Sugar-Modified Conjugated Diene Analogues of Adenosine and Uridine: Synthesis, Interaction with *S*-Adenosyl-L-homocysteine Hydrolase, and Antiviral and Cytostatic Effects

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Moffatt oxidation of 2′,3′-*O*-isopropylideneuridine (**1a**) and treatment of the crude 5′-aldehyde with formylmethylene-stabilized Wittig reagent gave the vinylogously extended 7′-aldehyde **2a**. Condensation of **2a** with ethoxycarbonyl- or dibromomethylene phosphorane reagents gave the conjugated dienes **6a** and **4a**, respectively. Deacetonization gave diene ester **7a** [5′(*E*),7′(*E*); with s-trans conformation] and dibromodiene **5a** [5′(*E*)], respectively. Analogously, 2′,3′-*O*-isopropylideneadenosine (**1b**) was Wittig-extended into the conjugated dibromodiene **5b** [5′(*E*)] and dienoic ester **7b** [5′(*E*),7′(*E*)]. Furthermore, palladium-catalyzed coupling of the vinyl 6′(*E*)-stannanes **14** with (*E*) and (*Z*) ethyl 3-iodoacrylate gave stereodefined access to dienoic esters **7** (*E*,*E*) and **16** (*E*,*Z*). Incubation of AdoHcy hydrolase with 100 μM of **5b** resulted in partial inhibition of the enzyme without any apparent change in the enzyme's nicotinamide adenine dinucleoside (NAD^+) content. In contrast, **7b** and **16b** produced time- and concentration-dependent inactivation of *S*-adenosyl-L-homocysteine (AdoHcy) hydrolase producing significant decreases in the enzyme's NAD^+ content. However, **7b** and **16b** upon incubation with AdoHcy hydrolase were not metabolized suggesting that these compounds are type I mechanism-based inhibitors. No specific antiviral activity was noted for **5a**,**b**, **7a**,**b**, and **16a**,**b** against any of the viruses tested; dibromodiene **5b** proved cytotoxic at a concentration ≥6.7 μM and cytostatic at ≥11 μM, while dienoic esters **16a**,**b** showed activity against both varicella-zoster virus (at 10 μM, **16a**) and cytomegalovirus (at 10 μM, **16a**; 18 μM, **16b**).

Introduction

The enzyme *S*-adenosyl-L-homocysteine (AdoHcy) hydrolase (EC 3.3.1.1) effects hydrolytic cleavage of AdoHcy to adenosine (Ado) and L-homocysteine (Hcy).[1] The cellular levels of AdoHcy and Hcy are critical since AdoHcy is a potent feedback inhibitor of crucial transmethylation enzymes,[1,2] and elevated plasma levels of Hcy in humans have been shown to be a risk factor in coronary artery disease.[3] A number of inhibitors that function as substrates for the "3′-oxidative activity" of AdoHcy hydrolase and convert the enzyme from its active form [nicotinamide adenine dinucleotide (NAD^+)] to its inactive form [reduced nicotinamide adenine dinucleotide (NADH), type I inhibition] have been prepared.[1] Inhibitors that function as substrates for the "5′/6′-hydrolytic activity" include the vinyl fluorides [9-(5-deoxy-5-fluoro-β-D-*erythro*-pent-4-enofuranosyl)adenine],[4] the homovinyl halides[5] **A** (Figure 1), and the oxime derivatives of adenosine 5′-aldehyde[6] among others, and they have been reviewed.[1]

The geminal (dihalohomovinyl)adenosines **B** were designed as putative new substrates for the hydrolytic activity of AdoHcy hydrolase.[7] Addition of an enzyme-sequestered water molecule across the 5′,6′-double bond of **B** (Y = F), followed by loss of bromide, was proposed to generate the homoAdo 6′-carboxyl fluoride at the active site of AdoHcy hydrolase.[7b] Nucleophilic attack by proximal amino acid functionalities caused type II (covalent binding) inhibition. Addition of water across the 5′,6′-triple bond of haloacetylenes[8] **C** followed by tautomerization of the hydroxyvinyl intermediates was postulated to generate similar reactive electrophiles at the enzyme active site.[8,9] The doubly homologated vinyl halides **D** and its acetylenic analogue with greater conformational flexibility at C5′ relative to vinylogous **B** or acetylenic **C** derivatives were not substrate for the hydrolytic activity of the AdoHcy hydrolase.[10]

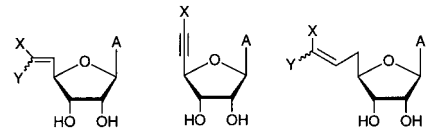

Figure 1.

Covalent inhibition of AdoHcy hydrolase with 5′-deoxy-5′-difluoromethylthioadenosine by the electrophilic entity (thioformyl fluoride) liberated from the substrate has been reported by Guillerm and coworkers.[11a,b] They also developed a new series of the 5′-thioadenosine analogues substituted at sulfur with

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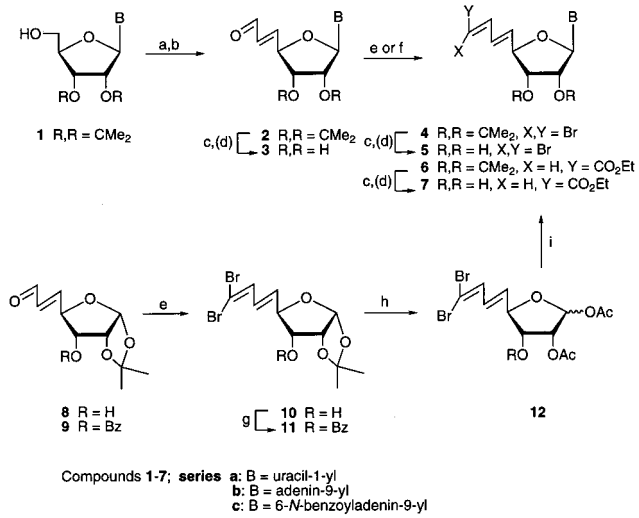
Scheme 1[a]



[a] Reagents: (a) DCC/DMSO/Cl_2CHCO_2H. (b) $Ph_3P{=}CHCHO$/CH_2Cl_2. (c) TFA/H_2O. (d) NH_3/MeOH. (e) $CBr_4/PPh_3/(Zn)CH_2Cl_2$. (f) $Ph_3P{=}CHCO_2Et/CH_2Cl_2$. (g) BzCl/pyridine. (h) (i) TFA/H_2O; (ii) Ac_2O/pyridine/DMAP. (i) Adenine/$SnCl_4/CH_3CN$.

allenyl and propynyl groups that are processed by hydrolytic activity of the enzyme causing its irreversible inactivation.[11c] The X-ray crystal structures of AdoHcy hydrolase revealed an unusual dual role for a catalytic water molecule at the active site.[12]

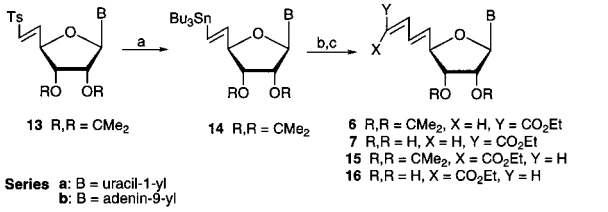
We now describe the syntheses of the first dibromodienes and dienoic esters of the uracil and adenine nucleosides; their antiviral, cytotoxic, and cytostatic activities; and the interaction of adenine analogues with AdoHcy hydrolase. The conjugated dienes derived from adenosine were designed as putative substrates of the hydrolytic activity of AdoHcy hydrolase. These derivatives with extended conjugation to the furanosyl ring should provide different stereochemical and conformational attributes for proper binding. Moreover, dienoic esters having a carboxylate group at C9′ might approach the binding site for the carboxylate function of AdoHcy hydrolase (C10′).

