General Approach to the Synthesis of Specifically Deuterium-Labeled Nucleosides

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Starting from D-(−)-ribose, a set of synthetic routes sharing common intermediates has been developed and exemplified in [5′-$^2H_2$]-, [4′-^{2}H]-, [1′-^{2}H]-, (5′*R*)-[5′-^{2}H]-, and (5′*S*)-[5′-^{2}H]-N^4-benzoylcytidine and, by deoxygenation, their corresponding 2′-deoxynucleosides. These syntheses provide convenient access to millimolar quantities of deuterium/tritium-labeled natural or unnatural nucleosides for direct use or automated oligonucleotide synthesis.

Introduction

The calicheamicins (CLM),[1] the esperamicins (ESP),[2] and dynemicin (DYN)[3] each contain a 10-membered cyclic diynene contained in a superstructure that provides binding affinity to double-stranded DNA and a ready reductive activation path. The antitumor antibiotic activity associated with these compounds, like that of the neocarzinostatin chromophore (NCS)[4] and the newly disclosed kedarcidin (KED)[5] and C-1027,[6] is attributed to their ability to bind in the minor groove of DNA and cleave one or both strands of the helix. The singular characteristic proposed for each of these drugs is a thiolate-induced activation and electrocyclic rearrangement to a highly reactive diradicaloid intermediate and homolytic abstraction of CH atoms from the deoxyribosyl backbone. The resulting carbon-centered DNA radical(s) combine with dissolved oxygen to lead on to strand cleavage by well-studied mechanisms. While the extents of sequence selectivity are quite variable in the DNA cleavages carried out by these compounds and their derivatives,[4f,5–8] experiments with NCS,[4f] CLM,[7d,e] and ESP[8c] reveal that 5′- and 4′-hydrogen abstractions are the principal events leading to DNA scission. These hydrogens are relatively exposed to solvent at the outer reaches of the minor groove and, hence, to drug approach. In contrast, H-1′ is the least accessible minor groove hydrogen located near the floor of the minor groove, and has been shown to be a minor site of reaction only for NCS.[9]

Central to our investigations of the proposed mechanism of DNA cleavage by CLMγ_1^I has been the preparation of oligodeoxynucleotides containing a TCCT sequence, which had been observed to be particularly favored for reaction.[7b] Dodecamers were to be synthesized such that the 2′-deoxycytidine (dC) would bear deuterium labels selectively at C-5′, C-4′, and C-1′, those positions that ultimately face into the minor groove on assembly into short helical fragments. The base sequences of the dodecamers were chosen to minimize reaction outside of the TCCT site (reduced finally to <5%[7e]) to provide, in principle, efficient transfer of deuterium to the spent form of the drug, CLMϵ. It would be possible to accurately identify major and minor sites of DNA hydrogen abstraction by the drug by NMR and mass spectrometric methods and establish the loci in CLMϵ to which isotope was transferred. From the latter the orientation(s) of the drug could be mapped in the minor groove[7d] and the dynamics of the atom transfer process could be examined on each strand in, for example, kinetic isotope effects.[7e] However, to obtain sufficient amounts

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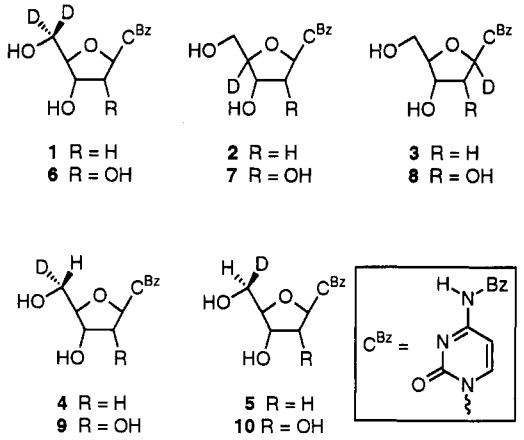
Scheme 1



1 R = H, **6** R = OH; **2** R = H, **7** R = OH; **3** R = H, **8** R = OH; **4** R = H, **9** R = OH; **5** R = H, **10** R = OH

of labeled nucleoside for automated DNA synthesis,[10] scales >50 mg of specifically labeled material were required.

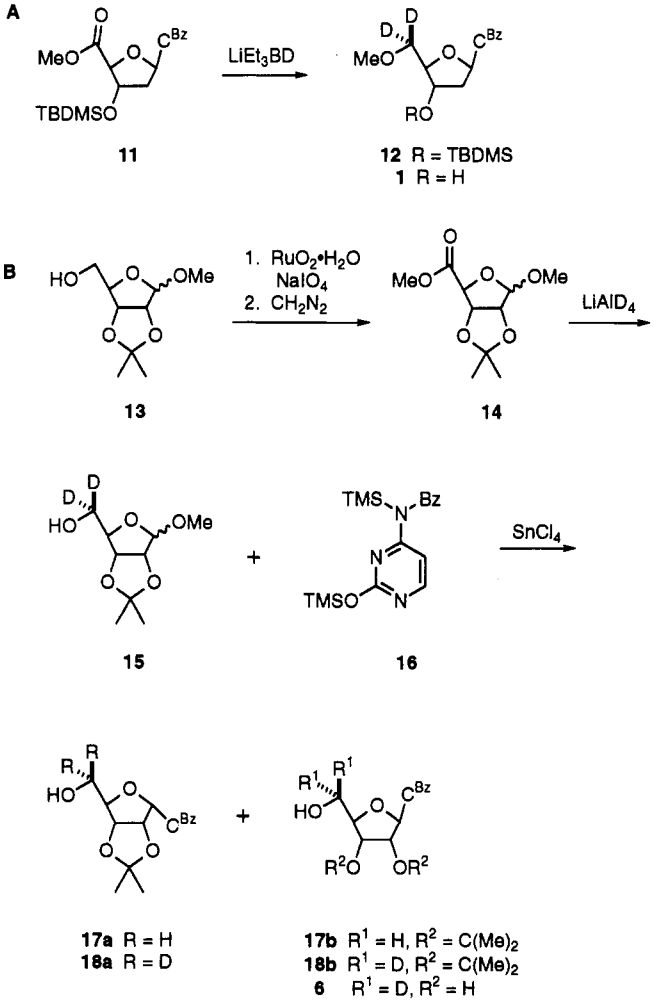
We have combined, extended and simplified known methods to achieve a set of syntheses that begin with inexpensive D-(−)-ribose and share common intermediates to yield specifically deuterium-labeled ribonucleotides or deoxyribonucleotides in quantities useful for automated oligonucleotide synthesis. The syntheses were designed to allow attachment of natural or unnatural bases relatively late in the overall process. Notably (5′*R*)- and (5′*S*)-[5′-^{2}H]-N^4-benzoylcytidine have been prepared, which, additionally, can find application as probes of DNA structure to overcome ambiguities in dihedral angle calculations based on vicinal coupling to H-4′.

Results and Discussion

Previous syntheses of labeled nucleosides have relied upon three principal strategies: (1) enzymatic transformations upon the intact nucleoside to introduce a heavy isotope at C-4′ and C-2′ of the ribosyl skeleton using *S*-adenosylhomocysteinase[11] and ribonucleotide triphosphate reductase,[11b] respectively, (2) chemical modification of an intact nucleoside to introduce deuterium at C-4′ [12] and C-5′,[13] and the most versatile strategy, (3) synthesis of isotopically labeled ribose suitably functionalized to undergo coupling with any of the purine and pyrimidine bases. This latter strategy was employed successfully in the synthesis of C-1′,[11b,14,15] and C-5′ monodeuteriated[16] and dideuteriated[17] labeled nucleosides.

[5′-^{2}H$_2$]-N^4-Benzoyl-2′-deoxycytidine. In an effort to obtain [5′-^{2}H$_2$]-N^4-benzoyl-2′-deoxycytidine (1) directly from 2′-deoxycytidine, we attempted to introduce two deuterium atoms at C-5′ by reduction of 11 (Scheme 2A),

Scheme 2



11; **12** R = TBDMS, **1** R = H; **13**; **14**; **15**; **16**; **17a** R = H, **18a** R = D; **17b** R^1 = H, R^2 = C(Me)$_2$, **18b** R^1 = D, R^2 = C(Me)$_2$, **6** R^1 = D, R^2 = H

available from 2′-deoxycytidine in four steps.[18] Preliminary trials to reduce **11** with NaB^{2}H$_4$ resulted in complex product mixtures and poor yields of the labeled nucleoside, in part due to the propensity of benzoylated pyrimidines to undergo reduction.[19] Reduction of **11** with LiEt$_3$B^{2}H gave the labeled nucleoside **1**, albeit in low yield (ca. 5%). While this route provided sufficient quantities of **1** for synthesis of the labeled oligomer,[7d,20] the low overall yield (ca. 2%) and tedious purifications caused us to formulate another route.

A more versatile synthesis of the labeled nucleoside began with with 2,3-*O*-(1-methylethylidene)-1-*O*-methyl-D-ribofuranose (**13**), itself readily available from D-ribofuranose (Scheme 2B).[21] Oxidation of **13** with $RuO_2 \cdot H_2O$ and $NaIO_4$[22] followed by in situ ester formation with diazomethane gave **14** in 92% yield. Reduction of the ester with LiAl^{2}H$_4$ gave **15** in 93% yield. Comparison of high field ^{1}H NMR spectra of **15** and **13** showed a complete absence of the resonances for the 5′-hydrogens (δ 3.61 and 3.68) of **15** demonstrating excellent incorporation of deuterium. In initial trials, **13** was converted to the 1,2,3,5-

(10) The labeled oligodeoxynucleotides were synthesized using standard phosphoramidite methodology. Each 10-μmol synthesis of DNA requires ca. 70 mg of the labeled nucleoside which had been prepared for use on an automated DNA synthesizer by known methods.[27]

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(18) Synthesis of **11** began by perbenzoylation of 2′-deoxycytidine followed by *O*-debenzoylation. The 5′-OH was temporarily masked as its dimethoxytrityl ether which allowed silylation of 3′-OH. The trityl group was removed by acid hydrolysis;[27] oxidation and esterification gave **11**.

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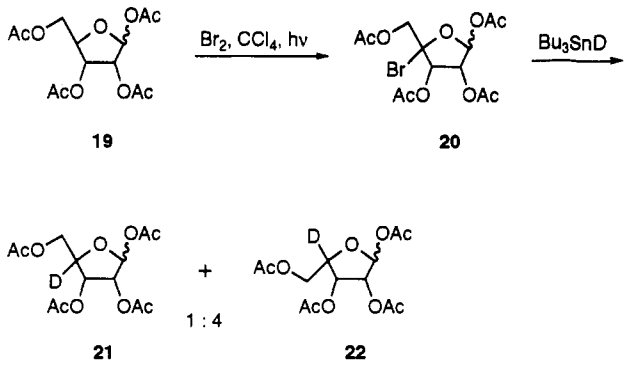
Scheme 3



tetra-*O*-acetyl-D-ribofuranose[23] followed by stereospecific addition of the activated derivative of N^4-benzoylcytidine (**16**) according to the proceedure of Vorbrüggen[24] but in poor yield (ca. 30% over three steps). Direct coupling of **13** to **16** gave a 74% combined yield of two isomers, **17a** (α-anomer) and **17b** (β-anomer) in a ratio of 1:1.4. The two isomers were easily separated by standard chromatographic techniques. Addition of **16** to the labeled sugar **15** under identical conditions gave **18a** and **18b** (1:1.6) in a 62% yield. To complete the synthesis of **1**, the acetonide was hydrolyzed with 95% aqueous TFA[25] to give [5′-$^2H_2$]-N^4-benzoylcytidine (**6**) in 93% yield. Deoxygenation[26] of **6** gave [5′-$^2H_2$]-N^4-benzoyl-2′-deoxycytidine (**1**), which was protected and activated for synthesis of the labeled DNA.[27] Deuterium content was estimated by mass spectrometric analysis to be 96 ± 1%/site.

