 60, found m/e 210.126 85.

Acknowledgment. We thank the National Institutes of Health (Grant No. CA23663) for generous financial assistance.

Registry No. 3a (isomer 1), 75233-76-0; **3a** (isomer 2), 75233-77-1; **3b**, 75233-78-2; **4**, 75247-61-9; **5**, 75233-79-3; **6**, 75233-80-6; **7**, 75233-81-7; **9**, 75233-82-8; **10**, 75233-83-9; **11a**, 75233-84-0; **11a** phosphonate, 75233-85-1; **11b**, 75247-62-0; **12a**, 75247-63-1; **12a** lactol, 75233-86-2; **12b**, 75233-87-3; **12b** lactol (isomer 1), 75233-88-4; **12b** lactol (isomer 2), 75233-89-5; **13**, 75233-90-8; **14**, 75233-91-9; **15**, 75233-92-0; **17**, 75233-93-1; **18**, 75233-94-2; **19**, 75233-95-3; **20**, 75233-96-4; 3-(hydroxymethyl)-3-buten-2-one, 73255-29-5; 1-acetoxy-3-methyl-1,3-butadiene, 17616-47-6; bromoacetyl bromide, 598-21-0; cyanoacetyl chloride, 16130-58-8.

Synthesis and Properties of 2′-Deoxy-2′-thiocytidine

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In order to assess the significance of the 2′-position of nucleosides, the nucleoside analogue 2′-deoxy-2′-thiocytidine was synthesized by using 2,2′-anhydro-1-β-D-arabinosylcytosine and P_2S_5 as starting materials. Several thiophosphorylated derivatives were also obtained as synthetic intermediates and characterized by NMR spectroscopy and elemental analysis. The main stable intermediate was 2′-deoxy-2′-thiocytidine 2′,3′-phosphorodithioate which was subjected to iodine oxidation, alkaline hydrolysis, and finally, a dephosphorylation step, yielding the title compound 2′-*S*-dCyd, or its disulfide. According to NMR and ORD data, the 2′-carbon of the nucleoside is in the endo orientation, and the rotation of the cytosine moiety is restricted. The most outstanding chemical property of 2′-*S*-dCyd is the lability of the glycosidic bond, owing to an intramolecular displacement reaction. The rate of decomposition could be conveniently studied by ORD spectroscopy as a function of pH, ionic strength, and temperature. Slightly different first-order kinetics were observed for the nucleoside and its 3′-phosphate.

In the past decade numerous 2′-substituted nucleoside analogues[1–4] have been prepared for the study of structure–function relationships in nucleic acids. Of these analogues, only the 2′-amino and thio substituents possess the capacity to hydrogen bond as hydrogen donors. Since the closest analogue of OH is the SH group, the thorough study of 2′-deoxy-2′-thionucleosides seems to be overdue. To this date work with 2′-deoxy-2′-thionucleosides has not progressed much beyond the point of successful or unsuccessful chemical synthesis.

Previous experiments[5–8] have shown that the classical chemical routes for the introduction of a 2′-thio (erythro) substituent, i.e., displacement on a nucleoside of a threo substituent with thioacetate or thiobenzoate, require protected nucleosides and deblocking procedures which result in cleavage of the glycosidic bond. Only one analogue, 2′-deoxy-2′-thiouridine was chemically characterized,[9,10] but it was not investigated biochemically or biologically. Derivatives of 2′-deoxy-2′-thioadenosine were prepared,[5–8] but the free nucleoside was too labile to be isolated.

We have concentrated our efforts on the synthesis of the cytidine analogue 2′-deoxy-2′-thiocytidine because of its expected stability and biological activity. Our attempts to obtain 2′-*S*-dCyd[11] in a manner analogous to Imazawa's method by reacting anhydro-araC[11] with thioacetate were not successful, and only cytosine was formed. We found it necessary to introduce a thio nucleophile as a 3′ neighboring group which could then react selectively with the 2′-carbon. The use of a thiophosphorylated precursor allowed the introduction of cis 3′-O, 2′-S substitution without the use of blocking groups. The conditions for the hydrolysis and oxidation of the phosphorodithioate esters were mild and yielded a stable disulfide of 2′-*S*-dCyd. This convenient storage form, in turn, could be quantitatively reduced to the thiol by using β-mercaptoethanol.