Chemistry

Moffatt oxidation[13] of 2′,3′-*O*-isopropylideneuridine (**1a**) and treatment of the crude 5′-aldehyde with formylmethylene-stabilized Wittig reagent gave the vinylogously extended 7′-aldehyde **2a** (61%; *E* isomer, $J_{5'-6'} = 15.8$ Hz) as demonstrated[14] for thymidine analogues (Scheme 1). Condensation of **2a** with ethoxycarbonylmethylene-stabilized Wittig reagent produced conjugated diene **6a** as a single product in 81% yield. Deacetonization [trifluoroacetic acid (TFA)/H_2O] and crystallization gave dienoic ester **7a**. The ^{1}H nuclear magnetic resonance (NMR) coupling constants for **6a** ($J_{5'-6'} = 15.3$ Hz, $J_{6'-7'} = 10.3$ Hz, and $J_{7'-8'} = 15.4$ Hz) and **7a** are indicative of 5′(*E*)/7′(*E*) stereochemical assignments with an s-trans conformation and are in harmony with the literature values for the conjugated dienoic esters.[15] Treatment of the 7′-aldehyde **2a** with generated in situ dibromomethylene phosphorane reagent (Ph_3P/CBr_4)[16] gave the conjugated 8′,8′-dibromodiene **4a** in 71% as a single isomer. Removal of the isopropylidene group yielded **5a**.

The 6-*N*-benzoyl-2′,3′-*O*-isopropylideneadenosine (**1c**) was converted into the vinyl 7′-aldehyde **2c** (48%) while

Scheme 2[a]



[a] Reagents: (a) Bu_3SnH/AIBN/toluene/Δ. (b) (*E* or *Z*) ethyl 3-iodopropenoate/$Pd(PPh_3)_4$/THF/Δ. (c) TFA/H_2O.

2′,3′-*O*-isopropylideneadenosine (**1b**) gave 7′-aldehyde **2b** in lower yield (22%). Attempted deprotection (NH_3/MeOH, TFA/H_2O) of **2c** failed to give pure **3b** (a vinylogous extension of the potent inhibitor adenosine 5′-aldehyde[6]). Treatment of **2c** with ethoxycarbonylmethylene Wittig reagent and successive deprotections of the resulting **6c** gave the diene **7b** (*E*,*E*) with the extended conjugation to an ethoxycarbonyl group at C8′ (42% overall from **2c**). Condensation of the aldehyde **2c** with (dibromomethylene)triphenylphosphorane and subsequent deprotections produced somehow unstable conjugated dibromodiene **5b** in low yield after tedious purifications. Therefore, the synthesis of **5b** from sugar precursors was investigated.

Selective hydrolysis of the 5,6-*O*-isopropylidene acetal from 1,2;5,6-di-*O*-isopropylidene-α-D-allofuranose and oxidative cleavage with periodic acid[17] gave the dehomologated 5-aldehyde, which was treated with formylmethylene Wittig reagent to give the vinylogous aldehyde **8** as *E*-isomer (68% overall). Successive Wittig type olefination with the dibromomethylene reagent gave **10** that was benzoylated to produce (dibromodiene)ribose **11** (53%; 36% total). Subjection of 3-*O*-benzoyl-1,2;5,6-di-*O*-isopropylidene-α-D-allofuranose to this double Wittig extension sequence gave **11** in higher yield (61% total). The isopropylidene group was removed from **11** (TFA/H_2O), and the product was acetylated to give the anomeric acetates **12** (44%; α/β, ∼1:4). Coupling ($SnCl_4$/CH_3CN)[18] of the anomeric mixture of **12** and adenine followed by deacylation gave the somehow unstable 8′,8′-dibromodiene adenosine **5b**.

Stille coupling[19] between the nucleoside vinyl 6′(*E*)-stannanes **14a** or **14b** [prepared[5,20] by stannyldesulfonylation of the readily available 6′(*E*)-tosylvinyl homonucleosides[21] **13**] and the corresponding vinyl halides was found to give stereodefined access to dienes. Thus, Pd-catalyzed coupling of **14a** or **14b** with ethyl *E*-3-iodopropenoate gave dienoic esters **6a** or **6b** as single isomers (*E*,*E*; Scheme 2). On the other hand, cross coupling of the **14a** or **14b** with ethyl *Z*-3-iodopropenoate afforded dienes **15a** and **15b** (*E*,*Z*; 70% yield). Coupling constant analysis in the ^{1}H NMR spectra ($J_{5'-6'} = 15.3$ Hz, $J_{6'-7'} = 11.4$ Hz, and $J_{7'-8'} = 11.3$ Hz) were diagnostic[15] for the 5′(*E*)/7′(*Z*) stereochemical assignment. The structure of dienes was also confirmed by correlation spectroscopy (COSY) and HETCOR experiments. Deprotection of **6a**,**b** and **15a**,**b** gave **7a**,**b** and **16a**,**b**, respectively.

Inactivation of *S*-Adenosyl-L-homocysteine Hydrolase

Incubation of AdoHcy hydrolase with 100 μM of dibromodiene **5b** resulted in partial inhibition of the

Table 1. Inhibition of AdoHcy Hydrolase with **5b**, **7b**, and **16b**

compds	% enzyme activity remaining[a]	% NAD^+ remaining[b]
5b	48 ± 1	93.1 ± 3.4
7b	3.6 ± 0.1	25.9 ± 3.4
16b	1.8 ± 0.6	36.2 ± 6.8

[a] AdoHcy hydrolase (210 nM) was incubated with 100 μM **5b**, **7b**, and **16b** in buffer A at 37 °C for 20 min, and the remaining enzyme activity was assayed as described in the Experimental Section. Data are the average of duplicate determinations. [b] AdoHcy hydrolase (5.8 μM) was incubated with 100 μM **5b**, **7b**, and **16b** in buffer A at 37 °C for 20 min, and the NAD^+/NADH content was assayed as described in the Experimental Section.

enzyme activity without any apparent change in the enzyme's NAD^+ content (Table 1). In addition, **5b** itself was not metabolized upon incubation with AdoHcy hydrolase (data not shown). These results suggest that **5b** is not a substrate for the enzyme's hydrolytic or 3′-oxidative activities. The compound appears to be a competitive inhibitor of AdoHcy hydrolase with weak inhibitory effects on the enzyme (Table 1).

Adenosine dienoic esters **7b** and **16b** were designed as putative substrates for the hydrolytic activity of AdoHcy hydrolase. Conceptually, enzyme-mediated addition of water might occur as a 1,2- or 1,4-process across the conjugated dienes in **7b** and **16b** resulting in the formation of metabolites, which should be detectable by high-performance liquid chromatography (HPLC). To test this hypothesis, compounds **7b** and **16b** were incubated with AdoHcy hydrolase and the incubation buffer was analyzed by HPLC for the formation of any metabolites of these compounds. No evidence for the disappearance of **7b** and **16b** or the appearance of metabolites was obtained (data not shown). These results suggest that **7b** and **16b** are not substrates for the enzyme's hydrolytic activity.

Incubation of compounds **7b** and **16b** at 100 M with AdoHcy hydrolase produced significant inhibition of the enzyme activity and partial depletion of its NAD^+ content (Table 1). Dienoic esters **7b** and **16b** produced time- and concentration-dependent inactivation of AdoHcy hydrolase (Figure 2A). The K_i and k_{inact} values for **7b** and **16b** (Table 2) were obtained using the Kitz and Wilson equation[22] $\{k_{obs} = k_{inact} \cdot [I]/(K_i + [I])\}$ (Figure 2B). These results, which are similar to those reported on doubly homologated dihalovinyl and acetylene analogues of adenosine,[10] suggest that inactivation of AdoHcy hydrolase by **7b** and **16b** involves a type I mechanism (cofactor depletion). However, we cannot totally rule out the possibility of a type II mechanism (covalent modification) where the products arising from the enzyme's hydrolytic activity are tightly bound to the enzyme and are not released into the solution.

