Molecular Design of a New Class of Spin-Labeled Ribonucleosides with *N-tert*-Butylaminoxyl Radicals

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We designed a new type of spin-labeled nucleosides with an *N-tert*-butylaminoxyl radical which is introduced to the nucleobase directly. Purine and pyrimidine ribonucleosides containing the aminoxyl radical such as **1a**−**d**, **2**, **3**, and **4** were synthesized to investigate the stability and behavior of the *N-tert*-butylaminoxyl radical on a nucleobase. Lithiation of tri-*O*-silylated 6-chloropurine ribonucleoside (**5**) followed by reaction with 2-methyl-2-nitrosopropane (MNP) gave the key compound **6a**, which was further converted to **6b**−**d**. Oxidation of the obtained **6a**−**d** and their triols (**7a**−**d**) with Ag_2O led to formation of the corresponding stable spin-labeled nucleosides (**8a**−**d** and **1a**−**d**), which were confirmed by EPR spectroscopy. Similarly, the precursors of spin-labeled pyrimidines (**13**, **20**, and **23**) were synthesized by site-selective lithiation of tri-*O*-protected pyrimidine derivatives (**9**, **18**, and **21**) followed by the reaction with MNP and deprotection. An EPR study showed that the aminoxyl radicals (**2**, **3**, and **4**) were stable and that their hyperfine structures were dependent on the position of the radical. Electron densities of pyrimidine also affected hyperfine structures.

Introduction

Spin-labeled nucleic acids have been used to study structures and dynamics of nucleic acids by electron paramagnetic resonance (EPR) spectroscopy. In the previous studies of spin-labeled nucleosides,[1] only 2,2,6,6-tetramethylpiperidine-1-oxyl (TEMPO) and related compounds were used as a spin source, which were connected to the nucleobase with the "linker" part.[2] We planned the synthesis of a new class of spin-labeled nucleosides such as purine derivatives (**1a**−**d**), a 6-substituted uridine derivative (**2**), a 6-substituted cytidine derivative (**3**), and a 5-substituted uridine derivative (**4**) in which a spin source, an *N-tert*-butylaminoxyl radical, is directly attached to the purine[3] and pyrimidine nucleus (Figure 1).

In these compounds, the unpaired electron of the aminoxyl radical can interact with electrons of the nucleobase. It is of interest to study the behaviors of aminoxyl radicals upon changes of electron density distribution in the nucleobase because those changes also might be caused by formation and breakdown of hydrogen bonding between complementary base pairs. Furthermore, direct introduction of a spin probe into the nucleobase decreases the complexity of EPR spectra as flexible linker confers the motion of aminoxyl radical independent of the DNA. Thus, it might be advantageous to use spin-labeled oligonucleotides containing 2′-deoxy and 2′-*O*-methyl derivatives of **1b**, **2**, **3**, and **4** to study structures of nucleic acids unless steric bulkiness of the spin source disrupts the structure of the nucleic acids. We anticipated that electronic properties of **1b**, **2**, **3**, and **4** were shared with 2′-deoxy and 2′-*O*-methyl analogues, which can be synthesized by conversion of the corresponding ribonucleosides by known procedures[4,5] or by procedures similar to those for spin-labeled ribonucleo-

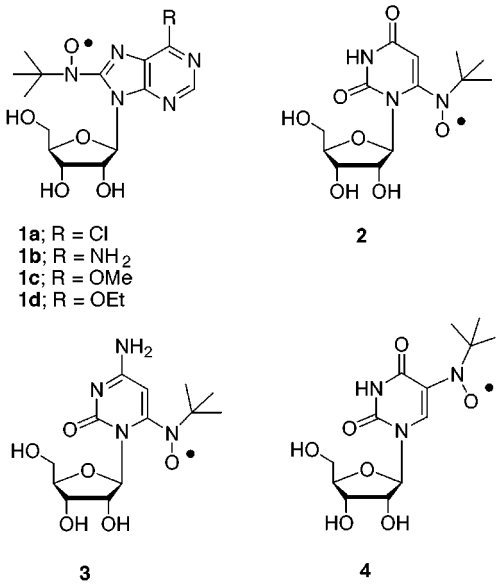

Figure 1. Spin-labeled ribonucleosides with *N-tert*-butylaminoxyl radicals.

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(1) (a) Duh, J.-L.; Bobst, A. M. *Helv. Chim. Acta* **1991**, *74*, 739 and references therein. (b) Zhdanov, R. I.; Porotikova, V. A.; Rozantsev, E. G. *Synthesis* **1979**, 267. (c) Gnewuch, T.; Sosnovsky, G. *Chem. Rev.* **1986**, *86*, 203.

(2) Spaltensen, A.; Robinson, B. H.; Hopkins, P. B. *Biochemistry* **1989**, *28*, 9484−9495.

(3) Preliminary communication of this research: Aso, M.; Norihisa, K.; Tanaka, M.; Koga, N.; Suemune, H. *J. Chem. Soc., Perkin Trans. 2* **2000**, 1637.

(4) Roelen, H. C. P. F.; Saris, C. P.; Brugghe, H. F.; van den Eist, H.; Westra, J. G.; van der Marel, G. A.; van Boom, J. H. *Nucleic Acids Res.* **1991**, *19*, 4361−4369.

(5) Shibahara, S.; Mukai, S.; Nishimura, T.; Inoue, H.; Ohtsuka, E.; Morisawa, H. *Nucleic Acids Res.* **1987**, *59*, 4403−4415.

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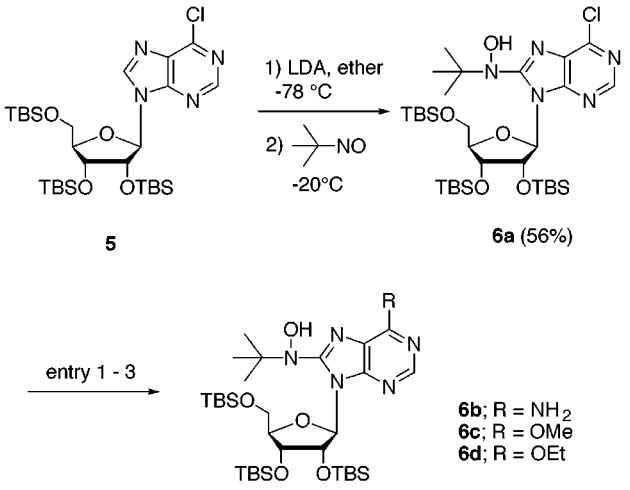
Scheme 1

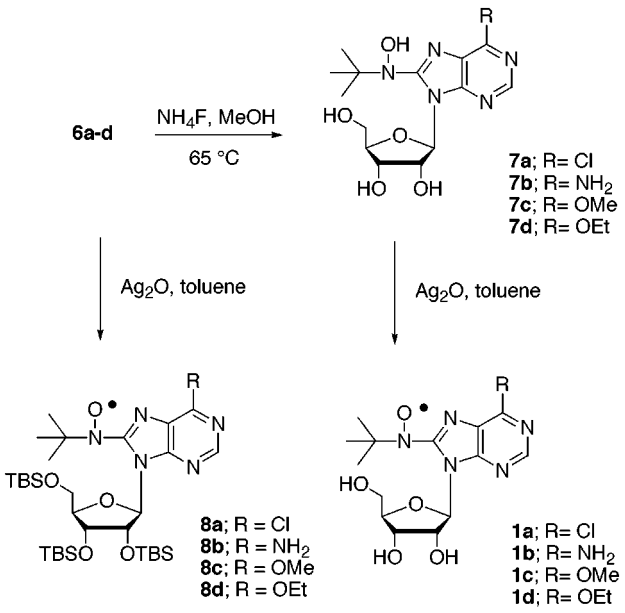


entry	reagent	solvent	temp.	product	R	yield (%)
1	NH_3	MeOH	60°C	**6b**	NH_2	82
2	NaOMe	MeOH	r.t.	**6c**	OMe	70
3	NaOEt	EtOH	r.t.	**6d**	OEt	76

sides. Thus, we synthesized **1a**−**d**, **2**, **3**, and **4** and studied their stabilities and electromagnetic behaviors by using EPR. The *N*-*tert*-butylaminoxyl radical, the choice of spin source in this study, can be obtained by oxidation of the corresponding hydroxylamino group as used for construction of stable high-spin poly(*N*-*tert*-butylaminoxyl radical)s containing the *m*-phenylene framework as organic super-high-spin molecules.[6] We are also interested in biological activities of ribonucleosides with an *N*-*tert*-butylhydroxylamino group and *N*-*tert*-butylaminoxyl radical as *N*-*tert*-butylhydroxylamine[7] and aminoxyl radical[8] are reported to be potent antioxidants.

