2-Amino-6-arylthio-9-[2-(phosphonomethoxy)ethyl]purine Bis(2,2,2-trifluoroethyl) Esters as Novel HBV-Specific Antiviral Reagents

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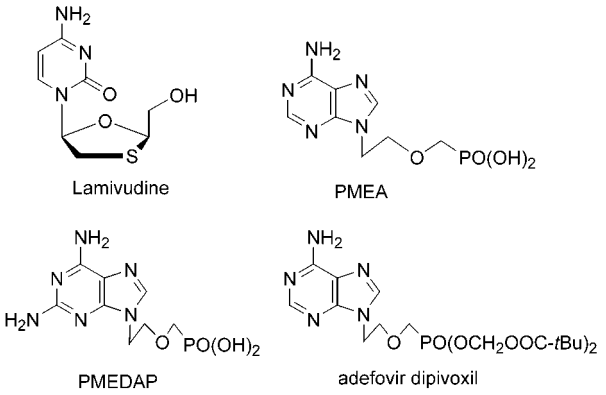
Novel 2-amino-6-arylthio-9-[2-(phosphonomethoxy)ethyl]purine bis(2,2,2-trifluoroethyl) esters were synthesized and evaluated for antihepatitis B virus (HBV) activity in vitro using HB611, HuH-6 cell line, stably transfected with the HBV genome. Among the compounds synthesized, 2-amino-6-phenylthio-9-[2-(phosphonomethoxy)ethyl]purine bis(2,2,2-trifluoroethyl) ester (**8**), 2-amino-6-(4-methoxyphenylthio)-9-[2-(phosphonomethoxy)ethyl]purine bis(2,2,2-trifluoroethyl) ester (**16**), 2-amino-6-(3-methoxyphenylthio)-9-[2-(phosphonomethoxy)ethyl]purine bis(2,2,2-trifluoroethyl) ester (**17**), and 2-amino-6-(2-methoxyphenylthio)-9-[2-(phosphonomethoxy)ethyl]-purine bis(2,2,2-trifluoroethyl) ester (**18**) showed considerably high anti-HBV activity, as represented by IC_{50} values of 0.05, 0.03, 0.04, and 0.08 μM, respectively, and exhibited low cytotoxicity, as represented by CC_{50} values of more than 1000 μM. It was suggested that these compounds did not have anti-HIV activity, and compound **8** showed only weak anti-HSV-1 activity. An antiviral agent, 9-[2-(phosphonomethoxy)ethyl]adenine (PMEA), which was used as a control in the present study, showed moderate anti-HBV activity, as represented by an IC_{50} value of 0.2 μM. Furthermore, compound **16** was administered orally to mice at a dose of 100 mg/kg in order to examine its gastrointestinal absorbability. Consequently, the main active metabolite was observed in mouse plasma, with especially high concentrations in the liver.

Introduction

Hepatitis B virus (HBV) is the causative agent of both acute and chronic hepatitis B infections. Treatment of HBV infection constitutes one of the current therapeutic challenges in virology. The number of chronic carriers is estimated to be more than 400 million worldwide, with roughly 4 million deaths annually from the resulting cirrhosis and hepatocellular carcinoma. Only a few drugs are currently available for the clinical treatment of hepatitis B. Interferon α appears effective but only in 10−30% of treated patients.[1,2] Another approved drug, lamivudine,[3,4] the 5′-triphosphate derivative of which inhibits HBV DNA polymerase, has the problem of inducing drug resistance. Several other nucleoside analogues have also been investigated. Yokota et al. have reported that phosphonomethoxyethylpurine analogues, especially 9-[2-(phosphonomethoxy)ethyl]adenine (PMEA) and 9-[2-(phosphonomethoxy)ethyl]-2,6-diaminopurine (PMEDAP), which are known to have a broad spectrum of activity against viruses, e.g., HIV[5] and HSV,[6] also exhibit anti-HBV activity.[7] However, phosphonomethoxyethylpurine analogues are minimally absorbed from the gastrointestinal tract because of the negative charge of phosphonic acid. To improve oral bioavailability, many PMEA prodrugs have been synthesized.[8] Among them, the dipivaloyloxymethyl ester of PMEA, adefovir dipivoxil, shows acceptable oral bioavailability.[9] The present study was conducted to explore novel 9-[2-(phosphonomethoxy)ethyl]purine analogues that might have more potent anti-HBV activity and less cytotoxicity. Since PMEDAP is an anti-HBV agent with higher efficacy compared to PMEA,[7] we synthesized various PMEDAP derivatives that have alkyl or aryl groups on the 6-amino group.[10] Most of the synthesized compounds showed anti-HBV activity in vitro but also exhibited toxicological changes, e.g., inhibited body weight gain, alopecia, and diarrhea, when administered to rats. However, 6-(methylphenylamino) analogue retained anti-HBV activity despite showing less toxicity. Therefore, we planned to introduce other bulky groups at the 6-position of purine ring. We describe herein the synthesis of various derivatives with arylthio groups at the 6-position of 2-amino-9-[2-(phosphonomethoxy)ethyl]purine. To increase oral bioavailability, the phosphonates of the relevant analogues were protected with the bis(2,2,2-trifluoroethyl) group, as is done with nucleoside phosphate prodrugs.[11] The present



