Syntheses of Puromycin from Adenosine and 7-Deazapuromycin from Tubercidin, and Biological Comparisons of the 7-Aza/Deaza Pair[1]

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Protection (O5′) of 2′,3′-anhydroadenosine with *tert*-butyldiphenylsilyl chloride and epoxide opening with dimethylboron bromide gave the 3′-bromo-3′-deoxy xylo isomer which was treated with benzylisocyanate to give the 2′-*O*-(*N*-benzylcarbamoyl) derivative. Ring closure gave the oxazolidinone, and successive deprotection concluded an efficient route to 3′-amino-3′-deoxyadenosine. Analogous treatment of the antibiotic tubercidin {7-deazaadenosine; 4-amino-7-(β-D-ribofuranosyl)-pyrrolo[2,3-*d*]pyrimidine} gave 3′-amino-3′-deoxytubercidin. Trifluoroacetylation of the 3′-amino function, elaboration of the heterocyclic amino group into a (1,2,4-triazol-4-yl) ring with *N*,*N*′-bis-[(dimethylamino)methylene]hydrazine, and nucleophilic aromatic substitution with dimethylamine gave puromycin aminonucleoside [9-(3-amino-3-deoxy-β-D-ribofuranosyl)-6-(dimethylamino)purine] and its 7-deaza analogue. Aminoacylation [BOC-(4-methoxy-L-phenylalanine)] and deprotection gave puromycin and 7-deazapuromycin. Most reactions gave high yields at or below ambient temperature. Equivalent inhibition of protein biosynthesis in a rabbit reticulocyte system and parallel growth inhibition of several bacteria were observed with the 7-aza/deaza pair. Replacement of N7 in the purine ring of puromycin by "CH" has no apparent effect on biological activity.

Introduction

The isolation of puromycin,[2] 9-{3-deoxy-3-[(4-methoxy-L-phenylalanyl)amino]-β-D-ribofuranosyl}-6-(dimethylamino)purine, the classic aminonucleoside antibiotic with activity in a broad range of organisms and tumors, was reported in 1952,[3] a year after publication of the first nucleoside antibiotic cordycepin.[4] As a structural mimic of the 3′-terminus of an aminoacyl-tRNA,[5] puromycin binds at ribosomal "acceptor" sites and participates in transpeptidation with the growing peptidyl-tRNA.[6] However, after the nascent polypeptide is transferred to the α-amino group of puromycin, further elongation is thwarted. Peptidyl-puromycin is released from the polyribosome−mRNA complex, which then dissociates, and protein biosynthesis is disrupted.[7] The isolation of other aminonucleoside antibiotics and biological studies have been summarized.[8]

The novel aminosugar structure of puromycin[9] was confirmed by the synthetic studies of Baker and coworkers[10] in which 3-acetamido-1-*O*-acetyl-2,5-di-*O*-benzoyl-3-deoxy-D-ribofuranose (prepared from D-xylose) was coupled with purine derivatives to give 3′-amino-3′-deoxyadenosine[10d] (20 steps, ∼3%) and puromycin.[10c] Although several subsequent syntheses of 3-amino-3-deoxy-D-ribose derivatives have been reported,[11] all suffer from numerous steps and low overall yields (e.g., one synthesis[12] of 3′-amino-3′-deoxyadenosine starting from adenosine had 12 steps and ∼3% overall yield, and even

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the most efficient routes, with 12 steps from D-xylose, gave the aminosugar nucleoside in only 10−15%[11d,13]).

We communicated an efficient nine-stage synthesis of 3′-amino-3′-deoxyadenosine from adenosine with reactions (*average* yields >93%) performed at or below ambient temperature.[14] We now report details of that synthesis (∼56% overall), improved hydrogenolysis conditions for *N*-debenzylation, and we demonstrate the generality of this approach by transformation of the antibiotic tubercidin[15] (7-deazaadenosine) into 3′-amino-3′-deoxytubercidin (∼52% overall). We also describe the conversion of these aminonucleosides into puromycin and 7-deazapuromycin with our methodology for functionalization and displacement of heterocyclic amino groups.[16]

Although a rich array of puromycin analogues with modified amino acid,[17] carbohydrate,[18] and/or base[19] moieties has been prepared and evaluated biologically, most modifications have been too extensive to allow definitive structure−activity correlations. Our 7-deazapuromycin is the first analogue with a single change in the purine ring. The purine N7 atom is known to function as a hydrogen-bond acceptor in Hoogsteen base pairing, so it was of interest to probe whether replacement of N7 by "CH" would alter association of 7-deazapuromycin with ribosomal proteins/nucleic acids. Also, N7 is significant in binding and/or catalytic steps with certain enzymes,[20] which might result in differential interactions with enzymes that are involved with antibiotic metabolism.[8a] Because the glycosyl bond of purine nucleosides is much more labile than that of 7-deazapurine analogues, 7-deazapuromycin should be more stable than puromycin.

Results and Discussion

Treatment of adenosine (**1a**) (Scheme 1) with α-acetoxyisobutyryl bromide in moist acetonitrile gave trans 2′,3′-bromohydrin acetates that were converted into 2′,3′-anhydroadenosine (**2a**) (93% from **1a**) with Dowex 1 × 2 (OH^-) resin in methanol.[21] Protection (O5′) of **2a** with TBDPSCl and treatment of the silylated epoxide with dimethylboron bromide[22] (−78 °C) gave the 3′-bromo xylo isomer **3a** (95%). This compound was treated with benzylisocyanate to give the 2′-*O*-(*N*-benzylcarbamoyl) derivative **4a** (89%), which underwent ring closure with sodium hydride (−20 °C), and the resulting oxazolidinone was desilylated (tetrabutylammonium fluoride, TBAF) to give **5a** (82%, two steps). Treatment of **5a** with aqueous sodium hydroxide gave 3′-*N*-benzyl-3′-amino-3′-deoxyadenosine[23] (92%), which was hydrogenolyzed[24] (Pearlman's catalyst) to give 3′-amino-3′-deoxyadenosine (**6a**) (92%). This conversion of adenosine into the target antibiotic **6a** (∼56% overall for the nine-stage sequence, >93% *average* yields) was even more efficient (>60% overall) when intermediates obtained by workup were carried forward without individual purification. We now have demonstrated the generality of this methodology by an analogous sequence with tubercidin (**1b**) to give 3′-amino-3′-deoxytubercidin (**6b**) (∼52% overall).

Noteworthy features of this sequence are: (1) All reactions were conducted at or below ambient tempera-

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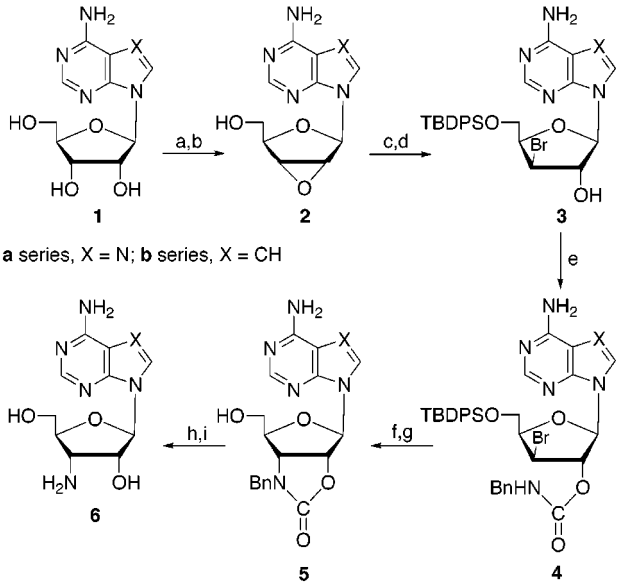
Scheme 1[a]

