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Synthesis and Fluorescent Properties of Bi- and Tricyclic 4-*N*-Carbamoyldeoxycytidine Derivatives

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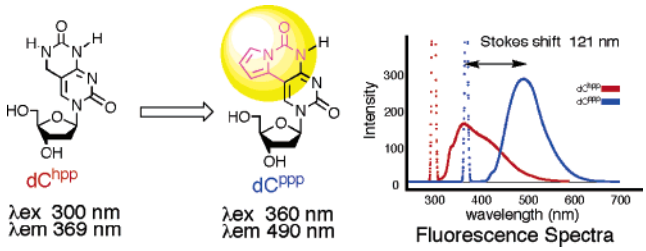

New bi- and tricyclic deoxycytidine derivatives (dC^{hpd}, dC^{mpp}, dC^{tpp}, dC^{ppp}) were synthesized as analogues of a fluorescent nucleoside, dC^{hpp}, previously reported. The carbamoyl group of dC^{hpd} and the 5-position of the cytosine ring are bridged via an ethylene linker so that the modified group forms a nonplanar structure with the cytosine ring. The fluorescent study of dC^{hpd} indicated that the coplanar structure between the carbamoyl group and the cytosine ring is of importance. *N*-Methylation of the carbamoyl group (dC^{mpp}) weakened the intensity of the fluorescence of dC^{hpp}, and the derivative (dC^{tpp}), which had a thiocarbamoyl group, lost its fluorescent property. Moreover, addition of a pyrrolo-ring (dC^{ppp}) to dC^{hpp} enhanced the intensity of fluorescence, and an emission light was observed with a marked Stokes shift of 120 nm.

Introduction

A number of base-modified nucleoside derivatives that can emit fluorescent light have been reported to date. Oligonucleotides incorporating such fluorescent nucleosides have been synthesized and utilized as bioprobes for various studies on detection, structural analysis, and location of DNA/RNA molecules.[1−12]

Recently, we have studied the synthesis and properties of a series of 4-*N*-carbamoyldeoxycytidine derivatives.[13,14] As the result, it was found that a geometrically unlocked 4-*N*-carbamoyldeoxycytidine derivative [dC^{cmy} (**1**)][13,14] and its 4-*N*-(*N*-alkylcarbamoyl) derivatives were nonfluorescent. In contrast, a conformationally locked bicyclic nucleoside derivative [dC^{hpp} (**2**)][13,14] was found to emit strong fluorescence at 360 nm upon UV irradiation at 300 nm.[15] Furthermore, dC^{hpp} incorporating into oligodeoxynucleotides showed base-discriminating properties, namely, that the fluorescence of dC^{hpp} (**2**) was greatly suppressed when a dC^{hpp}−dG base pair was formed; the fluorescence was maintained when a dC^{hpp}−dA base pair was formed.[15] These properties were similar to those of benzopyridopyrimidine (BPP) nucleoside derivatives reported by Okamoto et al.[3k,m] However, it is interesting that the conjugated

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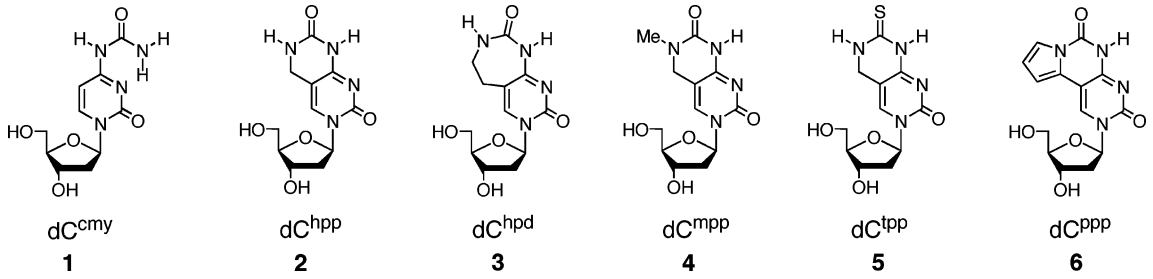
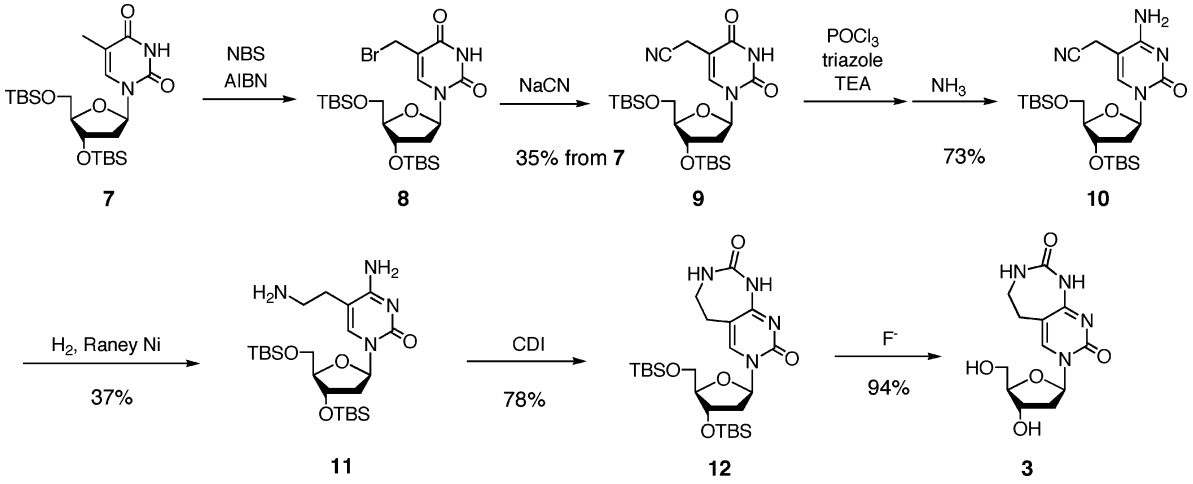


FIGURE 1. Chemical structures of dC^{cmy} (**1**), dC^{hpp} (**2**), dC^{hpd} (**3**), dC^{mpp} (**4**), dC^{tpp} (**5**), and dC^{ppp} (**6**).

