First Regioselective Enzymatic Acylation of Amino Groups Applied to Pyrimidine 3′,5′-Diaminonucleoside Derivatives. Improved Synthesis of Pyrimidine 3′,5′-Diamino-2′,3′,5′-trideoxynucleosides

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The chemistry of natural nucleosides and their analogues has been widely studied due to their potential as fungicidal, antitumor, and antiviral agents.[1] Consequently, extensive modifications have been made to both the heterocyclic base and the sugar moiety in order to avoid the drawbacks shown by nucleosides or analogues in certain applications.

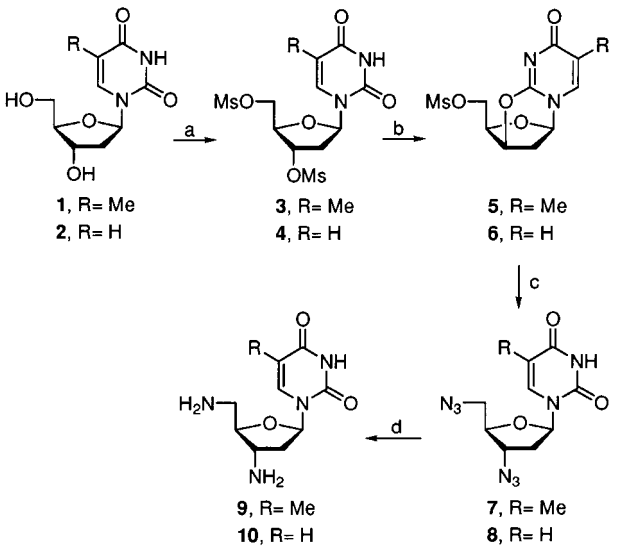
For organic chemists, enzymatic-catalyzed reactions have become standard procedures for nucleoside modification, since they avoid the time-consuming protection and deprotection steps required by the occurrence of various hydroxyl groups in the sugar skeleton of the natural nucleosides.[2]

The 3′,5′-diamino analogues of thymidine[3] and 2′-deoxyuridine[4] have been synthesized previously, and they showed a complete lack of either antiviral or antineoplastic activity. It is known that in some cases a simple acylation of one of the hydroxyl groups in a nucleoside can result in an increase of their biological activity compared with the unmodified derivative.[5]

Regioselective protection of one of the primary amino groups situated in the 3′- or 5′-positions is a very difficult task, since traditional chemical methods do not distinguish between them, and moreover, there are other reactive points on the molecule such as the nitrogen atoms on the bases.

It is the purpose of this paper to report the regioselective enzymatic acylation of the amino groups in the sugar moiety of pyrimidine 3′,5′-diaminonucleosides as well as to show improved syntheses of the starting materials, 3′,5′-diamino-3′,5′-dideoxythymidine and 3′,5′-diamino-2′,3′,5′-trideoxyuridine, on the basis of its fewer steps and high overall yields.

Scheme 1[a]



[a] Reaction conditions for B = T (in parentheses for B = U): (a) $MeSO_2Cl$, Py, 0 °C, 15 h, 89% (13 h, 91%); (b) Et_3N, EtOH, 80 °C, 18 h, 86% (20 h, 86%); (c) NaN_3, DMF 130 °C, 6 h 92% (120 °C, 5 h, 62%); (d) H_2, Pd/C, EtOH, rt, 24 h, 90% (21 h, 85%).

Treatment of thymidine (**1**) with methanesulfonyl chloride in pyridine at 0 °C afforded the disulfonate **3** (Scheme 1). When this dimesyl derivative was heated under reflux with an excess of Et_3N in EtOH solution, anhydronucleoside **5** was isolated in 86% yield. The diazido derivative **7** was obtained by treatment of **5** with sodium azide in DMF solution at 130 °C. Hydrogenation of **7** in EtOH in the presence of 10% Pd/C afforded 3′,5′-diamino-3′,5′-dideoxythymidine (**9**) through a four-step sequence with 63% overall yield.

The same strategy was applied to prepare 3′,5′-diamino-2′,3′,5′-trideoxyuridine (**10**). In this case, nucleophilic displacement with NaN_3 on anhydronucleoside **6** was carried out at 120 °C, since higher reaction temperatures gave decomposition products and lower ones provided just 5′-OMs displacement without anhydronucleoside opening. Despite moderate yield of this step, we obtained diamino nucleoside **10** in four steps with 41% overall yield, almost double that described in the literature.[4]

We have previously reported that oxime esters are good acylating agents in regioselective enzymatic acylations of nucleosides.[6] Since amines are much more nucleophilic than alcohols, they react nonenzymatically with oxime esters. To solve this problem, nonactivated esters, such as alkyl esters, were used. The studies of enzymatic acylation focused on 3′,5′-diamino-3′,5′-dideoxythymidine (**9**, Scheme 2).