In our first attempt[12] we used dithiophosphate as a thiophosphorylating agent, but the lability of this compound made it unsuitable for large-scale preparation of 2′-*S*-dCyd. In the same communication we also noted the felicitous peculiarity of anhydro-araC chemistry which features a reversal in the customary reactivities of the 5′ and 3′ OH groups. This becomes understandable in view of the X-ray diffraction data which reveal an interaction

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(11) Abbreviations: 2′-*S*-dCyd, 2′-deoxy-2′-thiocytidine; 2′-*S*-dCyd-2′,3′-P and 2′-*S*-dCyd-2′,2′-PS are the corresponding cyclic phosphorothioate and phosphorodithioate; anhydro-araC, 2,2′-anhydro-1-β-D-arabinosylcytosine.
(12) E. Bradbury and J. Nagyvary, *Nucleic Acids Res.*, 3, 2437–2443 (1976).

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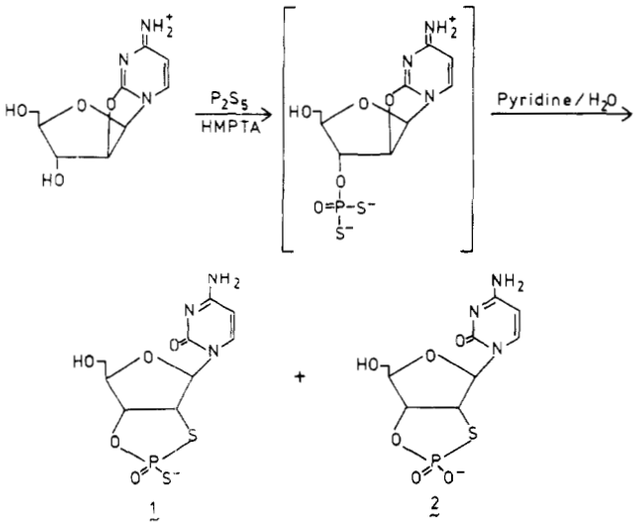
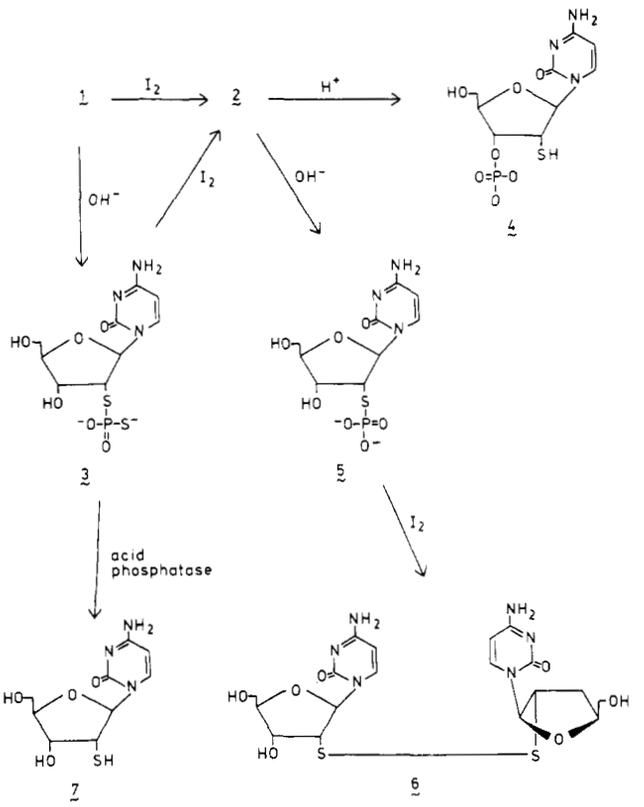
Scheme I



