

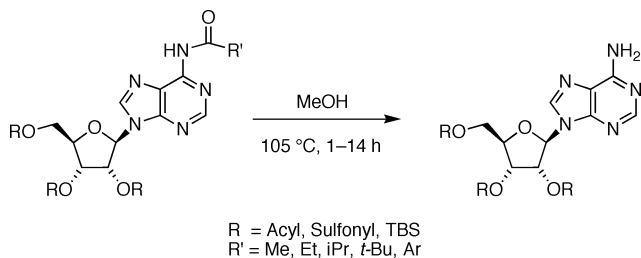
Nucleic Acid Related Compounds. 127. Selective N-Deacylation of N,O-Peracylated Nucleosides in Superheated Methanol¹

Ireneusz Nowak, Martin Conda-Sheridan, and
Morris J. Robins*

Department of Chemistry and Biochemistry,
Brigham Young University, Provo, Utah 84602-5700

morris_robins@byu.edu

Received June 18, 2005

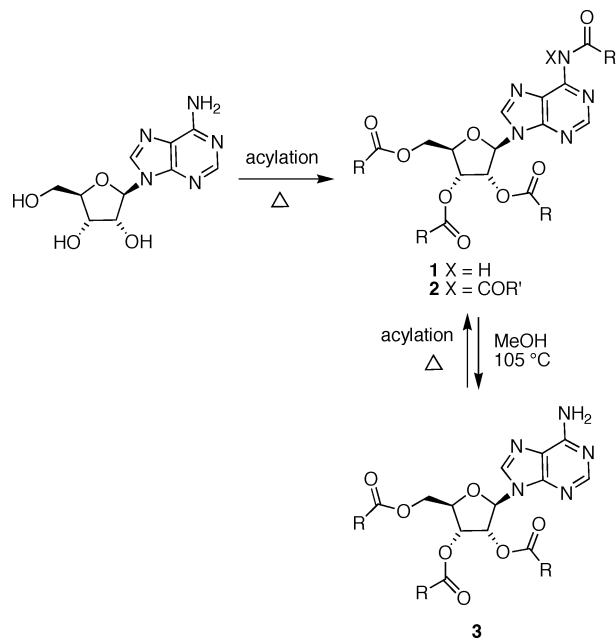


Solutions of peracylated adenosine, cytidine, and related nucleoside derivatives undergo selective N-deacylation upon heating at elevated temperatures (oil bath $\geq 105\text{ }^{\circ}\text{C}$) in methanol. An increase in the bulk of the *N*-acyl group has little effect on the rate of N-deacylation but increases the N/O selectivity ratio. Extended heating is required for N-deacylation with arylcarboxylic acid derivatives. Contamination with acidic or basic reagent residues is avoided.

Selective protection and deprotection methodologies are keystones of modern organic synthesis.² Numerous reports on manipulations of acylated nucleosides demonstrate their removal under conditions that are orthogonal to a variety of other protecting groups. Acyl groups have been removed by both chemical³ and enzymatic⁴ procedures. Selective N-acylation of nucleobases has been employed for temporary protection,⁵ especially during glycosylation coupling reactions.⁶ However, N-acylation is often encountered as an undesired side reaction during protection of the sugar portion of nucleosides that have an amino group on the nucleobase. Lowering the reaction temperature can attenuate N-acylation with the less reactive acid anhydrides but usually not with acid chlorides.

Various target structures require reactions with the free 6-amino group of adenosine. Current projects in our laboratory involve diazotization of an amino group⁷ and synthetic applications of adenosine *N*-oxides. We observed rapid loss of the *N*-acetyl group, relative to

SCHEME 1. N,O-Acylation and N-Deacylation of Adenosine



O-acetyl cleavage, upon heating a solution of 6-*N*-2',3',5'-tri-*O*-tetraacetyladenosine in MeOH in a sealed vessel at $105\text{ }^{\circ}\text{C}$. Holy reported that treatment of 4-*N*-2',3',5'-tri-*O*-tetraacetylcytidine with 80% aqueous acetic acid at reflux resulted in rearrangement of the acetyl group from N4 to N3 of the cytosine ring. Such treatment of 6-*N*-2',3',5'-tri-*O*-tetraacetyladenosine effected removal of the *N*-acetyl group without its rearrangement to N1.⁸ Heating a methanolic solution of 4-*N*-2',3',5'-tri-*O*-tetraacetylcytidine at reflux for 40 h was required to solvolyze the *N*-acetyl group.⁹ Cleavage of *N*-acyl groups with zinc bromide and alcohols has been noted.¹⁰ We now report the convenient and selective removal of *N*-acyl groups from peracylated nucleosides with superheated methanol.

