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Thermolytic Carbonates for Potential 5′-Hydroxyl Protection of Deoxyribonucleosides

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Thermolytic groups structurally related to well-studied heat-sensitive phosphate/thiophosphate protecting groups have been evaluated for 5′-hydroxyl protection of deoxyribonucleosides as carbonates and for potential use in solid-phase oligonucleotide synthesis. The spatial arrangement of selected functional groups forming an asymmetric nucleosidic 5′-*O*-carbonic acid ester has been designed to enable heat-induced cyclodecarbonation reactions, which would result in the release of carbon dioxide and the generation of a nucleosidic 5′-hydroxyl group. The nucleosidic 5′-*O*-carbonates **3**−**8**, **10**−**15**, and **19**−**21** were prepared and were isolated in yields ranging from 45 to 83%. Thermolytic deprotection of these carbonates is preferably performed in aqueous organic solvent at 90 °C under near neutral conditions. The rates of carbonate deprotection are dependent on the nucleophilicity of the functional group involved in the postulated cyclodecarbonation reaction and on solvent polarity. Deprotection kinetics increase according to the following order: **4** < **5** < **10** < **6** < **12** < **7** < **13** < **8** < **14** ≅ **19**−**21** and CCl_4 < dioxane < MeCN < *t*-BuOH < MeCN:phosphate buffer (3:1 v/v, pH 7.0) < EtOH:phosphate buffer (1:1 v/v, pH 7.0). Complete thermolytic deprotection of carbonates **7**, **8**, **13**, and **14** is achieved within 20 min to 2 h under optimal conditions in phosphate buffer−MeCN. The 2-(2-pyridyl)amino-1-phenylethyl and 2-[*N*-methyl-*N*-(2-pyridyl)]aminoethyl groups are particularly promising for 5′-hydroxyl protection of deoxyribonucleosides as thermolytic carbonates.

Introduction

Since the orthogonal protection of hydroxyl groups still presents a formidable challenge in the preparation of either natural or synthetic bioactive molecules, alkyl carbonates have found wide applications in such syntheses as protecting groups for alcohols.[1] In the context of nucleic acid synthesis, the isobutyloxycarbonyl group has been used to regioselectively protect the 5′-hydroxyl of thymidine to permit phosphorylation of the 3′-hydroxy function.[2] However, because of the strongly nucleophilic conditions that are required for its cleavage, the isobutyloxycarbonyl group is incompatible with the conventional *N*-acyl groups used for nucleobase protection, and has found limited applications as a hydroxyl protecting group in oligonucleotide synthesis. Carbonates have since been designed to provide 5′-hydroxyl protection while being compatible with the traditional nucleobase *N*-acyl protecting groups. These carbonates include the 9-fluorenylmethoxycarbonyl,[3] 2-(phenylsulfonyl)ethoxycarbonyl,[4] 2-(4-nitrophenyl)ethoxycarbonyl,[5] 2-dansylethoxycarbonyl,[6] and 1-phenyl-2-cyanoethoxycarbonyl groups.[7] These carbonates are sensitive to nonnucleophilic basic conditions and are generally compatible with 2′-*O*-acetal groups in the synthesis of oligoribonucleotides. Carbon-

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ates not requiring strong basic conditions for subsequent deprotection have also been developed for 5′-hydroxyl protection of nucleosides. Specifically, the (*p*-chlorophenoxy)carbonyl group has been successfully used in the solid-phase synthesis of an hexadecaoligodeoxyribonucleotide.[8] The iterative removal of the 5′-*O*-(*p*-chlorophenoxy)carbonate group throughout oligonucleotide synthesis is effected in less than 10 min upon treatment with a buffered peroxyanion solution.[8,9] The photosensitive α-methyl-6-nitropiperonyloxycarbonyl[10] and 2-(2-nitrophenyl)propyloxycarbonyl[11] groups have been employed for 5′-/3′-hydroxyl protection of deoxyribonucleoside phosphoramidites in the photolithographic in situ preparation of oligonucleotides on glass surfaces. These photolabile carbonate groups are typically cleaved under UV irradiation at 365 nm. The 3′,5′-dimethoxybenzoinyloxycarbonyl group for 5′-*O*-protection of deoxyribonucleoside phosphoramidites has similarly been tested in the photolithographic synthesis of oligonucleotides on arrays. Photochemical deprotection of this carbonate proceeded rapidly at 310 nm in a nonpolar solvent or without solvent.[12] While the photodirected approaches to the synthesis of oligonucleotide on microarrays exhibit attractive features, these methods have the potential to form undesirable side products or lead to unwanted secondary photoreactions with oligonucleotides. In this regard, the photogeneration of strong acids[13] for cleaving the terminal 5′-*O*-dimethoxytrityl group of growing oligonucleotide chains that are assembled via standard phosphoramidite chemistry[14] has also the propensity of being harsh to oligonucleotides and to glass surfaces.

Given our interest at improving the chemical synthesis of oligonucleotides on arrays, our strategy is to avoid photodeprotection reactions. We are now reporting our findings on the development of thermolabile carbonates for 5′-hydroxyl protection of deoxyribonucleosides, and evaluating the suitability of these groups for iterative deprotection reactions and for potential applications to the synthesis of oligonucleotides on arrayable glass surfaces.

Results and Discussion

The use of deoxyribonucleoside cyclic *N*-acylphosphoramidites in the *P*-stereodefined solid-phase synthesis of selected oligodeoxyribonucleoside phosphorothioates led to the formation of internucleotidic 2-[*N*-(2-fluoroacetyl)-amino]-1-phenylethyl thiophosphotriester linkages.[15] These phosphotriesters are thermolabile, as they are cleanly and efficiently converted to phosphorothioate diesters within 1 h upon heating at 85 °C in MeCN:H_2O (1:1 v/v). It was postulated that thiophosphate deprotection proceeded through a cyclodeesterification mechanism involving the participation of the amidic carbonyl group with concomitant formation of an oxazoline side product.[15] This novel approach to phosphate/thiophosphate deprotection led to a search for new thermolabile phosphate/thiophosphate protecting groups. Aside from the 2-[*N*-(2-fluoroacetyl)amino]-1-phenylethyl group, the 2-(*N*-acetylamino)ethyl,[16] 2-(*N*-acetyl-*N*-methylamino)ethyl,[16] 2-(*N*-formyl-*N*-methylamino)ethyl,[16] 2-(*N*,*N*-dimethylaminocarbonyloxy)ethyl,[16] 4-oxopentyl,[16,17] 3-(*N*-*tert*-butylcarboxamido)-1-propyl,[16,17b,18] 3-(*N*,*N*-dimethylcarboxamido)-1-propyl,[19] 3-(2-pyridyl)-1-propyl,[20] and several 2-benzamidoethyl groups[21] have also demonstrated thermolytic properties under neutral aqueous conditions. Given the relative ease with which these phosphate/thiophosphate protecting groups are removed from oligonucleotides, we decided to use structurally similar groups for the 5′-hydroxyl protection of deoxyribonucleosides. The thermolytic properties of these groups will be evaluated as carbonates so that release of carbon dioxide under the deprotection conditions may mimic phosphate/thiophosphate leaving groups. Several 3′-*O*-acetylthymidine 5′-*O*-carbonates were prepared to determine the structural parameters influencing the deprotection kinetics of each carbonate in the production of 3′-*O*-acetylthymidine.

Preparation of 3′-*O*-Acetylthymidine 5′-*O*-Carbonates. The synthesis of 3′-*O*-acetylthymidine 5′-*O*-carbonates begins with the condensation of 1,1′-carbonyldiimidazole (1.1 equiv) with 3′-*O*-acetylthymidine (**1**, 1.0 equiv) in anhydrous MeCN to afford the corresponding 3′-*O*-acetylthymidine 5′-*O*-(1-imidazolyl)carbamate **2** (Figure 1A) in yields exceeding 90% (TLC). To this solution is added a primary alcohol (1.5 equiv) followed by anhydrous 1,1,3,3-tetramethylguanidine (6.0 equiv). TLC analysis of the reaction shows that formation of the desired 3′-*O*-acetylthymidine 5′-*O*-carbonate (**3**−**8**) does not further progress beyond a 2 h reaction time. When secondary alcohols are employed in the preparation of 3′-*O*-acetylthymidine 5′-*O*-carbonates, 1,1′-carbonyldi-

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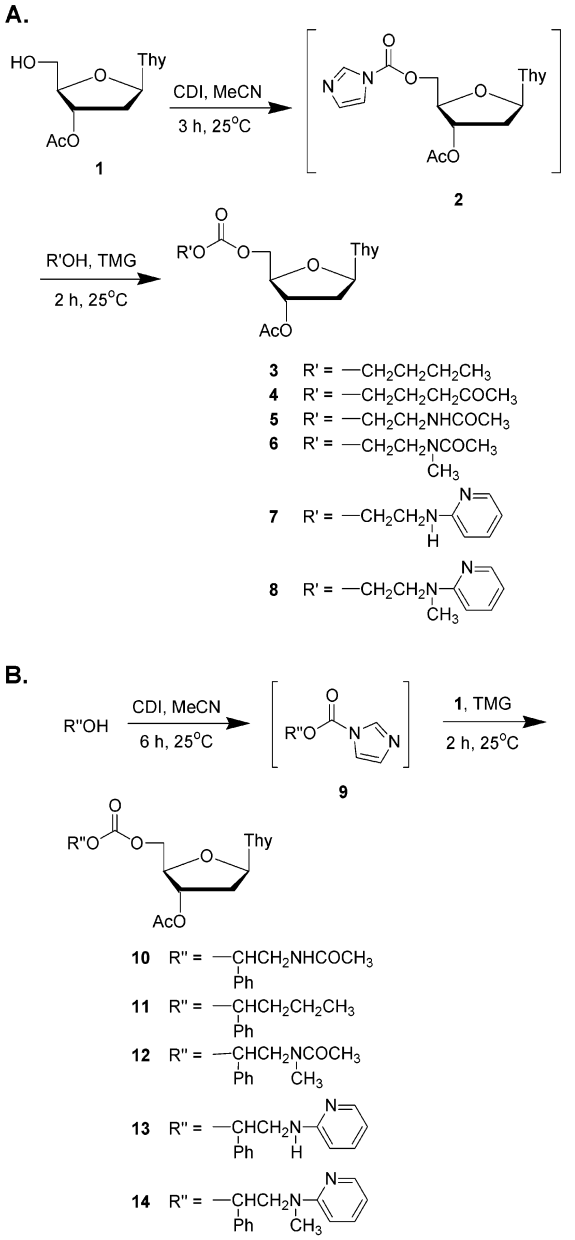


