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Synthesis of 1,2,3-Triazolo-carbanucleoside Analogues of Ribavirin Targeting an HCV in Replicon

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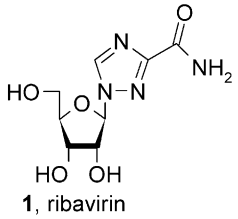
Abstract—The synthesis of carbocyclic and phosphonocarbocyclic analogues of ribavirin, an anti-HCV inhibitor, are described. Those compounds were evaluated against HCV but also against other important viruses in order to determine their spectrum of antiviral activity. Compounds **6** and **13** displayed a moderate IC_{50} against HIV-1 of 43.8 and 37 μM, respectively.

Introduction

Chronic hepatitis C (HCV) remains a major public health problem, with an estimated 200–300 million people infected in the world. Indeed, despite the mass screening program for early diagnosis and treatment of HCV infection, and the improvement of antiviral therapy[1] relying on the combination pegylated interferon[2] and ribavirin[3] [**1**, virazole®, (1-β-D-ribofuranosyl-1,2,4-triazole-3-carboxamide], 40–50% of patients are no responders to the best available treatments (Fig. 1). Recently, major knowledge has been gained about the mechanism of HCV replication, due to the development of HCV infection study models. The expression of enzymatically active nonstructural viral proteins required for HCV genome replication and the development of a replicon system allowing the replication of an HCV subgenome in hepatocyte culture should allow us to study new and specific inhibitors of HCV replication, as well as new targets for antiviral therapy. Of several putative viral enzyme targets, the NS5B polymerase has been intensively studied because of its central role in viral replication.[4] Recently Bressanelli et al.[5] reported a first co-crystallization of HCV NS5B with ribonucleotides, which lead to the identification of a specific and unique GTP-binding pocket outside the active site. Thus, specific inhibitors of this virus-encoded RNA-dependent RNA polymerase (RdRP) should serve as potent antiviral agents.

The pharmaceutical importance of carbocyclic nucleoside analogues has prompted the design and synthesis of many examples of these compounds showing activity against HIV, HBV, and HSV types 1 and 2.[6] As part of our drug discovery program, this paper provides a full account of the synthesis and biological evaluation of new carbocyclic analogues **4**–**6** of the anti-HCV agent ribavirin. We have chosen to introduce some 1,2,3-triazole heterocycles, as they have been considered an interesting component in terms of biological activity and are seen in many drugs.[7,8] Because it has been suggested that one of the active forms of ribavirin is the monophosphate, which inhibits inosine monophosphate dehydrogenase (IMPDH), decreasing the intracellular concentration of GTP and leading to a decrease of viral protein synthesis, we also turn our attention to the



1, ribavirin

Figure 1. Chemical structure of anti-HCV ribavirin.

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synthesis of the phosphonate carbocyclic analogues of ribavirin **11**, **12**, and **14**. We also evaluated the newly synthesized compounds against other important viruses in order to determine their spectrum of antiviral activity.

Chemistry

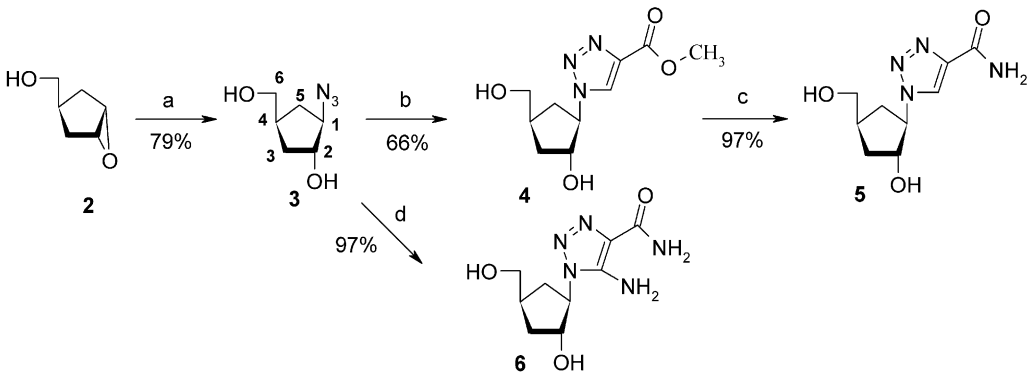
The preparation of the desired ribavirin analogues **4–6**, **11**, **13**, and **15** is illustrated in Schemes 1 and 2. Thus, starting with the known epoxide (**2**),[9] obtained from the 1-hydroxymethyl-3-cyclopentene,[10] new carbocyclic analogues **4–6** were synthesized through a series of efficient chemical transformations (Scheme 1). The key step for the preparation of 1,2,3-triazoles involves a 1,3-dipolar cycloaddition[11] between azides and alkynes. Thus, the opening of the epoxide ring of **2** by NaN_3 yielded **3** (79%), which was reacted with methylpropiolate to afford directly the (±)-1-(2-hydroxy-4-hydroxymethyl-cyclopentyl)-1H-[1,2,3]triazole-4-carboxylic acid methyl ester (**4**) in 66% yield. When using a solution of 2-cyanoacetamide on the azido compound (**3**), the (±)-5-amino-1-(2-hydroxy-4-hydroxymethyl-cyclopentyl)-1H-[1,2,3]triazole-4-carboxylic acid amide (**6**) was isolated in 97% yield. Ammonolysis of (**4**) yielded the amide analogue **5** (97%).