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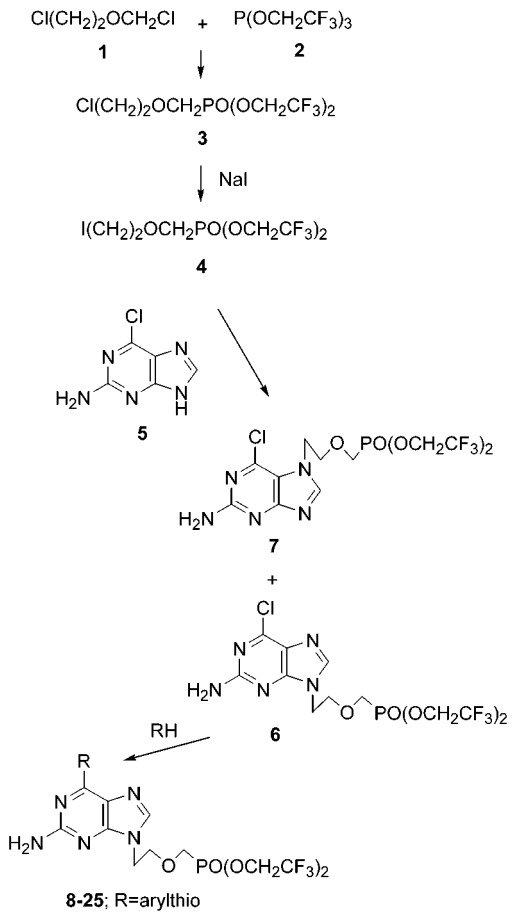
Scheme 1



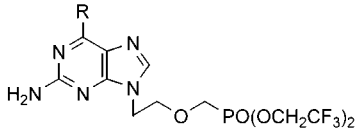
paper also describes the results of assays on antiviral activity of newly synthesized compounds and of a preliminary study on gastrointestinal absorption of a representative compound among the relevant analogues in mice.

Results and Discussion

Chemistry. 2-Chloroethyl methyl ether (**1**) was treated with tris(2,2,2-trifluoroethyl)phosphine (**2**) to quantitatively afford bis(trifluoroethyl) (2-chloroethoxy)methylphosphonate (**3**). Compound **3** was converted to bis(trifluoroethyl) (2-iodoethoxy)methylphosphonate (**4**) with sodium iodide. Subsequently, 2-amino-6-chloropurine (**5**) was treated first with 1,8-diazabicyclo[5,4,0]undec-7-ene (DBU) and then with compound **4**. 2-Amino-6-chloro-9-[2-(phosphonomethoxy)ethyl]purine bis(2,2,2-trifluoroethyl) ester (**6**) was obtained as the major product, and 2-amino-6-chloro-7-[2-(phosphonomethoxy)ethyl]purine bis(2,2,2-trifluoroethyl) ester (**7**) was obtained as a minor product. 2-Amino-6-arylthio-9-[2-(phosphonomethoxy)ethyl]purine bis(2,2,2-trifluoroethyl) esters (**8**−**25**) were synthesized by treating compound **6** with arylthiols, e.g., substituted/nonsubstituted benzenethiol and naphthalenethiol, in the presence of triethylamine (Scheme 1).

In Vitro Antiviral Assay. The anti-HBV activity of the compounds synthesized in the present study was evaluated according to a modified method of the original described by Ueda et al., in which HB611, HuH-6 cell line, stably transfected with the HBV genome, was used.[12] Anti-HBV activity and cytotoxicity are summarized in Table 1. 6-Phenylthio analogue **8** and 6-(methoxyphenylthio) analogues **16**−**18** showed high anti-HBV activity and low cytotoxicity. Compound **16** with the highest potency showed about a 10-fold higher efficacy than PMEA, as represented by the IC_{50} values listed in Table 1. The compounds with bulky alkoxy groups on their phenyl rings, **20**−**23**, showed reduced anti-HBV activity. The compounds with alkyl groups on their phenyl rings, **9**−**11**, as well as the 6-(2-naphthylthio) analogue **25**, showed high anti-HBV activity but also exhibited high cytotoxicity. The compounds with more polar groups on their phenyl rings (**14** and **15**) showed low anti-HBV activity.

