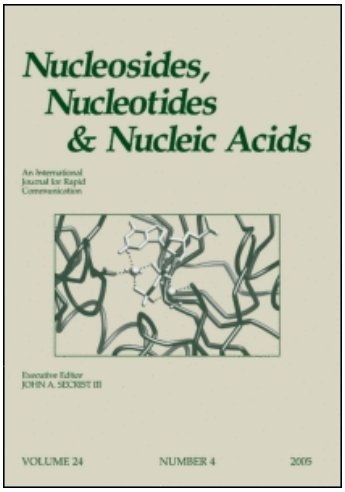

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Phosphoramidate Protides of Carbocyclic 2′,3′-Dideoxy-2′,3′-Didehydro-7-Deazaadenosine with Potent Activity Against HIV and HBV

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ABSTRACT

Synthesis of phosphoramidate protides of carbocyclic D- and L-2′,3′-dideoxy-2′,3′-didehydro-7-deazaadenosine by treatment of the nucleoside with phosphorochloridates in the presence of pyridine and t-BuMgCl is described. Several of these protides showed significantly improved antiviral potency over the parent nucleosides against both HIV and HBV.

Key Words: Carbocyclic 2′3′-dideoxy-2′3′-didehydro-7-deazaadenosine; Phosphoramidate protides; HIV; HBV.

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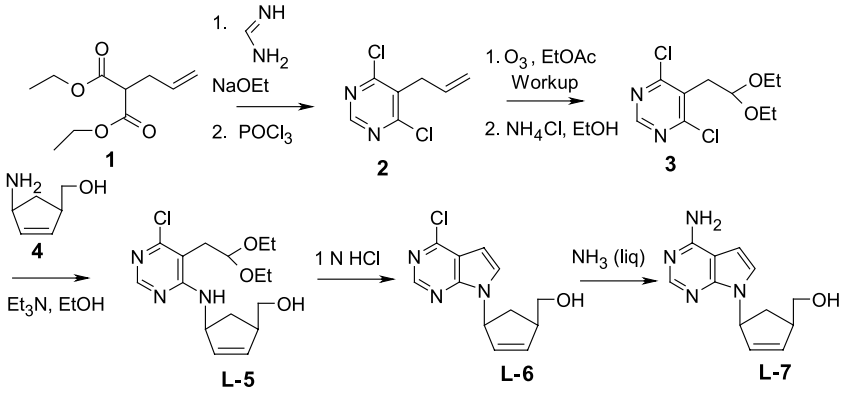
INTRODUCTION

2′,3′-Dideoxyribofuranonucleosides have received considerable attention as this class of nucleosides includes several compounds with potent anti-HIV activity (e.g. ddC, ddI and analogs).[1–3] Besides the synthesis of the natural purine and pyrimidine analogs, several heterocycle-modified 2′,3′-dideoxyribofuranonucleosides, such as pyrrolo[2,3-d]pyrimidines (7-deazaadenine) have been synthesized.[4–6] Several of these have demonstrated antiviral activity. The preclinical research leading to the selection of the carbocyclic nucleoside abacavir (Ziagen) involved the synthesis and evaluation of several racemic carbocyclic analogs, including carbocyclic 2′,3′-dideoxy-2′,3′-didehydro-7-deazaadenosine. As the racemic carbocyclic 2′,3′-dideoxy-2′,3′-didehydro-7-deazaadenosine showed modest HBV activity, we became interested in the synthesis of prodrug derivatives that could deliver its monophosphate intracellularly. Several prodrug strategies have been described in the literature, these include phosphoramidate derivatives,[7–14] cycloSal derivatives[15] and bis(SATE)phosphotriester derivatives.[16,17] All of these have been shown to significantly improve the potency in vitro of a variety of nucleosides against HIV and/or HBV.