For preparation of the phosphono carbanucleoside counterparts, **11**, **13** and **15**, the treatment of epoxide **2** with tosylchloride and the subsequent activation with sodium iodide provided **7** (77%), which was reacted with the lithium salt of diisopropyl methanephosphonate in THF to furnish the phosphono-epoxide **8** in 90% yield.[12]

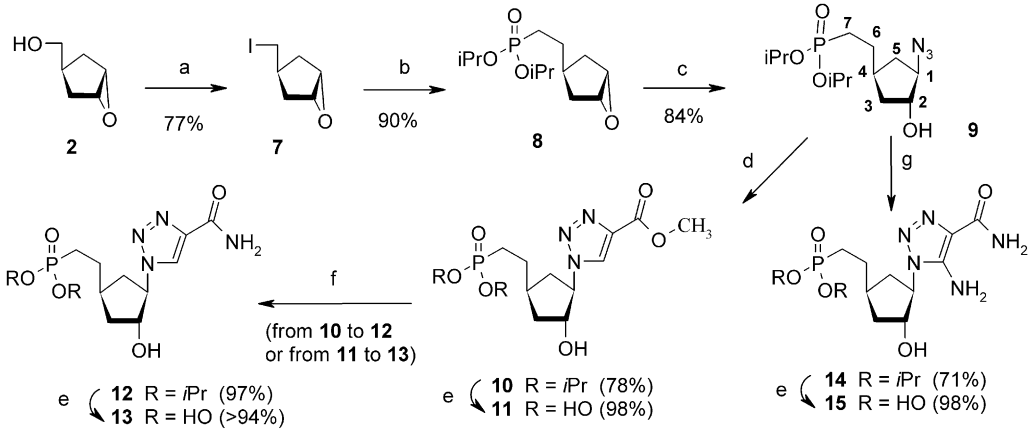
Reaction of phosphono-epoxide **8** with sodium azide in MeOH–H_2O (8:1) gave the azide **9** in 84% yield. The azido group of **9** was reacted with methylpropiolate and 2-cyanoacetamide to yield the (±)-1-{4-[2-(diisopropoxy-phosphoryl)-ethyl]-2-hydroxy-cyclopentyl}-1H-[1,2,3]triazole-4-carboxylic acid methyl ester (**10**) and (±)-{2-[3-(5-amino-4-carbamoyl-[1,2,3]triazol-1-yl)-4-hydroxy-cyclopentyl]-ethyl}-phosphonic acid diisopropyl ester (**14**), respectively. The final deprotection of **10** and **14** with a solution of bromotrimethylsilane in CH_2Cl_2 afforded the free phosphonic acid final compounds **11** (98%) and **15** (98%), respectively. The amide analogue **13** was obtained by a first ammonolysis of **10** (97%) and subsequent deprotection (99%), or directly from **11** (in 94%) with a solution of $MeOH/NH_3$. Compounds have been obtained in good yields and analyzed by HRMS.

Biological Assay

The synthesized compounds **4–6**, **11**, **13**, and **15**, along with the known antiviral compounds (acyclovir for



Scheme 1. Reagents and conditions: (a) NaN_3, MeOH, H_2O, rfx, 15 h; (b) methylpropiolate, 50 °C, 16 h; (c) NH_3/MeOH, 0 °C, 16 h; (d) 2-cyanoacetamide, K_2CO_3, DMSO, 50 °C, 16 h.



Scheme 2. Reagents and conditions: (a) TsCl, pyridine, 0 °C then NaI, acetone; (b) $(iPrO)_2P(O)CH_3$, *n*BuLi, THF, −78 °C; (c) NaN_3, MeOH; (d) methylpropiolate, 50 °C, 16 h; (e) Me_3SiBr, CH_2Cl_2, rt; (f) $MeOH/NH_3$, 0 °C, 16 h; (g) 2-cyanoacetamide, K_2CO_3, DMSO, rt.

Table 1. Evaluation of 1,2,3-triazolo-carbanucleoside analogue antiviral activity against human immunodeficiency virus (HIV), herpes simplex virus (HSV-1), hepatitis B hirus (HBV) and cytotoxicity against PBM, CEM and VERO cells in vitro, expressed in μM

Compd.	Anti-HIV-1 activity in human PBM cells		HSV-1 plaque reduction assay		HBV	Toxicity (IC_{50}) in:		
	EC_{50}	EC_{90}	EC_{50}	EC_{90}	EC_{90}	PBM	CEM	VERO
Acyclovir	>100	>100	0.11	0.69	>100	>100	>100	>100
3TC	0.005	0.002	>100	>100	0.24±0.28	>100	>100	>100
AZT	0.016	0.10	>10	>10	>10	>100	14.0	29.0
4	>100	>100	>100	>100	>10	>100	>100	>100
5	>100	>100	>100	>100	>10	>100	>100	>100
6	**43.8**	**89.0**	>100	>100	>10	>100	>100	>100
11	>100	>100	>100	>100	>10	>100	>100	>100
13	**37.0**	**=100**	>100	>100	>10	>100	>100	>100
15	>100	>100	>100	>100	>10	>100	>100	>100