Table 1. Anti-HBV Activity and Cytotoxicity of 6-Arylthio Analogues in Vitro



compd	R	IC_{50}[a] (μM)	CC_{50}[b] (μM)
8	SPh	0.05	>1000
9	SPh(4-Me)	0.06	110
10	SPh(3-Me)	0.09	150
11	SPh(2-Me)	0.08	340
12	SPh(4-Et)	0.4	170
13	SPh(4-*i*Pr)	0.98	170
14	SPh(4-NO_2)	0.52	380
15	SPh(4-Cl)	0.12	62
16	SPh(4-OMe)	0.03	>1000
17	SPh(3-OMe)	0.04	>1000
18	SPh(2-OMe)	0.08	>1000
19	SPh(4-OEt)	0.64	>1000
20	SPh(4-O−*n*Pr)	0.87	250
21	SPh(4-O−*i*Pr)	0.29	350
22	SPh(4-O−*n*Bu)	>1.0	260
23	SPh(4-O−*i*Bu)	0.84	100
24	SPh(4-O−CF_3)	0.06	820
25	S-(2-naphthyl)	0.09	198
PMEA		0.2	>1000
lamivudine		2.2	>1000

[a] Concentrations of compounds achieving 50% inhibition of cytoplasmic HBV-DNA synthesis. [b] Concentrations of compounds required for 50% extinction of HuH-6 cells.

Subsequently, the 6-arylthio derivatives with high anti-HBV activity (**8**, **16**−**18**) were examined for anti-HIV activity. None of these compounds showed significant anti-HIV activity at dose levels of up to 13, 34, 10, and 22 μM, respectively. Compound **8** showed only slight anti-HSV-1 activity (IC_{50} = 25 μM).

Gastrointestinal Absorption. Since the above-mentioned analogues were designed as prodrugs to allow absorption from the gastrointestinal tract, we measured concentrations of metabolites in the plasma and liver of mice given a single oral dose of 100 mg/kg of compound **16**, a representative compound among the relevant analogues. Blood was collected and the liver was removed at specified time points, and metabolites of compound **16** were analyzed by HPLC. A partially hydrolyzed monoester, 2-amino-6-(4-methoxyphenyl)-thio-9-[2-(phosphonomethoxy)ethyl]purine 2,2,2-trifluoroethyl ester (**26**), and a completely hydrolyzed phosphonic acid, 2-amino-6-(4-methoxyphenyl)thio-9-[2-(phosphonomethoxy)ethyl]purine (**27**), were used as the reference compounds. Since the main metabolite in the plasma and liver of mice was monoester **26**, which was also active against HBV in vitro (IC_{50} = 0.07 μM), the concentrations in the plasma and liver of mice were

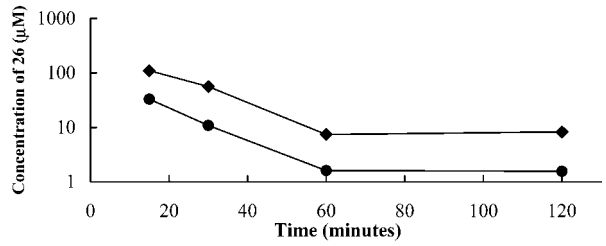
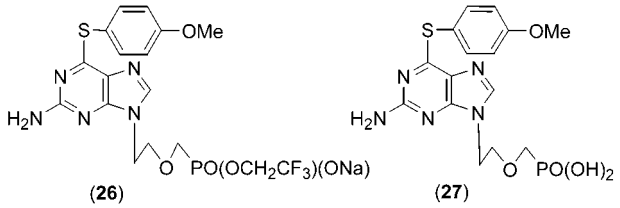


Figure 1. Concentrations of **26** in plasma (●) and in the liver (◆) after oral administration of **16** to mice.



measured on a time-course basis. The measurements, shown in Figure 1, indicated that compound **16** was absorbed from the gastrointestinal tract of mice after oral administration and active metabolite **26** was detected in the mouse plasma, with high concentrations in the liver.

Conclusions

We synthesized a number of novel 2-amino-6-arylthio-9-[2-(phosphonomethoxy)ethyl]purine bis(2,2,2-trifluoroethyl) esters. Among them, 6-phenylthio- and 6-(methoxyphenyl)thio derivatives showed potent HBV-specific antiviral activity in vitro, in contrast to PMEA and PMEDAP, which have a broad spectrum of activity against viruses. In a preliminary study, compound **16** was absorbed from the gastrointestinal tract when administered orally to mice, and active metabolite **26** was highly detected in the liver. These data led us to consider that some of the analogues examined in the present study have properties that might be suitable for hepatitis B chemotherapy.

Experimental Section

Melting points were measured with a Yanagimoto micro-melting-point meter and were not corrected. ^{1}H NMR spectra were recorded at 300 MHz on an ARX-300 or a DPX-300 Bruker NMR spectrometer using tetramethylsilane as an internal standard; chemical shifts (δ) were recorded in parts per million (ppm). Silica gel column chromatography was conducted with Wakogel C-300. Lamivudine, extracted from tablets of Epivir (Glaxo Wellcome), was purified. PMEA was prepared according to a published method.[13]