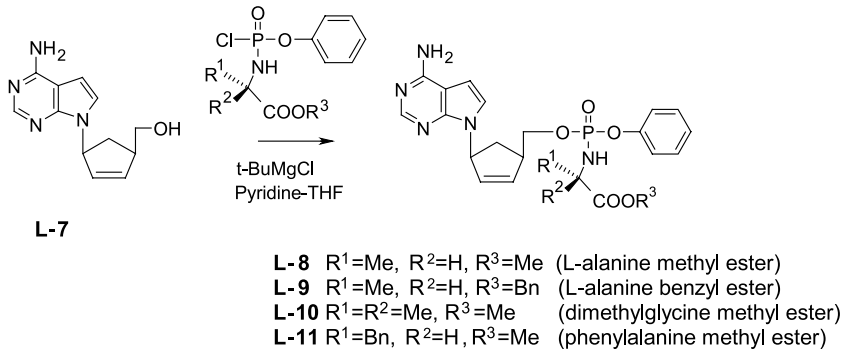
Herein we describe the synthesis of several phosphoramidate prodrugs of carbocyclic 2′,3′-dideoxy-2′,3′-didehydro-7-deazaadenosine along with their anti-HIV and HBV activity.

RESULTS AND DISCUSSION

Several different prodrug strategies have been designed to deliver monophosphates intracellularly. We decided to use the phosphoramidates as this approach is well validated and we have demonstrated that it improves the HBV activity of 7-(2′,3′-dideoxy-3′-fluoro-β-D-ribofuranosyl)pyrrolo[2,3-d]pyrimidine-4-amine.[18] An additional advantage of the phosphoramidates is that the nucleoside can be converted to the phosphoramidate prodrug in one chemical step. The D- and L-nucleosides (**7**) were



Scheme 1. Synthesis of carbocyclic L-2′,3′-dideoxy-2′,3′-didehydro-7-deazaadenosine (**L-7**, D-analogs were synthesized in a similar fashion).



Scheme 2. Synthesis of phosphoramidates.

synthesized as outlined in Scheme 1 using a synthetic strategy similar to the one previously described by Legraverend et al.[19,20] and Montgomery et al.[21]

We chose to synthesize four different phosphoramidate derivatives, the analogs of which had previously been reported to give good improvement in activity against HIV and HBV for other nucleosides.[7–14] Treatment of 2′,3′-dideoxy-2′,3′-didehydro-7-deazaadenosine with the phosphorochloridate reagents[7–14] in pyridine in the presence of t-BuMgCl (as outlined in Scheme 2) gave good yields of protides **8–11**.

The activity of the protide derivatives **8–11** along with the activity of the parent nucleosides (**L-7** and **D-7**) against HIV and HBV is shown in Tables 1 and 2, respectively.

Phosphoramidate delivery improves the anti-HIV activity of the L-nucleoside significantly, up to 3000 fold for the L-alanine methyl ester derivative **L-8**. Activity of the D-nucleoside against HIV was not improved by protide delivery. Significant

Table 1. Anti-HIV-1 (3B) activity and cytotoxicity in MT-4 lymphocytes[22] of carbocyclic 2′, 3′-dideoxy-2′,3′-didehydro-7-deazaadenosine derivatives and their corresponding phosphoramidates.

Compound number[a]	IC_{50} (μM)[b]	CC_{50} (μM)[b]	Selectivity index[b]
L-7 (nucleoside)	280	>500	>2
L-8	0.091	26	>285
L-9	0.175	>10	>57
L-10	0.3	>10	>33
L-11	0.3	31.5	105
D-7 (nucleoside)	>200	>200	–
D-8	>10	>25	~1
D-9	>78	78	~1
D-10	>490	490	~1
D-11	29	42	~1

[a]All protides were tested as 1:1 mixtures of diastereomers at phosphorus.
[b]IC_{50} is 50% inhibitory concentration, CC_{50} is 50% cytotoxic concentration, selectivity index is CC_{50}/IC_{50}, results are averages of two or more experiments, each in duplicate.

Table 2. Anti-HBV activity and cytotoxicity in HepG2-2.2.15 cells[23] of carbocyclic 2′, 3′-dideoxy-2′,3′-didehydro-7-deazaadenosine derivatives and the corresponding phosphoramidates.