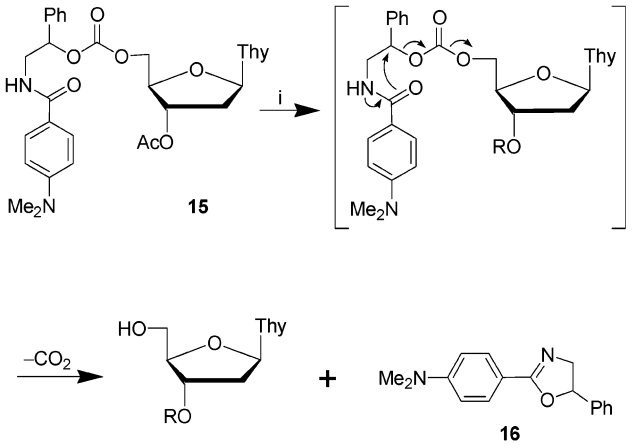
FIGURE 1. Synthesis of 3′-*O*-acetylthymidine 5′-*O*-carbonates. Key: CDI = 1,1′-carbonyldiimidazole; TMG = 1,1,3,3-tetramethylguanidine; Thy = thymin-1-yl.

imidazole is reacted first with the secondary alcohol (1.0 equiv) in dry MeCN (Figure 1B).

Formation of the corresponding (1-imidazolyl)carbamate intermediate **9** is near complete (TLC) within 6 h at 25 °C. Addition of solid 3′-*O*-acetylthymidine (1.0 equiv) to the solution followed by dry 1,1,3,3-tetramethylguanidine (6.0 equiv) produced the 3′-*O*-acetylthymidine 5′-*O*-carbonate product (**10**−**14**) within 2 h. The nucleosidic carbonates **3**−**8** were purified by silica gel chromatography and were isolated in yields ranging from 70 to 83%, whereas carbonates **10**−**14** were isolated in yields varying from 45 to 73%.

Structural Parameters Affecting the Thermostability of 3′-*O*-Acetylthymidine 5′-*O*-Carbonates. To systematically address the structural features affecting

SCHEME 1[a,b]



[a] Reaction conditions: (i) MeCN:phosphate buffer, pH 7.0, (3:1 v/v), 90 °C, 3 h. [b]Key: Thy = thymin-1-yl; R = acetyl and H.

the thermolytic stability of nucleosidic 5′-*O*-carbonate protecting groups, the carbonate **3** was dissolved in MeCN:phosphate buffer, pH 7.0 (3:1 v/v), and heated to 90 ± 2 °C in a tightly stoppered screw-cap glass vial. The thermostability of **3** was assessed by reverse phase (RP) HPLC to serve as a reference in a series of experiments. The 5′-*O*-butyl carbonate group was cleaved to the extent of ca. 2% within 6 h, as evidenced by the presence of **1** and thymidine (Table 1). In this experiment, 3′-*O*-deacetylation of **3** is more predominant than the cleavage of the 5′-*O*-butyl carbonate group. Incidentally, the 3′-*O*-acetyl group serves as an internal standard in estimating the stability of 5′-*O*-carbonates to potential cleavage caused by the buffer and the nucleophilic functional groups endogenous to the carbonates at elevated temperatures. In this context, the thermostability of 5′-*O*-(4-oxopentyl) carbonate **4** is very similar to that of **3** under identical conditions (Table 1). In contrast to the relatively rapid thermolytic cleavage of the 4-oxopentyl phosphate protecting group,[17a] the 4-oxo group of carbonate **4** was not sufficiently nucleophilic to induce significant release of carbon dioxide through a cyclodecarbonation mechanism analogous to the cyclodeesterification of heat-sensitive phosphate/thiophosphate protecting groups. To further assess the participation of nucleophilic groups to the putative intramolecular cyclodecarbonation mechanism, the 5′-*O*-amidoethyl carbonates **5** and **6** were selected for such an investigation. Under the conditions studied, the amidic carbonyl group of carbonate **5** is significantly more nucleophilic than the 4-oxo group of carbonate **4**, and results in more efficient 5′-*O*-decarbonation than 3′-*O*-deacetylation (Table 1). Furthermore, the significant decrease in thermostability of carbonate **6** is reflective of an increased nucleophilicity of the amidic carbonyl group, which led to a ca. 30-fold increased production of **1** and thymidine within 3 h relative to that produced from carbonate **4** (Table 1) within 6 h. Given the modest 3′-*O*-deacetylation detected in this experiment, it is likely that **1** is generated through the proposed intramolecular cyclodecarbonation mechanism (Scheme 1).

To further validate the postulated cyclodecarbonation mechanism operating in the thermolytic deprotection of selected 3′-*O*-acetylthymidine 5′-*O*-carbonates, the ther-

TABLE 1. RP-HPLC Analysis of 3′-*O*-Acetylthymidine 5′-*O*-Carbonates under Thermolytic Conditions[a]

carbonate	time (min)	recovered carbonate (area %)	3′-OH carbonate (area %)	**1** (area %)	thymidine (area %)	5′-*O*-carbonate species[b] (area %)	5′-OH species[c] (area%)
3	360	93.3	5.0	1.6	0.1	98.3	1.7
4	360	93.8	3.5	2.5	0.2	97.3	2.7
5	360	79.6	3.4	16.2	0.8	83.0	17.0
6	180	18.9	1.3	74.4	5.4	20.2	79.8
7	120	0.4	0.0	92.6	7.0	0.4	99.6
8	30	0.3	0.0	92.9	6.8	0.3	99.7
10	360	29.7	2.3	62.1	5.9	32.0	68.0
11	360	92.2	5.7	1.9	0.2	97.9	2.1
12	120	14.4	0.9	79.3	5.4	15.3	84.7
13	60	0.3	0.0	92.5	7.2	0.3	99.7
14	15	0.2	0.0	98.8	0.9	0.2	99.7

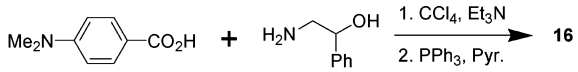
[a] The carbonates (~2 mg) were dissolved in MeCN:phosphate buffer (3:1 v/v, pH 7.0, 0.5 mL) and were heated to 90 ± 2 °C. RP-HPLC analyses of the deprotection reactions were performed with a 5 μm Supelcosil LC-18S column (25 cm × 4.6 mm) under the following conditions: starting from 0.1 M triethylammonium acetate pH 7.0, a linear gradient of 1% MeCN/min is pumped at a flow rate of 1 mL/min for 40 min and, then, held isocratically for 20 min. Retention times (R_T) of the 3′-*O*-acetylthymidine 5′-*O*-carbonates and related deprotection species are reported in the Supporting Information. [b] 5′-*O*-Carbonate species is the sum of recovered carbonate and 3′-OH carbonate expressed in area %. [c] 5′-OH species is the sum of **1** and thymidine expressed in area %.

mostability of carbonates **10**−**12** was determined (Table 1). Addition of a phenyl group to the carbon atom adjacent to the carbonate function generates an electrophilic benzylic center, which should favor a nucleophilic amidic carbonyl attack and release of carbon dioxide in accordance with the proposed cyclodecarbonation mechanism (see Scheme 1). As a consequence of this modification, the thermostability of carbonate **10** is considerably lower than that of carbonate **5** under identical conditions, and resulted in a 4-fold increase in the production of **1** and thymidine (Table 1). The carbonate **11** was subjected to the same thermolytic conditions to assess whether the creation of the benzylic center alone accounted for the decreased thermostability of **10**. Since the thermostability of **11** is similar to that of carbonate **3** (Table 1), the benzylic function is necessary but not sufficient to account for the lower thermostability of **10** relative to that of **5**, and further supports an operative cyclodecarbonation mechanism in the thermolysis of selected 3′-*O*-acetylthymidine 5′-*O*-amidoethyl carbonates. On the basis of the significant difference in thermostability observed between carbonates **5** and **6**, one can predict that the thermostability of **12** would be considerably lower than that of **10** under the same conditions. Indeed, the thermolytic release of **1** and thymidine from **12** proceeds to the extent of 85% within 2 h when compared with that of 68% in the case of **10** over a 6 h deprotection time (Table 1).

The carbonate **15** (Scheme 1) was prepared as shown in Figure 1B to unequivocally confirm the cyclodecarbonation mechanism operating in the thermolytic cleavage of 3′-*O*-acetylthymidine 5′-*O*-amidoethyl carbonates.[22] The carbonate **15** was designed to (i) produce the oxazoline **16** upon thermolytic cyclodecarbonation conditions, (ii) enable UV detection of **16** at 254 nm, and (iii) provide lipophilicity to **16** to facilitate its isolation by RP-HPLC.

As expected, RP-HPLC analysis of the thermolytic cyclodecarbonation of **15** indicated the formation of the oxazoline **16**, which is revealed as a peak having a

SCHEME 2



retention time (R_T) of 58 min.[23] Mass spectral analysis of RP-HPLC-purified **16** is consistent with its proposed structure. The identity of **16** was further corroborated by its synthesis (Scheme 2), which was adapted from a published method.[24]

The oxazoline **16** was isolated by silica gel chromatography and was shown to have the same RP-HPLC retention time as the oxazoline generated from the thermolytic cyclodecarbonation of **15** when using two different chromatographic gradients.[23] In addition, mass spectral analysis of these oxazolines revealed peaks corresponding to the correct mass expected for **16**. These findings strongly support the cyclodecarbonation of 3′-*O*-acetylthymidine 5′-*O*-amidoethyl carbonates under thermolytic conditions.