General Methods To Synthesize 2-Amino-6-arylthio-9-[2-(phosphonomethoxy)ethyl]purine Bis(2,2,2-trifluoroethyl) Ester Analogues. 2-Chloroethyl chloromethyl ether (87 g, 670 mmoL) and tris(2,2,2-trifluoroethyl)phosphite (200 g, 610 mmoL) were allowed to react at 160 °C for 7 h to quantitatively afford **3**. Compound **3** (206 g, 0.6 moL) and sodium iodide (270 g, 1.8 mol) were dissolved in methyl ethyl ketone (2 L), and the resultant solution was heated under reflux for 8 h. After completion of the reaction, the mixture was cooled to room temperature and concentrated to dryness. The residue, dissolved in a solution of chloroform/hexane (1:1 v/v), was absorbed on a silica gel column and was then eluted with a solution of chloroform/hexane (hexane concentration was gradually reduced from 50% to 0%) to quantitatively afford **4**. Compound **5** (15.0 g, 88 mmoL) was suspended in DMF (360 mL) and treated with DBU (13.9 mL, 93 mmoL) at 80 °C for 1 h. Subsequently, compound **4** (41.6 g, 97 mmoL) was added to the reaction mixture and subjected to 100 °C for 5 h. After completion of the reaction, the mixture was cooled to room temperature and concentrated to dryness. The residue was dissolved in chloroform, absorbed on a silica gel column, and eluted with chloroform containing 5% methanol to afford **6** (23.3 g, 49.4 mmoL, yield 56%). Arylthiol (30 mmoL) was added to a DMF solution (68 mL) of triethylamine (2.1 mL, 15 mmoL) and compound **6** (7.1 g, 15 mmoL), and the mixture was stirred at 100 °C for 2 h. The reaction mixture was cooled to room temperature and concentrated to dryness. The residue was dissolved in chloroform, absorbed on a silica gel column, and eluted with chloroform containing 5% methanol to afford the desired product.

2-Amino-6-phenylthio-9-[2-(phosphonomethoxy)ethyl]-purine Bis(2,2,2-trifluoroethyl) Ester (8): yield 61%; mp 105−106 °C (ethanol); ^{1}H NMR ($CDCl_3$) δ 3.91−3.95 (m, 4H, OCH_2P, NCH_2CH_2O), 4.25−4.29 (m, 2H, NCH_2CH_2O), 4.34−4.41 (m, 4H, OCH_2CF_3), 4.77 (b, 2H, NH_2), 7.41−7.45 (m, 3H, Ph), 7.61−7.66 (m, 2H, Ph), 7.72 (s, 1H, 8-H). Anal. ($C_{18}H_{18}F_6N_5O_4PS$) C, H, N.

2-Amino-6-(4-methylphenylthio)-9-[2-(phosphonomethoxy)ethyl]purine Bis(2,2,2-trifluoroethyl) Ester (9): yield 71%; mp 92.5−93 °C (diisopropyl ether); ^{1}H NMR ($CDCl_3$) δ 2.40 (s, 3H, CH_3), 3.89−3.96 (m, 4H, NCH_2CH_2O, NCH_2CH_2O), 4.26 (d, J = 5.1 Hz, 2H, OCH_2P), 4.39−4.47 (m, 4H, OCH_2CF_3), 4.79 (br, 2H, NH_2), 7.23 (d, J = 9.8 Hz, 2H, Ph), 7.31 (d, J = 9.8 Hz, 2H, Ph), 7.71 (s, 1H, 8-H). Anal. ($C_{19}H_{20}F_6N_5O_4PS$) C, H, N.

2-Amino-6-(3-methylphenylthio)-9-[2-(phosphonomethoxy)ethyl]purine Bis(2,2,2-trifluoroethyl) Ester (10): yield 66%; mp 75.5−76.5 °C (diisopropyl ether); ^{1}H NMR ($CDCl_3$) δ 2.39 (s, 3H, CH_3), 3.90−3.95 (m, 4H, OCH_2P, NCH_2CH_2O), 4.24−4.29 (m, 2H, NCH_2CH_2O), 4.34−4.41 (m, 4H, OCH_2CF_3), 4.77 (b, 2H, NH_2), 7.20−7.38 (m, 2H, Ph), 7.42−7.50 (m, 2H, Ph), 7.72 (s, 1H, 8-H). Anal. ($C_{19}H_{20}F_6N_5O_4PS$)) C, H, N.

2-Amino-6-(2-methylphenylthio)-9-[2-(phosphonomethoxy)ethyl]purine Bis(2,2,2-trifluoroethyl) Ester (11): yield 68%; mp 73−74 °C (diisopropyl ether); ^{1}H NMR ($CDCl_3$) δ 2.42 (s, 3H, CH_3), 3.90−3.94 (m, 4H, OCH_2P, NCH_2CH_2O), 4.24−4.29 (m, 2H, NCH_2CH_2O), 4.31−4.41 (m, 4H, OCH_2CF_3), 4.74 (b, 2H, NH_2), 7.24−7.40 (m, 3H, Ph), 7.62 (d, J = 7.9 Hz, 1H, Ph), 7.72 (s, 1H, 8-H). Anal. ($C_{19}H_{20}F_6N_5O_4PS$) C, H, N.