Adenosine was acylated with acetic, propionic, isobutyric, and pivalic anhydrides in pyridine. Typically, acetylation of adenosine at ambient temperature takes several hours and produces a mixture of *O*-tri- and *N,O*-tetraacetyl derivatives ($\sim 3:1$), which have similar chromatographic mobilities.¹¹ We effected complete N-acylation by heating the reaction mixtures overnight at 50 or $80\text{ }^{\circ}\text{C}$, which gave both tetra-, **1**, and pentaacylated, **2**, products (Scheme 1). (Because the pentaacylated compounds **2** are converted quickly to their tetraacyl analogues **1** in hot MeOH, we designate compounds **1** as starting materials even when mixtures of both **1** and **2** are present.) Treatment of **1** (1–2 mmol) with superheated MeOH was examined (Table 1).

(1) Paper 126 is: Janeba, Z.; Balzarini, J.; Andrei, G.; Snoeck, R.; De Clercq, E.; Robins, M. J. *J. Med. Chem.* **2005**, *48*, 4690–4696.

(2) Greene, T. W.; Wuts, P. G. M. *Protective Groups in Organic Synthesis*; Wiley: New York, 1999.

(3) (a) Asakura, J.-i. *Nucleosides Nucleotides* **1993**, *12*, 701–711 and references therein. (b) Ishido, Y.; Sakairi, N.; Okazaki, K.; Nakazaki, N. *J. Chem. Soc., Perkin Trans. 1* **1980**, 563–573.

(4) Singh, H. K.; Cote, G. L.; Sikorski, R. S. *Tetrahedron Lett.* **1993**, *34*, 5201–5204.

(5) Igolen, J.; Morin, C. *J. Org. Chem.* **1980**, *45*, 4802–4804.

(6) Vorbrüggen, H.; Ruh-Pohlentz, C. *Org. React.* **2000**, *55*, 1–630.

(7) Liu, J.; Robins, M. J. *Org. Lett.* **2005**, *7*, 1149–1151.

(8) Holy, A. *Collect. Czech. Chem. Commun.* **1979**, *44*, 1819–1827.

(9) Beranek, J.; Pitha, J. *Collect. Czech. Chem. Commun.* **1964**, *29*, 625–633.

(10) Kierzek, R.; Ito, H.; Bhatt, R.; Itakura, K. *Tetrahedron Lett.* **1981**, *22*, 3761–3764.

(11) Also indicated by the moderate yields and necessity for further purifications reported by: (a) Reist, E. J.; Calkins, D. F.; Fisher, L. V.; Goodman, L. *J. Org. Chem.* **1967**, *33*, 1600–1603. (b) Nair, V.; Chamberlain, S. D. *Synthesis* **1984**, 401–403.

TABLE 1. N-Deacylation of 1/2 Mixtures in Superheated Methanol^a

entry ^b	time ^c	R	R'	product	yield ^d
1 (a)	1	Me	Me	3a	84 (92)
2 (a)	12	Me	Me	3a	(45)
3 (b)	1	Et	Et	3b	(87)
4 (b)	1.5	Et	Et	3b	98
5 (b)	12	Et	Et	3b	(80)
6 (c)	1	ⁱ Pr	ⁱ Pr	3c	(56)
7 (c)	3	ⁱ Pr	ⁱ Pr	3c	98
8 (c)	12	ⁱ Pr	ⁱ Pr	3c	(92)
9 (d)	1	Me	<i>t</i> -Bu	3a	(77)
10 (d)	3	Me	<i>t</i> -Bu	3a	70
11 (e)	3	ⁱ Pr	<i>t</i> -Bu	3c	93
12 (f)	1	ⁱ Pr	Ph	3c	7
13 (f)	12	ⁱ Pr	Ph	3c	88
14 (g)	14	Ph	Ph	3d	84
15 (h)	14	MePh	MePh	3e	79
16 (h)	14 ^e	MePh	MePh	3e	95
17 (i)	14	ClPh	ClPh	3f	84

^a Reactions were performed by the general procedure. ^b Starting material in parentheses. ^c Hours. ^d Percent isolated or (% by ¹H NMR). ^e Temperature = 120 °C.

