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SYNTHESIS AND ANTIVIRAL EVALUATION OF 9-(S)-[3-ALKOXY-2-(PHOSPHONOMETHOXY)PROPYL]NUCLEOSIDE ALKOXYALKYL ESTERS: INHIBITORS OF HEPATITIS C VIRUS AND HIV-1 REPLICATION

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Abstract

We reported previously that octadecyloxyethyl 9-(*S*)-[3-hydroxy-2-(phosphonomethoxy)-propyl]adenine (ODE-(*S*)-HPMPA) was active against genotype 1b and 2a hepatitis C virus (HCV) replicons. This is surprising because acyclic nucleoside phosphonates have been regarded as having antiviral activity only against double stranded DNA viruses, HIV and HBV. We synthesized octadecyloxyethyl 9-(*S*)-[3-methoxy-2-(phosphonomethoxy)propyl]-adenine and found it to be active in genotype 1b and 2a HCV replicons with EC_{50} values of 1-2 μM and a CC_{50} of>150 μM. Analogs with substitutions at the 3′-hydroxyl larger than methyl or ethyl, or with other purine bases were less active but most compounds had significant antiviral activity against HIV-1 in vitro. The most active anti-HIV compound was octadecyloxyethyl 9-(*R*)-[3-methoxy-2-(phosphonomethoxy)propyl]guanine with an EC_{50} <0.01 nanomolar and a selectivity index of>4.4 million.

Keywords

antiviral prodrugs; hepatitis C virus; human immunodeficiency virus-1; alkoxyalkyl prodrugs; nucleoside phosphonate prodrugs

1. Introduction

Treatment of hepatitis C virus (HCV) infection remains an important unmet medical need due to the inadequacies of current interferon-based therapy.[1] In the United States there are 3 to 4 million persons with chronic HCV infection.[2] A number of agents are currently in clinical development for HCV including NS3 protease inhibitors, NS5A inhibitors[3] and NS5B antiviral nucleosides and non-nucleoside polymerase inhibitors.[4] Clinical and in vitro

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data indicate that resistance develops readily with protease and non-nucleoside polymerase inhibitors.[5-7] In contrast, nucleoside inhibitors, which target the catalytic site of the NS5B RNA dependent RNA polymerase, are active across different HCV genotypes and exhibit a higher barrier to resistance.[8]

Acyclic nucleoside phosphonates (ANPs) are an important group of broad spectrum antiviral agents with activity against double stranded DNA (dsDNA) viruses,[9] or viruses which rely on reverse transcription such as HBV[10] and HIV-1.[11] We previously reported that octadecyloxyethyl 9-(*S*)-[3-hydroxy-2-(phosphonomethoxy)propyl]adenine (ODE-(*S*)-HPMPA, **1**) exhibited antiviral activity against genotype 1b and 2a HCV replicons, the first report of significant antiviral activity of an acyclic nucleoside phosphonate against an RNA virus.[12] However, ODE-(*S*)-HPMPA has substantial cytotoxicity.[12-13] To identify additional anti-HCV compounds with less cytotoxicity, we synthesized several closely related analogs having methyl, ethyl or isopropyl substitutions at the 3′-hydroxyl, octadecyloxyethyl 9-(*S*)-[3-methoxy-2-(phosphonomethoxy)propyl]adenine (ODE-(*S*)-MPMPA, **15**), octadecyloxyethyl 9-(*R*)-[3-methoxy-2-(phosphonomethoxy)propyl]adenine (ODE-(*R*)-MPMPA, **16**), hexadecyloxypropyl 9-(*S*)-[3-methoxy-2-(phosphono-methoxy)propyl]adenine (HDP-(*S*)-MPMPA, **17**), hexadecyloxypropyl 9-(*R,S*)-[3-ethoxy-2-(phosphonomethoxy)propyl]adenine (HDP-(R,S)-EPMPA, **18**) and hexadecyloxypropyl 9-(*R,S*)-[3-isopropoxy-2-(phosphonomethoxy)propyl]-adenine (HDP-(R,S)-IPPMPA, **19**) (Scheme 1). We also prepared the HDP- or ODE- esters of (*R* and *S*)-MPMP-2,6 diaminopurine (**23-26**) (Scheme 2), guanine (**34-37**) (Scheme 3), cytosine (**40**), 6-methoxypurine (**43**) (Scheme 4) and 6-O-methylguanine (**44**) (Scheme 5). The antiviral activity of these compounds against HCV, HIV-1 and other viruses was compared with ODE-(*S*)-HPMPA (**1**).

2. RESULTS & DISCUSSION

2.1 Chemistry

Synthesis of the new ANP analogs was carried out using the synthon approach we developed previously for alkoxyalkyl esters of (S)-9-[3-hydroxy-2-(phosphonomethoxy)-propyl]adenine [(S)-HPMPA].[13] As detailed in Scheme 1, adenine reacted with various alkyl glycidyl ethers under basic conditions to give a series of 9-(3-alkoxy-2-hydroxypropyl)adenines (**2-5**). After protection of the amino group with monomethoxytrityl, compounds **6-9** could be alkylated with octadecyloxyethyl or hexadecyloxypropyl p-toluenesulfonyloxymethyl-phosphonate, then deprotected to provide adenine derivatives **15-19**. As shown in Scheme 2, 3-methoxy- and 3-ethoxy-2-phosphonomethoxypropyl analogs of 2,6-diaminopurine (**23 – 26**) were prepared using a similar strategy. In this case, acceptable yields (22 – 50%) of the target compounds were obtained without protection of the amino groups. Synthesis of the related guanine (Scheme 3, **34 – 37**) and cytosine (Scheme 4, **40**) analogs started from 6-O-benzylguanine and N^4-monomethoxytritylcytosine, respectively. Methoxypurine analogs (**43-44**) were prepared as shown in Scheme 5.

2.2 Biological Activity

Compounds were tested for anti-HCV activity in genotype 1b and 2a replicons as previously described[14] and their activity compared with that of ODE-(S)-HPMPA (Table 1). ODE-(S)-MPMPA (**15**) retained full activity against genotype 1b and 2a replicons with EC_{50} values of 1.43±0.38 and 2.38±1.09 μM while the (*R*) isomer (**16**) was slightly less active with EC_{50} of 4.65 and 5.33 μM. HDP-(S)-MPMPA (**17**) was slightly less active than the corresponding ODE ester with EC_{50} of 2.36 (1b) and 4.64 μM (2a). When ethyl or isopropyl substitutions were made at the 3′-hydroxyl instead of methyl, the anti-HCV activity dropped slightly with HDP-(*R,S*)-EPMPA (**18**) to EC_{50} of 7.59 (1b) and 8.87 μM (2a) suggesting that larger

substitutions are not favored. Cytotoxicity of ODE-(S)-MPMPA was substantially lower than that observed with ODE-(S)-HPMPA, CC_{50} >150 versus 35.6 μM. Of the various adenine analogs, ODE-(S)-MPMPA had the greatest selectivity index, >105 with genotype 1b and >63 with genotype 2a replicons. The anti-HCV activity of ODE-(S)-MPMPA is similar to that of 4′-azidocytidine and 2′-C-methylcytidine, EC_{50} 1.13 to 1.28 μM[15], and less than that of PSI-352938, a cyclic phosphate prodrug of β-D-2′-α-fluoro-2′-β-C-methylguanosine, EC_{50} 0.13 to 0.20 μM. [16]

We also prepared alkoxyalkyl MPMP esters of cytosine, guanine, 2,6-diaminopurine, 6-methoxypurine and 6-O-methylguanine. Among them, the most active anti-HCV compound was ODE-(*S*)-MPMPG (**34**) with EC_{50} values of 8.26 and 10.7 μM against genotype 1b and 2a, respectively; the (*R*) isomer (**35**) was slightly less active with EC_{50} of 12.6 and 12.4 μM. These compounds also had low cytotoxicity with CC_{50} values >150 μM. HDP-(*S*)-MPMPMP (**43**) also exhibited significant activity in the 8.9 to 12.5 μM range. HDP-(*S*)-MPMPOMG (**44**) and the ODE and HDP esters of both (*R*) and (*S*)-MPMPDAP (**23**-**25**) were less active with EC_{50} values ranging from 18 to 26 μM while ODE-(*S*)-MPMPC (**40**) was inactive.

We also evaluated the compounds in MT-2 cells infected with HIV-1 (Table 2). ODE-(S)-HPMPA (**1**) was highly active with an EC_{50} of 0.0001μM. However, the CC_{50} was 0.033 μM making it the most cytotoxic compound in the series. ODE-(*S*)-MPMPA (**15**) retained substantial antiviral activity with an EC_{50} of 0.03 μM and a CC_{50} of 22 μM (selectivity index 733). By way of comparison, our previous studies with adefovir and tenofovir in HIV-1 infected MT-2 cells showed EC_{50} values for these compounds of 1.1 and 0.65 μM, respectively. [17,18] HDP-(*S*)-MPMPA (**17**) was less active and ODE-(*R*)-MPMPA (**16**) was considerably less active and selective. Introduction of an ethoxy or isopropoxy at the 3′-hydroxyl position of the acyclic moiety (compounds **18**, **19**) resulted in a loss of antiviral activity (Table 2). Surprisingly, the most active compound was ODE-(*R*)-MPMPG (**35**), EC_{50} $<1\times10^{-5}$ μM and a selectivity index of >4.4 million. Interestingly, the (*S*) isomer (**34**) was substantially less active with an EC_{50} of 0.2 μM. The same pattern was observed with ODE-(*R*)-MPMPDAP (**24**) which was more active (EC_{50} = 0.4 μM) than the (*S*) isomer (**23**). As noted before with the adenine analogs, the HDP esters (**25**, **36**) were less active. Again, introduction of larger ethyl groups at the 3′-hydroxyl of the acyclic chain of these compounds (**26**, **37**) caused a large loss of anti-HIV activity. ODE-(*S*)-MPMPC (**40**) was less potent (EC_{50} = 12.7 μM) and HDP-(*S*)-MPMPMP (**43**) and HDP-(*S*)-MPMPOMG (**44**) were considerably less potent against HIV (EC_{50} >10μM).