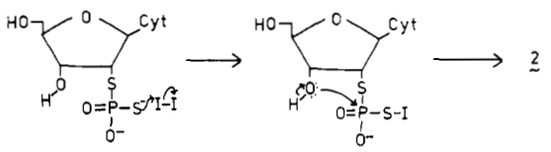
between the 5′-OH and the positively charged base in anhydro-araC.[13] We are now reporting on the more convenient use of phosphorus pentasulfide in effecting the completely regiospecific 3′ thiophosphorylation which is followed by rearrangement of the labile intermediate to form 2′-*S*-dCyd-2′,3′-PS. Our choice of P_2S_5 was in part motivated by the considerable interest in nucleoside phosphorothioates and their interactions with enzymes.[14-16]

Anhydro-araC tosylate was thiophosphorylated with 2 equiv of P_2S_5 in hexamethylphosphoramide in a reaction involving the 3′-OH group but not affecting the chromophore. The structure of the first intermediate (see Scheme I) is hypothetical, but it is an anhydro-araC derivative. The primary product, presumably a pentathiopyrophosphate, was hydrolyzed in pyridine–water to the dithiophosphate which subsequently underwent rearrangement to yield the stable products 2′-deoxy-2′-thiocyticine 2′,3′-phosphorodithioate (1) and phosphorothioate **2**. Since **1** contains an asymmetric phosphorus, it is undoubtedly a mixture of two diastereomers. Indeed, the minor peaks appearing upfield from the ^{13}C signals of C-2′ and C-3′ may be due to the shielding by the sulfur of the S-endo (axial) isomer. The two cyclic phosphates (**1** and **2**) were similar in electrophoretic mobility, but **1** was faster in chromatographic mobility (TLC) than **2** and, surprisingly, gave a positive test with Ellman's reagent.[17] The observed ratio of compounds **1** and **2** was a function of time allowed for the rearrangement of the 3′-phosphorodithioate intermediate to the cyclic phosphate in pyridine–H_2O. If the reaction was left for a few hours, the ratio of **1** to **2** was approximately 15:1. However, if the reaction was left for 24 h, the ratio dropped to as low as 3:1. The conversion of **1** to **2** also took place in distilled water but at a very slow rate. The cyclic phosphorodithioate **1** was easily oxidized by I_2 to the cyclic phosphorothioate **2** which itself was stable to the action of I_2. Upon treatment with dilute alkali (pH 12, 35 °C, 30 min) **1** was hydrolyzed to 2′-deoxy-2′-thiocytidine 2′-phosphorodithioate (**3**, Scheme II) which was stable in the pH range of 8 to 12 but could not be isolated in pure solid form. The oxidation of **3** with 1 equiv of I_2 led to the formation of **2**, via intramolecular phosphorylation, with only a minor amount of nucleoside **6** being formed. In contrast, we[16] showed previously that 2′-*S*-dCyd-2′-P was completely dephosphorylated by I_2 treatment.

Scheme II



Scheme III



The dominance of oxidative ring closure of the dithioate over oxidative hydrolysis was somewhat unexpected but can be explained mechanistically (Scheme III). As suggested by Cook et al.,[18] oxidative hydrolysis of *S*-phosphates possibly proceeds through initial attack on the sulfur by iodine to give a iodosulfonium ion, followed by rapid hydrolysis of the S–P bond. The preference of oxidative ring closure in the case of the dithiophosphate over oxidative hydrolysis can be accounted for by the fact that the anionic sulfur is more readily accessible to attack by iodine, and formation of the neutral SI intermediate is preferable to formation of the iodosulfonium intermediate. This does not exclude oxidative hydrolysis from occurring as evidenced by the production of a minor amount of the dinucleoside disulfide. It should be noted that while the 2′-*S*-phosphate is easily dephosphorylated by iodine, the same 2′-*C* S–P bond is stable to oxidative hydrolysis in the 2′,3′ cyclic phosphorothioate. Otherwise, the acid- and base-catalyzed openings of the cyclic phosphorodithioate **1** were similar to those found previously[12] in **2**, i.e., P–O cleavage with base and P–S cleavage in acid.

