Synthesis, in Vitro Antiviral Evaluation, and Stability Studies of Novel α-Borano-Nucleotide Analogues of 9-[2-(Phosphonomethoxy)ethyl]adenine and (*R*)-9-[2-(Phosphonomethoxy)propyl]adenine

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We describe here the synthesis of 9-[2-(Boranophosphonomethoxy)ethyl]adenine (**6a**) and (*R*)-9-[2-(Boranophosphonomethoxy)propyl]adenine (**6b**), the first α-boranophosphonate nucleosides in which a borane (BH_3) group substitutes one nonbridging oxygen atom of the α-phosphonate moiety. H-phosphinates **5a** and **5b** and α-boranophosphonates **6a** and **6b** were evaluated for their in vitro activity against human immunodeficiency virus (HIV) infected cells and against a panel of DNA or RNA viruses. Compounds **5a**, **5b**, **6a**, and **6b** exhibited no significant antiviral activity in vitro and cytotoxicity. To measure the chemical and enzymatic stabilities of the target compounds **6a** and **6b**, kinetic data of decomposition for derivatives **5a**, **5b**, **6a**, **6b**, and standard compounds were studied at 37 °C in several media. The α-Boranophosphonates **6a** and **6b** were metabolized in culture medium into H-phosphinates **5a** and **5b**, with half-live values of 5.3 h for **6a** and 1.3 h for **6b**.

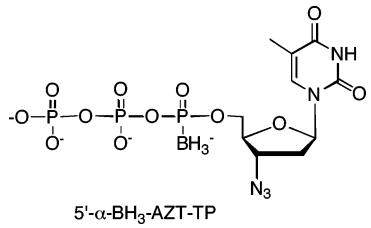
Introduction

Many antiviral drugs directed against HIV-1, the etiologic agent of acquired immunodeficiency syndrome (AIDS), focus on the inhibition of HIV-1 reverse transcriptase (RT), one of the key enzymes in the replicative cycle of the virus. Most of the available drugs targeting the HIV-1 RT are nucleoside and nucleotide analogues. Intracellular phosphorylation is required to convert a nucleoside into its active triphosphate form in order to be selectively incorporated into the viral DNA, leading to the antiviral effect by chain termination. Dideoxynucleosides such as zidovudine (AZT), stavudine (d4T), or lamivudine (3TC) have been extensively used as antiviral nucleosides targeting HIV RT.[1] However, under therapeutic pressure, the viral RT gene mutates and creates RT variants bearing amino acid substitutions responsible for the loss of nucleotide analogue efficacy. The majority of resistance-associated mutations are located near the RT active site, and these resistance mechanisms are now well-characterized.[2−4]

Nucleoside 5′-(α-P-borano)triphosphates are nucleotide analogues in which a borane (BH_3) group substitutes one of the nonbridging α-phosphate oxygens in nucleoside 5′-triphosphate (Chart 1).

Potential use of these derivatives was originally described in boron neutron capture therapy for cancer treatment and antisense technologies.[5−7] Later, some interesting results were obtained using borane−nucleotide analogues as inhibitors of HIV-1 RT,[8] which were capable to overcome resistance recovering sensitivity of the mutant RTs to these inhibitors.[9−12] Indeed, the presence of an α-boranophosphate group in the triphosphate form of clinically relevant compounds, such as AZT, d4T, or dideoxyadenosine (ddA), does not influence the binding of the analogue to the RT active site but specifically provides (or restores) a high incorporation rate of the analogue by wild-type and mutant HIV-1 RTs.[11,12]

Chart 1. Example of 5′-(α-*P*-Borano)triphosphate Nucleoside



5'-α-BH_3-AZT-TP

The concept of the acyclic nucleoside phosphonate (ANPs) has been used to design chain terminators[13,14] for antiviral therapy and proved to be valid.[15−17] Currently, tenofovir disoproxil fumarate [a bis-POC prodrug of (*R*)-9-(2-phosphonomethoxypropyl)adenine, (*R*)-PMPA][1] and adefovir dipivoxil [a bis-POM prodrug of 9-(2-phosphonomethoxyethyl)adenine, PMEA],[18] two ANPs, are used clinically as anti-HIV and anti-HBV (hepatitis B virus) drugs, respectively. There is considerable interest in phosphonates as biologically active mimics of natural phosphates. Phosphonate replacement is attractive, since the carbon−phosphorus bond is not susceptible to enzymatic cleavage by phosphatases, thus enhancing physiological stability. Moreover, the presence of a α-phosphonate allows circumventing the often rate-limiting first phosphorylation step.

According to the powerful antiviral activity of the acyclic nucleoside phosphonate analogues and the advantages granted to triphosphate nucleosides by the presence of an α-P-borane group, we have developed chimeric α-boranophosphonate nucleosides. We have explored the synthesis of 9-[2-(boranophosphonomethoxy)ethyl]adenine (**6a**) and (*R*)-9-[2-(boranophosphonomethoxy)propyl]adenine (**6b**), where one of the nonbridging oxygen atoms of the α-phosphonate group is replaced by a BH_3 group (Chart 2). The aim of this concept is to obtain synergy between α-*P*-borano derivative properties and

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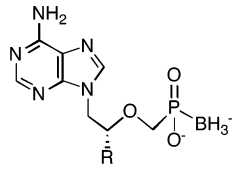
Chart 2. Designed α-Boranophosphonate Analogues **6a** and **6b**



6a R = H , 9-[2-(Boranophosphonomethoxy)ethyl]adenine
6b R = Me, (*R*)-9-[2-(Boranophosphonomethoxy)propyl]adenine

phosphonate derivative properties. These compounds are analogous to nucleoside α-boranophosphates with respect to the oxidation state of the phosphorus atom. They have not been described so far in the chemistry of nucleic acid analogues, and they have not been screened in vitro against viruses.

Results and Discussion

Chemistry. The strategy developed for the preparation of the α-boranophosphonate nucleosides is depicted in Scheme 1. Methods initially described for the preparation of α-boranophosphates via phosphoramidite[19] or H-phosphonate[20] cannot be applied for the synthesis of α-boranophosphonate derivatives. Instead, an efficient way via the boronation of an activated H-phosphinate intermediate was used to prepare the target compounds 9-[2-(boranophosphonomethoxy)ethyl]adenine (**6a**) and (*R*)-9-[2-(boranophosphonomethoxy)propyl]adenine (**6b**).

