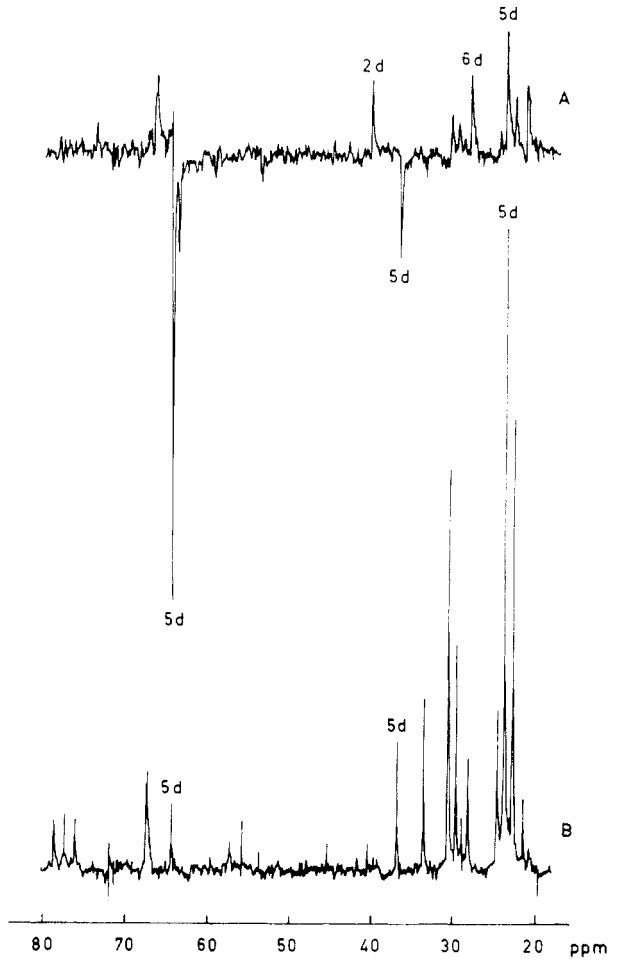

Figure 2. Reaction of 1 with **2d**. (A) ^{13}C NMR spectrum taken 3 min after initiation of the reaction in $CDCl_3$ in the presence of pyridine. (B) Spectrum taken after 10 min (complete reaction).

radical prior to reaction in chloroform as the solvent.[16]

Experimental Section

Melting points were determined on a Mettler FP-2 apparatus. ^1H NMR spectra were run on a Varian A-60 instrument. Chemical shifts (δ) are downfield from Me_4Si. ^{13}C NMR spectra were recorded on a Varian XL-100 spectrometer, chemical shifts (δ) are relative to the solvent $CDCl_3$ (δ = 77.0 ppm). Infrared spectra were recorded on a Unicam SP200 spectrophotometer.

Materials. The solvents were purified and dried by standard methods. *tert*-Butylsulfinyl chloride[21] [1: pale yellow oil; bp 58 °C (15 mm); NMR ($CDCl_3$) δ 1.40 (s)] and the *N*-hydroxysulfonamides **2a**,[6] **2b**,[22] **2c**,[23] and **2d**[22] were prepared according to literature procedures.

General Procedure for Reaction of 1 with 2a-d. A solution of 1 (0.007 mol) in acetone (40 mL) was added dropwise under N_2 to a stirred solution of **2a-d** (0.007 mol) and pyridine (0.014 mol) in acetone (40 mL) at 20 °C. After the solution was stirred for 2 h the acetone was evaporated (20 mm; 30 °C). The residue was dissolved in chloroform (150 mL), extracted once with 150 mL of water, and dried over Na_2SO_4. After removal of the solvent, the organic material was resolved into its components by column chromatography (silica gel, 60-120 mesh; $CHCl_3$). The components were identified by comparison of their boiling point or melting point and their NMR and infrared spectra with authentic samples. The aqueous layer contained pyridinium chloride, pyridinium *tert*-butylsulfonate,[10] and the water-soluble sulfonamides. The sulfonamides were obtained after extraction of the aqueous layer with three 150-mL portions of ethyl acetate and subsequent evaporation of the organic solvent in vacuo.