[4′-^{2}H]-N^4-Benzoyl-2′-deoxycytidine. Deuterium introduction at C-4′ is difficult by displacement or redox routes. Attempts to brominate[28,29] the intact deoxynucleoside at C-4′ for subsequent reduction with Bu_3Sn^2H failed. Photobromination of 1-*O*-acetyl-2,3,5-tri-*O*-benzoyl-β-D-ribofuranose (**19**), however, did take place in a stereospecific manner to give the 4-bromo derivative **20** with the D-*ribo* configuration.[30] Unfortunately, reduction of this bromide with Bu_3Sn^2H gave 1,2,3,5-tetraacetyl-D-ribofuranose (**21**) and its epimer, 1,2,3,5-tetraacetyl-D-lyxofuranose (**22**), in a 1:4 ratio. The poor stereoselectivity of the reduction could not be improved and caused us to abandon this approach.

A new strategy began by protection of 2,3-*O*-(1-methylethylidene)-D-ribose (**23**)[31] (Scheme 4). Masking the open form of the hemiacetal as an olefin was accomplished by the slow addition of *i*-propyltriphenylphosphorane[32] to a solution of **24** over a period of several hours to give (2*R*,3*S*,4*S*)-1-*O*-[(1,1-dimethylethylidene)dimethylsilyl]-2-hydroxy-6-methyl-3,4-*O*-(1-methylethylidene)hept-5-ene (**25**) in 62% yield (averaged over several trials). Oxidation of C-2 (C-4 in ribose) with the Dess–Martin periodinane[33] followed by chromatographic workup afforded **26** in 95% yield. A trial reduction of **26** with $LiAlH_4$ showed remarkably good stereoselectivity for the 2*R* isomer (**25**:**27** = 4.8:1). Reduction of **26** with $LiAl^2H_4$[34] gave a 69% yield of two isomers: **28a** (2*R*) and **28b** (2*S*) in an isolated ratio of 2.5:1. Simplification of resonances for H-1 and H-3 of **28a** and **28b** confirmed a high level of deuterium incorporation at C-2. Chromatographic separation of the two isomers was followed by ozonolysis of **28a** accompanied by reductive workup (Me_2S[35]) to give [4-^{2}H]-5-*O*-[(1,1-dimethylethyl)dimethylsilyl]-2,3-*O*-(1-methylethylidene)-D-ribose (**29**) in a nearly quantitative yield. Acetylation of **29** with acetic anhydride in pyridine gave **30** in 99% yield. Coupling of **30** and **16** as before gave a 60–70% yield of a mixture of **31a** and **31b**, isolated in ratio of 1:1.4. Following chromatographic separation of the two isomers, **31b** was treated with 95% TFA as before[25] to give [4′-^{2}H]-N^4-benzoylcytidine (**7**) in 91% yield. Deoxygenation[26] of **7** gave **2** whose deuterium content was determined by mass spectrometry to be 97 ± 1%.

[1′-^{2}H]-N^4-Benzoyl-2′-deoxycytidine. The synthesis of **3** followed an approach similar to that for **1** and **2**. Introduction of deuterium at C-1′ was to be performed by the partial reduction of a suitably derivatized form of D-ribonolactone.[36] In keeping with the synthesis of **2**, we chose to make 5-*O*-[(1,1-dimethylethyl)dimethylsilyl]-2,3-*O*-(1-methylethylidene)-D-ribonolactone (**32**)[37] from **24** (Scheme V). Oxidation at C-1 of **24** was conveniently carried out by the addition of an EtOAc[38] solution of **24** to an aqueous suspension of $RuO_2{\cdot}H_2O$ and $NaIO_4$ to give, after chromatography, 81% of **32**. It is worth noting that the usual solvent combination (CH_3CN/CCl_4 = 1:1)[22] generally employed with this reagent resulted in incomplete oxidation of **24**. A small amount of **32** formed shortly after the reagents were combined suggested that the ruthenium catalyst was somehow being removed from the catalytic cycle. It seems likely that EtOAc acts to release the lactone from the coordination sphere of the metal thereby facilitating propagation of the catalytic cycle. Reduction of **32**[39] was carried out by careful addition of an ethereal suspension of $LiAl^2H_4$ to a cold (−10 °C) solution of **32**.[40] It was essential to carefully control the reaction temperature and slowly add the reductant to routinely obtain good yields (58%) of the labeled sugar **33**.[11b,14] Acetylation of the hemiacetal gave **34**, which was coupled to **16** as above to give the usual mixture of isomers (**35a** and **35b** in 60% yield) in a ratio of 1:1.1. The syntheses of **8**[25] and **3**[26] were completed as before. Deuterium content of **3** was estimated at 98 ± 1% by mass spectrometric analysis.

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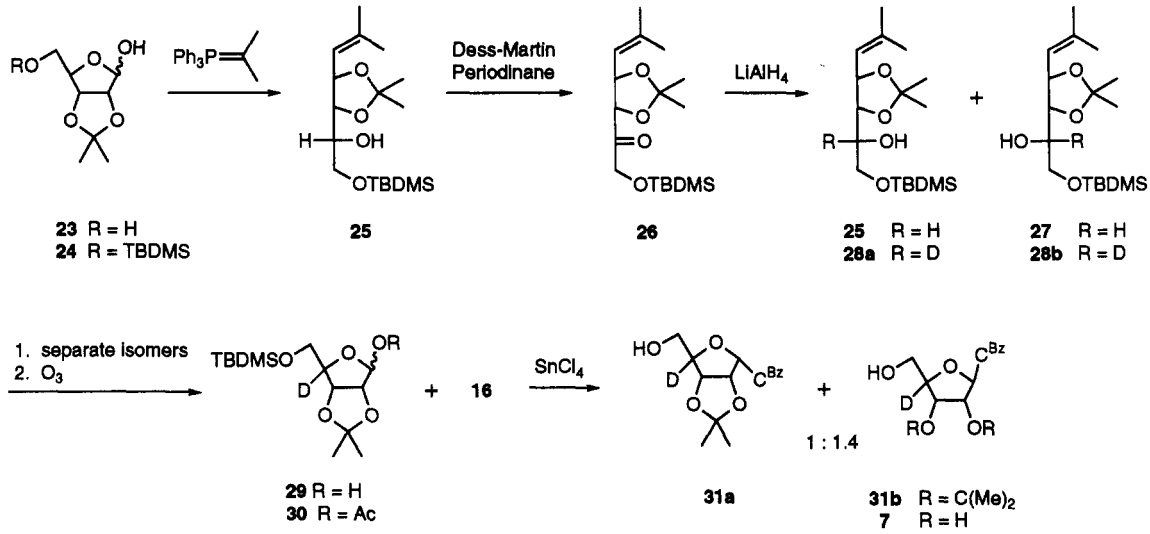
Scheme 4



23 R = H
24 R = TBDMS

25

26

25 R = H
28a R = D

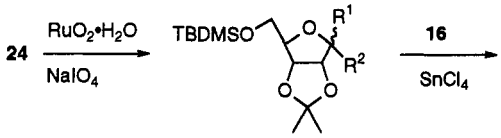
27 R = H
28b R = D

29 R = H
30 R = Ac

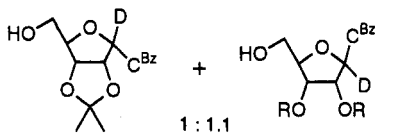
31a

31b R = $C(Me)_2$
7 R = H

Scheme 5



32 $R^1 = R^2 = O$
33 R^1 = OH, R^2 = D
34 R^1 = OAc, R^2 = D



35a

35b R = $C(Me)_2$
8 R = H

(5′*R*)- and (5′*S*)-[5′-^{2}H]-N^4-benzoyl-2′-deoxycytidine. To investigate the stereospecificity of atom abstraction at the 5′-carbon of the cytidine targeted by the drug,[7e] we synthesized both 5′*R*- and 5′*S*-[5′-^{2}H]-N^4-benzoyl-2′-deoxycytidine (**4** and **12**, respectively). The synthesis of (5′*R*)- and (5′*S*)-[-5′-^{2}H]adenosine from (5*R*)-[5-^{2}H]-1,5-anhydro-2,3-*O*-(1-methylethylidene)-D-ribofuranose (**37**) had been reported by Ohrui.[16a] The labeled anhydrosugar **37** was available from D-ribose in four steps by $LiEt_3B^2H$ displacement of the monobromide resulting from stereospecific photohalogenation of the protected anhydrosugar **36**.[16a,41] The diastereomeric excess (de) of the deuteriated product was judged to be >99% by high field ^{1}H NMR spectroscopy. In the original report,[16a] **37** was converted to (5*R*)- or (5*S*)-[5-^{2}H]-1,2,3-tri-*O*-acetyl-5-*O*-benzoyl-β-D-ribofuranose prior to the addition of adenine. We sought to obtain a similar synthetic intermediate and, in trial reactions, attempted to convert **36** to its corresponding 1,2,3,5-tetra-*O*-acetate. Unfortunately, peracetylation proceeded in only 18% yield over three steps. However, it had been noted at this time that nucleoside analogues could be obtained directly from 1,6-anhydro-2-deoxyglucopyranosides.[42] In a test reaction **36** and **16** were treated with TMS triflate in acetonitrile to give a moderately good yield (62%) of α- and β-2,3-*O*-(1-methylethylidene)-N^4-benzoylcytidine (**17a** and **17b**, respectively) in a ratio of 1:1. In an attempt to increase the amount of **17a** relative to **17b**, $SnCl_4$ was substituted for TMS triflate. This modification succeeded in changing the ratio of **17a** and **17b** to 1:1.3. Application of these conditions to **37** gave a 1:1.1 ratio of α- and β-(5′*R*)-[5′-^{2}H]-2,3-*O*-(1-methylethylidene) (**38a** and **38b**, respectively) in 69% yield. Syntheses of **9** and **4** were completed as before.[25,26]

Inversion at C-5′ to the *S*-configuration was investigated at two points in the synthesis of **4**. In the first, **38b** was reacted with 1.1 equiv each of PPh_3, DEAD, and 4-nitrobenzoic acid in HMPA[43] to give **39** in 50% yield and 80% de as judged by careful integration of the ^{1}H NMR resonances for the 5′*R* and 5′*S* hydrogens (δ 4.66 and 4.72, d_6-acetone). This stereochemical result is to be contrasted to the nearly complete inversion at C-5′ when the α-anomer **38a** was treated under identical conditions. We reason that for a small fraction of reactants (ca. 10%), the participation of the cytosine carbonyl oxygen (O-2) occurs at C-5′ through intramolecular displacement of the activated oxygen and is followed by displacement of the base by 4-nitrobenzoate with net retention of configuration at C-5′. This mechanism is supported by the known formation of N^4-benzoyl-O^2,5′-cyclocytidine in 71% yield when N^4-benzoylcytidine is reacted with PPh_3 and DEAD in the absence of the acidic component (i.e. 4-nitrobenzoic acid in this instance).[43b] A second and more convenient juncture for the inversion of stereochemistry at C-5′ was identified after the synthesis of **4** was complete. When **4** was combined with 1.1 equiv of PPh_3 and DEAD and 5 equiv of 4-nitrobenzoic acid, **41** was produced in 72% yield, a significant improvement over that obtained previously. More importantly, inversion at C-5′ took place in 94% de as judged by careful intergration of the signals for the 5′*R* and 5′*S* hydrogens (δ 4.53 and 4.63) in the ^{1}H NMR spectrum. Methanolysis of the ester was readily achieved by stirring **41** with NaOMe in MeOH/THF (1:1) for 7 min[44] to give (5′*S*)-[5′-^{2}H]-N^4-benzoyl-2′-deoxycytidine (**5**) in 75% yield. The deuterium content of both labeled