Reaction of adenine and 2-bromoethylbenzoate[21] in the presence of sodium hydride in *N*,*N*-dimethylformamide at 60 °C, followed by the cleavage of the benzoate protecting group in methanol saturated with ammonia gave the compound **1a** with 84% yield. Condensation of adenine with (*R*)-propylene carbonate in *N*,*N*-dimethylformamide at 140 °C for 16 h in the presence of sodium hydroxide in catalytic amount[22,23] afforded the pure alcohol **1b** isolated by crystallization directly from the reaction mixture with 81% yield. The resulting alcohols **1a** and **1b** were alkylated with diethyl *p*-toluenesulfonyloxymethanephosphonate **2** in the presence of sodium *tert*-butoxide in *N*,*N*-dimethylformamide,[22,24] which consistently gave diethyl phosphonates **3a** and **3b** with 27% and 29% yields, respectively. Efforts to improve these yields through use of an excess of reagent **2** or by reaction with the triflate analogue of the phosphonate **2** remained ineffective.[25]

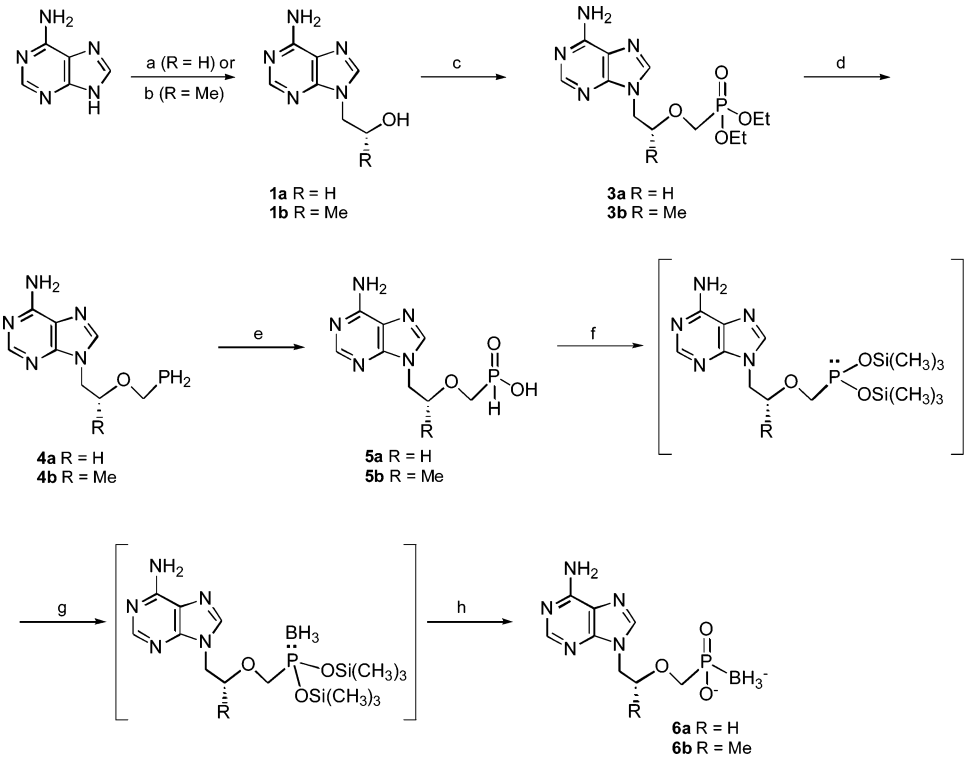
The original approach to obtain the desired compounds consists of the reduction of diethyl ester phosphonate by some of the methods described in the synthesis of primary phosphine.[26−28] Here, we achieved effective reduction of the diethyl phosphonates **3a** and **3b** using lithium aluminum hydride ($LiAlH_4$) and chlorotrimethylsilane (TMSCl). $LiAlH_4$ reduction of the two phosphonate diesters **3a** and **3b** in tetrahydrofuran mediated by the addition of a stoechiometric quantity of TMSCl afforded phosphines **4a** and **4b** with 78 and 65% yields, respectively. Phosphines **4a** and **4b** were easily isolated, purified, and characterized. Both phosphines were oxidized with 2 equiv of hydrogen peroxide in a mixture of water/tetrahydrofuran to afford the H-phosphinates **5a** and **5b** in quantitative yields.[29,30] Progress of the reaction was followed by ^{31}P NMR spectroscopy. Oxidation of **4a** and **4b** proved to be fast and went to completion within 1 h. Inspection of the ^{31}P NMR spectra showed that the signals from **4a** (δ: −144.67 ppm, tt, $^1J_{PH}$ = 199.0 Hz and $^2J_{PH}$ = 7.8 Hz) and **4b** (δ: −144.86 ppm, tt, $^1J_{PH}$ = 198.5 Hz and $^2J_{PH}$ = 7.3 Hz) were replaced by new resonances at 21.15 for **5a** (dm, $^1J_{PH}$ = 528 Hz) and 24.64 ppm for **5b** (dm, $^1J_{PH}$ = 528 Hz).

Because the phosphorus atom in the H-phosphinate form lacks a free electron pair and is not a suitable donor for the BH_3 group introduction, the boronation procedure requires an in situ intermediate activation of the H-phosphinate with a silylating agent,[31] leading to a disilyl phosphonite. H-phosphinates **5a** and **5b** in anhydrous tetrahydrofuran were activated in situ with *N*,*O*-bis(trimethylsilyl)acetamide (BSA) in 1 h into the corresponding disilyl phosphonite intermediates before the oxidation step. We tested several borane complexes in different solvents to optimize boronation conditions. Fast and relative efficient boronation could be achieved with borane−*N*,*N*-diisopropylethylamine complex (BH_3·DIPEA). Borane−tetrahydrofuran (BH_3·THF), borane−pyridine (BH_3·pyridine), and borane−dimethyl sulfide (BH_3·Me_2S) complexes required longer reaction times and/or gave lower yields. Moreover, in an attempt to minimize the formation of the diboronated product, described as the main product in some cases,[8] the amount of borane complex was reduced to a maximum of 2 equiv to reach the best compromise between the consuming of starting material and the appearance of side products. In situ boronation of the disilyl phosphonites from **5a** and **5b** with 2 equiv of BH_3·DIPEA resulted in the formation of bis(trimethylsilyl)-α-boranophosphonates.[32−34] Without isolation, the obtained intermediates were treated with concentrated ammonium hydroxide (30%) in methanol (1:1, v/v) to remove the trimethylsilyl group, yielding the α-boranophosphonates **6a** and **6b**. The presence of the P−B bond was confirmed by ^{31}P NMR spectra,[20,35] which showed a typical broad peak at 83 and 98 ppm, respectively. Pure α-boranophosphonates **6a** and **6b** were isolated by reverse phase chromatography in 28% and 32% yields, respectively.