The reaction was carried out with 8 equiv of ethyl acetate in THF at 40 °C in the presence of molecular sieves (4 Å) to avoid decomposition of the starting material (entry 1, Table 1). Among the enzymes tested [*Candida antarctica* lipase B (CAL-B), *Pseudomonas cepacia* lipase (PSL), immobilized *Pseudomonas cepacia*

(1) (a) MacCoss, M.; Robins, M. J. In *Chemistry of Antitumor Agents*; Wilman, D. E. V., Ed.; Blackie and Son: U.K., 1990; p 261. (b) Robins, R. K.; Kini, G. D. In *Chemistry of Antitumor Agents*; Wilman, D. E. V., Ed.; Blackie and Son: U.K., 1990; p 299. (c) Robins, R. K.; Revankar, G. R. In *Antiviral Drug Development*; De Clercq, E., Walker, R. T., Eds.; Plenum Press: New York, 1998; p 11.

(2) (a) Ferrero, M.; Gotor, V. *Monatsh. Chem.* **2000**, *131*, 585−616. (b) Ferrero, M.; Gotor, V. *Chem. Rev.* **2000**, *100*, 4319−4347.

(3) Lin, T.-S.; Prusoff, W. H. *J. Med. Chem.* **1978**, *21*, 109−112.

(4) Lin, T.-S.; Zhou, R.-X.; Scanlon, K. J.; Brubaker, W. F., Jr.; Lee, J. J. S.; Woods, K.; Humphreys, C.; Prusoff, W. H. *J. Med. Chem.* **1986**, *29*, 681−686.

(5) (a) Hamamura, E. K.; Prystasz, M.; Verheyden, J. P. H.; Moffat, J. G.; Yamaguchi, K.; Uchida, N.; Sato, K.; Nomura, A.; Shiratori, O.; Takese, S.; Katagiri, K. *J. Med. Chem.* **1976**, *19*, 654−662. (b) Hamamura, E. K.; Prystasz, M.; Verheyden, J. P. H.; Moffat, J. G.; Yamaguchi, K.; Uchida, N.; Sato, K.; Nomura, A.; Shiratori, O.; Takese, S.; Katagiri, K. *J. Med. Chem.* **1976**, *19*, 667−674.

(6) Gotor, V.; Moris, F. *Synthesis* **1992**, 626−628. (b) Moris, F.; Gotor, V. *J. Org. Chem.* **1993**, *58*, 653−660. (c) Moris, F.; Gotor, V. *Tetrahedron* **1993**, *49*, 10089−10098.

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Scheme 2

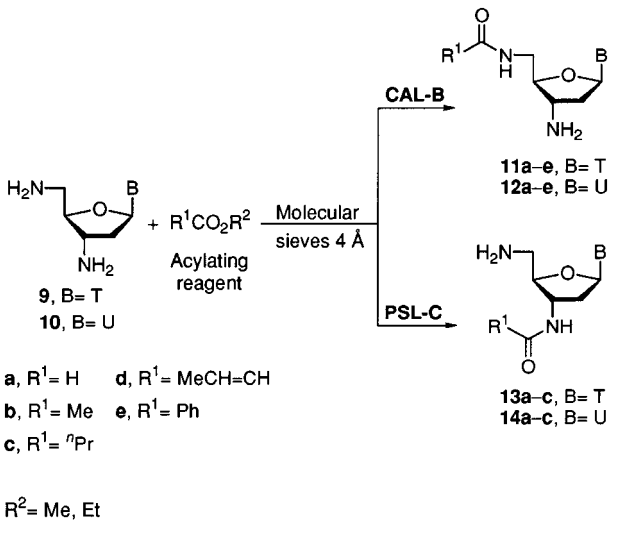
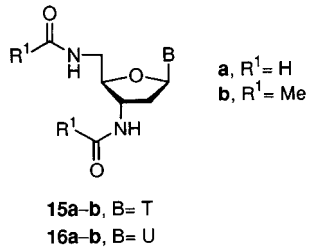


Table 1. Enzymatic Acylation of 3′,5′-Diaminothymidine 9 in THF

		acylating agent					isolated yields (%)		
entry	enzyme	R^1	R^2	equiv	T (°C)	t (h)	N-3′	N-5′	N-3′,5′
1	CAL-B	Me	Et	8	40	6		80	5
2	CAL-B	Me	Et	8	28	9		39	20
3	CAL-B	H	Et	2	40	8		55	20
4	CAL-B	*n*Pr	Et	10	40	32		68	
5	CAL-B	MeCH=CH	Me	20	60	31		83	
6	CAL-B	Ph	Me	30	60	75		67	
7	PSL-C	Me	Et	20	40	48	21[a]		
8	PSL-C	Me	Et	50	60	20	89		
9	PSL-C	H	Et	5	60	21	35	13	47
10	PSL-C	*n*Pr	Et	50	60	94	23	26	

[a] Starting material was recovered.