SCHEME 1. Synthesis of dC^{hpd} (3)



system of the cytosine ring of dC^{hpp} (**2**) is much smaller than those of BPP derivatives.

In this paper, we report the synthesis and fluorescent properties of new bicyclic [dC^{hpd} (**3**), dC^{mpp} (**4**), dC^{tpp} (**5**)] and tricyclic [dC^{ppp} (**6**)] 4-*N*-carbamoyldeoxycytidine derivatives. The chemical structures of these derivatives are shown in Figure 1. Among them, we found that dC^{ppp} (**6**) exhibited intriguing fluorescent properties with a sharp emission maximum at 490 nm and a significant Stokes shift of 120 nm when excited at 369 nm (UV absorption maximum).

Results and Discussion

The carbamoyl group of dC^{hpd} (**3**) and the 5-position of the cytosine ring were bridged via an ethylene linker so that the plane of the carbamoyl group is slightly twisted toward that of the cytosine ring. dC^{mpp} (**4**) is an *N*-methylated analogue of dC^{hpp} (**2**), and dC^{tpp} (**5**) is a thiocarbamoylated derivative corresponding to dC^{hpp} (**2**). dC^{ppp} (**6**) was designed to enhance the intensity and improve the visibility of the fluorescent properties by expansion of the conjugated system of dC^{hpp} (**2**).

The synthesis of dC^{hpd} (**3**) is outlined in Scheme 1. Radical bromination[16] of a 3′,5′-*O*-protected thymidine derivative (**7**) followed by treatment with NaCN gave the 5-cyanomethyl-T derivative **9** in 35% yield. The reaction of compound **9** with $POCl_3$−1*H*-1,2,4-triazole−Et_3N[17] followed by ammonia treatment gave the cytidine derivative **10** in 73% yield. Raney Ni-catalyzed hydrogenation of compound **10** gave the diamine **11**

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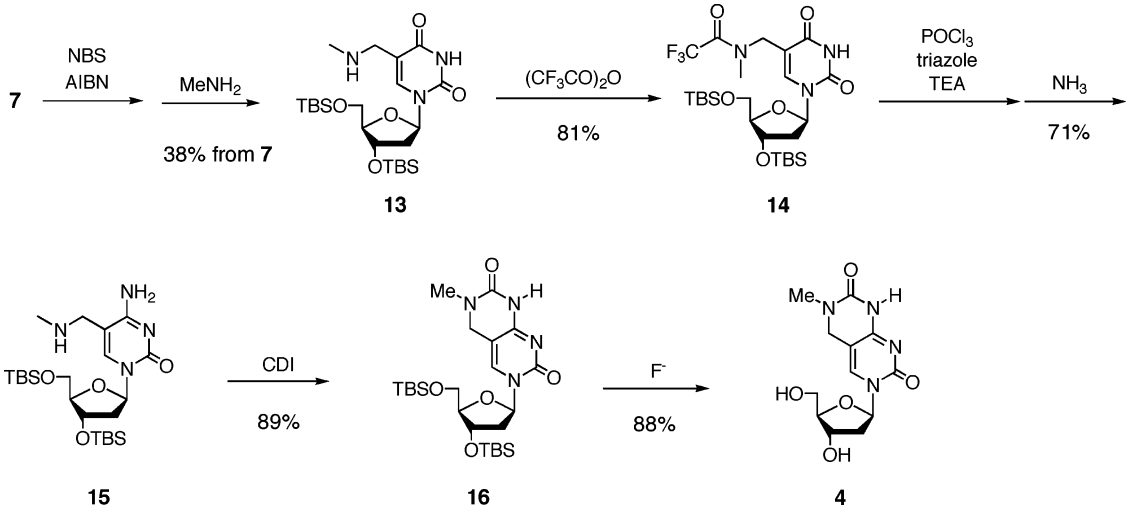
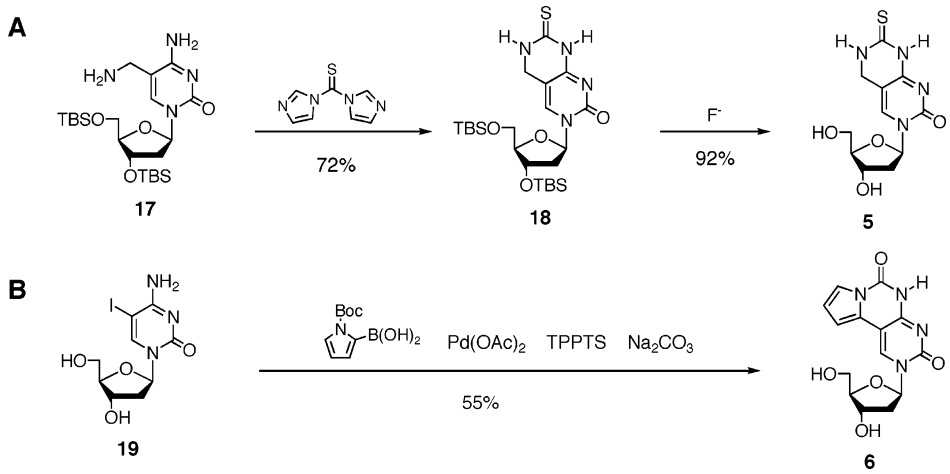
SCHEME 2. Synthesis of dC^{mpp} (4)



SCHEME 3. Synthesis of dC^{tpp} (5) and dC^{ppp} (6)



in 37% yield. Intramolecular cyclization of compound **11** with 1,1′-carbonyldiimidazole afforded the bicyclic derivative **12** in 78% yield. The desilylation of compound **12** with $Et_3N\cdot3HF$ gave dC^{hpd} (**3**) in 94% yield.