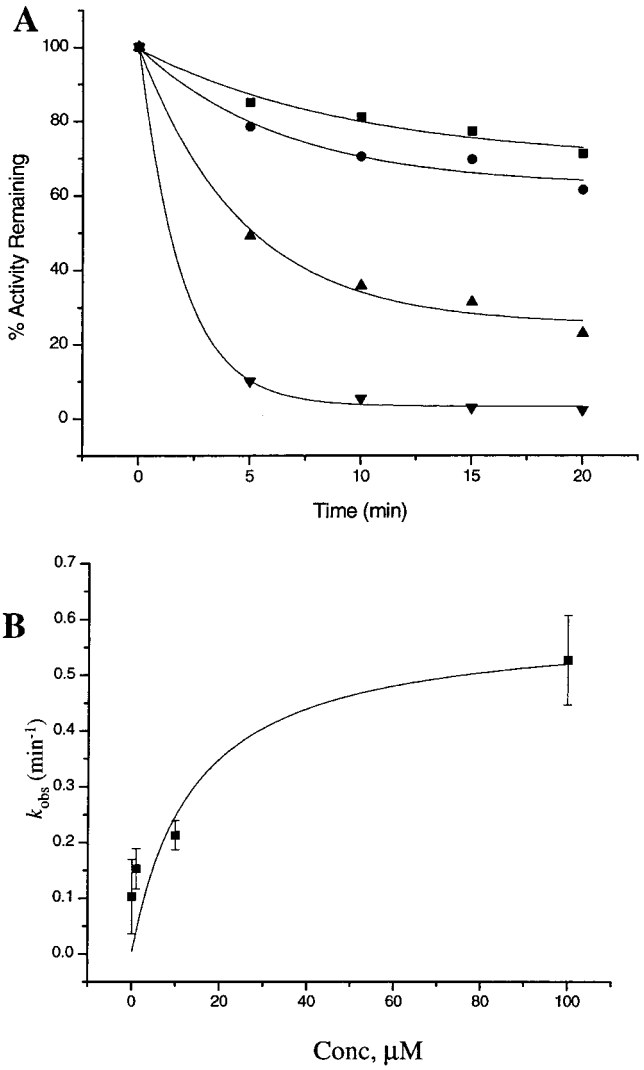


Figure 2. Time course of inactivation on AdoHcy hydrolase by **16b**. (A) AdoHcy hydrolase (210 nM) was incubated with **16b** (■, 0.1 M; ●, 1 M; ▲, 10 M; and ▼, 100 M) in buffer A at 37 °C. At the times indicated, enzyme activity was determined in the synthetic direction as described in the Experiemental Section. (B) This shows a plot of k_{obs} values, pseudo-first-order rate constant calculated from the data shown in A vs the concentration of **16b**. These data were then fitted to the Kitz and Wilson equation[22] to estimate the K_i and k_{inact} values for **16b**.

Table 2. K_i and k_{inact} Values for the Inhibitory Effects of **7b** and **16b** on AdoHcy Hydrolase

compds	K_i (μM)	k_{inact} (min^{-1})
7b	10 ± 6	0.7 ± 0.1
16b	13.7 ± 14	0.59 ± 0.1

Antiviral, Cytotoxic, and Cytostatic Activities

Uridine **5a**, **7a**, or **16a** and adenosine **5b**, **7b**, or **16b** diene analogues were examined for their antiviral, cytotoxic, and cytostatic activity in a broad spectrum of antiviral, cytotoxic, and cytostatic tests. When evaluated against human immunodeficiency virus (HIV-1, III_B strain; HIV-2, ROD strain) in T-lymphocytic MT-4 cells, no antiviral effects were noted with **5a**,**b**, **7a**,**b**, and **16a**,**b** at subtoxic concentrations. The 50% cytostatic concentration (CC_{50}) was 300 (**5a**), 11 (±5.5) (**5b**), >370 (**7a**), 175 (±33) (**7b**), 40 (±7.5) (**16a**), and 160 (±10) μM (**16b**), respectively.

In human embryonic lung (HEL) cells, the compounds showed minor activity against human cytomegalovirus (CMV, strains AD-169 and Davis) and varicella-zoster virus (VZV, strains YS, OKA, 07/1, and YS/R) at subtoxic concentrations. The minimum cytotoxic concentrations were >120 M for **5a** and **7a**,**b** and 45 μM for **5b**. The CC_{50} of **5b** for HEL cells was 54 μM. *E*,*Z*-Dienoic esters **16a**,**b** showed some activity against both VZV [50% effective concentration (EC_{50}): 10 μM, **16a**] and CMV (EC_{50}: 10 μM, **16a**; 18 μM, **16b**) albeit

concentrations that were at an average 5-fold below the cytotoxicity threshold.

In human embryonic skin muscle (E_6SM) cell cultures, again, **5b** was the most cytotoxic (minimum concentration required to cause a microscopically detectable alteration of normal cell morphology ≥6.7 μM). For the other compounds, it was ≥40 μM. No activity was noted against herpex simplex virus (HSV-1, KOS strain; HSV-2, G strain), vaccinia virus, or vesicular stomatitis virus at subtoxic concentrations.

Also, in HeLa cell cultures, no activity was noted with any of the compounds against vesicular stomatitis virus, respiratory syncytial virus, or Coxsackie B4 virus at a concentration of ≥35 μM (minimum cytotoxic concentration for **5b**) or ≥200 μM (**5a**, **7a**,**b**, and **16a**,**b**). In vero cell cultures, again, **5b** was cytotoxic at ≥35 mM (the other compounds at ≥200 μM), and no specific activity against parainfluenza virus type 3, reovirus type 1, Sindbis virus, Coxsackie B4 virus, or Punta Toro virus was noted at subtoxic concentrations.

Summary and Conclusions

The novel dibromodienes **5a**,**b** and dienoic esters **7a**,**b** and **16a**,**b** with the extended conjugation to the ribose ring were prepared from uridine and adenosine (or hexofuranose precursors) using Wittig type homologations or Stille cross-coupling procedures. The adenosine analogues **5b**, **7b**, and **16b**, with two possible sites for the hydrolytic interaction with the AdoHcy hydrolase via a 1,2- or 1,4-process, were found to be type I inhibitors of the enzyme. Dibromodiene **5b** was found to be a competitive inhibitor, whereas dienoic esters **7b** and **16b** produced time- and concentration-dependent inactivation of AdoHcy hydrolase with significant decreases in the enzyme's NAD^+ content. However, upon incubation of **7b** and **16b** with AdoHcy hydrolase, no metabolites of these dienes were detected suggesting that they are not substrates for the enzyme's hydrolytic activity. However, we cannot totally rule out the possibility that metabolites are formed but they are tightly bound to the enzyme and not released into the incubation media. No specific antiviral activity was observed with any of the compounds at subtoxic concentrations. Compound **5b** emerged as the most cytotoxic (cytotoxicity threshold: 6.7 μM).

Experimental Section

Uncorrected melting points were determined with a capillary apparatus. UV spectra were measured with solutions in MeOH. ^{1}H (400 MHz) and ^{13}C (100 MHz) NMR spectra were determined with solutions in $CDCl_3$ unless otherwise specified. Mass spectra (MS) were obtained with atmospheric pressure chemical ionization (APCI) except when CI (CH_4) is noted. Merck kieselgel 60-F_{254} sheets were used for thin-layer chromatography (TLC), and products were detected with 254 nm light or by development of color with $Ce(SO_4)_2/(NH_4)_6Mo_7O_{24}\cdot 4H_2O/H_2SO_4/H_2O$. Merck kieselgel 60 (230−400 mesh) was used for column chromatography. Preparative reversed-phase (RP)-HPLC was performed with a Supelcosil LC-18S column with a Perkin-Elmer LC 200 binary pump system (gradient solvent systems are noted) or analytical reversed-phase column (Vydac C18, 5 μm, 250 mm × 4.6 mm; Hesperia, CA) for enzymatic assays. Elemental analyses were determined by Galbraith Laboratories, Knoxville, TN. Reagent grade chemicals were used, and solvents were dried by reflux over and distillation from CaH_2 [except tetrahydrofuran (THF)/potassium] under argon. Sonication was performed with a Branson 5200 ultrasonic bath.