Sulfonimides 5b and 5d were prepared by adding a solution of the appropriate sulfonyl chloride (0.010 mol) in 50 mL of dry dimethoxyethane (DME) to a stirred solution of *N*-methyl-*tert*-butylsulfonamide (0.010 mol) and *n*-butyllithium (0.010 mol) in 50 mL of dry DME under N_2 at 20 °C. After evaporation of the solvent (20 mm; 50 °C), the crude reaction mixture was dissolved in CCl_4 (150 mL) and extracted twice with 150 mL of water. Removal of CCl_4 in vacuo afforded almost pure **5b** and **5d**. Crystallization from CCl_4 gave pure **5b** [mp 75-76 °C; IR (CCl_4) 1135, 1165, 1345, 1370 cm^{-1}; ^1H NMR (CCl_4) δ 1.49 (s, 9 H), 3.20 (s, 3 H), 3.28 (s, 3 H); ^{13}C NMR ($CDCl_3$) δ 24.0, 37.0, 40.9, 64.3. Anal. Calcd for $C_6H_{15}NO_4S_2$: C, 31.42; H, 6.59; N, 6.11; S, 27.96. Found: C, 31.19; H, 6.42; N, 6.02; S, 27.58] and pure **5d** [mp 97-98 °C; IR (CCl_4) 1140, 1175, 1350, 1380 cm^{-1}; ^1H NMR δ 1.50 (s, 9 H), 3.18 (s, 3 H), 7.4-8.1 (m, 5 H); ^{13}C NMR δ 24.2, 37.2, 64.6, 128.7, 128.8, 133.5, 137.9. Anal. Calcd for $C_{11}H_{17}NO_4S_2$: C, 45.34; H, 5.88; N, 4.81; S, 22.01. Found: C, 45.21; H, 5.80; N, 4.90; S, 21.79].

***N*-Methyl-*tert*-butylsulfonamide** was obtained by oxidation of *N*-methyl-*tert*-butylsulfinamide (prepared by passing 2 equiv of gaseous methylamine through a solution of 1 in ether) with 1 equiv of *m*-chloroperbenzoic acid in CH_2Cl_2 at 0 °C. Recrystallization of the crude product gave the pure sulfonamide: yield 50%; mp 83-84 °C; IR (CCl_4) 3410, 3300, 1320, 1130 cm^{-1}; ^1H NMR δ 1.40 (s, 9 H), 2.86 (d, J = 6 Hz, 3 H), 4.5 (br m, 1 H); ^{13}C NMR δ 23.7, 30.1, 59.3. Anal. Calcd for $C_5H_{13}NO_2S$: C, 39.71; H, 8.66; N, 9.26; S, 21.20. Found: C, 39.36; H, 8.56; N, 9.22; S, 21.23.

Sulfonimides 5a and 5c were prepared by a procedure similar to that for **5b** and **5d** by adding a solution of *tert*-butylsulfonamide and 1 equiv of *n*-BuLi in DME to a solution of the sulfonyl chloride in DME. The products obtained after removal of the inorganic material by filtration and evaporation of the DME were sufficiently pure to serve as reference compounds and were characterized by ^1H NMR. **5a**: NMR ($CDCl_3$) δ 1.45 (s, 9 H), 3.08 (s, 3 H). **5c**: NMR ($CDCl_3$) δ 1.47 (s, 9 H), 7.3-8.1 (m, 5 H).

***tert*-Butylsulfonamide** was prepared via a similar procedure as employed for the *N*-methyl derivative, mp 163-165 °C (lit.[2] mp 162-165 °C).

CIDNP Experiments. The ^1H CIDNP experiments (35 °C) were carried out by using solutions of **2a-d** (0.1 g) and 2 equiv of pyridine in 0.6 mL of acetone-d_6. The NMR spectrum was recorded immediately after initiating the reaction with 1 equiv of 1 and subsequently at suitable time intervals. For the ^{13}C CIDNP experiments solutions of **2d** (0.50 g) together with 2 equiv of pyridine in $CDCl_3$ (2 mL) were used. After the spectrometer was locked on $CDCl_3$, and equimolar amount of 1 was added carefully. CIDNP was observed after 7 times 27 pulses (acquisition time 0.8 s; 35 °C).