The synthesis of dC^{mpp} (**4**) is shown in Scheme 2.The in situ treatment of the intermediate **8** with methylamine gave the 5-methylaminomethyl-T derivative **13** in 38% yield. The acylation of **13** gave the *N*-trifluoroacetylated compound **14** in 81% yield. Compound **14** was converted to a cytidine derivative (**15**) in 71% yield via a two-step reaction. Intramolecular cyclization of compound **15** by use of 1,1′-carbonylimidazole afforded the cyclic compound **16** in 89% yield. Finally, removal of the TBS groups with TBAF gave the desired compound dC^{mpp} (**4**) in 88% yield.

The synthesis of dC^{tpp} (**5**) is shown in Scheme 3A. The 5-aminomethyldeoxycytidine derivative **17**[13,14] was cyclized with 1,1′-thiocarbonyldiimidazole to afford the bicyclic derivative **18** in 72% yield. Deprotection of the silyl groups with TEA·3HF gave the desired compound **5** in 92% yield. The synthesis of dC^{ppp} (**6**) is shown in Scheme 3B. The Suzuki−Miyaura coupling of 5-iodododeoxycytidine with *N*-BOC-pyrrol-2-ylboronic acid in the presence of TPPTS as a catalyst gave directly the tricyclic product **6** in 55% yield. It should be noted that the intramolecular cyclization simultaneously proceeded with elimination of *t*-BuOH.

The UV absorption spectra of compounds **1**−**5** in phosphate buffer (pH 7.0) are shown in Figures 2A and 3A. The spectrum of dC^{hpp} (**2**), which is different from those of dC and dC^{cmy} (**1**), exhibited an interesting absorption at 300 nm, which is the λ_{max} of the excitation spectra of dC^{hpp} (**2**). The UV absorption spectra of dC^{hpd} (**3**) and dC^{mpp} (**4**) maintained the absorption at 300 nm, so fluorescence properties similar to those of dC^{hpp} (**2**) were expected. In contrast, the UV absorption spectrum of dC^{tpp} (**5**), which has a broad band around 260−330 nm, was different from those of dC^{hpp} (**2**), dC^{hpd} (**3**), and dC^{mpp} (**4**). The excitation spectra (data not shown) of dC^{hpp} (**2**), dC^{hpd}, and dC^{mpp} (**4**) showed similar λ_{max} values at 300 nm; dC^{tpp} (**5**) was found to be a nonemissive nucleoside. The fluorescent spectra of dC^{hpp} (**2**), dC^{hpd} (**3**), and dC^{mpp} (**4**) excited at 300 nm are shown in

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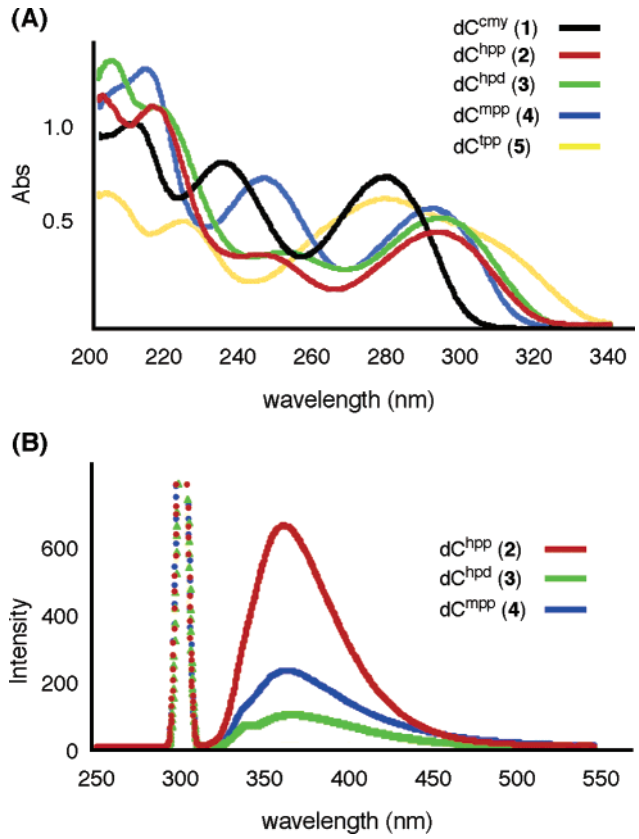
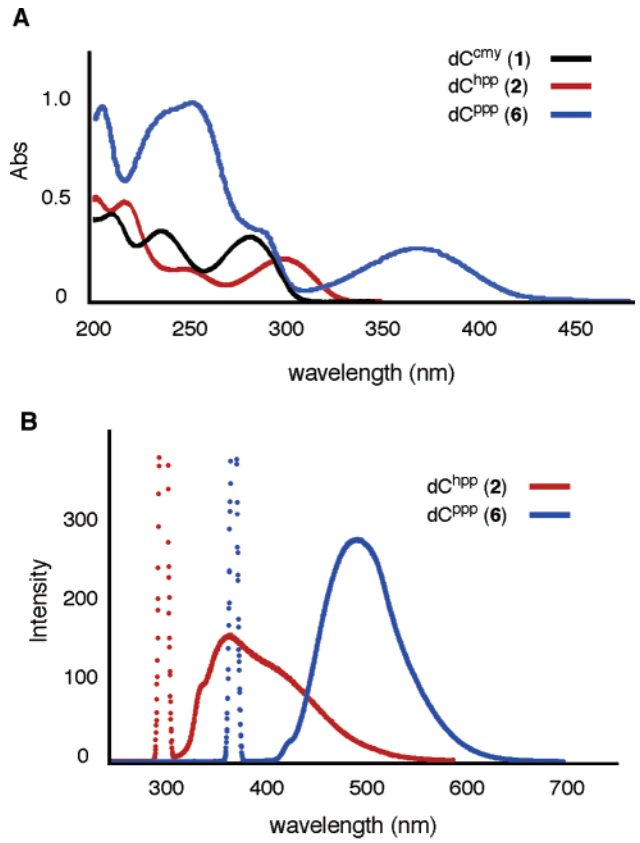


FIGURE 2. (A) UV absorption spectra of dC^{cmy} (**1**), dC^{hpp} (**2**), dC^{mpp} (**3**), dC^{hpd} (**4**), and dC^{tpp} (**5**): 80 μM nucleoside, 10 mM sodium phosphate, pH 7.0. (B) Fluorescence spectra of dC^{hpp} (**2**), dC^{hpd} (**3**), and dC^{mpp} (**4**): 5 μM nucleoside, 10 mM sodium phosphate, pH 7.0. Excitation was performed at 300 nm.