2-Amino-6-(4-ethylphenylthio)-9-[2-(phosphonomethoxy)ethyl]purine Bis(2,2,2-trifluoroethyl) Ester (12): yield 72%; mp 82.5−83 °C (diisopropyl ether); ^{1}H NMR ($CDCl_3$) δ 1.28 (t, J = 7.6 Hz, 3H, CH_3CH_2), 2.71 (t, J = 7.6 Hz, 2H, CH_3CH_2), 3.90−3.95 (m, 4H, OCH_2P, NCH_2CH_2O), 4.24−4.29 (m, 2H, NCH_2CH_2O), 4.34−4.41 (m, 4H, OCH_2CF_3), 4.77 (b, 2H, NH_2), 7.26 (d, J = 8.1 Hz, 2H, Ph), 7.53 (d, J = 8.1 Hz, 2H, Ph), 7.72 (s, 1H, 8-H). Anal. ($C_{20}H_{22}F_6N_5O_4PS$) C, H, N.

2-Amino-6-(4-isopropylphenylthio)-9-[2-(phosphonomethoxy)ethyl]purine Bis(2,2,2-trifluoroethyl) Ester (13): yield 24%; mp 119.5−120.5 °C (diisopropyl ether); ^{1}H NMR ($CDCl_3$) δ 1.29 (d, J = 6.9 Hz, 6H, $(CH_3)_2CH$), 2.96 (septet, J = 6.9 Hz, 1H, $(CH_3)_2CHO$), 3.85−3.98 (m, 4H, OCH_2P, NCH_2CH_2O), 4.18−4.30 (m, 2H, NCH_2CH_2O), 4.34−4.44 (m, 4H, OCH_2CF_3), 4.79 (b, 2H, NH_2), 7.28 (d, J = 8.2 Hz, 2H, Ph), 7.64 (d, J = 8.2 Hz, 2H, Ph), 7.75 (s, 1H, 8-H). Anal. ($C_{21}H_{24}F_6N_5O_4PS$) C, H, N.

2-Amino-6-(4-nitrophenylthio)-9-[2-(phosphonomethoxy)ethyl]purine Bis(2,2,2-trifluoroethyl) Ester (14): yield 34%; mp 121−122 °C (diisopropyl ether); ^{1}H NMR ($CDCl_3$) δ 3.91−3.96 (m, 4H, OCH_2P, NCH_2CH_2O), 4.27−4.31 (m, 2H, NCH_2CH_2O), 4.34−4.41 (m, 4H, OCH_2CF_3), 4.83 (b, 2H, NH_2), 7.76 (s, 1H, 8-H), 7.80 (d, J = 8.9 Hz, 2H, Ph), 8.24 (d, J = 8.9 Hz, 2H, Ph). Anal. ($C_{18}H_{18}F_6N_5O_4PS\cdot{}^1/_2H_2O$) C, H, N.

2-Amino-6-(4-chlorophenylthio)-9-[2-(phosphonomethoxy)ethyl]purine Bis(2,2,2-trifluoroethyl) Ester (15): yield 53%; mp 126−127.5 °C (diisopropyl ether); ^{1}H NMR ($CDCl_3$) δ 3.90−3.94 (m, 4H, OCH_2P, NCH_2CH_2O), 4.24−4.28 (m, 2H, NCH_2CH_2O), 4.31−4.44 (m, 4H, OCH_2CF_3), 4.77 (s, 2H, NH_2), 7.39 (d, J = 8.5 Hz, 2H, Ph), 7.56 (d, J = 8.5 Hz, 2H, Ph), 7.76(s, 1H, 8-H). Anal. ($C_{18}H_{17}ClF_6N_5O_4PS$) C, H, N.

2-Amino-6-(4-methoxyphenylthio)-9-[2-(phosphonomethoxy)ethyl]purine Bis(2,2,2-trifluoroethyl) Ester (16): yield 71%; mp 93−95 °C (diisopropyl ether); ^{1}H NMR ($CDCl_3$) δ 3.86 (s, 3H, OCH_3), 3.90−3.94 (m, 4H, OCH_2P, NCH_2CH_2O), 4.24−4.28 (m, 2H, NCH_2CH_2O), 4.34−4.41 (m, 4H, OCH_2CF_3), 4.75 (b, 2H, NH_2), 6.95 (d, J = 9.0 Hz, 2H, Ph), 7.53 (d, J = 9.0 Hz, 2H, Ph), 7.71 (s, 1H, 8-H). Anal. ($C_{19}H_{20}F_6N_5O_5PS$) C, H, N.

2-Amino-6-(3-methoxyphenylthio)-9-[2-(phosphonomethoxy)ethyl]purine Bis(2,2,2-trifluoroethyl) Ester (17): yield 87%; mp 62−63.5 °C (diisopropyl ether); ^{1}H NMR ($CDCl_3$) δ 3.82 (s, 3H, OCH_3), 3.90−3.94 (m, 4H, OCH_2P, NCH_2CH_2O), 4.25−4.29 (m, 2H, NCH_2CH_2O), 4.31−4.44 (m, 4H, OCH_2CF_3), 4.82 (b, 2H, NH_2), 6.92−7.00 (m, 1H, Ph), 7.18−7.40 (m, 4H, Ph), 7.72 (s, 1H, 8-H). Anal. ($C_{19}H_{20}F_6N_5O_5PS$) C, H, N.