Chart 1



lipase (PSL-C), *Candida rugosa* lipase (CRL), and subtilisin], CAL-B showed excellent regioselectivity toward the 5′-NH_2, **11b** being isolated with 80% yield after flash chromatography. The structure of this compound is clearly ascertained from its ^{1}H NMR (MeOH-d_4) spectrum, which showed a downfield shift corresponding to both $H_{5'}$ protons from 2.89 to 3.23 ppm in diaminonucleoside **9** to 3.72 ppm in **11b**. Meanwhile, $H_{3'}$ did not display any significant change. In addition, heteronuclear correlation ^{1}H−^{13}C experiments 2D HMBC showed a cross-peak between $H_{5'}$ and C=O, which corresponds to correlation $H_{5'}$-CNCO via $^3J_{CH}$. It is noteworthy that no N-3′ acylated derivative **13b** was formed, despite both amino groups being primary. Diacetylated derivative **15b** (Chart 1) was detected as a minor product (5%). To minimize the formation of this compound, the process was carried out at 28 °C. However, 20% of N-3′,5′-diacetylated **15b** was obtained contrary to our expectations (entry 2, Table 1).

Table 2. Enzymatic Acylation of 3′,5′-Diaminodeoxyuridine 10 in THF/Py (4.5:1, v/v)

		acylating agent					isolated yields (%)		
entry	enzyme	R^1	R^2	equiv	T (°C)	t (h)	N-3′	N-5′	N-3′,5′
1	CAL-B	Me	Et	8	40	7		75	
2	CAL-B	H	Et	2	40	7		48	20
3	CAL-B	*n*Pr	Et	10	40	7		70	
4	CAL-B	MeCH=CH	Me	20	60	21		67	
5	CAL-B	Ph	Me	30	60	69		65	
6	PSL-C	Me	Et	50	60	31	61	19	11
7	PSL-C	H	Et	5	60	31	23	24	40
8	PSL-C	*n*Pr	Et	50	60	94	18[a]	8	

[a] Starting material was recovered.

In an attempt to confer versatility to this enzymatic reaction, other acyl moieties, such as formyl, alkyl, alkenyl, or aryl, were introduced. Thus, only 2 equiv of the more reactive ethyl formate was necessary to acylate compound **9**. Due to its higher reactivity, a lower yield of N-5′ formyl **11a** was found, in addition to a considerable amount of N-3′,5′-diformyl **15a** (entry 3, Table 1). In contrast, complete regioselectivity toward the N-5′ position was achieved with high yield when ethyl butyrate, methyl crotonate, and methyl benzoate were employed (entries 4−6, Table 1). In these cases, due to the lower reactivity of the esters, the ratios of acylating agents were increased. Also, higher temperatures were used to bring conversions close to 100%.

Interestingly, PSL-C catalyzed the acetylation of **9** with total regioselectivity toward the N-3′ position. Fifty equivalents of EtOAc and 60 °C was necessary to afford compound **13b** with excellent yield (entry 8, Table 1). The ^{1}H NMR (D_2O) spectrum of this monoacylnucleoside presents a downfield shift of ca. 1 ppm on the $H_{3'}$ proton (from 3.34 ppm in **9** to 4.35 ppm in **13b**), whereas $H_{5'}$ almost did not change. Moreover, it was possible to confirm the structure by 2D HMBC that showed a cross-peak between $H_{3'}$ and C=O corresponding to correlation $H_{3'}$-CNCO via $^3J_{CH}$. Lower temperatures and a lesser amount of acylating agent led to the recovery of a substantial quantity of unreacted starting material (entry 7, Table 1).

On the other hand, when ethyl formate or butyrate was used, the process took place with lower selectivity (entries 9−10, Table 1). In the cases of methyl crotonate or benzoate, which have already shown low reactivity with CAL-B, the reaction did not occur.