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	dC^{hpp}	dC^{ppp}
excitation	300 nm	369 nm
emission	360 nm	490 nm
Stokes' shift	60 nm	121 nm

FIGURE 3. (A) UV absorption spectra of dC^{cmy} (**1**), dC^{hpp} (**2**), and dC^{ppp} (**6**): 40 μM nucleoside, 10 mM sodium phosphate, pH 7.0. (B) Fluorescense spectra of dC^{cmy} (**1**) and dC^{ppp} (**6**): 1 μM nucleoside, 10 mM sodium phosphate, pH 7.0 (dC^{hpp}, excitation at 300 nm; dC^{ppp} (**6**), excitation at 369 nm). (C) λ_{max} for excitation and emission spectra of dC^{hpp} (**2**) and dC^{ppp} (**6**) and their Stokes shifts are summarized.

Figure 2B. The spectra of dC^{mpp} (**4**) and dC^{hpd} (**3**) had λ_{max} values at 360 nm, which were similar to that of dC^{hpp} (**2**). These results suggest that the chromophores of these fluorescent nucleosides are common to that of dC^{hpp} (**2**).

In contrast to these results, it was found that dC^{ppp} (**6**) exhibited unique and fascinating behavior as a fluorescent molecule. To our surprise, this modified deoxynucleoside has a λ_{max} at 369 nm in its UV absorption spectrum and a sharp and strong emission at 490 nm upon excitation at 369 nm. The Stokes shift was evaluated to be 121 nm. Such a remarkable shift has not been reported among the modified nucleoside derivatives reported up to now. This excitation fluorescent property is useful for various studies that require clear-cut differences between wavelengths for excitation and emission. Fortunately, a convenient apparatus that generates a laser beam at 370 nm is now commercially available. The quantum yield of dC^{ppp} (**6**) was calculated to be 0.11 with an ϵ value of 4.76 $\times$ 10^3 in 10 mM sodium phosphate (pH 7.0). This quantum yield is similar to that of dC^{hpp} (**2**) (0.12) reported previously.[15] Therefore, dC^{ppp} (**6**) proved to show emission spectra with a preferable Stokes shift, keeping the quantum yield at the same level.

Several fluorescent cytosine derivatives have been reported to date. For example, benzopyridopyrimidine (BPP) was reported as a fluorescent cytosine base by Inoue H. This base exhibited emission at 388 nm upon excitation at 345 nm. Therefore, the Stokes shift was very small (43 nm). 4-Amino-1*H*-benzo[*g*]quinazoline-2-one, a cytosine derivative having a naphthalene ring attached to the 5,6 double bond, was reported by Goddle et al. This base has a fluorescent property of emission at 456 nm when excited at 260 nm. The Stokes shift was smaller (96 nm) than that of our compound. Recently, Okamoto reported naphthopyridopyrimidine (NPP), which fluoresced at 432−452 nm upon excitation at 364 nm. As exemplified by these precedents, it should be noted that dC^{ppp} (**6**) possessed an extraordinarily large Stokes shift as well as a great shift of emission wavelength to 490 nm.

Conclusion

In this study, it was suggested that the fluorescent property of C^{hpp} (**2**) arises from the coplanarity of the cytosine molecule as well as the presence of the carbonyl group. Addition of a pyrrolo-ring to C^{hpp} (**2**) resulted in a significant red shift of λ_{max} in its UV spectrum and a strong emission peak at 490 nm with a marked Stokes shift of 120 nm. These promising properties of dC^{ppp} (**6**) would be useful for bioscience and biotechnology based on the use of fluorescent-labeled oligonucleotides.

Experimental Section

3′,5′-*O*-Bis(*tert*-butyldimethylsilyl)-5-cyanomethyldeoxyuridine (9). Compound **7** (9.1 g, 19.4 mmol) was rendered anhydrous by repeated coevaporation with dry CH_3CN and finally dissolved in dry CCl_4 (100 mL). To the solution were added NBS (4.13 g, 23.2 mmol) and AIBN (164 mg, 1 mmol), and the resulting mixture was refluxed for 1 h. After cooling to room temperature, more NBS (690 mg, 3.88 mmol) and AIBN (100 mg, 0.61 mmol) were added, and the mixture was refluxed for 1 h. The mixture was diluted with $CHCl_3$ (150 mL), and the mixture was washed three times with brine (200 mL). The organic layer was collected, dried over Na_2SO_4, filtered off, and evaporated under reduced pressure. The residue was rendered anhydrous by repeated coevaporation with dry CH_3CN and finally dissolved in dry DMF (100 mL). To the solution was added NaCN (1.4 g, 29 mmol), and the resulting mixture was stirred at room temperature for 30 min. The residue was diluted with ethyl acetate (200 mL), and the mixture was washed with H_2O (300 mL) and saturated $NaHCO_3$ (200 mL). The organic layer was collected, dried over Na_2SO_4, filtered off, and evaporated under reduced pressure. The crude product was purified by NH silica gel chromatography with hexane−ethyl acetate to give the product **9** (3.34 g, 35%): ^{1}H NMR ($CDCl_3$) δ 0.07−0.10 (12H, m), 0.82−0.96 (18H, m), 1.96−2.06 (1H, m), 2.32−2.41 (1H, m), 3.41 (2H, s), 3.75−3.87 (2H, m), 3.96−4.03 (1H, m), 4.38−4.42 (1H, m), 6.26 (1H, dd, J = 6.0 Hz, J = 5.6 Hz), 7.78 (1H, s), 9.68 (1H, br); ^{13}C NMR ($CDCl_3$) δ −5.5, −5.4, −4.9, −4.7, 15.7, 17.9, 18.3, 25.7, 25.9, 41.6, 63.0, 72.5, 86.0, 88.3, 104.5, 116.2, 137.9, 149.8, 162.1. HRMS (ESI), m/z (M + H): calcd for $C_{23}H_{42}N_3O_5Si_2^+$, 496.2663; found, 496.2668.