As expected, the less sterically hindered *N*-(acetyl and propionyl) groups underwent the most rapid methanolysis (entries 1 and 3), followed by the *N*-(isobutyryl and pivalyl) amides (entries 6 and 9). Prolonged heating must be avoided, especially with *N,O*-acetates (entries 2 and 10), because the ester groups on the sugar moiety undergo slow methanolysis. The more hindered *N,O*-propionyl analogue **1b**, and especially the *N,O*-isobutyryl derivative **1c**, underwent highly selective *N*-deacylation in 1.5 and 3 h, respectively (entries 4 and 7). Hindered rotation of the bulky isobutyryl groups was evidenced by the appearance of twinned sets of signals (1:1) in the ¹H NMR spectrum of **1c**. More steric interference would occur with neighboring 2'- and 3'-*O*-pivalyl groups, and complete *O*-pivalylation of adenosine was not observed. The more bulky *N*-pivalyl group of 6-*N*-pivalyl-2',3',5'-tri-*O*-isobutyryl-adenosine (**1e**) (prepared by pivalylation of **3c**) underwent selective methanolysis relative to the less sterically hindered *O*-isobutyryl esters.

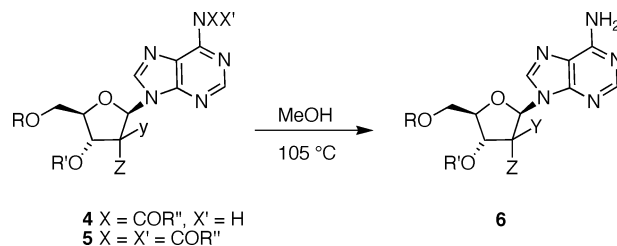
Loss of the *N*-pivalyl group was complete within 3 h (entries 10 and 11). The lower yield of the desired product from methanolysis of **1d** (entry 10) results from partial solvolysis of the *O*-acetyl groups on the sugar moiety. Benzoylation of **3c** with benzoyl chloride/pyridine gave the 6-*N,N*-dibenzoyl derivative **2f**. Methanolysis of **2f** at 105 °C proceeded slowly and required 12 h for completion (entries 12 and 13). The *N*-debenzoylated products **3d** and **3f** were obtained in lower yields (84%) from the perbenzoylated derivatives **2g** and **2i** (entries 14 and 17) because of competing *O*-debenzoylation. Methanolysis of the *p*-toluylated **2h** gave **3e** in 79% yield (entry 15). The incomplete *N*-detoluylation was possibly due to the inductive donating effect of the *p*-methyl group. Elevation of the bath temperature to 120 °C resulted in deprotection of **2h** to give **3e** in 95% yield (entry 16).

From a practical perspective, synthesis of the acetyl **3a**, propionyl **3b**, and isobutyryl **3c** derivatives are one-pot procedures. Isolation of intermediates **1a–c** and **2a–c** is not necessary; excess reagents are removed by evaporation in vacuo. Methanolysis of the crude reaction mixtures gives products that are clean or readily purified. Short reaction times for *N*-deprotection are advantageous

TABLE 2. N-Deacylation of 4/5 to Give 6 in Superheated MeOH^a

entry ^b	time ^c	R	R'	R''	Y	Z	yield ^d
1 (a)	1.5	Ac	Ac	Me	H	H	86 (93)
2 (b)	14	Tol	Tol	MePh	H	H	72
3 (c)	12	Bz	Bz	Ph	H	OTs	58
4 (d)	1.5	TBS	Ac ^e	Me	H	OTs	85
5 (e)	1.5	Ac	Ac	Me	OAc	H	76 (90)

^a Reactions were performed by the general procedure. ^b Starting material in parentheses. ^c Hours. ^d Percent isolated or (% by ¹H NMR). ^e Product **6d** (R' = H) had lost both the 6-*N*- and 3'-*O*-acetyl groups.

SCHEME 2. N-Deacylation of Some Adenosine Derivatives

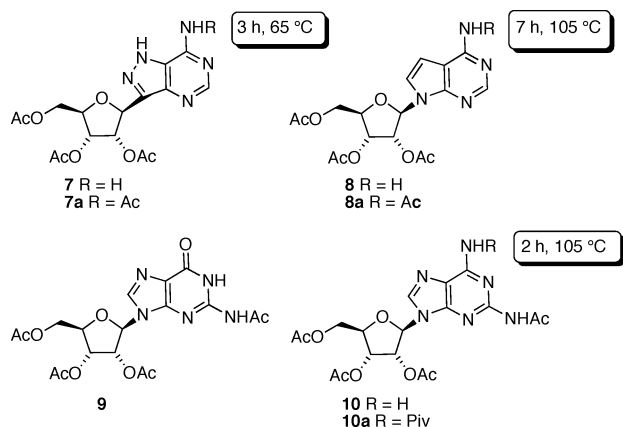
relative to prior acylations that require extended incubation periods and proceed with varied *N* versus *O* selectivities.