ODE-(S)-MPMPA was also tested against HCMV and HSV-1 using our previous methods.[19] We reported previously that ODE-(*S*)-HPMPA is a powerful inhibitor of the replication of orthopoxviruses, including variola,[20] vaccinia and cowpox,[21] and ectromelia,[22] as well as other dsDNA viruses including human cytomegalovirus (HCMV) and herpes simplex virus, type 1 (HSV-1).[13] We examined the effect of blocking the 3′-hydroxyl of HPMPA with 3′-methoxy on the compound's antiviral activity against dsDNA viruses including vaccinia, cowpox, HCMV and HSV-1 (Table 3). As we reported previously, ODE-(*S*)-HPMPA had potent antiviral activity against these viruses with EC_{50} values ranging from <0.1 to 20 nanomolar. However, ODE-(*S*)-MPMPA (**15**) exhibited a dramatic loss of antiviral activity with EC_{50} values >400 to >45 million times higher than those of ODE-(S)-HPMPA (Table 3). We reported previously that inhibition of vaccinia virus replication occurs by a unique mechanism in which (*S*)-HPMPA diphosphate is incorporated into DNA by the viral E9L polymerase. However, the vaccinia polymerase cannot copy across the drug lesion in HPMPA containing templates.[23] The EC_{50} for vaccinia inhibition by ODE-(*S*)-HPMPA is 20 nanomolar versus 18,300 nanomolar for ODE-(*S*)-MPMPA, a reduction of 915-fold (Table 3). These findings generally support the

principal mechanism which we described previously because incorporation of HPMPA into viral DNA, blocking further copying of the drug-containing chain is not possible with ODE-(*S*)-MPMPA, and any residual antiviral activity with the latter compound is due to obligatory chain termination. Presumably this is also the mechanism of action in cowpox which has a closely related DNA polymerase. The antiviral mechanism of action of HPMPA diphosphate has not been studied extensively for HCMV and HSV-1.

3. Conclusions

Prior studies identified ODE-(*S*)-HPMPA (**1**) as a compound with good antiviral activity against HCV.[12] However, this compound has significant toxicity both in vitro and in mice, in vivo. Surprisingly, replacement of the acyclic chain hydroxyl with a methoxy group greatly reduces toxicity while anti-HCV activity is preserved in the 1-2 μM range. Selectivity indexes for ODE-(S)-MPMPA (**15**) range from >63 to >105, a marked improvement over the selectivity of ODE-(*S*)-HPMPA (**1**). Interestingly, blocking the 3′-hydroxyl of ODE-(*S*)-HPMPA with a methyl results in a marked loss of antiviral activity against double stranded DNA viruses, including vaccinia, cowpox, HCMV and HSV-1. The ODE-MPMP- analogs of guanine, 6-methoxypurine and 6-O-methylguanine were also active against HCV while the ODE (*S*) and (*R*) isomers of MPMPDAP were somewhat less potent. ODE-(*S*)-MPMPC was inactive. ODE-(*R*)-MPMPG (**35**) showed striking antiviral activity against HIV-1 with an EC_{50} of <0.01 nanomolar and a selectivity index of >4.4 million. Our results support further evaluation of ODE-(*S*)-MPMPA (**15**) for use against chronic HCV infection and ODE-(*R*)-MPMPG (**35**) for HIV infection.

4. Experimental

4.1 General Chemistry Methods

All reagents were of commercial quality and used without further purification unless indicated otherwise. Chromatographic purification was done using the flash method with silica gel 60 (EMD Chemicals, Inc., 230–400 mesh). ^{1}H NMR spectra were recorded on Varian HG spectrophotometers operating at 400 MHz and are reported in units of parts per million (ppm) relative to internal tetramethylsilane at 0.00 ppm. Assignments of ^{1}H NMR signals are made using the numbering system shown in Scheme 1. Routine electrospray ionization mass spectra (ESI-MS) were recorded on a Finnigan LCQDECA spectrometer, and high resolution mass spectra (HRMS) on an Agilent 6230 Accurate-Mass TOFMS mass spectrometer in ESI negative mode. Both spectrometers are located at the small molecule facility, Department of Chemistry, University of California, San Diego and operated by Dr. Yongxuan Su. Purity of the target compounds was characterized by high performance liquid chromatography (HPLC) using a Beckman Coulter System Gold chromatography system. The analytical column was Phenomenex Synergi™ Polar-RP (4.6 × 150 mm) equipped with a SecurityGuard™ protection column. Mobile phase A was 95% water/5% methanol and mobile phase B was 95 % methanol/5% water. At a flow rate of 0.8 mL/min, gradient elution was as follows: 10 % B (0 – 3 min.); 10 % to 95 % B (3 – 20 min.); 95 % B (20 – 25 min.); 95 % to 10 % B (25 – 34 min.). Compounds were detected by ultraviolet light (UV) absorption at 274 nm. Purity of compounds was also assessed by thin layer chromatography (TLC) using Analtech silica gel-GF (250 μm) plates and the solvent system: $CHCl_3$/MeOH/con NH_4OH/H_2O (70:30:3:3 v/v). TLC results were visualized with UV light, phospray (Supelco, Bellefonte, PA, USA) and charring at 400 °C. The key synthons, hexadecyloxypropyl- and octadecyloxyethyl p-toluenesulfonyloxymethylphosphonate, were prepared as described previously.[13]

4.2 General procedure A. Synthesis of 3-alkoxy-2-hydroxypropyl nucleoside analogs

The preparation of various 3-alkoxy-2-hydroxypropyl nucleoside analogs was accomplished using the base catalyzed ring-opening reaction of alkyl glycidyl ethers with nucleobases as described by Brodfuehrer et al.[24] Sodium hydride (2 mmol) was added to a solution of the nucleic acid base (10 mmol) and an alkyl glycidyl ether (10 mmol) in anhydrous N,N-dimethylformamide (50 mL) and the mixture was stirred and heated to 100 °C for 6 hours. After cooling, the reaction was quenched with H_2O, the solvent was removed in vacuo and the residue was purified by flash column chromatography on silica gel. Elution of the column with 10% MeOH/CH_2Cl_2 gave the product.

4.2.1. (*S*)-9-[(3-methoxy-2-hydroxy)propyl]adenine (2)—was synthesized from adenine and (*S*)-methyl glycidyl ether (TCI America, Portland, OR). 75% yield. ^{1}H NMR ($CDCl_3$/methanol-d_4) δ 8.25 (s, 1H); 8.03 (s, 1H); 4.39-4,44 (m, 1H); 4.19-4-25 (m, 1H); 4.08-4.18 (m, 1H); 3.37-3.44 (m, 2H); 3.40 (s, 3H). MS (ESI): 224.14 $[M+H]^+$.

4.2.2. (*R*)-9-[(3-methoxy-2-hydroxy)propyl]adenine (3)—was synthesized from adenine and (*R*)-methyl glycidyl ether (TCI America, Portland, OR). 72% yield. ^{1}H NMR (methanol-d_4) δ 8.20 (s, 1H, H-8); 8.08 (s, 1H, H-2); 4.39 (dd, 1H, H-1′a, $J_{1'a,2'}$ = 3.4 Hz, J_{gem} = 14.2 Hz); 4.20 (dd, 1H, H-1′b, $J_{1'b,2'}$ = 8.0 Hz, J_{gem} = 14.2 Hz); 4.11 (m, 1H, H-2′); 3.42 (d, 2H, H-3′, $J_{3',2'}$ = 5.2 Hz); 3.37 (s, 3H, -OCH_3).

4.2.3. (*R,S*)-9-[(3-ethoxy-2-hydroxy)propyl]adenine (4)—was synthesized from adenine and (*R,S*)-ethyl glycidyl ether (TCI America) with 59% yield. ^{1}H NMR ($CDCl_3$/methanol-d_4), δ:8.24 (s, 1H); 8.04 (s, 1H); 4.40-4.65 (m, 1H); 4.20-4-27 (m, 1H); 4.10-4.15 (m, 1H); 3.55-3.57 (m, 2H); 3.46-3.54 (m, 2H); 1.21 (t, J=7Hz, 3H). MS (ESI): 238.09 $[M+H]^+$.

4.2.4. (*R,S*)-9-[(3-isopropoxy-2-hydroxy)propyl]adenine (5)—was synthesized from adenine and (*R,S*) isopropyl glycidyl ether (Aldrich Chem.). 33.8% yield. ^{1}H NMR ($CDCl_3$/methanol-d_4), δ 8.21 (s, 1H, H-8); 8.08 (s, 1H, H-2); 4.41 (dd, 1H, H-1′a, $J_{1'a,2'}$ = 3.8, J_{gem} = 14.2 Hz); 4.23 (dd, 1H, H-1′b, $J_{1'b,2'}$ = 7.6 Hz, J_{gem} = 14.4 Hz); 4.10 (m, 1H, H-2′); 3.60 (septet, 1H, -C*H*$(CH_3)_2$, J = 6.0 Hz); 1.16 (d, 6H, -CH(C*H*$_3)_2$).

4.3. General procedure B. Synthesis of 9-[(3-alkoxy-2-hydroxy)propyl]-N^6-monomethoxytrityladenine (6-9)

The monomethoxytrityl group was used to block the exocylic amino group of adenine and was introduced by the transient protection method described by Ti et al.[25] Bromotrimethylsilane (6.3 mmol) was added dropwise to a suspension of 9-[(2-hydroxy-3-alkoxy)propyl]adenine (**2**-**5**) (2.8 mmol) in dry pyridine (10 mL). The mixture was stirred 15 min. until it became clear, then monomethoxytrityl chloride (0.99 g, 3.2 mmol) and 4-(dimethylamino)pyridine (20 mg, 0.2 mmol) were added and stirring was continued overnight. The mixture was cooled with an ice bath and H_2O (1 mL) was added. Stirring was continued 10 min., then con. NH_4OH (1 mL) was added and the reaction was stirred 30 additional min. The mixture was allowed to warm to room temperature and filtered through a pad of celite. The filtrate was evaporated in vacuo and the residue was purified by flash column chromatography on silica gel. Gradient elution (100% hexanes to 100% ethyl acetate) afforded the N^6-monomethoxytrityl 9-[(2-alkoxy-3-methoxy)propyl]adenines (**6**-**9**).

4.3.1. (*S*)-9-[(3-methoxy-2-hydroxy)propyl]-N^6-monomethoxytrityladenine (6)—was synthesized from **2**. 98 % yield. ^{1}H NMR ($CDCl_3$/methanol-d_4), δ 8.17 (s, 1H, H-8); 8.13 (s, 1H, H-2); 7.39-7.75 (m, 14H, trityl); 4.50-4.59 (m, 1H); 4.31-4.39 (m, 1H);

4.22-4.30 (m, 1H); 3.45 (s, 3H); 3.50-3.60 (m, 2H); 3.55 (s, 3H). MS (ESI): 496.06 $[M+H]^+$, 518.13 $[M+Na]^+$.

4.3.2. (*R*)-9-[(3-methoxy-2-hydroxy)propyl] N^6-monomethoxytrityladenine (7)— was synthesized from **3**. 19 % yield. ^{1}H NMR ($CDCl_3$/methanol-d_4), δ 8.03 (s, 1H, H-8); 7.78 (s, 1H, H-2); 7.35-7.33 (m, 4H, trityl); 7.29-7.24 (m, 10H, trityl); 4.61 (d, 1H, H-1′a, $J_{1'a,2'}$ = 4 Hz); 4.39 (dd, 1H, H-1′b, $J_{1'b,2'}$ = 2.2 Hz, J_{gem} = 13.8 Hz); 4.16 (m, 1H, H-2′); 3.78 (s, 3H, Ar-OCH_3); 3.41 (dd, 1H, H-3′a, $J_{3'a,2'}$ = 5.2 Hz, J_{gem} = 9.2 Hz); 3.36 (s, 3H, CH_2-OCH_3); 3.33 (dd, 1H, H-3′b, $J_{3'b,2'}$ = 6.2 Hz, J_{gem} = 9.8 Hz).