3′,5′-*O*-Bis(*tert*-butyldimethylsilyl)-5-cyanomethyldeoxycytidine (10). 1*H*-1,2,4-Triazole (3.73 g, 54 mmol) was suspended in MeCN (30 mL). Phosphoryl chloride (1.1 mL, 12 mmol) was added dropwise at 0 °C, and the mixture was stirred for 10 min at 0 °C. After addition of triethylamine (7.5 mL, 54 mmol) over 15 min, the mixture was stirred at 0 °C for another 30 min. Compound **9** (1.98 g, 4.0 mmol) in MeCN (30 mL) was added, and the solution was stirred at room temperature for 2 h. To the mixture was added H_2O (10 mL), and the mixture was evaporated under reduced pressure. The residue was diluted with $CHCl_3$ (200 mL), and the mixture was washed with brine and saturated $NaHCO_3$. The organic layer was collected, dried over Na_2SO_4, filtered off, and evaporated under reduced pressure. The residue was dissolved in pyridine−NH_3(aq) (1:1, v/v, 50 mL). After being stirred for 3 h, the solution was evaporated under reduced pressure. The residue was diluted with $CHCl_3$ (100 mL), and the mixture was washed three times with saturated $NaHCO_3$ (100 mL). The organic layer was collected, dried over Na_2SO_4, filtered off, and evaporated under reduced pressure. The crude product was purified by C200 silica gel chromatography with CH_3Cl−MeOH to give the product **10** (1.44 g, 73%): ^{1}H NMR ($CDCl_3$) δ 0.04−0.78 (12H, m), 0.82−0.91 (18H, m), 1.88−1.98 (1H, m), 2.37−2.46 (1H, m), 3.48 (2H, s), 3.75−3.82 (2H, m), 3.93−3.99 (1H, m), 4.31−4.36 (1H, m), 6.20 (1H, dd, J = 6.6 Hz, J = 6.3 Hz), 7.17 (2H, br), 7.75 (1H, s); ^{13}C NMR ($CDCl_3$) δ −5.4, −5.4, −4.9, −4.6, 17.2, 18.0, 18.4, 25.7, 25.9, 42.1, 62.9, 72.1, 86.6, 88.1, 95.6, 115.9, 140.3, 155.4, 163.7. HRMS (ESI), m/z (M + H): calcd for $C_{23}H_{43}N_4O_4Si_2^+$, 495.2823; found, 495.2821.

3′,5′-*O*-Bis(*tert*-butyldimethylsilyl)-5-(2-aminoethyl)deoxycytidine (11). Compound **10** (247 mg, 0.5 mmol) was dissolved in *i*PrOH (10 mL). To the solution was added Raney Ni (Aldrich, 25 mg, wet), and the resulting mixture was vigorously stirred under H_2 gas for 4 h at 40 °C. The mixture was filtered using a celite bed, which was then repeatedly washed with boiling EtOH. The mixture was evaporated under reduced pressure, and the crude product was purified by recycling preparative HPLC with CH_3Cl−MeOH (4:1, v/v) containing 0.5% TEA to give the product **11** (94 mg, 37%): ^{1}H NMR ($CDCl_3$) δ 0.01−0.46 (12H, m), 0.82−0.88 (18H, m), 1.83−1.95 (1H, m), 2.26−2.38 (1H, m), 2.0−2.48 (2H, br), 2.83−2.92 (2H, br), 3.69−3.82 (2H, m), 3.83−3.88 (1H, m), 4.29−4.36 (1H, m), 6.23 (1H, dd, J = 6.6 Hz, J = 6.3 Hz), 7.39 (1H, s); ^{13}C NMR ($CDCl_3$) δ −5.36, −5.3, −4.8, −4.6, 18.0, 18.4, 25.7, 25.9, 31.6, 40.9, 41.9, 62.8, 71.9, 85.5, 87.3, 105.0, 138.3, 155.7, 165.4. HRMS (ESI), m/z (M + H): calcd for $C_{23}H_{47}N_4O_4Si_2^+$, 499.3136; found, 499.3135.

3-[3,5-*O*-Bis(*tert*-butyldimethylsilyl)-2-deoxy-β-D-ribofuranosyl)]-6,7-dihydro-3*H*-pyrimido[4,5-*d*][1,3]diazepine-2,8(5*H*,9*H*)-dione (12). Compound **11** (90 mg, 0.18 mmol) was rendered anhydrous by repeated coevaporation with dry CH_3CN and finally dissolved in dry DMF (18 mL). To the solution was added CDI (33 mg, 0.2 mmol), and the resulting mixture was stirred at room temperature for 1 h and then at 60 °C for 3 h. The mixture was diluted with ethyl acetate (10 mL), and the mixture was washed with H_2O (60 mL) and saturated $NaHCO_3$ (60 mL). The organic layer was collected, dried over Na_2SO_4, filtered off, and evaporated under reduced pressure. The crude product was purified by recycling preparative HPLC with MeOH to give the product **12** (74 mg, 78%): ^{1}H NMR ($CDCl_3$) δ 0.04−0.80 (12H, m), 0.86−0.89 (18H, m), 1.93−2.04 (1H, m), 2.46−2.53 (1H, m), 2.71−2.78 (2H, m), 3.40−3.43 (2H, m), 3.72−3.95 (2H, m), 3.96−3.98 (1H, m), 4.32−4.38 (1H, m), 6.24 (1H, dd, J = 6.3 Hz, J = 5.6 Hz), 7.86 (1H, s), 8.24 (1H, br), 9.22 (1H, br); ^{13}C NMR (DMSO-d_6) δ −5.3, −5.3, −4.8, −4.6, 25.8, 25.9, 30.8, 40.6, 62.9, 72.4, 85.8, 87.5, 105.6, 142.2, 154.1, 154.5, 161.0. HRMS (ESI), m/z (M + H): calcd for $C_{24}H_{45}N_4O_5Si_2^+$, 525.2928; found, 525.2927.