1-[5,6-Dideoxy-2,3-*O*-isopropylidene-*β*-D-*ribo*-hept-5(*E*)-enodialdo-1,4-furanosyl]uracil (2a). A solution of **1a** (2.84 g, 10 mmol) and dicyclohexylcarbodiimide (DCC; 7.21 g, 35 mmol) in dried Me_2SO (25 mL) was cooled (∼5 °C) under argon, Cl_2CHCO_2H (0.41 mL, 645 mg, 5 mmol) was added, and stirring was continued for 90 min at ambient temperature. The (triphenylphosphoranylidene)acetaldehyde (3.19 g, 10.5 mmol) was then added in one portion, and the resulting solution was stirred for 5 h. Oxalic acid dihydrate (3.15 g, 25 mmol) in MeOH (20 mL) was added, and after 20 min, the reaction mixture was concentrated (to ∼one-third volume), dicyclohexylurea was filtered and washed with cold MeOH, and the combined filtrates were evaporated (in vacuo). The residue was partitioned ($NaHCO_3/H_2O/CHCl_3$), and the organic layer was washed (H_2O, brine) and dried ($MgSO_4$), and volatiles were evaporated. Column chromatography of the residue (50 → 70% EtOAc/hexane) gave **2a** (1.88 g, 61%) as a syrup. ^{1}H NMR: δ 1.32 and 1.53 (2 × s, 2 × 3, 2 × Me), 4.74 ("t", J = 4.8 Hz, 1, H4′), 4.92 (dd, J = 4.3, 6.2 Hz, 1, H3′), 5.13 (d, J = 6.3 Hz, 1, H2′), 5.56 (s, 1, H1′), 5.72 (d, J = 7.9 Hz, H5), 6.22 (ddd, J = 1.5, 7.8, 15.8 Hz, 1, H6′), 6.93 (dd, J = 5.7, 15.8 Hz, 1, H5′), 7.27 (d, J = 8.0 Hz, 1, H6), 9.51 (d, J = 7.8 Hz, 1, H7′), 10.40 (br s, 1, NH). ^{13}C NMR: δ 25.56, 27.40, 84.81 and 85.06 (C2′ and C3′), 88.08 (C4′), 96.91 (C1′), 103.15 (C5), 115.05, 132.47 (C6′), 144.11 (C6), 150.75 (C2), 153.11 (C5′), 164.38 (C4), 193.91 (C7′). MS: m/z 309 (MH^+).

6-*N*-Benzoyl-9-[5,6-dideoxy-2,3-*O*-isopropylidene-*β*-D-*ribo*-hept-5(*E*)-enodialdo-1,4-furanosyl]adenine (2c). Oxidation of **1c**[13] (1.23 g, 3 mmol) and treatment of the crude 5′-aldehyde with (triphenylphosphoranylidene)acetaldehyde (as described for **2a**; column chromatography: $CHCl_3$ → 2% MeOH/$CHCl_3$) gave **2c** (625 mg, 48%). ^{1}H NMR: δ 1.39 and 1.53 (2 × s, 2 × 3, 2 × Me), 4.93 ("t", J = 4.5 Hz, 1, H4′), 5.25 (dd, J = 3.7, 6.1 Hz, 1, H3′), 5.55 (dd, J = 1.2, 6.2 Hz, 1, H2′), 6.08 (ddd, J = 1.5, 7.7, 15.8 Hz, 1, H6′), 6.22 (d, J = 1.3 Hz, 1, H1′), 6.83 (dd, J = 5.4, 15.8 Hz, 1, H5′), 7.44−8.05 (m, 6, H_{arom} and NH), 8.11 (s, 1, H2), 8.71 (s, 1, H8), 9.43 (d, J = 7.8 Hz, 1, H7′). MS: m/z 436 (100, MH^+).

Note. Subjection of **1b** (307 mg, 1 mmol) to this sequence and two column chromatographies ($CHCl_3$ → 4% MeOH/$CHCl_3$ and EtOAc → 2% MeOH/EtOAc) gave **2b** (73 mg, 22%; ∼10% contamination). ^{1}H NMR: δ 9.46 (d, J = 7.7 Hz, 1, H7′). MS: m/z 332 (100, MH^+).

Deprotection of 2c. Attempted Synthesis of 9-[5,6-Dideoxy-*β*-D-*ribo*-hept-5(*E*)-enodialdo-1,4-furanosyl]adenine (3b). Treatment of **2c** (44 mg, 1 mmol) with NH_3/MeOH and TFA/H_2O (as described for **7b**) followed by RP-HPLC purification (as described for **5b**) gave small quantities of **3b** (∼4 mg) contaminated (∼25%, ^{1}H NMR) by byproducts. ^{1}H NMR (MeOH-d_4): δ 6.36 (ddd, J = 1.5, 7.8, 15.8 Hz, 1, H6′), 7.14 (dd, J = 5.7, 15.8 Hz, 1, H5′), 9.60 (d, J = 7.8 Hz, 1, H7′).

1-[8,8-Dibromo-5,6,7,8-tetradeoxy-2,3-*O*-isopropylidene-*β*-D-*ribo*-oct-5(*E*)-7-dienofuranosyl]uracil (4a). The solution of **2a** (308 mg, 1 mmol) in CH_2Cl_2 (10 mL) was added into a stirring mixture containing (dibromomethylene)triphenylphosphorane [generated in situ by stirring CBr_4 (663 mg, 2 mmol) and Ph_3P (1.05 g, 4 mmol) in dried CH_2Cl_2 (20 mL) for 15 min at ∼0 °C (ice-bath) under N_2]. After 1 h (ice-bath was removed after 10 min), the reaction mixture was partitioned ($NaHCO_3/H_2O/CHCl_3$). The organic layer was washed (H_2O, brine) and dried ($MgSO_4$), and volatiles were evaporated. Column chromatography of the residue (50 → 65% EtOAc/hexane) gave **4a** (329 mg, 71%). ^{1}H NMR: δ 1.35 and 1.58 (2 × s, 2 × 3, 2 × Me), 4.62 (dd, J = 4.1, 7.5 Hz, 1, H4′), 4.86 (dd, J = 4.1, 6.1 Hz, 1, H3′), 5.13 (d, J = 6.3 Hz, 1, H2′), 5.57 (s, 1, H1′), 5.77 (d, J = 7.9 Hz, H5), 6.08 (dd, J = 7.5, 15.4 Hz, 1, H5′), 6.34 (dd, J = 10.2, 15.4 Hz, 1, H6′), 6.98 (d, J = 10.2 Hz, H7′), 7.24 (d, J = 8.0 Hz, 1, H6), 10.25 (br s, 1, NH). MS: m/z 467 (27, MH^+ [$^{81}Br_2$]), 465 (53, MH^+ [$^{81/79}Br_2$]), 463 (27, MH^+ [$^{79}Br_2$]).

1-[8,8-Dibromo-5,6,7,8-tetradeoxy-β-D-*ribo*-oct-5(*E*)-7-dienofuranosyl]uracil (5a). A solution of **4a** (46 mg, 0.1 mmol) in CF_3CO_2H/H_2O (9:1, 3 mL) was stirred at ∼0 °C (ice-bath) for 1 h. Volatiles were evaporated under oil-pump vacuum (<10 °C), coevaporated with toluene (3×), and kept under vacuum for 1 h. Column chromatography (EtOAc → 3% MeOH/EtOAc) of the residue and crystallization (MeOH/EtOAc) gave **5a** (26 mg, 62%); mp 167−169 °C. UV: max 252 nm (ε 24 400), min 222 nm (ε 7300). ^{1}H NMR (Me_2SO-d_6): δ 3.88 (q, *J* = 5.5 Hz, 1, H3′), 4.09 (q, *J* = 4.8 Hz, 1, H2′), 4.28 (t, *J* = 4.8 Hz, 1, H4′), 5.40 (d, *J* = 6.1 Hz, 1, OH3′), 5.55 (d, *J* = 5.3 Hz, 1, OH2′), 5.67 (d, *J* = 8.0 Hz, 1, H5), 5.76 (d, *J* = 4.2 Hz, 1, H1′), 6.24−6.27 (m, 2, H5′,6′), 7.29 (dd, *J* = 4.9, 6.3 Hz, 1, H7′), 7.60 (d, *J* = 8.1 Hz, 1, H6), 11.40 (br s, 1, NH). ^{13}C NMR (Me_2SO-d_6): δ 72.86 (C3′), 73.69 (C2′), 83.17 (C4′), 89.54 (C1′), 92.03 (C8′), 102.37 (C5), 128.06 (C6′), 136.21 (C5′), 136.54 (C7′), 141.37 (C6), 150.88 (C2), 163.37 (C4). MS: *m*/*z* 427 (47, MH^+ [$^{81}Br_2$]), 425 (100, MH^+ [$^{81/79}Br_2$]), 423 (48, MH^+ [$^{79}Br_2$]). Anal. [$C_{12}H_{12}Br_2N_2O_5$ (424.05)] C, H.