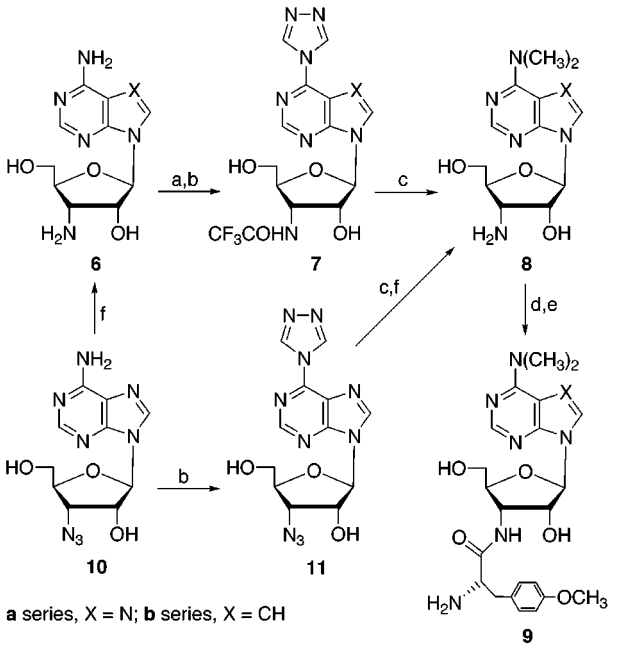


[a] (a) $Me_2C(OAc)COBr/MeCN/H_2O$. (b) Dowex 1 × 2 (OH^-)/MeOH. (c) *t*-BuSi(Ph)$_2$Cl/pyridine. (d) $Me_2BBr/Et_3N/CH_2Cl_2$/-78 °C. (e) $PhCH_2NCO/Et_3N$/THF/MeCN. (f) NaH/THF/-20 °C. (g) Bu_4-NF/THF. (h) $NaOH/H_2O$/THF. (i) $Pd(OH)_2$−C/NH_4HCO_2/MeOH/H_2O/Δ.

ture with readily available reagents and common laboratory conditions. (2) Dimethylboron bromide effected regiospecific epoxide ring opening to give the 3′-bromo xylo isomer **3** at −78 °C. (3) Conversion of the O5′ protected bromohydrin **3** into carbamate **4** with benzylisocyanate at ambient temperature occurred without a significant side reaction at the heterocyclic amino group. (4) Cyclization of bromohydrin carbamate **4** into the oxazolidinone with sodium hydride caused only minor formation of the 2′,3′-epoxide at −20 °C, whereas the epoxide was the major product at ambient temperature. The sequence also involved standard deprotection of O5′ (TBAF/THF) to give **5**, which underwent hydrolytic decarbonylation ($NaOH/H_2O$/THF). Alternative use of acylisocyanates gave analogous O2′ carbamates, and their cyclization into *N*-acyloxazolidinones also occurred readily. However, aqueous base first removed the *N*-acyl group, and the resulting oxazolidinone anion was refractory to decarbonylation. Hydrogenolysis of the benzyl−amino bond was highly sensitive to the batch of Pd−C catalyst,[14] but debenzylation has now been found to occur readily and repeatably with the Pearlman palladium hydroxide on carbon catalyst with ammonium formate[24] (hydrogen source) in MeOH/H_2O at reflux. Finally, the 3′-amino-3′-deoxynucleosides were purified by Dowex 1 × 2 (OH^-) chromatography and recrystallized to give **6a** and **6b**.

We previously described an efficient synthesis of 3′-azido-3′-deoxyadenosine[25] (**10**) (Scheme 2), which we now used to prepare puromycin aminonucleoside (**8a**). Treatment[16] of **10** with *N*,*N*′-bis[(dimethylamino)methylene]-hydrazine (BDMAMH) dihydrochloride[26] and TMSCl in pyridine gave 9-(3-azido-3-deoxy-β-D-ribofuranosyl)-6-(1,2,4-triazol-4-yl)purine (**11**) (77%), which was converted

Scheme 2[a]



[a] (a) (i) $(CF_3CO)_2O$/pyridine; (ii) Dowex 1 × 2 (OH^-)/MeOH. (b) (i) $Me_2CHNNCHNMe_2$·2HCl/Me_3SiCl/pyridine/100 °C; (ii) MeOH. (c) Me_2NH/H_2O/pyridine. (d) BOC(4-methoxy-L-phenylalanine)/DCC/*N*-hydroxysuccinimide/DMF. (e) (i) CF_3CO_2H; (ii) Dowex 1 × 2 (OH^-). (f) Ph_3P/NH_3/MeOH/pyridine.

quantitatively into 9-(3-azido-3-deoxy-β-D-ribofuranosyl)-6-(dimethylamino)purine with aqueous dimethylamine.[16] Staudinger reduction gave 9-(3-amino-3-deoxy-β-D-ribofuranosyl)-6-(dimethylamino)purine[11a] (**8a**) (95%).

An alternative route was applicable to both **6a** and **6b**. Trifluoroacetyl protection of the 3′-amino group of **6a** or **6b**, elaboration of the heterocyclic amine into the (1,2,4-triazol-4-yl) leaving group (BDMAMH·2HCl/TMSCl/pyridine), and triazole displacement/trifluoroacetyl removal (Me_2NH/H_2O) gave **8a** or **8b** (∼70% for three steps). Condensation of BOC-(4-methoxy-L-phenylalanine) with **8a** or **8b** (DCC/*N*-hydoxysuccinimide) gave the protected aminoacyl-aminonucleosides. BOC removal (TFA) from the purine intermediate (36%) completed our synthesis of puromycin (**9a**), but extensive glycosyl-bond cleavage lowered the yield. In contrast, BOC removal proceeded without difficulty with the more acid-stable 7-deaza analogue to give **9b** (78% from **8b**). No attempt was made to improve BOC removal conditions for puromycin, which served as a model for the synthesis of 7-deazapuromycin.

Inhibition of protein synthesis by puromycin (positive control) and 7-deazapuromycin was studied with a messenger-dependent mammalian translation system.[27] Messenger RNA, encoding for either pro or mature IL-1β protein, was added to rabbit reticulocyte lysates that contained all necessary translational-machinery components plus [^{35}S]methionine for radio-detection/quantitation of the synthesized IL-1β. Following incubation, translation products were denatured and separated by size with SDS polyacrylamide gel electrophoresis. No biosynthesis of IL-1β was detected at 50 μM concentrations of commercial (Sigma) puromycin, synthetic puromycin (**9a**), or 7-deazapuromycin (**9b**) with either pro or

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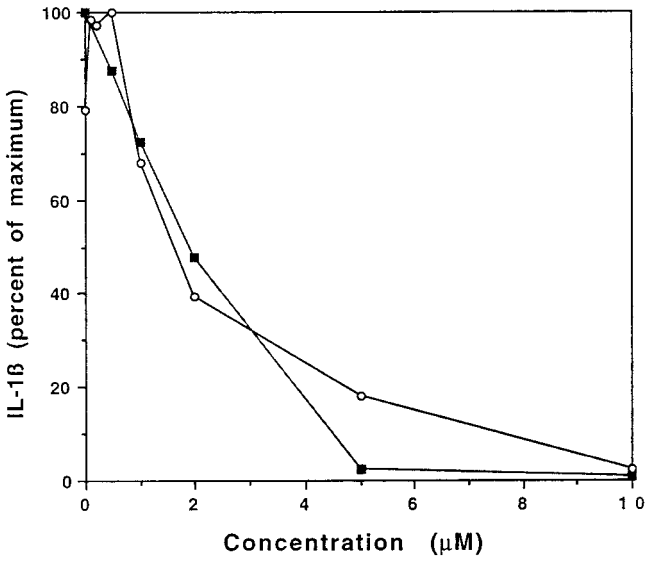

Figure 1. Inhibition of protein synthesis by puromycin or 7-deazapuromycin. Increasing concentrations of puromycin (Sigma) (○) or **9b** (■) were added to a rabbit reticulocyte translation system containing proIL-1β mRNA (see Experimental Section). After incubation (60 min), reaction mixtures were separated by SDS polyacrylamide gel electrophoresis, and the amounts of synthesized proIL-1β were quantitated (phosphorimager). Results are the percent of maximum proIL-1β from each lane.

mature IL-1β mRNAs (data not shown). Dose−response experiments (Figure 1) showed that both **9a** and **9b** inhibited protein synthesis at similar levels ($IC_{50} = 2 \pm 1$ μM) and with similar inhibition profiles.