Antiviral Activity. Compounds **5a**, **5b**, **6a**, and **6b** were evaluated for antiviral activity against HIV-1 in MAGI-CCR5 cells. PMEA and (*R*)-PMPA were chosen as standard compounds for this evaluation because there are effective derivatives against HIV-1.[13] Indeed, PMEA and (*R*)-PMPA exhibited in vitro activity against HIV-1 in this assay with 50% effective concentration (EC_{50}) values of 5.04 and 0.35 μM, respectively. None of the compounds **5a**, **5b**, **6a**, and **6b** exhibited any activity and cytotoxicity at concentrations as high as 400 μM. Related to the broad-spectrum antiviral profile of acyclic nucleoside phosphonate analogues, compounds **5a**, **5b**, **6a**, and **6b** were also evaluated for antiviral activity against other viruses, including hepatitis C virus (subgenomic replicon in Huh-5-2 cells), herpes simplex virus type 1 (HSV-1) and type 2 (HSV-2), vaccinia virus, vesicular stomatitis virus in HEL cells; Coxsackie B4 virus and respiratory syncytial virus in Hela cells; and para-influenza-3 virus, reovirus-1, sindbis virus, and Punta Toro virus in Vero cells. No significant cytotoxicities were reported for any of the compounds up to 200 μM. Compounds **5a**, **5b**, **6a**, and **6b** exhibited no significant in vitro antiviral activity up to 200 μM.

Stability Studies. Any effective antiviral compound has to be resistant enough to hydrolysis that might occur before it reaches the bloodstream and should be lipophilic enough to cross the gastrointestinal barrier. Therefore, to test the usefulness of α-boranophosphonates **6a** and **6b** and the intermediates H-phosphinates **5a** and **5b**, it is necessary to study their chemical and enzymatic stabilities. PMEA and (*R*)-PMPA were chosen as standard compounds. To measure the relative chemical and enzymatic stabilities of the compounds, kinetic data of decomposition for derivatives **5a**, **5b**, **6a**, **6b**, and standard compounds were studied at 37 °C (a) in several buffers (pH 1.2, 5.2, 7.4, 8.1, 9.0, 11.5), (b) in RPMI 1640, (c) in culture medium (RPMI 1640 containing 10% heat-inactivated fetal calf serum), and (d) in total cell extract (CEM-SS cell). These various media were thought to be valid in vitro models for the different types of

Scheme 1[a]



[a] Reagents and conditions: (a) 2-bromoethyl benzoate, NaH, DMF, 60 °C, 16 h then sat. NH_3/MeOH, 14 h; (b) (*R*)-propylene carbonate, NaOH, DMF, 140 °C, 16 h; (c) diethyl [[(*p*-toluenesulfonyl)oxy]methyl] phosphonate (**2**), sodium *tert*-butoxide, DMF, rt, 72 h; (d) TMSCl, $LiAlH_4$, THF, −78 °C then rt, 2 h; (e) H_2O_2, H_2O/THF, rt, 1 h; (f) BSA, THF, rt, 1 h (g) BH_3·DIPEA, THF, rt, 1 h; (h) NH_4OH:MeOH (1:1, v/v).

Table 1. Calculated Half-Lives of the Derivatives **5a**, **5b**, **6a**, and **6b** Compared to PMEA and (*R*)-PMPA in Several Chemical and Enzymatic Media

	$t_{1/2}$ (h)			
compd	buffers[a]	RPMI-1640	culture medium	total cell extracts
5a	stable[b]	>72	>72	>72
5b	stable	>72	>72	
6a	stable	>72	5.3[c]	
6b	stable	>72	1.3[c]	24[c]
PMEA	stable	>72	>72	>72
(*R*)-PMPA	stable	>72	>72	>72

[a] pH of the buffers: 1.2, 5.2, 7.4, 8.1, 9.0, 11.5. [b] "Stable" means less than 5% decomposition after 72 h. [c] Single product of decomposition: **5a** from **6a** and **5b** from **6b**.

decomposition that may affect the compounds in vivo. The RPMI 1640 represents a free enzyme system but a nucleophile-enriched medium.[36] The culture medium is correlated with an enzymatic nucleophile-enriched medium (extracellular medium mimic), and the conditions of total cell extract are used to mimic the intracellular medium. Crude aliquots of incubation mixtures were directly analyzed by using a described on-line HPLC cleaning method.[37] In this technique, a crude biological sample is directly injected by means of a buffer (ion pairing reagent) in a short reverse-phase precolumn that does not retain proteins but retains the analytes of interest. The precolumn is connected to an analytical column and when the strength of the eluent is increased, the analytes are transferred on to an analytical column and chromatographed; all metabolites could be easily detected. The amount of remaining parent compound at each time point was used to determine the half-live of the compound. Results are summarized in Table 1.

We observed that all compounds are stable in acidic, neutral, and basic buffers for more than 72 h. Indeed, compounds **5a**, **5b**, **6a**, and **6b** are stable for more than 72 h (less than 5% decomposition) in all buffers. The standard compounds PMEA and (*R*)-PMPA are stable in all nonenzymatic and enzymatic media. In nonenzymatic medium (RPMI 1640), the α-boranophosphonates **6a** and **6b** are also stable for more than 72 h. In culture medium, the half-life of **6a** and **6b** is approximately 15 and 50 times shorter, respectively, than in RPMI 1640. In both cases, the decomposition leads to the formation of a less lipophilic compound. Co-injections with authentic samples allowed us to attribute this signal to the corresponding H-phosphinate derivatives **5a** and **5b**, respectively. No other metabolite was observed in both cases. Indeed, the H-phosphinate intermediates **5a** and **5b** are very stable, as shown in Table 1. In total cell extracts, **6a** and **6b** are slowly hydrolyzed to give rise, as in culture medium, to the formation of **5a** and **5b**, with half-life values of 48 and 24 h, respectively. In general, compound **6a** was observed to be more stable than compound **6b** in culture medium and total cell extracts. The difference between decomposition rates of **6a** and **6b** in total cell extracts and in culture medium might be attributed to the difference in the enzymatic content between both media. Our results show that the observed metabolism of an α-boranophosphonate derivative consists of the conversion of the P−B bond into a P−H bond, which leads to the formation of a H-phosphinate derivative, the later being very stable in a large panel of chemical and enzymatic media. The unique degradation pathway of an α-boranophosphonate derivative is achieved via BH_3 group reduction and is mediated by an enzymatic nucleophile-enriched medium. In all tested media, we did not observe hydrolysis of the α-boranophosphonate group by nucleophilic attack on the phosphorus atom, yielding a phosphonate group. Moreover, breakage of the α-boranophosphonate linkage has not been observed under these conditions.