4.3.3. (*R,S*)-9-[(3-ethoxy-2-hydroxy)propyl] N^6-monomethoxytrityladenine (8)— was synthesized from **4**. 78 % yield. ^{1}H NMR ($CDCl_3$/methanol-d_4), δ 7.98 (s, 1H); 7.25-7.34 (m, 14H); 6.82 (s, 1H); 4.35-4.43 (m, 1H); 4.15-4.24 (m, 1H); 4.05-4.15 (m, 1H); 3.78 (s, 3H); 3.52-3.56 (m, 2H); 3.44-3.47 (m, 2H); 1.20 (t, J=7Hz, 3H). MS (ESI): 509.78 $[M+H]^+$.

4.3.4. (*R,S*)-9-[(3-isopropoxy-2-hydroxy)propyl] N^6-monomethoxytrityladenine (9)—was synthesized from **5.** 32 % yield. ^{1}H NMR (DMSO-d_6) δ 8.10 (s, 1H, H-8); 7.89 (s, 1H, H-2); 7.28-7.26 (m, 10H, trityl); 7.21-7.18 (m, 4H, trityl); 6.83 (d, 1H, -NH-); 5.16 (d, 1H, -OH); 4.23 (dd, 1H, H-1′a, $J_{1'a,2'}$ = 3.4 Hz, J_{gem} = 13.8 Hz); 3.98 (dd, 1H, H-1′b, $J_{1'b,2'}$ = 8.4 Hz, J_{gem} = 13.6 Hz); 3.92 (m, 1H, H-2′); 3.70 (s, 3H, Ar-OCH_3); 3.50 (septet, 1H, -C*H*$(CH_3)_2$); 3.35 (dd, 1H, $J_{3'a,2'}$ = 4.8 Hz, J_{gem} = 10 Hz); 3.27 (dd, 1H, H-3′b, $J_{3'b,2'}$ = 6, J_{gem} = 9.6); 1.04 (dd, 6H, - CH(C$H_3)_2$).

4.4. General procedure C. Alkylation of 9-[(3-alkoxy-2-hydroxy)propyl] derivatives (6-9, 20-22, 27-29, 38, 41-42) with alkoxyalkyl p-toluenesulfonyloxymethylphosphonate. Synthesis of (10-14, 23-26, 30-33, 39, 43-44)

Sodium *t*-butoxide (0.19 g, 2.0 mmol) was added to a solution of the 3-alkoxy-2-hydroxypropyl nucleoside (1.0 mmol) and the alkoxyalkyl p-toluenesulfonyloxymethylphosphonate[12] (2.0 mmol) in anhydrous N,N-DMF (20 mL). The mixture was heated to 80 °C and kept overnight. After cooling, the solvents were evaporated in vacuo and the residue was purified by flash column chromatography on silica gel. The column was eluted with a gradient: chloroform 100% - chloroform-methanol (20%) to give the products.

4.4.1. Octadecyloxyethyl (*S*)-9-[(3-methoxy-2-phosphonomethoxy)propyl] N^6-monomethoxytrityladenine, sodium salt (10)—was synthesized from (S)-9-[(3-methoxy-2-hydroxy)propyl]-N^6-monomethoxytrityladenine **(6)** and octadecyloxyethyl p-toluenesulfonyloxymethylphosphonate. 60% yield. ^{1}H NMR ($CDCl_3$/methanol-d_4), δ 8.18 (s, 1H); 7.98 (s, 1H); 7.22-7.55 (m, 14H); 4.38-4.50 (m, 2H); 4.12-4.37 (m, 2H); 4.00-4.08 (m, 1H); 3.82- 3.98 (m, 2H); 3.79 (s, 3H); 3.58-3.65 (m, 2H); 3.44-3.48 (m, 2H); 3.38-3.43 (m, 2H); 3.35 (s, 3H); 1.40-1.60 (m, 2H); 1.16-1.38 (m, 30H); 0.88 (t, J = 7Hz, 3H). MS (ESI): 886.48 $[M+H]^+$.

4.4.2. Octadecyloxyethyl (*R*)-9-[(3-methoxy-2-phosphonomethoxy)propyl] N^6-monomethoxytrityladenine, sodium salt (11)—was synthesized from (*R*)-9-[(3-methoxy-2-hydroxy)propyl]-N^6-monomethoxytrityladenine **(7)** and octadecyloxyethyl p-toluenesulfonyloxymethylphosphonate. 43% yield. ^{1}H NMR ($CDCl_3$/methanol-d_4), δ 8.20 (s, 1H); 7.98 (s, 1H); 7.22-7.37 (m, 14H); 4.42-4.50 (m, 1H); 4.28-4.37 (m, 1H); 3.91-3.98 (m, 2H); 3.82- 3.90 (m, 2H); 3.79 (s, 3H); 3.60-3.69 (m, 1H); 3.48-3.58 (m, 3H); 3.39-3.46 (m, 2H); 3.35 (s, 3H); 1.45-1.60 (m, 2H); 1.20-1.38 (m, 30H); 0.88 (t, J=7Hz, 3H). MS (ESI): 886.57 $[M+H]^+$.

4.4.3. Hexadecyloxypropyl (*S*)-9-[(3-methoxy-2-phosphonomethoxy)propyl] N^6-monomethoxytrityladenine, sodium salt (12)—

was synthesized from (*S*)-9-[(3-methoxy-2-hydroxy)propyl]-N^6-monomethoxytrityladenine **(6)** and hexadecyloxypropyl p-toluenesulfonyloxymethylphosphonate. 77% yield. ^{1}H NMR ($CDCl_3$/methanol-d_4), δ: 8.17 (s, 1H); 7.94 (s, 1H); 7.22-7.36 (m, 14H); 4.38-4.50 (m, 2H); 4.28-4.37 (m, 2H); 3.82- 3.98 (m, 2H); 3.79 (s, 3H); 3.58-3.65 (m, 1H); 3.38-3.58 (m, 6H); 3.34 (s, 3H); 1.78-1.87 (m, 2H); 1.44-1.60 (m, 2H); 1.10-1.40 (m, 26H); 0.88 (t, J=7Hz, 3H). MS (ESI): 870.33 $[M-H]^-$.

4.4.4. Hexadecyloxypropyl (*R,S*)-9-[(3-ethoxy-2-phosphonomethoxy)propyl] N^6-monomethoxytrityladenine, sodium salt (13)—

was synthesized from (R,S)-9-[(3-ethoxy-2-hydroxy)propyl]-N^6-monomethoxytrityladenine **(8)** and hexadecyloxypropyl p-toluenesulfonyloxymethylphosphonate. 80% yield. ^{1}H NMR ($CDCl_3$/methanol-d_4), δ: 8.19 (s, 1H); 7.98 (s, 1H); 7.20-7.36 (m, 14H); 4.42-4.65 (m, 1H); 4.28-4.37 (m, 1H); 3.80- 3.95 (m, 3H); 3.78 (s, 3H); 3.48-3.65 (m, 6H); 3.28-3.48 (m, 2H); 1.78-1.87 (m, 2H); 1.44-1.55 (m, 2H); 1.08-1.30 (m, 26H); 1.15 (t, J=7Hz, 3H); 0.88 (t, J=7Hz, 3H). MS (EI): 886.42 $(M+H)^+$.

4.4.5. Hexadecyloxypropyl (*R,S*)-9-[(3-isopropoxy-2-phosphonomethoxy)propyl] N^6-monomethoxytrityladenine, sodium salt (13)—

was synthesized from (R,S)-9-[(3-isopropoxy-2-hydroxy)propyl]-N^6-monomethoxytrityladenine **(9)** and hexadecyloxypropyl p-toluenesulfonyloxymethylphosphonate. 25% yield. ^{1}H NMR ($CDCl_3$/methanol-d_4) δ 8.15 (s, 1H, H-8); 7.88 (s, 1H, H-2); 7.30 – 7.25 (m, 4H, trityl); 7.20 – 7.12 (m, 10H, trityl); 4.66 (dd, 1H, H-1′a, $J_{1'a,2'}$ = 3.5 Hz, J_{gem} = 14.2 Hz); 4.47 (dd, 1H, H-1′b, $J_{1'b2'}$ = 6.2 Hz, J_{gem} = 14.0 Hz); 4.01 (m, 2H, -P-O-CH_2-); 3.88 (dd, 1H, -CH_a-P-, $J_{P,CHa}$ = 9.2 Hz, J_{gem} = 13.6 Hz); 3.71 (dd, 1H, - CH_b-P-, $J_{P,CHb}$ = 9.6 Hz, J_{gem} = 14.0 Hz); 3.75 – 3.55 (m, 3H, H-3′ + H-2′); 3.51 (t, 2H, -C*H*$_2$-O-CH_2-); 3.45 (t, 2H, -CH_2-O-C*H*$_2$-); 1.83 (pentet, 2H, -O-CH_2C*H*$_2$$CH_2$O-); 1.53 (m, 2H, - $CH_2(CH_2)_{15}$-); 1.27 (m, 26H, -$(CH_2)_{15}$-); 1.10 (d, 6H, -$CH(CH_3)_2$); 0.89 (t, 3H, -CH_3).

4.5. General procedure D. Synthesis of Alkoxyalkyl-9-[(3-alkoxy-2-phosphonomethoxy)propyl]adenine (15-19)

Alkoxyalkyl 9-[(3-alkoxy-2-hydroxy)propyl]-N^6-monomethoxytrityladenine **(10-14)** (0.60 mmol) was added to 80% acetic acid, stirred and heated to 60 °C for 2 hours. After cooling, the solvent was removed in vacuo and the residue purified by flash column chromatography on silica gel. Elution with 20% MeOH/ CH_2Cl_2 gave the products.

4.5.1. Octadecyloxyethyl (*S*)-9-[(3-methoxy-2-phosphonomethoxy)propyl]adenine, sodium salt (ODE-(*S*)-MPMPA) (15)—

Deprotection of **10** (procedure D) gave **15** in 73% yield as a white powder. ^{1}H NMR ($CDCl_3$/methanol-d_4) δ 8.35 (s, 1H, H-8); 8.22 (s, 1H, H-2); 4.53 (dd, H, H-1′a, $J_{1'a2'}$ = 3.3 Hz, J_{gem} 14.3 Hz); 4.37 (dd, 1H, H-1′b, $J_{1'b2'}$ = 6.6 Hz, J_{gem} 14.7 Hz); 4.01-3.98 (m, 3H, P-O-CH_2- + H-2′); 3.87 (dd, 1H, -CH_a-P-, $J_{P,CHa}$ = 9.2 Hz, J_{gem} = 13.2); 3.70 (dd, 1H, -CH_b-P-, $J_{P,CHb}$ = 9.0 Hz, J_{gem} = 13.0 Hz); 3.57 (t, 2H, -CH_2-O-); 3.44 (t, 2H, -O-CH_2-); 1.53 (m, 2H, -O-$CH_2CH_2(CH_2)_{15}$-); 1.26 (m, 30H, -$(CH_2)_{15}CH_3$); 0.89 (t, 3H, -CH_3, J = 7Hz). MS (ESI+): 614.41 $[M+H]^+$; HRMS (ESI-) calcd. for $C_{30}H_{55}N_5O_6P$ [M-H] 612.3895, found 612.3897 (E = 0.3 ppm). HPLC analysis: retention time 22.35 min., purity 96.12 %.