2-Amino-6-(2-methoxyphenylthio)-9-[2-(phosphonomethoxy)ethyl]purine Bis(2,2,2-trifluoroethyl) Ester (18): yield 63% (foam); ^{1}H NMR ($CDCl_3$) δ 3.80 (s, 3H, OCH_3), 3.89−3.94 (m, 4H, OCH_2P, NCH_2CH_2O), 4.23−4.27 (m, 2H, NCH_2CH_2O), 4.31−4.45 (m, 4H, OCH_2CF_3), 4.78 (b, 2H, NH_2), 6.96−7.04 (m, 2H, Ph), 7.43 (dd, J = 7.7, 1.5 Hz, 1H, Ph), 7.59 (dd, J = 7.7, 1.5 Hz, 1H, Ph), 7.69 (s, 1H, 8-H). Anal. ($C_{19}H_{20}F_6N_5O_5PS$) C, H, N.

2-Amino-6-(4-ethoxyphenylthio)-9-[2-(phosphonomethoxy)ethyl]purine Bis(2,2,2-trifluoroethyl) Ester (19): yield 32%; mp 61−64 °C (diisopropyl ether); ^{1}H NMR ($CDCl_3$) δ 1.45 (t, J = 7.0 Hz, 3H, CH_3CH_2O), 3.91−3.94 (m, 4H, OCH_2P, NCH_2CH_2O), 4.08 (q, J = 7.0 Hz, 2H, CH_3CH_2O), 4.24−4.26 (m, 2H, NCH_2CH_2O), 4.34−4.41 (m, 4H, OCH_2CF_3), 4.76 (b, 2H, NH_2), 6.94 (d, J = 8.4 Hz, 2H, Ph), 7.52 (d, J = 8.4 Hz, 2H, Ph), 7.71 (s, 1H, 8-H). Anal. ($C_{20}H_{22}F_6N_5O_5PS$) C, H, N.

2-Amino-6-(4-*n*-propoxyphenylthio)-9-[2(phosphonomethoxy)ethyl]purine Bis(2,2,2-trifluoroethyl) Ester of (20): yield 29% (foam); ^{1}H NMR ($CDCl_3$) δ 1.05 (t, J = 7.4 Hz, 3H, $OCH_2CH_2CH_3$), 1.84 (tq, J = 6.8, 7.4 Hz, 2H, $OCH_2CH_2CH_3$), 3.82−4.00 (m, 6H, $OCH_2CH_2CH_3$, OCH_2P, NCH_2CH_2O), 4.24−4.31 (m, 2H, NCH_2CH_2O), 4.34−4.44 (m, 4H, OCH_2CF_3), 4.78 (b, 2H, NH_2), 6.95 (d, J = 8.7 Hz, 2H, Ph), 7.51 (d, J = 8.7 Hz, 2H, Ph), 7.71 (s, 1H, 8-H). Anal. ($C_{21}H_{24}F_6N_5O_5PS$) C, H, N.

2-Amino-6-(4-isopropoxyphenylthio)-9-[2-(phosphonomethoxy)ethyl]purine Bis(2,2,2-trifluoroethyl) Ester (21): yield 51%; mp 68.5−70 °C (diisopropyl ether); ^{1}H NMR ($CDCl_3$) δ 1.37 (d, J = 6.0 Hz, 6H, $(CH_3)_2CHO$), 3.85−3.98 (m, 4H, OCH_2P, NCH_2CH_2O), 4.18−4.30 (m, 2H, NCH_2CH_2O), 4.34−4.44 (m, 4H, OCH_2CF_3), 4.60 (septet, J = 6.0 Hz, 1H, $(CH_3)_2CHO$), 4.77 (b, 2H, NH_2), 6.93 (d, J = 8.7 Hz, 2H, Ph), 7.51 (d, J = 8.7 Hz, 2H, Ph), 7.71 (s, 1H, 8-H). Anal. ($C_{21}H_{24}F_6N_5O_5PS$) C, H, N.

2-Amino-6-(4-*n*-butoxyphenylthio)-9-[2-(phosphonomethoxy)ethyl]purine Bis(2,2,2-trifluoroethyl) Ester (22): yield 80%; mp 89−92 °C (diisopropyl ether); ^{1}H NMR ($CDCl_3$) δ 0.99 (t, J = 7.5 Hz, 3H, $OCH_2CH_2CH_2CH_3$), 1.51 (tq, J = 8.1, 7.5 Hz, 2H, $OCH_2CH_2CH_2CH_3$), 1.79 (tt, J = 6.4, 8.1 Hz, 2H, $OCH_2CH_2CH_2CH_3$), 3.84−3.98 (m, 4H, OCH_2P, NCH_2CH_2O), 4.00 (t, J = 6.4 Hz, 2H, $OCH_2CH_2CH_2CH_3$), 4.20−4.28 (m, 2H, NCH_2CH_2O), 4.34−4.40 (m, 4H, OCH_2CF_3), 4.76 (b, 2H, NH_2), 6.94 (d, J = 8.8 Hz, 2H, Ph), 7.52 (d, J = 8.8 Hz, 2H, Ph), 7.71 (s, 1H, 8-H). Anal. ($C_{22}H_{26}F_6N_5O_5PS$) C, H, N.