Compound number[a]	IC_{50} (μM)[b]	CC_{50} (μM)[b]	Selectivity index[b]
L-7 (nucleoside)	2	50	25
L-8	0.09	230	>2500
L-9	0.1	>11	>110
L-10	0.05	>2	>40
L-11	0.055	>36	>650
D-7 (nucleoside)	>200	>200	–
D-8	54	81	~1
D-9	11	18	~1
D-10	73	64	~1
D-11	20	51	~2

[a]All protides were tested as 1:1 mixtures of diastereomers at phosphorus.
[b]IC_{50} is 50% inhibitory concentration, CC_{50} is 50% cytotoxic concentration, selectivity index is CC_{50}/IC_{50}, results are averages of two or more experiments, each in duplicate.

increases in HIV potency (above 3 orders of magnitude) for the L-isomers suggest that formation of the monophosphate is limiting for carbocyclic L-2′,3′-dideoxy-2′,3′-didehydro-7-deazaadenosine.

Similarly, the activity of the L-enantiomer (**L-7**) against HBV was significantly improved by protide delivery. Thus, all four protides improved activity against HBV about 20-fold. Again the D-isomers proved much less active than the corresponding L-isomers.

The improvements in potency are significantly greater for HIV than for HBV. The SAR for the protide enhancement in activity with variation of the amino acid and ester appear to differ between the HIV (L-alanine methyl ester phosphoramidate gave the best potency) and HBV assay (dimethylglycine methyl ester phosphoramidate gave the best potency), suggesting that different anabolic pathways predominate in liver versus lymphocytes and that the optimal protide structure will vary with the target cell type.

In conclusion, protides of L-2′,3′-dideoxy-2′,3′-7-deazaadenosine significantly improved activity against HIV-1 (about 3 orders of magnitude) and against HBV (about 1–2 orders of magnitude). This was not the case for the corresponding D-analogs. We continue to investigate improvement in activity of nucleosides by protide delivery.

EXPERIMENTAL

Chemistry

Nuclear magnetic resonance (NMR) spectra were obtained at 300 MHz on Varian Unity Plus NMR spectrophotometer. The chemical shifts are reported in parts per million (ppm) relative to tetramethylsilane as an internal standard. Elemental analysis

were performed by Atlantic Microlab Inc. Flash column chromatography was performed using Merck Silica gel 60 (230–400 mesh), and the stated solvent system under pressure. Mass spectra were obtained on Micromass Platform mass spectrometers from Micromass Ltd. Altrincham, UK, using Electrospray Ionization.

(+)-(1R,4S)-4-[4-Chloro-7H-pyrrolo(2,3-d)pyrimidin-7-yl]-2-cyclopentene-1-methanol (L-6): (+)-(1S, 4R)-4-amino-2-cyclopentene-1-methanol hydrochloride (**4**, 3.96 mmol), triethylamine (1.20 g, 11.8 mmol), and 4,6-dichloro-5-(2,2-diethoxy-ethyl)pyrimidine (**3**, 1.05 g, 3.96 mmol) were refluxed in absolute ethanol (5 mL) for 4 hours. The solution was cooled, 1 N NaOH (8 mL) added, and volatiles removed under reduced pressure. The residue was chromatographed on silica gel with 10% methanol-chloroform. Product-containing fractions (**L-5**) were combined and concentrated to an oil. This oil (**L-5**) was dissolved in dioxane (10 mL)—1 N hydrochloric acid (3 mL) and the solution stirred at ambient temperature for 2 days, neutralized with ammonium hydroxide, and volatiles evaporated under reduced pressure. The residue was chromatographed on silica gel and title compound (**L-6**) eluted with 5–10% methanol-chloroform as a light tan oil (0.60 g, 60%); ^{1}H-NMR (DMSO-d_6) δ: 8.67 (s, 1 H), 7.70 and 6.69 (two d, J = 3.65 Hz, 1 H each), 6.10 (m, 1 H), 6.0–5.8 (m, 2 H), 4.77 (m, 1 H), 3.5 (m, 2 H), 2.9 (m, 1 H), 2.7 (m, 1 H), 1.6 (m, 1 H); MS (API$^+$): 252(35), 250(100), 156(24), 154(73). $[\alpha]_{589}$+ 43.6° (c 0.0045, methanol).