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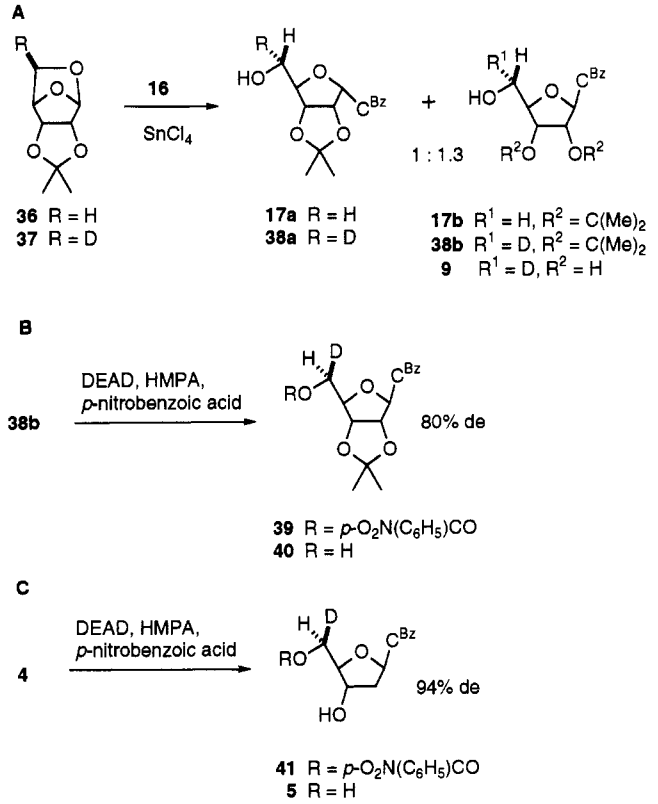
Table 1. Selected ^{1}H NMR Data[a] for Labeled N^4-Benzoylcytidines and N^4-Benzoyl-2′-deoxycytidines[b]

compound	C-1′		C-2′		C-3′		C-4′		C-5′*R*		C-5′*S*	
N^4-benzoylcytidine	5.81	d	4.02	d × d	3.98	m	3.91	d × d	3.61	d × d	3.76	d × d
6	5.81	d	4.02	d × d	3.98	d	3.91	d	–	–	–	–
7	5.81	d	4.01	d × d	3.98	d	–	–	3.61	d	3.75	d
8	–	–	4.03	d	3.98	m	3.91	d × d	3.61	d × d	3.75	d × d
9	5.82	d	4.02	d × d	3.99	t	3.91	d × d	–	–	3.73	d
N^4-benzoyl-2′-deoxycytidine	6.14	app t	2.06	d × d × d	4.24	m	3.88	q	3.59	d × d	3.64	d × d
			2.31	d × d × d								
1	6.14	t	2.06	d × d × d	4.24	d × t	3.87	d	–	–	–	–
			2.31	d × d × d								
2	6.14	t	2.06	d × d × d	4.23	d × d	–	–	3.58	d	3.63	d
			2.31	d × d × d								
3	–	–	2.05	d × d	4.24	m	3.87	q	3.58	d × d	3.63	d × d
			2.31	d × d								
4[c]	6.13	t	2.04	d × d × d	4.23	m	3.86	app t	–	–	3.61	d
			2.31	m								
5[c]	6.13	t	2.04	d × d × d	4.22	m	3.86	t	3.56	d	–	–
			3.31	m								

[a] The chemical shift of each resonance is reported and observed splitting pattern. Complete ^{1}H NMR data are located in the Experimental Section. [b] Top and bottom values refer to the C-2′β and C-2′α hydrogens, respectively. [c] Recorded at 300 MHz.

Scheme 6



diastereomers was determined to be 97 ± 1% by mass spectrometric measurements.

In conclusion, a related set of syntheses beginning with D-(−)-ribose has been described that provides nucleosides **6-9** and 2′-deoxynucleosides **1-5**, among others, in amounts sufficient for the automated preparation of oligonucleotides selectively labeled with one or more deuterium atoms. These syntheses share common intermediates and allow late base introduction, whether natural or unnatural, to afford high synthetic efficiency and versatility.

Experimental Section

Tetrahydrofuran (THF) and diethyl ether (Et_2O) were distilled from sodium and benzophenone. Dichloromethane (CH_2Cl_2), benzene, acetonitrile (ACN), toluene, diisopropylethylamine, 2,2-dimethoxypropane, hexamethylphosphoramide (HMPA), pyridine, and triethylamine (TEA) were distilled from calcium hydride. Carbon tetrachloride (CCl_4) and dimethylformamide (DMF) was successively dried (3×) over 4-Å molecular sieves. Methanol (CH_3OH) was distilled from its magnesium alkoxide under nitrogen.[45] *p*-Toluenesulfonic acid was dried by azeotropic removal of water with benzene followed by drying under reduced pressure. Dimethoxytrityl chloride was dried over phosphorus pentoxide in vacuo at 110 °C for no more than 12 h. Tributyltin hydride and phenyl chlorothioformate were distilled immediately prior to use. Unless otherwise noted, all other reagents were of the highest quality available and were used without further purification. Flash chromatography was carried out with Merck Kieselgel 60 and the mobile phase indicated in individual procedures as described by Still.[46] Melting points are uncorrected. Specific rotations were determined using a 1.0-dm quartz-window cell of 1.0-mL capacity; concentrations are indicated in g/100 mL. Proton and carbon NMR spectra chemical shifts are reported in ppm and were generally referenced to the solvent in which the sample was dissolved or to TMS. Coupling constants (*J*) are reported in hertz and peak multiplicities described as singlet (s), doublet (d), triplet (t), quartet (q), multiplet (m), apparent (app), or broad (br). High and low resolution mass spectra were obtained using the EI (70 eV) or CI (120 eV, NH_3) operating mode.

5-(Methoxycarbonyl)-2,3-*O*-(1-methylethylidene)-1-*O*-methyl-D-ribofuranose (14). A solution of **13** (15.0 g, 73.5 mmol) in 300 mL of CCl_4/ACN (1:1) was added to a vigorously stirring aqueous solution of $NaIO_4$ (10% w/v, 220 mL) and RuO_2H_2O (220 mg, 1.6 mmol).[22] The biphasic mixture was stirred at rt for 4 h. The reaction was diluted with water (200 mL) and CH_2Cl_2 (500 mL). The layers were separated and the aqueous phase was washed with CH_2Cl_2 (2 × 100 mL). The combined organic extracts were dried over anhyd $MgSO_4$, filtered, and evaporated under reduced pressure. The residue was dissolved in CH_3OH (100 mL) and treated with an excess of etheral diazomethane. After 10 min the reaction mixture was evaporated under reduced pressure and the title compound purified by filtration through a plug of silica gel (50% EtOAc in hexane) to yield 15.6 g (92%) of a colorless oil. A sample for analysis was obtained by kugelrohr distillation: air bath 50 °C, 1.5 mmHg (lit.[47] bp 116–118 °C, 0.3 mmHg); $[\alpha]^{25}_D$ −72.7° (*c* 2.0, $CHCl_3$); ^{1}H NMR (400 MHz, $CDCl_3$) δ 1.33 (s, 3 H), 1.48 (s, 3 H), 3.39 (s, 3 H), 3.76 (s, 3 H), 4.54 (d, 1 H, *J* = 6), 4.60 (s, 1 H), 5.03 (s, 1 H), 5.22 (d, 1 H, *J* = 6); MS (EI) *m/e* 217 (M−CH_3, 36%), 173 (M−CO_2CH_3, 22), 157 (17), 143 (35), 114 (27), 85 (22), 59 (45), 58 (22), 43 (100).

[5-$^2H_2$]-2,3-*O*-(1-Methylethylidene)-1-*O*-methyl-D-ribofuranose (15). A solution of the ester **14** (14.55 g, 62.7 mmol) in Et_2O (75 mL) was added to a suspension of $LiAl^2H_4$ (1.50 g, 17.5 mmol) and anhyd Et_2O (75 mL). The reaction mixture was heated to reflux for 5 h. An additional portion of $LiAl^2H_4$ (0.40 g, 4.4 mmol) was added to the reaction. The contents of the flask were cooled to rt and the excess hydride was quenched by the

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addition of water (2.0 mL), 3 N NaOH (2.0 mL), and water (6.0 mL).[48] The solids were removed by filtration through a pad of Celite and the filtrate was evaporated under reduced pressure to yield the title compound **15** as a colorless oil (12.02 g, 93%). A sample for analysis was obtained by vacuum distillation as described previously: bp 100 °C, 1.5 mmHg; $[\alpha]^{23}_D$ -76.6° (*c* 2.0, $CHCl_3$); ^1H NMR (400 MHz, $CDCl_3$) δ 1.32 (s, 3 H), 1.49 (s, 3 H), 3.22 (bd s, 1 H), 3.44 (s, 3 H), 4.43 (s, 1 H), 4.59 (d, 1 H, *J* = 6.0), 4.84 (d, 1 H, *J* = 6.0), 4.97 (s, 1 H); MS (EI) *m/e* 191 ($M-CH_3$, 18%), 173 ($M-CD_2OH$, 10), 159 (11), 131 (6), 88 (25), 70 (64), 59 (100), 43 (91).

5-*O*-[(1,1-Dimethylethyl)dimethylsilyl]-2,3-*O*-(1-methylethylidene)-D-ribofuranose (24). **23** (23.3 g, 122.4 mmol) was dissolved in anhyd CH_2Cl_2 (150 mL) and treated with TEA (18.8 mL, 134.6 mmol), DMAP (1.5 g, 12.2 mmol), and *tert*-butyldimethylsilyl chloride (20.3 g, 134.6 mmol), added as a solution in anhyd CH_2Cl_2 over a period of ca. 30 min. The reaction mixture was stirred overnight, washed with 1 N HCl (100 mL), saturated $NaHCO_3$ (100 mL), and brine (100 mL), dried over anhyd $MgSO_4$, filtered, and evaporated under reduced pressure. The residue was purified by flash chromatography (10% EtOAc in hexanes) to yield **24** as a clear oil that crystallized upon standing (21.6 g, 58%): mp 52–54 °C (lit.[49] mp 47 °C); $[\alpha]^{25}_D$ -17.0; ^1H NMR (400 MHz, $CDCl_3$) δ 0.14 (s, 3 H), 0.15 (s, 3 H), 0.93 (s, 9 H), 1.33 (s, 3 H), 1.49 (s, 3 H), 3.75 (d × d, 1 H, *J* = 11.1 2.1), 3.78 (d × d, 1 H, *J* = 11.1 2.1), 4.36 (m, 1 H), 4.50 (d, 1 H, *J* = 6.0), 4.70 (d, 1 H, *J* = 6.0), 4.77 (d, 1 H, *J* = 11.9), 5.28 (d, 1 H, *J* = 11.9); MS (EI) *m/e* 303 (M-1, 10%), 289 ($M-CH_3$, 4.1), 247 (11), 189 (8), 171 (23), 75 (100).