Ethyl 1,5,6,7,8-Pentadeoxy-2,3-*O*-isopropylidene-1-(uracil-1-yl)-β-D-*ribo*-non-5(*E*),7(*E*)-dienofuranuronate (6a). Method A. (Carbethoxymethylene)triphenylphosphorane (191 mg, 0.55 mmol) was added to a stirred solution of **2a** (154 mg, 0.5 mmol) in dried CH_2Cl_2 (10 mL) at ambient temperature under N_2. After 14 h, the reaction mixture was partitioned ($NaHCO_3/H_2O/CH_2Cl_2$), and the organic layer was washed (brine) and dried ($MgSO_4$), and volatiles were evaporated. Column chromatography (50 → 60% EtOAc/hexane) gave **6a** (153 mg, 81%). ^{1}H NMR: δ 1.32 (t, *J* = 7.1 Hz, 3, CH_3), 1.37 and 1.59 (2 × s, 2 × 3, 2 × Me), 4.22 (q, *J* = 7.1 Hz, 2, CH_2), 4.62 (dd, *J* = 4.3, 7.0 Hz, 1, H4′), 4.84 (dd, *J* = 4.4, 6.5 Hz, 1, H3′), 5.11 (dd, *J* = 1.3, 6.3 Hz, 1, H2′), 5.57 (d, *J* = 1.1 Hz, 1, H1′), 5.75 (d, *J* = 8.0 Hz, H5), 5.90 (d, *J* = 15.4 Hz, 1, H8′), 6.24 (dd, *J* = 7.1, 15.3 Hz, 1, H5′), 6.38 (dd, *J* = 10.3, 15.3 Hz, 1, H6′), 7.24 (d, *J* = 7.9 Hz, 1, H6), 7.28 (dd, *J* = 10.3, 15.4 Hz, H7′), 10.05 (br s, 1, NH). ^{13}C NMR: δ 14.64, 25.65, 27.49, 60.85, 84.85 and 85.14 (C2′ and C3′), 88.73 (C4′), 95.82 (C1′), 103.07 (C5), 114.99, 123.34 (C8′), 130.86 (C6′), 138.44 (C5′), 143.33 and 143.44 (C6 and C7′), 150.50 (C2), 164.16 (C4), 167.05 (C9′). MS: *m*/*z* 379 (MH^+).

Method B. Ethyl (*E*)-3-iodopropenoate[23] (49 μL, 80 mg, 0.35 mmol) was added to **14a**[20] (160 mg, 0.29 mmol) and $Pd(PPh_3)_4$ (15 mg, 0.013 mmol) in dried THF (7 mL), and the solution was gently heated (oil bath, 70 °C) for about 2.5 h. Volatiles were evaporated, and the residue was partitioned ($NaHCO_3/H_2O/CH_2Cl_2$). The organic layer was washed (diluted $Na_2S_2O_3$, brine), dried ($MgSO_4$), evaporated, and column-chromatographed (55 → 70% EtOAc/hexane or CH_2Cl_2 → 3% MeOH/CH_2Cl_2) to give **6a** (82 mg, 75%).

Note. Care has to be taken to avoid unnecessary heating and prolonged reaction time. The coupling reactions were usually ceased when TLC showed ∼90−95% consumption of vinyl stannanes **14** in order to avoid/minimize isomerization of dienes.

Ethyl 1-(Adenin-9-yl)-1,5,6,7,8-pentadeoxy-2,3-*O*-isopropylidene-β-D-*ribo*-non-5(*E*),7(*E*)-dienofuranuronate (6b). Treatment of **14b**[5] (55 mg, 0.093 mmol) with ethyl (*E*)-3-iodopropenoate[23] (14 μL, 25 mg, 0.11 mmol) (as described for **6a**, method B) gave **6b** (27 mg, 73%). ^{1}H NMR: δ 1.28 (t, *J* = 7.1 Hz, 3, CH_3), 1.41 and 1.63 (2 × s, 2 × 3, 2 × Me), 4.19 (q, *J* = 7.1 Hz, 2, CH_2), 4.78 (dd, *J* = 3.4, 6.2 Hz, 1, H4′), 5.12 (dd, *J* = 3.5, 6.1 Hz, 1, H3′), 5.56 (br d, *J* = 6.2 Hz, 1, H2′), 5.80 (d, *J* = 15.4 Hz, 1, H8′), 5.88 (br s, 2, NH_2), 6.11 (s, 1, H1′), 6.17 (dd, *J* = 6.4, 15.4 Hz, 1, H5′), 6.25 (dd, *J* = 10.5, 15.3 Hz, 1, H6′), 7.13 (dd, *J* = 10.5, 15.4 Hz, 1, H7′), 7.89 (s, 1, H2), 8.37 (s, 1, H8). MS: *m*/*z* 402 (MH^+). Depending on the experiment, diene **6b** was contaminated by isomer **15b** (5−20%; ^{1}H NMR).

Ethyl 1-(6-*N*-Benzoyladenin-9-yl)-1,5,6,7,8-pentadeoxy-2,3-*O*-isopropylidene-β-D-*ribo*-non-5(*E*),7(*E*)-dienofuranuronate (6c). Treatment of **2c** (287 mg, 0.66 mmol) with (carbethoxymethylene)triphenylphosphorane (243 mg, 0.7 mmol) (as described for **6a**, method A) gave **6c** (216 mg, 65%). ^{1}H NMR: δ 1.25 (t, *J* = 7.1 Hz, 3, CH_3), 1.39 and 1.62 (2 × s, 2 × 3, 2 × Me), 4.14 (q, *J* = 7.2 Hz, 2, CH_2), 4.79 (dd, *J* = 3.4, 6.2 Hz, 1, H4′), 5.10 (dd, *J* = 3.5, 6.1 Hz, 1, H3′), 5.56 (dd, *J* = 1.5, 6.2 Hz, 1, H2′), 5.80 (d, *J* = 15.4 Hz, H8′), 6.10 (dd, *J* = 6.4, 15.3 Hz, 1, H5′), 6.19 (s, 1, H1′), 6.22 (dd, *J* = 10.8, 15.4 Hz, 1, H6′), 7.10 (dd, *J* = 10.3, 15.4 Hz, H7′), 7.48−8.03 (m, 5, H_{arom}), 8.11 (s, 1, H2), 8.75 (s, 1, H8), 9.51 (br s, 1, NH). MS: *m*/*z* 506 (100, MH^+).

Ethyl 1,5,6,7,8-Pentadeoxy-1-(uracil-1-yl)-β-D-*ribo*-non-5(*E*),7(*E*)-dienofuranuronate (7a). A solution of **6a** (113 mg, 0.3 mmol) in CF_3CO_2H/H_2O (9:1, 5 mL) was stirred at ∼0 °C (ice-bath) for 1 h. Volatiles were evaporated under vacuum, coevaporated with toluene (3 ×), and kept under vacuum for 1 h. Column chromatography (EtOAc → 3% MeOH/EtOAc) and crystallization (MeOH) gave **7a** (69 mg, 68%) as white needles; mp 177−178 °C. UV: max 259 nm (ε 37 300), min 223 nm (ε 8500). ^{1}H NMR (Me_2SO-d_6): δ 1.22 (t, *J* = 7.1 Hz, 3, CH_3), 3.88 (q, *J* = 5.5 Hz, 1, H3′), 4.10 (q, *J* = 5.0 Hz, 1, H2′), 4.13 (q, *J* = 7.1 Hz, 2, CH_2), 4.32 (t, *J* = 5.7 Hz, 1, H4′), 5.42 (d, *J* = 6.0 Hz, 1, OH3′), 5.57 (d, *J* = 5.4 Hz, 1, OH2′), 5.66 (d, *J* = 8.0 Hz, 1, H5), 5.77 (d, *J* = 4.5 Hz, 1, H1′), 6.05 (d, *J* = 15.4 Hz, 1, H8′), 6.43 (dd, *J* = 6.1, 15.3 Hz, 1, H5′), 6.50 (dd, *J* = 9.8, 15.3 Hz, 1, H6′), 7.26 (dd, *J* = 9.9, 15.3 Hz, 1, H7′), 7.62 (d, *J* = 8.1 Hz, 1, H6), 10.40 (br s, 1, NH). ^{13}C NMR (Me_2SO-d_6): δ 14.50, 60.32, 73.08 and 73.92 (C2′ and C3′), 88.29 (C4′), 89.43 (C1′), 102.45 (C5), 122.21 (C8′), 129.67 (C6′), 140.48 (C5′), 141.39 (C6), 144.00 (C7′), 151.45 (C2), 164.00 (C4), 166.41 (C9′). MS: *m*/*z* 339 (MH^+). Anal. [$C_{15}H_{18}N_2O_7$ (338.33)] C, H, N.