Acknowledgment. We thank Professor R. Kaptein for valuable discussions.

Registry No. 1, 31562-43-3; **2a**, 50695-55-1; **2b**, 66110-38-1; **2c**, 599-71-3; **2d**, 7340-20-7; **5a**, 75975-41-6; **5b**, 75975-42-7; **5c**, 75975-43-8; **5d**, 75975-44-9; **6a**, 3144-09-0; **6b**, 1184-85-6; **6c**, 98-10-2; **6d**, 5183-78-8; 7, 75975-45-0; 8, 31562-41-1.

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Nitrogen-15 Nuclear Magnetic Resonance Spectroscopy of 5-Azacytidine[1]

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5-Azacytidine (4-amino-1-β-D-ribofuranosyl-1,3,5-triazin-2(1*H*)-one, 1)[3,4] has been investigated for its potential

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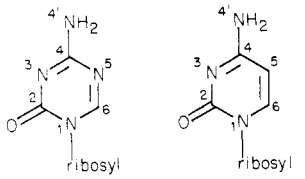


1 2

in leukemia therapy,[5] based on the finding, inter alia, that mRNA containing 5-azacytidylic acid residues is unable to function correctly in protein synthesis.[6] The compound is also of interest for comparison of its ^{15}N NMR characteristics with those of cytidine (**2**),[7,8] especially with regard to assignments of chemical shifts, differentiation between the N4′ protons, degree of restricted rotation about C4-N4′, and site of protonation of the ribonucleoside.

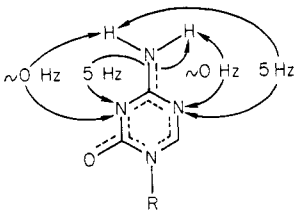
Experimental Section

A 1.1 M solution of 5-azacytidine (**1**)[9] in (methylsulfinyl)-methane was used to obtain natural-abundance ^{15}N NMR spectra with, and without, proton decoupling at 25 °C at 18.25 MHz in a 25-mm sample tube, using the Bruker WH-180 spectrometer and conditions previously described.[7]

Results and Discussion

Among the ^{15}N shifts observed for 5-azacytidine (**1**), namely, 207.9, 184.4, 159.2, and 278.4 ppm (all upfield relative to external 0.1 M $D^{15}NO_3$ in D_2O), the first and the last of the group could be assigned to N1 and N4′, respectively, by comparison with the corresponding values, 222.2 and 281.0 ppm,[7] observed for N1 and N4′ of cytidine (**2**).[8] In the proton-coupled ^{15}N spectrum of **1**, a triplet was observed at 278.4 ppm with a ^{15}N–H coupling of 91 Hz. A corresponding ^{15}N–H coupling of 90 Hz was reported for [4′-^{15}N]-1-methylcytosine.[10] In the proton-coupled ^{15}N spectrum of **1**, selective decoupling of the sole aromatic proton H6, δ 8.55, caused no change in multiplicity of the N4′ signal but caused the 5-Hz ^{15}N doublet at 207.9 ppm to become a singlet, confirming the N1 assignment. The selective decoupling effect on the doublet of doublets (5, 12 Hz) at 159.2 ppm was to convert this signal to a 5-Hz doublet, permitting its assignment as due to the N5, attached to H6. The remaining signal in the proton-coupled ^{15}N spectrum of **1**, a 5-Hz doublet at 184.4 ppm, which was not affected by selective decoupling of H6, δ 8.55, was assignable to N3.