The preference for *N* versus *O* deprotection in superheated MeOH is reversed relative to that noted by Khorana and co-workers for selective *O*-debenzoylation of perbenzoylated nucleosides with NaOH or NaOMe.¹² Jones' "transient protection" protocol also provides *N*-acyl derivatives by complete trimethylsilylation followed by acylation and selective removal of the *O*-silyl groups.¹³ Thus, the procedures of Khorana¹² and Jones¹³ are complementary to our deprotections in superheated methanol.

We reasoned that these mild solvolysis conditions with methanol should be compatible with 2'-deoxyadenosine derivatives, which are highly susceptible to both acid-catalyzed glycosyl-bond hydrolysis and base-promoted eliminations.¹² Indeed, the glycosyl bond in **4a** (Scheme 2) was stable at 105 °C for 2 h, and essentially pure **6a** was obtained (Table 2). However, a lower yield of **6b** (72%) was obtained from **4b** due to the loss of 6-*N*-toluyladenine. A tosyl ester at O2' was stable in superheated methanol (entries 3 and 4), but compound **6c** (58%) was obtained in a lower yield. An explanation became evident when the 3'-*O*-benzoyl ester in **5c** was replaced with the more labile 3'-*O*-acetyl group in **5d** (entry 4). The *N*-deacetylation of **5d** was accompanied by complete loss of the 3'-*O*-acetyl group. Methanolysis of the ester moiety at C3' was facilitated by the electron-withdrawing tosylate at C2'. It is noteworthy that the 5'-*O*-*tert*-butyldimethylsilyl group in **5d** was stable under these conditions. Such compatibility with silyl ether protecting groups enhances the practicality of this deprotection method. The arabino epimer **5e** also underwent

(12) (a) Rammler, D. H.; Khorana, H. G. *J. Am. Chem. Soc.* **1962**, *84*, 3112–3122. (b) Schaller, H.; Weimann, G.; Lerch, B.; Khorana, H. G. *J. Am. Chem. Soc.* **1963**, *85*, 3821–3827.

(13) Ti, G. S.; Gaffney, B. L.; Jones, R. A. *J. Am. Chem. Soc.* **1982**, *104*, 1316–1319.

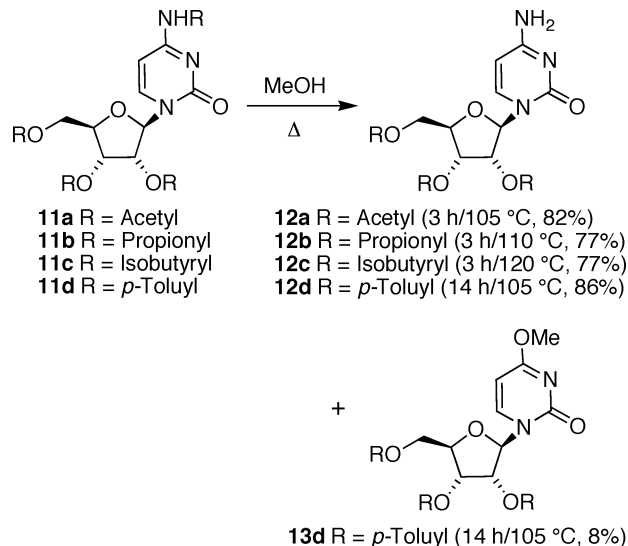
SCHEME 3. Related Nucleosides Subjected to MeOH/Δ


selective methanolysis to give a high yield of the N-deprotected **6e** (entry 5).

Tetraacetyl derivatives of formycin **7a**, tubercidin **8a**, guanosine **9**, and the pivalylated 2,6-diaminopurine nucleoside **10a** were subjected to our standard conditions to further probe its applicability (Scheme 3). The *N*-acetyl group was cleanly removed from the formycin derivative **7a** within 3 h at 65 °C (refluxing MeOH) to give 2',3',5'-tri-*O*-acetylformycin (**7**). It is likely that some **7a** also was formed during a previously reported acetylation of formycin¹⁴ and that facile *N*-deacetylation occurred during repeated addition and evaporation of ethanol. The tubercidin derivative **8a** was more stable and required 7 h at 105 °C for complete *N*-deacetylation. This treatment resulted in significant accompanying loss of sugar *O*-acetyl groups. The 2-*N*-acetyl group of guanosine derivative **9** was not removed by prolonged heating (12 h) at 105 °C. It is noteworthy that the diaminopurine derivative **10a** lost the more bulky 6-*N*-pivalyl group within 2 h; the 2-*N*-acetyl group remained unchanged after 3 h at 105 °C.