Table 2. Evaluation of 1,2,3-triazolo-carbanucleoside analogue antiviral activity against hepatitis C virus (HCV) Replicon activity, expressed in μM, except for IFN which is expressed as international units/mL

Compd.	Anti-HCV replicon activity (IC_{50})		Cell viability assays (CC_{50})
	RNA (−)	RNA (+)	
Interferon α-**2b**	79	74	>1000
Ribavirin	230	170	110
Mycophenolic acid	>40	40	7
4	>320	>320	>320
5	>320	>320	>320
6	>320	>320	>320
11	>320	>320	>320
13	>320	>320	>320
15	>320	>320	>320

HSV, IFN for HCV, 3TC for HBV and AZT for HIV), were tested for their antiviral activities in vitro, and the results are shown in Table 1. The procedures used were the same as described previously. Among these nucleoside analogues, only compounds (±)-5-amino-1-(2-hydroxy-4-hydroxy-methyl-cyclopentyl)-1H-[1,2,3]triazole-4-carboxylic acid amide (**6**) and the phosphonate (±)-{2-[3-(5-amino-4-carbamoyl-[1,2,3]triazol-1-yl)-4-hydroxy-cyclopentyl]-ethyl}-phosphonic acid diisopropyl ester (**14**) were found to exhibit moderate anti-HIV activity, with an EC_{50} of 43.8 and 37.0 μM, respectively. Both compounds showed no cytotoxicity against PBM, CEM or VERO cells. The carbocyclic analogues of ribavirin **4**, **5**, and **6** and their respective phosphonate derivatives **11**, **13**, and **15**, were reasoned to be potential inhibitors of the *inosine monophosphate dehydrogenase* (IMPDH), an enzyme involved in the metabolism of guanosine monophosphate (GMP) and a potential target for anti-HCV strategy. Therefore, these compounds, along with other anti-HCV compounds (interferon α-2b, ribavirin and mycophenolic acid [MPA]) were evaluated for their antiviral activities and toxicity in vitro using Huh7 cells harboring and replicating subgenomic HCV replicons, as described in the experimental section. The results are presented in Table 2. None of the synthesized compounds reached the inhibitory concentration 50 at the highest concentrations tested (e.g., 320 μM), indicating a lack of significant antiviral activity in our experimental conditions. It is worth noting that both ribavirin and MPA, two molecules known for their inhibitory effect on IMPDH, did not show any specific antiviral activity, suggesting that this type of inhibitor might be inactive in this in vitro system, in contrast with interferon α-2b used as a positive control. In the absence of any potent antiviral activity of **4**, **5**, and **6** and their phosphonate derivatives **11**, **13**, and **15**, it would be interesting to evaluate the di- and triphosphonate forms of **4**, **5**, and **6**, to determine whether they could inhibit the HCV polymerase activity in this replicon system.

Experimental

Commercially available chemicals and solvents were reagent grade and used as received. Dry tetrahydrofuran, pyridine and dichloromethane were obtained from distillation over CaH_2 or Na, *N,N*-dimethylformamide over BaO. The reactions were monitored by thin-layer chromatography (TLC) analysis using silica gel plates (Kieselgel 60 F_{254}, E. Merck). Compounds were visualized by UV irradiation and/or spraying with 20% H_2SO_4 in EtOH, followed by charring at 150 °C. Column chromatography was performed on Silica Gel 60 M (0.040–0.063 mm, E. Merck). Melting points were recorded on a Büchi (Dr. Tottoli) and were uncorrected. ^1H and ^{13}C NMR spectra were recorded on a Bruker AVANCE DPX 250 Fourier Transform spectrometer at 250 MHz for ^1H and 62.9 MHz for ^{13}C, respectively, using tetramethylsilane as the internal standard; signals are reported as s (singlet), d (doublet), t (triplet), q (quartet), m (multiplet). Mass spectra were recorded on a Perkin-Elmer SCIEX API-300 (heated nebullizer) spectrometer. High-resolution mass analyses (HRMS) were performed by the CRMPO, University of Rennes 1, and Fr using the Fast Atom Bombardment (FAB) or Electron Spray Ionization (ESI) mode. The nomenclature of the obtained compounds is in accordance with the IUPAC rules and was checked with Autonome.[13] The numbering and assignment of the chemical shifts for all described compounds are related to the corresponding ribose or carbanucleosides derivatives.

(±)-2-Azido-4-hydroxymethyl-cyclopentanol (3). A suspension of **2** (155 mg, 1.36 mmol), NaN_3 (176 mg, 2.72 mmol) in MeOH–H_2O (8:1, 35 mL) was refluxed overnight. After filtration, the organics were concentrated under reduced pressure, and the residue was purified by silica gel column chromatography (10:1 CH_2Cl_2–MeOH) to give **3** (79%, 170 g) as a colorless oil. ^1H NMR (CD_3OD–D_2O) δ 1.35–1.42 (m, 2H, 3-Hα, 5-Hα), 1.69 (m, 1H, 4-H), 2.25–2.34 (m, 2H, 3-Hβ, 5-Hβ), 3.44 (d, 2H, J = 6.5 Hz, 6-H), 3.67 (dd, 1H J = 5.4, 12.5 Hz, 2-H), 3.95 (dd, 1H, J = 5.9, 13.8 Hz, 1-H). ^{13}C NMR ($CDCl_3$) δ 31.9 (C5), 34.9 (C3), 37.0 (C4), 66.1 (C6), 68.6 (C2), 76.5 (C1); HR-FAB-MS m/z 158.1739 calcd for $C_6H_{12}N_3O_2$ $[M+H]^+$, found m/z 158.1736.