Even though the thermolytic cyclodecarbonation of **12** is 85% complete within 2 h, the use of such amidoethyl carbonates for 5′-hydroxyl protection toward oligonucleotide synthesis is not practical given the sluggish deprotection kinetics of these carbonates. Structural modifications of the amidoethyl carbonates were required to significantly improve their deprotection rates under thermolytic conditions. In this regard, the 2-(2-pyridyl)-aminoethyl carbonates **7** and **8** were designed to increase the electronic density on the nitrogen atom of the 2-pyridyl function in a manner analogous to that of the amidic carbonyl group of carbonates **5** and **6**, respectively. Syntheses of 2-(2-pyridyl)aminoethanol and 2-[*N*-methyl-*N*-(2-pyridyl)]aminoethanol, which are prerequisite to the preparation of carbonates **7** and **8** (Figure 1A), were

(22) The amido alcohol required for the preparation of **15** was obtained from the condensation of 4-(dimethylamino)benzoic acid with 1,1′-carbonyldiimidazole in THF followed by addition of 2-methylamino-1-phenylethanol. Details of the synthesis can be found in the Experimental Section.

(23) RP-HPLC analysis was performed with a 5 μm Supelcosil LC-18S column (25 cm × 4.6 mm) and the following gradient pumped at a flow rate of 1 mL/min: linear from 0% B to 50% B in 50 min, isocratic 50% B for 5 min, and linear from 50% B to 0% B in 5 min. However, when using the following gradient at a flow rate of 1 mL/min—linear from 0% B to 70% B in 50 min, isocratic 70% B for 5 min, and linear from 70% B to 0% B in 5 min—the retention time of **16** is 44 min. A: 0.1 M triethylammonium acetate, pH 7.0; B: MeCN.

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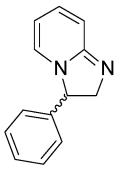
performed as recommended in the literature[25] by reacting 2-bromopyridine with 2-aminoethanol and 2-(methylamino)ethanol, respectively.

The thermostability of **7** and **8** was tested under conditions identical with those used for amidoethyl carbonates. As expected, the increased nucleophilicity of the 2-*N*-alkylaminopyridyl group in carbonate **7** resulted in the formation of **1** and thymidine to the extent of 99.6% within 2 h on the basis of RP-HPLC analysis of the deprotection reaction (Table 1). The deprotection kinetics of carbonate **7** is thus considerably faster than that of the analogous amidoethyl carbonate **5**, which produced **1** and thymidine to the extent of 17% within 6 h at 90 °C (Table 1). It is likely that the thermolytic deprotection of **7** proceeds through a cyclodecarbonation mechanism similar to that proposed for amidoethyl carbonates. Indeed, RP-HPLC analysis of the thermolytic deprotection reaction revealed a peak (R_T of 22 min) corresponding to the expected cyclodecarbonation side product **17** (Scheme 3) on the basis of NMR data (see the Experimental Section).

Consistent with the corresponding amidoethyl carbonate, thermolytic deprotection of carbonate **8** was rapid and produced **1** and thymidine to the extent of 99.7% within 30 min. To further improve the deprotection kinetics of carbonates **7** and **8**, the 1-phenyl-2-(2-pyridyl)-aminoethyl carbonate **13** and its *N*-methyl derivative **14** were prepared as outlined in Figure 1B. RP-HPLC analysis of the thermolytic deprotection of carbonates **13** and **14**, under the conditions described in Scheme 3, indicated that **1** and thymidine were both produced to the extent of 99.7% within 60 and 15 min, respectively (Table 1). The thermolysis of **13** also led to the formation of the cyclodecarbonation side product **18**, which exhibited a RP-HPLC retention time of 47 min due to increased lipophilicity relative to that of **17** (R_T of 22 min under identical chromatographic conditions). NMR and mass spectral analyses of **18** are in accordance with its proposed structure (see the Experimental Section).



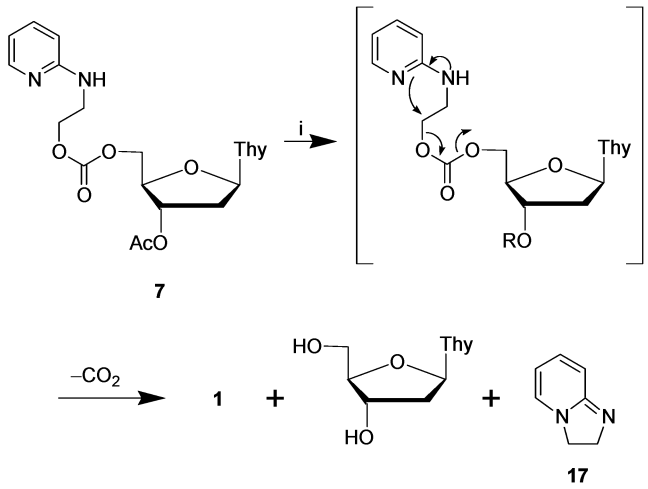
18

Solvent Effects on the Thermolytic Deprotection of 3′-*O*-Acetylthymidine 5′-*O*-Carbonate 14. While the thermolytic deprotection of **14** has been investigated with use of phosphate buffer−MeCN, it became imperative to evaluate its deprotection kinetics in other solvents. Thus, phosphate buffer−MeCN was replaced by CCl_4, dioxane, MeCN, *t*-BuOH, and phosphate buffer−EtOH in separate thermolytic experiments. The results of this study are presented in Table 2 and indicate that the rates of deprotection increase with solvent polarity, as the fastest rates being observed are those obtained in phosphate buffer−EtOH.

The *N*-protected-3′-*O*-acetyldeoxyribonucleoside 5′-*O*-carbonates **19**−**21** were then prepared as recommended for the synthesis of **14** to assess the generality of the thermolytic deprotection method.

(25) Weiner, N.; Kaye, I. A. *J. Org. Chem*. **1949**, *14*, 868−872.

SCHEME 3[a,b]

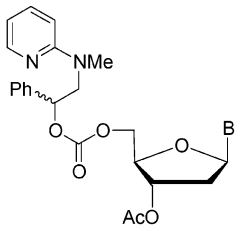


[a] Reaction conditions: (i) MeCN:phosphate buffer, pH 7.0, (3:1 v/v), 90 °C, 2 h. [b]Key: Thy = thymin-1-yl; R = acetyl and H.

TABLE 2. RP-HPLC Analysis of 3′-*O*-Acetylthymidine 5′-*O*-Carbonate 14 under Thermolytic Conditions in Various Solvents[a]

solvent	recovered carbonate (area %)	**1** (area %)	Thy (area %)
CCl_4	65.6	34.4	ND
dioxane	56.5	43.5	ND
MeCN	10.0	90.0	ND
t-BuOH	7.1	92.9	ND
A[b]	ND	93.4	6.6
B[c]	ND	98.6	1.4

[a] The carbonate (∼2 mg) was heated to 90 ± 2 °C in the specified solvent (0.5 mL) for 20 min or as indicated. RP-HPLC analyses of the deprotection reactions were performed under conditions identical with those described in Table 1. [b] A: EtOH:phosphate buffer (1:1 v/v, pH 7.0), 10 min. [c] B: MeCN:phosphate buffer (3:1 v/v, pH 7.0). ND, not detected.



19 B = 4-*N*-Benzoylcytosin-1-yl
20 B = 6-*N*-Benzoyladenin-9-yl
21 B = 2-*N*-Isobutyrylguanin-9-yl

Removal of the 5′-*O*-carbonate group from **19**−**21** is, like that of **14**, generally complete within 20 min in phosphate buffer−MeCN, and within 10 min in phosphate buffer−EtOH, as determined by RP-HPLC analysis of the deprotection reactions (Table 3). A more extensive 3′-*O*-deacetylation occurred in experiments performed in phosphate buffer−EtOH, presumably because of the buffer's propensity to induce transesterification at elevated temperature (Table 3). Noteworthy is the thermolytic deprotection of carbonate **20**, which produced

TABLE 3. RP-HPLC Analysis of *N*-Protected 3′-*O*-Acetyl-2′-deoxyribonucleoside 5′-*O*-Carbonates 19−21 under Thermolytic Conditions[a]

carbonate	buffer[b]	time (min)	recovered carbonate (area %)	*N*-protected 3′-OAc dN (area %)	*N*-protected dN (area %)	dN (area %)
19	A	5	2.1	95.3	2.6	ND
	A	10	ND	95.2	4.8	ND
	B	15	0.8	97.3	1.9	ND
	B	20	ND	97.3	2.7	ND
20	A	5	3.7	93.3	2.8	<0.1
	A	10	ND	93.8	6.1	0.1
	B	15	0.2	99.1	0.7	ND
	B	20	ND	99.4	0.6	ND
21	A	5	2.7	95.2	2.2	ND
	A	10	ND	95.1	4.9	ND
	B	15	0.3	98.1	1.6	ND
	B	20	ND	97.6	2.4	ND

[a] The carbonates (∼2 mg) were dissolved in buffer A or B (0.5 mL) and were heated to 90 ± 2 °C for specified periods of time. RP-HPLC analyses were performed with a 5 μm Supelcosil LC-18S column (25 cm × 4.6 mm) under the following conditions: Starting from 0.1 M triethylammonium acetate pH 7.0, a linear gradient of 1% MeCN/min is pumped at a flow rate of 1 mL/min for 40 min, and is held isocratically for 5 min. Then, a linear gradient of 2% MeCN/min is set for 10 min at 1 mL/min, and finally held isocratically for 20 min. Retention times (R_T) of the 3′-*O*-acetyl-2′-deoxyribonucleoside 5′-*O*-carbonates and related deprotection species are reported in the Supporting Information. [b] A: EtOH:phosphate buffer (1:1 v/v, pH 7.0). B: MeCN:phosphate buffer (3:1 v/v, pH 7.0). ND, not detected; dN = 2′-deoxyribonucleoside.

small amounts of 6-*N*-benzoyladenine (≤0.1%) and induced the loss of N^6-benzoyl protection (≤0.1%) only when phosphate buffer−EtOH was used as the solvent (Table 3).