3-[2-Deoxy-β-D-ribofuranosyl)]-6,7-dihydro-3*H*-pyrimido[4,5-*d*][1,3]diazepine-2,8(5*H*,9*H*)-dione (3). Compound **12** (40 mg, 76 μmol) was rendered anhydrous by repeated coevaporation with dry CH_3CN and finally dissolved in dry THF−CH_2Cl_2 (4:1, v/v, 1 mL). To the solution was added TEA·3HF (65 μL, 0.4 mmol), and the resulting mixture was stirred at 50 °C for 2 h. The mixture was diluted with H_2O (1 mL) and coevaporated with EtOH under reduced pressure. The resulting precipitate was collected by filtration, washed with cold EtOH, and dried in vacuo to give the product **3** (21 mg, 94%): ^{1}H NMR (DMSO-d_6) δ 1.93−2.09 (1H, m), 2.18−2.29 (1H, m), 2.75−2.81 (2H, br), 3.40−3.51 (2H, br), 3.63−3.72 (2H, m), 3.75−3.82 (1H, m), 4.19−4.22 (1H, m), 5.03 (1H, br), 5.30 (1H, br), 6.19 (1H, dd, J = 6.2 Hz, J = 6.2 Hz), 7.18 (1H, br), 7.89 (1H, s), 9.34 (1H, br); ^{13}C NMR (DMSO-d_6) δ 30.7, 40.9, 61.1, 70.0, 85.7, 87.8, 105.5, 142.8, 154.2, 154.6, 160.9. HRMS (ESI), m/z (M + H): calcd for $C_{12}H_{17}N_4O_5^+$, 297.1199; found, 297.1194.

3′,5′-*O*-Bis(*tert*-butyldimethylsilyl)-5-methylaminomethyldeoxyuridine (13). Compound **7** (4.7 g, 10 mmol) was rendered anhydrous by repeated coevaporation with dry CH_3CN and finally dissolved in dry CCl_4 (50 mL). To the solution were added NBS (1.96 g, 11 mmol) and AIBN (82 mg, 0.5 mmol), and the resulting mixture was refluxed for 1 h. The mixture was diluted with $CHCl_3$ (150 mL), and the mixture was washed three times with brine (200 mL). The organic layer was collected, dried over Na_2SO_4, filtered, and evaporated under reduced pressure. The residue was rendered anhydrous by repeated coevaporation with dry CH_3CN and finally dissolved in dry THF (75 mL). To the solution was added $MeNH_2$ (2.0 M, THF, 25 mL), and the resulting mixture was stirred at room temperature for 10 min. The mixture was evaporated under reduced pressure, and the residue was diluted with $CHCl_3$ (100 mL); the mixture was washed with brine and saturated $NaHCO_3$. The organic layer was collected, dried over Na_2SO_4, filtered off, and evaporated under reduced pressure. The crude product was purified by C200 silica gel chromatography with $CHCl_3$−MeOH to give the product **13** (1.9 g, 38%): ^{1}H NMR ($CDCl_3$) δ 0.05−0.89 (12H, m), 0.87−0.91 (18H, m), 1.93−2.04 (1H, m), 2.19−2.29 (1H, m), 2.36 (3H, s), 3.42 (3H, m), 3.70−3.84 (2H, m), 3.91−3.95 (1H, m), 4.37−4.41 (1H, m), 6.29 (1H, dd, J = 6.0 Hz, J = 6.0 Hz), 7.54 (1H, s); ^{13}C NMR ($CDCl_3$) δ −5.3, −5.2, −4.7, −4.5. 18.1, 18.5, 25.8, 26.1, 35.6. 41.3, 48.5, 63.0, 72.2, 84.9, 87.8, 112.6, 137.0, 149.9, 163.1. HRMS (ESI), m/z (M + H): calcd for $C_{23}H_{46}N_3O_5Si_2^+$, 500.2976; found, 500.2971.

3′,5′-*O*-Bis(*tert*-butyldimethylsilyl)-5-[(*N*-trifluoroacetyl)methylaminomethyl]deoxyuridine (14). Compound **13** (1.63 g, 3.3 mmol) was rendered anhydrous by repeated coevaporation with dry pyridine and finally dissolved in dry pyridine (30 mL). To the solution was added trifluoroacetic acid anhydrate (552 μL, 3.9 mmol), and the resulting mixture was stirred at room temperature for 30 min. The mixture was diluted with $CHCl_3$ (50 mL), and the mixture was washed three times with brine and saturated $NaHCO_3$. The organic layer was collected, dried over Na_2SO_4, filtered off, and evaporated under reduced pressure. The crude product was purified by C200 silica gel chromatography with hexane−$CHCl_3$ to give the product **14** (1.6 g, 81%): ^{1}H NMR ($CDCl_3$) δ 0.06−0.82 (12H, m), 0.87−0.90 (18H, m), 1.96−2.33 (1H, m), 2.25−2.33 (1H, m), 3.28 (3H, s), 3.68−3.80 (2H, m), 3.91−3.95 (1H, m), 4.10−4.27 (2H, m), 4.37−4.42 (1H, m) 6.23 (1H, dd, J = 6.9 Hz, J = 6.6 Hz), 7.81 (1H, s), 9.23 (1H, s); ^{13}C NMR ($CDCl_3$) δ −5.4−5.3, −4.8, −4.6, 18.1, 18.5, 25.8, 25.9, 26.0, 6.7, 36.7, 40.9, 46.2, 63.2, 72.2, 72.6, 85.5, 86.1, 88.0, 88.2, 108.6, 114.1, 118.3, 141.7, 149.7, 156.9, 163.4. HRMS (ESI), m/z (M + H): calcd for $C_{25}H_{45}F_3N_3O_6Si_2^+$, 596.2799; found, 596.2791.