Growth inhibition of bacterial cultures gave parallel results with puromycin (**9a**) or **9b**. The gram-positive bacteria *Bacillus cereus* NCTC 8035, *Bacillus subtilis* ATCC 6633, *Micrococcus flavus* ATCC 10240, *Micrococcus lysodeicticus* NCTC 2665, *Staphylococcus aureus*, *Sarcina lutea* ATCC 9241, and *Streptococcus faecalis* ATCC 8043 were inhibited at minimum inhibitory concentration (MIC) values of 5−10 μg/mL (data not shown). The gram-negative bacteria *Escherichia coli* NCIB 8743 and *Serratia marcescens* had MIC values of 5−40 μg/mL, whereas the gram-negative bacterium *Pseudomonas aeruginosa* and the yeasts *Candida albicans* and *Candida tropicalis* MUCL 28.180 had MIC values >40 μg/mL with both **9a** and **9b**.[28] The 7-deaza aminonucleoside **8b** was not active in any of these assays, which corresponds to the noted lack of activity of puromycin aminonucleoside against bacteria.[8a]

In summary, efficient procedures (>93% *average* yields) under mild conditions (at or below ambient temperature) have been developed for conversion of the naturally occurring ribonucleosides **1a** and **1b** into their 3′-amino-3′-deoxy analogues **6a** and **6b** (>52% overall). Elaboration of the heterocyclic amino group into a (1,2,4-triazol-4-yl) substituent provided a good leaving group for nucleophilic aromatic substitution with dimethylamine. Acylation of the 3′-amino group with BOC-(4-methoxy-L-phenylalanine) and deprotection completed our synthesis of the protein biosynthesis inhibitor puromycin (**9a**) and its 7-deaza analogue **9b**. This constitutes the first practical approach to the aminoacyl-aminonucleoside antibiotic class. The hybrid antibiotic **9b** is the first analogue that is singly modified (7-deaza) in the purine ring system. It is noteworthy that chemical properties of **9b** differ significantly from those of puromycin, but no loss of biological activity was observed. Protein biosynthesis in a rabbit reticulocyte system was inhibited almost identically by **9a** or **9b**, and MIC values for inhibition of several gram-positive and gram-negative bacteria also were parallel for the two analogues. Thus, N7 of the purine ring of puromycin must play little if any role in binding or other key interactions with ribosomal proteins or RNAs.

(28) De Clercq, E.; Robins, M. J., unpublished results.

Experimental Section

Uncorrected melting points were determined with a microstage block or a capillary tube apparatus. UV spectra were determined with solutions in MeOH unless otherwise noted. ^{1}H (200 or 400 MHz) and ^{13}C (50 MHz) NMR spectra were obtained with solutions in Me_4Si/Me_2SO-d_6 unless otherwise noted, and designations within quotation marks indicate apparent peak shapes when more complex splitting exists. Mass spectra were determined at 20 or 70 eV. TLC was performed with silica gel on aluminum plates, and chromatography was performed with silica gel columns unless otherwise noted. Reagent grade chemicals were used. Chlorotrimethylsilane (TMSCl) and all reaction solvents except toluene were distilled before use. Pyridine was dried by distillation from CaH_2. Reactions with *N*,*N*′-bis[(dimethylamino)methylene]hydrazine dihydrochloride (BDMAMH·2 HCl)[26] to elaborate triazole rings were conducted under an atmosphere of N_2 or Ar with a leak-proof Teflon sleeve on the condenser ground-glass joint. Volatile materials were flash evaporated at <35 °C under an aspirator or a mechanical oil pump vacuum. Solid products were dried in vacuo over P_4O_{10} for ≥24 h (stable products at 100−110 °C). "Diffusion crystallization" was performed as described.[29] Yields of some reactions were markedly lowered if reaction conditions were altered.

9-[3-Bromo-5-*O*-(*tert*-butyldiphenylsilyl)-3-deoxy-β-D-xylofuranosyl]adenine (3a). TBDPSCl (522 μL, 552 mg, 2.01 mmol) was added to **2a**[21c] (500 mg, 2.01 mmol) in dried pyridine (10 mL), and the solution was stirred for 12 h at ambient temperature. H_2O (1 mL) was added, stirring was continued for 30 min, and volatiles were evaporated. The residue was partitioned ($H_2O/CHCl_3$), and the organic phase was washed [(H_2O, 2 × 25 mL); $NaHCO_3/H_2O$; brine] and dried (Na_2SO_4). Volatiles were evaporated to give 9-[2,3-anhydro-5-*O*-(*tert*-butyldiphenylsilyl)-β-D-ribofuranosyl]adenine (quant, used in the next step) with ^{1}H NMR: δ 0.9 (s, 9H), 3.69 (dd, J = 6.0, 10.6 Hz, 1H), 3.94 (dd, J = 5.5, 10.6 Hz, 1H), 4.26 (d, J = 2.6 Hz, 1H), 4.35 ("t", J = 6.5 Hz, 1H), 4.48 (d, J = 2.6 Hz, 1H), 6.28 (s, 1H), 7.4 (m, 12H), 8.08 (s, 1H), 8.30 (s, 1H). ^{13}C NMR:[30] δ 58.52, 58.56, 63.8, 81.0, 82.9, 118.6, 139.6, 149.0, 152.8, 156.4.

Et_3N (8.2 μL, 6.0 mg, 0.059 mmol) and Me_2BBr/CH_2Cl_2 (0.33 M, 1.5 mL, 0.50 mmol) were added to a cold (−78 °C) solution of the protected epoxide (120 mg, 0.25 mmol) in CH_2Cl_2 (7 mL). Stirring was continued for 1 h, and the reaction mixture was poured into a stirred solution of saturated $NaHCO_3/H_2O$. The organic layer was separated, and the aqueous layer was extracted (CH_2Cl_2). The combined organic phase was washed (brine) and dried (Na_2SO_4). Evaporation of volatiles gave a white solid (136 mg, 97%) that was crystallized (THF/MeCN) to give **3a** (133 mg, 95%) with mp 210−211 °C. UV max 259 nm (ϵ 13 800), min 233 nm (ϵ 700). ^{1}H NMR: δ 1.20 (s, 9H), 4.40 (dd × 2, $J \approx 5$, 11 Hz, 2H), 4.54 ("q", $J \approx 5$ Hz, 1H), 4.62 (dd, $J \approx 4$, 5 Hz, 1H), 4.96 ("q", $J \approx 5$ Hz, 1H), 5.93 (d, $J \approx 5$ Hz, 1H), 6.41 (br d, 1H), 7.34 (s, 2H), 7.40−7.70 (m, 10H), 8.10

(29) (a) Brown, J. N.; Trefonas, L. M. *Org. Prep. Proced. Int.* **1970**, *2*, 317−319. (b) Robins, M. J.; Mengel, R.; Jones, R. A.; Fouron, Y. *J. Am. Chem. Soc.* **1976**, *98*, 8204−8213.

(30) ^{13}C NMR peaks for TBDPS and/or Ph of the benzyl group were usually within the ranges δ 18−19 (quaternary), 26−27 (CH_3), and 126−135 (Ph).

(s, 1H), 8.16 (s, 1H). ^{13}C NMR:[30] δ 53.0, 65.3, 79.80, 79.84, 88.4, 118.7, 138.1, 149.1, 152.6, 155.9. MS *m*/*z*: 510.0614 (M[^{79}Br] − *t*-Bu = 510.0597). Anal. Calcd for $C_{26}H_{30}BrN_5O_3Si$: C, 54.93; H, 5.32; N, 12.32. Found: C, 54.73; H, 5.12; N, 12.45.