(2*R*,3*S*,4*S*)-1-*O*-[(1,1-Dimethylethyl)dimethylsilyl]-2-hydroxy-6-methyl-3,4-*O*-(1-methylethylidene)hept-5-ene (25). A suspension of *i*-propyltriphenylphosphonium iodide[50] (30.4 g, 70.4 mmol) in anhyd THF (220 mL) was cooled to 0 °C and treated with *n*-BuLi (1.6 M in hexane; 44.0 mL, 70.4 mmol). **24** (10.2 g, 33.6 mmol) in anhyd THF (30 mL) was added via a syringe pump overnight. The reaction mixture was quenched with saturated aqueous $NaHCO_3$ (250 mL), diluted with EtOAc (500 mL), and filtered. The organic layer was washed with brine (200 mL), dried over anhyd $MgSO_4$, filtered, and evaporated to dryness. The residue was purified by flash chromatography (8% EtOAc in hexanes) to give the title compound (6.9 g, 62%) as a colorless oil: $[\alpha]^{25}_D$ 5.0° (*c* 1.0, $CHCl_3$); IR ($CHCl_3$) 3566, 3009, 2990, 2932, 2884, 2859, 1471, 1463, 1378 cm^{-1}; ^1H NMR (400 MHz, $CDCl_3$) δ 0.08 (s, 3 H), 0.09 (s, 3 H), 0.91 (s, 9 H), 1.35 (s, 3 H), 1.44 (s, 3 H), 1.74 (d, 3 H, *J* = 1.4), 1.79 (d, 3 H, *J* = 1.4), 3.66–3.71 (m, 2 H), 3.78–3.83 (m, 1 H), 3.98–4.02 (m, 1 H), 4.94 (d × d, 1 H, *J* = 6.1 9.5), 5.36 (d × septet, 1 H, *J* = 1.4 9.5); ^{13}C NMR (100 MHz, $CDCl_3$) δ -5.43, -5.38, 18.15, 18.34, 25.53, 25.88, 26.17, 28.08, 64.45, 69.80, 74.51, 77.39, 108.10, 120.14, 138.32; MS (EI) *m/e* 315 ($M-CH_3$, 0.2%), 215 (6), 197 (4), 126 (19), 117 (100); HRMS calcd for $C_{16}H_{31}O_4Si$ ($M-CH_3$) 315.1992, found 315.1996.

(3*S*,4*S*)-1-*O*-[(1,1-Dimethylethyl)dimethylsilyl]-2-hydroxy-6-methyl-3,4-*O*-(1-methylethylidene)hept-5-en-2-one (26). Dess–Martin periodinane[33] (9.3 g, 21.8 mmol) was added in portion-wise fashion to **25** (6.0 g, 18.2 mmol) in CH_2Cl_2 (100 mL) The mixture was stirred at rt for 2 h. The reaction was filtered through a plug of silica and and the silica was eluted with CH_2Cl_2. The solution was concentrated and the residue purified by flash chromatography (5% EtOAc in hexanes) to yield the title compound as a colorless oil (5.8 g, 97%). $[\alpha]^{25}_D$ 4.0° (*c* 1.0, $CHCl_3$); IR ($CHCl_3$) 3013, 2955, 2931, 2849, 1731, 1602, 1472, 1378 cm^{-1}; ^1H NMR (400 MHz, $CDCl_3$) δ 0.08 (s, 6 H), 0.91 (s, 9 H), 1.39 (s, 3 H), 1.59 (s, 3 H), 1.71 (d, 3 H, *J* = 1.5), 1.72 (d, 3 H, *J* = 1.5), 4.25 (d, 1 H, *J* = 18.9), 4.47 (d, 1 H, *J* = 18.9), 4.49 (d, 1 H, *J* = 7.6), 5.00 (d × septet, 1 H, *J* = 1.5 9.4), 5.51 (d × d, 1 H, *J* = 7.6 9.3); ^{13}C NMR (100 MHz, $CDCl_3$) δ -5.53, -5.46, 18.38, 24.87, 25.77, 25.92, 27.07, 68.51, 74.56, 81.36, 109.71, 119.17, 139.95, 206.43; MS (CI) *m/e* 346 (M + 18, 6%), 304 (24), 288 (100), 271 (69), 245 (15), 197 (8), 187 (53), 113 (9); HRMS calcd for $C_{17}H_{32}O_4SiNH_4$ 346.2414, found 346.2420.

Procedure A. (2*R*,3*S*,4*S*)-1-*O*-[(1,1-Dimethylethyl)dimethylsilyl]-2-hydroxy-6-methyl-3,4-*O*-(1-methylethylidene)hept-5-ene (25) and (2*S*,3*S*,4*S*)-1-*O*-[(1,1-Dimethylethyl)dimethylsilyl]-2-hydroxy-6-methyl-3,4-*O*-(1-methylethylidene)hept-5-ene (27). **26** (400 mg, 1.2 mmol) was dissolved in anhyd Et_2O (12 mL) and treated with $LiAlH_4$ (16 mg, 0.4 mmol). The reaction was stirred at rt for 30 min after which an additional amount of $LiAlH_4$ (5 mg, 0.12 mmol) was added. Excess hydride was quenched with water (31 μL), 3 N NaOH (31 μL), and water (93 μL).[48] The mixture was filtered through a pad of Celite and evaporated. **25** and **27** were separated by flash chromatography (10% EtOAc in hexanes) to yield 250 mg (63%) and 53 mg (13%), respectively. **25** exhibited spectroscopic properties identical to the material prepared previously. **27**: IR ($CHCl_3$) 3561, 3012, 2989, 2956, 2931, 2885, 2858, 1472, 1463, 1383; ^1H NMR (400 MHz, $CDCl_3$) δ 0.04 (s, 3 H), 0.05 (s, 3 H), 0.88 (s, 9 H), 1.39 (s, 3 H), 1.51 (s, 3 H), 1.70 (d, 3 H, *J* = 1.4), 1.77 (d, 3 H, *J* = 1.4), 2.38 (d, 1 H, *J* = 5.5), 3.51–3.61 (m, 3 H), 4.16 (d × d, 1 H, *J* = 4.0 6.8), 4.89 (d × d, 1 H, *J* = 6.8 9.4), 5.41 (d × septet, 1 H, *J* = 1.4 9.4); ^{13}C NMR (100 MHz, $CDCl_3$) δ -5.37, 18.20, 18.33, 25.04, 25.89, 26.09, 27.36, 64.17, 70.34, 73.99, 108.07, 120.53, 138.42; MS (EI) *m/e* 315 ($M-CH_3$, 0.1%), 215 (8), 197 (5), 126 (19), 117 (100); HRMS calcd for $C_{16}H_{31}O_4Si$ ($M-CH_3$) 315.1992, found 315.1990.

[2-^{2}H]-(2*R*,3*S*,4*S*)-1-*O*-[(1,1-Dimethylethyl)dimethylsilyl]-2-hydroxy-6-methyl-3,4-*O*-(1-methylethylidene)hept-5-ene (28a) and [2-^{2}H]-(2*S*,3*S*,4*S*)-1-*O*-[(1,1-Dimethylethyl)dimethylsilyl]-2-hydroxy-6-methyl-3,4-*O*-(1-methylethylidene)-hept-5-ene (28b). **28a** (2.82 g, 49%) and **28b** (1.14 g, 20%) were produced when **26** (5.72 g, 17.4 mmol) was reduced with $LiAl^2H_4$ (600 mg, 14 mmol) in anhyd Et_2O (100 mL) according to Procedure A. **28a**: ^1H NMR (400 MHz, $CDCl_3$) δ 0.076 (s, 3 H), 0.078 (s, 3 H), 0.90 (s, 9 H), 1.34 (s, 3 H), 1.43 (s, 3 H), 1.73 (d, 3 H, *J* = 1.4), 1.78 (d, 3 H, *J* = 1.4), 2.43 (br s, 1 H), 3.67 (d, 1 H, *J* = 10.1), 3.79 (d, 1 H, *J* = 10.1), 3.99 (d, 1 H, *J* = 6.0), 4.93 (d × d, 1 H, *J* = 6.0, 9.6), 5.34 (d × septet, 1 H, *J* = 1.4 9.6); MS (EI) *m/e* 316 ($M-CH_3$, 0.2%), 216 (8), 198 (5), 126 (29), 118 (100). **28b**: ^1H NMR (400 MHz, $CDCl_3$) δ 0.047 (s, 3 H), 0.052 (s, 3 H), 0.88 (s, 9 H), 1.39 (s, 3 H), 1.51 (s, 3 H), 1.70 (d, 3 H, *J* = 1.4), 1.77 (d, 3 H, *J* = 1.4), 3.53 (d, 1 H, *J* = 10.1), 3.56 (d, 1 H, *J* = 10.1), 4.15 (d, 1 H, *J* = 6.8), 4.91 (d × d, 1 H, *J* = 6.8, 9.4), 5.41 (d × septet, 1 H, *J* = 1.5 9.4); MS (EI) *m/e* 316 ($M-CH_3$, 0.4%), 216 (8), 197 (5), 126 (17), 118 (100).

NOTE: Alternatively, **28a** (2.05 g, 58%) and **28b** (588 mg, 17%) were obtained from **26** (3.50 g, 10.7 mmol), NaB^2H_4 (318 mg, 7.74 mmol), and D_2O (354 μL, 17.7 mmol) in anhyd THF (35 mL). The two isomers exhibited spectroscopic properties identical to those previously recorded.

Procedure B. 5-*O*-[(1,1-Dimethylethyl)dimethylsilyl]-2,3-*O*-(1-methylethylidene)-D-ribofuranose (24). A solution of **25** (212 mg, 0.64 mmol) in anhyd CH_2Cl_2 (15 mL) was cooled to -78 °C and treated with ozone until a blue-colored solution persisted. The ozonide was decomposed with $(CH_3)_2S$[35] (60 μL, 0.82 mmol) at -78 °C followed by warming to rt. The reaction mixture was evaporated under reduced pressure and **24** was isolated by flash chromatography (20% EtOAc in hexanes) as a colorless oil (196 mg, 99%). The product exhibited spectroscopic properties identical to those reported previously for **24**.

[4-^{2}H]-5-*O*-[(1,1-Dimethylethyl)dimethylsilyl]-2,3-*O*-(1-methylethylidene)-D-ribofuranose (29). Ozonolysis of **28a** (7.27 g, 22.0 mmol) according to Procedure B (CH_2Cl_2, 50 mL; $(CH_3)_2S$, 2.0 mL, 27 mmol) gave **29** (5.51 g, 82%): mp 45–47 °C; ^1H NMR (400 MHz, $CDCl_3$) β-anomer δ 0.13 (s, 3 H), 0.14 (s, 3 H), 0.92 (s, 9 H), 1.32 (s, 3 H), 1.48 (s, 3 H), 3.74 (d, 1 H, *J* = 11.4), 3.76 (d, 1 H, *J* = 11.4), 4.50 (d, 1 H, *J* = 6.0), 4.69 (d, 1 H, *J* = 6.0), 4.75 (d, 1 H, *J* = 11.7), 5.27 (d, 1 H, *J* = 11.7); α-anomer (<5%) δ 0.05 (s, 6 H), 0.88 (s, 9 H), 1.38 (s, 3 H), 1.54 (s, 3 H), 3.63 (d, 1 H, *J* = 10.9), 3.90 (d, 1 H, *J* = 11.5), 4.54 (d × d, 1 H, *J* = 4.0, 6.0), 4.72 (d, 1 H, *J* = 6.0), 5.44 (d × d, 1 H, *J* = 4.0, 11.5); MS (EI) *m/e* 290 ($M-CH_3$, 7%), 248 ($M-C(CH_3)_3$, 16), 190 ($M-C(CH_3)_3CSi(CH_3)_2$, 13), 172 (18), 144 (39), 129 (48), 118 (51), 75 (100), 73 (46).