(+)-(1R,4S)-4-[4-Amino-7H-pyrrolo(2,3-d)pyrimidin-7-yl]-2-cyclopentene-1-methanol (L-7): (+)-4-[4-Chloro-7H-pyrrolo(2,3-d)pyrimidin-7-yl]-2-cyclopentene-1-methanol (**L-6**, 600 mg, 2.36 mmol) was heated in liquid ammonia (50 mL) in a Parr bomb at 65°C for 3 days. Volatiles were evaporated with 1 N sodium hydroxide (2.3 mL) and the residue chromatographed on silica gel. Title compound was eluted with 10% methanol-chloroform as a colorless oil. Evaporation of an acetonitrile solution gave title compound (**L-7**) as white powder which was washed with hexanes (291 mg, 52%); m.p. 165–168°C; ^{1}H-NMR (DMSO-d_6) δ: 8.07 (s, 1H), 7.06 and 6.56 (two d, J = 3.5 Hz, 1 H each), 7.0 (br s, 2 H), 6.10 (m, 1 H), 5.80 (m, 2 H), 4.75 (m, 1 H), 3.45 (m, 2 H), 2.85 (m, 1 H), 2.6 (m, 1 H), 1.45 (m, 1 H) ; MS (CI): M + 1, 231(100), 163(37), 135(45). Anal. Calcd. for $C_{12}H_{14}N_4O \times 1/2\ H_2O$: C, 60.24; H, 6.32; N, 23.41. Found: C, 60.54; H, 5.96; N, 23.73. $[\alpha]_{589}$ + 42.9° (c 0.0028, methanol).

(1R,4S)-4-[4-Amino-7H-pyrrolo(2,3-d)pyrimidin-7-yl]-2-cyclopentene-1-methanol-*O*-[phenyl (methoxy L-alaninyl)]phosphoramidate (L-8): To (+)-(1R, 4S)-4-[4-amino-7H-pyrrolo(2,3-d)pyrimidin-7-yl]-2-cyclopentene-1-methanol (**L-7**, 0.19 g, 0.8 mmoles) was added anhydrous pyridine (4 mL) and anhydrous tetrahydrofuran (3 mL). Subsequently, tert-butylmagnesiumchloride (0.9 mL, 1 M solution in tetrahydrofuran) was added and the reaction stirred under nitrogen for 10 minutes at room temperature. A solution of phenyl methoxy-L-alaninyl phosphochloridate (1.2 mmol in anhydrous tetrahydrofuran) was added and the reaction stirred at room temperature for 12 hours. The resulting mixture was concentrated to a syrup under reduced pressure. This syrup was dissolved in dichloromethane (60 mL) and the organic phase washed with water (2 × 30 mL), dried (magnesium sulfate), filtered and concentrated to a foam. This foam was purified by flash column chromatography on silica gel. The title compound (**L-8**) was eluted with 5 % methanol in chloroform to give, after removal of volatiles,

290 mg (77%) of a white foam: ^{1}H-NMR ($CDCl_3$) δ: 8.23 (s, 1 H), 7.22 (m, 2 H), 7.10 (m, 3 H), 6.88 (2 × d, 1 H), 6.27 (d, 1 H), 5.98 (m, 1 H), 5.87 (m, 1 H), 5.80 (m, 1 H), 5.35 (bs, 2 H), 3.85–4.10 (m, 4 H), 3.61 (s, 3 H), 3.06 (m, 1 H), 2.7 (m, 1 H), 1.50 (m, 1 H), 1.28 (m, 3 H); ^{31}P-NMR ($CDCl_3$): 2.99 and 2.80; MS (EI): M + 23 (Na), 494. Anal. Calcd. for $C_{22}H_{26}N_5O_4P \times 3/2\ H_2O$: C, 54.77; H, 6.06; N, 14.52; Found: C, 54.32; H, 5.79; N, 14.22.