9-[2-*O*-(*N*-Benzylcarbamoyl)-3-bromo-5-*O*-(*tert*-butyldiphenylsilyl)-3-deoxy-β-D-xylofuranosyl]adenine (4a). Benzylisocyanate (220 μL, 234 mg, 1.76 mmol) and Et_3N (180 μL, 133 mg, 1.32 mmol) were added to **3a** (500 mg, 0.88 mmol) in THF/MeCN (1:1, 50 mL), and the solution was stirred for 48 h at ambient temperature (further reagent was added if necessary). EtOH (1 mL) was added, and stirring was continued for 30 min. Volatiles were evaporated, and the residue was chromatographed [silica gel; EtOH/$CHCl_3$ (1:19)]. The product was recrystallized (hexanes/$CHCl_3$) to give **4a** (0.55 g, 89%) with mp 178−179 °C. UV max 259 nm (ϵ 14 700), min 233 nm (ϵ 3100). ^{1}H NMR: δ 1.00 (s, 9H), 3.98 (dd × 2, *J* = 5.2, 10.8 Hz, 2H), 4.12 and 4.16 (dd × 2, *J* = 6.4, 16.0 Hz, 2H), 4.50 ("q", *J* = 4.8 Hz, 1H), 4.88 (dd, *J* = 4.2, 5.5 Hz, 1H), 5.86 ("t", *J* = 3.8 Hz, 1H), 6.14 (d, *J* = 4.0 Hz, 1H), 7.10−7.30 (m, 18H), 8.10 (s, 1H), 8.16 (s, 1H). ^{13}C NMR:[30] δ 44.0, 49.5, 65.5, 80.3, 81.1, 86.1, 118.3, 138.4, 149.5, 153.3, 155.0, 156.1. MS (FAB) *m*/*z*: 704 (M[^{81}Br] + 1), 702 (M[^{79}Br] + 1). Anal. Calcd for $C_{34}H_{37}BrN_6O_4Si$: C, 58.20; H, 5.32; N, 11.98. Found: C, 58.01; H, 5.25; N, 12.08.

9-[3-(Benzylamino)-3-*N*,2-*O*-carbonyl-3-deoxy-β-D-ribofuranosyl)adenine (5a). NaH (50% in mineral oil; 20 mg, 0.42 mmol) under N_2 in a dried three-neck round-bottom flask was rinsed with hexane, freshly distilled THF (40 mL) was added, and the suspension was cooled to −20 °C. A solution of **4a** (250 mg, 0.36 mmol) in THF (5 mL) was added, stirring was continued for 12 h, and the mixture was filtered (with a layer of Celite). Volatiles were evaporated, and the residue was used directly in the next step. A sample of this 9-[3-(benzylamino)-5-*O*-(*tert*-butyldiphenylsilyl)-3-*N*,2-*O*-carbonyl-3-deoxy-β-D-ribofuranosyl]adenine was purified by preparative TLC (EtOH/$CHCl_3$, 1:19) to give material with UV max 259, min 233 nm. IR ($CHCl_3$ film): 1763 cm^{-1} (C═O). ^{1}H NMR: δ 0.84 (s, 9H), 3.51 (dd, *J* = 6.1, 11.1 Hz, 1H), 3.60 (dd, *J* = 4.8, 10.5 Hz, 1H), 4.35 ("q", *J* = 4.5 Hz, 1H), 4.39 (d, *J* = 15.6 Hz, 1H), 4.55 (dd, *J* = 3.6, 8.8 Hz, 1H), 4.60 (d, *J* = 15.3 Hz, 1H), 5.91 (dd, *J* = 3.3, 8.1 Hz, 1H), 6.38 (d, *J* = 2.9 Hz, 1H), 7.36 (m, 17H), 8.00 (s, 1H), 8.27 (s, 1H). ^{13}C NMR:[30] δ 46.8, 60.6, 63.9, 79.6, 85.4, 89.6, 119.3, 140.9, 149.1, 153.1, 156.2, 156.7. MS (FAB) *m*/*z*: 621 (M + 1).

The crude TBDPS product (~0.36 mmol) was dissolved in freshly distilled THF (40 mL), TBAF/THF (1 M, 360 μL, 0.36 mmol) was added, and the solution was stirred for 1 h at ambient temperature. Volatiles were evaporated, the residue was partitioned (H_2O/$CHCl_3$), and the aqueous layer was extracted (2 × $CHCl_3$). The combined organic phase was washed ($NaHCO_3$/H_2O; brine) and dried (Na_2SO_4). Volatiles were evaporated, and the residue was chromatographed (MeOH/$CHCl_3$, 1:9) and diffusion-crystallized (MeOH/Et_2O) to give **5a** (112 mg, 82% from **4a**) with mp 229−230 °C. UV max 258 nm (ϵ 14 500), min 225 nm (ϵ 3300). IR ($CHCl_3$ film): 1752 cm^{-1} (C═O). ^{1}H NMR (360 MHz): δ 3.40 ("t", *J* = 3.5 Hz, 2H), 4.26 (dd, *J* = 3.8, 7.7 Hz, 1H), 4.32 (d, *J* = 15.3 Hz, 1H), 4.36 ("d", *J* = 2.8 Hz, 1H), 4.62 (d, *J* = 15.5 Hz, 1H), 5.26 (br s, 1H), 5.70 (dd, *J* = 3.5, 8.5 Hz, 1H), 6.32 (d, *J* = 3.5 Hz, 1H), 7.35 (m, 7H), 8.12 (s, 1H), 8.34 (s, 1H). ^{13}C NMR:[30] δ 46.5, 61.1, 61.6, 79.7, 85.6, 89.8, 119.2, 140.4, 149.2, 152.3, 156.3, 156.7. MS (FAB) *m*/*z*: 383 (M + 1). Anal. Calcd for $C_{18}H_{18}N_6O_4$: C, 56.54; H, 4.75; N, 21.98. Found: C, 56.20; H, 4.54; N, 21.74.

9-(3-Amino-3-deoxy-β-D-ribofuranosyl)adenine (3′-Amino-3′-deoxyadenosine) (6a). NaOH/H_2O (1 M, 5 mL) was added to **5a** (112 mg, 0.31 mmol) in THF (5 mL), and the solution was stirred for 48 h at ambient temperature. Dowex 50 (H^+) resin was added, and the mixture was stirred for 1 h and filtered. Volatiles were evaporated, and the residue was dissolved in H_2O and chromatographed [Dowex 1 × 2 (OH^-); H_2O and MeOH/H_2O (1:1)]. The product was recrystallized ($CHCl_3$/MeOH) to give 9-[3-(benzylamino)-3-deoxy-β-D-ribofuranosyl]adenine (96 mg, 92%) with mp 175−176 °C (lit.[23] mp 171 °C). UV max 259 nm (ϵ 15 700), min 229 nm (ϵ 3900). ^{1}H NMR: δ 3.35 (m, 1H), 3.54 (m, 1H), 3.70 (m, 1H), 3.72 and 3.78 (dd × 2, *J* = 6.0, 15.0 Hz, 2H), 3.92 (dt, *J* = 3.2, 6.2 Hz, 1H), 4.57 (dd, *J* = 4.1, 5.3 Hz, 1H), 5.27 ("t", *J* ≈ 6 Hz, 1H), 5.87 (br s, 1H), 5.96 (d, *J* = 3.9 Hz, 1H), 7.32 (m, 8H), 8.12 (s, 1H), 8.35 (s, 1H). ^{13}C NMR:[30] δ 51.8, 58.9, 62.3, 73.2, 84.7, 90.5, 119.7, 140.9, 149.4, 153.4, 156.5. MS *m*/*z*: 356.1603 (M^+ = 356.1597). Anal. Calcd for: $C_{17}H_{20}N_6O_3$: C, 57.29; H, 5.66; N, 23.59. Found: C, 57.01; H, 5.69; N, 23.70.