Procedure C. 1-*O*-Acetyl-5-*O*-[(1,1-dimethylethyl)dimethylsilyl]-2,3-*O*-(1-methylethylidene)-D-ribofuranose (42). **24** (392 mg, 1.29 mmol), anhyd pyridine (3.0 mL), and acetic anhydride (600 μL, 6.45 mmol) were stirred at rt for 4 h. The reaction mixture was diluted with EtOAc (30 mL) and washed

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with 1 N HCl (2 × 20 mL), 10% aqueous $CuSO_4$ (30 mL), saturated $NaHCO_3$ (30 mL), and brine (30 mL). The organic phase was dried over anhyd $MgSO_4$, filtered, and evaporated under reduced pressure to yield a colorless oil (442 mg, 99%) suitably pure for the next step. A sample for analysis was obtained by flash chromatography (10% EtOAc in hexanes): $[\alpha]^{25}_D$ −68° (*c* 1.0, $CHCl_3$) [lit.[51] $[\alpha]^{25}_D$ −101° (*c* 0.0186, CCl_4) for the β-anomer and $[\alpha]^{25}_D$ −10.8° (*c* 0.141, CCl_4) for the α-anomer]; IR ($CHCl_3$) 3025, 2943, 1743; ^1H NMR (400 MHz, $CDCl_3$) δ 0.07 (s, 6 H), 0.91 (s, 9 H), 1.34 (s, 3 H), 1.50 (s, 3 H), 2.05 (s, 3 H), 3.54 (d × d, 1 H, *J* = 10.6 8.0), 3.68 (d × d, 1 H, *J* = 10.6 5.0), 4.30 (d × d, 1 H, *J* = 8.0 5.0), 4.68 (d, 1 H, *J* = 5.8), 4.78 (d, 1 H, *J* = 5.8), 6.17 (s, 1 H); ^{13}C NMR (100 MHz, $CDCl_3$) δ −5.41, 18.28, 21.26, 25.16, 25.85, 26.55, 63.51, 81.70, 85.19, 88.11, 102.70, 112.82, 169.55; MS (EI) *m/e* 331 (M−CH_3, 6%), 289 (M−$C(CH_3)_3$, 9), 287 (M−CH_3COO, 6), 229 (10), 171 (54), 117 (100); HRMS calcd for $C_{15}H_{27}O_6Si$ (M−CH_3): 331.1577, found 331.1578.

[4-^{2}H]-1-*O*-Acetyl-5-*O*-[(1,1-dimethylethyl)dimethylsilyl]-2,3-*O*-(1-methylethylidene)-D-ribofuranose (30). **30** (2.14 g, 99%) was prepared from **29** (1.90 g, 6.22 mmol) and acetic anhydride (2.90 mL, 30.7 mmol) in anhyd pyridine (15 mL) using procedure C: $[\alpha]^{25}_D$ −65.8° (*c* 1.1, $CHCl_3$); ^1H NMR (400 MHz, $CDCl_3$) δ 0.07 (s, 6 H), 0.90 (s, 9 H), 1.34 (s, 3 H), 1.50 (s, 3 H), 2.04 (s, 3 H), 3.54 (d, 1 H, *J* = 10.5), 3.67 (d, 1 H, *J* = 10.5), 4.68 (d, 1 H, *J* = 5.9), 4.77 (d, 1 H, *J* = 5.9), 6.16 (s, 1 H); MS (EI) *m/e* 332 (M−CH_3, 4%), 290 (M−$C(CH_3)_3$, 5), 288 (M−CH_3COO, 5), 232 (M−$(CH_3)_3CSi(CH_3)_2$, 7), 230 (7), 172 (37), 117 (83), 75 (42), 73 (38), 43 (100).

5-*O*-[(1,1-Dimethylethyl)dimethylsilyl]-2,3-*O*-(1-methylethylidene)-D-ribonolactone (32). To a freshly prepared solution of 10% aqueous $NaIO_4$ (w/v, 333 mL) into which had been added $RuO_2·H_2O$ (142 mg, 1.1 mmol) was added a solution of **24** (10.0 g, 33.3 mmol) in EtOAc[38] (100 mL) over a period of ca. 20 min. The resulting two-phase mixture was stirred overnight. Excess oxidant was destroyed with 2-propanol (10 mL) (as indicated by the change in color of the mixture from yellow/orange to black) and the two layers were separated. The aqueous layer was washed with EtOAc (2 × 100 mL). The combined organic extracts were washed with brine (100 mL), dried over anhyd $MgSO_4$, filtered through a bed of Celite, and evaporated to dryness. **32** was isolated by flash chromatography (20% EtOAc in hexanes) to yield 8.1 g (81%) of a white crystalline solid. A sample for analysis was obtained by crystallization from pentane: mp 75–76 °C (lit.[39] mp 69–70 °C); $[\alpha]^{25}_D$ −48.4° (*c* 1.0, $CHCl_3$) [lit.[39] $[\alpha]^{25}_D$ −46.6° (*c* 0.8, $CHCl_3$)]; ^1H NMR (400 MHz, $CDCl_3$) δ 0.057 (s, 3 H), 0.073 (s, 3 H), 0.88 (s, 9 H), 1.39 (s, 3 H), 1.48 (s, 3 H), 3.80 (d × d, 1 H, *J* = 11.3 1.5), 3.89 (d × d, 1 H, *J* = 11.3 2.1), 4.60 (br t, 1 H, *J* = 1.7), 4.71 (d, 1 H, *J* = 5.6), 4.73 (d, 1 H, *J* = 5.6); MS (EI) *m/e* 287 (M−CH_3, 10%), 217 (34), 159 (12), 131 (20), 129 (42), 117 (100).

[1′-^{2}H]-5-*O*-[(1,1-Dimethylethyl)dimethylsilyl]-2,3-*O*-(1-methylethylidene)-D-ribofuranose (33). **32** (3.70 g, 12.20 mmol) in anhyd THF (10 mL) was cooled to −10 °C and reduced with $LiAl^2H_4$ (257 mg, 6.10 mmol) in anhyd THF (9.6 mL). Excess reductant was quenched with H_2O (6 μL), 3 N NaOH (6 μL), and H_2O (19 μL).[48] The solids were filtered through a bed of Celite. The filtrate was dried over anhyd $MgSO_4$, filtered, and evaporated under reduced pressure. **33** (2.16 g, 58%) was isolated as a mixture of α- and β-anomers by flash chromatography (15% EtOAc in hexanes): mp 48–51 °C (lit.[52] mp 47 °C); β-anomer ^1H NMR (400 MHz, $CDCl_3$) δ 0.13 (s, 3 H), 0.14 (s, 3 H), 0.93 (s, 9 H), 1.32 (s, 3 H), 1.48 (s, 3 H), 3.62-3.80 (m, 4 H), 4.35 (br t, 1 H), 4.50 (d, 1 H, *J* = 5.9), 4.70 (d × d, 1 H, *J* = 5.9 0.93); α-anomer ^1H NMR (400 MHz, $CDCl_3$) δ 0.06 (s, 6 H), 0.92 (s, 9 H), 1.39 (s, 3 H), 1.55 (2, 3 H), 3.62–3.80 (m, 4 H), 4.15 (br t, 1 H), 4.54 (d, 1 H, *J* = 6.2), 4.73 (d, 1 H, *J* = 6.2); MS (EI) *m/e* 304 (M−1, 4.1%), 290 (M−CH_3, 4.8), 248 (18), 190 (15), 172 (21), 143 (29), 75 (100).

[1′-^{2}H]-1-*O*-Acetyl-5-*O*-[(1,1-dimethylethyl)dimethylsilyl]-2,3-*O*-(1-methylethylidene)-D-ribofuranose (34). **34** (2.03 g, 99%) was obtained from the reaction of **33** (1.79 g, 5.87 mmol), acetic anhydride (2.80 mL, 29.3 mmol), and anhyd pyridine (10 mL) according to Procedure C: $[\alpha]^{25}_D$ −70.4° (*c* 1.3, $CHCl_3$); ^1H NMR (400 MHz, $CDCl_3$) δ 0.07 (s, 6 H), 0.90 (s, 9 H), 1.34 (s, 3 H), 1.50 (s, 3 H), 2.04 (s, 3 H), 3.55 (d × d, 1 H, *J* = 10.5 8.0), 3.68 (d × d, 1 H, *J* = 10.5 5.0), 4.30 (d × d, 1 H, *J* = 7.9 5.1), 4.68 (d, 1 H, *J* = 6.0), 4.78 (d, 1 H, *J* = 6.0); MS (EI) *m/e* 332 (M−CH_3, 5.7%), 289 (M−$C(CH_3)_3$, 3.2), 288 (M−CH_3COO, 3), 230 (11), 172 (44), 144 (15), 130 (20), 117 (100).

Procedure D. α-N^4-Benzoyl-2′,3′-*O*-(1-methylethylidene)cytidine (17a) and β-N^4-Benzoyl-2′,3′-*O*-(1-methylethylidene)-cytidine (17b). N^4-Benzoylcytosine (430 mg, 2.0 mmol) in anhyd ACN (3.0 mL) was treated with bis(trimethylsilyl)acetamide (BSA) (494 μL, 2.0 mmol) and warmed to ca. 45 °C for 15 min. The resulting clear solution was cooled to rt. $SnCl_4$ (351 mL, 3.0 mmol) was added. In a separate flask, **13** (204 mg, 1.0 mmol) in anhyd ACN (1.0 mL) was treated with BSA (247 μL, 1.0 mmol) at rt for ca. 15 min. The contents of the second flask were added by cannula to the first flask. The reaction was stirred at 50 °C for 2 h. The reaction was cooled to room temperature and poured into a vigorously stirring mixture of EtOAc (20 mL) and 1 N NaOH (8 mL). The solids were removed by filtering through a pad of Celite. The layers were separated and the organic layer was washed with brine (20 mL), dried over anhyd $MgSO_4$, filtered, and evaporated under reduced pressure. The residue was adsorbed onto silica gel and purified by flash chromatography (*i*-PrOH:EtOAc:hexanes = 1:4:5) to give **17a** (119 mg, 31%) and **17b** (166 mg, 43%). Samples for analysis of both isomers were obtained by crystallization from ethanol and hexane. **17a**: mp 220–222 °C; $[\alpha]^{25}_D$ −168° (*c* 1.0, CH_3OH); IR (KBr) 3363, 3606, 3128, 3107, 3070, 3007, 2989, 2977, 2938, 2913, 2861, 1964, 1953, 1625, 1602, 1556, 1500, 1482 cm^{-1}; ^1H NMR (400 MHz, DMSO-d_6) δ 1.24 (s, 3 H), 1.30 (s, 3 H), 3.59 (m, 2 H), 4.38 (t, 1 H, *J* = 3.0), 4.85–4.90 (m, 2 H), 5.18 (t, 1 H, *J* = 5.0), 6.18 (d, 1 H, *J* = 4.0), 7.40 (br d, 1 H, *J* = 7.6), 7.50 (app t, 2 H, *J* = 7.6), 7.62 (app t, 1 H, *J* = 7.3), 8.00 (app d, 2 H, *J* = 7.0), 8.04 (d, 1 H, *J* = 7.5), 11.18 (br s, 1 H); MS (EI) *m/e* 387 (M, 11%), 372 (M−CH_3, 3), 216 (25), 214 (14), 186 (10), 138 (18), 105 (100). Anal. Calcd for $C_{19}H_{21}N_3O_6$: C, 58.91; H, 5.46; N, 10.85. Found: C, 58.83; H, 5.48; N, 10.82. **17b**: mp 177–178 °C (lit.[53] mp 182 °C); $[\alpha]^{25}_D$ −1.6° (*c* 0.75, CH_3OH); ^1H NMR (400 MHz, DMSO-d_6) δ 1.30 (s, 3 H), 1.50 (s, 3 H), 3.59 (d × d, 1 H, *J* = 11.8 4.9, H-5′*R*), 3.66 (d × d, 1 H, *J* = 11.8 4.0, H-5′*S*), 4.21 (m, 1 H), 4.77 (d × d, 1 H, *J* = 6.2 3.0), 4.90 (d × d, 1 H, *J* = 6.2 2.0), 5.02 (br s, 1 H), 5.87 (d, 1 H, *J* = 2.0), 7.33 (br d, 1 H, *J* = 6.1), 7.51 (app t, 2 H, *J* = 7.7), 7.62 (app t, 1 H, *J* = 7.5), 8.01 (app d, 2 H, *J* = 7.6), 8.27 (br d, 1 H, *J* = 7.2), 11.18 (br s, 1 H); MS (EI) *m/e* 387 (M, 1.7%), 372 (M−CH_3, 4.3), 216 (37), 215 (36), 214 (14), 173 (5), 138 (12), 105 (100). Anal. Calcd for $C_{19}H_{21}N_3O_6$1/2 H_2O: C, 57.57; H, 5.59; N, 10.60. Found: C, 57.74; H, 5.54; N, 10.54.