The proton-coupled ^{13}C spectrum[11-13] of **1** at 45.28 MHz, which was obtained under the same conditions as the ^{15}N spectrum, showed chemical shifts for C2 at 152.7 (singlet), C4 at 165.1 (^3J = 13 Hz to H6),[4,14] and C6 at 155.7 ppm (^1J = 207 Hz to H6). Selective proton decoupling by irradiation of the proton resonance at δ 7.50 caused no change in the carbon splittings, while irradiation of H6 at δ 8.55 caused C4 and C6 to become singlets. Until now, the ^{15}N resonances reported[7,8] for proton-coupled spectra of cytidine and other nucleosides have shown splittings only with directly attached protons or with those on adjacent carbons. Thus, the source of the ^{15}N–H doublet couplings of 5 Hz for N3 at 184.4 ppm and of 5 Hz, following selective decoupling of H6, for N5 at 159.2, mentioned earlier, requires explanation. The 5-Hz couplings arise from the N4′ protons, because selective irradiation of the protons at δ 7.50 causes these splittings to disappear. If rotation were rapid about the C4-N4′ bond, the N3 and N5 resonances would be split into 1:2:1 triplets by the two protons on N4′. Because these resonances are not triplets at 25 °C, there must be sufficient double-bond character in the C4-N4′ bond to make the rotation slow on the NMR time scale. Then, of the pairs of three-bond ^{15}N–H couplings to N3 and N5, one should be 5 Hz and the other too small to be detected. Trans coupling of ^{15}N and ^1H in each pair, as shown in **3**, is most likely to account for the 5-Hz figure.



3

At 52 °C, the 5-Hz couplings to N3 and N5 disappear while the 91-Hz couplings to N4′ persist. This result is as expected if at 52 °C rotation about the C4-N4′ bond becomes rapid with respect to the time of observation. It should be pointed out, however, that an increased rate of intermolecular proton exchange with increasing temperature could wash out a 5-Hz coupling to the N3 and N5 nuclei without appreciably changing the N4′–H coupling. While it cannot be decided definitely, on the basis of the present results, which type of process leads to the loss of the two 5-Hz couplings to N3 and N5, the proton-coupled ^{15}N NMR spectrum does appear to serve for detection of restricted rotation. An analogous earlier ^{15}N application involved [4′-^{15}N]-1-methylcytosine and 9-ethylguanine in 1:1 (methylsulfinyl)methane/*N*,*N*-dimethylmethanamide solution which, in the ^1H NMR of the base-paired complex at −10 °C, showed nonequivalent N–H protons and therefore hindered rotation of the amino group.[15] Proton magnetic resonance[16] has been used previously to detect hindered rotation of NH_2, $NHCH_3$, and $N(CH_3)_2$ in various cytosine and cytidine derivatives[10,16-22] and their salts;[17,22]

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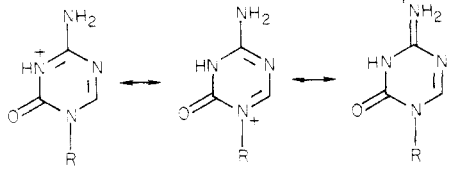
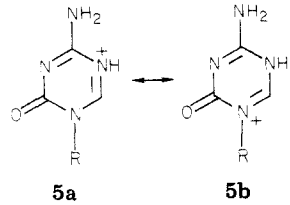
rotational rates have been estimated[10] and the influence of solvent on hindrance of rotation has also been examined.[19]

The $\Delta G^{\ddagger}$ value estimated from the ^{15}N spectra for the exchange process which averages the N3,N5 $^{3}J_{^{15}N-H}$ couplings in 1 is 17 kcal/mol. This value falls in the range of the values observed for rotation of the dimethylamino group of 1,4′,4′-trimethylcytosine and 4,4′-dimethyl-1-(2′,3′,4′,6′-tetra-*O*-acetyl-D-glucopyranosyl)cytosine in other solvents,[19] and this fact supports the interpretation of exchange being the result of speeding up of restricted rotation about the C4-N4′ bond. In any case, at 25 °C, the proton-coupled ^{15}N resonance of N4′ is not expected to be a precise 1:2:1 triplet if the protons attached to N4′ are magnetically nonequivalent, unless the difference between their one-bond couplings to N4′ is small. This appears to be the case with **1**, but in many similar situations, as with primary amides, such differences in one-bond couplings are well-known.[23]