Cytidine derivatives had reactivity patterns similar to those of adenosine. The tetraacetylcytidines **11a** (R = COMe), **11b** (R = COEt), and **11c** (R = COiPr) underwent *N*-deprotection within 3 h at 105–120 °C to give **12a** (82% by NMR, 61% isolated), **12b** (77% isolated), and **12c** (77% isolated). Heating the tetratoluylyl derivative **11d** (R = *p*-methylbenzoyl) for 14 h at 105 °C gave **12d** (86% isolated) plus 8% of the 4-methoxy-2-pyrimidinone¹⁵ derivative **13d** (methanolysis at C4 to produce *p*-toluamide) (Scheme 4). Concomitant loss of toluylyl ester groups from the sugar moiety was not observed. By comparison, removal of the *N*-acetyl group from tetraacetylcytidine had required 40 h at reflux (65 °C).⁹

A net shift of electron density from the exocyclic amino nitrogen atom into the π -deficient heterocyclic ring occurs with conjugated amidine systems in the pyrimidine rings of nucleobases. The resulting enhanced electrophilicity of the carbonyl carbon atom of amides formed with these amino groups allows deprotection of such amides in the presence of usually more stable esters. An estimate of

SCHEME 4. Methanolysis of Acylated Cytidine Derivatives


the relative electron delocalization into the π -deficient ring (i.e., away from the amino nitrogen) is provided by pK_a data. Amides derived by N6 acylation of adenosine ($pK_{a1} = 3.6^{16}$) undergo *N*-deacylation quite readily. Those derived from the more basic cytidine ($pK_{a1} = 4.2^{17}$) are less reactive with MeOH at 105 °C, and *N*-deacetylation of amide derivatives of tubercidin ($pK_{a1} = 4.7^{17}$) is even more difficult.

In conclusion, we have demonstrated that solutions of peracylated adenosine and cytidine derivatives in superheated methanol undergo selective *N*-deacylation. Contamination with acidic or basic residues is avoided with this convenient neutral procedure. An increase in the steric bulk of the *N*-acyl group has a limited effect on the rate of *N*-deacylation, but it significantly increases the *N*/*O* selectivity ratio (i.e., apparent retardation of the rate of *O*-deacylation). Removal of *N*-acyl groups derived from arylcarboxylic acids requires more prolonged heating but proceeds successfully. Judicious choices of temperature and *N*- versus *O*-acyl groups allow virtually complete selectivity for *N*-deacylation with superheated methanol. In contrast to the reactivity of amides at C6 of purine nucleosides, 2-acetamidopurine derivatives were completely resistant to methanolysis at 105 °C. This allows selective removal of the sterically hindered pivalyl group from N6 of a 2-acetamido-6-pivalamidopurine compound. Adenosine and cytidine derivatives with ester protection on the sugar moiety and a free amino group on the base are important intermediates. Our methodology makes such derivatives readily accessible.

Experimental Section

General. ¹H (500 MHz) and ¹³C (125 MHz) NMR spectra were recorded with solutions in CDCl₃ unless noted. ¹³C peaks with identical chemical shifts for more than one carbon are specified, and a shift range is indicated (ovlp) for overlapping peaks with

(14) Lindell, S. D.; Moloney, B. A.; Hewitt, B. D.; Earnshaw, C. G.; Dudfield, P. J.; Dancer, J. E. *Bioorg. Med. Chem. Lett.* **1999**, *9*, 1985–1990.

(15) Robins, M. J.; Naik, S. R. *Biochemistry* **1971**, *10*, 3591–3597.

(16) Dawson, R. M. C.; Elliott, D. C.; Elliott, W. H.; Jones, K. M. *Data for Biochemical Research*; Oxford University Press: New York, 1969.

(17) Ikehara, M.; Fukui, T.; Uesugi, S. *J. Biochem.* **1974**, *76*, 107–115.

multiple carbons. Electron impact (EI) mass spectra were obtained at 70 eV. Mallinckrodt anhydrous methanol was used for all "superheated MeOH" deprotection experiments. Other chemicals and solvents were of reagent quality.