Stability of the 3′-*O*-Acetyl-2′-deoxyribonucleoside 5′-*O*-Carbonates 14 and 19−21 in Anhydrous Solvents. The purification of **14** and *N*-protected-3′-*O*-acetyl-2′-deoxyribonucleoside 5′-*O*-carbonates **19**−**21** by silica gel chromatography was difficult. The purified carbonates were contaminated with variable amounts of 3′-*O*-acetylthymidine **1** or *N*-protected-3′-*O*-acetyl-2′-deoxyribonucleosides that were produced during chromatography. Thus, the carbonates **14** and **19**−**21** were not completely stable to the chromatographic conditions used for purification and raised questions on the stability of these carbonates in anhydrous MeCN, which is the preferred solvent used for deoxyribonucleoside phosphoramidites in the synthesis of oligonucleotide on solid supports or on planar glass surfaces. The stability of carbonates **14** and **19**−**21**, as 0.1 M solutions in dry MeCN, was tested at 25 °C. RP-HPLC analysis of the solutions revealed the presence of **1** and *N*-protected-3′-*O*-acetyl deoxyribonucleosides contaminating the carbonates to the extent of 10% after 24 h. Conversion of carbonate **14** to **1** in MeCN was reduced considerably when the solution was stored at 5 °C. At this temperature, the formation of 3′-*O*-acetylthymidine over a period of 24 h was minimal (1.4%). The formation of **1** was also minimized when **14** was dissolved in dioxane as a 0.1 M solution to be kept at 25 °C. Under these conditions, RP-HPLC analysis of the solution indicated that the formation of 3′-*O*-acetylthymidine reached the level of 2.5% within 24 h. While the 3′-*O*-phosphoramidite derivatives of **14** and that of **19**−**21** may not be as stable in MeCN as standard 5′-*O*-dimethoxytrityl deoxyribonucleoside phosphoramidites, it is, however, anticipated that these phosphoramidite derivatives, when dissolved in dry dioxane, may still find applications in the synthesis of short oligonucleotides (20−25 mers) given that most syntheses are complete within 5 h.

Conclusion

On the basis of our findings on thermolabile phosphate/thiophosphate protecting groups we have developed novel thermolytic carbonate groups for the 5′-hydroxyl protection of deoxyribonucleosides. Most 5′-*O*-carbonates are stable at ambient temperature in anhydrous aprotic solvents, such as MeCN, except for 5′-*O*-carbonates **14**, and **19**−**21**, which show a ∼10% deprotection over 24 h. With the exception of 5′-*O*-carbonates **3**, **4**, and **11**, thermolytic deprotection of the carbonates studied proceeds predominantly through a cyclodecarbonation mechanism (see Schemes 1 and 3) within 10 min to more than 6 h at 90 °C (see Tables 1 and 3). 3′-*O*-Deacetylation of nucleoside 5′-*O*-carbonates occurring during thermolytic deprotection indicates that hydrolysis and/or nucleophilic attack caused by endogenous functional groups may likely contribute to carbonate cleavage at elevated temperature. The nucleoside 5′-*O*-carbonates **8** and **13** are representative examples of 5′-hydroxyl protection. These compounds are easily purified by silica gel chromatography and yet undergo near complete thermolytic 5′-*O*-deprotection in less than 60 min at 90 °C under neutral conditions. The use of carbonates **8** and **13** in automated oligonucleotide synthesis on microarrays is, however, impractical for such an iterative process given the time required for thermolytic cleavage of the 5′-*O*-carbonates. The 2-(2-pyridyl)amino-1-phenylethyl and 2-[*N*-methyl-*N*-(2-pyridyl)]aminoethyl groups may nonetheless be useful for hydroxyl protection of nucleosides and carbohydrates, and that of any natural or synthetic products as carbonates, whenever orthogonal protection/deprotection of functional groups may be required.

The lesser stability of the 2-[*N*-methyl-*N*-(2-pyridyl)]-amino-1-phenylethyl group for 5′-hydroxyl protection of deoxyribonucleosides as a carbonate prompted its modification to improve its stability at 25 °C while retaining its rapid thermolytic deprotection kinetics. Preliminary results show that the placement of an electron-withdrawing group at *C*-4 of the pyridyl moiety provides adequate stability to the carbonate at ambient temperature to

permit oligonucleotide synthesis on glass surfaces. Efficient conversion of the electron-withdrawing group to an electron-donating group led to speedy thermolytic deprotection of the carbonate protecting group at temperatures much lower than 90 °C. The details on the use of such a versatile thermolytic carbonate for 5′-hydroxyl protection of deoxyribonucleosides will be reported elsewhere in due course.

Experimental Section

(±)-2-[(4-Dimethylamino)benzoyl]amino-1-phenylethanol. 4-(Dimethylamino)benzoic acid (3.30 g, 20.0 mmol) and 1,1′-carbonyldiimidazole (3.24 g, 20.0 mmol) were dissolved in anhydrous tetrahydrofuran (20 mL), and the magnetically stirred solution was refluxed for 1 h. (±)-2-Amino-1-phenylethanol (2.70 g, 20.0 mmol) and triethylamine (2.8 mL, 20 mmol) were then added to the stirred solution, which was kept under reflux for an additional 2.5 h. The solution was cooled to ambient temperature and evaporated to dryness under reduced pressure. The material was dissolved in chloroform (100 mL) and the solution was washed with a saturated solution of sodium bicarbonate (3 × 100 mL) followed by water (100 mL). The organic phase was dried over anhydrous magnesium sulfate, and then evaporated to dryness under low pressure affording a white amorphous solid (4.50 g, 15.8 mmol, 79%) that is pure enough (TLC) to be used without further purification.^{1}H NMR (300 MHz, DMSO-d_6:$CDCl_3$) δ 8.10 (m, 1H), 7.72 (d, J = 9.0 Hz, 2H), 7.38−7.19 (m, 5H), 6.65 (d, J = 9.0 Hz, 2H), 5.50 (d, J = 4.4 Hz, 1H), 4.77 (ddd, J = 4.4, 4.4, 8.0 Hz, 1H), 3.50 (ddd, J = 4.4, 6.2, 13.2 Hz, 1H), 3.28 (ddd, J = 5.2, 8.0, 13.2 Hz, 1H), 2.95 (s, 6H). ^{13}C NMR (75 MHz, DMSO-d_6:$CDCl_3$) δ 39.5, 47.6, 71.5, 110.5, 121.0, 125.7, 126.6, 127.7, 128.3, 143.7, 151.8, 166.5. FAB-HRMS: calcd for $C_{17}H_{21}N_2O_2$ $(M + H)^+$ 285.1603, found 285.1610.

(±)-2-(*N*-Acetyl)amino-1-phenylethanol. This compound was prepared in a manner identical with that described for the synthesis of (±)-2-[(4-dimethylamino)benzoyl]amino-1-phenylethanol. Characterization data were comparable to those reported in the literature.[26]

2-[*N*-Methyl-*N*-(2-pyridyl)]aminoethanol. 2-Bromopyridine (7.6 mL, 80 mmol) and 2-methylaminoethanol (12.8 mL, 160 mmol) were heated for 24 h in an oil bath kept at 160 °C. The reaction mixture was then allowed to cool to ambient temperature and chloroform (300 mL) was added. The solution was shaken with a saturated solution of potassium carbonate (200 mL). The organic layer was dried over anhydrous magnesium sulfate and evaporated to dryness. The material left was distilled under reduced pressure affording a liquid (bp 112−115 °C at 0.8 Torr) (6.4 g, 46 mmol, 58%). ^{1}H NMR (300 MHz, DMSO-d_6) δ 8.04 (ddd, J = 1.0, 2.0, 4.9 Hz, 1H), 7.46 (ddd J = 2.0, 7.0, 8.7 Hz, 1H), 6.58 (ddd J = 1.0, 2.0, 8.7 Hz, 1H), 6.51 (ddd J = 2.0, 4.9, 7.0 Hz, 1H), 3.56 (t, J = 1.9 Hz, 4H), 3.01 (s, 3H). ^{13}C NMR (75 MHz, DMSO-d_6) δ 36.7, 51.7, 58.5, 105.5, 110.9, 137.0, 147.3, 158.2. FAB-HRMS: calcd for $C_8H_{13}N_2O$ $(M + H)^+$ 153.1028, found 153.1034.

(±)-1-Phenyl-2-(2-pyridyl)aminoethanol. (±)-1-Phenyl-2-(2-pyridyl)aminoethanol was prepared according to the procedure of Gray et al.[27] and was isolated as a solid in 60% yield. ^{1}H NMR (300 MHz, DMSO-d_6) δ 7.96 (ddd, J = 1.0, 2.0, 5.0 Hz, 1H), 7.35 (m, 6H), 6.52 (ddd, J = 1.0, 2.0, 8.5 Hz, 1H), 6.47 (ddd, J = 2.0, 5.0, 7.0 Hz, 1H), 5.65 (d, J = 4.2 Hz, 1H), 4.75 (m, 1H), 3.51 (ddd, J = 4.3, 6.7, 13.4 Hz, 1H), 3.26 (ddd, J = 5.1, 7.9, 13.4 Hz, 1H). ^{13}C NMR (75 MHz, DMSO-d_6) δ 49.2, 71.5, 108.4, 111.5, 125.9, 126.7, 127.9, 136.5, 144.2, 147.2, 158.8. FAB-HRMS: calcd for $C_{13}H_{15}N_2O$ $(M + H)^+$ 215.1184, found 215.1192.