When I_2 oxidation of **3** was followed by alkaline hydrolysis of the cyclic phosphate, a second I_2 oxidation

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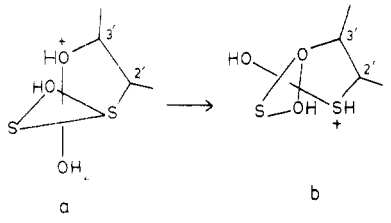
Table I. ^{13}C and ^{1}H NMR Data[a-c]

compd	C-2	C-4	C-5	C-6	C-1′	C-2′	C-3′	C-4′	C-5′
1	158.30	166.77	97.43	142.86	93.45	56.96	84.79 d (9.8)	82.99 d (3.6)	61.86
6	158.30	166.77	97.86	142.46	90.15	56.46	72.86	86.73	62.15

compd	H-5	H-6	H-1′	H-2′	H-3′	H-4′	H-5′
7	6.50 d ($J_{5,6}$ = 7.7)	8.15 d ($J_{6,5}$ = 7.7)	6.72 d ($J_{1',2'}$ = 8.5)	4.05 d ($J_{2',3'}$ = 6.0)	4.85 d ($J_{3',4'}$ = 2.7)	4.53 m	4.25 m

[a] Chemical shifts are given in parts per million and coupling constants are given in parentheses in hertz. [b] d and m indicate the multiplicities (d = doublet, m = multiplet). [c] Carbon signals characterized by J values appear as doubles due to ^{13}C–^{31}P coupling.

Scheme IV



yielded the desired dinucleoside disulfide (**6**). The disulfide was isolated and characterized by NMR (Table I). It is a stable storage form of 2′-deoxy-2′-thiocytidine (**7**) to which it can be converted by reduction with 2-mercaptoethanol. The title compound (**7**) was also obtained by enzymatic dephosphorylation of **3** by acid or alkaline phosphatase. Since the structure of **3** is unstable in acid due to the migration of the thiophosphoryl group from the 2′- to the 3′-position, the actual substrate of the acid phosphatase has yet to be determined. The remaining unhydrolyzed nucleotide, unlike the starting material, was labile at pH 10, suggesting the presence of a free 2′-SH. The migration of phosphate from the 2′-S into the 3′-O position can be interpreted as a result of protonation leading to P–S cleavage. According to the preference rules as applied for phosphorothioate hydrolysis by Usher et al.,[19] the electropositive sulfur remains in the basal position of the trigonal-bypyrimid transition state (Scheme IV, structure a). The specific P–O ring opening of **1** in alkaline medium confirms the preference of sulfur for the basal positions. Since the ester hydrolysis must take place via apical positions, protonation and pseudorotation of sulfur into the apex may be required (b, Scheme IV). The major product obtained after incubation of **3** at pH 4.8 contained a free 2′-SH group as evidenced by the hyperchromicity observed in an alkaline solution at 235 nm.

The ^{13}C NMR chemical shifts (Table I) are consistent with those for cytidine with exception of the 2′-C which is shifted upfield at 56.46 ppm (cytidine 2′-C, 70.60 ppm).[20] The upfield shift is due to shielding of the 2′-C by the thiol substituent. The splitting of C-4′ and C-3′ in **1** may be due to the presence of the phosphorus, but, surprisingly, C-1′ and C-2′ are not affected. A key point worth noting about the ^{1}H NMR assignments is that the protons of the ribose moiety are substantially shifted either upfield or downfield, relative to their assignments in ribocytidine. The coupling constants for $J_{1',2'}$ and $J_{3',4'}$ values suggest a 2′-endo (S-type)[21] or deoxyribo-like conformation.

The pK_a of the thiol was measured by spectrophotometric titration at 235 nm. For 2′-*S*-dCyd, we obtained a value of 7.5 which is in close agreement with that of 2′-deoxy-2-thiouridine (7.3).[10] The pK_a of 2′-*S*-dCyd-3′-phosphate was found to be 8.0.

Table II. Rates of Decomposition of Thionucleoside **7** and Thionucleotide **4** at 37 °C as a Function of pH[a]

	rate, s^{-1}	
pH	2′-*S*-Cyd	2′-*S*-Cyd-3′-P
10	5.25×10^{-2}	1.51×10^{-2}
9	2.70×10^{-2}	1.45×10^{-2}
8	6.10×10^{-3}	5.62×10^{-3}
7	7.83×10^{-4}	1.58×10^{-3}
6	1.69×10^{-4}	4.70×10^{-4}
5	2.74×10^{-5}	4.79×10^{-5}

[a] The rates at 25, 37, and 47 °C of the thionucleoside are 4.28×10^{-4}, 7.83×10^{-4}, and 1.65×10^{-3} s^{-1}, respectively, with a $\Delta H^{\ddagger}$ of 11.7 ± 0.5 kcal/mol and a $\Delta S^{\ddagger}$ of −34.6 cal/mol K at pH 7.0.