3′,5′-*O*-Bis(*tert*-butyldimethylsilyl)-5-methylaminomethyldeoxycytidine (15). 1*H*-1,2,4-Triazole (2.35 g, 34 mmol) was suspended in CH_3CN (20 mL). Phosphoryl chloride (685 μL, 7.6 mmol) was added dropwise at 0 °C, and the mixture was stirred for 10 min at 0 °C. After addition of triethylamine (4.8 mL, 34 mmol) over 15 min, the mixture was stirred at 0 °C for another 30 min. Compound **14** (1.5 g, 2.52 mmol) in CH_2Cl_2−CH_3CN (1:1, v/v, 20 mL) was added, and the solution was stirred at room temperature for 2 h. To the mixture was added H_2O (10 mL); the mixture was then evaporated under reduced pressure. The residue was diluted with $CHCl_3$ (100 mL), and the mixture was washed with brine and saturated $NaHCO_3$. The organic layer was collected, dried over Na_2SO_4, filtered off, and evaporated under reduced pressure. The residue was dissolved in MeOH saturated with NH_3. After being stirred for 6 h, the solution was evaporated under reduced pressure. The residue was diluted with $CHCl_3$ (100 mL), and the mixture was washed three times with saturated $NaHCO_3$. The organic layer was collected, dried over Na_2SO_4, filtered off, and evaporated under reduced pressure. The crude product was purified by C200 silica gel chromatography with CH_3Cl−MeOH to give the product **15** (890 mg, 71%): ^{1}H NMR ($CDCl_3$) δ 0.02−0.81 (12H, m), 0.84−0.93 (18H, m), 1.87−1.97 (1H, m), 2.34−2.43 (1H, m), 2.37 (3H, s), 3.49 (2H, s), 3.81−3.95 (3H, m), 4.28−4.37 (1H, m), 6.28 (1H, dd, J = 6.6 Hz, J = 6.3 Hz), 6.41 (1H, br), 7.53 (1H, br), 7.57 (1H, s); ^{13}C NMR ($CDCl_3$) δ −5.3, −5.2, −4.8, −4.5, 18.1, 18.5, 25.8, 26.0, 35.4, 42.2, 51.2, 62.8, 71.7, 85.9, 87.5, 103.0, 138.2, 155.6, 165.9. HRMS (ESI), m/z (M + H): calcd for $C_{23}H_{47}N_4O_4Si_2^+$, 499.3136; found, 499.3138.

6-(3,5-*O*-Bis(*tert*-butyldimethylsilyl)-2-deoxy-β-D-ribofuranosyl)-3,4-dihydro-3-methylpyrimido[4,5-*d*]pyrimidine-2,7(1*H*,6*H*)-dione (16). Compound **15** (250 mg, 0.5 mmol) was rendered anhydrous by repeated coevaporation with dry CH_3CN and finally dissolved in dry DMF (50 mL). To the solution was added CDI (90 mg, 0.55 mmol), and the resulting mixture was stirred at room temperature for 1 h and then at 60 °C for 3 h. The mixture was diluted with ethyl acetate (20 mL), and the mixture was washed with H_2O (200 mL) and saturated $NaHCO_3$ (200 mL). The organic layer was collected, dried over Na_2SO_4, filtered off, and evaporated under reduced pressure. The crude product was purified by recycling preparative HPLC with MeOH to give the product **16** (233 mg, 89%): ^{1}H NMR ($CDCl_3$) δ −0.06−0.02 (12H, m), 0.73−0.86 (18H, m), 1.85−1.95 (1H, m), 2.34−2.43 (1H, m), 2.89 (3H, s), 3.62−3.89 (3H, m), 4.03−4.17 (2H, m), 4.20−4.27 (1H, m), 6.11 (1H, dd, J = 6.2 Hz, J = 6.0 Hz), 7.85 (1H, s), 8.45 (1H, br); ^{13}C NMR ($CDCl_3$) δ −5.5, −4.7, 17.8, 18.2, 25.6, 25.8, 34.5, 42.3, 46.5, 62.3, 71,2, 86.9, 87.9, 96.4, 138.1, 151.8, 154.6, 159.6. HRMS (ESI), m/z (M + H): calcd for $C_{24}H_{45}N_4O_5Si_2^+$, 525.2928, found, 525.2931.

6-(2-Deoxy-β-D-ribofuranosyl)-3,4-dihydro-3-methylpyrimido-[4,5-*d*]pyrimidine-2,7(1*H*,6*H*)-dione (4: dC^{mpp}). Compound **16** (110 mg, 0.21 mmol) was rendered anhydrous by repeated coevaporation with dry CH_3CN and finally dissolved in dry THF−CH_2Cl_2 (4:1, v/v, 2 mL). To the solution was added TEA·3HF (171 μL, 1.1 mmol), and the resulting mixture was stirred at 50 °C for 2 h. The mixture was diluted with H_2O (1 mL), and coevaporated with EtOH under reduced pressure. The resulting precipitate was collected by filtration, washed with cold EtOH, and dried in vacuo to give the product **4** (55 mg, 88%): ^{1}H NMR (DMSO-d_6) δ 1.92−2.02 (1H, m), 2.38−2.45 (1H, m), 2.89 (3H, s), 3.58−3.93 (3H, m), 4.12−4.17 (2H, m), 4.31−4.35 (1H, m), 6.20 (1H, dd, J = 6.3 Hz, J = 6.0 Hz), 7.92 (1H, s), 8.96 (1H, br); ^{13}C NMR ($CDCl_3$) δ 34.1, 40.9, 45.7, 61.2, 70.2, 85.9, 87.9, 97.3, 138.9, 152.4, 154.6, 160.6. HRMS (ESI), m/z (M + H): calcd for $C_{12}H_{17}N_4O_5^+$, 297.1199; found, 297.1198.