α-[5′-$^2H_2$]-N^4-Benzoyl-2′,3′-*O*-(1-methylethylidene)cytidine (18a) and β-[5′-$^2H_2$]-N^4-Benzoyl-2′,3′-*O*-(1-methylethylidene)cytidine (18b). **18a** (1.57 g, 24%) and **18b** (2.50 g, 38%) were isolated from the reaction of **15** (3.50 g, 17.0 mmol), N^4-benzoylcytosine (7.30 g, 34.0 mmol), BSA (10.4 mL, 42.1 mmol), and $SnCl_4$ (6.0 mL, 51.3 mmol) in anhyd ACN (85 mL) according to procedure D. **18a**: mp 220–222 °C; $[\alpha]^{25}_D$ −161.6° (*c* 0.38, CH_3OH); ^1H NMR (400 MHz, DMSO-d_6) δ 1.25 (s, 3 H), 1.31 (s, 3 H), 4.38 (s, 1H), 4.87 (d, 1 H, *J* = 6.1), 4.89 (d × d, 1 H, *J* = 6.1 4.0), 6.19 (d, 1 H, *J* = 4.0), 7.41 (br d, 1 H, *J* = 7.0), 7.51 (app t, 2 H, *J* = 7.6), 7.63 (app t, 1 H, *J* = 7.4), 8.00 (app d, 2 H, *J* = 7.2), 8.05 (d, 1 H, *J* = 7.4), 11.22 (br s, 1 H); MS (EI) *m/e* 389 (M, 9%), 374 (M−CH_3, 3), 216 (26), 215 (24), 214 (13), 138 (17), 105 (C_6H_5CO, 100). **18b**: mp 179–180 °C; $[\alpha]^{25}_D$ −3.3° (*c* 0.76, CH_3OH); ^1H NMR (400 MHz, DMSO-d_6) δ 1.30 (s, 3 H), 1.50 (s, 3 H), 4.21 (d, 1 H, *J* = 3.1), 4.77 (d × d, 2 H, *J* = 6.2 3.1), 4.90 (d × d, 1 H, *J* = 6.2 2.0), 5.86 (d, 1 H, *J* = 2.0), 7.34 (br d, 1 H), 7.51 (app t, 2 H, *J* = 7.7), 7.63 (app t, 1 H, *J* = 7.4), 8.01 (app d, 2 H, *J* = 8.3), 8.30 (d, 1 H, *J* = 7.6), 11.30 (bd s, 1 H); MS (EI) *m/e* 389 (M, 1.4%), 374 (M−CH_3, 4), 216 (34), 215 (38), 214 (15), 186 (10), 175 (3), 138 (13), 105 (C_6H_5CO, 100).

Procedure E. 17a and 17b. N^4-Benzoylcytosine (620 mg, 2.9 mmol), suspended in anhyd ACN (7.0 mL), was treated with BSA (1.42 mL, 5.8 mmol) and $SnCl_4$ (0.51 mL, 4.3 mmol) as in procedure D. A solution of **42** (485 mg, 1.40 mmol) in anhyd ACN (3.0 mL) was added by cannula to this solution. The reaction mixture was stirred at 45 °C for 1 h, cooled to rt, and poured into

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a vigorously stirring mixture of EtOAc (50 mL) and 1 N NaOH (20 mL). The solids were removed by filtering through a pad of Celite. The filtrate was washed with brine (50 mL), dried over anhyd $MgSO_4$, filtered, and evaporated under reduced pressure. The residue was dissolved in THF (10 mL) and treated with TBAF (3.0 mL) for 1 h at rt. The reaction was diluted with EtOAc (50 mL) and washed with 1 N HCl (20 mL) and brine (20 mL), dried over anhyd $MgSO_4$, filtered, and evaporated under reduced pressure. **17a** (200 mg, 36%) and **17b** (211 mg, 38%) were obtained by chromatography (see procedure D) and exhibited spectroscopic properties identical to those reported above.

α-[4′-^{2}H]-N^4-Benzoyl-2′,3′-*O*-(1-methylethylidene)cytidine (31a) and β-[4′-^{2}H]-N^4-benzoyl-2′,3′-*O*-(1-methylethylidene)cytidine (31b). **31a** (813 mg, 35%) and **31b** (718 mg, 31%) were isolated from the reaction of **30** (2.07 g, 5.97 mmol), N^4-benzoylcytosine (2.59 g, 12.03 mmol), BSA (5.93 mL, 24.0 mmol), and $SnCl_4$ (2.11 mL, 18.0 mmol) in anhyd ACN (40 mL) using procedure E. **31a**: mp 220–222 °C; $[\alpha]^{25}_D$ −166.3° (*c* 1.0, CH_3OH); ^{1}H NMR (400 MHz, DMSO-d_6) δ 1.25 (s, 3 H), 1.31 (s, 3 H), 3.60 (app d, 2 H, *J* = 5.1), 4.87 (d, 1 H, *J* = 6.0), 4.89 (d × d, 1 H, *J* = 6.0 4.1), 5.19 (t, 1 H, *J* = 5.1), 6.19 (d, 1 H, *J* = 4.0), 7.41 (br d, 1 H, *J* = 7.0), 7.50–7.65 (m, 3 H), 8.00 (app d, 2 H, *J* = 7.6), 8.05 (d, 1 H, *J* = 7.4), 11.22 (br s, 1 H); MS (EI) *m/e* 388 (M, 5%), 373 (M−CH_3, 2), 216 (22), 215 (21), 214 (11), 186 (11), 174 (8), 138 (14), 105 (C_6H_5CO, 100). **31b**: mp 180–181 °C; $[\alpha]^{25}_D$ −1.7° (*c* 1.0, CH_3OH); ^{1}H NMR (400 MHz, DMSO-d_6) δ 1.30 (s, 3 H), 1.50 (s, 3 H), 3.57 (d, 1 H, *J* = 11.8, H-5′*R*), 3.65 (d, 1 H, *J* = 11.8, H-5′*S*), 4.76 (d, 1 H, *J* = 6.1), 4.90 (d × d, 1 H, *J* = 6.1 2.0), 5.86 (d, 1 H, *J* = 2.0), 7.33 (br d, 1 H), 7.52 (app t, 2 H, *J* = 7.8), 7.63 (app t, 1 H, *J* = 7.4), 8.01 (app d, 2 H, *J* = 7.4), 8.30 (d, 1 H, *J* = 7.3), 11.30 (br s, 1 H); MS (EI) *m/e* 388 (M, 1.5%), 373 (M−CH_3, 4), 216 (38), 215 (37), 214 (14), 186 (9), 174 (4), 138 (13), 105 (C_6H_5CO, 100).

α-[1′-^{2}H]-N^4-Benzoyl-2′,3′-*O*-(1-methylethylidene)cytidine (35a) and β-[1′-^{2}H]-N^4-benzoyl-2′,3′-*O*-(1-methylethylidene)cytidine (35b). **35a** (631 mg, 28%) and **35b** (728 mg, 32%) were obtained from **34** (1.87 g, 5.87 mmol), N^4-benzoylcytosine (2.53 g, 11.80 mmol), BSA (5.80 mL, 23.5 mmol), and $SnCl_4$ (2.10 mL, 17.9 mmol) in anhyd ACN (40 mL) using procedure E. **35a**: mp 220–221 °C; $[\alpha]^{25}_D$ −167.4° (*c* 1.0, CH_3OH); ^{1}H NMR (400 MHz, DMSO-d_6) δ 1.25 (s, 3 H), 1.31 (s, 3 H), 3.60 (m, 2 H), 4.38 (m, 1 H), 4.86 (d, 1 H, *J* = 6.0), 4.89 (d, 1 H, *J* = 6.0), 5.10 (br s, 1 H), 7.40 (br d, 1 H), 7.51 (app t, 2 H, *J* = 7.9), 7.72 (app t, 1 H, *J* = 7.4), 7.99–8.04 (m, 3 H), 11.10 (br s, 1 H). **35b**: mp 181–182 °C; $[\alpha]^{25}_D$ −1.5° (*c* 0.59, CH_3OH); ^{1}H NMR (400 MHz, $CDCl_3$) δ 1.30 (s, 1 H), 1.50 (s, 1 H), 3.58 (d × d, 1 H, *J* = 11.8 4.8, H-5′*R*), 3.65 (d × d, 1 H, *J* = 11.8 4.2, H-5′*S*), 4.20 (app q, 1 H, *J* = 4.0), 4.77 (d × d, 1 H, *J* = 6.2 3.3), 4.90 (d, 1 H, *J* = 6.2), 7.33 (br d, 1 H), 7.51 (app t, 2 H, *J* = 7.7), 7.62 (app t, 1 H, *J* = 7.4), 8.00 (app d, 2 H, *J* = 7.4), 8.27 (br d, 1 H, *J* = 7.6), 11.19 (br s, 1 H); MS (EI) *m/e* 388 (M^+, 0.62%), 373 (M−CH_3, 3.7), 217 (9.9), 216 (30), 215 (38), 214 (14), 138 (13), 105 (C_6H_5CO, 100).

Procedure F. 17a and 17b. This reaction is essentially that outlined in procedure E except that the residue isolated from the original reaction mixture did not require further treatment with TBAF. In addition, the reaction was conducted at 40 °C for 4 h. Thus, addition of **36** (1.8 g, 10.3 mmol) in anhyd ACN (40 mL) to a flask containing N^4-benzoylcytosine (2.7 g, 12.4 mmol), BSA (6.1 mL, 24.7 mmol), anhyd ACN (40 mL), and $SnCl_4$ (1.4 mL, 12.4 mmol) followed by the addition of a further amount of $SnCl_4$ (0.3 mL, 2.6 mmol) yielded after flash chromatography **17a** (1.03 g, 26%) and **17b** (1.34 g, 34%). Both compounds exhibited spectroscopic properties identical to those previously reported for **24a** and **24b**.