When similar processes were carried out with 2′-deoxyuridine derivative **10**, parallel behavior was observed. To favor solubility of the starting nucleoside, pyridine was added as cosolvent. CAL-B kept its excellent regioselectivity in the acylation of N-5′ position, isolating exclusively compounds **12**, except in case of ethyl formate in which 20% of diacyl derivative **16a** was obtained (entries 1−5, Table 2).

In contrast, PSL-C showed only moderate regioselectivity toward N-3′ in the case of acetylation (entry 6, Table 2) with an isolated yield of 61% for compound **14b**. The formylation occurred with no selectivity, and the main product was the N-3′,5′-diformyl nucleoside derivative **16a** (entry 7, Table 2). Although ethyl butyrate presented a degree of selectivity toward N-3′, the conversion of the process was low even with prolonged reaction times (entry 8, Table 2).

This enzymatic strategy allowed us to regioselectively synthesize, for the first time, N-3′- or N-5′-acylated

pyrimidine 3′,5′-diamino nucleoside derivatives by means of a very simple and convenient procedure using PSL-C or CAL-B as biocatalyst, respectively. Moreover, an improved synthesis of pyrimidine 3′,5′-diamino-2′,3′,5′-trideoxynucleosides has been described through a four-step sequence and with high overall yield. A series of N-monoacylated 3′,5′-diamino nucleosides were prepared as potential antiviral and/or antitumor agents. The biological activity of these derivatives will be tested, and the results will be reported in a due course.

Experimental Section[7]

General Methods. *C. antarctica* lipase B (CAL-B, 7300 PLU/g) was a gift from Novo Nordisk Co. Immobilized *P. cepacia* lipase (PSL-C, 783 U/g) was purchased from Amano Pharmaceutical Co.

General Procedure for the Enzymatic Acylation of 3′,5′-Diaminonucleosides. Corresponding ester (ethyl formate, ethyl acetate, ethyl butyrate, methyl crotonate, and methyl benzoate) was added to a suspension of diaminonucleoside (20 mg, 0.08 mmol, in the case of **10** it was previously dissolved in 1 mL of dry pyridine), lipase (10 mg of CAL-B or 130 mg of PSL-C), and molecular sieves 4 Å (20 mg) in dry THF (4.5 mL) under nitrogen, and the mixture was stirred at 250 rpm (temperature and reaction time are indicated in Tables 1 and 2). Then, the enzyme and molecular sieves were filtered off and washed with MeOH (3 × 5 mL). The filtrate was evaporated to dryness, and the crude residue was purified by flash chromatography column (gradient eluent 10% MeOH/EtOAc−MeOH for compounds **11a**−**e**, **12a**−**e** and gradient eluent 10% MeOH/EtOAc−MeOH−10% NH_3(aq)/MeOH for compounds **13a**−**c**, **14a**−**c**).

3′-Amino-5′-formylamino-3′,5′-dideoxythymidine (11a): ^{1}H NMR (MeOH-d_4, 300 MHz) δ 2.09 (s, 3H, H_7), 2.32−2.55 (m, 2H, $H_{2'}$), 3.55 (m, 1H, $H_{3'}$), 3.76 (m, 2H, $H_{5'}$), 3.90 (m, 1H, $H_{4'}$), 6.33 (dd, 1H, $H_{1'}$, $^3J_{HH}$ = 7.1 Hz, $^3J_{HH}$ = 4.8 Hz), 7.68 (s, 1H, H_6), and 8.33 (s, 1H, *H*CO); MS (ESI$^+$, *m*/*z*) 291 [(M + Na)$^+$, 100], and 269 [(M + H)$^+$, 76].

5′-Acetylamino-3′-amino-3′,5′-dideoxythymidine (11b): ^{1}H NMR (MeOH-d_4, 200 MHz) δ 2.09 (s, 3H, H_7), 2.15 (s, 3H, *Me*CO), 2.30−2.60 (m, 2H, $H_{2'}$), 3.55 (m, 1H, $H_{3'}$), 3.72 (m, 2H, $H_{5'}$), 3.88 (m, 1H, $H_{4'}$), 6.30 (dd, 1H, $H_{1'}$, $^3J_{HH}$ = 7.3 Hz, $^3J_{HH}$ = 4.8 Hz), and 7.68 (s, 1H, H_6); MS (FAB$^+$, *m*/*z*) 283 [(M + H)$^+$, 15], 245 (4), 180 (14), and 140 (47).