The ORD curve of 2′-*S*-dCyd is similar to the spectra of the nucleotides reported in our previous publication,[16] but the amplitude of molar rotation is much larger at $\Delta[\phi]$ = 56 000 deg L/mol cm. The large Cotton effect has been interpreted[22] as being a result of restricted rotation around the glycosidic bond caused by close contacts. It is remarkable that the molar rotation of 2′-*S*-dCyd surpassed even that of arabinosylcytosine, i.e., an analogue with 2′-β-OH. Since the disulfide **6** has a slightly higher ORD amplitude than that of **7**, the contribution of the substitution on the SH is not readily predictable. Hydrogen bonding of SH to the 2-CO group in **7** and base–base interaction in **6** may add to the rigidity of these structures with unusually high optical rotation.

The most intriguing chemical property of 2′-thio-substituted nucleosides is the unprecedented lability of the glycosidic bond at neutral and alkaline pH. In order to understand the reasons of this behavior, we have undertaken a kinetic study using both the nucleoside 2′-*S*-dCyd and its 3′-phosphate in hydrolysis experiments. Since nucleosides exhibit a Cotton effect between 300 and 220 nm in the ORD spectra but the sugar or base alone does not, the disappearance of optical rotation at 291.5 nm (α_{max}) served as a convenient assay method for the rate of decomposition. The rate of decomposition was measured as a function of pH, ionic strength, buffer concentration, and the temperature. These data, shown in Figures 1 and 2 and Table II, lead to the conclusion that the removal of the cytosine base is due to an intramolecular nucleophilic displacement and not hydrolysis. The first-order rate constants for the decomposition of 2′-*S*-dCyd and its 3′-phosphate are given in Table II. Both compounds were essentially stable below pH 5.0 ($t_{1/2}$ > 48 h) and decomposed at constant rates above pH 9.5. The rate of de-

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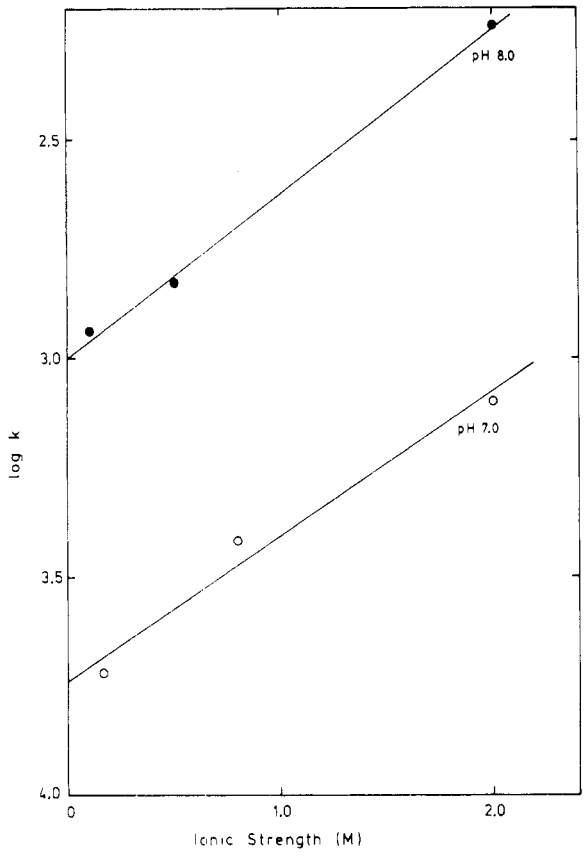


Figure 1. Ionic strength–rate profiles in 0.1 M sodium phosphate at pH 8.0 and 7.0 at 37 °C.