(1R,4S)-4-[4-Amino-7H-pyrrolo(2,3-d)pyrimidin-7-yl]-2-cyclopentene-1-methanol-*O*-[phenyl (benzyloxy L-alaninyl)]phosphoramidate (L-9). (+)-(1R, 4S)-4-[4-Amino-7H-pyrrolo(2,3-d)pyrimidin-7-yl]-2-cyclopentene-1-methanol (**L-7**, 0.20 g, 0.9 mmoles) was treated with phenyl benzyloxy-L-alaninyl phosphochloridate (2.5 mL of 1 M solution in tetrahydrofuran, 2.5 mmol) as described above to give after silica gel chromatography with 5% methanol in chloroform 330 mg (69%) of the title compound **L-9** as a white foam: ^{1}H-NMR ($CDCl_3$) δ:8.34 (s, 1 H), 6.9–7.4 (m, 10 H), 6.95(2 × d, 1 H), 6.35 (m, 1 H), 5.8 –6.1 (3 × m, 3 H), 5.45 (broad s, 2 H), 5.13 (m, 2 H), 4.0–4.2 (m, 4 H), 3.1 (m, 1 H), 2.75 (m, 1 H), 1.58 (m, 1 H), 1.4 (m, 3 H); ^{31}P-NMR ($CDCl_3$):3.2 and 3.0; MS (EI): M + 1, 548; Anal. Calcd. for $C_{28}H_{30}N_5O_5P \times 1/3\ H_2O$: C, 60.75; H, 5.58; N, 12.65; Found: C, 60.93; H, 5.65; N, 12.58.

(1R,4S)-4-[4-Amino-7H-pyrrolo(2,3-d)pyrimidin-7-yl]-2-cyclopentene-1-methanol-*O*-[phenyl (methoxy L-dimethylglycinyl)]phosphoramidate (L-10): (+)-(1R, 4S)-4-[4-Amino-7H-pyrrolo(2,3-d)pyrimidin-7-yl]-2-cyclopentene-1-methanol (**L-7**, 0.20 g, 0.9 mmoles) was treated with phenyl methoxy-L-dimethylglycinyl phosphochloridate (2.5 mL of 1 M solution in tetrahydrofuran, 2.5 mmoles) as described for preparation of **L-8** to give, after silica gel chromatography with 5% methanol in chloroform, 310 mg (73%) of the title compound **L-10** as a white foam: ^{1}H-NMR ($CDCl_3$) δ:8.35 (s, 1 H), 7.1–7.4 (m, 5 H), 6.97 (2 × d, 1 H), 6.38 (m, 1 H), 6.08 (m, 1 H), 5.97 (m, 1 H), 5.90 (m, 1 H), 5.40 (broad s, 2 H), 4.00–4.20 (m, 3 H), 3.73 (s, 3 H), 3.15 (m, 1 H), 2.8 (m, 1 H), 2.25 (s, 1 H), 1.55 (m, 6 H); ^{31}P-NMR ($CDCl_3$):1.73; MS (EI): M + 1, 486; Anal. Calcd. for $C_{23}H_{28}N_5O_5P \times 1/2\ H_2O$: C, 55.87; H, 5.91; N, 14.16; Found: C, 55.93; H, 5.91; N, 14.16.