3′-Amino-5′-butyrylamino-3′,5′-dideoxythymidine (11c): ^{1}H NMR (MeOH-d_4, 200 MHz) δ 1.14 (t, 3H, $H_{4''}$, $^3J_{HH}$ = 7.3 Hz), 1.83 (m, 2H, $H_{3''}$), 2.09 (s, 3H, H_7), 2.30−2.57 (m, 4H, $H_{2'}$ + $H_{2''}$), 3.55 (m, 1H, $H_{3'}$), 3.70 (m, 2H, $H_{5'}$), 3.87 (m, 1H, $H_{4'}$), 6.30 (dd, 1H, $H_{1'}$, $^3J_{HH}$ = 7.0 Hz, $^3J_{HH}$ = 4.8 Hz), and 7.65 (s, 1H, H_6); MS (ESI$^+$, *m*/*z*) 333 [(M + Na)$^+$, 82], and 311 [(M + H)$^+$, 100).

3′-Amino-5′-crothonylamino-3′,5′-dideoxythymidine (11d): ^{1}H NMR (MeOH-d_4, 300 MHz) δ 2.05 (m, 6H, $H_{4''}$ + H_7), 2.33−2.53 (m, 2H, $H_{2'}$), 3.55 (m, 1H, $H_{3'}$), 3.79 (m, 2H, $H_{5'}$), 3.90 (m, 1H, $H_{4'}$), 6.17 (dq, 1H, $H_{2''}$, $^3J_{HH}$ = 15.1 Hz, $^4J_{HH}$ = 1.4 Hz), 6.29 (dd, 1H, $H_{1'}$, $^3J_{HH}$ = 7.1 Hz, $^3J_{HH}$ = 4.3 Hz), 7.01 (dq, 1H, $H_{3''}$, $^3J_{HH}$ = 15.1 Hz, $^3J_{HH}$ = 6.8 Hz), and 7.64 (s, 1H, H_6); MS (ESI$^+$, *m*/*z*) 331 [(M + Na)$^+$, 100], and 309 [(M + H)$^+$, 75].

3′-Amino-5′-benzoylamino-3′,5′-dideoxythymidine (11e): ^{1}H NMR (MeOH-d_4, 300 MHz) δ 1.90 (s, 3H, H_7), 2.37−2.57 (m, 2H, $H_{2'}$), 3.62 (m, 1H, $H_{3'}$), 3.95 (m, 1H, $H_{4'}$), 4.02 (m, 2H, $H_{5'}$), 6.31 (dd, 1H, $H_{1'}$, $^3J_{HH}$ = 7.4 Hz, $^3J_{HH}$ = 4.3 Hz), 7.63−7.77 (m, 4H, H_6 + H_m + H_p), and 8.05 (m, 2H, H_o); MS (ESI$^+$, *m*/*z*) 383 [(M + K)$^+$, 7], 367 [(M + Na)$^+$, 100], and 345 [(M + H)$^+$, 94].

3′-Amino-5′-formylamino-2′,3′,5′-trideoxyuridine (12a): ^{1}H NMR (MeOH-d_4, 300 MHz) δ 2.35−2.55 (m, 2H, $H_{2'}$), 3.50 (m, 1H, $H_{3'}$), 3.77 (m, 2H, $H_{5'}$), 3.92 (m, 1H, $H_{4'}$), 5.90 (d, 1H, H_5, $^3J_{HH}$ = 8.0 Hz), 6.29 (dd, 1H, $H_{1'}$, $^3J_{HH}$ = 7.1 Hz, $^3J_{HH}$ = 4.6 Hz), 7.88 (d, 1H, H_6, $^3J_{HH}$ = 8.0 Hz), and 8.32 (s, 1H, *H*CO); *MS* (ESI$^+$, *m*/*z*) 293 [(M + K)$^+$, 12], 277 [(M + Na)$^+$, 100], and 255 [(M + H)$^+$, 77].

5′-Acetylamino-3′-amino-2′,3′,5′-trideoxyuridine (12b): ^{1}H NMR (MeOH-d_4, 200 MHz) δ 2.16 (s, 3H, *Me*CO), 2.30−2.60 (m, 2H, $H_{2'}$), 3.50 (m, 1H, $H_{3'}$), 3.70 (m, 2H, $H_{5'}$), 3.90 (m, 1H, $H_{4'}$), 5.90 (d, 1H, H_5, $^3J_{HH}$ = 7.9 Hz), 6.29 (dd, 1H, $H_{1'}$, $^3J_{HH}$ = 7.0 Hz, $^3J_{HH}$ = 4.8 Hz), and 7.89 (d, 1H, H_6, $^3J_{HH}$ = 7.9 Hz); MS (ESI$^+$, *m*/*z*) 291 [(M + Na)$^+$, 100], and 269 [(M + H)$^+$, 73).