Acylation of Adenosine with Acid Anhydrides. Adenosine (0.5 g, 1.87 mmol), pyridine (2 mL), and an excess of the acid anhydride were heated overnight at 50 or 80 °C (oil-bath temperature). Volatiles were evaporated in vacuo, and the residue was chromatographed [EtOAc/hexanes (1:2) → EtOAc and then EtOAc/MeOH (10:1)] to give quantitative yields of the 6-*N*,2',3',5'-tri-*O*-tetraacyladenines, **1**, and/or 6-di-*N*,2',3',5'-tri-*O*-pentaacyladenines, **2**.

General Procedure for Methanolysis of the Peracylated Adenosines. Compounds **1** and/or **2** (1–2 mmol) were dissolved in MeOH (10 mL) in a 30 mL pressure vessel equipped with a Teflon valve. The vessel was placed in an oil bath heated at 105 or 120 °C for the specified time. Volatiles were evaporated, and the residues were dried in vacuo. Compounds **3b** and **3c** were ¹H NMR pure (>98%). Compound **3a** was purified by crystallization, and **3d–f** were purified by chromatography. Larger scale methanolysis reactions required longer reaction times and/or higher temperatures. For example, *N*-deacetylation of a solution of **1a** (15.0 g, 34.5 mmol) in MeOH (150 mL) in a 250 mL vessel required heating for 6 h at 110 °C.

7-*N*,2',3',5'-Tri-*O*-tetraacetylformycin (7a). Formycin (1.0 g, 3.75 mmol), Ac₂O (2.0 mL, 2.16 g, 21.2 mmol), and pyridine (2 mL) were stirred for 1 h at ambient temperature. Volatiles were evaporated, and the residue was chromatographed (EtOAc) to give **7a** (1.62 g, quantitative): UV (MeOH) max 262, 295, 305 nm (ε 6200, 6800, 6700); min 247, 278, 301 nm (ε 5300, 4700, 6500). ¹H NMR δ 2.08, 2.10, 2.14, 2.80 (4 × s, 4 × 3H), 4.29–4.33 (m, 1H), 4.42–4.48 (m, 2H), 5.45 (d, *J* = 5.4 Hz, 1H), 5.68–5.70 (m, 1H), 5.99 (t, *J* = 5.4 Hz, 1H), 6.62 (br s, 1H), 8.24 (br s, 1H), 8.44 (s, 1H); ¹³C NMR δ 20.45, 20.46, 20.6, 23.0, 63.5, 71.9, 72.9, 75.7, 79.8, 120.9, 146.2, 147.9, 151.6, 155.3, 169.5, 169.6, 170.5, 172.0; FAB-MS *m/z* 436 (M + H⁺); HRMS calcd for C₁₈H₂₂N₅O₈ 436.1468, found 436.1472.

2',3',5'-Tri-*O*-acetylformycin¹⁸ (7). A solution of **7a** (1.62 g, 3.72 mmol) in MeOH (40 mL) was refluxed for 3 h (TLC). Volatiles were evaporated to dryness, and the residue was chromatographed [EtOAc → MeOH/EtOAc (1:10)] to give **7** (1.24 g, 84%): UV (MeOH) max 229, 293 nm (ε 6200, 9800); min 244 nm (ε 3200). ¹H NMR δ 2.05, 2.10, 2.11 (3 × s, 3 × 3H), 4.28 (dd, *J* = 4.9, 12.2 Hz, 1H), 4.34–4.37 (m, 1H), 4.59 (dd, *J* = 2.4, 11.7 Hz, 1H), 5.49 (d, *J* = 5.9 Hz, 1H), 5.64 (t, *J* = 5.4 Hz, 1H), 5.97 (t, *J* = 5.6 Hz, 1H), 6.64 (br s, 3H), 8.27 (s, 1H); ¹³C NMR δ 20.4 (2C), 20.7, 63.5, 71.7, 73.3, 75.7, 76.7, 79.7, 122.6 (br s), 139.5 (br s), 140.5 (br s), 150.8 (br s), 151.8, 170.06, 170.14, 171.6; FAB-MS *m/z* 416 (100%, M + Na⁺); HRMS calcd for C₁₆H₁₉N₅O₇-Na 416.1182, found 416.1178.