(±)-2-[*N*-Methyl-*N*-(2-pyridyl)amino]-1-phenylethanol. (±)-2-[*N*-Methyl-*N*-(2-pyridyl)]amino-1-phenylethanol was prepared in a manner identical with that of 1-phenyl-2-(pyridyl)aminoethanol, and was isolated as an oil in 55% yield. The crude oil was redistilled as recommended in the literature.[27] ^{1}H NMR (300 MHz, DMSO-d_6) δ 8.08 (ddd, J = 1.0, 2.0, 5.0 Hz, 1H), 7.47 (ddd, J = 2.0, 7.0, 8.8 Hz, 1H), 7.32 (m, 5H), 6.60 (m, 1H), 6.54 (ddd, J = 2.0, 5.0, 7.0 Hz, 1H), 4.85 (dd, J = 4.8, 7.8 Hz, 1H), 3.62 (ddd, J = 4.8, 7.8, 14.1 Hz, 2H), 2.90 (s, 3H). ^{13}C NMR (75 MHz, DMSO-d_6) δ 37.5, 58.0, 70.8, 105.6, 111.0, 125.8, 126.8, 127.8, 136.9, 144.1, 147.3, 158.1. FAB-HRMS: calcd for $C_{14}H_{17}N_2O$ $(M + H)^+$ 229.1341, found 229.1346.

General Procedure for the Preparation of the 3′-*O*-Acetylthymidine 5′-*O*-Carbonates 3−8. Dry 3′-*O*-acetylthymidine (**1**, 29.6 mg, 0.10 mmol) and 1,1′-carbonyldiimidazole (18 mg, 0.11 mmol) were placed in a flame-dried 4-mL glass vial, which was immediately stoppered with a rubber septum. Anhydrous MeCN (0.5 mL) was added to the solids by the use of a syringe through the rubber septum. The solution, which was obtained 5 min after adding MeCN, was left standing at 25 °C. Thin-layer chromatography (TLC) analysis of the reaction indicated near complete conversion of **1** to the corresponding 5′-*O*-carbonylimidazolide **2** within 3 h. Then, the selected primary alcohol (0.15 mmol) was added to the reaction mixture followed by 1,1,3,3-tetramethylguanidine (70 μL, 0.6 mmol). The solution was left at room temperature until the 5′-*O*-carbonylimidazolide **2** was completely consumed (∼2 h) and a new product was formed as evidenced by TLC. The reaction mixture was then applied onto one preparative TLC plate, which was developed once with dichloromethane/methanol (9:1 v/v) as the mobile phase. The desired UV-absorbing band was cut out and the product was eluted from silica gel with dichloromethane/acetonitrile (8:2 v/v). The solvent was removed by evaporation under reduced pressure[28] affording the 3′-*O*-acetylthymidine 5′-*O*-carbonates **3**−**8** in yields ranging from 70 to 83%.

5′-*O*-(1-Butyl)oxycarbonyl-3′-*O*-acetylthymidine (3). ^{1}H NMR (300 MHz, $CDCl_3$) δ 7.42 (q, J = 1.3 Hz, 1H), 6.44 (dd, J = 5.6, 8.7 Hz, 1H), 5.27 (ddd, J = 1.9, 4.3, 6.5 Hz, 1H), 4.45 (dd, J = 2.9, 11.9 Hz, 1H), 4.38 (dd, J = 2.7, 11.9 Hz, 1H), 4.20 (m, 3H), 2.42 (ddd, J = 1.9, 5.6, 14.1 Hz, 1H), 2.23 (ddd, J = 6.5, 8.7, 14.1 Hz, 1H), 2.12 (s, 3H), 1.93 (d, J = 1.3 Hz, 3H), 1.67 (m, 2H), 1.40 (m, 2H), 0.95 (t, J = 7.3 Hz, 3H). ^{13}C NMR (75 MHz, $CDCl_3$): δ 12.5, 13.5, 18.8, 20.8, 30.6, 37.3, 67.0, 68.5, 74.4, 82.2, 84.5, 111.7, 134.9, 150.5, 154.7, 163.5, 170.4. FAB-HRMS: calcd for $C_{17}H_{25}N_2O_8$ $(M + H)^+$ 385.1611, found 385.1613.

5′-*O*-(4-Oxo-1-pentyl)oxycarbonyl-3′-*O*-acetylthymidine (4). ^{1}H NMR (300 MHz, $CDCl_3$) δ 7.38 (q, J = 1.3 Hz, 1H), 6.40 (dd, J = 5.7, 8.7 Hz, 1H), 5.27 (ddd, J = 2.1, 4.3, 6.5 Hz, 1H), 4.45 (dd, J = 3.2, 11.9 Hz, 1H), 4.39 (dd, J = 2.9, 11.9 Hz, 1H), 4.21 (m, 3H), 2.55 (t, J = 7.1 Hz, 2H), 2.44 (ddd, J = 2.1, 5.7, 14.3 Hz, 1H), 2.24 (ddd, J = 6.5, 8.7, 14.3 Hz, 1H), 2.15 (s, 3H), 2.11 (s, 3H), 1.96 (m, 2H), 1.93 (d, J = 1.3 Hz, 3H). ^{13}C NMR (75 MHz, $CDCl_3$) δ 12.5, 20.8, 22.6, 29.8, 37.3, 39.3, 67.1, 67.8, 74.2, 82.1, 84.6, 111.6, 134.8, 150.4, 154.6, 163.5, 170.4, 207.0. FAB-HRMS: calcd for $C_{18}H_{25}N_2O_9$ $(M + H)^+$ 413.1560, found 413.1565.

5′-*O*-(2-*N*-Acetyl)aminoethyloxycarbonyl-3′-*O*-acetylthymidine (5). ^{1}H NMR (300 MHz, DMSO-d_6) δ 8.05 (t, J = 5.6 Hz, 1H), 7.51 (m, 1H), 6.19 (dd, J = 6.2, 8.4 Hz, 1H), 5.20 (ddd, J = 2.6, 6.7, 9.5 Hz, 1H), 4.36 (dd, J = 4.3, 11.5 Hz, 1H), 4.31 (dd, J = 5.5, 11.5 Hz, 1H), 4.17 (ddd, J = 4.3, 5.5, 9.5 Hz, 1H), 4.09 (m, 2H), 3.29 (q, J = 5.5 Hz, 2H), 2.40 (ddd, J = 6.7, 8.4, 14.4 Hz, 1H), 2.28 (ddd, J = 2.6, 6.2, 14.4 Hz, 1H), 2.07 (s, 3H), 1.80 (s, 3H), 1.79 (d, J = 1.4 Hz, 3H). ^{13}C NMR (75 MHz, DMSO-d_6) δ 12.0, 20.7, 22.4, 35.3, 37.5, 66.5, 67.1, 73.6, 80.7,

(26) Laïb, T.; Ouazzani, J.; Zhu, J. *Tetrahedron*: *Asymmetry* **1998**, *9*, 169−178.

(27) Gray, A. P.; Heitmeier, D. E.; Spinner, E. E. *J. Am. Chem. Soc.* **1959**, *81*, 4351−4355.

(28) Given the thermolytic properties of these carbonates it is recommended to remove the solvent without external source of heat to prevent premature 5′-*O*-deprotection.

84.0, 109.9, 135.7, 150.4, 154.2, 163.5, 169.4, 170.0. FAB-HRMS: calcd for $C_{17}H_{24}N_3O_9$ $(M + H)^+$ 414.1513, found 414.1524.

5′-*O*-[2-(*N*-Acetyl-*N*-methyl)]aminoethyloxycarbonyl-3′-*O*-acetylthymidine (6). ^{1}H NMR (300 MHz, $CDCl_3$) δ 7.28 (q, $J = 1.3$ Hz, 1H), 6.33 (dd, $J = 5.6$, 8.6 Hz, 1H), 5.22 (ddd, $J = 2.1$, 4.5, 6.5 Hz, 1H), 4.39 (dd, $J = 4.0$, 12.2 Hz, 1H), 4.33 (dd, $J = 3.3$, 12.2 Hz, 1H), 4.25 (m, 1H), 4.22 (t, $J = 5.6$ Hz, 1H), 4.21 (t, $J = 5.6$ Hz, 1H), 3.62 (t, $J = 5.6$ Hz, 1H), 3.57 (t, $J = 5.6$ Hz, 1H), 3.07 (s, 3H), 2.48 (ddd, $J = 2.1$, 5.6, 14.2 Hz, 1H), 2.18 (ddd, $J = 6.5$, 8.6, 14.2 Hz, 1H), 2.14 (s, 3H), 2.12 (s, 3H), 1.94 (d, $J = 1.3$ Hz, 3H). ^{13}C NMR (75 MHz, $CDCl_3$) δ 12.6, 20.8, 21.7, 29.6, 37.3, 37.5, 61.3, 62.2, 63.8, 74.0, 82.1, 84.8, 111.5, 134.4, 150.1, 163.2, 170.2, 170.3. FAB-HRMS: calcd for $C_{18}H_{26}N_3O_9$ $(M + H)^+$ 428.1669, found 428.1679.

5′-*O*-[2-*N*-(2-Pyridyl)]aminoethyloxycarbonyl-3′-*O*-acetylthymidine (7). ^{1}H NMR (300 MHz, DMSO-d_6) δ 7.94 (m, 1H), 7.46 (m, 1H), 7.34 (ddd, $J = 2.0$, 7.2, 8.4 Hz, 1H), 6.47 (m, 2H), 6.17 (dd, $J = 6.2$, 8.1 Hz, 1H), 5.19 (ddd, $J = 2.9$, 6.8, 9.7 Hz, 1H), 4.32 (m, 2H), 4.20 (m, 2H), 4.16 (m, 1H), 3.50 (m, 2H), 2.37 (ddd, $J = 6.8$, 8.1, 14.4 Hz, 1H), 2.26 (ddd, $J = 2.9$, 6.2, 14.4 Hz, 1H), 2.05 (s, 3H), 1.76 (d, $J = 1.0$ Hz, 3H). ^{13}C NMR (75 MHz, DMSO-d_6) δ 11.7, 20.5, 35.4, 66.6, 66.9, 73.5, 80.7, 84.0, 108.2, 109.8, 111.7, 135.5, 136.4, 147.2, 150.1, 154.1, 158.2, 158.3, 163.3, 169.8. FAB-HRMS: calcd for $C_{20}H_{25}N_4O_8$ $(M + H)^+$ 449.1672, found 449.1662.