Conclusion

Novel α-boranophosphonate derivatives have been synthesized: 9-[2-(boranophosphonomethoxy)ethyl]adenine (**6a**) and (*R*)-9-[2-(boranophosphonomethoxy)propyl]adenine (**6b**). Practical synthetic procedures have been developed. Target compounds were screened for their antiviral activity in vitro against HIV-1 and other DNA or RNA viruses. No significant cytotoxicities were reported for any of the compounds up to 200 μM. Compounds **5a**, **5b**, **6a**, and **6b** did not exhibit any in vitro activity up to 200 μM against all tested viruses. The effective activity of an antiviral acyclonucleotide requires a good stability in physiologic media, transport of the derivative across cell membranes, phosphorylation to the triphosphate analogue, and efficient, selective utilization of the triphosphate form by the viral DNA polymerase as an alternate substrate. Stability studies of α-boranophosphonates **6a** and **6b** in culture medium have shown a modest stability of these derivatives and a unique metabolism in enzymatic nucleophile-enriched medium via the reduction of the P−B bond into a P−H bond. The compounds **6a** and **6b** are metabolized in culture medium into H-phosphinates **5a** and **5b**, with half-live values of 5.3 h for **6a** and 1.3 h for **6b**. The lability of the BH_3 group of the α-boranophosphonates **6a** and **6b** in culture medium may be a major limitation of these derivatives. Therefore, it appears unlikely that the α-boranophosphonate can be uptaken in cells and that its active form can reach the target viral enzyme before its decomposition. Questions concerning the transport of α-boranophosphonates through cellular membranes remain unanswered and are to be considered. We are currently investigating (1) the propensity of the compounds **5a**, **5b**, **6a**, and **6b** to be phosphorylated by cellular kinases (adenylate kinases 1 and 2) and other kinases; (2) the synthesis of the potentially active triphosphate form of the derivatives **6a** and **6b**; and (3) the evaluation of the affinity and the incorporation of the triphosphate form by HIV-1 wild-type and mutant RTs. These results will be reported in due time.

Experimental Section

Chemistry. Melting points were determined in capillary tubes with a 9100 Electrothermal (Fisher Scientific) apparatus and are uncorrected. The ^{1}H, ^{13}C, and ^{31}P NMR spectra were determined with a BRUKER AMX at 250 MHz and the ^{11}B NMR spectra were determined with a BRUKER AMX at 400 MHz. Chemical shifts are expressed in ppm and coupling constants (*J*) are in hertz (s = singlet, bs = broad singlet, d = doublet, dd = double doublet, t = triplet, dt = double triplet, td = triple doublet, qd = quadruple doublet, m = multiplet, dm = double multiplet). FAB mass spectra (MS) and high-resolution mass spectra (HRMS) were obtained on a JEOL SX 102 mass spectrometer using a cesium ion source and a glycerol/thioglycerol matrix (GT). Preparative flash column chromatographies were performed using silica gel (Merck) G60 230−240 mesh. Analytical thin layer chromatographies (TLC) were performed on silica gel 60F 254 aluminum plates (Merck) of 0.2 mm thickness. The spots were examined with a UV light and Cericdip spray. Analytical high-performance liquid chromatography (HPLC) analyses were carried out on a Waters Associates unit equipped with a model 600E multisolvent delivery system, a model 600E controller system, a model Rheodyne sample injector, a 991 photodiode array detector, and an in-line degazer AF. Samples were eluted using a linear gradient of 0.05 M triethylammonium bicarbonate (TEAB) buffer in 100% water (pH 7.5) (buffer A) to 0.05 M TEAB in 50% acetonitrile (buffer B), programmed over a 60-min period with a flow rate of 1 mL/min and detection at 260 nm. All solvents were of HPLC grade and filtered prior to use. A 1 M solution of triethylammonium bicarbonate buffer was prepared by adding dry ice to a 1 M triethylamine solution until the pH reached 7.5. Triethylammonium bicarbonate solutions were made fresh by dissolving reagent-grade triethylammonium bicarbonate in HPLC-grade water prior to filtration. Analytical reverse-phase chromatography was carried out on a 4.6 × 100 mm Source 15RPC column. Preparative purifications of α-boranophosphonate derivatives were achieved on an ÄKTA*prime* FPLC (Amersham) using a reverse-phase Source 30RPC column (18 × 350 mm) and a linear gradient of 0.05 M TEAB buffer in 100% water (pH 7.5) (buffer A) to 0.05 M TEAB buffer in 50% acetonitrile (buffer B), programmed over a 6 h period with a flow rate of 2 mL/min and detection at 254 nm.

9-(2-Hydroxyethyl)adenine (1a). To a suspension of 60% sodium hydride in mineral oil (1.70 g, 42.57 mmol) in anhydrous *N*,*N*-dimethylformamide (120 mL) was added adenine (5.23 g, 38.70 mmol) under argon, and the mixture was heated at 60 °C for 1 h. 2-Bromoethyl benzoate (9.2 mL, 58.06 mmol) was added dropwise at 60 °C, and the reaction was stirred at this temperature for 16 h, under argon. The mixture was then filtered to remove insoluble material, and the filtrate was evaporated under reduced pressure and coevaporated three times with toluene. The residue was triturated with ethyl acetate and then filtered to give a white solid, which was immediately resuspended in methanol saturated with ammonia (400 mL). The reaction mixture was stirred for 14 h at room temperature, and then methanol was removed under reduced pressure. Recrystallization from ethanol afforded compound **1a** (5.87 g, 85%). Mp: 236 °C (lit.[38] mp: 238−239 °C). ^{1}H NMR (DMSO-d_6) δ: 8.13 (s, 1H, H-2), 8.10 (s, 1H, H-8), 7.23 (bs, 2H, NH_2), 5.05 (bs, 1H, OH), 4.19 (t, J = 5.2 Hz, 2H, CH_2O), 3.71 (t, J = 5.2 Hz, 2H, CH_2N). ^{13}C NMR (DMSO-d_6) δ: 155.79, 152.23, 149.44, 141.46, 118.55, 59.17, 45.61. MS (GT, FAB$^+$): 136 (adenine + 1H)$^+$, 180 (M + 1H)$^+$, 202 (M + Na)$^+$.

(*R*)-9-(2-Hydroxypropyl)adenine (1b). A solution of adenine (3.85 g, 28.49 mmol), (*R*)-propylene carbonate (2.7 mL, 31.34 mmol), and pulverized sodium hydroxide (60 mg, 1.42 mmol) in anhydrous *N*,*N*-dimethylformamide (80 mL) was heated at 140 °C with stirring for 16 h, under argon. After cooling, the mixture was then filtered to remove insoluble material, and the filtrate was evaporated under reduced pressure and coevaporated three times with toluene. The residue was triturated with ethyl acetate and then filtered to give a white solid which was immediately recrystallized in ethanol to afford compound **1b** (4.5 g, 81%). Mp: 193 °C (lit.[39] mp: 192−195 °C). ^{1}H NMR (DMSO-d_6) δ: 8.15 (s, 1H, H-2), 8.06 (s, 1H, H-8), 7.22 (bs, 2H, NH_2), 5.06 (bs, 1H, OH), 4.06 (m, 3H, CH_2N and CHO), 1.12 (d, J = 5.7 Hz, 3H, CH_3). ^{13}C NMR (DMSO-d_6) δ: 155.87, 152.21, 149.67, 141.44, 118.50, 64.57, 50.07, 20.80. MS (GT, FAB$^+$): 136 (adenine + 1H)$^+$, 194 (M + 1H)$^+$, 216 (M + Na)$^+$, 232 (M + K)$^+$, 387 (2M + 1H)$^+$.