3′-Amino-5′-butyrylamino-2′,3′,5′-trideoxyuridine (12c): ^{1}H NMR (MeOH-d_4, 300 MHz) δ 1.14 (t, 3H, $H_{4''}$, $^3J_{HH}$ = 7.4 Hz), 1.83 (m, 2H, $H_{3''}$), 2.35−2.55 (m, 4H, $H_{2'}$ + $H_{2''}$), 3.50 (m, 1H, $H_{3'}$), 3.71 (m, 2H, $H_{5'}$), 3.90 (m, 1H, $H_{4'}$), 5.89 (d, 1H, H_5, $^3J_{HH}$ = 8.0 Hz), 6.28 (dd, 1H, $H_{1'}$, $^3J_{HH}$ = 7.4 Hz, $^3J_{HH}$ = 4.6 Hz), and 7.91 (d, 1H, H_6, $^3J_{HH}$ = 8.0 Hz); MS (ESI$^+$, *m*/*z*) 319 [(M + Na)$^+$, 100], and 297 [(M + H)$^+$, 71].

3′-Amino-5′-crothonylamino-2′,3′,5′-trideoxyuridine (12d): ^{1}H NMR (MeOH-d_4, 300 MHz) δ 2.05 (dd, 3H, $H_{4''}$, $^3J_{HH}$ = 6.8 Hz, $^4J_{HH}$ = 1.7 Hz), 2.35−2.55 (m, 2H, $H_{2'}$), 3.50 (m, 1H, $H_{3'}$), 3.75 (m, 2H, $H_{5'}$), 3.92 (m, 1H, $H_{4'}$), 5.87 (d, 1H, H_5, $^3J_{HH}$ = 8.0 Hz), 6.16 (dq, 1H, $H_{2''}$, $^3J_{HH}$ = 15.1 Hz, $^4J_{HH}$ = 1.7 Hz), 6.28 (dd, 1H, $H_{1'}$, $^3J_{HH}$ = 7.1 Hz, $^3J_{HH}$ = 4.6 Hz), 7.00 (dq, 1H, $H_{3''}$, $^3J_{HH}$ = 15.1 Hz, $^3J_{HH}$ = 6.8 Hz), and 7.87 (d, 1H, H_6, $^3J_{HH}$ = 8.0 Hz); MS (ESI$^+$, *m*/*z*) 317 [(M + Na)$^+$, 100], and 295 [(M + H)$^+$, 50].

3′-Amino-5′-benzoylamino-2′,3′,5′-trideoxyuridine (12e): ^{1}H NMR (MeOH-d_4, 300 MHz) δ 2.39−2.59 (m, 2H, $H_{2'}$), 3.59 (m, 1H, $H_{3'}$), 3.93 (m, 2H, $H_{5'}$), 4.03 (m, 1H, $H_{4'}$), 5.79 (d, 1H, H_5, $^3J_{HH}$ = 8.0 Hz), 6.29 (dd, 1H, $H_{1'}$, $^3J_{HH}$ = 7.1 Hz, $^3J_{HH}$ = 4.0 Hz), 7.63−7.77 (m, 3H, H_m + H_p), 7.94 (d, 1H, H_6, $^3J_{HH}$ = 8.0 Hz), and 8.05 (m, 2H, H_o); MS (ESI$^+$, *m*/*z*) 369 [(M + K)$^+$, 15], 353 [(M + Na)$^+$, 100], and 331 [(M + H)$^+$, 97].

5′-Amino-3′-formylamino-3′,5′-dideoxythymidine (13a): ^{1}H NMR (D_2O, 200 MHz) δ 1.70 (s, 3H, H_7), 2.25−2.50 (m, 2H, $H_{2'}$), 3.04−3.29 (m, 2H, $H_{5'}$), 3.92 (m, 1H, $H_{4'}$), 4.42 (m, 1H, $H_{3'}$), 5.97 (dd, 1H, $H_{1'}$, $^3J_{HH}$ = 7.9 Hz, $^3J_{HH}$ = 4.7 Hz), 7.31 (s, 1H, H_6), and 7.92 (s, 1H, *H*CO); MS (ESI$^+$, *m*/*z*) 291 [(M + Na)$^+$, 2], and 269 [(M + H)$^+$, 100].