(±)-1-(2-Hydroxy-4-hydroxymethyl-cyclopentyl)-1H-[1,2,3]triazole-4-carboxylic acid methyl ester (4). The mixture of **3** (101 mg, 0.64 mmol) and methylpropiolate (300 μL) was heated at 50 °C for 16 h. The reaction mixture was then cooled to ambient temperature and concentrated in vacuo. The residue was purified by silica gel column chromatography (10:1 CH_2Cl_2-MeOH) to give **4** (66%, 102.6 mg) as a colorless oil: ^1H NMR (CD_3OD) δ 1.86 (m, 1H, 4′-H), 1.99 (m, 2H, 3′-H), 2.47 (m, 2H, 5′-H), 3.55 (d, 2H, J = 5.9 Hz, 6′-H), 3.90 (s, 3H, OMe), 4.42 (ddd, 1H, J = 7.1, 7.3, 7.5 Hz, 2′-H), 4.75 (ddd, 1H, J = 7.3, 7.3, 7.3 Hz, 1′-H), 8.61 (s, 1H, 5-H); ^{13}C NMR (CD_3OD) δ 34.3 (C5′), 35.9 (C3′), 37.8 (C4′), 52.5 (OMe), 66.5 (C6′), 70.1 (C2′), 77.4 (C1′), 128.9 (C5), 140.3 (C6), 162.4 (C=O); UV λmax (MeOH) 214 nm (ε 11000); HR-FAB-MS m/z 242.1141 calcd for $C_{10}H_{16}N_3O_4$ $[M+H]^+$, found m/z 242.1143.

(±)-1-(2-Hydroxy-4-hydroxymethyl-cyclopentyl)-1H-[1,2,3]triazole-4-carboxylic acid amide (5). A solution of **4** (42.6 mg, 0.18 mmol) in MeOH (2.5 mL) saturated with NH_3 was stirred at 0 °C for 16 h. After evaporation of the volatiles, the residue was purified by silica gel column chromatography (10:1 CH_2Cl_2-MeOH) to give **5** (97%, 38.6 mg) as a colorless waxy solid: ^1H NMR (CD_3OD) δ 1.88 (m, 1H, 4′-H), 2.00 (m, 2H, 3′-H), 2.47 (m, 2H, 5′-H), 3.55 (d, 2H, J = 5.7 Hz, 6′-H), 4.41 (ddd, 1H, J = 7.1, 7.3, 7.3 Hz, 2′-H), 4.74 (ddd, 1H, J = 7.1, 7.3, 7.3 Hz, 1′-H), 8.44 (s, 1H, 5-H); ^{13}C NMR (CD_3OD) δ 34.3 (C5′), 36.0 (C3′), 37.8 (C4′), 66.5 (C6′), 70.0 (C2′), 77.4 (C1′), 126.8 (C5), 143.5 (C6), 164.8 (C=O); UV λmax (MeOH) 210 nm (ε 12135); HR-FAB-MS m/z 227.1144 calcd for $C_9H_{15}N_4O_3$ $[M+H]^+$, found m/z 227.1145.

(±)-5-Amino-1-(2-hydroxy-4-hydroxymethyl-cyclopentyl)-1H-[1,2,3]triazole-4-carboxylic acid amide (6). To a solution of 2-cyanoacetamide (80.2 mg, 0.95 mmol) in DMSO (1.0 mL) at rt was added K_2CO_3 (131.8 mg, 0.95 mmol) under an argon atmosphere. The mixture was stirred at the same temperature for 1 h. After the addition of DMSO solution (1.0 mL) of **3** (50.0 mg, 0.32 mmol), the stirring was continued for 16 h at 50 °C. After evaporation of the solvent, the residue was purified by silica gel column chromatography (5:1 CH_2Cl_2–MeOH) to give **6** (97%, 38.6 mg) as a colorless solid: ^1H NMR (CD_3OD) δ 1.86 (m, 1H, 4′-H), 1.98 (m, 2H, 3′-H), 2.44 (m, 2H, 5′-H), 3.56 (d, 2H, J = 6.2 Hz, 6′-H), 4.42 (ddd, 1H, J = 7.0, 7.1, 7.1 Hz, 2′-H), 4.53 (ddd, 1H, J = 6.8, 7.0, 7.1 Hz, 1′-H); ^{13}C NMR (CD_3OD) δ 32.8 (C5′), 36.0 (C3′), 37.9 (C4′), 65.5 (C6′), 66.4 (C2′), 76.5 (C1′), 122.9 (C5), 146.7 (C6), 166.7 (C=O); UV λmax (H_2O, pH 7) 234 nm (ε 8900), 261 (ε 8600); HR-FAB-MS m/z 242.1253 calcd for $C_9H_{16}N_5O_3$ $[M+H]^+$, found m/z 242.1253.