Synthesis of 8-(*N*-*tert*-Butylhydroxylamino) Purine Derivatives. Miyasaka et al. reported that treatment of 6-chloropurine derivatives with LDA resulted in exclusive lithiation at the 8-position and subsequent reaction with electrophiles afforded 8-substituted purine nucleosides.[9] Introduction of an *N*-*tert*-butylhydroxylamino group, the precursor of the aminoxyl radical, into the purine nucleus at the 8-position was carried out according to their procedure; tri-*O*-silylated 6-chloropurine ribonucleoside (**5**), prepared from inosine, was lithiated with 6 equiv of LDA in dry ether at −78 °C and subsequent treatment with 2-methyl-2-nitrosopropane (MNP) at −20 °C gave the desired **6a** in 56% yield (Scheme 1). Purification of **6a** by silica gel chromatography led to its decomposition, but **6a** was purified by using alumina. The position of the *N*-*tert*-butylhydroxylamino group was determined by the ^{1}H NMR spectrum. The C2′-H of **6a** was observed at lower field (5.19 ppm) than that of **5** (4.59 ppm). The *syn*-glycosidic conformation of **6a** might cause an anisotropic deshielding of C2′-H by the nitrogen atom at the 3-position.[10] Direct synthesis of the 6-amino derivative (**6b**) by lithiation of the tri-*O*-silylated adenosine derivative followed by reaction with MNP was not successful, but **6b** was synthesized by a substitution reaction of the chloride group of **6a**. Heating **6a** in ammoniacal methanol in a sealed tube (60 °C, 4 days) gave **6b** in 82% yield. The *N*-*tert*-butylhydroxylamino function at the 8-position was not changed under these conditions. The 6-alkoxy derivatives, **6c** and **6d**, were also formed with ease by treatment of **6a** with sodium alkoxides at room temperature in 70% and 76% yields, respectively. In the ^{1}H NMR spectra, the C2′-H of **6b**−**d** were also observed at lower fields, similar to that of **6a** (**6b**, 5.35 ppm; **6c**, 5.19 ppm; **6d**, 5.21 ppm). The final step of the synthesis was desilylation of **6a**−**d**. The conditions for desilylation such as those with TBAF and HF did not effect formation of the corresponding triols (**7a**−**d**). Finally, **6a**−**d** were converted to **7a**−**d** in 87−98% yields by heating with ammonium fluoride in MeOH (Scheme 2).

Scheme 2



Formation of *N*-*tert*-Butylaminoxyl Radical at the C-8 Position. Oxidation of **6a**−**d** and **7a**−**d** with Ag_2O proceeded easily to afford the corresponding aminoxyl radicals (**8a**−**d** and **1a**−**d**) (Scheme 2). For example, the toluene solution of **7a** gradually turned red in color after addition of Ag_2O and TLC analysis of the reaction mixture showed formation of less polar, red aminoxyl radical. Attempts to isolate the radical **1a** were unsuccessful, but formation of the aminoxyl radical was confirmed by EPR spectroscopy. The EPR spectrum of **1a** in a toluene solution at room temperature showed a triplet with a hyperfine coupling constant of $a_N = 9.70$ G due to interaction between an unpaired electron and nitrogen nucleus of the aminoxyl radical (g value = 2.006, Figure 2a). The EPR spectrum of **1a** could be well simulated[11] by assuming hyperfine coupling constants (a_N = 9.70, 2.45, 1.06, 0.39, and 0.35 and a_H = 1.00 G) to indicate that the unpaired electron must be delocalized

(6) Fujita, J.; Tanaka, M.; Suemune, H.; Koga, N.; Matsuda, K.; Iwamura, H. *J. Am. Chem. Soc.* **1996**, *118*, 9347 and references therein.
(7) Atmna, H.; Martinez, A. P.; Ames, B. N. *J. Biol. Chem.* **2000**, *275*, 6741−6748.
(8) Krishna, M. C.; Russo, A.; Mitchell, J. B.; Goldstein, S.; Dafni, H.; Samuni, A. *J. Biol. Chem.* **1996**, *271*, 26026−26031.
(9) Kato, K.; Hayakawa, H.; Tanaka, H.; Kuramoto, H.; Shindoh, S.; Shuto, S.; Miyasaka, T. *J. Org. Chem.* **1997**, *62*, 6833.
(10) Pless, P.; Dudycz, L.; Stolarski, R.; Shugar, D. *Z. Naturforsch.* **1978**, *33c*, 902−907.
(11) Duling, D. R. *J. Magn. Reson., Ser. B* **1994**, *104*, 105−110.

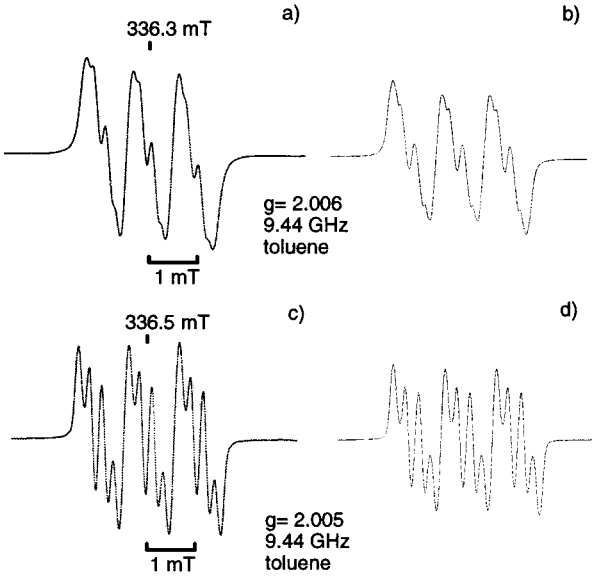


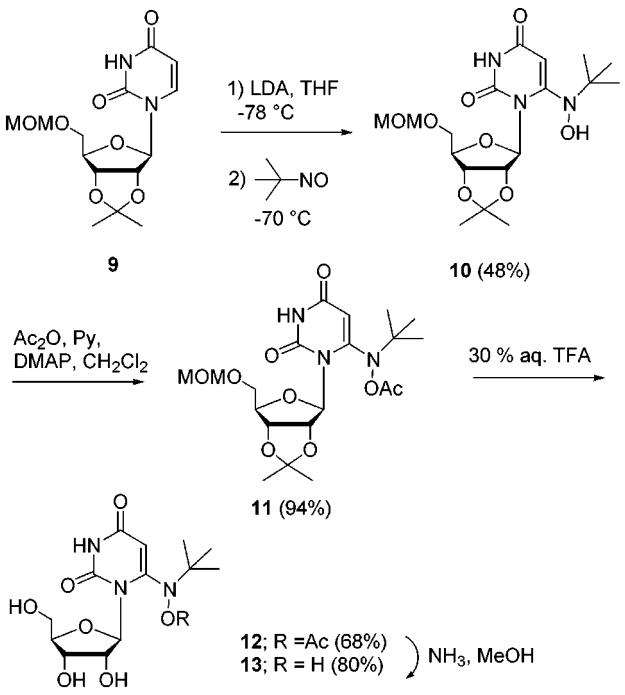
Figure 2. (a) EPR spectrum of **1a**. (b) Simulated spectrum of **1a** (a_N = 9.70, 2.45, 1.06, 0.39, 0.35 and a_H = 1.00 G). (c) EPR spectrum of **1b**. (d) Simulated spectrum of **1b** (a_N = 10.50, 2.68, 1.16, 0.40, 0.40 G).

into the purine ring (Figure 2b). The intensity of the signal decreased gradually, but the triplet due to the aminoxyl radical persisted for several days. The half-time period was estimated to be about 4 days at room temperature, which showed that **1a** was quite stable. The EPR spectrum of **1b** in toluene also showed a triplet with a hyperfine coupling constant of a_N = 10.5 G (g value = 2.005, Figure 2c) but the shape of each signal of the triplet was somewhat different from that of **1a** probably due to the difference of spin density distribution in the purine ring. The EPR spectrum of **1b** was well simulated by assuming hyperfine coupling constants a_N = 10.5, 2.68, 1.16, 0.40, and 0.40 G (Figure 2d). EPR spectra of the radicals (**1c**,**d** and **8a**−**d**) in toluene were also measured to show spectra similar to those of **1a** and **1b** (g value = 2.005−2.006, Supporting Information).

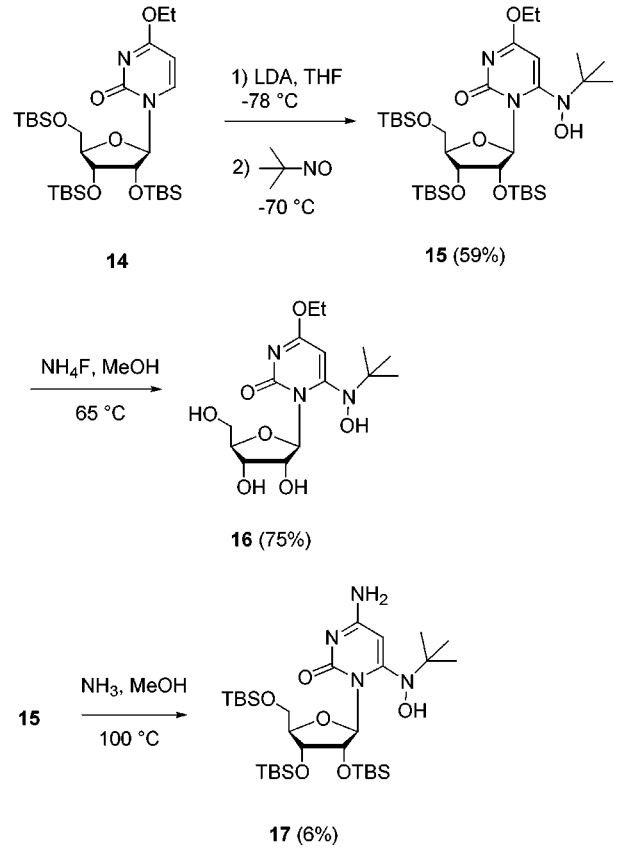
Synthesis of *N*-*tert*-Butylhydroxylamino Pyrimidine Derivatives. Lithiation reactions of pyrimidine compounds were also studied by Miyasaka extensively, and selective synthesis of 5- and/or 6-substituted pyrimidine derivatives are effected by using the appropriate base and protective group of the ribose moiety.[12] Lithiation of **9** with LDA followed by reaction with MNP gave **10** in 48% yield. X-ray analysis of **10** showed that the *N*-*tert*-butylhydroxylamino group was introduced into the 6-position of uracil (Scheme 3). Attempted deprotection of MOM and isopropylidene groups with 30% aqueous trifluoroacetic acid led to decomposition of **10**. However, treatment of *O*-acetylated **11**[13] under the same conditions gave the triol **12**, which was further converted to **13**.