3′-Acetylamino-5′-amino-3′,5′-dideoxythymidine (13b): ^{1}H NMR (D_2O, 300 MHz) δ 1.77 (s, 3H, H_7), 1.90 (s, 3H, *Me*CO), 2.31−2.52 (m, 2H, $H_{2'}$), 3.12−3.33 (m, 2H, $H_{5'}$), 3.99 (m, 1H, $H_{4'}$), 4.35 (m, 1H, $H_{3'}$), 6.04 (dd, 1H, $H_{1'}$, $^3J_{HH}$ = 7.7 Hz, $^3J_{HH}$ = 5.1 Hz), and 7.38 (s, 1H, H_6); MS (ES$^+$, *m*/*z*) 283 (M$^+$, 100), 241 (4), 158 (23), 99 (17), and 60 (21); MS (ESI$^+$, *m*/*z*) 283 [(M + H)$^+$, 100].

5′-Amino-3′-butyrylamino-3′,5′-dideoxythymidine (13c): ^{1}H NMR (MeOH-d_4, 300 MHz) δ 1.14 (t, 3H, $H_{4''}$, $^3J_{HH}$ = 7.4 Hz), 1.83 (m, 2H, $H_{3''}$), 2.10 (s, 3H, H_7), 2.38 (t, 2H, $H_{2''}$, $^3J_{HH}$ = 7.1 Hz), 2.42−2.64 (m, 2H, $H_{2'}$), 3.02−3.17 (m, 2H, $H_{5'}$), 3.91 (m, 1H, $H_{4'}$), 4.58 (m, 1H, $H_{3'}$), 6.37 (dd, 1H, $H_{1'}$, $^3J_{HH}$ = 7.1 Hz, $^3J_{HH}$ = 5.7 Hz), and 7.75 (s, 1H, H_6); MS (ESI$^+$, *m*/*z*) 349 [(M + K)$^+$, 15], 333 [(M + Na)$^+$, 33], and 311 [(M + H)$^+$, 100].

5′-Amino-3′-formylamino-2′,3′,5′-trideoxyuridine (14a): ^{1}H NMR (D_2O, 300 MHz) δ 2.52−2.73 (m, 2H, $H_{2'}$), 3.29−3.49 (m, 2H, $H_{5'}$), 4.16 (m, 1H, $H_{4'}$), 4.61 (m, 1H, $H_{3'}$), 5.91 (d, 1H, H_5, $^3J_{HH}$ = 8.3 Hz), 6.18 (dd, 1H, $H_{1'}$, $^3J_{HH}$ = 7.7 Hz, $^3J_{HH}$ = 5.1 Hz), 7.74 (d, 1H, H_6, $^3J_{HH}$ = 8.3 Hz), and 8.15 (s, 1H, *H*CO); MS (ESI$^+$, *m*/*z*) 277 [(M + Na)$^+$, 5], and 255 [(M + H)$^+$, 100].

3′-Acetylamino-5′-amino-2′,3′,5′-trideoxyuridine (14b): ^{1}H NMR (D_2O, 300 MHz) δ 1.89 (s, 3H, *Me*CO), 2.29−2.53 (m, 2H, $H_{2'}$), 3.07−3.26 (m, 2H, $H_{5'}$), 3.96 (m, 1H, $H_{4'}$), 4.33 (m, 1H, $H_{3'}$), 5.75 (d, 1H, H_5, $^3J_{HH}$ = 8.3 Hz), 6.03 (dd, 1H, $H_{1'}$, $^3J_{HH}$ = 7.7 Hz, $^3J_{HH}$ = 5.4 Hz), and 7.58 (d, 1H, H_6, $^3J_{HH}$ = 8.3 Hz); MS (ESI$^+$, *m*/*z*) 291 [(M + Na)$^+$, 3], and 269 [(M + H)$^+$, 100].

5′-Amino-3′-butyrylamino-2′,3′,5′-trideoxyuridine (14c): ^{1}H NMR (D_2O, 300 MHz) δ 0.89 (t, 3H, $H_{4''}$, $^3J_{HH}$ = 7.4 Hz), 1.60 (m, 2H, $H_{3''}$), 2.24 (t, 2H, $H_{2''}$, $^3J_{HH}$ = 7.4 Hz), 2.40−2.68 (m, 2H, $H_{2'}$), 3.23−3.42 (m, 2H, $H_{5'}$), 4.10 (m, 1H, $H_{4'}$), 4.48 (m, 1H, $H_{3'}$), 5.87 (d, 1H, H_5, $^3J_{HH}$ = 8.0 Hz), 6.14 (dd, 1H, $H_{1'}$, $^3J_{HH}$ = 6.2 Hz, $^3J_{HH}$ = 5.1 Hz), and 7.70 (d, 1H, H_6, $^3J_{HH}$ = 8.0 Hz); MS (ESI$^+$, *m*/*z*) 319 [(M + Na)$^+$, 7], and 297 [(M + H)$^+$, 100].