(±)-3-Iodomethyl-6-oxabicyclo[3.1.0]hexane (7). To a solution of **2** (1.02 g, 8.95 mmol) in anhydrous pyridine (10 mL) at 0 °C was added TsCl (2.21 g, 11.60 mmol). After being stirred for 2 h, the reaction mixture was filtered, washed with brine, dried over MgSO4 then concentrated in vacuo. The residue was purified by silica gel column chromatography (2:1 hexanes–EtOAc) to give the tosylate intermediate (90%, 2.16 g) as a yellow oil. ^1H NMR ($CDCl_3$) δ 2.01–2.10 (m, 4H, 3-H, 5-H), 2.46 (s, 3H, Me), 2.62 (m, 1H, 4-H), 3.94 (d, 2H, J = 7.2 Hz, 6-H), 5.62 (s, 2H, 1-H, 2-H), 7.37 (d, 2H, J = 8.1 Hz, H-aryl), 7.82 (d, 2H, J = 8.1 Hz, H-aryl). This compound was directly engaged into the next reaction. Thus, a suspension of tosylate intermediate (2.16 g, 8.07 mmol), NaI (1.57 g, 10.50 mmol) in anhydrous acetone (15 mL) was refluxed under Ar for 3 h. After filtration, the organics were concentrated under reduced pressure, and the residue was purified by silica gel column chromatography (2:1 hexanes–EtOAc) to give **7** (85%, 1.53 g) as a colorless oil. ^1H NMR ($CDCl_3$) δ 1.35–1.98 (m, 4H, 3-H, 5-H), 2.34 (m, 1H, 4-H), 3.26 (d, 2H, J = 6.3 Hz, 6-H), 3.50 (s, 2H, 1-H, 2-H); ^{13}C NMR ($CDCl_3$) δ 31.8 (C4), 33.6 (C3, C5), 56.7 (C1, C2), 58.2 (C6); HR-FAB-MS m/z 225.0395 calcd for $C_6H_{10}IO$ $[M+H]^+$, found m/z 225.0391.

***trans*-[2-(6-Oxa-bicyclo[3.1.0]hex-3-yl)-ethyl]-phosphonic acid diisopropyl ester (8)**. To a solution of diisopropylmethyl phosphonate (479 mg, 2.66 mmol) in THF (5.0 mL) at −78 °C was added dropwise 1.6 M *n*BuLi (1.66 mL, 2.66 mmol) in hexane under an argon atmosphere. The mixture was stirred at the same temperature for 1 h. After the addition of THF solution (4.0 mL) of **7** (542 mg, 2.42 mmol), the stirring was continued for another 2 h. The reaction mixture was poured into satd. aq NH_4Cl (10 mL), and extracted with CH_2Cl_2 (50 mL×3). The combined organic layers were washed with brine (50 mL), dried ($MgSO_4$), then concentrated in vacuo. The residue was purified by silica gel column chromatography (12:1 CH_2Cl_2-MeOH) to give **8** (90%, 601 mg) as a colorless oil: ^1H NMR ($CDCl_3$) δ 1.34 (d, 12H, J = 6.2 Hz, 4×Me), 1.15–2.07 (m, 7H, 3-H, 4-H, 5-H, 6-H), 2.26 (m, 2H, $\underline{CH_2}P$), 3.47 (s, 2H, 1-H, 2-H), 4.76 (m, 2H, 2 X $\underline{CH}(Me)_2$); ^{13}C NMR ($CDCl_3$) δ 24.0 (d, J = 2.9 Hz, $(\underline{Me})_2\overline{CH}$), 27.1 (d, J = 143 Hz, $\underline{CH_2}P$), 27.6 (C4), 33.4 (d, J = 18 Hz, C6), 31.8 (C3, C5), $\overline{56.7}$ (C1, C2), 68.7 (d, J = 6.5 Hz, $(Me)_2\underline{CH}$); HR-FAB-MS m/z 276.2931 calcd for $C_{13}H_{25}O_4P$ $[M+H]^+$, found m/z 276.2928.

(±)-[2-(3-Azido-4-hydroxy-cyclopentyl)-ethyl]-phosphonic acid diisopropyl ester (9). To a solution of **8** (228 mg, 0.83 mmol) in MeOH (24 mL) and H_2O (3.0 mL) was added NaN_3 (102 mg). After being stirred at refluxing temperature for 14 h, the reaction mixture was cooled to ambient temperature and concentrated in vacuo. The residue was diluted with CH_2Cl_2 (25 mL), and the