Introduction of the *N*-*tert*-butylhydroxylamino group into the 6-position of the 4-ethoxy pyrimidine derivative was carried out successfully under similar conditions

Scheme 3



Scheme 4



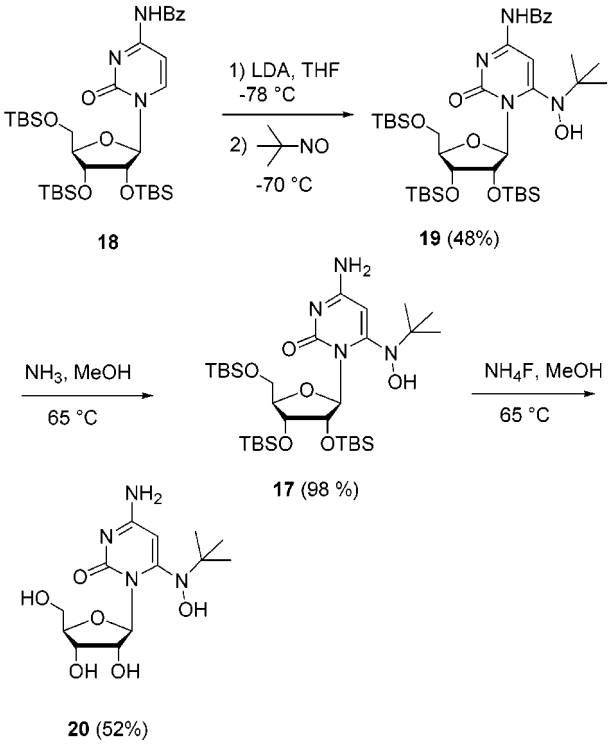
(Scheme 4). Treatment of the tri-*O*-silylated derivative **14**[14] with LDA and subsequent reaction with MNP gave 6-substituted **15** in 59% yield. The ^{1}H NMR spectrum of **15** did not show the signal of the C-6 proton and the C-5 proton was observed at 5.96 ppm. Desilylation of **15** with TBAF was accompanied by removal of the *N*-*tert*-butyl-

(12) (a) Tanaka, H.; Hayakawa, H.; Miyasaka, T. *Chem. Pharm. Bull.* **1981**, *29*, 3565−3572. (b) Hayakawa, H.; Tanaka, H.; Obi, K.; Itoh, M.; Miyasaka, T. *Tetrahedron Lett.* **1987**, *28*, 87−90. (c) Tanaka, H.; Hayakawa, H.; Iijima, S.; Haraguchi, K.; Miyasaka, T. *Tetrahedron* **1985**, *41*, 861−866.

(13) ^{1}H NMR spectra of **11** and **12** showed the presence of two isomers in a ratio of 2:1. Those isomers might be rotational isomers, and hydrolysis of the acetyl group of **12** afforded **13** as a single isomer in 80% yield.

(14) Ogilvie, K. K.; Beaucage, S. L.; Schifman, A. L.; Theriault, N. Y.; Sadana, K. L. *Can. J. Chem.* **1978**, *56*, 2768−2780.

Scheme 5



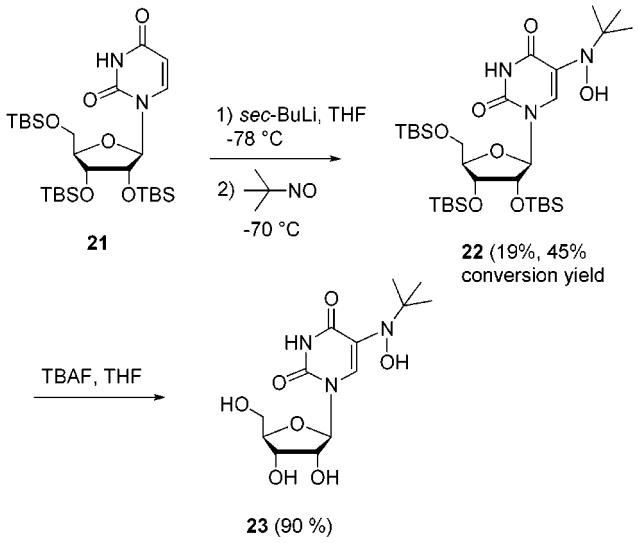
hydroxylamino group, and the triol **16** was obtained in low yield. However, heating **15** with ammonium fluoride in methanol gave **16** in 75% yield. Unfortunately, conversion of **15** to *N*-*tert*-butylhydroxylamino cytidine derivative **17** by heating in ammoniacal methanol[15] proceeded at only 6% (isolated yield, 33% conversion yield). Next, the synthesis of 6-substituted cytidine derivative **19** from **18**[16] was carried out. Lithiation of **18** with LDA and the following reaction with MNP gave **19** in 48% yield (Scheme 5). Heating **19** in ammoniacal methanol (65 °C, 6 h) afforded **17** in 98% yield. Desilylation of **17** also proceeded with ammonium fluoride to give the radical precursor **20** in 52% yield.

Introduction of the *N*-*tert*-butylhydroxylamino group into the 5-position of the uracil moiety was studied with **21**.[12c] Lithiation of **21** with *sec*-BuLi and subsequent reaction with MNP gave **22** albeit in low yield (19% isolated yield, 45% conversion yield, Scheme 6). In the ^{1}H NMR spectrum of **22**, the signal of the C-5 proton was not observed and the C-6 proton was observed at 7.61 ppm. For desilylation, treatment of **22** with TBAF gave **23** in 90% yield, whereas **23** was obtained in low yield (ca. 20%) by heating **22** with ammonium fluoride.

EPR Measurement of Spin-Labeled Pyrimidine Derivatives. Oxidation of pyrimidine derivatives containing the *N*-*tert*-butylhydroxylamino group (**13**, **20**, **16**, and **23**) with Ag_2O proceeded easily to afford the corresponding aminoxyl radicals (**2**, **3**, **24**, and **4**) (Figure 3).

Interestingly, hyperfine couplings of EPR spectra were dependent on the position of the *N*-*tert*-butylaminoxyl group. The EPR spectrum of **2** in CH_2Cl_2 containing 1% MeOH showed nine peaks with hyperfine coupling constants of a_N = 12.6, 2.55 G (*g* value = 2.005, Figure 4a). 6-Substituted **3** and **24** showed similar spectra (**3**, a_N = 12.9, 3.53 G; **24**, a_N = 12.7, 2.52 G, Figures 4b and 4c). In contrast, 5-substituted **4** showed six peaks with hyperfine coupling constants of a_N = 13.6, a_H =1.75 G (*g* value = 2.005, Figure 4d). The intensity of the signal decreased gradually but persisted for several weeks. Electron density of the amino group of the pyrimidine ring also affected hyperfine couplings. The EPR spectrum of cytidine derivative **25** showed nine peaks, whereas that of **26**, the amino group of which was benzoylated, showed six peaks (Figure 5).

Scheme 6



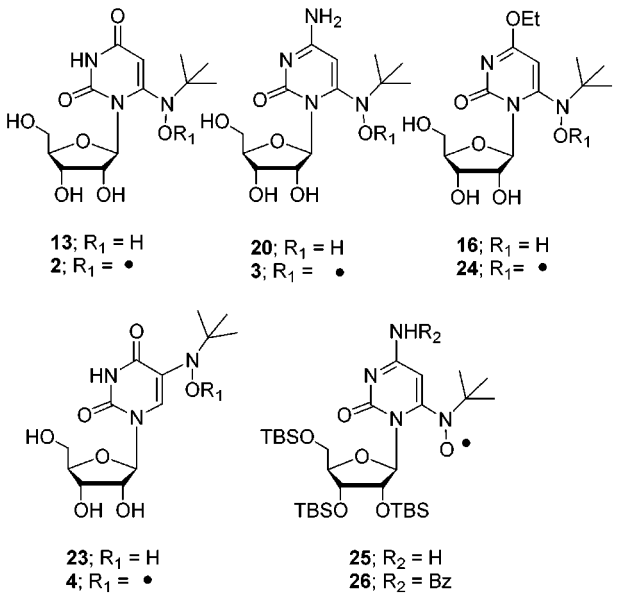


Figure 3. Pyrimidine ribonucleosides with *N*-*tert*-butylhydroxylamino groups and *N*-*tert*-butylaminoxyl radicals.

Discussion

Synthesis and Conformation of Ribonucleosides with *N*-*tert*-Butyhydroxylamino Group. As precursors of **1a**−**d**, **2**, **3**, and **4**, purine ribonucleosides (**6a**−**d**) and pyrimidine ribonucleosides (**13**, **20**, and **23**) were synthesized by site-selective lithiation and the following reaction with 2-methyl-2-nitrosopropane and deprotection. The conformational properties of the modified

(15) Matsuda, A.; Itoh, H.; Takenuki, K.; Sasaki, T.; Ueda, T. *Chem. Pharm Bull* **1988**, *36(3)*, 945−953.