2-Amino-6-(4-isobutoxyphenylthio)-9-[2-(phosphonomethoxy)ethyl]purine Bis(2,2,2-trifluoroethyl) Ester (23): yield 69%; mp 92.5−93 °C (diisopropyl ether); ^{1}H NMR ($CDCl_3$) δ 1.05 (d, J = 6.8 Hz, 6H, $(CH_3)_2CHCH_2O$), 2.14 (d and septet, J = 6.5 and 6.8 Hz, 1H, $(CH_3)_2CH$ CH_2O), 3.76 (d, J = 6.5 Hz, 2H, $(CH_3)_2CH$ CH_2O), 3.85−3.98 (m, 4H, OCH_2P, NCH_2CH_2O), 4.18−4.30 (m, 2H, NCH_2CH_2O), 4.34−4.44 (m, 4H, OCH_2CF_3), 4.77 (b, 2H, NH_2), 6.94 (d, J = 8.7 Hz, 2H, Ph), 7.51 (d, J = 8.7 Hz, 2H, Ph), 7.71 (s, 1H, 8-H). Anal. ($C_{22}H_{26}F_6N_5O_5PS$) C, H, N.

2-Amino-6-(4-trifluoromethoxyphenylthio)-9-[2(phosphonomethoxy)ethyl]purine Bis(2,2,2-trifluoroethyl) Ester (24): yield 25%; mp 127−127.5 °C (diisopropyl ether); ^{1}H NMR ($CDCl_3$) δ 3.86−3.98 (m, 4H, OCH_2P, NCH_2CH_2O), 4.22−4.30 (m, 2H, NCH_2CH_2O), 4.34−4.44 (m, 4H, OCH_2CF_3), 4.78 (b, 2H, NH_2), 7.27 (d, J = 8.7 Hz, 2H, Ph), 7.66 (d, J = 8.7 Hz, 2H, Ph), 7.73 (s, 1H, 8-H). Anal. ($C_{19}H_{17}F_9N_5O_5PS$) C, H, N.

2-Amino-6-(2-naphthylthio)-9-[2-(phosphonomethoxy)ethyl]purine Bis(2,2,2-trifluoroethyl) Ester (25): yield 71%; mp 100−101 °C (diisopropyl ether); ^{1}H NMR ($CDCl_3$) δ 3.91−4.00 (m, 4H, OCH_2P, NCH_2CH_2O), 4.25−4.32 (m, 2H, NCH_2CH_2O), 4.34−4.50 (m, 4H, OCH_2CF_3), 4.73 (b, 2H, NH_2), 7.50−7.62 (m, 2H, Ar), 7.62−7.71 (m, 1H, Ph), 7.74 (s, 1H, 8-H), 7.82−7.94 (m, 3H, Ar), 8.16 (s, 1H, Ar). Anal. ($C_{22}H_{20}F_6N_5O_4PS$) C, H, N.

Preparation of 2-Amino-6-(4-methoxyphenylthio)-9-[2-(phosphonomethoxy)ethyl]purine 2,2,2-Trifluoroethyl Ester Sodium Salt (26). Compound **16** (5.754 g, 10 mmoL) was dissolved in THF (30 mL). A 1 N NaOH aqueous solution (9.9 mL) was added to this solution, and the mixture was left at ambient temperature for 16 h. After THF was removed by evaporation, the residue was diluted with water (50 mL) and the solution was extracted with ethyl ether (30 mL, three times) to remove any remaining compound **16**. The water layer was freeze-dried to afford the desired material: yield 5.04 g (9.8 mmoL, 98%); ^{1}H NMR ($CDCl_3$) δ 3.30−3.39 (m, 2H, OCH_2P), 3.75−3.80 (m, 2H, NCH_2CH_2O), 3.80 (s, 3H, OCH_3), 4.01−4.07 (m, 2H, OCH_2CF_3), 4.09−4.18 (m, 2H, NCH_2CH_2O), 6.23 (b, 2H, NH_2), 7.01 (d, J = 8.70 Hz, 2H, Ph), 7.50 (d, J = 8.7 Hz, 2H, Ph), 8.01 (s, 1H, 8-H). Anal. ($C_{17}H_{18}F_3N_5NaO_5PS\cdot H_2O$) C, N. H calcd, 3.78; found, 3.31.