organic phase was washed with brine (30 mL), dried ($MgSO_4$), then evaporated. The residue was purified by silica gel column chromatography (10:1 CH_2Cl_2–MeOH) to give **9** (84%, 220 mg) as a colorless oil: ^1H NMR ($CDCl_3$) δ 1.20–1.36 (complex, 12H, 4×Me), 1.60–1.93 (complex, 9H, 3-H, 4-H, 5-H, 6-H, $\underline{CH_2}P$), 2.90 (brs, D_2O exchg, 1H, OH), 4.09 (ddd, 1H, $J=5.8$, 6.0, 7.5 Hz, 2-H), 3.71 (ddd, 1H, $J=5.8$, 6.0, 7.5 Hz, 1-H), 4.70 (dt, 2H, $J=6.1$, 12.5 Hz, 2 X $\underline{CH}(Me)_2$); ^{13}C NMR ($CDCl_3$) δ 24.0 (d, $J=4.2$ Hz, $(\underline{Me})_2CH$), 25.5 (d, $J=143$ Hz, $\underline{CH_2}P$), 29.1 (C4), 35.4 (C3), 35.6 ($J=3.6$ Hz, C6), 38.4 (C5), 68.5 (C2), 70.1 (d, $J=6.7$ Hz, $(Me)_2\underline{CH}$), 76.8 (C1); IR (film) ν_{max} 2101 (azide) cm^{-1}; HR-FAB-MS m/z 320.1739 calcd for $C_{13}H_{27}N_3O_4P$ $[M+H]^+$, found m/z 320.1736.

(±)-1-{4-[2-(Diisopropoxy-phosphoryl)-ethyl]-2-hydroxy-cyclopentyl}-1H-[1,2,3]triazole-4-carboxylic acid methyl ester (10). The mixture of **9** (245.6 mg, 0.77 mmol) and methylpropiolate (2.5 mL) was heated at 80 °C for 13 h. The reaction mixture was then cooled to ambient temperature and concentrated in vacuo. The residue was purified by silica gel column chromatography (10:1 CH_2Cl_2-MeOH) to give **10** (78%, 242.3 mg) as a colorless waxy solid. ^1H NMR ($CDCl_3$) δ 1.31 (d, 12H, $J=6.2$ Hz, 4×Me), 1.64–1.98 (complex, 7H, 3′-H, 4′-H, 5′-H, 6′-H), 2.35–2.56 (m, 2H, $\underline{CH_2}P$), 3.93 (s, 3H, OMe), 4.51 (m, 1H, 2′-H), 4.59–4.72 (complex, 3H, 1′-H, 2×$\underline{CH}(Me)_2$), 8.22 (s, 1H, 5-H); ^{13}C NMR ($CDCl_3$) δ 24.0 (d, $J=2.9$ Hz, $(\underline{Me})_2CH$), 25.4 (d, $J=143$ Hz, $\underline{CH_2}P$), 28.9 (C4′), 35.3 (d, $J=17.0$ Hz, C6′), 36.5 (C5′), 38.2 (C3′), 52.2 (OMe), 68.7 (C2′), 70.1 (d, $J=6.8$ Hz, $(Me)_2\underline{CH}$), 127.2 (C5), 141.4 (C6), 161.1 (C=O); HR-FAB-MS m/z 404.1951 calcd for $C_{17}H_{31}N_3O_6P$ $[M+H]^+$, found m/z 404.1944.

(±)-1-[2-Hydroxy-4-(2-phosphono-ethyl)-cyclopentyl]-1H-[1,2,3]triazole-4-carboxylic acid methyl ester (11): general procedure for deprotection. A solution of **10** (54.6 mg, 0.14 mmol) in CH_2Cl_2 (3.0 mL) was treated with bromotrimethylsilane (413.0 mg, 2.70 mmol) under argon atmosphere. The resulting solution was protected from light and stirred at room temperature for 72 h. The reaction mixture was concentrated to dryness and the residue was added MeOH (2 mL) and coevaporated. After evaporation of the solvent, the residue was triturated with Et_2O and dried to afford **11** (98%, 42.1 mg) as a waxy solid: ^1H NMR (CD_3OD) δ 1.70–1.95 (complex, 7H, 3′-H, 4′-H, 5′-H, 6′-H), 2.36 (m, 2H, $\underline{CH_2}P$), 3.91 (s, 3H, OMe), 4.45 (ddd, 1H, $J=6.0$, 6.1, 7.9 Hz, 2′-H), 4.77 (ddd, 1H, $J=6.1$, 6.8, 7.0 Hz, 1′-H), 8.63 (s, 1H, 5-H); ^{13}C NMR (CD_3OD) δ 28.6 (d, $J=133$ Hz, $\underline{CH_2}P$) 31.7 (C4′), 37.1 (d, $J=16.9$ Hz, C6′), 37.8 (C5′), 39.2 (C3′), 52.6 (OMe), 70.5 (C2′), 77.5 (C1′), 129.2 (C5), 140.0 (C6), 162.1 (C=O); UV λ_{max} (MeOH) 215 nm (ε 10,985); HR-ESI-MS m/z 341.0878 calcd for $C_{12}H_{19}N_2O_6NaP$ $[M+Na]^+$, found 341.0885.