(16) Ogilvie, K. K.; Schifman, A. L.; Penny, C. L. *Can. J. Chem.* **1979**, *56*, 2230−2238.

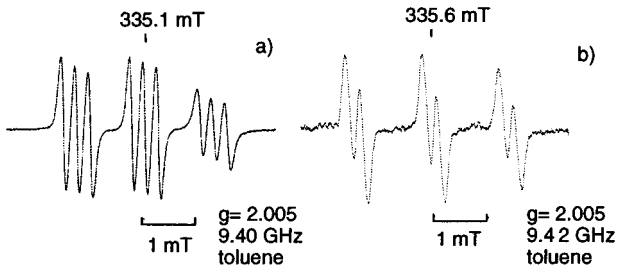


Figure 5. EPR spectra of (a) **25** (a_N = 12.8, 1.99 G) and (b) **26** (a_N = 14.3, a_N = 2.73 G).

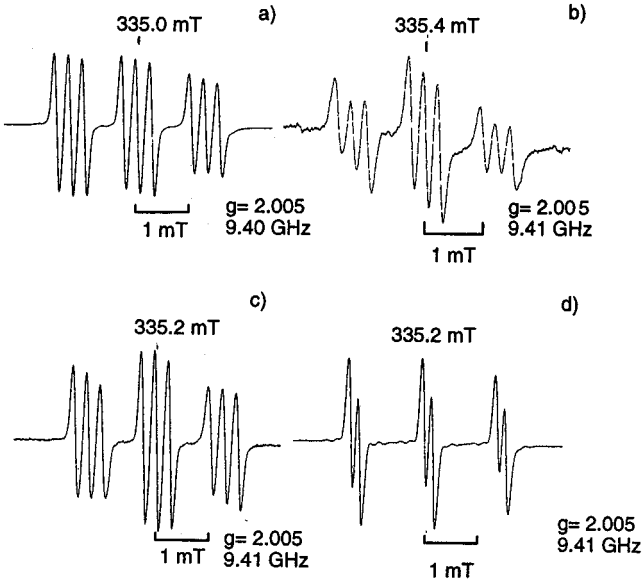


Figure 4. EPR spectra of (a) **2** (a_N = 12.6, 2.55 G), (b) **3** (a_N = 12.9, 3.53 G), (c) **24** (a_N = 12.7, 2.52 G), and (d) **4** (a_N = 13.6, a_H = 1.75 G). The spectra were obtained from solutions in CH_2Cl_2 containing 1% MeOH.

nucleosides are associated with their biological behaviors and conformations of oligonucleotides and polynucleotides containing them. It is known that introduction of a bulky substituent into the 8-position of purine and the 6-position of pyrimidine causes a shift of the *syn*/*anti* equilibrium to *syn* due to interaction of substituent and ribose. In ^{1}H NMR spectra, characteristic changes in chemical shifts of C1′-H, C2′-H, and C3′-H relative to those of unmodified nucleosides are observed. Especially, the chemical shifts of C2′-H are shifted downfield significantly when nucleosides have a high population of the *syn* form. These changes result from the anisotropic influence of the N(3) atom and the ring currents of the purine moiety or of the 2-keto group of the pyrimidine in the conformation *syn*. Therefore, the *syn*/*anti* conformational preference was determined by comparison of chemical shifts of ^{1}H NMR spectra in D_2O and DMSO-d_6.[10,17,18] We compared the chemical shifts of C2′-H of **7b** and those of adenosine and 8-bromoadenosine in DMSO-d_6 to estimate conformation of **7b** in solution because preferred conformation of adenosine is *anti* and that of 8-bromoadenosine is predominantly *syn*.[17] The chemical shift change of C2′-H of **7b** relative to adenosine was 0.28 ppm (C2′-H of **7b**; 4.89 ppm, C2′-H of adenosine; 4.61 ppm), which was smaller than that of 8-bromoadenosine relative to adenosine (0.48 ppm; C2′-H of 8-bromoadenosine; 5.09 ppm). It is likely that the *syn* population of **7b** is smaller than that of 8-bromoadenosine. For pyrimidine ribonucleosides, the chemical shift of C2′-H of 6-methyluridine is shifted downfield (4.81 ppm in D_2O) compared to that of uridine (4.32 ppm) to indicate the preferred *syn* conformation of 6-methyluridine in solution.[12a] We compared the chemical shift of C2′-H of **13** (4.70 ppm) and that of uridine. Compared to the chemical shift change of C2′-H of 6-methyluridine relative to uridine (0.49 ppm), that of **13** was smaller (0.38 ppm). The chemical shift data of **7b** and **13** indicate that steric bulkiness of an *N*-*tert*-butylhydroxylamino group introduced into the 8-position of purine and the 6-position of pyrimidine is smaller than that of bromide and methyl groups. In contrast, the C2′-H of 5-substituted **23** was observed at 4.32 ppm in D_2O which is comparable to that of uridine and **23** might exist in an *anti* conformation similar to 5-methyluridine. Introduction of *N*-*tert*-butylhydroxylamino group into the 5-position of pyrimidine might minimize structural disruption of the oligonucleotide duplex.

EPR Study of Spin-Labeled Purine and Pyrimidine Ribonucleosides. An EPR study of spin-labeled purine and pyrimidine ribonucleosides indicated that aminoxyl radicals attached directly to nucleobases were quite stable. In EPR spectra of **1a** and **1b**, the largest hyperfine coupling constants were 9.70 and 10.50 G (Figures 2a and 2c). These values are reasonable for an *N*-*tert*-butylaminoxyl radical on an sp^2 hybridized carbon.[19] The EPR spectra of **1a**−**1d** could be well simulated with five hyperfine coupling constants of nitrogens (for **1a**, a_N = 9.70, 2.45, 1.06, 0.39, and 0.35 and a_H = 1.00 G). This indicated that the unpaired electron of the aminoxyl radical might be delocalized into the purine ring (Figures 2b and 2d). In spin-labeled pyrimidine ribonucleosides, 6-substituted **2**, **3**, and **24** showed nine peaks with two hyperfine coupling constants of nitrogens (for **2**, a_N = 12.6, 2.55 G). In contrast, 5-substituted **4** showed six peaks with hyperfine coupling constants of a_N = 12.7, a_H = 3.53 G (Figure 4). It is likely that hyperfine couplings of spin-labeled pyrimidines are more influenced by the position of the radical than the structure of pyrimidines. In addition to its electronic effect, the steric influence of an *N*-*tert*-butylaminoxyl radical on the conformation including the dihedral angles between N−O bonds and the pyrimidine ring planes must be important because spin density distribution is dependent on interaction of the unpaired radical electron and electrons of pyrimidines. Influences of substituents of the purine and pyrimidine rings on hyperfine couplings were also observed. The EPR spectra of 6-substitued **26** with the benzoylated amino group a showed sharp contrast to that of **25**, probably due to the difference of electron densities of the pyrimidine ring (Figure 5). These data prompted us to study behaviors of 2′-*O*-methyl and 2′-deoxy analogues of **1b**, **2**, **3**, and **4** in oligonucleotides, and their synthetic study is in progress.

Experimental Section

Melting points were taken on a Yanagimoto melting point apparatus and are not corrected. ^{1}H NMR spectra were obtained on a JEOL GX-270 (270 MHz) spectrometer. Chemi-

(17) Jordan, F.; Niv, H. *Biochim. Biophys. Acta* **1977**, *476*, 265−271. They reported that the changes of chemical shifts in DMSO-d_6 showed the same trends in D_2O.

(18) Schweizer, M. P.; Bantam, E. B.; Witkowski, J. T.; Robins, R. K. *J. Am. Chem. Soc.* **1973**, *95*, 3770−3778.

(19) Buettner, G. R. *Free Rad. Biol. Med.* **1987**, *3*, 259−303.

cal shifts are reported as δ values with respect to tetramethylsilane (TMS) or 2,2-dimethyl-2-silapentane 5-sulfonate (DSS) as an internal standard or are referenced to a residual proton at 2.49 ppm for DMSO-d_6 or at 3.30 ppm for CD_3OD. ^{13}C NMR spectra were taken on a JEOL GX-270 (68 MHz) or a Varian UNITY-plus (125 MHz) spectrometer. IR spectra were taken on a JASCO IR A-100 infrared spectrophotometer. High-resolution mass spectra (HRMS) were determined on an SX-102 or a JMS-700T spectrometer. EPR spectra were recorded on a Bruker ESP 300 X-band (9.4 GHz) spectrometer equipped with a Hewlett-Packard 5350B microwave frequency counter. For thin-layer chromatography, precoated TLC plates (Merck, Kieselgel 60 F_{254}) were used. Column chromatography was carried out on silica gel 70−230 mesh (Merck, Kieselgel 60) or on alumina.