α-(5′*R*)-[5′-^{2}H]-N^4-Benzoyl-2′,3′-*O*-(1-methylethylidene)-cytidine (38a) and β-(5′*R*)-[5′-^{2}H]-N^4-benzoyl-2′,3′-*O*-(1-methylethylidene) cytidine (38b). **38a** (1.32 g, 34%) and **38b** (1.43 g, 37%) were isolated from the reaction of **37** (1.78 g, 10.3 mmol), N^4-benzoylcytosine (2.66 g, 12.4 mmol), BSA (6.11 mL, 24.7 mmol), and $SnCl_4$ (1.75 mL, 15.0 mmol) in anhyd ACN (80 mL) according to procedure F. **38a**: mp 225–226 °C; $[\alpha]^{25}_D$ −160.6° (*c* 0.3, CH_3OH); ^{1}H NMR (400 MHz, DMSO-d_6) δ 1.24 (s, 3 H), 1.30 (s, 3 H), 3.58 (d, 1 H, *J* = 3.1, H-5′$_S$), 4.37 (d, 1 H, *J* = 3.6), 4.86 (d, 1 H, *J* = 6.0), 4.89 (d × d, 1 H, *J* = 6.0 4.1), 5.14 (br s, 1 H), 6.18 (d, 1 H, *J* = 4.0), 7.40 (br d, 1 H, *J* = 7.1), 7.51 (app t, 2 H, *J* = 7.6), 7.62 (app t, 1 H, *J* = 7.6), 7.99 (app d, 2 H, *J* = 7.2), 8.04 (1, d H, *J* = 7.5), 11.20 (br s, 1 H); MS (EI) *m/e* 388 (M, 8.0%), 373 (M−CH_3, 2.0), 216 (16), 215 (20), 214 (12), 186 (10), 138 (15), 105 (C_6H_5CO, 100). **38b**: mp 178–179 °C; $[\alpha]^{25}_D$ −1.9° (*c* 1.0, CH_3OH); ^{1}H NMR (400 MHz, DMSO-d_6) δ 1.29 (s, 3 H), 1.49 (s, 3 H), 3.63 (t, 1 H, *J* = 4.8, H-5′*S*), 4.20 (t, 1 H, *J* = 3.7), 4.76 (d × d, 1 H, *J* = 6.1 3.3), 4.90 (d × d, 1 H, *J* = 6.1 2.1), 5.06 (d, 1 H, *J* = 5.1), 5.86 (d, 1 H, *J* = 2.1), 7.34 (br d, 1 H, *J* = 6.4), 7.51 (app t, 2 H, *J* = 7.7), 7.62 (app t, 1 H, *J* = 7.4), 8.00 (app d, 2 H, *J* = 7.1), 8.29 (br d, 1 H, *J* = 8.1), 11.17 (br s, 1 H); MS (EI) *m/e* 388 (M, 1.0%), 373 (M−CH_3, 2.6), 216 (22), 215 (28), 214 (12), 138 (11), 105 (C_6H_5CO, 100).

Procedure G. N^4-Benzoylcytidine. **17b** (546 mg, 1.4 mmol) was treated with 95% aqueous TFA (10 mL; precooled to −10 °C) for 20 min at −10 °C and then at room temperature for 30 min. The reaction mixture was evaporated under reduced pressure. The residue was triturated was Et_2O (5 × 15 mL). The resulting white solid was suspended in CH_3OH (30 mL) and stirred for 3.5 h. Evaporation of the CH_3OH under reduced pressure yielded a colorless powder (425 mg, 87%): mp 229–230 °C (lit.[54] 233–235 °C); ^{1}H NMR (400 MHz, DMSO-d_6) δ 3.61 (d × d, 1 H, *J* = 12.2 3.0, H-5′*R*), 3.76 (d × d, 1 H, *J* = 12.2 2.7, H-5′*S*), 3.92 (m, 1 H), 3.98 (br t, 1 H, *J* = 5.4), 4.02 (d × d, 1 H, *J* = 4.8 3.0), 5.81 (d, 1 H, *J* = 3.0), 7.32 (br d, 1 H, *J* = 6.8), 7.52 (app t, 2 H, *J* = 7.7), 7.63 (app t, 1 H, *J* = 7.4), 8.01 (app d, 2 H, *J* = 7.2), 8.50 (d, 1 H, *J* = 7.6), 11.26 (br s, 1 H); MS (CI) *m/e* 348 (M + 1, 24%), 245 (11), 244 (22), 218 (13), 216 (43), 150 (19), 139 (46), 122 (100).

[5′-^{2}H$_2$]-N^4-Benzoylcytidine (6). **6** (1.96 g, 93%) was recovered from the reaction of **18b** (2.35 g, 6.0 mmol) and 95% aqueous TFA (50 mL) as outlined in procedure G: mp 230–232 °C; ^{1}H NMR (400 MHz, DMSO-d_6) δ 3.91 (d, 1 H, *J* = 6.0), 3.98 (d × d, 1 H, *J* = 4.8 6.0), 4.02 (d × d, 1 H, *J* = 4.8 3.1), 5.81 (d, 1 H, *J* = 3.1), 7.34 (br d, 1 H), 7.51 (app t, 2 H, *J* = 7.6), 7.63 (app t, 1 H, *J* = 7.4), 8.00 (app d, 2 H, *J* = 7.2), 8.49 (d, 1 H, *J* = 7.5), 11.25 (br s, 1 H); MS (CI) *m/e* 350 (M + 1, 10%), 246 (13), 216 (11), 139 (35), 122 (100).

[4′-^{2}H]-N^4-Benzoylcytidine (7). **7** (404 mg, 91%) was isolated from the reaction of **31b** (497 mg, 1.28 mmol) and 95% aqueous TFA (10 mL) according to procedure G: mp 223–224 °C dec; ^{1}H NMR (400 MHz, DMSO-d_6) δ 3.61 (d, 1 H, *J* = 12.2, H-5′*R*), 3.75 (d, 1 H, *J* = 12.2, H-5′*S*), 3.98 (d, 1 H, *J* = 4.8), 4.01 (d × d, 1 H, *J* = 4.8 3.0), 5.81 (d, 1 H, *J* = 3.0), 7.33 (br d, 1 H), 7.52 (app t, 2 H, *J* = 7.8), 7.63 (app t, 1 H, *J* = 7.4), 8.01 (app d, 2 H, *J* = 7.6), 8.50 (d, 1 H, *J* = 7.5), 11.25 (br s, 1 H); MS (CI, NH_3) *m/e* 349 (M + 1, 9%), 247 (3), 246 (14), 245 (15), 218 (13), 216 (51), 151 (18), 149 (5), 139 (24), 133 (6), 122 ($C_6H_5CONH_3$, 100), 114 (33), 112 (92), 105 (C_6H_5CO, 45).

[1′-^{2}H]-N^4-Benzoylcytidine (8). **8** (533 mg, 92%) was isolated by treatment of **35b** (655 mg, 1.71 mmol) with 95% aqueous TFA (25 mL) according to procedure G: mp 230–231 °C dec; ^{1}H NMR (400 MHz, DMSO-d_6) δ 3.61 (d × d, 1 H, *J* = 12.1 3.1, H-5′*R*), 3.75 (d × d, 1 H, *J* = 12.1 2.6, H-5′*S*), 3.92 (m, 1 H), 3.98 (br t, 1 H, *J* = 5.5), 4.03 (d, 1 H, *J* = 4.9), 4.94 (br s, 1 H), 5.05 (br s, 1 H), 5.35 (br s, 1 H), 7.32 (br s, 1 H), 7.50 (app t, 2 H, *J* = 7.6), 7.62 (app t, 1 H, *J* = 7.4), 8.00 (app d, 2 H, *J* = 7.1), 8.45 (br d, 1 H, *J* = 6.5), 11.12 (br s, 1 H); MS (CI) *m/e* 349 (M + 1, 22%), 245 (37), 216 (3), 141 (5), 114 (6), 113 (6), 112 (100), 105 (11).

(5′*R*)-[5′-^{2}H]-N^4-Benzoylcytidine (9). **9** (164 mg, 74%) was recovered from the reaction of **38b** (248 mg, 0.64 mmol) and 95% aqueous TFA (25 mL) as outlined in procedure G: mp 225–226 °C dec; ^{1}H NMR (400 MHz, DMSO-d_6) δ 3.73 (d, 1 H, *J* = 2.8, H-5′*S*), 3.91 (d × d, 1 H, *J* = 5.7 2.8), 3.99 (t, 1 H, *J* = 5.4), 4.02 (d × d, 1 H, *J* = 5.1 3.3), 5.82 (d, 1 H, *J* = 3.3), 7.31 (br s, 1 H), 7.51 (app t, 2 H, *J* = 7.6), 7.62 (app t, 1 H, *J* = 7.3), 8.00 (app d, 2 H, *J* = 7.2), 8.45 (d, 1 H, *J* = 6.8), 11.14 (br s, 1 H); MS (CI) *m/e* 349 (M + 1, 5%), 245 (6), 139 (12), 129 (12), 114 (52), 112 (100).

Deoxygenation at C-2′. Each of the labeled N^4-benzoylcytidines was deoxygenated at C-2′ using the four-step synthetic protocol established by Robins.[26] Complete characterization of all intermediates is supplied in the supplementary material.

N^4-Benzoyl-2′-deoxycytidine (44). Deoxygenation of N^4-benzoylcytidine (**43**) gave N^4-benzoyl-2′-deoxycytidine (**44**) (71

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mg, 74%; 27% from **43**): mp >250 °C [lit.[55] mp 174–175 °C (soften), 194 °C (melted), lit.[37] 230 °C dec]; $[\alpha]^{25}_D$ 83.7° (*c* 0.25, MeOH); ^{1}H NMR (400 MHz, DMSO-d_6, signals except 3′- and 5′-OH given after D_2O exchange) δ 2.06 (d × d x d, 1 H, *J* = 13.4 6.5 6.5, H-2′β), 2.31 (d × d × d, 1 H, *J* = 13.4 6.5 3.9, H-2′α), 3.59 (d × d, 1 H, *J* = 12.0 4.0, H-5′*R*), 3.64 (d × d, 1 H, *J* = 12.0 4.0, H-5′*S*), 3.88 (q, 1 H, *J* = 4.0), 4.24 (m, 1 H), 5.08 (t, 1 H, *J* = 5.0), 5.28 (d, 1 H, *J* = 4.0), 6.14 (app t, 1 H, *J* = 6.5), 7.34 (br d, 1 H, *J* = 7.3), 7.51 (app t, 2 H, *J* = 7.6), 7.62 (app t, 1 H, *J* = 7.4), 8.00 (app d, 2 H, *J* = 7.2), 8.40 (d, 1 H, *J* = 7.3), 11.23 (br s, 1 H); MS (CI) *m/e* 332 (M + H, 7.5%), 316 (0.4), 216 (100), 122 (11), 114 (2), 112 (35), 105 (12), 98 (33), 81 (32).