(1R,4S)-4-[4-Amino-7H-pyrrolo(2,3-d)pyrimidin-7-yl]-2-cyclopentene-1-methanol-*O*-[phenyl (methoxy L-phenylalaninyl)]phosphoramidate (L-11): (+)-(1R, 4S)-4-[4-amino-7H-pyrrolo(2,3-d)pyrimidin-7-yl]-2-cyclopentene-1-methanol (**L-7**, 0.20 g, 0.9 mmoles) was treated with phenyl methoxy-L-phenylalaninyl phosphochloridate (2.5 mL of 1 M solution in tetrahydrofuran, 2.5 mmoles) as described above to give after silica gel chromatography with 5% methanol in chloroform 320 mg (67%) of the title compound **L-11** as a white foam: ^{1}H-NMR ($CDCl_3$) δ: 8.22 (s, 1 H), 6.9–7.3 (m, 10 H), 6.85(m, 1 H), 6.25 (2 × d, 1 H), 5.7–6.0 (m, 3 H), 5.35 (broad s, 2 H), 4.15 (m, 1 H), 3.55–4.0 (m, 2 H), 3.55 (2 × s, 3 H), 2.6–2.9 (m, 5 H), 1.4 (m, 1 H); ^{31}P-NMR ($CDCl_3$):3.0; MS (EI): M + 1, 548; Anal. Calcd. for $C_{28}H_{30}N_5O_5P \times 0.7$ MeOH: C, 60.48; H, 5.80; N, 12.29; Found: C, 60.49; H, 5.51; N, 11.98.

(−)-(1S,4R)-4-[4-Chloro-7H-pyrrolo(2,3-d)pyrimidin-7-yl]-2-cyclopentene-1-methanol (D-6): Spectral data identical to **L-6**; $[\alpha]_{589} - 44°$ (c 0.05, methanol).

(-)-(1S,4R)-4-[4-Amino-7H-pyrrolo(2,3-d)pyrimidin-7-yl]-2-cyclopentene-1-methanol (D-7): (−)-4-[4-Chloro-7H-pyrrolo(2,3-d)pyrimidin-7-yl]-2-cyclopentene-1-methanol (**D-6**, 4 g, 16 mmol) was treated as described above for **L-7** to give 2.5 g, 68% of **D-7** as a white solid: Spectral data identical to **L-7**. Anal. Calcd. for $C_{12}H_{14}N_4O$: C, 62.59; H, 6.13; N, 24.33. Found: C, 62.70; H, 6.25; N, 24.52. $[\alpha]_{589}$ − 43.2° (c 0.004, methanol).

(1S,4R)-4-[4-Amino-7H-pyrrolo(2,3-d)pyrimidin-7-yl]-2-cyclopentene-1-methanol-*O*-[phenyl (methoxy L-alaninyl)]phosphoramidate (D-8): To (−)-(1R, 4S)-4-[4-amino-7H-pyrrolo(2,3-d)pyrimidin-7-yl]-2-cyclopentene-1-methanol (**D-7**, 0.5 g, 2.2 mmoles) was added anhydrous pyridine (2 mL) and anhydrous tetrahydrofuran (10 mL). Subsequently, tert-butylmagnesiumchloride (2.8 mL, 1 M solution in tetrahydrofuran) was added and the reaction stirred under nitrogen for 10 minutes at room temperature. A solution of phenyl methoxy-L-alaninyl phosphochloridate (4.3 mmol in anhydrous tetrahydrofuran) was added and the reaction stirred at room temperature for 12 hours. Workup as described above gave 450 mg (44%) of **D-8** as a white foam: Spetral data identical to **L-8**. Anal. Calcd. for $C_{22}H_{26}N_5O_4P \times 3/2\ H_2O$: C, 54.77; H, 6.06; N, 14.52; Found: C, 54.62; H, 5.99; N, 14.13.

(1S,4R)-4-[4-Amino-7H-pyrrolo(2,3-d)pyrimidin-7-yl]-2-cyclopentene-1-methanol-*O*-[phenyl (benzyloxy L-alaninyl)]phosphoramidate (D-9): (−)-(1R, 4S)-4-[4-Amino-7H-pyrrolo(2,3-d)pyrimidin-7-yl]-2-cyclopentene-1-methanol (**D-7**, 0.28 g, 1.2 mmoles) was treated with phenyl benzyloxy-L-alaninyl phosphochloridate (1 M solution in tetrahydrofuran, 2.43 mmol) as described above to give after silica gel chromatography with 5% methanol in chloroform 539 mg (81%) of **D-9** as a white foam: Spectral data identical to **L-9**. Anal. Calcd. for $C_{28}H_{30}N_5O_5P \times H_2O$: C, 59.46; H, 5.70; N, 12.38; Found: C, 59.18; H, 5.41; N, 12.37.