6-Chloro-8-(*N*-*tert*-butylhydroxylamino)-9-(2,3,5-tris-*O*-TBDMS-β-D-ribofuranosyl)purine (6a). To a dry ether solution containing LDA (8.20 mmol) was added dropwise a solution of 6-chloro-9-(2,3,5-tris-*O*-TBDMS-β-D-ribofuranosyl)-purine (860 mg, 1.37 mmol) in 10 mL of dry ether at −78 °C under an Ar atmosphere, and the temperature was maintained for 1.5 h. A solution of 2-methyl-2-nitrosopropane (MNP) (360 mg, 4.1 mmol) in 5 mL of dry ether was added to the reaction mixture, and the temperature was raised to −40 °C. A solution of MNP (360 mg, 4.1 mmol) in 5 mL of dry ether was added again, and the temperature was raised to −20 °C. The reaction mixture was quenched by adding saturated aqueous ammonium chloride solution, and the organic layer was extracted with ether, washed with brine, and dried over Na_2SO_4. The solvent was evaporated under reduced pressure, and the residue was purified on alumina. Elution with hexane/EtOAc (10:1) gave **6a** (550 mg, 56%) as a colorless solid: mp 144 °C; IR ($CHCl_3$) 3400, 2900, 1590, 1250, 1160, 1130, 1070, 830 cm^{-1}; ^1H NMR (270 MHz, $CDCl_3$) δ 8.62 (s, 1H), 6.68 (brs, 1H, D_2O exchanged), 6.21 (d, J = 5.4 Hz, 1H), 5.19 (t, J = 5.1 Hz, 1H), 4.53 (t, J = 4.3 Hz, 1H), 4.08−3.72 (m, 2H), 3.68 (m, 1H), 1.46 (s, 9H), 0.96 (s, 9H), 0.86 (s, 9H), 0.78 (s, 9H), 0.17 (s, 3H), 0.16 (s, 3H), 0.06 (s, 3H), 0.04 (s, 3H), −0.10 (s, 3H), −0.34 (s, 3H); ^{13}C NMR (125 MHz, $CDCl_3$) δ 157.1 (s), 151.5 (s), 151.0 (d), 149.6 (s), 123.0 (s), 88.8 (d), 84.4 (d), 73.2 (d), 71.4 (d), 61.9 (t), 60.5 (s), 26.1 (q), 25.91 (q), 25.90 (q), 25.7 (q), 18.5 (s), 18.1 (s), 17.9 (s), −4.2 (q), −4.5 (q), −4.6 (q), −5.0 (q), −5.3 (q), −5.6 (q). Anal. Calcd for $C_{32}H_{62}ClN_5O_5Si_3$: C, 53.64; H, 8.72; N, 9.77. Found: C, 53.57; H, 8.70; N, 9.81.

6-Amino-8-(*N*-*tert*-butylhydroxylamino)-9-(2,3,5-tris-*O*-TBDMS-β-D-ribofuranosyl)purine (6b). Ammonia was bubbled through a solution of **6a** (400 mg, 0.56 mmol) in MeOH (40 mL) at −40 °C, and the resultant ammoniacal solution was heated at 60 °C in a sealed tube for 4 days. The solvent was evaporated under reduced pressure, and the residue was purified on alumina. Elution with hexane/AcOEt (10:1) gave **6b** (320 mg, 82%) as a colorless solid: mp 140−142 °C; IR ($CHCl_3$) 3410, 2900, 1620, 1240, 1150, 1120, 1070, 830 cm^{-1}; ^1H NMR (270 MHz, $CDCl_3$) δ 8.24 (s, 1H), 6.98 (brs, 1H), 6.14 (d, J = 4.9 Hz, 1H), 5.64 (brs, 2H), 5.29 (t, J = 5.0 Hz, 1H), 4.55 (t, J = 4.0 Hz, 1H), 4.08−3.97 (m, 2H), 3.71 (m, 1H), 1.36 (s, 9H), 0.93 (s, 9H), 0.85 (s, 9H), 0.77 (s, 9H), 0.15 (s, 3H), 0.13 (s, 3H), 0.08 (s, 3H), 0.02 (s, 3H), −0.10 (s, 3H), −0.30 (s, 3H); HRMS (FAB) m/z calcd for $C_{32}H_{65}ClN_6O_5Si_3$ ([M + H]$^+$) 697.4324, found 697.4312. Anal. Calcd for $C_{32}H_{64}ClN_6O_5Si_3$: C, 55.13; H, 9.25; N, 12.05. Found: C, 55.17; H, 9.22; N, 12.00.

6-Methoxy-8-(*N*-*tert*-butylhydroxylamino)-9-(2,3,5-tris-*O*-TBDMS-β-D-ribofuranosyl)purine (6c). To a solution of **6a** (65 mg, 0.09 mmol) in 3 mL of dry MeOH was added NaOMe (15 mg, 0.28 mmol) under an Ar atmosphere, and the mixture was stirred at room temperature. After 4 h, MeOH was removed under reduced pressure and the residue was diluted with AcOEt and washed with saturated aqueous ammonium chloride and brine. The organic layer was dried over Na_2SO_4 and concentrated under reduced pressure. The residue was purified on alumina. Elution with hexane/AcOEt (10:1) gave **6c** (40 mg, 70%) as a colorless solid: mp 106−108 °C; IR ($CHCl_3$) 3400, 2960, 2940, 2850, 1600, 1210 cm^{-1}; ^1H NMR (270 MHz, $CDCl_3$) δ 8.43 (s, 1H), 6.44 (brs, 1H), 6.17 (d, J = 5.0 Hz, 1H), 5.19 (t, J = 5.0 Hz, 1H), 4.55 (t, J = 4.6 Hz, 1H), 4.14 (s, 3H), 4.05−3.96 (m, 2H), 3.73 (dd, J = 13.4, 6.1 Hz, 1H), 1.39 (s, 9H), 0.94 (s, 9H), 0.83 (s, 9H), 0.76 (s, 9H), 0.15 (s, 3H), 0.13 (s, 3H), 0.02 (s, 3H), 0.00 (s, 3H), −0.12 (s, 3H), −0.35 (s, 3H); ^{13}C NMR (125 MHz, $CDCl_3$) δ 160.5 (s), 154.7 (s), 151.4 (s), 151.3 (d), 119.5 (s), 88.6 (d), 84.0 (d), 73.1 (d), 71.4 (d), 61.9 (t), 60.2 (s), 54.2 (q), 26.2 (q), 26.0 (q), 25.9 (q), 25.8 (q), 18.5 (s), 18.1 (s), 17.9 (s), −4.2 (q), −4.5 (q), −4.6 (q), −4.9 (q), −5.3 (q), −5.6 (q). Anal. Calcd for $C_{33}H_{65}N_5O_6Si_3$: C, 55.66; H, 9.21; N, 9.84. Found: C, 55.89; H, 9.13; N, 9.69.

6-Ethoxy-8-(*N*-*tert*-butylhydroxylamino)-9-(2,3,5-tris-*O*-TBDMS-β-D-ribofuranosyl)purine (6d). To a solution of **6a** (40 mg, 0.06 mmol) in 2 mL of dry EtOH was added NaOEt (11 mg, 0.17 mmol) under Ar, and the mixture was stirred at room temperature. After 4 h, EtOH was removed under reduced pressure and the residue was diluted with AcOEt and washed with saturated aqueous ammonium chloride solution and brine. The organic layer was dried over Na_2SO_4 and concentrated under reduced pressure. The residue was purified on alumina. Elution with hexane/AcOEt (10:1) gave **6d** (31 mg, 76%) as a colorless oil: IR ($CHCl_3$) 3400, 2960, 2940, 2850, 1600 cm^{-1}; ^1H NMR (270 MHz, $CDCl_3$) δ 8.43 (s, 1H), 6.37 (s, 1H), 6.18 (d, J = 5.0 Hz, 1H), 5.21 (t, J = 5.0 Hz, 1H), 4.66 (q, J = 7.0 Hz, 2H), 4.59 (t, J = 4.6 Hz, 1H), 4.06−3.97 (m, 2H), 3.79−3.72 (m, 1H), 1.50 (t, J = 7.0 Hz, 3H), 1.41 (s, 9H), 0.96 (s, 9H), 0.85 (s, 9H), 0.79 (s, 9H), 0.17 (s, 3H), 0.15 (s, 3H), 0.04 (s, 3H), 0.02 (s, 3H), −0.09 (s, 3H), −0.32 (s, 3H); ^{13}C NMR (125 MHz, $CDCl_3$) δ 160.4 (s), 154.7 (s), 151.5 (s), 151.4 (d), 119.7 (s), 88.6 (d), 84.1 (d), 73.0 (d), 71.7 (d), 63.1 (t), 62.1 (t), 60.3 (s), 26.2 (q), 26.0 (q), 26.0 (q), 25.8 (q), 18.4 (s), 18.1 (s), 17.9 (s), 14.6 (q), −4.2 (q), −4.4 (q), −4.6 (q), −4.9 (q), −5.3 (q), −5.6 (q); HRMS (FAB) m/z calcd for $C_{34}H_{68}N_5O_6Si_3$ ([M + H]$^+$) 726.4477, found 726.4489.