5′-*O*-[2-*N*-Methyl-*N*-(2-pyridyl)]aminoethyloxycarbonyl-3′-*O*-acetylthymidine (8). ^{1}H NMR (300 MHz, DMSO-d_6) δ 8.04 (ddd, $J = 1.0$, 2.0, 4.9 Hz, 1H), 7.48 (ddd, $J = 2.0$, 7.1, 8.6 Hz, 1H), 7.41 (m, 1H), 6.61 (ddd, $J = 1.0$, 2.0, 8.6 Hz, 1H), 6.55 (ddd, $J = 2.0$, 4.9, 7.1 Hz, 1H), 6.19 (dd, $J = 6.1$, 8.4 Hz, 1H), 5.15 (ddd, $J = 2.7$, 6.5, 9.3 Hz, 1H), 4.28 (m, 3H), 4.12 (ddd, $J = 4.8$, 7.6, 9.3 Hz, 1H), 3.81 (m, 3H), 2.99 (s, 3H), 2.32 (ddd, $J = 6.5$, 8.4, 14.4 Hz, 1H), 2.22 (ddd, $J = 2.7$, 6.1, 14.4 Hz, 1H), 2.06 (s, 3H), 1.74 (d, $J = 1.1$ Hz, 3H). ^{13}C NMR (75 MHz, DMSO-d_6) δ 12.5, 20.6, 35.3, 36.5, 47.6, 49.4, 49.8, 65.6, 67.1, 83.9, 105.5, 109.8, 111.5, 137.2, 137.8, 144.4, 147.3, 154.1, 154.6, 157.9, 169.9. FAB-HRMS: calcd for $C_{21}H_{27}N_4O_8$ $(M + H)^+$ 463.1829, found 463.1855.

General Procedure for the Preparation of the 3′-*O*-Acetylthymidine 5′-*O*-Carbonates 10−15. A selected secondary alcohol (0.10 mmol) and 1,1′-carbonyldiimidazole (18 mg, 0.11 mmol) were placed in a flame-dried 4-mL glass vial, which was immediately stoppered with a rubber septum. Anhydrous MeCN (0.5 mL) was added to the reactants with a syringe through the rubber septum. An immediate solution was obtained and was left standing at 25 °C. TLC analysis of the reaction indicated near complete conversion of the starting alcohol to the corresponding carbonylimidazolide **9** within 6 h. 3′-*O*-Acetylthymidine (**1**, 29.6 mg, 0.10 mmol) was then added to the reaction mixture followed by 1,1,3,3-tetramethylguanidine (70 μL, 0.6 mmol). The solution was left at room temperature until **9** was completely consumed (∼2 h) and a new product was formed as evidenced by TLC. The reaction mixture was then applied onto one preparative TLC plate, which was developed once with dichloromethane/methanol (9:1 v/v) as the mobile phase. The desired UV-absorbing band was cut out and the product was eluted from silica gel with dichloromethane/acetonitrile (8:2 v/v). The solvent was removed by evaporation under reduced pressure[28] affording the 3′-*O*-acetylthymidine 5′-*O*-carbonates **10**−**14** in yields ranging from 45 to 73%.

5′-*O*-(2-*N*-Acetyl)amino-1-phenylethyloxycarbonyl-3′-*O*-acetylthymidine (10). ^{1}H NMR (300 MHz, $CDCl_3$) δ 7.40 (m, 5H), 7.28 (q, $J = 1.3$ Hz, 1H), 6.32 (dd, $J = 5.7$, 8.6 Hz, 1H), 5.62 (t, $J = 8.7$ Hz, 1H), 5.22 (ddd, $J = 2.1$, 4.3, 6.7 Hz, 1H), 4.38 (dd, $J = 4.3$, 12.1 Hz, 1H), 4.33 (dd, $J = 3.4$, 12.1 Hz, 1H), 4.24 (m, 1H), 3.98 (t, $J = 8.7$ Hz, 1H), 3.54 (t, $J = 8.7$ Hz, 1H), 2.47 (ddd, $J = 2.1$, 5.7, 14.2 Hz, 1H), 2.17 (ddd, $J = 6.7$, 8.6, 14.2 Hz, 1H), 2.13 (s, 3H), 2.11 (s, 3H), 1.94 (d, $J = 1.3$ Hz, 3H). ^{13}C NMR (75 MHz, $CDCl_3$) δ 12.5, 20.7, 20.8, 29.6, 37.5, 48.3, 63.8, 74.1, 77.8, 82.1, 84.8, 111.4, 125.6, 128.8, 134.5, 138.4, 150.2, 159.8, 163.5, 170.1, 170.3. FAB-HRMS: calcd for $C_{23}H_{28}N_3O_9$ $(M + H)^+$ 490.1826, found 490.1828.

5′-*O*-(1-Phenyl)-1-butyloxycarbonyl-3′-*O*-acetylthymidine (11). ^{1}H NMR (300 MHz, $CDCl_3$) δ 7.38 (q, $J = 1.3$ Hz, 1H), 7.36−7.30 (m, 5H), 6.38 (dd, $J = 5.6$, 8.9 Hz, 1H), 5.59 (dd, $J = 6.5$, 7.7 Hz, 1H), 5.18 (ddd $J = 2.0$, 4.2, 6.5 Hz, 1H), 4.41 (dd, $J = 3.2$, 11.9 Hz, 1H), 4.35 (dd, $J = 2.8$, 11.9 Hz, 1H), 4.20 (m, 1H), 2.38 (ddd, $J = 2.0$, 5.6, 14.2 Hz, 1H), 2.13 (ddd, $J = 6.5$, 8.9, 14.2 Hz, 1H), 2.09 (s, 3H), 1.96 (m, 1H), 1.94 (d, $J = 1.3$ Hz, 3H), 1.80 (m, 1H), 1.34 (m, 2H), 0.92 (t, $J = 7.4$ Hz, 3H). ^{13}CNMR (75 MHz, $CDCl_3$) δ 12.5, 13.6, 18.7, 20.8, 29.7, 37.3, 38.2, 67.0, 74.4, 81.0, 82.2, 84.5, 111.6, 126.4, 128.5, 134.9, 139.5, 150.2, 154.2, 163.3, 170.3. FAB-HRMS: calcd for $C_{23}H_{29}N_2O_8$ $(M + H)^+$ 460.1924, found 461.1908.

5′-*O*-[2-(*N*-Acetyl-*N*-methyl)]amino-1-phenylethyloxycarbonyl-3′-*O*-acetylthymidine (12). ^{1}H NMR (300 MHz, $CDCl_3$) δ 7.40−7.30 (m, 6H), 6.34 (dd, $J = 5.7$, 8.8 Hz, 1H), 5.80 (dd, $J = 5.1$, 8.4 Hz, 0.5H), 5.75 (dd, $J = 5.1$, 8.4 Hz, 0.5H), 5.27 (ddd, $J = 2.0$, 4.4, 6.7 Hz, 0.4H), 5.15 (ddd, $J = 2.0$, 4.4, 6.7 Hz, 0.6H), 4.45 (ddd, $J = 2.6$, 3.1, 11.8 Hz, 1H), 4.35 (ddd, $J = 2.6$, 3.1, 11.8 Hz, 1H), 4.21 (m, 1H), 3.80−3.60 (m, 2H), 2.97 (s, 1.6H), 2.95 (s, 1.4H), 2.45 (ddd, $J = 2.0$, 5.7, 14.3 Hz, 0.5H), 2.38 (ddd, $J = 2.0$, 5.7, 14.3 Hz, 0.5H), 2.25 (ddd, $J = 6.7$, 8.8, 14.3 Hz, 0.5H), 2.17 (ddd, $J = 6.7$, 8.8, 14.3 Hz, 0.5H), 2.11 (s, 1.6H), 2.10 (s, 1.4H), 2.07 (s, 3H), 1.88 (d, $J = 1.3$ Hz, 2H), 1.80 (d, $J = 1.3$ Hz, 1H). ^{13}C NMR (75 MHz, $CDCl_3$) δ 12.46, 12.51, 20.8, 29.6, 37.7, 37.9, 67.1, 67.3, 74.0, 74.4, 78.5, 78.7, 82.0, 82.2, 84.6, 111.5, 111.6, 126.2, 126.3, 128.7, 128.8, 134.7, 134.8, 150.2, 150.3, 153.9, 154.0, 163.2, 163.4, 170.3, 170.4, 171.1, 171.2. FAB-HRMS: calcd for $C_{24}H_{30}N_3O_9$ $(M + H)^+$ 504.1982, found 504.1996.

5′-*O*-[2-(2-Pyridyl)]amino-1-phenylethyloxycarbonyl-3′-*O*-acetylthymidine (13). ^{1}H NMR (300 MHz, CD_3CN) δ 8.04 (ddd, $J = 1.0$, 2.0, 5.0 Hz, 1H), 7.40 (m, 6H), 7.33 (q, $J = 1.4$ Hz, 1H), 6.55 (ddd, $J = 2.0$, 5.0, 7.0 Hz, 1H), 6.47 (ddd, $J = 1.0$, 2.0, 8.4 Hz, 1H), 6.13 (dd, $J = 6.9$, 7.2 Hz, 1H), 5.80 (dd, $J = 4.3$, 8.2 Hz, 1H), 5.23 (m, 1H), 4.32 (ddd, $J = 3.3$, 4.6, 12.0 Hz, 2H), 4.17 (ddd, $J = 3.3$, 4.6, 8.0 Hz, 1H), 3.75 (ddd, $J = 4.3$, 8.2, 14.3 Hz, 1H), 3.65 (ddd, $J = 4.3$, 8.2, 14.3 Hz, 1H), 2.32 (m, 2H), 2.04 (s, 3H), 1.78 (d, $J = 1.4$ Hz, 3H). ^{13}C NMR (75 MHz, CD_3CN) δ 12.5, 21.1, 37.2, 47.1, 67.9, 74.7, 79.9, 82.6, 86.2, 108.9, 111.6, 113.7, 127.3, 129.4, 129.5, 136.8, 138.1, 139.2, 148.6, 151.5, 155.2, 159.5, 164.5, 171.3. FAB-HRMS: calcd for $C_{26}H_{29}N_4O_8$ $(M + H)^+$ 525.1985, found 525.1998.