4.5.2. Octadecyloxyethyl (*R*)-9-[(3-methoxy-2-phosphonomethoxy)propyl]adenine, sodium salt (ODE-(*R*)-MPMPA) (16)—

Deprotection of **11** gave **16** in 72% yield. ^{1}H NMR ($CDCl_3$/methanol-d_4), δ: 8.24 (s, 1H); 8.21 (s, 1H); 4.43-4.54 (s, 1H); 4.25-4.35 (m, 1H); 3.88-3.98 (m, 3H); 3.80-3.88 (m, 1H);

3.50-3.60 (m, 4H); 3.38-3.48 (m, 3H); 3.37 (s, 3H); 1.49-1.56 (m, 2H); 1.20-1.35 (m, 30H); 0.88 (t, J=7Hz, 3H). MS (ESI+): 614.55 $[M+H]^+$, 636.46 $[M+Na]^+$. HRMS (ESI-) calcd. for $C_{30}H_{55}N_5O_6P$ $[M-H]^-$ 612.3895, found 612.3900 (E = 0.8 ppm). HPLC analysis: retention time 21.82 min., purity 95.24 %.

4.5.3. Hexadecyloxypropyl (*S*)-9-[3-methoxy-2-phosphonomethoxy)propyl]adenine, sodium salt (HDP-(*S*)-MPMPA) (17)—

Deprotection of **12** gave **17** in 77% yield. ^{1}H NMR ($CDCl_3$/methanol-d_4), δ 8.28 (s, 1H); 8.23 (s, 1H); 4.48-4.61 (s, 2H); 4.32-4.37 (m, 2H); 3.91-3.96 (m, 2H); 3.80-3.86 (m, 1H); 3.58-3.64 (m,1H); 3.41-3.57 (m, 3H); 3.30-3.41 (m, 2H); 3.38 (s, 3H); 1.82-1.90 (m, 2H); 1.49-1.55 (m, 2H); 1.18-1.38 (m, 26H); 0.89 (t, J=7Hz, 3H). MS (ESI-): 598.29 $[M-H]^-$. HRMS (ESI-) calcd. for $C_{29}H_{53}N_5O_6P$ $[M-H]^-$ 598.3739, found 598.3737 (E = -0.3 ppm). HPLC analysis: retention time 22.43 min., purity 93.0 %.

4.5.4. Hexadecyloxyethyl (*R,S*)-9-[(3-ethoxy-2-phosphonomethoxy)propyl]adenine, sodium salt (HDP-(*R,S*)-EPMPA) (18)—

Deprotection of **13** gave **18** in 92% yield. ^{1}H NMR ($CDCl_3$/methanol-d_4), δ 8.28 (s, 1H); 8.21 (s, 1H); 4.48-4.53 (s, 1H); 4.34-4.39 (m, 1H); 3.90-4.00 (m, 3H); 3.80-3.86 (m, 1H); 3.58-3.64 (m,1H); 3.44-3.58 (m, 6H); 3.35-3.41 (m, 2H); 1.82-1.90 (m, 2H); 1.49-1.58 (m, 2H); 1.22-1.38 (m, 26H); 1.20 (t, J=7Hz, 3H); 0.89 (t, J=7Hz, 3H). MS (ESI): 612.44 $(M-H)^-$. HRMS (ESI-) calcd. for $C_{30}H_{55}N_5O_6P$ $[M-H]^-$ 612.3895, found 612.3898 (E = 0.5 ppm). HPLC analysis: retention time 22.60 min., purity 91.8 %

4.5.5. Hexadecyloxyethyl (*S*)-9-[(3-isopropoxy-2-phosphonomethoxy)propyl]adenine, sodium salt (HDP-(*R,S*)-IPPMPA) (19)—

Deprotection of **14** gave **19** in 75% yield. ^{1}H NMR (methanol-d_4) δ 8.35 (s,1H, H-8), 8.25 (s, 1H, H-2), 4.53, (dd, 1H, H-1′a, $J_{1'a2'}$ = 3.4 Hz, J_{gem} = 14.6 Hz), 4.39 (dd, 1H, H-1′b, $J_{1'b2'}$ = 6.6 Hz, J_{gem} = 14.6 Hz), 3.93 (t, 2H, P-O-CH_a, J = 6.8 Hz), 3.91 (t, 1H, P-O-CH_b, J = 6.4Hz); 3.85 (dd, -CH_a-P-, $J_{P,CHa}$ = 9.0 Hz, J_{gem} = 13 Hz), 3.66 (dd, 1H, -CH_b-P-, $J_{P,Hb}$ = 9.6 Hz, J_{gem} = 13.2 Hz); 3.59-3.48 (m, 3H, H-3′ + H-2′), 3.46 (t, 2H, -CH_2-O CH_2), 3.37 (t, 2H, -CH_2-O-CH_2-), 1.80 (pentet, 2H, -O-CH_2-CH_2-CH_2-O-), 1.51 (m, 2H, -O-CH_2- CH_2-$(CH_2)_{13}$-), 1.27 (m, 26H, -$(CH_2)_{13}$-), 1.13 (d, 6H, -CH(CH_3)$_2$), 0.89 (t, 3H, -CH_3); MS (ESI-): 626.69 $[M-H]^-$. HRMS (ESI-) calcd. for $C_{31}H_{57}N_5O_6P$ $[M-H]^-$ 626.4052, found 626.4053 (E = 0.2 ppm). HPLC analysis: retention time 21.95 min., purity 97.1 %.

4.6. 2,6-Diaminopurine derivatives

4.6.1 (S)-9-[(3-methoxy-2-hydroxy)propyl]-2,6-diaminopurine (20)—was synthesized from 2,6-diaminopurine (TCI America) and (S)-methyl glycidyl ether (procedure A). 27% yield (0.65 g). ^{1}H NMR ($CDCl_3$/methanol-d_4), δ 7.68 (s, 1H, H-8); 4.20-4.30 (m, 1H); 4.05-4.12 (m, 2H); 3.32-3.47 (m, 2H); 3.39 (s, 3H).

4.6.2. (*R*)-9-[(3-methoxy-2-hydroxy)propyl]-2,6-diaminopurine (21)—was synthesized from 2,6-diaminopurine and (*R*)-methyl glycidyl ether (procedure A). 41% yield. ^{1}H NMR ($CDCl_3$/methanol-d_4), δ 7.73 (s, 1H, H-8); 4.22 (d, 1H, H-1′a, J_{gem} = 12.4 Hz), 4.06-4.02 (m, 2H, H-1′b + H-2′); 3.39 (d, 2H, H-3′, J = 3.2 Hz); 3.36 (s, 3H, -OCH_3).

4.6.3. (R,S)-9-[(3-ethoxy-2-hydroxy)propyl]-2,6-diaminopurine (22)—was synthesized from 2,6-diaminopurine and (R,S)-ethyl glycidyl ether (procedure A). 71 % yield. ^{1}H NMR ($CDCl_3$/methanol-d_4) δ 7.77 (s, 1H, H-8); 4.22 (dd, 1H, J1′a,2′ = 3.6 Hz, J_{gem} = 12.4 Hz, H-1′a); 4.06-4.02 (m, 2H, H-1′b + H-2′); 3.88 (q, 2H, -OC$H_2$$CH_3$); 3.39 (d, 2H, H-3′, J = 3.2 Hz); 1.16 (t, 3H, -OCH_2CH_3).

4.6.4. Octadecyloxyethyl (S)-9-[(3-methoxy-2-phosphonomethoxy)propyl]2,6-diaminopurine, sodium salt (ODE-(S)-MPMPDAP) (23)—was synthesized (procedure C) from (S)-9-[(3-methoxy-2- hydroxy)propyl]2,6-diaminopurine and octadecyloxyethyl p-toluenesulfonyloxymethylphosphonate. 50% yield. ^{1}H NMR ($CDCl_3$/methanol-d_4), δ 7.72 (s, 1H); 4.00-4.20 (m, 5H); 3.80-3.90 (m, 1H); 3.58-3.65 (m, 2H); 3.50-3.58 (m, 2H); 3.40-3.50 (m, 1H); 3.44 (s, 3H); 3.30-3.38 (m, 2H); 1.50-1.60 (m, 2H); 1.18-1.38 (m, 30H); 0.88 (t, J=7Hz, 3H). MS (ESI): 627.48 $[M-H]^-$, 629.47 $[M+H]^+$. HRMS (ESI-) calcd. for $C_{30}H_{56}N_6O_6P$ $[M-H]^-$ 627.4004, found 627.4007 (E = 0.5 ppm). HPLC analysis: retention time 23.67 min., purity 91.9 %.

4.6.5. Octadecyloxyethyl (R)-9-[(3-methoxy-2-phosphonomethoxy)propyl]2,6-diaminopurine, sodium salt (ODE-(R)-MPMPDAP) (24)—was synthesized (procedure C)from (R)-9-[(3-methoxy-2- hydroxy)propyl]2,6-diaminopurine and octadecyloxyethyl p-toluenesulfonyloxymethylphosphonate with 49% yield. ^{1}H NMR ($CDCl_3$/methanol-d_4), δ: 7.75 (s, 1H);4.42-4.51 (m, 1H); 4.00-4.20 (m, 4H); 3.80-3.90 (m, 1H); 3.60-3.65 (m, 2H); 3.50-3.58 (m, 2H); 3.40-3.50 (m, 1H) 3.49 (s, 3H); 3.30-3.38 (m, 2H); 1.50-1.62 (m, 2H); 1.20-1.38 (m, 30H); 0.88 (t, J=7Hz, 3H). MS (ESI+): 629.55 $[M+H]^+$. HRMS (ESI-) calcd. for $C_{30}H_{56}N_6O_6P$ $[M-H]^-$ 627.4004, found 627.4007 (E = 0.5 ppm). HPLC analysis: retention time 23.67, purity 98.4 %.

4.6.6. Hexadecyloxypropyl (S)-9-[3-methoxy-2-phosphonomethoxy)propyl]-2,6-diaminopurine, sodium salt (HDP-(S)-MPMPDAP) (25)—was synthesized (procedure C) from (S)-9-[(3-methoxy-2-hydroxy)propyl]2,6-diaminopurine and hexadecyloxypropyl p-toluenesulfonyloxymethylphosphonate with 30% yield. ^{1}H NMR ($CDCl_3$/methanol-d_4), δ: 7.72 (s, 1H); 4.02-4.20 (m, 3H); 3.95-4.02 (m, 2H); 3.78-3.85 (m, 2H); 3.45-3.60 (m, 3H); 3.38-3.45 (m,6H); 1.82-1.95 (m, 2H); 1.45-1.60 (m, 2H); 1.20-1.38 (m, 26H); 0.88 (t, J=7Hz, 3H). MS (ESI+): 615.50 $[M+H]^+$, 637.45 $[M+Na]^+$. HRMS (ESI-) calcd. for $C_{29}H_{54}N_6O_6P$ $[M-H]^-$ 613.3848, found 613.3854 (E = 1.0 ppm). HPLC analysis: retention time 22.98 min., purity 90.3%.