3′,5′-Diformylamino-3′,5′-dideoxythymidine (15a): ^{1}H NMR (MeOH-d_4, 300 MHz) δ 2.10 (s, 3H, H_7), 2.47−2.70 (m, 2H, $H_{2'}$), 3.65−3.89 (m, 2H, $H_{5'}$), 4.11 (m, 1H, $H_{4'}$), 4.62 (m, 1H, $H_{3'}$), 6.34

(7) Compounds **3**,[8] **4**,[9] **5**,[8] **6**,[10] **7**,[3] **8**,[4] **9**,[3] and **10**[4] were previously reported; experimental procedures and ^{1}H and ^{13}C NMR data are given in the Supporting Information. For compounds **11**−**16**, full spectral data and copies of ^{1}H and ^{13}C NMR spectra are given in the Supporting Information. The level of purity is indicated by the inclusion of elemental analyses.

(8) Michelson, A. M.; Todd, A. R. *J. Chem. Soc.* **1955**, 816−823.
(9) Horwitz, J. P.; Chua, J.; Da Rooge, M. A.; Noel, M.; Klundt, I. L. *J. Org. Chem.* **1966**, *31*, 205−211.
(10) Horwitz, J. P.; Chua, J.; Noel, M.; Donatti, J. T. *J. Org. Chem.* **1967**, *32*, 817−818.

(dd, 1H, $H_{1'}$, $^3J_{HH}$ = 7.1 Hz), 7.73 (s, 1H, H_6), 8.28 (s, 1H, *H*CO), and 8.31 (s, 1H, *H*CO); MS (ESI$^+$, *m*/*z*) 335 [(M + K)$^+$, 10], 319 [(M + Na)$^+$, 100], and 297 [(M + H)$^+$, 1].

3′,5′-Diacetylamino-3′,5′-dideoxythymidine (15b): ^{1}H NMR (MeOH-d_4, 300 MHz) δ 2.10 (s, 3H, H_7), 2.16 (s, 6H, *Me*CO), 2.30−2.70 (m, 2H, $H_{2'}$), 3.60−3.80 (m, 2H, $H_{5'}$), 4.05 (m, 1H, $H_{4'}$), 4.50 (m, 1H, $H_{3'}$), 6.32 (dd, 1H, $H_{1'}$, $^3J_{HH}$ = 6.9 Hz, $^3J_{HH}$ = 6.0 Hz), and 7.70 (s, 1H, H_6); MS (ESI$^+$, *m*/*z*) 363 [(M + K)$^+$, 11], and 347 [(M + Na)$^+$, 100].

3′,5′-Diformylamino-2′,3′,5′-trideoxyuridine (16a): ^{1}H NMR (MeOH-d_4, 300 MHz) δ 2.53−2.70 (m, 2H, $H_{2'}$), 3.62−3.90 (m, 2H, $H_{5'}$), 4.11 (m, 1H, $H_{4'}$), 4.61 (m, 1H, $H_{3'}$), 5.92 (d, 1H, H_5, $^3J_{HH}$ = 8.3 Hz), 6.33 (dd, 1H, $H_{1'}$, $^3J_{HH}$ = 7.1 Hz), 7.92 (d, 1H, H_6, $^3J_{HH}$ = 8.3 Hz), 8.29 (s, 1H, *H*CO), and 8.30 (s, 1H, *H*CO); MS (ESI$^+$, *m*/*z*) 321 [(M + K)$^+$, 10], and 305 [(M + Na)$^+$, 100].

3′,5′-Diacetylamino-2′,3′,5′-trideoxyuridine (16b): ^{1}H NMR (MeOH-d_4, 200 MHz) δ 2.16 (s, 6H, *Me*CO), 2.47−2.62 (m, 2H, $H_{2'}$), 3.60−3.82 (m, 2H, $H_{5'}$), 4.10 (m, 1H, $H_{4'}$), 4.51 (m, 1H, $H_{3'}$), 5.92 (d, 1H, H_5, $^3J_{HH}$ = 8.3 Hz), 6.31 (dd, 1H, $H_{1'}$, $^3J_{HH}$ = 6.4 Hz), and 7.92 (d, 1H, H_6, $^3J_{HH}$ = 8.3 Hz); MS (ESI$^+$, *m*/*z*) 349 [(M + K)$^+$, 6], and 333 [(M + Na)$^+$, 100].

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Supporting Information Available: Experimental procedures and complete ^{1}H and ^{13}C NMR spectral data in addition to mp, IR, microanalysis, and MS data. This material is available free of charge via the Internet at http://pubs.acs.org.

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