[5′-^{2}H$_2$]-*N*^{4}-Benzoyl-2′-deoxycytidine (1). **1** (410 mg, 69%; 26% from **6**) was isolated from **6**: mp >250 °C; $[\alpha]^{25}_D$ 89.8° (*c* 0.26, CH_3OH); ^{1}H NMR (400 MHz, DMSO-d_6) δ 2.06 (d × d x d, 1 H, *J* = 13.5 6.4 6.2, H-2′β), 2.31 (d × d x d, 1 H, *J* = 13.5 6.2 3.5, H-2′α), 3.87 (d, 1 H, *J* = 3.5), 4.24 (d × t, 1 H, *J* = 6.4 3.5), 6.14 (t, 1 H, *J* = 6.2), 7.34 (br d, 1 H), 7.51 (app t, 2 H, *J* = 7.7), 7.62 (app t, 1 H, *J* = 7.4), 8.00 (app d, 2 H, *J* = 7.1), 8.40 (d, 1 H, *J* = 7.4), 11.23 (bd s, 1 H); MS (CI, NH_3) *m/e* 334 (M + H, 6%), 318 (1), 216 (4), 122 (5), 114 (5), 112 (100), 105 (7), 100 (14), 83 (23).

[4′-^{2}H]-*N*^{4}-Benzoyl-2′-deoxycytidine (2). **2** (123 mg, 74%; 31% from **7**) was isolated from **7**: mp >250 °C; $[\alpha]^{25}_D$ 82.1° (*c* 0.52, CH_3OH); ^{1}H NMR (400 MHz, DMSO-d_6) δ 2.06 (d × d x d, 1 H, *J* = 13.5 6.2 6.0, H-2′β), 2.31 (d × d x d, 1 H, *J* = 13.5 6.2 4.0, H-2′α), 3.58 (d, 1 H, *J* = 12.0, H-5′*R*), 3.63 (d, 1 H, *J* = 12.0, H-5′*S*), 4.23 (d × d, 1 H, *J* = 6.0 4.0), 6.14 (t, 1 H, *J* = 6.2), 7.34 (br d, 1 H), 7.51 (app t, 2 H, *J* = 7.7), 7.62 (app t, 1 H, *J* = 7.4), 8.00 (app d, 2 H, *J* = 7.1), 8.40 (d, 1 H, *J* = 7.3), 11.23 (br s, 1 H); MS (CI) *m/e* 333 (M + H, 7%), 317 (1), 216 (9), 122 (14), 114 (18), 112 (100), 105 (12), 98 (10), 81 (22).

[1′-^{2}H]-*N*^{4}-Benzoyl-2′-deoxycytidine (3). **3** (136 mg, 85%; 31% from **8**) was isolated from **8**: mp 200 °C (shrank) no mp below 250 °C; $[\alpha]^{25}_D$ 85.5° (*c* 0.25, CH_3OH); ^{1}H NMR (400 MHz, DMSO-d_6, signals except 3′- and 5′-OH given after D_2O exchange) δ 2.05 (d × d, 1 H, *J* = 13.3 6.0, H-2′β), 2.31 (d × d, 1 H, *J* = 13.3 3.5, H-2′α), 3.58 (d × d, 1 H, *J* = 11.9 4.0, H-5′*R*), 3.63 (d × d, 1 H, *J* = 11.9 3.7, H-5′*R*), 3.87 (app q, 1 H, *J* = 3.7), 4.24 (m, 1 H), 4.98 (t, 1 H, *J* = 5.1), 5.19 (d, 1 H, *J* = 4.3), 7.34 (d, 1 H, *J* = 7.3), 7.50 (app t, 2 H, *J* = 7.6), 7.61 (app t, 1 H, *J* = 7.4), 8.00 (app d, 2 H, *J* = 7.3), 8.37 (d, 1 H, *J* = 7.3), 11.10 (br s, 1 H); MS (CI) *m/e* 333 (M + 1, 9%), 229 (9), 216 (15), 122 (12), 112 (100), 99 (14), 82 (20).

(5′*R*)-[5′-^{2}H]-*N*^{4}-Benzoyl-2′-deoxycytidine (4). **4** (484 mg, 70%; 27% from **9**) was isolated from **9**: mp 201–202 °C; $[\alpha]^{25}_D$ 80.5° (*c* 0.25, CH_3OH); ^{1}H NMR (300 MHz, DMSO-d_6) δ 2.04 (d × d x d, 1 H, *J* = 13.1 6.5 6.3, H-2′β), 2.27–2.34 (m, 1 H, H-2′α), 3.61 (d, 1 H, *J* = 3.6, H-5′*S*), 3.86 (app t, 1 H, *J* = 3.6), 4.23 (m, 1 H,), 5.03 (br s, 1 H), 5.27 (br s, 1 H, OH), 6.13 (t, 1 H, *J* = 6.3,), 7.35 (br d, 1 H, *J* = 7.4), 7.50 (app t, 2 H, *J* = 7.5), 7.62 (app t, 1 H, *J* = 7.3), 7.99 (app d, 2 H, *J* = 7.2), 8.39 (br d, 1 H, *J* = 7.4), 9.57 (br s, 1 H); MS (CI) *m/e* 332 (M, 0.2%), 216 (4.4), 215 (16), 214 (7.7), 186 (12), 105 (100).

Procedure I. *N*^{4}-Benzoyl-2′-deoxy-5′-(4-nitrobenzoyl)-cytidine (45). *N*^{4}-Benzoyl-2′-deoxycytidine (**44**, 50 mg, 0.15 mmol) and Ph_3P (43.5 mg, 0.17 mmol) were dissolved in anhyd HMPA (0.5 mL). In a separate flask, 4-nitrobenzoic acid (125 mg, 0.75 mmol) was dissolved in anhyd HMPA (0.5 mL) followed by addition of DEAD (28 μL, 0.17 mmol). The second solution was added by cannula to the first. The reaction was stirred at room temperature for 5 h and then diluted with EtOAc (10 mL). The organic layer was washed with 50% saturated $NaHCO_3$ (2 × 10 mL), water (5 × 10 mL), and brine (10 mL), dried over anhyd $MgSO_4$, filtered, and evaporated under reduced pressure. The title compound (53 mg, 74%) was isolated by flash chromatography (CH_3OH:$CHCl_3$ = 1:9). Crystals which formed in the most concentrated fractions were collected and dried under vacuum: mp 140 °C (shrank), 150–151 °C (melted); $[\alpha]^{25}_D$ 77.2° (*c* 0.25, 5% CH_3OH in $CHCl_3$) ; IR ($CHCl_3$) 3400, 3027, 3015, 3008, 1730, 1558, 1531; ^{1}H NMR (300 MHz, DMSO-d_6) δ 2.24 (d × d x d, 1 H, *J* = 6.5 6.5 13.1, H-2′β), 2.41 (m, 1 H, H-2′α), 4.19 (app q, 1 H, *J* ca. 4.4), 4.38 (m, 1 H), 4.53 (d × d, 1 H, *J* = 12.1 5.5, H-5′*R*), 4.63 (d × d, 1 H, *J* = 12.1 3.6, H-5′*S*), 5.54 (br s, 1 H), 6.17 (t, 1 H, *J* = 6.2), 7.23 (br d, 1 H, *J* = 7.4), 7.50 (app t, 2 H, *J* = 7.5), 7.61 (app t, 1 H, *J* = 7.4), 7.99 (app t, 2 H, *J* = 7.3), 8.14–8.20 (m, 3 H), 8.33 (app d, 2 H, *J* = 9.0); MS (CI) *m/e* 481 (M + 1, 0.3%), 332 (M–ArCO, 2.3), 246 (12), 149 (21), 137 (25), 120 (74), 105 (100). Anal. Calcd for $C_{23}H_{20}N_4O_8{}^1/_2$ H_2O: C, 56.44%; H, 4.32%; N, 11.45%. Found: C, 56.47%; H, 4.35%; N, 11.46%.

(5′*S*)-[5′-^{2}H]-*N*^{4}-Benzoyl-2′-deoxy-5′-(4-nitrobenzoyl)cytidine (41). **41** (276 mg, 72%) was isolated from the reaction of **4** (278 mg, 0.84 mmol), PPh_3 (241 mg, 0.92 mmol), 4-nitrobenzoic acid (703 mg, 4.2 mmol), and DEAD (145 μL, 0.92 mmol) in anhyd HMPA (5.6 mL) using procedure I: mp 140–141 °C (shrank), 151–152 °C (melted); ^{1}H NMR (400 MHz, DMSO-d_6) δ 2.24 (d × d x d, 1 H, *J* = 6.5 6.5 13.5, H-2′β), 2.41 (m, 1 H, H-2′α), 4.19 (t, 1 H, J = 4.9), 4.39 (m, 1 H), 4.51 (d, 1 H, *J* = 5.7, H-5′*R*), 5.53 (br s, 1 H), 6.17 (t, 1 H, *J* = 6.2), 7.23 (br d, 1 H, *J* = 7.4), 7.50 (app t, 2 H, *J* = 7.5), 7.61 (app t, 1 H, *J* = 7.3), 7.99 (app d, 2 H, *J* = 6.8), 8.14–8.20 (m, 3 H), 8.33 (app d, 2 H, *J* = 9.0); MS (CI) *m/e* 482 (M + 1, 1.2%), 351 (17), 179 (11), 236 (13), 220 (18), 216 (100), 215 (7), 155 (33), 139 (99).

Procedure J. *N*^{4}-Benzoyl-2′-deoxycytidine (44). **45** (20 mg, 0.043 mmol) was dissolved in anhyd THF /CH_3OH (1 : 1; 1 mL) and treated with $NaOCH_3$ (ca. 5.25 M in CH_3OH; 33 μL, 0.17 mmol). The reaction was stirred for *exactly* 7 min and then poured into a flask containing Dowex 50W acidic resin (pyridinium form; 1 g) and water (2 mL). The mixture was stirred for 5 min then filtered. The resin was rinsed with CH_3OH (2 × 10 mL). The filtrate was evaporated under reduced pressure. The title compound was obtained by trituration of the residue with Et_2O (5 × 10 mL) followed by flash chromatography (CH_3OH:$CHCl_3$ = 15:85) to yield a white solid (7.4 mg, 52%) whose spectroscopic properties were identical to those previously reported for **52**.

(5′*S*)-[5′-^{2}H]-*N*^{4}-Benzoyl-2′-deoxycytidine (5). **5** (147 mg, 75%) was recovered from the reaction of **41** (276 mg, 0.59 mmol) and $NaOCH_3$ (ca. 5.25 M in CH_3OH; 450 μL, 2.36 mmol) in THF/CH_3OH (1:1; 40 mL) according to procedure J: mp >250 °C; $[\alpha]^{25}_D$ 88.3° (*c* 0.25, CH_3OH); ^{1}H NMR (400 MHz, DMSO-d_6) δ 2.04 (d × d × d, 1 H, *J* = 13.1 6.5 6.2, H-2′β), 2.27–2.34 (m, 1 H, H-2′α), 3.56 (d, 1 H, *J* = 3.8, H-5′*R*), 3.86 (t, 1 H, *J* = 3.8), 4.22 (m, 1 H), 5.05 (br s, 1 H), 5.26 (br s, 1H), 6.13 (t, 1 H, *J* = 6.2), 7.35 (br d, 1 H, *J* = 7.4), 7.50 (app t, 2 H, *J* = 7.5), 7.62 (app t, 2 H, *J* = 6.9), 7.99 (app d, 2 H, *J* = 7.1), 8.39 (br d, 1 H, *J* = 7.4), 9.57 (br s, 1 H); MS (CI) *m/e* 332 (M, 0.3%), 216 (7.6), 215 (17), 214 (7.8), 186 (14), 105 (100).

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Supplementary Material Available: Experimental procedures for 2′-deoxygenation of **6-10** correspondingly to **1-5** and ^{1}H NMR spectra of **25-27** (11 pages). This material is contained in libraries on microfiche, immediately follows this article in the microfilm version of the journal, and can be ordered from the ACS; see any current masthead page for ordering information.

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