5′-*O*-2-[*N*-Methyl-*N*-(2-pyridyl)]amino-1-phenylethyloxycarbonyl-3′-*O*-acetylthymidine (14). ^{1}H NMR (300 MHz, $CDCl_3$) δ 8.17 (ddd, $J = 1.0$, 2.0, 4.9 Hz, 0.5H), 8.15 (ddd, $J = 1.0$, 2.0, 4.9 Hz, 0.5H), 7.46 (ddd, $J = 2.0$, 7.1, 8.8 Hz, 0.5H), 7.43 (ddd, $J = 2.0$, 7.1, 8.8 Hz, 0.5H), 6.58 (ddd, $J = 2.0$, 4.9, 7.1 Hz, 0.5H), 7.35 (m, 6H), 6.56 (ddd, $J = 2.0$, 4.9, 7.1 Hz, 0.5H), 6.49 (ddd, $J = 1.0$, 2.0, 8.8 Hz, 0.5H), 6.47 (ddd, $J = 1.0$, 2.0, 8.8 Hz, 0.5H), 6.36 (dd, $J = 5.6$, 9.0 Hz, 0.5H), 6.33 (dd, $J = 5.6$, 9.0 Hz, 0.5H), 6.00 (dd, $J = 4.5$, 7.6 Hz, 0.5H), 5.95 (dd, $J = 4.5$, 7.6 Hz, 0.5H), 5.13 (ddd, $J = 1.8$, 6.4, 8.0 Hz, 0.5H), 5.10 (ddd, $J = 1.8$, 6.4, 8.0 Hz, 0.5H), 4.38 (dd, $J = 5.1$, 11.9 Hz, 1H), 4.28 (dd, $J = 2.7$, 11.9 Hz, 1H), 4.17 (m, 1H), 3.98 (dd, $J = 7.6$, 14.5 Hz, 1H), 3.91 (dd, $J = 4.5$, 14.5 Hz, 1H), 2.95 (s, 1.5H), 2.90 (s, 1.5H), 2.39 (ddd, $J = 1.8$, 5.6, 14.1 Hz, 0.5H), 2.34 (ddd, $J = 1.8$, 5.6, 14.1 Hz, 0.5H), 2.10 (s, 1.5H), 2.09 (s, 1.5H), 2.07 (ddd, $J = 6.4$, 9.0, 14.1 Hz, 0.5H), 2.02 (ddd, $J = 6.4$, 9.0, 14.1 Hz, 0.5H), 1.87 (d, $J = 1.3$ Hz, 1.5H), 1.78 (d, $J = 1.3$ Hz, 1.5H). ^{13}C NMR (75 MHz, $CDCl_3$) δ 12.4, 12.5, 20.8, 37.2, 37.5, 37.8, 38.5, 55.1, 55.5, 67.1, 67.2, 74.1, 74.5, 78.8, 78.9, 82.0, 82.1, 84.5, 105.5, 111.6, 112.1, 126.2, 126.4, 128.5, 128.6, 128.7 134.6, 134.7, 137.2, 137.5, 137.9, 147.8, 148.0, 150.6, 154.0, 158.0, 158.2, 163.8, 170.3. FAB-HRMS: calcd for $C_{27}H_{31}N_4O_8$ $(M + H)^+$ 539.2142, found 539.2169.

5′-*O*-[2-*N*-(4-Dimethylamino)benzoyl]amino-1-phenylethyloxycarbonyl-3′-*O*-acetylthymidine (15). ^{1}H NMR (300 MHz, $CDCl_3$) δ 7.62 (d, $J = 9.1$ Hz, 2H), 7.41−7.34 (m, 5H), 7.30 (q, $J = 1.3$ Hz, 0.3H), 7.23 (q, $J = 1.3$ Hz, 0.7H), 6.64 (d,

J = 9.1 Hz, 1.2H), 6.63 (d, J = 9.1 Hz, 0.8H), 6.35 (m, 1H), 6.30 (dd, J = 5.5, 8.8 Hz, 1H), 5.88 (dd, J = 4.2, 8.2 Hz, 0.3H), 5.84 (dd, J = 4.2, 8.2 Hz, 0.7H), 5.17 (ddd, J = 2.0, 6.5, 8.0 Hz, 1H), 4.49 (dd, J = 3.6. 11.8 Hz, 1H), 4.31 (dd, J = 2.9. 11.8 Hz, 1H), 4.19 (m, 1H), 3.96 (ddd, J = 4.2, 6.5, 14.3 Hz, 1H), 3.76 (ddd, J = 5.5, 8.2, 14.3 Hz, 1H), 3.01 (s, 6H), 2.39 (ddd, J = 2.0, 5.5, 14.2 Hz, 0.5H), 2.35 (ddd, J = 2.0, 5.5, 14.2 Hz, 0.5H), 2.18 (ddd, J = 6.5, 8.8, 14.2 Hz, 0.5H), 2.10 (ddd, J = 6.5, 8.8, 14.2 Hz, 0.5H), 2.08 (s, 2H), 2.07 (s, 1H), 1.86 (d, J = 1.3 Hz, 2H), 1.76 (d, J = 1.3 Hz, 1H). ^{13}C NMR (75 MHz, $CDCl_3$) δ 12.4, 12.5, 20.8, 36.9, 37.1, 40.0, 44.5, 44.7, 67.0, 67.3, 74.0, 74.4, 77.2, 79.4, 79.5, 82.0, 82.1, 84.7, 111.0, 111.6, 120.6, 120.7, 126.2, 126.4, 128.3, 128.4, 128.7, 128.8, 128.9, 134.8, 136.8, 137.1, 150.2, 152.6, 154.1, 163.3, 167.3, 167.4, 170.3. FAB-HRMS: calcd for $C_{30}H_{35}N_4O_9$ $(M + H)^+$ 595.2404, found 595.2377.

General Procedure for the Thermolytic Deprotection of 3′-*O*-Acetyl-2′-deoxyribonucleoside 5′-*O*-Carbonates. 3′-*O*-Acetyl-2′-deoxyribonucleoside 5′-*O*-carbonates (~2 mg) were placed in a 4-mL screw-cap glass vial. The material was dissolved by adding either MeCN:phosphate buffer pH 7.0 (3:1 v/v) or EtOH:phosphate buffer pH 7.0 (1:1 v/v) (0.5 mL) as indicated in Tables 1−3. The glass vial containing the solution was securely capped and then heated for a predetermined period of time (see Tables 1−3) to 90 ± 2 °C, using a heating block. The progress of the reaction was analyzed by RP-HPLC, using a 5 μm Supelcosil LC-18S column (25 cm × 4.6 mm) and a linear gradient of 1% MeCN/min, starting from 0.1 M triethylammonium acetate, pH 7.0, at a flow rate of 1 mL/min. The MeCN gradient was created by pumping a solution of 40% MeCN in 0.1 M triethylammonium acetate, pH 7.0. RP-HPLC retention times of the 5′-*O*-carbonates under study are reported in the Supporting Information.

Synthesis and Characterization of 2-(4-Dimethylamino)phenyl-5-phenyl-Δ^2-oxazoline (16). To a suspension of 4-(dimethylamino)benzoic acid (332 mg, 2.01 mmol) and 2-amino-1-phenylethanol (276 mg, 2.01 mmol) in MeCN (6 mL) was added, under an inert atmosphere, pyridine (7 mL), Et_3N (840 μL, 6.06 mmol), and CCl_4 (780 μL, 8.08 mmol). A solution of Ph_3P (1.57 g, 5.99 mmol) in MeCN:pyridine (1:1 v/v, 16 mL) was then added to the reaction mixture, dropwise, over a period of 5 min. The resulting clear yellow solution turned brown and cloudy within 1 h. The suspension was left stirring overnight and was then evaporated under reduced pressure to a brown residue. The material was dissolved in CH_2Cl_2 (60 mL) and was washed with ice-cold 2 N NaOH (50 mL). The aqueous layer was extracted with CH_2Cl_2 (2 × 60 mL). The combined organic phases were dried over anhydrous sodium sulfate and rotoevaporated to dryness. The residue was purified by silica gel chromatography with use of a linear gradient of ethyl acetate (2.5% to 15%) in toluene affording **16** (327 mg, 61%) as a light-brown solid (mp 106−108 °C). ^{1}H NMR (300 MHz, $CDCl_3$) δ 7.89 (d, J = 9.0 Hz, 2H), 7.37−7.32 (m, 5H), 6.69 (d, J = 9.0 Hz, 2H), 5.61 (dd, J = 7.8, 9.9 Hz, 1H), 4.44 (dd, J = 9.9, 14.4 Hz, 1H), 3.93 (dd, J = 7.8, 14.4 Hz, 1H), 3.03 (s, 6H). CI-MS: calcd for $C_{17}H_{17}N_2O$ $(M - H)^-$ 265, found 265.