(±)-{2-[3-(4-Carbamoyl-[1,2,3]triazol-1-yl)-4-hydroxy-cyclopentyl]-ethyl}-phosphonic acid diisopropyl ester (12). A solution of **10** (32.6 mg, 0.08 mmol) in MeOH (2.5 mL) saturated with NH_3 was stirred at 0 °C for 16 h. After evaporation of the volatiles, the residue was purified by silica gel column chromatography (10:1 CH_2Cl_2–MeOH) to give **12** (97%, 38.6 mg) as a colorless waxy solid: ^1H NMR ($CDCl_3$) δ 1.31 (d, 12H, $J=5.5$ Hz, 4×Me), 1.62–1.98 (complex, 7H, 3′-H, 4′-H, 5′-H, 6′-H), 2.33 (m, 2H, $\underline{CH_2}P$), 4.46 (m, 1H, 2′-H), 4.60–4.73 (complex, 3H, 1′-H, 2×$\underline{CH}(Me)_2$), 8.39 (s, 1H, 5-H); ^{13}C NMR ($CDCl_3$) δ 24.0 (d, $J=2.8$ Hz, $(\underline{Me})_2CH$), 25.4 (d, $J=143$ Hz, $\underline{CH_2}P$), 28.8 (C4′), 35.3 (d, $J=17.5$ Hz, C6′), 36.5 (C5′), 38.2 (C3′), 68.9 (C2′), 70.2 (d, $J=6.7$ Hz, $(Me)_2\underline{CH}$), 77.5 (C1′), 125.9 (C5), 142.2 (C6), 162.5 (C=O); HR-FAB-MS m/z 389.1954 calcd for $C_{16}H_{30}N_4O_5P$ $[M+H]^+$, found m/z 389.1958.

(±)-{2-[3-(4-Carbamoyl-[1,2,3]triazol-1-yl)-4-hydroxy-cyclopentyl]-ethyl}-phosphonic acid (13). **Method A.** A solution of **11** (22.1 mg, 0.06 mmol) in CH_2Cl_2 (3.0 mL) was treated with bromotrimethylsilane (166.4 mg, 1.09 mmol) under argon atmosphere. The resulting solution was protected from light and refluxed for 14 h. The reaction mixture was concentrated to dryness and the residue was added to MeOH (2 mL) and coevaporated. After evaporation of the solvent, the residue was triturated with Et_2O and dried to afford **13** (94%, 15.5 mg) as a waxy solid.

Method B. A solution of **12** (25.1 mg 0.08 mmol) in MeOH (2.5 mL) saturated with NH_3 was stirred at 0 °C for 14 h. After evaporation of the solvent, the residue was triturated with Et_2O and dried to afford **13** (99%, 23.7 mg) as a waxy solid: ^1H NMR (CD_3OD) δ 1.53–1.89 (complex, 7H, 3′-H, 4′-H, 5′-H, 6′-H), 2.28-(m, 2H, $\underline{CH_2}P$), 4.38 (ddd, 1H, $J=6.6$, 6.8, 7.1 Hz, 2′-H), 4.71 (ddd, 1H, $J=6.8$, 7.1, 7.1 Hz, 1′-H), 8.43 (s, 1H, 5-H); ^{13}C NMR (CD_3OD) δ 28.6 (d, $J=133$ Hz, $\underline{CH_2}P$), 31.7 (C4′), 37.1 (d, $J=16.9$ Hz, C6′), 38.2 (C5′), 39.5 (C3′), 70.4 (C2′), 77.7 (C1′), 126.8 (C5), 143.4 (C6), 164.8 (C=O); UV λmax (MeOH) 210 nm (ε 11935); HRESIMS m/z 305.1015 calcd for $C_{10}H_{18}N_4O_5P$ $[M+H]^+$, found 305.1018.

(±)-{2-[3-(5-Amino-4-carbamoyl-[1,2,3]triazol-1-yl)-4-hydroxy-cyclopentyl]-ethyl}-phosphonic acid diisopropyl ester (14). To a solution of 2-cyanoacetamide (27.1 mg, 0.32 mmol) in DMSO (1.0 mL) at rt was added K_2CO_3 (44.4 mg, 0.32 mmol) under an argon atmosphere. The mixture was stirred at the same temperature for 30 min. After the addition of DMSO solution (1.0 mL) of **9** (51.4 mg, 0.16 mmol), the stirring was continued for 5 h at 50 °C. After evaporation of the solvent, the residue was purified by silica gel column chromatography (15:1 CH_2Cl_2–MeOH) to give **14** (71%, 46.1 mg) as a colorless solid: ^1H NMR ($CDCl_3$) δ 1.33 (d, 12H, $J=6.2$ Hz, 4×Me), 1.60–1.93 (complex, 7H, 3′-H, 4′-H, 5′-H, 6′-H), 2.40–2.48 (complex, 2H, $\underline{CH_2}P$), 4.36–4.55 (complex, 2H, 1′-H, 2′-H), 4.65 (complex, 2H, 2×$\underline{CH}(Me)_2$); ^{13}C NMR ($CDCl_3$) δ 23.8 (d, $J=2.7$ Hz, $(\underline{Me})_2CH$), 25.6 (d, $J=142$ Hz, $\underline{CH_2}P$), 28.7 (C4′), 35.8 (d, $J=17.5$ Hz, C6′), 36.7 (C5′), 37.9 (C3′), 69.2 (C2′), 70.3 (d, $J=6.8$ Hz, $(Me)_2\underline{CH}$), 71.4 (C1′), 118.8 (C5), 142.2 (C6), 163.8 (C=O); HR-FAB-MS m/z 404.2063 calcd for $C_{16}H_{31}N_5O_5P$ $[M+H]^+$, found m/z 404.2065.