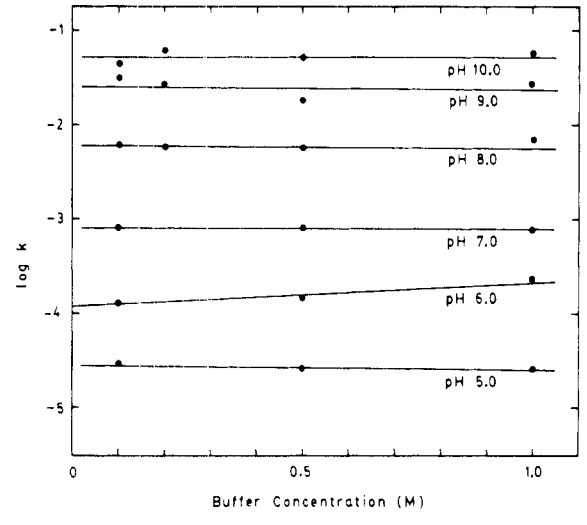
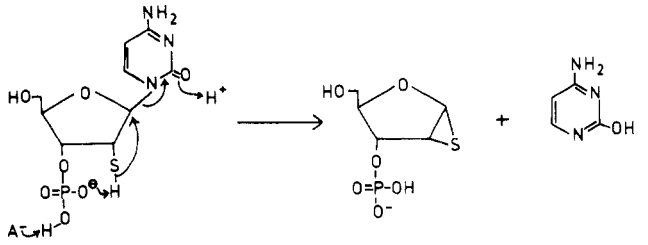


Figure 2. Dependence of rate on the concentration of the buffers, sodium phosphate (pH 5.0–7.5) and sodium bicarbonate (pH 8.0–10.0), at 2 M ionic strength at 37 °C.

composition is independent of buffer concentration; i.e., there is no general-base catalysis (Figure 2). In addition, the pH rate profile (Table II) indicates a dependence of the rate on the ionization of the SH, but the ionic strength (Figure 1) and temperature also have an effect. For practical purposes, the nucleoside is stable at 0 °C at neutral pH, but at 37 °C, under biologically relevant conditions, the nucleoside would not survive beyond a few hours. The observed tenfold increase in rate with ionic strength may be explained by the stabilization of the anionic species. The pH dependence of the relative stabilities of the nucleotide **4** and the nucleoside **7** is surprising. There is an increase in the rate of decomposition of the

Scheme V



glycosyl bond of the 3'-phosphate over that of the nucleoside below and at the pK_{a2} of the phosphate. However, there is a reversal in the order of decomposition (i.e., nucleoside > nucleotide) at about pH 8.0. The ratios K_4/K_7, as calculated from the data of Table II, are 1.0, 2.8, 1.0, and 0.54 for pH's of 5.0, 6.0, 8.0, and 9.0, respectively. Since the increased rate of decomposition of **4** falls into the pH range of phosphate dissociation, one could postulate the involvement of the phosphate dianion in the abstraction of the thiol proton. This mechanism depicted (Scheme V) would allow for the maintenance of constant charge density throughout the whole process. The theoretical interest in finding $K_4 > K_7$ is that it provides a chemical evidence for the interaction of the 3'-phosphate and the 2'-SH. The existence of such a hydrogen bond is supported by the analogous interaction of natural 3'-nucleotides and oligonucleotides which was based on NMR[23] and CD data.[24]

It is of considerable practical interest with regard to the biological functioning and disposition of 2'-*S*-dCyd that the nucleotide forms, including the units incorporated into RNA, are also expected to be labile. The prospect of self-destructing nucleic acids being synthesized is a unique aspect of 2'-thionucleosides which sets them apart from other 2'-analogues. The 1,2-episulfide (Scheme V) has not yet been isolated, and we have not explored the possibility that the reaction may be reversible. However, the episulfide represents an activated form of the anomeric carbon, and it may react with an excess of heterocyclic base. Such a reaction would be of interest for modifying base sequences in nucleic acids.

The extension of our research on 2'-thionucleotides will focus on the influence of structure on biochemical properties. Pharmacological exploration of 2'-*S*-dCyd as an antimetabolite is also warranted by preliminary results. (The nucleoside inhibits the growth of several cell lines in cultures.)