Isolation and Characterization of 2,3-Dihydroimidazo[1,2-*a*]pyridine (17). This compound was isolated from the thermal deprotection of carbonate **7** with RP-HPLC. The peak exhibiting a R_T of 22 min under the chromatographic conditions used for analysis of the deprotection reaction (vide supra) was collected and the eluate was evaporated to dryness under reduced pressure. The residue, generated from multiple RP-HPLC runs, was coevaporated several times with aqueous acetonitrile to remove residual triethylammonium acetate, and was then left exposed to high vacuum overnight prior to characterization. ^{1}H NMR (300 MHz, DMSO-d_6) δ 8.36 (ddd, J = 1.0, 2.0, 4.9 Hz, 1H), 8.07 (ddd, J = 1.0, 2.0, 8.6 Hz, 1H), 7.83 (ddd, J = 2.0, 7.2, 8.6 Hz, 1H), 7.13 (ddd, J = 2.0, 4.9, 7.2 Hz, 1H), 4.45 (m, 2H), 4.16 (m, 2H). ^{13}C NMR (75 MHz, DMSO-d_6) δ 43.7, 62.0, 112.0, 118.9, 137.8, 147.6, 154.6. FAB-HRMS: calcd for $C_7H_9N_2$ $(M + H)^+$ 121.0766, found 121.0771.

Isolation and Characterization of 3-Phenyl-2,3-dihydroimidazo[1,2-*a*]pyridine (18). This compound was isolated from the thermolytic deprotection of carbonate **13** by RP-HPLC. The peak corresponding to **18** (R_T of 47 min, vide supra) was collected and processed in a manner identical with that described for the isolation of **17**. ^{1}H NMR (300 MHz, DMSO-d_6) δ 8.36 (ddd, J = 1.0, 2.0, 4.9 Hz, 1H), 8.11 (ddd, J = 1.0, 2.0, 8.5 Hz, 1H), 7.86 (ddd, J = 2.0, 7.3, 8.5 Hz, 1H), 7.46 (m, 5H), 7.15 (ddd, J = 2.0, 4.9, 7.3 Hz, 1H), 5.77 (dd, J = 7.7, 8.6 Hz, 1H), 4.63 (dd J = 8.6, 10.4 Hz, 1H), 4.04 (dd, J = 7.7, 10.4 Hz, 1H). ^{13}C NMR (75 MHz, DMSO-d_6) δ 50.7, 74.3, 112.2, 119.1, 126.2, 128.7, 128.8, 138.0, 147.7, 150.6, 153.8. FAB-HRMS: calcd for $C_{13}H_{13}N_2$ $(M + H)^+$ 197.1079, found 197.1070.

Preparation of the *N*-Protected 3′-*O*-Acetyl-2′-deoxyribonucleoside 5′-*O*-Carbonates 19−21. These carbonates were prepared and purified in a manner similar to that described for the preparation of the 3′-*O*-acetylthymidine 5′-*O*-carbonates **10**−**15**, and were isolated in similar yields.

***N*^{4}-Benzoyl-5′-*O*-[2-*N*-methyl-*N*-(2-pyridyl)]amino-1-phenylethyloxycarbonyl-3′-*O*-acetyl-2′-deoxycytidine (19).** ^{1}H NMR (300 MHz, DMSO-d_6) δ 8.12−7.99 (m, 4H), 7.62 (m, 1H), 7.54−7.47 (m, 3H), 7.46 (ddd, J = 2.0, 4.9, 8.8 Hz, 1H), 7.40−7.30 (m, 5H), 6.59 (dd, J = 4.9, 6.8 Hz, 1H), 6.54 (dd, J = 2.0, 8.8 Hz, 1H), 6.17 (dd, J = 6.2, 7.4 Hz, 0.5H), 6.13 (dd, J = 6.2, 7.4 Hz, 0.5H), 5.87 (dd, J = 4.6, 8.4 Hz, 0.5H), 5.84 (dd, J = 4.6, 8.4 Hz, 0.5H), 5.16 (ddd, J = 2.5, 6.6, 9.2 Hz, 1H), 4.29 (m, 3H), 3.94 (dd, J = 4.6, 14.5 Hz, 1H), 3.89 (dd, J = 8.4, 14.5 Hz, 1H), 2.93 (s, 1.5H), 2.92 (s, 1.5H), 2.49 (m, 1H), 2.29 (ddd, J = 2.5, 7.4, 14.4 Hz, 1H), 2.06 (s, 3H). ^{13}C NMR (75 MHz, DMSO-d_6) δ 20.6, 32.3, 37.1, 37.3, 38.1, 54.3, 57.3, 64.4, 67.1, 77.9, 78.1, 105.6, 108.2, 111.7, 113.1, 126.07, 126.1, 127.7, 128.2, 128.4, 128.5, 129.2, 129.6, 136.5, 137.0, 137.19, 137.24, 137.8, 144.7, 147.4, 147.5, 153.5, 153.6, 154.3, 157.71, 157.75, 169.8. FAB-HRMS: calcd for $C_{33}H_{33}N_5O_8$ $(M + Cs)^+$ 760.1383, found 760.1384.

***N*^{6}-Benzoyl-5′-*O*-[2-*N*-methyl-*N*-(2-pyridyl)]amino-1-phenylethyloxycarbonyl-3′-*O*-acetyl-2′-deoxyadenosine (20).** ^{1}H NMR (300 MHz, DMSO-d_6) δ 8.74 (s, 0.5H), 8.73 (s, 0.5H), 8.64 (s, 0.5H), 8.63 (s, 0.5H), 8.11−8.05 (m, 2H), 8.03 (dd, J = 2.0, 4.9 Hz, 1H), 7.68−7.51 (m, 3H), 7.45 (ddd, J = 2.0, 7.1, 8.8 Hz, 1H), 7.38−7.30 (m, 6H), 6.61−6.47 (m, 3H), 5.82 (dd, J = 5.0, 7.9 Hz, 1H), 5.40 (ddd, J = 2.7, 6.2, 8.6 Hz, 1H), 4.33 (dd, J = 7.7, 12.0 Hz, 1H), 4.25 (dd, J = 4.5, 12.0 Hz, 1H), 4.23 (m, 1H), 3.95−3.79 (m, 2H), 3.12 (ddd, J = 6.2, 7.8, 14.4 Hz, 0.5H), 3.09 (ddd, J = 6.2, 7.8, 14.4 Hz, 0.5H), 2.89 (s, 1.5H), 2.86 (s, 1.5H), 2.60 (ddd, J = 2.7, 6.8, 14.4 Hz, 1H), 2.10 (s, 1.5H), 2.09 (s, 1.5H). ^{13}C NMR (75 MHz, DMSO-d_6) δ 20.7, 35.3, 37.1, 37.2, 54.3, 54.4, 57.3, 64.4, 67.0. 67.1, 73.9, 74.0, 77.8, 77.9, 81.2, 81.3, 83.5, 83.8, 85.2, 105.62, 105.64, 108.2, 111.7, 113.1, 125.8, 126.1, 127.7, 128.17, 128.19, 128.3, 128.4, 129.2, 129.5, 136.5, 136.6, 137.0, 137.2, 137.7, 137.8, 144.7, 147.4, 147.5, 151.5, 151.6, 153.50, 153.54, 157.7, 169.8. FAB-HRMS: calcd for $C_{34}H_{33}N_7O_7$ $(M + Cs)^+$ 784.1496, found 784.1505.

***N*^{2}-Isobutyryl-5′-*O*-[2-*N*-methyl-*N*-(2-pyridyl)]amino-1-phenylethyloxycarbonyl-3′-*O*-acetyl-2′-deoxyguanosine (21).** ^{1}H NMR (300 MHz, DMSO-d_6) δ 8.17 (s, 0.5H), 8.16 (s, 0.5H), 8.09 (dd, J = 2.0, 5.0 Hz, 0.5H), 8.07 (dd, J = 2.0, 5.0 Hz, 0.5H), 7.48 (ddd, J = 2.0, 6.9, 8.8 Hz, 1H), 7.38−7.27 (m, 5H), 6.58 (dd, J = 2.0, 6.9 Hz, 1H), 6.55 (ddd, J = 2.0, 5.0, 8.8 Hz, 1H), 6.23 (dd, J = 6.4, 8.5 Hz, 0.5 H), 6.20 (dd, J = 6.4, 8.5 Hz, 0.5 H), 5.82 (dd, J = 4.5, 8.1 Hz, 0.5H), 5.80 (dd, J = 4.5, 8.1 Hz, 0.5H), 5.26 (m, 1H), 4.23 (m, 3H), 3.91 (ddd, J = 4.5, 8.1, 14.6 Hz, 1H), 3.85 (ddd, J = 4.5, 8.1, 14.6 Hz, 1H), 2.92 (s, 1.5H), 2.89 (s, 1.5 H), 2.85 (m, 1H), 2.74 (qt, J = 6.7 Hz, 1H), 2.50 (ddd, J = 2.1, 6.2, 14.5 Hz, 1H), 2.07 (s, 3H), 1.11 (d, J = 6.7 Hz, 3H), 1.10 (d, J = 6.7 Hz, 3H). ^{13}C NMR (75 MHz, DMSO-d_6) δ 20.7, 32.3, 36.6, 37.1, 37.3, 38.1, 54.3, 54.4, 57.3, 64.4, 77.9, 78.0, 85.0, 105.6, 105.7, 108.3, 111.7, 113.1, 126.1, 127.7, 128.2, 128.4, 128.5, 129.2, 129.6, 136.5,

137.0, 137.21, 137.24, 137.7, 137.8, 144.7, 147.4, 147.5, 153.51, 153.54, 154.4, 157.7, 169.8, 169.9. FAB-HRMS: calcd for $C_{31}H_{35}N_7O_8$ $(M + Cs)^+$ 766.1601, found 766.1566.

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Supporting Information Available: Materials and methods, RP-HPLC retention times of 3′-*O*-acetyl-2′-deoxyribonucleoside 5′-*O*-carbonates and that of related deprotection species, and ^{1}H NMR spectra of (±)-2-[(4-dimethylamino)-benzoyl]amino-1-phenylethanol, 2-[*N*-methyl-*N*-(2-pyridyl)]-aminoethanol, (±)-1-phenyl-2-(2-pyridyl)aminoethanol, (±)-2-[*N*-methyl-*N*-(2-pyridyl)amino]-1-phenylethanol, **3**−**8**, **10**−**15**, and **17**−**21**. This material is available free of charge via the Internet at http://pubs.acs.org.

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