Ethyl 1-(Adenin-9-yl)-1,5,6,7,8-pentadeoxy-β-D-*ribo*-non-5(*E*),7(*E*)-dienofuranuronate (7b). (Step *a*). A solution of **6c** (126 mg, 0.25 mmol) in NH_3/MeOH (10 mL) was stirred overnight at ∼5 °C. Volatiles were evaporated, and the residue was flash chromatographed (50 → 70% EtOAc/hexane) to give **6b** (89 mg, 89%). (Step *b*). Treatment of **6b** (60 mg, 0.15 mmol) with TFA/H_2O (as described for **5a**) and crystallization (MeOH) gave **7b** (40 mg, 72%; 64% total); mp 117−119 °C. UV: max 259, 257 nm (ε 39 000, 39 100), min 258, 223 nm (ε 38 700, 9600). ^{1}H NMR (Me_2SO-d_6): δ 1.22 (t, *J* = 7.1 Hz, 3, CH_3), 4.14 (q, *J* = 7.2 Hz, 2, CH_2), 4.19 (q, *J* = 5.1 Hz, 1, H3′), 4.45 (t, *J* = 4.3 Hz, 1, H4′), 4.67 (q, *J* = 5.3 Hz, 1, H2′), 5.52 (d, *J* = 5.7 Hz, 1, OH3′), 5.57 (d, *J* = 5.6 Hz, 1, OH2′), 5.96 (d, *J* = 4.9 Hz, 1, H1′), 6.02 (d, *J* = 15.3 Hz, 1, H8′), 6.46−6.51 (m, 2, H5′,6′), 7.28 (dd, *J* = 10.1, 15.3 Hz, 1, H7′), 7.31 (br s, 2, NH_2), 8.13 (s, 1, H2), 8.32 (s, 1, H8). ^{13}C NMR (Me_2SO-d_6): δ 14.40, 60.25, 73.02 and 74.12 (C2′ and C3′), 84.75 (C4′), 89.10 (C1′), 119.95 (C5), 121.90 (C8′), 129.25 (C6′), 140.10 (C5′), 140.70 (C8), 143.96 (C7′), 149.65 (C4), 152.94 (C2), 156.20 (C6), 166.70 (C9′). MS: *m*/*z* 362 (100, MH^+). Anal. [$C_{16}H_{19}N_5O_5 \cdot H_2O$ (379.38)] C, H, N.

3-*O*-Benzoyl-5,6-dideoxy-2,3-*O*-isopropylidene-α-D-*ribo*-hept-5(*E*)-enodialdo-1,4-furanose (9). Treatment of 3-*O*-benzoyl-1,2;5,6-di-*O*-isopropylidene-α-D-allofuranose[8a] (3 g, 8.25 mmol) with H_5IO_6 (crude 5-aldehyde was dissolved in EtOAc and washed with $NaHCO_3/H_2O$) and $Ph_3P{=}CHCHO$ (2 h; as described for **10**, step *a*) gave **9** (2.1 g, 80%). ^{1}H NMR: δ 1.21 and 1.50 (2 × s, 2 × 3, 2 × Me), 4.75 (dd, *J* = 4.8, 9.4 Hz, 1, H3), 4.88 (ddd, *J* = 1.3, 4.9, 9.0 Hz, 1, H4), 4.94 (t, *J* = 4.1 Hz, 1, H2), 5.85 (d, *J* = 3.6 Hz, 1, H1), 6.33 (ddd, *J* = 1.3, 7.7, 15.8 Hz, 1, H6), 6.80 (dd, *J* = 4.9, 15.7 Hz, 1, H5), 7.36 (t)/7.49 (t)/7.97 (d, 5, H_{arom}), 9.50 (d, *J* = 7.8 Hz, H7). ^{13}C NMR: δ 26.81 and 26.94 (CMe_2), 76.50 (C2), 76.57 (C3), 78.76 (C4), 104.83 (C1), 113.7 (CMe_2), 128.90/129.24/130.88/133.98 (Bz), 132.90 (C6), 150.94 (C5), 165.80 (Bz), 193.23 (C7). HRMS (CI): *m*/*z* 319.1173 (100, MH^+ [$C_{17}H_{19}O_6$] = 319.1182).

8,8-Dibromo-5,6,7,8-tetradeoxy-1,2-*O*-isopropylidene-α-D-*ribo*-oct-5(*E*)-7-dienofuranose (10). (Step *a*). H_5IO_6 (2.19 g, 9.6 mmol) was added to a solution of 1,2;5,6-di-*O*-isopropylidene-α-D-allofuranose[8a] (2 g, 7.68 mmol) in dried EtOAc (80 mL) at ambient temperature, and stirring was continued for 2 h (TLC showed conversion into more polar 5-aldehyde). The mixture was filtered, the filter cake was washed (EtOAc), and the combined filtrate was evaporated. The residue was dissolved in dried CH_2Cl_2 (50 mL), (triphenylphosphoranylidene)acetaldehyde (2.33 g, 7.68 mmol) was

then added in one portion, and the resulting solution was stirred at ambient temperature overnight. The solution was washed ($NaHCO_3/H_2O$, brine) and dried ($MgSO_4$), and volatiles were evaporated. Chromatography (50 → 70% EtOAc/hexane) of the residue gave 5,6-dideoxy-2,3-*O*-isopropylidene-α-D-*ribo*-hept-5-enodialdo-1,4-furanose (**8**; 1.12 g, 68%). ^{1}H NMR: δ 6.34 (ddd, J = 1.5, 8.0, 15.7 Hz, 1, H6), 6.83 (dd, J = 4.5, 15.8 Hz, 1, H5), 9.51 (d, J_{7-6} = 8.0 Hz, 1, H7). (Step *b*). The solution of the above material (**8**; 1.12 g, 5.2 mmol) in CH_2Cl_2 (25 mL) was added via syringe into a mixture containing (dibromomethylene)triphenylphosphorane [generated in situ by stirring CBr_4 (3.02 mg, 9.1 mmol), Ph_3P (2.39 g, 9.1 mmol), and activated Zn (dust; 595 mg, 9.1 mmol) in dried CH_2Cl_2 (50 mL) for 6 h at ambient temperature under N_2; sonication was applied intermittently for a total of 30 min.]. After 12 h, the reaction mixture was partitioned ($NaHCO_3/H_2O$//$CHCl_3$), and the organic layer was washed (H_2O, brine) and dried ($MgSO_4$), and volatiles were evaporated. Column chromatography of the residue (40 → 60% EtOAc/hexane) gave **10** (1.37 g, 71%; 48% overall yield) as white crystals; mp 134−136 °C (dec). ^{1}H NMR: δ 1.29 and 1.50 (2 × s, 2 × 3, 2 × Me), 2.41 (d, J = 10.8 Hz, 1, OH3), 3.63 (ddd, J = 5.0, 8.9, 10.6 Hz, 1, H3), 4.14 (t, J = 7.6 Hz, 1, H4), 4.51 (t, J = 4.5 Hz, 1, H2), 5.78 (d, J = 3.8 Hz, 1, H1), 5.84 (dd, J = 6.3, 15.5 Hz, 1, H5), 6.36 (ddd, J = 1.1, 10.4, 15.4 Hz, 1, H6), 6.90 (d, J = 10.4 Hz, H7). ^{13}C NMR: δ 26.41 and 26.52 (CMe_2), 76.01 (C3), 78.32 (C2), 80.04 (C4), 92.57 (C8), 103.73 (C1), 112.73 (CMe_2), 129.42 (C6), 132.61 (C5), 135.96 (C7). HRMS (CI): m/z 371.9217 (50, M^+ [$C_{11}H_{14}{}^{81}Br_2O_4$] = 371.9220), 369.9254 (100, M^+ [$^{81/79}Br_2$] = 369.9239), 367.9276 (49, M^+ [$^{79}Br_2$] = 367.9259). Anal. [$C_{11}H_{14}Br_2O_4$ (370.04)] C, H.