Preparation of 2-Amino-6-(4-methoxyphenylthio)-9-[2-(phosphonomethoxy)ethyl]purine (27). 2-Amino-6-chloro-9-[2-(phosphonomethoxy)ethyl]purine (2.1 g, 6.83 mmoL), which was obtained according to a published method,[14] was dissolved in DMF (30 mL). 4-Methoxybenzenethiol (1 mL, 8.2 mmoL) and pyridine (2.7 mL, 32.8 mmoL) were added to this solution, and the solution was heated at 80 °C for 3 h. The solvent was removed by evaporation under reduced pressure. The residue was dissolved in water (10 mL), and acetone (60 mL) was added to afford crystals of the desired compound: yield 1.68 g (4.1 mmoL, 60%); mp 228−231.5 °C; ^{1}H NMR (Me_2SO-d_6) δ 3.19 (d, 2H, J = 8.70 Hz, OCH_2P), 3.80 (s, 3H, OCH_3), 3.80−3.85 (m, 2H, NCH_2CH_2O), 4.17−4.21 (m, 2H, NCH_2CH_2O), 7.02 (d, J = 8.4 Hz, 2H, Ph), 7.50 (d, J = 8.4 Hz, 2H, Ph), 7.95 (s, 1 H, 8-H). Anal. ($C_{15}H_{18}N_5O_5PS$) C, H, N.

Procedure To Assess Anti-HBV Activity. The anti-HBV activity of the above-mentioned compounds was evaluated according to a modified method of the original reported by K. Ueda et al.[12] HB611, an HBV-producing cell line, was maintained in Dulbecco's modified eagle's medium (DMEM) that was supplemented with 10% fetal bovine serum, 100 μg/mL streptomycin, 100 IU/mL penicillin, and 0.2 mg/mL Geneteicin (Life Technologies) at 37 °C in the presence of 5% CO_2. HB611 cells (2×10^4 cells/well) were cultured in a 24-well plate, and the medium was replaced with fresh medium on days 2 and 5 of culture. On day 8 of culture, the medium was replaced with another medium containing a test compound at final concentrations of 0.001−10 μM. Cells in three wells were similarly treated with a test compound of the same concentration. The cells were cultured for 9 more days, and then all DNA was collected from the cells. The amounts of HBV replication intermediates were determined by Southern blot analysis. The mean amount of HBV replication intermediates at each concentration was calculated, and the 50% inhibitory concentration (IC_{50}) of the test compound was determined. The experiment was repeated at least twice in the same manner. The 50% cytotoxic concentration (CC_{50}) of each compound was determined in HuH-6 cell line, the original for HB611 cell line. HuH-6 cells, applied to a 96-well plate at a density of 1×10^5 cells/mL, were cultured in DMEM, which was supplemented with 10% fetal bovine serum and each test compound, at 37 °C for 3 days in the presence of 5% CO_2. At the end of the culture, 3-(4,5-dimethylthiazol-2-yl)-5-(3-carboxymethoxyphenyl)-2-(4-sulfophenyl)-2*H*-tetrazolium (MTS) was added to the medium, and the plate was further incubated at 37 °C for 2 h. To determine cell viability, the absorbance, at 490 nm, of each well was measured with a microplate reader (NJ-2000: INTERMED).

Procedure for Anti-HIV-1 Assay. MT-4 cells (1×10^5 cells/mL) were infected with HIV-1 (HTLV-IIIB strain) at a multiplicity of infection (MOI) of 0.02. The cells were cultured with various concentrations of test compounds; 3′-azido-3′-deoxythymidine (AZT) was used as a control. After a 4-day incubation at 37 °C, the number of viable cells was counted according to the 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyl-2*H*-tetrazolium bromide (MTT) method, and the inhibitory concentration at which cell viability is inhibited by 50% (IC_{50}) was determined. The experiment was repeated twice in the same manner. The concentration required for 50% extinction of MT-4 cells (CC_{50}) was also determined.

Procedure for Anti-HSV-1 Assay. RPMI8226 cells (3×10^4 cells) were infected with HSV-1. The cells were cultured with various concentrations of test compounds; aciclovir was used as a control. After a 4-day incubation at 37 °C, the number of viable cells was counted according to the MTT method, and the inhibitory concentration at which cell viability is inhibited by 40% (IC_{40}) was determined. The concentration required for 50% extinction of RPMI8226 cells (CC_{50}) was also determined.

Estimation of Gastrointestinal Absorption in Mice. Compound **16** was administered by oral gavage to male C57B/6J mice as a single 100 mg/kg dose in 10 mL of 0.5% tragacanth in water. Blood, collected from two mice at given time points, was centrifuged (10000*g* for 3 min) to obtain plasma. Acetonitrile (0.1 mL) was added to the plasma (0.05 mL), the mixture was centrifuged (10000*g* for 3 min), and 0.05 mL of the supernatant was analyzed by HPLC. The liver was removed from two mice previously given compound **16** in the same manner as described above. Each liver was immersed in 2-fold (W/V) saline and was then homogenized with a homogenizer (Hitachi). Acetonitrile (0.1 mL) was added to the homogenate (0.05 mL), and the mixture was centrifuged (10000*g* for 3 min), and then 0.05 mL of the supernatant was analyzed by HPLC. HPLC was conducted using a D-7000 system (Hitachi) equipped with a Capcellpack C_{18} UD column (Shiseido, 4.6 mm × 250 mm). The detector wavelength, flow rate, and column temperature were set at 311 nm, 0.8 mL/min, and 30 °C, respectively. The mobile phase consisted of 35% acetonitrile in water containing 1.25 mM Pic A reagent (Waters).

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