The 3′-benzylamino compound (90 mg, 0.25 mmol), NH_4HCO_2 (107 mg, 1.7 mmol), and $Pd(OH)_2$−C (17 mg) were heated at reflux in H_2O/MeOH (1:10) for 90 min. The mixture was filtered, volatiles were evaporated, and the residue was chromatographed [Dowex 1 × 2 (OH^-); H_2O and MeOH/H_2O (1:1)]. The product was diffusion crystallized (MeOH/Et_2O) to give **6a** (62 mg, 92%) with mp 259−261 °C (lit.[10d] mp 260−261 °C dec). UV (H_2O) max 259 nm (ϵ 14 500), min 226 nm (ϵ 2100). ^{1}H NMR: δ 1.66 (br s, 2H), 3.47 (m, 1H), 3.57 (m, 1H), 3.73 (m, 2H), 4.28 (dd, *J* = 3.0, 5.3 Hz, 1H), 5.17 ("t", *J* = 4.8 Hz, 1H), 5.77 (br s, 1H), 5.94 (d, *J* = 2.7 Hz, 1H), 7.30 (s, 2H), 8.15 (s, 1H), 8.39 (s, 1H). ^{13}C NMR: δ 52.5, 61.2, 75.0, 85.7, 89.4, 119.2, 139.7, 149.0, 152.6, 156.1. MS *m*/*z*: 266.1130 (M^+ [$C_{10}H_{14}N_6O_3$] = 266.1127).

4-Amino-7-(3-amino-3-deoxy-β-D-ribofuranosyl)pyrrolo-[2,3-*d*]pyrimidine (3′-Amino-3′-deoxytubercidin) (6b). α-Acetoxyisobutyryl bromide (2.26 mL, 1.55 g, 7.42 mmol) and H_2O/Me_3CN (1:99, 7 mL) were added to a suspension of **1b** (1.0 g, 3.76 mmol) in dry Me_3CN (70 mL), and the mixture was stirred for 45 min at ambient temperature and neutralized ($NaHCO_3$/H_2O, 70 mL). The solution was extracted (2 × EtOAc), and the combined organic phase was washed (brine) and dried (Na_2SO_4). Volatiles were evaporated, and the white solid foam was dissolved (MeOH) and stirred with Dowex 1 × 2 (OH^-) (washed with MeOH) for 2 h at ambient temperature. The resin was filtered and washed (MeOH). Volatiles were evaporated to give 4-amino-7-(2,3-anhydro-β-D-ribofuranosyl)pyrrolo[2,3-*d*]pyrimidine (**2b**; quant, used in the next step) with UV max 269 nm. ^{1}H NMR: δ 3.44 (dd, *J* = 5.6, 11.0 Hz, 1H), 3.54 (dd, *J* = 6.2, 11.0 Hz, 1H), 4.10 (dd, *J* = 5.6, 6.2 Hz, 1H), 4.19 (d, *J* = 2.6 Hz, 1H), 4.27 (d, *J* = 2.6 Hz, 1H), 5.05 (br s, 1H), 6.29 (s, 1H), 6.60 (d, *J* = 3.7 Hz, 1H), 7.07 (s, 2H), 7.35 (d, *J* = 3.7 Hz, 1H), 8.08 (s, 1H). ^{13}C NMR: δ 58.2, 59.1, 61.4, 80.7, 82.1, 100.4, 103.0, 122.9, 150.2, 152.3, 157.8. MS *m*/*z*: 248 (M^+).

Treatment of **2b** with TBDPSCl (1.04 mL, 1.10 g, 4.00 mmol) in dry pyridine (20 mL) (as described for **2a**) gave a dark-green oil that was chromatographed [EtOH/$CHCl_3$ (1:19)] to give 4-amino-7-[2,3-anhydro-5-*O*-(*tert*-butyldiphenylsilyl)-β-D-ribofuranosyl)pyrrolo[2,3-*d*]pyrimidine as a white foam (1.64 g, 90%, used in the next step) with UV max 267 nm. ^{1}H NMR: δ 0.97 (s, 9H), 3.64 (dd, *J* = 6.2, 10.6 Hz, 1H), 3.83 (dd, *J* = 6.2, 10.6 Hz, 1H), 4.25 (m, 2H), 4.35 (d, *J* = 2.4 Hz, 1H), 6.28 (s, 1H), 6.51 (d, *J* = 3.6 Hz, 1H), 7.06 (br s, 2H), 7.14 (d, *J* = 3.6 Hz, 1H), 7.27−7.62 (m, 10H), 8.03 (s, 1H). ^{13}C NMR:[30] δ 57.8, 58.6, 63.9, 80.0, 82.5, 100.6, 115.7, 122.1, 150.1, 152.2, 157.8. MS *m*/*z*: 486 (M^+).

Et_3N (0.084 mL, 0.061 g, 0.6 mmol) and Me_2BBr (0.49 mL, 0.6 g, 5 mmol) were added to a cold (−78 °C) solution of the protected epoxide (1.22 g, 2.5 mmol) in dry CH_2Cl_2 (150 mL). The solution was stirred for 3 h at −78 °C, overnight at −15 °C, and was then poured into stirred $NaHCO_3$/H_2O. The organic layer was separated, and the aqueous layer was extracted (CH_2Cl_2). The combined organic phase was washed (brine) and dried (Na_2SO_4). Volatiles were evaporated, and the pale-green solid was chromatographed [EtOH/$CHCl_3$ (1:9)] and recrystallized (Me_2CO/CH_2Cl_2) to give 4-amino-7-[3-bromo-5-*O*-(*tert*-butyldiphenylsilyl)-3-deoxy-β-D-xylofuranosyl]pyrrolo-[2,3-*d*]pyrimidine (**3b**) (1.2 g, 84%) with mp ≈ 129 °C. UV max 266 nm. ^{1}H NMR: δ 1.05 (s, 9H), 3.95 ("d", *J* ≈ 5.0 Hz, 2H), 4.42 ("q", *J* ≈ 5 Hz, 1H), 4.60 (dd, *J* ≈ 4, 5 Hz, 1H), 4.71 ("q", *J* ≈ 4 Hz, 1H), 6.12 (d, *J* ≈ 4 Hz, 1H), 6.38 (d, *J* ≈ 5 Hz, 1H), 7.01 (br s, 2H), 6.60 (d, *J* = 3.7 Hz, 1H), 7.24 (d, *J* = 3.7 Hz, 1H), 7.35−7.75 (m, 10H), 8.10 (s, 1H). ^{13}C NMR:[30] δ 55.0, 65.5, 78.8, 81.5, 88.2, 100.6, 102.8, 121.3, 150.7, 152.3, 157.8. MS *m*/*z*: 510 (M[^{81}Br] − *t*-Bu + 1).

Treatment of **3b** (500 mg, 0.88 mmol) in THF/MeCN (1:1, 100 mL) with Et_3N (184 μL, 134 mg, 1.32 mmol) and benzyl-

isocyanate (217 μL, 234 mg, 1.6 mmol) (as described for **3a**) gave 4-amino-7-[2-*O*-(*N*-benzylcarbamoyl)-3-bromo-5-*O*-(*tert*-butyldiphenylsilyl)-3-deoxy-β-D-xylofuranosyl]pyrrolo[2,3-*d*]pyrimidine (**4b**) as an off-white foam (520 mg, 84%, used in the next step) with UV max 266 nm. ^{1}H NMR: δ 1.00 (s, 9H), 3.93 ("d", $J \approx 5$ Hz, 2H), 4.12 and 4.17 (dd × 2, $J \approx 6$, 16 Hz, 2H), 4.39 ("q", $J \approx 5$ Hz, 1H), 4.85 (dd, $J \approx 4$, 5 Hz, 1H), 5.64 ("t", $J \approx 4$ Hz, 1H), 6.32 (d, $J \approx 4$ Hz, 1H), 6.60 (d, $J \approx 3$ Hz, 1H), 7.05−7.35 (m, 19H), 8.10 (s, 1H). ^{13}C NMR:[30] δ 44.0, 51.3, 65.3, 79.2, 82.2, 85.7, 101.1, 102.7, 121.1, 150.5, 152.2, 155.1, 157.7. MS *m*/*z*: 562 (M − *t*-Bu − Br).