Diethyl [[(*p*-Toluenesulfonyl)oxy]methyl] phosphonate (2). Triethylamine (3.38 mL, 24.04 mmol) was added dropwise to a stirred solution of diethyl hydroxymethylphosphonate (3.85 g, 24.04 mmol) in anhydrous diethyl ether (30 mL). After the mixture had cooled to −10 °C, a solution of *p*-toluenesulfonyl chloride (4.58 g, 24.04 mmol) in anhydrous diethyl ether (10 mL) was added dropwise with the internal temperature maintained at −10 °C. After being stirred at 0 °C for 1 h, the mixture was allowed to warm to room temperature and was then stirred 16 h, under argon. Diethyl ether (80 mL) was added and the solid was filtered off. The solvents were removed under reduced pressure, and the oil was purified by silica gel flash chromatography [eluent, stepwise gradient of ethyl acetate (0−10%) in dichloromethane] to afford the compound **2** (5.57 g, 75%) as an colorless oil. ^{1}H NMR ($CDCl_3$) δ: 7.76 (d, J = 8.1 Hz, 2H, Ts), 7.33 (d, J = 8.1 Hz, 2H, Ts), 4.15 (m, 6H, CH_2 from $P(OEt)_2$ and CH_2P), 2.40 (s, 3H, Ts), 1.28 (t, J = 6.9 Hz, 6H, CH_3 from $P(OEt)_2$). ^{13}C NMR ($CDCl_3$) δ: 145.91, 132.05, 130.38, 128.53, 63.75 (d, J = 6.6 Hz), 62.76 (d, J_{CP} = 168.8 Hz), 21.99, 16.69 (d, J = 5.5 Hz). MS (GT, FAB$^+$): 155 (Ts)$^+$, 267 (M − 2Et)$^+$, 295 (M − Et)$^+$, 323 (M + 1H)$^+$, 645 (2M + 1H)$^+$.

9-[2-(Diethylphosphonomethoxy)ethyl]adenine (3a). To a solution of compound **1a** (1.48 g, 8.25 mmol) in anhydrous *N*,*N*-dimethylformamide (40 mL) was added, at room temperature, sodium *tert*-butoxide (1.38 g, 8.25 mmol). The mixture was stirred

40 min, under argon, and then a solution of diethyl phosphonate **2** (2.66 g, 8.25 mmol) in anhydrous *N*,*N*-dimethylformamide (10 mL) was added. After 72 h, the mixture was filtered and concentrated under reduced pressure. The residue dissolved in water was extracted with chloroform. The organic extracts were dried over magnesium sulfate, filtered, and concentrated under reduced pressure. The residue was purified by silica gel flash chromatography [eluent, stepwise gradient of methanol (0−10%) in dichloromethane]-to give **3a** (740 mg, 27%) as a white solid. ^{1}H NMR (CD_3OD) δ: 8.10 (s, 1H, H-2), 8.03 (s, 1H, H-8), 4.35 (t, J = 5.0 Hz, 2H, CH_2N), 3.93 (dq, J = 8.0 and J = 7.0 Hz, 4H, CH_2 from $P(OEt)_2$), 3.86 (t, J = 5.0 Hz, 2H, CH_2O), 3.76 (d, J_{PH} = 8.5 Hz, 2H, CH_2P), 1.11 (td, J = 7.0 Hz and J = 0.5 Hz, 6H, CH_3 from $P(OEt)_2$). ^{13}C NMR (CD_3OD) δ: 157.29, 153.71, 150.67, 143.34, 119.34, 72.15 (d, J = 11.9 Hz), 66.63 (d, J_{CP} = 166 Hz), 63.99 (d, J = 6.6 Hz), 44.54, 16.62 (d, J = 5.8 Hz). ^{31}P NMR (CD_3OD) δ: 21.56. MS (GT, FAB$^+$): 136 (adenine + 1H)$^+$, 330 (M + 1H)$^+$, 352 (M + Na)$^+$.

(*R*)-9-[2-(Diethylphosphonomethoxy)propyl]adenine (3b). To a solution of compound **1b** (1.44 g, 7.44 mmol) in anhydrous *N*,*N*-dimethylformamide (30 mL) was added, at room temperature, sodium *tert*-butoxide (1.25 g, 7.44 mmol). The mixture was stirred for 1 h, under argon, and then a solution of diethyl phosphonate **2** (2.40 g, 7.44 mmol) in anhydrous *N*,*N*-dimethylformamide (10 mL) was added. After 64 h, the mixture was filtered and concentrated under reduced pressure. The residue dissolved in water was extracted with chloroform. The organic extracts were dried over magnesium sulfate, filtered, and concentrated under reduced pressure. The residue was purified by silica gel flash chromatography [eluent, stepwise gradient of methanol (0−10%) in dichloromethane]-to give **3b** (740 mg, 29%) as a white solid. ^{1}H NMR (CD_3OD) δ: 8.23 (s, 1H, H-2), 8.15 (s, 1H, H-8), 4.41 (dd, J = 14.5 Hz and J = 3.2 Hz, 1H, CH_aN), 4.22 (dd, J = 14.5 Hz and J = 7.7 Hz, 1H, CH_bN), 4.10 (m, 1H, CHO), 4.03−3.69 (m, 6H, CH_2 from $P(OEt)_2$ and CH_2P), 1.33 (t, J = 7.1 Hz, 6H, CH_3 from $P(OEt)_2$), 1.27 (d, J = 6.2 Hz, 3H, CH_3). ^{13}C NMR (CD_3OD) δ: 157.30, 153.70, 150.89, 143.63, 119.70, 77.65 (d, J = 12.3 Hz), 64.32 (d, J_{CP} = 167 Hz), 64.08 (d, J = 6.6 Hz), 63.95 (d, J = 6.6 Hz), 49.18, 16.73 (d, J = 5.8 Hz), 16.71, 16.70 (d, J = 5.8 Hz). ^{31}P NMR (CD_3OD) δ: 22.11. MS (GT, FAB$^+$): 136 (adenine + 1H)$^+$, 344 (M + 1H)$^+$, 366 (M + Na)$^+$, 687 (2M + 1H)$^+$.