4.6.7. Hexadecyloxypropyl (R,S)-9-[3-ethoxy-2-phosphonomethoxy)propyl-2,6-diaminopurine, sodium salt (HDP-(R,S)-EPMPDAP) (26)—was synthesized (procedure C) from (R,S)-9-[(3-ethoxy-2- hydroxy)propyl]2,6-diaminopurine and hexadecyloxypropyl p-toluenesulfonyloxymethylphosphonate with 22% yield. ^{1}H NMR ($CDCl_3$/methanol-d_4), δ: 7.40 (s, 1H); 4.44-4.52 (m, 1H); 4.18-2.28(m, 1H); 3.91-4.10 (m, 3H); 3.80-3.90 (m, 1H); 3.45-3.60 (m, 5H); 3.38-3.45 (m, 4H); 1.82-1.95 (m, 2H); 1.45-1.60 (m, 2H); 1.15-1.38 (m, 28H); 0.88 (t, J=7Hz, 3H). MS (ESI-): 627.53 $[M-H]^-$. HRMS (ESI-) calcd. for $C_{30}H_{56}N_6O_6P$ $[M-H]^-$ 627.4004, found 627.4008 (E = 0.6 ppm). HPLC analysis: retention time 23.37 min., purity 96.4 %.

4.7. Guanine derivatives

4.7.1. (*S*)-9-[(3-methoxy-2-hydroxy)propyl]-6-O-benzylguanine (27)—was synthesized from 6-O-benzylguanine (APAC Pharmaceutical LLC, Columbia, MD) and (S)-methyl glycidyl ether (procedure A). 49% yield. ^{1}H NMR ($CDCl_3$/methanol-d_4), δ 7.75 (s, 1H); 7.49-7.51 (m, 2H); 7.29-7.40 (m, 3H); 5.55 (s, 2H); 4.23-4.32 (m, 1H); 4.05-4.14 (m, 2H); 3.39-3.41 (m, 2H); 3.39 (s, 3H).

4.7.2. (*R*)-9-[(3-methoxy-2-hydroxy)propyl] -6-O-benzylguanine (28)—was synthesized from 6-O-benzylguanine and (R)-methyl glycidyl ether (procedure A). 44% yield. ^{1}H NMR ($CDCl_3$/methanol-d_4), δ 7.74 (s, 1H); 7.47-7.51 (m, 2H); 7.29-7.40 (m, 3H); 5.55 (s, 2H); 4.40-4.60 (m, 1H); 4.05-4.14 (m, 2H); 3.32-3.45 (m, 2H); 3.39 (s, 3H).

4.7.3. (*R,S*)-9-[(3-ethoxy-2-hydroxy)propyl]-6-O-benzylguanine (29)—was synthesized from 6-O-benzylguanine and (*R,S*) ethyl glycidyl ether (procedure A). 51% yield. ^{1}H NMR ($CDCl_3$/methanol-d_4), δ 7.76 (s, 1H); 7.50-7.52 (m, 2H); 7.32-7.40 (m, 3H); 5.52 (s, 2H); 4.17-4.21 (m, 1H); 3.95-4.00 (m, 2H); 3.44-3.50 (m, 2H); 3.34-3.38 (m, 2H); 1.16 (t, J=7Hz, 3H).

4.7.4. Octadecyloxyethyl (S)-9-[(3-methoxy-2-phosphonomethoxy)propyl]-6-O-benzylguanine (30)—was synthesized (procedure C) from (S)-9-[(3-methoxy-2-hydroxy)propyl]-6-O-benzylguanine and octadecyloxyethyl p-toluenesulfonyloxymethylphosphonate. 26% yield. ^{1}H NMR ($CDCl_3$/methanol-d_4), δ 7.93 (s, 1H); 7.50-7.56 (m, 2H); 7.31-7.40 (m, 3H); 5.55 (s, 2H); 4.24-4.36 (m, 1H); 3.93-4.22 (m, 1H); 3.75-3.98 (m, 4H); 3.60-3.70 (m, 4H); 3.30-3.60 (m, 8H); 1.42-1.60 (m, 2H); 1.18-1.38 (m, 30H); 0.89 (t, J=7Hz, 3H). MS (ESI): 720.51 $[M+H]^+$.

4.7.5. Octadecyloxyethyl (*R*)-9-[(3-methoxy-2-phosphonomethoxy)propyl]-6-O-benzylguanine (31)—was synthesized (procedure C) from (*R*)-9-[(3-methoxy-2-hydroxy)propyl]-6-O-benzylguanine and octadecyloxyethyl p-toluenesulfonyloxymethylphosphonate. 83% yield. ^{1}H NMR ($CDCl_3$/methanol-d_4), δ 7.93 (s, 1H); 7.52-7.47 (m, 2H); 7.23-7.38 (m, 3H); 5.55 (s, 2H); 4.18-4.38 (m, 2H); 3.75-3.98 (m, 4H); 3.55-3.65 (m, 1H); 3.43-3.50 (m, 3H); 3.30-3.43 (m, 8H); 1.45-1.60 (m, 2H); 1.18-1.38 (m, 30H); 0.89 (t, J=7Hz, 3H). MS (ESI): 718.54 $[M-H]^-$.

4.7.6. Hexadecyloxypropyl (S)-9-[3-methoxy-2-phosphonomethoxy)propyl]-6-O-benzylguanine (32)—was synthesized (procedure C) from (S)-9-[(3-methoxy-2-hydroxy)propyl]-6-O-benzylguanine and hexadecyloxypropyl p-toluenesulfonyloxymethylphosphonate. 71% yield. ^{1}H NMR ($CDCl_3$/methanol-d_4), δ:7.94 (s, 1H); 7.49-7.55 (m, 2H); 7.24-7.40 (m, 3H); 5.55 (s, 2H);4.30-4.40 (m, 1H); 4.17-4.22 (m, 1H); 3.80- 3.92 (m, 3H); 3.72-3.92 (m, 1H); 3.55-3.62 (m, 1H); 3.40-3.52 (m, 4H); 3.28-3.40 (m, 2H); 3.37 (s, 3H); 1.75-1.85 (m, 2H); 1.44-1.60 (m, 2H); 1.16-1.38 (m, 26H); 0.89 (t, J=7Hz, 3H). MS (ESI): 706.50 $[M+H]^+$.

4.7.7. Hexadecyloxypropyl (*R,S*)-9-[3-ethoxy-2-phosphonomethoxy)propyl]-6-O-benzylguanine (33)—was synthesized (procedure C) from (R,S)-9-[(3-methoxy-2-hydroxy)propyl]- 6-O-benzylguanine and hexadecyloxypropyl p-toluenesulfonyloxymethylphosphonate. 42% yield). ^{1}H NMR ($CDCl_3$/methanol-d_4), δ 7.95 (s, 1H); 7.48-7.52 (m, 2H); 7.30-7.40 (m, 3H); 5.56 (s, 2H);4.34-4.40 (m, 1H); 4.19-4.26 (m, 1H); 3.77- 3.93 (m, 4H); 3.58-3.66 (m, 1H); 3.47-3.55 (m, 5H); 3.35-3.45 (m, 3H); 1.78-1.85 (m, 2H); 1.48-1.55 (m, 2H); 1.17-1.28 (m, 29H); 0.89 (t, J = 7 Hz, 3H). MS (ESI): 718.46 $[M-H]^-$.

4.7.8 General procedure E. Synthesis of Alkoxyalkyl-9-[(3-alkoxy-2-phosphonomethoxy)propyl]guanine (34-37)—The protected guanine compounds (**30-33**) (0.71 mmol) were added to 10% trifluoroacetic acid/CH_2Cl_2 and the mixture was stirred at room temperature for 2 days. The solvent was removed in vacuo and the residue purified by flash column chromatography on silica gel. The column was eluted with 20% MeOH/CH_2Cl_2 and the crude products were recrystallized from water to give the alkoxyalkyl 9-[(3-alkoxy-2-phosphonomethoxy)propyl]guanine derivatives **(34-37)**.

4.7.9. Octadecyloxyethyl (*S*)-9-[(3-methoxy-2-phosphonomethoxy)propyl]guanine (ODE-(*S*)-MPMPG) (34)—Deprotection of **30** gave **34** as a white powder. 93% yield. ^{1}H NMR ($CDCl_3$/methanol-d_4), 7.82 (s, 1H); 4.24-4.36 (m, 1H); 4.10-4.28 (m, 1H); 3.95-4.05 (m, 2H); 3.78-3.90 (m, 2H); 3.62-3.73 (m,

1H); 3.52-3.60 (m, 2H); 3.40-3.50 (m, 2H); 3.25-3.40 (m, 3H); 1.45-1.60 (m, 2H); 1.18-1.38 (m, 30H); 0.89 (t, J = 7Hz, 3H). MS (ESI): 628.44 $[M-H]^-$. HRMS (ESI-) calcd. for $C_{30}H_{55}N_5O_7P$ $[M-H]^-$ 628.3845, found 628.3846 (E = 0.2 ppm). HPLC analysis: retention time 21.10, purity 96.2 %.

4.7.10. Octadecyloxyethyl (*R*)-9-[(3-methoxy-2-phosphonomethoxy)propyl]guanine (ODE- (*R*)-MPMPG) (35)—

Deprotection of **31** gave **35** as a white powder. 67% yield. ^{1}H NMR ($CDCl_3$/methanol-d_4), 8.07 (s, 1H, H-8); 7.51 (s, 2H, $-NH_2$); 4.34 (dd, 1H, H-1′a, $J_{1'a2'}$ = 3.9 Hz, J_{gem} = 14.6 Hz); 4.13 (dd, 1H, H-1′b, $J_{1'b2'}$ = 6.4 Hz, J_{gem} = 14.3 Hz); 4.00 (m, 2H, -P-O-CH_2-); 3.87 (dd, 1H, -CH_a-P-, $J_{P,CHa}$ = 8.7 Hz, J_{gem} = 12.9 Hz); 3.68 (dd, 1H, -CH_b-P-, $J_{P,CHb}$ = 9.6 Hz, J_{gem} = 12.8Hz); 3.59 (t, 2H, -C*H*$_2$-O-CH_2); 3.46 (d + t, 4H, -CH_2-O-C*H*$_2$ + H-3′); 3.38 (s, 3H, - OCH_3); 1.50-1.62 (m, 2H, -O-CH_2C*H*$_2$(CH_2)$_{15}$-); 1.27 (m, 30H, -(CH_2)$_{15}$-); 0.89 (t, J = 7 Hz, 3H, -CH_3). MS (ESI+): 630.29 $[M+H]^+$; HRMS (ESI-) calcd. for $C_{30}H_{55}N_5O_7P$ $[M-H]^-$ 628.3845, found 628.3843 (E = -0.3 ppm). HPLC analysis: retention time 22.33 min., purity 92.9 %.