(1S,4R)-4-[4-Amino-7H-pyrrolo(2,3-d)pyrimidin-7-yl]-2-cyclopentene-1-methanol-*O*-[phenyl (methoxy L-dimethylglycinyl)]phosphoramidate (D-10): (−)-(1R, 4S)-4-[4-Amino-7H-pyrrolo(2,3-d)pyrimidin-7-yl]-2-cyclopentene-1-methanol (**D-7**, 0.28 g, 1.2 mmoles) was treated with phenyl methoxy-L-dimethylglycinyl phosphochloridate (1 M solution in tetrahydrofuran, 2.43 mmoles) as described above to give, after silica gel chromatography with 5% methanol in chloroform, 400 mg of **D-10** as a white foam: Spectral data identical to **L-10**. Anal. Calcd. for $C_{23}H_{28}N_5O_5P \times 1/2\ H_2O$: C, 55.87; H, 5.91; N, 14.16; Found: C, 55.45; H, 5.75; N, 14.39.

(1S,4R)-4-[4-Amino-7H-pyrrolo(2,3-d)pyrimidin-7-yl]-2-cyclopentene-1-methanol-*O*-[phenyl (methoxy L-phenylalaninyl)]phosphoramidate (D-11): (−)-(1R, 4S)-4-[4-amino-7H-pyrrolo(2,3-d)pyrimidin-7-yl]-2-cyclopentene-1-methanol (**D-7**, 0.41 g, 1.8 mmoles) was treated with phenyl methoxy-L-phenylalaninyl phosphochloridate (1 M solution in tetrahydrofuran, 3.58 mmoles) as described above to give after silica gel chromatography with 5% methanol in chloroform 350 mg (36%) of **D-11** as a white foam: Spectral data identical to **L-11**. Anal. Calcd. for $C_{28}H_{30}N_5O_5P \times H_2O$ C, 59.46; H, 5.70; N, 12.38; Found: C, 59.42; H, 5.36; N, 12.09.

ANTIVIRAL ASSAYS

HBV

Antiviral potency and growth inhibition potential of compounds was determined using the assay developed by Jansen et al.[a] Briefly, HepG2-2.2.15 cells constitutively producing HBV[24] were seeded into 96 well microtiter plates at a density of 5×10^3 per well and growth medium containing drug was replaced every other day for 9 days. Supernatants were then collected and analyzed for HBV content. Samples were tested in conjunction with both positive (0.448 fg/ul plasmid DNA) and negative (RPMI medium supplemented with 2mM L-glutamine and 10% fetal calf serum) controls. Data was normalized to non-drug treated cells, and expressed as a percent of control for analysis.

Evaluation of toxicity (i.e. growth inhibition) was made by fixing monolayers with 70% ethanol, and staining with bisbenzimide H33342 for 1 hour at 37°C. Fluorescence values of drug treated cells were compared to non-drug treated cells and expressed as a percentage of control. HBV detection (and hence efficacy determination) was performed by ''capturing'' virus from supernatants on anti-HBsAg coated plates, washing, denaturing to release HBV DNA, performing PCR with biotinylated primers, streptavidin capture of biotinylated PCR products with concomitant probe hybridization, addition of substrate, and reading optical densities of the colorimetric reaction. Dilutions of a standardized HBV-containing supernatant were included on every plate, and HBV DNA concentrations of test wells were calculated from this HBV standard curve.

HIV

Anti-HIV-1 (3B) activity and cytotoxicity in MT-4 lymphocytes was determined as previously described by Averett et al.[b]

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[a]Compounds were tested for anti-HBV activity according to the method described by: From Ref. [23].

[b]Compounds were assayed for HIV activity in MT-4 cells according to the method described by: [From Ref. 22].

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