9-[2-(Phosphanylmethoxy)ethyl]adenine (4a). Chlorotrimethylsilane (3.15 mL, 24.78 mmol) was added dropwise to a stirred solution of lithium aluminum hydride (940 mg, 24.78 mmol) in anhydrous tetrahydrofuran (50 mL) at −78 °C. The resulting mixture was allowed to warm to room temperature and stirred for 2 h, under argon. Compound **3a** (2.04 g, 6.19 mmol) in anhydrous tetrahydrofuran (200 mL) was added to the reducing mixture at −50 °C. The mixture was allowed to warm to room temperature and stirred for 2 h. The reaction was stopped by addition of water (10 mL) and sodium hydroxide (20% solution, 10 mL). The mixture was filtered through Celite. The organic layer was dried over magnesium sulfate, filtered, and concentrated under reduced pressure. A purification by silica gel flash chromatography [eluent, stepwise gradient of methanol (0−5%) in dichloromethane] yielded **4a** (1.08 g, 78%) as a white powder. HPLC purity: >98%. ^{1}H NMR (CD_3OD) δ: 8.10 (s, 1H, H-8), 7.99 (s, 1H, H-2), 4.30 (t, J = 5.1 Hz, 2H, CH_2N), 3.87 (m, 2H, CH_2P), 3.71 (t, J = 5.1 Hz, 2H, CH_2O), 3.07 and 2.81 (dm, $^1J_{PH}$ = 199.0 Hz, 2H, PH_2). ^{13}C NMR (CD_3OD) δ: 157.31, 153.68, 150.71, 143.32, 119.94, 70.23 (d, J = 2.8 Hz), 62.49 (d, J_{CP} = 12.6 Hz), 44.70. ^{31}P NMR (CD_3OD) δ: −144.67 (tt, $^1J_{PH}$ = 199.0 Hz and $^2J_{PH}$ = 7.8 Hz). MS (GT, FAB$^+$): 136 (adenine + 1H)$^+$, 226 (M + 1H)$^+$.

(*R*)-9-[2-(Phosphanylmethoxy)propyl]adenine (4b). Chlorotrimethylsilane (2.62 mL, 20.62 mmol) was added dropwise to a stirred solution of lithium aluminum hydride (782 mg, 20.62 mmol) in anhydrous tetrahydrofuran (40 mL) at −78 °C. The resulting mixture was allowed to warm to room temperature and stirred for 2 h, under argon. Compound **3b** (1.77 g, 5.15 mmol) in anhydrous tetrahydrofuran (150 mL) was added to the reducing mixture at −78 °C. The mixture was allowed to warm to room temperature and stirred for 2 h. The reaction was stopped by addition of water (10 mL) and sodium hydroxide (20% solution, 10 mL). The mixture was filtered through Celite. The organic layer was dried over magnesium sulfate, filtered, and concentrated under reduced pressure. A purification by silica gel flash chromatography [eluent, stepwise gradient of methanol (0−5%) in dichloromethane] yielded **4b** (795 mg, 65%) as a white powder. HPLC purity: >98%. ^{1}H NMR (CD_3OD) δ: 8.11 (s, 1H, H-8), 7.97 (s, 1H, H-2), 4.25−3.60 (m, 5H, CH_2N, CHO and CH_2P), 2.90 and 2.13 (dt, $^1J_{PH}$ = 198.5 Hz and J = 6.3 Hz, 2H, PH_2), 1.08 (d, J = 6.1 Hz, 3H, CH_3). ^{13}C NMR (CD_3OD) δ: 157.31, 153.68, 150.82, 143.67, 119.73, 75.91 (d, J = 2.3 Hz), 60.25 (d, J_{CP} = 12.0 Hz), 49.48, 17.16. ^{31}P NMR (CD_3OD) δ: −144.86 (tt, $^1J_{PH}$ = 198.5 Hz and $^2J_{PH}$ = 7.3 Hz). MS (GT, FAB$^+$): 136 (adenine + 1H)$^+$, 240 (M + 1H)$^+$.

9-[2-(Hydroxyphosphinylmethoxy)ethyl]adenine (5a). To a stirred solution of **4a** (1.08 g, 4.79 mmol) in water (30 mL) and tetrahydrofuran (30 mL) was added dropwise 2% aqueous hydrogen peroxide solution (10.8 mL). The mixture was stirred at room temperature for 1 h and concentrated under reduced pressure to give after freeze-drying compound **5a** (1.23 g, quant) as a white powder (lit.[40] mp: 229−230 °C). HPLC purity: >99%. ^{1}H NMR (DMSO-d_6) δ: 8.17 (s, 1H, H-8), 8.15 (s, 1H, H-2), 7.96 and 5.85 (dt, $^1J_{PH}$ = 528.0 Hz and J = 2.1 Hz, 1H, PH), 7.40 (bs, 2H, NH_2), 4.36 (t, J = 5.2 Hz, 2H, CH_2N), 3.92 (t, J = 5.2 Hz, 2H, CH_2O), 3.69 (dd, J = 7.2 Hz and J = 2.1 Hz, 2H, CH_2P). ^{13}C NMR (DMSO-d_6) δ: 155.44, 151.66, 149.35, 141.32, 118.46, 70.33 (d, J = 10.8 Hz), 69.04 (d, J_{CP} = 109.0 Hz), 42.39. ^{31}P NMR (DMSO-d_6) δ: 21.15 (dm, $^1J_{PH}$ = 528.0 Hz). MS (GT, FAB$^+$): 136 (adenine + 1H)$^+$, 258 (M + 1H)$^+$, 515 (2M + 1H)$^+$. HRMS (FAB): calcd for $C_8H_{13}N_5O_3P$ (M + H)$^+$ 258.0756, found 258.0765.

(*R*)-9-[2-(Hydroxyphosphinylmethoxy)propyl]adenine (5b). To a stirred solution of **4b** (795 mg, 3.32 mmol) in water (20 mL) and THF (20 mL) was added dropwise 2% aqueous hydrogen peroxide (7.5 mL, 2 equiv). The mixture was stirred at room temperature for 1 h and concentrated under reduced pressure to give after freeze-drying compound **5b** (900 mg, quant) as a white powder. HPLC purity: >99%. ^{1}H NMR (DMSO-d_6) δ: 8.23 (s, 1H, H-8), 8.20 (s, 1H, H-2), 7.95 and 5.85 (dt, $^1J_{PH}$ = 528.0 Hz and J = 2.2 Hz, 1H, PH), 7.59 (bs, 2H, NH_2), 4.38 (dd, J = 14.2 Hz and J = 3.9 Hz, 1H, CH_aN), 4.31 (dd, J = 14.2 Hz and J = 6.0 Hz, 1H, CH_bN), 4.10 (m, 1H, CHO), 3.75 (m, 2H, CH_2P), 1.13 (d, J = 6.8 Hz, 3H, CH_3). ^{13}C NMR (DMSO-d_6) δ: 155.21, 151.35, 149.56, 141.79, 118.17, 75.48 (d, J = 11.0 Hz), 67.19 (d, J_{CP} = 111.2 Hz), 46.64, 16.93. ^{31}P NMR (DMSO-d_6) δ: 24.64 (dm, $^1J_{PH}$ = 528.0 Hz). MS (GT, FAB$^+$): 136 (adenine + 1H)$^+$, 272 (M + 1H)$^+$, 543 (2M + 1H)$^+$. HRMS (FAB): calcd for $C_9H_{15}N_5O_3P$ (M + H)$^+$ 272.0913, found 272.0905.