6-Chloro-8-(*N*-*tert*-butylhydroxylamino)-9-(β-D-ribofuranosyl)purine (7a). To a solution of **6a** (100 mg, 0.14 mmol) in 5 mL of MeOH was added ammonium fluoride (52 mg, 1.40 mmol), and the mixture was heated at 65 °C. After 10 h, MeOH was removed under reduced pressure and the residue was purified on Florisil. Elution with $CHCl_3$/MeOH (5:1) gave **7a** (45 mg, 87%) as a colorless solid: mp 105−107 °C; IR (KBr) 3300, 2900, 1580, 1320 cm^{-1}; ^1H NMR (270 MHz, DMSO-d_6) δ 9.63 (s, 1H, D_2O exchanged), 8.64 (s, 1H), 6.11 (d, J = 5.6 Hz, 1H), 5.21 (m, 2H, D_2O exchanged), 5.00 (m, 2H, 1H was exchanged with D_2O), 4.32 (dd, J = 9.6, 5.0 Hz, 1H), 3.90 (m, 1H), 3.70 (m, 1H), 3.56 (m, 1H), 1.38 (s, 9H); ^{13}C NMR (125 MHz, DMSO-d_6) δ 157.3 (s), 151.5 (s), 150.3 (d), 146.8 (s), 129.5 (s), 89.1 (d), 85.2 (d), 71.3 (d), 70.2 (d), 61.7 (t), 59.8 (s), 25.8 (q); HRMS (FAB) m/z calcd for $C_{14}H_{21}N_5O_5Cl$ ([M + H]$^+$) 374.1231, found 374.1245.

6-Amino-8-(*N*-*tert*-butylhydroxylamino)-9-(β-D-ribofuranosyl)purine (7b). To a solution of **6b** (96 mg, 0.14 mmol) in 5 mL of MeOH was added ammonium fluoride (51 mg, 1.38 mmol), and the mixture was heated at 65 °C. After 10 h, MeOH was removed under reduced pressure and the residue was purified on Florisil. Elution with $CHCl_3$/MeOH (5:1) gave **7b** (46 mg, 94%) as a colorless solid: mp 187−189 °C; IR (KBr) 3350, 2980, 2940, 1640, 1600, 1360, 1330, 1300 cm^{-1}; ^1H NMR (270 MHz, DMSO-d_6) δ 8.89 (s, 1H, D_2O exchanged), 8.06 (s, 1H), 7.20 (brs, 2H, D_2O exchanged), 6.08 (d, J = 6.6 Hz, 1H), 5.87−5.83 (m, 1H, D_2O exchanged), 5.20 (d, J = 4.0 Hz, 1H, D_2O exchanged), 5.06 (d, J = 7.3 Hz, 1H, D_2O exchanged), 4.89 (dd, J = 12.5, 6.9 Hz, 1H), 4.20 (m, 1H), 3.94 (m, 1H), 3.66 (dm, J = 12.4 Hz, 1H), 3.58−3.49 (m, 1H), 1.29 (s, 9H); ^{13}C NMR (125 MHz, DMSO-d_6) δ 155.5 (s), 152.1 (s), 151.5 (d), 147.8 (s), 116.7 (s), 87.8 (d), 85.8 (d), 71.8 (d), 70.8 (d), 62.1 (t), 59.0 (s), 26.0 (q); HRMS (FAB) m/z calcd for $C_{14}H_{23}N_6O_5$ ([M + H]$^+$) 355.1730, found 355.1761.

6-Methoxy-8-(*N*-*tert*-butylhydroxylamino)-9-(β-D-ribofuranosyl)purine (7c). To a solution of **6c** (80 mg, 0.11 mmol) in 5 mL of MeOH was added ammonium fluoride (42 mg, 1.13 mmol), and the mixture was heated at 65 °C. After 10 h, MeOH was removed under reduced pressure and the residue was purified on Florisil. Elution with $CHCl_3$/MeOH (5:1) gave **7c** (40 mg, 98%) as a colorless solid: mp 115−117 °C; IR (KBr) 3350, 2990, 2870, 1600, 1580, 1480, 1350, 1320 cm^{-1}; ^1H NMR

(270 MHz, $CDCl_3$) δ 8.29 (s, 1H), 7.93 (brs, 1H, D_2O exchanged), 6.63 (brs, 1H, D_2O exchanged), 6.31 (d, J = 7.9 Hz, 1H), 5.15 (dd, J = 7.6, 5.0 Hz, 1H), 4.46 (d, J = 4.6 Hz, 1H), 4.28 (s, 1H), 4.15 (s, 3H), 3.93 (d, J = 11.9 Hz, 1H), 3.75−3.70 (m, 1H), 1.30 (s, 9H); ^{13}C NMR (68 MHz, $CDCl_3$) δ 160.1 (s), 154.4 (s), 151.0 (d), 149.5 (s), 119.1 (s), 89.6 (d), 87.0 (d), 77.2 (d), 72.9 (d), 63.3 (t), 61.3 (s), 54.5 (q), 25.9 (q); HRMS (FAB) m/z calcd for $C_{15}H_{24}N_5O_6$ ($[M + H]^+$) 370.1726, found 370.1691.

6-Ethoxy-8-(*N*-*tert*-butylhydroxylamino)-9-(β-D-ribofuranosyl)purine (7d). To a solution of **6d** (110 mg, 0.15 mmol) in 5 mL of MeOH was added ammonium fluoride (56 mg, 1.51 mmol), and the mixture was heated at 65 °C. After 12 h, MeOH was removed under reduced pressure and the residue was purified on Florisil. Elution with $CHCl_3$/MeOH (5:1) gave **7d** (52 mg, 90%) as a colorless solid: mp 102−104 °C; IR (KBr) 3300, 2940, 1600, 1450, 1320 cm^{-1}; ^{1}H NMR (270 MHz, $CDCl_3$) δ 8.26 (s, 1H), 7.97 (brs, 1H, D_2O exchanged), 6.56 (brd, J = 11.6 Hz, 1H, D_2O exchanged), 6.32 (d, J = 7.9 Hz, 1H), 6.07 (brs, 1H, D_2O exchanged), 5.24−5.20 (m, 1H), 4.67−4.46 (m, 3H), 4.29 (s, 1H), 4.07 (brs, 1H, D_2O exchanged), 3.92 (d, J = 12.6 Hz, 1H), 3.76 (t, J = 12.6 Hz, 1H), 1.52 (t, J = 7.1 Hz, 3H), 1.28 (s, 9H); ^{13}C NMR (68 MHz, $CDCl_3$) δ 159.8 (s), 154.2 (s), 151.1 (d), 149.5 (s), 119.0 (s), 89.6 (d), 87.0 (d), 73.0 (d), 72.8 (d), 63.9 (t), 63.4 (t), 61.5 (s), 25.9 (q), 14.3 (q); HRMS (FAB) m/z calcd for $C_{16}H_{25}N_5O_6$ ($[M + H]^+$) 384.1883, found 384.1861.

5′-Methoxymethyl-6-(*N*-*tert*-butylhydroxylamino)-2′,3′-*O*-isopropylideneuridine (10). To a soluiton of LDA in THF (6 mL), prepared from *n*-BuLi (6.2 mL, 9.68 mmol) in hexane and diisopropylamine (1.36 mL, 9.68 mmol), was added a solution of 5′-methoxymethyl-2′,3′-*O*-isopropylideneuridine (**9**)[12a] (530 mg, 1.61 mmol) in 11 mL of THF at −78 °C, and the mixture was stirred for 1 h at −70 °C. A solution of MNP (842 mg, 9.68 mmol) in 7.8 mL of THF was added dropwise, and the mixture was stirred for 3 h. A saturated NH_4Cl solution was added to the reaction mixture, and the organic layer was extracted with AcOEt, dried over Na_2SO_4, and concentrated under reduced pressure. The residue was purified by silica gel chromatography (AcOEt) to give **10** (263 mg, 48%) as colorless needles: mp 185 °C (recrystallized from hexane), ^{1}H NMR (270 MHz, $CDCl_3$) δ 8.95 (br, 1H, D_2O exchanged), 6.77 (br, 1H, D_2O exchanged), 6.59 (s, 1H), 5.78 (s, 1H), 5.09 (dd, J = 6.3, 1.0 Hz, 1H), 4.80 (t, J = 5.6 Hz, 1H), 4.73 (d, J = 6.6 Hz, 1H), 4.65 (d, J = 6.6 Hz, 1H), 4.32−4.29 (m, 1H), 3.87 (dd, J = 8.9, 3.6 Hz, 1H), 3.75 (t, J = 8.9 Hz, 1H), 3.38 (s, 3H), 1.55 (s, 3H), 1.33 (s, 3H), 1.24 (s, 9H); HRMS (FAB) m/z calcd for $C_{18}H_{30}O_8N_3$ ($[M + H]^+$) 416.2033, found 416.2053.

5′-Methoxymethyl-6-(*N*-*tert*-butylacetoxylamino)-2′,3′-*O*-isopropylideneuridine (11). To a solution of **10** (163 mg, 0.39 mmol) in CH_2Cl_2 (7 mL) were added pyridine (0.05 mL, 0.59 mmol), acetic anhydride (0.05 mL, 0.59 mmol), and DMAP (4.8 mg, 0.04 mmol), and the mixture was stirred at room temperature for 3 h. A saturated aqueous $NaHCO_3$ solution was added to the reaction mixture, and the organic layer was extracted with CH_2Cl_2, washed with brine, and dried over Na_2SO_4. After concentration, the residue was purified by silica gel chromatography (AcOEt) to give **11** (169 mg, 94%) as a colorless solid: ^{1}H NMR (270 MHz, $CDCl_3$) δ (major) 9.78 (bs, 1H), 6.97 (s, 1H), 5.73 (s, 1H), 5.14 (d, J = 6.6 Hz, 1H), 4.92 (m, 1H), 4.65 (dd, J = 7.8, 6.8 Hz, 2H), 4.31−4.24 (m, 1H), 3.76−3.73 (m, 2H), 3.35 (s, 3H), 2.08 (s, 3H), 1.56 (s, 3H), 1.34 (s, 3H), 1.27 (s, 9H), (minor) 9.59 (bs, 1H), 6.97 (s, 1H), 5.75 (s, 1H), 5.03 (d, J = 5.9 Hz, 1H), 4.92 (m, 1H), 4.71 (s, 2H), 4.31−4.24 (m, 1H), 3.76−3.73 (m, 2H), 3.35 (s, 3H), 2.08 (s, 3H),1.56 (s, 3H), 1.36 (s, 3H), 1.30 (s, 9H); HRMS (FAB) m/z calcd for $C_{20}H_{32}O_9N_3$ ($[M + H]^+$) 458.2139, found 458.2172.