Protonation of cytosine and 1-methylcytosine has been shown to occur on N3 by means of ^{1}H NMR spectroscopy.[17,22,25] The locus of protonation of cytidine (**2**) was shown in the ^{15}N NMR spectrum by the shift of the N3 resonance (166.1 ppm) upfield by 64.8 ppm on the addition of acid to **2** in Me_2SO.[7] Titration of 5-azacytidine (**1**) with trifluoroethanoic acid in (methylsulfinyl)methane and determination of the associated ^{15}N shift changes showed a striking parallelism to the changes for the corresponding nitrogens of **2**. Thus, for addition of 1.5 mol of trifluoroethanoic acid to 1 mol of ribonucleoside, the changes in parts per million for **1** are −1.8, N3, +64.5, and N4′, −10, and for **2** are N1, −1.1, N3, +64.8, and N4′, −12.0. The chemical shift of N5 of **1** moves by +5.6 ppm, which has the direction and magnitude expected for, at most, about 10% protonation on N5. Therefore, we conclude that N3 is the favored nitrogen for the protonation of **1**. This is consistent with expectations based on the relative favorableness of the contributing resonance structures of the possible conjugate acids of **1** by addition of a proton to N3 (**4a–e**), similar to **2** protonated, vs. N5 (**5a** and **5b**).



4a **4b** **4c**



5a **5b**

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Three-Membered Rings. 8. Reaction of 1-Halocyclopropane 1,2-Diesters with 1,8-Diazabicyclo[5.4.0]undec-7-ene. Unexpected Products

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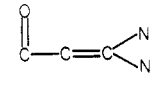
A general method for the preparation of substituted cyclopropane-1,2-dicarboxylic acids has been described.[1] A limitation of that method is that all cis-1,2,3-trisubstituted cyclopropanes and similar tetrasubstituted cyclopropanes with three substituents cis cannot be prepared. Potentially, there are several possible solutions to this problem. One of these would be the catalytic reduction of 1,2,3- or 1,2,3,3-substituted cyclopropenes. It was felt that dehydrohalogenation of the readily available 1-halocyclopropane 1,2-diesters[1] would provide the requisite cyclopropenes for the 1,2,3 case. Dehydrohalogenation of halocyclopropanes has been attempted before and has led commonly to isomerization or simple adduct formation with the basic reagent.[2] In spite of this, several bases not previously used in such dehydrohalogenations have been examined. The results with 1,8-diazabicyclo[5.4.0]undec-7-ene (DBU) are reported here.

Initial studies showed that an equimolar mixture of DBU with dimethyl 1-chloro-3-methylcyclopropane-1,2-dicarboxylate (**1**)[1] in ethyl acetate at room temperature resulted in the slow formation of DBU hydrochloride. No cyclopropene could be detected, but a small amount of crystalline product (**2**) was obtained. Elemental and spectral analysis showed **2** to be an adduct of DBU and the diester **1** minus the elements of hydrogen chloride *and* methanol. Subsequent work showed that this "adduct" could be obtained in moderate yield (47%) by using a threefold excess of DBU over diester **1**. A trapping experiment using furan as a solvent gave the same results as in ethyl acetate—**2** was produced, but no adduct of a presumed cyclopropene intermediate and furan was observed.

Compound **2** shows an infrared band at 1715 cm^{-1} expected for an ester. An even stronger, considerably broader band appears at 1550 cm^{-1}; in conjunction with a medium-strength band at 1615 cm^{-1}, this is suggestive of a highly polarized conjugated system and is consistent with a vinylogous urea-type structure (I). A shoulder at 3010



I

cm^{-1} is suggestive of a tertiary C–H in a cyclopropane. The remaining spectrum is rich in bands, but correlation with specific structural features is uncertain.

The ^{1}H NMR spectrum is a mess at 60 MHz and is not completely resolved at 200 MHz. However, the singlet (3 H) at δ 3.73 clearly belongs to a methyl ester, and the doublet (3 H) at δ 1.54 is a CH_3CH grouping. A doublet

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