4.7.11. Hexadecyloxypropyl (*S*)-9-[3-methoxy-2-phosphonomethoxy)propyl]guanine (HDP-(*S*)-MPMPG) (36)—

Deprotection of **32** gave **36** as a white powder. 93% yield. ^{1}H NMR ($CDCl_3$/methanol-d_4), 7.83 (s, 1H); 4.26-4.31 (m, 1H); 4.06-4.11 (m, 1H); 3.93- 3.98 (m, 2H); 3.81-3.86 (m, 2H); 3.60-3.63 (m, 2H); 3.48-3.57 (m, 3H); 3.35-3.44 (m, 2H); 3.37 (s, 3H); 1.84-1.89 (m, 2H); 1.52-1.58 (m, 2H); 1.15-1.40 (m, 26H); 0.88 (t, J=7Hz, 3H). MS (ESI): 616.45 $[M+H]^+$, 638.40 $[M+Na]^+$. HRMS (ESI-) calcd. for $C_{29}H_{53}N_5O_7P$ $[M-H]^-$ 614.3688, found 614.3687 (E = -0.2 ppm). HPLC analysis: retention time 20.55 min., purity 93.6 %.

4.7.12. Hexadecyloxypropyl (*R*,*S*)-9-[3-ethoxy-2-phosphonomethoxy)propyl]guanine (HDP-(*R*,*S*)-EPMPG) (37)—

Deprotection of **33** gave **37**. 88% yield. ^{1}H NMR ($CDCl_3$/methanol-d_4), 7.85 (s, 1H); 4.28-4.33 (m, 1H); 4.11-4.17 (m, 1H); 3.92- 3.97 (m, 2H); 3.79-3.86 (m, 2H); 3.63-3.92 (m, 1H); 3.47-3.56 (m, 5H); 3.34-3.42 (m, 3H); 1.83-1.90 (m, 2H); 1.52-1.57 (m, 2H); 1.22-1.40 (m, 26H); 1.20 (t, J=7 Hz, 3H); 0.88 (t, J=7 Hz, 3H). MS (EI): 628.40 $[M-H]^-$. HRMS (ESI-) calcd. for $C_{30}H_{55}N_5O_7P$ $[M-H]^-$ 628.3845, found 628.3848 (E = 0.5 ppm). HPLC analysis: retention time 20.92 min., purity 95.1 %.

4.8. Cytosines

4.8.1. (S)-1-[(3-methoxy-2-hydroxy)propyl]-N^4-monomethoxytritylcytosine (38)—

was synthesized from N^4-monomethoxytritylcytosine and (S)-methyl glycidyl ether (procedure A). 91% yield. ^{1}H NMR ($CDCl_3$/methanol-d_4), δ 7.45-7.67 (m, 14H); 7.17 (d, J=6Hz, 1H); 5.82 (d, J=6Hz, 1H); 4.30-4.40 (m, 2H); 4.12 (s, 3H); 3.80-3.92 (m, 1H); 3.65-3.75 (m, 2H); 3.65 (s, 3H).

4.8.2. Octadecyloxyethyl (S)-1-[(3-methoxy-2-phosphonomethoxy)propyl]-N^4-monomethoxytritylcytosine (39)—

was synthesized from (S)-9-[(3-methoxy-2-hydroxy)propyl]-N^4-monomethoxytritylcytosine (**38**) and octadecyloxyethyl p-toluenesulfonyloxymethylphosphonate (procedure C). 45% yield ^{1}H NMR ($CDCl_3$/ methanol-d_4), δ 7.10-7.40 (m, 14H); 6.85 (d, J=6Hz, 1H); 5.52 (d, J=6Hz, 1H); 4.20-4.39 (m, 2H); 3.77-4.02 (m, 4H); 3.55-3.65 (m, 1H); 3.43-3.52 (m, 3H); 3.30-3.45 (m, 8H); 1.45-1.65 (m, 2H); 1.18-1.40 (m, 30H); 0.89 (t, J=7 Hz, 3H). MS (ESI): 860.55 $[M-H]^-$.

4.8.3. Octadecyloxyethyl (*S*)-1-[(3-methoxy-2-phosphonomethoxy)propyl]cytosine, sodium salt (40) (ODE-(*S*)-MPMPC)—

Octadecyloxyethyl (S)-1-[(3-methoxy-2-phosphonomethoxy)propyl]-N^4-monomethoxytritylcytosine (**39**) (0. 26 g, 0.60 mmol) was added to 80% aq acetic acid and heated to 60 °C for 2 hours. After cooling, the solvent was removed in vacuo and the residue purified by flash column chromatography on silica gel. Elution with 20% MeOH/ CH_2Cl_2 gave the product (0.1 g, 55%). ^{1}H NMR ($CDCl_3$/methanol-d_4) δ 7.80 (d, J = 6 Hz, 1H); 6.00 (d, J = 6 Hz, 1H); 4.04-4.15 (m, 4H); 3.55-3.68 (m, 3H); 3.42-3.53 (m, 2H); 3.35-3.42 (m, 4H); 1.50-1.65 (m, 2H); 1.18-1.38 (m, 30H); 0.88 (t, J = 7 Hz, 3H). MS (ESI+): 590.33 [M +H]$^+$, 612.34 [M+Na]$^+$. HRMS (ESI-) calcd. for $C_{29}H_{55}N_3O_7P$ [M-H]$^-$ 588.3783, found 588.3784 (E = -0.2 ppm). HPLC analysis: retention time 22.75 min., purity 90.9 %.

4.9. 6-Methoxypurine derivatives

4.9.1. (S)-9-[(3-methoxy-2-hydroxy)propyl]-6-methoxypurine (41)—Reaction of 6-methoxypurine (TCI America, Portland, OR) with (S)-methyl glycidyl ether according to procedure A gave compound **41**. 67% yield. ^{1}H NMR (methanol-d_4) δ 8.51 (s, 1H, H-8), 8.24 (s, 1H, H-2), 4.74 (dd, 1H, $J_{1'a2'}$= 3.8 Hz, J_{gem}=14.2 Hz), 4.28 (dd, 1H, $J_{1'b2'}$ = 8 Hz, J_{gem} = 14.2 Hz), 4.18 (s, 3H, Ar-OCH_3), 4.14 (m, 1H, H-2′), 3.42 (d, 2H, $J_{3'2'}$ = 5.2 Hz), 3.37 (s, 3H, -CH_2- OCH_3).

4.9.2. (S)-9-[(3-methoxy-2-hydroxy)propyl]-6-O-methylguanine (42)—Reaction of 6-*O*-methylguanine (Aldrich Chem.) with (S)-methylglycidyl ether (Procedure A) gave compound **42**. 78% yield. ^{1}H NMR (methanol-d_4) δ 7.81 (s, 1H, H-8); 4.35 (dd, 2H, H-1′); 4.15 (m, 1H, H-2′); 4.05 (s, 3H, Ar-OCH_3); 3.39 (d, 2H, H-3′); 3.35 (s, 3H, -OCH_3). MS (ESI): m/z 254.08 [M+H]$^+$.

4.9.3. Hexadecyloxypropyl (*S*)-9-[(3-methoxy-2-phosphonomethoxy)propyl]-6-methoxypurine (HDP-(S)-MPMPMP) (43)—Reaction of compound **41** with hexadecyloxypropyl p-toluenesulfonyloxymethylphosphonate according to procedure C afforded compound **43.** 29% yield. ^{1}H NMR (methanol-d_4) δ 8.51 (s, 1H, H-8); 8.44 (s, 1H, H-2); 4.56 (dd, 1H, H-1′$_a$, $J_{1'a,2'}$ = 3.6 Hz, J_{gem} = 14.0 Hz); 4.44 (dd, 1H, H-1′$_b$, $J_{1'b,2'}$ = 6.6 Hz, J_{gem} = 14.6 Hz); 4.17 (s, 3H, Ar-OCH_3); 3.96 (m, 1H, H-2′); 3.86 (t, 1H, P-O-CH_a, J = 6.4 Hz); 3.84 (t, 1H, P-O-CH_b, J = 6.4 Hz); 3.78 (dd, 1H, -CH_a-P-, $J_{P,CH}$ = 9.2 Hz, J_{gem} = 12.8 Hz); 3.60 (dd, 1H, - CH_b-P-, $J_{P,CH}$ = 9.6 Hz, J_{gem} = 12.8 Hz); 3.44 (m, 1H, H-2′); 3.43 (t, 2H, -C*H*$_2$-O-CH_2-); 3.36 (t, 2H, -CH_2-O-C*H2*-); 3.32 (s, 3H, -OCH_3); 1.76 (pentet, 2H, -O-CH_2C*H*$_2$$CH_2$-O-); 1.49 (m, 2H, - CH_2-O-CH_2C*H*$_2$$(CH_2)_{13}$-); 1.28 (m, 26H, -$(CH_2)_{13}$-); 0.89 (t, 3H, -$CH_3$). MS (ESI-) m/z 613.46 [M-H]$^-$. HRMS (ESI-) calcd. for $C_{30}H_{54}N_4O_7P$ [M-H]$^-$ 613.3736, found 613.3739 (E = 0.5 ppm).

4.9.4. Hexadecyloxypropyl (*S*)-9-[(3-methoxy-2-phosphonomethoxy)propyl]-6-O-methylguanine (HDP-(S)-MPMPOMG) (44)—Reaction of compound **42** with hexadecyloxypropyl p-toluenesulfonyloxymethylphosphonate according to procedure C afforded compound **44**. 34% yield. ^{1}H NMR (methanol-d_4) δ 7.95 (s, 1H, H-8), 4.34 (dd, 1H, H-1′a, $J_{1'a2'}$ = 4 Hz, J_{gem} = 14.4 Hz); 4.22 (dd, 1H, H-1′b, $J_{1'b2'}$ = 6.4 Hz, J_{gem} = 14.4 Hz); 4.04 (s, 3H, Ar-OCH_3); 3.91 (m, 1H, H-2′); 3.87 (t, 1H, P-O-CH_a-, J = 6.4 Hz); 3.86 (t, 1H, P-O-CH_b-, J = 6.4 Hz); 3.75 (dd, 1H, -CH_a-P-, $J_{CH,P}$ = 9.4 Hz, J_{gem} = 13 Hz); 3.64 (dd, 1H, -CH_b-P-, $J_{CH,P}$ = 9.2 Hz, J_{gem} = 12.8 Hz); 3.44 (t, 2H, -CH_2-O-); 3.43 (d, 2H, H-3′); 3.35 (t, 2H, -O-CH_2); 3.34 (s, 3H, - OCH_3); 1.78 (pentet, 2H, -O-CH_2C*H*$_2$$CH_2$-O-); 1.50 (m, 2H, -$OCH_2$C*H*$_2$$(CH_2)_{13}$-); 1.27 (m, 26H, $(CH_2)_{13}$); 0.89 (t, 3H, -CH_3). MS (ESI): *m/z* 630.35 [M+H]$^+$; 628.37 [M-H]$^-$. HRMS (ESI-) calcd. for $C_{30}H_{55}N_5O_7P$ [M-H]$^-$ 628.3845, found 628.3847 (E = 0.3 ppm). HPLC analysis: retention time 21.60 min., purity 97.0 %.