6-*N*-*tert*-Butylacetoxylaminouridine (12). A mixture of **11** (21.4 mg, 0.05 mmol) and 30% aqueous trifluoroacetic acid (3 mL) was stirred at room temperature for 5 h. The reaction mixture was concentrated under reduced pressure, and the residue was purified by silica gel chromatography ($CHCl_3$:MeOH = 10:1) to give **12** (11.8 mg, 68%) as a colorless oil: ^{1}H NMR (270 MHz, CD_3OD) δ (major) 7.73 (s, 1H), 6.57 (s, 1H), 5.53 (5.59) (s, 1H), 4.33 (4.24) (s, 1H), 3.73−3.48 (m, 2H), 3.15−3.13 (m, 2H), 1.94 (s, 3H), 1.14 (s, 9H), (minor) 7.73 (s, 1H), 6.57 (s, 1H), 5.59 (s, 1H), 4.24 (s, 1H), 3.73−3.48 (m, 2H), 3.15−3.13 (m, 2H), 1.94 (s, 3H), 1.14 (s, 9H); HRMS (FAB) m/z calcd for $C_{13}H_{22}O_7N_3$ ($[M + H]^+$) 374.1563, found 374.1554.

6-*N*-*tert*-Butylhydroxylaminouridine (13). A mixture of **12** (11.8 mg, 0.03 mmol) and a saturated methanolic ammonia solution (2 mL) was stirred at room temperature for 30 min. The reaction mixture was concentrated under reduced pressure, and the residue was purified by silica gel chromatography ($CHCl_3$:MeOH = 4:1) to give **13** (8.4 mg, 80%) as a colorless oil: ^{1}H NMR (270 MHz, DMSO-d_6) δ 9.09 (s, 1H, D_2O exchanged), 6.18 (d, J = 2.0 Hz, 1H), 5.51 (s, 1H), 4.99 (d, J = 7.3 Hz, 1H, D_2O exchanged), 4.92 (d, J = 5.0 Hz, 1H, D_2O exchanged), 4.63 (m, 1H, D_2O exchanged), 4.36 (m, 1H), 4.18 (m, 1H), 3.58 (m, 2H), 1.15 (s, 9H), (D_2O) δ 6.45 (d, J = 3.1 Hz, 1H), 5.94 (s, 1H), 4.70 (dd, J = 6.7, 3.1 Hz, 1H), 4.42 (t, J = 6.7 Hz, 1H), 3.89−3.86 (m, 2H), 3.72 (dd, J = 12.8, 7.0 Hz, 1H), 1.25 (s, 9H); HRMS (FAB): m/z calcd for $C_{13}H_{22}O_7N_3$ ($[M + H]^+$) 332.1458, found 332.1459.

4-Ethoxy-1-(2′,3′,5′-tris-*O*-*tert*-butyldimethylsilyl-β-D-ribofranosyl)-2(1*H*)-pyrimidine (14). To a solution of 4-ethoxy-1-(β-D-ribofranosyl)pyrimidine[15] (1.12 g, 4.10 mmol) in dry DMF (22 mL) were added TBDMSCl (3.09 g, 20.5 mmol) and imidazole (1.40 g, 20.5 mmol), and the mixture was stirred at room temperature for 10 h. The reaction mixture was diluted with H_2O, and the organic layer was extracted with AcOEt, dried over Na_2SO_4, and concentrated under reduced pressure. The residue was purified by silica gel chromatography (hexane:AcOEt = 10:1) to give **14** (2.47 g, 98%) as a colorless solid: ^{1}H NMR (270 MHz, $CDCl_3$) δ 8.35 (d, J = 7.3 Hz, 1H), 5.72 (d, J = 7.6 Hz, 1H), 5.68 (s, 1H), 4.40−4.35 (m, 2H), 4.10−3.98 (m, 4H), 3.74 (d, J = 11.9 Hz, 1H), 1.31 (t, J = 7.1 Hz, 3H), 0.91 (s, 3H), 0.87 (s, 9H), 0.84 (s, 9H), 0.09 (s, 3H), 0.07 (s, 3H), 0.04 (s, 3H), 0.03 (s, 3H), −0.04 (s, 3H), −0.06 (s, 3H); HRMS (FAB) m/z calcd for $C_{31}H_{65}O_6N_4Si_3$ ($[M + H]^+$) 615.3681, found 615.3662.

4-Ethoxy-6-(*N*-*tert*-butylhydroxylamino)-1-(2′,3′,5′-tris-*O*-*tert*-butyldimethylsilyl)-2(1*H*)-pyrimidine (15). To a solution of LDA in THF (14 mL), prepared from *n*-BuLi (10.3 mL, 16.1 mmol) in hexane and diisopropylamine (2.26 mL, 16.1 mmol), was added a solution of **14** (2.47 g, 4.02 mmol) in 27 mL of THF at −78 °C under an Ar atmosphere, and the mixture was stirred for 1 h at −70 °C. A solution of MNP (1.75 g, 20.1 mmol) in 18 mL of THF was added dropwise, and the mixture was stirred for 3 h. A saturated NH_4Cl solution was added to the reaction mixture, and the organic layer was extracted with AcOEt, dried over Na_2SO_4, and concentrated under reduced pressure. The residue was purified by silica gel chromatography (AcOEt:hexane = 1:30) to give **15** (1.49 g, 53%) as a colorless oil: ^{1}H NMR (270 MHz, $CDCl_3$) δ 6.39 (d, J = 6.9 Hz, 1H), 6.35 (br, 1H), 5.96 (s, 1H), 5.28 (m, 1H), 4.45−4.35 (m, 2H), 4.29−4.26 (m, 1H), 4.00−3.90 (m, 2H), 3.89−3.69 (m, 1H), 1.34 (t, J = 7.3 Hz, 3H), 1.27 (s, 9H), 0.94 (s, 9H), 0.89 (s, 9H), 0.84 (s, 9H), 0.13 (s, 3H), 0.10 (s, 3H), 0.06 (s, 3H), 0.05 (s, 3H), 0.02 (s, 3H), −0.06 (s, 3H); HRMS (FAB) m/z calcd for $C_{33}H_{68}O_7N_3Si_3$ ($[M + H]^+$) 702.4365, found 702.4338.

4-Ethoxy-6-(*N*-*tert*-butylhydroxylamine)-1-(β-D-ribofranosyl)pyrimidine (16). To a solution of **15** (210 mg, 0.3 mmol) in 15 mL of dry MeOH was added ammonium fluoride (110.9 mg, 3.0 mmol) under an Ar atmosphere, and the mixture was heated at reflux for 30 min. After cooling, the reaction mixture was concentrated under reduced pressure and the residue was purified by silica gel chromatography ($CHCl_3$:MeOH = 7:1) to give **16** (178 mg, 75%) as a colorless solid: ^{1}H NMR (270 MHz, CD_3OD) δ 6.49 (d, J = 2.6 Hz, 1H), 6.08 (s, 1H), 4.61 (dd, J = 6.6, 3.0 Hz, 1H), 4.46 (t, J = 6.4 Hz, 1H), 4.39 (q, J = 6.9 Hz, 2H), 3.90−3.82 (m, 2H), 3.79−3.65 (m, 1H), 1.34 (t, J = 6.9 Hz, 2H), 1.24 (s, 9H); HRMS (FAB) m/z calcd for $C_{15}H_{26}O_7N_3$ ($[M + H]^+$) 360.1771, found 360.1820.

6-(*N*-*tert*-Butylhydroxylamino)-2′,3′,5′-tris-*O*-*tert*-butyldimethylsilylcytidine (17). Ammonia was bubbled into a solution of **15** (168 mg, 0.24 mmol) in 4 mL of MeOH at 0 °C for 15 min, and the mixture was heated at 100 °C in a sealed tube. After 48 h, the mixture was cooled to room temperature and concentrated under reduced pressure. The residue was

purified by silica gel chromatography (AcOEt) to give **17** (10 mg, 6% isolated yield; 33% conversion yield) as a colorless solid: ^{1}H NMR (270 MHz, $CDCl_3$) δ 6.51 (br, 1H), 6.37 (d, J = 6.9 Hz, 1H), 5.80 (s, 1H), 5.32−5.26 (m, 2H), 4.25 (d, J = 3.3 Hz, 1H), 3.98−3.86 (m, 2H), 3.74−3.72 (m, 1H), 1.26 (s, 9H), 0.93 (s, 9H), 0.89 (s, 9H), 0.85 (s, 9H), 0.13 (s, 3H), 0.10 (s, 3H), 0.08 (s, 3H), 0.05 (s, 3H), 0.03 (s, 3H), −0.04 (s, 3H); HRMS (FAB) m/z calcd for $C_{31}H_{65}O_6N_4Si_3$ ([M + H]$^+$) 673.4242, found 673.4219.