3-(3,5-*O*-Bis(*tert*-butyldimethylsilyl)-2-deoxy-β-D-ribofuranosyl)-5,6,7,8-tetrahydro-7-thioxopyrimido-[4,5-*d*]pyrimidin-2(3*H*)-one (18). Compound **17** (242 mg, 0.5 mmol) was rendered anhydrous by repeated coevaporation with dry CH_3CN and finally dissolved in dry DMF (30 mL). To the solution was added thiocarbonyl-*N*,*N*-bis(imidazole) (98 mg, 0.55 mmol), and the resulting mixture was stirred at room temperature for 1 h. The mixture was diluted with ethyl acetate (20 mL), and the mixture was washed with H_2O (100 mL) and saturated $NaHCO_3$ (100 mL). The organic layer was collected, dried over Na_2SO_4, filtered off, and evaporated under reduced pressure. The crude product was purified by recycling preparative HPLC with MeOH to give the product **18** (190 mg, 72%): ^{1}H NMR (DMSO-d_6) δ 0.05−0.07 (12H, m), 0.85−0.87 (18H, m), 2.05−2.12 (1H, m), 2.22−2.41 (1H, m), 3.72−3.76 (2H, m), 3.86−3.91 (1H, m), 3.99−4.28 (2H, m), 4.30−4.37 (1H, m), 6.18 (1H, dd, J = 6.2 Hz, J = 6.2 Hz), 7.85 (1H, s), 9.28 (1H, br), 11.05 (1H, br); ^{13}C NMR ($CDCl_3$) δ −5.4, −4.9, 4.6, 17.9, 18.3, 25.7, 25.9, 41.1, 42.4, 62.6, 71.6, 87.2, 88.3, 96.1, 139.2, 154.7, 156.5, 177.7. HRMS (ESI), m/z (M + H): calcd for $C_{23}H_{43}N_4O_4SSi_2^+$, 527.2544; found, 527.2539.

3-(2-Deoxy-β-D-ribofuranosyl)-5,6,7,8-tetrahydro-7-thioxopyrimido-[4,5-*d*]pyrimidin-2(3*H*)-one (5: dC^{tpp}). Compound **18** (160 mg, 0.3 mmol) was rendered anhydrous by repeated coevaporation with dry CH_3CN and finally dissolved in dry THF (3 mL). To the solution was added TEA·3HF (244 μL, 5 mmol), and the resulting mixture was stirred at room temperature for 8 h. The mixture was diluted with H_2O (1 mL) and coevaporated with EtOH under reduced pressure. The resulting precipitate was collected by filtration, washed with cold H_2O, and dried in vacuo to give the product **5** (82 mg, 92%): ^{1}H NMR (DMSO-d_6) δ 1.95−2.03 (1H, m), 2.21−2.50 (1H, m), 3.31−3.42 (2H, m), 3.61−3.68 (1H, m), 3.83−3.90 (1H, m), 4.20−4.32 (3H, m), 5.01 (1H, br), 5.22 (1H, br), 6.08 (1H, dd, J = 6.3 Hz, J = 6.0 Hz), 8.09 (1H, s), 9.28 (1H, br), 11.00 (1H, br); ^{13}C NMR (DMSO-d_6) δ 40.9, 61.2, 70.1, 86.1, 88.0, 96.3, 139.8, 154.6, 157.2, 177.7. HRMS (ESI), m/z (M + H): calcd for $C_{11}H_{15}N_4O_4S^+$, 299.0814; found, 299.0811.

2-(2-Deoxy-β-D-ribofuranosyl)pyrimido[5,4-*e*]pyrrolo[1,2,-*c*]-pyrimidine-3,6(2*H*,5*H*)-dione (6: dC^{ppp}). Compound **19** (177 mg, 0.5 mmol), palladium acetate (5.6 mg, 0.025 mmol), TPPTS (20 mg, 0.035 mmol), Na_2CO_3 (53 mg, 1.0 mmol), and *N*-BOC-pyrrole-2-boronic acid (106 mg, 0.5 mmol) were placed in a round-bottomed flask under argon. Degassed H_2O−CH_3CN (2:1, v/v, 5 mL) was added, and the mixture was stirred at 60 °C for 30 min. More palladium acetate (5.6 mg, 0.025 mmol), TPPTS (20 mg, 0.035 mmol), and *N*-BOC-pyrrole-2-boronic acid (106 mg, 0.5 mmol) were added, and the mixture was stirred for another 30 min at 60 °C. The mixture was diluted with H_2O (10 mL) and concentrated under reduced pressure to one-half of its volume. $CHCl_3$ (20 mL) was added, and the mixture was washed with brine and saturated $NaHCO_3$. The organic layer was collected, dried over Na_2SO_4, filtered, and evaporated under reduced pressure. The crude product was purified by C200 silica gel chromatography with $CHCl_3$−MeOH to give the product **6** (88 mg, 55%): ^{1}H NMR (DMSO-d_6)

δ 1.91−2.16 (1H, m), 2.22−2.36 (1H, m), 3.62−3.81 (2H, m), 3.82−3.86 (1H, m), 4.18−4.22 (1H, m), 5.06 (1H, br), 5.29 (1H, br), 6.11 (1H, dd, J = 6.3 Hz, J = 6.3 Hz), 6.44−6.49 (2H, m), 7.50−7.52 (1H, m), 8.53 (1H, s), (1H, br); ^{13}C NMR (DMSO-d_6) δ 41.2, 60.2, 68.7, 86.8, 87.8, 96.8, 103.4, 114.2, 115.8, 125.2, 138.5, 146.3, 153.3, 157.1. HRMS (ESI), m/z (M + H): calcd for $C_{14}H_{15}N_4O_5^+$, 319.1042; found, 319.1044.

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Supporting Information Available: General remarks and the ^{1}H and ^{13}C NMR data of all new compounds **3**−**6**, **9**−**16**, and **18**. This material is available free of charge via the Internet at http://pubs.acs.org.

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