4.10. Assays

4.10.1. HCV antiviral activity—HCV assays were carried out as previously described using 10,000 FEO replicon cells (BM4-5 or JFH-1 based) per well in 96-well plates.[14] Cells and compounds were incubated for 48 to 72 hours with all conditions run in triplicate. Additionally, three to four experimental replicates were completed per compound. Luciferase activity was determined using a micro-plate luminometer (Veritas Microplate Luminometer, Turner Biosystems) according to the manufacturer's instructions (Brightglo, Promega). Relative light units (RLU) for each condition were used to generate a dose response for each compound using Prism (version 4, GraphPad software). The cytotoxicity of each compound was determined using a fluorescent cell viability and death assay (MultiTox-Fluor, Promega). All compounds were tested at concentrations up to 100 μM (0.3% DMSO final concentration).

4.10.2. HIV-1 inhibition assays—MT-2 cells (AIDS Research and Reference Reagent Program, National Institute of Allergy and Infectious Diseases, National Institutes of Health) were maintained in RPMI 1640 supplemented with 10% FBS (JRH Biosciences, Lenexa, Kans.), 10 mM HEPES buffer, 50 IU of penicillin/ml, and 50 μg of streptomycin/ml. HIV-1_{LAI} was obtained from the AIDS Research and Reference Reagent Program. The antiviral activity of each compound was determined by inoculating MT-2 cells with HIV-1_{LAI} at a multiplicity of infection (MOI) of 0.001 $TCID_{50}$/cell, followed by incubation in the presence of threefold serial drug dilutions (three wells per dilution). Four days after infection, culture supernatants were harvested, lysed with 0.5% Triton X-100, and assayed for p24 antigen concentration using a commercial enzyme-linked immunosorbent assay (ELISA) (Perkin Elmer Life Sciences, Boston, MA). The antiviral activity of each compound is expressed as the EC_{50}, which is the concentration required to inhibit p24 antigen production by 50%. To assess cytotoxicity, MT-2 cells were incubated with drug for 72 hrs and harvested. Flow count beads (Beckman Coulter, Miami, FL) were added to the cell suspension followed by propidium iodide staining and analysis using an Epics Elite flow cytometer (Beckman Coulter). The 50% cytotoxic concentration (CC_{50}) was calculated from the cell counts and viability.[17]

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Abbreviations

(S)-HPMPA	9-(*S*)-[3-hydroxy-2-(phosphonomethoxy)propyl]adenine
(*S*)-MPMPA	9-(*S*)-[3-methoxy-2-(phosphonomethoxy)propyl]adenine
ODE	octadecyloxyethyl
HDP	hexadecyloxypropyl
ODE-(*S*)-MPMPA	octadecyloxyethyl 9-(*S*)-[3-methoxy-2-(phosphonomethoxy)propyl]adenine, ODE-(*R*)-MPMPA, octadecyloxyethyl 9-(*R*)-[3-methoxy-2-(phosphonomethoxy)propyl]adenine, HDP-(*S*)-MPMPA, hexadecyloxypropyl 9-(*S*)-[3-methoxy-2-(phosphonomethoxy)propyl]adenine, HDP-(*R*,*S*)-EPMPA, hexadecyloxypropyl 9-(*R*,*S*)-[3-ethoxy-2-

	(phosphonomethoxy)propyl]adenine, HDP-(*R*,*S*)-IPPMPA, hexadecyloxypropyl 9-(*R*,*S*)-[3-isopropoxy-2-(phosphonomethoxy)propyl]adenine
ODE-(*S*)-MPMPDAP	octadecyloxyethyl 9-(*S*)-[3-methoxy-2-(phosphonomethoxy)propyl]2,6-diaminopurine
HDP-(*R*,*S*)-EPMPDAP	hexadecyloxypropyl 9-(*R*,*S*)-[3-ethoxy-2-(phosphonmethoxy)propyl]2,6-diaminopurine
ODE-(*S*)-MPMPG	octadecyloxyethyl 9-(*S*)-[3-methoxy-2(phosphonomethoxy)propyl]guanine
ODE-(*S*)-MPMPC	octadecyloxyethyl 1-(*S*)-[3-methoxy-2-(phosphonmethoxy)propyl]cytosine
HDP-(*S*)-MPMPMP	hexadecyloxypropyl 9-(*S*)-[3-methoxy-2-(phosphonomethoxy)propyl]6-methoxypurine
HDP-(*S*)-MPMPOMG	hexadecyloxypropyl 9-(*S*)-[3-methoxy-2-(phosphonomethoxy)propyl]6-*O*-methylguanine

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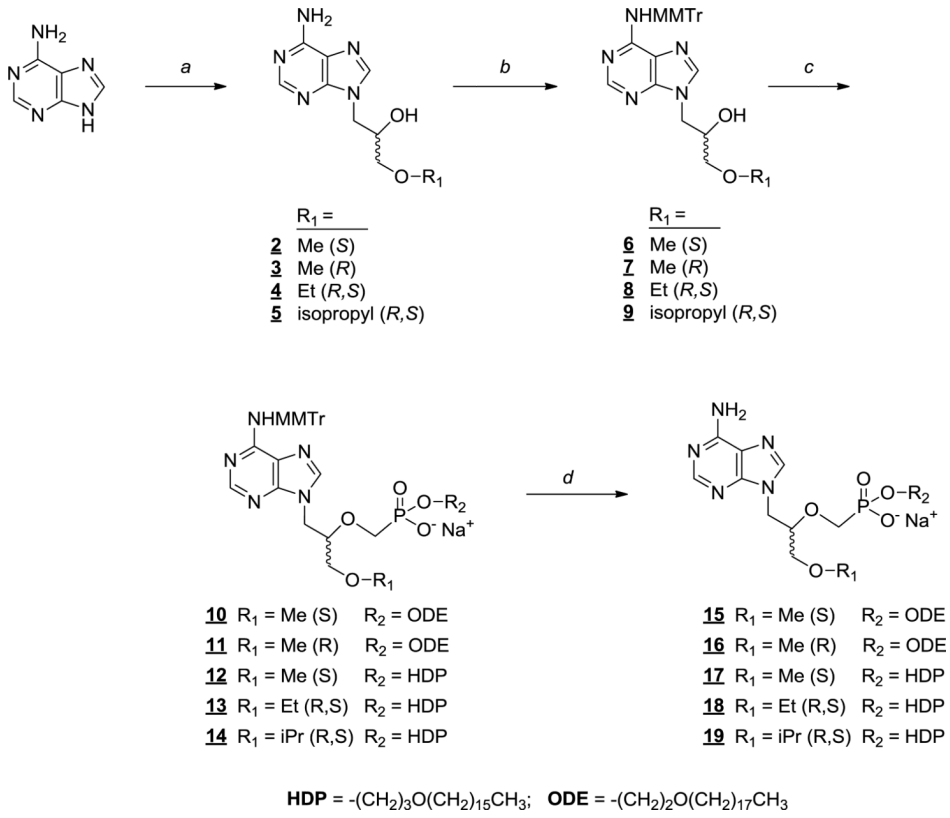
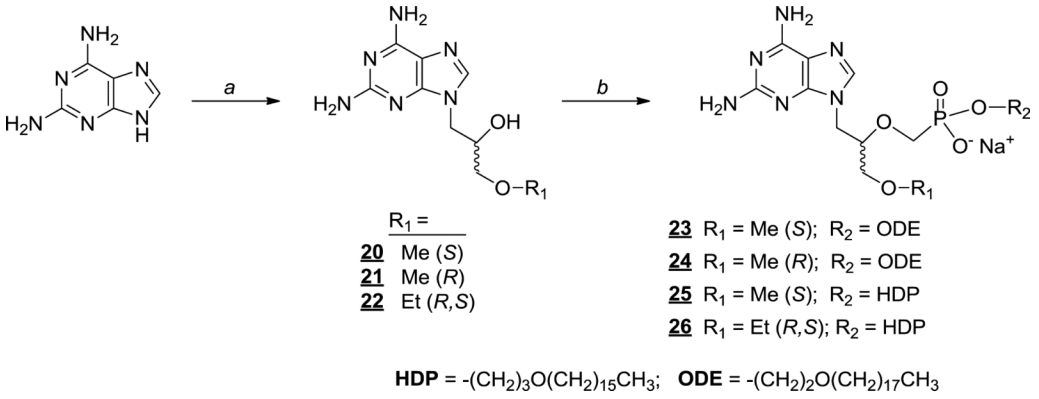


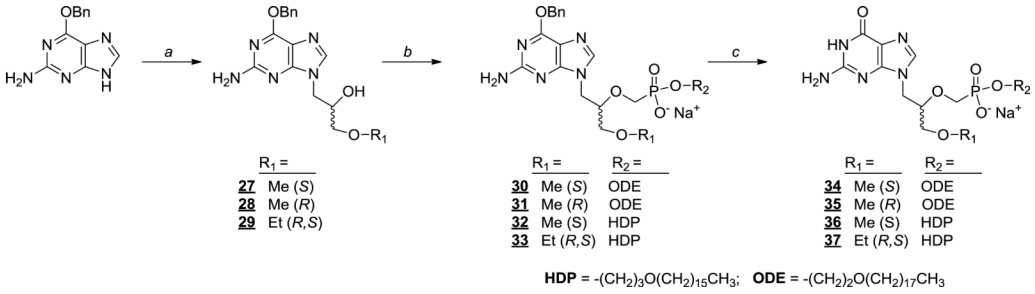
Scheme 1.

Synthesis of adenine derivatives (**15** – **19**). ***Reagents and Conditions***: a) NaH, alkyl glycidyl ether, N,N-DMF, 100 °C, 6h; b) TMSBr, MMTrCl, pyridine; c) sodium t-butoxide, hexadecyloxypropyl (HDP) or octadecyloxyethyl (ODE) p-toluenesulfonyloxymethylphosphonate, N,N-DMF, 80 °C, 16h; d) 80% aq acetic acid, 60 °C, 2h



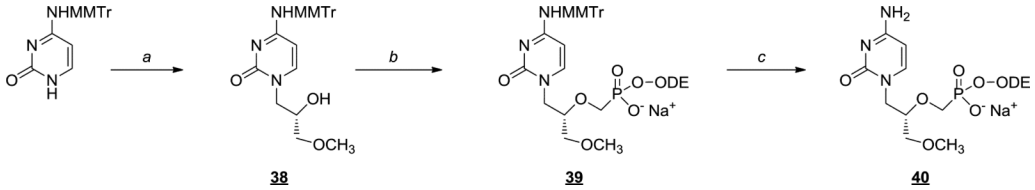
Scheme 2.