Experimental Section

Materials and Methods. 2,2'-Anhydroarabinosylcytosine tosylate was a gift from Dr. W. J. Wechter of the Upjohn Co. Potato acid phosphatase Type IV (EC 3.1.3.2) was purchased from Sigma Chemical Co. Phosphorus pentasulfide was a product of Eastman Kodak and was used without further purification. Analytical grade hexamethylphosphotriamide was dried over molecular sieves (type 4A). NMR spectra were recorded on a Varian FT-80Z spectrometer in D_2O with Me_4Si as the external standard. ^{13}C spectra were observed at 50.3 MHz on a Varian XL-200 instrument. Electrophoresis was performed on a Savant Model FP-22A flat-plate high-voltage electrophoresis system in 0.05 M $Et_3NH{\cdot}HCO_3$, pH 7.2. Thin-layer chromatography was carried out on Eastman chromatogram silica sheets in 1-butanol/0.5 M ammonium acetate/95% ethanol (72:16:12) and 1-butanol/water (85:15). Spectrophotometric titration was recorded on a Cary Model 15 spectrophotometer and the ORD spectra on

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a Cary Model 60 recording spectropolarimeter with a constant-temperature cell holder. The temperature was controlled by using a HETO-01-T6-230 temperature bath.

Thiophosphorylation of Anhydro-araC. The dry powder of anhydro-araC (1 g, 2.6 mmol) was added to a solution of phosphorus pentasulfide (1.5 g, 6.0 mmol) in dry hexamethylphosphorotriamide (25 mL), and the reaction was allowed to run in the dark for 48 h. The solution was added dropwise to 1.5 L of ether, which was then decanted, and the precipitate was dissolved in pyridine/water (9:1, 250 mL). After 16 h at room temperature, the solvent was evaporated in vacuo and the mixture applied to a DEAE cellulose column (4 × 70 cm, HCO_3^- form). After being washed with 1.0 L of H_2O, the column was eluted with a linear gradient of $Et_3NH{\cdot}HCO_3$ (0–0.25 M, 4 L). Two main peaks were eluted at 0.03 and 0.07 M $Et_3NH{\cdot}HCO_3$. The two peaks were each coevaporated from ethanol, yielding 12 600 (1.4 mmol) and 2700 (0.3 mmol) OD_{270} units, respectively, for a total yield of 61%. Both preparations were pure and homogenous on electrophoresis with a mobility of 0.63 relative to Cyd-3′-P. The material in peak 1 had an R_f of 0.5, while that of peak 2 had an R_f of 0.3 on TLC ($BuOH/H_2O/HAc$, 7:2:1). Samples for analysis were further purified by preparative electrophoresis on Whatman 3MM paper. They were converted to the Na salt by the addition of NaI in MeOH/acetone (1:1) followed by precipitation in acetone/ether (1:1). Compound 1 gave the following analysis for the formula $C_9H_{11}N_3O_5PS_2Na{\cdot}H_2O$. Anal. Calcd: P, 8.22; S, 16.97. Found: P, 8.21; S, 16.61. The ^{13}C NMR data (Table I) provide further proof for compound 1 as 2′-deoxy-2′-thiocytidine 2′,3′-phosphorodithioate. Compound 2 was identical with 2′-*S*-dCyd-2′,3′-P characterized in our earlier work.[12]

2′-Deoxy-2′-thiocytidine 2′-Phosphorodithioate (3). The Et_3NH^+ salt of compound 1 (4500 OD_{270} units, 0.5 mmol) was dissolved in dilute NaOH (pH 12), and after 30 min at room temperature the solution was neutralized with CO_2. Compound 3 could not be isolated in pure solid form but gave an electrophoretic mobility of 1.05 relative to Cyd-3′-P. TLC migrations were slightly faster than Cyd-3′-P, and the spots reacted positively with Ellman's reagent.[17]

Conversion of 3 into 2′-Deoxy-2′-thiocytidine 2′,3′-Phosphorothioate (2). Compound 3 (0.01 mmol) was reacted with a slight excess of I_2 in 50% methanol–H_2O containing 2 mg of $NaHCO_3$. The analysis of the reaction product by electrophoresis and TLC showed only 20% nucleoside. The major product was a cyclic phosphorothioate identical with 2′-*S*-dCyd-2′,3′-P. Alkaline hydrolysis of this cyclic phosphorothioate gave 2′-*S*-dCyd-2′-P (5) which was quantitatively dephosphorylated by I_2 treatment as described before. The nucleoside product was identified as the disulfide 6.