***N*-Benzoyl-2′,3′,5′-tris-*O*-*tert*-butyldimethylsilylcytidine (18).** Compound **18** was prepared according to Ogilvie's procedure.[16] ^{1}H NMR (270 MHz, $CDCl_3$) δ 8.71 (br, 1H), 8.60 (d, J = 7.26 Hz, 1H), 7.91 (d, J = 7.26 Hz, 2H), 7.47−7.63 (m, 4H), 4.04−4.17 (m, 4H), 3.82 (d, J = 10.89 Hz, 1H), 0.99 (s, 9H), 0.92 (s, 9H), 0.90 (s, 9H), 0.23 (s, 3H), 0.17 (s, 3H), 0.15 (s, 3H), 0.12 (s, 3H), 0.09 (s, 3H), 0.04 (s, 3H); HRMS(FAB) m/z calcd for $C_{34}H_{60}O_6N_3Si_3$([M + H]$^+$) 690.3790, found 690.3792.

6-(*N*-*tert*-Butylhydroxylamino)-*N*-benzoyl-2′,3′,5′-tris-*O*-*tert*-butyldimethylsilylcytidine (19). To a solution of LDA in THF (5.6 mL), prepared from *n*-BuLi (hexane solution; 6.2 mL, 9.68 mmol) and diisopropylamine (1.36 mL, 9.68 mmol), was added a solution of **18** (530 mg, 1.61 mmol) in 11 mL of THF at −78 °C under an Ar atmosphere, and the mixture was stirred for 1 h at −70 °C. A solution of MNP (842 mg, 9.68 mmol) in 8 mL of THF was added dropwise, and the mixture was stirred for 3 h. A saturated NH_4Cl solution was added to the reaction mixture, and the organic layer was extracted with AcOEt, dried over Na_2SO_4, and concentrated under reduced pressure. The residue was purified by silica gel chromatography (AcOEt:hexane = 1:2) to give **15** (263 mg, 48%) as a colorless solid: ^{1}H NMR (270 MHz, $CDCl_3$) δ 7.92−7.89 (m, 2H),7.69−7.48 (m, 4H), 6.38 (m, 2H), 5.26 (m, 1H), 4.32 (m, 1H), 3.94 (m, 2H), 3.73 (m, 1H), 1.37 (s, 9H), 0.94 (s, 9H). 0.90 (s, 9H), 0.86 (s, 9H), 0.14 (s, 3H), 0.11 (s, 3H), 0.08 (s, 3H), 0.07 (s, 3H), 0.04 (s, 3H), −0.05 (s, 3H); HRMS(FAB) m/z calcd for $C_{38}H_{69}N_4O_7Si_3$ ([M + H]$^+$).

17 from 19. Ammonia was bubbled into a solution of **19** (207 mg, 0.26 mmol) in 19 mL of MeOH at 0 °C for 15 min, and the mixture was heated at 65 °C in a sealed tube. After 6 h, the mixture was cooled to room temperature and concentrated under reduced pressure. The residue was purified by silica gel chromatography (AcOEt) to give **17** (175 mg, 98%).

6-(*N*-*tert*-Butylhydroxylamino)cytidine (20). To a solution of **17** (78.3 mg, 0.12 mmol) in dry MeOH (1.9 mL) was added ammonium fluoride (15 mg, 0.4 mmol), and the mixture was heated at reflux for 3.5 h. After cooling, the reaction mixture was concentrated under reduced pressure and the residue was purified by silica gel chromatography ($CHCl_3$:MeOH = 4:1) to give **20** (20 mg, 52%) as a colorless solid: ^{1}H NMR (270 MHz, DMSO-d_6) δ 8.97 (s, 1H, D_2O exchanged), 7.27−7.16 (m, 2H, D_2O exchanged), 6.23 (d, J = 2.0 Hz, 1H), 5.79 (s, 1H), 4.37 (m, 1H), 4.30 (m, 1H), 3.61−3.57 (m, 2H, D_2O exchanged), 3.38−3.34 (m, 4H, 1H was exchanged with D_2O), 1.13 (s, 9H), (D_2O) δ 6.50 (d, J = 3.1 Hz, 1H), 6.10 (s, 1H), 4.68 (dd, J = 6.7, 3.1 Hz, 1H), 4.43 (t, J = 6.7 Hz, 1H), 3.93−3.84 (m, 2H), 3.72 (dd, J = 12.2, 6.1 Hz, 1H), 1.22 (s, 9H); HRMS (FAB) m/z calcd for $C_{13}H_{23}N_4O_6$ ([M + H]$^+$) 332.1458, found 332.1459.

5-(*N*-*tert*-Butylhydroxylamino)-2′,3′,5′-tris-*O*-*tert*-butyldimethylsilyluridine (22). A mixture of *sec*-BuLi in THF (1.8 mL, 1.85 mmol) and TMEDA (0.28 mL, 1.85 mmol) in 6 mL of THF was stirred at −78 °C for 30 min. A solution of **21**[12c] (433 mg, 0.74 mmol) in 3 mL of THF was added dropwise at −70 °C. After 2 h, a solution of MNP (385 mg, 4.43 mmol) in 2 mL of THF was added to the reaction mixture and stirred for 2.5 h. The reaction was quenched by addition of a saturated aqueous NH_4Cl solution, and the organic layer was extracted with AcOEt, dried over Na_2SO_4, and concentrated under reduced pressure. The residue was purified by silica gel chromatography (AcOEt:hexane = 1:1) to give **22** (95 mg, 19% yield, 45% isolated yield) as a colorless oil: ^{1}H NMR (270 MHz, $CDCl_3$) δ 8.95 (brs, 1H, D_2O exchanged), 7.61 (s, 1H), 7.02 (br, 1H, D_2O exchanged), 5.97 (d, J = 7.3 Hz, 1H), 4.17−4.12 (m, 1H), 4.06−4.01 (m, 2H), 3.80 (dd, J = 8.6, 3.0 Hz, 1H), 3.73 (dd, J = 8.6, 3.0 Hz, 1H), 1.18 (s, 9H), 0.97 (s, 9H), 0.93 (s, 9H), 0.89 (s, 9H), 0.15 (s, 3H), 0.12 (s, 3H), 0.09 (s, 3H), 0.06 (s, 3H), 0.02 (s, 3H), −0.02 (s, 3H); HRMS (FAB) m/z calcd for $C_{31}H_{63}O_7N_3Na$ ([M + Na]$^+$) 696.3872, found 696.3892.

5-*N*-*tert*-Butylhydroxylaminouridine (23). To a solution of **22** (79 mg, 0.12 mmol) in 5 mL of THF was added TBAF (1 M solution in THF; 0.41 mL, 0.41 mmol), and the mixture was stirred at room temperature for 30 min. The reaction mixture was concentrated under reduced pressure, and the residue was purified by silica gel chromatography ($CHCl_3$:MeOH = 7:1) to give **23** (35 mg, 90%) as a colorless oil: ^{1}H NMR (270 MHz, DMSO-d_6) δ 8.27 (s, 1H, D_2O exchanged), 7.90 (s, 1H), 5.81 (d, J = 5.9 Hz, 1H), 5.37 (d, J = 5.9 Hz, 1H, D_2O exchanged), 5.09 (d, J = 4.6 Hz, 1H, D_2O exchanged), 5.03 (t, J = 4.5 Hz, 1H, D_2O exchanged), 4.01 (m, 1H), 3.95 (m, 1H), 3.86 (m, 1H), 3.51 (m, 2H), 1.03 (s, 9H), (D_2O) δ 8.06 (s, 1H), 5.95 (d, J = 4.3 Hz, 1H), 4.32 (t, J = 4.3 Hz, 1H), 4.22 (t, J = 5.5 Hz, 1H), 4.15 (m, 1H), 3.92 (dd, J = 12.8, 2.4 Hz, 1H), 3.80 (dd, J = 12.8, 3.4 Hz, 1H), 1.12 (s, 9H); HRMS(FAB) m/z calcd for $C_{13}H_{22}O_7N_3$ [M + H]$^+$) 332.1458, found 332.1463.

EPR Study of Aminoxyl Radicals. To toluene or CH_2Cl_2 containing a 1% MeOH solution of the appropriate hydroxylamine was added Ag_2O, and the mixture was left at room temperature. After 1 h, the supernatant liquid was taken from the reaction mixture and filtered. The solution containing the aminoxyl radical was placed in a 5 mm o.d. quartz tubes, degassed by three freeze-and-thaw cycles, and sealed. EPR spectra were taken at room temperature.

Simulation of EPR Fine Structure. The simulation of the EPR fine structure was performed by using Winsim program from P.E.S.T., Public EPR Software Tools, provided by National Institute of Environmental Health Science, National Institutes of Health. The line width of 0.5 G was used for simulation.

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Supporting Information Available: EPR spectra and simulated spectra of **1c**, **1d**, and **8a**−**d**. ^{1}H NMR charts of **6a**−**d**, **7a**−**d**, **10**, **11**, **12**, **13**, **15**, **16**, **17**, **19**, **20**, **22**, and **23**. X-ray crystallographic data for **10**. This material is available free of charge via the Internet at http://pubs.acs.org.

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