4-*N*,2',3',5'-Tri-*O*-tetraacetyltubercidin¹⁹ (8a). Tubercidin (1.0 g, 3.76 mmol), Ac₂O (2.0 mL, 2.16 g, 21.2 mmol), and pyridine (3 mL) were stirred for 2 h at 50 °C. Volatiles were evaporated, and the residue was chromatographed (EtOAc) to give **8a** (1.48 g, 91%): ¹H NMR δ 2.06 (s, 3H), 2.16 (s, 6H), 2.35 (br s, 3H), 4.35–4.45 (m, 3H), 5.59 (t, *J* = 4.9 Hz, 1H), 5.79 (t, *J* = 5.9 Hz, 1H), 6.55 (d, *J* = 5.9 Hz, 1H), 7.03 (d, *J* = 3.4 Hz, 1H), 7.34 (d, *J* = 5.9 Hz, 1H), 8.58 (s, 1H), 10.30 (br s, 1H); ¹³C NMR δ 20.2, 20.4, 20.6, 24.2, 63.2, 70.6, 72.8, 79.4, 85.1, 105.0, 109.1, 123.1, 150.3, 150.5, 153.0, 169.1 (br s), 169.2, 169.5, 170.1; FAB-MS *m/z* 457 (100%, M + Na⁺); HRMS calcd for C₁₉H₂₂N₄O₈-Na 457.1335, found 457.1341.

2',3',5'-Tri-*O*-acetyltubercidin (8): Method A. Tubercidin (1.0 g, 3.76 mmol), Ac₂O (2.0 mL, 2.16 g, 21.2 mmol), and pyridine (3 mL) were stirred for 1 h at ambient temperature. Volatiles were evaporated, and the residue was chromatographed (EtOAc) to give **8** (1.31 g, 89%): ¹H NMR δ 2.05, 2.147, 2.151 (3 × s, 3 × 3H), 4.32–4.41 (m, 3H), 5.38 (br s, 2H), 5.56

(dd, *J* = 3.9, 5.4 Hz, 1H), 5.74 (t, *J* = 5.9 Hz, 1H), 6.45 (d, *J* = 3.9 Hz, 1H), 6.46 (d, *J* = 5.9 Hz, 1H), 7.13 (d, *J* = 3.9 Hz, 1H), 8.34 (s, 1H); ¹³C NMR δ 20.3, 20.5, 20.7, 63.4, 70.7, 72.8, 79.3, 85.0, 100.0, 103.6, 121.2, 150.9, 152.1, 157.0, 169.5, 160.7, 170.3; FAB-MS *m/z* 415 (100%, M + Na⁺); HRMS calcd for C₁₇H₂₀N₄O₇-Na 415.1230, found 415.1231. **Method B.** A solution of **8a** (1.0 g, 2.30 mmol) in MeOH (20 mL) was heated at 105 °C for 7 h (TLC). Compound **8** was the major product (55–60%; ¹H NMR), but other compounds were present that had lost *O*-acetyl groups from the sugar moiety.

2-Acetamido-9-(2,3,5-tri-*O*-acetyl-β-D-ribofuranosyl)-6-pivalamidopurine (10a). A solution of 2-acetamido-9-(2,3,5-tri-*O*-acetyl-β-D-ribofuranosyl)-6-chloropurine²⁰ (2.9 g, 6.2 mmol) and Na₃ (2.0 g, 30.8 mmol) in DMF (60 mL) was stirred overnight at ambient temperature. Volatiles were evaporated, and the residue was chromatographed [hexanes/EtOAc (2:1) → EtOAc] to give 2-acetamido-9-(2,3,5-tri-*O*-acetyl-β-D-ribofuranosyl)-6-azidopurine (2.6 g, 88%). A solution of this material (2.0 g, 4.2 mol) in MeOH (40 mL) was stirred overnight under 1 atm of H₂ with Pd·C (5%, 180 mg). The catalyst was filtered and washed with MeOH, and volatiles were evaporated from the combined filtrate. The residue was chromatographed [MeOH/EtOAc (1:10)] to give 2-acetamido-9-(2,3,5-tri-*O*-acetyl-β-D-ribofuranosyl)adenine²¹ (**10**) (1.78 g, 94%) as a yellow foam. A solution of this material (400 mg, 0.89 mmol), pyridine (2 mL), and pivalic anhydride (1 mL) was stirred overnight at 80 °C. Volatiles were evaporated in vacuo, and the residue was chromatographed [hexanes/EtOAc (2:1) → EtOAc → MeOH/EtOAc (1:10)] to give **10a** (390 mg, 83%): ¹H NMR δ 1.39 (s, 9H), 2.09, 2.10, 2.16 (3 × s, 3 × 3H), 2.54 (br s, 3H), 4.37–4.51 (m, 3H), 5.74 (s, 1H), 5.90 (t, *J* = 5.1 Hz, 1H), 6.12 (d, *J* = 4.9 Hz, 1H), 8.04 (s, 1H), 8.48 (br s, 1H), 8.72 (br s, 1H); ¹³C NMR δ 20.0, 20.2, 20.4, 24.9, 26.9, 40.1, 62.8, 70.1, 72.7, 79.8, 86.2, 120.1, 140.7, 150.3, 151.9, 152.5, 169.1, 169.2, 170.0, 174.9; FAB-MS *m/z* 557 (100%, M + Na⁺); HRMS calcd for C₂₃H₃₀N₆O₉Na 557.1972, found 557.1967.