Synthesis of 2,6-diaminopurine derivatives (**23** – **26**). ***Reagents and Conditions***: a) NaH, alkyl glycidyl ether, N,N-DMF, 100 °C, 6h; b) sodium t-butoxide, alkoxyalkyl p-toluenesulfonyloxymethylphosphonate, N,N-DMF, 80 °C.



Scheme 3.

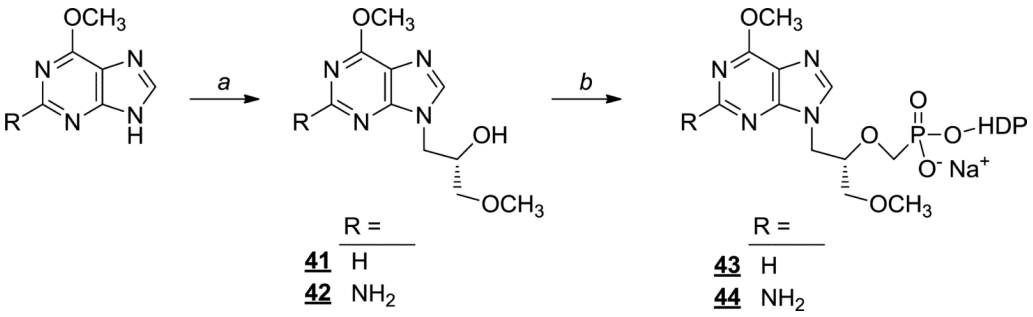
Synthesis of guanine derivatives (**34** – **37**). ***Reagents and Conditions***: a) NaH, alkyl glycidyl ether, N,N-DMF, 100 °C, 6h; b) sodium t-butoxide, hexadecyloxypropyl (HDP) or octadecyloxyethyl (ODE) p-toluenesulfonyloxymethylphosphonate, N,N-DMF, 80 °C; c) 10% CF_3COOH/CH_2Cl_2, rt, 2 days.



38 39 40

Scheme 4.

Synthesis of cytosine derivative (**40**). ***Reagents and Conditions***: a) NaH, (S)-methyl glycidyl ether, N,N-DMF, 100 °C, 6h; b) octadecyloxyethyl (ODE) p-toluenesulfonyloxymethylphosphonate; c) 80% aq acetic acid, 60 °C, 2h

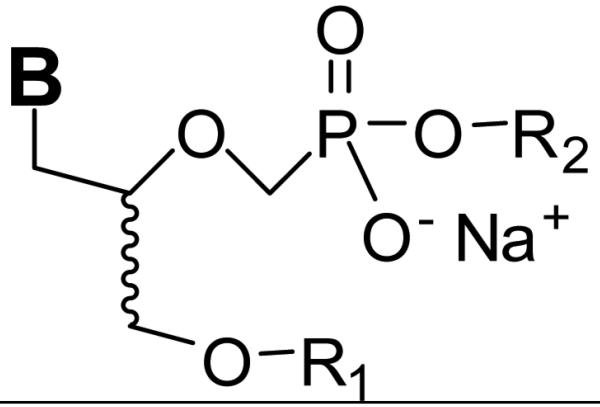


Scheme 5.

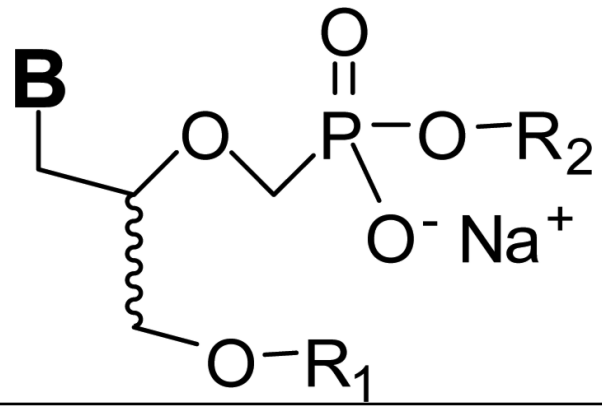
Synthesis of 6-methoxypurine derivatives (**43** – **44**). ***Reagents and Conditions***: a) NaH, (S)-methyl glycidyl ether, N,N-DMF, 100 °C, 6h; b) sodium t-butoxide, hexadecyloxypropyl (HDP) p-toluenesulfonyloxymethylphosphonate, N,N-DMF, 80 °C.

Table 1

HCV Replicon Activity and Cytotoxicity



Cmpd	Base	R_1	R_2	abbreviation	EC_{50} (μM)		CC_{50} (μM)	Selectivity Index BM4-5 (1b)	Selectivity Index JFH-1 (2a)
					BM4-5(1b)	JFH-1 (2a)			
1	adenine	H	ODE	ODE-(*S*)-HPMPA	1.55 ± 0.50	1.65 ± 0.33	35.6 ± 6.8	22.9	21.6
15		Me	ODE	ODE-(*S*)-MPMPA	1.43 ± 0.38	2.38 ± 1.09	>150	>105	>63
16		Me	ODE	ODE-(*R*)-MPMPA	4.65 ± 0.88	5.33 ± 0.92	>150	>32.3	>28.1
17		Me	HDP	HDP-(*S*)-MPMPA	2.36 ± 0.37	4.64 ± 1.26	>150	>63.6	>32.3
18		ethyl	HDP	HDP-(*R,S*)-EPMPA	7.59 ± 1.31	8.87 ± 2.05	99 ± 0.5	13.0	11.1
19		isopropyl	HDP	HDP-(*R,S*)-IPPMPA	51.2 ± 38.2	98.8 ± 20.8	100 ± 20.8	3.14	-
23	2,6-diaminopurine	Me	ODE	ODE-(*S*)-MPMPDAP	20.1 ± 1.98	21.4 ± 1.30	99.0 ± 13.4	4.94	4.62
24		Me	ODE	ODE-(*R*)-MPMPDAP	25.6 ± 4.23	25.8 ± 6.10	>150	>5.86	>5.81
25		Me	HDP	HDP-(*S*)-MPMPDAP	21.3 ± 3.10	23.6 ± 2.70	91.5 ± 17.8	4.30	3.87
26		ethyl	HDP	HDP-(*R,S*)-EMPDAP	18.2 ± 7.09	19.5 ± 1.48	>150	>8.2	>7.69
34	guanine	Me	ODE	ODE-(*S*)-MPMPG	8.26 ± 1.30	10.7 ± 1.33	>150	>18.2	>14.0
35		Me	ODE	ODE-(*R*)-MPMPG	12.6 ± 1.67	12.4 ± 3.45	>150	>11.9	>12.1
36		Me	HDP	HDP-(*S*)-MPMPG	22.0 ± 4.97	24.8 ± 7.00	>150	>6.82	>6.04
37		ethyl	HDP	HDP-(*R,S*)-EPMPG	25.5 ± 10.4	13.2 ± 3.45	>150	>5.88	>11.4
40	cytosine	Me	ODE	ODE-(*S*)-MPMPC	>150	>150	>150	-	-



Cmpd	Base	R_1	R_2	abbreviation	EC_{50} (μM)		CC_{50} (μM)	Selectivity Index BM4-5 (1b)	Selectivity Index JFH-1 (2a)
					BM4-5(1b)	JFH-1 (2a)			
43	6-methoxypurine	Me	HDP	HDP-(*S*)-MPMPMP	8.90 ± 1.22	12.5 ± 3.36	>150	8.04	5.72
44	6-O-methylguanine	Me	HDP	HDP-(*S*)-MPMPOMG	18.1 ± 0.27	17.4 ± 0.30	>150	4.98	5.18

Data are mean ± standard deviation (n=3).

Table 2

Antiviral Activity Against HIV-1 and Cytotoxicity in MT-2 Cells

Compound		EC_{50} (μM)	CC_{50} (μM)	Selectivity Index
1	ODE-(*S*)-HPMPA	0.0001 ± 0.000 (6)	0.033 ± 0.02 (3)	330
15	ODE-(*S*)-MPMPA	0.03 ± 0.015 (3)	22 ± 5 (3)	733
16	ODE-(*R*)-MPMPA	4.8 ± 3.0 (3)	26.7 ± 11.2 (3)	5.6
17	HDP-(*S*)-MPMPA	0.20 ± 0.26 (4)	32 ± 11 (3)	160
18	HDP-(*R,S*)-EPMPA	>10	23.6 ± 7.1 (3)	-
19	HDP-(*R,S*)-IPPMPA	>10	30.3 ± 12.5 (3)	-
23	ODE-(*S*)-MPMPDAP	0.23 ± 0.15	18.0 ± 5.3 (3)	78
24	ODE-(*R*)-MPMPDAP	0.04 ± 0.06 (4)	19.7 ± 9.5 (3)	493
25	HDP-(*S*)-MPMPDAP	4.6 ± 1.8 (3)	22.7 ± 6.8 (3)	4.9
26	HDP-(*R,S*)-EPMPDAP	>10	30.7 ± 11.7 (3)	-
34	ODE-(*S*)-MPMPG	0.20 ± 0.22 (4)	25 ± 13 (3)	125
35	ODE-(*R*)-MPMPG	$<1\times10^{-5}$ (3)	44 ± 5.6 (3)	$>4.4\times10^{6}$
36	HDP-(*S*)-MPMPG	2.03 ± 0.95 (4)	29.3 ± 3.1 (3)	14.4
37	HDP-(*R,S*)-EPMPG	>10	14.9 ± 6.7 (3)	-
40	ODE-(*S*)-MPMPC	12.7 ± 4.0 (3)	60.7 ± 16 (3)	4.8
43	HDP-(*S*)-MPMPMP	>10	22.0 ± 3.5 (3)	-
44	HDP-(*S*)-MPMPOMG	>10	34.3 ± 7.6 (3)	-

Antiviral and cytotoxicity methods as described previously (ref. 11). Data are mean ± std. deviation. The number of replicates is in parentheses.

Table 3

Comparative Antiviral Activity of ODE-(S)-HPMPA versus ODE-(S)-MPMPA Against dsDNA Viruses in vitro

	compound	EC_{50} (μM)			
		Vaccinia	**Cowpox**	**HCMV**	**HSV-1**
1	ODE-(*S*)-HPMPA	0.02 ± 0.01[a]	0.05 ± 0.04[a]	0.003 ± 0.001[a]	<0.0001
15	ODE-(*S*)-MPMPA	18.3 ± 2.4	>20.0	1.55 ± 0.4[b]	45.7 ± 10.1[b]
Fold change		915	>400	516	>45 million

[a] Data and methods from Beadle et al.(ref. 13)

[b] Data for ODE-(S)-MPMPA vs. HCMV and HSV-1 were obtained by plaque reduction assay in HFF cells as previously described by Prichard et al.(ref. 19).