3-*O*-Benzoyl-8,8-dibromo-5,6,7,8-tetradeoxy-1,2-*O*-isopropylidene-α-D-*ribo*-oct-5(*E*),7-dienofuranose (11). Method A. Treatment of the **9** (318 mg, 1 mmol) with $Ph_3P{=}CBr_2$ (1.75 mmol; as described for **10**, step *b*; column chromatography: 20 → 35% EtOAc/hexane) gave **11** (360 mg, 76%). ^{1}H NMR: δ 1.30 and 1.58 (2 × s, 2 × 3, 2 × Me), 4.70−4.74 (m, 2, H3,4), 4.92−4.96 (m, 1, H2), 5.88 (d, J = 3.5 Hz, 1, H1), 5.91 (dd, J = 5.6, 15.5 Hz, 1, H5), 6.36 (dd, J = 10.2, 15.5 Hz, 1, H6), 6.92 (d, J = 10.2 Hz, H7), 7.41 (t)/7.55 (t)/8.02 (d, 5, H_{arom}). ^{13}C NMR: δ 27.01 and 27.10 (CMe_2), 76.89 (C3), 77.72 (C2/4), 93.55 (C8), 104.54 (C1), 113.51 (CMe_2), 128.81/129.53/130.26/133.89 (Bz), 130.50 (C6), 132.68 (C5), 136.23 (C7), 171.48 (Bz). HRMS (CI): m/z 476.9560 (49, MH^+ [$C_{18}H_{19}{}^{81}Br_2O_5$] = 476.9563), 474.9595 (100, MH^+ [$^{81/79}Br_2$] = 474.9580), 472.9575 (49, MH^+ [$^{79}Br_2$] = 472.9599).

Method B. BzCl (0.23 mL, 280 mg, 2.0 mmol) was added to a solution of **10** (500 mg, 1.35 mmol) in dried pyridine (5 mL) at 0 °C, and the mixture was stirred for 4 h at ambient temperature under N_2. $NaHCO_3/H_2O$ (1 mL) was added, volatiles were evaporated in vacuo, and the residue was partitioned (EtOAc//$NaHCO_3/H_2O$). The organic layer was washed (1 M HCl/H_2O, $NaHCO_3/H_2O$, brine), dried (Na_2SO_4), and evaporated, and the residue was column chromatographed (15 → 30% EtOAc/hexane) to give **11** (474 mg, 74%).

9-[8,8-Dibromo-5,6,7,8-tetradeoxy-β-D-*ribo*-oct-5(*E*),7-dienofuranosyl]adenine (5b). Method A. (a) Acetolysis. A solution of **11** (474 mg, 1 mmol) in CF_3CO_2H/H_2O (9:1, 6 mL) was stirred at ∼0 °C (ice-bath) for 1 h. Volatiles were evaporated (<10 °C), coevaporated with toluene (3×), and kept under vacuum for 1 h. Pyridine (5 mL), DMAP (3 mg), and Ac_2O (0.47 mL, 510 mg, 5 mmol) were added, and the mixture was stirred at ∼5 °C overnight. Volatiles were evaporated, the residue was dissolved (EtOAc), and the solution was washed (dilute HCl/H_2O, saturated $NaHCO_3/H_2O$, brine), dried (Na_2SO_4), and evaporated. Chromatography (15 → 25% EtOAc/hexanes) of the residue resulted in partial separation of the anomers of **12** (228 mg, 44%; β/α, ∼4:1). ^{1}H NMR: δ 6.18 (s, 0.75, H1), 6.57 (d, J = 4.4 Hz, 0.25, H1). HRMS (FAB): m/z 542.9289 (52, MNa^+ [$C_{19}H_{18}{}^{81}Br_2O_7Na$] = 542.9276), 540.9297 (100, MNa^+ [$^{81/79}Br_2$] = 540.9297), 538.9318 (49, MNa^+ [$^{79}Br_2$] = 538.9317). (b) Coupling. $SnCl_4$ (0.118 mL, 260 mg, 1.0 mmol) was added dropwise to a suspension of adenine (84 mg, 0.6 mmol) and anomeric **12** (207 mg, 0.4 mmol) in dried CH_3CN (25 mL), and stirring was continued for 18 h at ambient temperature. Volatiles were evaporated, and the residue was partitioned ($NaHCO_3/H_2O$//$CHCl_3$). The organic layer was washed (brine), dried ($MgSO_4$), evaporated, and chromatographed (EtOAc → 1% MeOH/EtOAc) to give 2′-*O*-acetyl-3′*O*-benzoyl-**5b** [120 mg, 51%; contaminated (∼15%, ^{1}H NMR)]. (c) Deprotection. A solution of the above material (120 mg) in NH_3/MeOH (10 mL) was stirred for 5 h at ∼5 °C. Volatiles were evaporated, and the residue was chromatographed (EtOAc → 8% MeOH/EtOAc) and crystallized (MeOH) to give **5b** [19 mg, 11% (steps *b* and *c*)] as white crystals; mp 104−107 °C (soften), 172−174 °C (dec). UV: max 253 nm (ε 28 000), min 225 nm (ε 10 300). ^{1}H NMR (Me_2SO-d_6): δ 4.19 (q, J = 5.2 Hz, 1, H3′), 4.41 (t, J = 5.3 Hz, 1, H4′), 4.69 (q, J = 5.1 Hz, 1, H2′), 5.45 (d, J = 5.8 Hz, 1, OH3′), 5.58 (d, J = 5.6 Hz, 1, OH2′), 5.94 (d, J = 4.8 Hz, 1, H1′), 6.25 (dd, J = 9.3, 15.4 Hz, 1, H6′), 6.32 (dd, J = 5.8, 15.3 Hz, 1, H5′), 7.29 (br s, 2, NH_2), 7.30 (d, J = 9.2 Hz, 1 H7′), 8.16 (s, 1, H2), 8.27 (s, 1, H8). MS: m/z 450 (49, MH^+ [$^{81}Br_2$]), 448 (100, MH^+ [$^{81/79}Br_2$]), 446 (50, MH^+ [$^{79}Br_2$]). Anal. [$C_{13}H_{13}Br_2N_5O_3{\cdot}CH_3OH$ (479.14)] C, H, N. RP-HPLC purification (preparative LC-18S column, 20 → 50% CH_3CN/H_2O for 70 min at 2.5 mL/min) of the mother liquor gave an additional **5b** (14 mg, 8%; 19% total; t_R 66 min).

Method B. Treatment of **2c** (287 mg, 0.66 mmol) with $PhP_3{=}CBr_2$ (as described for **4a**) gave contaminated (∼15%, ^{1}H NMR) **4c** (86 mg, 22%). Debenzoylation with NH_3/MeOH followed by treatment with TFA/H_2O (as described for **7b**) and RP-HPLC purification gave **5b** (15 mg, 5% overall from **2c**). Compound **5b** was stable when kept at ∼5 °C for weeks. However, significant decomposition (∼10−15%, ^{1}H NMR) was observed after 4 months.

Ethyl 1,5,6,7,8-Pentadeoxy-2,3-*O*-isopropylidene-1-(uracil-1-yl)-β-D-*ribo*-non-5(*E*),7(*Z*)-dienofuranuronate (15a). Treatment of **14a**[20] (114 mg, 0.2 mmol) with ethyl (*Z*)-3-iodopropenoate[23] (31 μL, 54 mg, 0.24 mmol) (as described for **6a**, method B) gave **15a** (60 mg, 79%). ^{1}H NMR: δ 1.22 (t, J = 7.1 Hz, 3, CH_3), 1.37 and 1.59 (2 × s, 2 × 3, 2 × Me), 4.20 (q, J = 7.1 Hz, 2, CH_2), 4.70 (dd, J = 4.1, 7.1 Hz, 1, H4′), 4.85 (dd, J = 4.2, 6.1 Hz, 1, H3′), 5.10 (br d, J = 5.0 Hz, 1, H2′), 5.62 (s, 1, H1′), 5.74 (d, J = 11.9 Hz, 1, H8′), 5.76 (d, J = 8.4 Hz, 1, H5), 6.15 (dd, J = 7.3, 15.5 Hz, 1, H5′), 6.59 (t, J = 11.3 Hz, 1, H7′), 7.24 (d, J = 8.1 Hz, 1, H6), 7.28 (dd, J = 10.6, 15.4 Hz, 1, H6′), 9.42 (br s, 1, NH). MS: m/z 379 (MH^+).