Treatment of dried (in vacuo over P_4O_{10}, 40 °C, 48 h) **4b** (500 mg, 0.71 mmol) with NaH (34.3 mg, 1.43 mmol) in cold (−20 °C) THF (15 mL) (as described for **4a**) gave 4-amino-7-[3-(benzylamino)-5-*O*-(*tert*-butyldiphenylsilyl)-3-*N*,2-*O*-carbonyl-3-deoxy-β-D-ribofuranosyl]pyrrolo[2,3-*d*]pyrimidine as a white foam (430 mg, 97%, used in the next step) with UV max 268 nm. ^{1}H NMR: δ 0.85 (s, 9H), 3.52 (dd, $J \approx 5$, 11 Hz, 1H), 3.62 (dd, $J \approx 5$, 11 Hz, 1H), 4.29 ("q", $J \approx 5$ Hz, 1H), 4.35 (d, $J \approx 15$ Hz, 1H), 4.42 (dd, $J \approx 5$, 9 Hz, 1H), 4.61 (d, $J \approx 15$ Hz, 1H), 5.68 (dd, $J \approx 4$, 9 Hz, 1H), 6.33 (d, $J \approx 4$ Hz, 1H), 6.60 (d, $J = 3.5$ Hz, 1H), 7.05−7.25 (m, 18H), 8.00 (s, 1H). ^{13}C NMR:[30] δ 46.5, 59.8, 63.9, 79.3, 84.0, 89.2, 100.9, 103.2, 122.5, 150.1, 152.3, 156.6, 159.8. MS *m*/*z*: 619 (M^+).

Treatment of a solution of the protected oxazolidinone (0.40 g, 0.65 mmol) in freshly distilled THF (20 mL) with TBAF/THF (1M, 710 μL, 0.71 mmol) and purification (as described for **5a**) gave 4-amino-7-[3-(benzylamino)-3-*N*,2-*O*-carbonyl-3-deoxy-β-D-ribofuranosyl]pyrrolo[2,3-*d*]pyrimidine (**5b**) as a white foam (229 mg, 93%, used in the next step) with UV max 267 nm. ^{1}H NMR: δ 3.44 ("t", $J \approx 5$ Hz, 2H), 4.17 ("q", $J \approx 5$ Hz, 1H), 4.30 (dd, $J \approx 5$, 9 Hz, 1H), 4.33 and 4.62 (d × 2, $J \approx 15$ Hz, 2H), 5.26 (t, $J \approx 5$ Hz, 1H), 5.49 (dd, $J \approx 4$, 9 Hz, 1H), 6.28 (d, $J \approx 4$ Hz, 1H), 6.62 (d, $J = 3.5$ Hz, 1H), 7.18 (s, 2H), 7.30−7.45 (m, 6H), 8.05 (s, 1H). ^{13}C NMR:[30] δ 46.2, 60.6, 61.7, 79.2, 83.4, 84.4, 100.7, 103.2, 122.6, 150.1, 152.3, 156.6, 157.9. MS *m*/*z*: 381 (M^+).

NaOH/H_2O (1 M, 20 mL) was added to **5b** (280 mg, 0.73 mmol) in THF (20 mL), and the solution was stirred for 48 h at ambient temperature and was then neutralized (1 M HCl/H_2O). Volatiles were evaporated, and the residue was chromatographed [EtOH/$CHCl_3$ (1:9)] to give 4-amino-7-[3-(benzylamino)-3-deoxy-β-D-ribofuranosyl]pyrrolo[2,3-*d*]pyrimidine as a white foam (0.26 g, quant, used in the next step) with UV max 269 nm. ^{1}H NMR: δ 2.17 (br s, 1H), 3.26−3.29 (m, 1H), 3.36−3.39 (m, 1H), 3.66−3.67 (m, 1H), 3.72 and 3.77 (d × 2, $J \approx 13$ Hz, 2H), 3.84 (td, $J \approx 4$, 6 Hz, 1H), 4.39 (dd, $J \approx 4$, 5 Hz, 1H), 5.18 (t, $J \approx 5$ Hz, 1H), 5.82 (br s, 1H), 6.07 (d, $J \approx 5$ Hz, 1H), 6.55 (d, $J = 3.5$ Hz, 1H), 7.05 (br s, 2H), 7.20−7.40 (m, 6H), 8.25 (s, 1H). ^{13}C NMR:[30] δ 51.4, 59.3, 62.2, 72.8, 83.6, 89.3, 100.7, 103.2, 122.2, 149.8, 151.8, 157.8. MS *m*/*z*: 355 (M^+).

Hydrogenolysis of the 3′-benzylamino compound (260 mg, 0.73 mmol) in refluxing H_2O/MeOH (1:10) with NH_4HCO_2 (312 mg, 4.9 mmol) and $Pd(OH)_2$−C (42 mg) (as described for **6a**) and diffusion crystallization (MeOH/Et_2O) gave 3′-amino-3′-deoxytubercidin (**6b**) (175 mg, 90%) with mp 217−218 °C. UV max 271 nm. ^{1}H NMR: δ 2.61 (br s, 2H), 3.30−3.40 (m, 1H), 3.52−3.54 (m, 1H), 3.62−3.70 (m, 2H), 4.09−4.10 (m, 1H), 5.05 (t, $J \approx 5$ Hz, 1H), 5.62 (br s, 1H), 6.04 (d, $J \approx 4$ Hz, 1H), 6.56 (d, $J = 3.5$ Hz, 1H), 7.01 (br s, 2H), 7.35 (d, $J = 3.5$ Hz, 1H), 8.05 (s, 1H). ^{13}C NMR: δ 53.3, 61.7, 75.3, 85.2, 89.0, 99.7, 103.1, 122.1, 149.8, 151.9, 157.8. MS *m*/*z*: 265 (M^+). Anal. Calcd for $C_{11}H_{15}N_5O_3$ (265.3): C, 49.81; H, 5.70; N, 26.40. Found: C, 49.76; H, 5.81; N, 26.20.

9-(3-Azido-3-deoxy-β-D-ribofuranosyl)-6-(1,2,4-triazol-4-yl)purine (11). TMSCl (0.07 mL, 60 mg, 0.6 mmol) was added to **10** (43.7 mg, 0.150 mmol) and BDMAMH·2 HCl (112 mg, 0.522 mmol) in pyridine (1 mL) and the suspension was stirred for 24 h at 100 °C. The mixture was cooled to ambient temperature, TMSCl (0.06 mL) was added, and stirring was continued for 15 min. Volatiles were evaporated, the residue was partitioned {5 °C; CH_2Cl_2 (12 mL)/[brine (2 mL) + saturated $NaHCO_3/H_2O$ (1 mL)]}, and the organic layer was washed {5 °C; [brine (4 mL) + 2 M HCl/H_2O (2 mL)]} and filtered through a thin layer of Na_2SO_4. Volatiles were evaporated, the residue was stirred in MeOH (3 mL) for 4 days at ambient temperature, and volatiles were evaporated. The residue was suspended in cold MeOH (1 mL), filtered, washed (cold MeOH), and dried to give **11**·0.5 H_2O (40.4 mg, 77%) with mp 192−193 °C dec. UV max 275 nm (ε 14 000), min 236 nm (ε 3500). ^{1}H NMR: δ 3.58−3.82 (m, $J = 12.2$ Hz, 2H), 4.09 (ddd, $J = 3.5$, 3.7, 4.5 Hz, 1H), 4.35 (dd, $J = 4.5$, 5.3 Hz, 1H), 5.03 (ddd, $J = 5.3$ Hz, 1H), 5.31 (t, $J = 5.5$ Hz, 1H), 6.13 (d, $J = 4.9$ Hz, 1H), 6.41 (d, $J = 5.3$ Hz, 1H), 8.98 (s, 1H), 9.06 (s, 1H), 9.66 (s, 2H). ^{13}C NMR: δ 61.0, 61.4, 74.6, 83.1, 88.3, 122.9, 141.3, 142.9, 146.0, 152.3, 153.6. MS *m*/*z*: 345 ($M^+ + 1$). Anal. Calcd for $C_{12}H_{12}N_{10}O_3$·0.5 H_2O (353.3): C, 40.80; H, 3.71; N, 39.65. Found: C, 40.90; H, 3.79; N, 39.59.