4-*N*,2',3',5'-Tri-*O*-tetraacetylcytidine^{8,21} (11a). Cytidine (150 mg, 0.62 mmol) was stirred with Ac₂O (1 mL, 1.08 g, 10.6 mmol) and pyridine (3 mL) for 1 h at 80 °C. Volatiles were evaporated in vacuo, and the residue was dried under vacuum at 80 °C to give **11a** (0.25 g, 100%): ¹H NMR δ 2.09, 2.11, 2.16, 2.30 (4 × s, 4 × 3H), 4.39–4.44 (m, 3H), 5.33 (t, *J* = 5.9 Hz, 1H), 5.45 (dd, *J* = 4.4, 5.5 Hz, 1H), 6.09 (d, *J* = 3.9 Hz, 1H), 7.51 (d, *J* = 7.8 Hz, 1H), 7.93 (d, *J* = 7.3 Hz, 1H), 10.26 (s, 1H); ¹³C NMR δ 20.24 (2C), 20.6, 24.6, 62.5, 69.4, 73.5, 79.6, 89.1, 97.2, 144.0, 154.6, 163.3, 169.2, 169.3, 170.0, 171.3; FAB-MS *m/z* 434 (100%, M + Na⁺); HRMS calcd for C₁₇H₂₁N₃O₉Na 434.1175, found 434.1178.

2',3',5'-Tri-*O*-acetylcytidine^{9,21} (12a). A solution of **11a** (250 mg, 0.62 mmol) in MeOH (6 mL) was heated at 105 °C for 3 h. Volatiles were evaporated, and the residue [82% (¹H NMR) of **12a** plus other compounds with loss of *O*-acetyl groups from the sugar moiety] was chromatographed [EtOAc → MeOH/EtOAc (1:5)] to give **12a** (61%): ¹H NMR δ 2.09, 2.10, 2.13 (3 × s, 3 × 3H), 4.29–4.41 (m, 3H), 5.40 (t, *J* = 5.6 Hz, 1H), 5.46 (dd, *J* = 4.9, 5.9 Hz, 1H), 5.92 (d, *J* = 4.4 Hz, 1H), 5.96 (d, *J* = 7.8 Hz, 1H), 6.49 (br s, 1H), 7.40 (d, *J* = 7.3 Hz, 1H), 8.14 (br s, 1H); ¹³C NMR δ 20.38, 20.40, 20.7, 62.9, 69.8, 73.3, 78.9, 90.1, 96.2, 140.9, 155.6, 166.2, 169.5, 169.6, 170.4; FAB-MS *m/z* 392 (100%, M + Na⁺); HRMS calcd for C₁₅H₁₉N₃O₈Na 392.1070, found 392.1086.

Acknowledgment. We gratefully acknowledge NIH Grant GM029332, pharmaceutical company gift funds (M.J.R.), and Brigham Young University for support of this research.

Supporting Information Available: Experimental procedures, spectral data, and ¹³C NMR spectra. This material is available free of charge via the Internet at <http://pubs.acs.org>.

JO051256W

(18) Buchanan, J. G.; Stobie, A.; Wightman, R. H. *Can. J. Chem.* **1980**, *58*, 2624–2627.

(19) Wiley, P. F.; Johnson, J. H.; Hanze A. R. *J. Antibiotics* **1976**, *29*, 720–727. The site of *N*-acetylation (N4) and signal integration (5.3–5.9 ppm range) were reported incorrectly for **8a**.

(20) Kozai, S.; Yorikane, A.; Maruyama, T. *Nucleosides Nucleotides Nucleic Acids* **2001**, *20*, 1523–1531.

(21) Saladino, R.; Mincione, E.; Crestini, C.; Mezzetti, M. *Tetrahedron* **1996**, *52*, 6759–6780.