9-[2-(Boranophosphonomethoxy)ethyl]adenine (6a). Compound **5a** (200 mg, 0.78 mmol) was dried over phosphorus pentoxide under vacuum for 5 h and then dissolved in anhydrous tetrahydrofuran (10 mL). Anhydrous tetrahydrofuran was previously flushed with argon. BSA (960 μL, 3.89 mmol) was added by syringe and the solution was stirred for about 1 h at room temperature, under argon. DIPEA·BH_3 (270 μL, 1.56 mmol) was added, the solution was stirred for 1 h, and concentrated ammonium hydroxide (30%) in methanol (10 mL, 1:1, v/v) was added to the solution. After the solvents were evaporated under reduce pressure, the residue was purified by reverse-phase column chromatography (linear gradient 0−100% buffer B). Product fractions were collected and evaporated to dryness. An excess of triethylammonium bicarbonate was removed by repeated freeze-drying with deionized water to give **6a** (58 mg, 28%) as a white powder. HPLC purity: >98%. ^{1}H NMR (DMSO-d_6) δ: 8.16 (s, 1H, H-8), 8.08 (s, 1H, H-2), 7.12 (bs, 2H, NH_2), 4.22 (t, J = 5.2 Hz, 2H, CH_2N), 3.79 (t, J = 5.2 Hz, 2H, CH_2O), 3.33 (m, 2H, CH_2P), 0.5 to −0.10 (q, J = 88.0 Hz, 3H, BH_3). ^{31}P NMR (DMSO-d_6) δ: 83.04 (q, J_{PB} = 117.0 Hz). ^{11}B NMR (DMSO-d_6) δ: −31.38 (d, J_{PB} = 116.0 Hz). MS (GT, FAB$^-$): 270 (M)$^-$, 515 (M − BH_3)$^-$. HRMS (FAB): calcd for $C_8H_{14}N_5O_3PB$ (M)$^-$ 270.0927, found 270.0904.

(*R*)-9-[2-(Boranophosphonomethoxy)propyl]adenine (6b). Compound **5b** (200 mg, 0.74 mmol) was dried over phosphorus

pentoxide under vacuum for 5 h and then dissolved in anhydrous tetrahydrofuran (10 mL). Anhydrous tetrahydrofuran was previously flushed with argon. BSA (910 μL, 3.69 mmol) was added by syringe and the solution was stirred for about 1 h at room temperature, under argon. DIPEA·BH_3 (256 μL, 1.47 mmol) was added, the solution stirred for 1 h, and concentrated ammonium hydroxide (30%) in methanol (10 mL, 1:1, v/v) was added to the solution. After the solvents were evaporated under reduce pressure, the residue was purified by reverse-phase column chromatography (linear gradient 0−100% buffer B). Product fractions were collected and evaporated to dryness. The excess triethylammonium bicarbonate was removed by repeated freeze-drying from deionized water to give **6b** (66 mg, 32%) as a white powder. HPLC purity: >98%. ^{1}H NMR (D_2O) δ: 8.20 (s, 1H, H-8), 8.18 (s, 1H, H-2), 4.38 (dd, J = 15.0 Hz and J = 3.0 Hz, 1H, CH_aN), 4.21 (dd, J = 15.0 Hz and J = 7.7 Hz, 1H, CH_bN), 3.99 (m, 1H, CHO), 3.55 (m, 2H, CH_2P), 1.19 (d, J = 6.2 Hz, 3H, CH_3), 0.5 to −0.20 (qd, J = 90.0 Hz and J = 18.0 Hz, 3H, BH_3). ^{31}P NMR (D_2O) δ: 98.27 (q, J_{PB} = 115.0 Hz). ^{11}B NMR (D_2O) δ: −34.11 (d, J_{PB} = 113.0 Hz). MS (GT, FAB$^-$): 284 $(M)^-$, 270 $(M - BH_3)^-$. HRMS (FAB): calcd for $C_9H_{16}N_5O_3PB$ $(M)^-$ 284.1084, found 284.1118.

Biological Methods. Antiviral Assays. Anti-HIV Assays in MAGI-CCR5 Cells. 293T and MAGI-CCR5 cells were maintained in Dulbecco's modified Eagle's medium supplemented with antibiotics and 10% heat-inactivated fetal bovine serum. To obtain HIV-1 stocks, 293T cells were transiently transfected with an HIV-1NL4.3 molecular clone by the use of FuGENE 6 transfectant reagent (Roche) as recommended by the manufacturer. Two days post-transfection, the CAp24 antigen was quantitated in cell-free culture supernatants by HIV-1 p24 antigen capture assay kit (Coulter). MAGI-CCR5 cells (10^4 cells), containing a *LacZ* reporter under control of an integrated HIV promoter, were seeded on 96-well microliter culture plates and treated during 4 h with increasing amounts of PMEA, (*R*)-PMPA, **5a**, **5b**, **6a**, and **6b** (0−400 μM) before being infected with 100 ng of HIV-1 CAp24 antigen by spinoculation as previously described.[41] Cells were then washed and grown in the presence of each compound. Two days later, cells were stained for β-galactosidase activity and blue cells were counted. The 50% effective concentration (EC_{50}) corresponds to the compound concentration producing a 50% decrease of the number of blue cells in the virus-infected cell cultures. The 50% cytostatic concentration (CC_{50}) corresponds to the compound concentration required to inhibit cell proliferation by 50%.