9-(3-Amino-3-deoxy-β-D-ribofuranosyl)-6-(dimethylamino)purine (8a). Method A. A solution of **11** (51.7 mg, 0.15 mmol) in pyridine (4 mL) was treated with 40% $HNMe_2/H_2O$ (4 mL) and stirred for 40 min at ambient temperature. Volatiles were evaporated, and EtOAc was added and evaporated. The water-insoluble residue was chromatographed [Dowex 1 × 2 (OH^-); MeCN/H_2O (1:1) → MeOH/H_2O (1:1) → MeOH], product fractions were evaporated (cold), and the resulting white needles were dried at ambient temperature to give 9-(3-azido-3-deoxy-β-D-ribofuranosyl)-6-(dimethylamino)purine (47.6 mg, quant, used in the next step) with mp 199−201 °C dec. UV max 275 nm (ε 19 100), min 235 nm (ε 2100). ^{1}H NMR: δ 3.3−3.8 (m, $J = 12.4$ Hz, 8H), 3.99 (ddd, $J = 3.3$, 3.3, 3.7 Hz, 1H), 4.31 (dd, $J = 3.7$, 5.2 Hz, 1H), 4.99 ("t", $J = 5.8$ Hz, 1H), 5.58 (dd, $J = 4.7$, 6.8 Hz, 1H), 5.93 (d, $J = 6.0$ Hz, 1H), 6.26 (br s, 1H), 8.23 (s, 1H), 8.39 (s, 1H). ^{13}C NMR:[31] δ 61.6, 62.2, 74.0, 83.1, 88.0, 120.0, 138.8, 149.9, 152.0, 154.5. MS *m*/*z*: 320 (M^+). Anal. Calcd for $C_{12}H_{16}N_8O_3$ (320.3): C, 45.00; H, 5.04; N, 34.98. Found: C, 45.15; H, 5.14; N, 35.12.

NH_3/MeOH (1.5 mL) was added to this material (30.7 mg, 0.096 mmol) and Ph_3P (77.7 mg, 0.296 mmol) in pyridine (1.5 mL). The solution was stirred for 12 h at ambient temperature, and volatiles were evaporated. The residue was partitioned [Et_2O (30 mL)/H_2O (10 mL)], and the aqueous layer was concentrated and chromatographed [Dowex 1 × 2 (OH^-); H_2O → MeOH/H_2O (1:1) → MeOH]. Volatiles were evaporated (cold), and MeCN/MeOH (1:1) was added and evaporated (cold, 2×). The glistening white solid was dried to give **8a** (26.7 mg, 95%) with mp 213−215.5 °C (lit.[11a] mp 215−216 °C). UV max 275 nm (ε 18 700), min 235 nm (ε 2000). ^{1}H NMR:[32] δ 1.61 (br s, 2H), 3.3−3.7 (m, 8H), 3.7−3.8 (m, 2H), 4.23 (m, 1H), 5.12−5.17 (m, 1H), 5.79 (br s, 1H), 5.95 (d, $J = 2.6$ Hz, 1H), 8.22 (s, 1H), 8.42 (s, 1H). ^{13}C NMR:[31] δ 52.3, 60.9, 75.0, 85.5, 89.3, 119.8, 138.2, 149.7, 152.0, 154.5. MS *m*/*z*: 294 (M^+ [$C_{12}H_{18}N_6O_3$]) and fragmentations as reported.[33]

Method B. Trifluoroacetic anhydride (0.08 mL, 120 mg, 0.6 mmol) was added to a cold (0 °C) suspension of **6a** (51.9 mg, 0.195 mmol) in pyridine (2 mL). The mixture was stirred for 2 days at 5 °C, and volatiles were evaporated. MeOH was added and evaporated, and the residue was chromatographed [Dowex 1 × 2 (OAc^-); MeOH]. The purified solid was dissolved in pyridine (0.5 mL), BDMAMH·2 HCl (135 mg, 0.63 mmol) and TMSCl (0.20 mL, 170 mg, 1.6 mmol) were added, and the mixture was stirred for 31 h at 100 °C. TMSCl (3 drops) was added to the cooled reaction mixture, stirring was continued for 10 min at ambient temperature, and volatiles were evaporated. The residue was dissolved in ice-cold CH_2Cl_2 (15 mL), and the solution was washed {(5 °C) [brine (4 mL) + saturated $NaHCO_3/H_2O$ (2 mL)] and then [brine (4 mL) + 2 M HCl/H_2O (2 mL)]} and filtered through a layer of Na_2SO_4.

(31) Peaks for the 6-(dimethylamino) and/or benzylic carbon atoms were overlapped by solvent (Me_2SO_4-d_6) peaks[32d] (verified by HETCOR with **9a**).

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Volatiles were evaporated, and the residue was stirred in MeOH (1 mL) for 4 days at ambient temperature. The solid was filtered and washed (MeOH; Et_2O) to give **7a** (59.4 mg, 74%). A solution of **7a** (38 mg, 0.092 mmol) in pyridine (1 mL) was treated with 40% $HNMe_2/H_2O$ (1 mL) and stirred for 19 h at ambient temperature. Volatiles were evaporated, and the residue was chromatographed [Dowex 1 × 2 (OH^-); H_2O → MeOH/H_2O (1:1) → MeOH]. Product fractions were evaporated, and MeCN/MeOH (1:1) was added and evaporated. The glistening white solid was dried to give **8a** (25.8 mg, 96% from **7a**; 71% from **6a**) with mp 213−215 °C.

Puromycin (9a). DCC (73 mg, 0.354 mmol) was added to a cold (0 °C) solution of **8a** (99.5 mg, 0.338 mmol), BOC-(4-methoxy-L-phenylalanine) (103 mg, 0.35 mmol), and *N*-hydroxysuccinimide (41.3 mg, 0.359 mmol) in dried DMF (4 mL). The solution was stirred for 30 min in an ice-water bath and then for 25 h at ambient temperature. Dicyclohexylurea was filtered and washed (EtOAc, 17 mL), and the combined filtrate was evaporated. The residue was suspended in EtOAc (4 mL), sonicated, and the mixture was filtered. The white solid was washed (EtOAc) and dried to give the product with a trace of dicyclohexylurea contamination (178.5 mg, 92%). A sample of this material (143 mg, 0.25 mmol) was chromatographed [$CHCl_3$ → MeOH/$CHCl_3$ (1:99) → MeOH/$CHCl_3$ (2:98) → MeOH/$CHCl_3$ (4:96)]. Homogeneous product fractions were evaporated, and the solid was dried to give BOC-puromycin (121 mg, 62%, 85% recovery). A sample of this material (118 mg, 0.206 mmol) was stirred with TFA (1 mL) for 8 min under Ar at ambient temperature, volatiles were evaporated in vacuo, and MeCN was added and evaporated. The residue was dissolved in cold (4 °C) MeOH (10 mL) and stirred with Dowex 1 × 2 (OH^-) resin (4−6 mL, washed with MeOH). The resin was filtered and washed (MeOH), and the combined filtrate was evaporated. The residue was chromatographed [MeOH/CH_2Cl_2 (2:98)] to remove glycosyl cleavage byproducts. Evaporation of product fractions and drying gave **9a** (34.8 mg, 36% deprotection yield) with mp 175−179 °C dec (lit.[9a] 175.5−177 °C). UV max 275 nm (ϵ 20 400), min 240 nm (ϵ 3000). ^{1}H NMR:[32] δ 1.73 (br s, 2H), 2.5−2.6 (m, 1H), 2.91 (dd, J = 4.9, 13.4 Hz, 1H), 3.35−3.62 (m, 8H), 3.62−3.78 (m, 4H), 3.9−4.0 (m, 1H), 4.4−4.5 (m, 2H), 5.15 (t, J = 5.4 Hz, 1H), 5.98 (d, J = 1.8 Hz, 1H), 6.15 (d, J = 4.3 Hz, 1H), 6.84 (d, J = 8.5 Hz, 2H), 7.15 (d, J = 8.5 Hz, 2H), 8.05 (br s, 1H), 8.24 (s, 1H), 8.45 (s, 1H); peaks at δ 1.73, 5.15, 6.15, and 8.05 exchanged with D_2O. ^{13}C NMR:[31] δ 50.1, 55.2, 56.3, 61.0, 73.3, 83.6, 89.7, 113.9, 119.8, 130.5, 130.6, 138.2, 149.8, 152.1, 154.5, 158.0, 175.2, 177.8. MS *m*/*z*: 471 (M^+) and fragmentations as reported.[33] Anal. Calcd for $C_{22}H_{29}N_7O_5$ (471.5): C, 56.04; H, 6.20; N, 20.79. Found: C, 55.87; H, 6.12; N, 20.91.