(±)-{2-[3-(5-Amino-4-carbamoyl-[1,2,3]triazol-1-yl)-4-hydroxy-cyclopentyl]-ethyl}-phosphonic acid (15). A

solution of **14** (21.4 mg, 0.05 mmol) in CH_2Cl_2 (3.5 mL) was treated with bromotrimethylsilane (163.3 mg, 1.06 mmol) under argon atmosphere. The resulting solution was protected from light and refluxed for 14 h. The reaction mixture was concentrated to dryness and the residue was added to MeOH (3 mL) and coevaporated. After evaporation of the solvent, the residue was triturated with Et_2O and dried to afford **15** (98%, 16.6 mg, 0.05 mmol) as a waxy solid : ^{1}H NMR (CD_3OD) δ 1.75–1.94 (complex, 7H, 3′-H, 4′-H, 5′-H, 6′-H), 2.36–2.52 (m, 2H, $\underline{CH_2}$P), 4.40–4.56 (complex, 2H, 1′-H, 2′-H); ^{13}C NMR (CD_3OD) δ 28.8 (d, J = 132 Hz, $\underline{CH_2}$P), 31.4 (C4′), 36.8 (d, J = 16.9 Hz, C6′), 38.3 (C5′), 39.2 (C3′), 70.7 (C2′), 77.4 (C1′), 118.2 (C5), 142.9 (C6), 164.5 (C=O); UV λ_{max} (H_2O, pH 7) 235 nm (ε 9100), 260 (ε 9150); HR-ESI-MS m/z 320.1124 calcd for $C_{10}H_{19}N_5O_5P$ $[M+H]^+$, found 320.1112.

Antiviral and cytotoxicity assays for HIV-1 and HBV

Antiviral and cytotoxicity assays were conducted as described recently by Stuyver et al.[14] HIV and HBV assays were performed in activated primary human peripheral blood mononuclear cells (PBM) and AD38 cells (derived from 2.2.15HepG2 cells), respectively.

Antiviral and cytotoxicity assays for HCV

Cell culture system for HCV replication. Huh-7 cells harbouring the subgenomic HCV replicon BM4-5 were kindly provided by Dr. C. Seeger.[15] Cells were maintained in Dulbecco′s modified Eagle′s medium (DMEM) (Life Technologies) supplemented with 10% fetal bovine serum, 1% L-glutamine, 1% L-pyruvate, 1% penicillin and 1% streptomycin supplemented with 500 μg/mL G418 (Geneticin, Invitrogen). Cells were passaged every 4 days.

Cytotoxicity assays for HCV. Huh-7 cells were respectively seeded at a density of 3×10^4 cells/well in 96-well plates for the cell-viability assay, or at a density of 6×10^5 cells/well in six-well plates for the antiviral assay. Sixteen hours post seeding, cells were treated with the compounds of interest (**4**, **5**, **6**, **11**, **13**, **15**) at various concentrations (0; 0.312; 0.625; 1.25; 2.5; 5; 10; 20; 40; 80; 160; 320 μM) for 3 days. The administration of each drug was renewed each day. The medium used during the treatment phase did not contain the selective drug G418, as the latter could interfere with the assay. Other drugs, including ribavirin (ICN Pharmaceuticals, USA), mycophenolic acid (Sigma, USA), and interferon alpha-2b (IntronA) were used in the same conditions as positive controls. The concentrations used for these drugs were respectively: 0; 0.5; 1; 2; 4; 8; 16; 32; 64; 128; 256, and 512 μM for ribavirin, 0; 2.5; 5; 10; 20; 40 μM for mycophenolic acid, and 0; 0.1; 1; 10; 100 and 1000 UI/mL for IFN α-2b. At the end of treatment, cell viability assays were performed with the 96-well plates using Neutral Red assay (Sigma).

Antiviral Assays for HCV. Total RNA (tRNA) was extracted from six-well plates with the 'Extract All' reagent (Eurobio), which is a mixture of guanidinium thiocyanate–phenol–chloroform. Northern Blot analysis was then performed using the NorthernMax^{TM}-Gly (Ambion) kit, following manufacturer's instruction. Ten micrograms of tRNA was denatured in glyoxal buffer at 50 °C for 30 min and separated by agarose gel electrophoresis, then transferred for 12 h onto a charged nylon membrane (Biodyne B, Merck Eurolab). Hybridisation was carried out with three different [^{32}P]CTP-labelled riboprobes obtained by in vitro transcription (Promega). These probes were complementary to the NS5A region of the HCV genome, and to the cellular gene GAPDH, respectively. First, the blot was hybridized with two riboprobes directed against the negative strand of HCV RNA and the GAPDH mRNA, respectively. After one night of hybridization at 68 °C, the membrane was washed then exposed to X-ray film and a phosphor screen for quantitative analysis. The amount of GAPDH mRNA was used as an internal loading control to standardise the amount of HCV RNA detected. The same membrane was subsequently hybridized with a negative-sense riboprobe to determine the level of HCV-positive strand RNA using the same approach.

Antiviral and cytotoxicity assays for HSV. The newly synthesized nucleosides were evaluated for activity against HSV-1 (strain F) by plaque reduction assay in Vero cells, using methodologies described previously.[16] Cytotoxicity assays were conducted in rapidly dividing Vero cells, as previously described.[16] The median effective concentration was determined by the median effect method.[17]

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