Anti-HSV, Anti-Vaccinia Virus, and Anti-Vesicular Stomatitis Virus Assays in HEL Cells. Anti-Coxsackie Virus B4 and Anti-Respiratory Syncytial Virus Assays in HeLa Cells. Anti-Parainfluenza-3 Virus, Anti-Reovirus-1, Anti-Sindbis Virus, and Anti-Punta Toro Virus Assays in Vero Cells. Human embryonic lung (HEL) (ATCC-CCL 137), simian kidney (Vero), and human cervix carcinoma (HeLa) cells were propagated in minimal essential medium (MEM) supplemented with 10% heat inactivated fetal calf serum (FCS), 2 mM L-glutamine, and 0.075% bicarbonate. Herpes simplex virus type 1 (HSV-1) (KOS), HSV-2 (G), vaccinia virus, and vesicular stomatitis virus were assayed in HEL cell cultures; Coxsackie virus B4 and respiratory syncytial virus in HeLa cell cultures; and parainfluenza-3 virus, reovirus-1, Sindbis virus, and Punta Toro virus in Vero cell cultures. Reference compounds, acyclovir and zidovudine, were from Glaxo Smith Kline, ganciclovir was from Hoffmann-La Roche, and cidofovir was from Gilead. Cells were grown to confluency in microtiter trays and were inoculated with 100 times the 50% cell culture infective dose. Compounds, either alone or in combination, were added after a 1−2 h virus adsorption period. The virus-induced cytopathic effect (CPE) was recorded microscopically at 3 days postinfection and expressed as a percentage of the untreated controls. The 50% effective concentrations (EC_{50}) were derived from graphical plots. The minimal toxic concentration (MTC) was defined as the minimal concentration that resulted in a microscopically detectable alteration of cell morphology. The MTC was determined in uninfected confluent cell cultures that were incubated, akin to the cultures used for the antiviral assays, with serial dilutions of the compounds for the same time period. Cultures were inspected microscopically for alteration of cell morphology.

Evaluation of Antiviral Activity and Cytostatic Activities of Selected Compounds in HCV Genotype 1b Subgenomic Replicon Carrying Huh-5-2 Cells. Huh-5-2 cells (a cell line with a persistent HCV replicon I389luc-ubi-neo/NS3−3′/5.1; replicon with firefly luciferase−ubiquitin−neomycin phosphotransferase fusion protein and an EMCV-IRES driven NS3-5B HCV polyprotein) are cultured in RPMI medium (GIBCO) supplemented with 10% heat-inactivated fetal calf serum, 2 mM L-glutamine (Life Technologies), 1× nonessential amino acids (Life Technologies); 100 IU/mL penicillin and 100 μg/mL streptomycin and 250 μg/mL G418 (Geneticin, Life Technologies). Cells are seeded at a density of 7000 cells per well in 96-well View Plate (Packard) in medium containing the same components as described above, except for G418. Cells are allowed to adhere and proliferate for 24 h. At that time, culture medium is removed and five serial dilutions (5-fold dilutions starting at 100 μg/mL or 100 μM) of the test compounds are added in culture medium lacking G418. Interferon alfa 2a (500 IU) is included as a positive control in each experiment for internal validation. Plates are further incubated at 37 °C and 5% CO_2 for 72 h. Replication of the HCV replicon in Huh-5-2 cells results in luciferase activity in the cells. Luciferase activity is measured by adding 50 μL of 1× Glo-lysis buffer (Promega) for 15 min followed by 50 μL of the Steady-Glo Luciferase assay reagent (Promega). Luciferase activity is measured with a luminometer and the signal in each individual well is expressed as a percentage of the untreated cultures. The 50% effective concentrations (EC_{50}) are calculated from these datasets. Parallel cultures of Huh-5-2 cells, seeded at a density of 7000 cells/well of classical 96-well cell culture plates (Becton-Dickinson) are treated in a similar fashion except that no Glo-lysis buffer or Steady-Glo Luciferase reagent is added. The effect of the compounds on the proliferation of the cells is measured 3 days after addition of the various compounds by means of The CellTiter 96 AQ_{ueous} Non-Radioactive Cell Proliferation Assay (MTS, Promega). In this assay 3-(4,5-dimethylthiazol-2-yl)-5-(3-carboxymethoxyphenyl)-2-(4-sulfophenyl)-2*H*-tetrazolium (MTS) is bioreduced by cells into a formazan that is soluble in tissue. The number of cells correlates directly with the production of the formazan. The MTS stained cultures are quantified in a platereader.

Stability Studies. Media and Preparation of Cell Extracts. RPMI 1640 medium was purchased from GIBCO Life Technologies. Heat-inactivated fetal calf serum was purchased from PAN biotech. Culture medium was composed of RPMI 1640 containing 10% heat-inactivated fetal calf serum and stored at −80 °C. CEM-SS cell extract was prepared according to a published procedure.[37] Exponentially growing CEM-SS cells were recovered by centrifugation (500 *g*, 4 °C, 4 min), washed twice with PBS, and resuspended in 10 mM Tris-HCl, 140 mM KCl (pH 7.4), at the concentration of 30×10^6 cells/mL. Cells were lyzed by ultrasonic treatment and cellular debris were removed by centrifugation (10 000*g*, 4 °C, 20 min). The supernatant containing soluble proteins (3 mg/mL) was stored at −80 °C.

HPLC Analysis. We used a previously described on-line HPLC cleaning method.[37] The cleaning precolumn is a Guard-Pak insert (Delta-Pak C18, 100 Å) in a Guard-Pak holder, with a prefilter. The analytical column used is a Novapak C18, 3 μm, 100 Å, 4.6 × 150 mm. Samples were eluted using a linear gradient of 0.05 M TEAB buffer in 100% water (pH 7.5) (buffer A) to 0.05 M TEAB buffer in 50% acetonitrile (buffer B), programmed over a 60 min period with a flow rate of 1 mL/min and detection at 260 nm. The crude sample (50 μL, initial concentration of **5a**, **5b**, **6a**, **6b** of 1 mM) is injected into the precolumn and eluted with buffer A during 3 min. Then, the switching valve for connecting the precolumn to the column is activated and a linear gradient from 0% buffer B to 20% over 40 min is applied. The retention times are 21 min for **5a**, 26 min for **5b**, 24 min for **6a**, 30 min for **6b**, 19 min for PMEA, and 22 min for (*R*)-PMPA. Kinetic data of decomposition for derivatives **5a**, **5b**, **6a**, and **6b** compared to standard compounds (PMEA and (*R*)-PMPA) was studied at 37 °C (a) in several buffers (pH 1.2, 5.2, 7.4, 8.1, 9.0, 11.5), (b) in RPMI 1640, (c) in culture

medium (RPMI 1640 containing 10% heat-inactivated fetal calf serum, and (d) in total cell extract (CEM-SS cell). For each kinetic study, the compound solution is diluted with a freshly thawed aliquot of the considered medium to obtain a final concentration of 0.1 mM. The mixture is incubated at 37 °C, and for the required time, an aliquot (10% solution) is drawn and immediately frozen at −80 °C for further HPLC analysis. The amount of remaining parent compound at each time point was used to determine the half-life of the compound. The product of decomposition from parent derivative is determined by comparison with authentic samples and standard compounds.

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Supporting Information Available: Tables containing antiviral activities and cytotoxicities of the derivatives in cell cultures (Tables 1−5) and a table listing analytical data of target compounds (Table 6). This material is available free of charge via the Internet at http://pubs.acs.org.

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