7-(3-Amino-3-deoxy-β-D-ribofuranosyl)-4-(dimethylamino)pyrrolo[2,3-*d*]pyrimidine (8b). Trifluoroacetic anhydride (0.11 mL, 163 mg, 0.78 mmol) was added to an ice-cold suspension of **6b** (99.5 mg, 0.375 mmol) in pyridine (1 mL), and the mixture was stirred for 48 h at 5 °C under Ar. Volatiles were evaporated, the residue was stirred in MeOH (0.5 mL) for 10 min, this solution was chromatographed [Dowex 1 × 2 (OAc^-); MeOH], and the residue was dried (16 h). This material, BDMAMH·2 HCl (242 mg, 1.12 mmol), and TMSCl (0.38 mL, 3 mmol) were added to pyridine (0.8 mL), and the mixture was stirred for 24 h at 100 °C. Volatiles were evaporated, and the residue was partitioned [5 °C; H_2O (3 mL)/CH_2Cl_2 (15 mL)]. The organic phase was filtered through a thin layer of Na_2SO_4 and evaporated. The residue was stirred in MeOH (6 mL) for 19 h, volatiles were evaporated, and the residue was stirred in 40% ($HNMe_2/H_2O$)/pyridine (1:1, 12 mL) for 3 days at ambient temperature. Volatiles were evaporated, and the residue was chromatographed [Dowex 1 × 2 (OH^-); H_2O (50 mL) → MeOH/H_2O (1:1, 100 mL)] to give recovered **6b** (5.4 mg, 5%) and **8b** (76 mg, 69%) with mp 184.5−187 °C. UV max 283 nm (ϵ 15 300), min 246 nm (ϵ 2600). ^{1}H NMR: δ 1.60 (br s, 1H), 3.2−3.8 (m, 4H), 3.29 (s, 6H), 3.5−3.8 (m, 3H), 4.0−4.1 (m, 1H), 5.0−5.1 (m, 1H), 5.5−5.7 (m, 1H), 6.13 (d, J = 2.6 Hz, 1H), 6.70 (d, J = 3.7 Hz, 1H), 7.45 (d, J = 3.7 Hz, 1H), 8.13 (s, 1H). ^{13}C NMR:[31] δ 52.9, 61.4, 75.3, 84.9, 88.8, 102.5, 103.1, 121.4, 150.3, 151.0, 157.1. MS *m*/*z*: 293 (M^+). Anal. Calcd for $C_{13}H_{19}N_5O_3$ (293.3): C, 53.23; H, 6.53; N, 23.88. Found: C, 53.01; H, 6.49; N, 23.85.

7-{3-Deoxy-3-[(4-methoxy-L-phenylalanyl)amino]-β-D-ribofuranosyl}-4-(dimethylamino)pyrrolo[2,3-*d*]pyrimidine (7-Deazapuromycin) (9b). DCC (35.8 mg, 0.173 mmol) was added to a cold (0 °C) solution of **8b** (48.3 mg, 0.165 mmol), BOC-(4-methoxy-L-phenylalanine) (51.1 mg, 0.173 mmol), and *N*-hydroxysuccinimide (19.7 mg, 0.171 mmol) in dried DMF (2 mL). The solution was stirred for 30 min in an ice−water bath and then for 20 h at ambient temperature. Dicyclohexylurea was filtered and washed (EtOAc). The combined filtrate was evaporated, and the residue was dried (20 h) and chromatographed [$CHCl_3$ (~300 mL) → MeOH/$CHCl_3$ (1:99, ~100 mL) → MeOH/$CHCl_3$ (2:98, ~200 mL)]. Product fractions were evaporated, and the residue was stirred with TFA (0.8 mL) for 8 min at ambient temperature under Ar. Volatiles were evaporated in vacuo, and MeCN was added and evaporated. The residue was dissolved in cold (4 °C) MeOH (4 mL) and stirred with Dowex 1 × 2 (OH^-) (4 mL, washed with MeOH). The resin was filtered and washed (MeOH), and the combined filtrate was evaporated (cold) to give **9b**·H_2O (63 mg, 78%) with UV max 283 nm (ϵ 16 700), min 246 nm (ϵ 2550). ^{1}H NMR: δ 1.69 (br s, 2H), 2.45−2.60 (m, 1H), 2.92 (dd, J = 4.6, 13.5 Hz, 1H), 3.30 (s, 6H), 3.38−3.56 (m, 3H), 3.58−3.66 (m, 1H), 3.82−3.93 (m, 1H), 4.26−4.44 (m, 2H), 5.05 (t, J = 5.3 Hz, 1H), 6.00 (d, J = 4.4 Hz, 1H), 6.15 (d, J = 2.5 Hz, 1H), 6.74 (d, J = 3.8 Hz, 1H), 6.84 (d, J = 8.4 Hz, 2H), 7.15 (d, J = 8.4 Hz, 2H), 7.48 (d, J = 3.8 Hz, 1H), 8.06 (br s, 1H), 8.15 (s, 1H); peaks at δ 1.69, 5.05, 6.00, and 8.06 exchanged with D_2O. ^{13}C NMR:[31] δ 50.5, 55.2, 56.3, 61.4, 73.6, 83.0, 89.1, 102.8, 103.1, 113.9, 121.3, 130.5, 130.6, 150.4, 151.1, 157.2, 158.0, 175.2. MS *m*/*z*: 470 (M^+). Anal. Calcd for $C_{23}H_{30}N_6O_5$·H_2O (488.5): C, 56.55; H, 6.60; N, 17.20. Found: C, 56.71; H, 6.78; N, 17.50.

In Vitro Transcription and Translation of Mature or ProIL-1β. Plasmids (pHST215 or pHST202) containing a mature or pro interleukin-1β (IL-1β) cDNA insert with bacteriophage SP6 upstream promoter were transcribed in vitro as previously described.[27] The resulting mRNAs were translated in a nuclease-treated mRNA-dependent rabbit reticulocyte lysate (Promega) with [^{35}S]-methionine (Dupont-NEN) in the presence of commercial puromycin (Sigma), **9a**, or **9b**. RNasin (Promega) was added to each reaction mixture (4 units each) to minimize RNase activity. After incubation (60 min, 30 °C), translation products were boiled (3 min) in an SDS loading solution containing 2-mercaptoethanol (5%, Fisher Scientific) and separated on an SDS polyacrylamide gel (5−20% gradient) according to the manufacturer's instructions (BioRad). To ensure equal loading in each lane of the translation extracts, separated proteins were stained with Coomassie Blue R-250 by standard procedures.[34] No differences in protein content were observed, which indicated that differences in the newly synthesized IL-1β did not result from protein degradation or unequal loading. Translation products were detected by autoradiography of dried gels and were quantitated with a phosphorimager (Molecular Dynamics).

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