2′-Deoxy-2′-thiocytidine 3′-Phosphate (4). 2-Deoxy-2′-thiocytidine 2′,3′-phosphorodithioate (0.11 mmol, 1000 OD_{270} units) was treated with a slight excess of I_2, yielding the cyclic phosphorothioate 2. Compound 2 was hydrolyzed at pH 2 and 50 °C for 8 h, neutralized, and treated with a slight excess of I_2. The *S,S*-bis(2′-deoxy-2′-thiocytidine 3′-phosphate) was separated from other products on preparative electrophoresis on 3MM Whatman paper and had a mobility of 2.0, relative to the cyclic phosphorothioate (yield 0.043 mmol, 39%). Treatment with mercaptoethanol yielded the 2′-*S*-dCyd-3′-P.

2′-Deoxy-2′-thiocytidine (7). A solution of compound 3 (1 mmol) in 5 mL of 0.05 M sodium citrate, pH 4.8, was incubated with 5 mg of acid phosphatase at 37 °C for 16 h in the dark. The reaction mixture was reduced to dryness in vacuo and extracted with methanol, and the methanol extract evaporated onto 10 g of silica gel. This powder was placed on top of a silica gel column (4 × 35 cm, 70–325 mesh), and the nucleoside was eluted with $CHCl_3$/MeOH (1:1). The main fraction (8000 OD_{270} units) was passed through a Sephadex G-10 column (4 × 75 cm) with water as eluant. The main peak represented pure 2′-deoxy-2′-thiocytidine (7) which was obtained as a freeze-dried powder (850 OD_{270} units, 0.092 mmol). Thus, the yield of isolatable 2′-*S*-dCyd was about 0.74 mmol (74%), the rest being another neutral product (15%) and some nucleotide. Compound 7 reacted positively with Ellman's reagent, but after prolonged exposure to air it was oxidized to an Ellman negative compound. This compound was the same as the minor product observed on silica gel chromatography. It could be quantitatively converted to 7 by treatment with 2-mercaptoethanol; thus, its identity as the disulfide 6 was established. Compounds 6 and 7 were easily differentiated by TLC in $CHCl_3$/MeOH (7:3) with R_f values of 0.23 and 0.66, respectively. See Table I for the ^{13}C and ^1H NMR data. The elemental analysis of 7 gave the following for the formula $C_9H_{13}N_3O_4S{\cdot}H_2O$. Anal. Calcd: C, 38.99; H, 5.41; N, 15.16; S, 11.55. Found: C, 38.40; H, 5.14; N, 14.74; S, 12.02. The elemental analysis of 6 revealed some contamination by polysulfide and butanol. 2′-*S*-dCyd gave the expected UV maximum at 272 nm in the pH range above 6 and at 281 nm in acid. An increase of pH from pH 5 to 10 resulted in a hyperchromic shift at 235 nm with a midpoint of 7.5, corresponding to the pK of the 2′-SH group.

Lability of the *N*-Glycosidic Bond. The kinetics of decomposition of the glycosidic bond was studied by ORD over a pH range from 5.0 to 10.0 by using a phosphate buffer from pH 5.0 to 7.5 and bicarbonate buffer from pH 8.0 to 10.0. Optical rotation was measured at 291.5 nm through a 1-cm path length and a full-scale deflection of 0.1°. The cell was filled with 3 mL of buffer (0.1, 0.2, 0.5, 1.0 M; μ (ionic strength) = 2.0 M) containing 20 equiv of β-mercaptoethanol and kept for 5 min at the experimental temperature (25, 37, or 47 °C). Approximately 2.4 OD units of thionucleoside were added, and the change in rotation was recorded. Observed rotation (α) was converted to moles, and the data were plotted to determine the kinetic constants. $\Delta H^{\ddagger}$ and $\Delta S^{\ddagger}$ were calculated from the Eyring equation and are given in the legend to Table II.

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