Ethyl 1-(Adenin-9-yl)-1,5,6,7,8-pentadeoxy-2,3-*O*-isopropylidene-β-D-*ribo*-non-5(*E*),7(*Z*)-dienofuranuronate (15b). Treatment of **14b**[5] (135 mg, 0.23 mmol) with ethyl (*Z*)-3-iodopropenoate[23] (35 μL, 62 mg, 0.27 mmol) (as described for **6a**, method B) gave **15b** (72 mg, 78%). ^{1}H NMR: δ 1.27 (t, J = 7.1 Hz, 3, CH_3), 1.42 and 1.64 (2 × s, 2 × 3, 2 × Me), 4.18 (q, J = 7.2 Hz, 2, CH_2), 4.85 (br d, J = 7.1 Hz, 1, H4′), 5.10−5.14 (m, 1, H3′), 5.57 (br d, J = 6.2 Hz, 1, H2′), 5.62 (br s, 2, NH_2), 5.67 (d, J = 11.7 Hz, 1, H8′), 6.06 (dd, J = 7.2, 15.0 Hz, 1, H5′), 6.14 (s, 1, H1′), 6.45 (t, J = 11.2 Hz, 1, H7′), 7.53 (dd, J = 11.4, 15.3 Hz, 1, H6′), 7.91 (s, 1, H2), 8.37 (s, 1, H8). ^{13}C NMR: δ 14.64, 25.81, 27.52, 60.56, 84.70 and 85.11 (C2′ and C3′), 88.08 (C4′), 91.09 (C1′), 114.95, 119.83 (C8′), 120.75 (C5), 129.30 (C6′), 138.88 (C8), 140.47 (C5′), 143.06 (C7′), 149.82 (C4), 153.55 (C2), 155.83 (C6), 166.34 (C9′). MS: m/z 402 (100, MH^+).

Ethyl 1,5,6,7,8-Pentadeoxy-1-(uracil-1-yl)-β-D-*ribo*-non-5(*E*),7(*Z*)-dienofuranuronate (16a). Treatment of **15a** (57 mg, 0.15 mmol) with TFA/H_2O (as described for **7a**) gave **16a** (31 mg, 61%), which failed to crystallize; mp 167−169 °C (white solid from CH_3CN). UV: max 262 nm (ε 39 800), min 223 nm (ε 8100). ^{1}H NMR (Me_2SO-d_6): δ 1.21 (t, J = 7.1 Hz, 3, CH_3), 3.91 (q, J = 5.7 Hz, 1, H3′), 4.08−4.14 (m, 3, H2′ and CH_2), 4.35 (t, J = 5.6 Hz, 1, H4′), 5.39 (d, J = 6.0 Hz, 1, OH3′), 5.53 (d, J = 5.4 Hz, 1, OH2′), 5.65 (d, J = 8.0 Hz, 1, H5), 5.75 (d, J = 11.2 Hz, 1, H8′), 5.78 (d, J = 4.4 Hz, 1, H1′), 6.33 (dd, J = 6.6, 15.3 Hz, 1, H5′), 6.76 (t, J = 11.4 Hz, 1, H7′), 7.44 (dd, J = 11.4, 15.4 Hz, 1, H6′), 7.62 (d, J = 8.1 Hz, 1, H6), 11.40 (br s, 1, NH). ^{13}C NMR (Me_2SO-d_6): δ 14.96, 60.58, 73.59 and 74.45 (C2′ and C3′), 83.82 (C4′), 90.14 (C1′), 102.90 (C5), 118.96 (C8′), 127.88 (C6′), 141.75 and 141.96 (C5′ and C6), 144.48

(C7′), 151.45 (C2), 163.92 (C4), 166.87 (C9′). MS: *m*/*z* 339 (MH^+). Anal. [$C_{15}H_{18}N_2O_7$ (338.33)] C, H, N.

Ethyl 1-(Adenin-9-yl)-1,5,6,7,8-pentadeoxy-β-D-*ribo*-non-5(*E*),7(*Z*)-dienofuranuronate (16b). Treatment of **15b** (55 mg, 0.14 mmol) with TFA/H_2O (as described for **7a**) gave **16b** (35 mg, 71%); mp 158−160 °C (MeOH). UV: max 261 nm (ϵ 37 500), min 225 nm (ϵ 7000). ^{1}H NMR (Me_2SO-d_6): δ 1.17 (t, *J* = 7.1 Hz, 3, CH_3), 4.09 (q, *J* = 7.1 Hz, 2, CH_2), 4.20 (q, *J* = 5.3 Hz, 1, H3′), 4.47 (t, *J* = 5.6 Hz, 1, H4′), 4.66 (q, *J* = 5.1 Hz, 1, H2′), 5.48 (d, *J* = 5.5 Hz, 1, OH3′), 5.62 (d, *J* = 5.5 Hz, 1, OH2′), 5.73 (d, *J* = 11.3 Hz, 1, H8′), 5.96 (d, *J* = 4.7 Hz, 1, H1′), 6.40 (dd, *J* = 6.7, 15.3 Hz, 1, H5′), 6.77 (t, *J* = 11.4 Hz, 1, H7′), 7.32 (br s, 2, NH_2), 7.43 (dd, *J* = 11.4, 15.3 Hz, 1, H6′), 8.16 (s, 1, H2), 8.33 (s, 1, H8). ^{13}C NMR (Me_2SO-d_6): δ 14.92, 60.53, 73.86 and 74.92 (C2′ and C3′), 84.37 (C4′), 88.69 (C1′), 118.75 (C8′), 119.90 (C5), 127.71 (C6′), 140.57 (C8), 142.24 (C5′), 144.59 (C7′), 150.21 (C4), 153.56 (C2), 156.96 (C6), 166.23 (C9′). MS: *m*/*z* 362 (100, MH^+). Anal. [$C_{16}H_{19}N_5O_5$ (361.35)] C, H, N.

Inactivation of AdoHcy Hydrolase. Human AdoHcy hydrolase (2.5 μg) was incubated with 100 μM **5b**, **7b**, and **16b** in potassium phosphate buffer (250 μL; 50 mM, pH 7.2, containing 1 mM EDTA; buffer A) at 37 °C for 20 min. The remaining enzyme activity was assayed in the synthetic direction as described.[8b] The enzyme was incubated with 1 mM Ado and 1 mM Hcy in buffer A at 37 °C for 1 min followed by addition of 10 μL of 5 M $HClO_4$ to terminate the reaction. The reaction product, AdoHcy, was quantitatively measured using RP-HPLC with the detector monitoring at 258 nm.

Various concentrations of **7b** and **16b** were incubated with AdoHcy hydrolase (2.5 μg) in buffer A at 37 °C for different times (0−20 min). Remaining enzyme activities were determined as described above. These data were then used to calculate K_i and k_{inact} values by fitting to the Kitz and Wilson equation:[22] $k_{obs} = k_{inact} \cdot [I]/(K_i + [I])$.

Determination of the NAD^+ Content of AdoHcy Hydrolase after Incubation with Inhibitors 5b, 7b, and 16b. AdoHcy hydrolase (5.8 μM) was incubated with 100 μM inhibitor in buffer A at 37 °C for 20 min. The cofactors were then released from the enzyme by the addition of 10 μL of 5 N $HClO_4$. The concentration of NAD^+ was analyzed by RP-HPLC (C18 column, flow rate of 1 mL/min with a linear gradient of 2−98% solvent B over 25 min). Solvent A was 25 mM phosphate, pH 3.2, 10 mM heptane sulfonic acid, and solvent B was acetonitrile. The NAD^+ content was monitored at 258 nm.

Antiviral, Cytotoxic, and Cytostatic Activity Assays. The antiviral, cytotoxic, and cytostatic activity assays were performed according to well-established procedures.[24]

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